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Spinal subarachnoid injection of somatostatin causes neurological deficits and neuronal injury in rats *

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The tetradecapeptide somatostatin produced dose-related neurological deficits following subarachnoid injection in the lumbar spinal cords of rats. Lower pharmacological doses (1.6 and 3.1 nmol, i.t.) of somatostatin caused only transient deficits, while higher doses (6.2-25 nmol, i.t.) caused persistent deficits characterized by motor and sensory impairments in hindlimbs and tail, hindlimb edema, priapism, bladder atony with infarction, and urinary incontinence. Pretreatment with 0.3 nmol of the somatostatin receptor antagonist cyclo[7-aminoheptanoyl-Phe-D-Trp-Lys-Thr(Bzl)] blocked the hindlimb paralytic effects of 3.1 and 6.2 nmol of somatostatin, and significantly improved neurological recovery injection of 12.5 nmol of somatostatin. Higher doses of the antagonist produced hindlimb paralysis by itself. Neuroanatomical evaluations revealed extensive cell loss and necrosis in the lumbosacral spinal cords of rats paralyzed by 25 nmol of somatostatin. Collectively, these results suggest that through interactions with a receptor, somatostatin destroys neurons involved in diverse spinal cord functions.

Key words: Somatostatin; Somatostatin receptors; Spinal cord; Paralysis; (Injury, Intrathecal)

1. Introduction

The cyclic tetradecapeptide somatostatin was originally isolated from the hypothalamus and identified as a potent endocrine modulator on the basis of its inhibition of growth hormone secretion from dispersed rat pituitary cells (Brazeau et al., 1973). The subsequent detection of somatostatin immunoreactivity (Brownstein et al., 1975; Kobayashi et al., 1977; and Patel and Reichlin, 1978)

and somatostatin receptor binding sites (Reubi et al., 1981; Srikant and Patel, 1981; Epelbaum et al., 1982; Leroux and Pelletier, 1984) throughout the central nervous system prompted speculation that somatostatin-related peptides may have widespread neurobiological involvements. These peptides have now been linked to diverse functions, and have been implicated in the pathophysiology of several neurodegenerative diseases (for review, see Beal and Martin, 1986).

Somatostatin immunoreactivity is present in relatively high concentration in the spinal cord, and has a pattern of distribution and actions indicative of a role as a neurotransmitter for small primary afferent neurons (Stine et al., 1982; Tessler et al., 1986; Hökfelt et al., 1976; Forsmann, 1978; Dalsgaard et al., 1981). Moreover, as is consistent with its putative role as a primary sensory transmitter, noxious thermal stimuli selec-

* In conducting the research described in this report, the investigator(s) adhere to the 'Guide for the Care and Use of Laboratory Animals', as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The views of the author(s) do not purport to reflect the position of the Department of the Army or the Department of Defense (para 4-3, AR 360-5).

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tively increased the *in situ* release of somatostatin immunoreactivity from the rabbit dorsal horn (Kuraishi et al., 1985). Conversely, intrathecal (i.t.) injection of somatostatin has been shown to increase rat spinal flexion reflex excitability in response to these stimuli (Wiesenfeld-Hallin, 1985; 1986), and to cause behaviors indicative of nociceptive somatosensory function, such as caudally directed biting and hindlimb scratching (Seybold et al., 1982; Wiesenfeld-Hallin, 1985). Paradoxically, however, others have reported that i.t. somatostatin elevated pressure pain threshold in the rat (Chrubasik et al., 1984) and depressed dorsal horn neurons activated by comparable noxious stimuli in the cat (Randic and Miletic, 1978). In addition, with increases in dose, somatostatin was shown to totally depress rather than increase spinal flexion reflexes to noxious thermal stimuli (Wiesenfeld-Hallin, 1985). Thus, somatostatin may have multiple involvements in spinal cord nociceptive mechanisms. In addition, fibers, terminals and cell bodies containing somatostatin immunoreactivity have been described in other regions of the spinal cord, and suggest additional functional roles for this peptide (Hökfelt et al., 1976; Forsmann, 1978; Stine et al., 1982; Tessler et al., 1986).

