The ATP-Sensitive Potassium Channel Opener Alters MAC for Halothane but not for Isoflurane in Dogs

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Volatile anesthetics would exert their effects by acting at specific target proteins in the central nervous system. Neuronal membrane hyperpolarization brought about by increased potassium channel conductance is hypothesized to contribute to a mechanism of volatile anesthetic action. The purpose of this study was to determine whether the activation of ATP-sensitive potassium (K<sub>ATP</sub>) channels might play a potential role in the mechanism of anesthetic action. Fourteen dogs were anesthetized with halothane or isoflurane. Following determination of the control minimum alveolar anesthetic concentration (MAC) of either anesthetic by a tail-clamp technique, KRN2391, a novel K<sub>ATP</sub> channel opener, was infused intravenously at a dose of 3 µg·kg<sup>-1</sup>·min<sup>-1</sup> over 30 min and the MAC was determined again. The plasma concentration of KRN2391 was measured in all dogs. In additional 3 dogs the time courses of KRN2391 concentration in plasma and cerebrospinal fluid (CSF) were determined. The MAC for halothane was significantly reduced from 0.86±0.15% to 0.63±0.12% (P<0.01) by KRN2391 at a plasma level of 77 ng/ml. In contrast the MAC for isoflurane was not altered by this compound. The CSF concentration of KRN2391 increased gradually during intravenous administration. The results suggest that activation of K<sub>ATP</sub> channels would be involved in the mechanism of anesthetic action of halothane, whereas K<sub>ATP</sub> channels would not play a role in isoflurane anesthesia.

Key words: halothane, isoflurane, potassium channels, minimum alveolar concentration

Introduction

Volatile anesthetics would exert their effects by acting at specific target proteins in the central nervous system (CNS)\textsuperscript{1-4}. A large body of work is in favor of direct interactions between volatile anesthetics and channel proteins\textsuperscript{5-8}. The increase in potassium conductance via potassium channels bringing about membrane hyperpolarization of central neurons is thought to contribute to the anesthetic production\textsuperscript{9-12}. Recently, several lines of evidence suggest that ATP-sensitive potassium channels (K<sub>ATP</sub> channels) are widely distributed in CNS\textsuperscript{13} and play an important role in the modulation of CNS neurotransmitter release\textsuperscript{14,15}. Moreover, a few studies have demonstrated that the central administration of K<sub>ATP</sub> channel openers produced antinociception or potentiated morphine-induced antinociceptive effect, and that K<sub>ATP</sub> channel blockers antagonized morphine-induced antinociception\textsuperscript{16-18}. These investigations raise a question whether the activation of K<sub>ATP</sub> channels may play a substantial role in the mechanism of general anesthesia.

Zucker\textsuperscript{19} reported that minimum alveolar concentration (MAC) for isoflurane in rats was unaffected by intrathecal administration of two agonists of K<sub>ATP</sub> channels, cromakalim and pinacidil, concluding that activation of K<sub>ATP</sub> channels was unlikely to be a fundamental mechanism of anesthesia. On the other hand, Maclver and Kendig\textsuperscript{4} compared the effects of halothane, isoflurane and enfurane on the resting membrane potential and conductance of rat hippocampal CA1 neurons in vitro. They showed that anesthetic effects on resting membrane potential were voltage-dependent and agent-specific, suggesting that each volatile anesthetic might involve distinct ion channels. The present study was designed to clarify whether activation of K<sub>ATP</sub> channels might play a potential role in the mechanism of anesthetic action of halothane or isoflurane. We measured MAC of halothane and isoflurane in dogs in the absence or presence of a novel potassium channel opener, KRN2391 [N-cyano-N'(2-nitroxyethyl)-3-pyridine-carboximidamide monomethanesulfonate] via intravenous administration. KRN2391 is a novel vasodilator, which shows a similar magnitude in inducing 86Rb efflux via K<sub>ATP</sub> channels to that produced by cromakalim or by pinacidil, and has the property of crossing the blood brain barrier\textsuperscript{20,21}.

Methods

All experimental procedures and protocols described in this study were approved by the Animal Care Committee of Nagasaki University School of Medicine. General anesthesia was induced by one of two volatile anesthetics, halothane or isoflurane, in 100% oxygen via mask in 14 healthy mongrel dogs of either sex weighing 12-15 kg. Tracheal intubation was accomplished with a cuffed
endotracheal tube without the use of muscle relaxants. Mechanical ventilation was begun with a ventilator. End-tidal CO₂ concentration was measured continuously by an infrared gas analyzer (Ultima, USA) and maintained at levels of 35-40 mmHg by adjusting the respiratory rate. Catheters were inserted into the right femoral artery and vein for arterial blood sampling and pressure monitoring and for intravenous fluid and drug administration. A heating lamp and an electrical blanket were used to maintain esophageal temperature at 37.0-38.5°C. Lead II of ECG was monitored continuously. Both halothane and isoflurane concentrations were measured by means of an infrared gas analyzer calibrated automatically for each study according to the manufacturer’s specifications.

