Ketamine blocks $\text{Ca}^{2+}$-activated $\text{K}^+$ channels in rabbit cerebral arterial smooth muscle cells

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Han, Jin, Nari Kim, Hyun Joo, and Euiyong Kim. Ketamine blocks $\text{Ca}^{2+}$-activated $\text{K}^+$ channels in rabbit cerebral arterial smooth muscle cells. Am J Physiol Heart Circ Physiol 285: H1347–H1355, 2003; 10.1152/ajpheart.00194.2003.—Although ketamine and $\text{Ca}^{2+}$-activated $\text{K}^+$ ($\text{KCa}$) channels have been implicated in the contractile activity regulation of cerebral arteries, no studies have addressed the specific interactions between ketamine and the $\text{KCa}$ channels in cerebral arteries. The purpose of this study was to examine the direct effects of ketamine on $\text{KCa}$ channel activity using the patch-clamp technique in single-cell preparations of rabbit middle cerebral arterial smooth muscle. We tested the hypothesis that ketamine modulates the $\text{KCa}$ channel activity of the cerebral arterial smooth muscle cells of the rabbit. Vascular myocytes were isolated from rabbit middle cerebral arteries using enzymatic dissociation. Single $\text{KCa}$ channel activities of smooth muscle cells from rabbit cerebral arteries were recorded using the patch-clamp technique. In the inside-out patches, ketamine in the micromolar range inhibited channel activity with a half-maximal inhibition of the ketamine concentration value of $83.8 \pm 12.9 \, \mu\text{M}$. The Hill coefficient was $1.2 \pm 0.3$. The slope conductance of the current-voltage relationship was $320.1 \pm 2.0 \, \text{pS}$ between 0 and $+60 \, \text{mV}$ in the presence of ketamine and symmetrical $145 \, \text{mM}$ $\text{K}^+$. Ketamine had little effect on either the voltage-dependency or open- and closed-time histograms of $\text{KCa}$ channel. The present study clearly demonstrates that ketamine inhibits $\text{KCa}$ channel activities in rabbit middle cerebral arterial smooth muscle cells. This inhibition of $\text{KCa}$ channels may represent a mechanism for ketamine-induced cerebral vasoconstriction.

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conforms with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996). New Zealand White rabbits (0.8–1.2 kg) were anesthetized with pentobarbital sodium (10 mg/kg iv) and then exsanguinated. The middle cerebral arteries were removed and cleaned of extraneous connective tissue in a dissection solution that contained (in mM) 137 NaCl, 5.6 KCl, 0.42 Na2HPO4, 0.44 NaH2PO4, 4.17 NaHCO3, 1 MgCl2, 2.6 CaCl2, and 10 HEPES, which was adjusted to pH 7.3 with NaOH. The arteries were then transferred for 20 min into an isolation solution that contained (in mM) 55 NaCl, 6 KCl, 88 l-glutamic acid, 10 HEPES, and 10 glucose, which was adjusted to pH 7.3 with NaOH. The cerebral arteries were digested for 10 min in a solution that contained (in mg/ml) 1 albumin, 1 papain, and 1 diithioerythritol, and then were incubated for 10 min in 1 mg/ml albumin, 1 mg/ml collagenase type F, and 1 mg/ml hyaluronidase type I-S. The smooth muscle cells were isolated by gentle trituration with a 1 mg/ml albumin solution through a wide-bore pipette. The cells were stored at 4°C, and used within 12 h of preparation.

Electrophysiological methods. Single-channel currents were measured in both the inside-out and outside-out configurations of the patch-clamp system (20). Channel activity was measured using a patch-clamp amplifier (Axopatch-1D; Axon Instruments; Union City, CA). Gigaohm seals were obtained with the use of pipettes with 5 μm (model PP-83, Narishige; Tokyo, Japan). The tips were coated with Sylgard Apparatus; Kent, UK) with the use of a vertical puller (Digidata-1200, Axon Instruments). The patches were examined at holding potentials of between 0 and 70 mV, depending on the level of control channel activity. An equilibration period of ~5 min was allowed for each new patch; patches that showed large fluctuations in channel activity over this period were discarded. Thereafter, channel activity was recorded continuously until the end of the experiment. The effects of the drugs were observed for 5–10 min after their addition to the bath solution either via a pipette (to 1% of the bath volume) or via perfusion (in a volume that was at least 10 times the bath volume). Single-channel currents were digitized at a sampling rate of 48 kHz and stored in digitized format on digital audiotapes using the recorder (model DTR-1200, Biologic; Grenoble, France). The data were transferred to a personal computer (Pentium 4, IBM; Busan, Korea) for analysis with pCLAMP version 6.03 software (Axon Instruments) with the use of a analog-to-digital converter interface (Digidata-1200, Axon Instruments).