In preliminary experiments, we observed somatostatin to cause loss of nociceptive responsiveness and flaccid paralysis of the hindlimbs and tail immediately following spinal subarachnoid injection in rats (Long et al., 1987a). These responses to somatostatin closely resembled those described following i.t. injection of Dyn A-related peptides in rats (Przewlocki et al., 1983; Faden and Jacobs, 1984; Herman and Goldstein, 1985; Spampinato and Candelletti, 1985; Stevens and Yaksh, 1986; Long et al., *in press a,b*). In several cases, the persistence of motor and sensory deficits several days following somatostatin injection indicated possible injury to the spinal cord. These observations raise concern over the consequences of spinal cord exposure to somatostatin, particularly since i.t. somatostatin has recently been used for the treatment of chronic pain in humans (Chrubasik et al., 1984). Therefore, in these experiments we characterized these intrathecal actions of somatostatin in the rat. We report

that: (1) somatostatin caused dose-related neurological deficits, including loss of hindlimb motor function and nociceptive responsiveness, (2) the paralytic effects of somatostatin were blocked by pretreatment with the somatostatin receptor antagonist cyclo[7-aminoheptonyl-Phe-D-Trp-Lys-Thr(Bzl)] (Fries et al., 1982) and (3) persistent somatostatin-induced neurological deficits were correlated with neuroanatomical evidence of neuronal injury.

2. Materials and methods

2.1. Animal preparations

Injectations were made in halothane-anesthetized male Sprague-Dawley rats (300–350 g; Zivic Miller Laboratories, Allison Park, PA). Rats were secured in a stereotaxic apparatus and received dorsal midline incisions immediately rostral to the pelvic girdle. Using the vertebral processes as guides, 0.5 inch 30 gauge needles were carefully advanced to pass through intervertebral space into the subarachnoid space surrounding the cauda equina at L4 or L5 vertebral levels. Correct needle placement was verified by CSF flow from the catheter following its insertion. Peptides were dissolved in physiological saline and 15 μ l injections (peptide and cannula flush) were delivered over 20 s through PE 20 tubing secured to the distal end of the needle. Following these injections, incisions were treated with the topical antibacterial furazolidone, and closed with wound clips. Rapid recovery from halothane anesthesia enabled neurological evaluation of rats within several min following injections.

2.2. Animal evaluations

Neurological function was evaluated using a four point ordinal scale. Scores were assigned as follows: 3 = normal motor function; 2 = paraparesis, with ability to support weight and walk with slight impairment, or make walking movements without supporting weight; 1 = severe paraparesis, in which animals could make voluntary hindlimb movements but not walking move-

ments; 0 = total paralysis, with complete absence of any hindlimb movement.

For ED_{50} calculations, loss of the ability to walk (which was clearly distinguished by neurological scores of 0 or 1) was defined as a paralytic response to i.t. injection. Rats able to walk (receiving scores of 2 or 3 following i.t. injections) were regarded as non-responders. ED_{50} calculations were made using neurological scores obtained 5 min following injections (acute paralytic responses) and 24 h following injections (persistent paralytic responses).

Additional observations of drug effects on nociceptive and non-nociceptive function were made as described below.

2.2.1. Hindlimb flexion

Rats' hindlimbs were manually drawn back in a caudal extension and the flexion of limbs upon release was graded as follows: 2 = normal flexion; 1 = impaired flexion (slow weak response); 0 = absence of a flexion response.

2.2.2. Righting

Rats were placed in a supine position in a loose wood chip bedding and the ability of the animal to roll over and right itself within a 5 s period was recorded.

2.2.3. Tail flick response

Rats were gently wrapped in a towel. Using a light source focussed 2.5 cm from the tail tip, the latency for the rat to move its tail, and thereby terminate the nociceptive stimulus, was recorded. To prevent tissue damage, a 12 s maximal cutoff latency was used.

2.2.4. Response to paw pinch

Forepaws and hindpaws were pinched with a forceps and the presence or absence of a vocalization or limb flexion response was recorded.

2.3. Neuroanatomical methods

Four additional rats were anesthetized with ketamine (100 mg/kg i.m.). Two received L4-L5 vertebral level subarachnoid injections of somatostatin (25 nmol) and two were injected with the

saline vehicle. On the third postoperative day, these rats were euthanatized with sodium pentobarbital and perfused transcardially with physiological saline following immediately by 10% formalin. Brains and spinal cords were partially dissected and remained in situ for further fixation prior to microtomy and staining. Lumbar, sacral and coccygeal spinal segments were stained according to the method of Nissl and with hematoxylin and eosin.

