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Intrathecal Somatostatin in Rats: Antinociception Only in the Presence of Toxic Effects

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Effects of intrathecal (i.t.) somatostatin (SST) (10, 30, and 100 μg) on nociception, and autonomic and motor function were evaluated in rats with chronically implanted lumbar i.t. catheters. Doses of 10 and 30 μg SST i.t. had no effect on thermal cutaneous nociception, (hot plate and tail flick response). Thirty micrograms SST i.t. did not effect the visceral chemical evoked nociception (acetic acid writhing test) as compared to saline control groups. Rats treated with 100 μg SST i.t. invariably showed temporary or permanent hindlimb motor dysfunction, with flaccid paralysis in the most severe cases (motor function tests, electromyographic response). Blockade of the tail flick and foot pinch response was observed to a variable degree, and only in the presence of detectable motor impairment. Out of 40 animals injected with 100 μg SST i.t., 25% died within 10 min following injection. Effects of SST on the volume evoked micturition reflex were assessed in rats with chronically implanted bladder catheters. Three of the nine surviving animals receiving 30 μg SST i.t. and all animals receiving an additional dose of 60 μg SST i.t. showed a complete block of the micturition reflex and subsequent development of an overflow bladder. Histological examination of spinal cords revealed a mild inflammatory response in four out of five animals treated with 30 μg SST i.t. In spinal cords of animals, which had received 100 μg SST i.t. (n = 4), mild or severe nucleolysis of ventral and dorsal horns in the presence of inflammatory reaction was observed. Present experiments clearly demonstrate highly toxic effects of SST in rats with no margin of safety between antinociception and motor dysfunction. (Key words: Bladder: micturition reflex. Muscle: electromyography. Pain: experimental; measurement. Peptides: somatostatin. Spinal Cord: Nucleolysis; reflexes. Toxicity: somatostatin.)

SOMATOSTATIN (SST), a cyclic tetradecapeptide, may be involved in processing nociceptive information at the level of the spinal cord. Its functional role is, however, controversial. SST is present in small diameter cells of dorsal root ganglia,[1] associated with C-fiber afferents,[2] and in extrinsic and intrinsic dorsal horn fibers and terminals, with especially high concentrations in lamina II.[3] It is released from the spinal dorsal horn following noxious thermal stimulation,[4] and iontophoretic application of SST produces a suppression of dorsal horn neuronal activity.[5] It is not readily apparent whether exogenous SST, acting near spinal terminals containing SST, should intensify or alleviate pain. Thus, following intrathecal (i.t.) injection of SST in rats, increased excitability,[6] evidenced by prominent scratching and biting behavior, indicative of nociceptive sensation, has been reported. Alternatively, increased response latencies to tail pressure test,‡ indicating antinociceptive properties, have been observed. However, in the latter study, effects on motor activity were not systematically examined, and evidence of lethality was noted, at only slightly higher doses.‡ Despite these findings, SST has been given intrathecally and epidurally in humans for the management of acute postoperative and chronic cancer pain.[7,8] Prominent analgesia without negative side effects was reported in these clinical trials, which had been carried out without previous extensive toxicological animal studies. The present experiments were thus undertaken to systematically explore analgesic and possible toxicological effects of i.t. SST.

Materials and Methods

Animal Preparation

Experiments were performed in male Sprague-Dawley rats (250–300 g, Harlan Industries, Indianapolis, IN) that were housed in individual cages and had free access to food and water. The study was performed according to protocols approved by the Mayo Foundation Animal Care Committee.

In these experiments to be discussed, rats were implanted with: 1) an i.t. catheter only; 2) an i.t. catheter and a carotid artery catheter; 3) an i.t. catheter and a bladder catheter; or 4) a fourth ventricular cannula and carotid artery catheter.

Lumbar intrathecal catheters (n = 68) were implanted 3–5 days prior to the experiments under halothane anesthesia.[9] Through an insertion in the atlanto-occipital membrane, a PE-10 catheter was advanced 8 cm caudally, to position its tip at the thoracolumbar level of the spinal cord. The catheter was externalized on top of the skull and plugged with a piece of steel wire.

Cannulation of the fourth ventricle was performed 1 day prior to the experiment in rats (n = 7), not implanted with i.t. catheters. A guide cannula (24-gauge, thin-wall,

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‡ Ackerman E, Chrubasik J, Weinstock M, Wünsch E: Effects of intrathecal somatostatin on pain threshold in rats. Schmerz Pain Douleur 2:41–42, 1985.

stainless steel) was implanted stereotactically 9 mm caudally to the bregma at a depth of 5 mm,[10] so that the tip was positioned just superior to the fourth ventricle. On the day of the experiment, an injection cannula (29-gauge) was advanced through the guide cannula and positioned at a depth of 6.5 mm in the fourth ventricle. The position of the cannula was verified after termination of the experiment by injection of 10 μl Evans blue through the cannula.

Carotid artery catheters (PE-50) were implanted in rats having chronic i.t. catheters (n = 8) or fourth ventricular cannulae (n = 7) under halothane anesthesia 4 h prior to the experiment, to allow monitoring of arterial blood pressure (BP) and heart rate (HR) during the experiment (Grass® polygraph 7 model). Catheters were flushed with 100 U of heparin, plugged with steel wire and externalized on top of the head.

