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Short communication

Spinal transection increases the potency of clonidine on the tail-flick and hindlimb flexion reflexes

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Abstract

The effect of intrathecal clonidine on thermal nociception and hindlimb flexion was assessed in acute and chronic spinally transected rats. After an acute, 1-day spinalization, there was no change in the antinociceptive dose–response function to clonidine, relative to intact rats. However, there was a significant increase in potency 31 days after spinalization. Low doses of clonidine (0.25, 1, 4 and 20 μg) did not affect the nonnociceptive flexion reflex of acute spinal rats, but they elicited a dose-dependent response in chronic spinal rats. These data provide behavioral evidence of supersensitivity to α-adrenoceptor agonists in chronic spinal rats.

Keywords: Spasticity; Clonidine; Flexor reflex; Tail-flick; Antinociception

1. Introduction

Spinal cord injury often produces devastating functional impairment, including spastic paresis and chronic pain conditions (Yezierski, 1996; Christensen and Hulsebosch, 1997; Barbeau et al., 1998). Among the available medications for these disabling syndromes, agonists at α_2-adrenoceptors (Khan et al., 1999; Kamibayashi and Maze, 2000) may be especially useful because they (1) have dual clinical benefit for both spasticity (Yablon and Sipski, 1993; Barbeau et al., 1998; Kita and Goodkin, 2000) and a variety of pain states, including neuropathic pain arising from spinal cord injury (Glynn et al., 1986; Eisenach et al., 1995; Glynn and O'Sullivan, 1995; Mansikka et al., 1996; Rémy-Néris et al., 1999), (2) are not addictive, unlike opiate analgesics, which can also reduce spasticity, (Erickson et al., 1988), and (3) are effective after epidural or intrathecal administration, indicating that at least an important component of the analgesic and antispastic effects are spinally mediated, and therefore, less likely to elicit supraspinally mediated side effects (Hall et al., 2001).

In spite of these apparent advantages, few studies have described the dual effects of α_2-adrenoceptor agonists against pain and spasticity in patients who have sustained spinal cord injuries. Even studies using nonhuman models have usually limited their analyses to only one of these two conditions (Hao et al., 1996; Yezierski, 1996; Christensen and Hulsebosch, 1997; Barbeau et al., 1998; Chau et al., 1998). For this reason, we have developed the Chronic Spinal rat as an experimental model in which to assess the simultaneous effect of drugs on two spinal reflexes, the nociceptive tail withdrawal reflex (tail-flick), and the nonnociceptive hindlimb flexion reflex. Because chronic spinalization produces hindlimb spasticity (Parise et al., 1997; Duke and Advokat, 2000), it is possible to concurrently evaluate the antispastic and antinociceptive effect of drugs in this unanesthetized, in vivo preparation. We have previously described clinically relevant effects of baclofen (Advokat et al., 1999) and morphine (Advokat and Duke, 1999), on these two reflexes, in Chronic Spinal rats. The present study summarizes our results with intrathecal (i.t.) administration of the α_2-adrenoceptor agonist, clonidine.

2. Materials and methods

2.1. Subjects

A total of 71 (20 Intact; 37 Acute Spinal; 14 Chronic Spinal) male albino Sprague–Dawley rats (Holtzman Laboratories, Madison, WI), weighing an average of 281 (± 4) g when tested, were used in this study. All rats were singly

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housed in plastic cages in a colony room maintained on a 12:12 h light/dark cycle, with dark onset at 19:00 h, and had continuous access to food and water. The procedures for spinalization and postoperative care have been described in detail (Advokat and Duke, 1999; Advokat et al., 1999). Acute Spinal rats were tested 1 day, and Chronic Spinal rats an average of 31 ($\pm < 1$) days after surgery. At the end of the experiment, rats were euthanized by an anesthetic overdose or administration of CO2. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Louisiana State University (Baton Rouge, LA).

2.2. Behavioral testing

The tail-flick response was elicited by noxious thermal stimulation of the tail (iitcc, Woodland Hills, CA) and each score consisted of the mean of three trials, with a 14-s limit to prevent tissue damage.

