The Serotonin Antagonist Mianserin Improves Functional Recovery Following Experimental Spinal Trauma

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The ability of the serotonin antagonist mianserin to improve neurological recovery after graded impact trauma to the thoracic region of the spinal cord was compared to that of cyproheptadine and ketanserin in pentobarbital-anesthetized rats. Spinal cord injury was produced at T-10 by the weight-drop method and confirmed by the disappearance of the somatosensory-evoked response during the subsequent 15 minutes. In all experiments, drug or vehicle treatments were randomly administered as a single intravenous bolus 15 minutes after injury. Functional outcome was blindly assessed for 2 weeks after injury using a modified Tarlov scale, and in some cases, the Rivlin-Tator angleboard test. The survival of descending raphe-spinal axons was determined by the measurement of serotonin in postmortem spinal tissues located above and below the site of injury. In separate acute experiments, the physiological and hemodynamic correlates of a 50 gm cm injury and either mianserin or vehicle injection were examined, as were the effects on serotonin content and metabolism in spinal tissues harvested 30 minutes after injury. All doses of mianserin were associated with some index of improved recovery following a 50 gm cm injury, with a 1-mg/kg dose being clearly superior. Both ketanserin (0.1 mg/kg) and cyproheptadine (2 mg/kg) displayed marginal therapeutic actions for 50 gm cm injuries. In acute studies, mianserin at 1 mg/kg was associated with the preservation of posttraumatic spinal cord blood flow at T-12 as well as a pronounced alteration in postmortem spinal serotonin content and metabolism, in contrast to vehicle control treatments. These data lend further support to a serotonergic hypothesis of secondary spinal cord injury and suggest the potential use of mianserin for the acute treatment of spinal cord injury.

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The contribution of secondary autodestructive processes to the functional damage resulting from trauma in the nervous system is now widely accepted, and these processes are recognized to be a complex series of chemical and physiological events that occur in the vasculature and central nervous system (CNS) [1]. Thus, inflammatory responses and neurotransmitter alterations leading to ionic derangement are considered to be prominent components of secondary neurotrauma, and are implicated in the therapeutic properties of antioxidant [2], opioid antagonist [3], and thyrotropin-releasing hormone (TRH) analog treatments [4]. Although suspected, the interrelationships between these processes and treatments are not clear, nor are the relative contributions of vascular versus neural mechanisms to these responses and their treatment. Serotonin (5-HT) may be a vital link in this regard.

5-HT is predominantly localized in blood platelets in the periphery [5], and in raphe neurons that project throughout the CNS, including the spinal cord [6]. In the circulation and CNS, 5-HT is thought to mediate normal mechanisms of hemostasis [7] and hemodynamics [8] as well as inflammatory mechanisms leading to thrombosis [9], vasospasm [10], and traumatic extravasation [11]. In addition, 5-HT may interact with opioids colocalized in platelets [12]. In the spinal cord, 5-HT and its cotransmitter TRH are thought to mediate the raphe-spinal modulation of motor, sensory, and autonomic functions [6, 13], while 5-HT-opioid interactions are considered important for the sensory transmission of nociceptive information [14, 15].

We have reported rapid and robust increases in spinal 5-HT content following impact trauma in the rabbit [16] or distraction injury in the rat [17]. Similar findings have been reported in rats subjected to spinal impact trauma [18] and after spinal ischemia in rabbits [19]. Significantly, neuroprotective effects were noted

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for the 5-HT_2 antagonist cyproheptadine in the spinal ischemia model [20]. Protective actions in models of local cerebral ischemia were recently reported for the 5-HT_2 blocker ketanserin [21] and combined 5-HT_2 and calcium channel antagonist S-emopamil [22]. Although we reported the partial reversal of the 5-HT response to trauma by therapeutic dosage of TRH [23], the direct demonstration of 5-HT's mediation of neural damage after spinal impact trauma has yet to be forthcoming. The purpose of this study was to attempt such a demonstration by evaluating the therapeutic potential of mianserin, a potent and long-acting 5-HT antagonist with selectivity at 5-HT_2 and 5-HT_1 receptors [24]. Cyproheptadine and ketanserin, which are thought to possess selectivity for 5-HT_2 receptors only [25], were also evaluated.

Materials and Methods

Animals

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), weighing 250 to 300 gm, were used, at a minimum of 2 days after their arrival (n = 277). A 12-hour:12-hour light-dark cycle was used, and food and water were provided ad libitum. The research protocol and animal usage in these studies were approved by our Institutional Animal Care and Use Committee, and adhere to guidelines set forth by the US Department of Health, Education, and Welfare's "Guide for the Care and Use of Laboratory Animals."

Anesthesia

Anesthesia was induced in all animals using pentobarbital (60 mg/kg, intraperitoneally), with pure oxygen delivered intraoperatively through a tight-fitting mask. In all animals, rectal temperature was maintained between 37 and 38°C with a heating pad.