Data analysis and quantification of single channel activity. The threshold for judging the open state was set at 50% of the single-channel amplitude (13). The open-time histogram was derived from continuous recordings of >60 s. The open probability (P_o) was calculated with the use of the formula

\[ P_o = \frac{\sum_{j=1}^{n} t_j}{N} \]

where \( t_j \) is the time spent at current levels corresponding to \( j = 0, 1, 2, \ldots, n \) channels in the open state, \( T_d \) is the duration of the recording, and \( N \) is the number of channels that are active in the patch. The number of channels in a patch was estimated by dividing the maximum observed current by the mean unitary current amplitude. \( P_o \) was calculated over 30-s intervals.

Solutions and drugs. The solutions used in the whole cell experiments consisted of the following (in mM): 133.0 K-aspartic acid, 7.0 KCl, 2.5 Mg-ATP, 2.5 Na-ATP, 2.5 Tris-creatine phosphate, 2.5 Na-creatine phosphate, 5.0 HEPES, and 0.05 or 0.10 mM EGTA at pH 7.3 for the pipette, and 143.0 NaCl, 5.4 KCl, 5.0 HEPES, 0.33 Na2HPO4, 1.0 MgCl2, 16.6 glucose, and 0 or 1.8 CaCl2 (pH 7.4) for the bath.

For the inside-out patches, the bath solution was composed of (in mM) 135.0 KCl, 2.0 MgCl2, 1.2 KH2PO4, 1.8 or 2.2 CaCl2, 3.0 EGTA, and 10.0 HEPES, adjusted to pH 7.2 with KOH. The concentration of free Ca2+ in this solution was either 250 nM (1.8 mM CaCl2) or 500 nM (2.2 mM CaCl2). The pipette solution contained (in mM) 136.0 NaCl, 5.0 KCl, 10.0 HEPES, 1.0 MgCl2, and 1.8 CaCl2, adjusted to pH 7.4 with NaOH.

The bath solutions used in the outside-out patch experiments were as follows (in mM): 136.0 NaCl, 5.0 KCl, 10.0 HEPES, 1.0 MgCl2, and 1.8 CaCl2, adjusted to pH 7.4 with NaOH solution. The composition of the pipette solution was (in mM) 135.0 KCl, 2.0 MgCl2, 1.2 KH2PO4, 1.8 or 2.2 CaCl2, 3.0 EGTA, and 10.0 HEPES, adjusted to pH 7.2 with KOH. The concentration of free Ca2+ in this solution was either 250 nM (1.8 mM CaCl2) or 500 nM (2.2 mM CaCl2). The Ca2+ to-EGTA ratio was adjusted to give a pCa of 6 × 10−7, at which level the simultaneous opening of one to three channels was typically observed. All of the chemicals and drugs were obtained from Sigma (St. Louis, MO). All of the experiments were performed at a room temperature of 25 ± 2°C.

Solution exchange system. In most experiments, a superfusion system (model DAD-12, ALA Scientific Instrument; Westbury, NY) was used for the change of bath solution and drugs. The system is designed to simplify the application of various concentrations of drugs and solutions to cells. When pointed at the cell to be studied, if the cell remains within the stream of the solution, the cell is essentially immersed completely in the solution that is applied.

Statistics. The data are presented as means ± SE values where appropriate. Student’s t-test was used to calculate statistical significance. Differences of \( P < 0.05 \) were considered statistically significant.

RESULTS

Identification of \( \text{K}_{\text{Ca}} \) channels in cerebral arterial smooth muscle cells of rabbits. \( \text{K}_{\text{Ca}} \) channels have been characterized in a variety of cell types and are particularly abundant in smooth muscle cells (10). Furthermore, the pharmacological and electrophysiological properties of these channels have been well documented in smooth muscle cells (26, 28). Initially, we characterized the \( \text{K}_{\text{Ca}} \) channels in rabbit cerebral arterial smooth muscle cells.

