Differences in the Antinociceptive Effects of Alpha-2 Adrenoceptor Agonists in Two Substrains of Sprague-Dawley Rats

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ABSTRACT

In this study, we examined whether Sprague-Dawley rats obtained from two different vendors, Harlan and Sasco, differ with respect to the types of alpha-2 adrenoceptors in the spinal cord that mediate antinociception. This hypothesis was tested using two alpha-2 adrenoceptor agonists, dexmedetomidine and ST-91, which are relatively selective for alpha-2A and alpha-2B adrenoceptors, respectively, and two different measures of nociception, the tail-flick and the 55°C hot-plate test. Dexmedetomidine and ST-91 each increased tail-flick latency to a similar extent in both Harlan and Sasco rats, although dexmedetomidine was more efficacious than ST-91 in each substrain. However, the efficacy of these agonists was markedly different in Harlan and Sasco rats when the hot-plate test was used. For example, ST-91 was a full agonist in the hot-plate test in Harlan rats but a weak partial agonist in Sasco rats. Dexmedetomidine was a very weak partial agonist in Harlan rats and ineffective in the hot-plate test in Sasco rats. These findings suggest that (1) both spinal alpha-2A and alpha-2B receptors modulate nociceptive responses in the tail-flick test in both Harlan and Sasco rats; (2) hot-plate responses are mediated predominantly by alpha-2B adrenoceptors, with a minimal contribution by alpha-2A adrenoceptors in the Harlan rat and (3) hot-plate responses are not appreciably affected by either alpha-2A or alpha-2B adrenoceptors in the Sasco rat. These findings confirm previous reports that intrathecal administration of alpha-2 adrenoceptor agonists produces thermal antinociception in the rat. However, the magnitude of the antinociceptive effect is dependent on the receptor selectivity of the agonist used, cutaneous tissue stimulated to elicit nociceptive responses and substrain of rat.

It is well established that i.t. administration of alpha-2 adrenoceptor agonists such as dexmedetomidine (Fisher et al., 1991; Idänpäälä-Heikkilä et al., 1994; Kalso et al., 1991; Takano and Yaksh, 1992), clonidine (Ossipov et al., 1988; Reddy et al., 1980; Solomon et al., 1989; Takano and Yaksh, 1992) or ST-91 (Howe et al., 1983; Monaskey et al., 1990; Saeki and Yaksh, 1992; Takano and Yaksh, 1992) produces antinociception in rats (Kalso et al., 1991; Reddy et al., 1980; Takano and Yaksh, 1992), monkeys (Yaksh and Reddy, 1981) and humans (Filos et al., 1994; Gordh, 1988; Gordh and Tamsen, 1983; Tamsen and Gordh, 1984). Subsequent studies with idazoxan, yohimbine, prazosin and imiloxan indicated that these alpha-2 adrenoceptor antagonists differ in their rank order of potency to attenuate the antinociception produced by dexmedetomidine, clonidine and ST-91 in the Harlan Sprague-Dawley rat (Takano et al., 1992; Takano and Yaksh, 1992). Based on these differences, Yaksh and colleagues proposed that dexmedetomidine and clonidine produce antinociception by acting at alpha-2A adrenoceptors, whereas ST-91 acts at alpha-2B adrenoceptors (Takano et al., 1992; Takano and Yaksh, 1992).

The alpha-2 adrenoceptor agonists produce antinociception by inhibiting synaptic transmission in the rat spinal cord dorsal horn (Kalso et al., 1993; Murata et al., 1989; Sullivan et al., 1987, 1992). Stimulation of noradrenergic neurons in the rat brainstem also produces antinociception (Jones, 1992; Proudfit, 1992; Yeomans et al., 1992; West et al., 1993) by inhibiting the responses of dorsal horn neurons to noxious stimuli (Jones and Gebhart, 1986, 1987). Spinally projecting neurons in the A5, A6 (locus ceruleus) and A7 noradrenergic cell groups located in the pons are the predominant source of norepinephrine in the spinal cord of the rat (Nygren and Olson, 1976; Westlund et al., 1982, 1983). However, recent studies using more sensitive tract tracing and immunocytochemical methods have determined that the origin of the catecholaminergic projection to the dorsal horn differs signif-

ABBREVIATIONS: i.t., intrathecal; CL, confidence limit; HPL, hot-plate latency; ST-91, 2-(2,6-diethylphenylamino)-2-imidazoline; TFL, tail-flick latency.

