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Dexmedetomidine Injection into the Locus Ceruleus Produces Antinociception

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Background: **α_2-Adrenergic agonists such as clonidine and dexmedetomidine are known to produce sedation and analgesia in humans. The sedative effect of these agents is thought to occur through supraspinal pathways, involving the locus ceruleus (LC) and its projections in rats. While the antinociceptive response to α_2 agonists, given intrathecally, is mediated predominantly in the spinal cord, other sites of action have not been systematically studied. The authors examined whether α_2-adrenergic receptors in the LC mediate an antinociceptive effect.**

Methods: **For administration of different drugs into the LC, guide cannulas were placed with their tips in the LC in male Sprague-Dawley rats. Dexmedetomidine (3.5 μg/0.2 μl) was microinjected into the LC through the cannula, or given systemically by intraperitoneal injection (50 μg/kg). The antinociceptive effect of dexmedetomidine was measured using the tail-flick latency response. To determine the sites through which dexmedetomidine injection into the LC produces antinociception, the authors examined whether this response could be perturbed by the specific α_2-adrenergic antagonists atipamezole and L659,066 and pertussis toxin administered either into the LC or intrathecally before injection of dexmedetomidine systemically or directly into the LC. To eliminate the possibility that drug administered in one site (LC or intrathecal) could reach the other site, the dispositional characteristics of radiolabeled dexmedetomidine (LC) or atipamezole (intrathecal) were studied.**

Results: **Dexmedetomidine placed into the LC produces a dose-dependent increase in the tail-flick latency. This antinociceptive effect was blocked by pertussis toxin and by the α_2 antagonists atipamezole and L659,066 placed in the LC. Intrathecal administration of atipamezole and pertussis toxin also blocked the antinociceptive effect of dexmedetomidine placed in the LC. ^{3}H-dexmedetomidine introduced into the LC did not reach the spinal cord in pharmacologically active concentrations; also, intrathecally administered ^{3}H-atipamezole did not reach the LC in appreciable amounts. The systemic administration of dexmedetomidine produced an increase in tail-flick latency, and this effect was attenuated by the injection of atipamezole and L695,066 into the LC.**

Conclusions: **Part of the mechanism by which dexmedetomidine produces an antinociceptive effect is by an action directly on the LC, demonstrated by these studies in which antinociception produced by injection of this drug into the LC can be blocked by specific α_2 antagonists injected into the LC. Furthermore, the action of dexmedetomidine in the LC in turn may result in an increase in activation of α_2 adrenoceptors in the spinal cord, because the antinociceptive effect of LC dexmedetomidine injection also can be blocked by intrathecal injection of atipamezole and pertussis toxin. (Key words: Antinociception. Brain stem: locus ceruleus. Spinal cord. Sympathetic nervous system, α_2-adrenergic receptor agonists: dexmedetomidine.)**

ADRENERGIC agonists acting at the α_2-adrenergic receptor produce antinociception as well as sedation. These responses have been well documented in animals and in human clinical studies.[1] Systemic administration of the α_2-adrenergic agonists clonidine and dexmedetomidine produces sedation and antinociception, whereas intrathecal administration of these agonists produces antinociception only, leading to the conclusion that α_2 agonists modulate nociception through the spinal cord, and that supraspinal sites mediate the sedative effect.[2]

The noradrenergic innervation of the spinal cord arises from noradrenergic nuclei in the brain stem, including the locus ceruleus (LC, also characterized as the A6 group) and the A5 and A7 noradrenergic nuclei.[3,4] The activity of the noradrenergic A5, A6 (LC), and A7 neurons can be decreased by agonists acting at α_2-adrenergic receptors on their cell bodies.[5,6] Modulation of the activity of these neurons may therefore alter the activity of their axon terminals in the spinal

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cord. As a result, the action of α_2 agonists on these nuclei may modulate the descending noradrenergic effect on inhibition of nociception in the spinal cord.

The goals of the current study were: (1) to characterize the antinociceptive effect of dexmedetomidine administered into the LC, using the tail-flick latency (TFL) response in rats; and (2) to determine whether antinociception thus produced is mediated by α_2 receptors in the LC, spinal cord, or both.

Materials and Methods

The experimental protocol was approved by the Animal Care and Use Committee at the Palo Alto Veterans Administration Medical Center. Male Sprague-Dawley rats (Bantin and Kingman, Fremont, CA) weighing 270–340 g were used. All tests were performed between 9 AM and 4 PM. A total of 167 animals were used. Each animal was used for only one set of studies to eliminate possible interaction between different doses and routes of drugs.

