ORIGINAL

Effects of ketamine on nicorandil induced ATP-sensitive potassium channel activity in cell line derived from rat aortic smooth muscle

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Abstract : Purpose : Nicorandil opens adenosine triphosphate-sensitive potassium (K_{ATP}) channels in the cardiovascular system and is being increasingly used for the treatment of angina pectoris. In the present study, we tested whether intravenous anesthetic agent ketamine affected nicorandil-induced native vascular K_{ATP} channel activation. Methods : We used excised inside-out patch clamp configurations to investigate the direct effects of ketamine racemate and S-(+)-ketamine on the activities of K_{ATP} channels in cultured rat aortic smooth muscle cells. Furthermore, we also investigated whether intracellular MgADP could modulate ketamine inhibition. Results : Nicorandil significantly activated K_{ATP} channel activity, whereas this channel activity was completely blocked by glibenclamide, a specific K_{ATP} channel blocker. Ketamine racemate inhibited the nicorandil induced K_{ATP} channel activity (IC_{50}=34±1 μM, n=14), but S-(+)-ketamine was less potent than ketamine racemate in blocking nicorandil induced K_{ATP} channel activities (IC_{50}=226±7 μM, n=10). Application of MgADP to the intracellular side of the channel was able to decrease the inhibitory potency of ketamine racemate on nicorandil induced K_{ATP} channel activities. Conclusions : Our results indicate that ketamine inhibits nicorandil induced K_{ATP} channel activities in a dose dependent and stereoselective manner. Furthermore, increase of intracellular MgADP attenuates the inhibitory potency of ketamine racemate. J. Med. Invest. 57 : 237-244, August, 2010

Keywords **:** *intravenous anesthetics, ketamine, nicorandil, potassium channel, patch-clamp configuration.*

INTRODUCTION

Adenosine triphosphate-sensitive potassium (K_{ATP}) channels are widely distributed in various tissues, and respond to changes in the cell metabolism (1). In vascular smooth muscle cells, the opening of K_{ATP} channels causes vasodilation under physiological and pathophysiological conditions (2).

Nicorandil is a K_{ATP} channel opener with nitrate-like properties used in the management of angina pectoris (3, 4). Furthermore, a recent prospective randomized multicenter study demonstrated that prophylactic administration of nicorandil during operation reduced the frequency of cardiac events in patients undergoing noncardiac surgery (5). However, our previous studies have indicated that intravenous anesthetics, including ketamine directly inhibited K_{ATP} channel activities (6-8). Thus, it is

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important to understand whether significant interactions might occur between nicorandil and intravenous anesthetics when both drugs are administrated concurrently.

K_{ATP} channels are regulated by changes in intracellular ATP and ADP concentrations (1). Previous studies on native K_{ATP} channels have suggested that nicorandil action is also dependent on the presence of MgADP (9, 10). But whether the action of ketamine on nicorandil induced K_{ATP} channels depends on the presence of MgADP is currently unknown.

In the current study, we used patch clamp techniques to examine the electrophysiological effects of ketamine on nicorandil induced K_{ATP} channel activity in cultured rat aortic smooth muscle cells. We also determined the effects of intracellular MgADP on ketamine action.

MATERIALS AND METHODS

Cell culture

A continuous line of A10 vascular smooth muscle cells, derived from the thoracic aorta of fetal rats, was obtained from the American Type Culture Collection (ATCC). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (Life Technologies, Invitrogen, Carlsbad, CA, USA), 3.7 mg ml^{-1} $NaHCO_3$, and 100 μg ml^{-1} gentamycin at 37°C in a humidified atmosphere of 95% air and 5% CO_2. The medium was changed initially after 48 h and then every 2-3 days. When the cells had formed a confluent monolayer after 7-9 days, they were rendered quiescent by incubation in serum-free medium for 24 h and then harvested by the addition of 0.05% trypsin and 0.1% fetal bovine serum. Cells were used for experiment after 12 passages.