The MAC of halothane or isoflurane necessary to prevent purposeful movement in response to tail clamping was determined according to the method of Eger et al. Briefly, a 10-in hemostat was applied to the dog’s tail 4-in from the base to the full ratchet continuously for 60 sec or until purposeful movement in response to the tail-clamp stimulus. A positive was defined as gross movement of the head or extremities and did not include coughing, chewing, swallowing, or increased respiratory effort. The end-tidal halothane or isoflurane concentration was adjusted either up or down stepwise by approximately 0.05-0.10% with an equilibration period of at least 15 min before each tail clamping. MAC was calculated as the halothane or isoflurane concentration midway between that which allowed and that which prevented movement. A first baseline halothane or isoflurane MAC was determined after 1 h of anesthetic administration. A second halothane or isoflurane MAC was determined during KRN2391 (Kirin Brewery Co., Ltd., Gunma, Japan) administration. Following determination of the baseline MAC, the animal was anesthetized with halothane by the same manner as described above except for a fixed end-tidal concentration of 1%, and KRN2391 was administered intravenously at a dose of 3 μg · kg⁻¹ · min⁻¹. With the animal in the lateral position, the cranio-cervical area was shaved, and a 20-G spinal needle was inserted percutaneously into the subarachnoid space at the atlanto-occipital interspace. As clear CSF without blood mixing was identified, the infusion of KRN2391 was started, and samples from the two compartments, blood and CSF, were collected at 0, 15, 30 and 45 min after KRN2391.

Blood samples were centrifuged to separate plasma. The plasma and CSF samples were frozen and stored at −70 °C until assay. The plasma and CSF KRN2391 concentrations were performed using high-performance liquid chromatography (HPLC). The results were expressed as mean±SD and analyzed statistically by the two-tailed t test for paired data. P <0.05 was considered statistically significant.

Results

As shown in Fig. 1, the baseline MAC for halothane was 0.86±0.15%. KRN2391 reduced halothane MAC by 27% to 0.63±0.12% (P<0.01). In contrast, as shown in Fig. 2, the MAC for isoflurane was not significantly changed by KRN2391, i.e., 1.24±0.21% and 1.25±0.21% before and after KRN2391, respectively. Table 1. shows the plasma concentration of KRN2391, hemodynamics and acid base balance during halothane and isoflurane anesthesia. The mean plasma KRN2391 level at the time when the halothane MAC was determined was 77.6 ng/ml, and was similar to that of isoflurane group (78.4 ng/ml). The mean arterial pressure (MAP) significantly decreased by KRN2391 infusion during either anesthetic. However, there was no significant difference in the MAP between halothane and isoflurane anesthetized dogs, and there was no severe reduction in the MAP throughout the time course in either group (>70 mmHg). The acid base status and the respiratory condition were maintained in the normal range.

<table>
<thead>
<tr>
<th>Anesthetics</th>
<th>Time †</th>
<th>KRN2391 ‡</th>
<th>Mean Arterial Pressure § mmHg</th>
<th>Arterial Blood Gases ¶ mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td>ng/ml</td>
<td>Pre-</td>
<td>Post-</td>
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<tr>
<td>Halothane</td>
<td>80±21</td>
<td>77±28</td>
<td>101±14</td>
<td>78±12*</td>
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<tr>
<td>(n=7)</td>
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<td>7.41±0.03</td>
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<td>458±69</td>
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<td>36±2</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>83±11</td>
<td>78±37</td>
<td>109±18</td>
<td>82±10*</td>
</tr>
<tr>
<td>(n=7)</td>
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<td>7.39±0.04</td>
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<td>425±91</td>
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<td>37±1</td>
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Date are means±SD
† Elapsed time between the two MAC determinations.
‡ Plasma KRN2391 concentration at post-KRN2391 MAC.
* P<0.01 compared with MAP before KRN2391.
§ Mean arterial blood pressure before and after KRN2391 infusion.
¶ Arterial blood gases at post-KRN2391 MAC.
Fig. 1. Effects of KRN2391 on the MAC for halothane and isoflurane (mean±SD; n = 7 for each point). KRN2391 was infused at 3 μg·kg⁻¹·min⁻¹, and MAC was determined by the tail-clamp technique before (pre) and after (post) infusion of KRN2391 in dogs. * P<0.01 vs pre-KRN2391.

in either group, and there was no significant difference in pH, PO₂ or PCO₂ between the groups. The mean period of time between the pre- and post- MAC determination was 80.7 min in halothane and 83.6 min in isoflurane group, and there was no significant difference between the groups.

Fig. 2 shows the time course of plasma and CSF concentrations of KRN2391 infused at a dose of 3 μg·kg⁻¹·min⁻¹. The plasma KRN2391 reached near maximum level within 15 min, although the level increased gradually thereafter. KRN2391 was sufficiently detected in the CSF at 15-min infusion, and the level increased as time went by.