Cannulation for Drug Injection into Locus Ceruleus

Animals were anesthetized with halothane and placed in a stereotactic apparatus. The left LC was cannulated with a 24-G stainless steel cannula using the atlas of Paxinos and Watson[7] with the following coordinates: with bregma as reference, 1.2 mm lateral, 9.5 mm posterior, and 6.0 mm ventral to the skull surface. The cannula was fixed in position with methylmethacrylate resin, and the animal was allowed to recover for 3 days before the experiment.

To confirm correct placement of the cannula in the LC, a functional study was performed as follows. A 30-G stainless steel needle connected to polyethylene tubing was inserted into the cannula and positioned 1.0 mm below the tip. Dexmedetomidine (3.5 μg/0.2 μl; Farmos Research and Development, Turku, Finland), was injected using a pump (CMA/Microdialysis Acton, MA; model 100 microinjection pump) at a rate of 0.4 μl/min. Previous studies in our laboratory have demonstrated that placement of a cannula outside the LC fails to produce loss of righting reflex[8] and thus the functional effects correlate well with histologic findings.[9] Therefore, only rats in which the previous administration of dexmedetomidine through the cannula resulted in loss of righting reflex were used for these studies. Atipamezole (Farmos), a selective α_2 antagonist,[10] pertussis toxin (PTX; List Biological Laboratories, Campbell, CA), or the α_2 antagonist L659,066[10] (Merck Sharp & Dohme, West Point, PA) were also injected through the cannula in some experiments, using the same microinjection pump technique. L659,066 was used to test the dependence of the dexmedetomidine-mediated antinociceptive response on the α_2 adrenoceptor. This agent is relatively hydrophilic and is thought to be relatively impermeable and therefore has limited diffusion or spread after injection.[11] Atipamezole and L659,066 were dissolved in 5% dimethyl sulfoxide vehicle.

Intraventricular Administration of Drugs

To perform intraventricular administration of dexmedetomidine, a guide cannula was placed in the intraventricular space (lateral ventricle) in some rats. The animals were anesthetized and placed in the stereotactic frame. The guide cannula was placed using the following coordinates: 1.0 mm posterior to Bregma, 1.0 mm lateral, and 4.0 mm ventral to the skull surface. For injection of drug, a 30-G needle connected to polyethylene tubing was placed through the cannula, with its tip positioned 1.0 mm beyond the tip of the cannula. Injections of 3.5 μg/0.2 μl dexmedetomidine were made using the microinjection pump.

Intrathecal and Systemic Administration of Drugs

For intrathecal administration of atipamezole and pertussis toxin, animals were anesthetized with halothane, an incision was made over the cervical spine, and a small puncture made in the dura mater. Polyethylene tubing (0.28 mm ID) was threaded into the intrathecal space, 8.5 cm, so that the tip of the catheter was positioned at the lumbar level. This tubing was then sutured in place, and the skin was sutured together over the tubing. After the appropriate recovery time of 4–6 days, the desired agent was injected through the intrathecal cannula using the microinjection pump. For systemic administration of atipamezole and dexmedetomidine, the agent was given *via* the intraperitoneal route.

Distribution of ^{3}H-dexmedetomidine in Spinal Cord and Locus Ceruleus after Locus Ceruleus Injection

To determine whether the injection of dexmedetomidine into the LC resulted in significant levels of dexmedetomidine in the spinal cord, we performed a set

of studies using ^{3}H-dexmedetomidine injections in the LC, with subsequent measurement of its distribution in the LC and the spinal cord using liquid scintillation counting of these different tissue samples. ^{3}H-dexmedetomidine, 25 μCi, in methanol (Farmos) was placed in a microcentrifuge tube and the methanol was vaporized in a Savant Automatic Environmental SpeedVac (AES 1000, Savant, NY) at medium heat (43°C). One hundred microliters of nonlabeled dexmedetomidine (17.5 mg/ml) was added to the dried ^{3}H-dexmedetomidine to result in a specific activity of 10 μCi · .mmol^{-1}. Rats had cannulas placed into the LC, and an injection of 0.2 μl of ^{3}H-dexmedetomidine (3.5 μg total dexmedetomidine) was made into the LC using the technique of microinjection described earlier. Five min after injection of drug, the animal was anesthetized in carbon dioxide and decapitated. Immediately after decapitation, the brain and spinal cord were rapidly removed. The LCs from the injected and noninjected sides were harvested using a 0.8-mm diameter punch. The remaining pons and the medulla were harvested in separate pieces. The spinal cord was harvested and divided into eight sections including cervical, thoracic, and lumbar cord. To each harvested tissue sample, 0.5 ml tissue solubilizer (hyamine hydroxide, ICN, Aurora, OH) was added incubated overnight at 40°C. Five milliliters of cocktail (Cytoscint, ICN, Irvine, CA) was added to the solubilized tissue samples, and the samples were counted 48 h later (to allow time for any chemoluminescence to dissipate). Each sample was then counted in a Beckman LS 6000 liquid scintillation counter (Beckman, Fullerton, CA).