Bladder catheters[11] (n = 10) were implanted under halothane anesthesia 2 days prior to the insertion of the i.t. lumbar catheter to examine the effect of SST on bladder function. *Via* a lower median abdominal approach, a PE-100 tubing was advanced through a small incision at the vertex of the bladder. The catheter was sewed in place, the distal end was passed subcutaneously, externalized on top of the head, and plugged with stainless steel wire. To prevent infection, these animals received Amikacin sulfate (2.5 mg/kg, im) on a daily basis.

Nociceptive Tests

To assess the possible analgesic action of i.t. SST, its effects were assessed on a variety of nociceptive endpoints. The use of multiple assays allows consideration of whether the physiologic effect of a given drug is dependent upon a specific stimulus modality. This is particularly important in the face of negative data.

Foot Pinch. The response to pressure was evaluated by foot pinch. In this test, the presence of vocalization, agitation, or withdrawal to firm pinching of the hind foot with blunt forceps was noted. In our experience, normal rats react to this stimulus uniformly with an aversion reaction.

Hot Plate. The hot plate response was evaluated by placing the animal in a plexiglass enclosure on a metal surface with a constant temperature of 52.5° C. The response latency was recorded as the time the animal required to lick one of its hindpaws. Some animals which failed to show this specific behavior instead engaged in characteristic jumping behavior. In these animals, the endpoint was taken when both feet left the hot plate surface. Cutoff time in the absence of a response was 60 s.

Tail Flick. The tail flick response was evoked by placing the animal's tail over a rectangular slit above an illuminated projector lamp (Sylvania 300 W bulb). The response latency was the time required for the rat to vigorously remove its tail off the slit. To prevent tissue damage, the cutoff time was 6 s in the absence of a response.

Acetic Acid Writhing. Twelve rats with i.t. lumbar catheters were randomly assigned to a drug group (i.t. SST) or i.t. saline treatment. All animals were then injected with 0.5 ml of 9% acetic acid ip and placed randomly in individual plexiglass compartments of the observer chamber. The observer was blinded for the treatment groups, and scored individual rats over 5-min periods for 1 h. Grading scales were: 0 = normal exploratory behavior or normal posture with paws flat on floor; 1 = slight leaning to side or extended hindlimb; 2 = extension of both hindlimbs, body stretched and flat, often with pelvis rotated sideways; and 3 = active writhing, contraction of abdominal muscles with stretched hindlimbs and flattening abdomen against the floor. This test has been evaluated in our laboratory for reliability and reproducibility.[12] Animals were killed immediately at the end of the 1-h observation period by an anesthetic overdose.

Assessment of Motor Function

Motor function was evaluated by observation of specific response behavior and by electromyographic recording (EMG).

Placing/stepping Reflex. Drawing the dorsum of either hindpaw across the edge of the table elicits an upward lifting of the paw onto the surface of the table. When the plantar surface of the hindpaw is placed lightly on the surface of the table, an extension of the paw to make solid contact is evoked.

Righting Reflex. If a rat is placed horizontally with its back on the table, the normal rat will display a coordinated torsional twist around the body axis, to regain its normal position on its legs. This reflex can be separated into a cranial component, involving front leg activity, and a caudal component, involving hind leg activity.

Ability to Negotiate a 60° Inclined Plane. When placed upon a 60° inclined wire mesh surface, normal animals have no difficulties in ascending or descending on the plane.

Pinnae Reflex. Gently stroking the external auditory meatus with the point of a pencil evokes a brisk twitching of the pinnae.

Eye Blink Reflex. Touching the cornea with a blunt probe evokes closure of the eyelids.

EMG. Animals were placed in holding cages, which allowed unrestricted limb movement. Two monopolar platinum (10 mm × 100 μm diameter) electrodes were percutaneously inserted into the gastrocnemius muscle, and a third ground electrode was placed in the gastroc-

TABLE 1. Summary of Experimental Protocols

	Drug Injection	Cannulation	Examination	Number of Rats in Treatment Groups*					
					SST				
				SAL	10	30	60	100	Total
Single Injection	i.t.	—	HP, TF Motor function, histology	5	5	5	—	5	20
	i.t.	—	AAW	6	—	6	—	—	12
	i.t.	—	EMG	—	—	—	—	18	18
	i.t.	Carotid artery	BP, HR	—	—	—	—	8	8
	Fourth ventricle	Carotid artery	BP, HR	—	—	4	—	3	7
Repeated Injections	i.t.	Bladder	Bladder function	10	—	10	9	—	10

HP = hot plate response; TF = tail flick response; AAW = acetic acid writhing response; i.t. = intrathecal.
* Number of rats in the different treatment groups: saline (SAL) and somatostatin (SST) at 10, 30, 60, and 100 μg. For further details, see text.

nemius muscle of the other side. EMG signals were differentially amplified (Grass® P511K) and band-pass filtered from 10 Hz to 3 KHz. The resultant signal was displayed on a strip chart recorder (Grass® 7H25) calibrated to 50 μV/cm. The actual EMG signal was simultaneously full-wave rectified and integrated by a signal processor (Grass® 7P10E) which was calibrated so that the vertical pen travel per unit time on the chart recorder was proportional to the root-mean-squared power of the EMG.