Electrophysiological analysis

Channel currents were recorded with a patch clamp amplifier (CEZ 2200 ; Nihon Kohden, Tokyo, Japan) and stored in a personal computer (Aptiva ; International Business Machine Corporation, Armonk, NY, USA) with an analogue-to-digital converter (DigiData 1200 ; Axon Instruments, Foster City, CA, USA). pClamp version 7 software (Axon Instruments, Foster City, CA, USA) was used for data acquisition and analysis.

Electrophysiological measurements

Single channel currents were recorded in the inside-out configurations using a patch clamp amplifier as described previously (6-8). The intracellular solution contained 140 mM KCl, 2 mM EGTA, 2 mM $MgCl_2$, and 10 mM HEPES (pH=7.3). The pipette solution contained 140 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES (pH=7.4). Patch pipettes were pulled with an electrode puller (PP-830 ; Narishige, Tokyo, Japan). Recordings were made at 36± 0.5°C. The resistance of pipettes filled with internal solution and immersed in Tyrode's solution was 5-7 MΩ. The sampling frequency of the single-channel data was 5 KHz with a low-pass filter (1 KHz). The open probability (P_o) was determined from current amplitude histograms and was calculated as follows :

$$P_o = \frac{\left(\sum_{j=1}^{N} t_j \bullet j \right)}{T_d \bullet N}$$

where t_j is the time spent at current levels corresponding to j=0, 1, 2, N channels in the open state ; T_d is the duration of the recording ; and N is the number of the channels active in the patch. Recordings of 1-2 min were analyzed to determine Po. The channel activity was expressed as NPo. The NPo in the presence of drugs was normalized to the baseline NPo value obtained before drug administration and presented as relative channel activity.

Superfusates were directly applied to cells in the glass-bottom plastic cell bath (2-ml volume) at a rate of 2-2.5 ml/min using a plastic syringe (50-ml volume), vinyl chloride tubing (0.8-mm ID ; 50-cm length), and syringe pump (Terumo STC-525 ; Tokyo, Japan). When the concentration-dependent effects of drugs were studied, the superfusion was stopped for approximately 1 min at each concentration, and these drugs were injected into the cell bath using a glass syringe to five final concentrations in a cumulative manner (total volume injected was approximately 10-20 μl). Thus, the superfusion was stopped for total of approximately 5 min, and preliminary studies showed that stopping superfusion for approximately 5 min had no significant effects on electrophysiological measurements. The drug concentration needed to induce half-maximal inhibition of the channels (IC_{50}) and the Hill coefficients were calculated as follows :

$$y = \frac{1}{1 + ([D]/K_i)^H}$$

where y is the relative NPo, [D] is the concentration of drug, K_i is the IC_{50}, and H is the Hill coefficient.

Statistics

All data are presented as means± SE. Repeated-measures analysis of variance, followed by the Student-Newman-Keuls test, was applied to identify significant differences among the effects observed with different concentrations of ketamine racemate or S-(+)-ketamine. Paired statistical comparisons were made using paired t tests. In all comparisons, $P < 0.05$ was considered significant.

Drugs

Ketamine racemate, S-(+)-ketamine, glibenclamide, and nicorandil were obtained from Sigma-Aldrich Japan (Tokyo, Japan). Glibenclamide and nicorandil were dissolved in dimethylsulfoxide (DMSO, the final concentration of solvent in the cell bath was 0.01%). Preliminary studies showed that 0.02% of DMSO, a 2-fold higher concentration than used in the present study, had no significant effects on cultured rat aortic smooth muscle K_{ATP} channel currents.