Chronic Studies

SURGERY. After a sufficient depth of anesthesia was verified, the lateral tail vein was exposed and catheterized (24 gauge, Insyte, Becton Dickinson, Mountain View, CA) for the delivery of drug or vehicle solutions (n = 259). The animal was then positioned in a spinal stereotaxic apparatus (David Kopf, Tujunga, CA), with fixation at the ears. The skull was exposed, and stainless-steel jewelers' screws (Small Parts, Miami, FL) implanted for recording the somatosensory-evoked potential (SEP). As in previous work [26], screws were threaded into the skull near the union of the midline with bregma (positive) and lambda (negative) and in the nasal sinus (reference). Stimuli were delivered through a pair of platinum subdermal needle electrodes (Grass, Quincy, MA) inserted in the hindlimb near the medial malleolus and plantaris tendon. Constant voltage pulses were delivered at 3 Hz and at an intensity sufficient to elicit a slight twitch in the outer digits (10 to 15 V). SEPs were averaged over a 90-millisecond epoch using 256 trials and a bandpass between 3.2 and 3,200 Hz. The latency and amplitude of the major negative wave were then measured over three to five trials prior to laminectomy.

The thoracic region of the spine was then exposed, and a laminectomy performed at the T-10 level. The dura mater was left intact. The SEP was then reevaluated in order to confirm the presence of normal conduction after laminectomy. Animals were excluded from study if the SEP latency increased by more than 2 milliseconds or if the amplitude decreased by more than 50%. Animals with intact responses were then prepared for injury by applying additional fixation at the T-12 spinous process using a vertebral clamp.

SPINAL CORD INJURY. The injury device consisted of a hollow steel tube with a nylon impounder at the bottom (0.3-cm diameter at the tip). The impounder was free to move up into the tube but was restricted in its downward movement. After being secured to a micromanipulator, the device, with impounder tip fully extended, was lowered onto the exposed dura until contact with the cord caused the impounder to move precisely 0.2 cm up into the tube. At this point, either no weight was dropped (sham injury) or a 10-gm weight was dropped from a height of 5 or 10 cm (50 and 100 gm cm injuries). The SEP was then recorded 2, 5, 10, and 15 minutes later in order to verify the injured condition. Sham-injured rats were excluded if the SEP was significantly altered during any of these trials. Similarly, animals subjected to either injury level were excluded if the SEP response was not obliterated during the entire 15-minute period following weight-drop. Finally, only those animals surviving for 2 weeks after injury were included in the data pool (n = 145 included).

DRUG AND VEHICLE INJECTION. All solutions were injected intravenously 15 minutes after injury at a volume of 1 ml/kg and were preceded and followed by the injection of 0.3 ml of sterile saline. Solutions were prepared daily and sterilized by filtration (0.2 μm, Gelman Sciences, Ann Arbor, MI). Syringes were prepared by personnel not involved with the surgery or neurological evaluations and distributed in a randomized manner. Mianserin was dissolved in purified water (high-pressure liquid chromatography [HPLC] grade, Fisher Scientific, Pittsburgh, PA) at concentrations of 1, 5, or 10 mg/ml, as were cyproheptadine (2 mg/ml) and ketanserin (0.1 or 1 mg/ml). All drugs were purchased from Research Biochemicals (Natick, MA). The distribution of animals within these treatment groups is shown in Table 1.

NEUROLOGICAL EVALUATION. All assessments were performed in a blinded fashion. A modified Tarlov scale [27] was employed daily for 2 weeks. Each hindlimb was rated as follows:

0—total paraplegia of hindlimbs
1—no spontaneous movement but responds to hindlimb pinch
2—spontaneous movement but unable to stand
3—able to support weight but cannot walk on broad, flat surface
4—able to walk on broad, flat surface
5—able to walk on broad, flat surface and support weight on a 1.8-cm-wide ledge
6—able to walk on ledge

Table 1. Distribution of Animals in Chronic Studies

Injury	Vehicle[a]	Mianserin			Cyproheptadine (2 mg/kg)	Ketanserin	
		1 mg/kg	5 mg/kg	10 mg/kg		0.1 mg/kg	1 mg/kg
Sham							
No. of animals[b]	7 (8)	5 (6)	5 (6)	6 (6)	6 (7)	—	—
Median final neuroscore[c]	12	12	12	12	12		
50 gm cm							
No. of animals[b]	37 (68)	12 (20)	10 (22)	5 (22)	11 (16)	10 (13)	8 (10)
Median final neuroscore[c]	4.5	8	6.5	8	8	7.5	5.5
100 gm cm							
No. of animals[b]	5 (8)	4 (8)	5 (12)	3 (14)	5 (13)	—	—
Median final neuroscore[c]	4	5	3	4	4		

[a]Total number of vehicle-treated animals are shown (those included and those tested); however, drugs were not all examined concomitantly. Treatments were randomized in three sets of experiments: mianserin (all doses) and cyproheptadine, mianserin (1 and 5 mg/kg), mianserin and ketanserin. In all sets, vehicle was administered at a minimum frequency of every fifth animal.
[b]Number of animals included in treatment/injury group fulfilling somatosensory-evoked potential criteria and surviving 14 days. In parentheses are the total number of animals tested.
[c]Median final neuroscore at 2 weeks for animals included.