Evaluation of Bladder Function by Cystometrography

To assess the volume evoked micturition reflex, rats were placed in open wire restraining cages, which allowed a normal sitting posture, but prevented front-to-back rotation. Urine was collected directly into a lever arm-mounted cup connected to a volume-calibrated strain gauge. The bladder catheter was connected to a continuous infusion pump (250 μl saline/min) and to a transducer for the monitoring of bladder pressure. The slow saline infusion normally evokes regular bladder contraction with a voided volume of approximately 1 ml every 4 min. Bladder pressure and voided volume were continuously monitored on a two-channel pen writer. The following parameters of the volume-evoked micturition reflex were measured: baseline pressure, bladder opening pressure, peak pressure, and volume of urine expressed per bladder contraction (see[11] for other details of preparation).

Drug Injections and Treatment Groups

Table 1 summarizes the different experimental protocols.

Cyclic SST-14, purchased from Sigma (No. S-9129, Lot No. 114F-07982, 114F-01011) and Peninsula (No. 8001-GMP-111, Lot No. 011117, 011614), was prepared for i.t. injection immediately prior to the experiment by dilution in sterile 0.9% NaCl. SST was always delivered in a volume of 10 μl, which was cleared from the i.t. catheter by subsequent injection of 10 μl 0.9% NaCl (hand-driven injection pump).

Analgesic and behavioral effects of SST were evaluated in three different groups of rats (n = 5 in each group). These rats received a single i.t. injection of 10, 30, or 100 μg SST in random order and unblinded fashion, and results were compared to a saline control group (n = 5), which received 20 μl 0.9% NaCl i.t. To evaluate the effects of SST on chemical visceral evoked pain, six rats were injected with 30 μg SST i.t., the highest dose not evoking permanent or temporary hindlimb motor dysfunction. A higher dose (100 μg SST i.t.) would have clearly identified animals as belonging to the treatment group, and, thus, would have been incompatible with the objective scoring of writhing behavior. A group of 18 rats receiving 100 μg SST i.t. was subjected to EMG evaluation. Monitoring of BP and HR was performed during the injection of SST at the lumbar level (n = 8, SST 100 μg), and into the fourth ventricle (n = 3, SST 100 μg; n = 4, SST 30 μg). Due to the minimal dead space of the intraventricular cannula, the fourth ventricular injection of SST (10 μl) was not followed by a saline flush, as in all other (i.t.) cases. Rats with bladder catheters (n = 10) were studied only if they showed a normal volume evoked micturition reflex.[11] These animals received subsequently, with an interval of 30 min, 0.9% NaCl, 30 μg and 60 μg SST i.t., with individual injection volumes of 10 μl, each followed by a saline flush of 10 μl.

Data Analysis

Hot plate and tail flick tests were performed on day 1 at baseline prior to i.t. drug injection (saline, SST 10,

30, or 100 μg) and at 5, 15, 30, 60, 120, and 180 min post-injection. Tests were repeated once on each of the following 5 days. Response analysis to hot plate and tail flick was performed by calculating the maximum per cent effect (MPE) according to the following equation: MPE = (post-drug response latency − pre-drug response latency) × (cutoff latency − pre-drug response latency)$^{-1}$ × 100. Mean MPEs were calculated at individual time points for the different treatment groups. The highest MPE values achieved over a period of 5–180 min and during day 2 to day 6 in individual animals were compared between groups by one-way analysis of variance. One-way ANOVA was also carried out to assess changes in body weight during the observation period in these different groups. Response analysis of acetic acid writhing was performed by summing the individual 5-min writhing scores assigned to each animal over the 60-min observation period, yielding the cumulative writhing score. The mean cumulative writhing scores were calculated for each group, and possible group differences were assessed by unpaired *t* test, since this scoring approaches a normal distribution.[12] Analysis of EMG tracings was carried out by counting the peaks of the integrated EMG response tracing over 5-min periods, and expressing the EMG activity as peaks × min^{-1}. Time points of EMG evaluation were at baseline, after the animal was allowed a 15-min adaptation period in its restraining cage, and immediately after the i.t. injection of 100 μg SST (1–6 min). Some of the animals were again tested at 1 h and 2 or 3 days after the SST injection. EMG activities post-drug injection were compared to baseline values by paired *t* tests. The paired *t* test was also applied, where appropriate, to evaluate changes in hemodynamic parameters following SST administration. The overall survival rate in the SST i.t. treatment groups (10, 30, 100 μg) was analyzed by the Chi-square test. Evaluation of behavioral tests was performed on a ± scale for placing and stepping, negotiation of the 60° inclined plane, righting, and pinnae reflex. Analysis was performed by Chi-square test.

Histology

Animals in the four different treatment groups (saline, SST 10, 30, or 100 μg i.t.) previously subjected to nociceptive and behavioral tests were killed on day 6 by an overdose of pentobarbital. Spinal cords were immediately removed with the i.t. catheter in place and fixed in 10% buffered formalin phosphate. A segment of the lumbar spinal cord just distal to the tip of the i.t. catheter was embedded in paraffin and cut in 10-μm sections. Sections were stained with hematoxylin eosin and luxol fast blue. Histologic evaluation of these slides was performed by an observer blinded for the different treatment groups. Segments were scored with regard to nucleolysis and inflammatory response on a scale of 0 to 2 (0 = no pathologic findings; 1 = mild changes, normal and pycnotic nuclei present, some inflammatory cells; 2 = severe pathologic changes, necrosis of most neurons, massive invasion of grey matter with inflammatory cells).