Distribution of ^{3}H-atipamezole in Spinal Cord and Locus Ceruleus after Intrathecal Injection

To determine whether the injection of atipamezole into the intrathecal space resulted in significant cancer concentrations of atipamezole in the LC, we performed a set of studies using ^{3}H-atipamezole injections through an intrathecal cannula, with subsequent measurement of its distribution in the LC and the spinal cord using liquid scintillation counting of these different tissue samples. Three microCuries ^{3}H-atipamezole in methanol was placed in a microcentrifuge tube and the methanol was vaporized in a Savant Automatic Environmental SpeedVac (AES 1000) at medium heat (43°C). Nonlabeled atipamezole (200 μl; 1.4 mg/ml) was added to the dried ^{3}H-atipamezole, which resulted in a specific activity of 3 mCi · mmol^{-1}. Rats had intrathecal catheters placed, and an injection of 10 μl of the ^{3}H-atipamezole (14 μg total atipamezole) was made into the intrathecal catheter using the technique of microinjection described earlier. Fifteen minutes after injection of drug, the animal was anesthetized in carbon dioxide and decapitated. Immediately after decapitation, the brain and spinal cord were rapidly removed. Harvesting of tissue samples and sample preparation for scintillation counting were identical to the procedures described earlier for dexmedetomidine.

Antinociceptive Testing

The antinociceptive response was measured by the TFL response. A high-intensity light was focused on the rat's tail and the time for the rat to move its tail out of the light beam was automatically recorded (Tail-flick apparatus, Columbus Instruments, Columbus, OH) and referred to as TFL. A different patch of the tail was exposed to the light beam on each trial to minimize the risk of tissue damage. The animals were placed on a heating blanket to maintain body and tail temperature during the experiment. A cutoff time of 10 s was predetermined, at which time the trial was terminated if no response occurred. Each TFL data point consisted of a mean of three trials on an individual animal. Data are expressed as maximum percent effect (MPE) according to the following formula:

$$\text{MPE (\%)} = \frac{\text{(postdrug latency)} - \text{(basal latency)}}{\text{(cut-off latency)} - \text{(basal latency)}} \times 100\%$$

Statistical Analysis. Results were analyzed using factorial analysis of variance, and expressed as a mean ± standard error of the mean.

Hypnotic Testing

This was performed as described previously.[8] Briefly, hypnotic response was defined by the loss of the rat's righting reflex, and its duration was measured in minutes and referred to as sleep time. The duration of the loss of righting reflex was assessed as the time from the rat's inability to right itself when placed on its back until the time that it spontaneously reverted, completely, to the prone position. This operational measurement of hypnotic response corresponds well to a more sophisticated monitoring system involving continuous electroencephalogram, electromyogram, and locomotor activity by telemetry.[12]

Results

Dexmedetomidine microinjected into the LC produced antinociception as represented by increased TFL in a dose-dependent manner. The maximum increase in TFL, expressed as %MPE, occurred within 5 min of administration of drug (fig. 1). At 5 min, the %MPE in animals treated with 7.0 μg dexmedetomidine (94 ± 5%, n = 5) was significantly greater than the %MPE in animals treated with 3.5 μg dexmedetomidine (63 ± 12%, n = 5), and both were significantly greater than in animals treated with saline (6 ± 2%, n = 5).

To test whether the antinociceptive effect of dexmedetomidine given into the LC is mediated by α_2 adrenoceptors, the specific α_2 antagonist atipamezole was administered by several routes. First, 14 μg/0.2 μl atipamezole was microinjected into the LC. By itself, atipamezole had no effect on the TFL (control: 4.0 ± 0.1 s; atipamezole: 4.0 ± 0.1 s, n = 4 each group). When 14 μg/0.2 μl atipamezole was injected into the LC 1 min before 3.5 μg/0.2 μl dexmedetomidine was injected into the LC, the antinociceptive effect of dexmedetomidine was abolished. The %MPE of the TFL in atipamezole-pretreated animals (1 ± 3% n = 4) was significantly less than the %MPE in dexmedetomidine animals pretreated with the dimethyl sulfoxide vehicle only (56 ± 8%, n = 4; table 1).

