

## Chronic Desipramine Treatment Desensitizes the Rat to Anesthetic and Antinociceptive Effects of the $\alpha_2$ -adrenergic Agonist Dexmedetomidine

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**Introduction:** The effects of long-term administration of the tricyclic antidepressant agent desipramine on the hypnotic, antinociceptive, anesthetic-sparing, and central norepinephrine turnover suppressant action of short-term dexmedetomidine, a highly selective  $\alpha_2$ -adrenergic agonist, were studied in rats.

**Methods:** Rats were given a 3- or 4-week course of twice daily administration of desipramine, 10 mg/kg, or saline. The effect of a hypnotic dose of dexmedetomidine, 250  $\mu$ g/kg given intraperitoneally, on the duration of loss of righting reflex was determined. The tail flick latency response was determined before and after 50  $\mu$ g/kg dexmedetomidine. The minimum anesthetic concentration of halothane and the central norepinephrine turnover rate were determined before and after administration of 30  $\mu$ g/kg dexmedetomidine. Changes in the affinity and density of the  $\alpha_2$ -adrenergic receptor in locus coeruleus and spinal cord also were determined.

**Results:** Treatment with desipramine decreased dexmedetomidine-induced loss of righting reflex duration by 67% and eliminated the antinociceptive effect of dexmedetomidine. Dexmedetomidine produced a 55% decrease in minimum anesthetic concentration in the control group but no reduction in desipramine-treated rats. Desipramine did not change the receptor density or binding affinity of  $\alpha_2$  receptors at the site for hypnotic (locus coeruleus) or antinociceptive (spinal cord) responses. No decrement in the central norepinephrine turnover rate was noted in the locus coeruleus of dexmedetomidine after 3 weeks of treatment with desipramine. The  $\alpha_1$ -adrenergic antagonist prazosin at 1 or 5 mg/kg completely (minimum anesthetic concentration reduction), almost com-

pletely (antinociceptive), or partially (hypnotic) restored responsiveness to normal.

**Conclusions:** These data indicate that treatment with desipramine induces hyporesponsiveness to the hypnotic, analgesic, and minimum anesthetic concentration-reducing, but not to the suppression of central norepinephrine turnover, properties of dexmedetomidine. The hyporesponsiveness appears to involve an  $\alpha_1$ -adrenergic mechanism. (Key words: Analgesia; antidepressant; hypnosis; tricyclic; volatile.)

$\alpha_2$ -ADRENERGIC agonists are increasingly being used in the perioperative period for their sedative, analgesic, anesthetic-sparing, and sympatholytic effects.<sup>1</sup>  $\alpha_2$ -Adrenoceptors at noradrenergic synapses in the locus coeruleus (LC) are the site for hypnotic<sup>2</sup> and some of the analgesic<sup>3</sup> actions. The tricyclic antidepressant agents (e.g., imipramine, amitriptyline, and their *N*-demethyl derivatives) are another class of drug, and they exert their action at noradrenergic neurons.<sup>4</sup> In addition, tricyclic antidepressant agents are widely prescribed in the management of chronic pain states,<sup>5</sup> and recently  $\alpha_2$ -adrenergic agonists also have been advocated for use in the same setting.<sup>6,7</sup> Therefore, any potential interaction of these two classes of compounds, acting as they do at the same noradrenergic synaptic connections, needs to be well understood.

Earlier, several investigators reported that long-term treatment with tricyclic compounds reduces sensitivity to  $\alpha_2$ -adrenergic agonists for several types of responses, including inhibition of norepinephrine release,<sup>8,9</sup> exploratory behavior,<sup>10</sup> hypoactivity,<sup>11</sup> hypothermia,<sup>12</sup> and analgesia.<sup>13</sup> In many of these studies, clonidine, a relatively nonspecific and weak  $\alpha_2$ -adrenergic agonist was used; further, several other anesthetic effects (including the hypnotic and anesthetic-sparing responses) were not studied. We have examined the effect of long-term treatment with desipramine on the anesthetic and analgesic properties of dexmedetomidine, a highly selective  $\alpha_2$ -adrenergic agonist<sup>14</sup> in rats. Desipramine was chosen for this study because it preferentially blocks norepi-

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nephine transport over serotonin transport.<sup>4</sup> We also have characterized binding to  $\alpha_2$ -adrenoceptors and addressed the role that the activation of  $\alpha_1$ -adrenoceptors may play in the putative subsensitivity to stimulation of  $\alpha_2$ -adrenergic agonists after long-term administration of desipramine.

## Materials and Methods

Two hundred eighty-four male Sprague-Dawley rats weighing 200–300 g were chosen as the experimental model, and approval of the experimental protocol was obtained from the Animal Care and Use Committee at the Palo Alto Veterans Administration Health Care System. The rats in the control and treatment groups originated from the same litter. Rats were stratified to match the distribution of the rats' weights as closely as possible in the control and treatment groups.

