A STUDY OF THE ANTINOCICEPTIVE AND TOXICOLOGICAL EFFECTS OF INTRATHECAL DEXMEDETOMIDINE AND METHOXAMINE IN THE RAT

SUE ELLEN MAHER

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

A STUDY OF THE ANTINOCICEPTIVE AND TOXICOLOGICAL EFFECTS OF INTRATHECAL DEXMEDETOMIDINE AND METHOXAMINE IN THE RAT

by

Sue Ellen Maher

A thesis submitted to the School of Graduate Studies in

partial fulfilment of the requirements for the degree of

Master of Science

Specialization: Toxicology

School of Pharmacy

Memorial University of Newfoundland

St. John's, Newfoundland

1998

ABSTRACT

Alpha (α)-adrenergic agonists represent a novel drug class of spinal analgesics and are most commonly used in combination with local anesthetics and opioids. Spinal noradrenergic antinociception is mediated primarily by α_2-adrenoceptors, although α_1-adrenoceptor-mediated antinociception has never been disproven. As demonstrated vasoconstrictors, however, there are concerns regarding the safety of the spinal administration of α_1- and α_2-agonists, alone and in combination.

The purpose of this study was to determine if intrathecal (i.t.) methoxamine (MX) (α_1-agonist) potentiates i.t. dexmedetomidine (DX) (α_2-agonist)-induced antinociception, if i.t. DX, alone and in combination with MX, injected twice daily for four days, causes spinal neurotoxicity, and the effect of a sub-toxic combination of i.t. DX and MX on i.t. dynorphin-induced neurotoxicity. Male, Sprague-Dawley rats (300-400g) implanted with i.t. catheters (L1 termination) were used throughout. The i.t injection of dexmedetomidine (0.01-1 μg) produced dose-dependent antinociception in the tail flick (TF) and paw pressure (PP) tests (ED_{50} = 45 and 252 ng, respectively). The addition of a fixed dose of MX (10 μg i.t.), which produced <5% maximum percent effect (MPE) in the TF test and was inactive in the PP test, significantly shifted the DX dose response curve to the left (ED_{50} = 8.1 ng; TF test and 10 ng; PP test) but did not

prolong DX's duration of action. A fixed dose combination of DX (0.025 μg) + MX (10 μg) producing near maximal antinociception in the TF test and intermediate activity in the PP test, was near completely blocked by prazosin (10 μg i.t.) or Wyeth 27127 (0.5 μg i.t.).

Repeated i.t. injections of high dose DX (10 μg) and DX+MX (10 μg each) produced sedation but no motor dysfunction, no inflammation, haemorrhage or necrosis of the spinal cord. Immunohistochemical studies revealed no damage to or loss of calcitonin-gene-related peptide immunoreactivity(CGRP-IR) in either the dorsal or ventral horns or substance P immunoreactivity (SP-IR) in the ventral horn as compared to vehicle-treated rats. In contrast, dynorphin A_{1-13} (192 μg;120 nmol) produced: 1) immediate and irreversible hindlimb paralysis; 2) loss of the stepping and tail flick reflexes; and 3) delayed bladder and bowel dysfunction. Forty-eight hours after dynorphin, immunohistochemical examination revealed a marked depletion of CGRP-IR motor neurons in the lumbar ventral horn. CGRP-or SP-IR was unchanged in the dorsal horn.

To assess the effect of i.t. DX and MX, alone and in combination, on dynorphin neurotoxicity, rats were pretreated with i.t. DX, MX, a combination of DX+MX (10 μg each) or saline 15 min before i.t. dynorphin A_{1-13} (192 μg;120 nmol). In saline pretreated rats, dynorphin produced the same effects as described above. Pretreatment with i.t. DX, MX or DX+MX attenuated the hindlimb paralytic effect of dynorphin. Twenty-four hours after injection, motor

reflexes were preserved and accelerating rotarod (RR) scores were similar to controls (no dynorphin) in 4 out of 5 DX-, 3 out of 5 MX- pretreated and 4 out of 4 DX+MX-pretreated rats. Histological evaluation of the spinal cords obtained from these rats were consistent with these functional results, indicative of a neuroprotective effect. For DX and DX+MX pretreated rats, there was a corresponding decrease in rectal temperature (up to 3.4°C for DX alone). MX did not alter rectal temperature. These results of this study indicate that 1) a threshold dose of i.t. MX potentiates α_2-mediated antinociception in the rat; 2) repeated spinal administration of this drug combination has no detectable neurotoxic effect and 3) pretreatment with these drugs effects neuroprotection against i.t. dynorphin in the rat.

For Mom and Dad

Labor Omnia Vincit

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to my research supervisors, Drs. Christopher Loomis and Devhuti Vyas, for their expert guidance and encouragement throughout my research.

Dr. Detlef Beiger for allowing me the use of his laboratory and expertise in my immunohistochemical studies. Also sincerely appreciated are his kindness and humor.

Dr. Bruce Virgo for his guidance in my program and his open door.

Dr. Kantha Arunachalam for her technical advice and surgical training during the initial stages of my thesis research, as well as some of the preliminary data for this research.

Dr. Stephen Sherman for his generous help, constant support and friendship throughout my graduate program.

Janet Robinson for her skillful technical assistance and kindness.

Dr. Dale Corbett for allowing me the use of his photographic equipment. Also to Sue Evans, Drs. Suzanne Nurse and Fred Colbourne for their technical assistance.

Mr. John Bautista, Mr. Dongyuan Yao and Ms. Hemal Khandwala for their kind friendship and always good company.

I would like to thank Dr. Murray Brooker for his belief in me and constant encouragement.

Dr. Gerald Duncan for availing to me the services of the School of Pharmacy throughout my research.

The staff of Animal Care Services for their excellent care of our animals.

I am grateful to the Toxicology Program for their personal financial support during my degree program and especially Dr. Jean Finney-Crawley for her efforts on my behalf.

Medical Audiovisual, especially John and Terry, for their advice and help with

the immunohistochemical photographs.

I would also like to thank Orion Corporation Farmos for providing the dexmedetomidine HCL for these experiments.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

±	plus and minus, in addition to, subtracted from (about the mean)
>	greater than
≥	equal to or greater than
<	less than
≤	equal to or less than
°C	degree(s) Celsius
α	alpha, a Greek letter, a subtype of adrenergic receptor
α_1	alpha 1, a subtype of α-adrenergic receptor
α_{1A}, α_{1B}, α_{1C}	alpha 1A, B or C, subtypes of the α_1-adrenergic receptor
α_2	alpha 2, a subtype of α-adrenergic receptor
α_{2A}, α_{2B}, α_{2C}	alpha 2A, B or C, subtypes of the α_2-adrenergic receptor
β	beta, a Greek letter, a subtype of adrenergic receptor
δ	delta, a Greek letter, a subtype of opioid receptor
μ	mu, a Greek letter, used to indicate micro in metric units, also a subtype of opioid receptor
ad libitum	freely
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	analysis of variance
ATP	adenosine triphosphate
AVP	arginine vasopressin
Ca^{++}	calcium ion
$[Ca^{++}]_i$	intracellular calcium concentration
CGRP	calcitonin gene related peptide
CI	confidence interval
cm	centimeter, 10^{-3} meter, unit of distance
CNS	central nervous system
CSF	cerebrospinal fluid
DADTL	[D-Arg^1, D-$Trp^{7,9}$, Leu^{11}]-SP
DAG	diacylglycerol
DL	dextrorotatory, levorotatory, raecemic mixture of enantiomers
DMSO	dimethyl sulfoxide, an organic solvent
DNA	deoxyribonucleic acid
DPDPE	[D-Pen^2, D-Pen^5]enkephalin, a δ-opioid agonist
DPDT	[D-Pro^2, D-$Trp^{7,9}$]-SP
DRG	dorsal root ganglion
DX	(4)(5)-[2,3-dimethylphenyl]-imidazole
Dyn	dynorphin

EAA	excitatory amino acid(s)
ED_{50}	effective dose yielding a 50 percent response
e.g.	*exempli gratia* (for example)
et al.	et alia (and others)
g	gram, unit of mass
Glu	glutamate
Gi	inhibitory G-protein
h	hour(s)
^3H	tritium, radioactive isotype of hydrogen
H^+	hydrogen ion
Hg	mercury
H_2O_2	hydrogen peroxide
HCl	hydrochloric acid
IC_{50}	inhibitory dose decreasing a response by 50 percent
i.e.	*Id est,* that is
IgG	immunoglobulin G
in vitro	in glassware
in vivo	in the living body
i.t.	intrathecal(ly)
i.v.	intravenous(ly)
I, II	Roman numerals one and two, used to denote spinal lamina
I_2	imidazole receptor subtype
i.p.	Intraperitoneal
IP_3	inositol triphosphate
IR	immunoreactivity
I.U.	International units
K^+	potassium ion
KCl	potassium chloride
Kg	kilogram, 10^3 grams, unit of mass
LC	locus coeruleus
-LI	-like immunoreactivity
L1	lumbar vertebra number 1
log	logarithm (base 10)
m	meter, unit of distance
M	molar (moles/liter), unit of concentration
mg	milligram, 10^{-3} grams, unit of mass
mg/Kg	milligram/kilogram, unit of concentration
mg/mL	milligram/milliliter, unit of concentration
min	minute(s)
mL	milliliter, 10^{-3} liters, unit of volume

mL/h	milliliter/hour, rate of delivery
MK-801	(+)-5-methyl-10,11-dihydro-*5H*-dibenzo (*a,d*) cyclo heptene-5,10-imine maleate
mM	millimolar, 10^{-3} moles/liter, unit of concentration
mm	millimeter, 10^{-3}, unit of length
mm Hg	millimeters of mercury, unit of pressure
MPE	maximum possible effect
MX	methoxamine, 2-amino-1-(2,5-dimethylphenyl) ethyl] imidazole
N, n	number of determinations
NA	noradrenaline, a non-selective α-agonist
Na^+	sodium ion
ng	nanogram, 10^{-9} grams, unit of mass
NAD	nicotinamide adenine dinucleotide
NMDA	N-methyl-D-aspartate
nmol	nanomole(s), 10^{-9} moles, number of molecules
NO	nitric oxide
NOS	nitric oxide synthase
O_2^-	superoxide radical
p	probability of error
PAP	peroxidase anti-peroxidase
PCP	phenycyclidine
PE-10	polyethylene tubing (diameter ~0.61 mm)
per se	by or in itself
pH	$-\log_{10}[H^+]$
PKC	protein kinase C
PLD	phospholipase D
pmol	picomole, 10^{-12} moles, number of molecules
PP	paw pressure
PZ	prazosin, an α_1-antagonist
RR	rotarod
RVLM	rostral ventrolateral medulla
SAL	saline
SCBF	spinal cord blood flow
S.D.	standard deviation
sec	seconds
SEP	somatosensory evoked potentials
SP	substance P, a neurotransmitter
SST	somatostatin
ST	Saint
TF	tail flick

TRIM	1-(2-trifluoromethylphenyl) imidazole
u/mL	units/milliliter, unit of concentration
µg	microgram(s), 10^{-6} grams, unit of mass
µg/Kg	micograms/Kilogram, unit of concentration
µg/mL	microgram/milliliter, unit of concentration
µL	microliter(s), 10^{-6} litres, units of volume
µm	micrometer(s), 10^{-6} meters, unit of length
µmol	micromole, 10^{-6}, number of molecules
µM	micromolar, 10^{-6} M, unit of concentation
V	volts
VAF	virus antibody free
W	watts
WY	Wyeth 27127 (disulphonamino-benzoquinoline), an α_2-antagonist

1.0 INTRODUCTION

1.1 Statement of the Research Problem

With the elucidation of pontospinal noradrenergic pathways as components of the 'endogenous pain control system', and the subsequent pharmacological characterization of the adrenoceptor subtypes modulating nociceptive transmission in the spinal cord, it was quickly realized that α-agonists might exert a direct antinociceptive/analgesic effect. Indeed, the intrathecal (i.t.) administration of agonists with selectivity for α_2-adrenoceptors was shown to produce a dose-dependent, behaviourally-defined elevation of nociceptive threshold in a variety of experimental animals. Importantly, this antinociceptive effect occurs without demonstratable motor effects (Kuraishi et al., 1979; Reddy et al., 1980; Yaksh and Reddy, 1981; Howe et al., 1983). In contrast, doses of α_1-agonists necessary to achieve antinociception in the rat produced concurrent hyperreflexia, clonic flexion of the hindlimbs and serpentine movements of the tail (Howe et al., 1983). The failure of i.t α_1-agonists to discriminate between sensory and motor effects has been used as evidence against the role of α_1-adrenoceptors in spinal antinociception/analgesia. However, the contribution of this α-receptor subtype has never been disproven. If multiple α-adrenoceptor subtypes effect antinociception, then the co-administration of α_1- and α_2-agonists in threshold or sub-threshold doses should yield additive or potentially supra-additive antinociception. This pharmacological interaction has not been investigated.

While segmental and prolonged analgesia can be achieved with perispinal

drug administration, this technique carries with it an increased risk of drug-induced neurotoxicity. Experimental studies of selective drugs given epidurally or intrathecally to the rat have frequently noted an abrupt and sustained decrease in spinal cord blood flow prior to histopathological damage in the spinal ventral horn. Current evidence suggests that this neurotoxic effect arises from drug-induced ischemic injury. The pronounced vasoconstrictive effect of α_1- and α_2-agonists on central blood vessels raises important questions about their safety as spinal analgesics, especially when given in combination. The safety of such a combination alone, or in the presence of a known neurotoxin such as dynorphin, has not been assessed. The rationale for such an investigation lies in the increasing popularity of 'balanced anesthesia' in which a mixture of pharmacological agents, including α-agonists, with varying receptor selectivities, mechanisms of action and adverse effects, are co-administered to achieve more effective clinical anesthesia\analgesia.

In the present study, the effect of i.t. methoxamine (MX; α_1-agonist) on thermal (tail flick; TF) and mechanical (paw pressure; PP) antinociception elicited by i.t. dexmedetomidine (DX; α_2-agonist) was first investigated in the rat. A sub-acute neurotoxicological assessment using behavioural, morphological and immunohistochemical indices was subsequently undertaken to investigate the safety of this drug combination. Lastly, the effect of this combination on i.t. dynorphin-induced neurotoxicity was determined.

1.2 α-Agonists as Adjuvants to Local Anaesthetics

The spinal administration of a local anaesthetic for the purpose of surgery was first attempted in Germany in 1899 (Bier, 1899). Bier had noted the success of regional cocaine anaesthesia in lowering the requirement for general anaesthesia but he recognized the limitations of regional anesthesia for major surgical operations. In order to "render large areas of the body insensible to pain", Bier injected cocaine intrathecally in doses ranging from 5 to 15 milligrams (mg). A level of anaesthesia sufficient to allow major surgery was achieved in all six patients.

The use of epinephrine, a non-selective adrenergic agonist, to prolong spinal anaesthesia was introduced clinically in 1900 (Braun, 1914). Braun, Bier and other contemporary German clinicians began using epinephrine to prolong the duration of action of i.t. cocaine, thus obviating a significant disadvantage of spinal anaesthesia (Bier and Donitz, 1904; Heinke and Lawen, 1905; Braun, 1914). Between 1900 and 1940, the use of epinephrine with spinal anaesthesics enjoyed sporadic popularity but did not attain common acceptance because of an early report that i.t. epinephrine caused pronounced ischemia in the spinal cord of experimental animals (Biberfield, 1907). Using experimental animals under controlled conditions, Prickett, Gross and Cullen (1945) demonstrated that the addition of 1:10,000 and 1:30,000 (0.1mg/mL and 0.03 mg/mL) epinephrine could prolong the anaesthesic effect of i.t. procaine without permanent damage to

nervous tissue. This study stimulated the re-investigation of this technique. Indeed, recent clinical studies have shown that the addition of epinephrine (0.1 to 0.3 mg) prolongs the duration of action of spinal bupivacaine, lidocaine or tetracaine from 15 to 44% in patients undergoing abdominal, hip or elective surgery of the lower extremities (Leicht et al., 1986; Racle et al., 1987; Momose et al., 1994). In this regard, epinephrine is believed to counteract the arteriolar vasodilation induced by local anaesthetics in the spinal cord, thereby delaying absorption and increasing the residence time of the local anaesthetic near the site of injection. Indeed, epinephrine is a potent constrictor of cerebral (LoPacin and Rudy, 1983) and spinal (Partridge, 1991) blood vessels. Whether this prolongation is truly a pharmacokinetic interaction or an independent pharmacodynamic effect of epinephrine (see section 1.3) remains controversial (Fink et al., 1978; Denson et al., 1982, 1983, 1984; Ravindran et al.,1983). However, it is noteworthy that non-selective adrenergic (norepinephrine), α_1-selective (phenylephrine) and α_2-selective (clonidine) agonists have also proven effective in prolonging the duration of spinal anaesthesia (Mensink et al., 1987; Racle et al., 1987; Kishikawa et al., 1993; Fukuda et al., 1994).

1.3 Spinal Pharmacology of α-Adrenergic Antinociception

Noradrenaline (NA), released from bulbospinal neurons during exposure to noxious stimuli, is an important mediator of the endogenous pain control system.

Thus, focal stimulation of the rat locus coeruleus (LC), a major source of descending noradrenergic neurons, was shown to inhibit noxious evoked: a) neuronal activity in the dorsal horn (Jones and Gebhart, 1986b); b) spinal reflexes (Jones and Gebhart, 1986a; Janss et al., 1987) and c) complex nocifensive behaviours (Segal and Sandberg, 1977; Sandberg and Segal, 1978; Margalit and Segal, 1979). The concentration of NA and the NA metabolite, 3-methoxy-4-hydroxy-phenethylgycol was also significantly increased in CSF following electrical stimulation of the LC (Crawley et al., 1979). The i.t. administration of α-adrenoceptor antagonists significantly blocked the antinociceptive effect elicited by LC activation, suggesting a spinal site of antinociception (Jones and Gebhart, 1986a). Among the series of pharmacological antagonists used (phentolamine, yohimbine, prazosin, naloxone, methlysergide, atropine and bicuculline), only the non-selective α-antagonist, phentolamine, or the α_2-selective antagonist, yohimbine, significantly blocked the increase in nociceptive threshold elicited by LC stimulation. Similar results were reported by Miller and Proudfit (1990).

These early studies of endogenous pain modulation led researchers to examine the effect of exogenous NA and other α-agonists. When injected i.t., NA produced a significant dose-dependent increase in response latency in the hot plate and tail flick tests (Reddy and Yaksh, 1980; Reddy et al., 1980; Howe et al., 1983); an effect reproduced by other adrenergic agonists including DL-α-methylnorepinephrine; DL-epinephrine, ST-91, clonidine, L-phenylephrine, 3-4-

dihydroxytolazoline, oxymetazoline, methoxamine and cirazoline (Reddy et al., 1980; Reddy and Yaksh, 1980, Howe and Yaksh, 1982a,b; Howe et al., 1983). β-Agonists, such as isoproterenol, were without effect even at very high i.t. doses (Reddy et al., 1980).

While the i.t. administration of either α_1- or α_2-adrenoceptor agonists increased nociceptive thresholds in the rat, the doses of α_1-agonists necessary to achieve this effect produced concurrent dose-dependent motor effects such as hyperreflexia, clonic flexion of the hindlimbs and serpentine movements of the tail (Howe et al., 1983). In contrast, antinociceptive doses of either non-selective α-agonists or α_2-selective agonists inhibited nociceptive behaviours without demonstratable motor effects (Reddy et al., 1980; Reddy and Yaksh, 1980; Howe et al., 1983). Largely, on the basis of these observations, and on the variable effects of α_1-agonists on dorsal horn neurons, it was concluded that spinal noradrenergic antinociception is mediated by α_2-adrenoceptors. In support of this claim, adrenergic agonists with the greatest $\alpha_2\backslash\alpha_1$ receptor selectivity (e.g. DX) are the most effective in acute antinociceptive tests (Takano and Yaksh, 1993). Nevertheless, the contribution of α_1-adrenoceptors in spinal adrenergic antinociception/analgesia has never been disproven.

Early experiments demonstrated that α_1-selective agonists do effect antinociception following both systemic (Bentley et al., 1983; Hayes et al., 1986) and spinal administration (Howe et al., 1983; Yaksh, 1985). In turn,

pharmacological studies of NA-induced thermal antinociception, showed that the that α_1-antagonist, prazosin, was more effective than α_2-antagonists in inhibiting the antinociceptive effects of i.t. NA (Reddy et al., 1980; Howe et al., 1983). These data are supported by the results of radioligand binding studies and quantitative autoradiography demonstrating the presence of α_1-adrenoceptors throughout the dorsal grey matter of the rat spinal cord (Giron et al., 1985; Simmon and Jones, 1988; Roudet et al., 1993), and are consistent with the hypothesis that spinal α_1-adrenoceptors mediate at least some component of noradrenergic antinociception in the rat.

If both α_1- and α_2-adrenergic receptors effect spinal antinociception through distinct effector mechanisms, then co-administration of an α_1-agonist and an α_2-agonist in threshold or sub-threshold doses would be expected to yield an additive or potentially a supra-additive antinociceptive effect. Such an interaction *in vivo* would be consistent with the non-selective nature of NA as an agonist at α-adrenoceptors.

1.4 Neurotoxicity of Spinally Administered Drugs

As the practice of spinal analgesia has grown, there has been an increasing appreciation of the potential for adverse effects with this technique and the need for systematic neurotoxicological evaluation of drugs intended for spinal administration. This has proven to be particularly important for peptide drugs. For

example, 100 µg of i.t. somatostatin, a cyclic tetradecapeptide thought to be involved in the spinal processing of nociceptive information, produced severe hind limb dysfunction in the rat, up to the time of sacrifice 6 days later. There was also a corresponding loss of sensitivity to thermal and mechanical nociceptive stimuli. Histological (light microscopy) examination of the spinal cords removed from these animals revealed mild to severe nucleolysis of ventral and dorsal horn neurons with localized inflammation (Gaumann and Yaksh, 1988). Similarly, peptide analogues of substance P (developed as putative neurokinin receptor antagonists) given i.t. induced bilateral motor blockade in the hind limbs, and widespread neuronal necrosis in the lumbar region of the rat spinal cord (Post and Paulsson, 1985; Freedman et al., 1989). Comparable outcomes have been reported with i.t. endothelin (Hökfelt et al., 1989), an endothelium-derived vasoconstrictor peptide (Yanagisawa et al., 1988), i.t. dynorphin A, an endogenous kappa opioid agonist (Faden and Jacobs, 1984; Herman and Goldstein, 1985; Spampinato and Candeletti, 1985; Long et al., 1988; Stewart and Isaac, 1989) and arginine^{8}-vasopressin (Long et al., 1989b). Table 1 summarizes the neurotoxicological results with these peptides.

Although the exact mechanisms underlying this neurotoxicity remain unclear, these studies demonstrate: a) the severe and often irreversible damage that can occur in the spinal cord after the local administration of biologically active agents, including those found endogenously in neurons of the spinal cord; and b) the need

for careful toxicological evaluation of drugs intended for spinal use.

Table 1
Studies of Spinal Neurotoxicity Following Intrathecal Administration of Peptides in the Rat.

Drug/ Reference	Dose	Tests	Histology (Light or Immuno-)	Spinal Neurotoxicity
Somatostatin (SST) / Gaumann and Yaksh (1988)	10, 30 and 100 µg	AN, auto-nomic and motor func-tion	6 days after injection: 30 µg SST: mild inflammatory response in 4/5 rats. 100 µg SST: mild or severe nucleolysis of ventral and dorsal horns in the presence of inflammatory reaction.	100 µg: temporary or permanent* hindlimb motor dysfunction with flaccid paralysis in most severe cases, 25% of rats died 10 min. within injection. * permanent = up to day 6 when rats were sacrificed and perfused
Spantide/ Freedman et al., (1989)	2 µg	-	1,2, and 3 days after injection: no CGRP-IR in motoneurons, cresyl violet staining indicated a complete absence of motoneurons and a marked gliosis	Bilateral motor blockade of the hindlimbs that did not recover up to time of perfusion (1,2 or 3 days after injection).
DPDT and DADTL/ Post and Paulsson (1985)	2 µg	AN	3 days after injection: extensive necrosis of neuronal bodies in the ventral and dorsal horns	At half- maximal dose (1µg), all animals showed motor weakness of the hindlimbs. At 2 µg, all animals had pronounced motor impairment of the hindlimbs and non-responsiveness in the tail flick or pinching of hindlegs. This persisted up to time of perfusion, 3 days later.

Table 1 (cont'd)

Drug/ Reference	Dose	Tests	Histology (Light or Immuno-)	Spinal Neurotoxicity
Endothelin/ Hökfelt et al., (1989)	0.03 µg or 0.1 µg	-	24 h after injection: Fully paretic rats showed a marked loss of CGRP-IR in motoneurons in the ventral horn at the tip of the catheter. Single motoneurons appeared rounded without dendritic processes.	0.03 µg: All rats showed complete paresis after 5 min. All but one showed full recovery at 3 h. The one rat remained paralytic until perfusion. 0.1 µg: 3/5 rats died, 2 within 5 min., last within 3 h. Surviving rats showed complete paresis until perfusion, 24 h later.
Arginine^{8}-vasopressin / Long et al., (1989b)	0.5 - 1000 pmol	AN HD	No histochemical difference between control and experimental animals.	Dose-related loss of motor function in hindlimbs. Some rats died within 5 -10 min. of peptide injection due to pulmonary edema. Rats recovered hindlimb function within 30 min. of injection, even with 1.0 nmol dose. Flaccid hindlimb paralysis was accompanied by loss of flexor or vocal responses to pinching of the hindlimbs or tail.
Dynorphin A/ Spampinato and Candeletti (1985)	12.5 and 25 nmol	AN	-	25 nmol produced hindlimb paralysis and tail flaccidity lasting several hours.

Table 1 (cont'd)

Drug/ Reference	Dose	Tests	Histology (Light or Immuno-)	Spinal Neurotoxicity
Dynorphin/ Herman and Goldstein (1985)	14 nmol or greater	-	-	Long-lasting hindlimb paralysis (hindlimb and tail flaccidity) 14 nmol: paralysis wore off in 2 h 20 nmol: paralysis lasted for at least one week 50 nmol: paralysis lasted for at least one week
Dynorphin/ Faden and Jacobs (1984)	Dyn (1-17): 30 nmol; Dyn (1-13): 10,30,50 or 100 nmol Dyn (1-8): 30,50 or 100 nmol; α-neo-endorphin: 30, 50 or 100 nmol	-	-	All peptides produced dose-related flaccid hindlimb paralysis with the order of potency being DYN (1-17) > Dyn (1-13) > α NA ≈ Dyn (1-8). Spontaneously reversible over a 48 h period. However, at high doses (50 nmol), 1/3 of the animals developed paralysis that was irreversible.

Table 1 (cont'd)

Drug/ Reference	Dose	Tests	Histology (Light or Immuno-)	Spinal Neurotoxicity
Dynorphin A/ Long et al., (1988)	Dyn A (1-13): 3.1 -50 nmol Dyn A (3-13): 14 -56 nmol	AN	Dyn A (1-13) 25 nmol: Severe and extensive neuronal injury througout the lumbosacral enlargement.	Dyn A (1-13): Dose-related loss of motor function in hindlimbs and tail, loss of flexor or vocal responses to pinching of hindlimbs. Motor dysfunction ranged from transient, mild paraparesis to persistent, flaccid paralysis of hindlimbs and tail. Recovery was also dose-related. With low doses (3.1 and 6.2 nmol), recovery complete within 2 h. Slower and more limited recovery with 12.5 and 25 nmol. 50 nmol: rats did not recover neurologic function, flaccidity occasionally turned to spasticity by 4 to 7 days after injection. These animals also had loss of nociceptive responsiveness, hindlimb edema, bowel dysfunction, bladder distension with infarction and urinary incontinance. Dyn A (3-13): At 28 and 56 nmol, rats remained flaccidly paralyzed 24 h later.

Abbreviations: AN, antinociception; HD, hemodynamics; CGRP-IR, calcitonin gene related peptide-immunoreactivity; DPDT (D-Pro^2, D-Trp^{7,9})-substance P; DADTL (D-Arg^1, D-Trp^{7,9})-substance P (spantide)

1.5 Pathophysiology of I.T. Peptide-Induced Neurotoxicity

In experimental studies examining the spinal neurotoxic effects of diverse peptides such as dynorphin and endothelin (see Table I), two general observations have been consistently reported. First, each peptide induces an abrupt and sustained decrease in spinal cord blood flow (see Table II); an effect that precedes all behavioural, morphological and immunohistochemical measures of spinal neurotoxicity. Second, cell necrosis occurs primarily in motor neurons of the ventral horns. This latter observation is consistent with the difference in blood supply to ventral as compared to the dorsal horns of the spinal cord (see Figure 1A and B), and the known vulnerability of ventral horn neurons to disruptions in spinal cord blood flow (Cousins and Bridenbough, 1988). Indeed, prolonged aortic cross-clamping and the failure to re-establish the blood supply to the spinal cord is a major risk factor in the development of post-operative paraplegia in patients undergoing aneurysm repair (Marini and Cunningham, 1993).

Studies using radiolabelled microspheres to quantitate changes in blood flow have reported a decrease in the magnitude of spinal cord perfusion following the i.t. injection of selected neurotoxic peptides (Helke et al., 1987; Long et al., 1987; Thornehill et al., 1989). Because this technique does not allow for the continuous measurement of blood flow, the magnitude of change was assessed at time points chosen arbitrarily after i.t. drug administration. With the advent of laser doppler technology, both the magnitude and duration of change in spinal cord blood flow

could be followed in individual animals.