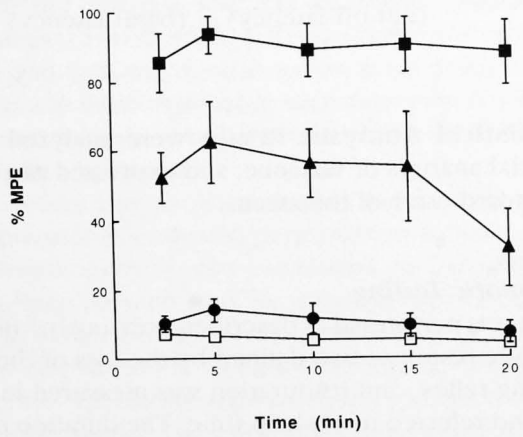

Fig. 1. Dexmedetomidine locus ceruleus injection in three doses: effect on percent of maximum possible effect and time course of effect. Dexmedetomidine was microinjected in a volume of 0.2 μl directly into the locus ceruleus at time 0. The percent of maximum possible effect defined as: (postdrug tail-flick latency − basal latency)/(cutoff latency − basal latency) × 100%) of the tail flick latency is reported as the mean ± SEM, n = 5 and 6 for each group. o = normal saline, 1 = dexmedetomidine 1.0 μg, Δ = dexmedetomidine 3.5 μg, and n = dexmedetomidine 7.0 μg.

Table 1. The Effect of Atipamezole, L659,066, or Pertussis Toxin on the Antinociceptive Action of Dexmedetomidine Administered Either into the Locus Ceruleus, Intraperitoneally or Intracerebroventricularly

Purpose of Experiment	Control	Treated	*P*
Effect of LC atipamezole on LC dexmedetomidine	56 ± 8	1 ± 3	0.0006
Effect of LC atipamezole on IP dexmedetomidine	72 ± 9	25 ± 2	0.0006
Effect of LC 1650,066 on LC dexmedetomidine	59 ± 7	15 ± 4	0.0001
Effect on LC 765.066 on IP dexmedetomidine	76 ± 11	25 ± 3	0.002
Effect of LC PTX on LC dexmedetomidine	60 ± 16	26 ± 23	0.002
Effect on IT PTX on LC dexmedetomidine	68 ± 12	10 ± 3	0.0001
Effect on ICV dexmedetomidine	3.7 ± 2	22 ± 6	0.02

LC = locus ceruleus; IP = intraperitoneally; PTX = pertussis toxin; IT = intrathecally; ICV = intracerebroventricularly.

Atipamezole injected into the LC also blocked the antinociceptive effect of dexmedetomidine given systematically (by intraperitoneal injection), although the effect was not as complete as the effect of atipamezole on the antinociceptive effect of dexmedetomidine injected into the LC. The intraperitoneal injection of 50/μg/kg dexmedetomidine, 40 min before tail-flick response testing, resulted in an %MPE of 72 ± 9% (n = 6). Injection of 14 μg/0.2 μl atipamezole into the LC 1 min before intraperitoneal dexmedetomidine injection resulted in a decrease in the %MPE to 25 ± 2%, (n = 6; table 1).

In a third set of experiments using atipamezole, the drug was injected through an intrathecal catheter. In these experiments, atipamezole was injected intrathecally 10 min before the injection of 3.5 μg/0.2 μl dexmedetomidine into the LC. Testing began 5 min after the injection of dexmedetomidine. Intrathecal administration of three doses of atipamezole in separate experiments demonstrated a dose-dependent antagonism of the antinociceptive effect of dexmedetomidine placed into the LC (fig. 2). Atipamezole (3.5 μg/10 μl) did not significantly change the %MPE in response to dexmedetomidine (atipamezole + dexmedetomidine, 51 ± 18% MPE; dexmedetomidine only, 46 ± 17%, MPE n = 4 each group). At a dose of 7.0 μg, atipamezole given intrathecally resulted in a %MPE (8 ± 3%MPE, n = 4) that was significantly less than that produced by dexmedetomidine in the LC only (52 ±

14 %MPE, n = 4). Intrathecal administration of 14 μg atipamezole also blocked the antinociceptive effect of dexmedetomidine injection into the LC (3.5 μl/0.2 μl dexmedetomidine only, 75 ± 13 %MPE, n = 6; dexmedetomidine and atipamezole 14 μg intrathecal, 4 ± 2 %MPE, n = 7). At this same dose (14 μg), atipamezole by itself or its vehicle (5% dimethyl sulfoxide) given intrathecally produced no change in the MPE compared to the same animals before injection (control TFL, 3.6 ± 0.1 s; atipamezole, 3.6 ± 0.1 s, n = 5 each group).