2.4. Chemicals

Somatostatin was purchased from the Sigma Chemical Co. (St. Louis, MO) and Peninsula Laboratories (Belmont, CA), and the somatostatin antagonist cyclo[7-aminoheptanoyl-Phe-D-Trp-Lys-Thr(Bzl)] was purchased from Bachem Inc. (Torrance, CA).

2.5. Data analysis

For neurological data, ED_{50} values and their 95% confidence intervals were determined using the computer program described by Tallarida and Murray (1981). Differences in neurological function among treatment groups were compared by means of the Kruskal Wallis test (Conover, 1980). Tail flick latencies were compared using one way analysis of variance with repeated measures. Differences among treatment groups were distinguished using the Newman-Keuls method (Winer, 1971).

3. Results

Injection of somatostatin into the rat spinal subarachnoid space produced neurological impairments within 5 min. In a dose-related manner, somatostatin caused loss of motor function in hindlimbs and tail (fig. 1), loss of flexor or vocal responses to pinch of the hindpaws (table 1), and elevated tail flick latencies (table 1). Motor dysfunction ranged from a transient, mild paraparesis to persistent flaccid paralysis of hindlimbs and tail (fig 1). The ED_{50} (and 95% confidence interval) for loss of the ability to walk 5 min following

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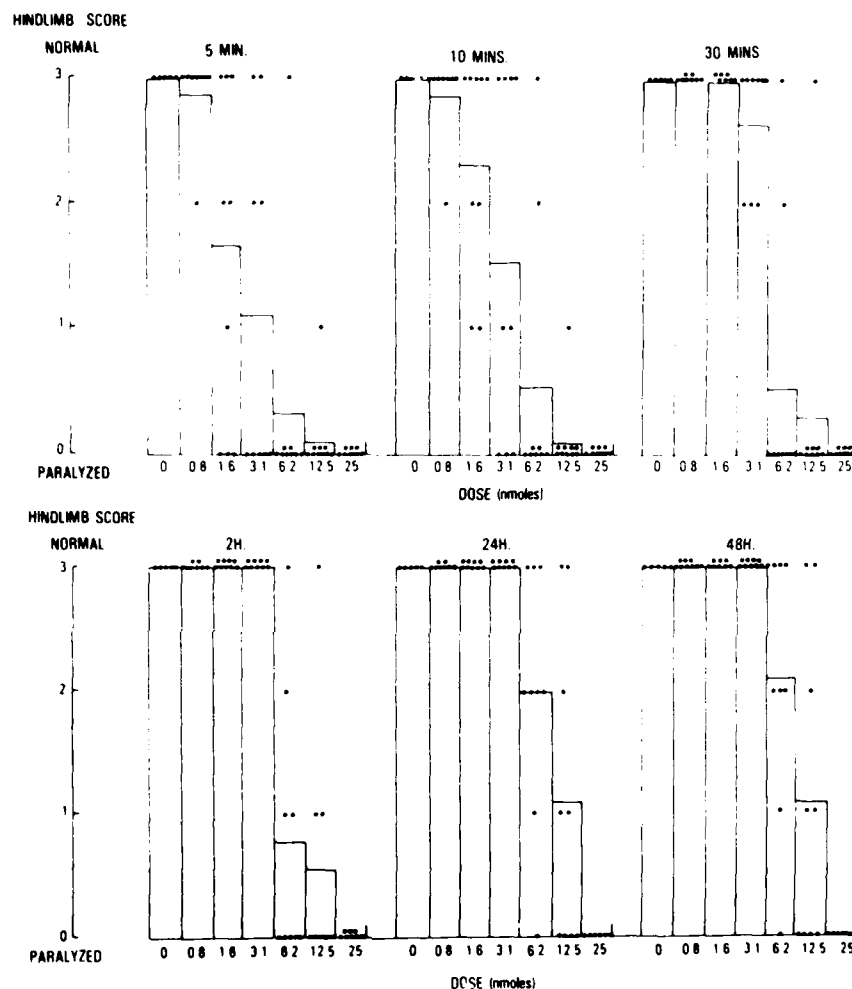


