Anesthesiology
79:571–579, 1993

J. B. Lippincott Company, Philadelphia

Effects of Ketamine on Contraction and Synthesis of Inositol 1,4,5-Trisphosphate in Smooth Muscle of the Rabbit Mesenteric Artery

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Background: **Ketamine acts directly on vascular smooth muscle, causing relaxation. It has been suggested that the mechanism underlying this action involves an interference with transmembrane Ca^{2+} influx and an inhibition of Ca^{2+} release from intracellular Ca^{2+} stores. In vascular smooth muscle cells, agonist-induced Ca^{2+} release is thought to be mediated by an intracellular second messenger, inositol 1,4,5-trisphosphate ($InsP_3$). To investigate the site at which ketamine acts on agonist-induced contraction, the authors studied the effects of ketamine on contraction and on the synthesis of $InsP_3$ in smooth muscles of the rabbit mesenteric artery.**

Methods: **Changes in isometric tension of smooth muscle fibers were measured by attaching a thin circular strip from the rabbit mesenteric artery to a strain gauge. To measure the norepinephrine (NE)-induced production of $InsP_3$, smooth muscle strips of the rabbit mesenteric artery were exposed to the agents and homogenized. Inositol 1,4,5-trisphosphate in the supernatant fractions was then assayed.**

Results: **Ketamine dose-dependently inhibited contractions induced by high K^+, NE, and histamine in normal Krebs solution. Ketamine also inhibited the NE- or histamine-induced contraction in Ca^{2+}-free solution containing 2 mM ethyleneglycol bis-(β-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA), indicating that this drug inhibits agonist-induced Ca^{2+} release from intracellular stores. Norepinephrine (10 μM) transiently increased the synthesis of $InsP_3$ in Ca^{2+}-free solution, and ketamine (0.1–1.0 mM) inhibited this effect, in a dose-dependent manner.**

Conclusions: **These results indicate that, in the rabbit mesenteric artery, ketamine inhibits agonist-induced Ca^{2+} release through its inhibitory action on the agonist-induced synthesis of $InsP_3$. Thus, it is possible that ketamine interferes with the synthesis of intracellular second messengers. (Key words: Anesthetics, intravenous: ketamine. Inositol 1,4,5-trisphosphate. Muscle, smooth: vasodilation.)**

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Received from the Department of Anesthesiology and Critical Care Medicine and the Department of Pharmacology, Faculty of Medicine, Kyushu University, Fukuoka, Japan. Accepted for publication May 8, 1993.

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THE intravenous anesthetic ketamine is widely used in patients with cardiovascular instability because of its cardiovascular stimulation. This stimulating effect has been ascribed to its sympathomimetic action *via* the central nervous system,[1] its modulation of the activities of the baroreceptors,[2] its inhibition of the neuronal uptake of catecholamines by sympathetic nerve endings,[3] and myocardial inotropy.[4] However, ketamine has been reported to produce biphasic blood-pressure responses (initially hypotensive, later hypertensive) or profound hypotension in animals.[2,5,6] The hypotension is thought to be caused by a direct vasodilating effect of ketamine.

In vascular smooth muscle tissues, contraction and relaxation depend, in part, on the intracellular free Ca^{2+} concentration. The intracellular free Ca^{2+} can be increased by an influx of external Ca^{2+} through a voltage-dependent Ca^{2+} channel, which can be modulated by receptor activation,[7] and also by the agonist-induced release of Ca^{2+} from intracellular stores (possibly the sarcoplasmic reticulum [SR]).[8] In the process of relaxation, some portion of the increased intracellular free Ca^{2+} is extruded to the extracellular space by plasmalemmal (Ca^{2+} + Mg^{2+})-ATPase and by Na^+-Ca^{2+} exchange, and the remainder undergoes reuptake into the intracellular stores. The mechanisms underlying the vasodilating effect of ketamine have been shown to involve both an interference with Ca^{2+} influx through the voltage-dependent Ca^{2+} channel[9,10] and an inhibition of Ca^{2+} release from the intracellular stores.[11]

The inhibitory effect of ketamine on Ca^{2+} influx through the voltage-dependent Ca^{2+} channel is thought to be exerted in the same manner as that of Ca antagonists. However, the mechanism underlying the inhibitory action of ketamine on agonist-induced Ca^{2+} release from intracellular stores has not been well investigated.

Activation of receptor by agonist in vascular smooth muscle tissues induces hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) by phospholipase C. As a result, inositol 1,4,5-trisphosphate ($InsP_3$) and 1,2-diacylglycerol (DAG) are produced, and these products play an essential role in contraction as second messengers. Particularly, $InsP_3$ is known to release Ca^{2+} from intracellular stores in vascular smooth muscle tissues.[12,13]

The current study was designed to clarify the effects of ketamine on agonist-induced $InsP_3$ synthesis in smooth muscle tissues of the rabbit mesenteric artery.

