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NEUROENDOCRINE AND AUTONOMIC REGULATION OF INGESTIVE BEHAVIOR IN THE GOLDEN HAMSTER

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NEUROENDOCRINE AND AUTONOMIC REGULATION OF INGESTIVE BEHAVIOUR IN THE GOLDEN HAMSTER (*Mesocricetus auratus*)

By

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ABSTRACT

Certain prandially released peptide hormones have been proposed to act as physiological satiety stimuli. It has been demonstrated in a variety of species that injection of these hormones reduces feeding in otherwise hungry animals. These peptides, however, were not previously tested in Syrian hamsters, a species known to have physiological feeding controls different from those of many other animals. The first series of experiments examined whether putative satiety hormones reduce feeding in this species. Feeding in fasted hamsters was measured after peripheral injections of cholecystokinin octapeptide (CCK-8), bombesin (BBS), thyrotropin releasing hormone (TRH), and calcitonin (CT). Each of these peptides reduced feeding in hamsters, but with varying degrees of specificity. CCK-8 and BBS, but not TRH and CT, appeared to reduce feeding specifically. Intraventricular CCK-8, BBS, and CT also decreased feeding in fasted hamsters. Effective central doses of BBS and CT were considerably lower than effective peripheral doses, thus indicating a central site of action for these peptides. Comparable amounts of CCK-8 were required to suppress feeding by either intraventricular or peripheral routes of administration.

In a second series of experiments it was found that peripheral CCK-8 injections also reduced gastric emptying. Since gastric distention is a well-known satiety stimulus which is enhanced by reduced gastric emptying, it was possible that CCK-8 reduced feeding indirectly by facilitating gastric distention. The sham-feeding paradigm, in which orally ingested liquid diet passes out of a gastric fistula (thereby eliminating gastric distention), was used to assess this possibility.

The relative efficacy of peripherally injected CCK-8 was tested in hamsters with chronic gastric fistulas during sham- and real-feeding (the fistula is closed and the stomach distends) sessions. Although CCK-8 did decrease sham-feeding, the magnitude of the decrement was small and not statistically reliable. In contrast, peripheral injections of CCK-8 produced a robust and reliable suppression of real-feeding. It is concluded that inhibition of gastric emptying and the ensuing gastric distention contribute to the satiety effect of peripherally administered CCK.

A third series of experiments investigated the role of the abdominal vagus in CCK-8's inhibition of feeding. Total abdominal vagotomy completely blocked feeding suppression by a relatively low intraperitoneal dose of CCK-8. This blocking effect appeared to be specific to CCK-8, as vagotomized hamsters showed the usual feeding suppression in response to peripherally administered doses of BBS, CT, and TRH. In a second experiment vagotomized hamsters were completely unresponsive to relatively low doses of CCK ($\leq$ 6.0 µg/kg) but did show feeding suppression in response to larger doses ($\geq$ 8.0 µg/kg). Selective gastric vagotomy also attenuated feeding suppression in response to CCK-8 but not as effectively as total abdominal vagotomy. These findings suggest that vagal fibres (probably afferents) are necessary for feeding suppression following low doses of exogenous CCK, and by inference, for feeding suppression by endogenously released gut-CCK. The gastric division of the vagus plays a major role in CCK's inhibition of feeding, but other abdominal divisions are also relevant. Extra-vagal sites of CCK action in the control of feeding (possibly in

the brain) are indicated, since vagotomized hamsters reduced feeding after the larger CCK-8 doses.

These findings in the hamster are discussed in relation to the sites and mechanisms of peptide action in the control of feeding in other species.

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INTRODUCTION

Cholecystokinin and Other Peptide Hormones as Putative Satiety Stimuli

The regulation of energy balance by organisms is an extraordinary biological function. The constancy of body weight (an index of energy regulation) in adults of many mammalian species, including people, over long periods is testimony to the accuracy with which energy input and expenditure are balanced (Le Magnen, 1983). That energy input and output can be regulated in spite of very different temporal characteristics on each side of the regulatory equation (i.e., tissues require a continuous supply of metabolic fuels whereas feeding is episodic) makes such regulation still more remarkable. It is appreciated that energy regulation is achieved by regulatory mechanisms on each side of the input/output equation. Mechanisms governing energy expenditure make a substantive contribution to the overall regulation of energy balance (e.g. quantitative and qualitative variations in food intake produce appropriate shifts in metabolic rates [Rothwell and Stock, 1980]). However, it is generally accepted that food intake is the primary effector of body energy balance (Le Magnen, 1983; Mrosovsky and Powley, 1980).

The study of the mechanisms that accomplish body energy regulation is a multidisciplinary endeavour. To physiologic.l psychologists behaviour is the principal concern. Physiological psychologists interested in energy balance have directed their experiments towards

revealing the physiological determinants of feeding behaviour. Historically, this endeavour has emphasized the study of stimuli which elicit feeding or putative stimuli for hunger. Despite a long experimental history, this approach has not yet clearly determined which physiological stimuli are sufficient and necessary for initiating feeding (Smith, 1982; Gibbs and Smith, 1984). Although stimuli arising from certain experimental manipulations (e.g. 2-deoxy-D-glucose induced glucodeprivation and insulin induced hypoglycemia) do reliably elicit feeding (at least in most species, see below), the relevance of these stimuli to episodic feeding has been questioned (Smith, 1982). It has been argued, for example, that feeding elicited by such stimuli is tantamount to an emergency response to conditions which rarely, or may never, confront animals (Mogenson and Phillips, 1975). It is therefore unlikely that stimuli arising from these extreme states of glucodeprivation have to do with the initiation of meals in animals feeding ad libitum. In view of the failure of a vast number of attempts at isolating the adequate stimuli for hunger (but see Louis-Sylvestre and Le Magnen, 1980), Smith (1982) suggests the study of the end point of feeding, satiety, as an alternative and perhaps more fruitful approach to examining how food intake is regulated.

In advocating the study of satiety, Smith and Gibbs (Smith, 1982; Gibbs and Smith, 1984) maintain that the primary advantage of the study of satiety over that of hunger, is that the sole stimulus for satiety, food, is known. This is somewhat of a misleading point because ingestion of food elicits a barrage of physiological responses, of which only a proportion may serve as intermediary satiety stimuli. However,

from an experimental point of view, the study of satiety does offer certain strategic advantages (Smith, 1982). For example, satiety has a short time course which not only makes it relatively easy to observe in the laboratory, but also makes possible a causal analysis of its antecedents. Moreover, satiety has the characteristics of a control system with a negative feedback loop. The advantage here is that the substrates and physiology of satiety can be studied within the conceptual framework of a reflex arc.

As noted above, food acting at various sites throughout the alimentary tract is capable of generating stimuli which could conceivably function as satiety signals. Isolating those physiological responses to food which are relevant to satiety is a formidable task. A variety of methods are now being used to investigate this problem (see discussions in Experiments 7, 8, and 9). The data available so far suggest that food-related stimuli of oropharyngeal, gastric, intestinal, and postabsorptive origins all contribute and interact to terminate feeding bouts (see Smith and Gibbs, 1979; Gibbs and Smith, 1984 for reviews). In recent years, work has focused on the role of peptide hormones, particularly those of gastrointestinal origin, as possible mediators of satiety. The fact that many of these peptides are released by specialized gut endocrine/paracrine cells in response to feeding (Bloom and Polak, 1981) make some of these peptides likely candidates for physiological negative feedback stimuli which curtail further feeding. A partial list of peptide hormones released into the local and general circulation, and suspected to play some role in satiety is provided in Table I. Aside from possibly regulating food intake and

TABLE I

Peripheral Sources of Candidate Satiety Peptides

Peptide	Site of Release*
Calcitonin	C Cells of the Thyroid
Pancreatic Polypeptide	Pancreas
Glucagon	Pancreas, Small Intestine, Large Intestine
Somatostatin	Pancreas, Stomach
Vasoactive Intestinal Peptide	Pancreas, Stomach, Small Intestine
Gastrin	Stomach
Cholecystokinin	Small Intestine
Gastric Inhibitory Peptide	Small Intestine
Secretin	Small Intestine
Neurotensin	Small Intestine
Gastrin Releasing Peptide/Bombesin	Stomach, Small Intestine, Large Intestine
Thyrotropin Releasing Hormone	Pancreas, Small Intestine, Large Intestine
Substance P	Small Intestine Large Intestine

*See Bloom and Polak (1981) for details.

satiety, these peptides have "classical" functions related to digestion and metabolism, including fluid and electrolyte secretion, digestive enzyme secretion, growth, endocrine secretion, and intestinal absorption.

An interesting development in the recent past has been the discovery that many of the peptide hormones initially isolated from the gut are also localized in the central nervous system (CNS). Conversely, many of established CNS peptides were subsequently discovered in peripheral tissues (e.g. thyrotropin releasing hormone, somatostatin). There is good evidence that some peptides may be synthesized in the CNS and function as neurotransmitters or neuromodulators. Thus, the possibility arises that peptide hormones from both sources could contribute to the control of feeding and satiety.

A first step in determining the relevance of these peptides to satiety is to test whether administration of exogenous peptide can hasten the onset of satiety by reducing food intake in hungry animals. Many peptides have been tested in this fashion. The results of representative studies in the rat are presented in abbreviated form in Table II. As can be seen in the table, a number of peptides reduce food intake in rats. In addition to peptides that reduce feeding, there is a growing body of evidence for a facilitative influence of opioid peptides on feeding (see Morley, Levine, Yim, and Lowy, [1983] and Olson, Olson, and Kastin [1983] for recent reviews).

Although tests of exogenous peptides on food intake serve as a useful screening device, the demonstration that administration of exogenous peptide can reduce feeding, taken alone, is not sufficient

TABLE II

Some Peptides That Reduce Feeding in Rats

and Proposed Site of Action

Peptide	Proposed Site of Action*	Reference
Cholecystokinin	Peripheral Central	Smith et al., 1981b Faris et al., 1983b
Bombesin/Gastrin Releasing Peptide	Peripheral and Central	Kulkosky et al., 1982b Stein and Woods, 1983
Calcitonin	Central	Twery et al., 1982
Somatostatin	Peripheral	Woods et al., 1981
Thyrotropin Releasing Hormone	Peripheral Central	Morley et al., 1982a Lin et al., 1983
Neurotensin	Central	Stanley et al., 1983
Glucagon	Peripheral	Geary and Smith, 1983

* i.e., localization of the receptors mediating the effect on feeding, central vs. peripheral receptors.

evidence for a physiological role of the peptide as a mediator of satiety. It is universally agreed that a peptide must be rigorously tested before it can be accepted as a physiological feedback stimulus for the cessation of feeding (see below). While the list of peptides that reduce feeding is long, only one peptide, cholecystokinin (CCK), has been subjected to intensive and rigorous testing.

CCK is a peptide hormone released from duodenal endocrine cells in response to food (amino and fatty acids in particular). CCK was originally discovered in the 1920's and named for its action on the gallbladder (Ivy and Oldberg, 1928). During the 1940's, CCK was rediscovered as pancreozymin for its ability to stimulate pancreatic enzyme secretion (Harper and Raper, 1943). It was only after it was isolated from porcine intestinal mucosa and sequenced as a 33 amino acid peptide in the 1960's, that it became apparent that CCK and pancreozymin were one and the same (Mutt and Jorpes, 1968). Since then, radioimmunological and chromatographical studies have indicated that CCK circulates in various molecular forms, those with 4, 8, 12, 33, and 39 amino acid residues (Maton, Selden, and Chadwick, 1982). CCK is also widely distributed in the CNS, predominantly as the carboxyl terminal octapeptide (CCK-8). CCK-containing perikarya and terminals are well represented in systems across the neuroaxis (Beinfeld, Meyer and Brownstein, 1981; Vanderhaeghen, Lotstra, Vierendeels, Gilles, Deschepper, and Verbanck, 1981). High affinity CCK receptor binding in the CNS generally conforms to the distribution of CCK-containing terminals (Zarbin, Innis, Wamsley, Snyder, and Kuhar, 1983). There is good neurophysiological and pharmacological evidence that CCK acts as a

neurotransmitter or neuromodulator at synaptic junctions (reviewed by Beinfeld, 1983). Its wide distribution within the CNS suggests a role for CCK in a variety of functions. Indeed, evidence presented thus far indicates that CCK may do much more than signal satiety and co-ordinate gut activity. Morley (1982) has reviewed the literature on classical and other CCK actions. Table III lists some of the more recently reported effects of exogenous CCK. Most of the behavioural work has focused on the role of CCK as a controller of food intake and mediator of satiety. Because CCK was among the first peptides to be studied in the context of feeding and because it has been the most extensively studied peptide, studies of CCK and feeding have become prototypes on which subsequent studies of other peptide hormones are based.

Several researchers have formulated similar sets of criteria that a peptide must meet before acceptance as a physiologically relevant and biologically significant mediator of satiety (e.g., Mueller and Hsiao, 1978; Smith and Gibbs, 1981). The pivotal criteria can be described as follows: The peptide should be released from endocrine cells as a consequence of feeding. Physiological amounts of exogenous peptide should reduce feeding, but more than feeding inhibition must be demonstrated before acceptance as a satiety peptide. Exogenous peptide administration should also facilitate the occurrence of satiety-related behaviours. A correlation between the endogenously released peptide and naturally occurring satiety must also be established. In this instance, manipulation of the release and the action of endogenously released peptide should produce appropriate shifts in feeding behaviour. Lastly, broad biological significance and practical utilization of knowledge

TABLE III

Recently Reported Effects of Cholecystokinin

on Physiology and Behaviour

Response	Species	Proposed Receptor Localization	Reference
Hypothermia	Rats	Central	Morley and Levine 1980
	Mice	Central	Zetler, 1982
Hyperthermia	Guinea Pigs	Central	Kandasmay and Williams, 1983
Increased respiratory activity	Cats	Central	Pagani et al., 1982
Decrease in the amplitude of rumen contractions	Sheep	Central	Della-Fera and Baile, 1980a
Accelerated gastric emptying	Dogs	Central	Papas et al., 1984
Decreased frequency of small intestine contraction	Rats	Central	Bueno and Ferre, 1982
Hyperglycemia	Rats	Central	Morley and Levine, 1980
Increased plasma corticosterone	Rats	Peripheral	Itoh et al., 1982a
Inhibition of prolactin release	Rats	Peripheral	Hodson et al., 1984

TABLE III (cont'd)

Response	Species	Proposed Receptor Localization	Reference
Increased plasma luteinizing hormone	Rats	Central	Kimura et al., 1983
Vasodilation and decreased renal-renin output	Rabbits	Peripheral	Calam et al., 1982
Depressed somato-motor reflex	Rats	Peripheral	Kawasaki et al., 1983
Analgesia	Rats	Central	Jurna and Zetler, 1981
Hyperalgesia	Rats	Central	Faris et al., 1983a
Antagonism of β-endorphin induced analgesia	Rats	Central	Itoh et al., 1982b
Enhanced passive avoidance learning	Rats	Central	Kadar et al., 1981
Reduced explor-atory and social behaviours	Mice Rats	Peripheral Peripheral	Crawley et al., 1981b Crawley et al., 1982
Antipsychotic effects in schizophrenics	Humans	Central	Nair et al., 1982

about satiety systems demand that these results be consistent across species. The necessity for these criteria will become clear below, in a detailed assessment of how well CCK has met them.

Obviously, if a hormone is to be a considered as a physiological satiety stimulus, it should be released during feeding. In species where it has been possible to measure circulating CCK, serum CCK levels show a dramatic rise within minutes after a meal (Maton et al., 1982, for humans; Fried, Odgen, Swierczek, Greeley, Rayford, and Thompson, 1983, for dogs). Thus, in certain species where circulating CCK levels have been measured, CCK has unambiguously met this criterion. In the species tested, peripheral administration of CCK suppresses feeding in a dose-related manner (see Table IV). Feeding suppression is specific to the biologically active sulfated C-terminal octapeptide or larger forms having this sequence. Desulfated CCK-8 has no, or a dramatically diminished, effect on feeding (e.g. Experiments 1 and 6). Thus, the effects of CCK on feeding show, at the very least, chemical specificity. However, in most species, for example the rat, it is not known whether the doses which reduce feeding are within physiological limits. For most species, it has been difficult or impossible to accurately assay total blood CCK levels because of the many forms of circulating CCK and because antibodies directed against CCK cross-react with gastrin, a structurally related peptide. Hence, an assessment of whether the amounts of exogenous CCK required to reduce feeding in small animals are physiological awaits the development of a sensitive assay that will provide crucial data on amounts released during feeding.

To know whether physiological amounts of exogenous CCK (or any

TABLE IV

Species in Which CCK is Reported to Reduce Feeding

Species	Route of Administration*	Reference
Rats	ip., i.v. i.c.	Gibbs and Smith, 1984 Faris et al., 1983b
Mice	i.p.	McLaughlin and Baile, 1981
Rabbits	i.v.	Houpt et al., 1978
Pigs	i.v. ICV	Houpt, 1983 Parrot and Baldwin, 1981
Sheep	i.v. ICV	Grovum, 1981 Della-Fera and Baile, 1979
Dogs	i.v.	Levine et al., 1984
Chickens	i.v. ICV	Savory and Gentle, 1983 Denbow and Myers, 1982
Hamsters	i.p. ICV	present investigation
Rhesus Monkeys	i.v.	Metzer and Hansen, 1983
Humans	i.v.	Stacher et al., 1982

* i.c. = intracranial

i.p. = intraperitoneal

i.v. = intravenous

ICV = intracerebroventricular

other peptide) effectively reduce feeding is the clearest means of establishing whether endogenously released CCK serves its hypothesized function. Although it is not yet known whether the doses that affect feeding in rats and other small mammals are physiological, other lines of research have established a link between endogenous CCK and satiety. It has been possible to manipulate the release of CCK and access of CCK to its receptor and measure changes in feeding in a manner consistent with its hypothesized role as a satiety peptide.

The release of endogenous CCK has been manipulated by several means. As noted earlier, endogenous CCK is released by proteins and fats contacting the duodenal mucosa. One of the classical actions of CCK is the release of pancreatic enzymes. The l- but not d-isomer of phenylalanine (L-PHE and D-PHE, respectively), is a potent releaser of pancreatic enzymes, its effect presumably mediated by the release of endogenous CCK (Meyer and Grossman, 1972). Presumptive stimulation of duodenal CCK release with gastric loads of L-PHE but not D-PHE, produced the predicted suppression of food intake in several rat strains (Anika, Houpt, and Houpt, 1977; McLaughlin, Peikin, and Baile, 1983a) and infrahuman primates (Gibbs, Falasco, and McHugh, 1976). Likewise, direct intraduodenal preloading (which circumvents the possible confounding effect of activation of gastric satiety mechanisms) with L-PHE reduces feeding in rats (Lew, Gibbs, and Smith, 1983). Other putative releasers of duodenal CCK have had similar effects on feeding. In pigs, intraduodenal infusions of sodium oleate or protein hydrolysate also reduced food intake (Anika, Houpt and Houpt, 1981). The addition of a local anaesthetic to the infusates, which would inhibit the release

of intraduodenal CCK, blocked or substantially diminished the satiety effect of both CCK secretagogues (Anika et al., 1981).

The release of CCK from the small intestine is subject to negative feedback from pancreatic enzymes (Schneeman and Lyman, 1975). One would expect that manipulation of these feedback mechanisms so as to alter the secretion of endogenous CCK would result in shifts in food intake appropriate with the view that CCK is a physiological satiety hormone. Gastric preloads with trypsin and pancrease (pancreatic enzymes which feedback to small intestine to inhibit further CCK release) have been shown to augment feeding in Zucker fatty and lean rats (McLaughlin et al., 1983a). It was also shown that preloading the stomach with inhibitors of trypsin (aprotinin and DGPM) had the reverse effects on feeding in Zucker rats (McLaughlin et al., 1983a; McLaughlin, Peikin, and Baile, 1983c).

Proglumide, a putative CCK and gastrin antagonist (Hahne, Jenson, Lemp, and Gardner, 1981), has also been shown to alter feeding in rats. Intraperitoneal proglumide blocks the satiety effect of exogenous CCK (e.g. Collins, Walker, Forsythe, and Belbeck, 1983); and administered intragastrically, proglumide increases spontaneous feeding (McLaughlin, Peikin, and Baile, 1983b). Further, Zucker rats auto-immunized against CCK eat more and consequently weigh more than non-immunized controls (McLaughlin, Buonomo, and Baile, personal communication). Collectively, the experiments cited above have consistently shown that animal feeding is sensitive to manipulations expected to alter the secretion of endogenous CCK or CCK's capacity to act on its receptor.

Postprandial satiety is associated with a complex of behaviours

that ordinarily occur at the end of a meal. In rats, hamsters, and other species these behaviours typically occur in the following sequence: grooming, exploratory behaviour, sedation, and sleep (see Smith and Gibbs, 1979 for further details). If a peptide hormone serves as a physiological link between the ingestion of food and satiety, one should expect the experimental administration of the peptide to yield the behaviours normally associated with satiety. In their classic paper, Antin, Gibbs, Holt, Young, and Smith (1975) presented a series of experiments showing that exogenous CCK not only suppressed feeding, but that the cessation of feeding was followed by the satiety sequence that ordinarily occurs after feeding. In contrast, when feeding was artificially curtailed by an aversive stimulus (quinine adulteration of the test diet), the constellation of satiety-related behaviours was not observed. These findings have since been confirmed and extended in other laboratories (Crawley, Rojas-Ramirez, and Mendleson, 1982; Mansbach and Lorenz, 1983).

Control of feeding by a peptide hormone should not be an idiosyncracy of one or a few species. Biological significance requires the peptide to operate in a consistent manner across species having comparable feeding habits and a similar digestive/neuroendocrine system synthesizing and releasing the hormone (Mueller and Hsiao, 1978; Anika et al., 1981; Smith and Gibbs, 1981; Smith, Gibbs, Jerome, Pi-Sunyer, Kissileff, and Thorton, 1982). It can be seen in Table IV, that CCK reduces feeding in a wide range of species.

A major problem frequently encountered in feeding studies is interpreting quantitative changes in feeding behaviour. Often, and

perhaps many times mistakenly so, subjective states like "hunger" and "satiety" are inferred from feeding data. The problem is not the question of whether or not animals "feel" hungry or satiated, but rather that animals may sometimes eat in the absence of hunger (e.g. stress-induced eating [Rowland and Marques, 1980]) and may stop eating for reasons other than satiety (e.g. as during gastrointestinal discomfort). It is exactly this that necessitated the formulation of many of the criteria used to assess whether or not a peptide hormone is a true mediator of satiety. Attuned to the problem of possible misinterpretation of peptide-induced feeding innibition, workers have generally shown appropriate caution in considering alternative explanations of peptide-produced feeding inhibition. For CCK's inhibition of feeding, Smith, Gibbs, and their associates at Cornell, have made painstaking attempts to prove that hypothetical alternatives to true satiety are not responsible for the observed suppression of feeding (e.g., Gibbs et al. 1973; Kraly, Carty, Resnick, and Smith, 1978). Some workers have nonetheless challenged the generally accepted interpretation that CCK-produced feeding suppression represents a facilitation of satiety. Critics of the CCK and feeding work have raised the possibility, that instead, CCK works to reduce feeding by inducing malaise and/or gastrointestinal discomfort (Deutsch, Thiel, and Greenburg, 1978; Swerdlow, van der Kooy, Koob, and Wenger, 1983). However, in the animal work, no investigator has reported that "reasonable" doses (in and above the suspected physiological range), which reduce feeding, produce overt symptoms of malaise. Of course, it is possible that CCK induces a subtle form of sickness that is not

directly visible to the experimenter.

The conditioned taste aversion paradigm has been used as one means of addressing the issue of the specificity of CCK's reduction of food intake. In such studies CCK is paired with the ingestion of a novel flavoured solution, and the avoidance of, or preference for, that flavoured solution is measured in subsequent tests. In some studies it has been shown that rats learn to avoid flavoured solutions previously paired with exogenous CCK (e.g. Deutsch and Hardy, 1977). Recently, Swerdlow et al. (1983) have demonstrated that rats also learn to avoid an experimental chamber in which they were previously treated with CCK. However, a number of other investigators have shown that CCK doses which reduced feeding, in their hands, did not support conditioned taste aversion learning (Gibbs, Young, and Smith, 1973 [rat]; Kraly et al., 1978 [rat]; Houpt, Anika, and Wolff, 1978 [rabbit]; Anika, Houpt, and Houpt, 1981 [pig]). The reasons for these discrepancies are not clear, but may be related to procedural differences among the studies (e.g. species and dose differences, variations in measurement of preference for the test solution, etc.). Conditioned taste aversion experiments have done little to resolve the matter of the specificity of CCK's effects on feeding. In view of the fact that CCK, under some conditions, can support flavour aversion learning, it is possible that CCK has aversive properties, aside from its capacity to reduce feeding. However, it remains to be proven that any possible aversive consequences of exogenous CCK and the accompanying reductions in food intake are causally related.

Other lines of evidence suggest that CCK's effects on feeding are

unlikely to be mediated by malaise. In rats, CCK does not appear to alter appetitive aspects of feeding behaviour. CCK-treated rats previously trained to lever press for food reinforcement do not reduce operant responding during extinction (Gosnell and Hsiao, 1981). Likewise, moderate doses which suppress feeding do not alter runway performance in animals previously trained to run for food (Cox, Toney, and Wiebe, 1983). These findings show that exogenous CCK does not alter rats' motivation to begin eating, but instead, accelerates the satiety process. One would expect a sick animal to be less motivated to eat. Furthermore, CCK's effects on ingestive behaviour, in most species, appear to be specific to feeding. For instance, intraperitoneal CCK in rats (Gibbs et al., 1973) and intraventricular CCK in sheep (Della-Fera and Baile, 1980a) do not reduce water intake in thirsty animals. One would expect a viscerally distressed animal to reduce its water consumption to the same degree that it reduces its food intake. Moreover, antagonism of CCK action by a variety of means (see above and Della-Fera, Baile, Schneider, and Grinker, 1981b) has consistently augmented food intake. It does not follow that this increase in food intake is a result of the animals becoming "unsick". Lastly, human subjects who received infusions of CCK and consequently ate less of a test meal, reported subjective feelings of satiety but no feelings of malaise or discomfort (Pi-Sunyer, Kisselif, Thornton, and Smith, 1982; Stacher, Steinringer, and Winklehner, 1982). Collectively, these studies provide a strong argument against the malaise interpretation of CCK-produced feeding suppression.

Physiological Control of Food Intake in Hamsters

In recent years there has been increasing interest in the hamster as an animal model for biobehavioural research. Recently, several researchers have focused attention on hamster feeding behaviour and its physiological controls. This work, reviewed below, has shown that patterns and physiological controls of ingestive behaviour in hamsters differ strikingly from those of the more commonly studied rodent, the rat. These studies have sparked considerable curiosity with regard to the signficance of these species differences, and whether different physiological stimuli control feeding in hamsters. As a comprehensive review of hamster feeding is forthcoming (Borer, in press), only the essential features of hamster feeding and how they differ from those of other species will be outlined below.

Normal adult hamsters generally eat between five and 12 g of standard rodent chow daily under typical laboratory conditions (unpublished observations; and see experiments that follow). Borer, Rowland, Mirow, Borer, and Kelch (1979) reported that hamsters eat an average of 0.9 g of chow during a meal, with an intermeal interval of about two hrs. These data were acquired in an observational study where hamsters broke a photobeam when their snouts poked into a food jar. Hamsters are notorious food hoarders (Lanier, Estep, and Dewsbury, 1974; Miceli and Malsbury, 1982), so that data on meal size and intermeal interval acquired by this means may be suspect. In several experiments (reported below) where hamsters' food rations were weighed at regular hourly intervals, over 90% of animals ate some food during each of the

hourly intervals. Thus, hamsters probably eat smaller meals and eat more frequently than previously reported. In view of the common use of hamsters in studies of biological rhythmicity, it is curious that conventionally housed hamsters show no diurnal feeding rhythms (Zucker and Stephan, 1973; Borer et al., 1979).

Hamster energy regulation is sensitive to seasonal variations in day length. During short photoperiods hamsters regulate energy balance at higher body weights (Wade, 1983). The effects of short photoperiods on hamster energy regulation are in part mediated by increased melatonin and decreased gonadal secretions (Bartness and Wade, 1984). Like other animals, hamsters also show dietary obesity (Fleming and Miceli, 1983; Wade, 1982). However, the underlying mechanisms of dietary obesity are quite different in hamsters, because it does not entirely result from increased caloric intake. Hamsters which self-select a high-fat diet in a cafeteria feeding paradigm (Fleming and Miceli, 1983) or feed exclusively on a high-fat diet (Wade, 1982) do show modest increments in caloric intake in comparison to control hamsters fed a high-carbohydrate chow diet, but this small increase does not entirely account for the large weight gains. Wade (1982; 1983) has presented evidence that hamsters feeding on high-fat diets decrease energy expenditure, presumably, by reducing thermogenesis in brown adipose tissue. In rats the reverse is true. High-fat and cafeteria diets stimulate thermogenesis in brown fat, which prevents rats from becoming as obese as they would otherwise be (Rothwell and Stock, 1980).

Hamsters are unusual in that they do not respond to stimuli which ordinarily elicit feeding in most other species. Hamsters do not eat in

response to glucoprivic challenges. Under a variety of conditions hamsters have failed to overeat after treatment with gluco-analogues such as 2-deoxy-D-glucose or 5-thioglucose (Di Battista, 1982; Ritter and Balch, 1978; Rowland, 1978; Sclafani and Eisenstadt, 1980; Silverman, 1978). Insulin induced hypoglycemia is a relatively weak feeding stimulus, as insulin treated hamsters do not show a marked increase in food intake (Di Battista, 1983; Rowland, 1978; 1983; Ritter and Balch, 1978).

The feeding response to glucoprivic stimuli is thought to be largely mediated by the release of norepinephrine (NE) within the medial hypothalamus (Leibowitz, 1980). For example, the feeding response to systemic or central 2-deoxy-D-glucose is virtually eliminated by intraventricular alpha-adrenergic receptor blockade (reviewed by Leibowitz, 1980). In the light of such a proposed interaction between glucoprivic stimuli and hypothalamic NE feeding systems, and the repeated failure of glucoprivic stimuli to elicit a reliable feeding response in the hamster, we were interested in determining whether central NE injections could elicit feeding in hamsters, as it does in a number of other species (Lee, Denbow, King, and Myers, 1982). We observed a weak and statistically unreliable feeding response following large intraventricular l-NE doses in hamsters offered a standard chow diet, and absolutely no response in hamsters offered a preferred diet of peanut butter and lard (LaCaille, Milway, and Miceli, unpublished). Comparable intraventricular l-NE doses have yielded robust feeding responses in other species like the rat (Leibowitz, Hammer, and Chang, 1983). Thus, our results on NE and feeding in hamsters do not challenge

the view that glucoprivic stimuli exert their effects on feeding via a medial hypothalamic NE feeding system. Our data suggest that lack of a feeding-relevant hypothalamic NE system may at least partially explain why glucoprivic stimuli fail to elicit feeding in hamsters. It was briefly noted in an earlier discussion that opioid peptides have facilitative effects on feeding. Opiate receptor agonists enhance food intake, while antagonists suppress feeding (Baile, Keim, Della-Fera, and McLaughlin, 1981; Morley et al., 1983). There is now evidence that the facilitative effects of opioid peptides on feeding are also (at least partially) dependent on the medial hypothalamic NE feeding system (Leibowitz and Hor, 1982). In this regard, it is interesting to note that hamster feeding is also insensitive to opioid receptor blockade with Naltrexone (Lowy and Yim, 1982; Morley et al., 1983).

Perhaps the most intriguing and challenging (to models of feeding behaviour) characteristic of hamster feeding is this animal's inability to adapt to intermittent food availabilty. Hamsters have consistently failed to show a compensatory postfast increase in food intake (Borer et al., 1979; Rowland, 1982; Silverman and Zucker, 1976), in spite of metabolic changes similar to those reported in rats after comparable periods of deprivation (Borer et al., 1979; Rowland, 1982; 1983). On prolonged intermittent feeding schedules, hamsters show severe weight loss, and eventually die (Silverman and Zucker, 1976). Upon return to ad lib feeding, hamsters regain weight at a rate proportional to the weight loss, but again, without significant increases in food intake (Borer et al., 1979). The re-establishment of body weight without accompanying hyperphagia is a likely result of increased metabolic

efficiency, perhaps mediated by the metabolic changes described by Wade (1982; 1983).

Does the fact that hamsters fail to show adequate compensatory postfast elevations in food intake suggest that they are not hungrier after deprivation? Other measures of feeding behaviour indicate that they are indeed hungry after fasting. Hamsters begin to eat with a shorter latency after a long fast (Di Battista, 1983); after fasting the size of the initial meal is larger than usual (Borer et al., 1979; see also experiments that follow); and food deprivation can potentiate the effectiveness with which other stimuli elicit feeding (Di Battista, 1983). Collectively, these observations indicate that food deprivation in hamsters does produce an initial propensity to overeat. However, the question remained as to why the deprivation-produced impetus to overeat is not translated to substantial compensatory increases in food intake. Borer et al. (1979) and Rowland (1982) have proposed that some omnipotent peripheral satiety signal triggered by feeding overrides the food-deprived hamster's initial propensity to overeat and prevents hamsters from adapting to intermittent feeding schedules (i.e., it makes hamsters incapable of ingesting a large proportion of their daily intake during a short time interval).

Since precise data on hamster meal parameters have yet to be documented, and since gastrointestinal and metabolic physiology have yet, or are only now beginning, to be investigated in the hamster, it is not possible to correlate aspects of hamster feeding behaviour with particular food-related stimuli. However, it is tempting to speculate that the peripheral satiety stimuli proposed to assume supranormal

significance in hamsters may be one, or a combination, of the putative satiety peptides described above.

A direct test of this hypothesis would be to determine whether hamsters are able (or better able) to adapt to intermittent feeding during prolonged antagonism of endogenously released putative satiety peptides. Unfortunately, for most peptides, good receptor antagonists are not currently available. An alternative approach would be to test directly the effects of the peptides on hamster feeding. This is a major objective of the work reported here. If hamster satiety is unique in being especially sensitive to the action of one or more of these peptides, one would predict more potent feeding effects relative to other species. A second objective of the present investigation is the determination of the site(s) and mechanisms of peptide action on hamster feeding. From a comparative aspect, since much more is known about the effects of CCK on feeding control in other species, most of the present work is concentrated on CCK's control of feeding in hamsters.

GENERAL METHODS

Animals

Unless otherwise stated, hamsters in these experiments were either purchased directly from the Lakeview (New Jersey) Hamstery or were laboratory bred descendants from that stock. Laboratory bred animals were weaned at approximately 21 days of age, at which time they were housed in sex segregated groups of 3-4.

Housing and Maintenance

During the experiments the animals were individually housed in polycarbonate tub cages or in hanging stainless steel cages with solid bottoms. In each type of cage, the animals were provided with wood shavings for bedding. Animal rooms were maintained at 22° C, with a reversed 14/10 hr light/dark cycle (but see Experiment 2 for exceptions). Except when otherwise indicated, animals had ad lib access to food (Purina Rat or Mouse Chow or Charles River Rodent Chow) and water. The diet used during each experiment is specified in the appropriate section.

Testing

Except for some groups of animals in Experiment 2, treatment commenced during the dark portion of the illumination cycle. In all experiments involving solid food, pellet rations were provided on the cage bedding. When it was necessary during the reweighing of food rations, the animals' cheek pouches were carefully examined for pellets or food bits. Hoarded food was gently expressed from the cheek pouches and added to the remaining ration. If food stored in the pouches was moist, it was air dried before weighing. Body weights were recorded to the nearest g, and food was weighed to the nearest 0.1 g.

EXPERIMENT 1: DOSE-RESPONSE AND DURATION OF ACTION OF PERIPHERAL CCK-8

In a preliminary study (Miceli and Malsbury, 1983) we reported that food-deprived hamsters decreased food intake to intraperitoneal CCK-8 in a dose-related manner. Although the threshold effective dose was somewhat higher than that reported in rats, the higher CCK-8 doses produced feeding suppressions comparable to those demonstrated in rats. However, in that experiment CCK-8 was tested under unusual conditions for the hamster because a rather long deprivation period (15 hrs) was used. In Experiment 1, I wished to further characterize the hamster's feeding response to peripheral CCK. In this experiment hamsters were tested with a wider range of CCK-8 doses and after less severe food deprivation. Lastly, food intake was monitored over a longer period to determine whether CCK can have more prolonged effects on hamster feeding as is sometimes reported in rats (McLaughlin and Baile, 1980a; 1980b).

Method

Experimentally naive female hamsters ranging from 110 to 140 g were prepared for this experiment by a 5.5 hr fast after which groups of animals received 0.1, 0.5, 0.7, 1.0, 2.0, 3.0, or 5.0 µg/kg sulfated CCK-8 (Peninsula, Lot # 002550), 2.0 µg/kg desulfated CCK-8 (Peninsula), or an equal volume (0.1 ml/100 g) of saline by intraperitoneal (i.p.) injection. Five min after the injection the animals were given a preweighed ration of food (Purina mouse chow) pellets. The ration was

re-weighed 1, 3, and 18 hrs later.

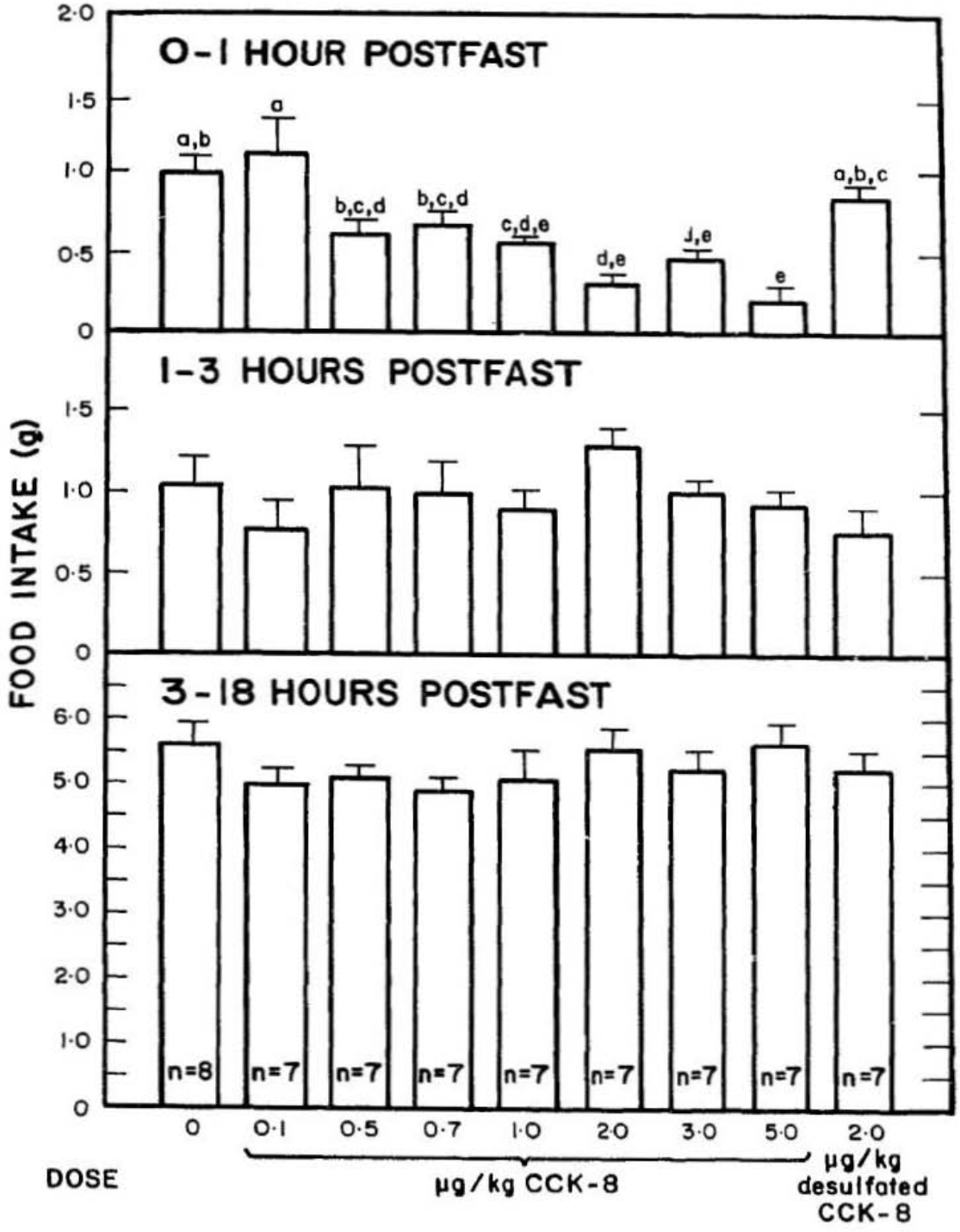
Results and Discussion

Food intakes during the first hr postfast, the second and third hrs postfast, and during the fourth through the eighteenth hrs postfast underwent separate one-way analyses of variance. Post-hoc comparisons of treatment means were made using the Newman-Keuls procedure. As shown in Figure 1, i.p. CCK-8 produced a dose-related suppression of food intake during the first hr, $F(8, 55) = 9.04$, $p < 0.001$. As previously reported (Miceli and Malsbury, 1983), the minimal effective dose producing a statistically reliable suppression of food intake was 1.0 µg/kg. Structural specificity of the CCK-8 molecule is also demonstrated in this experiment as desulfated CCK-8, at a dose which was effective in its sulfated form, did not suppress food intake. None of the animals treated with CCK-8 displayed abnormal behaviour or showed overt symptoms of malaise. No dose of CCK produced reliable changes in food intake beyond the first hr postfast.

EXPERIMENT 2: EFFECT OF CCK-8 DURING THE LIGHT AND DARK PORTIONS OF THE ILLUMINATION CYCLE

Rats have been reported to be considerably less responsive to CCK during the dark portion of the illumination cycle than during the light phase (Kraly, Cushin, and Smith, 1980; McLaughlin and Baile, 1980a; 1980b). The reason for this diurnal variation in responsiveness has not

FIGURE 1. Mean (± s.e.m.) food intake after i.p. injections of sulfated and desulfated CCK-8. Means without a common superscript (0-1 hr postfast) are significantly different, $p < 0.05$. No differences were observed beyond the first hr postfast.