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antly in Sprague-Dawley rats obtained from two different vendors. In Sprague-Dawley rats obtained from Harlan, locus ceruleus neurons project predominantly to the superficial laminae of the dorsal horn (Clark and Proudfit, 1992; Fritschy and Grzanna, 1990; Grzanna and Fritschy, 1991), whereas the axons of A7 neurons terminate in the ventral horn (Lyons et al., 1989). In contrast, in Sprague-Dawley rats obtained from Sasco, A7 neurons project mainly to laminae I–IV in the ipsilateral dorsal horn (Clark and Proudfit, 1991b), whereas locus ceruleus neurons project most heavily to the ipsilateral medial aspect of laminae VII–IX, with only a minor projection to the ventral part of the dorsal horn (Clark and Proudfit, 1991a; Proudfit and Clark, 1991). This substrain difference between Harlan and Sasco Sprague-Dawley rats has been replicated by other investigators (Sluka and Westlund, 1992). Initial studies of the ability of intrathecally administered alpha-2 adrenoceptor antagonists to attenuate the antinociception produced by electrical (West et al., 1993; Yeomans et al., 1992) or chemical (Yeomans and Proudfit, 1992) stimulation demonstrated significant functional differences between the catecholamine cell groups in these substrains. These results raised the possibility that the alpha-2 adrenoceptor subtypes that mediate antinociception in the spinal cord of these substrains may also be different.

The present experiments were done as part of a systematic study of the spinal cord alpha-2 adrenoceptors that modulate nociception in Sprague-Dawley rats obtained from Harlan and Sasco (Graham et al., 1995a, 1995b, 1996, 1997). In this aspect of the study, we report the effects of the alpha-2 adrenoceptor agonists dexmedetomidine and ST-91 on nociceptive responses determined using the tail-flick and 55°C hot-plate tests in both Harlan and Sasco Sprague-Dawley rats.

Methods

Surgical preparation. Male Sprague-Dawley rats (250–350 g) obtained from Harlan (Indianapolis, IN) and Sasco (Madison, WI) were anesthetized with halothane and implanted with i.t. catheters that terminated at the L4 segment of the spinal cord (Hammond, 1988; Yaksh and Rudy, 1976). The rats were allowed to recover for 10 days before testing and those exhibiting neurological deficits such as hemiparesis of fore paws or hindpaws were killed. Rats were used only once in this study, after which they were killed and the location and patency of each catheter were determined by direct visualization after an injection of India ink.

Behavioral testing. Thermal nociceptive thresholds were measured using the tail-flick (D’Amour and Smith, 1941) and hot-plate (Woolfe and MacDonald, 1944) tests. In the tail-flick test, a high-intensity light beam was focused on the dorsal surface of the rat’s blackened tail. The reflex removal of the tail from the light beam was electronically measured to the nearest 0.1 sec, and the average of two successive measurements was defined as the TFL. In the hot-plate test, the rat was placed on an enclosed 55°C copper surface, and the latency to a hindpaw lick or a jump off the surface was measured. One determination of the hot-plate response latency, measured to the nearest 0.1 sec, was made and defined as the HPL.

Drug-induced motor dysfunction can alter measurements of TFL or HPL and confound the interpretation of drug effects on nociception (Hammond, 1989). Motor function was therefore assessed by the righting reflex and the inclined plane test, having a rubber surface with horizontal ridges 0.1 cm in height. The angle of the inclined plane was adjusted in 5° increments to determine the largest angle at which the rat was able to maintain its position on the incline. Doses of drugs that produced significant motor deficits, as measured by the righting reflex and the inclined plane, were not included in the analysis of dose-response functions.

