**ATP-sensitive Potassium Channel Agonists Do Not Alter MAC for Isoflurane in Rats**

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The molecular mechanisms for general anesthesia are probably restricted to a sensitive set of target sites in the brain. Membrane hyperpolarization, brought about by increased potassium channel conductance, is coupled to opiate δ receptors, to α2-adrenoceptors, and to muscarinic M2 receptors, all of which have anesthetic-sparing effects. One type of potassium channel, the ATP-sensitive potassium channel (IKATP) has well-known agonists: cromakalim and pinacidil. The effects on isoflurane minimum alveolar concentration (MAC) of intracerebroventricular injection of these IKATP agonists and of the α2-adrenoceptor agonist clonidine were studied in rats. Baseline MAC was 1.60% (± 0.02 SEM) isoflurane in oxygen. 10 μg clonidine decreased MAC by 42% of baseline (P < 0.05); 20 μg clonidine decreased MAC by 58% of baseline (P < 0.01). Neither cromakalim (20 μg) nor pinacidil (20 μg) had any effect on MAC. The results imply that neither indiscriminate agonist action of volatile anesthetics at potassium channels nor indiscriminate inhibitory membrane hyperpolarization is likely to be a fundamental mechanism of anesthetics. Furthermore, potassium channels coupled to opiate δ receptors, to α2-adrenoceptors, and to muscarinic M2 receptors are probably not the IKATP type. (Key words: Anesthetic Action; theories. Anesthetics, volatile; isoflurane. Membrane, cell; potassium channel. Potency: MAC.)

**Several lines of evidence** suggest that potassium channels in the central nervous system are likely to be the ultimate target sites underlying the molecular basis of general anesthesia. Franks and Lieb have demonstrated a novel anesthetic-activated potassium current in a discrete subset of mollusc neurons.1 The half-maximal response of that potassium channel for halothane coincided with the halothane minimum alveolar concentration (MAC) in humans. Additional evidence for a possible role played by potassium channels comes from work with drugs that decrease anesthetic requirement and that act via potassium channels in the brain. α2-Adrenoceptor agonists decrease the requirement for several different types of anesthetic agents2-4 and also possess hypnotic-anesthetic action.5 In the locus coeruleus, stimulation of α2 adrenoceptors causes at least two separate mechanisms to work in concert. Two G protein–mediated postreceptor effector mechanisms produce inhibitory hyperpolarization: inhibition of adenylate cyclase through an inhibitory guanosine triphosphate binding protein (via Gi) to produce an opening of potassium channels, and a separate cAMP-independent (via Gs) opening of potassium channels.5 The identical two postreceptor G protein–mediated hyperpolarizing potassium channels are shared with opiate δ receptors in the locus coeruleus and probably elsewhere.6,7 A similar population of hyperpolarizing inwardly rectifying potassium channels is activated by agonist action at M2 muscarinic receptors.8,9 Like α2 agonists and opiate δ agonists, intracerebroventricular (icv) injection of an M2 muscarinic agonist reduces anesthetic requirement.10 Heteromultimeric potassium channels11 are expressed by a large gene family in mammalian brain12; such heteropolymerization and differential mRNA expression13 contribute to a functional diversity of potassium channels. Distinct ATP-sensitive potassium channels (IKATP) represent a type of potassium channel possibly involved in drug-induced inhibitory membrane hyperpolarization in the brain.14,15 Like the novel anesthetic-activated potassium channel identified by Franks and Lieb1 and the potassium channels coupled to opiate δ, α2-adrenergic, and M2 muscarinic receptors, IKATP is not appreciably voltage-gated.

Recently, two new drugs, cromakalim (BRL34915) and pinacidil (LY164021), have been identified as potent yet selective agonists of this IKATP.16,17 Both drugs hyperpolarize the cell membrane by increasing conductance through IKATP.18 To investigate whether inhibitory neuronal cell membrane hyperpolarization brought about by increased potassium channel conductance might play a role in the mechanism of general anesthesia, we measured the MAC for isoflurane in rats and examined the effect on MAC of icv injection with cromakalim and pinacidil. This study tested the hypothesis that icv administration of IKATP agonists would decrease MAC for isoflurane.

**Materials and Methods**

Male Sprague-Dawley rats weighing 295 g (± 3.7 SEM) were used in the study, which was approved by the Uni-
versity of Washington’s animal investigation committee. The rats were housed in the Medical School vivarium and maintained on a 12-h light–dark cycle and provided with food and water ad libitum. An intracerebral injection guide cannula was implanted in each of the rats at least 7 days prior to an experiment, and drugs dissolved in 1 μl sterile pyrogen-free physiologic saline at neutral pH were microinjected into the right ventricle of anesthetized rats as previously described. Sites of microinjection were later verified by histologic examination of the brains of animals by an observer unaware of the experimental findings.