FIGURE 1

A. Arterial Supply to the Spinal Cord. The arterial supply to the spinal cord originates from three main longitudinal systems: the ventral spinal artery and two dorsal spinal arteries. These systems extend along the length of the entire cord giving off a series of branches that extend into the spinal cord (central artery). Two additional longitudinal arterial channels are found on the surface of the cord with less frequency; the median dorsal spinal artery, situated at or close to the dorsal septum, and two lateral spinal arteries, situated about midway between the attachment of the dorsal and that of the ventral roots (Scremin, 1995). (Source: Figure 7-10, Cousins and Bridenbaugh, 1988)

B. Blood Supply of the Spinal Cord, Horizontal Distribution. The "central" area, supplied only by the ventral spinal artery, is predominately a motor area. This area is most vulnerable, since there is only one ventral artery as opposed to the dorsal spinal arteries of which there are two (only the left dorsal spinal artery is depicted). (Source: Figure 8-10, Cousins and Bridenbaugh, 1988)

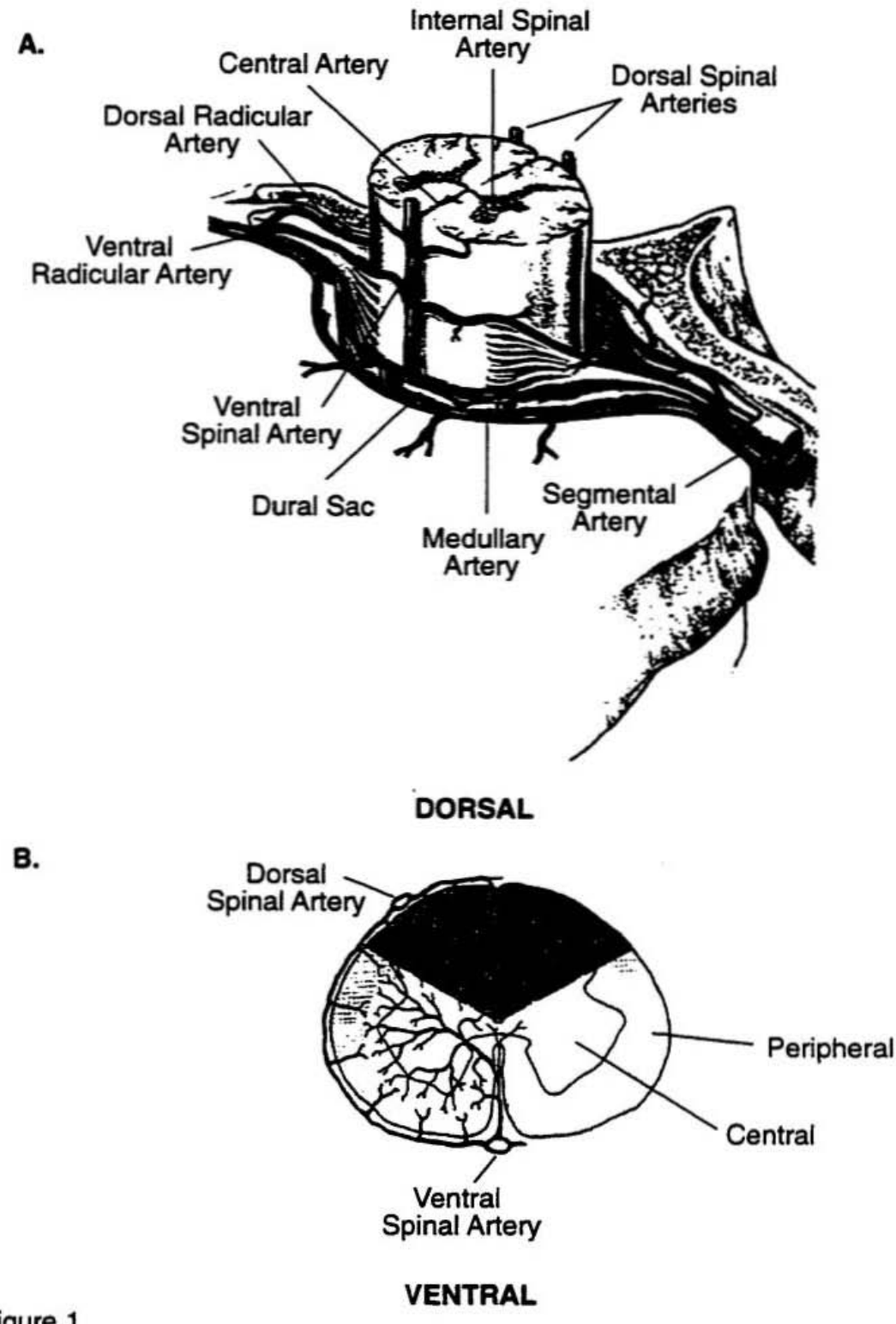

A.
Internal Spinal Artery
Central Artery
Dorsal Spinal Arteries
Dorsal Radicular Artery
Ventral Radicular Artery
Ventral Spinal Artery
Dural Sac
Segmental Artery
Medullary Artery
DORSAL
B.
Dorsal Spinal Artery
Peripheral
Central
Ventral Spinal Artery
VENTRAL

Figure 1

As shown in Table II, somatostatin, spantide, [D-Arg]-substance P, endothelin, dynorphin and arginine^{8}-vasopressin all caused an immediate decrease in spinal cord blood flow following i.t. administration. In the case of dynorphin A (1-13), the observed decrease in lumbosacral blood flow was also shown to be dose-related (Long et al.,1987). Using laser doppler flowmetry, Freedman et al., (1988) showed that i.t somatostatin and spantide decreased blood flow to 16 ± 4% and 30 ± 6% of control, respectively. This maximal effect occurred 10 to 20 min after injection. Comparable reductions in lumbosacral blood flow following i.t. [D-Arg]-spantide, dynorphin and AVP were reported using radiolabelled microspheres at similar time points (Helke et al., 1987; Long et al., 1987; Long et al., 1989a). The rapid onset of this hemodynamic effect was indicated by decreases of up to 50% of control two min after i.t. dynorphin A (1-13) (Thornehill et al., 1989).

In the case of somatostatin and spantide, a gradual but incomplete recovery of spinal cord blood flow was observed 60 min after injection (60 ± 14% and 53 ± 20% of the pre-injection baseline values, respectively) (Freedman et al., 1988). In contrast, i.t. endothelin induced a greater than 50% reduction in spinal cord blood flow for at least 3 h (Westmark et al., 1995). In the only radiolabelled microsphere study that looked at duration, blood flow returned to pre-treatment values 60 min after i.t. AVP (Long et al., 1989a).

These hemodynamic studies, and the biological diversity of peptides effecting spinal neurotoxicity, suggest a common physiological, rather than

pharmacological or direct toxicological, mechanism. That neurotoxicity is the result of an ischemic insult secondary to peptide-induced vasoconstriction in the spinal cord is also supported by the following experimental studies. In the rat, transient occlusion of the descending aorta suppling the spinal arteries produced behavioral and histopathological changes qualitatively similar to those induced by i.t. somatostatin, various substance P antagonists, dynorphin A and AVP (Marsala and Yaksh, 1994). Coadministration of the vasodilator hydralazine (1 μmol i.t.) with a paralytic dose of dynorphin A (20 nmol i.t.) blocked both the elevation in lactic acid concentration in spinal CSF and the neuropathological effect of dynorphin A compared to vehicle + dynorphin A-treated controls (Long et al., 1994). Lactic acid is a by-product of anaerobic glycolysis indicative of an ischemic state. Pretreatment with thryotropin-releasing hormone (2 mg/kg i.v.), 15 min before and immediately prior to the i.t. injection of spantide, prevented both the spinal vasconstrictive and neurotoxic effect of spantide in the rat (Freedman et al., 1988;1989). Similarly, the selective V_1-receptor antagonist [1-(β-mercapto-β, β-cyclopentamethylene proprioninic acid) 2- (O-methyl) tyrosine]-AVP blocked the decrease in lumbosacral perfusion (Long et al.,1989a)[1] and the neurotoxic effect of i.t. AVP in the rat (Long et al., 1989b). To test the possibility that dynorphin might have a direct neurotoxic

---

[1] It is important to note that the V_1 receptor antagonist did not alter SCBF when injected alone. This finding argues against a prominent role of endogenous AVP in the normal regulation of SCBF. However, AVP may be important in certain pathological conditions associated with vasospasm, compromised blood flow and ischemia.

mechanism, Long examined the effect of dynorphin A on cultured spinal cord neurons. Concentrations up to 1 mM did not alter the viability of these neurons *in vitro* (Long et al., 1994).

Vasoconstriction *per se* is not automatically detrimental to tissue. However, when such an event leads to a sustained imbalance between blood flow and metabolic demand, cellular injury and necrosis can occur. During normal perfusion, oxidative phosphorylation generates adenosine triphosphate (ATP), the major energy source of the cell. In the absence of adequate oxygenation and/or glucose delivery, neurons must resort to anaerobic glycolysis; an alternate and less efficient means of energy production. As ATP stores are depleted, vital energy-dependent mechanisms (i.e. ion pumps) are strained to maintain cell homeostasis. For example, it has been estimated that the ion gradients maintained by these pumps utilize 50 - 60% of the ATP supply (Vannucci, 1990). As these pumps fail and as sodium ions accumulate intracellularly, ischemic neurons become susceptible to depolarization. This, in turn, can lead to the accumulation of excitatory amino acids (EAA) and other neurotransmitters in the extracellular space of the ischemic field (Shimizu et al., 1993; Ueda et al., 1993). Indeed, a neurotoxic dose of i.t. dynorphin A produced a four-fold and three-fold increase in the concentration of glutamate and aspartate, respectively, in rat spinal CSF (Long et al., 1994). The EAA-evoked influx of calcium through N-methyl-D-aspartate (NMDA)-gated channels and sodium through kainate- and (±)-α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid

(AMPA)-gated channels (Vornow and Coyle, 1991) can further aggravate the unstable conditions initiated by ischemia. The rapid influx of ions may also cause edema and lysis of the cell membrane. The sensitivity of spinal neurons to EAA receptor agonists has been demonstrated *in vitro*. NMDA, AMPA and kainate receptor agonists each induced the concentration-dependent death of cultured murine spinal cord neurons (Regan, 1996).

Within the normal cell, calcium is tightly regulated by a combination of energy-dependent ion pumps and direct binding to the endoplasmic reticulum. When the pumping mechanisms fail, intracellular stores of calcium are released thereby increasing calcium in the cytosol ($[Ca^{++}]_i$) to abnormally high concentrations. In fact, the $[Ca^{++}]_i$ is inversely related to ATP concentration during periods of ATP depletion and resynthesis (Kim-Lee et al., 1993). Such a rise in $[Ca^{++}]_i$ can have serious metabolic consequences for the cell (see review by Krause et al., 1988). The activation of phospholipase A_2, triggered by a rise in $[Ca^{++}]_i$, initiates the arachidonic cascade. Prostaglandins, thromboxanes and leukotrienes arising from this pathway trigger the production of oxygen free radicals that have been implicated in lipid peroxidation and nucleic acid damage (Traystman et al., 1991). The activation of calcium-dependent nucleases can result in direct lethal injury to the cell's DNA (Tullis et al., 1982). Within seconds of the onset of ischemia, a calcium-dependent, proteolytic enzyme converts xanthine dehydrogenase from the "D" (NAD-reducing form) to the "O" oxidase form (McCord

et al., 1985). Upon reperfusion, the "O" form of this enzyme uses two products of ATP degradation, hypoxanthine or xanthine, and oxygen to produce a toxic superoxide radical (O_2^-). Of course, the inability to regulate $[Ca^{++}]_i$ will also promote the further release of EAA's (Figure 2).

Although our understanding of the mechanisms of neural injury triggered by ischemia has grown considerably, the number and complexity of events following ischemia has made elucidation of the exact sequence of events difficult. Nevertheless, it is clear that anything that compromises spinal cord blood flow, such as the i.t. injection of the peptides discussed above, has the *potential* to effect spinal neurotoxicity, especially in marginally perfused regions like the ventral horn.

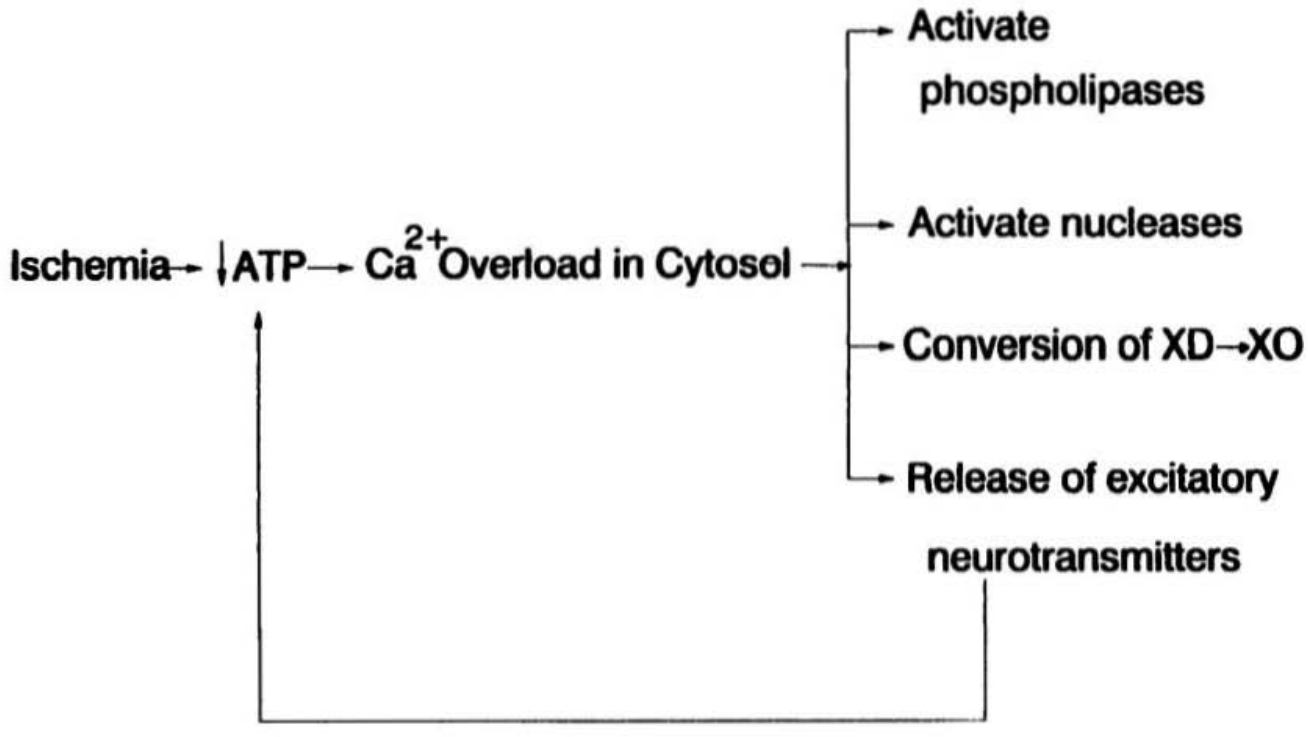


Figure 2 Proposed cycle of ischemia-mediated neurotoxicity.

Table 2
Studies of Spinal Cord Blood Flow Following Intrathecal Administration of Peptides in the Rat.

Drug/ Reference	Dose	Technique for Measuring Blood Flow	Change in Spinal Cord Blood Flow	Time Course
Somatostatin (SST) /Freedman et al., (1988)	30 µg	laser doppler	Blood flow decreased immediately after injection. The maximum reduction of 16 ± 4 % of the pre-injection baseline value occurred at 10 min.	At 60 min, blood flow returned to 60 ± 14 % of the pre-injection baseline value.
Spantide/ Freedman et al., (1988)	2 µg	laser doppler	Blood flow dropped immediately after injection and at 20 min was 30 ± 6% of the pre-injection baseline value.	There was a gradual but incomplete recovery of spinal cord blood flow. At 60 min, blood flow was 53 ± 20 % of the pre-injection baseline value.
D-Arg^{1}-D-Pro^{2}-D-Trp^{7,9}, Leu^{11}-substance P ([D-Arg]-SP)/Helke et al., (1987)	3.3 nmol	radiolabelled microspheres (reference sampling method)	Blood flow decreased 41% in the thoracic and 17 % in the lumboscaral spinal cord, 15 to 20 min after injection.	-
AVP/ Long et al., (1989a)	1, 10, 50 and 100 pmol	radiolabelled microspheres (reference sampling method)	At 10 min after injection, AVP reduced lumbosacral blood flow from 37 to 87 % with doses of 10 and 100 pmol, respectively.	Lumbosacral blood flow remained significantly decreased 30 min after the 100 pmol dose. Recovery to pre-treatment levels occurred by 60 min.

Table 2 (cont'd)

Drug/ Reference	Dose	Technique for Measuring Blood Flow	Change in Spinal Cord Blood Flow	Time Course
Endothelin/ Westmark et al., (1995)	0.37 mM	laser doppler	The decrease was rapid in onset achieving a greater than 50 % reduction from baseline.	A greater than 50 % reduction in blood flow was maintained for at least 3 h when the animal was anesthetised and perfused for light microscopic examination of the spinal cord.
Dyn A (1-13) Thornehill et al., (1989)	20 nmol	radiolabelled micospheres (reference sampling method)	Two minutes after injection, blood flow was decreased by up to 50 % in segments 2 cm caudal and 1 cm rostral to the tip of the catheter.	-
Dyn A (1-13) and Dyn A (3-13)/ Long et al., (1987)	Dyn A (1-13): 12.5, 25 and 50 nmol Dyn A (3-13): 50 nmol	radiolabelled microspheres	Dyn A (1-13): Dose-related reductions in lumbosacral blood flow. Lumbosacral blood flow was decreased 13 - 75 % and thoracic blood flow was decreased 38% at 10 min post-injection.. Dyn A (3-13): There was an approximately 40 % decrease in lumbosacral blood flow 10 min after injection.	-

Abbreviations: i.t., intrathecal; Dyn, dynorphin; AVP, arginine ^{8}-vasopressin

1.6 The Vascular Effects of α- Agonists

In light of the presumed mechanism of toxicity of i.t. peptides described above, and the the fact that perispinal routes of administration can expose the spinal cord to concentrated drug solutions (mM range), it is important to note that both α_1- and α_2-adrenergic agonists are potent constrictors of central blood vessels. In a study using isolated cerebral arteries from the monkey, phenylephrine and noradrenaline produced a dose-dependent contraction of vascular smooth muscle (Toda, 1983). The maximum responses were comparable for both drugs although the ED_{50} of phenylephrine ($1.11 \pm 0.41 \times 10^{-6}$ M) was 5.6 times greater than that of norepinephrine. Although, NA is a non-selective α-agonist, the contractions induced by NA using isolated human and monkey cerebral arteries appear to be mediated by α_1-receptors. Treatment with prazosin (3×10^{-10} and 10^{-9} M) significantly attenuated the contractile response to NA (10^{-7} to 10^{-5} M). Yohimbine was without effect in concentrations up to 10^{-6} M (Toda, 1983). Coughlan et al. (1992) showed that isolated canine middle cerebral arteries exhibit dose-related contractions to the highly selective α_2-agonist, dexmedetomidine (DX; α_2 to α_1 ratio of 1620:1 versus 200:1 for clonidine; Virtanen et al., 1988). At a concentration of $3 \times 10^{-3.9}$ M, the maximum concentration of DX that could be used without detectable α_1-mediated vasoconstriction, DX induced a response equivalent to 72.8 ± 5.7% of the maximal effect evoked by 40 mM KCl.

Large systemic doses of α-agonists can cause cerebral vasoconstriction.

For example, Zornow et al. (1990) demonstrated a 45% decrease in cerebral blood flow in isoflurane-anesthetized dogs following a systemic dose of DX (10 μg/kg i.v.). Karlsson et al. (1990) also demonstrated a 34% decrease in cerebral blood flow in halothane-anesthetized dogs using an identical dose of DX. The spinal administration of α_1- or α_2-agonists also impairs spinal cord blood flow. Phenylephrine (0.3, 0.5%), injected into the lumbar subarachnoid space of the dog, decreased blood flow by 28% and 44%, respectively, 30 minutes after drug administration (Dohi et al., 1984). In the rat, doses of 20, 100 and 400 nmol of i.t. clonidine each reduced SCBF 15 min after injection, but gray and white matter responded differently. In the grey matter of the spinal cord, the largest decreases occurred with the 20 and 100 nmol doses (29 - 44% compared to control). The 400 nmol dose produced significant decreases of a lesser magnitude (12 - 27%). In the white matter, the 100 and 400 nmol doses produced reductions of 17 - 39%, while the 20 nmol dose was without effect (Crosby et al., 1990). Epidural clonidine (10 and 30 μg/kg) also affected spinal cord blood flow (SCBF) in the pig (Gordh et al., 1986a). Reductions of 25% to 35% were observed in the lumbar and thoracic segments 45 minutes after drug administration. These data clearly indicate that α-adrenergic agonists can impair blood flow through cerebral and spinal vessels.

A direct comparison of the hemodynamic effect of neurotoxic peptides with that of α-agonists is complicated by the use of the different methodologies, species and spinal routes of drug administration. Nevertheless, both classes of drugs have

been shown to decrease spinal cord blood flow to a comparable degree within the first 30 min of i.t. drug administration. The 25-35% decrease in blood flow recorded 45 min after epidural clonidine (Gordh et al.,1986a) suggests that α-agonists have a sustained effect, and that they may induce a substantial disturbance in spinal perfusion. Unfortunately, there are no time-course data by which to judge the actual duration of this disturbance.

The adrenergic nerve supply to most blood vessels originates in the pre- or para-vertebral ganglia of the sympathetic nervous system. In the brain, the innervation of some blood vessels may originate from central catecholaminergic neurons (Edvinsson et al., 1973). There is great variation in the pattern and density of the sympathetic innervation of vascular smooth muscle (Burnstock, 1975). Large elastic arteries generally show a sparse pattern of innervation while capillaries, venules and small veins have virtually no adrenergic nerve supply. In contrast, smaller arteries display an increased density of innervation. They are surrounded by a hair-like network of sympathetic postganglionic nerve fibers influencing smooth muscle cells. Indeed, each terminal branch of a postganglionic fiber courses across the surface of one or more vascular smooth muscle cells, releasing norepinephrine from the vesicles within its multiple varicosites.

Vasoconstriction is mediated by post-junctional α_1- and α_2-adrenoceptors. Whereas larger aterioles and venules are regulated by both α_1- and α_2-adrenoceptors, vasoconstriction in the terminal vessels is mediated primarily by α_2-

adrenoceptors (Faber, 1988). α_1-Adrenoceptor mediated contraction of smooth muscle relies on the release of intracellular, rather than on extracellular, stores of Ca^{++}. Thus, agonist occupation of vascular α_1-adrenoceptors activates phospholipase C which acts on phosphotidylinositol bisphosphate, a membrane phospholipid, to produce two second messengers. Inositol triphosphate (IP_3) is released into the cytosol, and diacylglycerol (DAG) remains in the membrane and activates protein kinase C (PKC). IP_3 evokes release of Ca^{++} from internal stores within the sacroplasmic reticulum of the muscle cell; PKC sensitizes the contractile apparatus to Ca^{++}. The net effect of α_1-adrenoceptor activation is contraction of vascular smooth muscle without the movement of ions across the cell membrane and with a minimal change in the membrane potential.

The contraction of vascular smooth muscle mediated by α_2-adrenoceptors depends almost totally on extracellular Ca^{++} and an increase in the membrane permeability to this ion. It has been proposed that α_2-adrenoceptor activation may be coupled, via a pertussis toxin-sensitive G - protein (G_i) , to phospholipid hydrolysis involving phospholipase D (PLD) and the formation of phosphatidic acid (Aburto et al., 1995). Once formed, phosphatidic acid may give rise to DAG, via the action of phosphatidate phosphohydrolase, leading to increase of activity of PKC activity and subsequent contraction. Basal influx of extracellular Ca^{++}, and an as yet unidentified tyrosine kinase, are critical regulators of α_2-adrenoceptor coupling to phospholipase D.

Clearly, the smooth muscle of central blood vessels contains both α_1- and α_2-adrenoceptors. Each of these receptor subtypes is coupled to a distinct effector mechanism that mediates vasoconstriction. The spinal administration of either α_1- or α_2-agonists could be neurotoxic if prolonged vasoconstriction and thus a sustained ischemic state were to arise.

As summarized in Table III, current experimental evidence suggests that the spinal administration of individual α-adrenergic agonists is without neurotoxicological effects. However, the safety of a spinal α_1- + α_2-agonist combination, effecting independent vasoconstrictor mechanisms, has not been investigated.

Table 3
Studies of Spinal Neurotoxicity Following Intrathecal or Epidural Administration of α-Adrenoceptor Agonists.

Drug/ Route/Reference	Species	Dose	Test	Histology	Spinal Neurotoxicity
clonidine/epidural/ Eisenach et al. (1984)	sheep	daily dose on weekly schedule from 0.1 to 0.6 mg/kg/day	SEP, HD	Light	No
clonidine/epidural/ Eisenach et al. (1984)	sheep	total dose of 2.0 to 2.8 mg/kg	AN, HD, BG	Light	No
clonidine/epidural/ Coombs et al. (1984a)	sheep	daily dose on weekly schedule from 0.1 to 0.6 mg/kg/day	SEP, HD	Light	No
clonidine/intrathecal/ Gordh et al. (1984)	dog	12.5 or 25 µg/kg once daily for 14 days	Behaviour Body Weight	Light	No
clonidine/ intrathecal/ Gordh et al. (1986b)	rat	1.63 or 16.3 µg once daily for 14 days	Behaviour Locomotion	Light + EM	No
ST-91/ intrathecal/ Yaksh and Reddy (1981)	cat	2000 µg once daily for 7 days	-	Light	No
ST-91/intrathecal/ Yaksh and Reddy (1981)	monkey	4000 µg	AN Behaviour*	-	-

*Tendon reflexes, locomotion, muscle strength, food and water consumption

Table 3 (cont'd)

Drug/ Route/Reference	Species	Dose	Test	Histology	Spinal Neurotoxicity
guanfacine/ intrathecal/ Gordh et al. (1986b)	rat	16.3 or 75 µg once daily for 14 days	-	Light + EM	No
oxymetazoline/ intrathecal/ Loomis et al. (1992c)	rat	100 nmol twice daily for three days or 200 or 300 nmol once daily for three days	AN	Light + Immunohisto-chemistry	No

Abbreviations: AN, antinociception; BG, blood gases; HD, hemodynamics; SEP, somatosensory evoked potentials

1.7 Combination Drug Therapy

While spinal opioids and local anesthetics can achieve clinically effective acute pain control, none do so without attendant adverse effects. Spinal opioids can produce respiratory depression (immediate or delayed), neurological abnormalities (i.e. confusion), urinary retention and pruritis. Spinal local anesthetics cause postural hypotension, bradycardia and urinary retention. Elderly patients or patients with chronic obstructive pulmonary disease are also at risk of local anesthetic-induced respiratory depression (Cousins and Bridenbaugh, 1988). To minimize the problem of adverse effects and toxicity, alternate strategies have evolved in acute pain management. One example is the concept of balanced spinal anesthesia. This is achieved by administering low dose combinations of the analgesic drugs, each with separate and potentially synergistic mechanisms of action. In appropriate doses, these combinations produce a level of anesthesia/analgesia comparable to that of a large dose of a single drug while minimizing their individual adverse effects.

α-Adrenergic agonists represent a novel drug class of spinal analgesics. Used alone, analgesic doses produce pronounced hypotension and bradycardia. Additionally, the variability in the analgesic efficacy of α-agonists in humans makes their independent use unreliable. However, when used in combination with local anesthetics and opioids, the spinal administration of α-agonists has been demonstrated to have positive pharmacokinetic and pharmacodynamic effects. For

example, i.t. clonidine, at doses which were otherwise inactive, prolonged the duration of action of tetracaine in laboratory animals (Bedder et al., 1986; Mensink et al., 1987) and of bupivacaine in humans (Racle et al., 1987; Racle et al., 1988). This effect, which was dose-related (Bonnet et al., 1992), is suggestive of a pharmacokinetic interaction. In contrast, i.t. clonidine or norepinephrine significantly reduced the ED_{50} of i.t. morphine or DPDPE in mice (Roerig et al., 1992). Intrathecal metomidine and oxymetazoline produced a similar interaction with i.t. morphine in rats (Sherman et al., 1988; Ossipov et al., 1990a). In humans, the combination of epidural clonidine and fentanyl produced additive analgesia in a study of cesarean section (Eisenach et al. 1994). Clinical studies have also shown a synergism between opioids and α_2-agonists in the treatment of opioid refractory cancer pain (Polati et al., 1996). These results suggest a pharmacodynamic interaction between α-agonists and opioids, consistent with their antinociceptive/analgesic activity. Interestingly, clonidine has been shown to prolong the duration of analgesia of epidural morphine and fentanyl in humans (Motsch et al., 1990; Rostaing et al., 1991). These data suggest that both pharmacodynamic and pharmacokinetic factors underly the interaction of α-agonists with spinal opioids.

The combination of low dose local anesthestics and opioids in spinal anesthesia/analgesia has also become popular (Miller, 1994). For example, Cullen et al. (1985) studied a mixture of morphine (0.01%) and bupivacaine (0.1%) in the

control of major post-operative pain. In this double-blind study, pain scores in the morphine alone and the morphine-bupivacaine combination groups were significantly reduced compared to those of bupivacaine alone. Mixtures of epidural sufentanil (0.1-0.2 μg/ml) or fentanyl (1 μg/ml) with 0.125% bupivacaine are now commonly used during labour (Youngstrom et al., 1984; Vertomman et al., 1991). Interestingly, the addition of epinephrine (diluted 1:800,000 to a final concentration of 1.45 μg/ml) prolonged the duration of action of a bupivacaine-fentanyl combination, thereby reducing the need for supplementary doses to sustain pain control (Grice et al., 1990).

1.8 Rationale and Specific Objectives

Low dose combinations of analgesic and/or anesthetic drugs are being used increasingly to optimize pain control while minimizing the problem of adverse effects. α-Agonists such as epinephrine and phenylephrine are routinely used in combination with spinal local anesthetics (Cousins and Bridenbaugh, 1988) and α_2-agonists are being used adjunctively with spinal opioids. Thus, it is increasingly likely that α-agonists with varying selectivity for α_1- and α_2-adrenoceptors will be administered perispinally (either concurrently or serially) as components of a multiple drug approach to pain control.

Current evidence suggests that α_1- and α_2-adrenergic agonists possess discrete antinociceptive mechanisms (see section 1.3). Thus, their co-

administration in the spinal cord raises several important pharmacological questions. Does the combination of i.t. α_1- and α_2-adrenergic agonists effect an additive or supra-additive antinociceptive interaction? Can a spinal antinociceptive combination of α_1- and α_2- agonists be used without risk of ischemic toxicity given their potent vasoconstrictive effects and the overt neurotoxicity induced by i.t. peptides that cause localized vasocontriction in the spinal cord? Could an α_1- and α_2-agonist combination exacerbate the adverse effect of a known spinal neurotoxin? The purpose of this research was to investigate these questions in an animal species (rat) whose spinal blood supply resembles that of humans, and with a known vulnerability to ischemic neurotoxicity. The specific objectives of this research were:

1) To determine the dose-response relationship of i.t. DX, alone and in combination with a fixed dose of MX (10 μg), in the tail flick and paw pressure tests.

2) To determine if a combination of i.t. DX and MX, injected twice a day for four consecutive days, induces spinal neurotoxicity.

3) To determine the effect of a combination of i.t. DX and MX on i.t. dynorphin-induced neurotoxicity.

2.0 METHODS AND MATERIALS

2.1 Animals

All experiments were conducted using virus antibody free (VAF), male, Sprague-Dawley rats weighing between 300 to 400 grams at the time of experimentation (Charles River Inc., St. Constant, Que., Canada). Animals were housed in a climate controlled room (22 °C) with a 12 hour (h) light-dark cycle (lights on at 07:00 h). Purina® laboratory rat chow and tap water were provided *ad libitum*. Rats were housed in group cages prior to surgery for a minimum of one day. All procedures were approved by the Animal Care Committee of Memorial University in accordance with the Guidelines of the Canadian Council on Animal Care.

