Anesthetic and Hemodynamic Effects of the Alpha_2-adrenergic Agonist, Azepebole, in Isoflurane-anesthetized Dogs

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The authors studied the reduction in anesthetic requirement (MAC) and the hemodynamic effects of the highly selective alpha_2-adrenergic agonist azepebole in isoflurane-anesthetized dogs. Eleven male beagles were anesthetized with isoflurane in oxygen. After a 2-h equilibration period, they determined isoflurane MAC and baseline hemodynamic function. Azepebole (at 0.1, 0.3, and 1.0 mg/kg) was administered via a right arterial port over 15 min, while each dog was given isoflurane at the MAC dose for that animal. Twenty minutes after the end of infusion, at a time when hemodynamic variables were stable, they reassessed hemodynamic function. They then determined isoflurane MAC again. In other experiments, dogs were pretreated with either idazoxan (the alpha_2-adrenergic antagonist; n = 5) or naloxone (the opioid antagonist; n = 7) prior to the administration of azepebole. Isoflurane MAC was determined before and after each dose of azepebole. Isoflurane MAC decreased as the dose of azepebole increased, to the extent that at the highest dose (1 mg/kg) the decrement in MAC was more than 85%. This reduction in MAC caused by azepebole could be completely prevented by pretreatment with idazoxan, while naloxone was without effect. Azepebole did not change mean arterial blood pressure, but caused heart rate and cardiac output to progressively decrease. The MAC-reducing effect of azepebole appears to be mediated by alpha_2 adrenoceptors. Given the extent of the reduction in MAC, it is unlikely that inhibition of central noradrenergic neurotransmission through agonism of presynaptic alpha_2 adrenoceptors is the sole explanation, since complete disruption of central noradrenergic tracts decreases MAC by only 40%. The authors speculate that the recently demonstrated central postsynaptic alpha_2 adrenoceptors may play a role in the anesthetia-enhancing effect of these compounds. (Key words: Anesthesitcs, volatile; Isoflurane. Antagonists, alpha_2 adrenoceptor; idazoxan. Antagonists, opioid naloxone. Potency: MAC. Receptors, adrenoceptors: alpha_2, postsynaptic; alpha_2, presynaptic. Sympathetic nervous system, agonist, alpha_2: azepebole; 2-amino-6-ethyl-4,5,7,8-tetrahydro-6H-oxazolo-(5,4-d)-azepin dihydrochloride.)

**ALPHA_2 ADRENERGIC AGONISTS** exert a sympatholytic effect by stimulating the presynaptic alpha_2 adrenoceptors in the sympathetic nervous system. When activated, these receptors inhibit release of the neurotransmitter norepinephrine. Clinicians have used this pharmacologic property to advantage in settings which require an attenuation of noradrenergic neurotransmission. Clonidine, the prototypical alpha_2-adrenergic agonist, was introduced more than a decade ago into clinical practice for the treatment of mild to moderate hypertension. Subsequently, this drug has also been used in other hypernoradrenergic conditions, including those associated with opiate, alcohol, and tobacco withdrawal; chronic pain; and anxiety syndromes. Because of indirect evidence linking a decrease in central noradrenergic neurotransmission with an increased sensitivity to anesthetic agents, clonidine was also tried in the anesthetic paradigm. Clonidine reduces the dose requirements for anesthesia and analgesia during surgical stimulation. Most recently, clonidine was reported to attenuate hemodynamic responses to laryngoscopy and cardiopulmonary bypass surgery.

The anesthetic-sparing effect of clonidine has been ascribed to a decrease in central release of norepinephrine. However, other endogenous neuromodulators, especially endorphins, might be responsible for the central nervous system effects of clonidine. While clonidine is now being advocated as a supplemental agent for anesthesia or analgesia, it may not be the most appropriate of the generic class of alpha_2-agonists for use in this supplemental role. Unfortunately, a "ceiling effect," followed by a reversal of the initial effect, is obtained at higher clonidine doses. This property has been attributed either to its mixed agonist-antagonist effect at the alpha_2 adrenoceptor, or to an alpha_2 agonist action. Azepebole (B-HT 933) is a full alpha_2 agonist, and is more selective than clonidine for the alpha_2 adrenoceptor. Because of these properties, azepebole should be more efficacious than clonidine in its anesthetic-sparing effect, and should provide a selective probe to investigate the mechanism of this effect. In this study, we have 1) investigated the effect of azepebole upon the MAC of isoflurane in dogs; 2) characterized the hemodynamic effects of azepebole during isoflurane anesthesia; and 3) defined the contribution of the endogenous endorphin system to the MAC-reducing effect of the alpha_2 adrenergic agonists.