The %MPE after injection of L659,066 (350 μg/1.0 μl) into the LC and dexmedetomidine 50 μg/kg intraperitoneally (25 ± 3%, n = 5; L659,066 was injected 1 min before dexmedetomidine injection, and testing began 40 min after dexmedetomidine injection) was significantly less than the %MPE resulting from the injection of dexmedetomidine and vehicle (5% dimethyl sulfoxide) only (76 ± 11%, n = 5; table 1. Likewise, the %MPE after injection of L659,066 (350 μg/1.0 μl) followed 5 min later by injection of dexmedetomidine (3.5 μg/0.2 μl) into the LC (15 ± 4%, n = 8) was significantly less than the %MPE after dexmedetomidine and vehicle (5% dimethyl sulfoxide) injection into the LC (59 ± 7%, n = 8). Testing began 5 min after the injection of dexmedetomidine. In addition, the hypnotic response to dexmedetomidine (7.0 μg/0.2 μl) injection into the LC was completely blocked by the injection of L659,066 (200 μg/1.0 μl) into the LC 5 min before the injection of dexmedetomidine. That is, dexmedetomidine alone resulted in a mean sleep time of 62 ± 13 min. (n = 5), and pretreatment with L659,066 resulted in *no* loss of righting reflex in that group (table 1).

To confirm that the antinociceptive effect of dexmedetomidine administered into the LC was mediated by α_2 adrenoceptors coupled to G-protein mediators, PTX was administered in another set of experiments. In the first set of studies, PTX (0.5 μg/1.0 μl) was microinjected through a cannula into the LC 7 days before injection of dexmedetomidine (3.5 μl/0.2 μl) into the LC with testing of antinociceptive response 5 min later. In PTX-pretreated animals, the %MPE (26 ± 22, n = 6) was significantly less than in non-pretreated animals (%MPE 60 ± 16, n = 5; table 1).

In a second set of studies, PTX (0.5 μg/10 μl) was microinjected through an intrathecal cannula 7 days before testing. In animals treated with PTX, the %MPE after injection of dexmedetomidine (3.5 μg/0.2 μl) into the LC (10 ± 3%, n = 8) was significantly less than the %MPE in animals injected with vehicle (normal saline) into the intrathecal catheter and dexmedetomidine into the LC (68 ± 12% n = 8; table 1). Intrathecal administration of PTX (0.5 μg) did not affect the hypnotic response of 7.0 μg dexmedetomidine given into the LC (sleep time, defined as the duration of loss of righting reflex, for dexmedetomidine only, 79 ± 10 min, n = 7; sleep time for PTX-pretreated animals, 62.0 ± 9.9 min, n = 7).

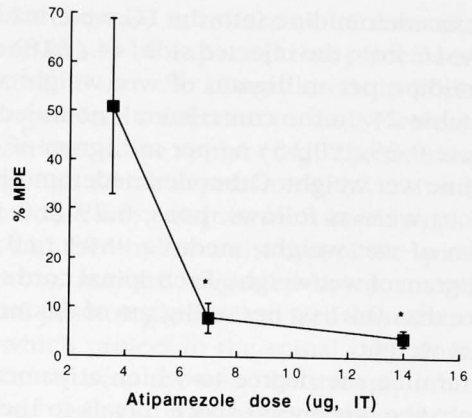

Fig. 2. Analgesic effect of three doses of atipamezole (3.5 μg/10 μl, 7.0 μg/10 μl, and 14.0 μg/10 μl) injected intrathecally on tail-flick latency resulting from dexmedetomidine 3.5 μg · 0.2 μl injection into the locus ceruleus. Data expressed as mean percent of maximum possible effect ± SEM.