Discussion

The present findings of a decrease in MAC with intravenous administration of a potassium channel opener, KRN2391, for halothane but not for isoflurane in dogs suggest that activation of KATP channels would be involved in halothane-induced general anesthesia, whereas KATP channels would not play a role in the anesthetic action of isoflurane.

KATP channels were originally described in cardiac muscle, but have now been well characterized in a variety of peripheral tissues. Recently, these channels have also been investigated in the CNS. KATP channels are present in a wide variety of neurons in CNS, including the substantia nigra, hippocampus, globus pallidus, motor neocortex, and spinal dorsal horn, and appear to be involved in the regulation of neuronal excitability.

It was demonstrated that opioid and adenosine produce anesthetic-sparing effects through both μ- and δ- opioid receptors and A₁-adenosine receptors, respectively. The molecular mechanisms involved in the analgesic state are believed that stimulation of μ- or δ-opioid receptors and A₁-adenosine receptors open potassium channels resulting in membrane hyperpolarization in several areas of the CNS. Recently, some studies have indicated that KATP channels are coupled to several central neuronal pathways, and regulate some neurotransmitter releases and transmitter-mediated responses. The analgesic effects caused by activating opioidergic pathways have been demonstrated to involve the opening of KATP channels.

Ocana et al observed that the administration of cromakalim dose-dependently potentiated the antinociceptive action of morphine in tail-flick test in mice, and that KATP channel antagonists abolished the antinociception. Likewise, recent evidence has also demonstrated a link between adenosine receptors and KATP channels in myocytes and brain. Li and Henry showed that neuronal membrane hyperpolarization produced by adenosine via A₁-adenosine receptors was depressed by glibenclamide in vitro. The antinociception induced by (-)-N6-(2-phenylisopropyl)-adenosine, an adenosine A₁ receptor agonist, was shown either to be potentiated by cromakalim or to be antagonized by KATP blocker, gliquidone. These studies together with our results suggest that the action of isoflurane is unlikely related to an action on KATP channels in the CNS. In contrast, the activation of KATP channels would be involved in the anesthetic action of halothane.

Maciver and Kendig compared the effects of halothane and isoflurane on resting membrane potential and conductance of hippocampal CA1 neurons in vitro. At the equianesthetic levels, halothane increased membrane con-
KRN2391 does not appear to be confined exclusively to
suggesting that KRN2391 agonizes both KATP channels and
only by glibenclamide but also by TEA and 4-AP.21' sug-
vasorelaxation caused by KRN2391 was antagonized not
halothane.

Therefore, the moderate hypotension induced by KRN2391
ate reduction but was kept over 70 mmHg in both groups.
present study, the arterial blood pressure showed a moder-
except the mean blood pressure below 50 mmHg.3') In the
moderate systemic hypotension per se does not alter MAC
was not changed by KRN2391 in spite of a similar
isoflurane would not affect KATP channels.
possible that halothane would activate KATP channels and
channel openers may contribute to the mechanism involved
in the altering effects of halothane. It is considered
that halothane would activate KATP channels and
KRN2391 potentiates the halothane effect, whereas
KRN2391 potentiates the halothane effect, whereas
isoflurane would not affect KATP channels.
It is unlikely that halothane MAC reduction may be due
to the hypotension caused by KRN2391, because isoflurane
MAC was not changed by KRN2391 in spite of a similar
decrease in blood pressure. It has been suggested that
moderate systemic hypotension per se does not alter MAC
except the mean blood pressure below 50 mmHg.20'. In the
present study, the arterial blood pressure showed a moder-
ate reduction but was kept over 70 mmHg in both groups.
Therefore, the moderate hypotension induced by KRN2391
is not a tenable explanation for the alteration of MAC for
halothane.

There was a considerable evidence to show that the
vasorelaxation caused by KRN2391 was antagonized not
only by glibenclamide but also by TEA and 4-AP.20', sug-
suggesting that KRN2391 agonizes both KATP channels and
voltage-gated potassium channels. Thus the action of
KRN2391 does not appear to be confined exclusively to

KATP channels. Either KATP channels or voltage-gated
potassium channels or both may be involved in the mecha-
nism of the KRN2391-induced halothane MAC reduction.
However, Franks and Lieb28' have summarized from avail-
able evidence that voltage-gated potassium channels are
very resistant to clinical concentrations of general anes-
thesics, and appear unlikely to play a potential role in the
production of anesthetic state.

In conclusion, as a proof of the hypothesis that different
anesthetic agents might produce a common endpoint by
differential mechanisms via own specific targets, the
present study demonstrates that activation of KATP chan-
nels would be involved in the mechanism of anesthetic
action of halothane, whereas KATP channels would not play
a role in isoflurane anesthesia.

Acknowledgements

Authors would like to thank Drs Y. Jinno and H. Sato,
The Pharmaceutical Research Laboratory, Kirin Brewery
Co., Ltd. for supplying KRN2391, and for technical
assistance for KRN2391 assay.

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