**Materials and Methods**

The experimental protocol was approved by the Animal Care and Use Committee of the Palo Alto VA Med-

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Anesthesia was induced by inhalation of isoflurane in oxygen from a mask in 11 male beagles (8–11 kg). Following tracheal intubation ventilation was controlled to maintain normocarbia (CO$_2$-ET = 4.5%). Catheters were inserted percutaneously for: 1) intra-arterial blood gas determination and pressure recording (femoral artery); 2) pulmonary arterial and central venous pressure monitoring and cardiac output (thermodilution) assessment; and 3) intravenous fluid and drug administration. End-tidal isoflurane and CO$_2$ concentrations (infrared analysis, Puritan-Bennett), heart rate and rhythm (lead II of the EKG), systemic arterial pressure, central venous pressure, pulmonary arterial pressure, and four lead surface EEG were continuously displayed and recorded. Core temperature was maintained at 37° C with insulating blankets and heating lamps. After a 2-h equilibration period, MAC for isoflurane was determined as previously described, and baseline hemodynamic function (mean arterial blood pressure, heart rate, central venous pressure, pulmonary arterial diastolic pressure, pulmonary artery occluded pressure, cardiac output, and derived systemic vascular resistance) was assessed. Azepekoxe, at three doses (0.1, 0.3, and 1.0 mg·kg$^{-1}$) was administered via the right atrial port over 15 min while maintaining the dog at its individual MAC for isoflurane. Ten minutes after termination of the infusion (at which time the hemodynamic profile was stable), hemodynamic function was reassessed. Also at this time, arterial blood was sampled for: 1) measurement of gas tensions and acid/base status; and 2) norepinephrine. The new MAC was then determined, following which the isoflurane concentration was increased to the baseline MAC value; the successive higher azepekoxe dose was given, and the above cycle was then repeated.

In separate experiments, dogs were prepared as described above and MAC determined. Thereafter, the animals were pretreated with either idazoxan (alpha$_2$-adrenoreceptor antagonist; n = 5) 0.3 mg·kg$^{-1}$, or naloxone (opiate antagonist; n = 7), 3 mg·kg$^{-1}$ bolus followed by an infusion of 0.025 mg·kg$^{-1}$·min$^{-1}$. This regimen has previously been shown to effectively block opiate receptors. MAC determination was then repeated, and again following each dose of azepekoxe.

Data were compared by ANOVA for repeated measurements and, subsequently, by paired t test with Bonferroni correction. A P value of <0.05 was considered the level for statistical significance.

**Results**

Arterial blood gas analysis confirmed no aberrations in acid-base status, ventilation, or oxygenation throughout the studies.

**Isoflurane MAC**

Following azepekoxe administration, the MAC for isoflurane progressively decreased such that, at the highest dose, the anesthetic requirement was decreased by almost 90% (fig. 1). The reduction of MAC by azepekoxe could be completely prevented by pretreatment with the selective alpha$_2$ adrenoreceptor antagonist idazoxan (fig. 2). Conversely, pretreatment with the opiate antagonist naloxone did not affect the MAC-reducing action of azepekoxe (fig. 2).
Hemodynamic Affects of Azepexole

At 1.0 MAC isoflurane, azepexole administration resulted in a progressive decrease in heart rate (fig. 3) and cardiac output (fig. 4). Mean arterial pressure (fig. 3), central venous, and pulmonary arterial pressures were unaffected by azepexole administration. While the derived systemic vascular resistance tended to increase after azepexole administration, this did not achieve statistical significance. Circulating norepinephrine levels decreased progressively (fig. 5) with increasing doses of azepexole such that, at the highest dose, plasma norepinephrine was below the detectable limit for our assay (<10 pg/ml) in some of the dogs.

The bradycardia following azepexole was manifested during naloxone, but not during idazoxan administration (table 1).

Discussion

Three major results follow from this study. First, azepexole caused a dose-dependent decrease in isoflurane MAC without an apparent “ceiling” effect. This effect of azepexole upon MAC could be completely prevented by pretreatment with the alpha2-adrenergic agonist idazoxan, but was unaffected by naloxone. Second, circulating norepinephrine was decreased significantly after successive treatments with increasing doses of azepexole. Third, mean arterial blood pressure did not change, but heart rate and cardiac output decreased progressively as the dose of azepexole was increased.

Mechanism for the Anesthetic-Sparing Effect of Azepexole

The firing rate of noradrenergic neurons has been correlated with different phases of the sleep-wake cycle in mammalian brain.29 Although the anesthetic state and the sleep phase of the sleep-wake cycle are not entirely analogous, they are remarkably similar in the manner in which they are affected by neurotoxics30 and neurolytic31 destruction of noradrenergic pathways. Nearly total depletion of norepinephrine stores resulted in a 40% reduction in MAC for volatile anesthetics.36,31 Other indirect evidence links central noradrenergic neurotransmission with depth of the anesthetic state. For example, acute administration of cocaine32 or amphetamine33 decreases the sensitivity of the brain to vol-

FIG. 3. Effect of azepexole on heart rate and blood pressure in isoflurane-anesthetized dogs. The heart rate — ■ — ■ and the mean arterial blood pressure — ○ — ○ (MAP) were measured in 11 isoflurane-anesthetized dogs at 1 MAC before (basal) and after azepexole 0.1, 0.3, and 1.0 mg/kg iv. *P < 0.05.

FIG. 4. Effect of azepexole on cardiac output in isoflurane-anesthetized dogs. Cardiac output (thermodilution technique) was measured in 11 isoflurane-anesthetized dogs at 1 MAC before (basal) and after azepexole 0.1, 0.3, and 1.0 mg/kg iv. *P < 0.05.