Results

General Behavioral Characteristics of Intrathecal SST

Injection of 100 μg SST i.t. consistently evoked severe agitation with vocalization during and immediately after injection, and was followed by a period of intense hindlimb scratching of flank and abdomen. Though not quantified, milder signs of agitation were transiently observed following 30 and 10 μg of SST i.t. No acute effects were observed with vehicle alone (saline). In the 100-μg SST i.t. group, severe hindlimb motor dysfunction developed between 3 and 5 min post-injection. These rats were unable to perform any behavioral tests involving the hindlimbs, whereas the pinnae reflex was consistently present (see below). Motor function showed varying degrees of return in some animals after periods ranging from 30 min to 6 days (see below). In general, as soon as motor function could be assessed, the hot plate latency displayed baseline values and the animal would show a distinct response to foot pinch. The tail flick latency, however, consistent with a continued motor weakness often remained prolonged. When hindlimb motor function was regained, a characteristic change in the pattern of moving was noted. Thus, body weight was not supported, as normally, by posing the hindpaws flat on the ground, but by making contact to ground only by standing on the toes. With this atypical "toe-walk," all motor function tests could still be performed. In animals in which motor dysfunction did not recover, hindlimb paralysis frequently changed from an initial flaccidity to spasticity over the following days. Animals receiving 100 μg i.t. SST frequently developed urinary retention, and clear evidence of hemorrhagic cystitis.

Effects of Intrathecal SST on Nociception and Motor Function

As indicated in figure 1, animals receiving saline, 10 and 30 μg SST i.t., displayed no change in the tail flick and hot plate latencies at intervals up to 30 min after injection. In the 100-μg SST i.t. group, the increase in tail flick latency was invariably accompanied by severe hindlimb dysfunction, and the hot plate test could not

be performed due to an inability to support weight on the hindpaws. Similarly, while pinch applied to the forepaw would elicit a vigorous squeaking and withdrawal, hindpaw pinch was without effect in animals showing motor dysfunction. This suggests motor impairment was accompanied by a total loss of somatic sensitivity.

As indicated in table 2, loss of motor function could be demonstrated with a variety of qualitative endpoints, including the loss of the placing and stepping reflex, inability to negotiate a 60° inclined plane, and the caudal righting response. The apparent limitation of the drug to the caudal dermatomes was also suggested by the preservation of the pinnae reflex, representing intact cervical sensory and cranial motor function.

SST at 30 μg i.t. did not alter the acetic acid evoked writhing response. Rats that received 30 μg SST i.t. had cumulative writhing scores of 14.5 ± 2.1 (mean ± SE) during the 1-h observation period, as compared to i.t. saline treated animals (22.7 ± 3.3). This value did not reach significance ($P > 0.05$).

Changes in body weight (day 1 to day 6) were inconsistent, with no differences between treatment groups.

In 18 rats receiving 100 μg SST i.t., hindlimb motor activity from the gastrocnemius muscle was recorded by EMG. Within 6 min following the injection of 100 μg SST i.t., a significant decrease in EMG activity (1.55 ± 0.33 peaks/min, mean ± SE) compared to baseline (2.71 ± 0.37) was observed, which was still present 1 h post-injection (2.06 ± 0.39). Integrated EMG values obtained on either day 2 or 3 were not different from baseline. At this time, hindleg spasticity with intermittent tremor had often developed, thus adding nonfunctional activity to the EMG.

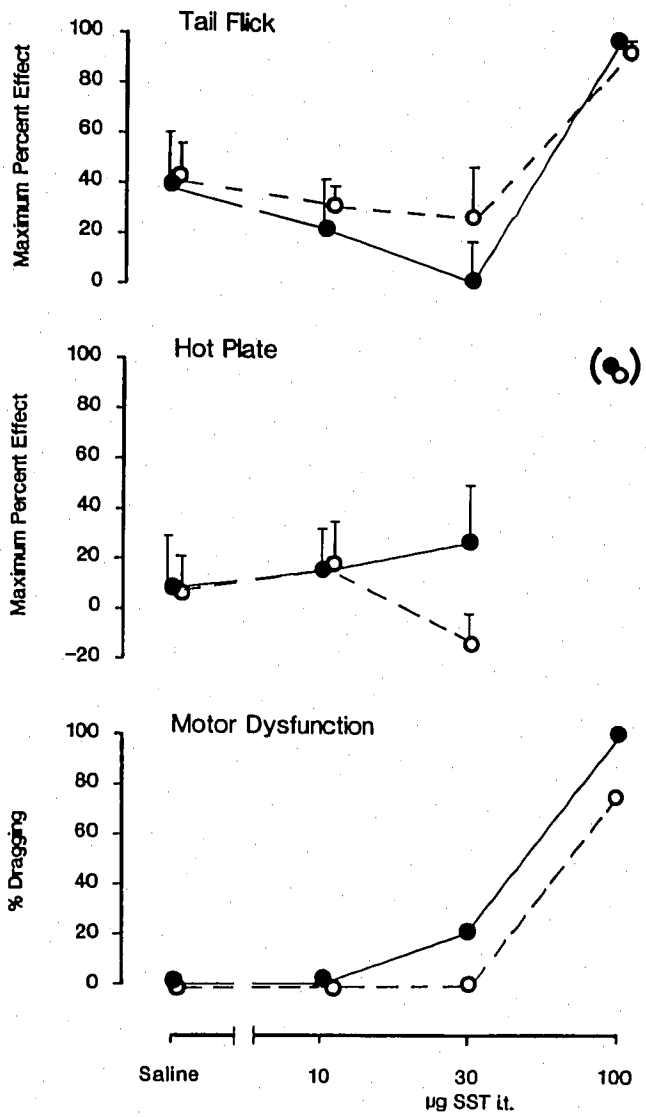

FIG. 1. Effects of intrathecal (i.t.) saline and 10, 30, and 100 μg somatostatin (SST) in rats on tail flick and hot plate response latencies expressed as maximum percent effect (mean ± SE) and corresponding motor dysfunction expressed as percent of animals dragging their hindlegs (% dragging) at 5 min (— ● —) and 30 min (- - O - -) following drug injection. Data from five animals each in saline, 10 and 30 μg SST group, from four animals in the 100 μg SST group, since one rat in this group died within 5 min following drug injection. Due to severe motor dysfunction in the 100-μg SST group, hot plate latencies could not be evaluated.

