Dexmedetomidine-Induced Decreases in Accumbal Dopamine in the Rat Are Partly Mediated via the Locus Coeruleus

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We have demonstrated previously that the systemic administration of the selective α2-adrenoceptor agonist dexmedetomidine (Dex) decreases extracellular dopamine (DA) levels in the rat nucleus accumbens (NAcc). Because the locus ceruleus (LC) is a noradrenergic center linked to several of the pharmacological effects of Dex, we investigated the role of the LC in Dex-induced modulation of accumbal DA. Microdialysis probes were implanted in the NAcc and LC of Sprague-Dawley rats, and Dex 5 mM (Dex-High, n = 6), Dex 0.5 mM (Dex-Mid, n = 5), Dex 5 μM (Dex-Low, n = 6), or artificial cerebrospinal fluid (control, n = 5) was administered in the LC via retrograde microdialysis for 45 min. Extracellular DA levels were continuously measured in the NAcc dialysates using high-performance liquid chromatography coupled to electrochemical detection. Dex produced significant decreases in extracellular DA in the NAcc. Accumbal DA decreased maximally to 68.9% ± 8.8%, 75.1% ± 6.5%, and 77.04% ± 12.8% of baseline in the Dex-High, Dex-Mid, and Dex-Low groups, respectively. No significant decrease in extracellular DA was observed in the control group. The coadministration of the highly selective α2-adrenoceptor antagonist (n = 6) RS 79948 20 mM prevented the Dex-induced decrease in accumbal DA. These data suggest that the LC plays a role in Dex-induced modulation of mesolimbic DA and support the hypothesis that noradrenergic systems can regulate remote dopaminergic sites in the central nervous system.

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We have demonstrated previously that systemic administration of Dex decreased extracellular DA concentrations in the rat NAcc in a dose-dependent manner, supporting the concept that $\alpha_2$-adrenoceptor agonists can significantly modify mesolimbic DA (17). Moreover, these Dex-induced decreases in accumbal DA were observed to be receptor-specific, in that the decreases were prevented by pretreatment with the highly selective $\alpha_2$-adrenoceptor antagonist atipamazone. Given that the LC appears to play a pivotal role in mediating several of the pharmacological effects of Dex and that the LC has been shown to modulate mesolimbic DA, the purpose of this study was to determine the role of the LC in mediating decreases in accumbal DA after the administration of a sedative dose of Dex.

**Methods**

The experimental protocol was approved by the Columbia University Animal Care and Use Committee and, in accordance with National Institutes of Health guidelines, adequate measures were taken to minimize pain and discomfort. Adult, male Sprague-Dawley rats, initially weighing 275–300 g, were purchased from a commercial breeder (Charles River Laboratories, Wilmington, MA) and used in these studies. The rats were housed individually in a temperature-controlled room at 22°C and were kept on a 12-h light/dark cycle. All animals had access to food and water *ad libitum* and underwent an acclimatization period for a minimum of 24 hours before being used in the study protocol.

Dex HCl was a gift from Orion Pharmaceuticals Farmos (Turku, Finland). RS 79948 HCl was purchased from Tocris Cookson Incorporated (Ellisville, MO). For the Dex studies, a 5 mM stock solution of Dex hydrochloride was prepared by dissolving the drug in artificial cerebrospinal fluid (CSF), purchased from Harvard Apparatus (Holliston, MA), containing sterile water and electrolytes in the following concentrations: $Na^+ 150$ mM, $K^+ 3.0$ mM, $Ca^{2+} 1.4$ mM, $Mg^{2+} 0.8$ mM, $PO_4^{3-} 1.0$ mM, $Cl^- 155$ mM. For the antagonist studies, a solution containing 5 mM Dex HCl and 20 mM RS 79948 HCl was prepared by dissolving both drugs in artificial CSF. Perchloric acid, ortho-phosphoric acid, and 1-octanesulfonic acid were purchased from Fluka Chemie AG (Milwaukee, WI), and EDTA was purchased from Sigma (St. Louis, MO). Citric acid monohydrate, NaOH, and methanol were purchased from Fisher Scientific (Fair Lawn, NJ).