Table 2. Percent Mortality/Exclusion

Injury	Vehicle	Mianserin			Cyproheptadine (2 mg/kg)	Ketanserin	
		1 mg/kg	5 mg/kg	10 mg/kg		0.1 mg/kg	1 mg/kg
Sham							
Animals that died[a]	0 (12.5)	0 (16.7)	16.7 (0)	0 (0)	0 (14.3)	—	—
Animals excluded[b]	(0)	(0)	(0)	(0)	(0)		
50 gm cm							
Animals that died[a]	3 (32.3)	0 (25)	4.5 (27.2)	31.8 (27.2)	0 (12.5)	0 (15.4)	0 (10)
Animals excluded[b]	(10.3)	(10)	(22.7)	(18.2)	(18.7)	(7.7)	(10)
100 gm cm							
Animals that died[a]	0 (37.5)	12.5 (37.5)	33.5 (25)	50 (21.4)	7.7 (23.1)	—	—
Animals excluded[b]	(0)	(0)	(0)	(7.1)	(30.8)		

[a]Percent of animals that died during or immediately following drug or vehicle injection. There was a significant correlation between mianserin dosage and injury severity ($p < 0.0001$, chi-square test). In parentheses are the percent of animals that died during the follow-up period prior to 2 weeks. There were no differences in mortality between treatment groups.
[b]Percent of animals excluded from study based on postinjury somatosensory-evoked potential criteria (see text for details).

Both the final and cumulative scores were noted, as was the ability to walk at 2 weeks. In later experiments, the Rivlin-Tator angleboard test [28] was performed before the animals were killed. The maximum angle maintained for 5 seconds or longer in both horizontal directions was measured, and averaged to yield a final value. These tests were performed on a subset of mianserin- and vehicle-treated animals and all animals given ketanserin. In addition, the acute lethal toxicity of each treatment was determined, as was the mortality rate for animals surviving between 1 and 13 days after injury (Table 2). These animals were not included in the data pool for the determination of treatment effects.

SPINAL SEROTONIN ANALYSIS. Following the 2-week follow-up, animals were killed by decapitation (between 1 and 3 PM for all animals, in order to avoid the effects of circadian variation of monoamine content). The spinal cord was rapidly, but carefully, exposed at its ventral surface, and the injury site identified by its position relative to the dorsal laminectomy. A 5-mm length of tissue centered at the injury site was then dissected free, and the 5-mm portions of tissue located rostrally and caudally then obtained. Tissues were weighed and then frozen at −70°C.

The concentrations of 5-HT and 5-hydroxyindole-3-acetic acid (5-HIAA) were determined in duplicate from filtered supernatants obtained after sonic disruption of tissues in 0.5-ml volumes of 0.1 *N* perchloric acid. As in previous work [29], liquid chromatography with dual electrochemical detection and automated data analysis was employed. The ratio of 5-HT concentration in the caudal to that in the rostral segment was calculated.

Acute Studies

Several additional intraoperative measures were combined with SEP analyses in these experiments. Arterial blood pressure and local spinal cord blood flow (SCBF) were measured continuously, while arterial blood gases, pH, and bicarbonate (HCO_3^-) levels were determined 10 minutes prior to and after injury, and 10 minutes after drug or vehicle injection. All animals were killed 30 minutes after injury, and their spinal cords harvested for 5-HT/5-HIAA analysis as mentioned above.

Blood pressure was monitored in the catheterized ventral tail artery using a pressure transducer (Statham P-23, Gould Inc, Oxnard, CA) and a bridge/amplifier channel on a polygraph (Grass 78D). Blood gases, pH, and bicarbonate values were determined in 0.15-ml samples withdrawn through this line using an automated analyzer (Corning M178, Waltham, MA). The mean arterial pressure (MAP) was calculated as the average of systolic and diastolic readings each minute for the 15 minutes before and after injury and injection. Local SCBF was measured using laser Doppler flowmetry (BPM 403, TSI Inc, St Paul, MN). The SCBF probe was sutured in place above a second laminectomy site at T-12 (approximately 2 to 3 mm above the dural surface) and was positioned directly over the dorsal vein. This method samples approximately 1 mm^3 of tissue. Minute by minute values were calculated as for the MAP.

All animals were subjected to a 50 gm cm impact injury and were treated 15 minutes later with water (1 ml/kg) or mianserin (1 or 5 mg/kg) (n = 6 for each treatment).

Statistical Analyses

For the assessment of treatment effects, only those animals that both fulfilled the criteria for injury based on the early SEP and survived for 14 days after surgery were included in the data pool. Postmortem neurochemical analyses were accomplished in fresh frozen spinal tissues harvested within minutes after these animals were killed. Differences between treatment groups were analyzed using the Kruskal-Wallis analysis of variance (ANOVA) followed by the Mann-Whitney *U* test for all measures except walking frequency, which was analyzed using Fisher's exact test. Acute lethality of drug administrations and postoperative mortalities among treatment groups as well as incline angle frequencies above 55 degrees were compared using chi-square tests. In addition, a one-way ANOVA and a Wilcoxon signed-rank matched pairs test or Mann-Whitney *U* test was run on the results of acute studies. A p value of less than 0.05 for all tests was considered significant. In cases where both the Kruskal-Wallis and Mann-Whitney analyses were performed, differences were considered significant only if p was less than 0.05 for both tests; however, only the Mann-Whitney results are reported. It should be noted that although nonparametric statistical methods were employed, in some cases data are displayed in a parametric format. This was done in order to avoid cumbersome graphic presentations while still providing information regarding the variability of the data.