For the flexion reflex, two recording leads were inserted percutaneously into the biceps femoris/semitendinosus muscle of one hindlimb, one ground lead was inserted subcutaneously in the thigh, and a pair of fine subcutaneous pin electrodes for stimulating were placed in the skin between the toes. The reflex was elicited by 5 square wave shocks, at 500 Hz, and 0.2-ms duration. All stimulation and recording procedures were performed with a Nicolet Viking IV D system (Nicolet Instrument, Madison WI). Stimulus intensity was set at 2.5 × threshold and five responses were elicited at approximately 30-s intervals. Each response was rectified and integrated, within a time window of 200 ms, providing an index of the area under the curve (AUC) in mV × ms as the measure of reflex magnitude. The response was defined as the mean of the five elicited flexion reflexes for each rat.

2.3. Drug administration and data analyses

After baseline measures, clonidine was injected in 10 μl of saline, under light isoflurane anesthesia, into the lumbar intrathecal space with a 26-gauge needle attached to a Hamilton microsyringe, as previously developed and described by Mestre et al. (1994). In the Acute Spinal condition, one group received an injection of saline only. All animals were tested 30, 60 and 90 min after i.t. injection.

For the tail-flick reflex, scores at each time point were converted to Percent Maximal Possible Effect, with the formula: postdrug latency − predrug latency/maximum latency (14 s) − predrug latency × 100. For the flexor reflex, the effect of clonidine was quantified as percent (%) of baseline with the formula: post-drug score/pre-drug score × 100. For both reflexes, the area under each time–effect curve (AUC) was calculated for each rat with the computer program PHARM/PCS (MicroComputer Specialists, Philadelphia, PA). Because of the 14-s cutoff for the tail-flick, the maximum possible AUC for that response was 9000. With this transformation, dose–response curves were obtained for both reflexes and, where possible, the ED_{50} value (± 95% confidence intervals, CI) was calculated using the Litchfield–Wilcoxin method (PHARM/PCS, MicroComputer Specialists). Dose–response functions were also analyzed by one-way Analyses-of-Variance, and compared with each other using two-way Analyses-of-Variance (Sigma-Stat, Jandel, San Rafael, CA). For all statistics, results were considered significant at $P < 0.05$.

3. Results

Prior to drug administration, the mean baseline tail-flick scores of the experimental groups were as follows: Intact, 4.4 to 5.0 s; Acute and Chronic Spinal, 4.0 to 4.6 (in each case).

The effect of i.t. clonidine on the tail-flick reflex, in each of the three conditions, is summarized in Fig. 1. The AUC for the saline group of Acute Spinal rats (which is not shown to improve clarity) was 131 ± 87. There was a significant dose effect in Intact rats ($F(3, 16) = 22.14$, $P < 0.001$; $n = 5$ in each case) and in Acute Spinal rats ($F(6, 36) = 8.41$, $P < 0.001$; $n = 6$ for saline and 4 μg and 5 for all other doses). When the two conditions were compared with each other across the four doses administered in common (4, 20, 40 and 60 μg), there was no difference between the Intact and Acute Spinal rats ($F(1, 33) = 0.024$), and no interaction, although the dose response effect was retained ($F(3, 33) = 24.82$, $P < 0.001$).

There was also a significant dose response effect in Chronic Spinal rats ($F(3, 10) = 6.22$, $P = 0.012$; $n = 4$ for