Materials and Methods

Preparation

In accordance with institutional Animal Care Committee standards, male albino rabbits (2.0–2.5 kg) were given sodium pentobarbital (40 mg/kg intravenously) and exsanguinated. The mesentery in the jejunal region was dissected out and the mesenteric artery rapidly excised in a dissecting chamber filled with oxygenated Krebs solution under a binocular microscope. After the fat and connective tissues had been carefully removed, thin circular strips (0.3–0.5 mm long, 0.05–0.08 mm wide, and 0.02–0.03 mm thick) were prepared for tension recording under a binocular microscope using fine forceps and small knives made from fragments of razor blade. For the measurement of $InsP_3$, longitudinally opened strips (15–20 mm long and 1.5–3.0 mm wide) were prepared. In all experiments, the endothelium was carefully removed by rubbing the intimal surface with a cotton ball moistened with Krebs solution.[14]

Recording of Mechanical Activity

Smooth muscle strips of rabbit mesenteric artery were prepared for measurement of their mechanical activities by attaching a circular strip to a strain gauge (UL-2, Minebea, Tokyo). A fine silk fiber was tied to each end of the strip and then fixed to a piece (about 1 mm × 1 mm) of Scotch double-sided adhesive tape (3M, St. Paul, MN). One tape was fixed to a wall of the chamber and the other tape to the strain gauge. The chamber had a volume of 0.9 ml. The solutions were rapidly injected using a syringe from one end of the chamber and simultaneously aspirated by a water pump from the other end. The tissue was not superfused continuously, but remained exposed to the solution in the chamber until the next solution was injected.[15]

Assays of $InsP_3$ (fig. 1)

After connective tissue and endothelium had been carefully removed, longitudinally opened muscle strips (15–20 mm long, 1.5–3.0 mm wide, and 0.1 mm thick) were allowed to equilibrate in flasks containing Krebs solution for over 2 h at 32° C. After this, the strips were transferred to Ca^{2+}-free solution containing 2 mM ethylenglycol-bis-(β-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA) with or without ketamine for 3 min. Norepinephrine (NE, 10 μM) was then applied in Ca^{2+}-free solution for 10–180 s. The reaction was stopped by the addition of a large amount of ice-cold trichloroacetic acid (TCA, final concentration 8%) and the strips were homogenized in a glass homogenizer. The homogenate was centrifuged at 3,000 rpm for 15 min and the supernatant fractions were treated with ether three times. The *p*H of the supernatant fractions

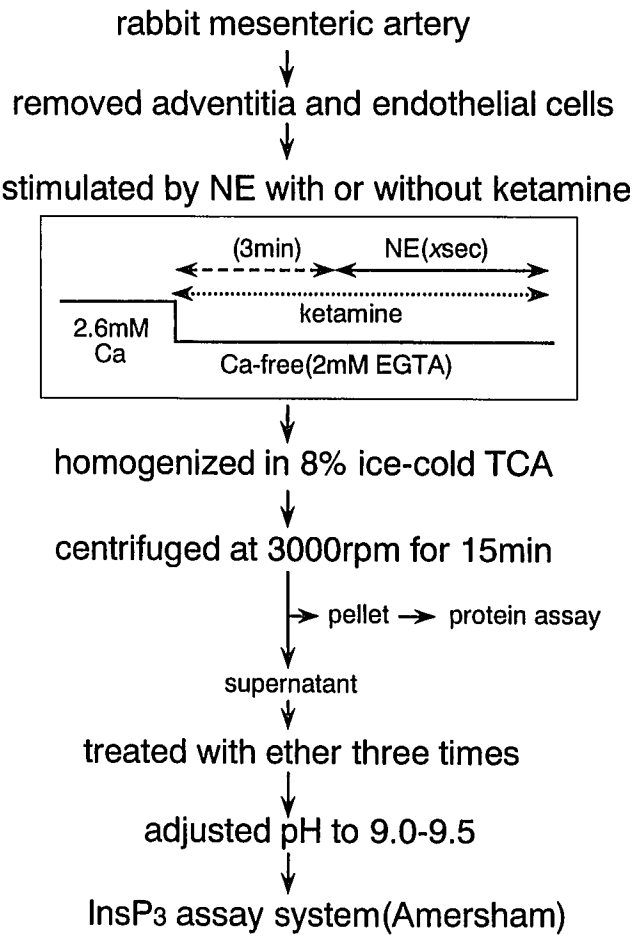

Fig. 1. Experimental design for assays of inositol 1,4,5-trisphosphate ($InsP_3$). TCA = trichloroacetic acid.

was adjusted to 9.0–9.5 by adding KOH in the presence of universal indicator and the amounts of $InsP_3$ in these fractions was measured using an $InsP_3$ [^{3}H] assay system from Amersham International (Little Chalfont, U.K.).