Figure 1A shows a representative large-conductance unitary current from a normal cell at a positive membrane potential. The channels responsible for these events appeared to be \( K^+ \) selective (data not shown) and had a single-channel conductance of 321.1 ± 3.4 nA (Fig. 1B, open circles, \( n = 5 \) patches), as measured by the slope of the current-voltage relationship between +10 and +60 mV in symmetrical 145 mM K+. Figure 1B shows the unitary current-voltage relationship recorded at membrane potentials from +10 to +70 mV. The channel activity of the open probability (\( N P_o \)) was voltage dependent in the positive voltage range between +30 and +50 mV (Fig. 1C; open circles, \( n = 7 \) patches). Figure 1D shows the relationship between the relative channel activities and membrane potentials.
We used outside-out patches to assess the effects of KCa channel blockers on single channel activities of this channel. From a holding potential of -50 mV, depolarization elicited single-channel events that were clearly distinguishable from noise, which demonstrated the existence of unitary currents that were responsible for the macroscopic currents that were obtained under the same conditions. Figure 2A shows the unitary KCa currents that were recorded from patches in the absence or presence of added tetraethylammonium (TEA; 100 μM). External TEA decreased the Po of the KCa channel and the mean single channel current. We found that TEA increased the open channel noise. Figure 2B shows the relationship between the unitary KCa currents and membrane potential.

**Fig. 1.** Ca²⁺-activated K⁺ channels (KCa) currents in rabbit middle cerebral arterial smooth muscle cells. **A**: single channel recordings from the patch in the inside-out mode. The membrane potentials are indicated on the top of each trace. C, zero current level. The data were sampled at 48 kHz and filtered at 2 kHz. **B**: current-voltage (I-V) relationships of KCa channels, as plotted for each channel in A. The indicated lines are based on the linear least-square fit to give the slope conductance. **C and D**: open probability (Po) of KCa channels increases with the membrane potential in the inside-out patch configuration (n = 7 patches).

**Fig. 2.** Effects of KCa channel blockers on channel activity in rabbit middle cerebral arterial smooth muscle cells. **A**: unitary KCa currents were recorded from the outside-out patches in the absence (expanded region a at bottom) and presence (expanded region b at bottom) of the external tetraethylammonium (TEA; 100 μM). **B**: I-V relationship at different TEA concentrations (0.1–0.5 mM TEA). **C**: block of KCa channels by iberiotoxin (IbTX). Original records from an outside-out patch obtained in the presence of 0, 10, 100, and 200 nM IbTX. **D**: histogram showing the effect of IbTX on the KCa channel activity of Po (NPo). The data are presented as means ± SE for NPo (n = 5 patches). *P < 0.05 compared with the control (in the absence of IbTX). The solution exchange protocols used for TEA and IbTX are shown above the current traces. All recordings were held at +50 mV. The data were sampled at 48 kHz and filtered at 2 kHz.
Fig. 3. Relationship between the activities of the KCa channels and intracellular solution Ca²⁺ concentration ([Ca²⁺]ᵢ) in the inside-out patches. A: examples of channel activity at different [Ca²⁺], recorded at +50 mV. Data were sampled at 48 kHz and filtered at 2 kHz. B: Pᵦ as a function of [Ca²⁺], the channel activity for each [Ca²⁺] is normalized against the value at 0.1 μM Ca²⁺. The relationship between [Ca²⁺], and the normalized channel activity is fitted to the Hill equation \(Pᵦ = 1/(1 + (Kd/[Ca²⁺])n_H)\), where \(Kd\) is the half-maximal ketamine activation concentration, \(n_H\) is the Hill coefficient. The values shown are the means ± SE for 7 patches. *P < 0.05, significantly different from control.

Fig. 4. A: effect of ketamine on the KCa channel in the inside-out patch membrane that was exposed to symmetrical 145 mM K⁺ and held at a membrane potential of +50 mV. The solution exchange protocol used for ketamine is shown above the current traces. The data were sampled at 48 kHz and filtered at 2 kHz (a–d represent the time periods). B: histogram showing the effect of ketamine on the KCa channel activity. The data are presented as means ± SE for \(NPᵦ\), under the following conditions: control (a), 1,000 μM ketamine (b), washout of 1,000 μM ketamine (c), and reapplication of 1,000 μM ketamine (d). *P < 0.05.