Several investigators have described widespread distribution of drug after administration into discrete brain regions.[13–15] This raises the question as to whether the volume of dexmedetomidine injected into the LC may travel in the cerebrospinal fluid or systematically to reach the spinal cord and exert its effect directly on the spinal cord; therefore, two separate sets of experiments were performed. Firstly dexmedetomidine was injected directly into the cerebrospinal fluid through an intracerebroventricular cannula. In these experiments, 3.5 μl dexmedetomidine in a volume of 0.2 μl (the same dose used for experiments in which the drug was injected into the LC) was microinjected through the intracerebroventricular cannula. In animals treated with dexmedetomidine in this manner, the %MPE (22 ± 6%, n = 8) was significantly greater than the TFL produced in control animals (4 ± 1.8%, n = 8), but the difference was not as great as seen in animals where the drug was injected directly into the LC. Therefore, dexmedetomidine injected into the ventricle has a weak antinociceptive effect compared to its injection discretely into the LC.

To determine the degree to which dexmedetomidine injected into the LC travels to the spinal cord, injections

of ^{3}H-dexmedetomidine into the LC were made (n = 4). In the LC from the injected side, 44 (±18) ng dexmedetomidine per milligram of wet weight was detected (table 2). In the contralateral, noninjected LC, there were 0.25 (±0.15) ng per milligram of dexmedetomidine wet weight. Other dexmedetomidine concentrations were as follows: pons, 0.29 (±0.15) per milligram of wet weight; medulla, 0.03 (±0.01) ng per milligram of wet weight. Each spinal cord segment had more than 0.01 ng per milligram of dexmedetomidine wet weight.

To determine the degree to which atipamezole injected into the intrathecal space travels to the LC, injections of ^{3}H-atipamezole into the intrathecal space were made (n = 5). In the LC from both sides, there were 0.06 (±0.03) ng dexmedetomidine per milligram of wet weight (Table 3). Other amounts were as follows: pons, 0.08 (±0.02) ng per milligram of wet weight; medulla, 0.09 (±0.03) ng milligram of wet weight; cervical spinal cord, 0.19 (±0.11) ng/mg wet weight; thoracic spinal cord, 0.69 (0.15) ng per milligram of wet weight; lumbar spinal cord, 2.04 (0.42) ng per milligram of wet weight. Therefore, there is a greater than 30-fold decrease in the atipamezole concentration between the site of administration in the spinal cord and the LC.

Table 3. Concentration of Atipamezole after Intrathecal Injection at the Level of Lumbar Spine

Tissue Site	Mean (ng/mg)	SE
Locus ceruleus (bilateral)	0.06	0.03
Pons	0.08	0.02
Medulla	0.09	0.03
Spinal cord segment		
1	0.12	0.04
2	0.19	0.11
3	0.37	0.14
4	0.69	0.15
5	1.40	0.37
6	2.04	0.42
7	1.81	0.42
8	1.03	0.37

Discussion

The results of this study suggest that α_2-adrenergic agonists acting within the LC produce antinociception in the rat. We have shown that dexmedetomidine placed directly into the LC produces an antinociceptive response, as measured by TFL, in a dose-related manner. The α_2 agonist dexmedetomidine injected directly into the LC must alter LC activity through the α_2 receptor, because the antinociceptive effect is blocked by the specific α_2 antagonist atipamezole and the impermeant antagonist L659,066 and by PTX placed directly into the LC. Previous studies have characterized the hypnotic effect of dexmedetomidine applied to the LC.[8] We have shown that the hypnotic effect of dexmedetomidine injected into the LC is blocked by atipamezole (an imidazoline α_2-adrenergic antagonist) given both locally and systemically,[8] and by systemically administered yohimbine (a non-imidazoline α_2 antagonist).[16] The current study demonstrates that a second effect is antinociception.

We also have provided data indicating that the antinociception that results from placement of dexmedetomidine into the LC is consistent with a mediating role for α_2-adrenergic receptors in the spinal cord. Spinal application of the α_2 antagonists atipamezole and PTX can block the antinociception produced by dexmedetomidine placed in the LC. The hypnotic effect, which is mediated supraspinally, is not blocked by the intrathecal administration of PTX.

The noradrenergic innervation of the spinal cord, which forms the anatomic framework through which α_2 agonists produce antinociception, arises from the noradrenergic nuclei in the brain stem, including the LC (also characterized as the A6 group) and the A5 and A7 noradrenergic nuclei.[3,4] Neuroanatomic techniques demonstrate that each noradrenergic nucleus innervates the spinal cord in a specific pattern. The LC axons course through the medial ventral funiculus to termi-

Table 2. Concentration of Dexmedetomidine after Injection into the Locus Ceruleus