### Study Design

Rats were administered desipramine subcutaneously, 10 mg/kg dissolved in saline, twice daily for 3 or 4 weeks. These doses and durations were selected from previous studies.<sup>8,15</sup> The earliest behavioral and norepinephrine turnover testing occurred 16 h after the last dose of desipramine. In some experiments, testing occurred 4 days or 1 or 2 weeks after the last dose of desipramine.

Dexmedetomidine, dissolved in saline, was administered either intraperitoneally or directly into the LC. In a separate series of experiments, rats were pretreated with prazosin, 1 or 5 mg/kg dissolved in distilled water, 15 min before administration of dexmedetomidine. Previous studies indicated that this compound at this time effectively blocked the  $\alpha_1$ -adrenergic receptor.<sup>16</sup> Observers were not blinded to the drug treatment. For all behavioral studies, the body temperatures of the rats were maintained to within 1°C of normal temperature by placing them on a heating blanket.

### Behavioral Testing

Hypnotic response to intraperitoneally administered dexmedetomidine was defined by the loss of righting reflex (LORR) in the rat, and its duration, measured in minutes, is referred to as sleep-time. Duration of LORR was assessed from the time the rat could be placed easily on its back to the time it spontaneously and completely reverted to the prone position. All hypnotic testing was performed between 10:00 AM and 6:00 PM as

described previously.<sup>16</sup> The dose of dexmedetomidine, 250  $\mu$ g/kg, was selected because it is on the linear portion of the dose-response curve.<sup>17</sup> In some studies, 7  $\mu$ g dexmedetomidine, a dose that reliably caused LORR,<sup>18</sup> was injected directly into the LC in chronically cannulated rats as previously described.<sup>2</sup>

The antinociceptive response of dexmedetomidine was measured by the tail-flick latency response. The animal was habituated to its surroundings for 1 week before testing. The rat was restrained in an acrylic cone, and a beam of light (intensity no. 8) was focused on the middle third of the tail from a distance of 4.5 cm (Tail Flick Analgesia Tester; Columbus Instruments, Columbus, OH). The latency between the exposure to the radiant heat source and the movement of the tail away from the focused beam is referred to as tail-flick latency. A cutoff of 10 s was selected. Data are expressed as percent of maximal possible effect:

$$\frac{\text{TFL with drug} - \text{baseline latency}}{\text{cutoff time (i.e. 10 sec)} - \text{baseline latency}} \times 100$$

The dose of dexmedetomidine, 50  $\mu$ g/kg, was selected because it produces 50% of the maximal possible effect.<sup>19</sup>

Minimum anesthetic concentration (MAC) of halothane was determined to investigate whether long-term treatment with desipramine attenuated the anesthetic-sparing action of  $\alpha_2$ -adrenergic agonists. Minimum anesthetic concentration was determined as previously described.<sup>20</sup> To examine the MAC-reducing effects of an  $\alpha_2$ -adrenergic agonist, the dose of dexmedetomidine, 30  $\mu$ g/kg, was selected because it was previously shown to decrease the MAC for halothane by 50%.<sup>21</sup>

### Suppression of Central Norepinephrine Turnover

Norepinephrine turnover was assessed in the LC. The MHPG/norepinephrine ratio is a means of assessing noradrenergic neuronal activity because the concentration of norepinephrine should be inversely proportional to the rate of norepinephrine neuronal activity, whereas the concentration of MHPG should be directly proportional.<sup>22</sup> This assumes that the experiments are performed during steady-state conditions and that monoamine neuronal reaction kinetics are first order. During such conditions, it can be assumed that formation and elimination rates of the monoamines and metabolites are proportional to their concentrations, with the constants of proportionality varying with the different steady-state conditions that groups of experimental ani-

mals might be in. In the case of norepinephrine, the rate of change of MHPG concentration is:  $d(\text{MHPG})/dt = -k_1(\text{MHPG}) + k_2(\text{NE})$ , where  $k_1$  is the rate constant for the elimination of MHPG,  $k_2$  is the rate constant for the conversion of norepinephrine to MHPG, and (NE) and (MHPG) are the concentrations of the norepinephrine and its metabolite, respectively. In the steady state,  $(\text{MHPG})/(\text{NE}) = k_2/k_1$ , and the ratio  $(\text{MHPG})/(\text{NE})$  is proportional to the rate constant for the conversion of norepinephrine to MHPG.

Animals were injected with saline or dexmedetomidine before being exposed to 30 s of  $\text{CO}_2$  to induce narcosis, and decapitation followed. The brain was removed, and 1-mm coronal slices of the brain stem region containing the LC were made. Circular punches (1-mm diameter) of the LC region were obtained from these slices. Punches were sonicated in an ice-cold 5% perchloric acid solution and centrifuged to precipitate proteins and membranes. The supernatant was filtered to exclude molecules exceeding 5,000 Da. These samples were stable for up to 3 months when stored at  $-80^\circ\text{C}$ .