Drugs. The drugs tested were the relatively selective alpha-2 adrenoceptor agonist dexmedetomidine (molecular weight = 236.7; Orion Corp., Orion-Farmons, Turku, Finland) and ST-91 (molecular weight = 253.8; Boehringer Ingelheim, Ridgefield, CT). Dexmedetomidine was injected in doses ranging from 1.3 to 12.7 nmol, and ST-91 was injected in doses ranging from 3.9 to 39.4 nmol. All drugs were dissolved in normal saline; pH of each solution was adjusted to 7.0 and administered intrathecally in a volume of 10 μl, followed by 10 μl of normal saline to flush the catheter. The injection volume was monitored by following movement of an air bubble through a calibrated length of tubing.

Experimental design. On the day before testing, the rats were brought to the testing environment from the animal care facility, their tails were blackened and they were allowed to acclimate to the testing room for several hours. The following day, they were returned to the testing environment and allowed to acclimate for ≥1 hour. Rats with mean baseline TFL values of >5.2 sec or baseline HPL values of >15 sec or those in which the inclined plane angle was <40° were excluded from further testing. Dexmedetomidine, ST-91, a mixture of dexmedetomidine and ST-91 or a saline control solution was then injected i.t., and response latencies were redetermined 10, 20, 30, 45 and 60 min later. Motor function was reassessed using the inclined plane and righting reflex tests at 30 min, which corresponds to the time of peak antinociceptive effect. Animals that did not respond by 14 sec in the tail-flick test or 40 sec in the hot-plate test after drug administration were assigned that cutoff latency. The tests were conducted in tandem, with the tail-flick test conducted before the hot-plate test. These experiments were conducted under a protocol approved by the Institutional Animal Care and Use Committee of the University of Chicago and in accordance with the “Guide for Care and Use of Laboratory Animals” of the National Institutes of Health.

Statistical analysis. The effect of each drug dose was compared with that of saline using a two-way analysis of variance for repeated measures in which one factor was drug treatment and the repeated factor was time. Multiple post hoc comparisons among individual mean values were made using the Newman-Keuls test (Keppel, 1973). ED50 values for each agonist were determined from dose-response curves generated by least-squares linear regression of response latency values at the time of peak effect from individual animals. Fieller’s theorem was used to determine 95% CIs (Finney, 1964). The ED50 was defined as the dose that produced one half of the maximal possible increase in response latency; this value was 9 sec for the tail-flick test and 25 sec for the hot-plate test. Dose-response curves were compared for parallelism and for differences in ED50 values by analysis of covariance (Zar, 1974). A value of P ≤ .05 was considered to be statistically significant.

Results

Effects of intrathecally administered dexmedetomi-
dine on TFL in Sasco and Harlan Sprague-Dawley rats. Intrathecal administration of dexmedetomidine produced a dose-dependent increase in TFL in both Sasco Sprague-Dawley rats (figs. 1A and 2A) and Harlan Sprague-Dawley rats (figs. 1B and 2A). This effect was apparent within 10 min, and the peak effect was achieved by 20 min (fig. 1). The increase in TFL persisted for ≥60 min for all drug doses. The ED50 (95% CL) of dexmedetomidine was 4.3 (2.9–6.0) nmol in Sasco Sprague-Dawley rats and 3.4 (1.9–5.3) nmol in Harlan Spra-
gue-Dawley rats. The difference between these values was not statistically significant (fig. 2A; \( P > .05 \)).