Fig. 1. Hindlimb motor function during 48 h following direct intervertebral subarachnoid injection of somatostatin. Points depict neurological scores assigned to individual rats. Bar heights represent mean scores for dose groups. Paralytic ED_{50} s (and 95% confidence intervals) were 2.4 (1.5–3.8) and 9.5 (6.9–13) nmol at 5 min and 24 h following injection, respectively. Kruskal-Wallis comparison of motor responses at 5 min and 24 h postinjection revealed significant differences among dose groups ($H(5,55) = 30.6$ at 5 min postinjection; $H(5,49) = 35.1$ at 24 h post-injection).

somatostatin injection was 2.4 (1.5–3.8) nmol. Rats were also unable to flex their hindlimbs from a caudal extension and right themselves from a supine position (table 1). No rats showed forelimb impairments. Additionally, in contrast to the somatostatin-treated rats, in no case did rats injected with saline vehicle show signs of altered neurological functions.

Rats losing hindlimb motor function also lost anal sphincter reflexes, frequently ejaculated within 10–20 min of somatostatin injection, and occasionally displayed respiratory dyspnea. Several rats died within 15–30 min of somatostatin injection, apparently as a result of pulmonary edema. Fluid was discharged from the nostrils at the time of death, and was evident in the lungs and trachea

TABLE 1

Effects of somatostatin on righting, hindlimb flexion, pinch response and tail flick latencies.

	Somatostatin dose (nmol)					
	0.8 (8) ^a	1.6 (9)	3.1 (9)	6.2 (9)	12.5 (10)	25 (9)
(1) Righting from supine position						
5 min	100 ^b	33	33	11	0	0
2 h	100	100	100	11	10	0
24 h	100	100	100	44	22	0
(2) Hindlimb flexion						
5 min						
2	88	33	33	11	0	0
1	12	22	11	0	10	0
0	0	44	56	89	90	100
2 h						
2	100	100	100	11	11	0
1	0	0	0	11	11	0
0	0	0	0	78	78	100
24 h						
2	100	100	100	67	22	0
1	0	0	0	11	22	0
0	0	0	0	22	56	100
(3) Hindlimb pinch						
5 min	100	67	44	11	10	0
2 h	100	100	100	44	33	0
24 h	100	100	100	89	56	0
(4) Tail flick latencies ^c						
Preinjection	3.1 ± 0.2	3.4 ± 0.2	3.6 ± 0.4	3.1 ± 0.3	3.2 ± 0.2	3.5 ± 0.3
5 min	3.7 ± 0.4	9.0 ± 1.3 ^d	10.3 ± 1.2 ^d	12 ^d	12 ^d	12 ^d
2 h	3.4 ± 0.3	4.9 ± 0.6	8.4 ± 1.1 ^d	11.3 ± 0.7 ^d	11.3 ± 0.7 ^d	12 ^d
24 h	3.0 ± 0.3	3.2 ± 0.2	3.8 ± 0.5	10.5 ± 0.8 ^d	12 ^d	12 ^d

^a Population in parentheses. ^b Percentage responders in each treatment group. ^c Seconds. ^d $P < 0.05$ when compared to preinjection latencies. F (treatment dose) = 69.87, $df = 5,48$; $P < 0.0001$.

during post-mortem examination. Rats with persistent loss of hindlimb motor function frequently displayed multiple convulsive episodes of short duration during a 2–6 h period after somatostatin injection.

Elevated tail flick latencies were seen with doses of somatostatin causing motor impairment (table 1). Dissociations between motor and nociceptive alterations were occasionally seen in which elevated tail flick latencies persisted following recovery of motor function. Non-paralytic doses of somatostatin did not cause elevated tail flick latencies.

Recovery from somatostatin-induced impairments was also dose-related. The ED_{50} for somatostatin-induced hindlimb paralysis at 24 h post-injection was 9.5 (6.9–13) nmol. As seen in fig. 1,

rats generally regained motor and sensory function in hindlimbs and tail within 2 h of injection of low doses (0.8–3.1 nmol) of somatostatin. In contrast, slower, limited recovery was observed following injection of 6.2 and 12.5 nmol doses of somatostatin. For example, of the eight rats losing the ability to walk following injection of 6.2 nmol of somatostatin, only one could walk 2 h after injection, whereas six of these rats could walk 24–48 h later. Following injection of 25 nmol of somatostatin, rats did not appreciably recover neurological function over 48 h of observation, and flaccidity persisted or occasionally changed to spasticity by 4–7 days following injection. Along with persistent motor impairment, these rats were characterized by continued loss of nociceptive responsiveness, hindlimb edema, bowel dysfunction

(as evidenced by diarrhea or aggregated dried stools), bladder atony with infarction, and urinary incontinence.

