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Sympatholytic and Minimum Anesthetic Concentration-sparing Responses are Preserved in Rats Rendered Tolerant to the Hypnotic and Analgesic Action of Dexmedetomidine, a Selective α_2-adrenergic Agonist

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Background: **The development of tolerance to the sympatholytic and anesthetic-reducing effects of α_2 agonists after prolonged administration of dexmedetomidine and how the number of available α_2 adrenoceptors affects these dexmedetomidine-induced responses was studied.**

Methods: **The sympatholytic action of acute and chronic (3 and 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ for 7 days) dexmedetomidine, was assessed by the decrease in norepinephrine turnover in the locus coeruleus and hippocampus. The anesthetic-reducing effect of chronic (7 days) dexmedetomidine (5 and 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$) was studied by determining the minimum alveolar concentration (MAC) for halothane that prevented rats from responding to a supramaximal noxious stimulus of dexmedetomidine (10 or 30 $\mu g \cdot kg^{-1}$), doses in the steep part of the dose-response curve.**

The receptor reserve for the norepinephrine turnover and anesthetic-sparing responses to dexmedetomidine was delineated with 0.3–1.0 $mg \cdot kg^{-1}$ N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, an irreversible alkylating agent.

Results: **After chronic administration of dexmedetomidine at both doses, acute dexmedetomidine significantly decreased norepinephrine turnover in the hippocampus and locus coeruleus. The baseline minimum anesthetic concentration (MAC) and the MAC-sparing effect to acutely administered dexmedetomidine were preserved after chronic dexmedetomidine treatment. In the N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline experiments, the dexmedetomidine-induced norepinephrine turnover effect required less than 20% and greater than 4% α_2 adrenoceptor availability in the locus coeruleus and the dexmedetomidine induced MAC-sparing effect required less than 40% and greater than 20% α_2 adrenoceptor availability in the locus coeruleus.**

Conclusion: **Tolerance does not develop for either the sympatholytic or MAC-sparing actions of dexmedetomidine, although it is present for the hypnotic response. The durable quality of the sympatholytic and MAC-sparing responses to dexmedetomidine after chronic treatment is explained by a comparatively larger receptor reserve than is needed for the hypnotic and analgesic responses, which are blunted by the same drug treatment regimen. (Key words: Brain: hippocampus; locus coeruleus. Sympathetic nervous system, α_2-adrenergic agonists: dexmedetomidine. Sympathetic nervous system, catecholamines: norepinephrine. Receptors: α_2 adrenoceptor. Treatment: chronic.**

THE antihypertensive action of α_2 agonists is exerted at many levels including their ability to inhibit the release of norepinephrine in both central and peripheral adrenergic pathways. This inhibitory action on norepinephrine release is referred to as their sympatholytic action.

Recent demonstrations of therapeutic indications for α_2-adrenergic agonists have led to multiple uses of this class of drug in the perioperative period.[1] For example, α_2-adrenergic agonists are efficacious for anxiolysis,[2] preoperative sedation,[3] and decreasing anesthetic requirements for volatile,[4] opioid,[5] and hypnotic induction agents.[6] Additionally, α_2-adrenergic agonists minimize hemodynamic fluctuations,[7] facilitate induced hypotensive techniques,[8] decrease postoperative shivering,[3,9] decrease oxygen utilization,[10] and may even be effective in protecting against ischemic damage to the brain[11] and heart.[12]

Because of the perceived benefit of these compounds

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at each stage in the surgical patient's care, we have advocated that subacute administration of α_2 agonists should encompass the entire perioperative period.[13] The regular practice of prolonged use of α_2 agonists in the management of chronic hypertension has clarified certain alterations in behavioral effects that accompany extended administration. While the beneficial antihypertensive effect is sustained for a period of years; the unwanted side effects, such as sedation, are relatively short-lived.[14] This differential sensitivity to chronic drug treatment can be explained by differences in the efficiency of signal transduction. The greater the efficiency with which a signal is transduced, the fewer the receptors that need to be activated to achieve a desired response. The most efficient responses have a built-in reserve that sustains the response even when the transduction mechanism is disrupted.

The development of tolerance is a ubiquitous biologic process in which responsiveness decreases with continuing exposure. While this has been particularly well characterized for responses mediated by adrenoceptors of the β and α_1 subclasses,[15] little is known about responses mediated by the α_2 adrenoceptors. We recently demonstrated that tolerance develops to both the hypnotic[16] and analgesic[17] effects of α_2 agonists after chronic treatment with dexmedetomidine. Whether tolerance develops similarly for other potentially useful perioperative effects of α_2 agonists is not known. We now report on the sympatholytic and anesthetic-sparing actions of dexmedetomidine after chronic administration.

Methods

Animal Preparation

Two hundred twenty-three male Sprague-Dawley rats (weighing 250–300 g) were chosen as the experimental model after approval of the experimental protocol by the Animal Care and Use Committee at the Palo Alto Veterans Affairs Medical Center. Rat littermates were stratified to match weight distribution in the control and treated groups as closely as possible. All behavioral tests and animal deaths occurred between 9 AM and 1 PM.