0-1 HOUR POSTFAST
1-3 HOURS POSTFAST
3-18 HOURS POSTFAST
FOOD INTAKE (g)
2·0
1·5
1·0
0·5
0
6·0
5·0
4·0
3·0
2·0
1·0
a,b
a
b,c,d
b,c,d
c,d,e
d,e
d,e
e
a,b,c
n=8
n=7
n=7
n=7
n=7
n=7
n=7
n=7
n=7
0
0·1
0·5
0·7
1·0
2·0
3·0
5·0
2·0
DOSE
µg/kg CCK-8
µg/kg desulfated CCK-8

been adequately explained, although some have suggested decreased responsiveness to endogenous CCK as a partial explanation for nocturnal hyperphagia (McLaughlin and Baile, 1980a). In the previous study of the hamster (Miceli and Malsbury, 1983) and Experiment 1, hamsters were tested during the dark phase of day/night cycle as hamsters do not show diurnal feeding rhythms (Borer et al., 1979; Zucker and Stephan, 1973). It is possible, however, that a cross-species unresponsiveness to CCK during the night is not related to diurnal feeding rhythms. Thus, the following experiment was undertaken to determine whether hamsters show diurnal fluctuations in responsiveness to CCK. Since females were used exclusively in our previous study (Miceli and Malsbury, 1983) and in Experiment 1, a comparison of CCK's effectiveness in male and female animals was also made.

Method

Twenty animals (10 females and 10 males) ranging from 100-120 g were used in this experiment. Upon arrival at the laboratory (47 days prior to testing) all the animals were housed in an animal room with a reversed 14/10 hr light/dark cycle (lights on at 1800 hr). Half of the animals were subsequently (two weeks prior to testing) housed in a separate room with a nonreversed 14/10 hr light/dark cycle (lights off at 1800 hr). The animals underwent a series of three tests. On day 0, the animals were tested with saline (0.4 ml/100 g bw); on day 2 with 1.5 µg/kg CCK (Squibb, Batch # 556159-2F722); and on day 4 with 3.0 ug/kg CCK-8. In preparation for each test, the animals were food deprived for

four hrs (water was available) at which point they were given the i.p. injection. Immediately after the injection, the animals were given a preweighed food ration (Purina rat chow). Food intake was determined for the first, second and third hrs postfast. The animals housed under the two lighting conditions were tested simultaneously (within 10 min of each other) beginning between 1300 and 1400 hrs. Thus testing for both groups of animals was begun 4-5 hrs prior to the scheduled evening change in illumination. Cumulative food intakes for the first, second and third hrs postfast underwent separate three-way (lighting x sex x dose) analyses of variance with repeated measures on one factor (dose). Group means at each dose were later compared using the Newman-Keuls procedure for repeated measures designs.

Results and Discussion

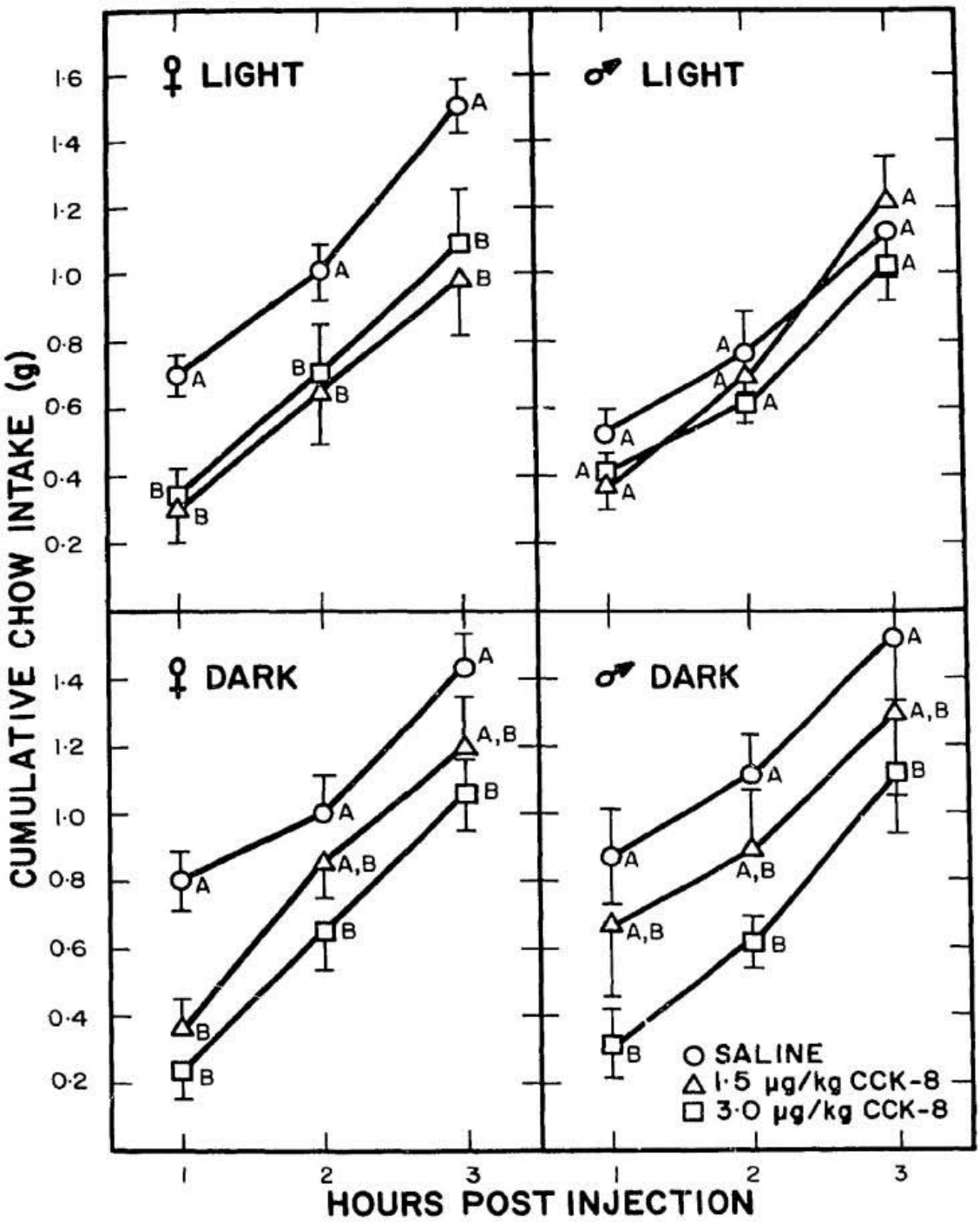
Animals tested during the dark, the males in particular, tended to eat more than animals tested during the light portion of the illumination cycle. This trend, however, was not significant ($p = 0.095$ for lighting effects, and $p = 0.17$ for the lighting x sex interaction). In most groups food intakes during the first hr postfast were reduced after CCK-8, $F(2, 32) = 15.71$, $p < 0.001$, and remained reduced for the remainder of the testing period, $F(2, 32) = 7.16$, $p < 0.003$ (second hr cumulative food intake) and $F(2, 32) = 5.02$, $p < 0.02$ (third hr cumulative food intake). Although the lighting x sex x dose interactions were not statistically reliable (p's > 0.09), subsequent post-hoc comparisons of the group means showed that CCK-8 effectively

reduced intakes in all but the group of males tested during the light period (see Figure 2).

Also of interest in this experiment is the finding that the majority of animals ate at each of the hourly intervals. For example, after saline injection, 100% of the animals ate a meal (defined as $\geq$ 0.2 g of chow) during the first hr, 90% during the second hr and 90% during the third hr. These findings contrast with a previous study of freely feeding hamsters in which two hrs was described as the typical intermeal interval. Thus, at least under the conditions described above, hamsters take smaller, more frequent meals than previously described (Borer et al., 1979).

Under baseline conditions (i.e., after i.p. saline), food intakes did not vary between female hamsters tested during the light and dark phases of the illumination cycle. Also, in females, doses of CCK-8 tested during the day and night were equally effective in reducing food intake. However, the same pattern did not hold true for male hamsters. In males tested during the day, food intake during the first hr postfast was approximately half of that consumed by males tested at night Subsequent hourly intakes did not differ between males tested during the day and night. The fact that females tested during the day and night and males tested during the night eat approximately twice as much during the first hr after deprivation as during subsequent hourly intervals (also see Experiments 3, 9 and 11; and Borer et al. [1979]) suggests that under certain conditions hamsters make a small initial postfast compensatory increase in food intake. That only intakes during the first hr postfast were smaller in males tested during the day further

FIGURE 2. Mean (± s.e.m) cumulative food intake in male (right) and female (left) hamsters given i.p. injections of CCK-8 during the light (top) and dark (bottom) portions of the illumination cycle. Means without a common superscript are significantly different, $p < 0.05$.


♀ LIGHT
♂ LIGHT
♀ DARK
♂ DARK
CUMULATIVE CHOW INTAKE (g)
HOURS POST INJECTION
SALINE
1·5 µg/kg CCK-8
3·0 µg/kg CCK-8

suggests that, for reasons not yet clear, male hamsters are unable or less able to make such a compensatory increase during the day. Also for unknown reasons, a CCK-8 produced reduction of food intake in males tested during the day was not demonstrated. At any rate, hamsters clearly respond to CCK-8 quite differently from rats, as there was no indication that hamsters are less responsive to CCK-8 at night.

EXPERIMENT 3: EVALUATION OF PROGLUMIDE, A PUTATIVE CCK RECEPTOR BLOCKER

Proglumide, a glutaramic acid derivative (DL-4-benzamido-N,N-dipropyl-glutaramic acid), has been reported as a competitive, specific and reversible CCK/gastrin receptor antagonist. Proglumide's ability to antagonize the action of CCK has been proven on several CCK-receptive systems. For example, proglumide produced a rightward shift in the dose-response curve for CCK stimulated pancreatic amylase secretion and displaced binding of tritiated CCK to its receptor on pancreatic acini (Hahne et al., 1981). Similarly, proglumide antagonized CCK-induced contractions of isolated guinea pig gall bladder and ileum segments (Davison and Najafi, 1982) and isolated rat gastric antral muscle (Collins and Gardner, 1982). There is neurophysiological and behavioural evidence that proglumide may also antagonize CNS-intrinsic CCK (Chiodo and Bunney, 1983; Watkins, Kinscheck, and Mayer, 1984; White and Wang, 1984).

Recently, proglumide has been shown to block real- and "sham-feeding" (see Experiment 8 for explanation) suppression by exogenous CCK

(Collins et al., 1983; Collins and Weingarten, 1984) and to augment spontaneous feeding in rats (McLaughlin et al., 1983; Schillabeer and Davison, 1984). Thus, in rats, it appears that proglumide blocks peripheral CCK receptors mediating satiety. In the following experiment it was of interest to determine whether proglumide could affect spontaneous feeding and CCK-induced feeding suppression in hamsters.

Method

Experimentally naive male and female hamsters (100-140 g) were randomly assigned to sex-matched groups. The animals were prepared for the feeding test by a five hr fast (water was available *ad lib*), at which time they received 200 or 400 mg/kg proglumide (A. H. Robins Co.) in a NaOH (pH = 8.0) solution or an equal volume of saline (1 ml/100 g) by i.p. injection. Fifteen minutes after this injection, animals were given either 4.0 µg/kg of freshly prepared CCK-8 (Squibb, Batch # 556159- F722) or saline (0.4 ml/ 100 g) i.p., and immediately after, given a preweighed food ration (Purina rat chow). The ration was reweighed 1, 2, 3, and 24 hrs later. Food intakes during each of these intervals underwent separate three-way (sex x dose 1 [saline vs proglumide doses] x dose 2 [saline vs CCK-8]) analysis of variance.

Results and Discussion

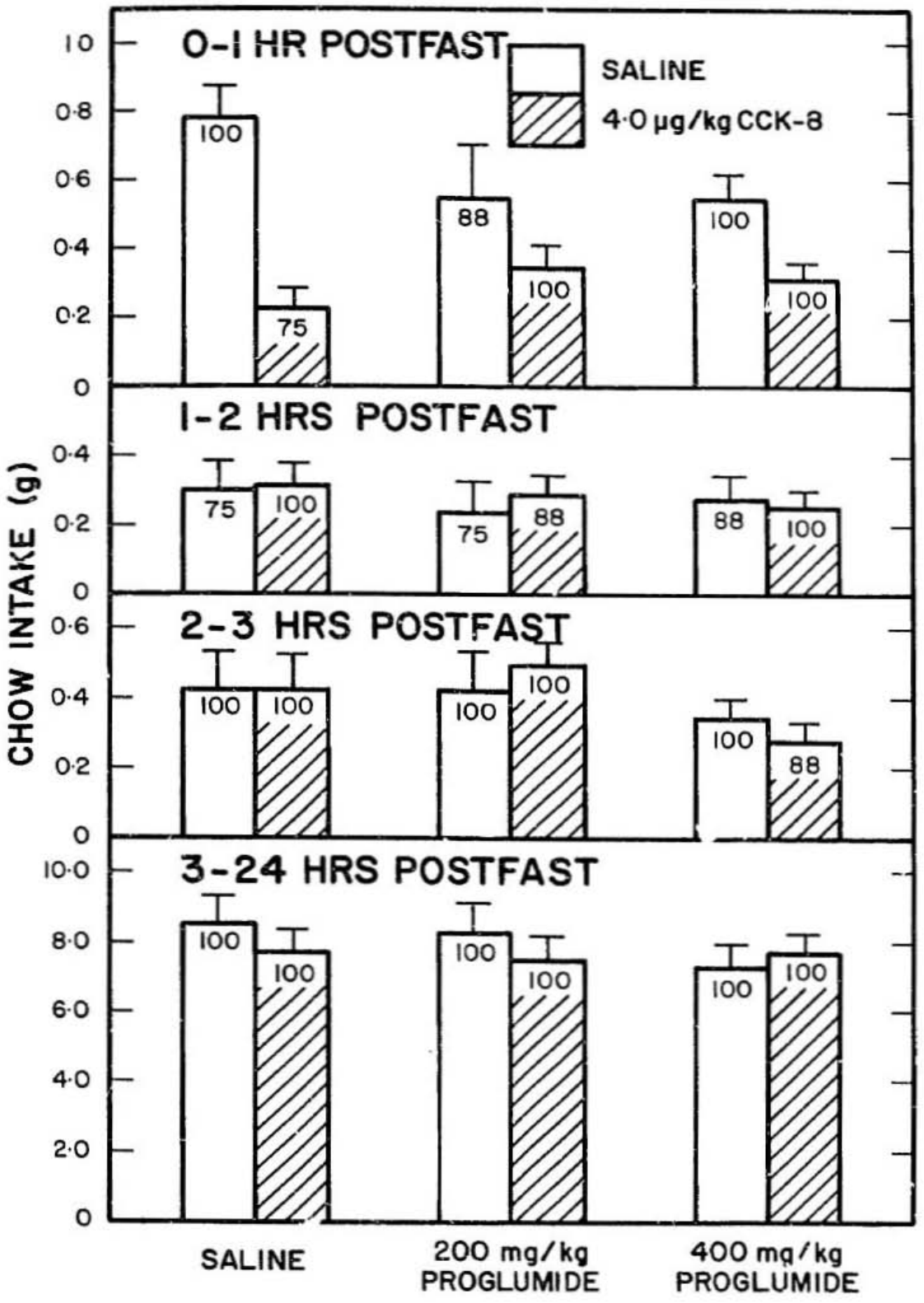
Male hamsters ate significantly less than females during the first hr and hrs 4 through 18 postfast, $F(1, 36) = 4.12$, $p < 0.05$ and $F(1, 36)$

= 13.22, $p < 0.001$, respectively. However, since sex did not interact with any of the other factors, data collected for male and female animals were collapsed for presentation in Figure 3. During the first hr postfast, animals treated with proglumide followed by saline tended to eat less than animals in the control group (saline followed by saline), but this difference was not significant ($p > 0.4$). Neither dose of proglumide significantly reversed the suppressive effect of CCK-8 ($p > 0.11$ for the dose 1 x dose 2 interaction). CCK-8 produced a strong feeding suppression during the first hr postfast, $F(1, 36) = 18.78$, $p < 0.0001$, and the magnitude of the suppression was consistent regardless of the dose of proglumide that preceded it. No group differences were significant beyond the first hr postfast. In summary, proglumide, alone did not augment food intake; and in combination with CCK-8, did not attenuate CCK's suppression of food intake.

Although only two proglumide doses were tested in this experiment, these doses are equal to or greater than those shown to be effective in antagonizing exogenous and endogenous CCK actions on rat feeding (Collins et al., 1983; McLaughlin et al., 1983). It is unlikely that procedural differences account for the discrepancies between the present experiment and the rat studies, as the present study was similar in design to that of the rat studies. In the absence of alternative explanations, the above data indicate that CCK receptors mediating CCK effects on hamster feeding are not blocked by proglumide.

The results of the present experiment are not the first to suggest that proglumide may not be an effective CCK antagonist in all CCK-receptive systems. Recent studies have indicated that proglumide is not

FIGURE 3. Mean (± s.e.m.) food intake in hamsters given i.p. injections of saline or proglumide prior to treatment with CCK-8. Numbers in parentheses denote the (approximate) percentage of animals in the group eating (≥ 0.2 g of food) during that time interval.


0-1 HR POSTFAST
SALINE
4·0 µg/kg CCK-8
1-2 HRS POSTFAST
2-3 HRS POSTFAST
3-24 HRS POSTFAST
CHOW INTAKE (g)
SALINE
200 mg/kg PROGLUMIDE
400 mg/kg PROGLUMIDE

an effective antagonist on all gut CCK/gastrin receptive systems (Fried, Begliner, Koeler, Whitehouse, Varga and Gyr, 1984; Bueno, Honde, and Fioramonti, 1984). Lateral ventricular infusions of proglumide in sheep did not antagonize endogenous brain CCK to increase feeding (Della-Fera and Baile, personal communication) whereas, dibutyryl cyclic GMP, another putative CCK antagonist, did (Della-Fera, Baile, and Peikin, 1981). Similarly, iontophoretic application of proglumide did not antagonize CCK-8's effects on unit activity in the medullary dorsal motor nucleus of the vagus (Ewart and Wingate, 1983). Lastly, it has recently been reported that proglumide did not displace binding of tritiated pentagastrin to putative CCK receptors in rat brain (Gaudreau, Quirion, St.-Pierre, and Pert, 1983)

EXPRIMENT 4: DOSE-RESPONSE AND DURATION OF ACTION OF PERIPHERAL INJECTIONS OF BOMBESIN, CALCITONIN, AND THYROTROPIN RELEASING HORMONE

Collectively, the data reported in Experiments 1, 2, and 3 (and in Miceli and Malsbury, [1983]) indicate that feeding is no more sensitive to the effects of peripherally administered CCK in hamsters than in other species. For example, rats respond significantly to CCK doses well below the minimally effective doses reported for the hamster (e.g. Gibbs, Fauser, Rowe, Rolls, Rolls, and Maddison, 1979; McLaughlin and Baile, 1980a; 1980b). At higher doses CCK produces comparable percent feeding suppressions in rats and hamsters. As noted in the general introduction, CCK is but one of many peptides proposed to be

important in the control of hunger and satiety (see Table II). The possibility remained that one or more of these other peptides may contribute to the hypothesized (Borer et al., 1979) overcontrol of postprandial satiety in this species. Although it was not feasible to test all peptide hormones suggested to play a role in the regulation of appetite, in the following experiments, hamsters were tested for feeding responsiveness to peripheral injections of bombesin (BBS), calcitonin (CT), and thyrotropin releasing hormone (TRH). These peptides were selected because they are representative of the peripheral endocrine systems producing peptide hormones (see Table I); because their effects on feeding in other species is well documented; and because these particular peptides are believed to influence feeding via different mechanisms (Levine and Morley, 1981; Morley, Levine, Kneip, and Grace, 1982a; Morley, Levine, Murray, Kneip, and Grace, 1982b).

Methods

Groups of female hamsters ranging between 120-145 g were food deprived for 5.5 hrs prior to testing. At the end of the deprivation period groups of hamsters (Part A) were given an i.p. injection of 0.5, 1.0, 5.0 or 10.0 µg/kg BBS (Bachem, Lot # R5911). In Part B, groups of hamsters were given 2.0, 4.0, 8.0, or 12.0 µg/kg CT (synthetic salmon CT, Bachem, Lot # R2164) by subcutaneous (s.c.) injection. In Part C groups of females were injected i.p. with 5.0, 10.0, 25.0, or 100.0 µg/kg TRH (Calbiochem, Lot # 702132) by i.p. injection. These peptides were initially dissolved in sterile physiologic saline, aliquoted and

stored at -50° C. Aliquots were thawed and diluted to the appropriate concentration just prior to use. Since Parts A, B, and C were run concurrently, each peptide was tested against the same control group. Six of the eight control animals received equal volumes of saline (0.4 mL/100 g b.w.) by i.p. injection, and two, by s.c. injection. Five min after the injection, the animals were given a preweighed ration of Purina mouse chow pellets, which was reweighed at regular intervals. The data for each of these experiments underwent statistical analyses as described in Experiment 1.

Results

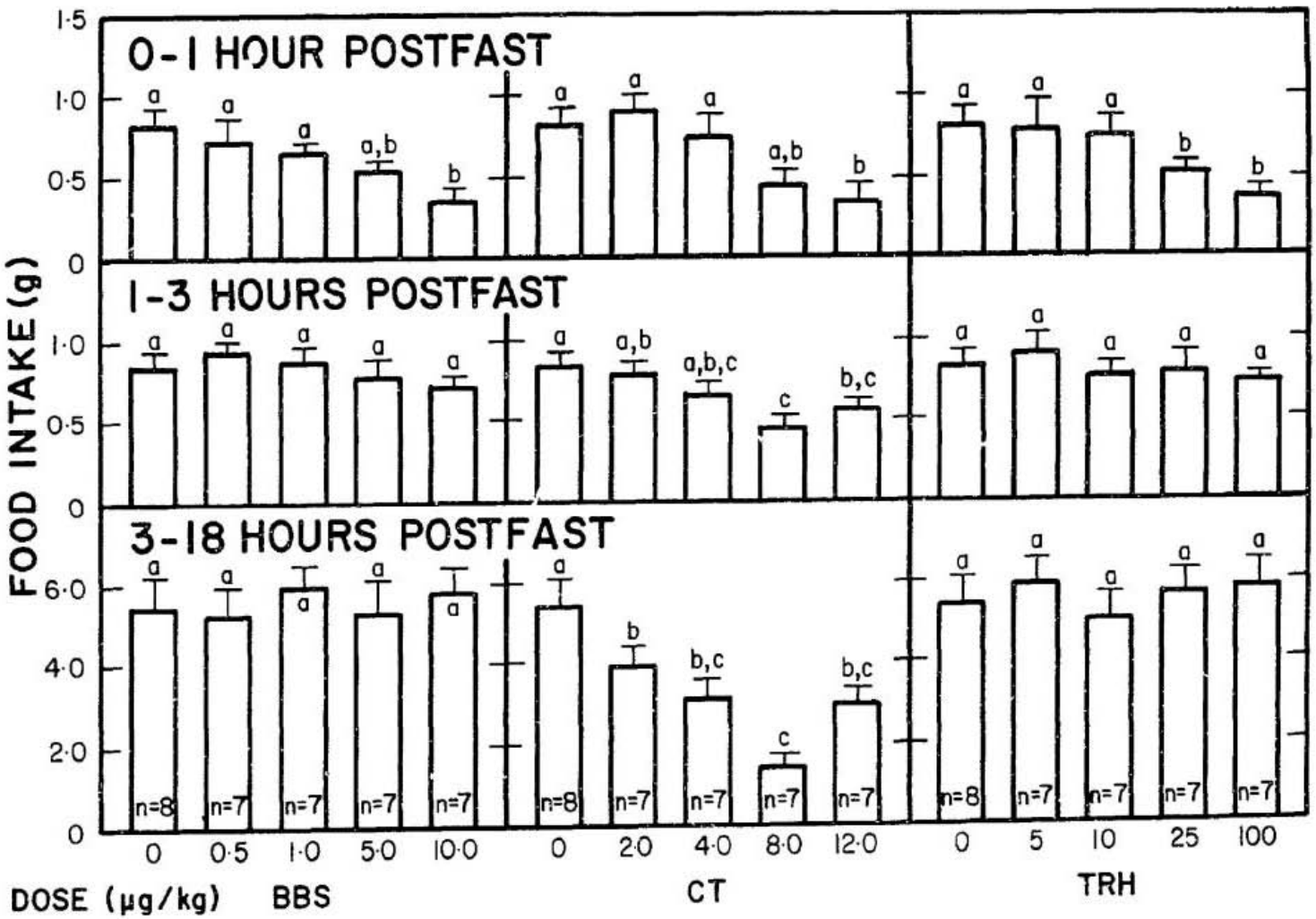
Part A - BBS

As can be seen in Figure 4 (left), the larger BBS doses were effective at reducing food intake during the first hr postfast, $F(4, 31) = 4.16$, $p < 0.009$. BBS's, like CCK-8's, effects were limited to the first hr postfast. None of the BBS doses appeared to produce toxic symptoms or abberant behaviour.

Part B - CT

Unlike peripherally administered CCK-8, BBS, and TRH (see below), CT produced a prolonged suppression of food intake (see Figure 4, middle). Significant effects were observed at each of the three measurement intervals, $F(4, 31) = 4.20$, $p < 0.008$, $F(4, 31) = 5.30$, p

FIGURE 4. Mean (± s.e.m.) food intake after i.p. or s.c. injections of BBS, CT, or TRH. Means without a common superscript are significantly different, $p < 0.05$.


0-1 HOUR POSTFAST
1-3 HOURS POSTFAST
3-18 HOURS POSTFAST
FOOD INTAKE (g)
1·5
1·0
0·5
0
6·0
4·0
2·0
n=8
n=7
0
0·5
1·0
5·0
10·0
2·0
4·0
8·0
12·0
5
10
25
100
DOSE (µg/kg)
BBS
CT
TRH

< 0.003, and $F(4,31) = 9.07$, $p < 0.001$, respectively. It is interesting that the lower CT doses that did not affect food intake initially (1 through 3 hrs postfast) were subsequently potent in reducing food intake. CT also differed from BBS and CCK-8, in that it produced behavioural changes that were clearly uncharacteristic of postprandial satiety. Although no behavioural measurements were taken in this series of experiments, hamsters treated with the larger CT doses were clearly hyperactive and hyperreactive. Such treated animals displayed apparently high levels of ambulation, rearing, scrabbling, and cage climbing. These animals also showed an exaggerated startle response when accidently touched by the experimenter's hand during the weighing of the food pellets. This syndrome was noted shortly after the injection and persisted to the end of the observation period.

Part C - TRH

TRH also reduced food intake during the first hr postfast, $F(4, 31) = 3.56$, $p < 0.02$, but only at high doses. The initial reduction of food intake after the larger TRH doses was also accompanied by abnormal behaviour. Shortly after the larger TRH injections, the animals assumed a characteristic extended-prone position, remaining motionless except when occasionally crawling. The animals remained in this position for approximately 15 min after the injection. Subsequently, the animals appeared normal for the remainder of the observation period.

Discussion

It is concluded from these experiments that hungry hamsters reduce food intake in response to a number of putative satiety peptides. However, there was no evidence of hamster feeding being more sensitive to the effects of these peptides (in terms of either the potency or duration of the effects) than that of other species for which data are available. In some cases hamsters appeared to be less sensitive to peptide effects than other animals. For example, the threshold i.p. BBS dose for hamsters was above 5 µg/kg; whereas rats tested under similar conditions significantly reduce their food intake to i.p. doses of BBS as low as 2.0 µg/kg (Gibbs et al., 1979). Likewise, considerably larger quantities of TRH are required to suppress feeding in hungry hamsters than in hungry rats (e.g. Gibbs, Gray, Martin, Lhamon and Stuckey, 1980). CT, on the other hand, seems to be equally effective in hungry hamsters and rats (Freed, Perlow, and Wyatt, 1979).

These studies of the hamster also demonstrate how peptides can have specific and non-specific effects on feeding. For example, the doses of TRH that reduced feeding clearly debilitated the animals. Similarly, suppression of feeding by TRH in rats was always accompanied by abnormal behaviours (Gibbs et al., 1980). The specificity of CT produced suppression of feeding in hamsters is more difficult to evaluate. By casual observations, it was evident that CT treated hamsters were hyperactive and/or hyperreactive, but it is not clear how this syndrome might contribute (if at all) to the long lasting suppression of food intake. It is interesting to note that in rats, CT can reduce feeding while having effects on locomotor behaviour opposite to those seen in

hamsters. CT is reported to suppress both spontaneous and amphetamine stimulated locomotor activity (Twery, Cooper, and Mailman, 1983b; Twery, Cooper, Lewis and Mailmain, 1983a). These divergent results between rats and hamsters suggest, but do not prove, that CT's effects on feeding may be independent of CT-produced alterations in locomotor behaviour. Lastly, BBS in hamsters, as in rats (Gibbs et al., 1979), reduced feeding without any apparent changes in other behaviour.

EXPERIMENT 5A: EFFECT OF CCK-8 ON DRINKING IN THIRSTY HAMSTERS

In the previous experiments, observations of the animals treated with putative satiety peptides during and after feeding suggest that exogenous CCK-8 and BBS can reduce feeding without altering other behaviour. These observations are consistent with the idea (but do not prove) that CCK-8 and BBS suppress feeding specifically and directly. By contrast, TRH appeared to reduce feeding indirectly and nonspecifically by temporarily debilitating the hamsters. For CT, it was more difficult to assess the specificity and directness of the feeding suppression. One specificity test used in rat studies has been to determine whether peptide doses that reduce feeding in hungry animals also reduce drinking in thirsty animals. If a peptide (or any other agent) can selectively reduce feeding (or drinking), then a degree of specificity is demonstrated because a gross (or even subtle) form of debilitation can be ruled out. Since it is now axiomatic that agents that suppress both drinking and feeding should be suspected of doing so

by inducing distress and/or sickness (e.g. Epstein, 1982), testing for possible peptide effects on drinking behaviour would further provide some index of whether certain peptides reduce feeding by inducing a subtle form of malaise. Experiment 5a was designed to determine whether doses of CCK that reduce feeding in hamsters also affect drinking.

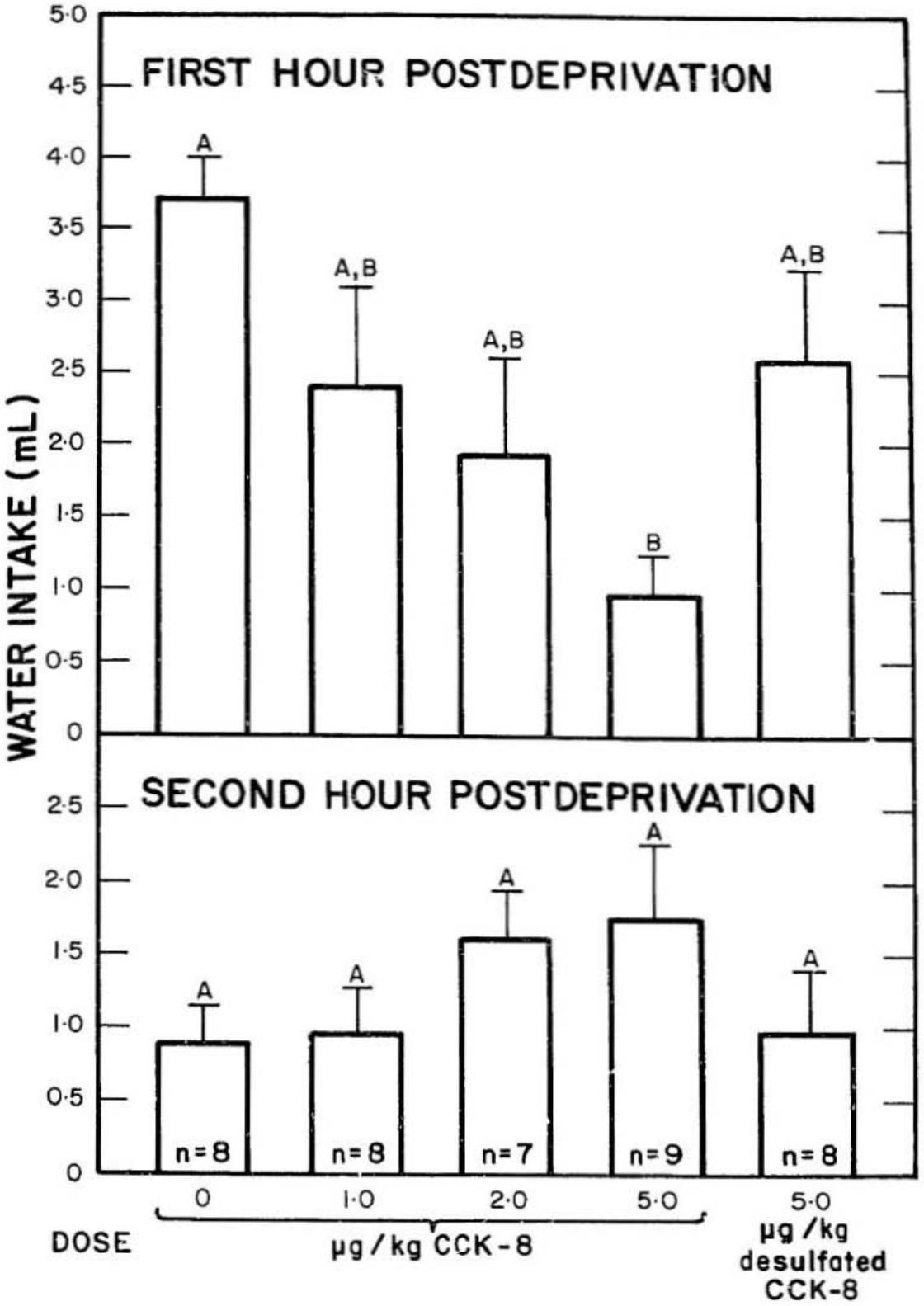
Method

Animals were 4-6 month old female hamsters that were previously bred once or twice, but were otherwise experimentally naive. These animals were tested at least one month after weaning of the last litter. On the morning of the day of testing the hamsters were water deprived for six hrs at which time they received either saline (0.1 mL/100 g b.w.), 1.0, 2.0, 5.0 µg/kg CCK-8 (Peninsula, Lot # 002550), or 2.0 µg/kg of desulfated CCK-8 (Peninsula) by i.p. injection. The water bottle was returned five min after the injection and water intakes were determined for the first and second hrs thereafter. Food was available ad lib throughout. Water intakes during the first and second hrs underwent analysis of variance and treatment means subsequently underwent post-hoc comparisons using the Newman-Keuls procedure.

Results and Discussion

As shown in Figure 5, CCK-8 produced a dose-related suppression of drinking during the first hr postdeprivation, $F(4, 35) = 4.20$, $p < 0.008$. However, the post-hoc analyses of treatment means revealed that

FIGURE 5. Mean (± s.e.m.) water intake in thirsty hamsters after i.p. injections of sulfated and desulfated CCK-8. Means without a common superscript are significantly different, $p < 0.05$.


FIRST HOUR POSTDEPRIVATION
SECOND HOUR POSTDEPRIVATION
WATER INTAKE (mL)
5·0
4·5
4·0
3·5
3·0
2·5
2·0
1·5
1·0
0·5
0
A
A,B
A,B
B
A,B
A
A
A
A
A
n=8
n=8
n=7
n=9
n=8
0
1·0
2·0
5·0
5·0
DOSE
µg/kg CCK-8
µg/kg desulfated CCK-8

only the largest dose produced a significant drinking decrement. The same large dose of desulfated CCK-8 did not reliably reduce water intake during the first hr. What appear to be dose-related increases in water intake during the second hr were not statistically significant ($p > 0.3$).

Although CCK-8 did reduce water intake in hamsters, the minimal effective dose to reliably do so was five times larger than that which reliably reduces food intake. It might be argued that the lack of significant results with the lower doses may be attributed to the relatively large within group variances, and that differences between the saline injected controls and animals tested with the lower CCK doses would have been significant if a-priori comparisons of treatment means were made instead of the more conservative post-hoc comparisons. The post-hoc comparisons were appropriate because there was no a priori reason to believe that CCK-8 either would or would not affect drinking. Moreover, if a priori comparisons of treatment means were made in Experiment 1, the minimal dose reliably reducing feeding would have been smaller than that which was reported.

In hamsters, like rats, a certain proportion of daily water intake is consumed prandially. In this laboratory, under the housing and maintenance conditions described in the previous experiments, water intake is strongly correlated to food intake, $r = 0.58$, $p < 0.01$, (Miceli, unpublished). The ratio of daily water intake to food intake of 1.2 mL:1 g that I typically find, is what might be expected from an animal feeding on a hard and dry diet. It should be noted that food was available throughout Experiment 5a, and that some portion of the saline

treated animals' water consumption may have been taken prandially. This is an important consideration because intake of dry chow may have been indirectly inhibited during water deprivation and hence, feeding and prandial drinking may have increased after deprivation. It follows that the decreased water intake after i.p. CCK-8 may have been the indirect result of the peptides' effects on feeding. To test this possibility, water deprived animals were treated with CCK-8 and water intake was subsequently measured in the absence of food. In subsequent experiments (Experiment 10; and Miceli, unpublished) where food and water intakes were measured concurrently, there was always a trend (though not always significant) for drinking to be reduced along with feeding after peripheral administration of BBS, TRH and CT. Thus, the possible antidipsogenic effects of these peptides were tested in the absence of food as well. As there is considerable (within group) variability in drinking data, to increase the sensitivity of the statistical analyses, possible peptide effects on water intake were studied in a repeated measures design where each animal served as its own control.

EXPERIMENT 5B: EFFECTS OF CCK-8, BBS, CT, AND TRH ON DRINKING IN THIRSTY HAMSTERS

Method

Ten experimentally naive (five females, five males) animals were randomly selected from the colony for this experiment. The animals underwent a series of five tests spaced five days apart. The animals

were prepared for each test by 15 hrs of water deprivation (food was available ad lib), at which time they received 5.0 µg/kg CCK-8 (Squibb, Batch # NN020NC) i.p., 10.0 µg/kg BBS (Bachem, Lot # R5911) i.p., 8.0 µg/kg CT (synthetic salmon, Bachem, Lot # 2164) s.c., 25.0 µg/kg TRH (Calbiochem Lot # 702132) i.p. or equal volumes (0.4 mL/100 g b.w.) of saline (4 animals s.c., 6 animals i.p.). Peptides and saline were tested in random order. Immediately after each injection, the hamsters were transferred to clean cages with empty food hoppers. Five min later they were given access to tap water, and water intake was monitored at regular intervals. Water intakes one hr, between one and five hrs, between five and nine hrs, and between nine and 24 hrs postdeprivation underwent separate two-way (sex x treatment) analyses of variance with repeated measures on the treatment factor. When appropriate, mean intakes after peptide treatments were compared with mean intake after saline.

Results and Discussion

Water intakes did not differ between the sexes, and sex did not interact with the treatment at any time interval. First hr postdeprivation water intakes tended to be lower after peptide treatment than after saline, but these differences did not reach the level of statistical significance, $F(4, 32) = 2.19$, $p = 0.09$. Intakes were significantly different at the 1-5 hrs and 9-24 hrs intervals, $F(4, 32) = 4.39$, $p < 0.005$ and $F(4, 32) = 2.70$, $p < 0.05$, respectively. These differences were attributed to the suppressive effects of CT (see Table

V).

In this, and the previous experiment, water intake was measured after treatment with peptides in an attempt to provide some measure of specificity for peptide effects on feeding. In this experiment the validity of drinking tests as a specificity measure was also assessed by determining whether TRH, a peptide that visibly debilitates hamsters, would reliably reduce drinking.

Under the testing conditions outlined above neither CCK-8 (at a dose which was previously effective), BBS, nor TRH reliably reduced water intake. The fact that TRH did again debilitate the animals (the initial drinking bout was terminated by a collapse into the extended-prone position described in the previous experiment), while not significantly reducing water intake suggests that drinking tests may not be (at least in hamsters) a reliable feeding specificity assay.

In rats, tests of CCK specificity using drinking as a control measure have yielded less ambiguous results. Doses of CCK-8 shown to reduce food intake failed to decrease, and sometimes augmented water intake in thirsty animals tested in the absence of food (e.g. Gibbs et al., 1973; McLaughlin and Baile, 1980a). On the other hand, McLaughlin and Baile (1980a) tested thirsty Zucker lean rats while food was available (as in Experiment 4a) and found that impure CCK and CCK-8 reduced water intake. They too suggested that this reduction was the indirect result of reduced food intake. The effects of CCK on drinking have also been studied in other species. Koopmans, Deutsch, and Branson (1972) found that mice treated with crude porcine gut extracts containing CCK reduced their water intake. However, it is not clear

TABLE V

Water Intakes After Treatment With Peptides

Time (Hrs) After Water Deprivation	Mean (± S.E.M.) Water Intakes (mL) Following:				
	Saline	5.0 µg/kg CCK-8	10.0 µg/kg BBS	25.0 µg/kg TRH	8.0 µg/kg CT
0-1	2.47 (0.58)	n.s. 1.66 (0.39)	n.s. 1.51 (0.24)	n.s. 1.27 (0.28)	n.s. 2.55 (0.77)
1-5	2.20 (0.50)	n.s. 2.19 (0.26)	n.s. 1.84 (0.26)	n.s. 2.41 (0.38)	* 0.75 (0.22)
5-9	1.80 (0.34)	n.s. 1.38 (0.27)	n.s. 1.29 (0.24)	n.s. 1.62 (0.29)	n.s. 0.77 (0.41)
9-24	5.04 (1.43)	n.s. 3.15 (0.60)	n.s. 3.20 (0.58)	n.s. 3.69 (0.88)	* 2.34 (1.02)

* lower than after saline, $p < 0.05$.

whether this reduction was mediated by the CCK or some impurity in the extract. Moreover, it was not stated in the study whether or not food was available during the drinking session. Doses of CCK-8 which reduced feeding also reduced operant responding for water in thirsty pigs (Baldwin, Cooper, and Parrot, 1983). These authors too failed to indicate whether or not food was available during water deprivation and the testing session.

Peripherally administered BBS at doses effective in reducing food intake failed to affect drinking in thirsty rats (Gibbs et al., 1979; Kulkosky et al., 1981). To my knowledge, possible effects of peripherally administered BBS on drinking in other species have not been reported. In the present studies, significant effects of peripheral BBS on drinking when food is available (Experiment 10), and the lack of a reliable effect when animals are tested in the absence of food (Experiment 5a), suggest that BBS's effects on drinking, when observed, may also be secondary to BBS's effects on feeding.

In contrast to CCK-8, BBS, and TRH, CT injections produce prolonged and robust reductions of water intake (e.g. Experiment 10), even when animals are tested in the absence of food (Experiment 5b). These results also differ from those of rat studies. In rats, systemically administered CT actually increased water intake (Perlow et al., 1980). Elevated water intake in CT-treated rats of that study was associated with pronounced diuresis. The reasons for these species differences are not clear. One possible explanation for these opposite effects in rats and hamsters is that CT may exert a strong antidiuretic action in hamsters (as demonstrated in the rat kidney after large pharmacological

doses of CT [Carney, Morgan, and Thompson, 1983]), and in this way reduce water intake. Alternatively, CT in hamsters may stimulate or facilitate behaviours (e.g. locomotor behaviours) which are incompatible with both feeding and drinking.