RESULTS

Nicorandil induced K_{ATP} channel activities in A10 cell derived from rat aortic smooth muscle

The effects of nicorandil on K_{ATP} channel in inside-out patch clamp configuration were recorded (Figure 1A). Single channel activities were observed infrequently before treatment of nicorandil (NPo<

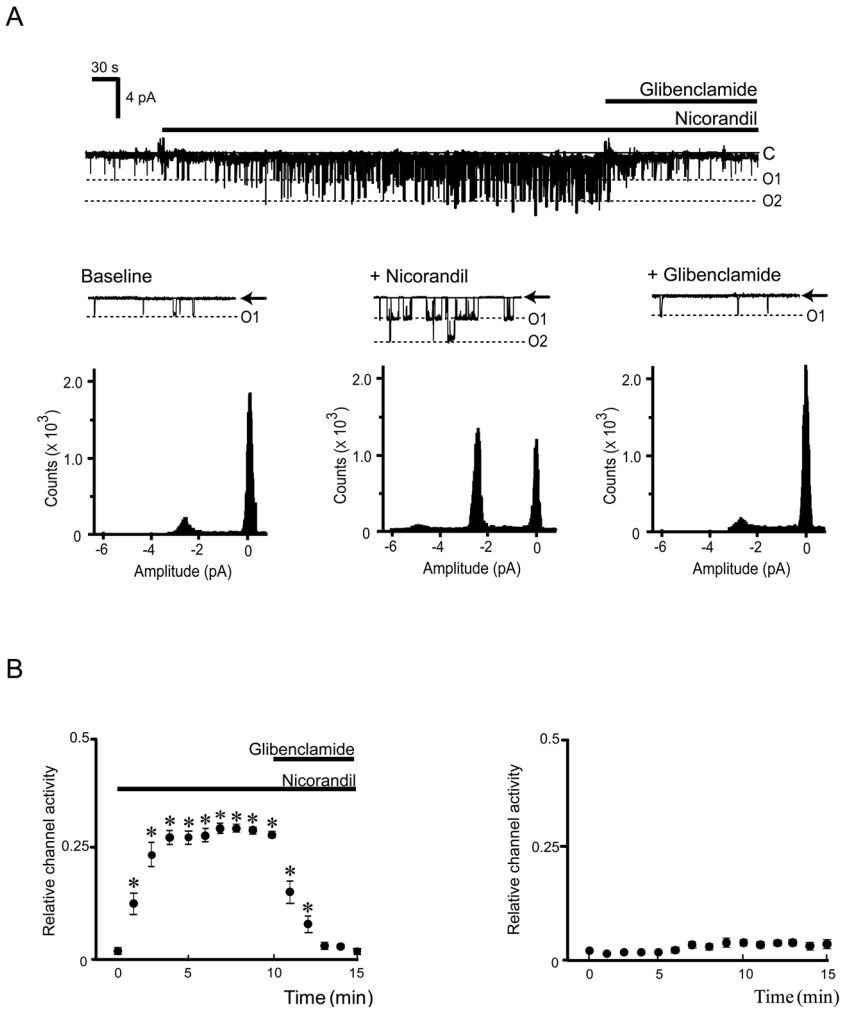

Figure 1. Shingle-channel characteristics of adenosine triphosphate-sensitive potassium channels in inside-out patch clamp configuration. (A) Representative traces of channel currents recorded from A10 cells are shown. Membrane potentials were clamped at -60 mV. Nicorandil (100 μM) and glibenclamide (0.5 μM) were added to the intracellular solution as indicated by the horizontal bar. C, closed channel level ; O1 and O2, open channel levels with one or two channels open simultaneously, respectively. Amplitude histograms were plotted by binning the data from the original recordings. The relative peaks of the histogram indicate the periods the channel resides in each conductance level. (B) The relationship between NPo and time after application of nicorandil (100 μM, left panel) and no application of nicorandil (time control, right panel). Each vertical bar represents measurements from 12 patches (mean± SE). * $p < 0.05$ versus baseline.