The biogenic amines were assayed with high-pressure liquid chromatography and reverse-phase chromatography on an HR-80 (ESA, Bedford, MA) column (70 mm) containing 3-mm spherical octadecylsilane beads. Compounds were quantified using an integrator (HP3396A, Hewlett Packard, Palo Alto, CA) from the peak areas generated by known standards. The limit of detection with this technique was 25 fmol. The electroactive biogenic amines and their metabolites were quantified by a high-pressure liquid chromatography system consisting of a Coulchem II detector (ESA, Bedford, MA) and a high-sensitivity cell detector maintained at 0.35 V. The mobile phase (Cat-A; ESA) was pumped (Beckman 110B, Fullerton, CA) at a flow rate of 1.4 ml/min.

#### *Radiolabeled Ligand Binding*

Animals were killed by decapitation during  $\text{CO}_2$  narcosis, and both LCs and the spinal cord were harvested. The tissue was homogenized in volumes (LC in 500  $\mu\text{l}$  and spinal cord in 10 ml) of ice-cold Tris (50 mM) and EDTA (0.8 mM) buffered to pH 7.5. After centrifugation at  $500 \times g$  (Sorvall RC-5B, rotor SS-34 rotor, Oklahoma City, OK) for 5 min, the supernatants were collected and centrifuged at  $44,000 \times g$  (20 min). The pellets were then washed once with the same buffer. The final pellets were stored at  $-80^\circ\text{C}$  in ice-cold potassium phosphate, 50 mM buffer, pH 7.65, for no longer than 3 weeks. On the day of the assay, aliquots of membranes

were thawed at room temperature and resuspended in Tris 50 mM buffer, pH 7.6, containing 10 mM  $\text{MgCl}_2$ . The membrane preparation was incubated for 15 min at  $37^\circ\text{C}$  and centrifuged at  $44,000 \times g$  for 20 min. This washing procedure was repeated, and the final membrane pellet was resuspended in 150  $\mu\text{l}$  (LC) or 10 ml (spinal cord) of 50 mM Tris-HCl, 10 mM  $\text{MgCl}_2$ , and 1 mM EGTA, pH 7.6. The binding procedures on the LC and spinal cord were adapted from Baron and Siegel<sup>23</sup> and Uhlen and Wikberg,<sup>24</sup> respectively. The radioligands used were [ $^{125}\text{I}$ ]iodoclonidine for the LC and [ $^3\text{H}$ ]clonidine for the spinal cord. Nonspecific binding was determined by the addition of 10  $\mu\text{M}$  rauwolscine.

#### *Data Analysis*

Data were analyzed by analysis of variance with *post hoc* Scheffé's test when appropriate. In some experiments, chi-square analysis was performed. A significant difference was considered to have occurred when the *P* value was  $<0.05$ . Data are expressed as mean  $\pm$  SD.