The rat was chosen as the experimental model for this thesis as the rat spinal cord arterial supply bears an almost exact resemblance to that of humans (Scremin, 1995). Given the focus of the thesis and the experimental use of dexmedetomidine in clinical trials, extrapolation of the results of the neurotoxicological evaluation of this drug to the use in humans would be best achieved using an animal model that closely resembled the human condition. Additionally, in the choice of a positive control for this thesis, it was important to select both a model and a drug, that had been well documented in the literature, against which to gauge the neurotoxicity of the drug(s) being evaluated. As, the majority of spinal neurotoxicity studies has been done in the rat, it was chosen as the model for the experimental work.

2.2 Surgical Procedures

2.2.1 Implantation of Intrathecal Catheters

Rats were implanted with intrathecal catheters using the method of Yaksh and Rudy (1976) as modified by Sherman et al (1988). Intrathecal catheters were prepared from polyethylene tubing (PE 10) stretched to approximately 1.5X its original length. A small loop was made approximately 4 cm from one end of the catheter and fixed with adhesive. The section of the catheter below the loop was trimmed to a length of 7.5 cm. Immediately prior to implantation, the catheter was flushed with sterile (0.9%) saline (Astra Pharma Inc.).

Anaesthesia was induced with 4% halothane (Halocarbon Laboratories, River Edge, N.J.) in oxygen (100%) and the rat was placed in a stereotaxic apparatus (Narishige Type SN-2, Tokyo, Japan). Anaesthesia was maintained with 2.0-2.5% halothane. A transverse cut was made at the base of the head and the atlanto-occipital membrane was exposed. The catheter was inserted through a small slit in the cisternal membrane, and guided carefully through the spinal subarachnoid space so that the tip was positioned near the lumbar enlargement. The catheter loop was sutured to the overlying muscle and the rostral end of the catheter was then passed under the skin and externalized on the top of the skull. The catheter was flushed with 10 µl of sterile saline to clear the catheter of any blood or debris, and sealed with a stainless steel plug. The incision was then closed with sutures and halothane was discontinued.

Animals were housed individually after surgery and allowed to recover for at least four days before experimentation. The i.t. catheter were flushed the next day with sterile saline and the surgical incision was cleansed with 10% hydrogen peroxide (H_2O_2) and treated with an anti-inflammatory cream (Viaderm K.C. Cream (Taro)). Only animals with normal posture and gait, and exhibiting normal eating, drinking and grooming behaviour were used for experiments.

2.2.2 Implantation of Intravenous Catheters

Jugular vein catheters were made from two sizes of Silastic® tubing, 0.020" X 0.037" X 0.0085" and 0.040" X 0.085" X 0.0225". Two 7 millimeter (mm) segments of the larger tubing were cut and each was threaded with a 3 centimeter (cm) length of nonabsorbable surgical suture (B-186 Ethicon, Peterborough, Ontario, Canada). A 12-cm length of the smaller tubing was then threaded through the two larger tubing segments. The first segment was positioned 4-cm from the end of the smaller tubing. Animals were anaesthetized with sodium pentobarbital (50mg/kg i.p.) and the left jugular vein was exposed. The proximal end of the smaller tubing was inserted into the jugular vein in the direction of the heart. The position of the catheter, in the vein, was then secured by tying the sutures of the overlying tubing segment to the surrounding musculature. As the tubing exited the jugular, a half-loop was formed so that the catheter could be passed subcutaneously to the back of the neck and externalized on the back of the skull. The loop was secured by positioning and tying the second overlying tubing segment

to the nearby musculature. The externalized portion of the catheter was trimmed to a final length of 3-cm and sealed with a stainless steel plug. Animals were allowed to recover for 48 h prior to the experiment and patency of the catheter was maintained by twice daily injections of 50 µl of sterile saline containing heparin (100 I.U./ml).

2.3 Antinociceptive Testing

Antinociception was assessed using the tail flick (TF) and paw pressure (PP) tests. For both of these tests, rats were lightly restrained by hand using a cloth to cover the head. This minimized exploratory behaviour and visual stimuli during testing. Rats were acclimatized to the TF and PP apparati the day before experimentation began. The mean of three consecutive measurements in the TF and PP tests was determined at each time point.

In the TF test (D'Amour and Smith, 1941), the ventral portion of the tail was placed over the 1-cm opening of the tail flick analgesia meter (Muromachi Kikai Company, Ltd. Tokyo, Japan). Radiant light from an 8V 50W light bulb, focussed using a reflector through the opening, was used as the heat source. The time between the onset of the heat source and reflex withdrawal of the tail (termed the "response latency") was automatically recorded. The light intensity was adjusted to yield control responses of 3-5 seconds (sec). In the absence of a response, the noxious stimulus was terminated after 10 sec and the cutoff latency recorded.

Paw pressure threshold (PP) was measured using a Ugo Basile Analgesy Meter (Ugo Basile, Italy) according to the method of Randall and Selitto (1957). The non-inflamed hind paw was placed on a Teflon plinth having a very low friction coefficient. A cone-shaped plunger was placed directly above the dorsal surface of the rat's paw. The force exerted on the paw was gradually increased at a fixed rate until a complete withdrawal or withdrawal attempt was made. The force eliciting a reflex withdrawal was then recorded. A cutoff pressure of 1250 grams was used in all experiments to prevent injury and inflammation.

2.4 Motor Function Testing

To assess motor function and coordination, animals were tested using an accelerating rotarod (RR) treadmill for rats (Ugo Basile, Italy) according to the method of Jones and Roberts (1968). The apparatus consists of four 7-cm diameter drums, machined to provide suitable grip for the rats. The drum was divided by five flanges enabling four rats to be tested simultaneously. The drum accelerated from 4 to 40 r.p.m. in a period of 5 minutes (min). The duration on the rota-rod (in sec) was automatically recorded for each animal. Animals were trained on the apparatus for one day prior to experimentation (one training trial in the morning and one in the afternoon). Animals were considered trained when they could remain on the rota-rod for a minimum of 60 sec on two consecutive training trials; a cutoff of 300 sec was used. Rota-rod performance was calculated as the mean of two consecutive rota-rod measurements.

2.5 Immunohistochemistry

At the end of the experimental period, rats were anaesthetized with pentobarbital (100 mg/Kg, injected intra-hepatically) and perfused transcardially with 50 mL of ice-cold heparinized saline (5 I.U. heparin/ mL) followed by 200 mL of 4% paraformaldehyde at 120 mm Hg. The spinal cord was excised and examined for signs of inflammation and haemorrhage, and the position of the i.t. catheter was determined. Animals were excluded if the tip of the catheter was not

found in the subarachnoid space near the L1 segment.

Spinal cords were post-fixed in the same fixative solution at 4°C for 16 to 24 h. The segment of the cord adjacent to the tip of the catheter (L1) was taken for immunohistochemical staining. Forty-µm thick transverse sections of the spinal cord were processed for substance P (SP)- or calcitonin gene related peptide (CGRP)-like immunoreactivity (IR), using the peroxidase-antiperoxidase (PAP) method (Sternberger, 1979).

Sections were sequentially incubated in: 1) 10% normal goat serum containing 0.3% H_2O_2 and 0.4% Triton X-100; 2) either rabbit antiserum to SP (1:2000 dilution; Incstar Corp.) or CGRP (1:10,000 dilution; Amersham); 3) 1:150 dilution of goat-anti-rabbit-IgG serum (Boehringer Mannheim Biochemicals); 4) 1:300 dilution of rabbit PAP (Sternberger Meyer Immunochemicals); and 5) 3',3' diamino-benzidine HCL (0.5 mg/mL), glucose oxidase (3.8 U/mL, Aspergillus niger type V; Sigma Chemicals) and D-glucose (2 mg/mL) in 0.1 M phosphate buffer (pH 7.2). Between incubations, sections were washed three times each for 15 min in phosphate buffered saline. Sections were then mounted on gelatin-coated slides.

Representative photomicrographs of each treatment group are presented in the thesis.

2.6 Drugs and Drug Administration

2.6.1 Drugs

The following drugs were generously provided by their manufacturers: dexmedetomidine HCL (Orion Corp. Farmos, Turku, Finland); methoxamine HCL (Burroughs Wellcome Ltd., Kirkland, P.Q., Canada); prazosin HCL (Pfizer Canada Inc., Kirkland, P.Q., Canada); and Wyeth 27127 HCL (Wyeth Ltd., Philadelphia, PA, USA). Porcine dynorphin A (1-13) was obtained from Peninsula Laboratories Inc. (Belmont, California, USA). Dexmedetomidine (DX; (4)(5)-[2,3-dimethylphenyl) ethyl]-imadazole) was selected as the α_2-agonist as it is a highly potent and selective α_2-agonist (α_2 to α_1 ratio of 1620: 1 versus 200:1 for clonidine; Virtanen et al., 1988). Methoxamine (MX; 2-amino-1-(2,5-dimethoxyphenyl) propan-1-ol hydrochloride) was chosen as the α_1-agonist because it produces fewer motor effects than the other α_1-agonists available and because it has a slightly greater α_1/α_2 selectivity ratio in displacing [^{3}H] prazosin and [^{3}H]p-aminoclonidine from binding studies in rat spinal cord membranes (Simmons and Jones, 1988). Wyeth 27127, a disulphonamido-benzoquinolizine compound which is equipotent with idazoxan in blocking α_2-adrenoceptors in isolated smooth muscle preparation, was used because its α_2:α_1 selectivity ratio is considerably greater than idazoxan (Bill et al., 1986). Prazosin is a classical α_1-selective antagonist which has been used extensively to characterize adrenergic binding sites and functional α-adrenoceptors. The neurotoxin used in this study was dynorphin A (1-13), a

naturally occurring thirteen amino acid peptide known to produce behavioral and histological neurotoxicity following i.t. injection in the rat (Faden and Jacobs, 1984; Herman and Goldstein, 1985; Spampinato and Candeletti, 1985; Long et al., 1988; Stewart and Isaac, 1989). All drugs were dissolved in sterile saline (Astra Pharma) except prazosin which was dissolved in dimethyl sulfoxide (DMSO). All drugs were prepared on the day of the experiment. Doses are expressed as the salt.

2.6.2 Drug Administration

All the i.t. drugs, except the pre-treatment injections in the dose response experiments and dynorphin, were injected through the i.t. catheter in a volume of 10 µL followed by 10 µL of saline using a hand-held 10-µL Hamilton syringe. For the i.t. pretreatment experiments, the adrenergic antagonists were injected in a volume of 5.0 µL followed by a 10 µL saline flush using a 10 µL Hamilton syringe. Dynorphin was injected in a volume of 5 µL, using a hand-held 5-µL dead volume Hamilton syringe, followed by a 10 µL saline flush. Injectates were gradually infused over 30 sec to minimize the increase in CSF pressure. Intravenous drug injections were given in a volume of 0.1 mL followed by a 0.1-mL saline flush. For all drug injections, rats were placed in a cloth restrainer (Owen et al., 1984) to which they had been previously acclimatized.

2.7 Experimental Protocols

2.7.1 Dose-Response Experiments

The effect of i.t. administration of dexmedetomidine (DX), in doses ranging from 10 ng to 1 μg, on tail flick latency and paw pressure threshold was examined. Tail flick latency and paw pressure threshold were determined immediately prior to and 15, 30, 60 and 90 min following the i.t. injection.

The dose response experiment was repeated using a fixed dose of 10 μg of methoxamine (MX) with the various doses of i.t. DX. Tail flick latency and paw pressure threshold were again determined at the same time points as the initial experiment.

To characterize the pharmacological interaction between i.t. DX and MX, α-adrenergic antagonists were given 10 min before a fixed dose combination of 0.025 μg DX + 10 μg MX. This dose combination was selected for the antagonism experiments because it yielded a near maximal effect (88% ± 19.2% maximum possible effect (MPE); mean ± SD; n=19) in the tail flick test, and an intermediate effect (41.9% ± 30.5% MPE; mean ± SD; n=19) in the paw pressure test. Tail flick latency and paw pressure threshold were determined immediately prior to the injection of the α-antagonist and 15, 30, 60 and 90 min following the injection of the DX/MX combination.

2.7.2 Single and Repeated Intrathecal Dosing with DX and DX + MX

As part of the neurotoxicological assessment, tail flick latency and rota-rod

performance were determined after each of eight consecutive i.t. injections. For the purpose of neurotoxicological assessment, maximal drug doses over and above that of pharmacological indices would have to be administered. The doses chosen were 10 µg DX and 10 µg DX + 10 µg MX which represent a 100 and 25 fold increase over the TF and PP ED_{50}'s, respectively. The injections were done twice a day (one in the morning and one in the afternoon) for four consecutive days. The treatments were injected in a 10 µL volume followed by a 10 µL saline flush. The i.t. injections were gradually infused over 30 sec to minimize the increase in CSF pressure. Tail flick latency was determined immediately prior to and 15 and 60 min after the injections. Rota-rod performance was determined immediately prior to and 15, 30, 60 and 90 min after the i.t. injections.

2.7.3 Acute Intravenous Study

The effect of i.t doses given by i.v. injection was also determined in rats fitted with indwelling jugular vein catheters. The treatment groups were saline, 0.025 µg DX + 10 µg MX, 0.25 µg DX + 10 µg MX and 10 µg DX + 10 µg MX. The treatments were injected in a 0.1 mL volume followed by a 0.1 mL saline flush. Both tail flick latency and rota-rod performance were determined immediately prior to and 15 and 60 min after the i.v. injections.

2.7.4 Pre-treatment Study

The effect of pre-treatment of rats with i.t. DX, MX or DX + MX before an i.t. injection of dynorphin A, a known neurotoxin, was examined. Saline, 10 µg DX, 10

μg MX, or 10 μg DX + 10 μg MX were intrathecally injected 15 min before an i.t. injection of dynorphin A (1-13). Initial dose response experiments using 60, 80, 120 and 150 nmol of i.t. dynorphin, were done to establish a neurotoxic dose for this study. A dose of 60 nmol (96 μg) produced transient hindlimb paralysis, reversible loss of the tail flick reflex and no histological evidence of spinal neurotoxicity. At 120 nmol (193 μg), dynorphin consistently produced irreversible hindlimb paralysis and loss of the stepping and tailflick reflexes (data not shown). The pre-treatments were injected in a 10 μL volume followed by a 10 μL saline flush while dynorphin A was injected in a 5 μL volume followed by a 10 μL saline flush.

Tail flick latencies were determined immediately prior to the pre-treatment, 15 min after the pre-treatment (and immediately prior to the dynorphin injection), 5 min and 24 h after the dynorphin injection. Rota-rod performance was determined immediately prior to the pre-treatment injections and 24 h after the dynorphin injection.

Motor function was assessed using the rotarod and stepping reflex tests. Testing was done immediately prior to the injections and 24 h following the injection.

2.7.5 Hypothermia Study

The effect of i.t. administration of DX and DX + MX on body temperature was examined. 10 μg DX , 10 μg MX or 10 μg DX + 10 μg MX was injected i.t. The treatments were injected in a 10 μL volume followed by a 10 μL saline flush.

Rectal temperature was recorded using a Harvard Homothermic Blanket Control Unit rectal temperature probe. Temperature was recorded immediately prior to injection and 15, 30, 60, 90,120, 150, 180, 210 and 240 min.

2.7.6 Neurotoxicity Study

As part of the neurotoxicological assessment, the spinal cords of rats in both the sub-chronic intrathecal and the pre-treatment studies were excised and immunohistochemical staining for substance P (SP) and calcitonin gene related peptide (CGRP) done. From the sub-chronic i.t. behavioural study, processing of the rats, for staining, began 12 h after the last injection of saline, DX and DX + MX and 48 h after the injection of dynorphin. From the pre-treatment behavioural study, processing of <u>all</u> animals began 48 h after the injection of dynorphin. (See Section 2.5 for Immunohistochemistry details).

2.8 Data Analysis

Both the tail flick and paw pressure results were expressed as percent of % MPE which was calculated as

$$\% \ MPE = \frac{post\ drug\ response - pre\ drug\ control}{cut\ \ off\ time - pre\ drug\ control} \ X\ 100$$

Rotarod results were also plotted using the 15 time point values and are expressed as percent of the baseline (% baseline) which was calculated as

$$\% \ baseline = \frac{post\ drug\ response}{pre\ drug\ response} \ X\ 100$$

The mean and standard deviations of the TF, PP and RR results were calculated. Dose response curves were constructed from the time course data using the maximum response obtained in the tail flick and paw pressure tests which occurred at the 15 min time point. ED_{50} values and 95% confidence intervals for the dose response curves were calculated using the method of Tallarida and Murray (1986). For the graphs for the repeated i.t. doses and the i.v. studies, means and standard deviations of the MPE's and % baseline's at the 15 min time point were used.

For the dose response and pre-treatment studies, statistical analysis across the treatment groups was conducted using one-way analysis of variance (ANOVA) followed by Newman-Keuls test for a significant F-ratio ($p<0.05$). For comparisons between two treatment groups, the student's t-test was used.

For the repeated i.t dosing study, statistical differences were determined using the Mann-Whitney two sample test.

For the i.v. study, between group repeated measures ANOVA followed by Newman-Keuls and within group repeated measures ANOVA followed by Newman-Keuls were used to determine statistical significance.

3.0 RESULTS

3.1 Dose-Response Relationships of DX and MX in the Tail Flick and Paw Pressure Tests

Intrathecal DX (10 ng - 1 µg) significantly increased tail flick latency and paw pressure threshold. The time of peak antinociceptive activity ranged from 15 to 30 min after injection, depending on the dose (data not shown). The duration of action for a near maximal dose (0.75 µg) was 90 min. As shown in Figure 3, the antinociceptive effect of i.t. DX was dose-dependent. The ED_{50}'s and 95% confidence intervals of i.t. DX in the tail flick and paw pressure tests are summarized in Table IV.

TABLE IV **ED_{50}'s and 95% Confidence Intervals (ng) of Intrathecal Dexmedetomidine Alone and in Combination with Intrathecal Methoxamine.**

TREATMENT	TAIL FLICK TEST	PAW PRESSURE TEST
Dexmedetomidine	44.5 (17.9 - 110)	252 (149 - 425)
Dexmedetomidine + Methoxamine	8.12 (6.56 - 10.1)	10 (9.0 - 11.6)

The co-administration of a fixed dose of i.t. MX (10 µg i.t.) with DX produced a significant leftward shift in the DX dose-response curve (Figure 3), and a corresponding 5.5-fold increase in the potency of DX in the tail flick test, and a 25-fold increase in the paw pressure test (see Table IV). Methoxamine (10 µg i.t.) itself yielded less than a 5% increase in the maximum percent effect (MPE) in the tail flick

test, and no change (0% MPE) in the paw pressure test (data not shown). Mild hypereflexia and occasional serpentine tail movements were observed immediately after i.t. MX but these generally lasted less than 5 min.

Intrathecal prazosin (2.5 or 10 µg) significantly attenuated the antinociceptive effect of the DX + MX combination compared to dimethyl sulfoxide (DMSO)-pretreated rats (Figure 4). Indeed, complete blockade was observed with the higher dose of prazosin in the paw pressure test. Intrathecal DMSO, the injection vechicle for prazosin, had no effect on the interaction when compared to naive or saline-pretreated rats. The α_2-antagonist, Wy 27127 (0.5 µg i.t.) completely blocked the antinociceptive effect of i.t. DX + MX in both tests. The residual effect observed after prazosin (10 µg) blockade in the tail flick test was equivalent to that of 0.025 µg DX alone (see figure 3). These data are consistent with the predominate role of α_2-adrenoceptors in the adrenergic modulation of spinal nociceptive transmission, and the invovlement of spinal α_1-receptors in the dexmedetomidine + methoxamine interaction.

3.2 Effect of Single and Repeated Dosing of DX + MX on Rota-Rod Performance and Tail Flick Latency

The i.t. injection of DX (10 µg) completely inhibited rota-rod performance in

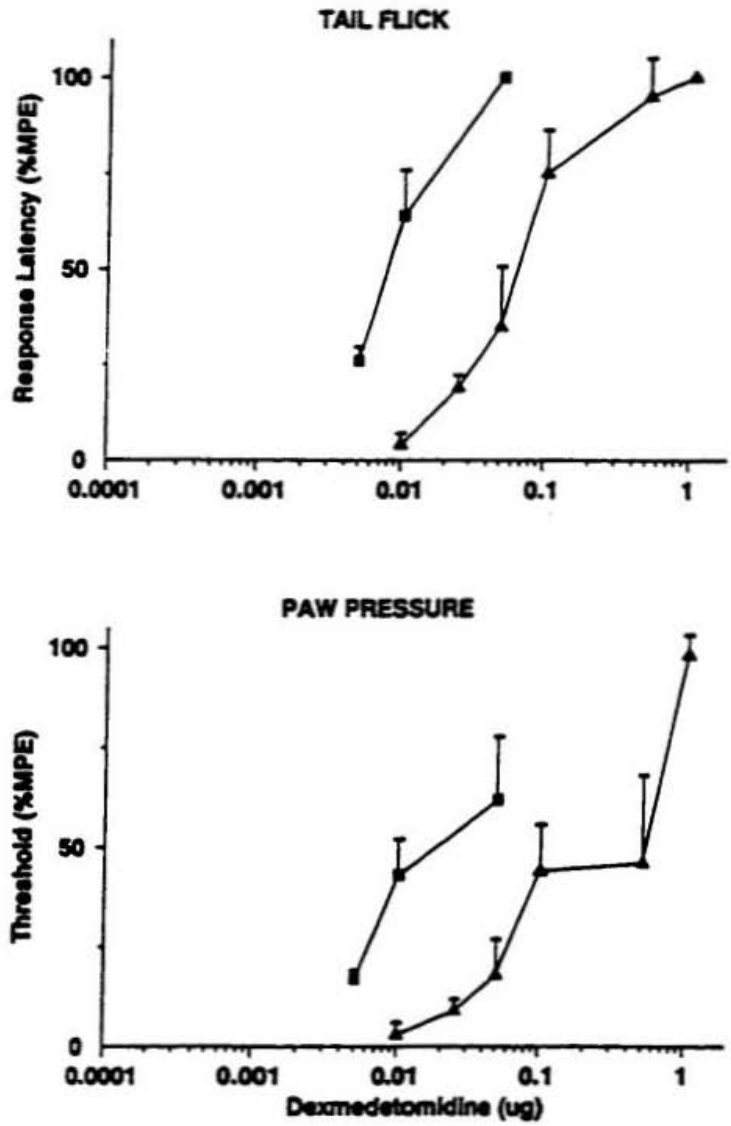


Figure 3 Log dose-response curves of intrathecal dexmedetomidine alone (▲), and in combination with a fixed dose of intrathecal methoxamine (10 µg; ■) in the tail flick and paw pressure tests. Data are expressed as the mean ± S.D. of 5 - 10 rats. ED_{50}'s and 95% confidence intervals are sumarized in Table IV. Methoxamine itself yielded < 5% MPE in the tail flick test, and no change (0%) in the paw pressure test (data not shown).

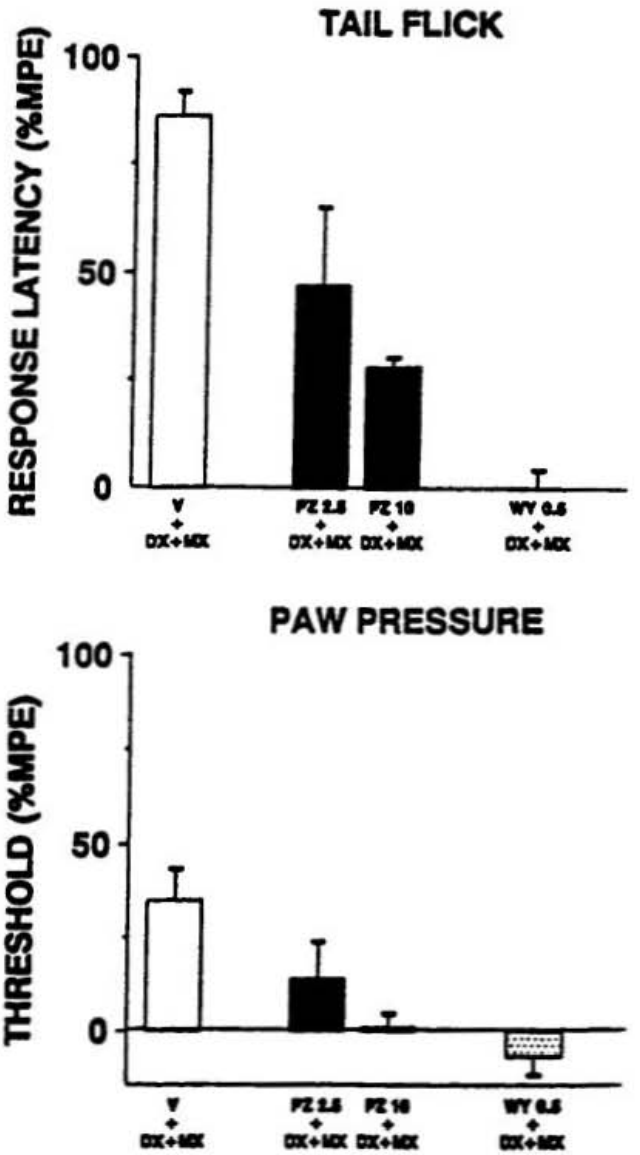


Figure 4 The effect of i.t. prazosin (PZ; 2.5 and 10 µg), Wyeth 27127 (WY; 0.5 µg) or DMSO (vehicle) injected 10 min before i.t. dexmedetomidine (DX; 0.025 µg) + methoxamine (MX; 10 µg). Data are expressed as the percent of maximum possible effect (%MPE) determined 30 min after α-agonist injection and represent the mean ± SEM of 5 -19 rats. All pretreatments were significantly different from V + DX + MX as determined using one-way ANOVA and Neuman-Keuls ($p < 0.05$).

this study (Figure 5), and produced a maximal increase in tail flick latency (Figure 6). Rats were clearly sedated (absence of a righting reflex, no startle reflex) at this dose with recovery occurring 120 min after injection. There was no evidence of permanent motor dysfunction or other adverse effects as the drug treatment effects were completely reversible. Beginning at approximately 120 min post injection, the animals regained the righting and startle reflexes, normal body posture and resumed grooming, feeding and drinking and exploratory behaviour. These effects were unchanged by the addition of MX (10 µg i.t.) (Figures 7 and 8).

Repeated dosing with DX and with DX + MX produced the same effects as the initial injections. Rotarod performance was completely inhibited (see Figure's 5 and 7) and a maximal increase in tail flick latency was produced (see Figure's 6 and 8). The effects were completely reversible after each dose of either DX or DX + MX. While the animals exhibited the same magnitude of effects in the RR and TF tests between the first and the eighth injections, the duration of the effects decreased suggesting some degree of tolerance (refer to the upper two graphs in Figures 5 to 8).

There was no evidence of cumulative toxicity as the appearance, behaviour and overall activity of the animals was the same in the saline animals and those animals that had received both DX and DX + MX, even after a full course of eight injections. Animals looked healthy and exhibited normal grooming, feeding and drinking and locomotor behaviours.

In summary, each i.t. injection of 10 µg DX and 10 µg DX+ 10 µg MX elicited the expected pharmacological effects as assessed using sensory and motor tests. Although, this testing might seem unnecessary given that the doses chosen represent a 100 fold (tail flick) and 25 fold (paw pressure) increase over the ED_{50}'s (see Table IV), it was necessary to ascertain that each i.t. injection was exerting a pharmacological effect. The animals exhibited the same magnitude of effects in the RR and TF tests between the first and eighth injections for both the DX and DX + MX treated groups. There appeared to be some small measure of tolerance as the recovery from the injections was faster after the eighth injection as compared to the first injection. In the assessment of the animals' behaviour, the repeated dosing did not appear to result in any cumulative toxicological effects as the animals appeared healthy and exhibited normal grooming, eating and drinking and exploratory behaviour.

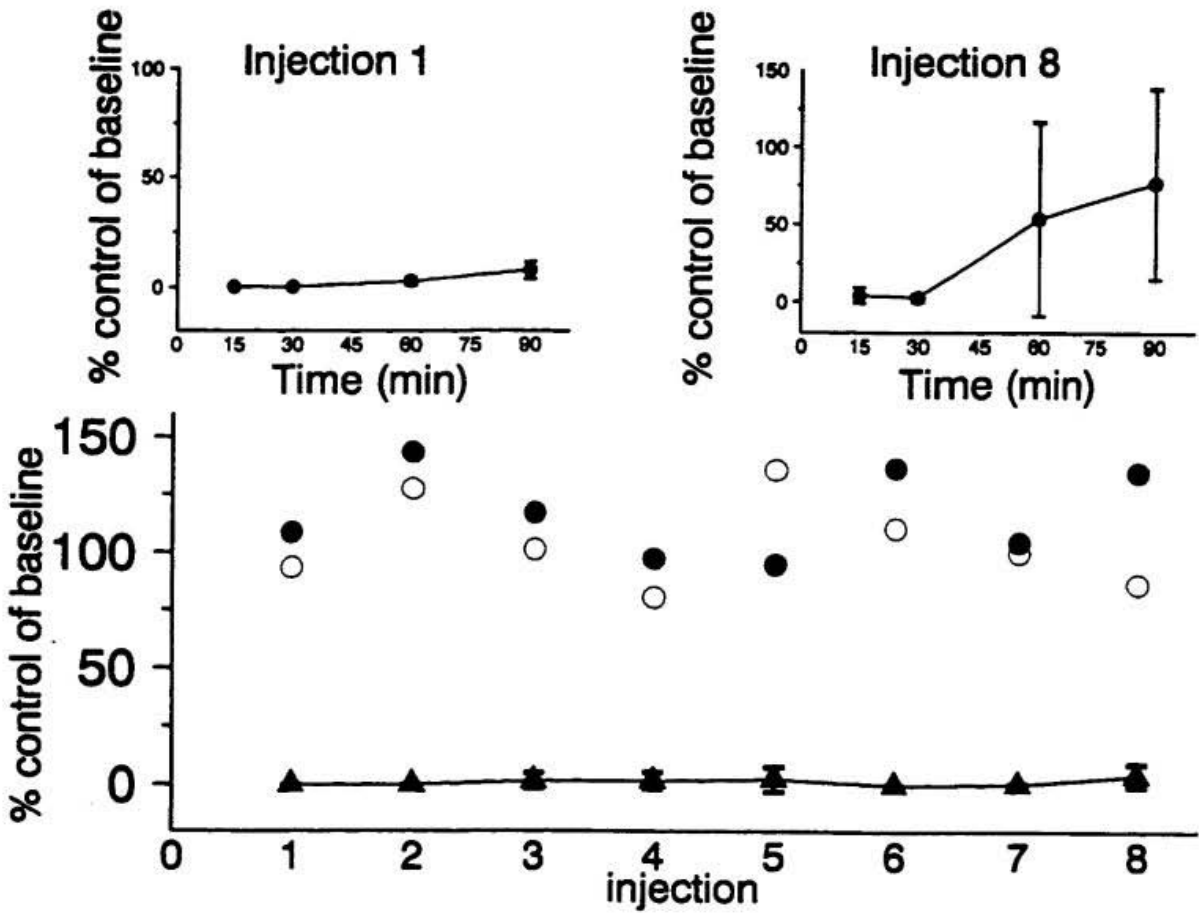


Figure 5 The effect of i.t dexmedetomidine on rota-rod performance. Lower figure: The effect of i.t. dexmedetomidine (10 μg) or saline on rota-rod performance determined 15 min after each of eight consecutive injections (two injections per day for 4 days; one in the morning and one in the afternoon). Data are expressed as the percent of baseline performance (e.g. rota-rod performance before each injection). The solid triangles represent the mean ± S.D. of 5 rats; the open and solid circles represent individual saline-treated controls. All time points for the dexmedetomidine treated animals were significantly different ($p < 0.05$) from the saline treated animals (Mann-Whitney Two Sample test). Upper figure: The time course of rota-rod performance after the first and last injection of i.t. dexmedetomidine (10 μg).