EXPERIMENT 6: EFFECTS OF CENTRAL CCK-8, CT, AND BBS INJECTIONS ON HAMSTER FEEDING AND DRINKING

There is considerable controversy regarding where systemically administered CCK acts to produce its effects on feeding, as CCK peptides are found in both the periphery and CNS. CCK is a large acidic molecule, and it is therefore unlikely that appreciable amounts of CCK released from the gut passively cross the blood-brain barrier (BBB) to act on CNS receptors (but see Faris et al., [1983a] and Hommer, Skirboll, and Palkovits [1983] for contradictory findings). It is possible that circulating CCK may passively cross the BBB at some privileged portal, for example the circumventricular organs such as the area postrema, or that it may cross the BBB via an active transport mechanism (Pardridge, 1983). However, evidence for either of these possibilities has yet to be presented. Thus, the current consensus is that duodenal CCK is not released in sufficient quantities to stimulate target sites in the CNS (Gibbs and Smith, 1984; Lorenz and Goldman, 1982).

Currently, attention has been directed at the possibility that CCK endogeneous to the brain may play a role in the regulation of feeding. CCK, predominantly in its octapeptide form, is abundant in the brain as

determined by radioimmunological and immunohistochemical methods (Beinfeld et al., 1981; Vanderhaegen et al., 1981). The distribution of immunoreactive CCK within the CNS is not homogeneous. For example, the cortex and hypothalamus are particlarly rich in CCK peptides (Beinfeld et al., 1981; Beinfeld, 1983). The distribution of putative CCK receptors (binding sites) corresponds to the distribution of CCK immunoreactivity in the brain and spinal cord (Zarbin et al., 1983).

Throughout the neuroaxis CCK-containing perikarya and terminals have been described in neural systems associated with feeding. In the forebrain, CCK is localized in cell bodies and terminals within the medial preoptic area/hypothalamus (Kiss, Beinfeld, Williams, and Palkovits, 1983; Zaborszky, Beinfeld, Palkovits, and Heimer, 1983); and in the brainstem, substantial CCK immunoreactivity has been reported in the parabrachial region (the pontine taste area) and the medullary dorsal vagal complex (Kubota, Inagaki, Shiosaka, Cho, Tateishi, Hashimura, Hamaoka, and Tohyama, 1983; Zaborszky et al., 1983). Thus, the distribution of CCK within the brain is not inconsistent with the idea that brain CCK may also be important for the regulation of feeding and satiety.

There are now several lines of evidence supporting the idea that brain CCK is involved in the regulation of satiety. In sheep, continuous lateral cerebral ventricular infusion of minute amounts of CCK decreases food intake in hungry animals (Della-Fera and Baile, 1979; 1980a; 1980b). Since similar amounts of CCK infused into the circulation were ineffective, Della-Fera and Baile (1980a) concluded that CCK delivered through the lateral ventricle was acting directly on

brain receptors. Della-Fera and Baile (1980a) further demonstrated that the effects of their ventricular infusions in sheep were specific to feeding. Doses of CCK-8 which reduced feeding did not affect water intake in thirsty animals or alter core temperature. More importantly, these workers demonstrated that antagonizing the action of brain CCK stimulated feeding in satiated sheep. Food intakes were increased during ventricular infusion of CCK antisera (Della-Fera et al., 1981b) and during infusion of dibutyryl cyclic GMP, a putative CCK antagonist (Della-Fera, Baile, and Peikin, 1981a). Thus, in sheep a role for brain CCK in the control of feeding has been clearly demonstrated.

In rats, bolus injections (Grinker, Schneider, Ball, Cohen, Strohmayer, and Hirsch, 1980; Morley et al., 1982a; Lorenz and Goldman, 1982) or prolonged infusion (Della-Fera and Baile, 1979) of CCK-8 into the lateral cerebral ventricle have repeatedly failed to produce significant changes in food intake. One interpretation of these negative findings is that there may be species variations in the permeability of a cerebrospinal fluid (CSF)-brain barrier, and that in rats CCK delivered through the CSF may not gain access to the relevant brain receptors. This is a likely possibility because CCK delivered directly into brain tissue has produced effects on rat feeding. CCK injected directly into the rat medial hypothalamus attenuated spontaneous feeding (Faris, Scallet, Olney, Della-Fera and Baile, 1983b) and also attenuated feeding elicited by local NE injections (McCaleb and Myers, 1980).

Lesions of the CNS have been made in an effort to localize central systems necessary for the expression of CCK effects on feeding. In an

early study it was reported that localized injections of caerulein, the decapeptide CCK analogue, into the ventromedial hypothalamus (VMH) suppressed feeding in hungry rats and that VMH lesions abolished the reduction of food intake after systemic administration of caerulein (Stern, Cudillo, and Kruper, 1976). Since systemically injected radiolabelled caerulein was found to bind to putative receptors in the VMN, Stern et al. (1976) suggested that CCK peptides might act on CNS receptors to control feeding. However, Jerome, Kulkosky, Simansky and Smith (1981) failed to confirm that VMH lesioned rats are unresponsive to caerulein, and were unable to demonstrate a suppressive effect of intraventricular caerulein. Rats with VMH lesions were also responsive to the suppressive effects of systemically administered CCK-8 when tested with a standard chow diet (Kulkosky, Brechenridge, Krinsky, and Woods, 1976; Simansky, Jerome and Smith, 1980), but were less responsive than controls when tested with a more palatable diet (Krinsky, Lotter and Woods, 1979). More recently, Bellinger, Bernardis and Williams (1983) reported that weanling rats with lesions of the dorsomedial hypothalamus were not responsive to the effects of systemic CCK-8 on feeding. Although these findings in the rat do not necessarily suggest that circulating CCK peptides gain access to, and act on hypothalamic receptors to influence feeding, they do tentatively suggest that under certain conditions, the integrity of the hypothalamus may be necessary for the expression of CCK's effects on feeding.

Another strategy of evaluating the role of brain CCK in the control of feeding and satiety has been to study CCK content and receptor binding in specific brain regions under fed-fasted and lean-obese

conditions. In early studies, Straus and Yalow (1978; 1979) reported decreased immunoreactive CCK in the cortex of genetically obese mice in comparison to lean littermates, and decreased cortical CCK levels in mice after a fast. Several groups have since failed to confirm these findings. Schneider, Monahan, and Hirsch (1979) and Finkelstein, Steggles, Lotstra, and Vanderhaeghen (1981) found no significant differences in immunoreactive CCK in whole hypothalami or cortices in comparisons of genetically obese-lean rat or mice pairs; and no differences in these regions in comparisons of fasted and satiated pairs of rats and mice. Similarly, Scallet, Della-Fera, Beinfeld and Baile (1984) compared CCK content in specific hypothalamic nuclei in fed and 24 hr fasted rats and found no differences. Dupont, Merand, Savard, LeBlanc and Dockray (1982) did report a dramatic increase in CCK content of the anterior olfactory nucleus in rats fed on a highly palatable cafeteria diet. This particular increase did not result from overeating, as increased CCK content was also noted in the anterior olfactory nuclei of cafeteria-fed animals whose caloric intakes were yoked to that of chow-fed controls. The significance of this finding to central CCK control of feeding, if any, remains to be explained.

Studies of CCK binding sites (putative CCK receptors) have been more informative. Saito, Williams, and Goldfine (1981a) reported increased binding sites in the hypothalamus (and other brain loci) after mice were fasted for 42 hrs. Hayes, Goodwin, and Paul (1981) failed to find quantitative differences in putative CCK receptors in the hypothalami of fasted rats and mice, but recently, Finkelstein, Steggles, Martinez, and Praissman (1983), using a more sensitive

radioreceptor assay, were able to confirm elevated CCK binding sites in the hypothalami of fasted rats. Saito, Williams, Waxler, and Goldfine (1982) also demonstrated a rise in hypothalamic CCK binding sites in mice made obese by treatment with goldthioglucose. No quantitative changes in binding sites were reported in the sheep hypothalamus after fasting (Della-Fera, Solomons, and Baile, 1983).

These local increases in putative CCK receptors are thought to reflect decreased local CCK release (Saito et al., 1982), as CCK has been shown to down regulate its own receptor (Saito, Goldfine, and Williams, 1981a). The difficulty in demonstrating local changes in CCK content may stem from the fact that CCK and other peptides are present in the CNS in very small amounts and are released at a limited number of synapses. Very local (at the synaptic level) changes, which could conceivably have a major impact on feeding behaviour, are difficult or impossible to measure with the presently available techniques.

Intracerebroventricular (ICV) CCK injections have also been tested in other species. In pigs ICV injections of CCK-8, in doses below those which are effective peripherally, reduced food intake in hungry animals, but not water intake in thirsty animals (Parrot and Baldwin, 1981). Similarly, ICV CCK-8 injections reduced food intake in chicks (Denbow and Myers, 1982). In our preliminary study of the hamster (Miceli and Malsbury, 1983), we reported that hamsters reduced their food intake in response to 50 ng of ICV CCK-8. This amount appeared to be smaller than the doses which were effective peripherally. A larger dose (100 ng) was even more potent. However, during the course of follow-up work an important factor that could have confounded the results of that study

was uncovered. It was discovered that hamsters show large and prolonged reductions of food and water intake after ICV injection of alkaline solutions (artificial CSF at pH $\geq$ 8.4) and that, for reasons not yet clear, dissolving lyophilized CCK-8 in small amounts of saline or artificial CSF (pH at 7.4) results in a basic solution. Thus, it is not clear whether the reduction of food intake after ICV CCK-8 that we initially reported was a direct result of the action of the CCK molecule, or whether it was an indirect result of the high pH of the injectate. Although others have reported effects of CSF injections (e.g. Della-Fera and Baile, 1980c) and pH effects (Twery et al., 1982) on feeding in other species, the mechanisms underlying these effects are not clear. Nonetheless, it was important to determine whether ICV CCK-8 could reduce feeding in hamsters under conditions in which the pH of the injection vehicle was carefully controlled. In the following experiment CCK-8 was tested under conditions as near optimal as possible. The pH of each injection solution was adjusted to near 7.4. The CCK solutions were prepared just prior to ventricular injections, and a wide range of doses was tested, since in some systems CCK can produce dose-related biphasic effects (Ellingwood, Rockwell, and Wagoner, 1983; Faris et al., 1983a).

In contrast to ICV CCK, ICV BBS and CT have potent suppressive effects on feeding in rats (see discussion below). The fact that BBS and CT-like immmunoreactivity (Flynn, Margules, and Cooper, 1981; Panula, Yang, and Costa, 1982; Roth, Weber, and Barches, 1982) and binding sites (Henke, Tobler, and Fischer, 1983; Wolf, Moody, O'Donohue, Zarbin, and Kuhar, 1983) have been demonstrated in the rat CNS, and that

the central doses of CT and BBS required to suppress feeding (in rats) are many times smaller than effective peripheral doses, has suggested that these peptides may affect feeding by acting directly on central receptors (see below). In view of these findings in the rat, it was considered appropriate to also test ICV BBS and CT in hamsters.

Methods

Animals

Laboratory bred male and female hamsters weighing 110-150 g were used in this study.

Surgery

Under pentobarbital (65 mg/kg i.p.) anaesthesia each animal was implanted with a chronic 23 ga stainless steel guide cannula aimed for the lateral ventricle. The tip of the guide cannula was stereotaxically lowered 2.7 mm beneath the dura at 1.1 mm anterior to bregma and 1.7 mm lateral to the midline. The cannula was anchored to the skull with stainless steel screws and dental acyrlic. The guide cannula was kept patent by an indwelling 30 ga obturator, whose tip was flush with that of the guide cannula.

Preparation of the Injection Solutions

Freshly prepared artifical CSF (124 mM NaCl; 5.0 mM KCl; 2.4 mM $CaCl_2.2H_2O$; 1.24 mM KH_2PO_4; 1.3 mM $MgSO_4$; 26 mM $NaHCO_3$) served as the vehicle for ICV peptide injections. The pH of the CSF was adjusted to 7.4 by dropwise addition of 1.0 N NaOH or 30% acetic acid. The CSF was passed through a 0.2 um Millipore filter prior to dissolving peptides. Sulfated CCK-8 (Calbiochem, Lot # 186001) solutions were prepared just prior to ICV injection. After dissolving CCK in CSF, the pH of the solution was rechecked, and if necessary (for the more concentrated solutions), the pH was readjusted to 7.2-7.6 with 10% acetic acid. Desulfated CCK-8 (Squibb, Batch # NN002NA) solutions were similarly prepared. BBS (Bachem, Lot # 5911) and CT (synthetic salmon, Bachem, Lot # R6236) were prepared as highly concentrated solutions in sterile saline, aliquoted, and stored at -50° C. Prior to ICV injection, the aliquots were thawed and diluted to the appropriate concentration with artificial CSF. The pH of the final solution was checked and if necessary (the more concentrated BBS solutions) was adjusted to 7.2-7.6.

Testing Procedure

All peptide and vehicle doses were delivered in a volume of 2 µL at room temperature. The indwelling obturator was replaced with a 30 ga injector cannula whose tip extended 0.5 mm beyond the tip of the guide cannula, while the animal was restrained by hand. The injector cannula was connected to a Hamilton microliter syringe with PE-10 tubing. Peptide and vehicle solutions were administered over 10 sec. The

injector cannula was kept in place for another 10-15 sec before it was removed from the guide cannula and replaced with the obturator.

Animals were prepared for each test with a three hr fast, during which, water was available ad lib. Five to 10 min after the ICV injection, food rations were presented to the animals. Food and water intakes were monitored at regular intervals up to 24 hrs.

Part A: CCK-8

After at least a four day period of recovery from surgery, animals were prepared for the first series of tests. On day 0 animals were given a mock injection. The guide cannula obturator was replaced with an injector cannula for 30 secs, but no solution was delivered. Food and water intakes were monitored over the next 24 hrs. Two days later, animals were similarly tested with either CSF alone, 0.25, 1.0, 2.5. 10, 100, 150, 250, 350 or 500 ng of sulfated CCK-8 or either 250 or 500 ng desulfated CCK-8.

Parts B and C: BBS AND CT

At least four days after the test with CSF or CCK-8, each hamster was again tested after a mock injection. At least two days after the second mock injection hamsters were randomly assigned to groups to be tested with either BBS (Part B), CT (Part C), or CSF (controls for both Part B and C). For Part B, groups were tested with 1.0, 10.0, 50.0, 100.0 or 250.0 ng BBS. In Part C groups were tested with 1.0, 10.0,

50.0, 100.0, or 250.0 ng CT. Since Parts B and C were run concurrently, both peptides were tested against the same control group of animals receiving CSF alone.

Histology

Many of the animals in this experiment were later used in Experiment 7. All of the animals were sacrificed by decapitation. The brains were removed and stored in Formalin until sectioned on a freezing microtome. Coronal sections through the region of the guide cannula were taken to verify the position of the cannula tip. Only those animals (approximately 95%) whose cannula tip was within the lateral ventricle were included in the data analyses.

Results

Food and water intakes after mock injections were typical of intact hamsters of the same age and weight. No significant differences in water and food intakes were found between the first and second mock injection trials, and there were no mock trial differences in food and water intakes among groups which were to receive peptides or CSF on the subsequent trials. To facilitate comparisons, food and water intakes during a given time interval after treatment with peptides or CSF are expressed as a precentage of intakes during the same time interval after the preceding mock injection. Percentage intakes during three time intervals (0-1, 1-7, and 7-24 hrs postfast) underwent separate one-way

analyses of variance, afterwhich, pairwise comparisons of treatment means were made using the Newman-Keuls procedure.

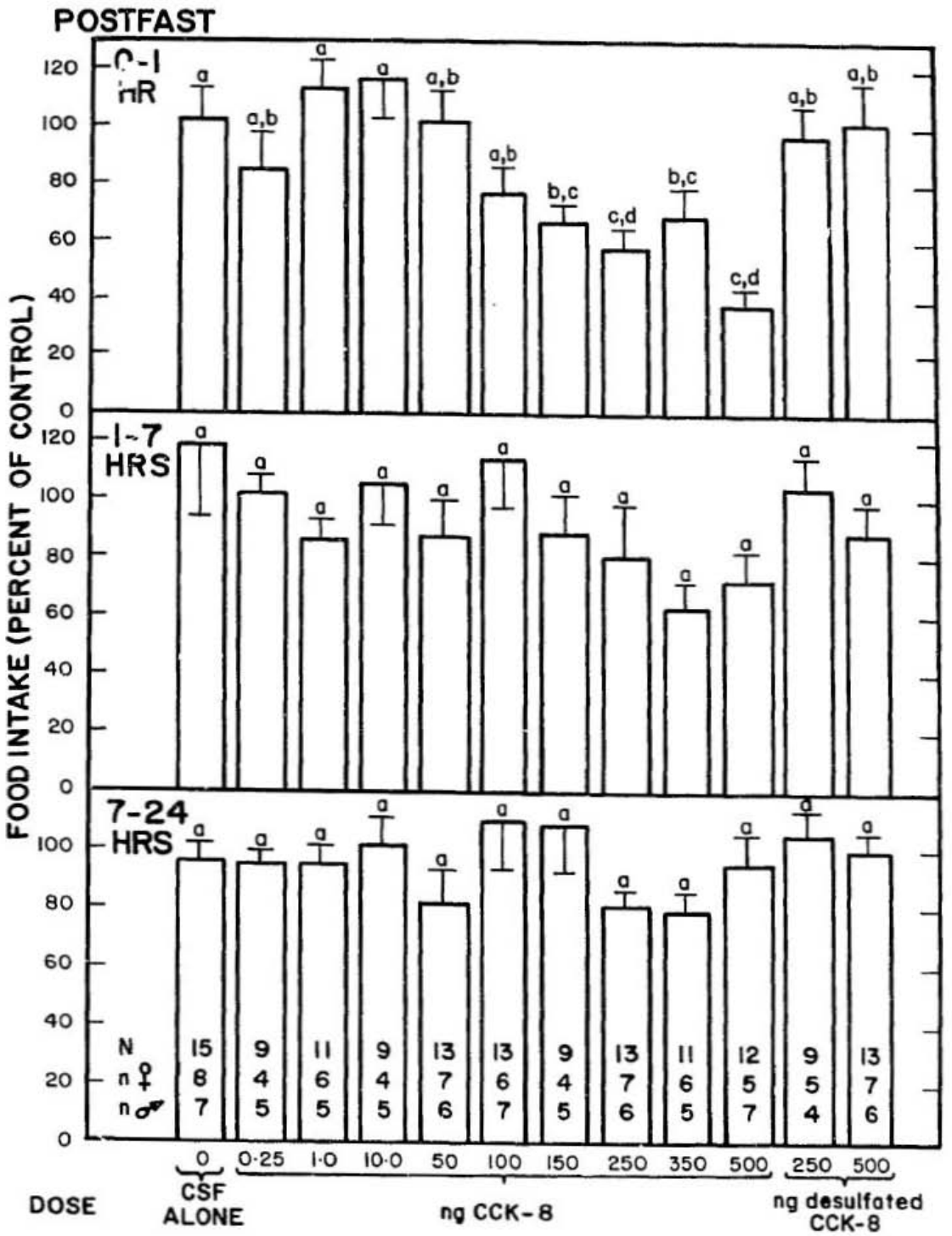
PART A: CCK-8

As shown in Figure 6, ICV injection of sulfated CCK-8 produced a dose-related suppression of food intake during the first hr postfast, $F(11, 125) = 5.16$, $p < 0.001$. As with peripherally administered CCK-8, the feeding suppression response was specific to the sulfated form of CCK-8. Large doses of desulfated CCK-8 failed to reduce food intake. The effects of sulfated CCK-8 on feeding did not extend beyond the first hr postfast. Water intakes were not at all affected by any dose of CCK-8 at any of the time intervals. No additional behavioural changes were observed after ICV CCK-8.

PART B: BBS

BBS produced a dose-dependent reduction of food intake during the first hr postfast, $F(5, 58) = 4.68$, $p < 0.002$. Although some of the larger BBS doses (50 and 100 ng) appeared to also reduce water intake during the same period, these differences did not reach statistical significance ($p = 0.12$) owing to the large within group variability (see Figure 7). Food intakes between one and seven hrs appeared to be reduced in BBS treated animals, but not in a dose-related fashion. The analysis of variance for percentage of control food intakes during this interval failed to show a significant BBS effect $F(5, 59) = 2.16$, $p =$

FIGURE 6. Mean (± s.e.m.) percent of control food intake after ICV injections of sulfated and desulfated CCK-8. Means without a common superscript are significantly different, $p < 0.05$.


POSTFAST
0-1 HR
1-7 HRS
7-24 HRS
FOOD INTAKE (PERCENT OF CONTROL)
0
20
40
60
80
100
120
a
a,b
a
a
a,b
a,b
b,c
c,d
b,c
c,d
a,b
a,b
a
a
a
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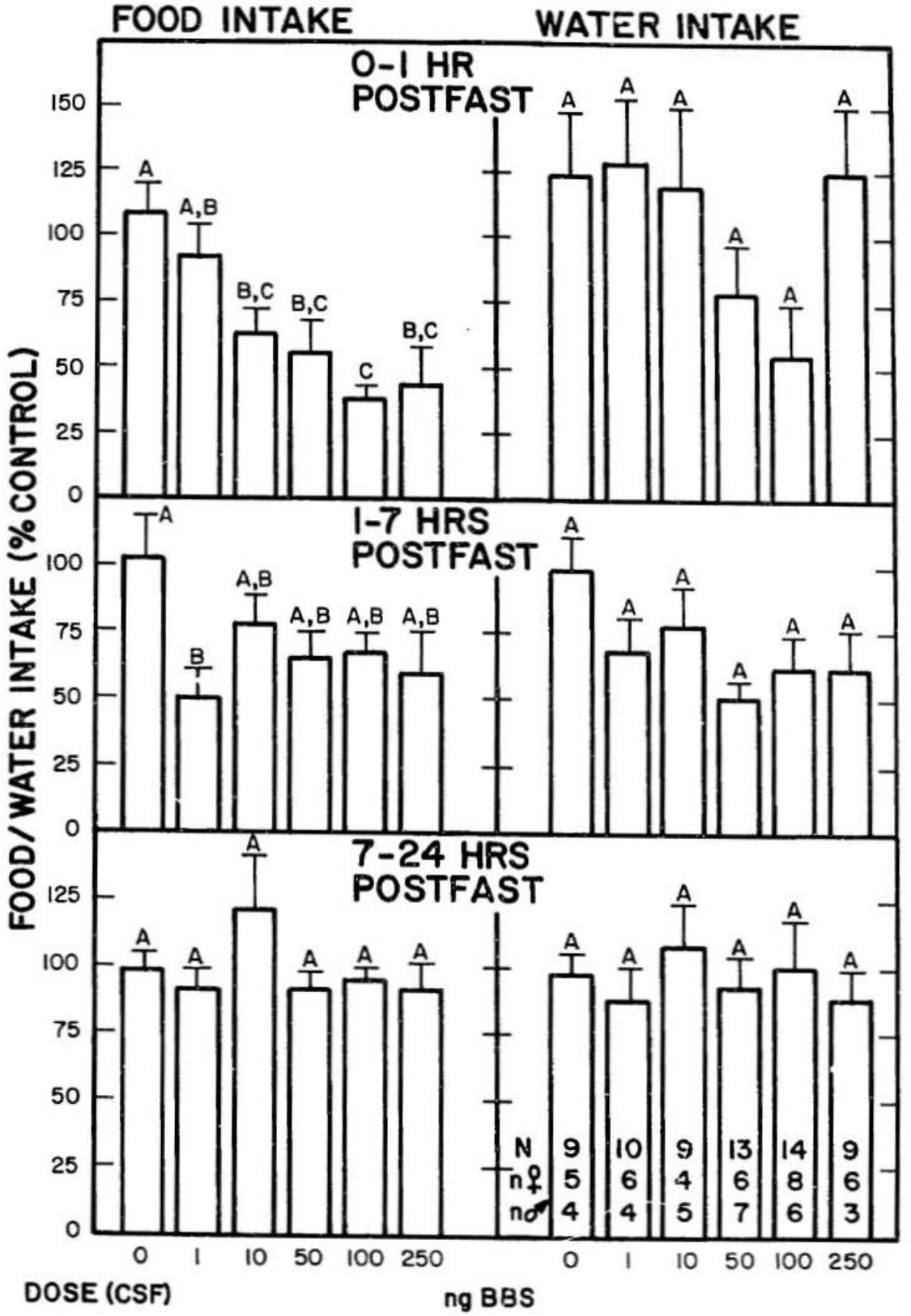
N 15 9 11 9 13 13 9 13 11 12 9 13
n♀ 8 4 6 4 7 6 4 7 6 5 5 7
n♂ 7 5 5 5 6 7 5 6 5 7 4 6
0
0·25
1·0
10·0
50
100
150
250
350
500
250
500
DOSE
CSF ALONE
ng CCK-8
ng desulfated CCK-8

FIGURE 7. Mean (± s.e.m.) percent of control food (left) and water (right) intakes after ICV injections of BBS. Means without a common superscript are significantly different, $p < 0.05$.

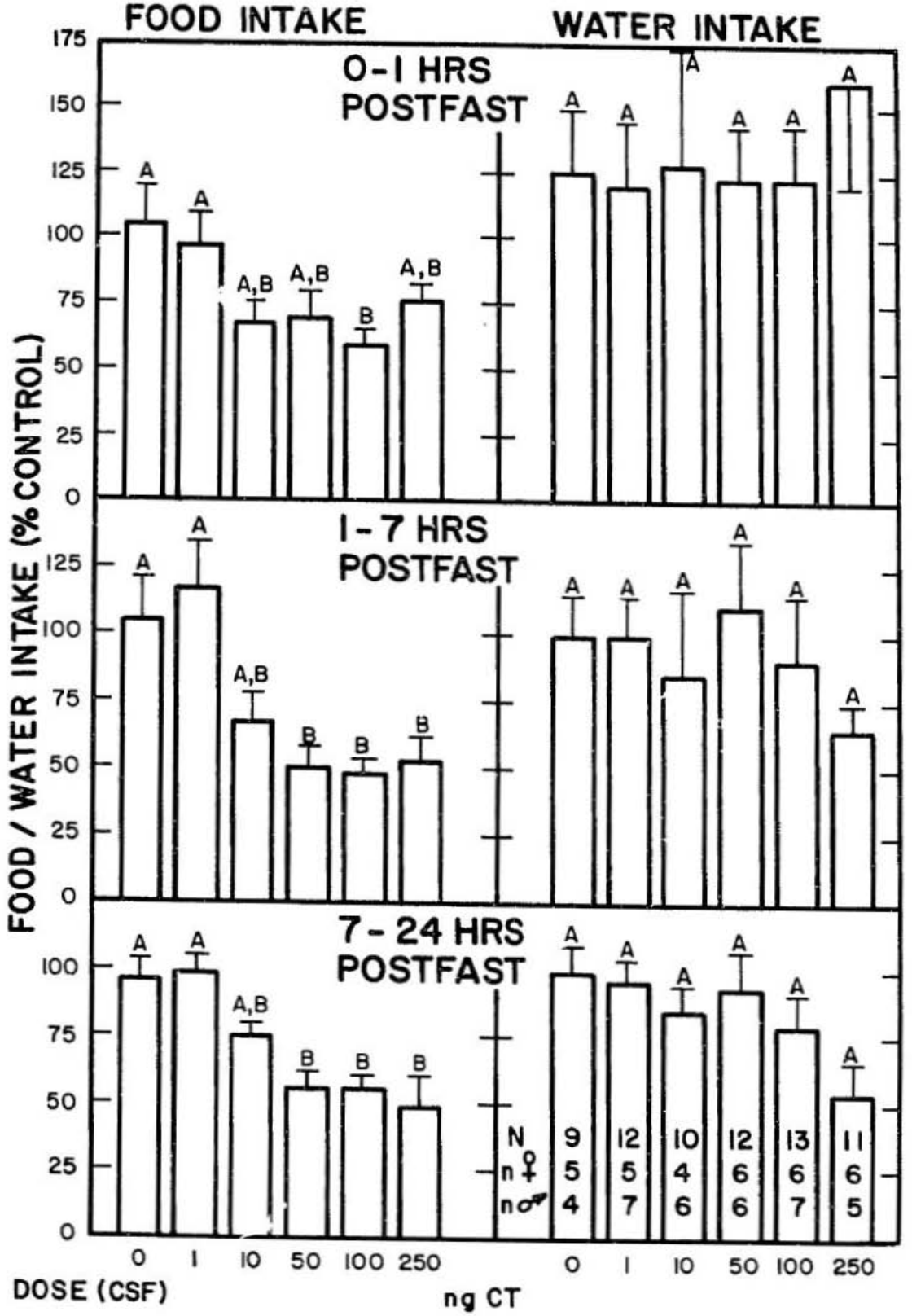

FOOD INTAKE
WATER INTAKE
0-1 HR POSTFAST
1-7 HRS POSTFAST
7-24 HRS POSTFAST
FOOD/WATER INTAKE (% CONTROL)
150
125
100
75
50
25
0
A
A,B
B,C
B,C
C
B,C
A
A
A
A
A
A
B
A,B
A,B
A,B
A,B
A
A
A
A
A
A
A
A
A
A
A
A
A
A
A
A
A
A
N 9 10 9 13 14 9
n♀ 5 6 4 6 8 6
n♂ 4 4 5 7 6 3
0 1 10 50 100 250
0 1 10 50 100 250
DOSE (CSF)
ng BBS

0.07, but comparisons of the treatment means (Newman-Keuls procedure at the 0.05 significance level) did indicate significantly lower percent of control intakes in animals treated with BBS in comparison to the group treated with CSF. Water intakes also tended to be reduced during the same period, but not reliably so. Food and water intakes were not affected by BBS during the 7-24 hr interval. Although food and water intakes were the only measures recorded during these tests, ICV BBS did produce striking changes in other behaviour. There was a marked increase in stereotypical grooming and scratching in animals treated with the larger BBS doses (50 ng and above). This grooming response lasted approximately 1-2 hrs after the injection.

PART C: CT

ICV CT also reduced food intake, but unlike CCK-8 and BBS, the effects were longer lasting; $F(5, 61) = 2.89$, $p < 0.02$, $F(5, 61) = 3.85$, $p < 0.01$, and $F(5, 61) = 9.60$, $p < 0.0001$, respectively for percent of control food intakes during the 0-1, 1-7, and 7-24 hrs postfast time intervals. Water intakes did not differ among groups at any time interval (see Figure 8). Activity measures were not taken in this study. However, animals treated with CT (50 ng and above) were observably hyperactive as described after s.c. administration of CT (see Experiment 4).

FIGURE 8. Mean (± s.e.m.) percent of control food (left) and water (right) intakes after ICV injections of CT. Means without a common superscript are significantly different, $p < 0.05$.


FOOD INTAKE
WATER INTAKE
0-1 HRS POSTFAST
1-7 HRS POSTFAST
7-24 HRS POSTFAST
FOOD / WATER INTAKE (% CONTROL)
175
150
125
100
75
50
25
0
A
A
A,B
A,B
B
A,B
A
A
A
A
A
A
A
A
A,B
B
B
B
A
A
A
A
A
A
A
A
A,B
B
B
B
A
A
A
A
A
A
N
n♀
n♂
9
5
4
12
5
7
10
4
6
12
6
6
13
6
7
11
6
5
0
1
10
50
100
250
DOSE (CSF)
ng CT

Discussion

Although ICV CCK-8 did produce a dose related suppression of food intake during the first hr, the minimal effective dose in the present study (150 ng) was three times larger than that which we previously reported (Miceli and Malsbury, 1983). Since in the present study, the pHs of the injection solutions were adjusted to near 7.4, and were not in our initial study, it is likely that some, if not all, of the effect we initially attributed to the action of CCK-8 was a result of the basic pH of the CCK-8 solution.

The central CCK-8 doses which now appear necessary to reduce food intake in hamsters correspond with those which are effective peripherally. In absolute quantities of the peptide, the amount of CCK-8 given i.p. to the group of animals in Experiment 1 that responded significantly to a dose of CCK-8 (the threshold effective dose of 1 ug/kg) was between 125 and 158 ng, which corresponds to the minimal amount required to suppress feeding by ICV administration. This poses an interpretative problem in that CCK-33 conjugated to a radiolabelled Bolton-Hunter reagent given by ICV injection in rabbits quickly appears in the general circulation (Passaro et al., 1982). It is possible that sufficient amounts of ICV delivered CCK-8 seeped into the general circulation to act on peripheral receptors and produce the observed effects on feeding. This suggestion rests on the assumption that CCK-8 delivered into the hamster CSF is carried to the general circulation as readily as CCK-33-radiolabelled Bolton-Hunter delivered in the rabbit ventricular system. Evidence from studies of other species suggests

that this may not necessarily be the case. For example, CCK-8 administered through the lateral ventricle in rats apparently does not seep into the general circulation since ICV injection of CCK-8 at doses considerably larger (1.0 to 6.4 ug) than peripherally effective doses have failed to produce effects on feeding (Grinker et al., 1980; Lorenz and Goldman, 1982; Morley et al., 1982a).

Another interpretative problem concerns the specificity of the effects of centrally administered CCK. As listed in Table III, there are a number of other effects of centrally administered CCK, some of which could conceivably mediate the observed effects on feeding. Yet, it should be noted that ICV CCK injections which produce these effects in rats, have no effect on rat feeding. Although one could argue that feeding in the hamster may be more susceptible to these factors than it is in rats, it remains to be seen whether central CCK injections do indeed have these other effects in hamsters, and in turn, whether these can alter hamster feeding.

The above arguments aside, the most important consideration is whether the ICV CCK doses are physiologically relevant. Presently, no data are available on concentrations of endogenous CCK in hamster CSF or brain. However, based on the CSF and brain CCK concentrations in other species (e.g. Della-Fera, Baile and Beinfeld [1982] report a basal value of 10-15 pg/ml CCK for sheep CSF, and Beinfeld [1983] reports a total of 1 ug of CCK peptides in the rat brain), the ICV doses which reduced feeding in hamsters greatly exceed the concentrations that might be expected in hamster CSF or brain. Assuming that brain CCK does in fact play a role in food intake regulation in hamsters, one of two

explanations may account for the need of apparently supraphysiological ICV doses to significantly reduce feeding.

Della-Fera and her colleagues (1980a; 1982) have proposed that in sheep, brain CCK works more as a hormone than as a classical neurotransmitter in the control of feeding. They suggest that CCK is released into some rostral component of the ventricular system during feeding and is transported via the CSF to periventricular receptor sites to produce satiety. If CCK works in the same way in hamsters, then the method of CCK delivery chosen for this experiment may not have been appropriate for demonstrating a physiological role of brain CCK in the control of feeding. Delivering CCK in one bolus injection may not mimic natural hormonal events, as some hormones are released in small quantities and act on target receptors over prolonged periods. In this regard it is interesting to note that in sheep, 30.6 pmoles of CCK-8 administered intraventricularly as a 45 min continuous infusion reduced food intake by 80%, whereas, the same amount given as one, four, or eight bolus injections, was without effect (Della-Fera and Baile, 1980b). In sheep, therefore, the method of CCK-8 delivery is critically important for its effect on feeding. It remains to be seen whether continuous infusions of smaller quantities of CCK-8 will effectively reduce food intake in hamsters.

Alternatively, CCK may act more as a classical neurotransmitter to influence hamster feeding and satiety. In such a case, the relevant receptors may not be in close proximity to the ventricular ependyma. Large ventricular doses of CCK-8, as used in the present experiment, would then be required to achieve appropriate concentrations at sites

where it might be active. This hypothesis could be tested by injecting smaller quantities of CCK directly into brain regions where it is thought to work.

Although the data reported here do not clearly demonstrate a physiological role for central CCK and CCK receptors in the control of hamster feeding and satiety, the fact that hamsters do show a feeding suppression in response to a bolus injection of relatively large amounts of CCK, while other species like the rat do not, and that a proposed peripheral site of action (the vagus nerve) does not entirely explain the actions of peripherally administered CCK on hamster feeding (see Experiment 11 and 12) does suggest that brain CCK and CCK receptors may be relevant to hamster feeding. Further studies along the lines suggested above should provide more definitive data on the role of brain CCK in the control of feeding in hamsters.

As reported in other species (Avery and Calisher, 1982; Kulkosky, Gibbs and Smith, 1982a;1982b; Baile and Della-Fera, 1981; Parrot and Baldwin, 1982), ICV BBS produced robust feeding suppressions in hamsters. It is further shown that effective central doses were lower than effective peripheral doses, which suggests a direct central action. Hamsters appear to be more sensitive to ICV BBS than are rats. Hamsters showed a significant reduction in food intake in response to doses of BBS as low as 10 ng. In rats 100 ng (Kulkosky et al., 1982a) or 250 ng (Avery and Calisher, 1982) of BBS were reported as the threshold effective doses. As shown in Figure 7, food intakes of animals treated with BBS were also slightly decreased during the 1-7 hrs postfast interval. Other workers have not examined food intake over such long

periods after ICV BBS injections in rats, but Avery and Calisher (1982) did report that in a one hr test, ICV BBS-produced feeding reductions were limited to the first half hr of the test. Thus, hamsters do appear to be slightly more sensitive to ICV BBS than rats, in that smaller amounts are sufficient to reduce feeding, and the feeding suppression may be of a longer duration.

As in rats (Kulkosky et al., 1982a; 1982b), ICV BBS elicited excessive grooming in hamsters. In rats, the minimal ICV dose to effectively increase grooming behaviour (10 ng) is considerably less than that required to suppress feeding (100 ng). By contrast, in hamsters a larger ICV BBS dose is required to induce excessive grooming (50 ng) than is required to reduce food intake (10 ng). Water intakes also appeared to be reduced during the 0-1 and 1-7 hrs postfast intervals. Again, because of the large within group variability in water intakes, group differences failed to reach statistical significance. Thirsty pigs given ICV BBS showed clear cut reductions of water intake which were dose-equivalent to those in a feeding experiment (Parrot and Baldwin, 1982). In rats, ICV BBS's effects on water intake are less consistent. Kulkosky et al. (1982a) reported that ICV BBS also reduced drinking in 24 hr water deprived rats. On the other hand, Avery and Calisher (1982) reported no reliable effect of a large ICV BBS dose in 6 hr water deprived rats.

The fact that the reduction of food intake following ICV BBS is accompanied by abberant behaviours (excessive grooming in rats and transient vomiting in pigs) and that drinking behaviour is also affected by the same doses has raised considerable doubt about the specificity of

BBS action. The fact that low doses of BBS elicit excessive grooming in rats without affecting food intake (Kulkosky et al., 1982a) and that a low dose can reduce feeding without eliciting excessive grooming in hamsters (see above) suggests that at least some of the ICV BBS effects on feeding may be independent of other behavioural changes (or at least, independent of the excessive grooming behaviour). This, however, does not preclude the possibility that the reductions in food intake after ICV BBS may only be secondary to other (and less conspicuous) central BBS effects; for example, effects on glucoregulation (Brown, Rivier and Vale, 1977), temperature (Avery and Calisher, 1982), and intragastric pressure and motility (Young, Deutsch and Tom, 1981). That many physiological and behavioural functions are altered by central BBS (and other peptide and biochemical) injections reflects on the ICV mode of drug administration. In this particular case, it is likely that many periventricular systems subserving different physiological and behavioural functions are simultaneously stimulated by BBS to produce an array of responses. Clearly, more localized peptide administration should serve not only to localize the relevant target cells, but also to test the specificity of peptide action. In this regard it is interesting to note that BBS administered directly into the lateral hypothalamic region reduced food intake in rats without eliciting excessive grooming behaviour (Stuckey and Gibbs, 1982).

As previously reported in rats (Perlow et al., 1980; Twery et al., 1982), ICV CT at doses considerably lower than effective peripheral doses, produced significant food intake reductions in hamsters. Also similar to rats, was the finding that ICV CT effects on feeding were

rapid in onset and long lasting. Curiously, there was no trend for decreased drinking in hamsters after ICV CT. This contrasts with the Twery et al. (1982) study of rats whose food and water intakes were measured concurrently after ICV CT injections. They found dose-related reductions of drinking that paralleled the reductions of eating. Apparently, different routes of CT administration can have opposite effects on rat and hamster drinking. Subcutaneous CT reduces drinking in hamsters (see Experiments 5b and 10), while producing an immediate (Perlow et al., 1980) or delayed (Twery et al., 1982) increase in rats; whereas ICV CT has no appreciable effect on drinking in hamsters, but decreases it in rats (Twery et al., 1982).

The minimal ICV dose of CT to significantly reduce food intake was also comparable to that in the rat. In the present study 24 hr food intake of hamsters treated with 50 ng CT ICV was approximately 57% of control intake. Twenty-four hr intake of rats treated with the miminally effective CT dose (75 ng or 0.35 U) was approximately 42% of control values. At higher doses, however, hamsters are considerably less responsive to ICV CT than rats. In the present study, within the range of doses tested, no further reductions were seen at doses higher than the minimally effective dose; i.e., 100 and 250 ng CT also reduced intake by about 50%. In rats, 300 ng CT ICV virtually abolished feeding and drinking over the following 24 hrs (Twery et al., 1982).

That considerably lower doses than those required by s.c. administration are effective in reducing food intake by the ICV route strongly suggests a central site of CT action in rats and hamsters. In rats, CT's effects on feeding have been shown to be independent of CT

effects on calcium metabolism (e.g. Twery et al., 1982), and this may also hold true in hamsters. There is however, controversy regarding the source of endogenous CT that might ordinarily limit feeding. Freed et al. (1979) have suggested a role for thyroidal CT, because circulating levels of CT rise during the course of a meal (Gray and Munson, 1969). But, as pointed out by Twery et al. (1982), the doses necessary to reduce feeding by either the s.c. or ICV route are many fold greater than the amount the thyroid is capable of supplying. The more realistic hypothesis, favoured by Twery et al. (1982), is that a CT-like peptide synthesized and released within the CNS may be more relevant to the control of feeding. The demonstration of CT-like immunoreactivity in the hypothalamus and pituitary (e.g. Flynn et al., 1981) and the presence of high affinity CT binding sites throughout the CNS (Henke et al., 1983; Koida, Nakamota, Furakawa and Orlowski, 1980) are not inconsistent with this idea.