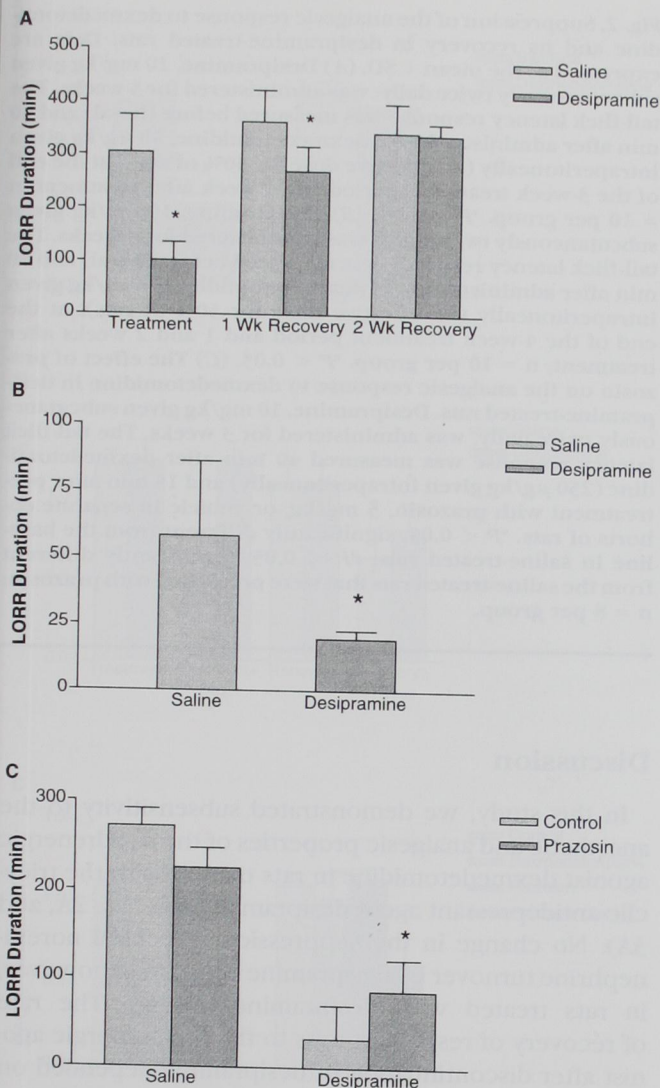
## Results

#### *Hypnotic Response*

The hypnotic response to a dose of dexmedetomidine that always caused LORR was significantly ( $P < 0.001$ ) reduced but never eliminated in the desipramine-treated rats. After a 1-week withdrawal period, the duration of LORR in the desipramine-treated rats was still significantly shorter ( $P < 0.002$ ) than in the saline-treated animals. The LORR duration did return to normal after a 2-week withdrawal period (fig. 1A). The desipramine-treated rats were significantly less responsive to the hypnotic effects of dexmedetomidine, even when the drug was injected directly into the LC at a dose (7  $\mu\text{g}$ ) that normally caused LORR in all rats, produced a shorter LORR in only 43% of the desipramine-treated rats (fig. 1B). Pretreatment with prazosin did not alter the hypnotic response to dexmedetomidine in the saline-treated animals. Although pretreatment with prazosin significantly increased the dexmedetomidine-induced sleep-time in desipramine-treated rats, it did not completely reverse the effects of treatment with desipramine (fig. 1C).

#### *Antinociceptive Response*

The baseline tail-flick latency for the 3-week desipramine-treated animals was similar to the latencies of

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**Fig. 1.** Hypnotic response to dexmedetomidine in desipramine-treated rats. Desipramine, 10 mg/kg was administered subcutaneously and twice daily for 3 weeks. Data are expressed as the mean  $\pm$  SD. (A) The duration of loss of righting reflex or sleep-time was measured after dexmedetomidine, 250  $\mu$ g/kg given intraperitoneally 16 h after the last injection of the 3-week treatment, 1 week after treatment, and 2 weeks after treatment in separate cohorts;  $n = 10$  per group. (B) Treatment with desipramine also reduced the sleep-time of the animals that lost their righting reflex;  $n = 3-6$  per group. (C) The effect of prazosin on the hypnotic response to dexmedetomidine in desipramine-treated rats. Desipramine, 10 mg/kg given subcutaneously twice daily, was administered for 3 weeks. The duration of loss of righting reflex (sleep-time), was measured after administration of dexmedetomidine (250  $\mu$ g/kg) 15 min after pretreatment with prazosin, 5 mg/kg given intraperitoneally, or saline in separate cohorts of rats. \* $P < 0.05$ ;  $n = 6$  per group.

saline-treated animals ( $4.4 \pm 0.8$  vs.  $4.0 \pm 0.6$  s, respectively). After administration of dexmedetomidine, 50  $\mu$ g/kg, the tail-flick latency of the saline-treated animals significantly increased to 54% of the maximal possible effect ( $P < 0.001$ ; fig. 2A). There was no change in the tail-flick latency after administration of dexmedetomidine in rats that had received desipramine for 3 weeks. When the rats were tested 1 week after discontinuing desipramine, dexmedetomidine induced similar changes in the tail-flick latency in the two groups (fig. 2A). When rats were treated with desipramine for 4 weeks, recovery of the analgesic response to dexmedetomidine only occurred at 2 weeks after discontinuation of desipramine (fig. 2B). Pretreatment with prazosin significantly enhanced the analgesic effect of dexmedetomidine in desipramine-treated rats although this response was still significantly less than that seen with saline-treated rats that were pretreated with prazosin (fig. 2C).