Four days before the study, the rats were anesthetized with ketamine (35 mg/kg intraperitoneal [i.p.]) and pentobarbital (35 mg/kg i.p.), a microdialysis guide cannula (CMA/12, CMA, North Chelmsford, MA) was placed in the NAcc, and a second CMA/12 guide cannula was positioned in the area adjacent to the ipsilateral LC. The cannulae were stereotaxically implanted using the following coordinates, according to the atlas of Paxinos and Watson (18): for the NAcc, anterior-posterior (AP) +1.7 mm from the bregma, lateral (L) −1.4 mm from the midline, and dorsal-ventral (DV) −6.0 mm from the top of the skull; for the LC, AP −9.9 mm from the bregma, L −1.6 mm from the midline, and DV −6.8 mm from the top of the skull (Fig. 1).

In addition, we performed another group of microdialysis studies using coordinates for the LC previously used and validated by Kawahara et al. (KW) (19). The coordinates for these cannulae were: AP −12 mm from the bregma, L −1.3 mm from the midline,
and DV –7.3 mm from the top of the skull with the cannula at a 15-degree angle with respect to the vertical axis in the sagittal plane. These coordinates for the LC were also used because the direct administration of α₂-agonists in this location has been demonstrated to functionally produce LC activation, resulting in pharmacologically induced changes in extracellular neurotransmitter levels at remote sites from drug administration (19). In addition, this location is further removed from the parabrachial nucleus (PBN), a structure located in the vicinity of the LC that also contains α₂-adrenoceptors (20) (Fig. 1).

On the day of the study, a CMA/12 cerebral microdialysis probe (1-mm membrane length, 0.5-mm outer diameter) was placed near the LC of the awake rat, followed by the insertion of another CMA/12 probe (2-mm membrane, 0.5-mm outer diameter) in the NAcc. The LC probe was used for drug administration by retrograde microdialysis, whereas the NAcc probe was used to collect dialysates for extracellular DA level determinations. The probes were perfused with artificial CSF at a rate of 2 μL/min using a gas-tight syringe pump (Beestinger®; BAS Inc., West Lafayette, IN). The awake rat was placed in a transparent study cage (BeeKeeper; BAS Inc.) equipped with a rotating swivel, and the cage was mounted on a rotating platform (Ratum; BAS Inc.), which allowed the rat to move about freely throughout the study.

Two hours after probe insertion, NAcc dialysate samples were collected at 15-min intervals, throughout the study, in vials pretreated with 5 μL of 0.1 M HClO₄ to minimize DA degradation. The dialysates were immediately analyzed using high-performance liquid chromatography with electrochemical detection. Once a stable baseline was obtained (three samples with <10% variation from their respective average), the study drugs were administered.

To evaluate the effects of local Dex administration in the LC on accumbal DA, initial studies were performed in which the rats received one of the following treatments: artificial CSF (control, n = 6), Dex 5 μM (Dex-Low, n = 6), Dex 0.5 mM (Dex-Mid, n = 6), or Dex 5 mM (Dex-High, n = 6). Two additional groups of rats received artificial CSF (Control-KW, n = 5) or Dex 5 mM (Dex-High-KW, n = 4) through the LC probe placed according to the coordinates previously used by Kawahara et al. (19). The LC probe was perfused with the CSF or Dex solution at a rate of 2 μL/min for 45 min. After the 45-min perfusion period with the CSF or Dex solution, the perfusion of the LC probe with artificial CSF was resumed and continued for another 90 min. These dosing regimens were based on previous studies (21) and were selected such that changes in accumbal DA could be evaluated after LC administration of sedative doses of Dex. The dosing regimens were also based on our pilot studies, which demonstrated that these doses produced sedation without significant respiratory depression, and on previous studies demonstrating that millimolar concentrations of compounds are often necessary to achieve clinically relevant brain concentrations during drug administration by retrograde microdialysis (19,22).

To determine whether the effects of Dex on extracellular DA in the NAcc were specifically mediated by α₂-adrenoceptors in the LC, RS 79948 HCl, a highly selective α₂-adrenergic antagonist, was coadministered with Dex 5 mM via the LC probe to a separate group of rats (Dex-RS, n = 6). After a stable baseline for DA was achieved, the LC probe was perfused for 45 min with a solution containing 20 mM RS 79948 HCl and Dex 5 mM, and then the perfusion with artificial CSF was resumed for an additional 90 min. Dialysate samples for NAcc DA were continuously collected at 15-min intervals.