GENERAL DISCUSSION OF EXPERIMENTS 1-6

As indicated in the introduction (and also see Experiment 12), hamsters, unlike other species, do not make adequate postfast compensatory food intake increases and are therefore unable to consume their required daily intakes during intermittent food availability. Several workers have suggested that the hamster's inability to adapt to intermittent food access may reflect overcontrolled postprandial satiety (Borer et al., 1979; Di Battista, 1983; Rowland, 1982). Since postprandial satiety in hamsters had not been previously studied in

depth, and since a number of peptide hormones have been proposed to serve as physiological satiety stimuli in other species, a series of normative studies was undertaken to determine the hamster's feeding responses to putative satiety peptide hormones. By comparing the degree to which feeding in hamsters and in other species are affected by exogenous hormones, it was possible to asses whether exaggerated responsiveness to one or more of these hormones contributes to the omnipotent satiety mechanisms proposed to operate in hamsters. Feeding in hamsters was therefore studied after peripheral administration of peptide hormones representative of those released by various components of the digestive system, specifically; BBS (whose mammalian analogue, gastrin releasing peptide, is a gastric hormone), CCK-8 (an intestinal hormone), TRH (a hormone found throughout the gut). and CT (a thyroidal hormone). Since these hormones are (or may be) also synthesized and released within the CNS, and since it is possible that peptides intrinsic to the CNS play a part in the control of feeding and satiety, hamster feeding was also studied after central administration of peptides.

It was demonstrated, that feeding in hamsters showed differential sensitivity to peripheral and central administration of these peptides, that the peptides suppressed feeding with varying degrees of specificity, and that these effects were of different durations. These results in the hamster differed only in minor ways from those reported in studies of other species. That is, feeding in hamsters did not appear to be more sensitive to the effects of these peptides than feeding in other animals for which data is available. These findings,

however, in no way challege the proposition that peripheral negative feed-back stimuli exert stronger control over feeding in hamsters than in other species studied. Peptides released as a consequence of feeding are but one class of (putative) satiety stimuli. The possibility remains that other classes of satiety stimuli (e.g. see Experiment 8) may have exaggerated control of feeding in hamsters. Further, the data collected in Experiments 1-6 do not preclude the possibility that peptide-related satiety mechanisms exert a stronger influence in hamster feeding than in other species. Although the present data show that a given quantity of exogenous peptide reduces feeding in hamsters and in other species (e.g. rats) to more or less the same extent, it is posssible that, in hamsters, more of the peptide is released prandially, and over a more prolonged period.

The similarity with which the tested peptides affected feeding in hamsters and rats is in itself a noteworthy finding in view of the many previously reported species differences in physiological control of feeding. The present findings in the hamster are therefore important because they add primal universality to putative peptidergic satiety mechanisms. These experiments further indicate that the hamster may be an appropriate animal model for elucidating peptidergic satiety mechanisms, which are currently poorly understood. Why should the hamster be preferred for this purpose, or studied at all in this context, for that matter? There are three justifications. Hamsters, of course, are intrinsically interesting to study; species differences can be revealing; and in some instances hamsters should be preferred for this type of study because they tolerate certain experimental manipulations better than other animals (e.g. see Experiments 10-12).

Thus, the following experiments were undertaken to determine the mechanisms of CCK action in the control of hamster feeding.

EXPERIMENT 7: EFFECT OF CCK-8 ON GASTRIC EMPTYING

The fact that meals end while most of the ingested food is still within the upper gastrointestinal tract is evidence that preabsorptive stimuli contribute to ending a meal. Preabsorptive stimuli arise from food acting at oropharyngeal, gastric, and upper intestinal sites. Stimuli generated at each of these sites contribute to postprandial satiety (Smith and Gibbs, 1979). In recent years, workers have focused their attention on gastric and intestinal mechanisms of satiety. There is now strong evidence that gastric stimuli play an important role in the control of postprandial satiety. For example, when ingested food is prevented from entering the small intestine by a pyloric noose or cuff, meal size is normal in rats (Gonzalez and Deutsch, 1980; Kraly and Smith, 1978). This indicates that in rats, orosensory and gastric stimuli may be sufficient to trigger satiety in the absence of postgastric stimuli. Other studies have further shown that gastric satiety has chemosensory and mechanosensory components (Deutsch, Young, and Kalogeris, 1978). That is, signals arising from nutrients chemically stimulating the stomach mucosa and from accumulated food stretching the stomach wall, act in concert to limit meal size. The idea that gastric distention plays an important role in controlling feeding was originally developed by Cannon and Washburn (1915) early in the century and has since bee supported in numerous experiments using

different experimental strategies (e.g. McCann and Stricker, 1983).

It has long been known that food acting on the small intestine produces stimuli which feed-back to the stomach and pylorus to reduce the rate of gastric emptying (see Bloom and Polak, 1981). This effect is partially attributed to the action of duodenally released CCK, as exogenous CCK has been shown to reduce gastric emptying in a number of species (Anika, 1982 [rats]; Mangel and Koegel, 1984 [rats]; Moos, McLaughlin, and Baile, 1982 [rats]; Debas, Farrooq, and Grossman, 1975 [dogs]; Moran and McHugh, 1982 [rhesus monkeys]). Since gastric distention can curtail feeding (e.g. Wirth and McHugh, 1983), and since one of the physiological actions of CCK is to decrease the rate of gastric emptying (thereby enhancing gastric distention), McHugh (1979) suggested that the effects of CCK on feeding may be mediated by its capacity to facilitate gastric distention. This hypothesis, with its elegant simplicity, has been subject to some criticism. For instance, in rats, other peptide hormones that also reduce the rate of gastric emptying (e.g. secretin) do not reduce feeding (Gibbs and Smith, 1984). Perhaps more damaging to McHugh's hypothesis are the results of studies of sham-feeding rats. In the sham-feeding preparation, ingested liquid diet is drained from a gastric fistula before appreciable amounts accumulate in the stomach, hence circumventing gastric distention. Exogenous CCK has been repeatedly shown to inhibit sham-feeding in rats with open gastric fistulas (reviewed in Smith and Gibbs, 1979; Gibbs and Smith, 1984). Evidently, in rats, exogenous CCK can reduce intake of a liquid diet in the absence of stomach distention. Similarly, when physiological amounts of nutrients are infused into the duodenum,

thereby stimulating the release of endogenous CCK, sham-feeding is also reduced in rats (Reidelberger, Kalogeris, Leung and Mendel, 1983). The rat, however, may not necessarily be a representative species with regard to how CCK works to control feeding. For example, in physiological amounts, nutrient loads introduced into the small intestine fail to reduce sham-feeding in rhesus monkeys (Wirth and McHugh, 1983). Furthermore, the suppressive effect of exogenous CCK in rhesus monkeys, particularly at low doses, was shown to be critically dependent on preloading the stomach with saline so that it distended sooner during the course of the feeding session (Moran and McHugh, 1982).

In summary, although it appears that CCK may reduce feeding in rats through mechanisms other than gastric distention, the rhesus monkey experiments suggest that this may not hold true in all species. Therefore, it seemed appropriate to assess the role of gastric distention in the mediation of CCK's effects on hamster feeding. The first experiment of the series that follows was designed to determine whether, in fact, CCK inhibits gastric emptying in hamsters.

Method

Animals with chronically implanted intracerebroventricular cannulae (Experiments 6a-c) were randomly selected for this experiment. At least six days after the last test with intraventricular peptide or CSF injection, hamsters were prepared for this experiment by a 48 hr fast in suspended wire bottom cages. Water was available ad lib during this

period. The fast was ended by giving animals a preweighed food ration of Purina rat chow pellets for 30 min. After 30 min of food access, the remaining food was removed and weighed and the animals were assigned to groups matched for 30 min food intakes. Within three min of removing the food, animals were then injected i.p. with either saline, 4.0 µg/kg desulfated CCK-8 (Squibb, Batch # NN002NA), 0.5, 1.0, 2.0, or 4.0 µg/kg sulfated CCK-8 (Squibb, Batch # 556159-F722). Fifteen min after the i.p. injection, the animals were sacrificed by decapitation. The peritoneal cavity was opened and the pylorus and lower oesophagus quickly ligated to prevent postmortem gastric emptying. The pylorus and oesophagus were then cut distal to the ligatures and the stomach removed from the intraperitoneal cavity. The stomach was then cut along the greater curvature and its contents gently expressed and allowed to air dry for 24 hrs. Dried stomach contents were weighed, and as a measure of gastric empyting, this amount was expressed as a percentage of what the animal ate during the 30 min postfast. In pilot experiments, it was determined that there is some residual food remaining in the hamster stomach after 48 hrs of food deprivation, which on the average, amounts to approximately 20% of what two-day food deprived hamsters will eat in the first 30 min postfast. It was not possible to test hamsters after substantially longer deprivation periods, as animals become anorectic after 72 hrs of deprivation.

Results and Discussion

As shown in Table VI, sulfated CCK-8 inhibited gastric empyting,

TABLE VI

Gastric Retention After Intraperitoneal CCK-8 Injections

Dose µg/kg CCK-8	N	n females	n males	$\bar{X}$ (± s.e.m.) 30 min Food Intake	$\bar{X}$ (± s.e.m.) % of Food Remaining In Stomach*
0 (saline)	10	5	5	0.52 (0.04)	58.5 (6.2)[a]
0.5	15	7	8	0.49 (0.06)	70.5 (6.6)[a, b]
1.0	10	5	5	0.49 (0.07)	90.2 (8.0)[b]
2.0	10	5	5	0.52 (0.05)	75.7 (5.9)[a, b]
4.0	10	5	5	0.52 (0.03)	83.2 (4.0)[b]
4.0 de-sulfated	10	5	5	0.51 (0.08)	68.2 (6.1)[a]

* Means without a common superscript are significantly different, $p < 0.05$.

$F(5, 59) = 2.83$, $p < 0.02$, whereas a large dose of desulfated CCK-8 had no effect. It is interesting to note that the doses of CCK-8 needed to inhibit gastric emptying in hamsters parallel those which suppressed feeding in hungry animals (see Figure 1).

The finding that comparable doses of CCK can reduce both food intake and gastric emptying suggests that enhanced gastric distention may account for some or all of CCK's effects on hamster feeding. However, in rats, comparable doses can also reduce feeding and gastric emptying by similar degrees (Anika, 1982; Moos et al., 1982), yet CCK appears to reduce feeding in rats in the absence of gastric distention. Thus, it remained to be determined whether CCK could do the same in hamsters. To this end, the first study of the sham-feeding hamster was undertaken.

EXPERIMENT 8: ANALYSIS OF SHAM-FEEDING IN HAMSTERS

Methods

Surgery

Hamsters were purchased from Canadian Hybrid Farms (Halifax, N.S., Canada), whose colony is derived from Lakeview animals. Prior to surgery the animals weighed 120-145 g and were experimentally naive.

The animals were implanted with a chronic gastric fistula under sodium pentobarbital anaesthesia The fistula was a version of the one described by Weingarten and Powley (1980), scaled down for the hamster

stomach. The fistula was a 10.7 mm long stainless steel tube (6.4 mm o.d., 4.3 mm i.d.) flanged at both ends. The inside of the fistula was tapped to accomodate a stainless steel set screw which kept the fistula closed. A 3 cm midline incision was made beginning just below the sternum. The stomach was then exteriorized and two concentric purse-string sutures were sewn along the greater curvature of the forestomach. A small incision was made on the stomach wall encircled by the sutures. One of the flanged ends of the fistula was inserted through the incision into the forestomach, and the sutures tied to secure the fistula. A 15 mm diameter circle of polypropylene surgical mesh was then sewn to the shaft of the fistula so that the mesh rested against the portion of the stomach through which the fistula was inserted. The mesh served to promote the growth of connective tissue which would provide further support for the fistula and a seal preventing leakage from the stomach. Another abdominal wall incision was then made to the left of the first, and the free end of the fistula pulled through it so that the stomach occupied as normal a position as possible. A purse-string suture was sewn into the abdominal muscle wall to secure the exteriorized fistula. The initial abdominal wall incision was closed with silk sutures and wound clips. A second 10 mm diameter circle of polypropylene mesh was then fastened to the shaft of the fistula between the abdominal wall muscle and skin. Another purse-string suture was sewn into the skin surrounding the exteriorized end of the fistula and tied securely. In most of the animals the skin around the exteriorized end of the fistula retracted at about 3-5 days postoperatively, exposing most of the surgical mesh which had firmly adhered to the muscle wall. To correct

this problem these animals were lightly re-anesthetized with ether and dental cement was applied over the mesh and around the exteriorized fistula flange. Immediately after each surgical procedure and periodically (at the end of each test session) thereafter, the wounds were treated with a topical antibiotic (Bacitracin).

Procedure

Animals were allowed at least a 9 day surgical recovery period prior to the start of the test sessions. During the recovery period, the animals were accustomed to the liquid test diet by making it accessible to them in their home cages for 8 hrs on two consecutive days (chow and water were also available). The test diet consisted of 50% Carnation brand evaporated milk, 37% distilled water, 12% sucrose and 1% artificial almond extract. In my experience, this liquid diet is palatable to hamsters. During the recovery period and between test sessions, the animals had ad lib access to Purina rat chow pellets and water. Seventeen hrs prior to each of the test sessions, the animals were transferred from their home cages (plastic, solid bottom) to suspended wire bottom test cages. Prior to each test session, the set screw normally closing the fistula was removed and the fistula and stomach were rinsed with a spray of isotonic saline at approximately 37^{o} C to remove food debris that would otherwise clog the fistula. For test sessions during which the animals were "real-feeding", the set screw was immediately replaced. In sham-feeding sessions, the set screw was replaced at the end of the session.

The animals underwent a series of test sessions in which three parameters were varied. Hamsters were tested under food deprived and non-deprived conditions; with the fistula open or closed; and after i.p. saline or CCK-8 (Squibb, Batch # 556159-2F2722) injections given less than five min before offering the feeding bottle. A detailed protocol for the experiment is provided in Table VII. On each session, intake of the liquid diet offered in a calibrated drinking bottle was recorded to the nearest mL, 5, 15, 30, 60, 120, 180 and 240 min after the drinking bottle became accessible. In pilot studies it was discovered that measuring drainage from the stomach during sham-feeding sessions was not feasible. Hamsters would not tolerate being tethered to a coil-wrapped collecting tube connected to an internal fistula that screwed into the chronically implanted fistula. In addition, drainage collected by this method would not provide an accurate measurement of the amount of liquid diet passing out of the fistula because of the small amounts ingested over a relatively long period of time. That is, most of the drippings from the fistula would cling and harden along the inner walls of the collecting tube before draining. Thus, in sham feeding sessions, orally ingested food was allowed to drip onto bedding trays beneath the cages. No attempts were made to measure these drippings. Except during occasional grooming of the area around the fistula, the hamsters did not ingest diet passing out of the fistula during sham-feeding sessions.

Results

Twenty five animals were initially implanted with gastric fistulas.

TABLE VII

Protocol for Experiment 8

Session #	Day	N	n females	n males	Hrs Deprivation	$\bar{X}$ (± S.E.M.) % of Control Body Weight	Fistula	Treatment
1	0	18	11	7	0	101.0 (0.6)	Closed	None
2	3	18	11	7	0	97.3 (0.7)	Open	None
3	7	18	11	7	17	94.1 (0.9)	Closed	None
4	10	18	11	7	17	89.2 (1.0)	Open	None
5	14	18	11	7	17	86.0 (1.2)	Open	None
6	18	18	11	7	17	84.9 (1.4)	Open	Saline
7	21	18	11	7	17	83.3 (1.6)	Open	4.0 µg/kg CCK-8
8	25	15	9	6	17	85.6 (1.6)	Closed	Saline
9	28	15	9	6	17	84.0 (1.7)	Closed	4.0 µg/kg CCK-8

In 18, the fistula remained patent at least until the end of the seventh session. Seven of the 25 were dropped from the study for the following reasons: five had leaks develop around the fistula; one developed a severe skin and muscle infection around the fistula; and one animal did not adequately adapt to the deprivation schedule and became totally aphagic. These animals were sacrificed when the problem was first noted, and any data collected for these animals were discarded. Later in the experiment, three other animals also developed leaks around the fistula. They too were immediately sacrificed, but any data collected for the animals up to the seventh session were included in the statistical analyses (see n's in Table VII). Animals whose data were included in analyses were judged to be in good health.

Part A: Initial Response to Sham Feeding

Cumulative intakes during the first through fourth sessions underwent three-way analysis of variance ([closed vs open fistula] x [0 vs 17 hrs of deprivation] x [within session time]) with repeated measures on each factor. Intakes tended to be higher after 17 hrs of food deprivation, but this difference did not reach statistical significance ($p = 0.096$). In addition, intakes were not reliably increased during the first two sham-feeding sessions (see Figure 9, top). None of the interactions among factors were significant.

Part B: Effects of Experience on Sham Feeding

FIGURE 9. Top: Mean (± s.e.m.) cumulative intake of liquid diet during real- and sham-feeding in hamsters tested under *ad lib* feeding conditions and after food deprivation (sessions 1-4). Bottom: Mean (± s.e.m.) cumulative intake of liquid diet in food-deprived hamsters across sham-feeding sessions. Means without a common superscript are significantly different, $p < 0.05$.

As shown in Figure 9 (bottom), there were dramatic increases in sham-fed liquid diet intake over the next two 17 hr deprivation - fistula open sessions (i.e., the fifth and sixth sessions compared to the fourth session). Although Figure 9 (bottom) may suggest that intakes during the fifth and sixth sessions were continuous, feeding was clearly episodic, with the animals taking more frequent and larger meals. Direct observations indicated that the animals failed to show normal postprandial satiety behaviour (i.e., resting and sleep) during these sham-feeding sessions.

To determine whether increased sham feeding over sessions was a result of some trial (e.g. learning) effect or due to the trend towards decreasing body weight (or some factor associated with it), a two-way analysis of covariance with repeated measures was conducted on the cumulative food intake data collected on the fourth, fifth, and sixth sessions. Sessions and time were the repeated measures, and percent of preoperative body weight at each session was the covariate. Intakes were not at all related to weight loss ($p = 0.31$). Overall intakes clearly increased over sessions 4 to 6, $F(2, 37) = 16.33$, $p < 0.0003$ independently of weight changes, and the magnitude of between session differences also increased significantly with within session time, $F(12, 204) = 12.75$, $p < 0.0001$ (for the sessions x time interaction).

Part C: Responsiveness to CCK-8 During Real and Sham Feeding

A three-way factorial analysis (open vs closed fistula x saline vs CCK-8 x time) was not possible because of missing data for three animals

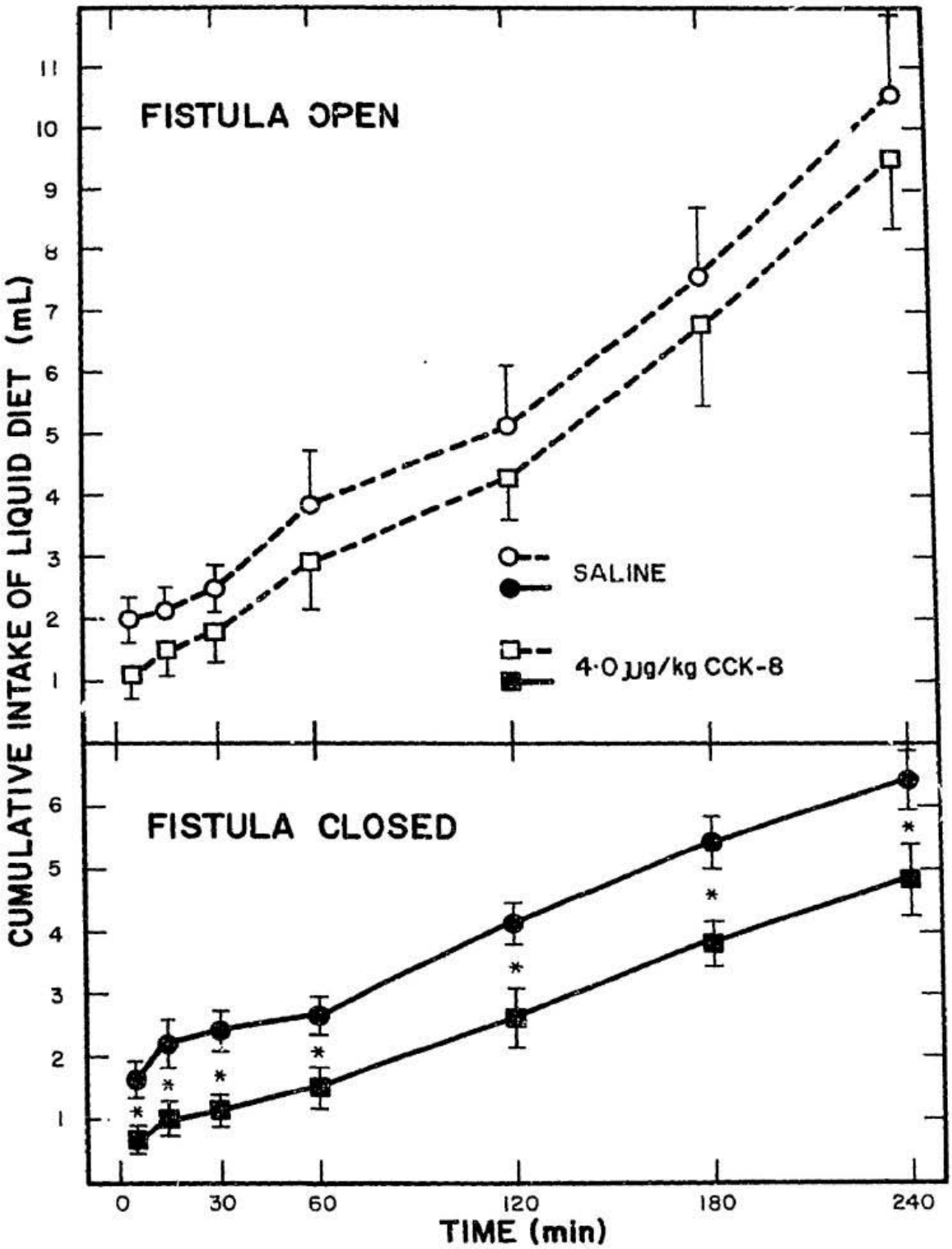
which had developed leaks around the fistula after the fistula open - CCK-8 session. Thus, intakes after saline and CCK-8 were compared in separate analyses for the fistula open and closed conditions. CCK-8 did not reliably reduce sham-feeding ($p = 0.32$). However, the same dose was effective in the subsequent session with the fistula closed, $F(11, 14) = 11.91$, $p < 0.004$ (see Figure 10). These results are not biased by the exclusion of three animals in the "fistula closed" analysis. Excluding the data of the same three animals from the "fistula open" analysis did not alter the conclusion that CCK-8 did not reliably reduce intake.

In summary, nondeprived hamsters did not increase their intakes when orally ingested liquid diet drained from the gastric fistula. Sham-feeding hamsters did not immediately increase their oral intakes after deprivation. Enhanced sham-feeding, however, was seen in subsequent sessions, indicating some practice effect. CCK-8 did not reliably reduce intake during sham-feeding, but was effective during real-feeding.

Discussion

It may be justly argued that several features of the experiment pose some interpretative problems and limit the generalizability of the above findings. Firstly, since ingested diet was not collected and compared against oral intake during the sham-feeding sessions, it is not absolutely certain that some food did not accummulate in the stomach, distend it to some degree, and empty into the duodenum. This possibility is unlikely because observations of the animals during sham-

FIGURE 10. Mean (± s.e.m.) cumulative intake of liquid diet after CCK-8 during sham-feeding (top) and real-feeding (bottom) sessions. * Means are significantly different, $p < 0.05$.


FISTULA OPEN
FISTULA CLOSED
SALINE
4·0 µg/kg CCK-8
CUMULATIVE INTAKE OF LIQUID DIET (mL)
TIME (min)
0
30
60
120
180
240
1
2
3
4
5
6
7
8
9
10
11

feeding indicated that diet began draining from the fistula shortly after oral ingestion commenced and continued to drain throughout the course of the meal. Moreover, X-ray (see Weingarten and Watson, 1982) and volumetric (Kraly, Carty, Resnick, and Smith, 1978) studies in rats have shown that orally ingested liquid diet drains freely out of gastric fistulas similar in design to the one used here. Another methodological problem is that hamsters may only ingest a fraction of a mL during a feeding bout, but the calibrated drinking tubes only read to a one mL accuracy. This problem became evident when animals were observed drinking during a given time interval, but the small amount did not register as a change on the drinking tube. Although this probably reduced the sensitivity of the analysis by increasing within treatment variability, (early in the sessions in particular), there are no a-posteriori reasons to believe that this problem biased the data in any particular direction. Another problem is related to the repeated measures design of the experiment in which different treatments were tested against a background of increasing weight loss. For the analysis of the data in Part B, this problem was corrected statistically by conducting an analysis of covariance which showed that intakes were not related to the amount of weight loss. Since weight loss reached an asymptotic level by the fifth session (see Table VII), weight loss and any factors associated with it, did not pose interpretative problems in subsequent analyses.

In sham-feeding rats, the amount of liquid diet and the rate at which it is ingested are a function of a complex interaction among many factors including; properties of the diet, for example, palatability

(Weingarten and Watson, 1982) and liquid diet osmolality (Mook, Culberson, Gelbart and McDonald, 1983); experience with sham-feeding and the diet (Mook et al., 1983); and level of deprivation (Young, Gibbs, Antin, Holt and Smith, 1974; see also Smith and Gibbs, 1979). In view of the high degree of variability in the reported rat studies, appropriate caution is taken not to generalize the above findings beyond the conditions under which the data were collected. For the purpose of this experiment, parameters believed to optimize sham-feeding in hamsters were chosen. Since, to my knowledge, this is the first sham-feeding study conducted in the hamster, these parameters were selected on the basis of what has been reported in rats. The diet was a sweet milk solution that is readily sampled by undeprived hamsters. The hamsters were familiarized with this diet prior to testing and were allowed to experience sham-feeding over several sessions to further enhance intake prior to testing CCK-8. Lastly, a deprivation period that maximally enhances sham-feeding in rats (Young et al., 1974) was selected for the present study. Thus, under a comparable set of testing conditions, it is possible to compare the present data with findings in rats.

A most surprising finding was the complete absence of increased oral intakes during the first sham-feeding session after 17 hrs of deprivation. Although rats do progressively increase oral intakes across sham-feeding sessions (e.g. Young et al., 1974), particularly when hypertonic nutrient solutions are used (Mook et al., 1983), elevated intakes are nonetheless seen during first experience with sham-feeding. In rats, increased oral intakes over sham-feeding sessions is

thought to reflect the animals' learning to dissociate oropharyngeal cues from the 'expected' postingestive consequences (i.e., satiety). Thus conditioned (or otherwise) orosensory cues normally play some role in satiety in normally feeding rats (Booth, 1972); and limit oral intakes during initial sham-feeding sessions (Mook et al., 1983). The lack of increased intakes by the hamsters during the first two sham-feeding sessions suggests that orosensory stimuli exert greater control in limiting sham- feeding in hamsters than in rats. This may also serve as a partial explanation for the normally feeding, intact hamster's inability to increase food intake after a fast. In this regard, it is important to note that total cumulative intakes during the last two sessions (eight and nine) with the fistula closed were double the amounts consumed during the first four sessions (compare the top of Figure 9 with the bottom of Figure 10). This, the first demonstration of hamsters substantially increasing their food intake after deprivation, suggests a loss or partial loss of orosensory control over feeding as a result of experience of sham-feeding.

Another major difference between sham-feeding rats and hamsters was the magnitude and pattern of sham-feeding. Even after considerable experience with sham-feeding, hamsters did not ingest copious amounts. Furthermore, while the hamsters did appear to take progressively more, and larger, meals across the sham-feeding sessions, feeding occurred in discrete bouts separated by 10 or more min. This contrasts with the behaviour of sham-feeding rats tested under comparable conditions, which will sham-feed to exhaustion if permitted (see Smith and Gibbs, 1979); and sham-feed almost continuously with feeding interrupted only by brief

pauses (e.g. Young et al., 1974). However, just like sham-feeding rats (Young et al., 1974), during the second and third sham-feeding sessions after 17 hrs of deprivation (sessions 5 and 6) the hamsters failed to show the usual postprandial behaviour. The hamsters were continuously aroused, and were never seen resting or sleeping. Thus, even though hamsters did not show the spectacular level of sham-feeding typical of rats, they clearly did not behave as if satiated during these sessions. It is clear that quantitative measures of satiety-related behaviour will add power to future studies of sham-feeding in the hamster.

The major objective of this experiment was to determine whether CCK-8, in hamsters, could reduce liquid diet intake in the absence of gastric distention. The data suggest not. However, this conclusion must be considered tentative in view of the issues raised below.

In rats, it has been shown that the effects of CCK on feeding are dependent on other preabsorptive stimuli, orosensory stimulation, for example (Gosnell and Hsiao, 1981; Waldbillig and Bartness, 1982). In one pertinent study of the sham-feeding rat, CCK was only effective in reducing oral intake when it was given some time after the animals began sham-feeding (Antin, Gibbs, and Smith, 1978), thus demonstrating that prior orosensory stimulation was necessary for CCK to reduce sham-feeding. It could be argued, therefore, that CCK failed to reduce sham-feeding in hamsters because it was administered prior to the animals having had orosensory stimulation. It is not known whether CCK administered later in the sham feeding session would have reliably reduced subsequent oral intake. Yet, it is clear that under the same conditions in which it failed to significantly reduce sham-feeding, CCK

was effective when the fistula was closed and the stomach able to distend.

One could also object to the fact that only one dose of CCK-8 was tested. The chosen dose was one that has been shown to reliably reduce food intake of hamsters in the foregoing experiments. Under the conditions in which the animals were tested, it is unlikely that smaller doses would have reduced feeding under the fistula-open condition, and any larger doses tested would have been a further upwards departure from physiological amounts. A related argument is that by virtue of endogenous CCK released by the duodenum during real-feeding, hamsters tested under the fistula open and fistula closed conditions were not tested under "dose-equivalent" conditions. This is clearly a valid argument. Evidently, only future studies that circumvent this confounding factor (see General Discussion of Experiments 7-9) will reveal whether, under conditions of equivalent circulating levels, CCK is more effective at reducing real- feeding than sham-feeding. There are, however, alternative means of addressing the issue of whether CCK's suppressive effect on feeding is mediated by gastric distention. One possible means is to examine CCK's effect on feeding in animals in which the mechanism through which CCK influences gastric emptying has been manipulated.

CCK's effects on gastric emptying are likely a result of a constellation of CCK actions on gastric and pyloric smooth muscle. By acting directly on smooth muscle receptors, and by activating extrinsic (e.g. vagal) and intrinsic (i.e., intramural plexus) neural loops, the net effect of CCK action is decreased glandular stomach motility and

contraction of the pylorus (Scheurer, Varga, Drack, Burki and Hulter, 1983; Behar, Biancani, and Zabinski, 1979; Debas, Farrooq, and Grossman, 1975). Although it is not possible to attribute CCK's effects on gastric emptying to its action on a particular muscular (or neuromuscular) system, Smith, Moran, Coyle, Kuhar, O'Donohue, and McHugh (1984) have suggested that the pylorus is the primary site through which CCK inhibits gastric emptying. This conclusion is based on their observation that, within the upper gastrointestinal tract, specific binding of radiolabelled CCK was restricted to the circular smooth muscle layer of the pylorus.

If Smith et al.'s (1984) hypothesis that CCK inhibits gastric emptying by acting primarily on the pylorus were correct, then experimentally blocking the expected pyloric response to CCK should abolish or attenuate CCK inhibition of gastric emptying. In the absence of such a pyloric mechanism it would then be possible to test whether the reduction of food intake after CCK is secondary to a reduced rate of gastric emptying and facilitation of stomach distention. A relatively simple means of limiting or eliminating CCK-stimulated contraction of the pylorus is to render this sphincter incompetent by pyloroplasty. In the following experiment, feeding after treatment with CCK was tested in hamsters which had undergone pyloroplasty and in sham-operated animals.

EXPERIMENT 9: ROLE OF THE PYLORUS IN THE INHIBITION OF GASTRIC EMPTYING IN RESPONSE TO CCK-8

Methods

Surgery

Laboratory bred hamsters derived from the Lakeview stock were selected for this experiment. The animals were four to five months old and weighed between 140-164 g. Pyloroplasties (N = 12; 6 females, 7 males) were performed under sodium pentobarbital anaesthesia. A midline incision was made as described for the gastric fistula implant. The stomach and upper portion of the small intestine were then exteriorized and kept moist by periodic application of sterile saline with surgical gauze. The pylorus was readily identified by applying gentle traction on the duodenum with a cotton-stick applicator. A small purse-string suture was then sewn along the ventral surface of the pylorus and a 1.5 mm longitudinal incision was made in the region encircled by the suture. The incision was probed with the wooden end of a cotton stick applicator to ensure the completeness of the incision. The suture was then tied to produce a 'pucker'. The stomach, pylorus, and duodenum were returned to the abdominal cavity, the abdominal muscle wall closed with sutures, and the skin wound closed with surgical clips. Sham operates (N = 10; 5 females, 5 males) were treated identically to the point of touching the pylorus with a cotton stick applicator.

Procedure

After a three week recovery period, a series of three tests separated by at least four days was begun. The animals were food

deprived (water was available ad lib) for three hrs prior to each test. On the first test, animals were given an i.p. injection of saline (0.4 ml/100 g) five min prior to allowing access to a preweighed food ration (Purina rat chow). Food intakes were calculated at hourly intervals for the next three hrs. The same protocol was followed on the subsequent tests except that hamsters were injected with 2.0 (second test) or 4.0 µg/kg (third test) CCK-8 (Squibb, Batch # 556159-2F722), respectively. One week after the third feeding test the animals were fasted for 48 hrs and underwent a gastric empyting test according to the protocol described in Experiment 7. Approximately half of the animals were tested with saline and the remainder with the same batch of CCK-8 (4.0 ug/kg).

Results and Discussion

There were no body weight differences between animals with pyloroplasty and surgical controls over the three week recovery period. Further, 24 hr food intakes sampled during the last three recovery days did not differ between the groups. Food intake after 3 hrs of deprivation and i.p. saline also did not differ between the groups. As can be seen in Table VIII, the groups did not respond differentially to the two doses of CCK-8. The lack of (surgical) treatment effect was so obvious, that statistical analyses were not conducted on the data. Table IX shows that animals treated with CCK-8 retained a greater proportion of ingested solid food in their stomachs than saline-injected controls $F(1, 19) = 16.49$, $p < 0.001$. Thus, again, CCK-8 reliably

TABLE VIII

Food Intake After Intraperitoneal Injections of CCK-8

in Hamsters With Pyloroplasty

	$\bar{X}$ (± S.E.M.) Food Intake (g) and [% of Animals Eating]*					
Group	Sham Surgery (n = 10)			Pyloroplasty (n = 13)		
Dose	0	2	4	0	2	4
	µg/kg CCK-8			µg/kg CCK-8		
Time (Hrs Postfast)						
0-1	1.15 (0.09) [100]	0.63 (0.07) [100]	0.38 (0.03) [90.0]	0.99 (0.10) [100]	0.57 (0.04) [100]	0.44 (0.04) [100]
1-2	0.38 (0.17) [90.0]	0.51 (0.07) [100]	0.55 (0.11) [100]	0.40 (0.06) [100]	0.53 (0.05) [100]	0.55 (0.06) [100]
2-3	0.53 (0.16) [100]	0.41 (0.04) [100]	0.40 (0.07) [100]	0.48 (0.09) [100]	0.55 (0.04) [100]	0.46 (0.05) [90.0]

* 0.2 or more g during a given time interval

TABLE IX

Effect of CCK-8 on Gastric Emptying in Hamsters with or without Pyloroplasty

Group	N	Dose	$\bar{X}$ (± S.E.M.) % of Food Remaining in Stomach
Sham	5	Saline	54.0 (5.5)
	5	4.0 µg/kg CCK-8	87.7 (8.6)
Pyloroplasty	6	Saline	49.5 (5.0)
	7	4.0 µg/kg CCK-8	82.3 (10.4)

inhibited gastric emptying. Under basal conditions (i.e., after saline) gastric retention was not altered by pyloroplasty; and CCK-8 inhibited gastric emptying in animals with pyloroplasty to the same degree that it did in intact hamsters.

In this experiment pyloroplasty was used as a possible way of blocking CCK produced inhibition of gastric emptying, thereby offering a possible means of studying CCK's actions on feeding behaviour in the absence of gastric distention. In short, the experiment sought to answer the question as to whether CCK's reduction of food intake is an indirect result of a facilitative effect on gastric distention. Of course, the validity of this approach rested on whether pyloroplasty was sufficient to block, or at least attenuate, CCK's actions on gastric emptying. As shown in Table IX, pyloroplasty did not affect gastric emptying in untreated animals, and did not alter the usual inhibition of gastric emptying after exogenous CCK.

Pyloroplasty is effectively used in humans undergoing gastric vagotomy (for ulcer treatment) to compensate for loss of gastric propulsive activity. Pyloroplasty was also found to restore gastric draining in rats with abdominal vagotomy (Mordes, el Lozy, Herrera and Silen, 1979). Thus, disruption of the normal mechanics of the pyloric circular musculature by pyloroplasty can have profound effects on gastric emptying in other species. It is not entirely clear why pyloroplasty did not at least attenuate the inhibition of gastric empyting produced by CCK. However, the results of the only other study (of which I am aware) examining the mechanisms of CCK action on gastric clearance may shed some light on this matter. In dogs, it was found

that although the destruction of the pyloric mechanism (by either pyloroplasty or antrectomy), reduced the inhibitory effect of low CCK-8 doses on gastric emptying, larger doses were as effective as in intact dogs (Yamagishi and Debas, 1978). These workers therefore concluded that the actions of CCK on the pylorus are indeed important for the peptide's effect on gastric emptying, but that additional mechanisms contribute to this response. They suggested that CCK's inhibition of gastric emptying is a net result of pyloric contraction and proximal stomach relaxation. It is not known whether the pyloroplasty would have abolished the inhibition of gastric emptying produced by lower doses of CCK in hamster. It is clear, however, that at the tested dose of CCK-8, gastric emptying in animals with pyloroplasty was inhibited to the same degree as in intact animals. Consequently, this procedure, as used under conditions outlined above, was not a suitable means of assessing the role of gastric distention in CCK's inhibition of food intake.

GENERAL DISCUSSION OF EXPERIMENTS 7-9

The question of whether the inhibition of gastric emptying and the resulting increased gastric distention mediate CCK's suppressive effect on hamster feeding or whether CCK influences feeding more directly was not answered entirely satisfactorily. Exogenous, and presumably, endogenous, CCK does have potent suppressive effects on gastric emptying in hamsters. In the sham-feeding preparation, CCK-8 produced a slight reduction in intake of liquid diet while the gastric fistula was open, but the magnitude of the effect was not statistically reliable. In

contrast, when animals were tested with the fistula closed and the stomach allowed to distend during feeding, CCK produced a reliable reduction of liquid diet intake. However, when animals were tested with the fistula closed, ingested food was allowed to stimulate the release of endogenous CCK. Therefore, since animals were not tested under "dose-equivalent" conditions, the experiment d'd not provide conclusive evidence that in hamsters, enhanced gastric distention mediates (either totally or partially) CCK's effects on feeding. Pyloroplasty was not used successfully as a further means of addressing this question.

Although it may appear that resolution of this issue (for the hamster) is at an impasse, all the possible experimental strategies for further probing this issue have not been exhausted. For example, it remains to be seen whether exogenous CCK is more potent in reducing food intake when the hamster stomach is preloaded with non-nutritive bulk so as to facilitate gastric distention. Further, the relative efficacy with which intestinal nutrient infusions suppress s. am- and real-feeding in hamsters also remains to be determined. In such tests, the effects of equivalent amounts of endogeneous CCK (and other intestinal satiety stimuli) on food intake could be evaluated with or without the occurrence of gastric distention. Lastly, the effects of CCK could be compared in real- and sham-feeding hamsters during temporary occlusion of the pylorus.

EXPERIMENT 10: ROLE OF THE ABDOMINAL VAGUS IN MEDIATING FEEDING RESPONSES TO PUTATIVE SATIETY PEPTIDES

The initial failures to find feeding effects following central CCK administration in rats (see Experiment 6 discussions), in combination with renewed interest in visceral nervous system control of feeding, provided the impetus for the search for a peripheral site of CCK action in the control of appetite. The subdiaphragmatic vagus was an appropriate candidate for a feeding-related peripheral CCK target site because it is rich in putative CCK receptors (Zarbin, Wamsley, Innis, and Kuhar, 1981), because it innervates visceral smooth muscle on which CCK acts directly or indirectly (Scheurer et al., 1983), and because abdominal vagotomy in rats blocked the satiety effect of intraduodenal infusions of the CCK secretagogues, fats and amino acids (Novin, Sanderson, and Gonzalez, 1979). In an early study, it was reported that feeding in rats with bilateral total subdiaphragmatic vagotomy remained responsive to systemic CCK-8 injections (Anika et al., 1977). More recently, Smith and his colleagues found that rats with virtually the same type of vagotomy were totally unresponsive to a range of CCK-8 and caerulein doses (Jerome et al., 1981; Smith, Jerome, Eterno, and Cushin, 1980; Smith, Jerome, Cushin, Eterno, and Simansky, 1981). Workers in different laboratories have since corroborated the blocking effect of abdominal vagotomy on CCK-induced feeding suppression (Lorenz and Goldman, 1982; Morley et al., 1982a) and have extended this finding by showing that abdominal vagotomy blocks the effects of CCK on satiety-related behaviour (Crawley, Hayes, and Paul, 1981) and eliminates augmented feeding after injections of proglumide, a putative CCK antagonist (Shillabeer and Davison, 1984). In fact, the vagotomy blocking effect in rats now appears so reliable that investigators have

begun to use tests of CCK on feeding to verify the functional completeness of vagotomy (Edwards and Ritter, 1983).

Although the importance of the vagus nerve for the CCK satiety effect has been firmly established in the rat, little is known about the role of the vagus nerve in other species that show reduced feeding in response to exogenous systemic CCK. The only other species in which CCK has been tested in vagotomized animals are rabbits and dogs, and in both species vagotomy was found not to alter feeding responses to CCK (Houpt et al., 1978; Levine, Morley, Siever, Gosnell and Silvis, 1984). In view of these species differences it was important to evaluate the role of the abdominal va_us in CCK feeding inhibition in hamsters.