#### Minimum Anesthetic Concentration-reducing Response

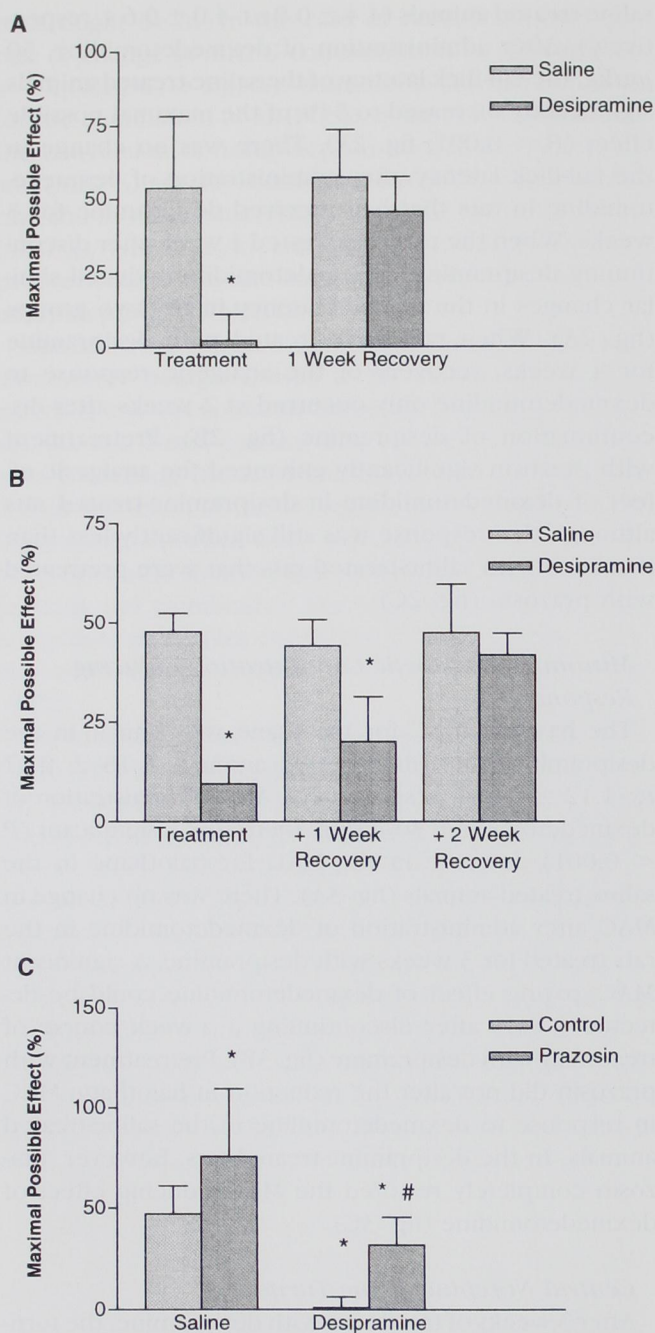
The baseline MAC for halothane was similar in the desipramine- and saline-treated animals ( $1.18 \pm 0.07$  vs.  $1.12 \pm 0.07\%$ , respectively). After administration of dexmedetomidine, 30  $\mu$ g/kg, there was a significant ( $P < 0.001$ ) decrease in the MAC for halothane in the saline-treated animals (fig. 3A). There was no change in MAC after administration of dexmedetomidine in the rats treated for 3 weeks with desipramine. A significant MAC-sparing effect of dexmedetomidine could be detected 4 days after discontinuing a 4-week course of treatment with desipramine (fig. 3B). Pretreatment with prazosin did not alter the reduction in halothane MAC in response to dexmedetomidine in the saline-treated animals. In the desipramine-treated rats, however, prazosin completely restored the MAC-reducing effect of dexmedetomidine (fig. 3C).

#### Central Norepinephrine Turnover

After 3 weeks of treatment with desipramine, the turnover of norepinephrine was unaffected in the LC (fig. 4). The ability of 30  $\mu$ g/kg dexmedetomidine to inhibit norepinephrine turnover was preserved in rats treated with desipramine.

#### Radiolabeled Ligand Binding Studies

$\alpha_2$ -Adrenoceptors in the spinal cord (site for antinociceptive response) and LC (site for hypnotic and antinociceptive responses) were not changed after 3 weeks