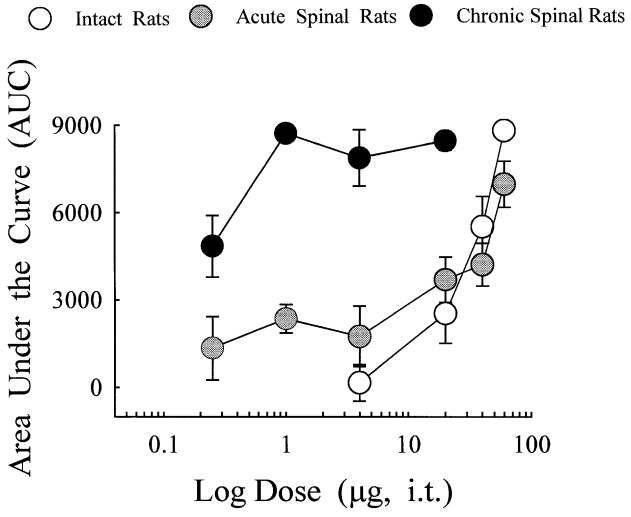

Fig. 1. Dose–response to intrathecal clonidine on the tail flick test, in Intact (open circles), Acute Spinal (shaded circles) and Chronic Spinal rats (solid circles). The data represent the mean ± S.E.M. of the area under the time–effect curve (at 30, 60 and 90 min) for separate groups of rats ($n = 3$ to 6), tested after administration of the indicated doses of clonidine (0.25 to 60 μg). There was a significant dose-dependent effect of clonidine in each of the three experimental conditions. Clonidine-induced antinociception in Intact and Acute Spinal rats did not differ, however, this effect was significantly increased in Chronic Spinal rats.

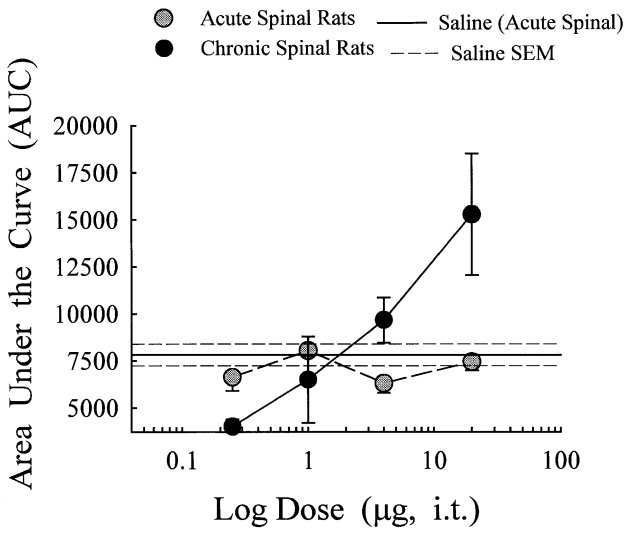

Fig. 2. Dose–response to intrathecal clonidine (0.25, 1, 4 or 20 μg) on the flexor reflex of Acute (shaded circles) and Chronic (solid circles) Spinal rats. The data were obtained from the same animals whose tail flick test results are summarized in Fig. 1. There was no difference among the doses in Acute Spinal rats, and no group differed from the saline condition. However, the same four doses produced a significant dose-dependent effect in Chronic Spinal rats.

0.25 and 1 μg, $n = 3$ for 4 and 20 μg). A Chronic Spinal saline condition was not included in this study, primarily for humane reasons, because the effect of saline has previously been shown to be the same as in Acute Spinal rats, i.e. no effect, under the same conditions (Advokat et al., 1999 and references therein). Comparison between Acute and Chronic Spinal rats across their four common doses indicated a significant difference ($F(1, 27) = 64.91$, $P < 0.001$) and a significant dose effect ($F(3, 27) = 4.16$, $P = 0.015$), with no interaction. It should be noted that, at doses ≥ 1 μg, the AUC scores of Chronic Spinal rats often reached the maximum value. Because this effect was so (unexpectedly) large, it was not considered necessary to increase the number of animals subjected to this manipulation. A separate, one-way analysis across the four common (lowest) doses in Acute Spinal rats was not significant.

The effect of clonidine on the flexion reflex in Acute and Chronic Spinal rats is summarized in Fig. 2. There was no dose–response effect across the four doses in Acute Spinal rats, and no group differed from the saline condition. However, the same doses produced a significant dose dependent effect ($F(3, 10) = 5.85$, $P = 0.014$) in Chronic Spinal rats, with an increase in response magnitude at the higher doses.

4. Discussion

Our results support previous reports of the dose-dependent antinociceptive effect of clonidine, and the well-established role of α_2-adrenoceptor agonists in spinal adrenergic analgesia (Khan et al., 1999; Asano et al., 2000; Kamibayashi and Maze, 2000). But these are the first data to show that the effect is not altered by acute spinalization, indicating that it is mediated post-synaptically to descending pathways. Recent reports suggest that the α_{2A}-adrenoceptor is the relevant subtype, because this subtype is not found on descending adrenergic terminals, and is located predominantly on primary afferent terminals in the dorsal horn (Stone et al., 1998).