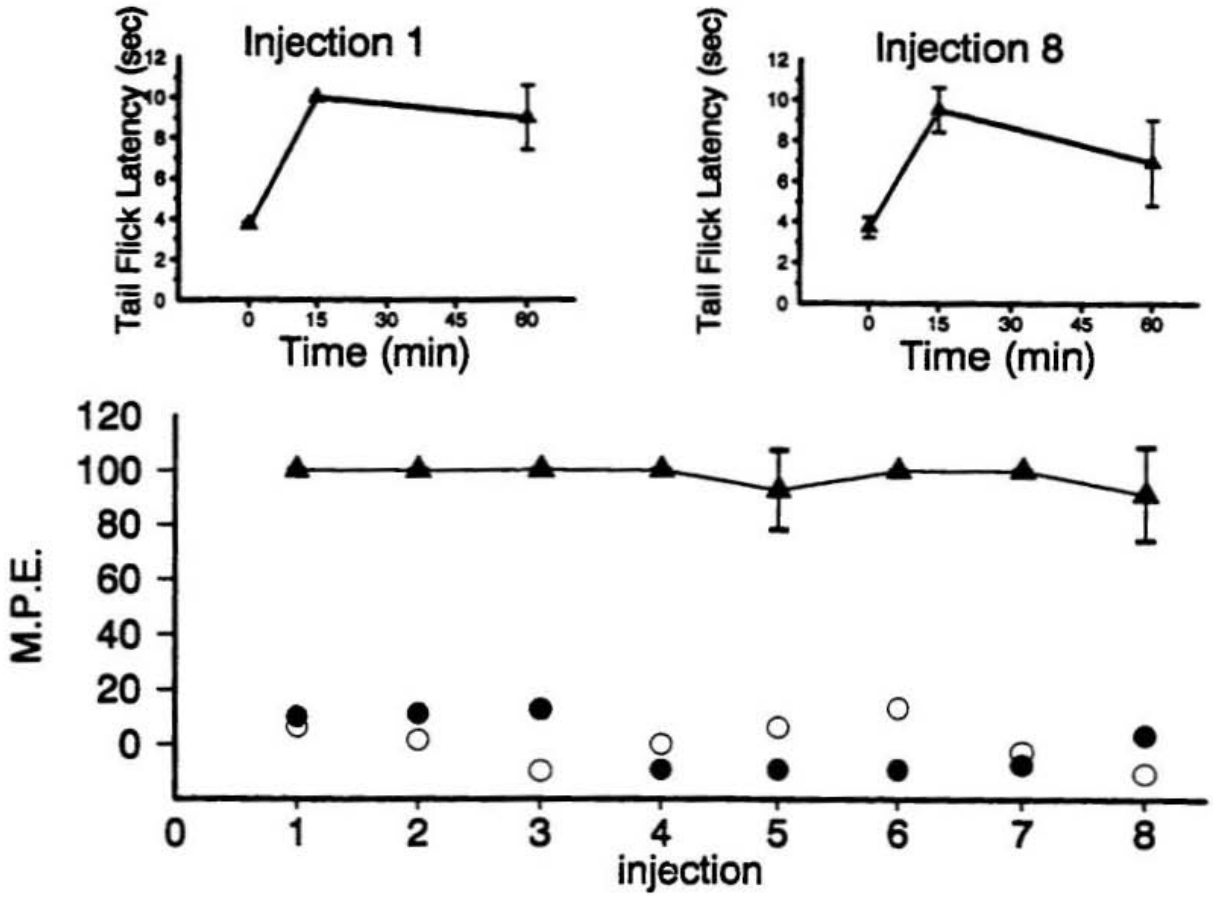


Figure 6 The effect of i.t dexmedetomidine on tail flick latency. Lower figure: The effect of i.t. dexmedetomidine (10 µg) or saline on tail flick latency determined 15 min after each of eight consecutive injections (two injections per day; one injection in the morning and one in the afternoon). Data are expressed as the maximum percent effect (% MPE). The solid triangles represent the mean ± S.D. of 4 rats; the open and solid circles represent individual saline-treated controls. All time points for the dexmedetomidine treated animals were significantly different ($p < 0.05$) from the saline treated animals (Mann-Whitney Two Sample test). Upper figure: The time course of tail flick latency after the first and last injection of i.t. dexmedetomidine (10 µg). Data are expressed as the mean of the raw tail flick scores with the cutoff time being 10 sec.

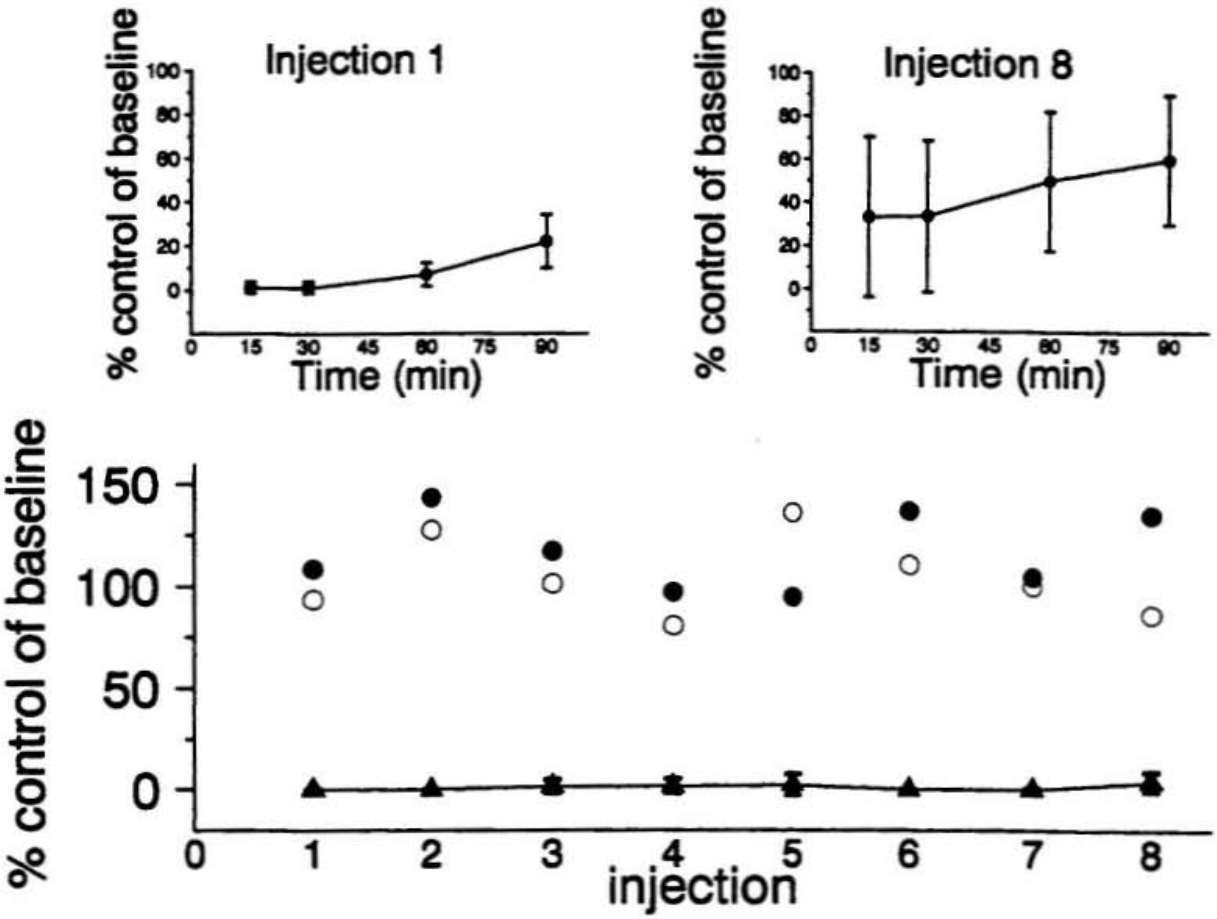


Figure 7 The effect of i.t dexmedetomidine and methoxamine on rota-rod performance. Lower figure: The effect of i.t. dexmedetomidine (10 µg) + methoxamine (10 µg) or saline on rota-rod performance determined 15 min after each of eight consecutive injections (two injections per day for 4 days; one in the morning and one in the afternoon). Data are expressed as the percent of baseline performance (e.g. rota-rod performance before each injection). The solid triangles represent the mean ± S.D. of 8 rats; the open and solid circles represent individual saline-treated controls. All time points for the dexmedetomidine and methoxamine treated animals were significantly different ($p < 0.05$) from the saline treated animals (Mann-Whitney Two Sample test). Upper figure: The time course of rota-rod performance after the first and last injection of i.t. dexmedetomidine (10 µg) + methoxamine (10 µg).

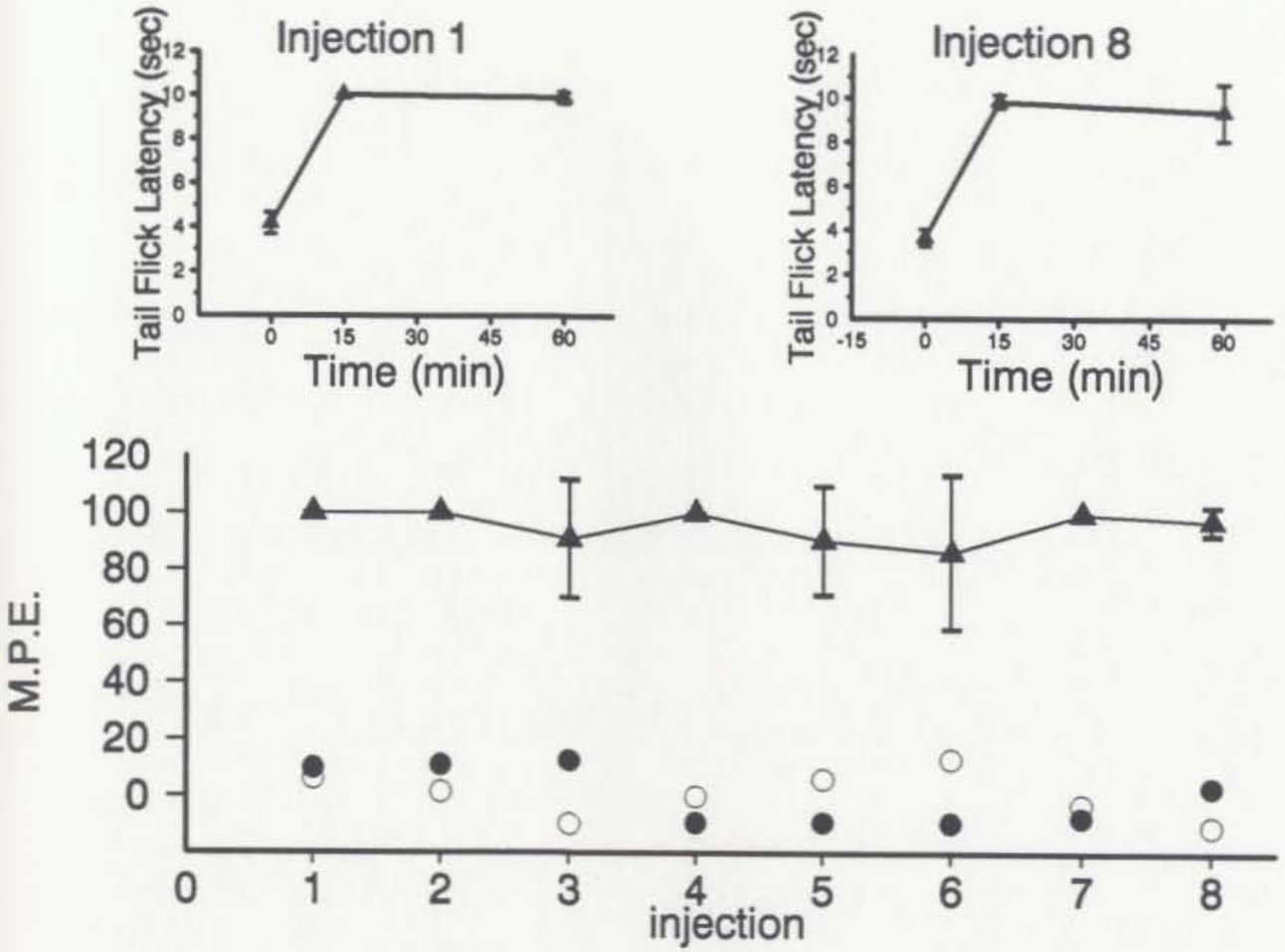


Figure 8 The effect of i.t dexmedetomidine and methoxamine on tail flick latency. Lower figure: The effect of i.t. dexmedetomidine (10 µg) + methoxamine (10 µg) or saline on tail flick latency determined 15 min after each of eight consecutive injections (two injections per day; one injection in the morning and one in the afternoon). Data are expressed as the maximum percent effect (% MPE). The solid triangles represent the mean ± S.D. of 4 rats; the open and solid circles represent individual saline-treated controls. All time points for the dexmedetomidine and methoxamine treated animals were significantly different ($p < 0.05$) from the saline treated animals (Mann-Whitney Two Sample test). Upper figure: The time course of tail flick latency after the first and last injection of i.t. dexmedetomidine (10 µg) + methoxamine (10 µg). Data are expressed as the mean of the raw tail flick scores with the cutoff time being 10 sec.

3.3 Effect of i.v. Dexmedetomidine + Methoxamine on Tail Flick Latency and Rota-Rod Performance

To assess the possibility of peripheral antinociceptive and motor effects of the DX + MX combination, i.t. doses (0.025 µg DX + 10 µg MX, 0.25 µg DX + 10 µg MX, 10 µg DX + 10 µg MX) were injected i.v. and tail flick latency (Figure 9) and rota-rod performance (Figure 10) determined. Low doses of DX (0.025-0.25 µg) in combination with MX (10 µg) had no effect on tail flick latency or rota-rod performance. In contrast, high dose DX (10 µg) in combination with MX (10 µg) almost completely inhibited rota-rod performance (Figure 10) and significantly increased tail flick latency (Figure 9).

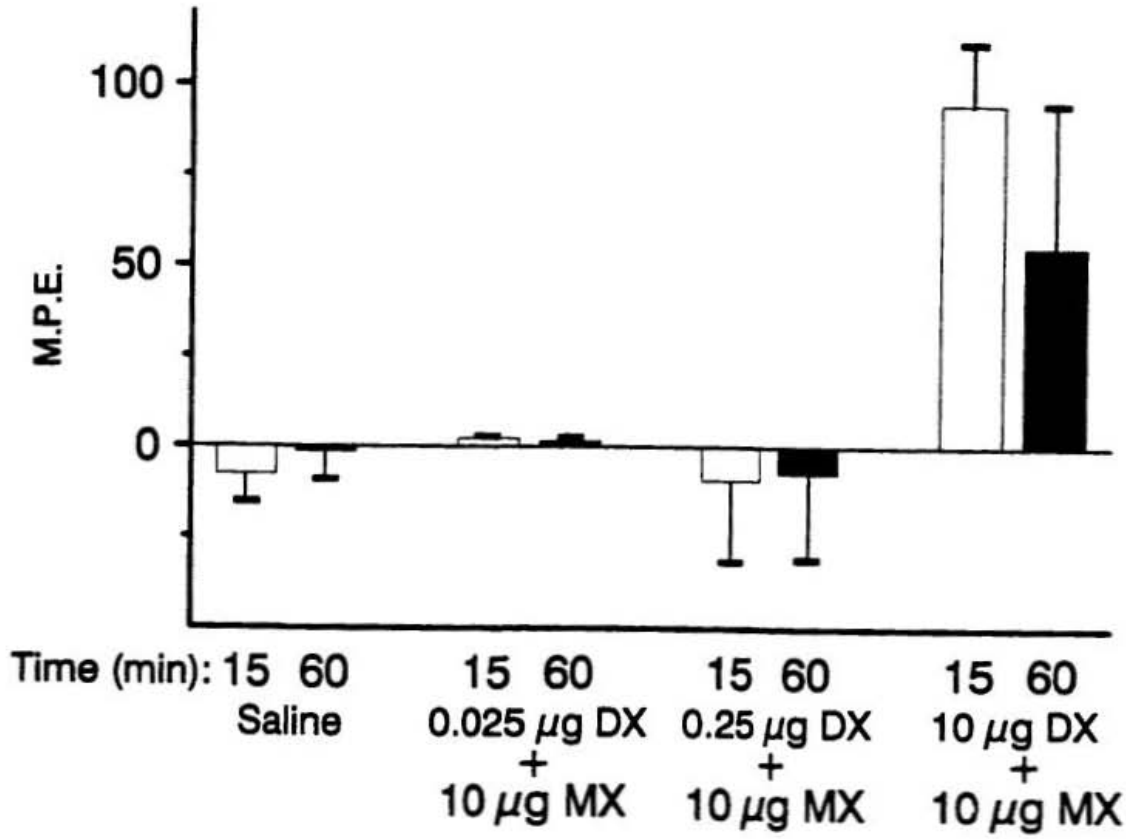


Figure 9 The effect of i.v. saline or dexmedetomidine + methoxamine on tail flick latency. Doses of 0.025 μg DX + 10 μg MX, 0.25 μg DX + 10 μg MX, 10 μg DX + 10 μg MX or saline was injected intravenously and tail flick latency determined 15 and 60 min after injection. Data are expressed as a percent of the maximum possible effect with each bar representing the mean ± S.D. of 5 - 10 rats. Statistics: (1) Between groups repeated measures ANOVA followed by Neuman-Keuls. The 15 and 60 min readings of the 10 μg DX + 10 μg MX dose are significantly different from the three other groups (ANOVA:$p<0.0001$; Neuman-Keuls:99% confidence). (2) Within group repeated measures ANOVA followed by Neuman-Keuls. Only the 10 μg DX + 10 μg MX's 15 and 60 min values are different from the baseline (ANOVA: $p<0.0001$; Neuman-Keuls:99% confidence).

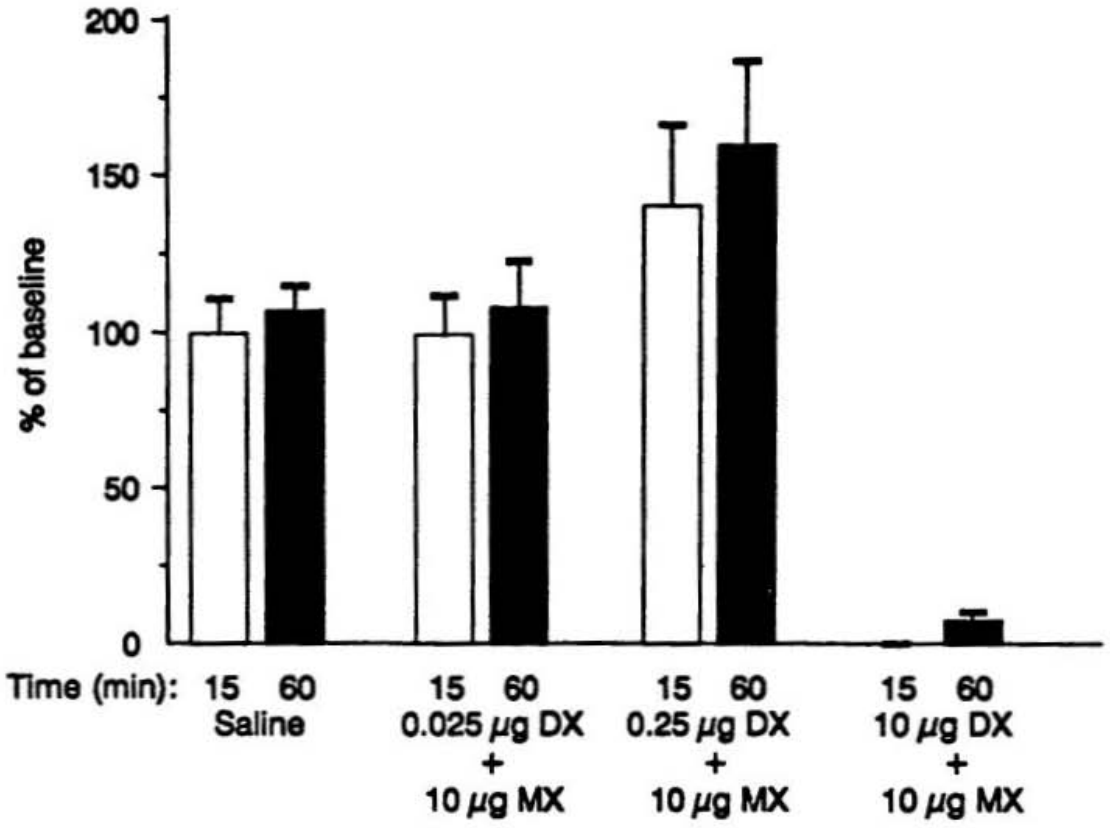
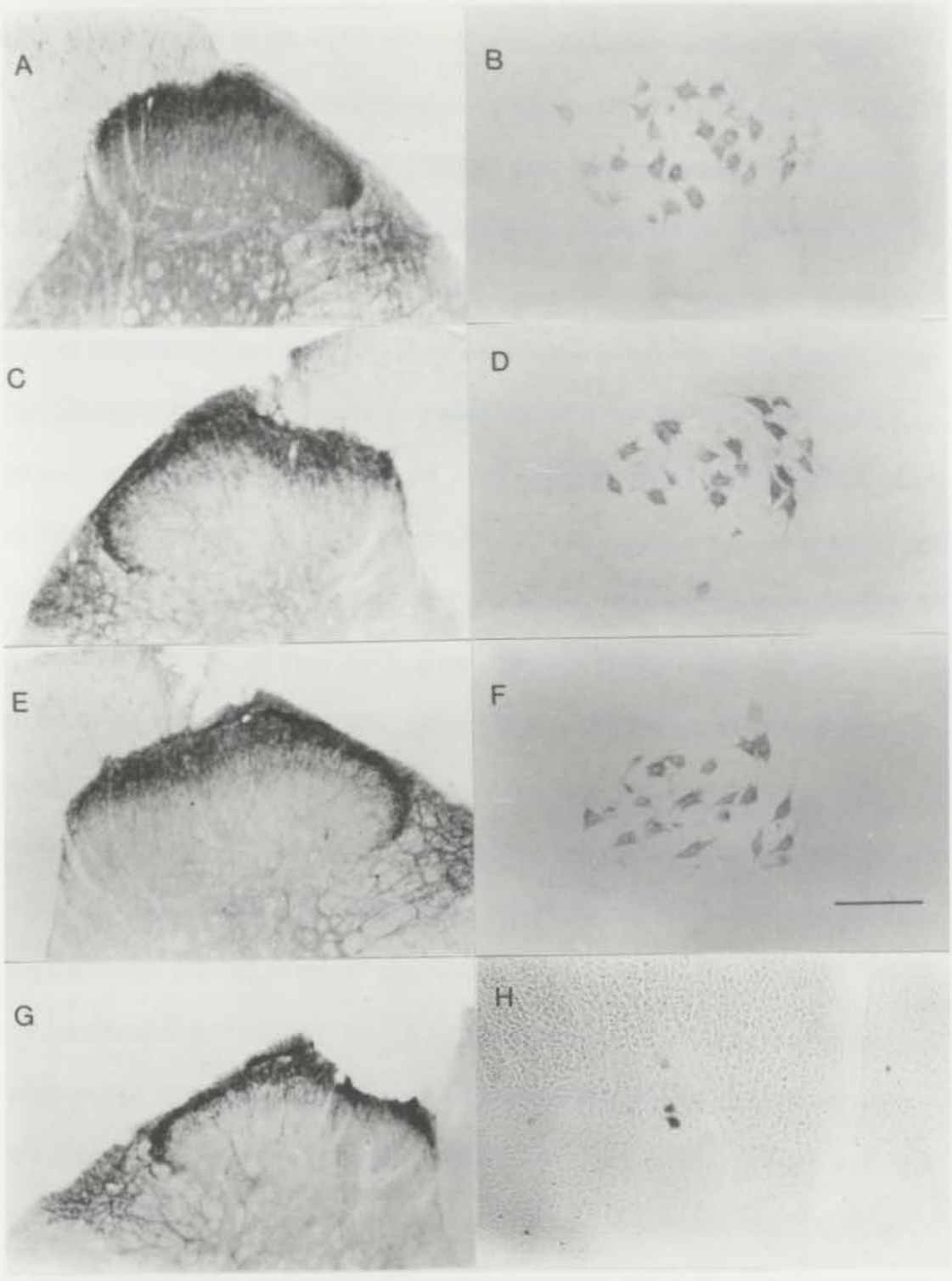


Figure 10 The effect of i.v. saline or dexmedetomidine + methoxamine on rota-rod performance. Doses of 0.025 µg DX + 10 µg MX, 0.25 µg DX + 10 µg MX, 10 µg DX + 10 µg MX or saline was injected intravenously and rota-rod performance determined 15 and 60 min after injection. Data are expressed as a percent of the baseline performance (e.g. rota-rod performance before injection) with each bar representing the mean ± S.D. of 5 - 10 rats. Statistics: (1) Between groups repeated measures ANOVA followed by Neuman-Keuls. The 15 and 60 min readings of the 10 µg DX + 10 µg MX dose are significantly different from the three other groups (ANOVA:$p<0.0001$; Neuman-Keuls:99% confidence). (2) Within group repeated measures ANOVA followed by Neuman-Keuls (a) 0.25 µg DX + 10 µg MX: The 60 min reading is significantly different from the baseline reading (ANOVA: $p < 0.05$; Neuman-Keuls; 99%). (b) 10 µg DX + 10 µg MX: the 15 and 60 min readings are significantly different from the baseline (ANOVA: $p<0.0001$; Neuman-Keuls: 99% confidence).

3.4 Evaluation of Spinal Cords Following Repeated I.T. Dexmedetomidine and Methoxamine Administration.

Inspection of the spinal cords from rats treated with i.t. saline, dexmedetomidine (10 µg) or dexmedetomidine (10 µg) + methoxamine (10 µg) twice daily for four days revealed no evidence of inflammation, haemmorrhage or necrosis near L1 (adjacent to the tip of the i.t. catheter). There was no detectable change in the staining pattern or intensity of substance P-immunoreactive (SP-IR) structures in the dorsal horn of dexmedetomidine or dexmedetomidine + methoxamine treated rats as compared to saline controls and no loss or damage to calcitonin gene related peptide-immunoreactive (CGRP-IR) motor neurons in the ventral horn (Figure 11). However, 48 h after a single i.t. injection of dynorphin A (1-13) (120 nmol) there was extensive damage to and loss of CGRP-IR neurons. The motor neurons remaining in the ventral horn were abnormally shaped, small in size and weakly staining for CGRP. SP-IR staining of fibers in laminae I and II of the dynorphin treated rats was unchanged from that of the saline-treated rats indicating a neurotoxicity that was selective for the motor neurons in the ventral horn only. Hindlimb paralysis and irreversible loss of the tail flick reflex were observed almost immediately after the dynorphin injection in these animals.

Figure 11 Photomicrographs of substance P-immunoreactivity in the dorsal horn (left column) and calcitonin gene related peptide (CGRP)-immunoreactivity in motor neurons (right column) of spinal cord sections (L1 segment) from animals treated with: A and B) intrathecal (i.t.) saline twice daily for four days; C and D) i.t dexmedetomidine (10 μg) twice daily for four days; E and F) i.t dexmedetomidine (10 μg) + i.t. methoxamine (10 μg) twice daily for four days and G and H) a single injection of dynorphin A (1-13) (120 nmol, 193 μg). Magnification bar is 200 μm. There was extensive damage to and loss of CGRP-IR motor neurons in the ventral horn of i.t. dynorphin - treated rats. There was no detectable change in the staining pattern in SP-IR structures in the dorsal horn of either the α-agonist or dynorphin treated rats as compared to the saline controls.


A
B
C
D
E
F
G
H

3.5 Effect of DX or DX + MX on i.t. Dynorphin-Induced Neurotoxicity.

Initial dose-response experiments using 60, 80, 100, 120 and 150 nmol of i.t. dynorphin showed that a 60 nmol (96 µg) dose produced no evidence of behavioural or immunohistochemical evidence of neurotoxicity. At 120 nmol (193 µg), dynorphin produced irreversible hindlimb paralysis, loss of the stepping and tail-flick reflexes and loss of CGRP-IR neurons in the spinal cord (see Section 3.4). The 150 nmol dose was lethal. In testing the effect of pre-treatment with i.t. α-agonists on dynorphin toxicity, we observed evidence of neuroprotection against the 120 nmol dose. Thus all saline pre-treated rats injected with dynorphin (120 nmol) exhibited irreversible hind limb paralysis, making them incapable of performing the rotarod test 24 h after injection (Figure 12). There was a corresponding loss of the stepping reflex (Figure 12) and the TF reflex (data not shown) that preceded the development of bladder and bowel dysfunction. In contrast, pre-treatment with i.t. DX, MX or DX + MX (10 µg each) attenuated the hind limb paralytic effect of i.t dynorphin. These doses were selected from the previous single and repeated i.t. dosing study.

As shown in Figure 12, the 24 h rotarod scores for all three α-agonist pre-treated groups were only marginally below their baseline values (or those of saline-treated rats that did not receive dynorphin), and significantly different from the saline-dynorphin group. All four rats receiving the DX + MX combination, three of five rats receiving DX, and three of four rats receiving MX had a normal stepping

reflex, 24 h after dynorphin (Figure 12). A similar trend was observed with the TF reflex (data not shown). In rats pre-treated with i.t. DX, the rectal temperature decreased by 3.3 ± 0.4°C below baseline 5 min after injection and remained below baseline for up to 4 h. In rats pre-treated with i.t. DX + MX, the rectal temperature decreased by 3.4 ± 0.3 °C. Methoxamine did not decrease rectal temperature.