Neuroanatomical study revealed that extensive spinal cord injury was associated with the persistent neurological deficits produced by 25 nmol of somatostatin (fig. 2b). Cell loss, necrosis, and cavitation of the gray matter was evident through many lumbosacral segments. Marked loss of nerve cells was widespread bilaterally in all nuclear groups. In contrast, no signs of neuronal injury

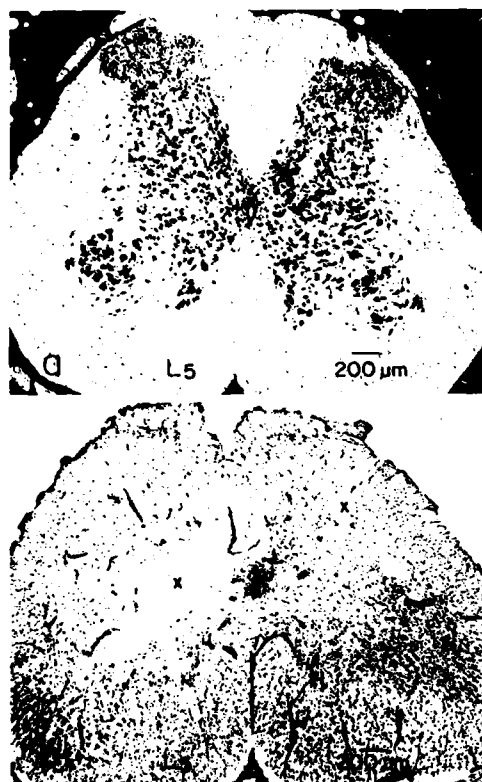


Fig. 2. Photomicrograph of Nissl stained spinal cord sections from rats injected with saline vehicle (a) or 25 nmol of somatostatin (b). Spinal cords were removed 72 h following intervertebral subarachnoid injections. Cells and nuclear groups of control spinal cords (a) were completely normal in appearance. By comparison, spinal cords of rats rendered paraplegic by somatostatin (b) were characterized by bilateral loss of neurons from all spinal cord nuclear groups, together with necrosis and cavitation (indicated by Xs) in the gray matter.

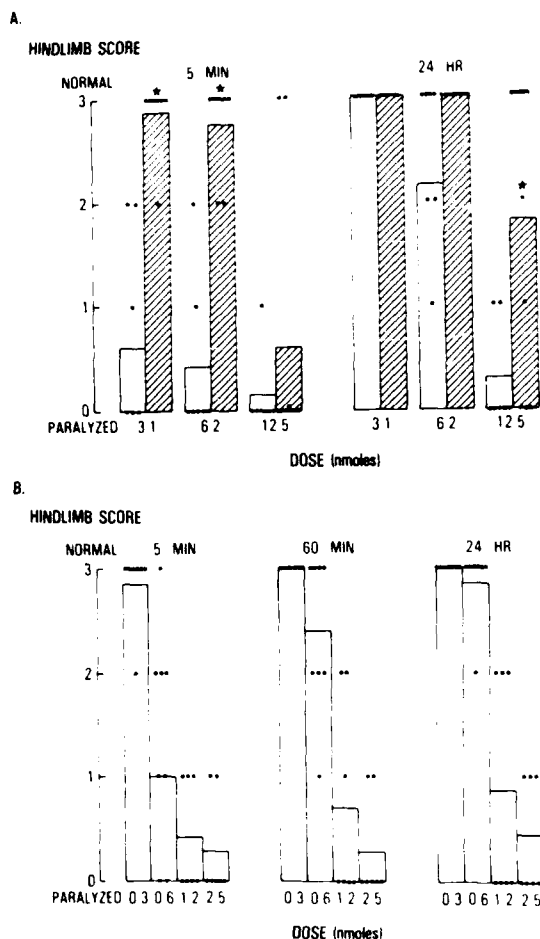


Fig. 3. (A) Effects of somatostatin receptor antagonist pretreatment on hindlimb paralytic actions of somatostatin. Rats were i.t. injected with saline vehicle (open bars) or 0.3 nmol of the antagonist (cross hatched bars) 5 min prior to injection of somatostatin (3.1, 6.2 or 12.5 nmol, i.t.). (B) Dose-related hindlimb paralytic actions of the somatostatin antagonist alone. * $P < 0.05$ when compared to rats pretreated with saline vehicle (Kruskal Wallis test; $H(5,45) = 28.8$ and 27.5 at 5 min and 24 h post-injection, respectively).