Continuous Drug Administration. Rats were exposed to the same conditions that were demonstrated to induce tolerance to the hypnotic action of dexmedetomidine, an α_2-adrenergic agonist.[16,17] Briefly, dexmedetomidine was chronically administered using Alzet osmotic minipumps (Model 2002 or 1007D Alza, Palo Alto, CA), which discharge their contents at a mean pumping rate of $0.48 \pm 0.02\ \mu l \cdot h^{-1}$. The pumps were loaded to deliver 3, 5, or 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ for 7 days. The pumps were inserted subcutaneously into the dorsal thoracic region during halothane anesthesia. In the initial experiments control animals also had the osmotic pumps containing only the vehicle implanted. This group did not differ in behavioral response from sham-operated control animals. Therefore, the latter were used.

Depletion of α_2 Adrenoceptor Reserve. Depletion of α_2 adrenoceptors was produced with N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), which covalently binds and permanently inactivates α_2 adrenoceptors; therefore, responsiveness to α_2 agonists is dependent on the recovery of the newly expressed receptor population.[18] This compound also is capable of rendering serotonergic and dopaminergic receptors dysfunctional *via* alkylation. Rats received 0.3 or 1.0 $mg \cdot kg^{-1}$ EEDQ subcutaneously.

Halothane Minimum Alveolar Concentration Testing. As previously described, the sensitivity to halothane was determined by measuring the MAC, which prevents a response to a supramaximal stimulus.[19] Briefly, halothane was vaporized in oxygen at a flow rate of $5\ l \cdot min^{-1}$ and introduced into a methyl methacrylate polymer exposure chamber. The response of animals to supramaximal noxious stimulus was assessed by applying a 6″ hemostat to the first ratchet position on the middle portion of the tail for 1 min. A positive response was noted if the rat moved purposefully in response to tail clamping. The individual testing for MAC was unblinded to the treatment.

Neurochemical Measurement. To measure monoamine turnover, animals received the vehicle or 10 and 30 $\mu g \cdot kg^{-1}$ dexmedetomidine intraperitoneally and were killed 30 min later by decapitation after narcosis induced by a 30-s exposure to carbon dioxide. The locus coeruleus (LC) was removed from each side and the hippocampus from the left side of the freshly harvested brain. Samples 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 daltons. These samples were stable for up to 3 months when stored at −80°C. The biogenic amines were assayed with high performance liquid chromatography with electrochemical detection, as previously described.[20]

The ratio of the concentrations of the major me-

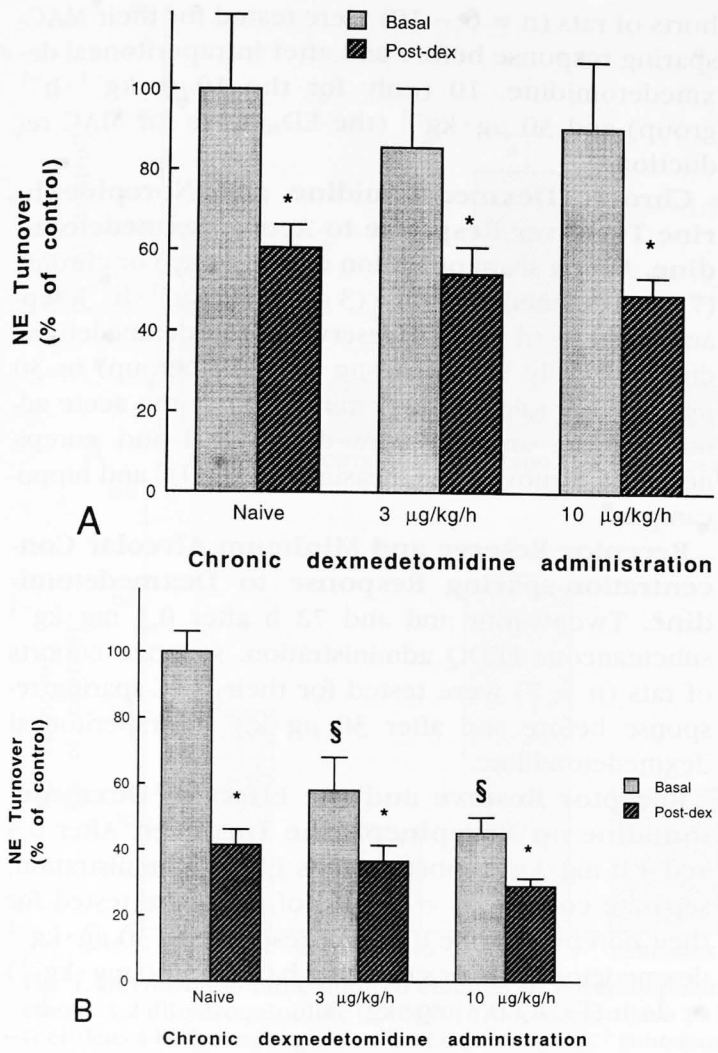

Fig. 1. Effect of chronic dexmedetomidine administration on norepinephrine turnover ± acute dexmedetomidine in the (*A*) locus coeruleus and (*B*) hippocampus. Rats were implanted with osmotic pumps set to deliver 3 or 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ (tolerant groups) or sham-operated (naive group). On the 7th day, rats were administered 30 $\mu g \cdot kg^{-1}$ dexmedetomidine intraperitoneally and the turnover of norepinephrine was determined. Data are expressed as a percentage of control animals normalized to 100%. ANOVA: $P < 0.0001$ (hippocampus) and $P < 0.005$ (locus coeruleus) $^*P < 0.05$ compared with basal state (Fisher test) $\S P < 0.0001$ compared with naive group. n = 7–8 per group.