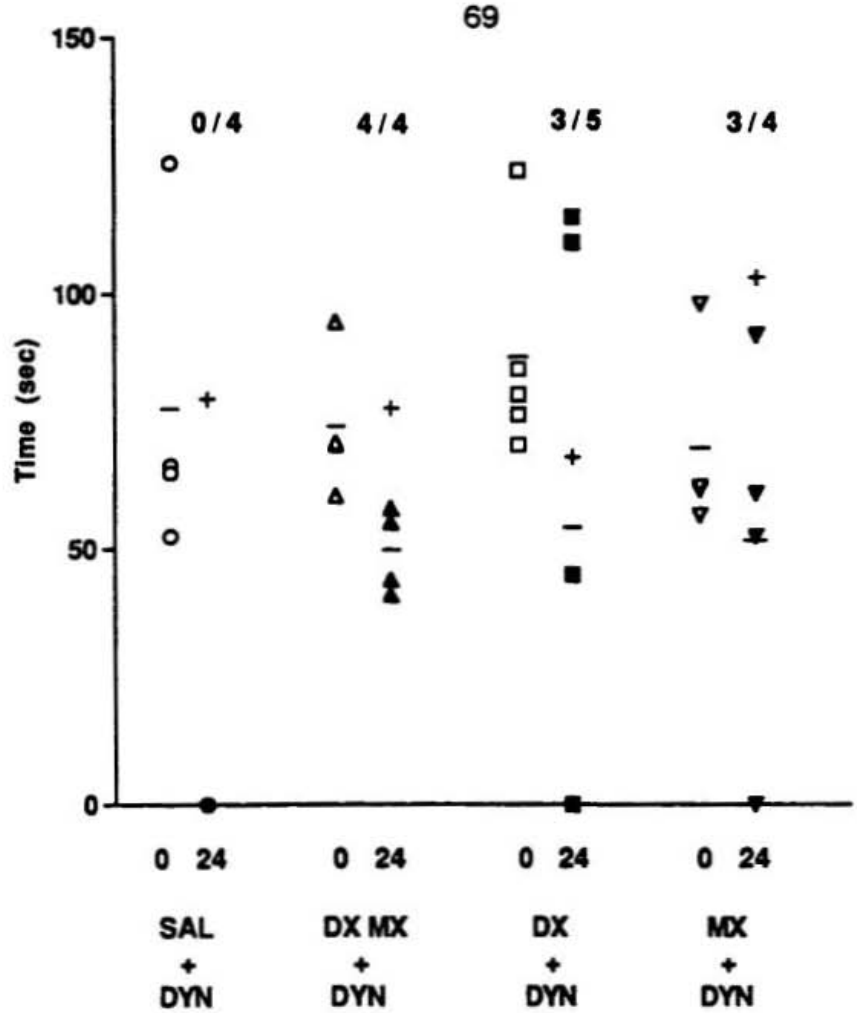


Figure 12 The effect of intrathecal dynorphin A (1-13) (DYN; 193 µg; 120 nmol) on rota-rod performance and the stepping reflex in rats pretreated with: i.t. saline (SAL); dexmedetomidine (DX; 10 µg) + methoxamine (MX; 10 µg); DX alone (10 µg) or MX alone (10 µg). Rota-rod performance was determined immediately before (open symbols; 0) and 24 h (closed symbols; 24) after dynorphin administration. Separate symbols are used for each treatment group and each symbol represents an individual animal score. There were no differences in baseline rota-rod scores among the four treatment groups (ANOVA; $p = 0.68$). All pretreatments were injected 15 min before dynorphin. The + symbol over the 24 h data represents the mean rota-rod performance in a separate group of rats that received only the pre-treatment (no dynorphin). The numbers above the treatment groups at the top of the figure represent the ratio of animals exhibiting a normal stepping reflex after drug administration. The mean baseline and 24 h rota-rod scores are indicated by the - symbol.

Table V

Effect of pre-treatment with intrathecal saline, dexmedetomidine and methoxamine, alone and in combination, on dynorphin treated rats.

	Saline/ Saline (n =3)	Saline/ Dynorphin (n = 2)	DX+MX/ Dynorphin (n = 4)	DX/ Dynorphin (n = 5)		MX/ Dynorphin (n = 4)	
				Protected (n = 3)	Non-Protected (n = 2)	Protected (n = 3)	Non-Protected (n = 1)
Mean TF Latency at 15 min (MPE)	5.4 ± 5.0	2.9 ± 0.42	100 ± 0	100 ± 0	100 ± 0	18.9 ±23.1	46.3
Mean TF Latency at 20 min (MPE)	2.1 ± 2.3	100 ± 0	94.8 ± 10.3	100 ± 0	100 ± 0	48.3 ± 25.7	100
Mean TF Latency at 24 hrs (MPE)	-2.5 ± 12.8	100 ± 0	14.4 ± 18.4	2.9 ± 2.5	100 ± 0	17.3 ± 13.1	100
Mean RR at 24 hrs (% baseline)	70.7 ± 17.1	0 ± 0	69.9 ± 22.3	100.9 ± 44.5	0 ± 0	95.1 ± 3.3	0
Hind limb paralysis	–	+ (48 hrs)	–	–	weakness (48 hrs)	–	+

Table 5 (cont'd)

Stepping reflex at 24 hrs	+	–	+	+	–	+	–
Bladder dysfunction	–	+	–	–	+	–	+

Tail flick latency (TF) and motor function (rotarod (RR), hind limb function, stepping reflex and bladder function) are used in the assessment of effects.
+ present in all animals
- absent in all animals

MPE = maximum percent effect (see Section 2.9 for calculation formula)
% baseline (see Section 2.9 for calculation formula)

3.6 Immunohistochemistry of Dynorphin Treated Rats Pretreated With Dexmedetomidine and Methoxamine.

Compared to saline controls (no dynorphin, Figure 13-A), there was a near total loss of CGRP-IR in the ventral horn of rats receiving saline + dynorphin (Figure 13-B). The few remaining CGRP-IR fibers were punctate in appearance, consistent with the severe motor dysfunction observed in these animals. Dynorphin-induced neurotoxicity was restricted to spinal motor neurons. SP-IR fibers in the dorsal horn of rats treated with saline + dynorphin (Figure 14-B) were normal in appearance and indistinquishable from those in saline controls (Figure 14-A).

Pretreatment with i.t. DX + MX, DX, or MX (Figures 13-C, D and E, respectively) attenuated the neurotoxic effect of i.t. dynorphin. The number of CGRP-IR neurons and the intensity of staining in the ventral horn were intermediate between that of saline + saline and saline + dynorphin groups. These results are consistent with the behavioural data for these groups (Table V). SP-IR fibers in the dorsal horn of dyn-pretreated rats treated with i.t. DX + MX, DX or MX were normal in appearance and indistinquishable from those in saline controls (Figures 14-C, D, and E respectively).

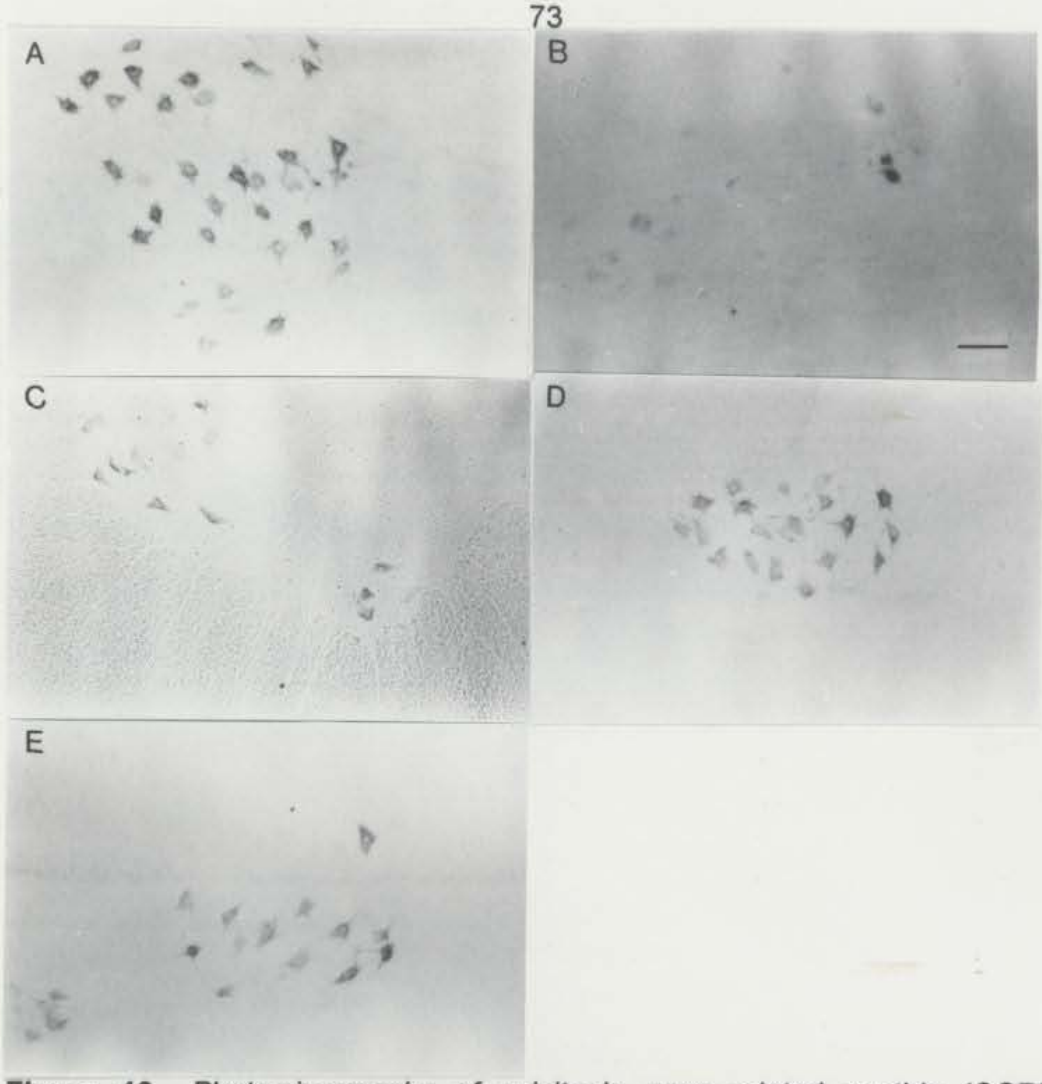


Figure 13 Photomicrographs of calcitonin gene related peptide (CGRP)-immunoreactivity in motor neurons (L1 segment) in the ventral horn after the intrathecal (i.t.) injection of saline (plate B), dexmedetomidine (10 µg) + methoxamine (10 µg) (plate C), dexmedetomidine (10 µg) (plate D) or methoxamine (10 µg) (plate E) followed 15 minutes later by the i.t. injection of dynorphin A (1-13) (193 µg, 120 nmol). Control rats (plate A) were pretreated with i.t. saline followed 15 minutes later by a second injection of i.t. saline. Rats were perfused 48 h after the dynorphin injection. Scale bar = 100 µm. Photomicrographs A and C-E were prepared using TMAX (Kodak) film. Because of the severe toxicity with dynorphin in group B, photomicrograph B was prepared using Technical Pan (Kodak) to provide better contrast.

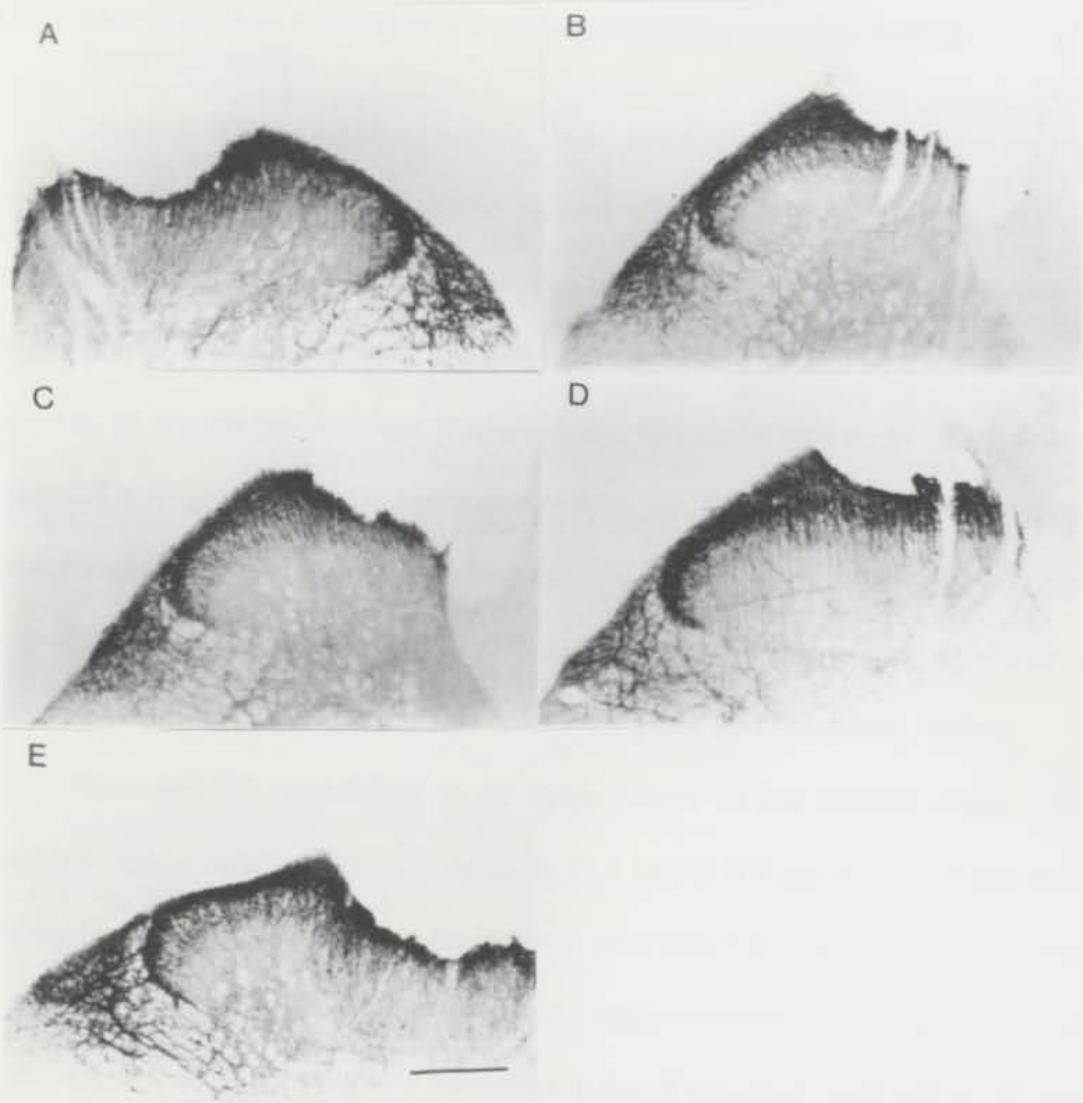


Figure 14 Photomicrographs of substance P (SP)-immunoreactivity in the dorsal horn (L1 segment) of the rat spinal cord after the intrathecal (i.t.) injection of saline (plate B), dexmedetomidine (10 µg) + methoxamine (10 µg) (plate C), dexmedetomidine (10 µg) (plate D) or methoxamine (10 µg) (plate E) followed 15 minutes later by the i.t. injection of dynorphin A (1-13) (193 µg, 120 nmol). Control rats (plate A) were pretreated with i.t. saline followed 15 minutes later by a second injection of i.t. saline. Rats were perfused 48 h after the dynorphin injection. Scale bar = 100 µm. All photographs were prepared using TMAX (Kodak) film.

4.0 DISCUSSION

4.1 Methoxamine Potentiates Dexmedetomidine-Induced Spinal Antinociception

Intrathecal DX produced potent, dose-dependent antinociception in both thermal and mechanical nociceptive assays with ED_{50}s of 0.044 and 0.25 µg, respectively. At doses ≤ 1 µg, this effect was observed without behavioral evidence of sedation or motor dysfunction. Qualitatively similar results with i.t. DX have been reported in the rat tail flick test (Fisher et al., 1991), as well as in other animal species (i.e. dog) using different nociceptive tests (i.e. thermal skin twitch response latency and paw withdrawal to mechanical pinch) (Sabbe et al., 1994). These data are consistent with the well recognized and selective effect of i.t. α_2-agonists on spinal nociceptive transmission (see review by Maze and Tranquilli, 1991).

However, DX was found to be more potent in the present study. For example, ED_{50}s ranging from 1.8 µg (0.2- 11.6 µg) to 4.3 µg (1.7 -10.8 µg) were reported by Sabbe et al. (1994) in the thermal skin twitch and paw withdrawal tests in the dog. In the rat, Fisher et al. (1991) observed that 3 or 10 µg of i.t. DX increased hot plate and tail flick latencies to cutoff values (6 sec) within 15 min. Animals receiving doses of 1 µg did not exhibit any significant antinociception compared to control. Similar results were reported by Kalso et al. (1991). Maximal increases in the rat tail flick latency (5 sec cut-off - 100% MPE) were induced by i.t. doses of ≥ 3 µg. In the rat hot plate (52.5°C) test, Takano and Yaksh (1991) found the ED_{50} (95% CI) of i.t. DX to be 3.1 (0.50 - 17) µg, compared to that of clonidine

and ST-91 with ED_{50}'s of 27.6 (12-71.3) and 5.3 (0.33-60.9) µg, respectively. The order of magnitude difference between the potency of DX in the TF assay in this study and that of Kalso et al. (1991) and Fisher et al. (1991) might be explained by differences in instrumentation, apparent stimulus intensities (as reflected by the baseline latency) and maximum duration of exposure (as determined by the cut-off value) used to determine the tail flick response. Factors such as radiant heat temperatures are known to significantly affect baseline latency and the observed antinociceptive activity (Kawakita, 1987; d'Amore et al., 1992). In the present study, all experments were conducted using the same instrument under indentical testing conditions. Therefore, while differences exist in the absolute potency of DX between these studies, the significant potentiation of DX by MX in the present study is unlikely to be an artifact of the experimental protocol.

The addition of a fixed dose of i.t. MX (10 µg) significantly increased the potency of i.t. DX as illustrated by the leftward shift in the dose-response curve. This corresponded to a 5.5-fold and 25-fold increase in the potency of DX in the tail flick and paw pressure tests, respectively. Methoxamine was chosen for this study for two reasons. First, it produced fewer motor effects in the spinal cord than phenylephrine. Second, it has a slightly greater α_1/α_2 selectivity ratio as determined by the displacement of [^{3}H] prazosin and [^{3}H] p-aminoclonidine from rat spinal cord membranes (Simmons and Jones, 1988). It is important to note that a near-inactive dose of MX was used. By itself, MX (10 µg i.t.) yielded less than 5% MPE in the tail

flick test and was completely inactive (0% MPE) in the paw pressure test. This helped to minimize the unwanted motor effects induced by i.t. α_1-agonists (Howe et al., 1983; Yaksh, 1985), and to distinguish between a possible additive or supra-additive interaction with DX[2]. The results obtained are consistent with: 1) a supra-additive interaction between DX and MX; and b) a spinal site of action. Thus, pretreatment with either i.t. prazosin (2.5 µg) or Wyeth 27127 (0.5 µg) significantly attenuated the interaction between DX and MX confirming the involvement of both major α-adrenoceptor subtypes and presumably the antinociceptive mechanisms to which these two receptor subtypes are coupled. The failure of the low i.t. doses of DX and MX (0.025 µg DX + 10 µg MX and 0.25 µg DX + 10 µg MX), given i.v., to effect antinociception strongly suggests a spinal site of action. This conclusion was further supported by the ability of low dose i.t. antagonists to blunt the potentiation between i.t. DX and MX.

The mechanisms by which α_2-agonists inhibit nociceptive transmission in the spinal cord have been extensively investigated. For example, spinal α_2-adrenoceptors have been shown to inhibit glutamate release from the central terminals of nociceptive primary afferent fibers in the rat (Ueda et al., 1995), and to depress high threshold stimulus-evoked activity in nociceptive specific and/or wide

---

[2] Isobolographic analysis is the preferred method for determining the nature and magnitude of drug interactions but it requires a reliable estimate of the ED_{50} and 95% CI for each drug. Unfortunately, the motor dysfunction induced by i.t. α_1-agonists precludes an accurate determination of the ED_{50} in behavioural tests of nociception.

dynamic range neurons in the cat dorsal horn (Fleetwood-Walker et al., 1985; Omote et al., 1991). Activation of neuronal α_2-adrenoceptors results in the opening of outwardly directed K^+ channels. This causes a hyperpolariation of the cell, which results in the suppression of neuronal firing (Nakamura et al., 1981; Egan et al., 1983). As well, α_2-adrenoceptor activation can inhibit voltage sensitive calcium channels (Williams and North, 1985), thereby suppressing Ca^{++} influx and the fusion of neurotransmitter-filled vesicles with a synaptic membrane. These actions on ion channels are believed to be mediated through a G-protein (Dunlap et al., 1987). As well, a common feature of all α_2 adrenergic receptors is the ability, when activated, to inhibit adenylate cyclase. This latter effect also appears to be important in transmembrane signaling.

In contrast, the mechanism(s) underlying α_1-adrenoceptor mediated antinociception is less clear. α_1-Adrenoceptors have been shown to regulate the level of excitability of neurons rather than participating in the transmission of rapid signals. Activation of α_1-adrenoceptors increases intracellular concentrations of Ca^{++} by several distinct mechanisms. The best understood involves the G-coupled activation of phospholipase C with the subsequent hydrolysis of two second messengers - diacylglycerol and inositol-1,4,5 triphosphate (IP_3). The latter compound stimulates release of intracellular Ca^{++} which mediates a broad range of cellular responses (Goodman and Gilman, 1990). As α_1-adrenoceptors are excitatory in the CNS (Aghajanian and Rogawski, 1983), the ability of α_1-agonists

to effect spinal antinociception strongly suggests that they activate, or at least facilitate, an inhibitory neural input on primary afferent and/or projection neurons in nociceptive pathways. Enkephalin-containing neurons are the most likely candidate for this inhibitory neuron. Enkephalin, one of the three major endogenous opioid peptides, is known to selectively modulate nociceptive transmission in the spinal cord (Yaksh, 1993). Additionally, enkephalin-containing neurons are present in a dense concentration within lamina I and II, which, significantly correspond to the synaptic termination of nociceptive C fibers in the spinal cord (Hunt et al., 1980; Bresnahan et al., 1984). The well-demonstrated potentiation of opioid spinal analgesia, including that mediated with enkephalin analogues, with α-agonists in both animal (Ossipov et al., 1990a; 1990b; Omote et al., 1991; Roerig et al., 1992) and human studies (Motsch et al., 1990; Gordon et al., 1992; Siddall et al., 1994) lend support to the possibility of an interaction between α_1-adrenoceptors and enkephalin neurons in the modulation of nociception. Indeed, Bautista (1996) has recently demonstrated that i.t. NE, but not DX, exhibits cross-tolerance to i.t. DADLE (a δ-selective opioid agonist) in the rat, implicating an spinal enkephalin-dependent mechanism in α_1-mediated anitnociception. Overall, these results indicate that α_1- and α_2-adrenoceptors utilize distinct antinociceptive mechanisms thereby fulfilling a critical requirement for a supra-additive interaction.

Recent molecular biological data point to the existence of multiple subtypes

of α_1- and α_2-adrenoceptors (i.e. $\alpha_{1A, 1B \text{ and } 1C}$, $\alpha_{2A, 2B, \text{ and } 2C}$) (Bylund, 1985, 1988). In this regard, pharmacological studies have shown that spinal antinociception is mediated by more than one α_2-adrenoceptor subtype. For example, i.t. DX appears to effect antinociception through α_{2A} receptors while the effects produced by ST-91 appear to be mediated by α_{2B} receptors (Takano et al., 1992). It was not the intention of the present study to elucidate the specific receptor subtypes underlying the antinociceptive interaction. Of relevance to this discussion, however, is the report that prazosin does exhibit antagonist activity at certain α_2-adrenoceptors ($\alpha_{2\text{-non-A}}$) (Takano and Yaksh, 1992). However, this effect was observed at an i.t. ED_{50} dose of 38 µg for ST-91 and doses exceeding 100 µg for DX and clonidine (Takano and Yaksh, 1992); doses 3 - 10 times greater than that used here. While this is admittedly a relatively small difference in absolute terms, the range of effective doses that can be administered i.t. is limited. Regardless, the fact that we are using a low dose of MX, a selective α_1-agonist not known to bind to α_2-adrenoceptors (Timmermans et al., 1984) does suggest an interaction between distinct subtypes of α_1- and α_2-adrenoceptors. However, these data do not exclude the possibility of an antinociceptive interaction between subtypes of the α_2-adrenoceptor. This remains to be explored.

While it is unknown if the supra-additive interaction observed in this study also occurs in humans, these results suggest that there could be merit in using a combination of α_1- and α_2-agonists for spinal analgesia. At the very least, this

combination would allow a reduction in the analgesic dose of the α_2-agonist; an important advantage given the marked bradycardia and hypotension that routinely occur after the i.t. or epidural administration of α_2-agonists (Pettinger, 1980; Redmond, 1981; Virtanen, 1985). Equally important is the potential for improved analgesic effectiveness with such a combination. This effect might even be further improved by the addition of a low dose opioid or other non-adrenergic analgesic drug. The use of a long acting non-selective α-agonist, although more convenient, is unlikely to be as effective as using a combination of α_1- and α_2-agonists. The relative contribution of α_1- and α_2-activity would effect the nature of the analgesic interaction. This factor would be extremely difficult to control using a single non-selective agonist. These issues remain to be investigated and could be the subject of future research.

4.2 Repeated Doses of Intrathecal DX, Alone and in Combination with MX, Do Not Cause Spinal Neurotoxicity

In view of the safety issues related to spinal drug administration, the effect of repeated i.t. injections of DX, alone and in combination with MX, on gross motor function and spinal immunohistochemistry was investigated. This concern was predicted on the following facts. DX is a potent vasoconstrictor. Coughlan et al., (1992) demonstrated dose-dependent constriction of dog middle cerebral arteries (72.8 ± 5.7% of the maximal response induced by 40 mM KCl). Interestingly, the

selective α_2-adrenergic antagonist atipamezole only partially blocked this effect at DX concentrations > 10^{-5} M, suggesting some activation of α_1-adrenergic receptors. Rat cerebral blood vessels contain post-junctional α_1-adrenoceptors that mediate contraction of vascular smooth muscle (McCulloch and Edvinsson, 1984). Moreover, α_1- and α_2-adrenoceptors cause vasoconstriction through independent effector mechanisms (see Section 1.6); an important fact considering the possible use of α_1- and α_2-agonist combinations. The physiological relevance of this effect is illustrated by the >45% reduction ($p< 0.05$) in cerebral blood flow induced by DX, with little or no effect on the cerebral metabolic oxygen demand in anesthetized dogs (Zornow et al., 1990). At the spinal level, α-adrenoceptor agonists decrease SCBF in experimental animals by as much as 44% (see Section 1.6), and non-adrenergic agents that cause a comparable reduction in SCBF are known to produce sensory, autonomic and motor dysfunction as well as histopathological damage to spinal cord cells (see Sections 1.4 and 1.5). For example, i.t. endothelin (0.03 µg) induced complete motor paralysis with a marked loss of CGRP-IR in spinal motoneurons of the rat (Hökfelt et al., 1989). Finally, cells in the spinal ventral horn are especially sensitive to interruptions in SCBF since the left and right sides of the ventral grey matter are supplied by only one spinal artery (anterior spinal artery) (Cousins and Bridenbaugh, 1988).

Twice daily injections of i.t. DX or DX + MX for four days produced no behavioral evidence of spinal neurotoxicity in the rat. Thus, rats exhibited normal

posture, gait and motor reflexes 2 h after the last (8th) injection of DX or DX+MX, as compared to saline controls. While both treatments (10 µg DX and 10 µg DX + 10 µg MX) produced severe sedation (100% MPE in the tail flick test and an inability to perform the rota-rod test), animals completely recovered after each injection. The same doses, given i.v., yielded a similar effect. These results, and the fact that sedation is mediated supra-spinally, indicate a vascular redistribution of DX from the spinal cord. DX is highly lipophillic (Savola et al., 1986) and therefore is able to readily cross blood brain barrier.

Gross examination of the spinal cords from these animals revealed no evidence of inflammation, haemmorrhage or necrosis near L1 (adjacent to the tip of the catheter). Subsequent immunohistochemical staining revealed no detectable change in either the pattern or intensity of SP- and CGRP-IR in the dorsal horn of DX or DX + MX treated rats as compared to the saline-treated controls. CGRP-IR in motoneurons in the ventral horn also remained unchanged from that of the saline controls. In contrast, a single neurotoxic dose of i.t. dynorphin produced irreversible loss of the stepping and tail flick reflexes. Gross examination of the spinal cords from these rats revealed haemmorrhaging around the L1 segment. Although SP- and CGRP-IR fibers in the dorsal horn of rats treated with dynorphin were normal in appearance and indistinquishable from those in saline controls, there was a near total loss of CGRP-IR motor neurons. The few remaining CGRP-IR fibers were punctate in appearance, consistent with the severe motor

dysfunction. Intrathecal dynorphin is believed to cause neurotoxicity through a deleterious vasoconstrictive effect (Long et al., 1987; Thornehill et al., 1989; Long et al., 1994) and therefore was a suitable positive control for this study. The results with dynorphin confirm our ability to detect spinal neurotoxicity following i.t. drug administration, and to localize the injury to a discrete region and type of neuron.

Indeed, localized decreases in CGRP-IR, reflecting damage to specific CGRP-containing neurons, have been observed in the DRG and/or spinal cord of experimental animals following dorsal rhizotomy, (Pohl et al., 1990), lumbar sympathectomy (Sekiguchi et al., 1996), transection of the sciatic nerve (Groves et al., 1996), chronic constriction injury or crushing of a peripheral nerve (Sommer and Myers, 1995), or treatment with selected neurotoxins (capsaicin - Pohl et al., 1990; cisplatin - Whitaker-Azmitia et al., 1995). Similar changes in spinal CGRP-IR have been reported in humans who sustained traumatic spinal cord injury (Melinek et al., 1994). Thus, CGRP-IR has been used as an immunohistochemical marker of neuropathy and neurotoxicity arising from a range of insults. Of relevance to the present study is the reported decrease in CGRP-IR in spinal motoneurons after local vasoconstrictive injury (Hökfelt et al., 1989).