Tissue Site	Mean (ng/mg)	SE
Ipsilateral locus ceruleus	47.14	18.21
Contralateral locus ceruleus	0.25	0.15
Pons	0.29	0.15
Medulla	0.03	0.01
Spinal cord segment		
1	<0.01	<0.01
2	<0.01	<0.01
3	<0.01	<0.01
4	<0.01	<0.01
5	<0.01	<0.01
6	<0.01	<0.01
7	<0.01	<0.01
8	<0.01	<0.01

nate in the ventral horn laminae VII and VIII, the motoneurons of lamina XI, and lamina X, with sparse innervation of the dorsal horn.[3] The neurons of the A5 group innervate the deep dorsal horn lamina IV–VI, the intermediate zone (lamina VII), lamina X, and the intermediolateral cell column.[17] The A7 group innervates the superficial dorsal horn, laminae I–IV.[18] Although the majority of studies have supported the conclusions described earlier, it has recently been found that the LC predominantly innervates the superficial dorsal horn, and does not significantly innervate the ventral horn, in one substrain of rat (Harlan Sprague-Dawley).[3,19] Neuroanatomic studies have determined that in the Bantin and Kingman Sprague-Dawley rats, which were used in the current studies, noradrenergic innervation of the spinal cord dorsal horn arises from both the LC and the A5 and A7 noradrenergic nuclei.[4]

Neurophysiologic studies provide evidence that the spinal cord projections of these noradrenergic nuclei modulate nociception in the spinal cord (reviewed by Jones).[20] Electrical stimulation of the LC inhibits the spinal nociceptive tail-flick reflex in the rat.[21] However, intrathecal administration of yohimbine (a specific α_2 antagonist) reverses the effect in rats of a substrain (Harlan Sprague-Dawley) in which the LC innervates the dorsal horn, and not in other strains in which the LC innervates the ventral horn.[19] Similarly, electrical stimulation of the LC inhibits responses of dorsal horn neurons to noxious peripheral stimuli in rat,[22] cat, and primate.[3] Activation of the A7 group also produces antinociception in the rat, and this effect is reversed with intrathecal yohimbine.[23]

The possibility that modulation of nociception by these descending noradrenergic projections is mediated by α_2-adrenergic receptors in the spinal cord is demonstrated by numerous studies.[20] Adrenergic agonists administered intrathecally produce strong antinociceptive effects.[24,25] Investigation of the potency of α agonists with differing selectivity for the α_1 *versus* α_2 adrenoceptors revealed that the α_2 adrenoceptor mediates the antinociceptive effect.[2] At the cellular level, specific α_2 agonists inhibit excitation of dorsal horn neurons in response to noxious stimuli[26] and inhibit activity of the ascending spinal wide dynamic range neurons in the cat.[27] Subsequent clinical studies corroborated these animal studies and demonstrated that intrathecal or epidural administration of clonidine results in analgesia.[28]

Previous studies demonstrate that α_2 agonists, including dexmedetomidine, cause inhibition of LC neurons and a decrease in release of NE from the LC.[6,29] Therefore, it may be expected that placement of dexmedetomidine into the LC may result in inhibition of LC, and therefore a decrease in the NE released from LC projections in the spinal cord. The neuroanatomic route through which depression of LC activity by dexmedetomidine may result in *increased* spinal cord NE release, and thereby the activation of the spinal α_2 adrenoceptors, may be suggested by recent neuroanatomic and physiologic studies. Neurons of the A5 cell group, which project to the spinal cord dorsal horn, have recently been shown to be strongly inhibited by clonidine.[5] Other studies have demonstrated that the A5 group receives projections from the LC.[17,30] From these results, it may be suggested that in the normal rat, LC activity could tonically inhibit the activity of A5 neurons. Theoretically, when dexmedetomidine is applied to the LC, the neurons of the LC would be inhibited and A5 neurons would be released from inhibition. Therefore, if A5 neurons are disinhibited, this would result in spinal cord NE release and antinociception. Because most noradrenergic neurons of the brain are inhibited by α_2 agonists, it is probable that the neurons of the A7 group are also tonically inhibited by the LC, and when the LC is inhibited by dexmedetomidine, the A7 neurons would also be disinhibited and release NE into the dorsal horn superficial lamina to produce antinociception. We speculate that application of dexmedetomidine into the LC may decrease LC activity and may cause increased A5 and A7 activity and increased release of NE into the spinal cord. In this way, NE released in the spinal cord in turn would act at α_2-adrenergic receptors to produce antinociception.