tabolite of a brain monoamine neurotransmitter to brain monoamine itself is used as an index of the overall turnover rate of the synaptic monoamine neurotransmitter. Underlying this ratio is the understanding that high concentrations of the monoamine neurotransmitter such as norepinephrine will reduce neuronal activity by acting as an autoregulator at presynaptic α_2 receptors. Conversely, increased concentrations of the neuronal metabolite, such as 3,4-dihydroxyphenylglycolaldehyde or 3-methoxy-4-hydroxyphenylglycol (MHPG), are associated with increased neuronal firing. The foregoing assumes that the formation and elimination of the monoamines and their metabolites are proportional to their concentrations.[21]

Receptor Density. α_2 Receptor density was measured in the LC 4 or 24 h after EEDQ administration. Animals were killed by decapitation under carbon dioxide narcosis and the LCs (2 LCs/rat) were harvested. Tissue was homogenized in 500 μl ice-cold Tris, 50 mM, EDTA 0.8 mM buffer *p*H 7.5. After centrifugation

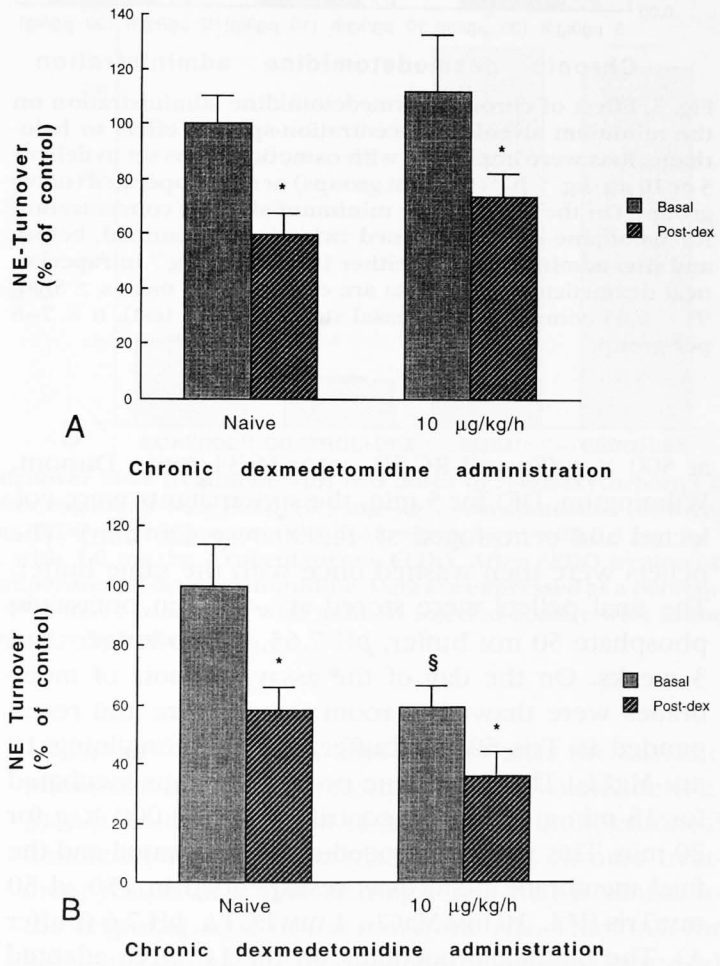

Fig. 2. Effect of chronic dexmedetomidine administration on norepinephrine turnover ± acute dexmedetomidine in the (*A*) locus coeruleus and (*B*) hippocampus. Rats were implanted with osmotic pumps set to deliver 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ (tolerant groups) or sham-operated (naive group). On the 7th day, rats were administered 10 $\mu g \cdot kg^{-1}$ dexmedetomidine intraperitoneally and the turnover of norepinephrine was determined. Data are expressed as a percentage of control animals normalized to 100%. ANOVA: $P < 0.05$ (hippocampus) and $P < 0.05$ (locus coeruleus) compared with basal state (Fisher test) $\S P < 0.05$ compared with naive group. n = 7–8 per group.