To assess possible neurotoxic injury to primary afferent terminals in the dorsal horn, changes in both SP-IR and CGRP-IR were examined. However, as SP-IR sensory fibers have been demonstrated, *in vitro,* to be more senstitive to capsaicin than CGRP-IR sensory fibers (Jeftinija et al., 1992), SP-IR was chosen

as the primary marker to examine the potential neurotoxicity of α-agonists to primary afferent fibers. Substance P is a undecapeptide that is densely localized in the superficial laminae of the dorsal horn of the spinal cord (Morhland, 1982), specifically in small diameter sensory afferent fibers (Hökfelt et al., 1975, Hunt, 1983). Substance P levels in the dorsal horn are dramatically reduced following dorsal root rhizotomy (Pohl et al., 1990), unilateral dorsal root ganglionectomy (Howe et al., 1987) or exposure to the selective neurotoxin, capsaicin (Nagy et al., 1983; Pohl et al., 1990) changes that correlate with disturbances in nociception (Abelli et al., 1993). CGRP is also present in small diameter primary afferent fibers and, like SP, is depleted from these fibers by pretreatment with capsaicin (Pohl et al, 1990). Thus, injury to small diameter primary afferent fibers by sub-acute administration of α-agonists should have been detectable using these immunohistochemical markers.

The absence of toxicity in this study was not due to problems of spinal drug delivery. Indeed, antinociception and motor function were assessed after each dose confirming effective drug administration and consistent pharmacological activity throughout the treatment schedule. Additionally, there appeared to be an absence of significant tolerance to the multiple injections of DX and DX + MX. There was a modest reduction in the time course of motor effects (rota-rod test) but the apparent magnitude of antinociception remained unchanged. However, the dose of DX used in the neurotoxicity study exceeded 100% MPE in the tail flick test

thereby limiting the detection of tolerance on the basis of magnitude. The apparent lack of tolerance in this study is in agreement with a recent study by Hayashi et al. (1995). After seven days of continuous subcutaneous infusion of DX, the analgesic response to an median effective dose of DX in the tail flick test remained unaltered compared to that of saline-infused rats. Thus, the absence of pharmacodynamic tolerance to twice daily injections of i.t. DX for four days in the present study is not surprising.

Drug dose is a critical factor in any neurotoxicity study. There were no published neurotoxicity data for α-agonists (see Section 1.6) from which to select a dose of i.t. DX for this study. Exposure of experimental animals to toxic agents in high doses is a necessary and valid method of discovering possible hazards in humans (Klaassen, 1995). Therefore, a dose of 10 µg, equivalent to 100 times the ED_{50} in the tail flick test, and 25 times the ED_{50} in the paw pressure test, was used. This satisfied the requirement for a high dose while remaining within a realistic range of clinical analgesic doses of i.t. clonidine (calculated per kg of body weight) (Racle et al., 1988). Of course, the absence of toxicity at 10 µg does not exclude its possibility at higher doses. However, the latter would certainly exceed the upper range for clinical analgesia of α-agonists and so the relevance of such studies would be questionable. Since it was not our intention to asess the safety of i.t. MX *per se*, but rather to investigate the safety of a threshold dose of MX co-injected with i.t. DX, the dose of MX in the neurotoxicity study remained unchanged at 10

μg. The absence of toxicity with this combination suggests that the co-activation of α_1- and α_2-adrenoceptors, at least at these doses, does not exceed a threshold for vasocontriction-induced spinal neurotoxicity in the rat.

4.3 Pretreatment With Intrathecal DX, MX and DX + MX Protects Against Dynorphin-Induced Spinal Neurotoxicity

The demonstrated lack of neurotoxicity with i.t. DX and MX in this animal model raised an important issue; namely the safety of these α-agonists in combination with a drug known to adversely affect SCBF and neuronal viability. Thus, the influence of i.t. DX and MX on a neurotoxic dose of i.t. dynorphin was investigated using the same behavioral and immunohistochemical indices as used in DX and MX neurotoxicity study.

In saline pretreated rats, i.t. dynorphin resulted in a permanent loss of the tail flick and stepping reflexes, permanent hindlimb paralysis rendering the rats unable to perform the rota-rod test, and marked cellular necrosis in the ventral horn. These results were identical to those observed in the DX and MX neurotoxicity study. In contrast, pretreatment with i.t. DX, MX or DX+MX significantly attenuated the neurotoxic effect of dynorphin. All four rats receiving the DX+MX combination, three of the five receiving DX and three of four receiving MX had a normal stepping reflex and normal gait, 24 h after dynorphin. In those animals exhibiting protection from dynorphin toxicity, their 24 h rota-rod scores were only marginally below their

baseline values and significantly different from those of the saline-dynorphin group. A similar trend was observed with the tail flick reflex. Immunohistochemically, the number of CGRP-IR neurons and the intensity of staining in the ventral horn were intermediate between that of the saline+saline and saline+dynorphin groups. CGRP- and SP-IR in the dorsal horn was equivalent among all the treatment groups. Thus, rather than exacerbating the neurotoxicity, pre-treatment with i.t. DX and/or MX blunted the insult inflicted by i.t. dynorphin up to 48 h after injection.

In view of the well-established effect of α-agonists on vascular smooth muscle, there was a real possibility that i.t. DX and MX might augment the vasoconstrictive and thus neurotoxic effect of i.t. dynorphin. Therefore, an attempt was made to identify a threshold dose of dynorphin that would induce minimal but detectable neurotoxicity. In a preliminary dose-response experiment, 60 nmol (96 µg) failed to produce any behavioural or immunohistochemical evidence of toxicity. The 80 (128 µg) dose produced transient hindlimb paralysis, reversible loss of the tail flick reflex but no histopathological evidence of spinal toxicity. The 100 (160 µg) dose resulted in hindlimb dyfunction that lasted up to the time of perfusion (48 h), irreversible loss of the tail flick reflex but still no imunohistochemical evidence of toxicity. At a dose of 120 nmol (193 µg), i.t. dynorphin resulted in paralysis that persisted up to the time of perfusion 48 h later, irreversible loss of the stepping and tail flick reflexes and an attenuation of CGRP-IR neurons in the vental horn. These data indicate the absence of a distinct dose-response relationship using both

behavioral and immunohistochemical indices. Rather, dynorphin neurotoxicity appears to be an all-or-none phenomenon, consistent with the proposed mechanism(s) underlying this outcome (see section 4.5).

4.4 Absence of Neurotoxicity With Intrathecal DX and MX

There are several possible explanations that might account for the absence of spinal neurotoxicity with these potent vasoconstrictors. One is that the degree of vasoconstriction induced by i.t. DX and MX was not sufficient to trigger the secondary events that underly neurotoxicity. The threshold decrease in SCBF (extent/duration) required to elicit spinal neurotoxicity has not yet been defined in hemodynamic studies. In studies of cerebral blood flow, it has been suggested that any perifocal tissue with a blood flow of <50% of control is at risk of injury/death (Siesjö, 1992a). However, these results are based on short term (1 -7 day) studies. As reviewed in Section 1.6, antinociceptive doses of i.t. α-agonists decrease SCBF by up to 39%; an effect lasting for up to 45 min (Gordh et al., 1986a; Crosby et al. 1990). These changes are comparable in magnitude to those caused by neurotoxic doses of i.t. dynorphin and suggest that the ventral spinal cord may be more sensitive to decreases in SCBF than either the dorsal spinal cord or cerebral tissue.

Alternatively, α-agonists could benefit from physiological compensatory mechanisms (autoregulation of blood flow) and/or concurrent pharmacological

effects (hypothermia, direct inhibition of neuronal activity) that prevent the metabolic disturbances leading to ischemic cell death. Cerebral and spinal blood vessels have the inherent capacity to regulate blood flow in the face of changing arterial blood pressure (West, 1985). This phenomenon is known as autoregulation. In the rat, autoregulation of SCBF occurs between 60 to 120 mm Hg (mean arterial pressure) (Hickey et al., 1986). This is consistent with autoregulation of cerebral blood flow in the range of 70 to 150 mm Hg (Rapela and Green, 1964; Agnoli et al., 1968; Strandgaard et al., 1974; Hernández-Perez et al., 1975). As a result, local autoregulatory mechanisms could compensate for α-agonist induced changes in vascular tone, thereby maintaining adequate tissue perfusion and cell homeostasis.

The two most obvious pharmacological effects of α_2-agonists that would prevent ischemic injury are: a) hypothermia; and b) direct neuronal inhibition. The influence of temperature on ischemic brain injury and the protective effect of hypothermia are well recognized (see review by Colbourne et al., 1997). For example, brief post-ischemic decreases of 3-4°C (rectal temperature) in the gerbil yielded histological evidence of cerebral protection in the CA1 region of the hippocampus up to 7 days post-ischemia (Buchan and Pulsinelli, 1990; Chopp et al., 1991). Of relevance to the present study is the well known neuroprotective effect of mild to moderate intra-ischemic hypothermia (Busto et al., 1987; Chopp et al., 1989; Minamisawa et al., 1990a,b; Welsh et al., 1990; Iwai et al., 1993). Indeed, intra-ischemic hypothermia (30°C brain temperature) unequivocally

protected neurons in the CA1 region of the gerbil on the basis of behavioral, physiological and histological endpoints (Nurse and Corbett, 1994). Protection was still evident 3 weeks after carotid artery occlusion (Nurse and Corbett, 1994). Using histological indices, Dietrich et al. (1993) reported significant neuroprotection with intra-ischemic hypothermia for up to two months in the rat. In a similar study, permanent neuroprotection was reported in the rat using a battery of behavioral and functional tests (Green et al., 1992). In this regard, the systemic administration of α_2-agonists has been shown to cause dose-dependent hypothermia in rodents (Minor et al.,1989; Menon et al., 1990; MacDonald et al., 1991). Decreases in rectal temperature ranged from 3-6°C and persisted for up to 3 h after injection. That this effect is centrally mediated is indicated by the failure of the peripherally-selective α_2-antagonist, L-659,066 to block i.p. clonidine-induced hypothermia (Menon et al., 1990). In the present study, i.t. DX, alone and in combination with MX, decreased rectal temperature by 3.4 ± 0.3°C and 3.3 ± 0.4°C, respectively for up to 4 h. Such temperature changes should be sufficient to attenuate any adverse vasoconstrictive effects induced by the perispinal administration of α-agonists, based on the neuroprotective effect of intra- and post-ischemic hypothermia in animal models of global ischemia.

In the CNS, the activation of α_2-adrenoceptors hyperpolarizes the cells upon which they reside, thereby inhibiting voltage sensitive calcium channels, Ca^{++} influx, and neuronal firing (see Section 4.1). That an elevation in cytosolic calcium during

ischemia contributes to cell injury is well recognized (Choi et al., 1990a,b; Siesjö, 1992a,b). Therefore, attenuation of this calcium overload would be expected to ameliorate neuronal injury. The activation of α_2-adrenoceptors has been shown to inhibit calcium conductance in neurons (Dunlap and Fichbach, 1981; Lipscomb et al., 1989; Boehm and Huck, 1991), and to decrease the duration of the calcium-dependent action potentials *in vitro* (Dunlap and Fichbach, 1981; Canfield and Dunlap, 1984). Of relevance to the present study is the report by Bickler and Hansen (1996) that the α_2-agonists, mivazerol (1 μM) and clonidine (0.1 μM), significantly inhibited the early rise in cytosolic calcium concentration in rat hippocampal brain slices evoked by glutamate (3 mM) during hypoxia; conditions used to mimic those of ischemia. Mivazerol and clonidine also decreased glutamate release and cellular damage as assessed by leakage of lactate dehydrogenase. In an *in vivo* study, the i.p. administration of 10 and 100 μg/kg DX reduced the infarct size in a rat model of incomplete cerebral ischemia (Hoffman et al., 1991b). Normal brain temperature (37°C) was maintained throughout the experiment thereby excluding hypothermia-mediated neuroprotection and a supporting a role for direct α_2-mediated neuronal inhibition. These results, and those of the present study indicate that α_2-adrenergic agonists elicit at least two concurrent pharmacological effects that are capable of offsetting adverse hemodynamic changes in the CNS.

4.5 Mechanisms of Dynorphin-Induced Spinal Neurotoxicity

The necrosis arising from the spinal injection of dynorphin does not appear to be a direct toxic effect of the drug. Addition of dynorphin to cultured spinal cord neurons (yielding a final concentration of up to 1 mM) did not alter cell viability (Long et al., 1994). Rather, dynorphin neurotoxicity appears to result from drug-induced spinal cord ischemia. Using radiolabelled microspheres, Long et al. (1987) reported a dose-related decrease in rat SCBF 10 min after the i.t. injection of dynorphin A (1-13). The greatest reduction occurred in the spinal segments closest to the tip of the i.t. catheter (lumbosacral, 37-75% and thoracic, 38%). In a similar study, 20 nmol of i.t. dynorphin A (1-13) significantly decreased SCBF (up to 50%) in segments 2 cm caudal and 1 cm rostral to the tip of the catheter (Thornehill et al., 1989). These hemodynamic changes were temporally associated with a 2.6-2.8 fold increase in lactate concentration in the spinal CSF (Long et al., 1994), indicative of an increase in anaerobic metabolism. Interestingly, co-administration of the vasodilator hydralazine reduced the elevation in lactic acid concentration and eliminated the paralytic and neuropathological effect of dynorphin A (Long et al., 1994).

A sustained imbalance between blood flow and metabolic demand in the CNS elicits a cascade of events that results in cell death. One of these events is the accumulation of excitatory amino acids in the extracellular fluid. The CSF concentration of glutamate increased 4-fold (6.4 μM) and aspartate 3-fold (6.3 μM)

in rat spinal cord following a neurotoxic dose (20 or 40 nmol) of i.t dynorphin A (Long et al., 1994). This increase was not due to the proteolysis of exogenous dynorphin as glutamate is not a constituent of this peptide. Even if it were, a 20-40 nmol dose of dynorphin A would not yield a concentration of glutamate in the μM range, a conservative estimate at best. Dynorphin could enhance the release of EAAs by a direct interaction with presynaptic neurons and/or as a result of local ischemia caused by dynorpin-induced vasoconstriction. However, the extracellular concentration of glutamate and aspartate remained unchanged in spinal cord cell cultures exposed to 100 and 500 μM of dynorphin A (Long et al., 1994). These results indicate that exposure to dynorphin *in vivo*, but not *in vitro*, triggers the release of EAAs thereby suggesting that accumulation is an ischemic-related event.

Excitatory amino acids have been implicated in many forms of spinal cord injury through their activation of N-methyl- D-aspartate (NMDA) receptors (Meldrum and Garthwaite, 1990; Long et al., 1994). In this regard, the motor dysfunction and neuropathology induced by i.t. dynorphin appears to be mediated, at least in part, by NMDA-receptors. This is indicated by the long term protective effect afforded by a series of competitive and non-competitive NMDA-receptor antagonists (ketamine, dextromethorphan or MK-801) against dynorphin toxicity in the rat. None of these antagonists altered the effect of i.t. dynorphin on lumbosacral blood flow or the subsequent increase in lactic acid concentration. Thus, in the absence of any temperature change (body temperature was not monitored by Long et al., 1994), the

neuroprotective effect is best explained by the blockade of NMDA-receptors whose excessive activation by EAAs elicits secondary pathophysiological events. The binding of EAA's to NMDA-receptors causes a rise in intracellular calcium and sodium (Vornow and Coyle, 1991; see section 1.5). Such a change would clearly aggravate an already unstable condition initiated by ischemia (i.e. ATP depletion, degradation of macromoleclues important to membrane and cytoskeletal integrity, production of metabolic acids) leading to cellular edema and lysis.

There are three other possible ways by which dynorphin may effect neurotoxicity via the NMDA-receptor-channel complex. Dynorphin may bind to a site on the complex to increase EAA binding at the NMDA receptor (i.e. an allosteric effect) thereby opening the ion channel. In fact, dynorphin has been shown to enhance the binding of the NMDA-selective ligand ([^{3}H]CGP-39653) to rat brain membranes *in vitro* (Dumont and Lemaire, 1994). If a comparable effect occurs with glutamate/aspartate *in vivo*, dynorphin would not only lead to the accumulation of EAAs, but would also enhance their binding to the NMDA-receptor (and presumably their ability to open the ion channel). Alternatively, dynorphin may facilitate the opening of the channel by binding directly to the NMDA receptor. Topical application of dynorphin to the spinal cord potentiated a C-fiber evoked, NMDA-mediated reflex in the rat (Caudle and Isaac, 1988). This effect is known to precede: a) the permanent loss of this reflex (Caudle and Isaac, 1988); b) motor paralysis (Skilling et al., 1992); and c) the extracellular accumulation of EAAs

(Skilling et al., 1992) induced by dynorphin. Given that dynorphin A_{1-13} is also known to displace L-[^{3}H] glutamate binding from rat cortical membranes *in vitro* (Massardier and Hunt, 1989), these results are consistent with a direct interaction of dynorphin at the NMDA-receptor. Lastly, dynorphin may facilitate channel opening by binding to a separate non-opioid (PCP?) site on the NMDA-receptor ion channel complex. Fragments of dynorphin (e.g. A_{2-17}) that do not bind to κ-opioid receptors induce NMDA-sensitive spinal neurotoxicity in the rat (Bakshi and Faden, 1990). Although these results do not differentiate between NMDA- versus other non-opioid sites on the complex, *in vitro* binding data support an interaction between dynorphin and the PCP receptor (Shukla et al., 1992). The relevance of these data to dynorphin toxicity is supported by the observation that metaphit, a PCP receptor antagonist, blocked the motor dysfunction induced by dynorphin (Shukla et al., 1992). These possible interactions are illustrated in Figure 15.

In summary, current evidence suggests that the pathophysiological effects triggered by i.t. dynorphin are related to some modulation of spinal NMDA receptor activity. However, the nature of the interaction between dynorphin and the NMDA-receptor ion channel complex, and thus the precise mechanism(s) of neurotoxicity have not yet been determined.

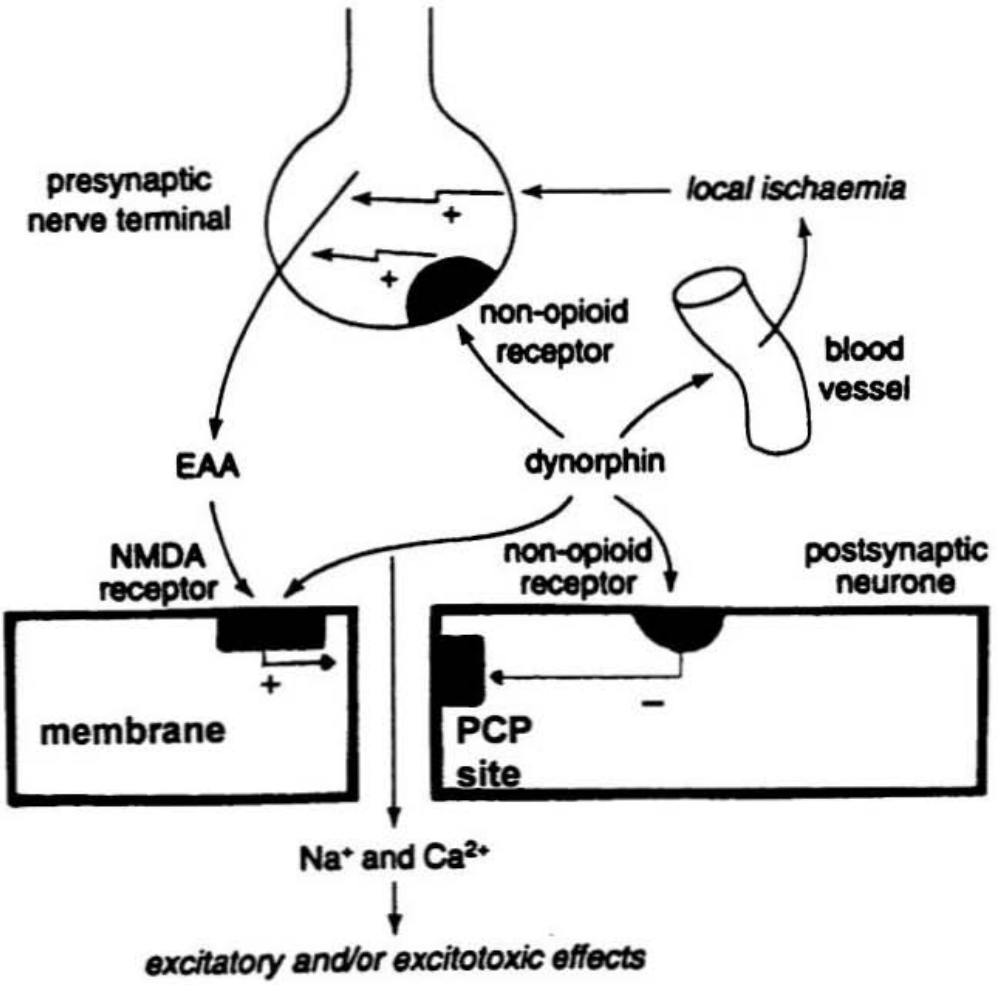


Figure 15 Possible mechanisms of action of dynorphin on the NMDA receptor complex. The facilitatory effects of dynorphin A and related peptides on NMDA receptor-mediated activity may be due to a direct interaction of the peptide with the receptor complex. Dynorphin was found to interact with both phenycyclidine (PCP) and NMDA receptors on the postsynaptic neuron. Dynorphin may also enhance the release of glutamate either by a direct interaction with presynaptic neurons or as a result of local ischemia caused by the constriction of small blood vessels. EAA, excitatory amino acids. (Source: Figure 2, Shukla and Lemaire, 1994)

4.6 Possible Neuroprotective Mechanisms of i.t. DX and MX Against Dynorphin-Induced Spinal Neurotoxicity

Although i.t. DX and MX proved free of neurotoxicity when given alone (see section 4.4), there was concern that these drugs, as vasoconstrictors, might exacerbate the neurotoxic effect of i.t. dynorphin. Surprisingly, pretreatment with i.t. DX and/or MX provided functional and histological protection for up 48 h after i.t. dynorphin. The mechanisms by which DX and MX might ameliorate i.t. dynorphin toxicity may or may not be the same as those responsible for the apparent safety of these drugs when given alone (autoregulation of blood flow, hypothermia, hyperpolarization - see section 4.4).

Normal autoregulatory mechanisms are clearly overwhelmed by i.t. dynorphin. A sustained decrease in SCBF and the accumulation of lactate acid and excitatory amino acids in spinal CSF have been consistently observed in i.t. dynorphin-treated animals (see section 4.5). As vasoconstrictors, α-agonists would be expected to place additional stress on this compensatory process. Thus, autoregulation of blood flow is unlikely to play a role in the neuroprotective effect of α-agonists.

4.6.1 Hypothermia

Hypothermia, a side-effect of α_2-agonist administration, is a possible mechanism for the α_2-agonist mediated neuroprotection from dynorphin toxicity

(see section 4.4). α_2-Agonists induce hypothermia in rodents by altering peripheral blood flow and by decreasing non-shivering thermogenesis (LoPachin and Rudy, 1983). These effects are centrally mediated. In the present study, i.t. DX and DX + MX decreased rectal temperature by 3.4 ± 0.3°C and 3.3 ± 0.4°C, respectively. The neuroprotective effect of hypothermia is well recognized (section 4.4) and has been used clinically for spinal cord protection during aortic occlusion (Pontius et al., 1954) and following spinal surgery (Albin et al., 1968). The precise mechanisms underlying this neuroprotection are unknown. However, hypothermia has been shown to: a) increase SCBF by 24-37% in pentobarbital anesthetized rats (Iwai et al., 1991); b) decrease glutamate and aspartate release in the peri- and/or post-ischemic period (Globus et al., 1988; Busto et al., 1989; Baker et al., 1992); c) reduce metabolic activity, thereby decreasing the rate of ATP depletion and the associated accumulation of inorganic phosphate and lactic acid (Sutton et al., 1991; Yager and Asselin, 1996); d) inhibit certain intracellular calcium-activated enzymes such as lipases and proteases; and e) preserve the activity of other calcium-dependent enzyme systems (e.g. calcium/calmodulin dependent protein kinases, protein kinase C) (Simon et al., 1984; Vematsu et al., 1988; Churn et al., 1990a; 1990b; Cardell et al., 1991; Scheinberg, 1991) and the synthesis of ubiquitin, an important factor in the disposal of proteins arising from hypoxia and ischemia (Yamashita et al., 1991). All these effects would serve to protect cells from ischemic injury.

4.6.2 Hyperpolarization

Direct inhibition of neuronal activity is also a possible mechanism by which α_2-agonists may protect against dynorphin toxicity. The pharmacological activation of presynaptic α_2-adrenoceptors has been found to inhibit the release of excitatory amino acids in rat spinal cord slices (Ueda et al., 1995) by inhibiting voltage sensitive Ca^{++} channels (Williams and North, 1985). Thus, co-administration of DX with dynorphin would be expected to inhibit the release of EAA that is known to occur with dynorphin-induced ischemia. Post-synaptically, α_2-adrenoceptors hyperpolarize cells by opening outwardly directed K^+ channels (Williams and North, 1985). Both of these effects would serve to attenuate the potentially catastrophic influx of Na^+ and Ca^{++} evoked by the accumulation of glutamate and aspartate (see section 4.4).

Stimulation of presynaptic α_2-adrenoceptors by DX has also been shown to inhibit catecholamine release (Johansson and Ehrenstrom, 1988). During cerebral ischemia, there is increased release of norepinephrine and dopamine (Globus et al., 1988; Globus et al., 1989; Gustafson et al., 1991), and NE, applied topically to the CNS, has a direct toxic effect on cortical neurons (Stein and Cracco, 1982). If the ischemia induced by i.t. dynorphin triggers a toxic release of catecholamines, then DX would be expected to attenuate both the release and the associated toxicity. Pretreatment with i.p. DX (30 min before carotid artery ligation and hemorrhagic hypotension) improved neurological outcome in the rat, and

decreased plasma catecholamine concentration in a dose-dependent manner (Hoffman et al., 1991b). This neuroprotective effect was blocked by co-administration of atipamezole, an α_2-adrenergic antagonist. Postischemic administration of DX (10 min after arterial occlusion) also had a protective effect in a rabbit model of focal cerebral ischemia however the relevant receptors were not identified in this study (Maier et al., 1993).

4.6.3 Imidazoline Receptor Mechanism(s)

The neuroprotective effect of DX could be mediated by receptors other than those of the α_2-subtype. Hoffman and colleagues (1991a,b) demonstrated that pretreatment with i.v. clonidine or i.p. DX improves the neurologic outcome in rats subjected to incomplete global ischemia, an effect replicated by the α_2-antagonist, idazoxan (Gustafson et al., 1989; 1990). This apparent paradox could be reconciled by the fact that idazoxan, clonidine and DX have affinity for the imidazole-preferring receptor. Moreover, activation of this non-adrenergic receptor has been shown to exert a protective effect against focal cerebral ischemia in the rat (Maiese et al., 1992). Interestingly, the selective non-imidazole α_2-antagonist SKF 86466 did not protect against ischemia (Maiese et al., 1992). The imidazole receptor complex is indeed functionally coupled as demonstrated by the hypotension evoked by the binding of clonidine, idazoxan or rilmenidine to imidazole receptors in the RVLM (Reis et al., 1992). [^{3}H] Dexmedetomidine has

also been shown to bind to a novel imidazole binding site in the adult rat spinal cord (Savola and Savola, 1996). Thus, the imidazole-preferring receptor, and not α_2-adrenoceptors, may elicit the neuroprotective effect of DX and DX+MX in the spinal cord.

The exact mechanisms underlying the neuroprotective effect are unknown but at least two possibilities exist. Imidazole compounds such as 1-(2-trifluoromethylphenyl) imidalzole (TRIM) have been shown to inhibit mouse cerebellar neuronal nitric oxide synthase (NOS) activity *in vitro* (IC_{50} = 0.32 μM)(Handy et al., 1996; Handy and Moore, 1997). Under the experimental conditions used, TRIM appeared to compete with the substrate L-arginine for NOS. This observation could be important given the putative role of NO in neurotoxicity following excessive NMDA receptor activation (Dawson et al., 1991,1993) and the demonstrated neuroprotective effect of NOS inhibition (Maiese et al., 1997). Alternatively, imidazole compounds activating mitochondrial I_2 receptors in astrocytes may reduce Ca^{++} uptake into neurons by stimulating the uptake of Ca^{++} into astrocytes (Reis et al., 1994). The deleterious effects of $[Ca^{++}]_i$ are summarized in section 1.5.

4.6.4 Possible Neuroprotective Mechanisms of MX

Unlike the protection afforded by pretreatment with DX and DX+MX, the neuroprotection observed with MX against dynorphin's spinal toxicity is surprising

and difficult to explain. MX is an α_1-selective adrenoceptor agonist (Simmons and Jones, 1988) with a predominantly depolarizing effect on neurons. Thus, direct post-synaptic neuronal inhibition as induced by α_2-agonists cannot account for the protective effect of MX. However, indirect inhibition secondary to the facilitation of other inhibitory inputs (e.g. enkephalinergic inhibition) by MX is possible. Indeed, i.t. MX-induced facilitation of spinal enkephalinergic inputs has been implicated in the potentiation of DX antinocieption (see section 4.1; Loomis et al., 1992a,b). Such a facilitation could modulate the toxic release of excitatory amino acids in the spinal cord. The activation of μ/δ-opioid receptors by enkephalin has also been shown to hyperpolarize neurons in the substantia gelatinosa of rat spinal cord slices (Yoshimura and North, 1983).

The central administration of MX does not reduce body temperature in experimental animals (see Results). Thus, MX lacks a putative and presumably important neuroprotective feature of DX. In addition, drugs lacking an imidazole moiety like MX have extremely low affinities for the I_2-imidazole receptor (Hosseini et al., 1997). This observation argues against a non-adrenergic imidazole-mediated neuroprotective effect like that attributed to α_2-agonists like DX.

As introduced in Section 1.5, the metabolic consequences of ischemia are numerous and complex. Thus, the amelioration of dynorphin toxicity by MX could occur at one or more of the secondary events arising from ischemia (see Figure 2). To our knowledge, this is the first report describing a neuroprotective effect with

centrally administered α_1-agonists. The results provide no information about the mechanism(s) underlying this effect and there is a paucity of information in the literature to account for such a result. Before detailed mechanistic studies are undertaken, it will be necessary to test the effects of MX and other α_1-agonists in selected animal models of ischemia. These experiments would determine: 1) the duration of neuroprotection afforded by these drugs; 2) if the neuroprotection is unique to i.t. dynorphin or if it is effective against general ischemic injury; and 3) if the neuroprotective effect extends to all α_1-agonists with differing physicochemical properties. Given the multitude of potential sites of action of MX, the elucidation of the exact mechanism(s) is likely to involve a significant investment of time and resources.