To examine the Ca²⁺ sensitivity of this channel in rabbit cerebral arterial smooth muscle cells, single channel currents were recorded with the use of inside-out patches. The activity of KCa channels, which was measured as \(NPᵦ\), correlated with changes in the intracellular concentration of Ca²⁺ ([Ca²⁺]ᵢ). The channel activity showed a very steep dependence on the [Ca²⁺], and complete saturation occurred at [Ca²⁺] > 0.01 μM. Figure 3 shows the concentration-response relations (KCa channel activity vs. [Ca²⁺]). The channel activities at six selected concentrations of Ca²⁺ were normalized against the channel activity recorded at 0.1 μM Ca²⁺ (maximum). At a low [Ca²⁺] (0.03 μM), the channel activity was 48.9% of the maximum level. The concentration-response relationship showed a good fit to the Hill coefficient (n_H). In normal cells, the half-maximum activation concentration of ketamine (Kd) of Ca²⁺ was 31.6 ± 0.4 nM (n = 7), and n_H was 4.1 ± 0.3. These results indicate that the channel activities observed in the present study were those of KCa channels.

Effect of ketamine on the KCa channel activity of cerebral arterial smooth muscle cells of rabbit. We used the inside-out patch configuration to investigate the effects of ketamine on KCa channel activities in cerebral arterial smooth muscle cells. The KCa channels was observed when inside-out patches formed (Fig. 4A). The application of 1,000 μM ketamine to the bath induced a marked and reversible decrease in KCa channel activity. The \(NPᵦ\) value was reduced from 1.40 to 0.05. This inhibition by ketamine was partially reversed by the removal of ketamine (\(NPᵦ = 1.37\)). Similar results were observed for eight other patches (Fig. 4B).

Figure 5 shows the concentration-dependent effects of ketamine on KCa channel activity. Ketamine inhibited KCa channel activity at concentrations as low as 10 μM ketamine.
**KETAMINE BLOCKS K_Ca CHANNELS**

**DISCUSSION**

One of the major findings of the present study was that ketamine inhibits the K_Ca channel activity in rabbit cerebral arterial smooth cells. This may be the first evidence to indicate that ketamine depolarizes cerebral vascular smooth muscle cells via the inhibition of the K_Ca channel, and thereby induces vasoconstriction.

Figure 6A shows the representative K_Ca current traces from inside-out patches in the presence of 100 µM ketamine. In Fig. 6B, the single-channel conductance, which represents the slope of the current-voltage relationship, was 320.1 ± 2.0 pS (n = 5) between +10 and +60 mV in symmetrical 145 mM K⁺. This finding was confirmed by the analysis of the amplitude histogram (data not shown). These results indicate that although channel activity is inhibited by ketamine, the amplitude of unitary current is not affected. In addition, ketamine had no effect on the voltage dependency of K_Ca channel activity (data not shown).

To further examine the inhibitory action of ketamine on K_Ca channel activity, the kinetic properties of the K_Ca channel were analyzed by measuring the open and closed times before and after the application of ketamine. The data were fitted using the nonlinear least-squares method, where the relative channel activity was 1/[1+(ketamine)/Kd] where [ketamine], is the ketamine concentration of the internal solution.

**Fig. 5.** A: representative tracing showing concentration-dependent inhibition of K_Ca channel activity by ketamine in the inside-out patch that was held at +50 mV. The bars above the trace indicate the periods of application of the various test solutions. The data were sampled at 48 kHz and filtered at 1 kHz. B: concentration-response relationship for the inhibitory action of ketamine. Each data point represents the mean ± SE of seven patches. The data were fitted to the Hill equation using the nonlinear least-squares method, where the relative channel activity was 1/[1+(ketamine)/Kd]^n where [ketamine], is the ketamine concentration of the internal solution.

μM and exhibited further inhibitory effects in a concentration-dependent manner. The K_Ca channel activity was inhibited maximally at 1 mM ketamine. To obtain the intracellular ketamine concentration ([ketamine]), -K_Ca channel activity relationship, we determined the effect of a given concentration of ketamine on each inside-out patch (Fig. 5B). A plot of relative channel activity as a function of [ketamine], was derived using data from nine patches, and the data were fitted to the n_H equation using the nonlinear least-squares method, where the relative channel activity was 1/[1+(ketamine)/Kd]^n where [ketamine], is the ketamine concentration of the internal solution.

*P < 0.05, significantly different from control.