EFFECTS OF INTRATHECAL SST ON BLADDER FUNCTION

Figure 2 shows the effects of i.t. SST on the volume evoked micturition reflex with representative cystometrographic tracings obtained in one animal. Under baseline conditions and following injection of i.t. saline, constant infusion of saline (250 μl/min) through the indwelling bladder catheter evoked regular bladder contractions and the simultaneous emission of urine (fig. 2A). Following injection of 30 μg SST i.t., a few rapid bladder contractions with decreased peak pressures, still leading to the emission of urine, were observed. This was followed by a period (8 min) of complete blockade of urinary outflow, despite the occurrence of bladder contractions as evidenced by peak pressures (fig. 2B). After this temporary loss of bladder function, a regular, though less frequent, volume evoked bladder reflex developed again. Thirty minutes later, 60 μg SST i.t., administered in the same animal, led to complete and prolonged block of the micturition reflex (fig. 2C). Immediate urinary retention, lasting approximately 10 min, was observed, despite bladder contractions with high peak pressures during the initial 5 min. Subsequently, all bladder activity was lost and a slow increase in intravesicular pressure, due to the continuous infusion of saline, was observed. As the bladder

TABLE 2. Effects of Intrathecal Somatostatin (SST) on Test of Motor Function in Rats

Tests of Motor Function*	Saline/SST 10 μg (n = 5)		SST 30 μg (n = 5)		SST 100 μg (n = 4)	SST 100 μg (n = 3)	Significant Trend‡	
	Day 1†	Day 6	Day 1†	Day 6	Day 1†	Day 6	Day 1	Day 6
Pinnae reflex	5/5	5/5	5/5	5/5	4/4	3/3	—	—
Caudal righting response	5/5	5/5	4/5	5/5	0/4	2/3	§	—
Placing/stepping	5/5	5/5	4/5	5/5	0/4	2/3	§	—
60° Inclined plane	5/5	5/5	4/5	5/5	0/4	2/3	§	—

* Test of motor function evaluated on a ± basis. Numbers refer to animals showing a positive response (= left side of dividing slash) *versus* total number of animals in that group (= right side of dividing slash).

† The worst score during a 5–180-min evaluation post-intrathecal injection is presented.

‡ Significant (§) difference between treatment groups analyzed by Chi-square test.

pressure reached a critical level (40 cm H_2O), continuous emission of urine (dribbling) occurred, indicating an overflow bladder. Similar results were obtained in the other animals subjected to this protocol. Out of ten rats receiving 30 μg SST i.t., one died within 10 min following drug injection. Three of the remaining nine rats acutely developed an overflow bladder with dribbling, whereas the others showed a tendency to an increase in baseline and bladder opening pressures and a decrease in peak pressures and voided volumes, indicating some degree of urinary retention. Subsequent administration of 60 μg SST i.t. led to the death of three animals within 10 min. Surviving animals showed a prolonged and complete block of the micturition reflex with development of an overflow bladder.

Effects of Intrathecal SST on Hemodynamic Parameters

In eight animals, HR and BP were continuously monitored during the injection of 100 μg SST i.t. Four of these animals died within 6–10 min due to rapid cardiovascular collapse, which was preceded by an initial significant increase in mean arterial BP (186 ± 10 mmHg, *versus* 136 ± 2 mmHg at baseline, mean ± SE, n = 4), and followed by respiratory arrest. Previous studies from this laboratory showed that i.t. injection of other analgesic drugs administered in the same volume (10 μl) of normal saline did not evoke any hemodynamic changes (see[13]).

TABLE 3. Immediate Death Rate following Somatostatin (SST) Administration

Injection Site	SST 10 μg	SST 30 μg	SST 100 μg
i.t.	0%	6.25%	25%
Fourth ventricle	—	50%	33%

Death rates expressed as percent of animals dying within 10 min following the administration of SST intrathecally (i.t.) or into the fourth ventricle. Calculations are based on numbers presented in table 1.

Effects of Ventricular SST on Hemodynamic Parameters and Behavior

To assess the possible role of central sites in the development of cardiovascular collapse, in three rats with carotid catheters, 100 μg SST was injected into the fourth ventricle. During the initial excitation period, all of the rats showed an immediate marked increase in BP with a mean value of 210 mmHg, *versus* 155 mmHg at baseline. Within 3 min, one of these animals died due to overt respiratory failure. Four animals received 30 μg SST into the fourth ventricle, and two of these animals also died after an initial excitation period followed by apparent respiratory failure.