**Effects of intrathecally administered dexmedetomidine on HPL in Sasco and Harlan Sprague-Dawley rats.** Unlike the tail-flick test, i.t. administration of dexmedetomidine to Sasco Sprague-Dawley rats in doses as high as 12.7 nmol did not significantly increase response latencies in the hot-plate test compared with values in saline-treated rats (fig. 3A). The administration of a higher dose of 42.2 nmol produced profound sedation and prostration, which interfered with the measurement of response latencies on the hot-plate test. In contrast, i.t. administration of 8.4 or 12.7 nmol of dexmedetomidine to Harlan Sprague-Dawley rats produced a modest increase in HPL that occurred within 20 min and persisted for \( \geq 60 \) min. Although a dose of 4.2 nmol of dexmedetomidine also increased HPL, this effect was not apparent until 45 min after drug administration (fig. 3B). No dose of dexmedetomidine increased HPL beyond the criterion value of 25 sec in either Sasco or Harlan Sprague-Dawley rats, which precluded the calculation of an ED_{50} value for dexmedetomidine in this test (fig. 2B).

**Effects of intrathecally administered ST-91 on TFL in Sasco and Harlan Sprague-Dawley rats.** Intrathecal administration of ST-91 produced a dose-dependent increase in TFL in both Sasco Sprague-Dawley rats (fig. 4A) and Harlan Sprague-Dawley rats (fig. 4B). Mean TFL values were significantly increased within 20 min, reached peak values by 30 min and remained elevated for at least 60 min (fig. 4, A and B). ST-91 appeared to be a partial agonist in the tail-flick test in Sasco Sprague-Dawley rats (figs. 4A and 5A), as well as in Harlan Sprague-Dawley rats (figs. 4B and 5A). Thus, mean TFL values increased to \( \approx 9 \) to 10 sec after i.t. administration of 11.8 nmol of ST-91, but no further increase in TFL occurred after i.t. administration of 39.4 nmol, a 3-fold higher dose, in either substrain (figs. 4, A and B, and 5A). The administration of ST-91 in a higher dose of 118.2 nmol produced spontaneous serpentine tail movements in Sasco Sprague-Dawley rats that prevented accurate measurements of TFL. Because ST-91 did not increase TFL beyond the criterion value of 9 sec, it was not possible to...
determine an ED$_{50}$ value and 95% CL for this agonist in either substrain (fig. 5A).

**Effects of intrathecally administered ST-91 on HPL in Sasco and Harlan Sprague-Dawley rats.** The i.t. administration of 11.8 or 39.4 nmol of ST-91 to Sasco Sprague-Dawley rats increased mean HPL to 20 sec within 30 min of drug injection (fig. 6A). The antinociceptive effect of a higher dose of ST-91 could not be determined due to the presence of spontaneous motor effects as noted above. No dose of ST-91 increased HPL beyond the criterion value of 25 sec in Sasco Sprague-Dawley rats, which precluded the calculation of an ED$_{50}$ value (fig. 5B).

In contrast, i.t. injection of ST-91 in Harlan Sprague-Dawley rats in a dose of 11.8 or 39.4 nmol increased HPL in a dose-dependent manner, with the peak effect observed at 30 min (fig. 6B). ST-91 injected in the lowest dose of 3.9 nmol also increased HPL, but this effect achieved statistical significance only at the 20-min time point. The ED$_{50}$ value (and 95% CL) of ST-91 in Harlan Sprague-Dawley rats was 15.5 (8.2–35.3) nmol (fig. 5B).

**Effects of combined i.t. administration of ST-91 and dexmedetomidine on TFL in Sasco and Harlan Sprague-Dawley rats.** In both Harlan and Sasco rats, dexmedetomidine was a full agonist, whereas ST-91 was a partial agonist in the tail-flick test. If these agonists act at the same receptor, then coadministration of the low-efficacy agonist ST-91 with the high-efficacy agonist dexmedetomidine should produce an antinociceptive effect that is less than that produced by dexmedetomidine alone. However, coadministration of ST-91 did not attenuate the antinociceptive effects of dexmedetomidine but rather produced a greater increase in TFL than dexmedetomidine alone at several time points in both Sasco and Harlan rats (P < .05; fig. 7, A and B).