The sedative effects of Dex were also monitored throughout the study. Because the microdialysis animal preparation precluded the use of righting reflex testing as a means of assessing the level of sedation, the onset and duration of sedation were determined by observing the response of the rats to manual tail and whisker stimulation. Specifically, the onset of sedation was defined as the moment at which the animal no longer attempted to move or blink its eyes in response to either gentle tail or whisker stimulation. The end of the period of sedation was defined as the moment at which the rat started to move or blink in response to either of these stimulations.

At the end of the study (90 min after the end of LC drug administration), the animals were killed with an overdose of pentobarbital administered via i.p. injection. The brain was immediately harvested and preserved in a 10% formalin and 15% sucrose solution. Coronal and sagittal sections were obtained (50 μm), and the probe placement was verified using the atlas of Paxinos and Watson (18). Only data obtained from animals with proper probe placement were analyzed.

The chromatographic analysis of DA was based on our highly sensitive DA assay, which achieves high sensitivity without the need for miniaturization (23). In brief, a mobile phase consisting of an ion-pairing phosphate-citrate buffer (50 mM H₃PO₄, 50 mM citric acid, 65 mg/L sodium 1-octanesulfonate, 40 mg/L EDTA, adjusted to pH 4.5 with NaOH), and 6% (v/v) methanol was delivered at a flow rate of 0.27 mL/min through a SPHER C18 column (100 × 2 mm, 5-μm particle size; Princeton Chromatography Inc., Cranbury, NJ) maintained at 30°C. A 10-μL injection was performed using a 9725i PEEK injector (Rheodyne, Cotati, CA) with a 20-μL loop. DA quantitation was achieved with an INTRO amperometric detector (Antec Leyden, Zoeterwoude, The Netherlands) fitted with a VT-03 flow cell with a glassy carbon working
Cerebrospinal fluid containing HClO4 in the same concentration as the dialysates (i.e., 5 μL of 0.1 M HClO4 per 30 μL of dialysate). The limit of detection for DA was 0.06 nM, whereas the within-day and between-day precision in the above calibration range were 0.5%–2.4% and 2.1%–4.3%, respectively.

Variations in basal neurotransmitter concentrations among animal groups are not unusual during in vivo sampling studies. Because there can normally be significant variability in basal DA concentrations among the animal groups, changes in extracellular DA over time were expressed as a percentage of each group’s respective average baseline concentration (baseline = 100%). The microdialysis data (DA% of baseline over time) were analyzed using two-way ANOVA (with the Dex dose and time as the two factors) followed, when appropriate, by Bonferroni’s multiple comparisons post hoc test. Statistical calculations were performed using InStat® statistical analysis software (GraphPad Software, Inc., San Diego, CA), and all data are reported as mean ± sd. A value of $P < 0.05$ was considered statistically significant.

**Results**

Proper probe location was observed in all animals and representative probe placements for the probe locations used in the experiments are depicted in Figure 1. All doses of Dex produced sedation; however, only 50% of the animals in the Dex-Low group and 66% of the animals in the Dex-Mid group became sedated compared with 100% of the animals in the Dex-High and Dex-High-KW groups. Of those animals that became sedated, the onset of sedation was significantly delayed in the Dex-Low group ($P < 0.001$) compared with all other groups that received Dex (Table 1). No significant difference in the duration of sedation was observed among the groups ($P = 0.35$).

Compared with the control group, local administration of Dex into the LC produced significant decreases in accumbal DA (Fig. 2). The onset of the decrease was dependent on the dose of Dex administered; consequently, a significant decrease in extracellular DA occurred sooner in the Dex-High group ($P < 0.05$), at 45–60 min compared with 90–105 min after Dex administration in the Dex-Mid and Dex-Low groups ($P < 0.05$). However, the magnitude of the decrease in extracellular DA at the end of the study was similar in all groups: 68.9% ± 8.8% ($P < 0.01$), 75.1% ± 6.5% ($P < 0.05$), and 77.0% ± 12.8% ($P < 0.05$) of baseline in Dex-High, Dex-Mid, and Dex-Low, respectively. In contrast, no significant decrease in extracellular DA was observed in the control (CSF) group during the study.