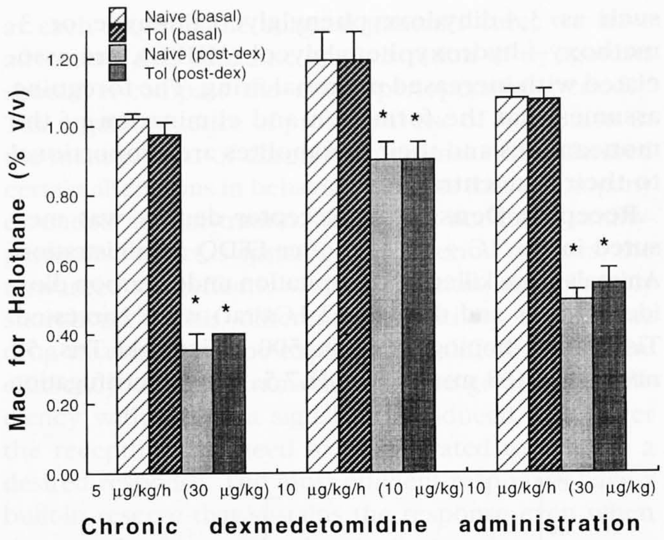

Fig. 3. Effect of chronic dexmedetomidine administration on the minimum alveolar concentration-sparing effect to halothane. Rats were implanted with osmotic pumps set to deliver 5 or 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ (tolerant groups) or sham-operated (naive group). On the 7th day, the minimum alveolar concentration for halothane was determined twice in each animal, before and after administration of either 10 or 30 $\mu g \cdot kg^{-1}$ intraperitoneal dexmedetomidine. Data are expressed as means ± SEM. *$P < 0.05$ compared with basal state (paired *t* test). n = 7–8 per group.

at 500 × *g* (Sorvall RC-5B, rotor SS-34 rotor, Dupont, Wilmington, DE) for 5 min, the supernatants were collected and centrifuged at 44,000 × *g* (20 min). The pellets were then washed once with the same buffer. The final pellets were stored at −80°C in potassium phosphate 50 mM buffer, *p*H 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, *p*H 7.6, containing 10 mM $MgCl_2$. The membrane preparation was incubated for 15 min at 37°C and centrifuged at 44,000 × *g* for 20 min. This washing procedure was repeated and the final membrane pellet was resuspended in 150 μl 50 mM Tris-HCL, 10 mM $MaCl_2$, 1 mM EGTA, *p*H 7.6 (buffer A). The binding procedures on the LC were adapted from Baron and Siegel.[22]

Experimental Regimens

Chronic Dexmedetomidine and the Minimum Alveolar Concentration-sparing Response to Acute Dexmedetomidine. After a sham operation (naive group) or chronic (7 days) dexmedetomidine administration (5 and 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$), separate cohorts of rats (n = 6 − 10) were tested for their MAC-sparing response before and after intraperitoneal dexmedetomidine, 10 (only for the 10-$\mu g \cdot kg^{-1} \cdot h^{-1}$ group) and 30 $\mu g \cdot kg^{-1}$ (the ED_{50} dose for MAC reduction).[19]

Chronic Dexmedetomidine and Norepinephrine Turnover Response to Acute Dexmedetomidine. After a sham operation (naive group) or chronic (7 days) dexmedetomidine (3 or 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$), separate cohorts (n = 7 − 8) reserved either dexmedetomidine, 10 (only for the 10-$\mu g \cdot kg^{-1} \cdot h^{-1}$ group) or 30 $\mu g \cdot kg^{-1}$, or saline. Thirty minutes after the acute administration, animals were decapitated and norepinephrine turnover was measured in the LC and hippocampus.

Receptor Reserve and Minimum Alveolar Concentration-sparing Response to Dexmedetomidine. Twenty-four and and 72 h after 0.3 $mg \cdot kg^{-1}$ subcutaneous EEDQ administration, separate cohorts of rats (n = 7) were tested for their MAC-sparing response before and after 30 $\mu g \cdot kg^{-1}$ intraperitoneal dexmedetomidine.

Receptor Reserve and the Effect of Dexmedetomidine on Norepinephrine Turnover. After 0.3 and 1.0 $mg \cdot kg^{-1}$ subcutaneous EEDQ administration, separate cohorts (n = 5 − 8) of rats were tested for their norepinephrine turnover response to 30 $\mu g \cdot kg^{-1}$ dexmedetomidine or saline at 4 h (EEDQ 1.0 $mg \cdot kg^{-1}$) or 24 h (EEDQ 0.3 $mg \cdot kg^{-1}$).

Statistics

Data are expressed as mean ± SEM. The results of multiple groups were analyzed by analysis of variance followed by Fisher's *post hoc* test. Comparison between two groups was performed by *t* test for unpaired data. A *P* value < 0.05 was considered statistically significant.

Results

Norepinephrine Turnover and Chronic Dexmedetomidine. In the LC (fig. 1A), chronic administration of both dexmedetomidine doses (3 and 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$) fails to alter the MHPG/norepinephrine ratio. Acute administration of dexmedetomidine (30 $\mu g \cdot kg^{-1}$ intraperitoneal) to animals receiving chronic treatment, decreased ($P < 0.05$) the MHPG/norepinephrine ratio.