Results

Mianserin

INTERACTION BETWEEN INJURY SEVERITY AND MIANSERIN TREATMENT. In initial studies, the effects of cyproheptadine (2 mg/kg, intravenously) or mianserin (1, 5, or 10 mg/kg, intravenously) were compared to vehicle treatment in rats subjected to sham, 50 gm cm, or 100 gm cm impact forces during pentobarbital anesthesia. All sham-injured animals displayed fully normal neurological function throughout the 2-week follow-up, irrespective of the treatment or dosage (data not shown). Mianserin at 1 and 5 mg/kg reduced the functional damage after 50 gm cm injury, in comparison to vehicle treatment, but did not affect the recovery from 100 gm cm injuries. The effects of vehicle or mianserin treatment (1 and 5 mg/kg) were retested after 50 gm cm injuries only. The protective effect of mianserin was preserved at both dosages. Comparison of vehicle and mianserin treatment effects in the two groups did not reveal significant differences in the final or cumulative Tarlov scores, such that the data from these experiments were combined.

TARLOV SCORES. The final neurological outcome as measured by the modified Tarlov ratings is shown in Figure 1 (for all animals who survived 14 days after a 50 gm cm impact sufficient to obliterate the SEP for at least 15 minutes after injury). Vehicle-treated animals displayed an average final score of 5, reflecting the presence of spontaneous movement in both hindlimbs, weight-bearing ability in one hindlimb, but the absence of locomotor function. Only 25% of vehicle-treated animals regained walking ability by 2 weeks. In contrast, mianserin at 1 mg/kg was associated with an average final score of 8, indicating the presence of locomotor function in both hindlimbs, with 69% of animals regaining the ability to walk. Both measures were significant with respect to the vehicle group (final Tarlov score, $p < 0.05$ Mann-Whitney *U* test; walking ability, $p < 0.01$, Fisher's exact test). Mianserin at 5 mg/kg

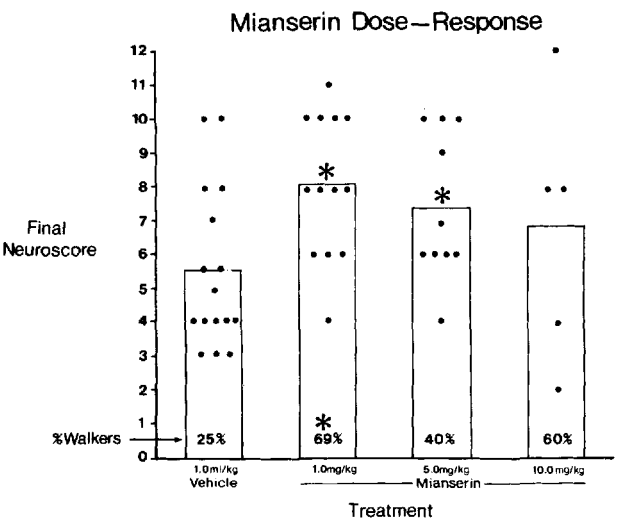
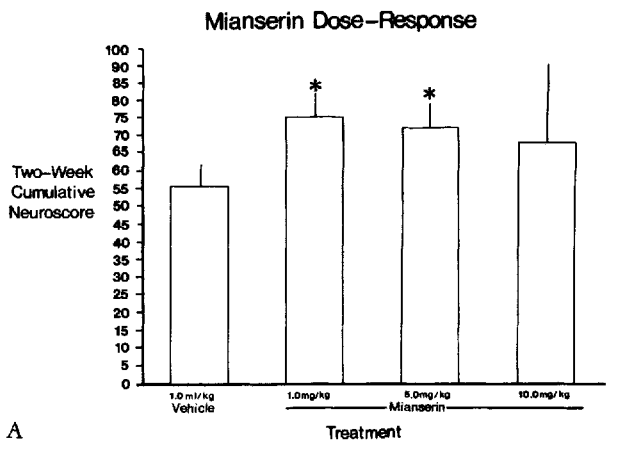


Fig 1. Mean final neuroscores (Tarlov ratings) at 2 weeks after injury are shown (bars), *along with individual scores* (dots), *and the percentage of animals regaining locomotor function in both hindlimbs* (% Walkers) *in vehicle- or mianserin-treated animals (1, 5, and 10 mg/kg doses). Neuroscores were significantly greater for the 1- and 5-mg/kg mianserin-treated groups* (asterisks, $p < 0.05$, *Mann-Whitney* U *test) than for the vehicle group, but only the low dose was associated with a significant increase in locomotion* ($p < 0.05$, *Fisher's exact test).*

resulted in a similar improvement in final Tarlov scores ($p < 0.05$, Mann-Whitney U test) and increased the frequency of walking ability, compared to the vehicle treatment, but not in a statistically significant manner. The 10-mg/kg dosage did not significantly improve either measure.

These results were confirmed by the analysis of the 14-day cumulative Tarlov scores (Fig 2A), indicating that the improved final outcome associated with mianserin was not a result of late improvements, and that a 2-week follow-up period was appropriate for these studies. The beneficial effects of mianserin were generally evident between 5 and 7 days after injury.