In the animals that received Dex in the LC using microdialysis probe placement coordinates previously used by Kawahara et al. (19), 5 mM Dex (Dex-High-KW) also produced a significant decrease in extracellular DA in the NAcc compared with its respective control group (control-KW) (Fig. 3). The maximum decrease in accumbal DA in Dex-High-KW was to 57.9% ± 8.7% of baseline ($P < 0.01$), and this occurred 90–105 min after the start of Dex administration. Furthermore, the onset of the significant decrease in accumbal DA occurred sooner in the Dex-High-KW group compared with Dex-High group: 15–30 min versus 45–60 min after Dex administration, respectively. However, once a significant decrease in NAcc DA in Dex-High and Dex-High-KW occurred, there was no significant difference between the groups in terms of the magnitude of the decrease.

To confirm that the observed decrease in NAcc DA was mediated by a $\alpha_2$-adrenoceptor specific response to Dex, the animals were treated with RS 79948 HCl 20 mM, a highly selective $\alpha_2$-adrenoceptor antagonist. The administration of Dex 5 mM in the presence of RS 79948 HCl 20 mM (Dex-RS) reversed the decrease in

<table>
<thead>
<tr>
<th>Group</th>
<th>Animals sedated (%)</th>
<th>Onset (min)</th>
<th>Duration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dex-Low (5 μM)</td>
<td>50</td>
<td>90 ± 12*</td>
<td>45 ± 21</td>
</tr>
<tr>
<td>Dex-Mid (0.5 mM)</td>
<td>66</td>
<td>50 ± 14†</td>
<td>50 ± 26</td>
</tr>
<tr>
<td>Dex-High (5 mM)</td>
<td>100</td>
<td>22 ± 6</td>
<td>64 ± 17</td>
</tr>
<tr>
<td>Dex-High-KW (5 mM)</td>
<td>100</td>
<td>29 ± 11</td>
<td>44 ± 11</td>
</tr>
</tbody>
</table>

*After the administration of a 2 μL/min infusion of artificial cerebrospinal fluid (control, $n = 5$), dexmedetomidine 5 μM (Dex-Low, $n = 6$), dexmedetomidine 0.5 mM (Dex-Mid, $n = 5$) or dexametomidine 5 mM (Dex-High, $n = 6$) immediately lateral to the locus coeruleus (LC) for 45 min. Dexmedetomidine 5 mM (Dex-Low-KW, $n = 4$) was also administered at the inferior border of the LC for 45 min. Sedation was measured by observing the animal’s movement in response to either gentle tail or whisker stimulation. Values are expressed as mean ± sd and *$P < 0.001$ compared with all other groups. †$P < 0.01$ compared with Dex-High group.
extracellular DA in the NAcc, previously observed in the Dex-High group (Fig. 4). Indeed, the maximum decrease in accumbal DA observed in the Dex-RS group was to 87.0% ± 10.6% of baseline, which was not statistically different from the control (CSF) animals, 94.6% ± 8.7%; however, this decrease in the Dex-RS group was significantly different from the maximum decrease observed in the Dex-High group, 68.9% ± 8.8% of baseline (P < 0.05).

Discussion

The role of the LC in mediating Dex-induced decreases in accumbal DA was examined in conscious rats using dual-probe cerebral microdialysis. We have demonstrated previously that the systemic administration of Dex decreased extracellular DA in the rat NAcc (17); however, the role of the LC in mediating these changes was not evaluated. Because the LC appears to play an integral part in mediating several of the pharmacological effects of Dex (13,14), we hypothesized that the direct, coerulean administration of Dex would modulate extracellular DA in the NAcc. In the present study, the direct administration of Dex into the LC produced a significant decrease in extracellular DA, thus supporting the hypothesis that the LC can modulate the levels of mesolimbic DA.