In the hippocampus (fig. 1B), chronic administra-

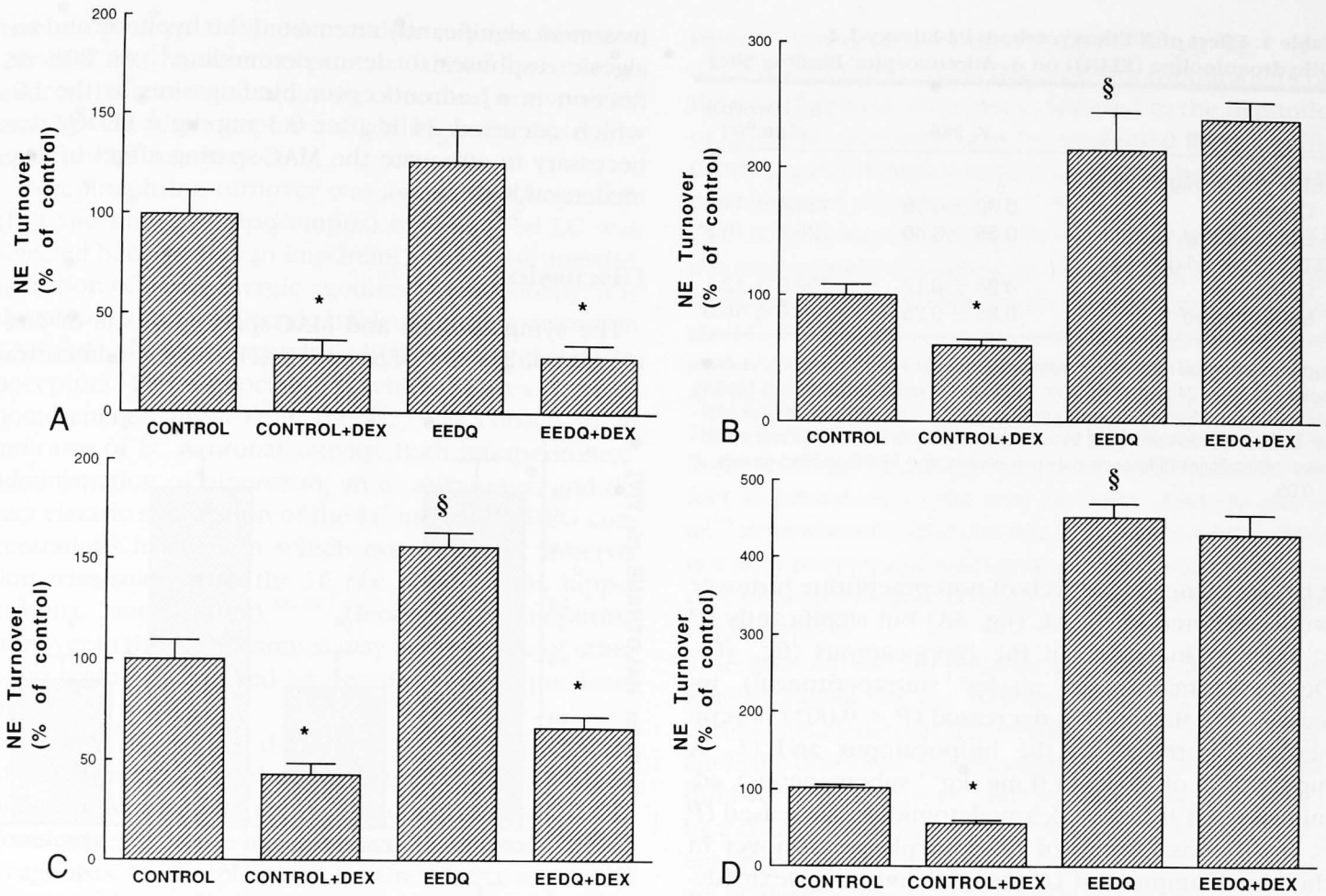

Fig. 4. Dexmedetomidine-induced changes in norepinephrine turnover after treatment with two doses of N-ethoxycarbonyl-2-ethoxy-1,2 dihydroquinoline (EEDQ). (A) locus coeruleus 24 h after treatment with EEDQ (0.3 mg · kg^{-1} subcutaneous), (B) locus coeruleus 4 h after treatment with EEDQ1.0 mg · kg^{-1} subcutaneous EEDQ, (C) hippocampus 24 h after treatment with 0.3 mg · kg^{-1} subcutaneous EEDQ, and (D) hippocampus 4 h after treatment with 1.0 mg · kg^{-1} subcutaneous EEDQ. After EEDQ treatment, rats were injected with saline or dexmedetomidine30 μg · kg^{-1} intraperitoneal dexmedetomidine. Data are expressed as a percentage of control animals normalized to 100%. ANOVA: $P < 0.001$ *$P < 0.001$ compared with animals injected acutely with saline (Fisher test) §$P < 0.001$ compared with non-EEDQ injected group. n = 5–8 per group.

tion of both dexmedetomidine doses (3 and 10 μg · kg^{-1} · h^{-1}) reduces ($P < 0.0001$) the MHPG/norepinephrine ratio compared to naive animals. Acute administration of dexmedetomidine (30 μg · kg^{-1} intraperitoneal) to animals receiving chronic treatment, results in a further reduction ($P < 0.05$) in the MHPG/norepinephrine ratio.