**Effects of combined i.t. administration of ST-91 and dexmedetomidine on HPL in Sasco and Harlan Sprague-Dawley rats.** In Sasco rats, dexmedetomidine was ineffective and ST-91 produced only a modest increase in HPL (fig. 8A). Coadministration of the low-efficacy agonist dexmedetomidine with the high-efficacy agonist ST-91 increased HPL to nearly 40 sec in this substrain (fig. 8A). In Harlan rats, dexmedetomidine produced a modest increase in HPL, whereas ST-91 produced a much greater increase in HPL (fig. 8B).
The coadministration of dexmedetomidine with ST-91 in this substrain also produced a large increase in HPL that was significantly greater than that of ST-91 alone (fig. 8B).

Discussion

The antinociceptive effects of dexmedetomidine and ST-91 differ in Harlan and Sasco Sprague-Dawley rats. The present study provides evidence that Harlan Sprague-Dawley and Sasco Sprague-Dawley rats differ with respect to the types of alpha-2 adrenoceptors in the spinal cord that mediate thermal antinociception. If the spinal alpha-2 adrenoceptors that mediate antinociception in each substrain are the same, then the antinociceptive effects of dexmedetomidine should be the same in each substrain. Similarly, the antinociceptive effects of ST-91 an alpha-2B adrenoceptor agonist should be the same in each substrain. This was not the case in the present study. The most marked difference between the substrains was observed in the hot-plate test. Dexmedetomidine increased HPL to 18 to 20 sec in Harlan rats but was ineffective in Sasco rats (table 1 and fig. 2). ST-91 increased HPL to 30 sec in Harlan rats but was much less effective in Sasco rats (table 1 and fig. 5). These differences suggest that the neural circuitry that subserves responses in the hot-plate test in Harlan rats differs from Sasco rats in (1) the number or distribution of alpha-2 adrenoceptors in the dorsal horn, (2) the relative densities of the different subtypes of alpha-2 adrenoceptor or (3) the efficiency of the coupling of alpha-2 adrenoceptors to subcellular effectors such as G proteins (Summers and McMartin, 1993). In contrast to the hot-plate test, there were no significant differences between the two substrains in the antinociceptive effects of either alpha-2 adrenoceptor agonist in the tail-flick test. Dexmedetomidine increased TFL with similar potency and to a maximal value of 12 to 13 sec in both Harlan and Sasco rats (table 1 and fig. 2). ST-91 also increased TFL with similar potencies in both Harlan and Sasco rats, although to maximal values of only 10 sec (table 1 and fig. 5). The similarity of the effects of dexmedetomidine and ST-91 in both substrains in the tail-flick test suggests that the distribution, density and types of alpha-2 adrenoceptors that mediate antinociception in the tail-flick test are not significantly different between the two substrains.
test are similar in both Harlan and Sasco Sprague-Dawley rats.

The antinociceptive effects of dexmedetomidine and ST-91 result from actions at different \( \alpha-2 \) adrenoceptors. Dexmedetomidine and ST-91 exhibited substantially different antinociceptive efficacies in the tail-flick and hot-plate tests in both substrains of rat. For example, in the tail-flick test dexmedetomidine was more potent and appeared to be a full agonist (fig. 2 and table 2), whereas ST-91 was less potent and appeared to be a partial agonist in Harlan and Sasco rats (fig. 5 and table 2). Even larger differences in the efficacy of these two \( \alpha-2 \) adrenoceptor agonists were observed in the hot-plate test, although in this test ST-91 was more efficacious than dexmedetomidine in each substrain. There are three possible explanations for such differences in the apparent potency and efficacy of dexmedetomidine and

| TABLE 1 | Summary of the antinociceptive effects of dexmedetomidine and ST-91: Comparison between Harlan and Sasco rats
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<td>ST-91</td>
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Fig. 7. Time course of the increases in TFL produced by i.t. administration of saline (○), 39.4 nmol of ST-91 (●), 12.7 nmol of dexmedetomidine (▲) or a mixture of 39.4 nmol of ST-91 and 12.7 nmol of dexmedetomidine in Sasco Sprague-Dawley rats (A) and Harlan Sprague-Dawley (B) rats. The TFL values for saline, ST-91 and dexmedetomidine were taken from figures 1 and 4. Each value is the mean ± S.E.M. TFL value of determinations in 6 to 15 animals. *P < .05, **P < .01, values that are significantly greater than those in dexmedetomidine-treated rats at the corresponding time point.