Incremental amounts of drug (5, 10, and 20 μg) were used. Drug treatment was initiated at 5 μg in a group of experimental animals. If no treatment effect was observed, the experiment was repeated in the next group of animals; the same strategy of incremental amounts (5, 10, and 20 μg) was superimposed on each animal of the group. The following drugs were injected ivc: clonidine hydrochloride and 4-aminopyridine (4-AP, a tertiary amine with potassium and other ion channel-blocking properties) obtained from Sigma Chemical Company (St. Louis, MO); cromakalim (BRL34915) obtained from Beecham Pharmaceuticals Research Division (Betchworth, Surrey); pinacidil monohydrate (LY164021) obtained from Lilly Research Laboratories (Indianapolis, IN); or an equivalent volume of saline.

Fifteen hours before the experiment, rats were moved from the vivarium to the research laboratory to be weighed, inspected for any gross evidence of sickness, and allowed to habituate themselves to the experimental environment. Only healthy rats with a weight gain of 10–15 g for the week following surgery were included in the study. All experiments were performed at the same time each day (between 8 AM and 2 PM). At the start of the experiment, the animals were placed in a clear Plexiglas box and exposed to a gas mixture of 5% isoflurane in oxygen until anesthetized 3–4 min later. They were then removed from the box and placed in a specially constructed apparatus that included an oxygen delivery system with an isoflurane vaporizer in line. The gas was delivered to a hollow cylindrical 250-ml vessel with a hole in the side near the proximal fresh gas inlet. This hole was sealed with a rubber diaphragm and an aperture that fit over the snout and mouth of a rat. The fresh gas flow of 3 l/min of oxygen was exhausted passively from the distal end. At each anesthetic concentration delivered, the gas in the apparatus was sampled by withdrawal into a glass syringe and then assayed for isoflurane concentration on a calibrated gas chromatograph. Isoflurane concentration in the apparatus correlated closely (r = .965, P < .001 [two-tail]) with the concentration of isoflurane set for delivery at the vaporizer. The entire apparatus was housed in a fume hood.

Spontaneously breathing anesthetized rats were placed in the apparatus and exposed to inspired isoflurane concentration of 2.25% in oxygen for 5 min. The inspired anesthetic concentration was then reduced in a stepwise fashion until MAC for that animal was determined. MAC was determined by the rat’s response to the nontraumatic application of a spring-loaded alligator clamp for 2 min at the base of the tail. Purposeful movement of head, trunk, or legs was considered a response. Stiffening, hyperventilation, or vocalizing was not considered to be a response. Animals without response had their anesthetic concentration reduced stepwise by 0.125% until they responded. Following each change in anesthetic concentration, 15 min was allowed for stabilization of the gas concentrations. Body temperature of the rats was maintained at 37° C by means of a warming blanket attached to a servomechanism that recorded temperature from a rectal lubricated temperature probe inserted 3 cm. Once baseline MAC had been determined for each animal, the inspired isoflurane concentration was readjusted to 2.25%, and the ivc injections performed. Following the injection, MAC was determined again. After the final MAC concentration was maintained for 15 min, blood was drawn from the artery at the base of the tail and placed on ice for measurement of blood gas tensions within 10 min.

Groups of 10–15 animals were allocated for study with each drug manipulation. However, the final number analyzed in each group varied because of attrition from illness, lost cannulae, and ivc injection sites that were not acceptable. Baseline MAC, MAC after treatment, change in MAC between treatments, and blood gas values in the different treatment groups were compared with values in their saline-treated control group by one-way analysis of variance. In addition, differences between the baseline MAC and MAC after treatment within each treatment group were compared with paired t tests after confirming normality of distribution of the data. P values of less than 0.05 were regarded as significant.

Results

Figure 1 illustrates the effect of treatments on MAC. Baseline MAC for isoflurane in oxygen averaged 1.60% (± 0.02 SEM) and was the same in all treatment groups. MAC after treatment with ivc saline (n = 13) or with sham injections (cannulae were plugged, n = 17) was the same as baseline. In contrast, treatment with 10 μg clonidine (n = 12) significantly decreased MAC by 42% of baseline (P < 0.05); 20 μg clonidine (n = 10) decreased MAC by 58% of baseline (P < 0.05).

