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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_{ATP}) 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_{ATP} channel opener, was infused intravenously at a dose of 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ 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_{ATP} channels would be involved in the mechanism of anesthetic action of halothane, whereas K_{ATP} 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)[1-4]. A large body of work is in favor of direct interactions between volatile anesthetics and channel proteins[5-10]. The increase in potassium conductance via potassium channels bringing about membrane hyperpolarization of central neurons is thought to contribute to the anesthetic production[11,12]. Recently, several lines of evidence suggest that ATP-sensitive potassium channels (K_{ATP} channels) are widely distributed in CNS[13] and play an important role in the modulation of CNS neurotransmitter release[14,15]. Moreover, a few studies have demonstrated that the central administration of K_{ATP} channel openers produced antinociception or potentiated morphine-induced antinociceptive effect, and that K_{ATP} channel blockers antagonized morphine-induced antinociception[16-18]. These investigations raise a question whether the activation of K_{ATP} channels may play a substantial role in the mechanism of general anesthesia.

Zucker[19] reported that minimum alveolar concentration (MAC) for isoflurane in rats was unaffected by intrathecal administration of two agonists of K_{ATP} channels, cromkalim and pinacidil, concluding that activation of K_{ATP} channels was unlikely to be a fundamental mechanism of anesthesia. On the other hand, MacIver and Kendig[4] compared the effects of halothane, isoflurane and enflurane 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_{ATP} 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 ^{86}Rb efflux via K_{ATP} channels to that produced by cromakalim or by pinacidil, and has the property of crossing the blood brain barrier.[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

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endotracheal tube without the use of muscle relaxants. Mechanical ventilation was begun with a ventilator. End-tidal CO_2 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℃. 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*[22]. 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 received intravenous KRN2391 dissolved in saline at a dose of $3\mu g \cdot kg^{-1} \cdot min^{-1}$. The KRN2391 was infused over 30 min to achieve a pharmacologic effect. When halothane or isoflurane MAC was determined, a blood sample was drawn to determine the plasma concentration of KRN2391.

A separate group of three dogs was used to determine the time course of plasma and cerebrospinal fluid (CSF) concentrations of KRN2391 during continuous infusion. 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 $3\mu g \cdot kg^{-1} \cdot min^{-1}$. 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 atlanta-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 ℃ 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. 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 1. Effects of KRN2391 on hemodynamics and acid base balance during halothane and isoflurane anesthesia

Anesthetics	Time †	KRN2391 ‡	Mean Arterial Pressure § mmHg		Arterial Blood Gases ¶ mmHg		
	min	ng/ml	Pre-	Post-	pH	Po_2	Pco_2
Halothane (n=7)	80±21	77±28	101±14	78±12*	7.41±0.03	458±69	36±2
Isoflurane (n=7)	83±11	78±37	109±18	82±10*	7.39±0.04	425±91	37±1

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.

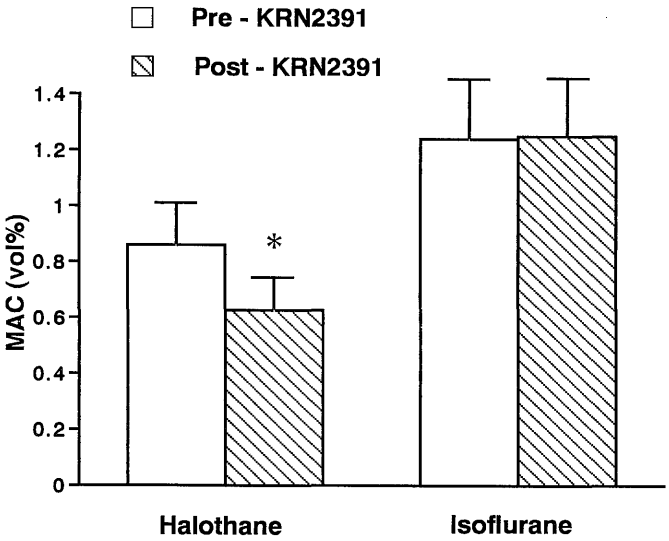

Fig. 1. Effects of KRN2391 on the MAC for halothane and isoflurane (mean±SD ; n = 7 for each point). KRN2391 was infused at $3\mu g \cdot kg^{-1} \cdot min^{-1}$, and MAC was determined by the tail-clamp technique before (pre) and after (post) infusion of KRN2391 in dogs.
*P<0.01 vs pre-KRN2391.