Just as relatively little is known about the stimuli that regulate feeding in hamsters, there is little information on stimuli that control drinking in this species. The few studies of physiological control of drinking behaviour in hamsters reported to date have indicated that stimuli which control drinking in other species, like the rat, may not be important controls in hamsters. In rats and other species, hypovolaemia is a potent stimulus for drinking (see Fitzsimons, 1980 for a review). However, colloid dialysis in hamsters, which induced a 30% depletion of intravascular volume, did not make the animals hyperdipsic (Fitts, Corp, and Simpson, 1982). In certain species, like the rat, the effects of hypovolaemia on drinking are believed to be mediated mainly by blood-borne angiotensin II (AII), which is generated in the plasma in response to vascular volume deficits and the associated hypotension (Fitzsimons, 1980). Consistent with this idea are numerous studies showing that systemic injections of AII mimic the physiological and

behavioural effects of hypovolaemia, including polydipsia (Fitzsimons, 1980; Lind and Johnson, 1982). Experiments directed at assessing whether blood-bourne AII is dipsogenic in hamsters have not yet been reported. It is not certain whether the 30% depletion of intravascular volume in the Fitts et al. (1982) study was sufficient to produce a rise in the level of circulating AII, because this was not measured directly. There was, however, indirect evidence of elevated blood AII levels, as their experimental hamsters exhibited other physiological and behavioural AII-mediated efffects (increased sodium appetite and increased renal water and sodium conservation). Thus, the Fitts et al. data suggest that renal renin-AII formation may have been activated by experimentally induced hypovolaemia, but that circulating AII is not an effective dipsogen. To test more directly whether circulating AII is a stimulus for drinking in the hamster, water intake was measured after systemic administration of exogenous AII.

In the rat, the dipsogenic effect of circulating AII appears to be partly mediated by its direct or indirect actions on vagal afferent fibres, because total or selective section of subdiaphragmatic vagal branches attenuates drinking to systemic injections of AII (Jerome and Smith, 1982b; Rowland, 1980; Simansky and Smith, 1983; Smith and Jerome, 1983). If intact hamsters were to drink more following peripheral injections of AII, it would be of interest to determine whether vagotomy would block or attenuate this response.

Methods

Surgery

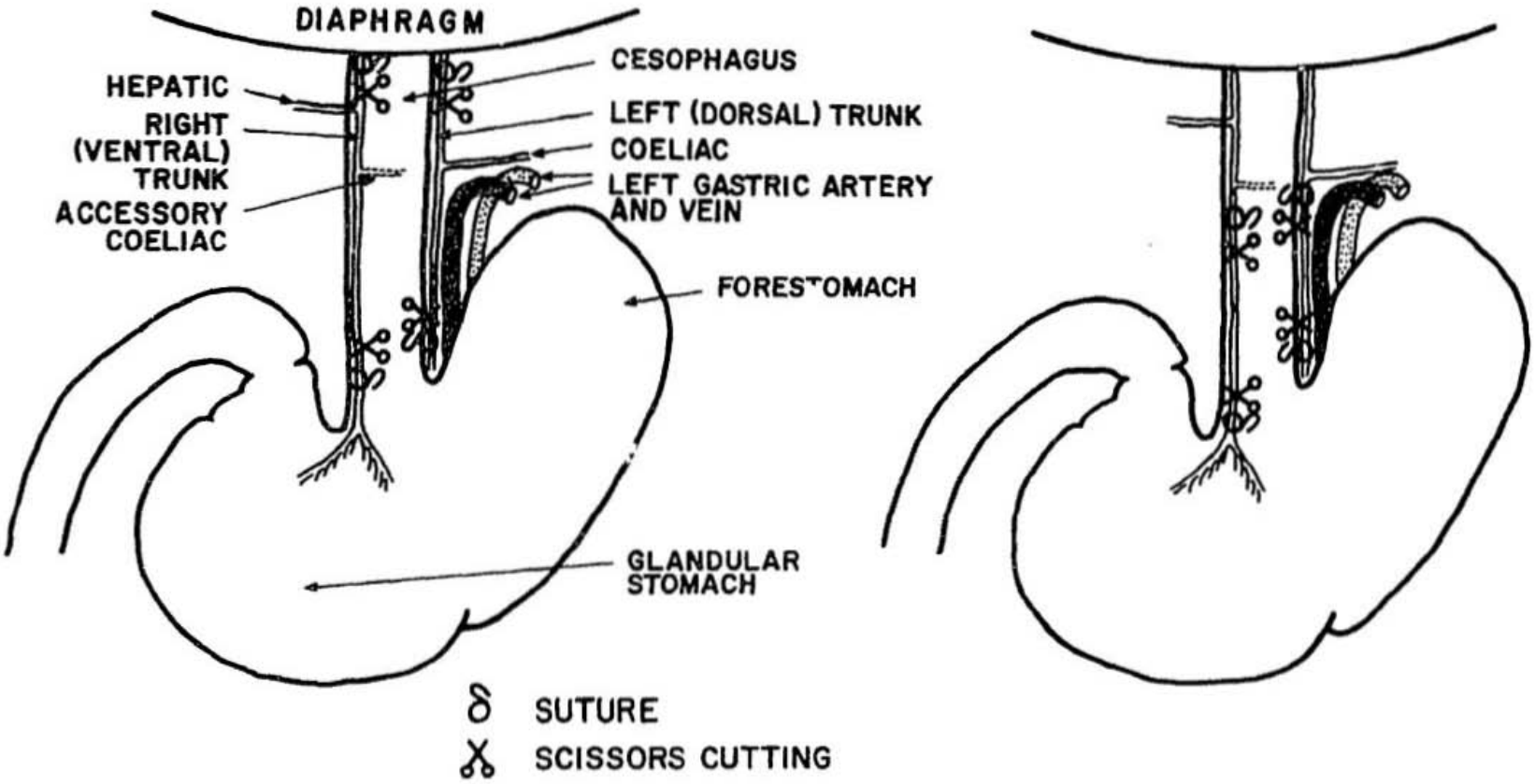
Twenty laboratory bred hamsters (10 female, 10 male) underwent total subdiaphragmatic vagotomy. Parasympathetic innervation of the liver, pancreas, oesophagus, and gastrointestinal tract arises from vagal fibres branching from the abdominal vagal trunks. In hamsters, hepatic and coeliac vagal fibres branch from the main abdominal trunks at points similar to those described in rats (compare Figure 11 with figures in Powley, Prechtl, Fox, and Berthoud [1983] and Tordoff and Novin [1982]). The only major difference was that, in hamsters, an accessory coeliac branch was not always visible under the dissecting microscope. This branch is very fine and in the cases where it was apparently absent, it may have been obscured by manipulation and traction of the stomach and oesophagus (Powley et al., 1983).

Vagotomies and sham surgeries were performed under sodium pentobarbital anaesthesia. A 3 cm midline incision was made just below the sternum. The stomach was exteriorized and covered with surgical gauze soaked in sterile saline. The lobes of the liver were gently deflected to the side to provide an unobstructed view of the oesophagus and vagi. With the aid of a 10x dissecting microscope, a 3-0 silk suture was tied around the right (or ventral) vagal trunk just below the diaphragm. Another suture was tied around the right trunk just above the point were it divides into gastric branches. Similarly, the left (or dorsal) trunk was sutured just below the diaphragm and just below the coeliac branch (see Figure 11). Approximately 3 cm of each trunk was removed between the sutures. Scissors and fine forceps were used to

FIGURE 11. Schematic representation of the abdominal vagi and their branches in the hamster. The figure denotes where the vagi were ligated and cut for each type of vagotomy performed in the behavioural (Experiments 10-12) and anatomical (Appendix) studies.

TOTAL VAGOTOMY

GASTRIC VAGOTOMY



strip away the nerve sections from the oesophagus. All fine communicating fibres and connective tissue on the outer oesophageal wall between the sutures were also removed with fine forceps. The sutures served to aid in the postmortem visual verification of the vagotomies and to prevent the possible regeneration of the sectioned nerves. The wounds were closed with silk sutures and surgical clips. The surgical procedure for sham surgery (5 female, 5 male) was identical, to the point of touching each trunk with a cotton-stick applicator. In pilot work, it was found that hamsters tolerate this vagotomy surprisingly well. Thus, no preventative or precautionary measures were necessary to ensure 100% survival.

Procedure

Animals were weighed at least weekly throughout the course of the experiment. While food and water were available ad lib prior to and during testing, the animals were given 1.0 mg/kg of synthetic human AII (Sigma, Lot # 72F-0462) or equal volumes of saline by s.c. injection. Following the injection the animals were returned to their cages and water intake over the next 90 min was recorded. Half of the hamsters were tested with AII on the 14th day after surgery and with saline on the 16th; and the other half received treatments in reversed order.

A series of five feeding tests was begun on day 29 postsurgery. The animals were prepared for each test by a 15 hr fast during which water was available ad lib. At the end of the fast the animals were injected with either 4.0 µg/kg CCK-8 (Squibb, Batch # NN0020NC) i.p.,

8.0 µg/kg BBS (Bachem, Lot # R5911) i.p., 25.0 µg/kg TRH i.p., 10.0 µg/kg CT (Bachem, Lot # R2164) s.c., or saline (i.p. or s.c.). Five min after the injection the animals were given a preweighed ration of Charles River rodent chow, after which, food and water intakes were measured at regular intervals up to 24 hrs postfast. The hamsters were tested in this way every fourth day. Peptide treatments and saline were given in random sequence.

Within two weeks of the last feeding test, the animals were fasted for 24 hrs and then sacrificed by decapitation. Immediately after decapitation a large thoracic and abdominal incision was made and the stomach and oesophagus were exposed. The oesophagus (above the diaphragm) and duodenum were ligated and cut distal to where they were ligated. The stomach and strip of oesophagus were stored in 10% Formalin for later inspection.

Results

Body Weights

Body weights on the day of surgery and on Days 7, 28, 35, and 56 postsurgery underwent three-way analysis of variance (surgery x sex x days) with repeated measures on the days factor. Overall, females were heavier than males, $F(1, 26) = 9.08$, $p < 0.006$, even though all the animals in this experiment were born within 10 days of each other. All groups lost weight over time, $F(4, 104) = 19.43$, $p < 0.001$. Weight loss appeared to be greater in males than females but this difference was not

statistically reliable (p = 0.071, for the sex x time interaction). Body weights of vagotomized males and females never differed significantly from that of their controls (see Figure 12).

Drinking Tests

The behaviour of the animals was notably changed after this dose of AII. Within two min of administration of this dose, the animals assumed an extended-prone position as described after large doses of TRH. This response lasted from five to 10 min, after which, animals appeared to be normal. Water intakes 30 min after, and between 31 and 120 min after s.c. saline or AII underwent separate two-way analyses of variance (surgery x treatment) with repeated measures on the treatment factor. Water intakes during the first 30 min after 1.0 mg/kg AII were greater than after saline, $F(1, 28) = 4.57$, $p < 0.05$. Vagotomized and sham operated animals were equally responsive to this dose of AII. Subsequent (31-120 min postinjection) water intakes were also increased after AII (see Table X), but this difference was not reliable (p = 0.08).

Feeding Tests

Food and water intakes during the first hr postfast, from the second through the fifth hrs, the sixth through the ninth, and tenth through the 24th hrs postfast underwent separate three-way analysis of variance (surgery x sex x treatment) with repeated measures on the treatment

FIGURE 12. Mean (± s.e.m.) body weights of intact and vagotomized hamsters throughout Experiment 10.

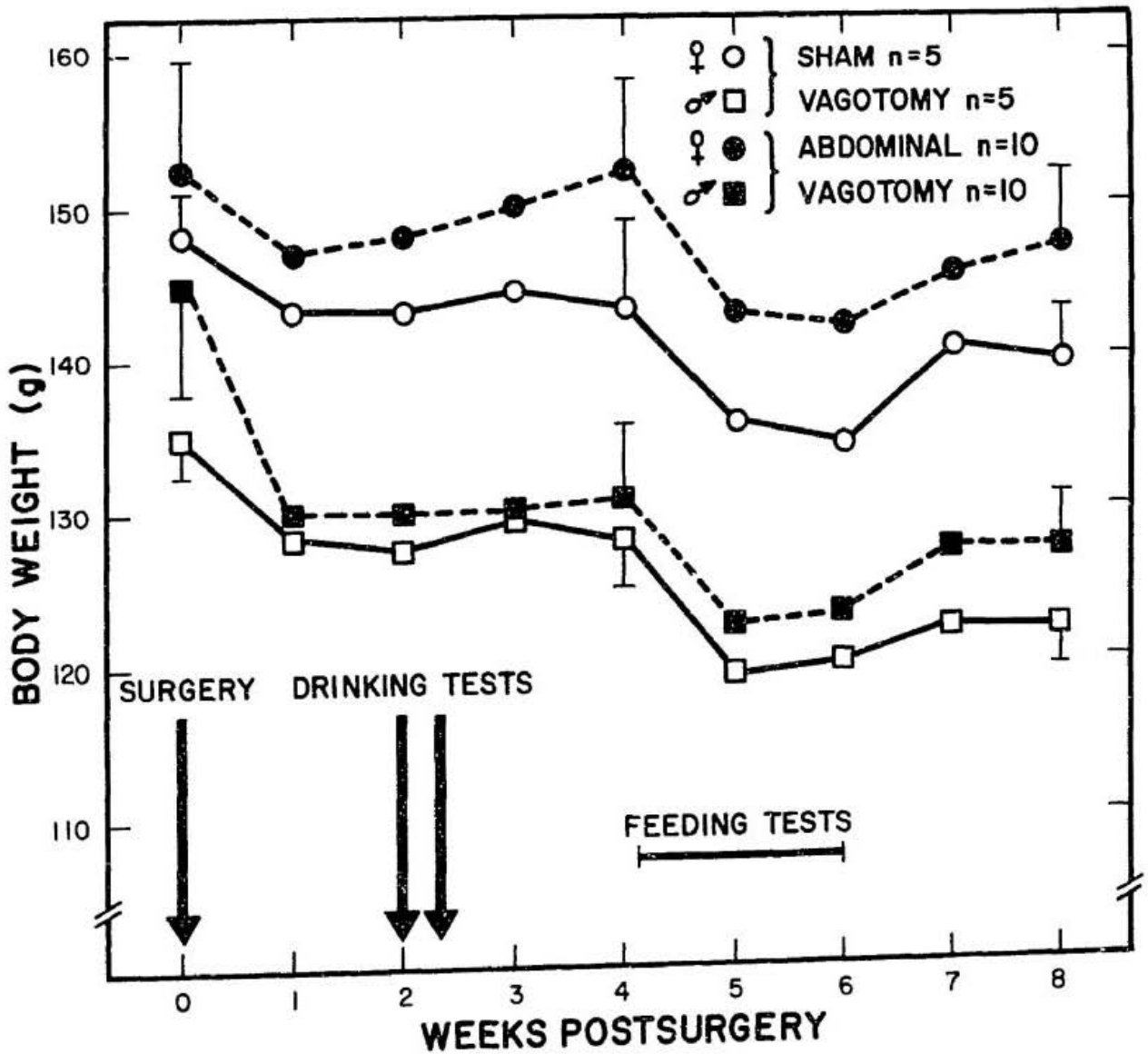

♀ ○ } SHAM n=5
♂ □ } VAGOTOMY n=5
♀ ● } ABDOMINAL n=10
♂ ■ } VAGOTOMY n=10
BODY WEIGHT (g)
160
150
140
130
120
110
SURGERY
DRINKING TESTS
FEEDING TESTS
0
1
2
3
4
5
6
7
8
WEEKS POSTSURGERY

TABLE X

Water Intake After High Dose Angiotensin II Injection

In Intact and Vagotomized Hamsters

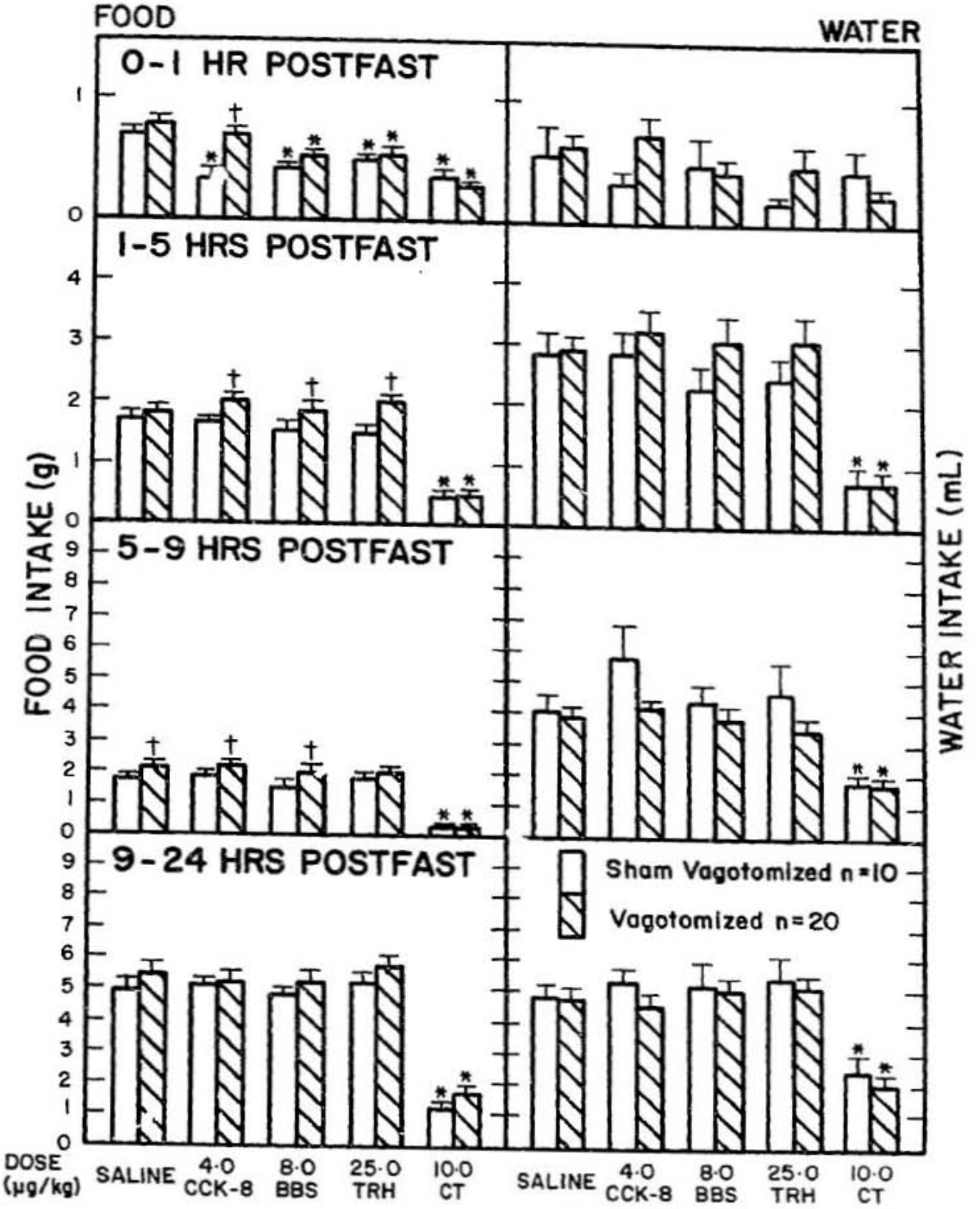
Group	n	$\bar{X}$ (± S.E.M) Water Intake (mL)			
		Isotonic Saline		1.0 mg/kg Angiotensin II	
		0-30 min Postinjection	31-120 min Postinjection	0-30 min Postinjection	31-120 min Postinjection
Sham	10	0.54 (0.21)	1.06 (0.26)	0.79 (0.32)	1.64 (0.34)
Vagotomy	20	0.55 (0.23)	1.09 (0.25)	1.33 (0.28)	1.26 (0.13)

factor. Where significant treatment effects were found, mean intake after peptide treatment for each group was compared to the corresponding mean intake after saline (paired t-test). Where significant treatment x surgery interactions were found, tests were made on simple main effects.

No significant differences were found between the sexes, and sex did not interact with any of the other factors. Thus, the data for male and female hamsters were collapsed for presentation in Figure 13. Food intakes during the first hr postfast were reduced after treatment with each of the peptides, $F(4, 112) = 12.43$, $p < 0.001$. However, sham operated and vagotomized animals responded differentially to peptide treatments. Although BBS, TRH, and CT produced comparable reductions in first hr postfast intakes in shams and vagotomized animals, CCK-8 was only effective in shams, $F(4, 112) = 4.25$, $p < 0.004$ (for the surgery x treatment interaction). Subsequent food intakes were also reduced by peptide treatment, $F(4, 112) = 52.47$, $p < 0.001$, $F(4, 112) = 28.66$, $p < 0.0001$, $F(4, 112) = 113.56$, $p < 0.001$, for intakes during 1-5, 5-9, and 9-24 hrs postfast, respectively. As shown in Figure 13, these differences are attributed to the long lasting suppressive effect of CT. Vagotomized animals ate more than shams during the 1-5 and 5-9 hrs postfast intervals, $F(1, 28) = 6.82$, $p < 0.02$ and $F(1, 28) = 6.97$, $p < 0.04$, respectively; but only on certain test (treatment) days $F(4, 112) = 3.58$, $p < 0.03$ and $F(4, 112) = 2.89$, $p < 0.05$ (corresponding group x treatment interaction). Food intakes during the 9-24 hrs postfast interval did not differ between groups.

Water intakes during the first hr postfast were not affected by

FIGURE 13. Mean (± s.e.m.) food (left) and water (right) intakes of intact and vagotomized hamsters after peripheral injections of CCK-8, BBS, TRH, and CT. * Mean intake is lower than mean intake after saline, $p < 0.05$. + Group (sham vs. vagotomy) means are significantly different, $p < 0.05$.


FOOD
WATER
0-1 HR POSTFAST
1-5 HRS POSTFAST
5-9 HRS POSTFAST
9-24 HRS POSTFAST
FOOD INTAKE (g)
WATER INTAKE (mL)
Sham Vagotomized n=10
Vagotomized n=20
DOSE (µg/kg)
SALINE
4·0 CCK-8
8·0 BBS
25·0 TRH
10·0 CT
SALINE
4·0 CCK-8
8·0 BBS
25·0 TRH
10·0 CT

peptide treatments. However, subsequent intakes were reduced after treatment with CT, $F(4, 112) = 22.75$, $p < 0.001$ (1-5 hrs postfast), $F(4, 112) = 28.66$, $p < 0.001$ (5-9 hrs postfast) and $F(4, 112) = 25.76$, $p < 0.001$ (9-24 hrs postfast). Water intakes never differed significantly between the groups.

Postmortem Anatomical Inspection

At the time of sacrifice, after 24 hrs of food deprivation, the oesophagus and stomach of vagotomized animals were abnormally distended. There were no obvious signs of vagal regeneration when the oesophageal strips were examined under the dissecting (20x) microscope.

Discussion

Several major findings arise from this experiment. Firstly, vagotomy is not debilitating in hamsters as it is in other species. Secondly, hamsters show only a modest increase in water intake after a large systemic dose of AII, and this modest response is not blocked or attenuated by total abdominal vagotomy. Thirdly, abdominal vagotomy blocked the satiety effect of the tested dose of CCK-8. Fourthly, the vagotomy blocking effect was specific to feeding suppression produced by CCK-8, as vagotomy did not reduce the suppression produced by BBS, TRH, and CT at the doses used.

As noted above, hamsters tolerate vagotomy surprisingly well. Although the vagotomized males tended to lose more weight than their

controls, this trend was not significant. In contrast, vagotomized females appeared to be better able to maintain the preoperative body weight during the feeding tests, but this too was not statistically reliable. Food intakes after saline and peptide treatments in vagotomized animals were never below that of their controls. In fact, during some time intervals, vagotomized animals ate slightly more than controls (see Figure 13). This pattern of ingestive and regulatory normalcy in vagotomized hamsters is in sharp contrast to the pattern in vagotomized rats and rabbits which are chronically hypophagic and whose body weights remain 15-30% below that of controls (Mordes et al., 1979; Rezek, VanderWeele, and Novin, 1975). In rats, the effects of abdominal vagotomy on feeding and body weight are so reliable that prolonged hypophagia and weight loss are often prerequisites for inclusion in functionally vagotomized groups (e.g. Lorenz and Goldman, 1982; and see Louis-Sylvestre, 1983). The reason for the lack of a debilitating effect of vagotomy in hamsters is not clear at present. The answer to this question awaits a better understanding of the mechanisms underlying vagotomy-induced hypophagia and weight loss in other species, as it would be almost impossible to unravel what is "not wrong" in vagotomized hamsters in the absence of such information.

Systemic injections of AII temporarily debilitated the animals in a manner similar to TRH. Upon recovery, a small increment in water intake was observed. As can be seen in Table X, the magnitude of this effect is trivial in view of the fact that intact rats given the same dose by the same route drink copiously (Jerome and Smith, 1982b; Simansky and Smith, 1983). Thus, as suggested by the study of Fitts et al. (1982),

it would appear that hamster drinking is relatively insensitive to circulating AII. However, it is possible that the pressor response to AII (which possibly accounted for the initial behavioural debilitation after this dose of AII) inhibited AII drinking that would have otherwise occurred. Although systemically administered AII does produce a vigorous drinking response in rats, the effect is substantially amplified when the accompanying pressor response to AII is blocked pharmacologically (Robinson and Evered, 1983). To test this possibility, water intakes were measured after administration of a smaller, and presumably less devastating (with respect to pressor activity), dose of AII in Experiment 11, below.

The major objective of Experiment 10 was to determine whether the subdiaphragmatic vagus is necessary for the satiety effect of peripheral CCK injections in hamsters. As shown in Figure 13, total abdominal vagotomy blocked the first hr postfast feeding suppression to CCK-8. In contrast, vagotomized hamsters remained normally responsive to the suppressive effects of other peptides. Vagotomy in rats is similarly selective in that it blocks the feeding inhibitory effects of only certain putative satiety peptides and substances. Vagotomized rats remain responsive to treatment with BBS, CT, and naloxone (Gibbs, Kulkosky, and Smith, 1981; Morley et al., 1982a) but show no, or an attenuated, respsonse to glucagon (Geary and Smith, 1983), somatostatin (Levine and Morley, 1982), TRH (Morley et al., 1982a), and of course, CCK (Smith et al., 1981b; Lorenz and Goldman, 1982). The available data indicate that vagotomized hamsters differ from their rat counterparts in only one respect; they remain responsive to TRH.

This experiment indicates that the abdominal vagus in hamsters, as in rats, is important for feeding suppression to exogenous CCK, and by inference, for feeding inhibition to duodenally released CCK. However, sometime after the completion of this experiment, Corp, Fitts, and Woods (1983) published an abstract describing a similar experiment in which vagotomized hamsters reduced their food intake to a dose of CCK to the same degree as controls. Although a number of procedural differences could account for these discrepant results, the most notable is that Corp et al. (1983) tested their hamsters with a large dose of CCK-8 (12.0 µg/kg), whereas the animals in the above experiment were tested with a relatively small dose (4.0 µg/kg). To determine whether dose could account for the contradictory findings, vagotomized and intact hamsters in the following experiment were tested with a wide range of systemic CCK-8 doses.

EXPERIMENT 11: EFFECTS OF ABDOMINAL VAGOTOMY ON RESPONSIVENESS TO VARIOUS DOSES OF CCK-8

Method

One male and eleven female laboratory bred hamsters were vagotomized as described in Experiment 10. One male and eight females served as sham-operated controls.

One week after surgery, the animals underwent a series of drinking tests as described in Experiment 10, except that a lower dose of All (100.0 µg/kg, s.c.) was used. Three weeks after vagotomy or sham-

surgery, each animal was implanted with a chronic intracerebroventricular cannula following the procedures described in Experiment 6, and underwent a series of feeding tests with intraventricular saline and CCK-8 after 15 hrs of food deprivation (data not presented here). A series of weekly feeding tests was begun on the sixth week after the initial surgery. The animals were prepared for each of these tests by a 3 hr fast (water was available ad lib). The animals were tested weekly with different CCK-8 (Squibb, Batch # 556159- 2F722) doses in the following order: saline, 2.0, 4.0, 8.0, 6.0, and 12.0 µg/kg by i.p. injection. Five min after the injection the animals were given a preweighed ration of Purina rat chow and intakes of both food and water were measured regularly over the next 24 hrs.

Upon completion of the feeding tests, the animals were food deprived for 24 hrs and then sacrificed by decapitation. The animal's abdomen and thorax were then quickly opened to expose the stomach and oesophagus. The pylorus and oesophagus were ligated with sutures and cut. The stomach and oesophagus were removed from the peritoneal and thoracic cavity. The stomach was cut along the greater curvature and its contents were gently expressed. The expressed food was dried and later weighed. The stomach and oesophagus were stored in 10% Formalin for later inspection.

Results

Body Weight

Preoperative body weight and body weights on Days 7, 21, 35, 42, and 77 postvagotomy (or sham vagotomy) underwent two-way analysis of variance (group x days) with repeated measures on the days factor. As shown in Figure 14, hamsters in both groups lost weight over time, $F(5, 95) = 33.94$, $p < 0.001$, but the weight loss was greater in shams, $F(5, 95) = 4.10$, $p < 0.003$. Further analysis (tests on simple main effects) revealed that although the groups were matched for body weight preoperatively, the vagotomized hamsters became heavier than controls by the sixth week postsurgery and remained so thereafter ($p < 0.05$).

Drinking Tests

The behavioural changes described in the previous experiment were not observed after this dose of AII. Drinking data underwent statistical analysis as described in Experiment 10. There was no reliable increase in water intake after this dose of AII (in either group) at any of the time intervals (see Table XI).

Feeding Tests

Food and water intakes during the first, second, third, and fourth through the 24th hrs postfast underwent separate two-way analysis of variance (group x dose) with repeated measures on the dose factor. Subsequent analyses of treatment means were made as described in Experiment 10.

As demonstrated in the previous experiments, all doses of CCK-8

FIGURE 14. Mean (± s.e.m.) body weights of intact and vagotomized hamsters throughout Experiment 11. * Means are significantly different, $p < 0.05$.

TABLE XI

Water Intake After Low Dose AII Injection

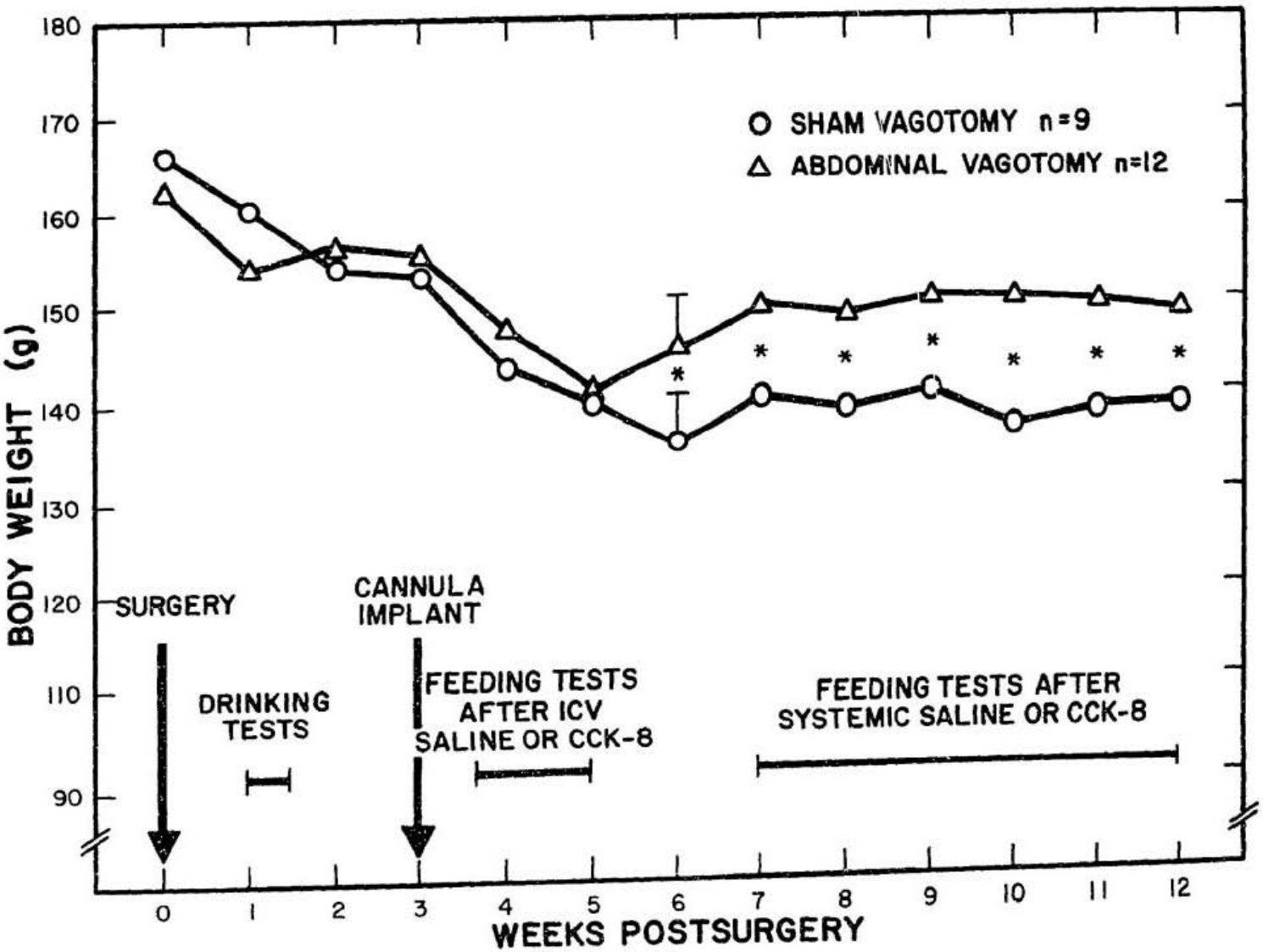
In Intact and Vagotomized Hamsters

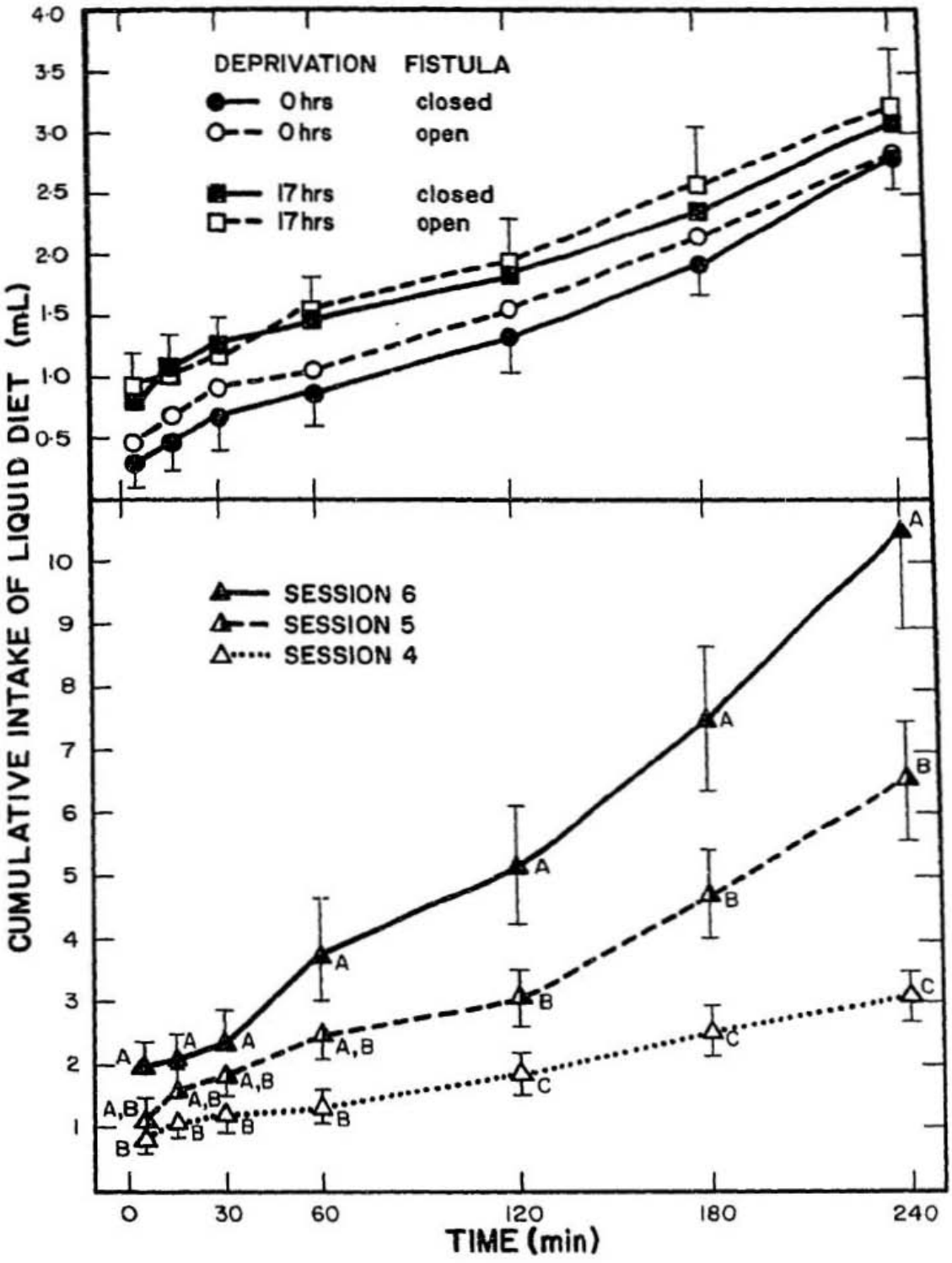
Group	n	$\overline{X}$ (± S.E.M) Water Intake (mL)			
		Isotonic Saline		100 µg/kg Angiotensin II	
		0-30 min	31-120 min	0-30 min	31-120 min
		Postinjection		Postinjection	
Sham	9	0.28 (0.15)	0.94 (0.32)	0.73 (0.21)	0.62 (0.27)
Vagotomy	12	0.67 (0.42)	1.01 (0.23)	0.75 (0.55)	0.56 (0.49)

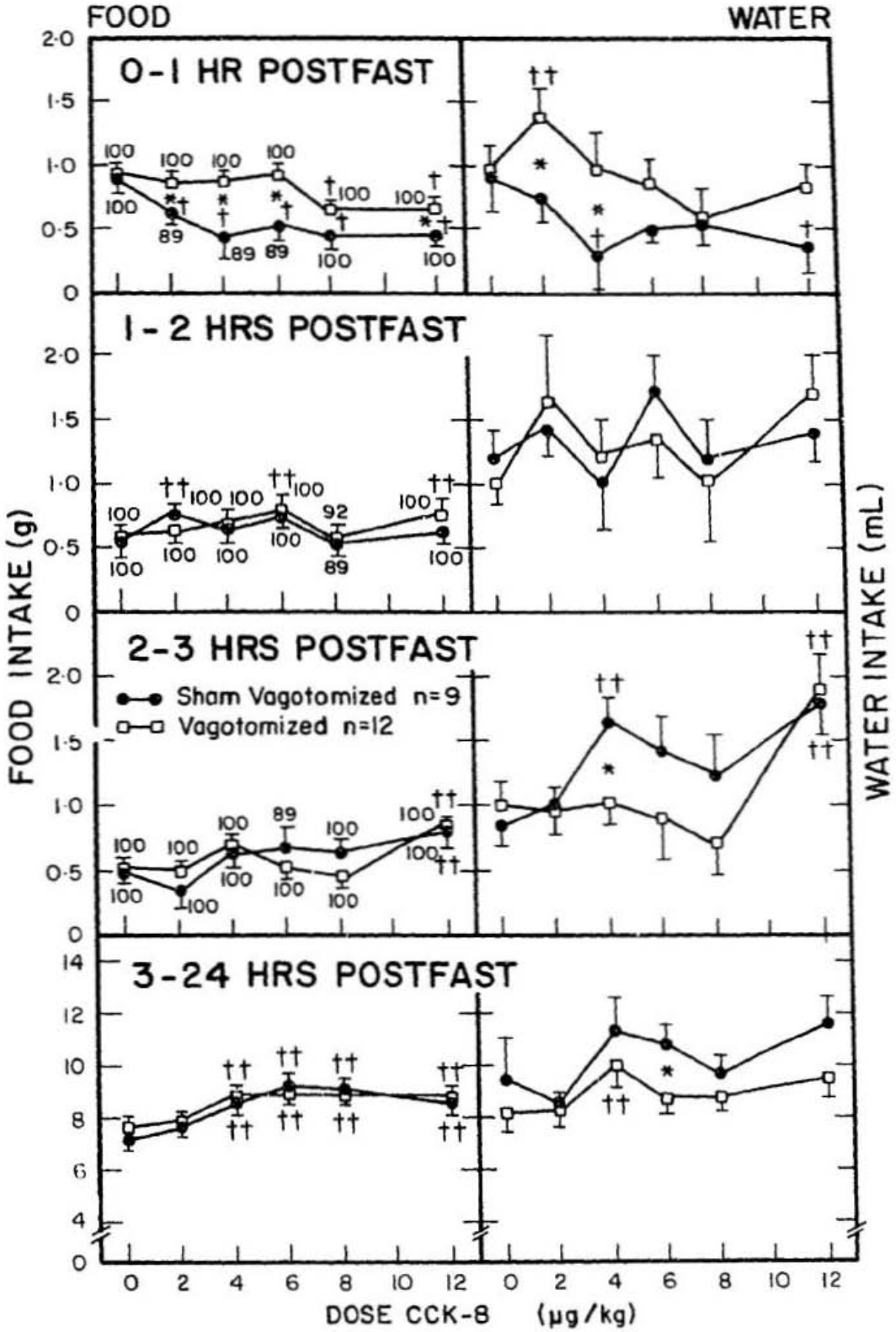
reduced feeding in intact hamsters during the first hr postfast, $F(5, 95) = 6.24$, $p < 0.003$. No aberrant behaviour was observed after even the largest dose of CCK-8. Figure 15 shows that subdiaphragmatic vagotomy again blocked feeding suppression to CCK-8, but only at lower doses $F(5, 95) = 3.16$, $p < 0.02$ (group x dose interaction). As shown in the figure, first hr food intakes of vagotomized hamsters were significantly reduced after 8.0 and 12.0 µg/kg CCK-8. Subsequent food intakes were increased after some doses of CCK-8, $F(5, 95) = 4.14$, $p < 0.01$ (for intakes between the second and third hrs postfast), and $F(5, 95) = 3.89$, $p < 0.01$ (food intakes between three and 24 hrs postfast). These increases are apparently a result of some trial or practice effect, insofar as similar increases were not seen in previous experiments where vagally intact hamsters received CCK-8 only once.

First hr postfast water intakes showed significant changes after certain doses of CCK-8, $F(5, 95) = 4.03$, $p < 0.01$. That is, water intakes during this period were reduced in shams after some doses of CCK-8, but increased in vagotomized animals after one dose of CCK-8 (see Figure 15), $F(5, 95) = 2.78$, $p < 0.05$ (group x dose interaction). As with food intakes, there were significant increases in subsequent water intakes during some dose trials, these increases being more pronounced in intact animals $F(5, 95) = 2.69$, $p < 0.04$ (dose effect for intakes during the third hr postfast); $F(5, 95) = 2.94$, $p < 0.04$ (corresponding group x dose interaction); $F(5, 95) = 3.89$, $p < 0.03$ (dose effect for water intake during 9-24 hrs postfast); $F(5, 95) = 1.87$, $p = 0.052$ (corresponding group x dose interaction).

