Dexmedetomidine Diminishes Halothane Anesthetic Requirements in Rats Through a Postsynaptic Alpha2 Adrenergic Receptor

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The effect of 4(5)-1-(2,3-dimethylphenyl)ethyl]imidazole (medetomidine), the alpha2 adrenergic agonist, on anesthetic requirements was investigated in rats anesthetized with halothane. Halothane MAC was determined before and after either dexmedetomidine (d-enantiomer) or levomedetomidine (l-enantiomer) 10, 30, and 100 μg/kg or vehicle ip. There was a dose-dependent decrease in MAC with the d-, but not the l-, stereoisomer. At the highest dose of dexmedetomidine (100 μg/kg), halothane could be discontinued for up to 30 min with no response to tail clamping. To determine whether alpha2 adrenoceptors mediated this effect of dexmedetomidine on MAC, cohorts of rats were pretreated with idazoxan, 10 mg/kg ip, a highly selective alpha2 antagonist. This completely prevented the reduction of MAC caused by dexmedetomidine. To determine whether the reduction of MAC caused by dexmedetomidine was mediated in part through either opiate or adenosine receptors, groups of rats were pretreated with either naltrindole, 5 mg/kg ip, an opiate antagonist, or 8-phenyltheophylline, 2 mg/kg ip, an A1 adenosine antagonist. These two pretreatments did not alter the reduction of MAC by dexmedetomidine. To determine whether postsynaptic mechanisms mediate the anesthetic effect of dexmedetomidine, rats were depleted of central catecholamine stores with either n-(2-chloroethyl)-n-ethyl-2-bromobenzylamine (DSP-4) or reserpine and alpha-methyl-para-tyrosine and MAC was determined before and after each dose of dexmedetomidine. While the catecholamine-depleted rats had a lower basal MAC than the vehicle controls, there was still a profound reduction in halothane MAC after administration of dexmedetomidine. The reduction of MAC by dexmedetomidine was blocked with idazoxan in the catecholamine depleted rats. These data indicate that the reduction of MAC caused by dexmedetomidine is mediated through alpha2 adrenoceptors with no apparent involvement of either opiate or A1 adenosine receptors. Data from catecholamine-depleted rats suggest that the mediating mechanism must involve site(s) other than or in addition to the presynaptic alpha2 adrenergic receptors on noradrenergic neurons. The authors conclude that central postsynaptic alpha2 adrenergic receptors mediate a significant part of the reduction of anesthetic requirements caused by dexmedetomidine. (Key words: Anesthetics, volatile; halothane. Potency: MAC. Sympathetic nervous system, receptors, Alpha2 agonist: 4(5)-1-(2,3-dimethylphenyl)ethyl]imidazole; dexmedetomidine; levomedetomidine. Sympathetic nervous system, receptors: postsynaptic; presynaptic.)

Alpha2 adrenergic agonists exert a sympatholytic effect by stimulating presynaptic alpha2 adrenoceptors, thereby inhibiting release of norepinephrine, in the sympathetic nervous system. Clonidine, the prototypical alpha2 adrenergic agonist, has been used in a variety of clinical situations in which attenuation of noradrenergic neurotransmission is desired. These include: treatment of hypertension; opiate withdrawal; chronic pain; and anxiety syndromes. Alpha2 adrenergic agonists have also been shown to reduce anesthetic requirements during surgery as well as attenuating hemodynamic responses to laryngoscopy and cardiopulmonary bypass.

Manipulations that decrease central noradrenergic neurotransmission have been associated with a decrease in anesthetic requirements as reflected by a lower MAC. Clonidine has been shown to decrease central noradrenergic neurotransmission in the same setting in which sensitivity to anesthetic agents was increased. However, in studies with azepoxide, a more selective alpha2 adrenergic agonist, we demonstrated a greater than 85% reduction in anesthetic requirements. It is unlikely that the presynaptic inhibition of central noradrenergic neurotransmission by alpha2 adrenergic agonists is enough to explain this profound reduction in anesthetic requirements, since MAC is reduced by no more than 40% when noradrenergic pathways are disrupted.

Other endogenous neuromodulators, especially endorphins and purines, might also be responsible for the central nervous system action of alpha2 adrenergic agonists. Adenosine, the purine nucleoside, inhibits central noradrenergic neurons via presynaptic purinergic receptors of the A1 subtype. Some evidence now supports the contention that clonidine is functionally synergistic with adenosine as an inhibitory neuromodulator. Also, clonidine increases central release of endorphin and can...
attenuate the hypernoradrenergic state in naloxone-induced withdrawal of morphine-dependent rats. We have demonstrated that the hypotensive effect of clonidine in rats with pheochromocytoma can be attenuated and reversed by naloxone.

More recently, we have utilized dexmedetomidine (fig. 1), a more potent alpha₂ agonist that is available in enantiomeric forms and is even more efficacious than azepoxide at reducing MAC in dogs. In the present study, we characterize the: 1) stereospecificity, 2) type of receptor, and 3) synaptic site at which the alpha₂ adrenergic agonist dexmedetomidine decreases anesthetic requirements in rats.