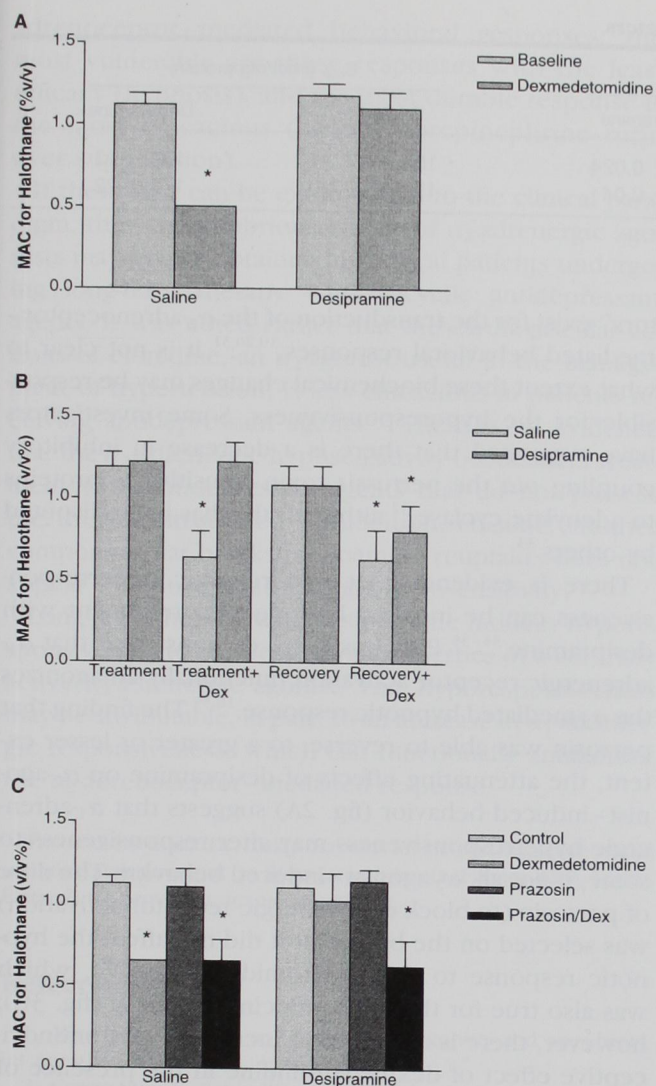


of treatment with desipramine as evidenced by similar  $B_{max}$  and  $K_d$  values between the desipramine-treated and naïve rats (table 1). The correlation coefficients of individual binding curves were: spinal cord control,  $0.994 \pm 0.006$ ; spinal cord desipramine treated,  $0.992 \pm 0.007$ ; LC control  $0.938 \pm 0.076$ ; and LC desipramine  $0.986 \pm 0.009$ .

**Fig. 2.** Suppression of the analgesic response to dexmedetomidine and its recovery in desipramine-treated rats. Data are expressed as the mean  $\pm$  SD. (A) Desipramine, 10 mg/kg given subcutaneously twice daily, was administered for 3 weeks. The tail-flick latency response was measured before (basal) and 40 min after administration of dexmedetomidine, 50  $\mu$ g/kg given intraperitoneally (an effective dose for 50% of rats), at the end of the 3-week treatment period and 1 week after treatment;  $n = 10$  per group.  $*P < 0.05$ . (B) Desipramine, 10 mg/kg given subcutaneously twice daily, was administered for 4 weeks. The tail-flick latency response was measured before (basal) and 40 min after administration of dexmedetomidine, 50  $\mu$ g/kg given intraperitoneally (an effective dose for 50% of rats), at the end of the 4-week treatment period and 1 and 2 weeks after treatment;  $n = 10$  per group.  $*P < 0.05$ . (C) The effect of prazosin on the analgesic response to dexmedetomidine in desipramine-treated rats. Desipramine, 10 mg/kg given subcutaneously twice daily, was administered for 3 weeks. The tail-flick latency response was measured 40 min after dexmedetomidine (250  $\mu$ g/kg given intraperitoneally) and 15 min after pretreatment with prazosin, 5 mg/kg, or vehicle in separate cohorts of rats.  $*P < 0.05$ , significantly different from the baseline in saline-treated rats;  $\#P < 0.05$ , significantly different from the saline-treated rats that were pretreated with prazosin;  $n = 8$  per group.

## Discussion

In this study, we demonstrated subsensitivity to the anesthetic and analgesic properties of the  $\alpha_2$ -adrenergic agonist dexmedetomidine in rats treated with the tricyclic antidepressant agent desipramine (figs. 1A, 2A, and 3A). No change in the suppression of central norepinephrine turnover by desipramine was noted, however, in rats treated with desipramine (fig. 4). The rate of recovery of responsiveness to the  $\alpha_2$ -adrenergic agonist after discontinuation of desipramine depended on the behavioral response that was being assessed. The MAC-sparing effect recovered after 4 days (fig. 3C), whereas during the same desipramine treatment conditions the antinociceptive response to dexmedetomidine was still abnormal 1 week after discontinuation (fig. 3B). During similar treatment conditions, the hypnotic response recovered even more slowly (fig. 1A) than did the antinociceptive response to dexmedetomidine (fig. 2A). The decrease in sensitivity to dexmedetomidine was not attributable to pharmacokinetic reasons because the hypnotic response was attenuated even when dexmedetomidine was administered directly into the LC (fig. 1B), the effect site for the hypnotic response.<sup>2</sup> Although no change was noted in  $\alpha_2$ -adrenoceptor binding function at the effect site for the hypnotic and antinociceptive responses (table 1), we were able to restore responsiveness partially (figs. 1C and 2C) or completely