4.7 Summary

The results of the present study raise a number of other important questions. Is the duration of neuroprotection afforded by α-agonists permanent or do they only delay the development of neurotoxicity? How soon must these drugs be given after dynorphin to modify the toxicity? What is their spectrum of activity against other forms of ischemic insult? What are the relative contributions of direct inhibitory versus indirect hypothemic effects of α_2-agonists in preserving neuronal function? Additionally, does the lack of neurotoxicity extend to other species, including humans? Answers to these question could provide important clues to the design

of other neuroprotective agents, or combinations of agents in the treatment of ischemic injury. At the very least, the results of this study provides further evidence for the safety of i.t. α-agonists using an animal model that is vulnerable to ischemic injury in the spinal cord, and for their potential use as neuroprotective agents.

REFERENCES

Abelli, L., P. Geppetti and C.A. Maggi. 1993. Relative contribution of sympathetic and sensory neurves to thermal nociception and tissue trophism in rats. Neuroscience. **57(3)**:739-745.

Agnoli, A., C. Fieschi, L. Bozzao, N. Battistini and M. Prencipe. 1968. Autoregulation of cerebral blood flow. Studies during drug-induced hypertension in normal subjects and in patients with cerebral vascular diseases. Circ. Res. **38**:800-812.

d'Amore, A., F. Chiarotti and P. Renzi. 1992. High-Intensity nociceptive stimuli minimize behavioural effects induced by restraining stress during the tail flick test. J. Pharmacol. Toxicol. Methods. **27(4)**:197-201.

Aburto, T., A. Jinsi, Q-B. Zhu and R.C. Deth. 1995. Involvement of protein kinace C activation in α_2-adrenoceptor-mediated contractions of rabbit saphenous vein. European J. Pharmacol. **277**:35-44.

Aghajanian, A.G. aand M.A. Rogawski. 1983. The physiological role of α-adrenoceptor in the CNS: new concepts from single cell studies. Trends in Pharmacol. Sci. **4**:315-317.

Ahuja, B.R. 1983. Analgesic effect of intrathecal ketamine in rats. Br. J. Anaesth. **55**: 991-995.

Albin, M.S., R.J. White, G. Acosta-Rus and D. Yashon. 1968. Study of functional recovery produced by delayed localized cooling after spinal cord injury in primates. J. Neurosurg. **29**:113-120.

Baker, C.J., S.T. Onesti, K.N.M. Barth, C.J. Prestigiacomo and R.A. Solomon. 1991. Hypothermic protection following middle cerebral artery occlusion in the rat. Surg. Neurol. **36**:175-180.

Baker, A.J., M.H. Zornow, M.R. Grafe et al., 1992. Hypothermia prevents ischemia-induced increases in hippocampal glycine concentrations in rabbits. Stroke. **22**:666-673.

Bakshi and Faden. 1990. Competitive and non-competitive NMDA-antagonists limit dynorphin A-induced rat hindlimb paralysis. Brain Research. **507**:1-5.

Bautista, J.C. 1996. A study of the antinociceptive interaction between intrathecal opioids and α-adrenergic agonists in the spinal cord of the rat. M.Sc. Thesis. Memorial University, St. John's, Newfoundland, Canada.

Bedder, M.D., Kozody, R., Palahniuk, K.J. Cumming, M.O., Pucci, W.R. 1986.

Clonidine prolongs canine tetracaine spinal anaesthesia. Can Anaesth. Soc. J. **33**:591-596.

Bentley, G.A., S.H. Newton and J. Starr. 1978. Studies in the antinociceptive action of α-agonist drugs and their interactions with opioid mechanisms. Br. J. Pharmacol. **79**:125-134.

Berthelson, S. and W.A. Pettinger. 1977. A functional basis for classification of α-adrenergic receptors. Life Science **21**:595-606.

Biberfield, J. 1907. Uber die Dosierung des in den Wirbelkanal Gesprtizen Suprarenins, Deutsche med. Wchnschr. **33**: 549. Quoted by Braun, p. 143.

Bickler, P.E. and B.M. Hansen. 1996. α_2-Adrenergic agonists reduce glutamate release and glutamate receptor-mediated calcium changes on hippocampal slices during hypoxia. Neuropharmacology. **35(6)**:679-687.

Bier, A. 1899. Ver. uber cocainisierung des ruckenmark. Deutsche Zeitschrift fur Chirurgie **51**: 361. Translated in "Classical File", Survey of Anesthesiology. 1962. **6**:352-358.

Bier, A. and A. Donitz. 1904. Ruckenmarksanasthesie. München med. Wchnschr. **1**:593-596.

Bill, S.J., A. Boniface, F. Haroun, R.P. McAdams, N. Lattimer and K.F. Rhodes. 1986. Some pharmacological properties of WY 27127 a more selective alpha$_2$:alpha$_1$-adrenoceptor antagonist than idazoxan in vitro. Naunyn-Schmiedeberg's Arch. Pharmacol.**334**:418-422.

Boehm, S. And S. Huck. 1991. Modulation of calcium currents via α_2-adrenoceptors in embryonic chick sympathetic neurons. Naunyn-Schmideberg's Arch. Pharmacol. **344**:382-385.

Bonnet, F., Liu, N., Delaunay, L. 1992. Combination of α-adrenergic agonists and local anaesthetics. Proceedings of Third Joint ESRA-ASRA Congress, 201-210.

Braun, H. 1914. Lokal Anesthesie, ed. 1, Leipsig, J.A. Barth, 1905. Braun, H.:Local Anesthesia, ed.3, (translated by P. Shields) p. 143, Philadelphia, Lea and Febiger.

Bresnahan, J.C., R.H. Ho and M.S. Beattie. 1984. A comparison of the ultrastructure of substance P and enkephalin-immmunoreactive elements in the nucleus of the dorsal lateral funiculus and laminae I and II of the rat spinal cord. J. Comp.

Neurol. **229**:497-511.

Brock-Utne, J.G., Kallichurum, S., Mankowitz, E., Maharaj, R.J., Downing, J.W. 1981. Effects of intrathecal saline and ketamine with and without preservative on the spinal roots of monkeys. S. Afr. Med.J. **81**:360-361.

Buchan, A. and W.A. Pulsinelli. 1990. Hypothermia but not the M-methyl-d-aspartate antagonist, MK-801, attenuates neuronal damage in gerbils subjected to transient global ischemia. J. Neurosci. **10**:311-316.

Burnstock, G. 1975. Innervation of vascular smooth muscle: histochemistry and electron microscopy. Clinical and Experimental Pharmacology and Physiology. **Suppl.2**:7-20.

Busto, R., W.D. Dietrich, M.Y.-T. Globus, I. Valdes, P. Scheinberg and M.D. Ginsberg. 1987. Small differencec in intra-ischemic brain temperature critically determine the extent of ischemic neuronal injury. J. Cereb. Blood Flow Metab. **7**:729-738.

Busto, R., M.Y.-T. Globus, W.D. Dietrich, et al., 1989. Effect of mild hypothermia on ischemia-induced release of neurotransmitters and free fatty acids in rat brain. Stroke. **20**:904-910.

Bylund, D.B. 1985. Hetereogeneity of alpha-2-adrenergic receptors. Pharmacol. Biochem. Behav. **22**:835-843.

Bylund, D.B. 1988. Subtypes of α_2-adrenoceptors: pharmacological and molecular biological evidence converge. Trends in Pharmacological Science **(9)**:356-361.

Canfield, D.R. and K. Dunlap. 1984. Pharmacological characterization of amine receptors on embryonic chick sensory neurons. British Journal of Pharmacology. **82**:557-563.

Carafoli, E., Penniston, J.T. 1985. The calcium signal. Sci. Am. **253**: 70-

Cardell, M., F. Boris-Möller and T. Wieloch. 1991. Hypothermia prevents the ischemia-induced translocation and inhibition of protein kinase C in rat striatum. 1991. J. Neurochem. **57**:1814-1817.

Carriedo, S.G., Yin, H.Z., Weiss, J.H. 1996. Motor neurons are selectively vulnerable to AMPA/ kainate receptor-mediated injury in vitro. Journal of Neuroscience. **16(13)**:4069-4079.

Caudle, R.M. and L. Isaac. 1987. Intrathecal dynorphin (1-13) results in an irreversible

loss of the tail flick reflex in rats. Brain Res. **435**:1-6.

Caudle, R.M. and L. Isaac. 1988. Influence of Dynorphin (1-13) on spinal reflexes in the rat. The Journal of Pharmacology and Experimental Therapeutics. **246(2)**:508-513.

Chavkin, C., James, I.F., Goldstein, A. 1982. Dynorphin is a specific endogenous ligand of the kappa opioid receptor. Science. **215**:413-415.

Choi, D.W. 1990a. Cerebral hypoxia: Some new approaches and unanswered questions. J. Neurosci. **10(8)**:2493-2501.

Choi, D.W. 1990b. Limitations of *in vitro* models of ischemia. Prog. Clin. Biol. Res. **361**:291-299.

Chopp, M., R. Knight, C.D. Tidwell, J.A. Helpern, E. Brown and K.M.A. Welch. 1989. The metabolic effects of mild hypothermia on global cerebral ischemia and recirculation in the cat: comparison to normothermia and hyperthermia. J. Cereb. Blood Flow Metab. **9**:141-148.

Chopp, M., H. Chen, M.O. Dereski and J.H. Garcia. 1991. Mild hypothermic intervention after graded ischemic stress in rats. Stroke. **22**:37-43.

Churn, S.B., W.C. Taft, M.S. Billingsley et al. 1990a. Temperature modulation of ischemic neuronal death and inhibition of calcium\calmodulin-dependent protein kinase II in gerbils. Stroke. **21**:1715-1721.

Churn, S.B., W.C. Taft and R.J. DeLorenzo. 1990b. Effects of ischemia on multifunctional calcium\calmodulin protein kinase type II in the gerbil. Stroke. **21(Suppl III)**:112-116.

Colbourne, F., G. Sutherland and D. Corbett. 1997. Postischemic Hypothermia. A critical appraisal with implication for clinical treatment. Molecular Neurobiology. **14(3)**:171-201.

Coombs, D.W., Allen, C., Meier, F., Fratkin, J. 1984a. Chronic intraspinal clonidine in sheep. Reg. Anesth. **9**:47-48

Coombs, D.W., Saunders, R., Gaylor, M., LaChance, P., and Jensen, L., 1984b. Clinical trial of intrathecal clonidine for cancer. (Abstract) Reg. Anes. **9**:28

Coughlan, M.G., Lee, J.G., Zeljko, Bosnjak, Z.J., Schmeling, W.T., Kampine, J.P. and D.C. Warltier, Direct coronary and cerebral vascular responses to

dexmedetomidine, Anesthesiology, 1992, **77**, 998-1006.

Cousins, M.J. and P.O. Bridenbaugh. 1988. Neural Blockade in Clinical Anesthesia and Management of Pain, 2nd ed., J.B. Lippencott, Philadelphia. 267-272.

Cousin, M.J. and L.E. Mather. 1984. Intrathecal and epidural administration of opioids. **61**:276-310.

Crawley, J.N., R.H. Roth and J.W. Mass. 1979. Locus coeruleus stimulation increases noradrenergic metabolite levels in rat spinal cord. Brain Research. **166**:180-184.

Crosby, G., Russo, M.A., Szabo, M.D., Davies, K.R. 1990. Subarachnoid clonidine reduces spinal cord blood flow and glucose utilization in conscious rats. Anesthesiology **73**:1179-1185.

Cullen, M.L., E.D. Staren, A. El-Ganzouri et al. 1985. Continuous epidural infusion for analgesia after major abdominal operations: a randomized, prospective, double-blind study. Surgery. **98**: 718-728.

Davies, J. And J.C. Watson. 1983. Role of excitatory amino acid receptors and polysynaptic activation in the rat spinal cord. Exp. Brain Res. **49**:280-296.

Dawson, V.L., T.M. Dawson, E.D. London, D.S. Bredt and S.H. Synder . 1991. Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. Proc. Nat. Acad. Sci. U.S.A. **88**:6368-6371.

Dawson, V.L., T.M. Dawson, D.A. Bartley, G.R. Uhl and S.H. Synder. 1993. Mechanisms of nitric oxide-mediated neurotoxicity in primary brain cultures. J. Neurosci. **13**:2651-2661.

Denson, D.D., Bridenbough, P.O., Turner, P.A., Phero, J.C. and P.P. Raj. 1982. Neural blockade and pharmacokinetics following subarachnoid lidocaine in the Rhesus monkey. I. Effects of epinephrine. Anesth. Analg., **61**: 746-750.

Denson, D.D., Bridenbough, P.O., Turner, P.A.and J.C. Phero. 1983. Comparison of neural blockade and pharmacokinetics after subarachnoid lidocaine in the Rhesus monkey. II. Effects of volume, osmolarity, and baricity. Anesth. Analg. **62**: 995-1001.

Denson, D.D., Turner, P.A., Bridenbough, P.O. and G.A.Thompson. 1984. Pharmacokinetics and neural blockade after subarachnoid lidocaine in the Rhesus monkey. III. Effects of phenyephrine. Anesth. Analg. **63** 129-133.

Dietrich, W.D., R. Busto, O. Alonso, M.Y.-T. Globus and M.D. Ginsberg. 1993. Intraischemic but not postischemic brain hypothermia protects chronically following global forebrain ischemia in rats. J. Cereb. Blood Flow Metab. **13**:541-549.

Dohi, S., Matsumiya, N., Takeshima, R., Naito, H. 1984. The effects of subarachnoid lidocaine and phenyephrine on spinal cord and cerebral blood flow in dogs. Anesthesiology **61**:238-244.

Dumont, M. and S. Lemaire. 1994. Dynorphin potentiation of [^{3}H]CGP-39653 binding to rat brain membranes. European Journal of Pharmacology. **271**:241-244.

Dunlap, K. and G.D. Fischback. 1981. Neurotransmitters decrease the calcium conductance activated by depolarization of embryonic chick sensory neurons. Journal of Physiology. **317**:519-535.

Dunlap, K., G.G. Holz and S.G. Rane. 1987. G proteins as regulators of ion channel function. Trends Neuroscience. **10**:241-244.

Edvinsson, L., M. Lindvall, K.C. Nielson and Ch. Owman. 1973. Are brain vessels innervated also by central (non-sympathetic) neurones? Brain Research. **63**:496-499.

Egan, T.M., G. Henderson, R.A. North and J.T. Williams. 1983. Noradrenaline-mediated synaptic inhibition in rat locus coeruleus neurones. J. Physiol. (Lond.) **10**:241-244.

Eisenach, J.C., R. Angelo, C. Taylor, and D.D. Hood. 1994. An isobolographic study of epidural clonidine and fentanyl after cesarean section. Anesth. Analg. **79**:285-290.

Eisenach, J.C., D.W. Dewan, J.C. Rose, and J.M. Angelo. 1984. Epidural clonidine produces antinociception, but not hypotension, in sheep. Anesthesiology. **66**:496-501.

Eisenach, J.C., S.Z. Lysak and C.M. Viscomi. 1989a. Epidural clonidine analgesia following surgery: Phase I. Anesthesiology. **71**:640-646.

Eisenach, J.C., R.L. Rauk, C. Buzzanell and S.Z. Lysak. 1989b. Epidural clonidine analgesia for intractable cancer pain: Phase I. Anesthesiology. **71**:647-652.

Espinoza, M.I. and J.T. Parer. 1991. Mechanisms of asphyxial brain damage and possible pharmacologic interventions, in the fetus. American Journal of

Gynecology. **164 (6, Part 1)**:1582-1581.

Faber, J.E. 1988. In-situ analysis of α-adrenoceptors on arteriolar and venular smooth muscle in rat skeletal muscle microcirculation. Circ. Res. **62**: 37- 50.

Faden, A.I. and T.P. Jacobs. 1984. Dynorphin-related peptides cause motor dysfunction through a non-opiate action. Br. J. Pharm. **81**:271-276.

Fink, B., Aasheim, G.M. and B.A. Levy. 1978. Neural pharmacokinetics of epinephrine. Anesthesiology. **48**:263-266.

Fisher, B., M.H. Zornow, T.L. Yaksh and B.M. Peterson. 1991. Antinociceptive propertis of intrathecal dexmedetomidine in rats. Eurpoean Journal of Pharmacology. **192**:221-225.

Fleetwood-Walker, S.M., R. Mitchell, P.J. Hope, V. Molony and A. Iggo. 1985. An α_2 receptor mediates the selective inhibition by noradrenaline of nociceptive responses of identified dorsal horn neurons. Brain Research. **334**:243-254.

Freedman, J., Post, C., Kåhrström, J., Öhlen, A., Mollenholt, P., Owman, C., Alari, L., and T. Hökfelt. 1988. Vasoconstrictor effects in spinal cord of the substance P antagonist [D-Arg^1, D-Trp^{7,9}, Leu^1] -substance P (Spantide) and somatostatin and interaction with thyrotropin releasing hormone. Neuroscience. **27(1)**: 267-278.

Freedman, J., Hökfelt, T., Post, C., Brodin, E., Sundström, E., Jonsson, G., Terenius, L., Leander, S., Fischer, J.A. and A. Verhofstad. 1989. Immunohistochemical and behavioural analysis of spinal lesions induced by substance P antagonist and protection by thyrotropin releasing hormone. Experimental Brain Research. **74**:279-292.

Fukuda, T., Dohi, S., and H. Naito. 1994. Comparisons of tetracaine spinal anesthesia with clonidine or phenylephrine in normotensive and hypertensive humans. Anesth-Analg. **78(1)**:106-111.

Gaumann, D.M. and T.L. Yaksh. 1988. Intrathecal somatostatin in rats: antinociception only in the prescence of toxic effects. Anesthesiology. **68**:733-742.

Gaumann, D.M., Yaksh, T.L., Post, C., Wilcox, G.L., Rodriguez, M. 1989. Intrathecal somatostatin in cat and mouse: studies on pain, motor behaviour and histopathology. Anesth. Analg. **68**:623-632.

Giron, L.T., S.A. Maccann and S.G. Crist-Orlando. 1985. Pharmacological

characterization and regional distribution of α_1-noradrenergic binding sites of rat spinal cord. Eur. J. Pharmacol.**115**:285-290.

Globus, M.Y.-T., R. Busto, W.D. Dietrich, E. Martinez, I. Valdes and M.D. Ginsberg. 1988. Intra-ischemic extracellular release of dopamine and glutamate is associated with striatal vulnerability to ischemia. Neurosci. Letter. **91**:36-40.

Globus, M.Y.-T., R. Busto, W.D. Dietrich, E. Martinez, I. Valdes and M.D. Ginsberg. 1989. Direct evidence for acute and massive norepinephrine release in the hippocampus during transient ischemia. J. Cereb. Blood Flow Metab. **9**:892-896.

Goodman and Gilman'sThe Pharmacological Basis of Therapeutics. 1990. 8th ed. Eds. A. Goodman Gilman, T.W. Rall, A.S. Nies and P. Taylor. Pergamon Press. New York.

Gordh, T.E., Ekman, S. and Lagerstedt, A.S. 1984. Evaluation of possible spinal neurotoxicity of clonidine. Upsala J. Med. Sci., **89**: 266-273.

Gordh, T. Jr., Feuk, U., Norlen, K., 1986a. Effect of epidural clonidine on spinal cord blood flow and regional and central hemodynamics in pigs. Anesth. Analg. **65**: 1312-1318.

Gordh, T. Jr., Post, C., and Olsson, Y. 1986b. Evaluation of the toxicity of subarachnoid clonidine, guanfacine, and a substance P-antagonist on rat spinal cord and nerve roots: light and electron microscopic observations after chronic intrathecal administration. Anesth. Analg. **65**:1303-1311.

Gordon, N.C., Heller, P.H. and J.D. Levine. 1992. Enhancement of pentazocine analgesia by clonidine. Pain. **48**: 162-169.

Grosby, G. and M.A. Russo. 1989. Spinal cord blood flow during tetracaine spinal anesthesia in conscious rats. Anesth. Analg. **68**:S64.

Grossman, M.L., A.I. Basbaum and H.L. Fields. 1982. Afferent and efferent connections of the tail flick reflex (a model used to analyze pain control mechanisms). J. Comp. Neurol. **206**:9-16.

Green, E.J., W.D. Dietrich, F. van Dijk, R. Busto, C.G. Markgraf, P.M. McCabe, M.D. Ginsberg and N. Schneiderman. 1992. Protective effects of brain hypothermia on behaviour and histopathology following global ischemia in rats. Brain Res. **580**:197-204.

Grice, S.C., J.C. Eisenach and D.M. Dewan. 1990. Labor analgesia with epidural

bupivacaine plus fentanyl: enhancement with epinephrine and inhibition with 2-chloroprocaine. Anesthesiology. **72**:623-628.

Groves, M.J., Y.W. Ng, A.Ciardi and F. Scaravilli. 1996. Sciatic nerve injury in the adult rat: comparison on oligosaccharide, CGRP and GAP43 immunoreactivity in primary afferents following two types of trauma. J. Neurocytol. **25(3)**:219-231.

Gustafson, I., Y. Miyauchi and T.W. Wieloch. 1989. Postischemic administration of idazoxan, an α_2-adrenoceptor antagonist, decreases neuronal damage in the rat brain. J. Cereb. Blood Flow and Metab. **9**:171-174.

Gustafson, I,. E. Westerberg and T. Wieloch. 1990. Protection against ischemia-induced neuronal damage by the α_2-adrenoceptor antagonist idazoxan: Influence of time of administration and possible mechanisms of action. J. Cereb. Blood Flow and Metab. **10**:885-894.

Gustafson, I., E.J. Westberg and T. Wieloch. 1991. Extracellular brain cortical levels of noradrenaline in ischemia: Effects of desipramine and postischemic administration of idazoxan. **86**:555-561.

Handy, R.L., H.L. Harb, P. Wallace, Z. Gaffen, K.J. Whitehead and P.K. Moore. 1996. Inhibition of nitric oxide synthase by 1-(2-trifluoromethylphenyl) imidazole (TRIM) *in vitro:* antinociceptive and cardiovascular effects. Br. J. Pharmacol. **119(2)**:423-431.

Handy, R.L. and P.K. Moore. 1997. Mechanism of the inhibition of neuronal nitroc oxide synthase by 1-(2-trifluoromethylphenyl) imidazole (TRIM). Life Science. **60(25)**:PL389-394.

Hayashi, Y., T.Z. Guo and M. Maze. 1995. Densensitization to the behavioural effects of alpha-2-adrenergic agonists in the rats. Anesthesiology. **82(4)**:954-962.

Hayes, A.G., M. Skingle and M.B. Tyers. 1986. Alpha-adrenoceptor-mediated antinociception in the rat and dog. Neuropharmacology. **25**:391-396.

Heinke, H. and A. Lawen. 1905. Experimentelle Untersuchungen und Klinische Erfahrungen uber die Verwertbarkeit von Novokain fir die Artliche Anasthesie. Deutsche Zschr. F. Chir. **80**:180-195.

Helke, C.J., Phillip, E.T. and J.T. O'Neill. 1987. Intrathecal administration of a substance P receptor antagonist: Studies on peripheral and central nervous system hemodynamics and on specificity of action. J. Pharmacol. Exp. Ther. **242(1)**:131-136.

Herman, B.H. and A. Goldstein. 1985. Antinociception and paralysis induced by intrathecal dynorphin A. J. Pharmacol. Exp. Ther. **232**:27-32.

Hernández-Perez, M.J., M.E. Raichle and H.L. Stone. 1975. The role of the peripheral sympathetic nervous system in cerebral vascular diseases. Stroke. **6**:284-292.

Hickey, R., M.S. Albin, L. Bunegin and J. Gelineau. 1986. Autoregulation of spinal cord blood flow: Is the cord a microcosm of the brain? Stroke. **17(6)**:1183-1189.

Hoffman, W.E., M.A. Cheng, C. Thomas, V.L. Baughman and R.F. Albrecht. 1991a. Clonidine decreases plasma catecholamines and improves outcome from incomplete ischemia in the rat. Anesth. and Analg. **73(4)**:460-464.

Hoffman, W.E., E. Kochs, C. Werner, C. Thomas and R.F. Albrecht. 1991b. Dexmedetomidine improves neurologic outcome from incomplete ischemia in the rat. Reversal by the α_2-adrenergic antagonist atipamezole. Anesthesiology. **75(2)**:328-332.

Hökfelt, T., J.O. Kellerth, G. Nilsson and B. Pernow. 1975. Substance P: localization in the central nervous system and in some primary sensory neurons. Science. **190**, 889-890.

Hökfelt, T., C. Post, J. Freedman, J.M. Lunberg and L. Terenius. 1989. Endothelin induces spinal lesions after intrathecal administration. Acta. Physiol. Scand. **137**:555-556.

Hosseini, A.R., P.R. King, W.J. Louis and A.L. Gundlach. 1997. [3H]2-(2-Benzofuranyl)-2-imidazoline, a highly selective radioligand for I2-imidazoline receptor binding sites. Studies in rabbit kidney membranes. Naunyn Schmiedeberg's Arch Pharmacol. **355(1)**:131-138.

Howe, J.R., J-Y Wang and T.L. Yaksh. 1983. Selective antagonism of the antinociceptive effect of intrathecally applied alpha adrenergic agonists by intrathecal prazosin and intrathecal yohimbine. J. Pharm. Exp. Ther. **224**:552-558.

Howe, J.R., T.L. Yaksh, and V.L.W. Go. 1987. The effect of unilateral dorsal root gaglionectomies or ventral rhizotomies on α_2-adrenoceptor binding to, and the substance P, enkephalin, and neurotensin content of, the cat lumbar spinal cord. Neuroscience. **21(2)**:385-394.

Howe, J.R. and W. Zieglgänsberger. 1987. Responses of rat dorsal horn neurons to natural stimulation and to iontophoretically applied norepinephrine. J. Comp.

Neurol. **255(1)**:1-17.

Hunt, S.P., Cytochemistry of the spinal cord. 1983. In P.C. Emson (Ed.), **Chemical Neuroanatomy**. Raven Press, New York. pp. 53-84.

Hunt, S.P., J.S. Kelly and P.C. Emson. 1980. The electron microscopic localization of methionine-enkephalin within the superficial layers (I and II) of the spinal cord. Neuroscience. **5**:1871-1890.

Iwai, A., W.W. Monafo and S.G. Eliasson. 1991. Effect of adrenalectomy or sympathectomy on spinal cord blood flow in hypothermic rats. Am. J. Physiol. **260**:H827-H831.

Iwai, T., M. Niwa, H. Yamada, M. Nozaki and K. Tsurumi. 1993. Hypothermic prevention of the hippocampal damage following ischemia in Mongolian gerbils comparison between intraischemic and brief postischemic hypothermia. Life Science. **52**:1031-1038.

Janss, A.J., S.L. Jones and G.F. Gebhart. 1987. Effect of spinal norepinephrine depletion on descending inhibition of the rat tail flick reflex from the locus coeruleus and lateral reticular nucleus in the rat. Brain Research. **400**:40-52.

Jeftinijia, S., F. Liu, K. Jeftinijia and L. Urban. 1992. Effect of capsaicin and resiniferatoxin on peptidergic neurons in cultured dorsal root ganglion. Regulatory Peptides. **39**: 123-135.

Johansson, P. and F. Ehrenstrom. 1988. Presynaptic aplha-adrenoceptor regulation of transmitter release in the conscious rat. Comp. Biochem. Physiol. **89**:65-69.

Jones, S.L. and G.F. Gebhart. 1986a. Characterization of coeruleospinal inhibition of the nociceptive tail-flick reflex in the rat: Mediation by spinal α_2-adrenoceptors. Brain Research. **364**:315-330.

Jones, S.L. and G.F. Gebhart. 1986b. Quantitative characterization of coeruleospinal inhibition of nociceptive transmission in the rat. Journal of Neurophysiology. **56**:1397-1410.

Kalso, E.A., R. Pöyhiä and P.H. Rosenberg. 1991. Spinal antinociception by dexmedetomidine, a highly selective α_2-adrenergic agonist. Pharmacology and Toxicology. **68**:140-143.

Karlsson, B.R., Forsman, M., Roald, O.K., Heier, M.S., Steen, P.A. 1990. Effect of dexmedetomidine, a selective and potent alpha$_2$ -agonist, on cerebral blood flow

and oxygen consumption during halothane anesthesia on dogs. Anesthesia and Analgesia. **71**:125-129.

Kawakita, K. And M. Funakoshi. 1987. A quantitative study on the tail flick test in the rat. Physiol. Behav. **39(2)**:235-240.

Kchachatarian, H., M.E. Lewis, M.K.H. Schafer and S.J. Watson. 1985. Anatomy of the CNS opioid system. Trends Neurosci. **8**:111-119.

Kim-Lee, M.H., Stokes, B.T. and D.K. Anderson. 1993. Intracellular calcium dynamics and cerebral injury: Modeling various insults in vitro. Brain Research. **613**:156-159.

Kishikawa, K., Namiki, A., Harada, Y., Sato, Y. And K. Omote. 1993. Norepinephrine prolongs tetracaine spinal anesthesia in surgical patients: a preliminary study. Anesth-Analg. **76(4)**:772-777.

Klaassen, C.D. ed. 1995. Casarett & Doull's Toxicology. The Basic Science of Poisons. McGraw-Hill. New York.

Kobinger, W. and L. Pichler. 1975. Investigation into some imidazoline compounds with respect to peripheral α_1-adrenoceptor stimulation and depression of cardiovascular centers. Naunyn-Schmeideberg's Arch. Pharmacol. **291**:175-191.

Kobinger, W. and L. Pichler. 1980. Relation between central sympathoinhibitory and peripheral pre- and postsynaptic α-adrenoceptors as evaluated by different clonidine-like substances, in rats. Naunyn-Schmiedeberg's Arch. Pharmacol.**315**:21-27.

Krause, G.S., White, B.C., Aust, S.D., Nayini, N.R., Kumar, K. 1988. Brain cell death following ischemia and reperfusion: a proposed biochemical sequence. Crit. Care Med. **16**:717-726.

Kuraishi, Y., Y. Harada and H. Takagi. 1979. Noradrenaline regulation of pain-transmission in the spinal cord mediated by α-adrenoceptors. Brain Research. **174**:333-336.

Langer, S.Z. 1974. Presynaptic regulation of catecholamine release. Biochem. Pharmacol. **23(13)**:1793-1800.

Lawhead, R.G., H.S. Blaxall, D.B. Bylund. 1992. Alpha-2A is the predominate alpha-2 adrenergic receptor subtype in human spinal cord. Anesthesiology. **77(5)**:983-91.

Lees, G.J. 1991. Inhibition of sodium-potassium-ATPase: a potentially ubiquitous mechanism contributing to central nervous system neuropathology. Brain Res. Reviews. **16**:283-300.

Leicht, C.H. and Carlson, S.A. 1986. Prolongation of spinal anesthesia with epinephrine and phenylephrine. Anesth. Analg. **65**:365-9.