**Materials and Methods**

The study protocol was approved by the Animal Care and Use Committee at the Palo Alto Veterans Administration Medical Center.

Male Sprague-Dawley rats (150–200 g) were acclimatized for 1 week before experimentation. They were housed in groups of two and maintained on a 12 h light:12 h dark cycle with food and water continuously available. For the MAC studies, halothane was vaporized in oxygen at a flow rate of 5 l/min, and the anesthetic concentration was monitored continuously by infrared spectral analysis and was confirmed at intervals by gas chromatography and mass spectroscopy. Rectal temperature was continuously monitored and maintained at 37.5 ± 1.0°C with heating blankets. Rats were placed in a 120 l plexiglass box in which the halothane concentration was maintained at approximately 1.0 MAC. This concentration was maintained for 2.5 h prior to determining MAC. Rats were then stimulated by clamping a 6-inch hemostat to the first rachet position on the middle portion of the tail. If the rat made a purposeful movement to the tail clamp within 1 min, a positive response was scored. If more than 50% of the animals responded, the halothane concentration was increased by approximately 10% of the initial concentration and stimulation was repeated after allowing 30 min for re-equilibration. Conversely, if more than 50% of the animals did not respond purposefully to this supramaximal stimulus, the halothane concentration was decreased by approximately 10% and, after 30 min, the testing sequence was repeated. When there was an equal number of positive and negative responses in the group, 1.0 MAC of halothane was established. If an equal number of responders and non-responders were not obtained within a group, the MAC value was determined by interpolating between “crossover” concentrations. MAC was determined for each group of ten rats in replicate experiments (the number is stated in the legends to the figures). MAC values were determined after administration of the control and treatment compounds (fig. 2). Thus, each group of rats served as its own control. To determine the dose-dependency and stereospecificity of the effect of medetomidine on MAC for halothane, rats were treated with dexmedetomidine (the d-isomer of medetomidine) or levomedetomidine (the l-isomer of medetomidine) at three doses; 10, 50, and 100 mg/kg or an equal volume of the vehicle ip. MAC was determined 30 min after injection. The involvement of alpha₂ adrenergic receptors in mediating the MAC reducing effects of dexmedetomidine was then investigated in separate experiments. Groups received dexmedetomidine at each of the three doses, with coadministration of idazoxan 10 mg/kg ip, a selective alpha₂ adrenergic antagonist. The control group received vehicle and idazoxan. MAC was determined before and after treatment. Involvement of either the opiate or A₁ adenosine receptor were examined by utilizing the antagonists naltrexone 5 mg/kg ip and 8-phenyltheophylline (8-PT), 2.5 mg/kg ip using the

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**Fig. 1. Structure of 4(S)-[1-(2,3-dimethylphenyl)ethyl]-imidazole, medetomidine.**

**Fig. 2. Schema for MAC determinations and drug perturbations used in the MAC studies.** Rats were maintained at approximately 1.0 MAC for 2 h. MAC was determined 30 min after administration of the control compound. MAC was determined 30 min after the administration of the treatment compound(s). Except for the norepinephrine and monoamine depletion studies, each group of rats served as its own control.
same experimental design as with idazoxan. To determine whether postsynaptic receptors were involved in the MAC reducing effects of dexmedetomidine, rats were treated with N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) or reserpine and alpha-methyl-para-tyrosine (AMPT). DSP-4, 50 mg/kg ip, was used to deplete endogenous norepinephrine in the central nervous system. Ten days later, MAC for halothane was determined in rats that received DSP-4 and control rats, before and after treatment with the three doses of dexmedetomidine. Reserpine, 5 mg/kg ip, and two doses of AMPT, 300 mg/kg ip, were used to deplete endogenous monoamines in the central nervous system. The dose of reserpine and a single dose of AMPT were administered 18 h prior to the experiment. The second dose of AMPT was administered 2 h before the experiment. Control rats received the vehicle ip. The MAC for halothane was determined in AMPT-treated or control rats, before and after administration of the three doses of dexmedetomidine. The effect of idazoxan treatment on the MAC-reducing action of dexmedetomidine was also tested in DSP-4 and AMPT-treated rats. In these rats, idazoxan, 10 mg/kg ip, was coadministered with dexmedetomidine and MAC was determined. At the conclusion of the experiments involving the catecholamine-depleted rats, animals were decapitated and the pons-medulla and remaining brain were rapidly removed, weighed, and stored at -70 C. These two brain regions were then analyzed for norepinephrine and dopamine content by HPLC with electrochemical detection as previously described. Since only inspired anesthetic concentration was measured, a correction was used to estimate alveolar concentration for halothane. Each group contained ten rats. The dose-dependency and stereospecificity experiments were done in quadruplicate; all other experiments were performed in triplicate. The mean and standard errors were calculated from the MAC determinations from group values. To test the stability of our model, MAC was determined repeatedly in a group of animals over the time course of a typical experiment. MAC did not change significantly over this period of time.

Data were analyzed by paired and unpaired t test with Bonferroni correction. Values of P < 0.05 were termed significant.