Electrical stimulation of the LC produces antinociception in several species.[19,26] This result may seem to be in opposition to the results seen here, where we show that the inhibition of the LC with application of an α_2 agonist results in antinociception. However, the effect of electrical stimulation of LC is not blocked by intrathecal α_2 antagonists, except in the substrain of rats in which the LC projects to the dorsal horn of the spinal cord.[19] In other rats, in which LC projects to the ventral horn and intermediate lamina, LC stimulation may activate other antinociceptive pathways that project to the spinal cord and use different neurotransmitters.[19] Therefore, the results of the current study may be considered as consistent with those of these other studies.

The question arises as to whether the drug applied to the LC may travel systemically or through the ce-

rebrospinal fluid to exert its effect directly on adjacent nuclei such as the dorsal raphe, parabrachial nuclei,[31] or adjacent cholinergic laterodorsal tegmental nucleus,[32] or in the spinal cord rather than in the LC itself. The deposition of dexmedetomidine into the intracerebral ventricles results in a very weak antinociceptive effect in this study. This antinociception may result from activation of α_2-adrenergic receptors in the brain stem adrenergic nuclei, including LC, A5, and A7. If the microinjection of dexmedetomidine directly into the LC resulted in dispersion of the drug through the cerebrospinal fluid to the spinal cord, it would be expected that intracerebroventricular injection of the same dose of dexmedetomidine should result in an increased level of antinociception, not a very weak antinociceptive effect, as we have shown here. Also, radiolabeled dexmedetomidine was barely detectable in the spinal cord segments after injection into the LC (table 2). In addition, PTX is a large protein, and local injection of PTX into the LC will result in very minimal spread of this substance to other areas.[33] Therefore, since α_2 antagonists injected into the LC successfully antagonize the antinociception produced by dexmedetomidine injected into the LC, the α_2-adrenergic receptor in the LC is involved in the antinociceptive effect. Also, because PTX blocks this effect, the receptor probably couples to a G protein. Thus, our results suggest that only a very small proportion of the dexmedetomidine injected into the LC may travel to the A5 and A7 nuclei or to the spinal cord to produce its effect directly on spinal cord, the major effect of dexmedetomidine placed into LC occurs through action on LC itself. Similarly, intrathecally administered atipamezole does not achieve pharmacologic concentrations in the LC. Therefore, dexmedetomidine and atipamezole interact with adrenoceptors at the site of injection and not at a remote location.

A previous study using injection of medetomidine into the LC bilaterally suggested that the antinociceptive effect resulted from spread of the drug into the spinal cord, because the effect persisted in rats in which the spinal cord had been severed.[34] However, in that study, the volume of drug injected into the LC was 1.0 μl, injected bilaterally for a total volume of 2.0 μl. In our study, a volume of 0.2 μl was injected unilaterally, decreasing the probability of significant systemic or intrathecal spread of the drug. Also, it must be considered that severing the spinal cord may have an effect on the binding characteristics and density of the α_2-adrenergic receptors in the spinal cord below the lesion. In rats in which noradrenergic innervation of the spinal cord was depleted with 6-hydroxydopamine, there was upregulation of the α_2 adrenoreceptor.[35] A similar upregulation may occur in spinally transected rats, so that even a small amount of adrenergic agonist reaching the spinal cord may have an exaggerated effect.

In conclusion, the current studies demonstrate that the α_2 adrenergic agonist dexmedetomidine produces an antinociceptive effect in the rat when this drug is injected directly into the LC. This antinociceptive effect is mediated by α_2 adrenoceptors in the LC, as shown by the antagonism of the antinociceptive effect when the α_2-adrenergic antagonists atipamezole and L659,066 are injected into the LC before the injection of dexmedetomidine. The effect is mediated by α_2 receptors coupled to a PTX-sensitive G-protein, as demonstrated by the studies in which PTX injected into the LC antagonized the antinociceptive effect. Furthermore, the antinociceptive effect of dexmedetomidine placed into the LC is also mediated by the activation of α_2 adrenoceptors coupled to PTX-sensitive G-proteins in the spinal cord, as shown by the antagonism of the antinociceptive effect by the intrathecal injection of atipamezole and PTX prior to the injection of dexmedetomidine into the LC. The hypnotic effect of LC injection of dexmedetomidine is not antagonized by the intrathecal injection of atipamezole or PTX, however. Therefore, the antinociceptive effect produced by activation of α_2 adrenoceptors in the LC, by application of dexmedetomidine, is produced by a direct effect on the neurons of the LC. We postulate that the direct effect of α_2 adrenoceptor activation on LC activity in turn results in activation of α_2 adrenoceptors in the spinal cord, through which the antinociceptive effect is mediated.

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