Similar findings were seen in both the LC (fig. 2A) and the hippocampus (fig. 2B) after acute administration of dexmedetomidine (10 μg · kg^{-1} intraperitoneal) to animals receiving chronic treatment (10 μg · kg^{-1} · h^{-1} dexmedetomidine)

The Minimum Alveolar Concentration-sparing Effect and Chronic Dexmedetomidine. Chronic administration of both dexmedetomidine doses (5 and 10 μg · kg^{-1} · h^{-1}) for 7 days did not alter the baseline minimum anesthetic concentration for halothane (fig. 3). Acute administration of 30 μg · kg^{-1} intraperitoneal dexmedetomidine to animals receiving chronic treatment results in a MAC-sparing effect that is similar to the response seen in the naive animals. The MAC for halothane after acute dexmedetomidine was decreased ($P < 0.0001$) in both the tolerant and naive groups. Analogous findings were seen after acute administration of 10 μg · kg^{-1} intraperitoneal dexmedetomidine to animals receiving chronic dexmedetomidine treatment (10 μg · kg^{-1} · h^{-1}).

Receptor Reserve and the Effect of Dexmedetomidine on Norepinephrine Turnover. After EEDQ (0.3 mg · kg^{-1} subcutaneous), administration 24

Table 1. Effect of N-Ethoxycarbonyl-2-Ethoxy-1,2-Dihydroquinoline (EEDQ) on α_2-Adrenoceptor Binding Sites

Group	K_d (nM)	B_{max} (fmol/mg of protein)
EEDQ (0.3 mg/kg at 24 h)		
Control	0.32 ± 0.16	126.4 ± 14.6
EEDQ-treated	0.35 ± 0.46	25.7 ± 16.3*
EEDQ (1.0 mg/kg at 4 h)		
Control	0.24 ± 0.12	109.2 ± 12.1
EEDQ-treated	0.34 ± 0.25	4.6 ± 16.3*

Separate groups of rats were administered EEDQ 0.3 and 1.0 $mg \cdot kg^{-1}$ subcutaneously, and, 24 and 72 h later, respectively radiolabeled-ligand binding studies were performed. Results are B_{max} and K_d = B1 95% confidence interval. Each value represents the tissue from 10 animals pooled into one experiment. * Significant difference between control and EEDQ-treated groups, $P < 0.05$.

h before saline, basal levels of norepinephrine turnover were unaltered in the LC (fig. 4A) but significantly ($P < 0.0001$) increased in the hippocampus (fig. 4C). Dexmedetomidine (30 $\mu g \cdot kg^{-1} \cdot$ intraperitoneal), injected 24 h after EEDQ, decreased ($P < 0.001$) norepinephrine turnover in the hippocampus and LC. A higher dose of EEDQ (1.0 $mg \cdot kg^{-1}$ subcutaneous), administered 4 h before dexmedetomidine, increased ($P < 0.001$) basal levels of norepinephrine turnover in the hippocampus and LC (figs. 4B and 4D). Dexmedetomidine (30 $\mu g \cdot kg^{-1} \cdot$ intraperitoneal) injected after this EEDQ treatment failed to decrease norepinephrine turnover. Four hours after 1.0 $mg \cdot kg^{-1}$ EEDQ (table 1), there was a 96% reduction in α_2 adrenoceptor binding sites in the LC.

Receptor Reserve and the Halothane MAC-sparing Effect of Dexmedetomidine. EEDQ (0.3 $mg \cdot kg^{-1}$ subcutaneous), administered 72 and 24 h before testing, did not alter the baseline MAC for halothane (figs. 5A and 5B). Dexmedetomidine's MAC-sparing effect was completely blocked in rats treated with EEDQ 24 h before acute administration of 30 $\mu g \cdot kg^{-1}$ intraperitoneal dexmedetomidine. In naive rats or rats treated with EEDQ 72 h earlier, the MAC-sparing effect to acute administration of dexmedetomidine remained intact ($P < 0.0001$).

Treatment with EEDQ produced a dose-dependent reduction in α_2 adrenoceptor binding sites in the LC (table 1) without affecting the binding affinity (dissociation constant). The dose-dependent reduction in α_2 adrenoceptor binding sites for α_2 adrenoceptors in the LC was reduced by 60% in rats 72 h after 0.3 $mg \cdot kg^{-1}$ EEDQ injection.[17] Previously, we reported that this treatment significantly attenuated the hypnotic and analgesic responses to dexmedetomidine.[17] An 80% reduction in α_2 adrenoceptor binding sites in the LC, which occurred 24 h after 0.3 $mg \cdot kg^{-1}$ EEDQ, was necessary to attenuate the MAC-sparing effect of dexmedetomidine.