FIGURE 15. Mean (± s.e.m.) food (left) and water (right) intakes of vagotomized and control hamsters after various i.p. doses of CCK-8. + Mean intake is lower than after saline. ++ Mean intake is higher than after saline. * Group (sham vs. vagotomy) differences are significant, p's < 0.05. Numbers adjacent to each mean denote the approximate percentage of animals in the group eating ($\geq$ 0.2 g of chow) during that time interval.


180
170
160
150
140
130
120
110
90
BODY WEIGHT (g)
O SHAM VAGOTOMY n=9
Δ ABDOMINAL VAGOTOMY n=12
SURGERY
DRINKING TESTS
CANNULA IMPLANT
FEEDING TESTS AFTER ICV SALINE OR CCK-8
FEEDING TESTS AFTER SYSTEMIC SALINE OR CCK-8
0 1 2 3 4 5 6 7 8 9 10 11 12
WEEKS POSTSURGERY


DEPRIVATION FISTULA
0 hrs closed
0 hrs open
17 hrs closed
17 hrs open
SESSION 6
SESSION 5
SESSION 4
CUMULATIVE INTAKE OF LIQUID DIET (mL)
TIME (min)
0
30
60
120
180
240


FOOD
WATER
0-1 HR POSTFAST
1-2 HRS POSTFAST
2-3 HRS POSTFAST
3-24 HRS POSTFAST
Sham Vagotomized n=9
Vagotomized n=12
FOOD INTAKE (g)
WATER INTAKE (mL)
DOSE CCK-8 (µg/kg)

Postmortem Anatomical Inspection

As in the previous experiment, the oesophagi and stomachs of vagotomized hamsters were abnormally distended after a 24 hr fast. There was no evidence of vagal regeneration under the dissecting microscope. Vagotomized hamsters retained a mean of 1.19 ($\pm$ 0.05) g of food in the stomach; whereas sham-operates retained a mean of 0.23 ($\pm$ 0.04) g. This difference is highly significant t (19) = 14, $p < 0.001$. This measure in hamsters is a good functional test of the completeness of vagotomy (at least for the gastric efferent division of the vagus) because the vagotomized hamster retaining the least amount of food in the stomach (0.84 g) had twice as much as the sham-operated hamster retaining the greatest amount (0.42 g).

Discussion

The results of the present experiment confirm the previous experiment's observation that total abdominal vagotomy completely eliminates feeding suppression to a relatively low dose of CCK-8. This experiment further demonstrates that at higher doses, vagotomized hamsters are almost as responsive to CCK-8 as sham-operates. In addition, it was shown that hamster drinking is completely insensitive to a low peripheral dose of AII. Lastly, it was observed that although both groups lost weight over the course of the experiment, weight loss was less pronounced in vagotomized hamsters.

Determination of the completeness of vagotomy in animal experiments

and clinical studies has proven difficult because the various tests involve a fair proportion of false-positives and false-negatives (see Louis-Sylvestre, 1983). In rats, the gastric retention test is moderately successsful at discriminating between intact rats and rats with gastric vagotomy. Under conditions similar to those outlined above for the hamster, rats with gastric vagotomy retain approximately twice as much food as intact animals (e.g. Sawchenko, Gold, and Farrazano, 1977). The same test is considerably more sensitive in hamsters. Hamsters with total or gastric vagotomy retained from five (Experiment 11) to 15 (see Experiment 12) times more food in the stomach than controls. Hence the gastric retention test is highly recommended for use in future studies of the gastric vagus in hamsters.

The low dose of AII used here produced no obvious debilitation, yet was completely ineffective in augmenting drinking. Again, this same dose, administered by the same route is dipsogenic in intact rats (Jerome and Smith, 1982b). The possibility still remains that a pressor response to exogenous AII prevents hamsters from increasing water intake. This is an important consideration because elevated titres of AII, under more natural conditions such as during severe loss of extracellular fluid, could possibly exert an influence on drinking in the absence of accompanying hypertension. This possibility, however, seems remote in view of the Fitts et al. (1982) study in which the authors presented indirect evidence of increased blood AII levels in hypovolaemic hamsters whose water intakes did not differ from their controls. It may be argued that the lack of a drinking effect in response to the peripheral doses of AII tested in Experiments 10 and 11

is not conclusive evidence against a role for blood-borne AII in the control of hamster drinking because a wider range of doses was not tested. It seems unlikely that hamsters would respond to doses smaller than the one tested in Experiment 11. Doses larger than the 1.0 mg/kg tested in Experiment 10 (2-5 mg/kg) have also been administered to intact hamsters, but produced no greater response than that seen in Experiment 10. These higher doses only increased water intake by 70-90%, which is trivial considering the degree of extracellular fluid depletion that would be necessary to produce comparable blood levels. Lastly, the lack of substantial drinking after these doses cannot be attributed to the particular form of AII (human synthetic) chosen for these experiments or to a bad lot of AII, as nanogram range doses of the same batch of AII delivered into the hamster CSF produced a brisk drinking response (Miceli, unpublished) as described previously (Miceli and Malsbury, 1983). Hence, it is almost certain that blood-borne AII is not an important stimulus for drinking in hamsters.

The elucidation of the neural mechanisms underlying AII-induced drinking in the rat has been complicated by the fact that there are multiple AII receptor systems relevant to thirst and drinking. A mechanistic analysis of AII-induced drinking is further complicated by virtue of the existence of two distinct angiotensin producing systems. In addition to the renal renin-angiotensin system noted earlier, Ganten and his colleagues have firmly established that angiotensin is also produced in the brain (Ganten, Fuxe, Pillips, Mann, and Ganten, 1978). Rats and other species respond to both central and peripheral injections of AII (see below). Problems associated with the development of

mechanistic analyses of AII's dipsogenic action have stemmed from unravelling which source of angiotensin and which angiotensin receptors are relevant to thirst and drinking. Workers studying the rat have found two CNS populations of angiotensin-sensitive neurones relevant to drinking. The subfornical organ (SFO), a forebrain circumventricular organ lying outside the blood-brain barrier, has been identified as one central site of the dipsogenic action of circulating AII (Simpson, Epstein, and Camardo, 1978; Mangiapane and Simpson, 1980). The region of the anteroventral third ventricle (AV3V) has been identified as the other forebrain site of dipsogenic action of AII (Johnson and Buggy, 1978). Although the organum vasculosum of the lamina terminalis (OVLT), which lies outside the blood-brain barrier, is contained within the AV3V area, it is believed that AV3V area neuronal elements responsive to the dipsogenic action of AII lie within the blood-brain barrier. This conclusion is based on studies (reviewed in Lind and Johnson, 1982) showing that rats with lesions of the SFO do not drink in response to systemic AII injections, but remain responsive to central (ICV) AII injections. In summary, the available evidence suggests that in the rat, a population of neurones in the AV3V have thirst-related receptors for brain isorenin-angiotensin (Lind and Johnson, 1982). Thirst-related receptors for circulating AII in the periphery are coupled to abdominal vagal afferents (Simansky and Smith, 1983), and in the brain, are found on neurones of the SFO (Lind and Johnson, 1982).

It is difficult to reconcile the recent findings on AII and drinking in hamsters with the status quo in the rat. Hamsters are responsive to the dipsogenic action of centrally administered (ICV) AII

to nearly the same degree as rats (Miceli, unpublished; Miceli and Malsbury, 1983). On the other hand, hamster drinking is minimally responsive to peripheral injections of large doses of AII, and this response is resistant to abdominal vagotomy. Under somewhat more physiological conditions, hamster drinking is completely unaffected by blood-borne AII that would be expected to be formed as a consequence of vascular volume deficits (Fitts et al., 1982). In this regard hamsters may be similar to sheep. Intracarotid infusions of AII in sheep produce thirst and drinking only at doses resulting in blood concentrations well above (approximately 10x) the physiological range (Abraham, Barker, Blaine, Denton, and McKinley, 1975; Abraham, Denton, McKinley, and Weisinger, 1976). However, ICV infusions of AII were effective at doses that resulted in CSF concentrations that approximated physiological concentrations (Abraham et al., 1975).

Although there are a number of possible explanations as to why hamsters respond to central but not peripheral administration of AII, the most parsimonious is that hamsters lack peripheral (vagal) and central (SFO) thirst-related receptors sensitive to circulating AII, but do have a brain isorenin-angiotensin system relevant to thirst. Although an isorenin-angiotensin system has not yet been reported in the hamster brain, the fact that hamster drinking is sensitive to ICV AII injections and that specific AII binding sites have been described at extracircumventricular regions of the hamster brain (Harding, Stone, and Wright, 1981) suggest that a brain isorenin-angiotensin system relevant to drinking may indeed exist in this species. It is further proposed that when drinking is observed after large, and no doubt

pharmacological, peripheral doses of AII, it is likely to occur as a consequence of abnormally large amounts of circulating AII distributing into the interstitial space of the OVLT and diffusing within the surrounding AV3V region to stimulate nearby AII-sensitive units. Of course, only further study can support or refute these interpretations.

Experiment 11 replicated the results of the previous experiment, as vagotomy blocked feeding suppression to lower ($\leq$ 6.0 µg/kg) doses of CCK-8, and further confirmed the prediction that vagotomized hamsters would show feeding suppression to larger doses of CCK-8. Vagotomized and intact rats have also been compared for their responsiveness to a wide range of CCK-8 doses. The results of such studies have varied somewhat, but have been consistent in showing that vagotomy blocks feeding suppression to a wider range of CCK-8 doses than does vagotomy in hamsters. For example, Lorenz and Goldman (1982) tested intact and totally (subdiaphragmatic) vagotomized rats with doses of CCK-8 ranging from approximately 0.9 to 29.2 µg/kg (or 20 to 640 Ivy Dog Units/kg), and found that vagotomized rats were completely unresponsive to any of the doses. Smith et al. (1980) however, reported that gastric vagotomy did not totally abolish feeding suppression to exogenous CCK-8, but instead produced a rightward shift in the dose-response curve. The magnitude of the shift was such that intact animals were six times as responsive to a given dose as vagotomized animals. These studies show unequivocally that vagotomized rats are insensitive to a much wider range of CCK-8 doses than are vagotomized hamsters.

On the basis of the studies showing that abdominal vagotomy abolishes or markedly attenuates rat feeding suppression to exogenous

CCK, Smith and his colleagues have argued for a peripheral, not central, site of action of duodenally released CCK. Though much the same can be argued for the hamster, the fact that vagotomized hamsters will show a significant feeding suppression to relatively large doses remains to be explained. Although the amounts required to suppress feeding in vagotomized hamsters likely produce circulating levels of CCK well beyond the amounts expected to be released during feeding, the fact that they do reduce feeding requires sites of action other than the vagus or sites innervated by the vagus. This finding in vagotomized hamsters is not incompatible with the idea presented earlier that brain CCK-receptive systems may also play a role in regulating feeding. It is possible, though not proven, that when plasma levels of CCK become abnormally high, sufficient amounts gain access to the CSF (via the interstitial space of the circumventricular organs, which lack a BBB) to stimulate central receptors related to appetite control. The argument here is not that prandially released duodenal CCK ordinarily gains access to such brain receptors, but that such brain systems (ordinarily receptive to CCK intrinsic to the brain) may indeed exist.

EXPERIMENT 12: ROLE OF THE GASTRIC DIVISION OF THE ABDOMINAL VAGUS

In order to define a more restricted abdominal site at which peripherally released CCK acts in rats, Smith and his associates (1981a) examined the effects of more selective abdominal vagotomies on feeding responsiveness to exogenous CCK-8. They found that rats with selective coeliac or hepatic, or combined coeliac and hepatic vagotomies showed

normal responsiveness to various doses of CCK-8. In contrast, selective gastric vagotomy blocked the satiety effect of CCK-8 as effectively as total abdominal vagotomy, thus indicating that gastric vagal branches are necessary and sufficient for the satiety action of CCK. Smith et al. (1981a) concluded that duodenally released CCK acts at some abdominal site innervated by the gastric vagus (very likely the stomach or upper intestine). Because all the subdiaphragmatic components of the hamster vagus were removed in Experiments 10 and 11, it was of interest in the following experiment to determine whether selective gastric vagotomy in the hamster reduces feeding suppression to exogenous CCK-8 as effectively as total abdominal vagotomy.

Recent studies in rats and people have implicated a major and previously unsuspected role for the subdiaphragmatic vagus in the drinking response elicited by injections of hypertonic saline (Jerome and Smith, 1982a; Schoon, Gortz, Smith, and Kral, 1984; Simansky, Jerome, Santucci, and Smith, 1982; Smith and Jerome, 1983). Hamsters too are polydipsic after injections of hypertonic saline (Lowy and Yim, 1982). It was therefore of interest to determine in the following experiment whether branches of the abdominal vagus are necessary for the hamster's drinking response to hypertonic saline.

It was shown in Experiment 11 that vagotomized hamsters, although weighing slightly less than controls preoperatively, eventually outweighed their controls. As these body weight shifts occurred against a background of deprivation schedule-induced weight loss, it is not clear whether vagotomized hamsters would have outweighed their controls if the animals had been allowed ad lib food access throughout the

experiment, or whether the vagotomized hamsters were simply better able to cope with repeated periods of deprivation. Furthermore, it is not clear whether group differences in body weight in Experiment 11 arose from vagotomized hamsters having eaten more than controls during some period, or whether metabolic changes accounted for these differences. In the following experiment it was of interest to examine long-term regulation of body weight and food intake in free-feeding vagotomized hamsters. To test the hypothesis that vagotomized hamsters are better able to cope with a repeated deprivation schedule, intact and vagotomized animals were subsequently challenged with a prolonged period of intermittent food access.

Methods

Animals were 120-140 day old hamsters purchased directly from the breeder (Lakeview). Groups of male and female hamsters matched for body weight underwent either total subdiaphragmatic vagotomy, selective gastric vagotomy, or laparotomy (shams). Total subdiaphragmatic and sham vagotomies were performed as described in Experiment 10. The gastric vagotomies were selective because they spared innervation of the upper oesophagus, liver, and pancreas (see Figure 11).

Animals were weighed at two or four day intervals throughout the experimental period. Forty-eight hr food intakes were sampled from day 3 to day 14 and from day 30 to day 44 postsurgery. On days 58 and 62 postsurgery, the animals' water intakes were measured after s.c. injections of isotonic (0.9%) or hypertonic (12.0%) saline (0.8 ml/100

g). Each concentration was in a solution containing 2.5% procaine hydrochloride (Sigma). Water intakes were measured over the four hrs after the injection. The animals were water repleted prior to each test and food was available throughout. Approximately half of the animals were tested with isotonic saline on day 58 and with hypertonic saline on day 62 postsurgery. The remaining animals received treatments in reversed order.

Beginning on day 68 postsurgery, the animals underwent a series of feeding tests. The animals were food deprived for three hrs prior to each test, at which time they received 0.4 ml/100 g b.w. saline (day 68), 4.0, and 10.0 ug/kg CCK-8 (Calbiochem, Lot # 386045) on days 72 and 76, respectively. Food was provided within 5 min of the i.p. injection, and food and water intakes during the next hr were measured.

Starting on day 82 postsurgery, the animals were maintained on a food deprivation schedule for seven consecutive days. During this time food was withheld for 9 hrs (from 0900 to 1800 hrs), and food intakes and body weights were recorded daily. Food intakes and body weights were also recorded on the eight days after reinstating 24 hr ad lib food access. At the end of the experimental period, the animals were fasted for 24 hrs and then sacrificed by decapitation. Gastric contents were removed, dried and weighed. Oesophageal strips were examined under the dissecting microscope to verify the vagotomies.

Results

Body Weight and Food Intake Under Ad Libitum Feeding Conditions

Preoperative body weights and body weights on days 20, 58, and 78 postsurgery underwent three-way analysis of variance with repeated measures on the days factor. As shown in Figure 16, hamsters gained weight over time, $F(3, 93) = 19.54$, $p < 0.001$. The weight gains were comparable across surgery and sex groupings, as the sex x time, surgery x time, and surgery x sex x time interactions were not significant. Food intakes over 48 hr time blocks (days 3-4, 5-6, 9-10, 39-40) similarly underwent three-way analysis of variance with repeated measures on the time factor. Just as body weights increased over time, there was a significant trend towards increased food intakes over time, $F(3, 93) = 75.58$, $p < 0.0001$. Although there were no overall group or sex differences in food intake, sex x time and sex x surgery x time interactions were statistically reliable, $F(3, 93) = 3.37$, $p < 0.01$ and $F(6, 93) = 6.35$, $p < 0.001$, respectively. As indicated in Figure 17, these effects are a result of decreased intakes in males with total abdominal or gastric vagotomies during days 3-4 and 5-6 postsurgery ($p < 0.05$). However, by day 9 postsurgery, food intakes in vagotomized male hamsters were equal to those of their controls.

Drinking Tests

Water intakes during the first two and second two hrs after injections of isotonic or hypertonic saline underwent separate three-way analysis of variance with repeated measures on the dose factor. No sex

FIGURE 16. Mean (± s.e.m.) body weights of male and female hamsters with gastric, total abdominal, or sham vagotomies during 24 hr *ad lib* feeding.

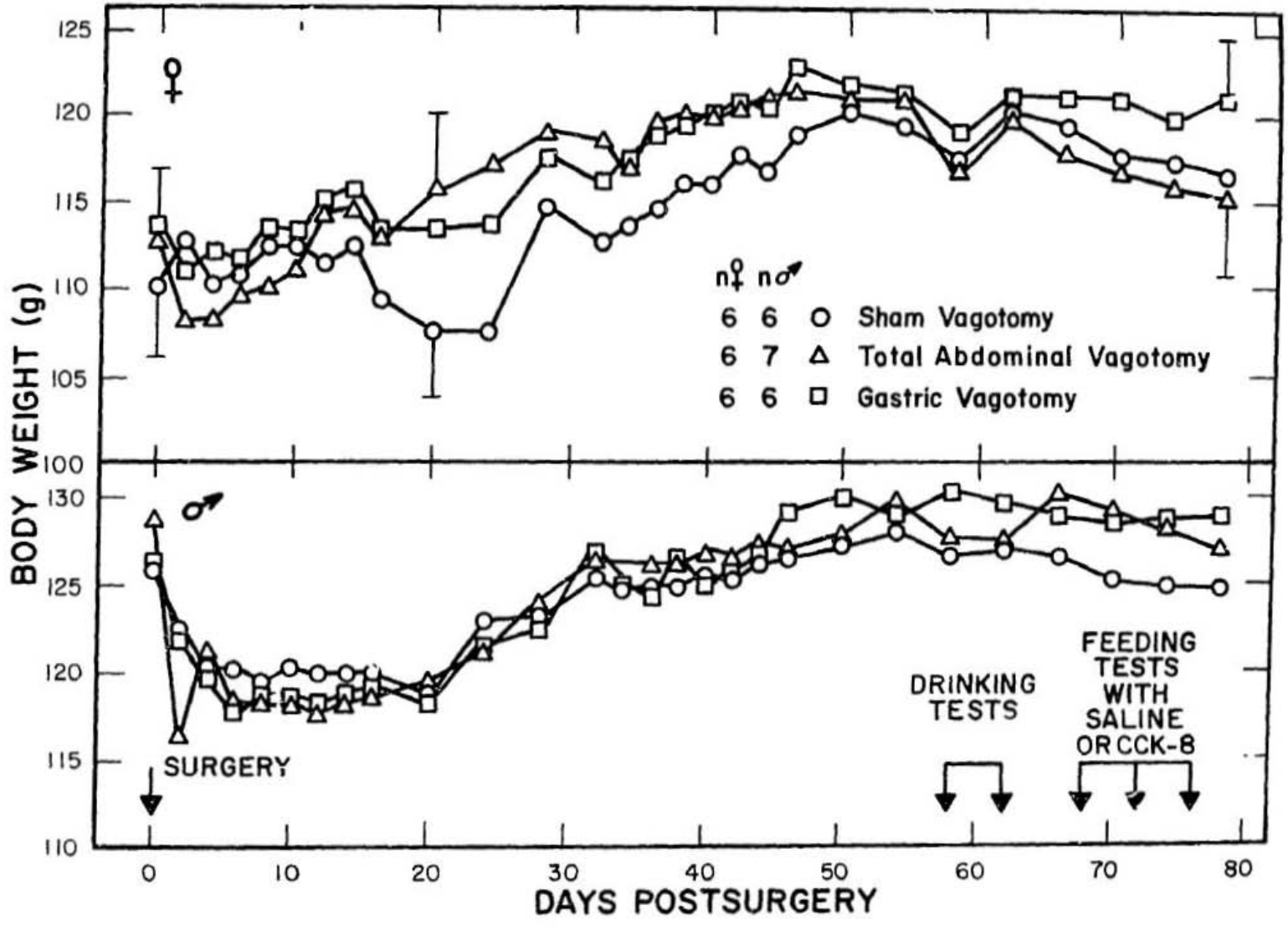
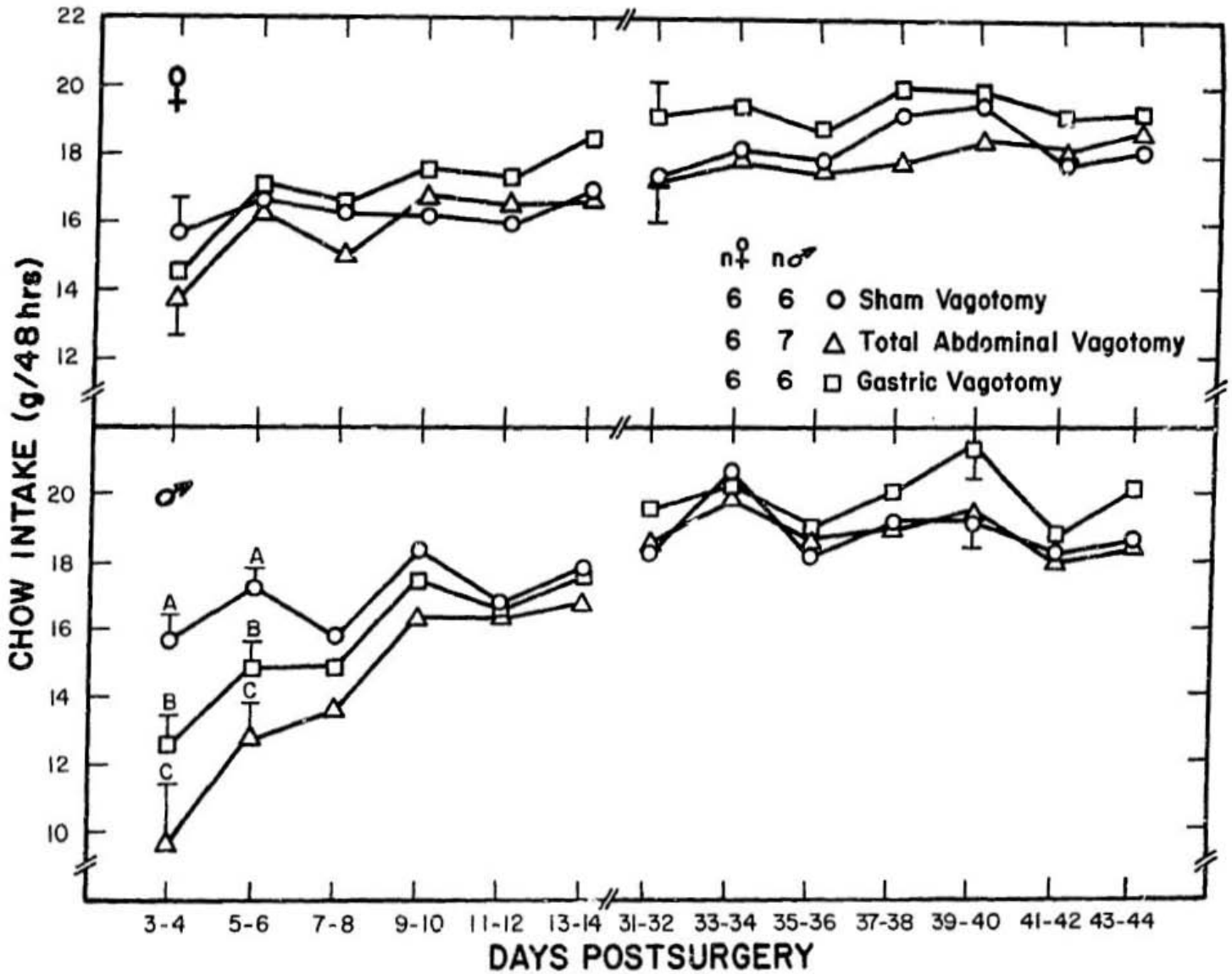

BODY WEIGHT (g)
♀
♂
n♀ n♂
6 6 Sham Vagotomy
6 7 Total Abdominal Vagotomy
6 6 Gastric Vagotomy
SURGERY
DRINKING TESTS
FEEDING TESTS WITH SALINE OR CCK-8
DAYS POSTSURGERY

FIGURE 17. Mean (± s.e.m.) food intakes of male and female hamsters with gastric, total abdominal, or sham vagotomies during 24 hr *ad lib* feeding.


22
20
18
16
14
12
♀
n♀ n♂
6 6 O Sham Vagotomy
6 7 △ Total Abdominal Vagotomy
6 6 □ Gastric Vagotomy
♂
20
18
16
14
12
10
A
B
C
CHOW INTAKE (g/48hrs)
3-4 5-6 7-8 9-10 11-12 13-14 31-32 33-34 35-36 37-38 39-40 41-42 43-44
DAYS POSTSURGERY

differences were found in these measures, and sex did not interact with any of the other factors. Thus, data for males and females were collapsed for presentation in Table XII. Water intakes during both time intervals were increased after hypertonic saline injection, $F(1, 34) = 25.38$, $p < 0.001$ (for water intakes during the first two hrs after injection) and $F(1, 34) = 6.83$, $p < 0.01$ (for intakes during the second two hrs after injection). As can be seen in Table XII, the magnitude of these increments was consistent across groups.

Food and Water Intakes after Intraperitoneal Injections of CCK-8

Food and water intakes during the first hr after the injection underwent three-way analysis of variance with repeated measures on the dose factor. Again, there were no sex differences in either of these two measures. Food intakes were reduced after CCK-8 injections, $F(2, 68) = 88.95$, $p < 0.0001$. However, the groups responded differentially to the doses of CCK-8, $F(4, 68) = 6.84$, $p < 0.001$ (for the surgery x dose interaction). Subsequent post-hoc analyses (tests on simple main effects for group comparisons at a given dose, and paired t-tests for comparisons of mean intakes after CCK-8 doses against mean intake after saline) revealed that hamsters with total abdominal vagotomy were completely unresponsive to 4.0 ug/kg CCK-8, but did show significant feeding suppression to the larger dose. In contrast, hamsters with selective gastric vagotomy were responsive to both doses, although the suppression to the smaller dose was of a smaller magnitude than that in sham-operates (see Figure 18).

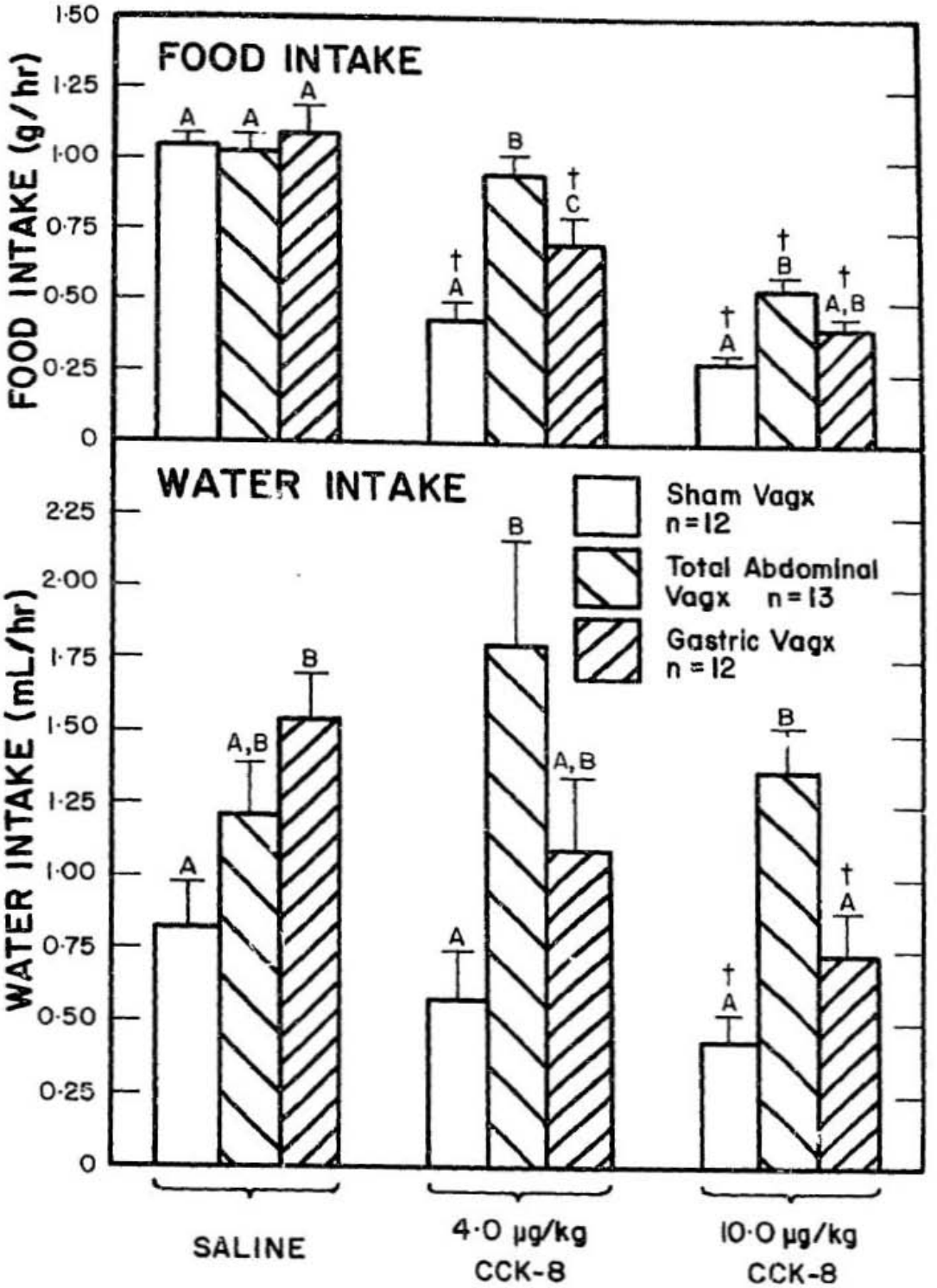
TABLE XII

Water Intake After Hypertonic Saline Injection

In Intact and Vagotomized Hamsters

Group	n	$\bar{X}$ (± S.E.M) Water Intake (mL)			
		Isotonic Saline		Hypertonic (12%) Saline	
		0-2 hrs Postinjection	2-4 hrs	0-2 hrs Postinjection	2-4 hrs
Sham	12	0.89 (0.18)	2.01 (0.32)	3.00 (0.39)	3.26 (0.34)
Total Abdominal Vagotomy	13	0.53 (0.21)	1.48 (0.34)	2.99 (0.33)	2.65 (0.32)
Gastric Vagotomy	12	0.88 (0.21)	1.67 (0.30)	3.72 (0.46)	2.96 (0.31)

FIGURE 18. Mean (± s.e.m.) food and water intakes in hamsters with total abdominal, gastric, or sham vagotomies after i.p. CCK-8 injections. Group means without a common superscript are significantly different, $p < 0.05$. + Mean intake is lower than mean intake after saline, $p < 0.05$.


FOOD INTAKE
FOOD INTAKE (g/hr)
1·50
1·25
1·00
0·75
0·50
0·25
0
A
A
A
†A
B
†C
†A
†B
†A,B
WATER INTAKE
WATER INTAKE (mL/hr)
2·25
2·00
1·75
1·50
1·25
1·00
0·75
0·50
0·25
0
A
A,B
B
A
B
A,B
†A
B
†A
Sham Vagx n=12
Total Abdominal Vagx n=13
Gastric Vagx n=12
SALINE
4·0 µg/kg CCK-8
10·0 µg/kg CCK-8

Water intakes were also reduced after CCK-8, but only after the larger dose, and only in the sham-operates and animals with gastric vagotomy $F(2, 68) = 4.42$, $p < 0.02$ (dose) and $F(4, 68) = 3.21$, $p < 0.05$ (surgery x dose interaction).

Food Intake and Body Weight During a Deprivation Schedule Challenge

Food intakes during the day prior to implementing the deprivation schedule, during the first and last days of the deprivation schedule, and during the first and seventh day after reinstating 24 hr ad lib feeding underwent three way analysis of variance with repeated measures on the days factor. Animals did not consume their normal 24 hr intakes during the daily 15 hrs of food availability. Upon return to 24 hr ad lib feeding, the animals did show a transient elevation of daily intake but it did not persist beyond the first day. These changes in food intake were highly significant $F(4, 124) = 272.5$, $p < 0.0001$, but the magnitude of the changes did not vary as a function of surgery. However, females (regardless of the type of surgery) were better able to adapt to the deprivation schedule as they ate slightly (but significantly) more than males during this time $F(4, 124) = 2.76$, $p < 0.04$ (sex x time interaction).

Body weights on the first and last day of the deprivation schedule and on the eighth day after reinstating 24 hr ad lib feeding underwent similar statistical analysis. All animals lost weight over the deprivation schedule and regained weight upon return to 24 hr ad lib feeding, $F(2, 629) = 241.2$, $p < 0.0001$. Males lost more weight than

females, $F(2, 62) = 10.06$, $p < 0.001$, and sham-operates (both sexes) lost more weight than animals with each type of vagotomy, $F(4, 62) = 3.09$, $p < 0.03$ (see Figure 19).

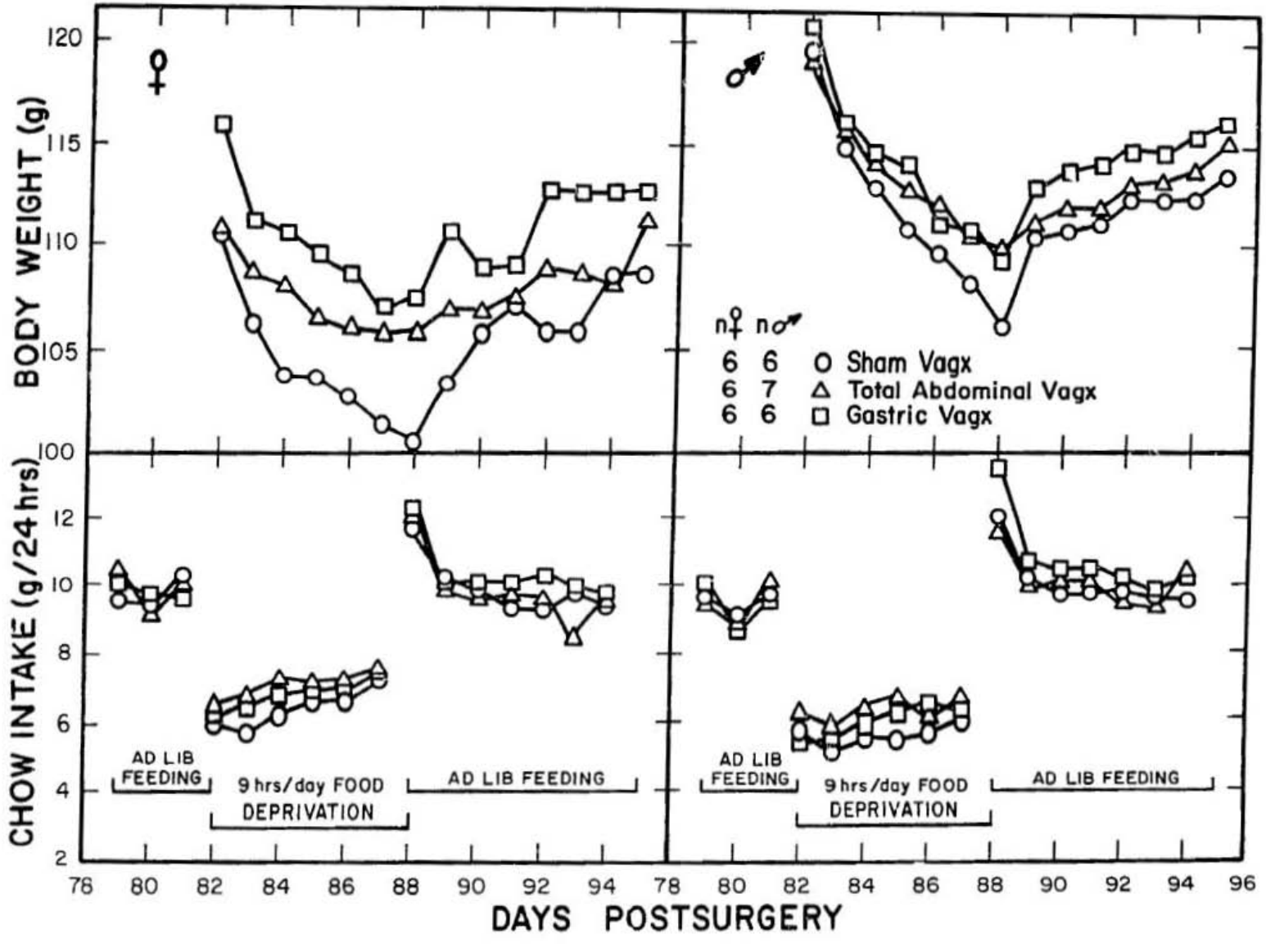
Gastric Retention and Postmortem Inspection

Even after a long survival period (98 or more days), there was no evidence of vagal regeneration under the dissection microscope. After a 24 hr fast, shams and animals with total abdominal and gastric vagotomies retained a mean (± s.e.m.) of 0.06 (0.02), 0.94 (0.07), and 0.88 (0.07) g of food in the stomach, respectively. The increased gastric retention in the vagotomized groups is significant, $F(2, 36) = 64.59$, $p < 0.0001$.

Discussion

Experiment 12 confirms and extends the findings of Experiments 10 and 11. With the exception of male hamsters during the first week after surgery, food intakes and body weights of vagotomized hamsters did not deviate from controls under *ad lib* feeding conditions. However, vagotomized animals lost less weight than controls during the repeated deprivation schedule, though not as a result of increased feeding during this period. Hamsters drank more after hypertonic saline injection, but this response was not dependent on the integrity of the abdominal vagus. Lastly, it was shown that although selective gastric vagotomy did attenuate feeding suppression to a low dose of exogenous CCK-8, it was

FIGURE 19. Mean (± s.e.m.) body weights and food intakes of intact and vagotomized male and female hamsters during intermittent food access.


BODY WEIGHT (g)
CHOW INTAKE (g/24 hrs)
DAYS POSTSURGERY
♀
♂
n♀ n♂
6 6 Sham Vagx
6 7 Total Abdominal Vagx
6 6 Gastric Vagx
AD LIB FEEDING
9 hrs/day FOOD DEPRIVATION
AD LIB FEEDING

not as effective as total abdominal vagotomy.

The effects of vagotomy on body weight have varied somewhat in these experiments. In Experiment 10 there was a tendency, though not statistically reliable, for vagotomized females to outweigh their controls and for vagotomized males to lose more weight than their controls. During the course of Experiment 11, vagotomized hamsters lost less weight than controls. In both these experiments between group body weight comparisons were made as the animals were steadily losing weight as a result of repeated peptide testing after deprivation. In Experiment 12 body weight and food intake were studied over a long period in vagotomized and control animals that were otherwise unmanipulated. Under these conditions, each type of vagotomy did not reliably alter long term regulation of food intake and body weight in hamsters of either sex. Vagotomy, however, did enable the animals to better tolerate repeated periods of food deprivation. As shown in Figure 19, shams lost more weight over the deprivation schedule than vagotomized animals. Although this weight loss differential was small, it was consistent across animals and statistically reliable. Figure 19 suggests that mean body weights of the intact and vagotomized groups would have further diverged had the deprivation schedule been prolonged. Evidently, this vagotomy effect was not mediated by increased feeding during the deprivation schedule. The reasons underlying differential weight loss during repeated deprivation remain to be elucidated.

Experiment 12 confirms Lowy and Yim's (1982) observation that hamsters increase their water intake after hypertonic saline injections. Unlike rats (Smith and Jerome, 1983) and people (Schoon et al., 1984),

this drinking response is not blocked or attenuated by subdiaphragmatic vagotomy. Thus, the abdominal vagus does not appear to be important for osmotic thirst in hamsters. In rats, vagotomy reduces spontaneous daily water intakes (Kraly, Gibbs, and Smith, 1975), in addition to reducing drinking to experimental challenges such as AII and hypertonic saline injections. The fact that the abdominal vagus is not necessary for drinking elicited by osmotic stimuli may partially account for why spontaneous 24 hr daily intakes (see water intakes after saline injections in Figures 13 and 15) are normal in vagotomized hamsters.

The present experiment confirms the observation made in the previous experiment in that total abdominal vagotomy completely blocks feeding suppression to low, but not high, doses of CCK-8. It was further found that selective gastric vagotomy was not as effective as total abdominal vagotomy. It is unlikely that partial regeneration, and hence partial recovery of gastric vagal function accounts for the differential effectiveness of the two types of vagotomies. There was no evidence of partial regeneration at postmortem anatomical inspection of oesophageal strips. Moreover, gastric clearance in animals with gastric vagotomy was as slow as in animals with total abdominal vagotomy, thus indicating a lack of regeneration of gastric efferents, and very likely, afferents. It is therefore concluded that although the gastric division of the abdominal vagus is important for the CCK satiety effect, other divisions (hepatic, coeliac, or both) must also be relevant.

GENERAL DISCUSSION OF EXPERIMENTS 10-12

The major objective of the last series of experiments was to provide a detailed analysis of the role of the subdiaphragmatic vagus in mediating CCK effects on hamster feeding. The results indicated that vagotomized hamsters are completely unresponsive to the suppressive effects of the lower doses of exogenous CCK-8 on feeding. The blocking effect of vagotomy was specific to CCK-8, since vagotomized and intact hamsters were equally responsive to other peptide hormones that reduced feeding. The absence of feeding suppression to lower doses of CCK-8 by hamsters with total abdominal vagotomy is partly mediated by denervation of the stomach and lower gastrointestinal tract, but other divisions of the abdominal vagus must also be important because the blocking effect of selective gastric vagotomy was not as robust as that of total abdominal vagotomy. Hamsters with either type of vagotomy decreased feeding in response to relatively large doses of exogenous CCK-8, which indicates that at high concentrations, CCK is active at extravagal sites. It was suggested that after large periperal doses, some amount of circulating CCK gains access to its receptors in brain feeding systems that are independent of the abdominal vagus.