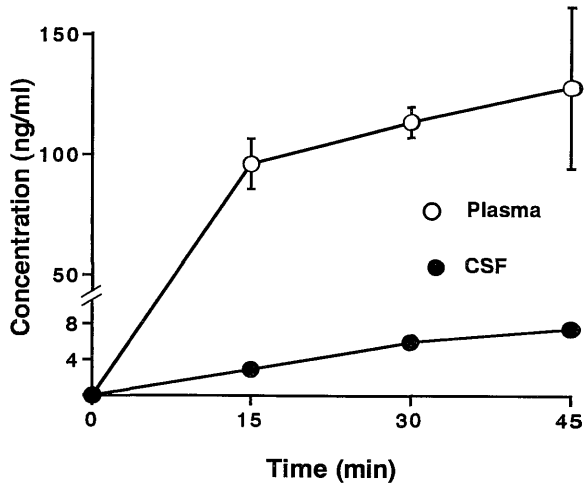

Fig. 2. Time course of the concentration of KRN2391 in the plasma and cerebrospinal fluid (CSF) (mean±SD; n = 3 for each point). KRN2391 was infused at $3\mu g \cdot kg^{-1} \cdot min^{-1}$ starting at time 0.

in either group, and there was no significant difference in pH, PO_2 or PCO_2 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 $3\mu g \cdot kg^{-1} \cdot min^{-1}$. 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 K_{ATP} channels would be involved in halothane-induced general anesthesia, whereas K_{ATP} channels would not play a role in the anesthetic action of isoflurane.

K_{ATP} channels were originally described in cardiac muscle[23], but have now been well characterized in a variety of peripheral tissues. Recently, these channels have also been investigated in the CNS. K_{ATP} channels are present in a wide variety of neurons in CNS, including the substantia nigra[13], hippocampus[13], globus pallidus[13], motor neocortex[24], and spinal dorsal horn[25], and appear to be involved in the regulation of neuronal excitability[26].

It was demonstrated that opioid[27] and adenosine[28] produce anesthetic-sparing effects through both μ- or δ-opioid receptors and A_1-adenosine receptors, respectively. The molecular mechanisms involved in the analgesic state are believed that stimulation of μ- or δ-opioid receptors[29] and A_1-adenosine receptors[28] open potassium channels resulting in membrane hyperpolarization in several areas of the CNS. Recently, some studies have indicated that K_{ATP} 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 K_{ATP} channels. Ocana et al[18]. observed that the administration of cromakalim dose-dependently potentiated the antinociceptive action of morphine in tail-flick test in mice, and that K_{ATP} channel antagonists abolished the antinociception. Likewise, recent evidence has also demonstrated a link between adenosine receptors and K_{ATP} channels in myocytes[30] and brain[32]. Li and Henry[32] showed that neuronal membrane hyperpolarization produced by adenosine via A_1-adenosine receptors was depressed by glibenclamide in vitro. The antinociception induced by (-)-N6-(2-phenylisopropyl)-adenosine, an adenosine A_1 receptor agonist, was shown either to be potentiated by cromakalim or to be antagonized by K_{ATP} blocker, gliquidone[31]. These studies together with our results suggest that the action of isoflurane is unlikely related to an action on K_{ATP} channels in the CNS. In contrast, the activation of K_{ATP} channels would be involved in the anesthetic action of halothane.

MacIver and Kendig[4] compared the effects of halothane and isoflurane on resting membrane potential and conductance of hippocampal CA1 neurons in vitro. At the equi-anesthetic levels, halothane increased membrane con-

ductance and produced hyperpolarization, whereas isoflurane decreased conductance and did not show significant effect on the membrane potential. Their results show that even structurally similar agents do not exert common hyperpolarizing effects on resting membrane potential or common changes in conductance. Our results are consistent with their studies supporting the view that different anesthetic agent might produce a common endpoint by multiple mechanisms via own selective ion channels. In the present study, KRN2391 was detected in the CSF, although there was a relatively large gradient in the concentration between plasma and CSF. This gradient might explain the relatively small magnitude of the reduction of halothane MAC by intravenous KRN2391.

General anesthetics would exert their effects by acting directly on specific target proteins in the CNS, in which a class of potassium channels seems to be particularly sensitive to volatile anesthetics. Nicoll and Madison[11] showed that halothane directly hyperpolarizes both frog motoneurons and rat hippocampal neurons at concentrations in the clinical range, possibly by an increase in potassium conductance. Reports of the hyperpolarizing actions of anesthetics in mammalian neurons[12], and the finding that low concentrations of anesthetics hyperpolarize and inhibit spontaneous activity of neurons in the pond snail Lymnaea stagnalis[33] suggest that potassium channels in the CNS are presumably the target sites underlying the molecular basis of volatile anesthetics. However, Stanford and Lacey[14] indicated that the firing rate of single substantia nigra pars reticulata neurons recorded extracellularly in brain slices was unaffected by lemakalim in normal physiological conditions. Thus, it is unlikely that direct neuronal hyperpolarization by K_{ATP} channel openers may contribute to the mechanism involved in the altering effects of halothane. It is considered possible that halothane would activate K_{ATP} channels and KRN2391 potentiates the halothane effect, whereas isoflurane would not affect K_{ATP} 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[34]. In the present study, the arterial blood pressure showed a moderate 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[21], suggesting that KRN2391 agonizes both K_{ATP} channels and voltage-gated potassium channels. Thus the action of KRN2391 does not appear to be confined exclusively to K_{ATP} channels. Either K_{ATP} channels or voltage-gated potassium channels or both may be involved in the mechanism of the KRN2391-induced halothane MAC reduction. However, Franks and Lieb[35] have summarized from available evidence that voltage-gated potassium channels are very resistant to clinical concentrations of general anesthetics, 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 K_{ATP} channels would be involved in the mechanism of anesthetic action of halothane, whereas K_{ATP} channels would not play a role in isoflurane anesthesia.

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