FIG. 5. Effect of azepexole on circulating norepinephrine levels in isoflurane-anesthetized dogs. Plasma norepinephrine levels were measured by HPLC-EC in 11 isoflurane-anesthetized dogs at 1 MAC before (basal) and after azepexole 0.1, 0.3, and 1.0 mg/kg iv. *P < 0.05.
Table 1. Effect of Azepeoxide on Mean Arterial Pressure and Heart Rate in Isoflurane-anaesthetized Dogs Pretreated with Idazoxan or Naloxone. For Comparison, Data (Mean [x] ± Standard Deviation [SD]) from Non-pretreated Animals (Fig. 3) are Included

<table>
<thead>
<tr>
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<th>Mean Arterial Pressure (mmHg)</th>
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<td></td>
<td>Basal</td>
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<tr>
<td>Idazoxan (%)</td>
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<td>(±SD)</td>
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<tr>
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<td>(±SD)</td>
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<td>13</td>
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<tr>
<td>No Rₐ (%)</td>
<td>91</td>
<td>—</td>
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<tr>
<td>(±SD)</td>
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* Significantly different from basals values by paired t test (P < 0.05).

null atile anesthetics, while both drugs increase norepinephrine at the neuroeffector junction in central noradrenergic pathways. Since circulating norepinephrine levels were decreased following azepeoxide administration, it is possible that azepeoxide inhibits central noradrenergic neurotransmission. However, norepinephrine release in the central nervous system was not directly assessed.

Idazoxan, the alpha₂ adrenoceptor antagonist, completely prevented the reduction in MAC caused by azepeoxide confirming the selectivity of this action to the alpha₂-adrenergic receptor. As reported earlier, the alpha₂ antagonist alone did not alter isoflurane MAC. This suggests that endogenous alpha₂-adrenergic ligands do not contribute to the depth of anesthesia in the basal state. It is unlikely that the putative inhibitory effect of azepeoxide on central noradrenergic neurotransmission is enough to explain the greater than 85% reduction in MAC that was seen in these experiments, because MAC is reduced no more than 40% when noradrenergic pathways are completely disrupted. Other endogenous neuromodulators, including endorphin and purine systems, might also be responsible for the central nervous system effects of alpha₂-adrenergic agonists. Clonidine increases central release of endorphin and the same final common pathway mediates the intracellular action of opiates and alpha₂-adrenergic agonists. However we were not able to block the MAC reduction following azepeoxide with opiate antagonists; therefore, an interaction at the level of the opiate receptor cannot be the cause of the effect of azepeoxide upon MAC.

Most of the alpha₂-adrenergic receptors in the central nervous system are located postjunctionally. Their functional significance at this site has yet to be determined, although it has been suggested that they play a role in behavioral responses, such as sedation. Therefore, it is important to consider that postjunctional alpha₂ adrenoceptors may mediate the reduction in anesthetic requirements caused by azepeoxide.

**HEMODYNOmic EFFECTS OF AZEPEOXIDE AT 1 MAC ISOFLURANE**

While mean arterial pressure was unaffected, there was a progressive decrease in heart rate and cardiac output concomitant with a tendency for the systemic vascular resistance to rise. In functionally denervated dogs and rats, the central hypotensive action of alpha₂-adrenergic agonists is not seen. Conversely, in the functionally denervated animal, an increase in systemic vascular resistance may occur through the vasoconstrictive effect, which is mediated by postsynaptic alpha₂-adrenoceptors located extrajunctionally on the peripheral vasculature. However, volatile anesthetic agents, especially halothane, may blunt the alpha₂-mediated vasconstrictive response. This may explain the lack of change in systemic arterial, central venous, and pulmonary arterial pressures after azepeoxide administration in animals anesthetized with isoflurane. We speculate that, in the functionally denervated isoflurane-anaesthetized dogs, neither the centrally mediated hypotensive effect nor the peripherally mediated hypertensive effect is manifested.

Azepeoxide decreases heart rate dose-dependently. In common with other alpha₂-adrenergic agonists, azepeoxide exerts both a centrally mediated vagomimetic effect and a sympathetic effect via the central ansa subclavian ("cardiac accelerator") nerve. Our study did not distinguish between these two possible mechanisms, although other studies suggest that the vagomimetic effect predominates.

There are many possible reasons for the decrease in cardiac output that was noted. First, the bradycardia might have resulted in a progressive decrease in cardiac output. Second, the modest increase in afterload could have contributed, in part, to the fall in cardiac output. Lastly, we speculate that the oxygen requirements and, hence, cardiac output might have decreased as anesthetic depth was increased with azepeoxide.
At lower doses of azepoxide, relatively modest changes in cardiovascular parameters were seen, suggesting that clinical trials to evaluate azepoxide as a supplement to general anesthesia can be conducted. Further studies to define: 1) the role of other endogenous neuromodulators; 2) the contribution from pre-versus postjunctional alpha_2 adrenoceptors; 3) the neuroanatomic site for this action; and 4) the clinical utility of such agents are being pursued.

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