During the collection of the above data it was also found that abdominal vagotomy in hamsters does not produce long lasting feeding and drinking deficits as it does in other species. Vagotomized hamsters are not hypophagic and do not regulate body weight at lower levels, and under certain conditions such as repeated deprivation, may actually outweigh intact animals. Drinking was also unaffected by vagotomy, as lesioned animals showed normal 24 hr water intakes and normal drinking in response to a hypertonic saline challenge.

Although these experiments clearly show a role for the abdominal vagus in mediating the effects of exogenous and presumably, endogenous CCK, it is not clear whether vagal afferents or efferents are critical. In rats, several lines of evidence indicate that vagal afferents are crucial for CCK feeding suppression. CCK receptors have been reported in the rat vagus nerve (Zarbin et al., 1981). Systemic injections of exogenous CCK in rats increase discharge rate of gastric and coeliac vagal afferents and decrease discharge rate of hepatic vagal afferents (Niijima, 1983). These findings demonstrate that CCK is capable of acting directly on vagal afferent fibres to influence feeding; however, it is not certain whether the vagal afferents containing CCK receptors or the afferent fibres activated or inhibited by systemic CCK are relevant to feeding and satiety. Cholinergic receptor blockade with atropine did not block the usual feeding inhibition to exogenous CCK in rats (Smith et al., 1981). This again suggests, but does not prove, that (cholinergic) fibres of vagal preganglionic motor neurones are not necessary for CCK-feeding suppression. The most direct and compelling evidence for the importance of vagal afferents comes from a recent study by Smith, Jerome, and Norgren (1983). By combining unilateral sectioning of vagal sensory rootlets as they enter the dorsolateral medulla with a unilateral section of the abdominal vagus on the same side, they were able to totally interrupt vagal sensory input from the gut to the brainstem, but spare approximately half of the vagal motor innervation of the gut (see Appendix). They found that this combination blocked feeding suppression to CCK as effectively as bilateral abdominal vagotomy. A unilateral abdominal vagotomy which, in my estimation,

would interrupt 40-60% of abdominal vagal afferents (depending on which trunk), did not block feeding inhibition to exogenous CCK. A unilateral abdominal vagotomy combined with an ipsilateral interruption of motor rootlets as they leave the ventral medulla, which would interrupt all or 80% of the vagal motor innervation of the gut (again, depending on which side the surgeries were performed) but only interrupt approximately half of the sensory vagal innervation, was similarly ineffective at blocking feeding suppression to CCK—8. These findings indicate that, in the rat, total lesions of vagal afferents are necessary and sufficient to block feeding inhibition to exogenous CCK. Although only further experiments along these lines can establish whether the same applies to the hamster, it would appear that vagal afferents also mediate CCK feeding suppression in hamsters.

The present experiments and the studies in rats have provided strong evidence that the abdominal vagus is the primary avenue through which peripherally administered exogenous CCK reduces feeding in these species. It is inferred from these studies that the vagus is necessary for the satiety effect of intestinal CCK. Two possible modes of CCK-vagus interaction may operate to generate a satiety signal. The first mechanism is the most simple. Food contacting the duodenal mucosa stimulates the release of CCK into the mesenteric circulation where it may gain access to its receptors on various branches of the abdominal vagus. However, direct activation of vagal CCK receptors need not arise from locally circulating CCK, as CCK released from the mucosal cells in the duodenum may act locally on nearby vagal afferent terminals (i.e., paracrine rather than endocrine stimulation) embedded in duodenal smooth

muscle (Mei, 1983). In either case, CCK is proposed to act directly on the vagus to activate an afferent limb of a satiety reflex. Alternatively, CCK may activate satiety-related vagal afferents indirectly by inhibiting gastric emptying. As noted in Experiments 7-9, gastric distention is a classic example of a satiety stimulus. Activity in a population of vagal afferent fibres and dorsal medullary units is phase-locked to stomach contraction and distention (Barber and Burks, 1983; Ewart and Wingate, 1983). Gastric distention in rats reduces feeding, and this effect is abolished by vagotomy (Gonzalez and Deutsch, 1981; but see Kraly and Gibbs, 1980). CCK constricts the pylorus and reduces the motility of the proximal stomach to reduce the rate of gastric emptying and facilitate gastric distention. Thus, it is possible that the vagus merely codes the effects of CCK on gastric distention to influence feeding. These mechansims of CCK-vagus interaction may not be mutually exclusive within a given species, and the possibility remains that they may operate synergistically to limit feeding.

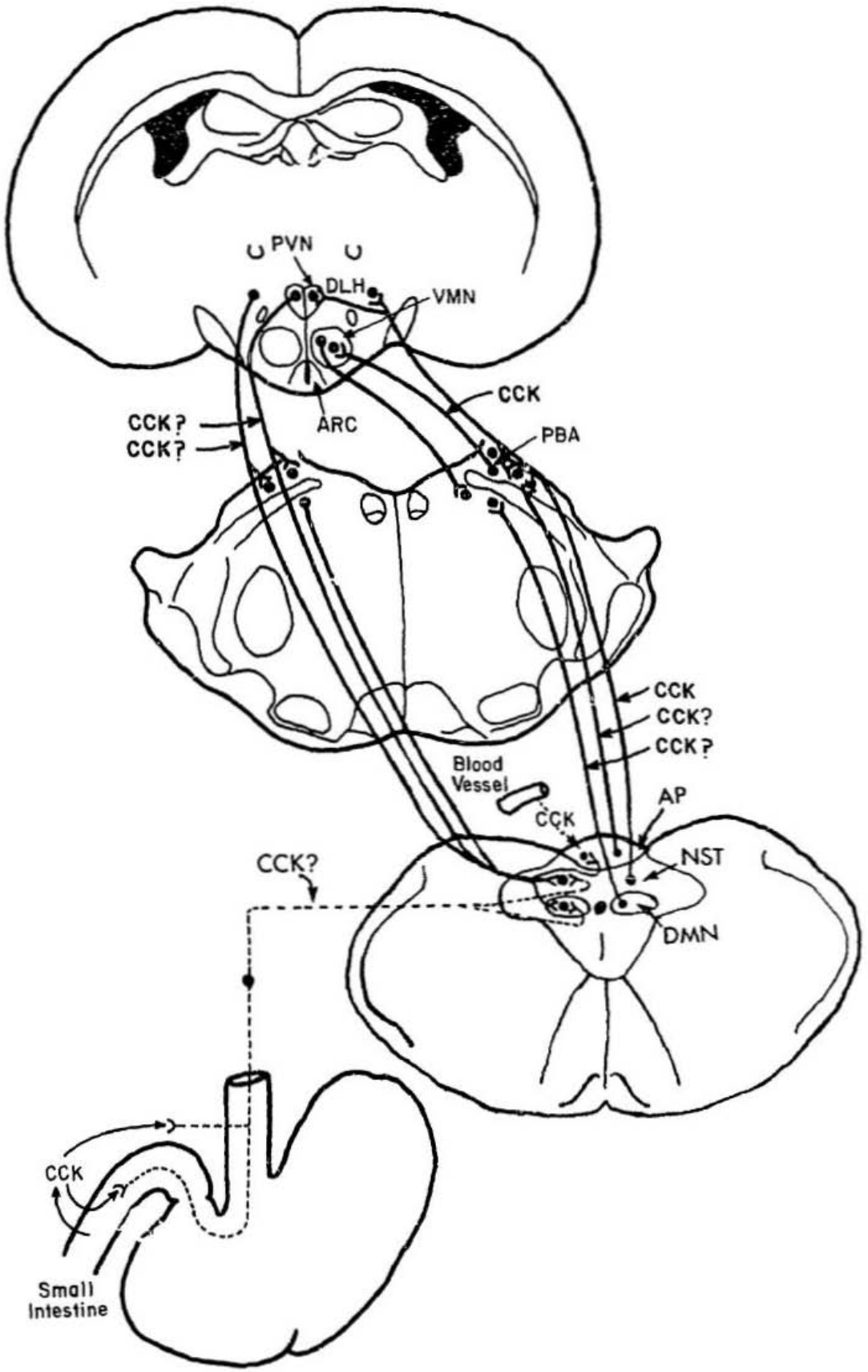
The demonstration that the vagus is necessary for CCK's effect on feeding in rats and hamsters is important because it specifies a possible link between the viscera and brain through which prandially released duodenal CCK generates and transmits a centrally directed satiety signal. Recently, Crawley and Schwaber (1984) and van der Kooy (1984) reported that lesions in the dorsal vagal complex (DVC) of the rat, which destroy the first central relay neurones of the ascending vagal viscerosensory pathway (see Appendix), block the effects of systemic CCK injections on feeding inhibition and on satiety-related behaviour. These recent findings, of course, contribute to the

understanding of the neural pathways necessary for CCK-initiated satiety, but only scratch the surface. Much more work is needed to arrive at a more complete understanding of the underlying brain circuitries. In view of the increasing interest in this area, it is likely that future studies of CCK and feeding will be directed at further tracing of the underlying CNS pathways.

A more challenging task for future investigations will be the elucidation of how brain CCK systems might contribute to the control of feeding. Figure 20 is a schematic representation of known and possible CCK-containing/CCK-receptive neural elements in brain systems implicated in the control of feeding. These anatomical connections (irrespective of neurotransmitter content) are well described in the rat (e.g. van der Kooy, Koda, McGinty, Gerfen, and Bloom, 1984) and other species, including the hamster (Miceli, unpublished). The importance of some of these pathways for the control of feeding has also been established. For example, Kirchgessner and Sclafani (1983) have presented strong evidence that hypothalamic hyperphagia and obesity are (at least in part) mediated by damage to the descending projections of the hypothalamic paraventricular nucleus. The paraventricular nucleus is rich in CCK-containing neurones (Faris et al., 1983b), some of which have long descending projections through the medial forebrain bundle (Kiss et al., 1983). CCK-containing neurones are also abundant in the dorsolateral hypothalamus (P. L. Faris, personal communication), and some of these may also be projection neurones. Although it has yet to be demonstrated, it is likely that a sub-population of neurones in the paraventricular nucleus and dorsolateral hypothalamus with projections

FIGURE 20. Schematic representation of possible modes of interaction between peripheral and central CCK systems in the control of feeding. These neuroanatomical pathways are well characterized in rats. Some of these pathways are known to contain CCK; others are likely to contain CCK, but have yet to be reported as CCK-containing. The medullary dorsal vagal complex is shown to be a possible site at which basal forebrain and visceral CCK-containing/CCK-receptive systems converge to control feeding. See text for further explanation.
Abbreviations:

AP	area postrema
ARC	arcuate nucleus
DLH	dorsolateral hypothalamus
DMN	dorsal motor nucleus
NST	nucleus of the solitary tract
PBA	parabrachial area
PVN	paraventricular nucleus
VMN	ventromedial nucleus


PVN
DLH
VMN
ARC
CCK
CCK?
CCK?
PBA
CCK
CCK?
CCK?
Blood
Vessel
CCK
AP
NST
CCK?
DMN
CCK
Small
Intestine

to the DVC contain CCK. There are additional sources of CCK input to the DVC. Units in the area postrema, which lie outside the BBB, may be sensitive to circulating CCK. Vagal afferent fibres also contribute to the immunoreactive-CCK present in DVC (Rehfeld and Lundberg, 1983). It is conceivable that abdominal vagal afferents activated by duodenal CCK may contain CCK. Lastly, CCK-immunoreactive cells have been described throughout the DVC. Some of these are interneurones, and some, projection neurones (Kubota et al., 1983; Mantyh and Hunt, 1984). Thus, the DVC may serve as an interface betweeen CCK systems functionally related to long-term (hypothalamic) and short-term (humoral/visceral nervous system) control of feeding. Brain CCK-containing systems may also be involved in the transmission of viscerosensory information to forebrain feeding-control systems. Some CCK-containing neurones in the region of the nucleus of the solitary tract receiving input from the abdominal vagus (see Appendix) project to the pontine parabrachial area, which in turn has CCK-containing neurones with projections to the medial basal hypothalamus. Of course, it is mere speculation that these CCK-containing neural elements have functional implications for the control of feeding and satiety. The anatomy of brain CCK systems, however, does serve as a conceptual starting point for designing experiments to clarify the contribution of CNS CCK to the control of feeding.

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APPENDIX

ANATOMICAL PROJECTIONS OF THE HAMSTER VAGUS NERVE

To further validate the vagotomy procedure used in the feeding experiments and to provide a description of the anatomical projections of the hamster vagus (none is currently available for this species), a horseradish peroxidase (HRP) study of the hamster lower abdominal and cervical vagus was undertaken. In these experiments HRP was applied to the proximal end of the sectioned vagus, where it was taken up by cut axons and transported retrogradely to reveal brainstem perikarya of origin and, transganglionically to reveal brainstem afferent projections.

In the rat, vagal afferents project to a limited group of second order sensory neurones in the dorsal medulla. The dorsal medullary regions receiving primary viscerosensory vagal input include the area postrema (AP) and possibly, the dorsal motor nucleus of the vagus (DMX), but the nucleus of the solitary tract (NST), its caudal aspect in particular, is the primary target of vagal afferents (Contreras, Beckstead, and Norgren, 1982; Kalia and Sullivan, 1982). The NST is also a central terminus for gustatory and somatosensory afferent axons of the glossopharyngeal and facial nerves and the trigeminal complex (Norgren, 1983). The NST extends throughout most of the length of the medulla, some three or more mm in the rat, and about 1.5-2.0 mm in the hamster. Although the NST is relatively compact and well delineated against neighbouring medullary structures, it has been subdivided, and

the corresponding subdivisions named, nearly as many times as it has been studied by neuroanatomists. Consequently, there are a number of different and often conflicting classification and nomenclature systems for the subdivisions of the NST in single species, like the rat. The reasons for the inconsistent nomenclature and subdivisions have already been discussed in detail (Kalia and Sullivan, 1982; Contreras et al., 1982). However, the foremost is that cytoarchitectonic boundaries within the NST are not distinct in Nissl-stained material. This is also true in Nissl-stained sections of the hamster medulla. At the rostrocaudal levels at which the NST receives sensory input from the cervical and gastric vagus, only three subdivisions could be clearly delineated in thionin-stained sections. A medial division, medial to the solitary tract (ST) and dorsal to the lateral aspect of the dorsal motor nucleus (DMN) of the vagus, can be readily distinguished in Nissl-stained material (see Figure 21). The medial division, as defined by Kalia and Sullivan (1982) and van der Kooy et al. (1984), in the hamster assumes a position comparable to that in the rat, although slightly more lateral. The second division is located ventrolateral to the solitary tract and lateral to the medial division, and may correspond to the rat lateral solitary nucleus described by van der Kooy et al. (1984). A cell-sparse commissural division in the midline dorsal medulla is also distinguishable in Nissl-stained hamster material.

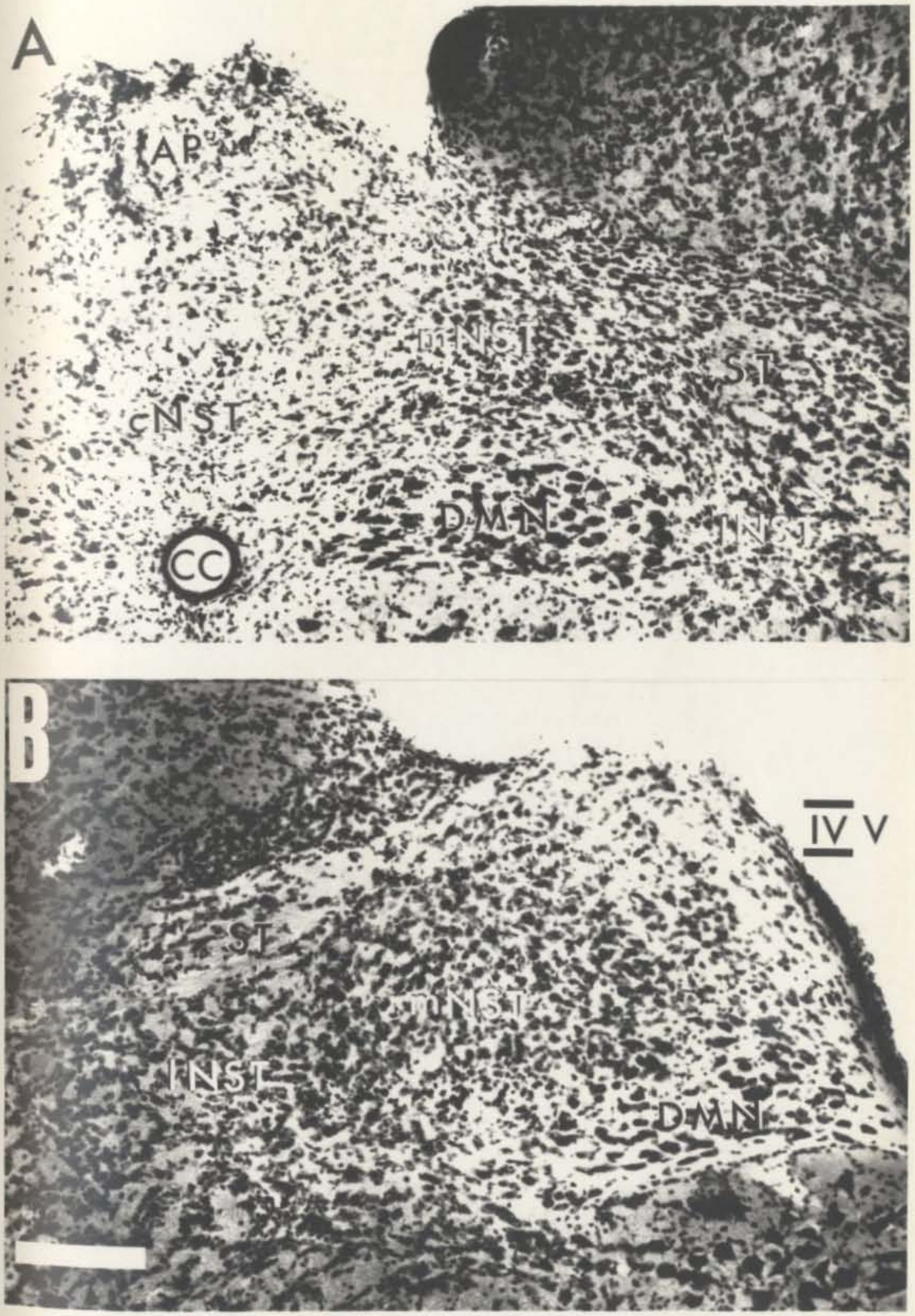
Methods

Fifteen male and female hamsters ranging between 130 and 140 g were

FIGURE 21. Thionin-stained coronal (50 µm) sections of the hamster dorsal medulla illustrating the divisons of the more caudal aspects of the NST. A: Section of the dorsal medulla at the level of the AP. B: The dorsal medulla at a more (approximately 200 µm) rostral level. Bar = 200 um.

Abbreviations for Figures 21-31:

AP	area postrema
CC	central canal
CG	commissural grey of the spinal cord
DMN	dorsal motor nucleus of the vagus
MLF	medial longitudinal funiculus
NA	nucleus ambiguus
NASN	nucleus of the accessory spinal nerve
NST V	nucleus of the spinal tract of the trigeminal nerve
N XII	hypoglossal nucleus
cNST	commissural division of the nucleus of the solitary tract
lNST	lateral division of the nucleus of the solitary tract
mNST	medial division of the nucleus of the solitary tract
ST	solitary tract
ST V	spinal tract of the trigeminal nerve
IV V	fourth ventricle


A
AP
cNST
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ST
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IV V
ST
INST
DMN

used for this study. Animals underwent either unilateral cervical vagotomy or abdominal vagotomy under pentobarbital anaesthesia. For the cervical vagotomy a skin incision was made to the side of the midline throat and the muscles overlying the carotid artery were retracted or cut by blunt dissection. As in the rat, the cervical vagus adheres to the dorsolateral aspect of the carotid. The nerve was carefully dissected free from the carotid sheath with fine forceps, and gently elevated with sutures. The nerve was then placed over a small sheet of Parafilm and cut with microdissection scissors approximately 0.5-1.0 mm below the nodose ganglion. Crystalline HRP (Sigma, Type VI) was then applied with fine forceps to the proximal end of the cut nerve. The HRP was dissolved to a thick consistency by the extracellular fluid surrounding the nerve. While taking care not to crush or mangle the nerve, the Parafilm beneath the vagus was then folded around the proximal stump and the ends of the Parafilm were pinched and sealed together with forceps so as to make a loose pocket around the nerve. The nerve within the pocket was then secured by loosely sewing the Parafilm pocket to the adjacent muscles. The muscles were sewn in layers and the skin wound closed with surgical clips. In two animals (1 male, 1 female), this procedure was successfully performed on the left cervical vagus; and in 3 animals (2 female, 1 male) on the right cervical vagus.

The procedure for abdominal (i.e., gastric) vagotomy was similar to that described in the behavioural studies. The right abdominal vagal trunk was cut above were it bifurcates into gastric branches. The left abdominal trunk was cut below the point where the coeliac branch

enters/leaves the trunk. A small sheet of Parafilm was placed between the proximal end of the cut nerve and the oesophagus, and crytalline HRP was applied to the proximal nerve stump. The Parafilm sheet was folded over and sealed to form a protective pocket. The nerve and pocket were then loosely secured around the oesophagus with sutures. Four (2 females, 2 males) animals underwent a "labelled" left abdominal vagal trunk and three (2 females, 1 male) animals underwent a labelled right abdominal trunk. In one of the labelled left abdominal cases, the right trunk was previously (during the same surgery) sectioned just below the diaphragm. The proximal end of this trunk was sealed by dripping paraffin wax over it and the surrounding upper oesophagus. The purpose of cutting and sealing the right trunk in this case was to provide unequivocal evidence against the possibility that inadvertent spread of HRP to the surrounding oesophagus and subsequent up-take from stray terminals of the right trunk accounted for the bilateral retrograde labelling observed in the other left abdominal cases. In two control animals with intact vagi (1 female, 1 male), crystalline HRP, in substantial excess of the amounts used in experimental animals, was applied over the lower oesophagus (at points where the vagi would ordinarily be cut) and superior aspects of the stomach. During the application of HRP care was taken not to accidentally damage vagal fibres or connective tissue. In a third control animal (female) with intact cervical vagi, crystalline HRP was applied to the musculature surrounding the cervical vagus and carotid.

After a survival period of 40-64 hrs the animals were overdosed with pentobarbital and perfused intracardially with saline followed by

500 mL of ice cold 1% formaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer followed by 500 mL of buffer or 10% sucrose in buffer. The brains were stored in buffer or 10% sucrose-buffer at 4° C until sectioning. The brains were blocked just rostral to the pontomedullary junction perpendicular to a skull-flat plane. Frontal sections of the lower brainstem and upper levels of the cervical spinal cord were taken at 50 µm on either a freezing microtome or vibratome. Alternate series of sections were mounted onto chrome-alum subbed slides and allowed to air dry at which point they were prepared for HRP histochemistry. The sections were reacted using tetramethylbenzidine as the chromagen according to standard procedures (Mesulam, 1982). One series of sections was immediately dehydrated in alcohol, cleared with xylene and cover slipped. The alternate series was counterstained with formal-thionin. The sections were examined with bright and dark field light microscopy.

Results

Cervical Vagotomy Experiments

a) Vagal afferents to the brainstem.

After exposing the central end of the cervical vagal stump to HRP, afferent fibres could be seen entering the ipsilateral dorsolateral medulla at its more rostral aspect. These fibres traversed the spinal tract and nucleus of the spinal tract of the V nerve in 4-5 fascicles

(see Figures 22 and 23). These fascicles could be seen heading medially and caudally towards the solitary tract (ST). The fascicles merged in the ST, where they continued caudally. At medullary levels caudal to where labelled afferents join the ST, extraperikaryal HRP, presumptive anterogradely labelled terminal varicosities, were seen in the ipsilateral NST. The anterograde labelling in the ipsilateral NST was restricted to a rostrocaudal zone (approximately 1 mm) centred at about the level of the area postrema (AP). Within this zone, extraperikaryal HRP grains were distributed throughout most of the NST. The heaviest anterograde labelling, however, was restricted to the medial division (See Figures 24 and 25). Labelled afferent fibres were also seen crossing the midline in the commissural NST to invade the contralateral NST. Sparse to moderate anterograde labelling in contralateral NST was mainly in the medial division and was confined to a narrower rostrocaudal zone (approximately 0.5 mm) which was also centred at about the level of the AP. Substantial anterograde label was also distributed ipsi-and contralaterally along the NST-AP border which included the so-called external and internal solitary zones (van der Kooy et al., 1984) and the inner rim of the AP. Extraperikaryal HRP grains within the core of the AP were sparse. Any possible labelling in the ipsilateral DMN was obscured by heavily labelled dendrites of the labelled motor neurones. However, extraperikaryal HRP grains were observed along the dorsal and ventral borders of the contralateral DMN, but not in the core of the nucleus.

b) Retrograde labelling.

FIGURE 22. Camera lucida drawings of sections of the hamster medulla and cervical spinal cord demonstrating the trajectories of vagal afferents (large dots) and efferents (thin dashes) and the rostrocaudal extent of anterograde (small dots) and retrograde (diamonds) labelling after applying crystalline HRP to the left cervical vagus.

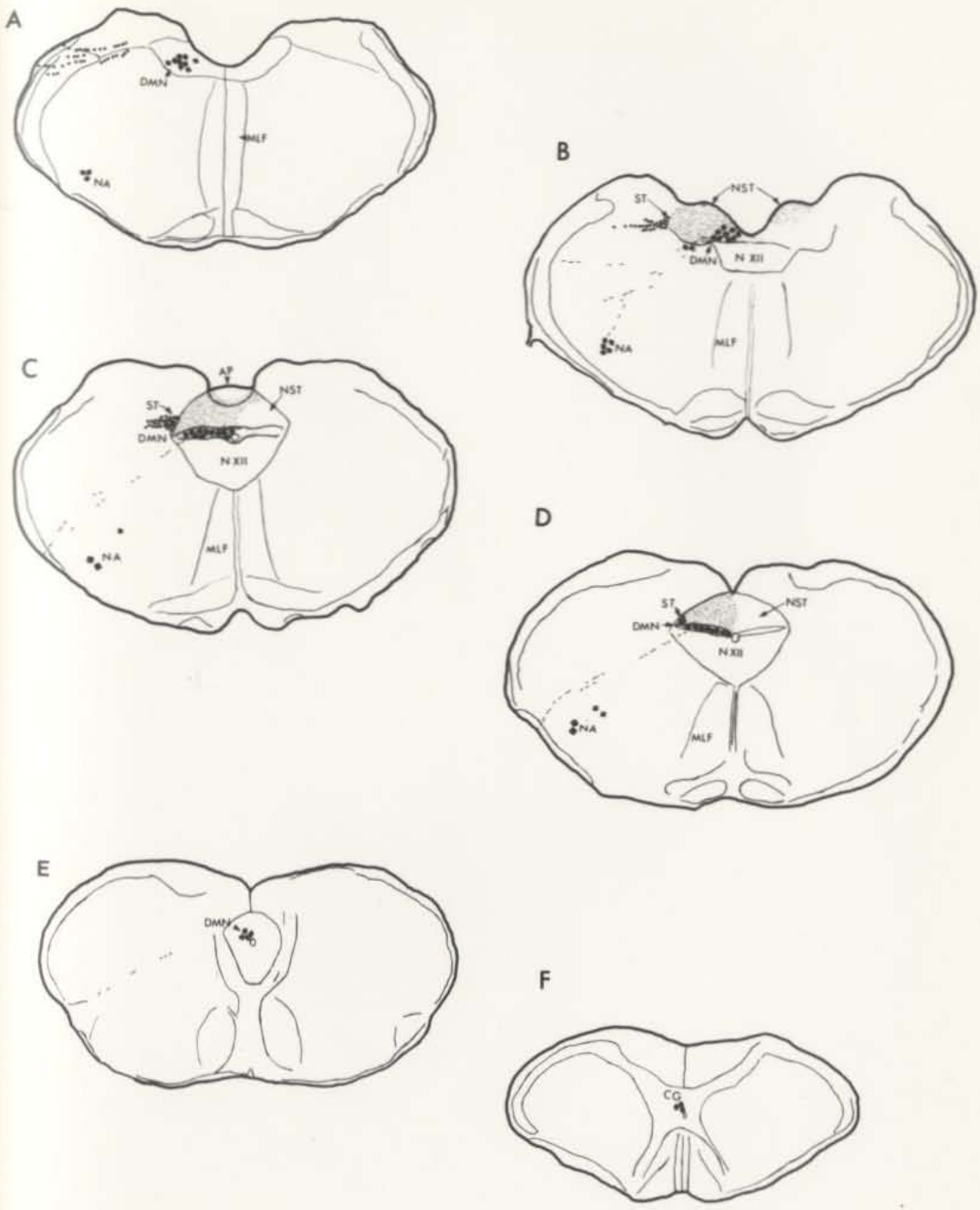

A
DMN
MLF
NA
B
ST
NST
DMN
N XII
NA
MLF
C
AP
NST
ST
DMN
N XII
NA
MLF
D
ST
NST
DMN
N XII
NA
MLF
E
DMN
F
CG

FIGURE 23. A: Darkfield photomicrograph of afferent fascicles fro: he right cervical vagus as they traverse the ST V and the NST V. Dorsa .s towards top and medial is towards left. B: Darkfield photomicrograph of afferent fibres from the right gastric vagus heading to the ST. Dorsal is towards top, medial is towards right. Bar = 100 µm.

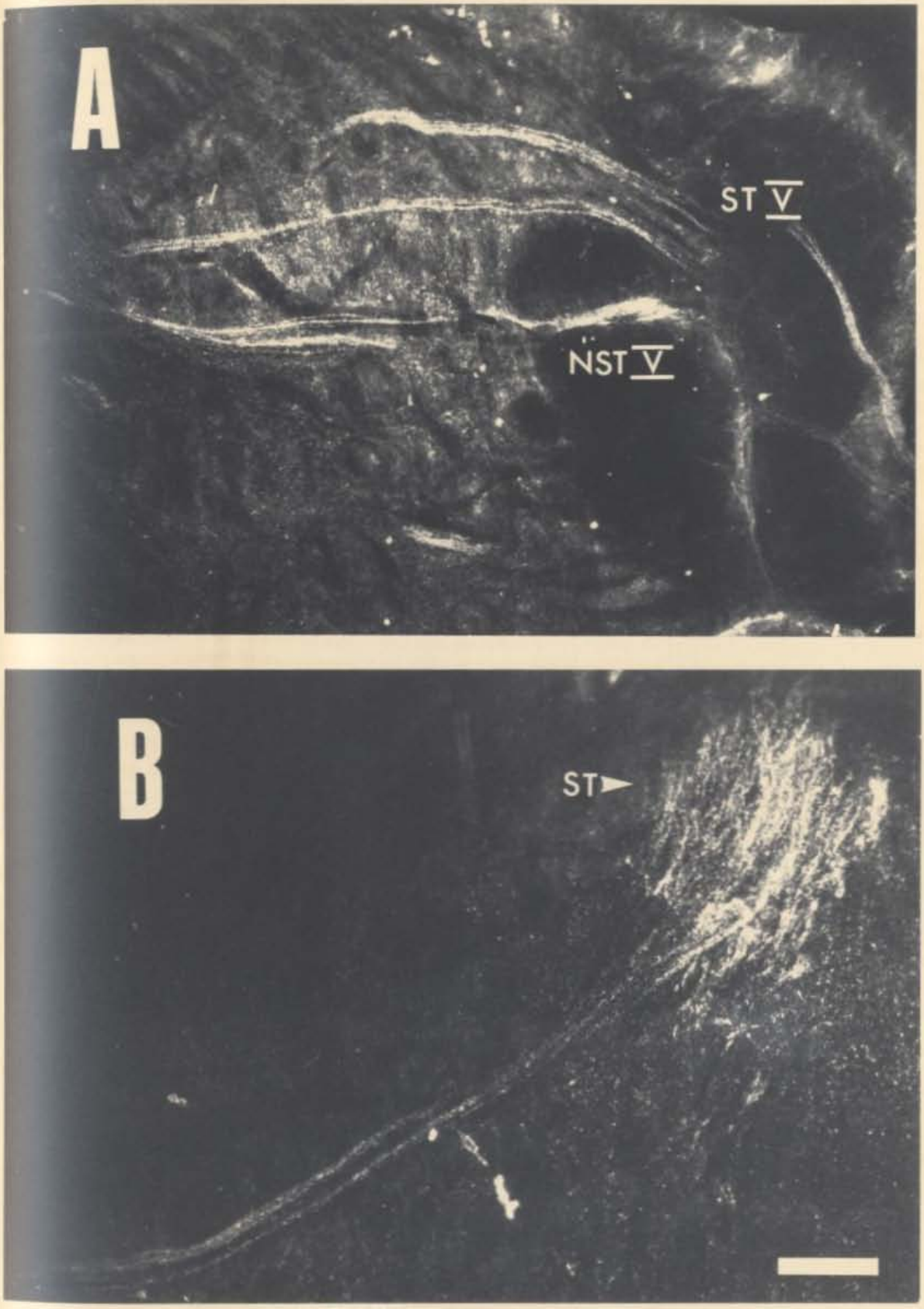

A
ST V
NST V
B
ST

FIGURE 24. Darkfield photomicrograph of the dorsal medulla at the level of the AP showing anterograde labelling in the NST and AP and retrograde labelling in the DMN region after HRP was applied to the right cervical vagus. The black dashed lines are at approximately the ventral border of the lateral DMN. Labelled perikarya below this line are in the N XII. Bar = 150 µm.

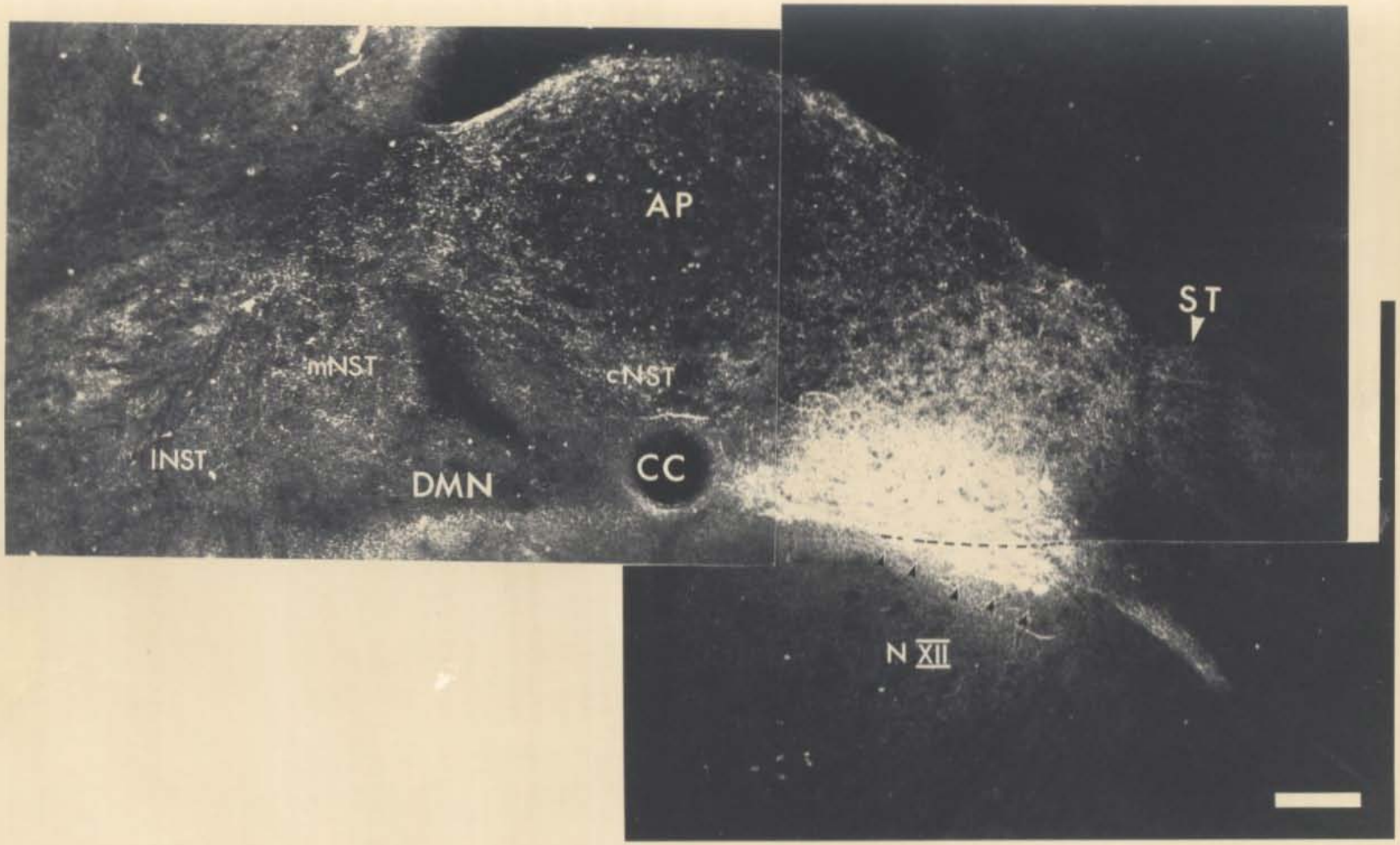
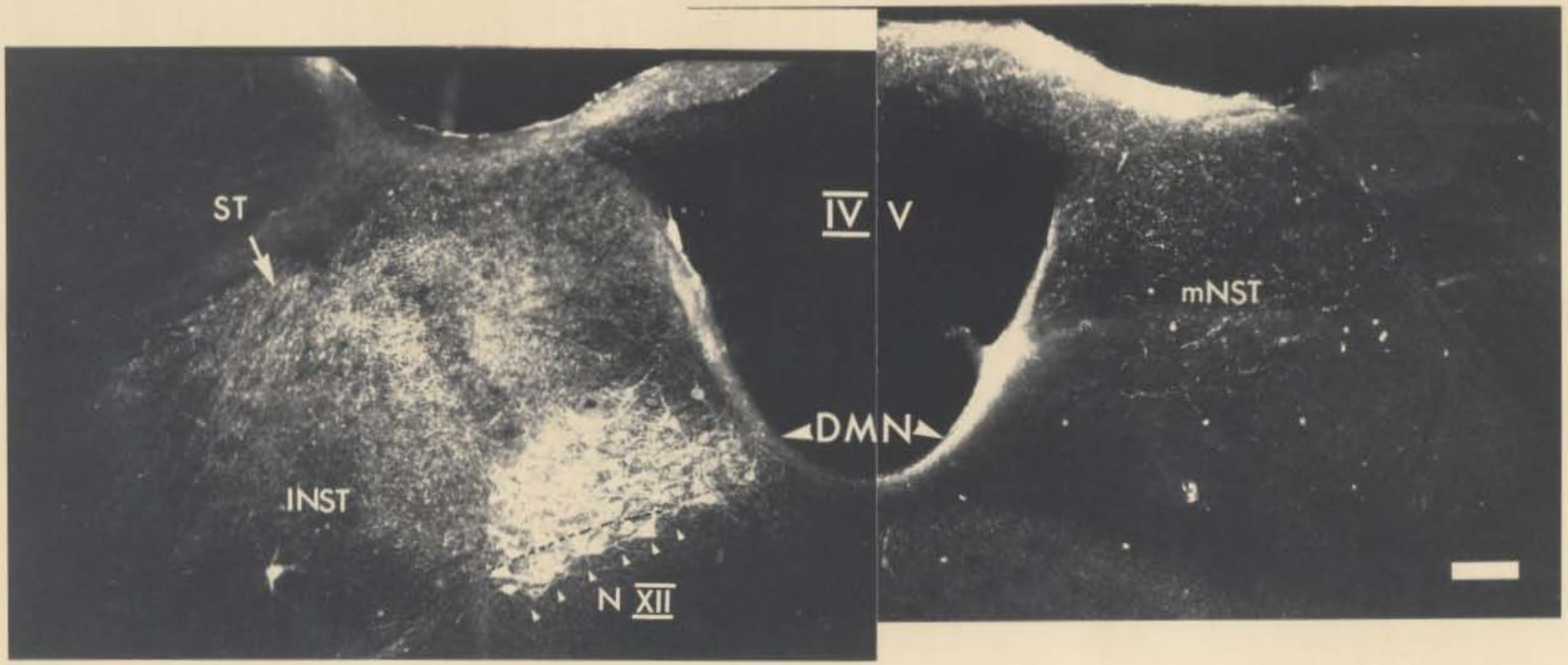

AP
ST
mNST
cNST
lNST
DMN
CC
N XII

FIGURE 25. Darkfield photomicrograph of the dorsal medulla rostral to the level of the AP showing heavy anterograde labelling in the NST and retrograde labelling in the DMN area after sectioning and applying HRP to the left cervical vagus. The dashed lines indicate approximately the ventral border of the DMN. Labelled perikarya below this line are in the N XII. Bar = 100 μm.


ST
IV
V
mNST
DMN
INST
N XII

Following HRP labelling of the cervical vagus, it appeared that all the perikarya in the ipsilateral DMN (throughout its rostrocaudal span) were densely filled with HRP reaction product. In addition to the labelled cells in the DMN proper, there were a few scattered labelled cells around the DMN, in the NST and N XII. In two cases there was substantial perikaryal labelling in the dorsolateral N XII, just ventral to the lateral aspect of the DMN. That all or almost all the cells in the DMN were filled with HRP reaction product prevented a morphological description of individual cells in the cervical cases. However, it was possible to pick out morphological detail of DMN neurones in the abdominal cases, since fewer were labelled. DMN neurones were mainly medium sized and polymorphic. Fusiform cells were the most predominant, but there were also a substantial number of multipolar, oval, and pyramidal cells. Labelled cells in the DMN formed a longitudinally-oriented column that extended caudally to the commissural grey (CG) in the upper levels of the cervical spinal cord (see Figure 26). Many large and medium sized multipolar labelled cells were also observed in the nucleus ambiguus (NA) and the region surrounding the NA (see Figure 27). An occasional labelled cell (1 or 2 per section) was sometimes seen in the medullary reticular formation between the labelled cell groups in the DMN and the NA areas. In the cervical spinal cord, labelled perikarya were also noted in the spinal nucleus of the accessory nerve (SNAN) and in the ventral horn, dorsolateral to the group in the SNAN. In all cervical cases, retrograde labelling was restricted to the side of the medulla/spinal cord ipsilateral to the cut vagus.

FIGURE 26. Darkfield photomicrophraph of a coronal section of the upper cervical spinal cord showing retrograde labelling in three spinal groups after applying HRP to the (right) cervical vagus. Cells in the CG are small in comparison to those in the NASN and in the "retroambiguus" region (arrow). Bar = 200 μm.