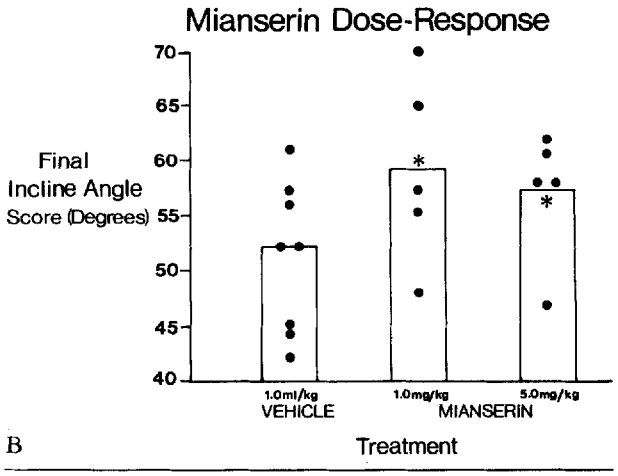


Fig 2. (A) Mean (± standard error of mean) cumulative neuroscores (Tarlov ratings) are shown for the groups described in Figure 1. As for the final values, mianserin at 1 and 5 mg/kg resulted in a significant improvement, compared to vehicle treatment (asterisks, $p < 0.05$, *Mann-Whitney U test), indicating the absence of late neurological improvement in mianserin's action. (B) Mean and individual final incline angle scores (Rivlin-Tator test) are shown in a subset of vehicle- or mianserin-treated animals (1- and 5-mg/kg doses). Despite the limited number of animals tested, both doses of mianserin significantly improved the frequency of incline scores above 55 degrees, compared with vehicle treatment* (asterisks, $p < 0.001$, *2 × 2 chi square with Yates correction for small numbers).*

RIVLIN-TATOR ANGLEBOARD SCORES. Despite the small number of mianserin-treated animals tested, both the 1- and 5-mg/kg dosages resulted in a significant improvement in angleboard score, compared with vehicle-treated animals (Fig 2B). Eighty percent of animals treated with mianserin maintained angles of 55 degrees or greater, in contrast to only 37.5% of the vehicle-treated group ($p < 0.001$, 2 × 2 chi-square test with Yates correction for small numbers).

LETHALITY AND MORTALITY. At a dosage of 10 mg/kg, and to a lesser extent 5 mg/kg, postinjury mianserin administration resulted in an immediate and severe respiratory disruption, usually leading to death. This effect was related to both the dosage of mianserin and the severity of injury ($p < 0.0001$, Cramer's phi = 0.446, contingency coefficient = 0.533). Thus, only 1 of 18 sham-injured animals given mianserin at any dose, and 1 of 34 animals given 1 mg/kg at any injury level, were thus affected. In contrast, 10 mg/kg was the LD_{50} dose of mianserin for animals subjected to 100 gm cm injuries. Significant lethality was not associated with either cyproheptadine or ketanserin. In animals surviving at least 24 hours, but less than 2 weeks, mortality rates were statistically similar among treatment groups ($p = 0.2678$, Cramer's phi = 0.139, contingency coefficient = 0.138). These data are summarized in Table 2.

POSTMORTEM SPINAL 5-HT DISTRIBUTION. The ratio of 5-HT concentration in spinal tissue below the injury site to that above this site was analyzed to assess the state of axoplasmic transport of 5-HT in descending raphe-spinal neurons and to provide a quantitative measure of injury severity. This ratio varied between 1.0 and 1.5 in sham-injured animals as shown in Figure 3B, in agreement with findings from extensive past studies of normal and sham-operated rats [16, 17, 23, 26]. This ratio is plotted against the final Tarlov outcome category for all animals in this study in Figure 3B. The ratio clearly delineates functional injury from the recovery of locomotion, as well as from normal function. In Figure 3A the ratio is compared among treatment groups after a 50 gm cm impact. Mianserin treatment at 1 mg/kg resulted in a 5-HT ratio consistent with impaired function but recovered locomotion, while higher doses of mianserin were often associated with 5-HT ratios either well above or below the norm. Thus, only the 1-mg/kg dose restored the 5-HT ratio in a significant manner, compared to the vehicle treatment.

INTRAOPERATIVE AND ACUTE MEASURES. Although the administration of all doses of mianserin were accompanied by an average increase in SEP amplitude, these effects were not significant (data not shown). This was