Mounting evidence suggests that noradrenergic pathways have modulatory effects on dopaminergic systems. Previous dual-probe microdialysis studies in rats have demonstrated that norepinephrine (NE)-DA interactions do exist between the LC and the frontal cortex (19), which is a terminal projection site for extracellular DA in the NAcc, previously observed in the Dex-High group (Fig. 4). Indeed, the maximum decrease in accumbal DA observed in the Dex-RS group was to 87.0% ± 10.6% of baseline, which was
dopaminergic and noradrenergic neurons. There is also evidence that these NE-DA interactions may not be limited to cortical brain regions but may also involve subcortical regions such as the NAcc. For example, Lategan et al. (6) demonstrated in rats that chemical-induced lesions of LC projections, using the noradrenergic neurotoxin DSP-4 [N(2-chloroethyl)-N-ethyl-2-bromobenzylamine], decreased basal mesolimbic DA overflow by 28%. Furthermore, Häidkind et al. (5) demonstrated in conscious rats that denervation of the LC via pretreatment with DSP-4 significantly reduced DA release in the NAcc in response to KCl-induced depolarization. The Dex-induced 23%–31% reduction in accumbal DA observed in our present study strongly supports the findings of these investigators by suggesting that mesencephalic areas receive a significant amount of modulatory input from the LC (5,7). Whereas these earlier studies used relatively nonselective noradrenergic lesioning of the LC, our study demonstrates that the changes in accumbal DA in conscious rats are related to a process that appears to partly involve $\alpha_2$-adrenoceptors.

Indeed, the observation that Dex-mediated decreases in accumbal DA were absent in the presence of the selective $\alpha_2$-adrenoceptor antagonist RS 79948 is highly supportive of the concept that LC-mediated regulation of mesolimbic DA is dependent, at least in part, on $\alpha_2$-adrenoceptor function. Nevertheless, the possibility that other receptor systems may be involved cannot be completely excluded, because $\alpha_2$-adrenoceptor agonists have functional effects on imidazoline receptors (24). It is noteworthy that imidazoline binding sites have been demonstrated to be present in the LC (25), and the stimulation of these imidazoline receptors with the relatively nonselective $\alpha_2$-adrenoceptor agonist clonidine has been associated with LC neuronal stimulation (24). We acknowledge that our current findings do not eliminate the potential contribution of imidazoline receptors in altering LC neuronal firing and the subsequent modulation of accumbal DA. However, our data suggest that, in the presence of functioning $\alpha_2$-adrenoceptors, coerulean administration of Dex leads predominantly to $\alpha_2$-adrenoceptor-mediated neuronal inhibition in the LC, resulting in a subsequent decrease in accumbal DA. Further studies are planned to examine the contribution of the imidazoline receptor system in mediating these changes in mesolimbic DA.

Another interesting finding in this study is the observation that a significant decrease in accumbal DA after Dex administration was not evident until 15–30 min and 45–60 min in the Dex-High-KW and Dex-High groups, respectively. Moreover, the faster onset in the DA response after Dex administration in the Dex-High-KW group seems to suggest that these coordinates are probably optimal for retrograde drug administration in the LC region. Although the mechanism underlying this delayed response is unclear, a possible reason for this may be that the drug was not delivered directly into the LC; rather, it was administered adjacent to this brain region to prevent probe-induced damage to the LC. During drug administration via retrograde microdialysis, variations in diffusion properties as well as tissue penetration have been described and may have played a significant role in delaying the effect of Dex on accumbal DA (22). Another possible explanation for the observed delay may be that the afferent input to the NAcc from the LC could be initially relayed via another major dopaminergic center, such as the ventral tegmental area (VTA).