Discussion

The sympatholytic and MAC-sparing effects of dexmedetomidine are preserved after chronic administra-

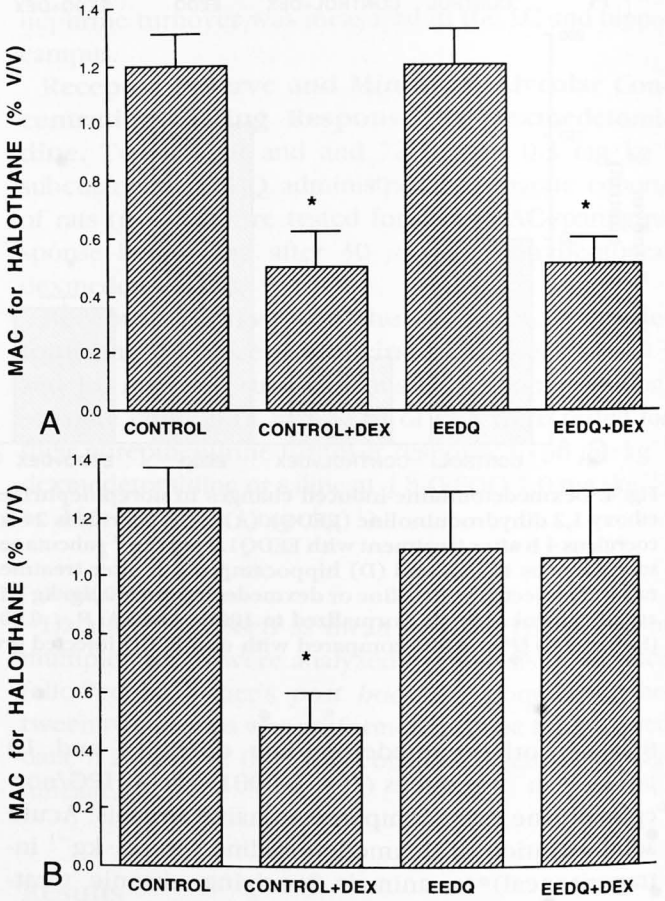

Fig. 5. The halothane minimum alveolar concentration-sparing effect of dexmedetomidine after treatment with N-ethoxycarbonyl-2-ethoxy-1,2dihydroquinoline. In separate groups of rats, the minimum alveolar concentration for halothane was determined (*A*) 72 h after treatment with 0.3 mg · kg subcutaneous N-ethoxycarbonyl-2-ethoxy-1,2dihydroquinoline and (*B*) 24 h after treatment with 0.3 mg · kg subcutaneous N-ethoxycarbonyl-2-ethoxy-1,2dihydroquinoline. The minimum alveolar concentration was determined twice in each animal, before and after administration of 30 $\mu g \cdot kg^{-1}$ intraperitoneal dexmedetomidine. Data are expressed as means ± SEM. *$P <$ 0.0001 compared with basal state (paired t test). n = 7 per group.

tion of the drug. Dexmedetomidine requires between 20% and 40% α_2 adrenoceptor availability in the LC to express its anesthetic-reducing effect and between 4% and 20% α_2 adrenoceptor availability to decrease norepinephrine turnover.

Norepinephrine turnover was assessed in brainstem (LC) and limbic (hippocampus) regions. The LC was selected because it is an important site for the anesthetic action of α_2-adrenergic agonists.[23] Additionally, it is the principal noradrenergic nucleus in the mammalian CNS and a discrete area with a high density of α_2 adrenoceptors. The hippocampus, which derives all of its noradrenergic input from the LC, was chosen as an indicator of LC neuronal activity. Both intraperitoneal administration of piperoxan, an α_2 antagonist, and direct electric stimulation of the LC increase MHPG concentrations in areas in which noradrenergic innervation arise solely from the LC (*i.*e., cerebellum, hippocampus, and cortex).[24-26] Hence, norepinephrine turnover in the hippocampus may be reflective of other brain regions subserved by noradrenergic input from the LC.

We have shown that the central sympatholytic effect of acutely administered dexmedetomidine is sustained under the same conditions in which animals were rendered tolerant to the hypnotic and analgesic effects of α_2 agonists. One explanation for the relative resistance to desensitization may relate to a large receptor reserve for autoreceptor function. Several studies have demonstrated a large surplus of spare autoreceptors in various systems including dopaminergic,[27] serotonergic,[28] and α_2-adrenergic.[29-31] Thus, it is possible that even if a large fraction of the α_2 autoreceptors are rendered nonfunctional, through uncoupling or internalization, the response transduced by the remaining receptors may still be intact. In animals treated with EEDQ, we demonstrated that greater than 4% and less than 20% of the α_2-adrenoceptor population was required to produce the norepinephrine turnover effect of dexmedetomidine.

Previously, we have shown a correlation between receptor reserve and resistance to the induction of tolerance for α_2-adrenergic responses.[17] The hypnotic response to dexmedetomidine, which has a receptor reserve of 23%, was decreased after 2 days of 3 $\mu g \cdot kg^{-1} \cdot h^{-1}$ dexmedetomidine administration. In contrast, the tail-flick analgesic response, which has a 40% receptor reserve, was decreased after 7 days of 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ dexmedetomidine, and the sympatholytic effect, which has a greater than 80% receptor reserve, was unaffected after 7 days of 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ dexmedetomidine. Thus, the comparative durability of the sympatholytic response compared to the hypnotic or analgesic response can be explained by a significantly greater receptor reserve owing to more efficient coupling and transduction.