0.001, n=10). Application of 100 μM nicorandil to the bath solution (intracellular side) significantly activated K^+-selective channel activities. These channel activities were completely blocked by 0.5 μM glibenclamide, a specific K_{ATP} channel inhibitor (Figure 1A, n=10), suggesting that the activity is indeed due to K_{ATP} channel currents. Figure 1B shows the relationship between nicorandil-induced NPo values and time for the traces. Approximately 4-5 min after application of 100 μM nicorandil, NPo reached steady-state (0.26± 0.02, n=10). Identical control experiments in the absence of nicorandil resulted in no K_{ATP} channel activation or inhibition during the same time periods (Figure 1B, n=5). In addition, the single channel conductance was 29.4± 1.2 pS (Figure 2, n=17), as measured by the current-voltage relationship between -80 and +60 mV membrane potential under conditions of symmetrical 145 mM K^+.

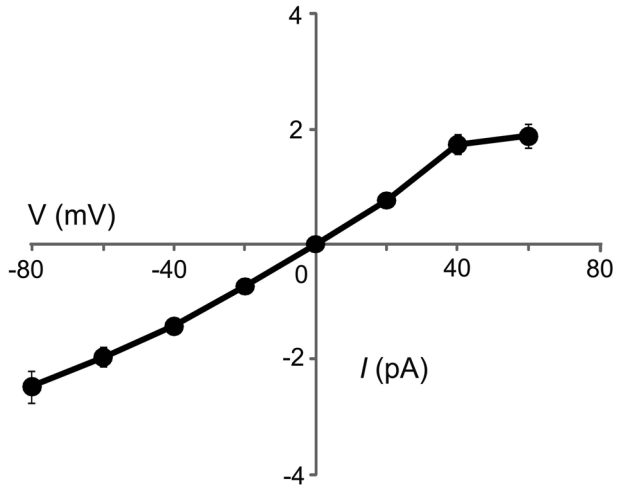

Figure 2. The current-voltage relationship for adenosine triphosphate-sensitive potassium channel currents. Each vertical bar represents measurements from 17 patches (mean± SE).

Effects of ketamine racemate on nicorandil induced K_{ATP} channel activities

To assess the effects of ketamine on nicorandil induced K_{ATP} channel activities in the absence of intracellular ADP, we measured single channel currents on inside-out patches in the presence of ketamine racemate. Application of 1-1000 μM ketamine racemate to the intracellular membrane surface inhibited the nicorandil induced K_{ATP} channel currents in a dose dependent-manner. As indicated in Figure 3A the inhibitory effects of ketamine racemate on

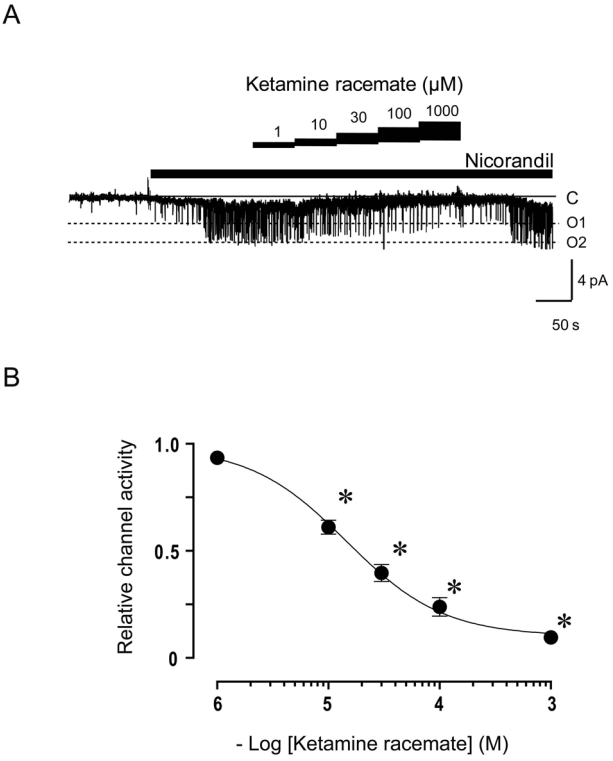

Figure 3. Effects of ketamine racemate on nicorandil induced adenosine triphosphate-sensitive potassium channel currents in the inside-out patch clamp configuration. (A) Representative example of nicorandil induced channel currents obtained before and after the application of ketamine racemate (1-1000 μM) is shown. Nicorandil (100 μM) and ketamine racemate were added to the intracellular solution as indicated by the horizontal bar. C, closed channel level ; O1 and O2, open channel levels with one or two channels open simultaneously, respectively. (B) Dose-dependent effects of ketamine racemate on the nicorandil induced channel activities. Each vertical bar constitutes measurements from 14 patches (mean± SE). *$p< 0.05$ versus baseline.