Fig. 8. Time course of the increases in HPL produced by i.t. administration of saline (○), 39.4 nmol of ST-91 (●), 12.7 nmol of dexmedetomidine (▲) or a mixture of 39.4 nmol of ST-91 and 12.7 nmol of dexmedetomidine in Sasco Sprague-Dawley rats (A) and Harlan Sprague-Dawley (B) rats. The HPL values for saline, ST-91 and dexmedetomidine were taken from figures 3 and 5. Each value is the mean ± S.E.M. HPL value of determinations in 6 to 15 animals. *P < .05, **P < .01, values that are significantly greater than those in ST-91-treated rats at the corresponding time point.
ST-91 in Sasco and Harlan rats. First, dexmedetomidine and ST-91 could act at the same \(\alpha-2\)-adrenoceptor in both substrains, one of which has fewer \(\alpha-2\)-adrenoceptors in the dorsal horn. Second, these two \(\alpha-2\)-adrenoceptor agonists could act at the same \(\alpha-2\)-adrenoceptor in both substrains, one of which has a lower intrinsic activity due to less efficient receptor coupling to subcellular effectors. Third, dexmedetomidine and ST-91 could act at different \(\alpha-2\)-adrenoceptor subtypes. The first two possibilities merit consideration because shifts in the dose-response curves for dexmedetomidine and ST-91 produced by the irreversible antagonist N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (Takano and Yaksh, 1991) or by variation in the intensity of noxious stimuli (Saeki and Yaksh, 1992) suggest that dexmedetomidine has lower fractional receptor occupancy requirements and greater intrinsic activity than ST-91. To resolve these possibilities, dexmedetomidine and ST-91 were coadministered. The administration of a full agonist with a partial agonist that acts at the same receptor will produce an effect that is less than that produced by the full agonist alone because the partial agonist, which exhibits a lower efficacy, competes with the full agonist for available receptor binding sites (Kenakin, 1993). However, in both the tail-flick and hot-plate tests, coadministration of the two \(\alpha-2\)-adrenoceptor agonists consistently enhanced, rather than attenuated, the antinociceptive effect of the full agonist. This finding suggests that dexmedetomidine and ST-91 do not have different intrinsic activities at the same \(\alpha-2\)-adrenoceptor but rather act at different subtypes of the \(\alpha-2\)-adrenoceptor. This conclusion is consistent with the results of earlier studies that concluded that dexmedetomidine acts predominantly at \(\alpha-2\)-A receptors and ST-91 acts predominantly at \(\alpha-2\)-B receptors (Takano et al., 1992; Takano and Yaksh, 1992). It is also supported by our recent finding that coadministration of dexmedetomidine and ST-91 in a 1:3 fixed dose ratio increases TFL and HPL in Sasco and Harlan rats in a synergistic manner (Graham et al., 1997), as would be expected of two agonists that act at different receptors.

The nociceptive responses of the feet and tail are modulated by different \(\alpha-2\)-adrenoceptors. If the same \(\alpha-2\)-adrenoceptors modulate responses in the tail-flick and hot-plate tests, then the rank order of potency of dexmedetomidine and ST-91 should be the same in both tests. This was not the case in the present study. For example, dexmedetomidine was a full agonist in the tail-flick test but a very weak partial agonist in Harlan rats and ineffective in Sasco rats in the hot-plate test. By comparison, ST-91 was a partial agonist in the tail-flick test, but in the hot-plate test it was a full agonist in Harlan rats and a weak partial agonist in Sasco rats. These differences in the relative efficacies of the \(\alpha-2\)-adrenoceptor agonists on nociceptive responses of the feet, determined using the hot-plate test, compared with nociceptive responses of the tail suggest that different \(\alpha-2\)-adrenoceptors modulate nociceptive responses of the feet and tail.