Animals that survived the initial phase displayed a peculiar pattern of behavior and changes in motor function which were very distinct from those observed after the injection of i.t. SST at the lumbar level. In the initial period of excitation (~2 min) following the fourth ventricular SST injection, animals displayed "barrel rolling" behavior. Severe hyperalgesia was present for a period of 10–45 min, as indicated by a marked vocalization response to skin brush and mild footpinch, as well as a prolonged and intensified pinnae reflex. Eye blink reflexes were absent. Subsequently, hind and front legs displayed spontaneous and symmetrical inward rotation, and an overall hunched shape with downward flexion of the head and marked curvature of the back developed (fetal posture). In this condition, animals still showed slow withdrawal of front and hind legs to pinch, but the righting reflex was lost. Maintaining the fetal posture, animals resumed spontaneous walking approximately ½ h following the SST injection.

Survival

Overall death rates which occurred within 10 min following the administration of SST i.t. or into the fourth ventricle are summarized in table 3. A 25% mortality rate was observed in animals treated with 100 μg SST i.t.; however, this value did not reach statistical

significance. Rats with fourth ventricular SST injections also showed a high incidence of death.

Histology

Spinal cords of rats in the different treatment groups (saline, SST, 10, 30, and 100 μg i.t., n = 5 in each group) were subsequently examined for histological changes after the 6-day observation period. Due to the acute death of one animal that received SST 100 μg, only four spinal cords were examined in this group, one of which was removed shortly after the spontaneous death of an animal at 48 h post-drug injection. Figure 3 presents histological sections (Luxol fast blue stains) of spinal cord lumbar segments, taken below the tip of the i.t. catheter, in two rats treated with 10 μg SST i.t. (fig. 3A) or 100 μg SST i.t. (fig. 3B). No clear pathologic changes occurred in saline and SST 10 μg treated animals. A mild inflammatory response throughout the gray matter without apparent signs of nucleolysis was observed in four out of five animals that had received 30 μg SST i.t. In the 100-μg SST i.t. group, mild (n = 2) to severe (n = 2) nucleolysis and inflammatory response were observed. Nucleolysis was equally present in dorsal and ventral horn segments, and mainly affected the large cells, leaving the smaller cells intact.

Discussion

Injection of intrathecal SST evoked the following characteristic responses: 1) an initial excitatory response (3 min) characterized by agitation, biting, scratching, and an increase in BP; 2) followed by a period of suppression marked by hindlimb motor dysfunction, loss of the micturition reflex, antinociception, and, in some cases, cardiovascular collapse (3–10 min); 3) an extended recovery period (from 30 min up to 6 days), during which motor and bladder function, as well as nociception, might be regained; and 4) obvious chromatolytic reactions in the spinal gray matter. Importantly, all of these effects were clearly dose dependent. No effects were observed with 10 μg SST i.t., whereas 100 μg SST i.t. invariably evoked the above-mentioned responses, although expressed to different degrees in any given animal.

Stimulating Effects of i.t. SST

Our observations during the initial excitatory response in part correspond to reports by Seybold *et al.*[14] and Wiesenfeld-Hallin[6] where i.t. SST (1, 5, and 20 μg) elicited 10–20-min biting, licking, and scratching responses in rats. Under present experimental conditions, however, marked scratching behavior, which was of shorter duration (5 min), was mainly observed at higher dosages (30 and 100 μg SST i.t.). The characteristic scratching and biting behavior may be explained by the perception of nociceptive stimulation evoked by the lumbar spinal administration of SST, which points to the involvement of SST as a neurotransmitter in pain pathways.[6,14]