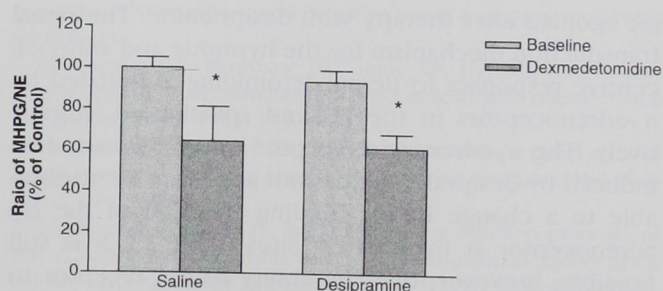
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**Fig. 3.** Anesthetic-sparing effect of dexmedetomidine in desipramine-treated rats. Data are expressed as the mean  $\pm$  SD. (A) Desipramine, 10 mg/kg given subcutaneously twice daily, was administered for 3 weeks. The minimum anesthetic concentration (MAC) of halothane was tested before (basal) and after administration of dexmedetomidine, 30  $\mu$ g/kg given by intraperitoneal injection (an effective dose for 50% of rats), at the end of the treatment period;  $n = 10$  per group;  $^*P < 0.05$ . (B) Recovery of the anesthetic-sparing effect of dexmedetomidine in desipramine-treated rats. Desipramine, 10 mg/kg given subcutaneously twice daily, was administered for 4 weeks. The MAC of halothane was tested before (basal) and after injection of the effective dose for 50% of rats for MAC-sparing effect for dexmedetomidine, 30  $\mu$ g/kg given intraperitoneally, at the end of the treatment period and 4 days after discontinuing treatment with desipramine;  $n = 10$  per group;  $^*P < 0.05$ , significantly different from baseline. (C) The effect of prazosin on the anesthetic-sparing action of dexmedetomidine in desipramine-treated rats. Desipramine, 10 mg/kg, given subcutaneously twice daily, was administered for 4 weeks. The MAC of halothane was tested before (basal) and after administration of dexmedetomidine, 30  $\mu$ g/kg given by intraperitoneal injection, at the end of the treatment period in the presence and absence of prazosin, 5 mg/kg, administered intraperitoneally 15 min before dexmedetomidine;  $n = 8$  per group;  $^*P < 0.05$ .

are required to transduce the hypnotic effect of dexmedetomidine.<sup>19</sup> It is notable that in this current study of  $\alpha_2$ -adrenergic receptor hyporesponsiveness, again the hypnotic response is the last to recover. At the other end of the spectrum, it was not possible to induce tolerance to the central norepinephrine turnover suppressant effect of dexmedetomidine after long-term treatment with the agonist<sup>26</sup> because only 4% of  $\alpha_2$ -adrenoceptors are required to transduce this response. Using similar paradigms, the MAC-reducing action of dexmedetomidine requires  $>20\%$  of the  $\alpha_2$ -adrenocep-

(fig. 3B) to normal by pretreatment with prazosin, an  $\alpha_1$ -adrenoceptor antagonist.

In a series of animal studies designed to investigate modulation of the behavioral and norepinephrine turnover suppressant effects of dexmedetomidine, we demonstrated that the facility with which we were able to attenuate the various anesthetic effects differed markedly. The hypnotic response is the least durable, disappearing quickly after long-term treatment with dexmedetomidine and requiring prolonged withdrawal from dexmedetomidine before the hypnotic response returned.<sup>25</sup> In studies using N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline that were designed to determine receptor occupancy requirements for each response, we established that more than 80% of  $\alpha_2$ -adrenoceptors



**Fig. 4.** The effect of dexmedetomidine on norepinephrine turnover in desipramine-treated rats. Desipramine, 10 mg/kg given subcutaneously twice daily, was administered for 3 weeks. Thirty minutes after administration of 30  $\mu$ g/kg dexmedetomidine, the animals were killed, and the ratio of MHPG to norepinephrine in the locus coeruleus was determined.

Table 1. Effect of Desipramine on  $\alpha_2$  Adrenoceptor Binding Parameters

	$K_d$ (nM)		$B_{max}$ (fmol/mg protein)	
	Control	Desipramine	Control	Desipramine
Spinal cord	$0.43 \pm 0.05$	$0.44 \pm 0.024$	$153 \pm 17$	$159 \pm 13$
Locus ceruleus	$0.22 \pm 0.04$	$0.20 \pm 0.04$	$174 \pm 30$	$174 \pm 22$

tors to be available, whereas the figure for the antinociceptive effect is  $>40\%$ . Again, in this study, the recovery data support this rank order of responsiveness, with  $MAC\text{-reduction} < \text{antinociception} < \text{hypnosis}$ .

Among possible reasons for the  $\alpha_2$ -adrenergic receptor subsensitivity noted after treatment with desipramine, one may consider a drug-drug interaction. In this case, the lingering presence of desipramine could functionally antagonize the  $\alpha_2$ -adrenoceptor-mediated response. Desipramine has no affinity for the  $\alpha_2$ -adrenoceptor, however, as evidenced by radiolabeled ligand binding studies.<sup>27</sup> In addition, the concentration of desipramine in plasma will have declined to zero by the end of the first week,<sup>11,28</sup> at which time the sedative-hypnotic response to  $\alpha_2$ -adrenergic agonists was still attenuated. Another possible drug-drug effect could be attributable to an alteration in the disposition of dexmedetomidine through the action of desipramine. Thus, desipramine could induce a kinetic alteration resulting in a change in the volume of distribution or clearance of dexmedetomidine, thereby changing its drug concentration at the effect site. The finding that the desipramine-treated rats were still hypo-responsive to LC-administered dexmedetomidine, however, precludes a pharmacokinetic mechanism for this end point.