The present results provide some insight into the relative importance of \(\alpha-2\)-adrenoceptors in modulating the nociceptive responses of the feet and the tail. For example, both dexmedetomidine and ST-91 were effective in the tail-flick test in both Harlan and Sasco rats. Because dexmedetomidine and ST-91 act preferentially at \(\alpha-2\)-A and \(\alpha-2\)-B adrenoceptors, respectively, these findings suggest that both \(\alpha-2\)-A and \(\alpha-2\)-B adrenoceptors modulate nociceptive responses in the tail in both Sasco and Harlan rats. It cannot be stated at this time which subtype of \(\alpha-2\)-adrenoceptor is of greater importance in modulating responses of the tail. Although ST-91 was not as efficacious as dexmedetomidine in the tail-flick test, it also has a higher fractional receptor occupancy and lower intrinsic activity than dexmedetomidine at \(\alpha-2\)-adrenoceptors (Saeki and Yaksh, 1992; Takano and Yaksh, 1991). Clarification of the relative importance of \(\alpha-2\)-A and \(\alpha-2\)-B receptors in the tail-flick test must therefore await the identification of \(\alpha-2\)-B adrenoceptor agonists with higher intrinsic activity than ST-91. Nevertheless, these observations suggest that the tail-flick test is a suitable measure for investigations of the potency or efficacy of \(\alpha-2\)-A or \(\alpha-2\)-B adrenoceptor ligands regardless of substrain.

In the hot-plate test, dexmedetomidine was a very weak partial agonist in Harlan rats and ineffective in Sasco rats, whereas ST-91 was a full agonist in Harlan rats and a weak partial agonist in Sasco rats. These findings suggest that in Harlan rats, responses in the hot-plate test are predominantly mediated by the \(\alpha-2\)-B adrenoceptor with a minimal contribution by \(\alpha-2\)-A receptors. In Sasco rats, the contribution of either \(\alpha-2\)-B or \(\alpha-2\)-A adrenoceptors appears to be minimal and much less than that in Harlan rats. These results suggest that the hot-plate test is not an appropriate measure for studies of the antinociceptive potency or efficacy of \(\alpha-2\)-A adrenoceptor ligands in either substrain. However, the hot-plate test is more suitable for studies of the antinociceptive effects of \(\alpha-2\)-B adrenoceptor agonists, particularly when determined using Harlan Sprague-Dawley rats.

These differences in the efficacy of \(\alpha-2\)-adrenoceptor agonists in reducing nociceptive responses of the foot in the two substrains, as well as differences between the tail and the feet in each substrain, are relevant to the use of recently developed rodent models of persistent inflammatory pain and neuropathic pain (Bennett and Xie, 1988; Hargreaves et al., 1988; Kim and Chung, 1992). That is, the choice of substrain is likely to be a critical factor in establishing the therapeutic efficacy of different classes of \(\alpha-2\)-adrenoceptor agonists in these models in which the hindpaw, rather than the tail, is the site of stimulation.