K_{ATP} channel activities were readily reversible.

The dose-dependent effects of ketamine racemate on nicorandil induced K_{ATP} channel activities in the absence of intracellular ADP are shown in Figure 3B. The IC_{50} and Hill coefficient (h) of ketamine racemate for the K_{ATP} channels are 34± 1 μM and 0.93 (n=14), respectively. The average percent recovery of K_{ATP} channel activities after washout of ketamine racemate was 92± 2% of the NPo measured before drug treatment.

Effects of S-(+)-ketamine on nicorandil induced K_{ATP} channel activities

In contrast to ketamine racemate, S-(+)-ketamine at 1-30 μM did not significantly inhibit nicorandil induced K_{ATP} channel currents (Figure 4A, n=11), indicating that the inhibitory effect of ketamine on

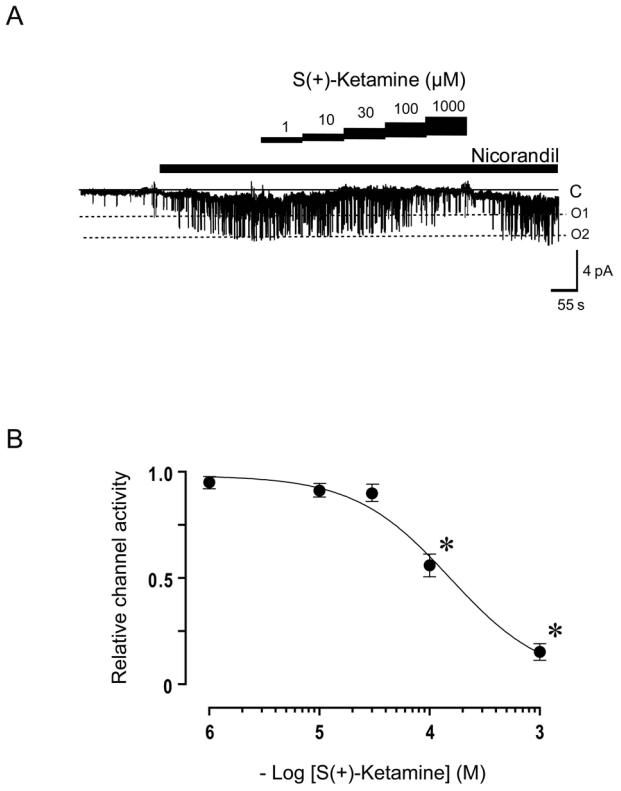

Figure 4. Effects of S-(+)-ketamine on nicorandil induced adenosine triphosphate-sensitive potassium channel currents in the inside-out patch clamp configuration. (A) A representative example of nicorandil induced channel currents obtained before and after the application of S-(+)-ketamine (1-1000 μM) is shown. Nicorandil (100 μM) and S-(+)-ketamine were added to the intracellular solution as indicated by the horizontal bar. C, closed channel level ; O1 and O2, open channel levels with one or two channels open simultaneously, respectively. (B) Dose-dependent effects of S-(+)-ketamine on the nicorandil induced channel activities. Each vertical bar constitutes measurements from 14 patches (mean± SE). *p< 0.05 versus baseline.

nicorandil induced K_{ATP} channel activities may be stereoselective.