**Fig. 6.** Effect of ketamine on the unit current of the K_Ca channels. A: traces of the unit current from the patch in the inside-out mode at various membrane potentials (+10 to +60 mV). The data were sampled at 48 kHz and filtered at 2 kHz. B: I-V relationships of the unit current of K_Ca channels after the application of ketamine at membrane potentials between +10 and +70 mV.
The induction of anesthesia with ketamine is often associated with increases in cardiac output, arterial blood pressure, and heart rate (1). It is generally believed that direct stimulation of the central nervous system, which leads to increased sympathetic nervous outflow, is the primary mechanism for cardiovascular stimulation by ketamine (27, 56). In addition, the inhibition of norepinephrine uptake into postganglionic sympathetic nerve endings, which represents depression of baroreceptor reflex activity, and adrenocortical stimulation have been suggested as ketamine activities (45, 49). In support of this notion, ketamine fails to produce cardiovascular stimulation or severe systemic hypotension when the sympathetic nervous system is either inhibited (55) or stimulated maximally (61). It is known that increases in CBF or ICP induced by ketamine are blunted in the presence of other anesthetics (1, 2, 14, 37, 57). Dawson et al. (14) observed that ketamine significantly increased the CBF in dogs, and this effect was blocked by pretreatment with thiopental sodium. In humans, Strebel et al. (57) observed that during normoventilation under light isoflurane anesthesia, ketamine increased blood velocity in the middle cerebral artery, and this effect was prevented by prior administration of midazolam. In patients with mildly increased ICP due to intracranial pathologic abnormalities, ketamine did not increase the ICP, and it decreased electroencephalogram activity under isoflurane. Because arterial smooth muscle cells are known to contain 1,000–10,000 KCa channels per cell (40), it is not surprising that membrane hyperpolarization via K+ channel activity provides an important mechanism to regulate arterial contraction. Four distinct types of KCa channels have been identified in arterial smooth muscle: KCa channels (9), delayed rectifier KCa channels (48), inward rectifier KCa channels (7), and ATP-sensitive KCa channels (8). However, few attempts have been made to investigate the role of KCa channel activation in ketamine activity. In the present study, we show for the first time that ketamine regulates KCa channel activity in rabbit middle cerebral arterial smooth muscle cells. The KCa channels are widespread and appear to occur at a high density in cell membrane of arterial smooth muscle (40). Thus the opening of only a few of these channels can produce a significant rise in membrane KCa conductance, which is a major regulator of membrane potential and hence arterial tone.

Asano et al. (3) suggested that the resting tone of cerebral arteries is determined by at least two components with opposite activities, i.e., contraction due to increased basal Ca2+ influx (probably through activation of the myosin light chain kinase), and relaxation due to the activation of KCa channels and other Ca2+- extraction systems. The net balance of these two components determines the degree of contraction. An increase in basal Ca2+ influx produces contraction and activates KCa channels or other Ca2+-extraction systems, bringing about hyperpolarization and Ca2+ extraction. The relationship between Ca2+ influx through voltage-dependent Ca2+ channels and membrane potential is precipitous; a 3-mV depolarization or hyperpolarization produces a twofold increase or decrease, respectively, in Ca2+ influx. Physiological or pharmacological agents that alter membrane potential may cause a significant change in blood vessel diameter. Because arterial smooth muscle cells are known to contain 1,000–10,000 KCa channels per cell (40), it is not surprising that membrane hyperpolarization via
activation of the \( K_{\text{Ca}} \) channels represents a powerful mechanism to lower blood pressure through vasodilation. In physiological concentrations of extracellular \( K^+ (~5 \text{ mM}) \), the equilibrium potential is approximately \(-85 \text{ mV} \), and passive \( K^+ \) movement is in the outward direction. The opening of \( K_{\text{Ca}} \) channels in the cell membranes of smooth muscle cells in arteries increases \( K^+ \) efflux, which causes membrane hyperpolarization. This closes the voltage-dependent \( Ca^{2+} \) channels, thus decreasing \( Ca^{2+} \) entry and leading to vasodilation. Because the \( K_{\text{Ca}} \) current is activated by both membrane depolarization and intracellular \( Ca^{2+} \), it represents an ideal candidate for negative feedback.

The conductance of \( K_{\text{Ca}} \) channels in rabbit middle cerebral artery smooth muscle cells in our study is similar to those reported previously in both excitable and nonexcitable cells (33–35, 44, 60). The \( K_{\text{Ca}} \) channels in our study had a conductance of 320 pS. Ottolia et al. (44) reported conductance ~310 pS for the channel in rat cerebellum. Similar conductances have been reported in human myometrium (60), a human embryonic kidney cell line (HEK293) expressing BKca channel \( \alpha \)-subunit hSlo (34, 35), and cochlear spiral lumen fibrocytes (33). \( K_{\text{Ca}} \) channels in many types of smooth muscle cells had single-channel conductances ranging between 200 and 250 pS (6, 11, 25, 36, 38, 39, 59). On the one hand, the single channel conductance was 231 pS in rat cerebral artery smooth muscle cells (47). In guinea pig basilar artery, the conductance was 260 pS (54). There are novel aspects of our work compared with other studies in cerebral arterial smooth muscle cells, such as the following: 1) other researchers worked primarily on rat or guinea pig cerebral arteries, whereas we used the rabbit cerebral artery; and 2) they worked on the basilar artery, whereas we worked on the middle cerebral artery. To our knowledge, no studies to date address the characteristics of \( K_{\text{Ca}} \) channels in rabbit middle cerebral arterial smooth muscle cells.