were evident in spinal cords of rats injected with the saline vehicle (fig. 2a).

Ten minute pre-treatment with the somatostatin receptor antagonist (0.3 nmol i.t.) blocked hindlimb paralytic actions of 3.1 and 6.2 nmol doses of somatostatin (fig. 3a). This dose of antagonist failed to block the hindlimb paralysis

seen 5 min following injection of 12.5 nmol of somatostatin; however, neurological recovery at 24 h post-injection was significantly improved in these rats (fig. 3a). Other aspects of neurological function (table 1) were similarly preserved following pretreatment with this dose of the somatostatin antagonist (results not shown). Although hindlimb motor function was not appreciably altered following injection of 0.3 nmol of the antagonist alone, at higher doses (0.6–2.5 nmol i.t.) it produced hindlimb paralysis by itself (fig. 3b).

4. Discussion

Following its injection into the rat lumbar spinal subarachnoid space, somatostatin caused persistent motor, sensory and autonomic dysfunction. Collectively, these deficits indicate that somatostatin caused a generalized injury to the lumbosacral spinal cord (*vide infra*). Previous reports addressing i.t. actions of somatostatin in the rat did not describe these signs of neurological impairment, and instead noted behaviors suggestive of nociceptive functions, such as scratching and caudally directed biting (Seybold et al., 1982; Wiesenfeld-Hallin, 1985), or potent antinociception (Chrubasik et al., 1984). We did not observe these behaviors following direct intervertebral injection of somatostatin in halothane-anesthetized rats, and suspect that these responses may have been obscured by the transient, halothane-induced anesthesia in our model.

The failure to observe hindlimb paralytic actions of somatostatin in previous studies may have resulted in part from the i.t. injection technique used. We used direct intervertebral needle injections in these experiments to avoid potential complications associated with the passage of a catheter through the length of the spinal cord subarachnoid space. In contrast, in earlier studies injections were made through indwelling i.t. catheters which were implanted a minimum of 7 days before experiments. As previously noted with other peptides (Long et al., submitted), while comparable potencies were observed in rats injected through intervertebral needles and indwelling i.t. catheters implanted one day preceding injection,

the paralytic potency of somatostatin was observed to diminish as the post-catheterization interval increased (unpublished observation). Thus, there is a loss of i.t. somatostatin potency in rats with chronically implanted i.t. catheters. Due to this loss of potency, it is not unexpected that doses of 0.6–12.2 nmol of somatostatin did not cause hindlimb dysfunction when injected through chronically implanted catheters in previous experiments (Seybold et al., 1982). Nonetheless, in accordance with our findings, Wiesenfeld-Hallin (1985) noted that in contrast to the caudally directed scratching and heightened hindlimb flexion reflex activity caused by lower somatostatin doses (6.1 and 0.6 nmol, respectively), 15.3 nmol of somatostatin caused reversible paralysis of the hindlimbs lasting 2–6 h, and 6.1 nmol totally blocked reflex activity.

In contrast to nociceptive behaviors, Chrubasik et al. (1984) reported that an i.t. bolus injection of somatostatin induced a brief, but significant increase in pain threshold in the rat. We observed increased tail flick latencies and loss of vocal responses to hindlimb pinch only with doses of somatostatin causing at least transient motor disruptions. The general co-occurrence of motor and sensory deficits suggests that these nociceptive alterations result from generalized disruptive actions of the peptide, rather than from specific antinociceptive mechanisms.