The dose-dependent effects of S-(+)-ketamine on nicorandil induced K_{ATP} channel activities in the absence of intracellular ADP are shown in Figure 4B. The IC_{50} and Hill coefficient (h) of S-(+)-ketamine for the nicorandil induced K_{ATP} channels are 226± 7 μM and 1.01 (n=10), respectively. The average percent recovery of K_{ATP} channel activities after washout of S-(+)-ketamine was 94± 3% of the NPo measured before drug treatment.

Effects of ketamine racemate on nicorandil induced K_{ATP} channel activities in the presence of intracellular ADP

Recent studies have demonstrated that the effects of K_{ATP} channel agonists (9, 10) and ketamine racemate (8) on K_{ATP} channels are modified by interaction of MgADP with sulphonylurea receptors (SURs). Therefore, we next examined whether the inhibitory effects of ketamine racemate on nicorandil induced K_{ATP} channel currents in rat aortic smooth muscle cells are affected by the presence of intracellular MgADP.

Figure 5A and 5B shows the effects of 30 μM ketamine racemate on nicorandil induced K_{ATP} channel activities in the absence or presence of a physiological concentration of MgADP (100 μM). Application of MgADP to the intracellular side of the channels significantly reduced the ketamine racemate inhibition of nicorandil induced K_{ATP} channel activities. In addition, the inhibitory potency of ketamine racemate on nicorandil induced K_{ATP} channel

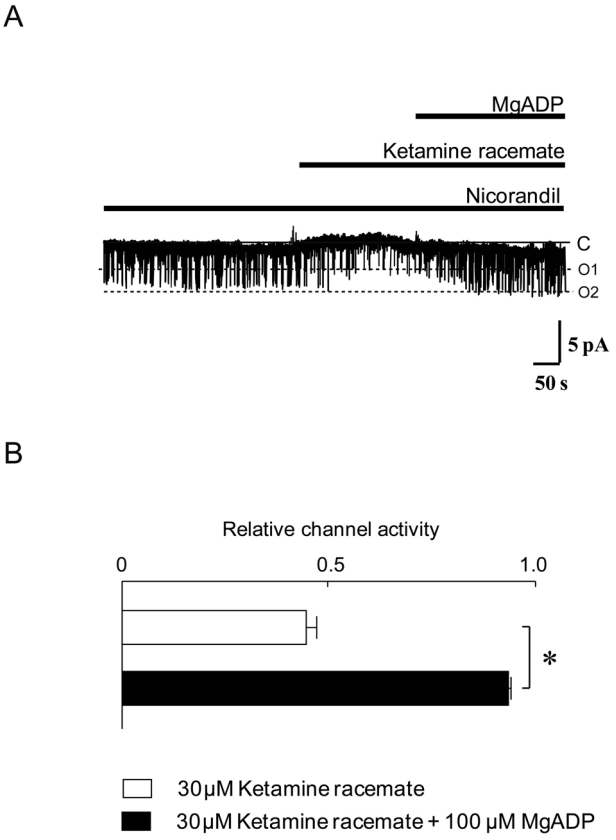

Figure 5. Effects of ketamine racemate and MgADP on nicorandil induced adenosine triphosphate-sensitive potassium channels in the excised inside-out configuration. (A) Representative example of nicorandil induced channel currents in the absence or presence of ketamine racemate and MgADP is shown. Nicorandil (100 μM), ketamine racemate (30 μM) and MgADP (100 μM) were added to the intracellular solution as indicated by horizontal bars. C, closed channel level ; O1 and O2, open channel levels with one or two channels open simultaneously, respectively. (B) The relative channel activities in the presence of ketamine racemate without MgADP (open columns) or with MgADP (closed columns). The horizontal bars indicate the mean± SE (n=12), respectively. *p< 0.05 versus without MgADP value.

activities was attenuated in the presence of intracellular MgADP (Figure 6A and 6B) ; ketamine racemate significantly inhibited nicorandil induced K_{ATP} channel activities only at the highest concentration (1000 μM, n=8, $p<0.05$ *vs.* baseline).