Neither treatment with cromakalim (20 μg, n = 16), nor treatment with pinacidil (20 μg, n = 14) had any no-
Effect of Treatment on Isoflurane MAC

![Graph showing the effect of treatment on isoflurane MAC](image)

**Fig. 1.** The columns represent the percentage of isoflurane in oxygen (+SEM) that was MAC for the following groups of rats: 1. Baseline MAC (n = 155); 2. repeat MAC after intracerebroventricular (icv) saline injection (n = 13); 3. repeat MAC after sham injection (n = 17); 4. repeat MAC after 10 μg icv clonidine injection (n = 12); 5. repeat MAC after 20 μg icv clonidine injection (n = 10); 6. repeat MAC after 20 μg icv cromakalim injection (n = 16); 7. repeat MAC after 20 μg icv pinacidil injection (n = 14); 8. repeat MAC after 20 μg icv 4-aminopyridine injection (n = 12). *P < 0.05 compared with group 1 and with group 2.

ticeable effect on MAC redetermined within 60 min after icv drug injection. For both drugs, this 20-μg dose used was calculated to exceed the effective in vitro dose by several hundred-fold. Similarly, treatment with 4-AP (20 μg, n = 12) had no effect on the value of repeat MAC measurement. MAC values of animals treated with 10 μg icv clonidine and 5 min later with additional icv cromakalim (20 μg) or with additional pinacidil (20 μg) were no different from the MAC values of animals treated with 10 μg icv clonidine. (Neither were they different from MAC values of animals treated with icv clonidine and 5 min later with additional icv saline).

None of the icv treatments (saline, clonidine, cromakalim, pinacidil, or 4-AP) produced any significant effect on recorded temperature. Arterial blood analyses for pH (7.393 ± 0.008 SEM), P CO₂ (45.9 ± 1.17 SEM), P O₂ (382.3 ± 14.05 SEM), and base excess (2.92 ± 0.29 SEM) were no different in the treatment groups.

**Discussion**

Many anesthetic agents, such as γ-aminobutyric acid-mimetic chloride channel agonists, opioids, and α₂-adrenoceptor agonists hyperpolarize neuronal cell membranes by a variety of mechanisms. Similarly, agonist action at IKATP also hyperpolarizes cell membranes. In the brain, IKATP is localized to the presynaptic position on a heterogeneous distribution of neuronal cells. The icv drug injections used in this study presumably distributed the drugs throughout the brain at sufficiently high concentration to have an effect on all potential sites of action in the brain. Several conclusions can be drawn from the current observation that the MAC for isoflurane in rats was unaffected by icv treatment with two agonists of IKATP, cromakalim, and pinacidil. First, indiscriminate agonist action at potassium channels per se is unlikely to be a fundamental mechanism of anesthesia. Second, indiscriminate inhibitory membrane hyperpolarization is also unlikely to be a fundamental mechanism of anesthesia.

The results do not rule out the possibility that inhibitory membrane hyperpolarization or potassium channel agonism play a role in the mechanism of anesthesia. However, if neither of these two mechanisms were to play a role, the results imply that they must be discretely localized either to a particular brain region, to a particular set of neurons, or to a particular type of potassium channel in order to produce anesthetic effects. Another conclusion that can be drawn from these results concerns the potassium channels known to be coupled to opiate μ receptors, to α₂-adrenoceptors, and to muscarinic M2 receptors. While agonist action at these latter receptors has anesthetic-sparing effects, agonist action at IKATP in the brain did not have anesthetic-sparing effects. The potassium channels coupled to these receptors are therefore unlikely to be predominantly of the IKATP type.

MAC was also unaffected by icv treatment with 4-AP, a tertiary amine capable of blocking a variety of ion channels, including IKATP. These results are consistent with a previous report by Maze and co-workers who nevertheless concluded that the basis for the anesthetic action of dexmedetomidine (an α₂-adrenoceptor agonist) was related to an action at a coupled 4-AP sensitive potassium conductance channel. However, the effect of 4-AP on dexmedetomidine's apparent potency does not necessarily imply that dexmedetomidine acts via a coupled 4-AP sensitive channel. For example, 4-AP blockade of non-voltage-gated potassium channels that are independent of α₂-adrenoceptors or blockade of other ion channels such as the voltage-gated potassium channels with resulting compensatory excitatory neurotransmitter release could be just as likely an explanation. Unpublished data from our laboratory show that veratridine, which mimics the depolarizing effect of high extracellular potassium concentrations, attenuated the anesthetic-sparing effect of clonidine. Therefore, the mechanism of action in the brain responsible for the anesthetic-like effect of α₂-agonists may turn out to be surprisingly similar to that responsible for the effect of opioids. They may both decrease neurotransmitter release from presynaptic terminals by action at similar or shared postreceptor effector mechanisms.
In summary, we studied a class of non-voltage-gated potassium channels, similar to a class of potassium channels coupled to receptors that have anesthetic sparing properties. Agonist action at these potassium channels, known to be well represented in the brain, did not result in any change in MAC for isoflurane in rats. Neither indiscriminate agonist action at potassium channels nor indiscriminate inhibitory membrane hyperpolarization is likely to be a fundamental mechanism of anesthesia.

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