It is noteworthy that chronic administration of dexmedetomidine resulted in a significant decrease in norepinephrine turnover in the hippocampus but not the LC. This finding concurs with Rochette *et al.*[32] who showed that chronic clonidine fails to diminish the norepinephrine turnover, as judged by the rate of decline of norepinephrine after α-methyltyrosine, in the brain stem and hypothalamus, while this inhibitory effect is preserved in the rest of brain. Also, Koulu *et al.*[33] demonstrated that chronic dexmedetomidine does not alter norepinephrine turnover, as measured by the rate of accumulation of dopa, in brain stem nuclei including the LC.

Because we did not measure the concentration of dexmedetomidine in the different brain regions during chronic dexmedetomidine infusion, we are unable to exclude the possibility that the difference in norepinephrine turnover between the LC and the hippocampus is caused by a change in the distribution of the drug in these different brain regions. We assume, however, that similar levels are attained in the LC and hippocampus because all tissues should be at equilibrium with the central compartment after seven days of dexmedetomidine infusion. Recent studies using a similar infusion regimen revealed that plasma concentrations have stabilized by the second day of drug administration.[16]

We should also consider the possibility that the α_2 adrenoceptor-effector mechanism in the hippocampus, but not the LC, is refractory to changes in sensitivity. Perhaps this difference in the sensitivity of the receptors is a function of their location in different brain regions. This explanation is supported by the observation that receptor reserve may be region specific. In studies with EEDQ, α_2 adrenoceptors regulating LC activity are characterized by a larger receptor reserve or are less sensitive to the influence of alkylation than are the population of α_2 adrenoceptors regulating norepinephrine utilization.[31] Moreover, regional variation in receptor sensitivity may be due to different subtypes of the α_2 receptor,[34] each with their respective intrinsic properties. This supposition is supported by the finding that chronic treatment with norepinephrine differentially downregulates α_{2A} and α_{2C}-adrenoceptor subtypes.[35]

Anesthetic Requirements and Chronic Dexmedetomidine Infusion

Chronic dexmedetomidine infusion neither changed the MAC for halothane nor norepinephrine turnover in the LC. This lack of effect may be causally related. Initial theories maintained that the MAC for anesthesia reflected the summation of changes in many systems, including the noradrenergic system.[36] More recent hypotheses have asserted that alterations in neurotransmission in discrete brain regions, as opposed to a widespread depression of neurotransmission, are responsible for the dose of drug required to produce the anesthetic state. This idea was supported by the demonstration that both cyclopropane and halothane significantly and selectively changed neurotransmitter content in a few brain regions.[37] Consonant with this hypothesis was evidence showing the regional specificity of metabolic changes, namely cerebral glucose utilization, induced by anesthetic agents.[38,39] Both of the aforementioned studies note that the LC is one of the few discrete brain regions with significant, distinctive changes in neurotransmitter content and metabolic activity after halothane anesthesia.

Studies linking the LC and volatile anesthetics are further supported by a substantial body of evidence implicating noradrenergic neurons in the modulation of anesthetic requirements. Depletion of monoamines with central neurotoxins[19,40] and elevation of central amine levels with release enhancers, dextroamphetamine[41] or cocaine,[42] have produced corresponding decreases and increases in anesthetic potency. Therefore, it is expected that the absence of change in the MAC for anesthesia would be accompanied by unaltered norepinephrine turnover in the LC. The change in norepinephrine turnover that was noted in the hippocampus would not be expected to change MAC for anesthesia because this region has been physiologically linked to memory but not to consciousness.[43] Other sites in the central nervous system that have been suggested as possible sites for volatile anesthetic action include the spinal cord,[44] although the LC remains an important candidate site for the MAC-sparing effect, if only because of its role on the hypnotic[45,46] and analgesic[47] actions of α_2-adrenergic agonists.

Analogous to the sympatholytic effect, the anesthetic-sparing effect of acutely administered dexmedetomidine was preserved in animals rendered tolerant to the hypnotic effect of the drug by chronic administration.[16] The comparatively refractory nature of dexmedetomidine's MAC-sparing effect may be explained by the existence of spare receptors: transduction of this behavioral response requires a relatively small fraction of the α_2 receptor population, less than 40% in the LC, such that it is unaffected by the reduction in receptor reserve elicited by chronic drug administration. Thus, the refractory quality of dexmedetomidine's MAC-sparing effect may be elucidated by the empirically established link between halothane anesthesia and neuronal activity in the LC, a discrete brain region with an unusual abundance of spare α_2-adrenergic receptors.[31]

In summary, both the sympatholytic and MAC-sparing responses dexmedetomidine persist in rats rendered tolerant to the hypnotic and analgesic properties of dexmedetomidine. While this may be partially attributed to differences in receptor subtypes that mediate these responses, it is significant that there are large differences in receptor coupling and transduction for the individual responses. The result of this is that chronic treatment with α_2 agonists will cause efficacy to change for some but not all responses.

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