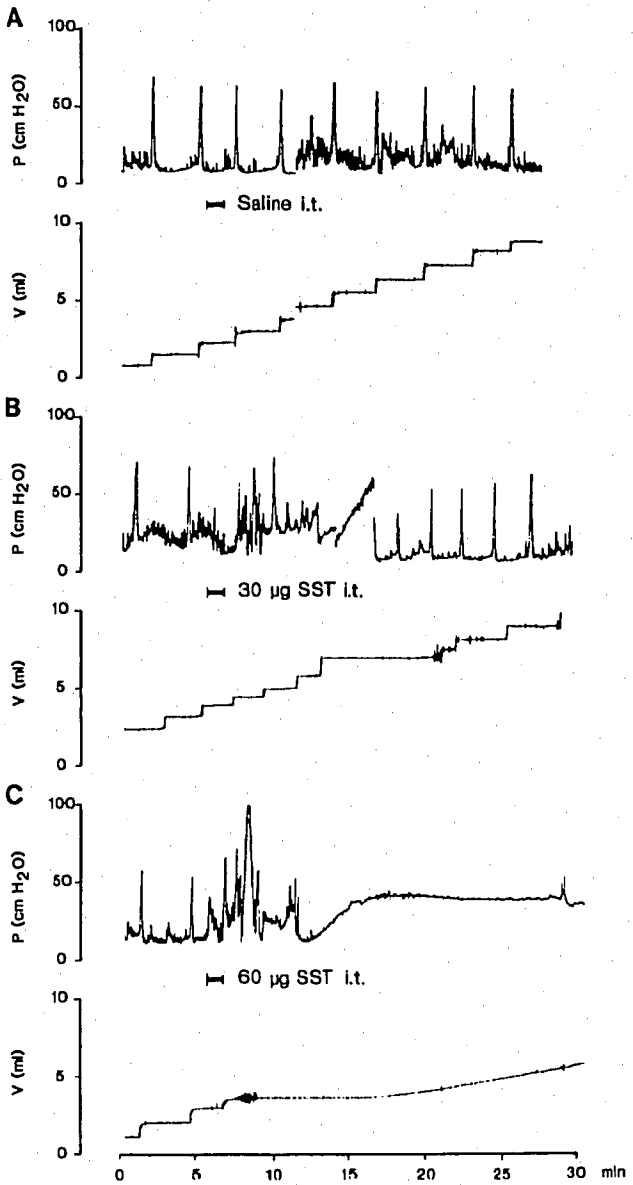

FIG. 2. Representative cystometrogram in one rat subjected to subsequent intrathecal (i.t.) injections, each 30 min apart, of saline (*A*) and somatostatin (SST) at 30 μg (*B*) and 60 μg (*C*) during continuous bladder infusion (250 μl saline/min). Top tracings represent bladder pressure (P), lower tracings volume of urine (V) in collection cup as function of time (min). No changes in regular bladder contractions and simultaneous urine emission is observed following i.t. saline administration, whereas 30 μg SST i.t. evokes an intermittent, and 60 μg SST i.t. a complete block of the volume-evoked micturition reflex.

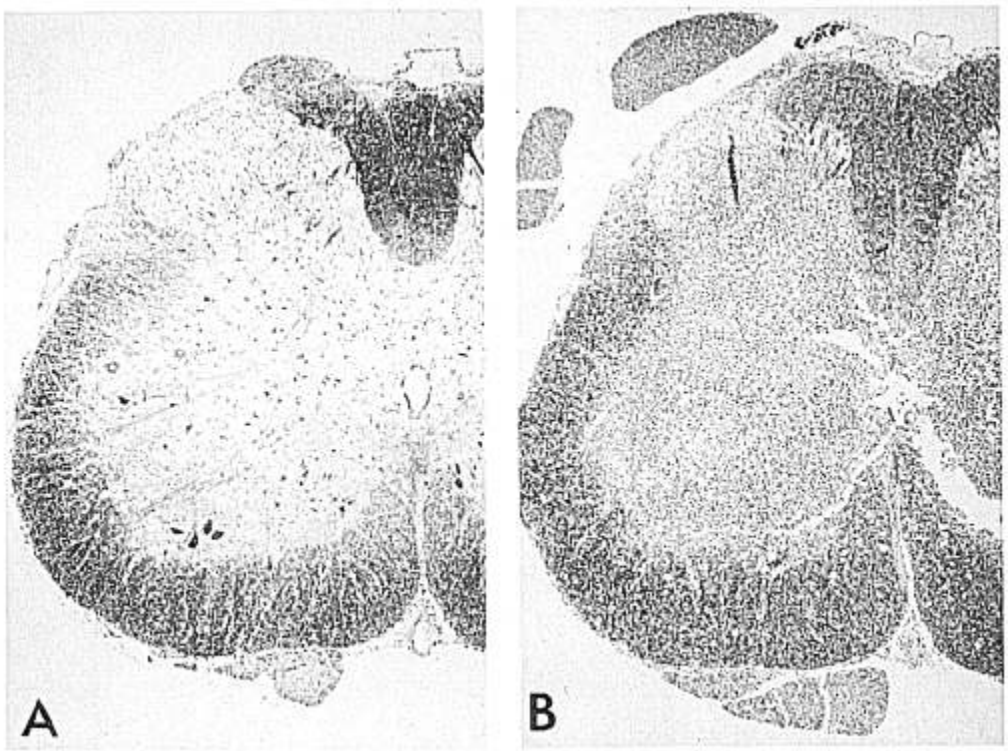

FIG. 3. Luxol fast blue stains of rat spinal cord sections taken below the tip of the intrathecal catheter on day 6 after the injection of somatostatin 10 μg i.t. (*A*) and 100 μg (*B*). Normal spinal cord histology in *A*, complete loss of ganglion cells and massive invasion throughout the gray matter with inflammatory cells in *B*.

ANTINOCICEPTIVE EFFECTS OF I.T. SST

With regard to "analgesia," in our hands, there was no separation between the behavioral effects to nociceptive measures and the motor effects of SST. Thus, changes in the response to tail flick and foot pinch were only observed in the presence of overt hindlimb motor dysfunction. Histological examination of lumbar spinal cord segments, just distal to the site of i.t. SST application, clearly demonstrates prominent effects on both dorsal and ventral neurons. This leads us to presume that the observed antinociceptive effects are due to a general toxic effect of SST on many populations of neurons, including, probably, those neurons involved in pain transmission.

EFFECTS OF I.T. SST ON MOTOR FUNCTION

Shortly after injection, rats receiving high concentrations of SST displayed agitated motor response consisting of biting and scratching. Though we interpret this as evidence of pain, we cannot exclude the possibility that some of the motor component may represent a reflex evoked scratching response (*e.g.*, a spinal motor reflex). The subsequent course, evidenced by a loss of EMG activity, suggests a loss of motor horn outflow, which recovered after lower doses and, variably, after higher doses. Given the correlation with cell damage, we conclude that the long-term effect reflects destruction of motor horn cells. It should be noted that simple measurement of the EMG revealed, even in paralyzed animals, some spontaneous EMG activity. In several cases, spasticity became evident, suggesting that total loss of motor horn cells did *not* occur. Parenthetically, we consider these effects to represent a local spinal action of SST, as the fourth ventricular injection led to distinguishably different syndromes. Thus, the presence of the hunched (fetal) posture with inward rotation of the extremities and loss of eye blink reflexes following fourth ventricular SST injection appears functionally equivalent to decerebration.