Indeed, strong evidence suggests that the LC projects to the VTA (1,2), a major mesencephalic dopaminergic center that ultimately supplies efferent projections to the NAcc. Specifically, it has been also shown that the $\alpha_2$-adrenoceptor agonist clonidine can modulate dopaminergic cell firing in the VTA (1). Although we did not examine the VTA in this study, the observation that clonidine significantly modulates VTA neuronal firing makes it likely that Dex would have similar effects in this area. Moreover, if $\alpha_2$-adrenoceptor-mediated changes in VTA neuronal firing are required before any changes in extracellular accumbal DA are observed, then this could potentially lead to a delay in extracellular accumbal DA outflow. The importance of the VTA in mediating changes in accumbal DA has been further supported by a recent study in mice demonstrating that at the level of the NAcc, $\alpha_2$-adrenoceptors regulate the release of NE but not DA (26). Moreover, these investigators also concluded that release in the NAcc appears to be regulated by an $\alpha_2$-adrenoceptor-mediated effect on DA neurons in the VTA. It is noteworthy that this delay in extracellular neurotransmitter changes has been observed previously. Specifically, using a similar multi-probe microdialysis rat model to study interactions between the LC and NAcc, Kawahara et al. (19), observed that it took between 30 and 60 min for the coerulean administration of carbachol to produce a significant increase in extracellular NE in the NAcc, thus suggesting that LC-mediated changes in extracellular neurotransmitter may indeed be delayed.

We also acknowledge that other investigators have previously failed to demonstrate a noradrenaline-DA interaction between the LC and NAcc (19). Specifically, using multiprobe microdialysis in rats, these investigators, demonstrated an increase of approximately 200% in NAcc NE after LC neuronal stimulation using the muscarinic agonist carbachol; however, they did not observe an effect on NAcc DA during the same period of carbachol-induced LC stimulation (19). In contrast, all of the doses of Dex used in our study produced a significant decrease in extracellular DA in...
the NAcc, albeit at different points in time. Moreover, the fact that this decrease was prevented by the administration of the α2-adrenoceptor antagonist RS 79948 lends additional support to the notion that NE-DA interactions do exist at the level of the LC and NAcc. Although the exact mechanism underlying this difference in the observed LC-mediated changes in accumbal DA is unclear at present, a possible factor may be experimental differences in LC neuronal firing. That is, as opposed to conditions that produce cholinergic-mediated LC stimulation, the LC-NAcc interaction may be more clearly evident after conditions that produce α2-adrenoceptor-mediated decreases in LC neuronal firing, as is the case with Dex.

We also recognize that, given the dimensions of the microdialysis probes and the relatively confined anatomic location of the LC, we cannot completely exclude the possibility that some of the observed effects in this study may be secondary to α2-adrenoceptor-mediated effects of Dex at other pontomedullary structures near the LC. For example, the PBN is a pontine group of neurons rich in α2-adrenoceptors (20) that has also been demonstrated to project to the VTA (27). Although this is a possibility, evidence suggests that after the administration of drugs via retrograde microdialysis, many compounds have rather slow diffusion rates as well as variable tissue penetration (22). These same authors have also concluded that relatively high drug concentrations, in the 1–10 mM range, often are necessary to produce substantial brain concentrations at a distance 1 mm from the infusion probe. In addition, we conclude that our data indeed support the concept that the LC has a partial role in producing the Dex-mediated changes in accumbal DA observed in this study, for several reasons. First, we observed sedative effects of the drug; because the LC is the region of the brain linked to the sedative-hypnotic effects of Dex, this suggests that α2-adrenoceptors in the LC were indeed activated, (14). Furthermore, in this study, we also used coordinates previously demonstrated to produce LC α2-adrenoceptor activation and remote changes in extracellular monoamines as measured via dual-probe microdialysis (19). Moreover, these coordinates were further removed from the PBN, yet the onset of drug effect was the fastest of any of the groups studied. In summary, although we cannot eliminate the possibility that α2-adrenoceptors in the PBN were activated, given the close proximity of the LC to our probe location, the observed changes in sedation level, and the diffusion factors described by Westerink and DeVries (22), we can conclude that the drug effects observed in this study were at least partly mediated by the LC.

In conclusion, the direct coerulean administration of the highly selective α2-adrenoceptor agonist Dex, at doses that produce clinically relevant sedation, decreases extracellular DA in the NAcc via a mechanism that is dependent, at least in part, on α2-adrenoceptors in the LC. Furthermore, these interactions between adrenergic compounds and the mesolimbic dopaminergic system should continue to be elucidated, particularly because several clinical pathological states, such as depression (7), Parkinson’s disease (28), and cocaine-induced seizure activity (29), may involve interactions between these two distinct neurotransmitter systems.

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References