Direct comparisons between the tail-flick and hot-plate
test should be made cautiously because the tail-flick test involves a reflexive response and the hot-plate test involves a behaviorally integrated response. Moreover, the neural circuitry that subserves these responses is different. The tail-flick reflex is produced by activation of C-fiber afferents (Fleischer et al., 1983; Handwerker et al., 1987; Necker and Hellon, 1978), which terminate in the S3-Co1 segments of the spinal cord (Grossman et al., 1982). By comparison, the primary afferents that innervate the plantar surface of the hindlimb travel predominantly in the tibial nerve to terminate in the L2–5 segments of the spinal cord (Molander and Grant, 1985, 1986). Activation of α5- as well as C-fiber afferents mediates the foot withdrawal response (Fleischer et al., 1983, Leem et al., 1993). Recent studies that used pseudorabies virus, a transneuronal tracer, to map the tail-flick reflex pathway indicated that there is one (and possibly more) interneuron in the tail-flick reflex pathway and that many of these are bilaterally distributed (Jasmin et al., 1997). Much less is known about the interneurons in the neural circuitry that subserves the hot-plate response; however, this pathway is also likely to be polysynaptic with a predominantly ipsilateral distribution of interneurons (Jasmin et al., 1997; Rotto-Percelay et al., 1992). The observed differences in the effects of dexmedetomidine and ST-91 in the tail-flick and hot-plate tests could result from a differential distribution of alpha-2A and alpha-2B adrenoceptors in the neural circuitry that underlies nociceptive responses in the tail-flick and hot-plate tests. Segmental differences in the density and distribution of spinal alpha-2A and alpha-2B adrenoceptors could explain the different efficacies of dexmedetomidine and ST-91 in the tail-flick test compared with the hot-plate test in each strain. However, the density of alpha-2 binding sites identified by [3H]rauwolscine does not differ among the cervical, thoracic, lumbar and sacral segments of the spinal cord (Roudet et al., 1994). Similarly, differences between Harlan and Sasco rats in the efficacy of dexmedetomidine or ST-91 in the hot-plate test could reflect differences between these two substrains in the density and distribution of alpha-2A and alpha-2B adrenoceptors in the lumbar spinal cord. Quantitative autoradiographic studies of the segmental distribution of alpha-2A and alpha-2B adrenoceptors in each substrain will be required to resolve these possibilities. Finally, the sites at which the alpha-2 adrenoceptor agonists may act to suppress nociceptive transmission are many and may include (1) terminals of the α5 or C fiber primary afferents, (2) first-through last-order interneurons and (3) wide dynamic range or nocispecific spinothalamic, spinoreticular or spinomesencephalic neurons that comprise the afferent pain pathways of the tail and foot. Agonists for receptors that are present on multiple components of the afferent pain pathways could conceivably exhibit greater antinociceptive efficacy and potency than agonists for receptors that are preferentially expressed by only one component (e.g., only one class of primary afferent). Definitive identification of the sites at which dexmedetomidine and ST-91 act to suppress nociception will require in situ hybridization studies or immunocytochemical studies that localize alpha-2A and alpha-2B adrenoceptors to immunocytochemically identified primary afferents, first-through last-order interneurons and projection neurons at the light and electron microscopic level.

Summary. The results of this study suggest that the Harlan and Sasco substrains of Sprague-Dawley rat differ with respect to the subtypes of α2 adrenoceptors that mediate antinociception. This work complements previous investigations that identified differences between these two substrains in the origin of the noradrenergic innervation of the spinal cord dorsal horn. The results of this study suggest that both spinal α2A and α2B receptors modulate responses in the tail-flick test in both Harlan and Sasco rats and that this noradrenergic mechanism appears to be “conserved” between substrains. The tail-flick test is therefore a suitable model for examining the actions of α2 adrenoceptor agonists and antagonists in spinal cord. These two substrains do differ importantly with respect to the spinal α2 adrenoceptor subtypes that modulate responses in the hot-plate test. Specifically, responses in the hot-plate test in Harlan Sprague-Dawley rats are mediated predominantly by α2B adrenoceptors with a minimal contribution by α2A adrenoceptors. In contrast, responses in the hot-plate test in Sasco Sprague-Dawley rats do not appear to be mediated appreciably by either α2A or α2B adrenoceptors. These findings confirm previous reports that intrathecally administered α2 adrenoceptor agonists produce antinociception in models of thermal nociception in the rat. However, the magnitude of the antinociceptive effect depends on the receptor selectivity of the agonist used, cutaneous tissue stimulated to elicit nociceptive responses and substrain of rat.

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