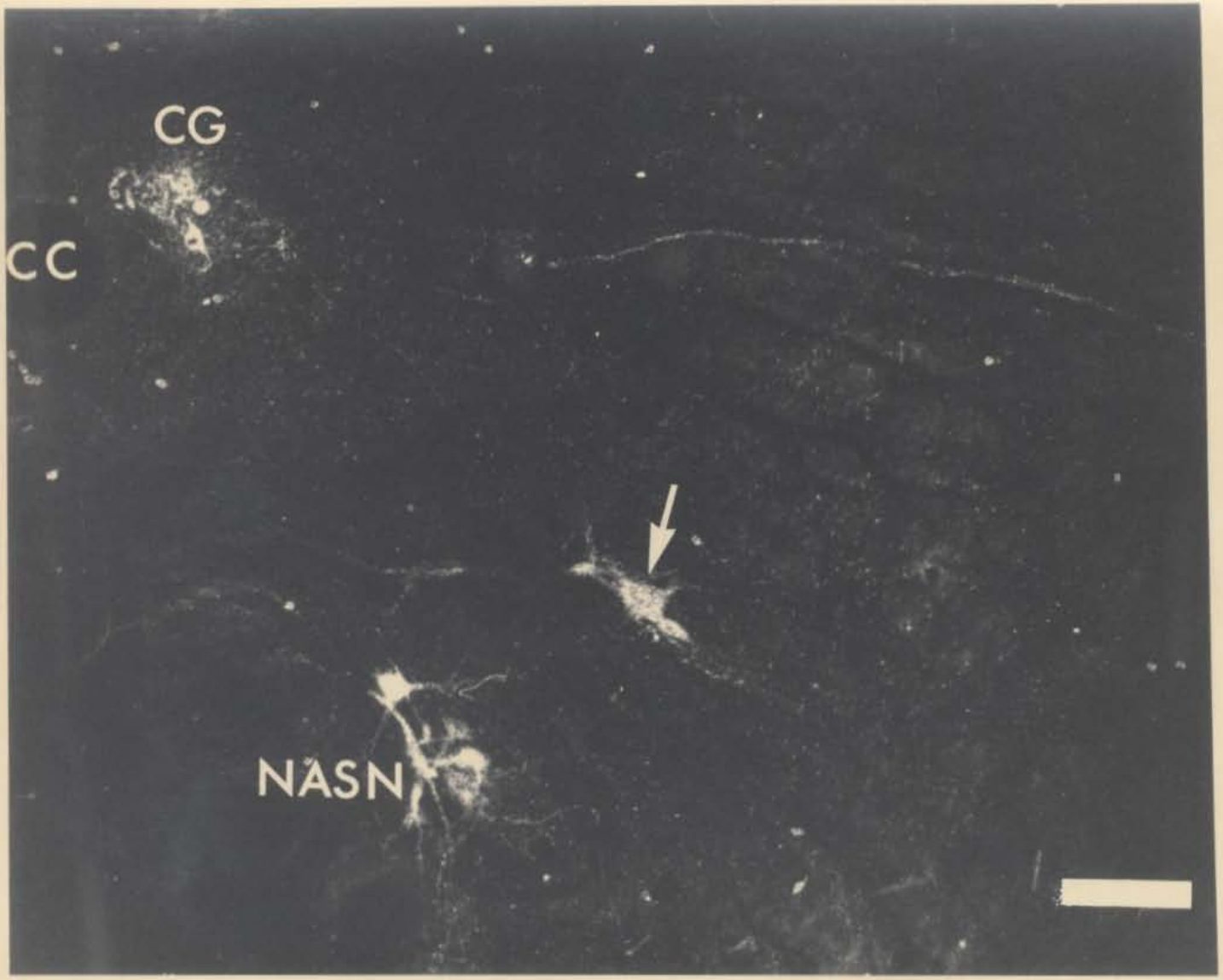
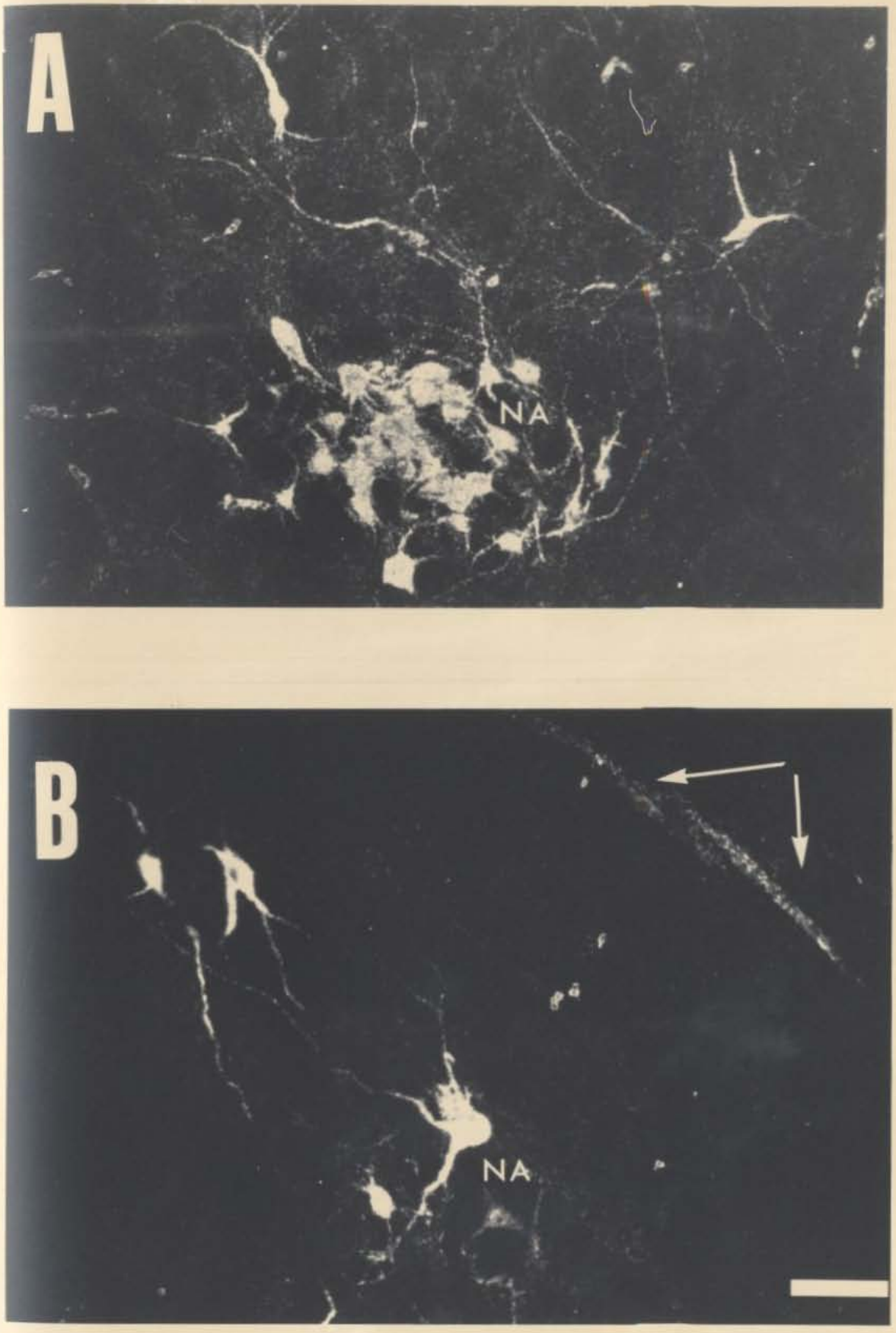

CG
CC
NASN

FIGURE 27. Darkfield photomicrographs of labelled cells in and around the NA after cutting and applying HRP to the right cervical (A) or left abdominal (B) vagus. The arrows in B indicate efferent fascicles from the DMN. Bar = 100 µm.


A
NA
B
NA

Axons of the DMN neurones could be seen traversing the medullary reticular formation in multiple fascicles. DMN axonal groups were seen throughout a wide rostrocaudal extent, beginning rostrally at the level of the anterior pole of the DMN and extending caudally to upper cervical levels of the spinal cord, the latter motor axons emerging from the CG (see Figures 22 and 26). There was no overlap in the rostrocaudal extent at which vagal afferent rootlets enter the dorsolateral medulla and at which vagal efferents leave the ventral medulla. That is, afferents enter the medulla and join the ST at rostrocaudal positions rostral to those at which efferents begin to leave the DMN. Labelled axons from the NA region took a circuitous route out of the brainstem. They could be seen travelling first dorsomedially and then making a loop to join efferents from the DMN as they headed ventrally out of the medulla.

Abdominal Vagus

Although fewer fascicles were observed, brainstem pathways of vagal efferents and afferents following transection and HRP labelling of the abdominal vagus were indistinguishable from those seen after transection and labelling of the cervical vagus. The intensity and distribution of extraperikaryal labelling and the number of labelled cells were reduced in comparision to the cervical cases. However, these quantitative differences were smaller than might be expected in view of the vagal components spared by abdominal vagotomy (innervation of the pancreas, upper oesophagus, liver, thoracic organs, and tracheal musculature).

Unlike the cervical vagotomy cases, where brainstem projections were symmetrical and predominantly uncrossed, brainstem origins and projections of the abdominal vagus were asymmetrical and crossed. Thus, labelling in the left and right abdominal cases will be described separately.

a) Right abdominal vagus.

Exposing the central end of the sectioned right abdominal vagal trunk to HRP resulted in retrograde labelling of motor neurones in the DMN, NA, and CG on the contralateral side. In the cervical spinal cord retrograde labelling was limited to the CG, as no labelled perikarya were noted in the NASN or dorsolateral to it. Although labelled cells in the DMN and CG were densely packed with HRP reaction product, there were fewer labelled cells than in the cervical cases. Quantitative differences in retrograde labelling between the cervical and right abdominal cases were particularly noticeable in the NA (see Figure 27). Fascicles from the DMN were also noticeably fewer in the abdominal cases. In two of the three cases, retrograde labelling was strictly unilateral. In the one case, a few labelled cells (1-2) were seen in the right DMN in a few sections. In the same case, labelling in the NA and the CG was strictly unilateral.

Two to three afferent fascicles were seen entering the left dorsolateral medulla and heading mediocaudally to join the ST. In all of the right abdominal cases, no afferent fibres were seen in the right side of the medulla and the right ST was devoid of label.

Extraperikaryal HRP grains were observed in the NST on the left side of the medulla. Weak anterograde labelling was also seen in the right NST. Extraperikaryal HRP in the NST in these (and the left abdominal cases) was observed throughout the rostrocaudal zone reported in the cervical cases. However, the distribution of anterograde label within the NST was more restricted after a labelled right abdominal vagotomy. Anterograde labelling in the NST was predominantly in the medial division, with some sparse to moderate labelling in the commissural region. Anterograde labelling in the lateral division was negligible or sparse. HRP grains were noted along the inner border of the AP and the dorsal and ventral borders of the right DMN, but labelling here was less pronounced than in the cervical cases. Often, the limited anterograde labelling in these regions of the dorsal vagal complex could only be seen in freshly reacted tissue (as the HRP reaction product, anterograde label in particular, faded over time), and in one of the seven abdominal cases, labelling in these areas was not observed at all.

b) Left abdominal vagus.

In contrast to the right abdominal cases where labelled perikarya were observed almost exclusively in the DMN, CG, and NA of the left medulla/spinal cord (i.e., unilaterally), application of HRP to the central end of the sectioned left abdominal vagus resulted in bilateral retrograde labelling in each of the preganglionic motor neuron groups. Although retrograde labelling in these areas was predominantly on the right, labelling on the left was substantial. The estimated ratio of

the right/left distribution of labelled neurones is 7:3. Labelled efferents from motor neurones in these cell groups were seen leaving the lower brainstem and spinal cord bilaterally (see Figures 28 and 29).

In all left abdominal cases vagal afferents were seen approaching the ST in the right medulla only. Anterograde labelling in the right NST had a distribution and density similar to that seen in the left NST in the right abdominal cases. However, significantly more afferent fibres crossed the midline in the commissural NST to distribute to the opposite NST in the left abdominal cases than in the right abdominal cases.

Controls

No anterograde or retrograde labelling was seen in the dorsal medulla after applying crystalline HRP over the musculature surrounding the cervical vagus. There was, however, some limited anterograde and retrograde labelling in the dorsal medulla after large amounts of crystalline HRP were smeared over the lower oesophagus and stomach of vagally intact animals. A sparse distribution of extraperikaryal HRP grains in the NST was barely visible in freshly reacted tissue examined under darkfield microscopy. A few retrogradely labelled cell bodies (0-6 cells/section) were seen bilaterally in the DMN and CG, but in contrast to the experimental cases, these perikarya were not densely packed with HRP reaction product (see Figure 30). Retrograde labelling was never seen in the NA region in any of these cases. It should be noted that the brain of each control animal was processed in parallel

FIGURE 28. Darkfield photomicrograph of the dorsal medulla at the level of the AP showing bilateral retrograde labelling in the DMN in a left abdominal case. Retrograde labelling in the DMN is predominantly on the right. Bar = 150 um.

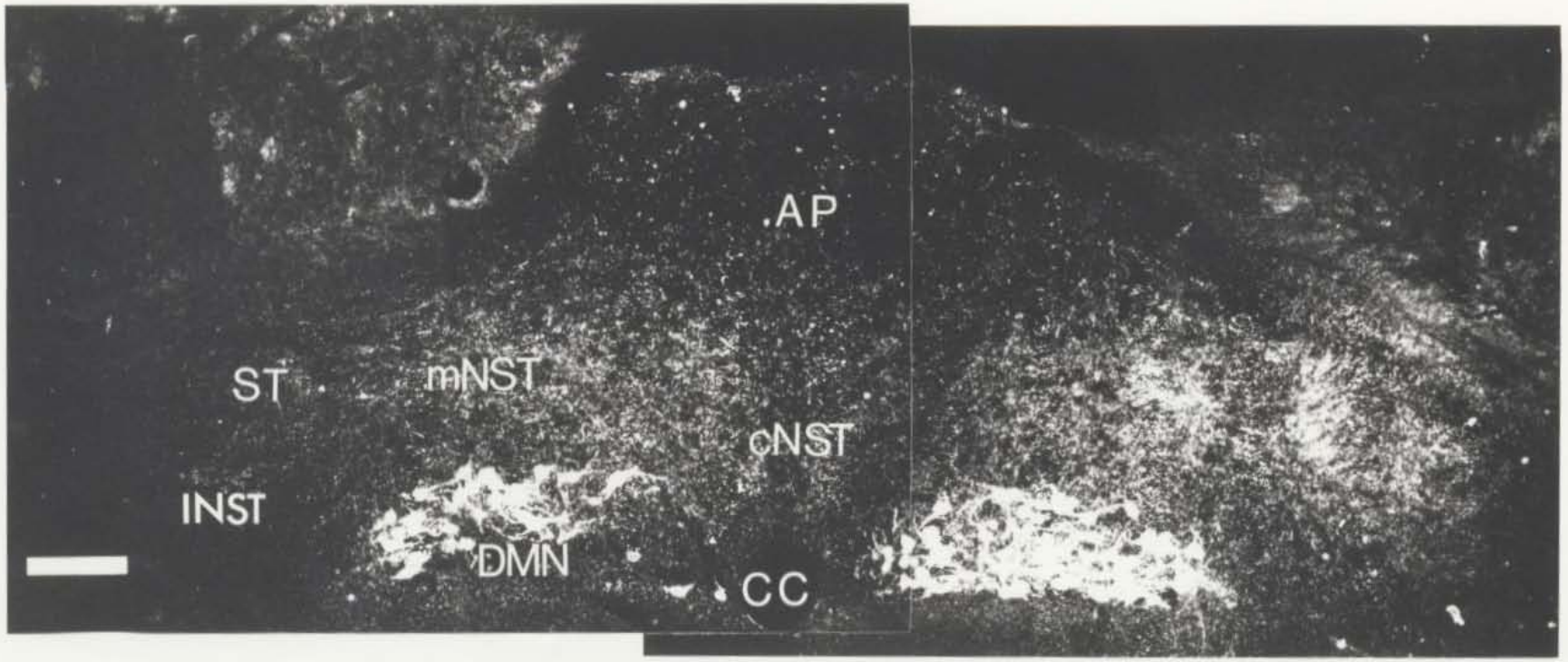

AP
ST
mNST
cNST
INST
DMN
CC

FIGURE 29. Darkfield photomicrograph of the dorsal medulla rostral to the level of the AP in a left abdominal case. Bar = 100 µm.

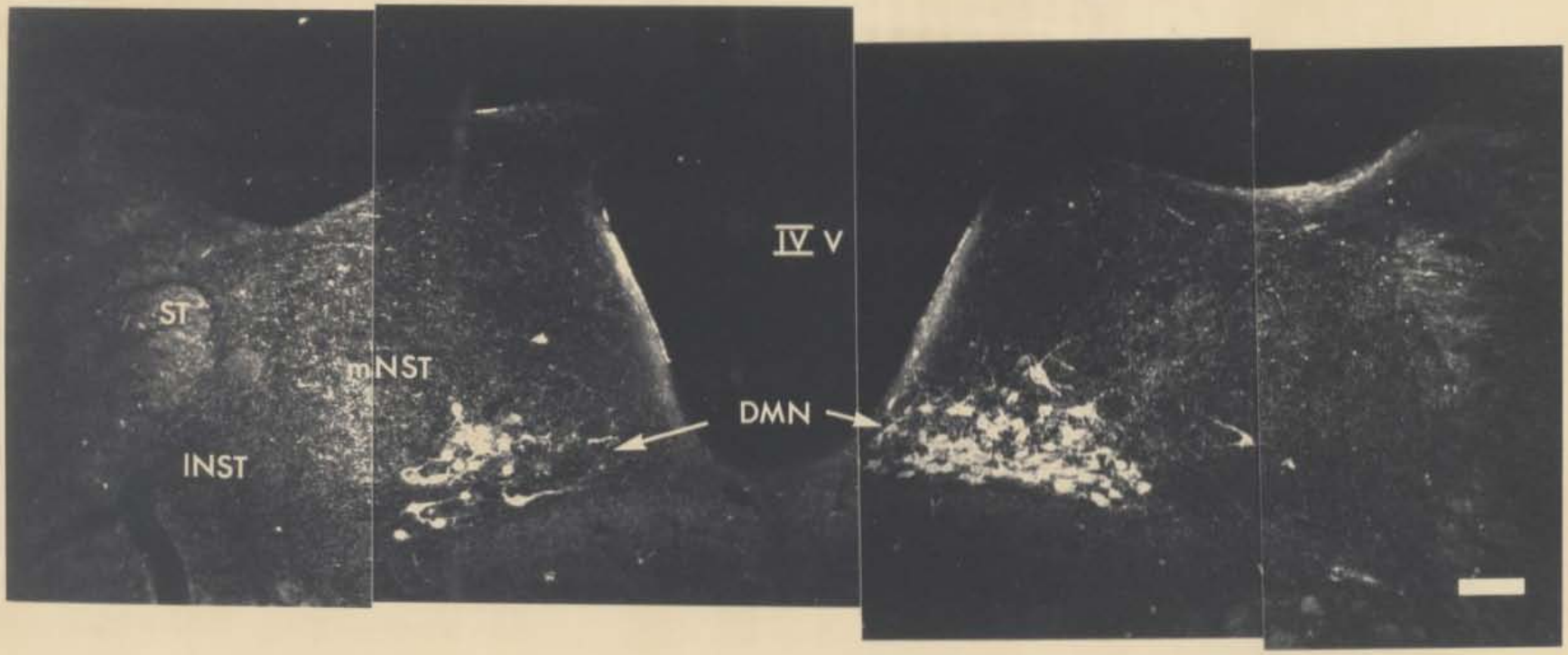
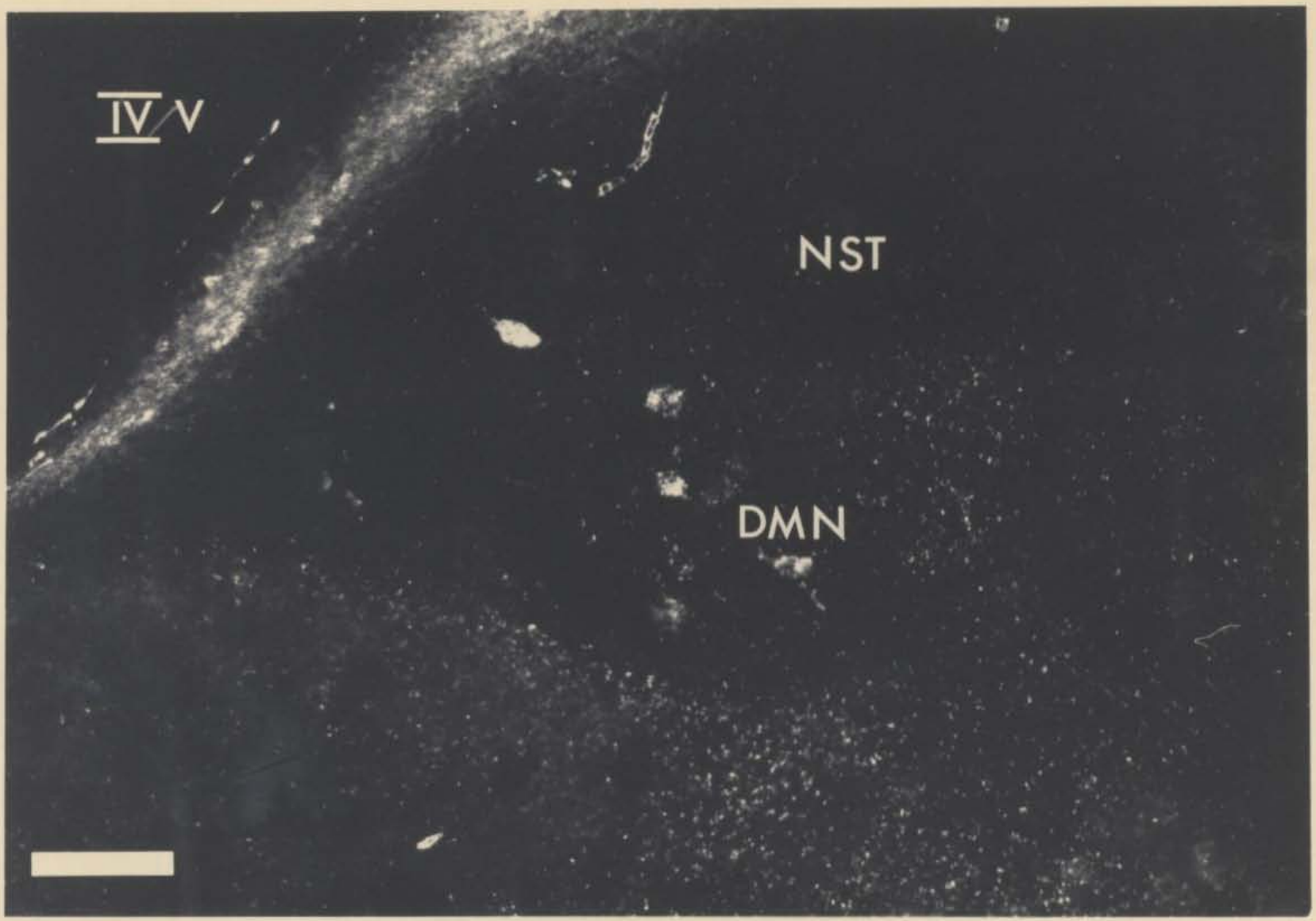

ST
INST
mNST
IV v
DMN

FIGURE 30. Darkfield photomicrograph of the dorsal medulla of a control hamster. Crystalline HRP was applied over the oesophageal and gastric surfaces, but the vagi remained intact. In this case, the largest number of labelled perikarya was in the section shown. As can be seen, the majority of the cells were not densely filled with HRP reaction product. Bar = 100 um.


IV/V
NST
DMN

with a brain of an experimental animal; that is, controls were treated with the same batch of HRP and perfused with the same batch of fixative as experimental animals. Moreover, each control brain was reacted together with an experimental brain in the same reaction dish. Thus, the labelling reported in cases where HRP was applied to the central end of a cut vagal trunk was, by and large, the result of up-take by the cut nerve.

Discussion

HRP histochemistry was used here to describe vagal afferent projections to the dorsal medulla, and the distribution of preganglionic motor neurones in the lower brainstem/upper spinal cord contributing efferent fibres to the cervical vagus and the gastric division of the abdominal vagus. The technique described here is different from those described in other reports in that transganglionic (anterograde) and retrograde transport were successfully demonstrated without prolonged incubation of the central end of the cut nerve in liquid HRP or repeated application of HRP over the nerve stump over many hrs. A single application of the enzyme marker was sufficient for up-take and transport by the nerve and thus, for the visualization of cells of origin and presumptive terminal fields. Loosely folding and sealing Parafilm around the proximal stump served to prevent the spread and up-take of HRP by terminals in the surrounding musculature. The possibility of leakage and spread of HRP from the Parafilm pocket around the nerve did not pose interpretative problems, as large amounts of HRP

applied over the musculature surrounding the cervical vagus, and over the lower oesophagus/stomach region produced very limited perikaryal and extraperikaryal labelling. Thus, it can be safely concluded that the vast majority of labelling in the experimental cases arose from up-take from the cut nerve and retrograde and transganglionic transport. Lastly, a comparison of the cervical and abdominal vagal cases made it possible to determine whether labelling of the vagus at its latter course would produce a more restricted and perhaps viscerotopic pattern of labelling. In this regard, it should be remembered that, since the the abdominal vagus was transected below the coeliac and hepatic branches, the abdominal vagotomy reported here represents a selective gastric vagotomy.

Brainstem Trajectory of Vagal Afferents and Efferents

Sensory and motor fibres of the vagus enter and leave the medulla in multiple fascicles. In the coronal sections taken from brains blocked perpendicular to a skull-flat plane, afferent fascicles were seen entering the dorsolateral medulla at some distance rostral to where motor fibres leave the ventrolateral medulla. Vagal afferents head dorsomedially into the ST and descend within the ST for some distance before distributing into the dorsal vagal complex. Motor fibers of the DMN and CG course ventrolaterally in numerous fascicles and are joined by efferents of the NA.

Motor Components of the Cervical and Abdominal Vagus

Efferent fibres of the cervical vagus arise from motor neurones in the ipsilateral NA and DMN of the medulla, and in the CG, NASN and dorsolateral ventral horn of the cervical spinal cord. A portion of these motor axons serve tracheal and thoracic musculature, while some continue downward in the abdominal vagal trunks. Motor fibres continuing in the lower abdominal trunk, arise exclusively from the DMN, NA, and CG, as no retrograde labelling was observed in the NASN and dorsolateral ventral horn of the abdominal cases. Efferent fibres of the right cervical vagus from motor neurones of the ipsilateral medulla and spinal cord, cross completely at some point between below the neck and above the diaphragm, to form the efferent component of the left abdominal trunk. Motor fibres of the left cervical vagus cross incompletely. While more fibres appear to cross and form the efferent component of the right abdominal trunk, a substantial number do continue uncrossed in the left abdominal trunk.

Sensory Components of the Cervical and Abdominal Vagus

Sensory fibres in the right abdominal trunk cross completely at some point above the diaphragm and join the left cervical vagus. Conversely, gastric vagal afferents in the left abdominal trunk cross completely to join the right cervical vagus. Afferents of the cervical vagus enter the more anterior aspects of the dorsolateral medulla on the ipsilateral side.

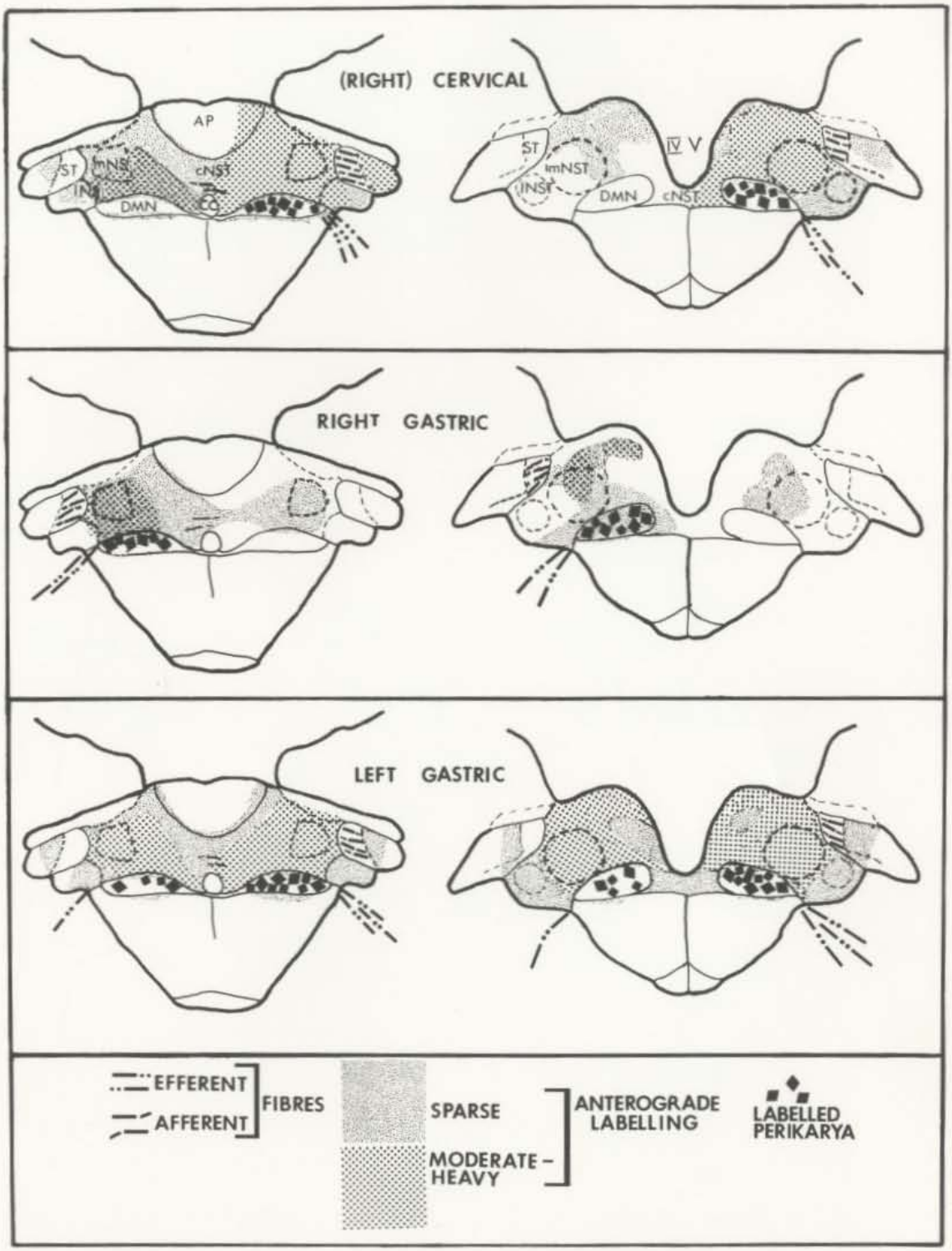
Afferent Projections to the Dorsal Medulla

Afferents from the cervical vagus project selectively to the caudal half of the NST. However, within this region, both the lateral and medial divisions of the NST receive afferent input from the cervical vagus, although this input is more dense in the medial division. The ventral and lateral portions of the AP also receive substantial input from the cervical vagus. It was not possible to determine whether the ipsilateral DMN, particularly the core of the nucleus, received input from the cervical vagus. However, that the dorsal and ventral borders of the contralateral DMN were lightly labelled with extraperikaryal HRP, suggests that tne ipsilateral DMN may receive considerable ·nput. Afferents from the gastric division of the abdominal vagus preferentially innervate the medial NST and the regions immediately adjacent to it. Anterograde labelling in the AP and the adjacent internal and external solitary zones, and along the dorsal and ventral borders of the DMN was sparse to moderate in most cases. In one case, labelling in these regions was not detectable. Figure 31 provides a schematic summary of the above observations.

Comparative Considerations

The source of preganglionic neurones contributing to, and terminal projection sites of, the vagus nerve have been studied in several species including, rat, cat, dog and monkey (Gwyn, Leslie, and Hopkins, 1979; Husten, 1924; Leslie, Gwyn, and Hopkins, 1982; Karim, Shaikh, Tan,

FIGURE 31. Schematic summary of the patterns of anterograde and retrograde labelling in the dorsal medulla after applying HRP to the left and right cervical and abdominal vagi. The labelling pattern observed in the left cervical cases mirrored that illustrated for the right cervical cases. Retrograde labelling in the NA region and CG followed the pattern shown for retrograde labelling in the DMN.


(RIGHT) CERVICAL
AP
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LEFT GASTRIC
EFFERENT
AFFERENT
FIBRES
SPARSE
MODERATE - HEAVY
ANTEROGRADE LABELLING
LABELLED PERIKARYA

and Ismail, 1984) by degeneration, autoradiographic, HRP histochemical, and electrophysiological techniques (Contreras et al., 1983; Husten, 1924; Kalia and Sullivan, 1982; Sauter, Niijima, Berthoud, and Jeanrenaud, 1983). Significant species differences have been reported, details of which have recently been presented elsewhere (Kalia and Sullivan, 1982; Karim et al., 1984). Since the present observations in the hamster resemble closely those reported in rat studies, only a comparison of the hamster and rat will be presented.

The pattern of labelling observed in the hamster experiments strongly resembles that observed in the rat. The few differences between these two species are of a quantitative nature, and most of these may be explained on the basis of technical or procedural differences.

Rats and hamsters appear to differ from other species (e.g. cats) in having separate brainstem trajectories for the efferent and afferent components of the vagus. As was observed in this study, after intranodose HRP injection in rats, Kalia and Sullivan (1982) reported that vagal afferents enter the dorsolateral medulla and course dorsomedially towards the ST, and that efferent fibres of the DMN and NA traverse the medulla through a more ventral pathway. The pathway of vagal efferents in the hamster is also similar to that in rats (Kalia and Sullivan, 1982) in that axons leave the major cell groups of origin in multiple fascicles and do so over a considerable length of the medulla and spinal cord. Hamsters, however, differ from rats in that fewer afferent fascicles were seen in the cervical cases than were reported in the rat after intranodose ganglion HRP (Kalia and Sullivan,

1982) or tritiated amino acid (Contreras et al., 1982) injections. Moreover, in the labelled cervical cases, vagal afferents were seen entering the dorsolateral medulla from a comparatively more restricted rostrocaudal zone than in rats. Whereas these afferents were seen entering the medulla in approximately two to four 50 um sections in hamster cases, Kalia and Sullivan (1982) reported that the entry point of vagal afferents in rats and their pathway to the ST spanned a considerable length of the rostral medulla. The present observations in the hamster further differ from observations in the rat, in that there was very little or no overlap in the rostrocaudal zone at which afferents traversed the dorsal medulla towards the ST and at which efferents from the DMN and NA coursed ventrolaterally to leave the medulla (cf. Fig. 6 in Kalia and Sullivan, 1982).

A long column of cells concentrated in and around the classically defined DMN contribute efferent fibres to the cervical and lower abdominal vagus in both the hamster and rat (Coil and Norgren, 1979; Dennison, Merritt, Aprison, and Felton, 1981a; Dennison, O'Connor, Aprison, Merritt, and Felton, 1981b). A few scattered HRP-positive cells were reported in the N XII after incubation of the rat cervical vagus in HRP (Dennison et al., 1981a). In two of the five hamster cervical cases, there was a particularly dense cluster of labelled hypoglossal cells ventrolateral to the DMN. It appears that in (some) hamsters more hypoglossal neurones send axons through the cervical vagus than in rats. In the rat, the DMN cell column tapers caudally, where it becomes continuous with the cell group in the CG of the cervical spinal cord. The hamster does not deviate from the rat in the longitudinal

span of this column. The second major cell group contributing motor fibres to the cervical and abdominal vagus is in and around the NA. In rats, after intranodose ganglion HRP injection, or HRP incubation of the proximal stump of the vagus, a cluster of labelled cells was seen in the NA region throughout its rostrocaudal extent. In rats, the labelled NA cell column extended rostrally to the retrofacial area, and caudally to the dorsolateral ventral horn in the upper segments of the cervical spinal cord, (the latter sometimes referred to as the retroambiguus nucleus; Kalia and Sullivan, 1982). In the hamster cervical cases, labelling in the NA column appeared to be more restricted. Retrograde labelling in the ventrolateral medulla did not extend rostrally to the level of the retrofacial nucleus. Caudally, the posterior-most region in which labelled perikarya were seen in the ventrolateral medulla was just rostral to the level of the pyramidal decussation. In the cervical cases, where labelled perikarya were observed in the retroambiguus region (i.e., the group dorsolateral to the NASN), there was a considerable zone in the caudal aspects of the ventrolateral medulla and in the upper segments of the cervical spinal cord devoid of retrograde labelling. This contrasts with the rat where labelled cells in the NA formed a long column that caudally, was continuous with the retroambiguus group (Kalia and Sullivan, 1982). Lastly, the presence of labelled perikarya in the NASN in the cervical cases is consistent with rat findings (Kalia and Sullivan, 1982; Leslie et al., 1982).

The distribution of retrograde labelling after sectioning the abdominal vagal trunks reported here for the hamster is in fair agreement with similar rat studies. Coil and Norgren (1979) reported

that labelled perikarya within the DMN region were restricted to the left hemisphere after the right abdominal (gastric) vagal trunk was incubated in HRP. In contrast, labelled perikarkya were noted in both the left (approximately 40% of the total) and right (60%) DMN after incubating the left trunk. Dennison et al. (1981b) noted a similar distribution in the right and left DMN after incubating the lower left abdominal trunk. However, they noted a few scattered HRP-positive cells in the right DMN, in addition to the large group in the left DMN after incubating the lower right trunk. The pattern of labelling in the DMN produced by applying HRP to the hamster's lower left abdominal trunk conforms to that reported in rats, although the left-right difference tends to be more pronounced in the hamster. Perikaryal labelling in the right and left DMN after HRP was applied to the hamster's lower right abdominal trunk was consistent with that reported in both the Coil and Norgren (1979) and Dennison et al. (1981b) study of the rat. That is, in two of the three hamster cases where HRP was applied to the right abdominal trunk, retrograde labelling in the DMN was limited to the left side, and in one case a few scattered HRP- positive cells were also noted in the right DMN region. These differences are likely to reflect individual-animal anatomical variation. In contrast to what some have reported in rats (Dennison et al., 1981b; Takayama, Ishikawa, and Miura, 1982), the pattern of labelling in the DMN in the hamster abdominal cases did not conform to some viscerotopic organization. Although there were fewer labelled DMN neurones after abdominal vagal section, these cells were seen throughout the rostrocaudal span of the DMN and CG and were not confined to a particular region within the DMN. The hamster

also appears to differ from rats with respect to the contribution of NA neurones to fibres in the abdominal trunks. Coil and Norgren (1979) reported that cells in and about the NA on each side give rise to fibres in both the left and right abdominal trunks. Although fibres in the hamster's left abdominal trunk arise from the left and right NA, NA motor axons in the right trunk arise exclusively from NA cells in the left side.

The most apparent difference between this study of the hamster and similar studies in the rat is in the anterograde labelling. By comparing the material collected in this study with the photomicrographs presented in rat studies (e.g. Kalia and Sullivan, 1982; Leslie et al., 1982), it would appear that vagal sensory projections to the dorsal vagal complex are not as dense in the hamster as in the rat. This difference may be most easily explained by a difference in experimental protocol. Although the exact way in which the tracer enzyme is applied to the nerve may contribute to quantitative differences in anterograde labelling, it is believed that the crucial difference between this and the aforementioned rat studies was that part of the fixative (the formaldehyde) used in the present study contained 10-15% methanol as stabilizer. Methanol is reported to reduce the sensitivity of HRP reactions, particularly anterograde labelling (Mesulam, 1982). Thus, the comparatively less intense extraperikaryal labelling may not necessarily reflect a species difference, but rather suboptimal anterograde labelling in the present study. Despite this quantitative difference, the distribution of vagosensory projections to the dorsal medulla in the hamster is quite similar to that reported in the rat

(Contreras et al., 1982; Kalia and Sullivan, 1982; Leslie et al., 1982).

The observation of vagal projections to the caudal NST and AP reported here for the hamster are in agreement with observations in the rat (Contreras et al., 1982; Leslie et al., 1982; Norgren and Smith, 1983). A modest projection from the cervical vagus distributing along the dorsal and ventral borders of contralateral DMN is also reported for the hamster. Evidence of direct vagal sensory projections to the DMN of the rat is presently equivocal. Contreras et al. (1982) reported no terminal labelling in the DMN after injection of tritiated amino acids into the nodose ganglion. Although Kalia and Sullivan did observe granular HRP along the dorsolateral and ventrolateral borders of the DMN contralateral to the injected nodose ganglion, Leslie et al. (1982) failed to confirm such a projection using similar procedures.

The distribution of primary sensory afferents from the vagus, facial, and trigeminal nerves within the NST are comparable in rats and hamsters. In each species, viscerosensory input from the vagus is restricted to the more caudal aspect of the NST, the medial zone of the NST being the primary recipient of this innervation (Contreras et al., 1982 [rat]; Leslie et al., 1982 [rat]; present investigation [hamster]). In contrast, gustatory afferents from the facial and glossopharyngeal nerves supply the lateral NST division, and at a level rostral to that at which the vagus supplies the NST (Contreras et al., 1982 [rat]; Whitehead and Frank, 1983 [hamster]). Although somatosensory inputs from the trigeminal system impinge on the NST at the rostrocaudal level supplied by vagal afferents, these inputs are confined to the lateral division (Contreares et al., 1982 [rat]; Whitehead and Frank, 1983

[hamster]).

In view of the fact that despite a similar anatomical organization of the vagus nerve, abdominal vagotomy produces near catastrophic deficits in alimentary behaviour in rats, while having relatively little behavioural effect in hamsters (see Experiments 10-12), it would be of interest to determine the electrophysiological properties of the hamster abdominal vagus. In other species, evidence has been presented for mechanoreceptors (McHugh, 1983), thermoreceptors (Gupta, Nier, and Hensel, 1979), insulin and glucoreceptors (Niijima. 1983), amino acid receptors (Jeanningros, 1982), pH receptors (Clarke and Davison, 1978), and osmoreceptors (Garnier and Mei, 1982) coupled to afferent fibres of the abdominal vagus. Most of these stimuli-specific afferent limbs have been suggested to be involved in complex mechanisms controlling homeostasis and alimentary behaviour (Mei, 1983; Novin, 1983; Norgren, 1983; Smith and Jerome, 1983). It is possible that the hamster abdominal vagus lacks afferent fibres receptive (or responsive) to one or more classes of stimuli (e.g. receptors for circulating AII, osmoreceptors). If such were the case, one would expect abdominal vagotomy to be less debilitating since fewer controlling afferent systems are destroyed.