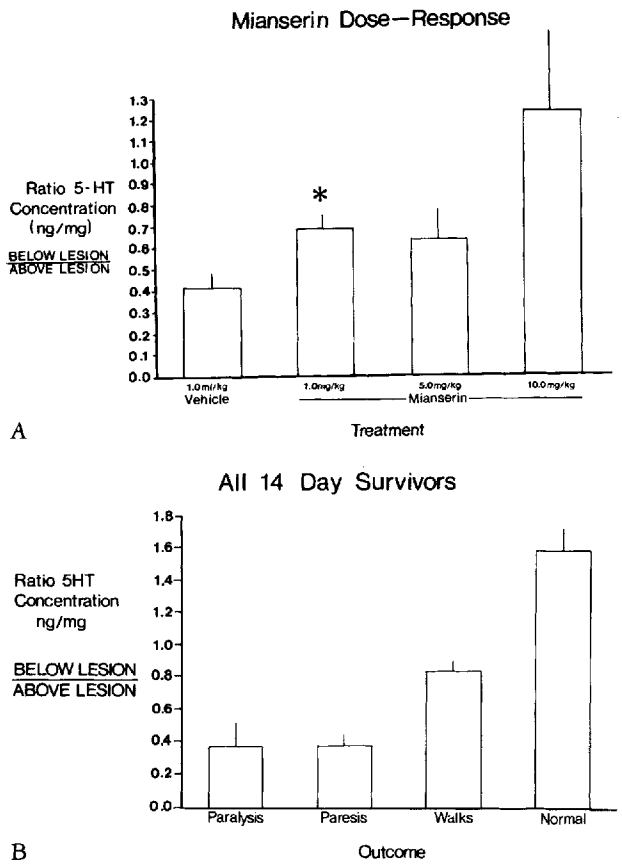


Fig 3. Mean (± standard error of mean) serotonin (5-HT) *concentrations (ng/mg of tissue wet weight) are shown as a ratio in the 5-mm segments of tissue located below and above the lesion site. Impact injury damage results in a loss of 5-HT below, its accumulation above the lesion, and a decrease in the calculated ratio. (A) All doses of mianserin were associated with higher ratios than was vehicle treatment, but only the 1-mg/kg dose was statistically significant* (asterisk, $p < 0.05$, *Mann-Whitney* U *test). (B) Ratios for all 14-day survivors, regardless of treatment and including sham-injured rats displaying fully normal function throughout the postinjury period. These animals displayed the normal ratio (approximately 1.5) reflecting the rostral-caudal increasing gradient in 5-HT content seen in the normal spinal cord. Injured animals that recovered locomotor function displayed a lower ratio (approximately 0.8) similar to that seen in the group treated with 1 mg/kg of mianserin. The ratio does not distinguish paralysis from paresis, but is nevertheless diminished (approximately 0.4) when compared with the ratio for walkers, and is similar to that seen in vehicle-treated rats.*

partially due to variability and the apparent ability of vehicle injections to also restore SEP amplitudes.

In acute experiments, arterial blood pressure, pH, oxygen (Po_2) and carbon dioxide pressure (Pco_2), and HCO_3^- values were within the normal range both before and after injury, as well as after drug or vehicle injections (data not shown). Mianserin treatment was associated with signs consistent with a significant acute enhancement of spinal 5-HT synthesis and metabolism

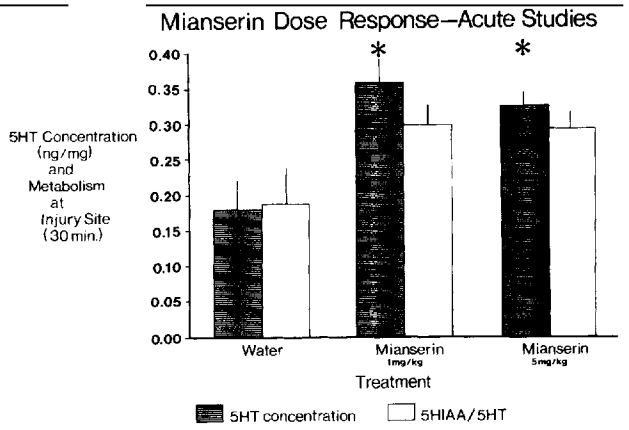


Fig 4. Mean (± standard error of mean) concentration of serotonin (5-HT) (dark bars) *and ratio of 5-hydroxyindole-3-acetic acid* (5-HIAA)/*5-HT in the 5-mm segment of spinal cord centered at the impact site and harvested 30 minutes after injury, and 15 minutes after the injection of vehicle (water) or mianserin (1 or 5 mg/kg). Consistent with a presynaptic action on 5-HT-containing neurons, mianserin resulted in elevated 5-HT concentration* (asterisks, $p < 0.05$, *by analysis of variance and Mann-Whitney* U *test), and a trend toward increased metabolism* (5-HIAA/*5-HT ratio).*

(Fig 4) and a trend toward nonsignificant restoration of local blood flow to preinjury levels (Fig 5).

Cyproheptadine

Cyproheptadine treatment resulted in a marginal improvement in the final neuroscore, but a significant increase in walking frequency 2 weeks after a 50 gm cm impact (Fig 6). Both the frequency and the extent

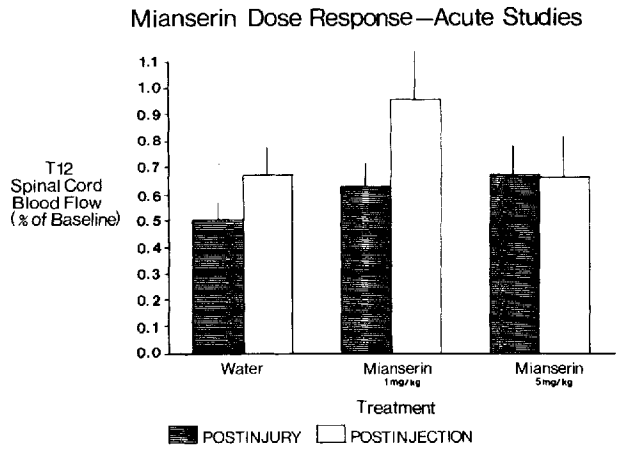


Fig 5. Laser Doppler spinal cord blood flow (LD-SCBF) response to 50 gm cm impact (dark bars) *and treatment* (white bars) *with vehicle, or 1 mg/kg or 5 mg/kg of mianserin. Values are expressed as a percentage of preinjury levels with the mean (± standard error of mean) percentage shown. Only the 1-mg/kg dose of mianserin appeared to restore LD-SCBF (measured each minute for 15 minutes after injection) to baseline levels* ($p < 0.05$ *Wilcoxon signed-rank matched pairs test).*