Pretreatment with 0.3 nmol of the somatostatin receptor antagonist described by Fries et al. (1982) blocked the paralytic effects of low doses of somatostatin and lessened the severity of dysfunction caused by 12.5 nmol of somatostatin (as reflected by improved neurological recovery 24 h post-injection). These data indicate that these effects may have resulted from interaction with a receptor or recognition site at which this compound is an antagonist. However, the somatostatin antagonist at higher doses caused hindlimb dysfunction by itself, and was in fact a much more potent paralytic agent than somatostatin (figs. 1 and 3). Therefore, this compound may actually be a potent partial agonist at this receptor site. This possibility is supported by previous indications of partial agonist effects of this somatostatin analog (Fries et al., 1982). Alternatively, the paralytic

actions of the somatostatin antagonist may be independent of somatostatin receptors, and result from mechanisms not involving receptor interactions, or actions at a distinct receptor site.

A variety of peptides including dynorphin A (Herman et al., 1980; Przewlocki et al., 1983; Faden and Jacobs, 1984; Herman and Goldstein, 1985; Spampinato and Cadelletti, 1985; Long et al., in press a,b), [Arg⁸]vasopressin (Kruse et al., 1977; Millan et al., 1984;), substance P antagonists (Piercy et al., 1982), and the δ opioid antagonist ICI 174864 (Long et al., in press a) share identical neurological effects with somatostatin, which, in addition to hindlimb paralysis following i.t. injection, include 'barrel rolling' following intracerebroventricular injection (Cohn and Cohn, 1975). Since receptor antagonists have predictable selectivities in blocking these actions, the shared effects are apparently mediated through multiple recognition sites. For example, the somatostatin receptor antagonist was selective for the i.t. actions of somatostatin and did not alter the paralytic actions of either dynorphin A-(1-13) or [Arg⁸]vasopressin (results not shown). Although initiated through interactions with distinct receptors, these consistently shared effects point to commonalities or convergences in the spinal and supraspinal mechanisms of action of this diverse collection of peptides.

The persistence and diversity of the neurological deficits induced by i.t. somatostatin indicated that it was causing widespread neuronal injury in the lumbosacral spinal cord. Neuroanatomical evaluations confirmed that the paraplegia produced by 25 nmol of somatostatin was accompanied by severe and extensive neuronal injury throughout the lumbosacral enlargement. The pronounced loss of neurons coupled with the prominent areas of necrosis and cavitation in the gray matter correlated well with the degree and persistence of the neurological deficits observed in these rats. The widespread pattern of injury contrasted with the discrete distribution of endogenous somatostatin and its receptors in the spinal cord (Hökfelt et al., 1976; Forsmann, 1978; Dalsgaard et al., 1981; Uhl et al., 1985). Thus, although apparently mediated at least in part through somatostatin receptors, the deleterious ef-

fects of i.t. somatostatin were not restricted to neuronal elements bearing receptors for somatostatin. These results suggest that a chain of events initiated by the receptor actions of somatostatin resulted in a generalized deleterious outcome throughout the cord. One mechanism by which i.t. somatostatin might induce a broad spectrum of neurological deficits is through ischemic injury to the lumbosacral spinal cord. This possibility is supported by the observation that paralytic i.t. doses of somatostatin caused striking reductions in rat lumbosacral spinal cord blood flow which were blocked by the somatostatin receptor antagonist (Long et al., 1987b). Thus, either directly or indirectly, somatostatin may be acting on the vasculature, restricting perfusion, and causing widespread ischemic damage.

→ Intrathecal administration of somatostatin has recently been clinically investigated as a means to treat intractable pain in humans (Chrubasik et al., 1984). Significant reductions in patient pain scores were recorded following bolus i.t. injections of 152.6 nmol of somatostatin, followed by i.t. infusions of 6.1–30.5 nmol/h. Obviously extrapolation between species is speculative due to potential differences in species sensitivities and the considerable differences in spinal cord sizes and volumes of distribution. Nevertheless, the present findings signal a need for caution and more comparative data before somatostatin is further used as a spinal analgesic in humans.

In summary, i.t. injections of somatostatin in the rat caused neurological impairments and neuronal damage. The neurological effects of somatostatin were dose-dependent and appeared to be receptor-mediated, since they were to some degree blocked by a somatostatin receptor antagonist. These results indicated an obvious need for caution in interpreting experimental responses to i.t. somatostatin and additionally point to the potential pathophysiological relevance of this neuropeptide.

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Con'd pg 297

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