Because of a large unitary conductance, the opening of a few \( K_{\text{Ca}} \) channels has a significant impact on membrane potential (29, 40, 46). Gokina et al. (19) demonstrated that the blockade of \( K_{\text{Ca}} \) channels by charybdotoxin and TEA results in membrane depolarization, an increase in the amplitude and duration of action potentials, and a marked contraction of smooth muscle cells in human pial arteries. In the present study, although ketamine had little effect on the kinetic properties of \( K_{\text{Ca}} \) channels, it decreased the \( P_o \), which suggests that the inhibition of \( K_{\text{Ca}} \) channels by ketamine may induce vasoconstriction. Moreover, this may be a direct effect of ketamine on \( K_{\text{Ca}} \) channels because ketamine decreased the \( P_o \) of \( K_{\text{Ca}} \) channels in isolated patch configurations in the present study.

The effects of ketamine on the other potassium channels have been evaluated. Dreixler et al. (17) reported that ketamine blocked recombinant \( Kv2.1 \) (31) and small-conductance \( K_{\text{Ca}} \) channels from the mammalian brain. They suggested that the inhibition of these channels causes long-term sleep disturbances, hyperexcitability, changes in learning ability, seizures, and neuronal degeneration. Other studies have found that ketamine inhibited transient outward \( K^+ \) channels (18, 30), \( K_{\text{IR}} \) channels (4), and sarcolemmal and mitochondrial \( K_{\text{ATP}} \) channels (21, 63), resulting in prolonged action potential duration and abolishing the cardioprotective effect of ischemic preconditioning in the heart. In the blood vessels, the direct effects of ketamine on \( K^+ \) channels have not been well studied. In vitro isometric tension experiments have shown that ketamine inhibits \( K_{\text{ATP}} \) channel-mediated vasorelaxation in canine pulmonary artery (53) and the rat aorta (15), suggesting that ketamine may inhibit \( K_{\text{ATP}} \) channels in vascular smooth muscle cells. Because \( K_{\text{ATP}} \) channels appear to be expressed in the blood vessels, previous results obtained from a conduit artery may have relevance to ketamine action in resistance blood vessels, such as cerebral arterioles. On the one hand, a recent study on \( K_{\text{Ca}} \) channels has demonstrated that ketamine attenuated pulmonary vasorelaxant responses to bradykinin and acetylcholine by inhibiting both nitric oxide and \( K_{\text{Ca}} \) channel-mediated components of the response (42). However, it was unclear whether ketamine can inhibit \( K_{\text{Ca}} \) channels in vascular smooth muscle cells because ketamine can also impair vasorelaxation via the inhibition of endothelial \( Ca^{2+} \) increase (42). Taken together with previous studies, it is suggested that ketamine action on \( K^+ \) channels may play a role in generating clinical effects or side effects of ketamine.

In humans, the peak plasma concentration of ketamine during induction of general anesthesia is \( \sim 110 \mu\text{M} \) with an intravenous administration of 2 mg/kg, whereas the free plasma concentration is likely to be <110 \( \mu\text{M} \) because 20% of ketamine is bound to plasma protein (16). We found that ketamine inhibited the \( K_{\text{Ca}} \) channel activities at micromolar range with the half-maximal inhibitory concentration of 83.8 \( \mu\text{M} \) in inside-out patches. Thus the inhibitory effects of ketamine on this channel activity reported in this study appear to be significant at clinically relevant concentrations. However, it is difficult to extrapolate the present results to the human because of the in vitro model and possible species differences.

Our results suggest that ketamine may contribute, at least in part, to cerebral vasoconstriction. The mechanism of ketamine-induced vasoconstriction in rabbit middle cerebral arterial smooth muscle cells may involve the inhibition of \( K_{\text{Ca}} \) channel activities. To our knowledge, this study provides the first evidence of a direct inhibitory effect of ketamine on \( K_{\text{Ca}} \) channels.

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DISCUSSIONS

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