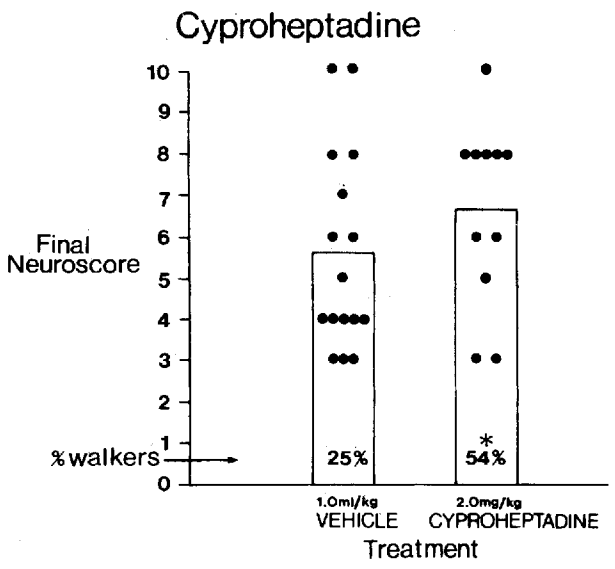


Fig 6. Mean and individual final neuroscores for cyproheptadine-treated animals, tested concurrently with mianserin- and vehicle-treated animals. Cyproheptadine resulted in a nonsignificant improvement in neuroscore, but did significantly enhance locomotor recovery (asterisk, $p < 0.05$, *Fisher's exact test).*

of functional recovery associated with cyproheptadine were less than that seen following 1-mg/kg mianserin treatment. Moreover, 5-HT ratios were not improved (data not shown).

Ketanserin

Ketanserin treatment at 1 mg/kg was ineffective (Fig 7A); however, 0.1-mg/kg dosages resulted in a nonsignificant trend toward improvement of walking frequency and final incline board scores, when compared with vehicle treatment (Fig 7B). The final and cumulative Tarlov scores, as well as the postmortem spinal 5-HT ratio, were not affected (data not shown).

Discussion

The results of these experiments demonstrate the therapeutic efficacy of mianserin treatment after experimental spinal cord injury (SCI) in the rat, and support the hypothesis that the acute elevation in spinal 5-HT concentration observed after trauma due to various causes in several species [16–19] represents an important component of secondary autodestructive processes. The relative importance of 5-HT actions in the vasculature versus the CNS after neural injury was not precisely determined; however, evidence for both hemodynamic and central effects of mianserin treatment is presented. A role for 5-HT as a link between the vascular and neural responses to traumatic SCI remains a distinct possibility.

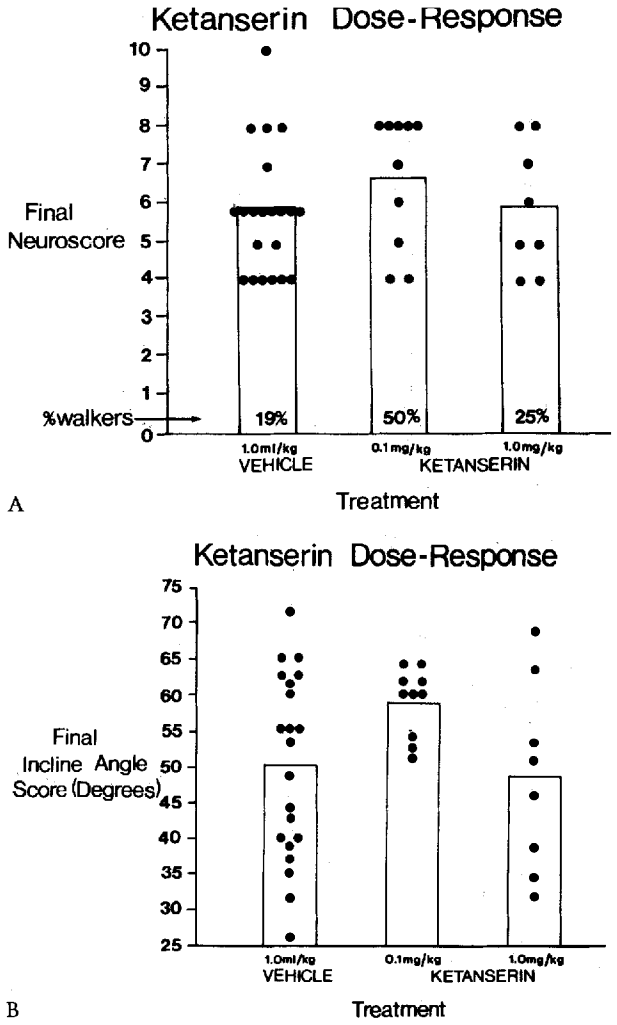


Fig 7. (A) Mean and individual final neuroscores for ketanserin-treated rats (0.1 and 1.0 mg/kg) versus vehicle-treated rats (tested concurrently). (B) The mean and individual final incline angleboard scores. Although neuroscores were not improved by either dose of ketanserin (A), rats treated with 0.1 mg/kg tended to display enhanced locomotor recovery (A) and tended to maintain their balance at greater incline angles, compared with vehicle-treated rats (B). Neither of these improvements, however, reached statistical significance.

All intravenous doses of mianserin tested were associated with at least one sign of improvement, but only the 1-mg/kg dosage was consistently accompanied by significant improvement for all measures. Thus, a single intravenous injection of 1 mg/kg of mianserin at 15 minutes after a 50 gm cm spinal impact resulted in significantly improved final and cumulative Tarlov scores and Rivlin-Tator angleboard scores as well as preserved long-tract survival below the lesion. From the functional standpoint, this dose of mianserin was associated with the restoration of walking ability in 69% of animals, as compared to 25% of vehicle-treated animals.