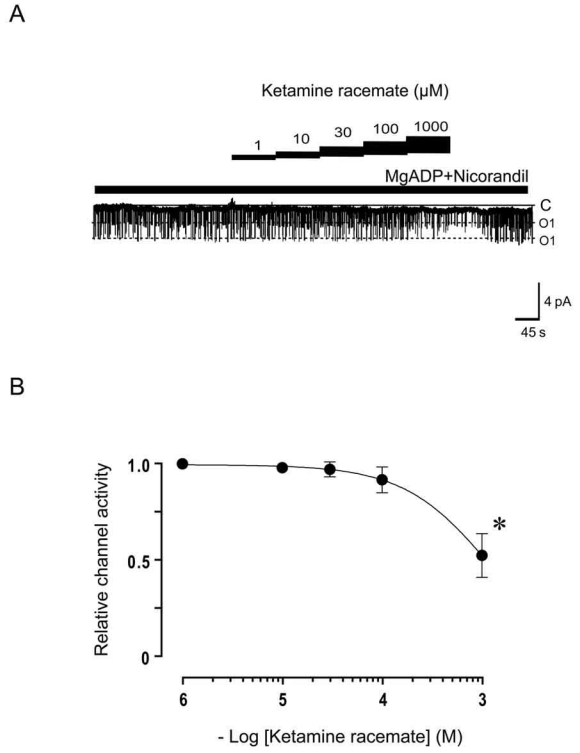

Figure 6. Effects of ketamine racemate on nicorandil induced adenosine triphosphate-sensitive potassium channels in the excised inside-out configuration under intracellular MgADP containing condition. (A) Representative example of nicorandil and MgADP induced channel currents before and after the application of ketamine racemate is shown. Nicorandil (100 μM) with MgADP (100 μM) and ketamine racemate (1-1000 μM) were added to the intracellular solution as indicated by horizontal bars. C, closed channel level ; O1 and O2, open channel levels with one or two channels open simultaneously, respectively. (B) Dose-dependent effects of ketamine racemate on the nicorandil induced channel activities in the presence of MgADP. Each vertical bar constitutes measurements from 8 patches (mean± SE). *$p<0.05$ versus baseline.

DISCUSSION

In the present study, we recorded single K_{ATP} channel currents using an inside-out patch clamp configuration and demonstrated that ketamine racemate and S-(+)-ketamine inhibited these channel activities induced by nicorandil in a concentration-dependent manner. Furthermore, the inhibitory effects of ketamine racemate had approximately 7-fold higher potency than those of S-(+)-ketamine. Since ketamine racemate is a mixture of S-(+) and R-(-)-ketamine stereoisomers, this observation would suggest that block of K_{ATP} channels by ketamine enantiomers may be at least partly stereoselective. Our results also demonstrated that intracellular MgADP restored the inhibitory action of ketamine racemate on nicorandil induced K_{ATP} channel activities.

K_{ATP} channels, which are widely distributed in many tissues, respond to alterations in the metabolic activity of the cell and thereby act as sensors of glucose and oxygen availability (1, 11). In the cardiovascular system, the sarcolemmal K_{ATP} channels are present both in cardiomyocytes and vascular smooth muscle cells (1). Recent physiological studies on mice lacking different K_{ATP} channel subunits have begun to clarify the roles of cardiac and vascular K_{ATP} channels in cardiovascular pathophysiology. Cardiac K_{ATP} channel ($Kir6.2^{-/-}$)-deficient mice had a number of cardiac abnormalities during myocardial ischemia or severe stress, including impaired ischemic preconditioning and attenuated electrocardiographic ST changes (12). Impaired vascular smooth muscle function was a feature of Kir6.1-deficient and SUR2-deficient mice and manifested as episodic coronary artery vasospasm and a high rate of sudden death (13, 14). Nicorandil is a potent cardiac and vascular K_{ATP} channel opener (9). In clinical practice, therefore, this drug is widely used in the treatment of ischemic heart disease (4). A recent study further suggested that prophylactic administration of nicorandil proved useful in the perioperative prevention of cardiac complications (5).