These data also show that clonidine-induced antinociception is more potent in Chronic than in Acute Spinal rats, indicating significant changes in adrenoceptor sensitivity following chronic spinalization. Other interpretations, such as a (hypothetical) change in skin temperature produced by spinal transection, or a vasoactive effect of clonidine, would not explain this pattern of results. First, the baseline tail-flick responses of the Acute and Chronic groups of spinal rats, while lower than that of Intact rats, were the same, which would not account for the difference between these groups in clonidine's antinociceptive effect. Second, the effect of clonidine in Acute Spinal rats was the same as in Intact rats, which would not be expected if these groups differed in either skin temperature or in their vasoactive response to clonidine. Third, a similar potency increase has been reported in other nonhuman pain models, such as inflammation-induced mechanical hyperalgesia (Mansikka et al., 1996, and references therein) and mechanical allodynia produced by spinal cord ischemia (Hao et al., 1996). Finally, the flexion response was also altered in Chronic Spinal rats, in a manner consistent with a receptor-mediated interpretation.

However, clonidine does not appear to be more potent in clinical, neuropathic, pain states relative to nonneuropathic conditions, with approximately 150 μg as a common dose across a variety of conditions (Glynn et al., 1986; Eisenach et al., 1995; Glynn and O'Sullivan, 1995; Armand et al., 1998). Although epidural clonidine is helpful for patients with spinal cord injury (Glynn et al., 1986), this effect may be modest depending on how well the drug reaches the site of the lesion (Siddall et al., 2000). Further investigation is needed to determine the reasons for this difference, e.g. the nature or location of the spinal lesion, the characteristics of the nociceptive stimulation, and the corresponding effect on motor function after spinal administration of adrenergic agents for chronic pain (Glynn and O'Sullivan, 1995).

Like analgesia, the depressive effect of clonidine and other α_2-adrenoceptor agonists on the hindlimb flexion response in intact animals is also well established. However, unlike analgesia, this effect is greatly altered by acute spinal transection. Immediately after spinalization, the same systemic doses of clonidine that depress flexion in the intact state become facilitatory, and increase the response. It has been shown that this effect is due to an action of α_1-adrenoceptors in the spinal cord, which is presumably masked in the intact animal by a supraspinal action at α_2-adrenoceptors (Kehne et al., 1985; Chen et al., 1987).

We did not see facilitation with i.t. clonidine in Acute Spinal rats, probably because the doses were too low. However, as with antinociception, the same doses had a greater effect in Chronic Spinal rats in that they decreased the flexion reflex at low doses and increased it at higher doses. That is, chronic spinalization produced an adaptation of α-adrenoceptors that was expressed as an antispastic action of clonidine. These results are consistent with the work of Barbeau, Rossignol and colleagues, in acute and chronic spinal cats, and patients with spinal cord injury (Barbeau et al., 1998; Chau et al., 1998; Denys et al., 1998; Giroux et al., 1999; Rémy-Néris et al., 1999). They have shown that low doses of i.t. clonidine (30–90 μg) reduced spasticity in spinally injured patients, by activation of spinal α_2-adrenoceptors, while the combination of facilitatory, α_1, and inhibitory, α_2-adrenoceptor activation improved various functional parameters involved in walking. They argue that the combined effects of multiple, α-adrenoceptor activation could be a useful treatment for spinal cord injury.

Of particular relevance to the present study is a case report in which a patient with a chronic spinal cord injury sustained severe rectal pain from anal sphincter spasms that blocked bowel function (Middleton et al., 1996). Both the pain and spasm resolved after 20 μg of i.t. clonidine, with no recurrence after 2 years of chronic infusion. In that discussion, it was appreciated that the beneficial action of clonidine could have been due to either a motor effect, with reduction in spasm leading to a reduction in pain, or an antinociceptive effect, with a subsequent decrease in spasm. Perhaps, similar studies of tolerance in chronic spinal rats might help to distinguish between these alternatives. With this model, it may be possible to address such questions and to better differentiate the therapeutic action of drugs for the relief of pain and spasticity in spinal cord injury.

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