Lipscombe, D., S. Kongsemut and R.W. Tsien. 1989. α-Adrenergic inhibition of sympathetic neurotranmitter release mediated by modulation of N-type calcium-channel gating. Nature. **340**:639-642.

Liu, D. 1994. An experimental model combining microdialysis with electrophysiology, histology and meurochemistry for studying excitotoxicity in spinal cord injury. Effect of NMDA and kainate. Mol-Chem-Neuropathol. **23(2-3)**:77-92.

Long, J.B., J.M. Petras, W.C. Mobley and J.W. Holaday. 1988. Neurological dysfunction after intrathecal injection of dynorphin A (1-13) in the rat. II. Nonopioid mechanisms mediate loss of motor, sensory and autonomic function. J. Pharmacol. Exp. Ther. **246(3)**:1167-1174.

Long, J.B., Kinney, R.C., Malcolm , D.S., Graeber, G.M. and J.M. Holaday. 1987. Intrathecal dynorphin A_{1-13} and dynorphin A_{5-13} reduce rat spinal cord blood flow by non-opioid mechanisms. Brain Research. **436**:374-379.

Long, J.B., Martinez-Arizala, A., Johnson, S.H. and J.W. Holaday. 1989a. Arginine^{8}-Vasopressin reduces spinal cord blood flow after spinal subarachnoid injection in rats. J. Pharmacol. and Exp. Ther. **249(2)**:499-506.

Long, J.B., A. Martinez-Arizala, D.D. Rigamonti and J.W. Holaday. 1989b. Hindlimb paralytic effects of arginine vasopressin and related peptides following spinal subarachnoid injection in the rat. Peptides. **9**:1335-1344.

Long, J.B., J.M. Petras, W.C. Mobley and J.W. Holaday. 1988. Neurological dysfunction after intrathecal injection of dynorphin A (1-13) in the rat. II. Nonopioid mechanisms mediate loss of motor, sensory and autonomic function. J. Pharm. Exp. Ther. **246**:1167-1174.

Long, J.B., Rigamonti, D.D., Oleshansky, M.A., Wingfield, C.P. and A. Martinez-Arizala. 1994. Dynorphin A - induced rat spinal cord injury: Evidence for excitatory amino acid involvement in a pharmacological model of ischemic spinal cord injury. Journal of Pharmacology and Experimental Therapeutics. **269(1)**: 358 - 366.

Loomis, C.W., K.D. Arunachalam, B. Milne and J. Bautista. 1993. Spinal α_1 - adrenoceptors mediate an enkephalin-dependent potentiation of spinal α_2-antinociception in the rat. Proceedings of the Symposium: α_2-Adrenergic Mechanisms in Spinal Analgesia (Lucerne, Switzerland), Regional Anesthesia, **18** (4S) (1993) 35.

Loomis, C.W., K.D. Arunachalam, D. Vyas and B. Milne. 1992a. Intrathecal (i.t.) methoxamine potentiates dexmedetomidine spinal antinociception in the rat: Evidence of a role for α_1-adrenoceptors and endogenous opioids. Society for Neuroscience. **18**:428.8.

Loomis, C.W., K.D. Arunachalam, D. Vyas and B. Milne. 1992b. Potentiation of intrathecal (i.t.) dexmedetomidine antinociception by i.t. methoxamine in the rat: evidence for an endogenous opioid link. Can. J. Anaesth. **39(5)**:A13.

Loomis, C.W., K. Jhamandas, B. Milne and F.W. Cervenko. 1987. Monoamine and opioid interactions in spinal analgesia and tolerance. Pharmacol. Biochem. **26**:445-451.

Loomis, C.W., Vyas, D., Sherman, S.E. 1992c. Intrathecal oxymetazoline does not produce neurotoxicity in the spinal cord of the rat. Brain Research. **599**: 73-82.

LoPachin, R.M. and T.A. Rudy. 1983. Sites and mechanism of action for the effects of intrathecal noradrenaline on thermoregulation in the rat. J. Physiol. **341**:527-544.

MacDonald, E., M. Scheinin, H. Scheinin and R. Virtanen. 1991. Comparison of the behavioural and neurochemical effects of the two optical enantiomers of metomidine, a selective alpha-2-adrenoceptor agonist. J. Pharmacol. Exp. Ther. **259**:848-854.

Maier, C., G.K. Steinberg, G.H. Sun. G.T. Zhi and M. Maze. 1993. Neuroprotection by the alpha$_2$-adrenoceptor agonist dexmedetomidine in a focal model of cerebral ischemia. Anesthesiology. **79**:306-312.

Maiese, K., L. Pek, S.B. Berger and D.J. Reis. 1992. Reduction in focal cerebral ischemia by agents acting at imidazole receptors. J. Cereb. Blood Flow and Metab. **12**:53-63.

Maiese, K., M. TenBroeke and I. Kue. Neuroprotection of lubeluzole is mediated through the signal transduction pathways of nitric oxide. J. Neurochem. **68(2)**:710-714.

Malvinovsky, J.M. and J.M. Bernard. 1996. Spinal clonidine fails to provide surgical

anesthesia for transurethral resection of prostate. A dose-finding pilot study. Reg-Anesth. **21(5)**:419-423.

Margalit, D. and M. Segal. 1979. A pharmacological study of analgesia produced by stimulation of the nucleus locus coeruleus. Psychopharmacology. **62**:169-173.

Marini, C.P. and J.N. Cunningham Jr. 1993. Issues surrounding spinal cord protection. Adv. Card. Surg. **4**:89-107.

Marsala, M. and T.L. Yaksh. 1994. Transient spinal ischemia in the rat: Characterization of behavioral and histopathological consequences as a function of the duration of aortic occlusion. Journal of Cerebral Blood Flow and Metabolism. **14**:526-535.

Massardier, D. and P.F. Hunt. 1989. A direct non-opiate interaction of dynorphin-(1-13) with the N-methyl-D-aspartate (NMDA) receptor. Eur. J. Pharmacol.**170(1-2)**:125-126.

Mattson, M.P. and S.W. Scheff. 1994. Endogenous neuroprotection factors and traumatic brain injury mechanisms of action and implication for therapy. Journal of Neurotrauma. **11**:3-20.

Maze, M. and W. Tranquilli. 1991. Alpha-2 adrenoceptor agonists:Defining the role in clinical anesthesia. **74**:581-605.

McCord, J.M. 1985. Oxygen-derived free radicals in post-ischemic tissue injury. N. Eng. J. Med. **312**:159-163.

McCulloch, J., and L. Edvinsson, Cerebrovascular smooth muscle reactivity: A critical appraisal of in vitro and in situ techniques, J. Cereb. Blood Flow and Metab., 1984, **4**, 29-139.

Medgett, I.C., McCulloch, M.W., Rand, M.J. 1978. Partial agonist action on prejunctional and postjunctional α-adrenoceptors. Naunyn Schmeidebergs Arch. Pharmacol. **304**:215-221.

Meldrum, B. and J. Garthwaite. 1990. Excitatory amino acid neurotoxicity and neurodegenerative disease. Trends in Pharmacological Science. **11(9)**:379-387.

Melinek, R., Holets, V.R., Puckett, W.R., Kreger, H. and Bunge, R.P. 1994. Calcitonin gene-related peptide (CGRP)-like immunoreactivity in motoneurons of the human spinal cord following injury. **11(1)**:63-71.

Menon, M.K., R.L. Lloyd, L.J. Fitten. 1990. Antagonism of the hypothermic effect of clozapine in mice by centrally-acting alpha-2-adrenergic antagonists and alpha-1-adrenergic agonists. Psychopharmacology-Berl., **101**:67-72.

Mensink, F.J., Kozody, R., Kehler, C.H. and Wade, J.G. 1987. Dose-response relationship of clonidine in tetracaine spinal anesthesia. Anesthesiology. **67**:717-721.

Miller, R.D. 1994. Anesthesia. 4th ed., Churchill Livingstone, New York.

Miller, J.F. and H.K. Proudfit. 1990. Antagonism of stimulation-produced antinociception from ventrolateral pontine sites by intrathecal administration of α-adrenergic antagonists and naloxone. Brain Research. **530**:20-34.

Minamisawa, H., M.-L. Smith and B.K. Siesjö. 1990a. The effect of mild hyperthermia and hypothermia on brain damage following 5, 10, and 15 minutes of forebrain ischemia. Ann. Neurol. **28**:26-33.

Minamisawa, H., C.-H. Nordström, M.-L. Smith and B.K. Siesjö. 1990b. The influence of mild body and brain hypothermia on ischemic brain damage. J. Cereb. Blood Flow Metab. **10**:365-374.

Minor, B.G., W. Danyaz, G. Jonnson, A.K. Mohammed, C. Post and T. Archer. 1989. Adaptive changes in alpha-2 adrenoceptor mediated responses: analgesia, hypothermia and hypoactivity. Pharmacol-Toxicol. **65(2)**:143-151.

Mollenholt, P., Post, C., Paulsson, I. and N. Rawal. 1992. Intrathecal somatostatin in the guinea pig: effects on spinal cord blood flow, histology and motor function. Pain. **51(3)**:343-347.

Mollenholt, P., Post, C., Rawal., N., Freedman, J., Hökfelt, T., and I. Paulsson. 1988. Antinociceptive and "neurotoxic" actions of somatostatin in rat spinal cord after intrathecal administration. Pain. **32**:95-105.

Momose, K., Shima, T., Satoh, K., Andoh, K., and Y. Hashimoto. 1994. Effect of epinephrine added to spinal anesthesia with lidocaine and tetracaine. Masui. **43(6)**:880-884.

Motsch, J.M. Gräber, E. and K. Ludwig. 1990. Addition of clonidine enhances postoperative analgesia from epidural morphine: a double-blind study. Anesthesiology. **73**:1067-1073.

Morhland, J.S. 1982. **Pain Pathways: Potential Sites for Analgetic Action**, Central

Analgetics. Daniel Lednicer, ed. John Wiley & Sons, New York. pp.11-14.

Nagy, J.I., Iversen, L.L., Goedert, M., Chapman, D. And Hunt, S.P. 1983. Dose-dependent effects of capsaicin on primary afferent sensory neurons in the neonatal rat. **3(2)**:399-406.

Nakamura, S., J.M. Tepper, S.J. Young and P.M. Groves. 1981. Neurophysiological consequences of presynaptic receptor activation: changes in noradrenergic terminal excitability. Brain Res. **226**:155-170.

Novack, T.A., M.C. Dillon and W.T. Jackson. 1996. Neurochemical mechanisms in brain injury and treatment: A review. Journal of Clinical and Experimental Neuropsychology. **18(5)**:685-706.

Nurse,S. and D. Corbett. 1994. Direct measurement of brain temperature during and after intraischemic hypothermia: Correlation with behavioural, physiological and histological endpoints. Journal of Neuroscience. **14(12)**:7726-7734.

Omote, K., Kitahata, L.M., Collins, J.G., Nakatani, K. And I. Nakagawa. 1991. Interaction between opiate subtype and alpha-2-adrenergic agonists in suppression of noxiously evoked activity of WDR neurons in the spinal dorsal horn. Anesthesiology. **74**:737-743.

Ossipov, M.H., S. Harris, P. Lloyd, E. Marineo, B.S. Lin and J. Bagley. 1990a. Antinociceptive interaction between opioids and medetomidine: Systemic additivity and spinal synergy. Anesthesiology. **73**:1227-1235.

Ossipov, M.H., Lozito, R., Messineo, E., Green, J., Harris, S. and P. Lloyd. 1990. Spinal antinociceptive synergy between clonidine and morphine, U69593, and DPDPE: isobolographic analysis. Life Science. **46**:PL-71-PL-76.

Ossipov, M.H., J.L. Suarez and T.C. Spaulding. 1989. Antinociceptive interactions between α_2-adrenergic and opiate agonists at the spinal level in rodents. Aneth. Anal. **68**:194-200.

Partridge, B.L. 1991. The effects of local anesthetics and epinephrine on rat sciatic nerve blood flow. Anesthesiology. **75**:243-251.

Pettinger, W.A. 1980. Pharmacology of clonidine. J. Cardiovasc. Pharmacol. **2(Suppl. 1)**:S21-S28.

Pohl, M. J.J. Benoliel, S. Bourgoin, M.C. Lombard, A. Mauborgne, H. Tacquet, A. Carayon, J.M. Besson, F. Cesselin and M. Hamon. 1990. Regional distribution of

calcitonin gene-related peptide-, substance P-, chloecystokinin-, met^5-enkephalin-, and dynorphin A (1- 8)- like materials in the spinal cord and dorsal root ganglia of adult rats: Effects of dorsal rhizotomy and neonatal capsaicin. Journal of Neurochemistry. **55**:1122-1130.

Polati, E., A.M. Pinaroli and S. Ischia. 1996. Balanced spinal analgesia in the treatment of oncologic pain. Review of the literature. Minerva-Anestesiol. **62(11)**:363-375.

Pontius, R.G., H.L. Brockman, D.A. Cooley and M.E. DeBakey. 1954. The use of hypothermia in the prevention of paraplegia following temporary aortic occlusion: experimental observation. Surgery. **36**:33-38.

Post, C. and I. Paulsson. 1985. Antinociceptive and neurotoxic actions of substance P analogues in the rat's spinal cord after intrathecal administration. Neuroscience Letters. **57**:159-164.

Przewlocki, R., A. Gramsh and A. Herz. 1983. Characterization and localization of immunoreactive dynorphin, α-neo-endorphin, met-enkephalin and substance P in the human spinal cord. Brain Research. **280(1)**: 95-103.

Prickett, M.D., Gross, E.G. and S.C. Cullen. 1945. Spinal anesthesia with solutions of procaine and epinephrine; Preliminary report of 108 cases. Anesthesiology. **6**: 469-474.

Racle, J.P., Benkhadra, A., Poy, J.Y. and Gleizal, B. 1987. Prolongation of isobaric bupivacaine spinal anesthesia with epinephrine and clonidine for hip surgery in the elderly. Anesth. Analg. **66**: 442-6.

Racle, J.P., Poy, J.Y., Benkhadra, A., Joudren, L., Fockenier, F. 1988. Prolongation de la rachianesthésie à la bupivacaine hyberbare par l'adrénaline et la clonidine chez le viellard. Ann. Fr.Anaesth. Reanim. **7**:139-144.

Rapela, C.E. and H.D. Green. 1964. Autoregulation of canine cerebral blood flow. Circ. Res. **14** and **15**. Suppl. I:205-211.

Ravindran, R.S., Viegas, O.J., Pantazis, K.L. and Baldwin, S.J. 1983. Serum lidocaine levels following spinal anaesthesia with lidocaine and epinephrine in dogs. Reg. Anaesth. **8**:6.

Rawal, N. Spinal Antinociception: Clinical Aspects. 1995. Annals of Medicine. **27**:263-268.

Reddy, R.S.V., J.L. Maderdrut and T.L. Yaksh. 1980. Spinal cord pharmacology of

adrenergic agonist-mediated antinociception. J. Pharmacol. Exp. Ther. **213**:525-533.

Reddy, S.V.R. and T.L. Yaksh. 1980. Spinal noradrenergic terminal system mediates antinociception. Brain Research. **189**:391-401.

Redmond, D.E. Jr. 1981. Clonidine and the primate locus coeuruleus: evidence suggesting anxiolytic and anti-withdrawal effects. Prog. Clin. Biol. Res. **71**:147-163.

Regan, R.F. 1996. The vulnerability of spinal cord neurons to excitotoxic injury: Comparison with cortical neurons. Neuroscience Letters. **213**: 9-12.

Reis, D.J., S. Regunathan, E.V. Golanov and D.L. Feinstein. 1994. Protection of focal cerebral ischemic infraction by rilmenidine in the animal: evidence that interaction with central imidazoline receptors may be neuroprotective. Am. J. Cardiol **74(13)**:25A-30A.

Reis, D.J., S. Regunathan and M.P. Meeley. 1992. Imidazole receptors and clonidine-displacing substance in relationship to control of blood pressure, neuroprotection, and adrenomedullary secretion. Am. J. Hypertens. **5(4 Pt 2)**:51S-57S.

Ritchie, J.M. and N.M. Greene. 1980. Local anesthetics. In the Pharmacological Basis of Therapeutics, eds. A.G. Gilman, L.S. Goodman and A. Gilman. MacMillan, New York, pp. 300-320.

Roerig, S.C., Lei, S., Kitto, K., Hylden, J.K.L. and G. Wilcox. 1992. Spinal interactions between opioid and noradrenergic agonists in mice: multiplicity involves delta and alpha-2-receptors. Journal of Pharmacology and Experimental Therapeutics. **262**:365-374.

Rostaing, S., Bonnet, F., Levron, J.C., Vodinh, J., Pluskwa, F., Saada, M. 1991. Effect of epidural clonidine on analgesia and pharmacokinetics of epidural fentanyl in post-operative patients. Anesthesiology. **75**: 420-425.

Roudet, C., M. Savasta and C. Feuerstein. 1993. Normal distribution of alpha-1 adrenoceptors in the rat spinal cord and its modification after noradrenergic denervation: A quantitative autoradiography. J. Neurosci. Res. **34**:44-53.

Sabbe, M.J., J.P. Penning, G.T. Ozaki and T.L. Yaksh. 1994. Spinal and systemic action of the α_2 receptor agonist dexmedetomidine in dogs. Anesthesiology. **80**:1057-1072.

Sandberg, D.E. and M. Segal. 1978. Pharmacological analysis of analgesia and self-stimulation elicited by electrical stimulation of catecholamine nuclei in the rat brain. Brain Research. **152**:529-542.

Savola, J.-M, H. Ruskoaho, J. Duurunen, J.S. Salonen and N.T. Kärki. 1986. Evidence for medetomidine as a selective and potent agonist at alpha-2- adrenoceptors. J. Autonom. Pharmac. **5**:275-284.

Savola, M.K. and J.M. Savola 1996. [^{3}H] Dexmedetomidine, an α_2-adrenoceptor agonist detects a novel imidazole binding site in adult rat spinal cord. Eur. J. Pharmacol. **306(1-3)**:315-323.

Scheinberg, P. 1991. The biologic basis for the treatment of acute stroke. Neurology. **41**:1867-1873.

Scheinin, H., R. Virtanin, E. Macdonald, R. Lammintausta and M. Scheinin. 1989. Medetomidine- A Novel α_2-adrenoceptor agonist: A review of its pharmacodynamic effects. Prog. Neuro-Psychopharmacol. and Biol. Psychiat. **13**:635-651.

Scremin, O.U. 1995. Cerebral Vascular System in The Rat Nervous System. 2nd ed. Academic Press, N.Y. ed. By George Paxinos.

Segal, M. and D. Sandberg. 1977. Analgesia produced by electrical stimulation of catecholamine nuclei in the rat brain. Brain Research. **123**:369-372.

Sekiguchi, Y., Y. Konnai, S. Kikuchi and Y. Sugiura. 1996. An anatomic study of neuropeptide immunoreactivities in the lumbar dura mater after lumbar sympathectomy. Spine.**21(8)**:925-930.

Sherman, S.E., C.W. Loomis, B. Milne and F.W. Cervenko. 1988. Intrathecal oxymetazoline produces analgesia via α-adrenoceptors and potentiates spinal morphine. Eur. J. Pharmacol. **148**:371-380.

Shimizu, H., Graham, S.H., Chang, L.H., Mintorovitch, J., James, T.L., Faden, A.I. and P.R. Weinstein. 1993. Relationship between extracellular neurotransmitter amino acids and energy metabolism during cerebral ischemia in rats monitored by microdialysis and *in vivo* magnetic resonance spectroscopy. Brain Research. **605**:33-42.

Shukla, V.K., M. Bansinath, M. Dumont and S. Lemaire. 1992. Selective involvement of kappa opioid and phencyclidine receptors in the analgesic and motor effects of dynorphin-A-(1-13)-Tyr-Leu-Phe-Asn-Gly-Pro. Brain Research. **591**:176-180.

Siddall, P.J., Gray, M., Rutkowski, S. and M.J. Cousins. 1994. Intrathecal morphine and clonidine in the management of spinal cord injury pain: a case report. Pain. **59**:147-148.

Siesjö, B.K. 1992a. Pathophysiology and treatment of focal cerebral ischemia. Part I. Pathophysiology. J. Neurosurg. **77(2)**:169-184.

Siesjö, B.K. 1992b. Pathophysiology and treatment of focal cerebral ischemia. Part II. Mechanisms of damage and treatment. J. Neurosurg. **77(3)**:337-354.

Simmons, R.M. and D.J. Jones. 1988. Binding of [^{3}H] prazosin and [^{3}H] p-aminoclonidine to α-adrenoceptors in rat spinal cord. Brain Research. **445**:338-349.

Simon, R.P., T. Griffiths, M.C. Evans et al., 1984. Calcium overload in selectively vulnerable neurons in the hippocampus during and after ischemia: an electron microscopy study in rats. J. Cereb. Blood Flow Metab. **4**:350-361.

Simpson, R.K., Robertson, C.S. and J.C. Goodman. 1990. Spinal cord ischemia-induced elevation of amino acids: Extracellular measurement with microdialysis. Neurochemical Res. **15(6)**:635-639.

Skilling, S.R., X. Sun, H.J. Kurtz and A.A. Larson. 1992. Selective potentiation of NMDA-induced activity and release of excitatory amino acids by dynorphin: possible roles in paralysis and neurotoxicity. Brain Research. **575**:272-278.

Spampinato, S. and S. Candeletti. 1985. Characterization of dynorphin A-induced antinociception at spinal level. Eur. J. Pharm. **110**:21-30.

Sommer, C. and R.R. Myers. 1995. Neurotransmitters in the spinal cord dorsal horn in a model of painful neuropathy and in nerve crush. Acta-Neuropayhol-Berl. **90(5)**:478-485.

Stein, S.C. and R.Q. Cracco. 1982. Cortical injury without ischemia produced by topical monoamines. Stroke. **13**:74-83.

Stewart, P., and L. Isaac. 1989. Localization of dynorphin-induced neurotoxicity in rat spinal cord. Life Science. **44**:1505-1514.

Strandgaard, S., E.T. MacKenzie, D. Sengupta, J.O. Rowan, N.A. Lassen and A.M. Harper. 1974. Upper limit of autoregulation of cerebral blood flow in the baboon. Circ. Res. **34**:435-440.

Sullivan, A.F., M.R. Dashwood and A.H. Dickenson. 1987. α_2-adrenoceptor modulation of nociception in rat spinal cord: location, effects and interactions with morphine. European J. Pharmacol. **138**:169-177.

Sutton, L.N., B.J. Clark, C.R. Norwood et al. 1991. Global cerebral ischemia in piglets under conditions of mild and deep hypothermia. Stroke. **22**:1567-1573.

Takano, Y., M. Takano and T.L. Yaksh. 1992. The effect of intrathecally administered imiloxan and WB4101: possible role of α_2-adrenoceptor subtypes in the spinal cord. European J. Pharmacol. **219**:465-468.

Takano, Y. and T.L. Yaksh. 1991. Relative efficacy of spinal alpha-2-agonists, dexmedetomidine, clonidine and ST-91, determined *in vivo* by using N-ethoxycarbonyl-2-ethoxy-1,2-dihydro-quinoline, an irreversible antagonist. Journal of Pharmacology and Exp. Therapeutics. **258(2)**:438-446.

Takano, Y. and T.L. Yaksh. 1992. Characterization of the pharmacology of intrathecally administered alpha-2 agonists and antagonists in rats. Journal of Pharmacology and Exp. Therapeutics. **261(2)**:764-772.

Thornehill, J.A., L. Gregor, R. Mathison and Q. Pittman. 1989. Intrathecal dynorphin A administration causes pressor responses in rats associated with an increased resistance to spinal cord blood flow. Brain Res. **490**:174-177.

Toda, N. 1983. Alpha adrenergic receptor subtypes in human, monkey and dog cerebral arteries. J. Pharmacol. Exp. Ther. **226(3)**:861-868.

Traystmann, R.J., J.R. Kirsch and R.C. Koehler. 1991. Oxygen radical mechanisms of brain injury following ischemia and reperfusion. Journal of Applied Physiology. **71**:1185-1195.

Ueda, Y., Obrenovitch, T.P., Lok, S.Y., Sarna, G.S. and L. Symon. 1993. Changes in extracellular glutamate concentration produced in the rat striatum by repeated ischemia. Stroke. **23**: 1125-1130.

Ueda, Y., T. Oyama, Y. Kuraishi, A. Akaike and M. Satoh. 1995. Alpha2-adrenoceptor-mediated inhibition of capsaicin-evoked release of glutamate from rat spinal dorsal horn slices. Neurosci. Lett. **188**: 137-139.

Uhlén, S. and J.E.S. Wikberg. 1991a. Delineation of three pharmacological subtypes of α_2-adrenoceptors in the kidney. Br. J. Pharmacol. **104**:657-664.

Uhlén, S. and J.E.S. Wikberg. 1991b. Rat spinal cord α_2-adrenoceptors are of the α_{2A}-

subtype: Comparison with α_{2A} and α_{2B}-adrenoceptors in rat spleen, cerebral cortex and kidney using ^{3}H-RX821002 ligand binding. Pharmacol. Toxicol. **69**:341-350.

Uhlén, S. and J.E.S. Wikberg. 1991c. Delineation of rat kidney α_{2A}- and α_{2B}-adrenoceptors with [^{3}H] RX821002 radioligand binding: computer modelling reveals that guanfacine is an α_{2A}-selective compound. European J. Pharmacol. **202**:235-243.

Unnerstall, J.R., T.A. Kopajtic and M.J. Kuhar. 1984. Distribution of α_2 agonist binding sites in the rat and human central nervous system: Analysis of some functional, anatomic correlates of the pharmacologic effects of clonidine and related adrenergic agents. Brain Research Reviews. **7**:69-101.

Vaino, O. 1989. Introduction to the clinical pharmacology of medetomidine. Acta. Vet. Scand. Suppl. **85**:85-88.

Vematsu, D., J.H. Greenberg, M. Reivich and A. Karp. 1988. In vivo measurement of cystolic free calcium during cerebral ischemia and reperfusion. Ann. Neurol. **24**:420-428.

Vertommen, J.D., E. Vandermeulen, H. Van Aken, L. Vaes, M. Soetens, A. Van Steenberge, P. Mourisse, J. Willaert, H. Noorduin, H. Devlieger and A.F. Van Assche. 1991. The effects of the addition of sufentanil to 0.125% bupivacaine on the quality of analgesia during labor and on the incidence of instrumental deliveries. Anesthesiology. **74**:809-814.

Virtanen, R. 1985. Detomidine and MPV-785, two sedative/analgesic imidazole derivatives with different species selectivity. Abstracts of the 3rd EAVPT congress, Ghent, Belgium. 39.

Virtanen, R., J-M Savola, V. Saano and L. Nyman. 1988. Characterization of the selectivity, specificity and potency of medetomidine as an α_2-adrenoceptor agonist. Eur. J. Pharmacol. **150**:9-14.

Vannucci, R.C. 1990. Experimental biology of cerebral hypoxia-ischemia: Relation to perinatal brain damage. Pediatric Research. **27**:317-326.

Vornov, J.J. and J.T. Coyle. 1991. Enhancement of NMDA receptor-mediated neurotoxicity in the hippocampal slice by depolarization and ischemia. Brain Research. **555**:99-106.

Watkins, L.R., Suberg, S.N., Thurston, C.L. and Culhane, E.S. 1986. Role of spinal

cord neuropeptides in pain sensitivity and analgesia: Thyrotropin releasing hormone and vasopressin. Brain Research. **362**:308-317.

Weiner, N. Norepinephrine, epinephrine and the sympathomimetic amines in The Pharmacological Basis of Therapeutics. Edited by Gilman, A.G., Goodman, L.S. and Gilman, A. New York: MacMillan Publishing Co. Inc. (1980) Chapter 8, 138-75.

Welsh, F.A., R.E. Sims and V.A. Harris. 1990. Mild hypothermia prevents ischemic injury in gerbil hippocampus. J. Cereb. Blood Flow Metab. **10**:557-563.

West, J.B. (Ed.) 1985. Best and Taylor's Physiological Basis of Medical Practice. Eleventh ed. Williams & Wilkins. Baltimore.

Westmark, K.R., Noble, L.J., Fukuday, K., Aihara, N., McKenzie, A.L. 1995. Intrathecal administration of endothelin-1 in the rat: impact on spinal cord blood flow and the blood-spinal cord barrier. Neurosci. Lett. **192(3)**:173-176.

Whitaker-Azmitia, P.M., M. Raio, D, Raio and A. Borella. 1995. A 5-HT3 receptor antagonist fails to prevent cisplatin-induced toxicity in immature rat spinal cord. Eur. J. Pharmacol. **275(2)**: 139-143.

Williams, J.T. and R.A. North. 1985. Catecholamine inhibition of calcium action potentials in rat locus coeruleus neurones. Neuroscience. **14**:103-109.

Yaksh, T.L. 1981. Spinal opiate analgesia: Characteristics and principles of action. Pain II. 293-346.

Yaksh, T.L. 1985. Pharmacology of spinal adrenergic systems which modulate spinal nociceptive processing. Pharmacol. Biochem. Behav. **22**:845-858.

Yaksh, T.L. 1993. New horizons in our understanding of the spinal physiology and pharmacology of pain processing. Semin. Oncol. 2 Suppl 1. **20**:6-18.

Yaksh, T.L. and Reddy, S.V.R., 1981. Studies in the primate on the analgetic effects associated with intrathecal actions of opiates, α-adrenergic agonists and baclofen. Anesthesiology **54**: 451-467.

Yaksh, T.L. and T.A. Rudy. 1976. Chronic catheterization of the spinal subarachnoid space. Physiol. Behav. **17**:1031-1036.

Yamashita, K., Y. Eguchi, K. Kajiwara and H. Ito. 1991. Mild hypothermia ameliorates ubiquitin synthesis and prevents delayed neuronal death in the gerbil

hippocampus. Stroke. **22**:1574-1581.

Yanagisawa, M., Inoue, A., Ishikawa, T., Kasuya, Y., Kimura, S., Kumagaye, S.I., Nakajima, K., Watanabe, T.X., Sakakibara, S., Goto, K., and Masaki, T. 1988. Primary structure, synthesis, and biological activity of rat endothelin, an endothelium-derived vasoconstrictor peptide. Proc. Natl. Acad. Sci., USA. **85**:6964-6967.

Yoshimura, M. and R.A. North. 1983. Substantia gelatinosa neurones hyperpolarized *in vitro* by enkephalin. Nature. **305**:529-530.

Youngstrom, P., D. Eastwood, H. Patel et al. 1984. Epidural fentanyl and bupivacaine in labor: double-blind study, abstracted. Anesthesiology. **61**:A414.

Zornow, M.H., J.E. Fleischer, M.S. Scheller, K. Nakakimura and J.C. Drummond, Dexmedetomidine, an α_2-agonist decreases blood flow in the isoflurane-anesthetized dog, Anesth. Analg, 1990, **70**, 624-630.