CARDIOVASCULAR EFFECTS OF I.T. SST

The hemodynamic effects observed after i.t. SST may be due to actions at different levels. Thus, an initial rise in BP may derive from SST action on spinal nociceptive neurons and subsequent stimulation of the sympathetic system. Alternatively, it may derive from a direct SST action on sympathetic neurons in the intermediolateral cell column, since SST afferent fibers have been found in close contact to sympatho-adrenal neurons.[15] Subsequent suppressive effects of SST on intermediolateral cell function might have evoked the ultimate fall in BP. Unpredictable supraspinal redistribution of SST might account for the variable hemodynamic effects observed after i.t. injection. SST could inhibit the descending sympatho-adrenal response[16] and/or result in a direct parasympathetic stimulation *via* activation of vagal motor neurons.[17] When a high

concentration of SST was administered at the lumbar level of the spinal cord, hemodynamic depression appeared to precede total respiratory arrest. In contrast, the injection of SST into the fourth ventricle, with more rapid access to medullary centers, evoked an overt respiratory arrest in three out of seven animals prior to hemodynamic deterioration. While the mechanism of the effects of SST on cardiovascular function is not known, it is conceivable that the acute physiological toxicity results from a concurrent effect on spinal and supraspinal centers.

Effects of i.t. SST on Micturition

Inhibitory effects of SST (30 and 60 μg i.t.) on the volume evoked micturition reflex might be explained by direct actions on sacral parasympathetic nuclei. These preganglionic parasympathetic neurons representing the efferent limb of the micturition reflex arch are richly innervated with SST containing fibers.[18]

Species Specificity

The prominent toxic effects of SST are self-evident in view of the behavioral, physiological, and histological findings. These results are somewhat disturbing, because SST has been administered i.t. and epidurally in humans, sometimes for prolonged periods.[7,8] In these clinical trials, in contrast to our experiments in rats, good analgesia without adverse side effects has been routinely reported. The fact that no adverse effects were observed in humans might be explained on the basis of the relatively low SST doses administered with single injections (250 μg). The human data suggesting no toxicity would thus correspond to our data in rats, where no toxicity was observed in the low-dose range (10 μg SST i.t.). However, a wide difference with regard to the analgesic properties of SST exists between humans and rats, which might be explained by species differences. Therefore, we recently conducted experiments in cats, chronically implanted with i.t. lumbar catheters. In this cat model, SST (2 mg i.t.) caused pronounced hindlimb motor dysfunction and was devoid of analgesic effects (D. Gaumann, T. Yaksh: unpublished observations).

Mechanisms of i.t. SST Toxicity

The question remains, what are the underlying mechanisms by which SST exerts its effects? Apart from nonspecific toxic mechanisms, SST may exert its effects by action on specific receptors. As noted, excitatory,[19,20] as well as inhibitory and hyperpolarizing, effects[5,21] of SST on central neurons have been reported. A clue to potential analgesic effects of SST was given by Randić.[22] In these cat studies, selective depressant actions of SST on nociceptive dorsal horn neurons were observed, while non-nociceptive mechanoreceptors were not affected or weakly activated. The apparent contradictory findings of SST actions may be explained on the basis of complex interactions of several messengers evoking the final cell response. Thus, Mancillas[23] observed depressant effects when SST alone was applied iontophoretically on rat hippocampal and cortical cells. However, in the presence of acetylcholine (ACH), SST caused an enhancement of the ACH-induced neuronal excitation. This effect was dose dependent, and inhibitory effects on ACH facilitation were observed, when SST was applied with higher currents. Experiments conducted by Delfs[24] in rat cortical cell cultures showed unusual dose-response characteristics of SST. At lower concentrations (500 pM), mainly excitatory, and, at higher concentrations (500 nM), mainly inhibitory effects were observed. The variability of the effects induced by SST may explain why, in our experiments, the severity in behavioral, motor and hemodynamic effects would vary between individual animals in the different dosage groups (30, 100 μg, SST i.t.). As pointed out by Delfs,[24] SST applied on cultured cortical neurons above inhibitory concentrations (10 μM to 1 mM) evoked long-lasting, and often irreversible, neuronal depolarization, indicative of neuronal membrane damage. The same mechanism may have been responsible for the consistent neurotoxic effects at high dosages (100 μg SST) observed in present experiments. It should be noted that neurolysis is not uncommonly reported following the application of excitatory agents, such as glutamate or capsaicin.[25] Application of such agents leads to a receptor-mediated long-term depolarization of the cell membrane, resulting in an initial excitation followed by a blockade. Such long-term depolarization may give rise to various changes in the intracellular milieu (increased intracellular Ca^{++}; elevated phospholipase activity, generation of superoxides) which may result in a more or less permanent cellular dysfunction. We can only speculate that this represents a possible explanation for the toxic effects of SST. However, SST toxicity may not only be limited to actions on the cell membrane,[24] but effects on cell metabolism[26] and/or spinal cord blood flow (see[27]) may also be hypothesized. SST thus joins the group of endogenous substances and/or their derivatives already reported to exhibit neurotoxic effects, including excitatory amino acids,[25] dynorphin A,[28] and substance P analogues.[29]

In conclusion, we have substantiated that severe toxic effects in rats occur following the i.t. administration of SST with no margin of safety between nociceptive and neurotoxic effects. Prior to the i.t. or epidural administration in humans for the management of pain, the tox-

icological and antinociceptive properties of SST should be more closely evaluated in a variety of animal models, and its mechanism of toxicity defined.

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