Our previous study demonstrated that ketamine racemate directly inhibited sarcolemmal K_{ATP} channels that formed by subunits expressed in COS-7 cells (8). The present study indicates that ketamine racemate also inhibits nicorandil induced K_{ATP} channel activities in native vascular smooth muscle cells (VSMCs). During surgical anaesthesia, clinical plasma concentrations for ketamine racemate are 20-50 μM, with approximately 12% of the drug being bound to plasma proteins (15). In current study, the threshold concentrations of ketamine racemate which inhibits nicorandil induced K_{ATP} channel activities (>10 μM) are within this range, but the concentrations of S-(+)-ketamine required for inhibition were very high (>100 μM). It is likely, therefore, that ketamine racemate inhibits K_{ATP} channel activities induced by nicorandil at clinically relevant concentrations. These results suggested that ketamine racemate could impair nicorandil induced pharmacological organ-protection against intraoperative

ischemic or hypoxic injury mediated by K_{ATP} channels. Indeed, racemic ketamine, but not the S-(+)-ketamine stereoisomer, was found to block early and late preconditioning in rabbit hearts (16, 17) and inhibit vasorelaxation induced by a K_{ATP} channel opener, levocromakalim (18).

A previous study reported that acute ischemic preconditioning in the rabbit heart is observed with nicorandil treatment prior to ischemia, but not after myocardial ischemia (19). These results indicate that activation of K_{ATP} channels during metabolic stress is essential for pharmacological organ protective effects of nicorandil.

During metabolic stress such as hypoxia or ischemia, the concentration of intracellular ADP rises while the levels of intracellular ATP drop. Similar to intracellular ATP, other magnesium nucleotides can modulate K_{ATP} channel activity, with the most dramatic effects being caused by MgADP, which antagonizes the inhibitory effects of ATP (1, 9). Our recent electrophysiological results suggested that application of intracellular MgADP enhances the inhibitory effects of ketamine racemate on the activity of reconstituted K_{ATP} channels expressed in COS-7 cells (8). We therefore hypothesised that intracellular MgADP also may enhance the inhibitory effects of ketamine racemate on nicorandil induced K_{ATP} channel activities. Surprisingly, our results demonstrated that the inhibitory effect of ketamine racemate at a clinically relevant concentration (30 μM) on nicorandil induced K_{ATP} channel activities was almost completely abolished by application of intracellular MgADP. These data suggest that the therapeutic effectiveness of nicorandil might not be impaired by ketamine racemate under metabolic stress conditions. The exact mechanisms of these effects have not been clearly identified. A recent study, however, indicated that metabolic inhibition significantly increased the potency of nicorandil on pig coronary artery relaxation (20). These results suggest that MgADP may enhance nicorandil induced K_{ATP} channel activities. Thus, our results suggest that the MgADP enhancement of K_{ATP} channel activation of nicorandil could mask the inhibitory effects of ketamine racemate.

The inherent limitations of this study model must be addressed. First, the concentration of nicorandil we used in the current study (100 μM) should be much higher than the clinically relevant concentrations (approximately 1 μM) (21). Therefore, it may be difficult to directly extrapolate our results to *in vivo* conditions. Second, we performed patch clamp experiments in cultured, not freshly isolated, VSMCs to avoid using damaged cells. Although cultured cells may undergo a variety of phenotypic changes, the functions of vascular K_{ATP} channels (e.g. sensitivity of K_{ATP} channel modulators and single channel conductance) were consistent with those of K_{ATP} channels previously recorded in freshly isolated VSMCs (22).

In conclusion, our results demonstrated that ketamine inhibits nicorandil induced vascular K_{ATP} channels activity in a dose-dependent and stereoselective manner. However, our results further indicated that increase of intracellular MgADP markedly attenuated the inhibitory effect of ketamine. These results suggested the possibility that ketamine might not impair the beneficial vasodilative effects of nicorandil as a K_{ATP} channel opener under conditions of metabolic inhibition.

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