The diminished efficacy of mianserin at 10 mg/kg (and to a lesser extent at 5 mg/kg) appeared to be related to a pulmonary toxicity. Acute respiratory distress was frequently produced during the administration of 10 mg/kg of mianserin to injured animals, with immediate respiratory failure and death occurring in 50% of animals subjected to 100 gm cm injuries. These effects were also seen with 5-mg/kg doses, with a mortality rate of approximately 33% after 100 gm cm impact forces. In contrast, respiratory abnormalities were rarely observed after 1 mg/kg of mianserin, and no acute mortalities resulted from its administration to 50 gm cm injured animals. The mechanisms behind these toxic actions are not known, but they are clearly proportional to the dosage and severity of injury and are probably related to the well-known presence of 5-HT receptors in lung tissue.

Mianserin's pharmacological actions are thought to exist predominantly at 5-HT receptor sites, where pure antagonistic effects have been noted [24, 30]. A selectivity of mianserin for 5-HT_2 over 5-HT_1 [24] over adrenergic and histaminergic receptor blockade is apparent [30]. There is evidence that the 5-HT_2 sites bound by mianserin may be distinct from those bound by the closely related agent ketanserin [31]. Vascular 5-HT_2 antagonistic effects are thought to mediate the beneficial actions of cyproheptadine [20] and ketanserin [21] in ischemic models of neural injury. These agents, however, were much less effective than mianserin in the model of traumatic injury studied in this report. There are several possible explanations for this apparent discrepancy.

The relatively long half-life of mianserin (33 hours) [32] was an important factor in the decision to examine the neuroprotective potential of a single dose given shortly after injury. Furthermore, it has been reported that cerebral 5-HT_2 receptors are down-regulated up to 7 days after a single administration of mianserin [33]. Thus, this agent possesses both a long duration of action as well as potent residual effects long after its elimination. On the other hand, both cyproheptadine and ketanserin possess similar metabolic half-lives, and produce similar secondary receptor effects at comparable dosages to those tested in this study [33]. Moreover, the side effects of all three agents are similar. While it is possible that the optimum neuroprotective dosage of cyproheptadine or ketanserin differs from those tested, identical postinjury doses have been reported effective in models of spinal [20] and cerebral ischemia [21]. Alternatively, this discrepancy may reflect fundamental differences between traumatic and ischemic neural injury. Indeed, the results presented suggest that the blockade of central 5-HT_1 receptors by mianserin constitutes an important component of its therapeutic action. Because neither cyproheptadine nor ketanserin possess significant activity at these sites, it is possible that combined antagonism of 5-HT_2 and 5-HT_1 receptors is optimal for the effective treatment of traumatic spinal cord injury.

Certain subclasses of 5-HT_1 binding sites are thought to comprise central 5-HT autoreceptors [34]. These presynaptic binding sites are implicated in the negative feedback regulation of raphe-spinal 5-HT synthesis and release. We previously hypothesized [16, 23] that a pathological stimulation of these processes occurs during the acute posttraumatic phase as a result of 5-HT extravasation, and that this release inhibition extends to other raphe-spinal cotransmitters (i.e., TRH). In this laboratory (unpublished observations, S. K. Salzman, 1989), a deleterious effect on neurological recovery from 50 gm cm injuries was demonstrated following pretreatment with the 5-HT_{1A} agonist (±)-8-hydroxydipropylaminotetralin. By this reasoning, mianserin administration could block the autoreceptor, thus preventing presynaptic inhibition induced by extravasated 5-HT. Our data support this conclusion, as mianserin was associated with elevated 5-HT and 5-HIAA concentrations in the acutely injured cord in contrast to vehicle treatment (see Fig 4). It must be noted, however, that mianserin at 1 or 5 mg/kg produced identical effects in this regard, despite the greater therapeutic efficacy of the lower dose. In contrast, a tendency toward the restoration of postinjury local SCBF was observed with only the lower dose (see Fig 5). This effect may be centrally mediated [8], but is also likely to reflect a vascular mechanism of action mediated by 5-HT_2 receptors [35].

In summary, the data presented suggest an action of mianserin at 5-HT_2 vascular sites leading to the prevention of posttraumatic ischemia. In the spinal cord, mianserin treatment resulted in an elevated concentration and oxidative metabolism of 5-HT consistent with a presynaptic facilitation of 5-HT synthesis and turnover mediated by the blockade of raphe-spinal 5-HT_1 autoreceptors. Thus, intravenous mianserin may act at both vascular and neural receptor sites to protect the acutely injured spinal cord. Further studies in progress are aimed at characterizing the 5-HT receptor sites and classes of greatest relevance to secondary mechanisms of neural trauma.

With respect to clinical applications, mianserin may be preferable to methylprednisolone [36] for the treatment of human spinal cord injury. The therapeutic actions of a single dose of mianserin in this study were comparable to those seen after an intensive methylprednisolone dosing regimen following spinal compression trauma in cats [2]. Studies in progress are examining an extended dose-response range and comparing mianserin efficacy to that of an opioid antagonist [3] and TRH analog [4]. Given positive results, mianserin should be considered a prime candidate for future clinical trials.

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