**Results**

Dexmedetomidine, the d-stereoisomer, decreased MAC for halothane in a dose-dependent fashion so that, at 100 μg/kg, halothane could be discontinued for up to 30 min without eliciting a purposeful response to tail-clamping (fig. 3). In contrast, treatment with levomedetomidine, the l-stereoisomer, did not change MAC (fig. 3). Idazoxan completely blocked the MAC-reducing action of dexmedetomidine, even at 100 μg/kg (fig. 4). Neither naltrexone nor 8-PT cotreatment changed the MAC reducing action of dexmedetomidine (fig. 4). DSP- 4 treatment decreased endogenous norepinephrine in the whole brain by 41% (P < 0.05) (table 1). This decrease in norepinephrine resulted in a significant (P < 0.05) reduction of the basal MAC in the DSP-4 treated rats (fig. 5). However, after dexmedetomidine treatment, there was a further reduction in halothane MAC (fig. 5) such that there was no difference in the MAC-reducing effect of 100 μg/kg of dexmedetomidine when rats receiving DSP-
Table 1. Effect of Treatment with DSP-4 or Reserpine and Alpha-methyl-para-tyrosine on Catecholamine Stores in the Rat Brain (Mean ± SD ng/g; [n])

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DSP-4</th>
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<tbody>
<tr>
<td>Whole brain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>564 ± 32</td>
<td>334 ± 188*</td>
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<tr>
<td>[10]</td>
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<tr>
<td>Pons-medulla</td>
<td></td>
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<tr>
<td>Norepinephrine</td>
<td>827 ± 134</td>
<td>573 ± 213*</td>
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<td>[10]</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>Whole brain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>516 ± 58</td>
<td>0*†</td>
</tr>
<tr>
<td>[10]</td>
<td></td>
<td></td>
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<tr>
<td>Dopamine</td>
<td>3044 ± 273</td>
<td>0*†</td>
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<tr>
<td>[10]</td>
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<tr>
<td>Pons-medulla</td>
<td></td>
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</tr>
<tr>
<td>Norepinephrine</td>
<td>848 ± 153</td>
<td>1 ± 4*</td>
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<tr>
<td>[10]</td>
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<td></td>
</tr>
<tr>
<td>Dopamine</td>
<td>88 ± 11</td>
<td>0*†</td>
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* P < 0.05 compared to control.
† These values were below the detection limit of the assay (0.1 ng/g).

From these studies, we may deduce that: 1) the d-isomer is stereospecific for the reduction of MAC caused by medetomidine in rats (fig. 5), 2) the reduction of MAC by dexmedetomidine is mediated through alpha2 adrenoceptors with no apparent involvement of either opiate or A1 adenosine receptors (fig. 4), and 3) the mechanism responsible for the above must involve site(s) other than...
the auto-inhibitory presynaptic α2adrenergic receptors on noradrenergic neurons because, in rats depleted of norepinephrine, MAC could still be reduced by dexmedetomidine (figs. 5, 6). The stereospecificity of the MAC-reducing property of dexmedetomidine suggests that this effect is mediated through a homogeneous receptor population. Indeed, we have confirmed the exclusive involvement of α2adrenergic receptors for the anesthetic action of dexmedetomidine with no apparent role for either purinergic or opioid receptors. α2adrenergic receptors are located presynaptically on all catecholaminergic pathways and, when stimulated, may inhibit both norepinephrine or dopamine release. Therefore, in our studies, we depleted both norepinephrine and dopamine (with AMPT) or norepinephrine nerve terminals alone (with DSP-4) to be certain that we had produced a lesion in each of the presynaptic catecholamine-containing nerve terminals that may be pertinent to the anesthetic response.

Apart from their presynaptic sites, α2adrenergic receptors are also located postsynaptically in the central nervous system, although their precise function at this site is not well understood. α2adrenergic agonists hyperpolarize locus coeruleus slices in the rat by increasing K+ conductance. Earlier, Nicoll had suggested that general anesthesia is characterized by hyperpolarization through an increase in K+ conductance and consequent depression of neuronal excitability. Thus, it is possible that α2adrenergic agonists supplement the anesthetized state by an increase in K+ conductance at postsynaptic sites in the central nervous system. The profound reduction in anesthetic requirements with dexmedetomidine raises the possibility that α2adrenergic agonists may be considered anesthetic agents in their own right, rather than adjunctive or supplemental drugs. If this is confirmed, the use of an anesthetic agent with a precisely known site and mechanism of action may advance the clinical practice of anesthesia in a number of ways, including: 1) the development of more selective and potent α2adrenergic agonists that are specific for a particular receptor location (i.e., pre-versus postsynaptic), and 2) the development of highly selective antagonists for this receptor site to rapidly reverse the anesthetic action. Further studies to investigate the clinical utility of this α2adrenergic agonist should take into consideration the fact that only the d-stereoisomer is active. In recently reported dog studies with dexmedetomidine, we observed little change in blood pressure and arterial blood gases. Therefore, we believe serious consideration should be given to performing early clinical studies with the pure d-stereoisomer.

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