We favor a pharmacodynamic mechanism to explain the change in end-organ responsiveness to  $\alpha_2$ -adrenergic agonists after therapy with desipramine. The signal transduction mechanism for the hypnotic and antinociceptive responses to dexmedetomidine is initiated by  $\alpha_2$ -adrenoceptors in the LC and spinal cord, respectively. The  $\alpha_2$ -adrenergic receptor hypo-responsiveness induced by desipramine does not appear to be attributable to a change in the binding function of the  $\alpha_2$ -adrenoceptor at these effect sites (table 1). It is still possible, however, that the ability of the receptor to couple to the G protein and to initiate transmembrane signaling may be dysfunctional after treatment with desipramine. Several groups of investigators have reported a downregulation in the number of  $\alpha_2$ -adrenoceptors.<sup>29,30</sup> Because of the possibility that "spare recep-

tors" exist for the transduction of the  $\alpha_2$ -adrenoceptor-mediated behavioral responses,<sup>19,26,31</sup> it is not clear to what extent these biochemical changes may be responsible for the hypo-responsiveness. Some investigators have suggested that there is a decrease in inhibitory coupling *via* the pertussis toxin-sensitive G proteins to adenylate cyclase,<sup>32</sup> although this has been disputed by others.<sup>33</sup>

There is evidence that  $\alpha_1$ -adrenergic hyperresponsiveness can be induced by long-term treatment with desipramine.<sup>34-38</sup> Previously, we demonstrated that  $\alpha_1$ -adrenergic receptor activation functionally antagonizes the  $\alpha_2$ -mediated hypnotic response.<sup>16,39</sup> The finding that prazosin was able to reverse, to a greater or lesser extent, the attenuating effects of desipramine on  $\alpha_2$ -agonist-induced behavior (fig. 2A) suggests that  $\alpha_1$ -adrenergic hyperresponsiveness may alter responsiveness to some, if not all,  $\alpha_2$ -agonist-induced behavior. The dose of prazosin (to block  $\alpha_1$ -adrenergic receptor activation) was selected on the basis that it did not affect the hypnotic response to dexmedetomidine (fig. 1C), which was also true for the MAC-reducing response (fig. 3C); however, there is a significant increase in the antinociceptive effect of dexmedetomidine in the presence of this dose of prazosin (fig. 2C). Although the normalizing effect of prazosin on the MAC-sparing and hypnotic actions of dexmedetomidine are probably attributable to attenuation of  $\alpha_1$ -adrenergic hyperresponsiveness after treatment with desipramine, this is more ambiguous in the case of changes in antinociceptive responsiveness.

We hypothesize that after long-term treatment with desipramine, transmembrane signaling of  $\alpha_1$ -adrenergic responses is sensitized. This could occur because the tricyclic antidepressant agents, which exhibit  $\alpha_1$ -antagonist activity,<sup>40</sup> can upregulate  $\alpha_1$ -adrenoceptors. In the naive setting, the weak  $\alpha_1$ -adrenoceptor affinity of dexmedetomidine<sup>39,41</sup> is not enough to attenuate  $\alpha_2$ -adrenoceptor-mediated behavioral responses. In the  $\alpha_1$  sensitized state, however, dexmedetomidine can "functionally antagonize" its  $\alpha_2$ -

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adrenoceptor-mediated behavioral responses; the most vulnerable are those responses with the least efficacy (hypnosis), and the most durable response is the most efficacious (central norepinephrine turnover suppression).

If these data can be extrapolated to the clinical paradigm, then the salubrious effects of  $\alpha_2$ -adrenergic agonists may not be obtained in surgical patients undergoing long-term therapy with tricyclic antidepressant agents. It was noted earlier that the cardiovascular actions of clonidine, an  $\alpha_2$ -agonist useful in the management of hypertension, is less efficacious in patients receiving antidepressant agents.<sup>42</sup> There is no evidence that the  $\alpha_2$ -adrenergic hyposensitivity occurs after treatment with antidepressant agents that do not possess the tricyclic structure.<sup>43</sup> In addition, cocaine, another compound that blocks monoamine reuptake, does not appear to induce  $\alpha_2$ -adrenergic hyposensitivity.<sup>44</sup>

Long-term therapy with desipramine induces hyporesponsiveness to the behavioral properties of a centrally active  $\alpha_2$ -adrenergic agonist. This hyporesponsiveness may be attributable, in part, to an increase in  $\alpha_1$ -adrenergic responsiveness, which can functionally antagonize the  $\alpha_2$ -adrenoceptor-mediated response.

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