Anesthetic and Hemodynamic Effects of the Alpha₂-adrenergic Agonist, Azepeoxole, 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 alpha₂ adrenergic agonist azepeoxole 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. Azepeoxole (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 alpha₂ adrenergic antagonist; n = 5) or naloxone (the opiate antagonist; n = 7) prior to the administration of azepeoxole. Isoflurane MAC was determined before and after each dose of azepeoxole. Isoflurane MAC decreased as the dose of azepeoxole increased, to the extent that the highest dose (1 mg/kg) the decrement in MAC was more than 85%. This reduction of MAC caused by azepeoxole could be completely prevented by pretreatment with idazoxan, while naloxone was without effect. Azepeoxole did not change mean arterial blood pressure, but caused heart rate and cardiac output to progressively decrease. The MAC-reducing effect of azepeoxole appears to be mediated by alpha₂ adrenoceptors. Given the extent of the reduction of MAC, it is unlikely that inhibition of central noradrenergic neurotransmission through agonism of presynaptic alpha₂ 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 alpha₂ adrenoceptors may play a role in the anesthesia-enhancing effect of these compounds. (Key words: Anesthetics, volatile: Isoflurane. Antagonists, alpha₂ adrenoceptor: idazoxan. Antagonists, opiate: naloxone. Potency: MAC. Receptors, adrenoceptors: alpha₂, postsynaptic; alpha₂, presynaptic. Sympathetic nervous system, agonist, alpha₂: azepeoxole; 2-amino-6-ethyl-4,5,7,8-tetrahydro-6H-oxazolo-(5,4-d)azepin dihydrochloride.)

ALPHA₂ ADRENERGIC AGONISTS exert a sympatholytic effect by stimulating the presynaptic alpha₂ 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 alpha₂-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 anesthetics 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 alpha₂ 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 alpha₂ adrenoceptor, or to an alpha₁ agonist action. Azepeoxole (B-HT 933) is a full alpha₂ agonist, and is more selective than clonidine for the alpha₂ adrenoceptor. Because of these properties, azepeoxole 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 azepeoxole upon the MAC of isoflurane in dogs; 2) characterized the hemodynamic effects of azepeoxole during isoflurane anesthesia; and 3) defined the contribution of the endogenous endorphin system to the MAC-reducing effect of the alpha₂ adrenergic agonists.

Materials and Methods

The experimental protocol was approved by the Animal Care and Use Committee of the Palo Alto VA Med.
Fig. 1. Effect of azepoxe on isoflurane MAC. The MAC for isoflurane in oxygen was determined in dogs (n = 11) before (basal) and after azepoxe 0.1, 0.3, and 1.0 mg/kg iv. *P < 0.05.

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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. Azepoxe, 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 azepoxe 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 naloxyone (opioid 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 opioid receptors. MAC determination was then repeated, and again following each dose of azepoxe.

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 azepoxe 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 azepoxe could be completely prevented by pretreatment with the selective alpha$_2$ adrenoreceptor antagonist idazoxan (fig. 2). Conversely, pretreatment with the opioid antagonist naloxyone did not affect the MAC-reducing action of azepoxe (fig. 2).
Hemodynamic Effects of Azepepxole

At 1.0 MAC isoflurane, azepepxole administration resulted in a progressive decrease in heart rate (fig. 3) and cardiac output (fig. 4). Mean arterial (fig. 3), central venous, and pulmonary arterial pressures were unaffected by azepepxole administration. While the derived systemic vascular resistance tended to increase after azepepxole administration, this did not achieve statistical significance. Circulating norepinephrine levels decreased progressively (fig. 5) with increasing doses of azepepxole 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 azepepxole was manifested during naloxone, but not during idazoxan administration (table 1).

Discussion

Three major results follow from this study. First, azepepxole caused a dose-dependent decrease in isoflurane MAC without an apparent “ceiling” effect. This effect of azepepxole upon MAC could be completely prevented by pretreatment with the alpha-2-adrenergic agonist idazoxan, but was unaffected by naloxone. Second, circulating norepinephrine was decreased significantly after successive treatments with increasing doses of azepepxole. Third, mean arterial blood pressure did not change, but heart rate and cardiac output decreased progressively as the dose of azepepxole was increased.

Mechanism for the Anesthetic-Sparing Effect of Azepepxole

The firing rate of noradrenergic neurons has been correlated with different phases of the sleep-wake cycle in mammalian brain. 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 neurotoxicity and neurolytic destruction of noradrenergic pathways. Nearly total depletion of norepinephrine stores resulted in a 40% reduction in MAC for volatile anesthetics. Other indirect evidence links central noradrenergic neurotransmission with depth of the anesthetic state. For example, acute administration of cocaine or amphetamine decreases the sensitivity of the brain to vol-

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

Fig. 4. Effect of azepepxole 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 azepepxole 0.1, 0.3, and 1.0 mg/kg iv. *P < 0.05.

Fig. 5. Effect of azepepxole 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 azepepxole 0.1, 0.3, and 1.0 mg/kg iv. *P < 0.05.
TABLE 1. Effect of Azepeoxone on Mean Arterial Pressure and Heart Rate in Isoflurane-anesthetized Dogs Pretreated with Idazoxan or Naloxone. For Comparison, Data (Mean [SD] ± Standard Deviation [SD]) from Non-pretreated Animals (Fig. 3) are Included

<table>
<thead>
<tr>
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<th>Mean Arterial Pressure (mmHg)</th>
<th>Heart Rate (BPM)</th>
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<td></td>
<td>Basal</td>
<td>Post R₄</td>
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<td></td>
<td>0.1</td>
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<td>Idazoxan (s)</td>
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<td>104</td>
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<tr>
<td>(±SD)</td>
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<td>Naloxone (s)</td>
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<td>(±SD)</td>
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<td>13</td>
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<tr>
<td>No R₄ (s)</td>
<td>91</td>
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<tr>
<td>(±SD)</td>
<td>16</td>
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* Significantly different from basal values by paired t test (P < 0.05).

Hemodynamic Effects of Azepeoxone 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-adrenoreceptors located extrajunctionally on the peripheral vasculature. However, volatile anesthetic agents, especially halothane, may blunt the alpha₂-mediated vasoconstrictive response. This may explain the lack of change in systemic arterial, central venous, and pulmonary arterial pressures after azepoxone administration in animals anesthetized with isoflurane. We speculate that, in the functionally denervated isoflurane-anesthetized dogs, neither the centrally mediated hypotensive effect nor the peripherally mediated hypertensive effect is manifested.

Azepeoxone decreases heart rate dose-dependently. In common with other alpha₂-adrenergic agonists, azepoxone exerts both a centrally mediated vagomimetic effect and a sympatholytic action via the ventral ansa subclaviana (“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 azepoxone.
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 alpha2 adrenolectors; 3) the neurotransmitter site for this action; and 4) the clinical utility of such agents are being pursued.

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