Solutions

The composition of the Krebs solution was as follows (mM concentrations): Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.6, HCO_3- 15.5, H_2PO_4- 1.2, Cl- 134.4, and glucose 11.5. The solution was bubbled with 95% O_2/5% CO_2 and the *p*H adjusted to 7.3–7.4. High-K solution was prepared by isosmotically replacing NaCl with KCl. For Ca^{2+}-free solutions, $CaCl_2$ was replaced with $MgCl_2$ and 2 mM EGTA was added.

Drugs

The chemicals used were: ketamine hydrochloride from Sankyo (Tokyo, Japan), norepinephrine (NE) and histamine from Sigma (St. Louis, MO), caffeine from Wako Pharmaceutical (Tokyo, Japan), and ethyleneglycol-bis-(β-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA) from Dojin Laboratories (Kumamoto, Japan).

Statistics

The experimental values are expressed as mean ± SD (n = number of observations, rabbits) and statistical significance between ketamine-treated group and control group was determined using Student's *t* test. To evaluate the dose dependency of ketamine, regression analysis with replication[16] and Spearman rank correlation coefficient were conducted. $P < 0.05$ was considered to be significant.

Results

In preliminary experiments, we found that ketamine produced similar inhibitory effects on the contractions induced by high K^+ and NE whether endothelium was, or was not, present. Thus, in the current study, all experiments were carried out on endothelium-denuded tissues. We also found that, in larger parts of the rabbit mesenteric artery that were used for $InsP_3$ measurements, ketamine inhibited the contraction induced by high K^+ or NE in a similar extent to that in smaller parts of the artery.

Effects of Ketamine on Contractions Induced by High K^+, NE, and Histamine in Krebs Solution

In the rabbit mesenteric artery, application of excess concentrations of K^+ induces depolarization of the plasma membrane of vascular smooth muscle cells. This membrane depolarization activates Ca^{2+} influx through the voltage-dependent Ca^{2+} channel in the plasma membrane, and, thus, intracellular Ca^{2+} is increased. The increased cytoplasmic Ca^{2+} produces contraction through an activation of contractile systems. The high K^+-induced contraction comprised a rapid phasic and a sustained tonic component (fig. 2A). Ketamine (30 μM–1 mM) inhibited these two components to the same extent (fig. 2C).

In the rabbit mesenteric artery, application of various concentrations of NE (over 0.3 μM) produced contraction, the maximum contraction being obtained with 10 μM. The maximum amplitude of contraction evoked by 10 μM NE was 0.58 ± 0.15 times that evoked by 128 mM K^+ (n = 5). Histamine (10 μM) also induced contraction in this tissue, although the maximum amplitude was smaller than for 10 μM NE, being 0.43 ± 0.15 times that induced by 128 mM K^+ (n = 5). Ketamine inhibited these NE- and histamine-induced contractions in a dose-dependent manner ($P < 0.01$ in both agonists-induced contractions by regression analysis with replication, $P < 0.01$ by Spearman rank correlation coefficient; figs. 2A, B, and D).

Effects of Ketamine on Contractions Induced by NE, Histamine, and Caffeine in Ca^{2+}-Free Solution

In the rabbit mesenteric artery, NE and histamine each produce a transient contraction in Ca^{2+}-free solution, and this has been presumed to be caused by agonist-induced release of Ca^{2+} from intracellular Ca^{2+} stores.[17] Caffeine also evokes a contraction because of release of Ca^{2+} from the same intracellular stores in this tissue.[18] We further investigated the effects of ketamine on the contractions induced by these agonists and by caffeine in Ca^{2+}-free solution.

After the Ca^{2+} stored in the smooth muscle cells had been completely depleted by repetitive applications of 20 mM caffeine in Ca^{2+}-free solution containing 2 mM EGTA, the tissues were exposed to 2.6 mM Ca^{2+} in Krebs solution for 20 min. This procedure is thought to completely fill the intracellular Ca^{2+} stores. Thereafter, the tissues were again superfused with Ca^{2+}-free solution for 3 min and agonists or caffeine subsequently applied for 3 min (fig. 3A). The amplitude of contraction induced by 10 μM NE was similar to that with 10 μM histamine. These were, respectively, 0.35 ± 0.05 times and 0.32 ± 0.05 times the amplitude of the 128 mM K^+-induced contraction in Krebs solution (n = 5).

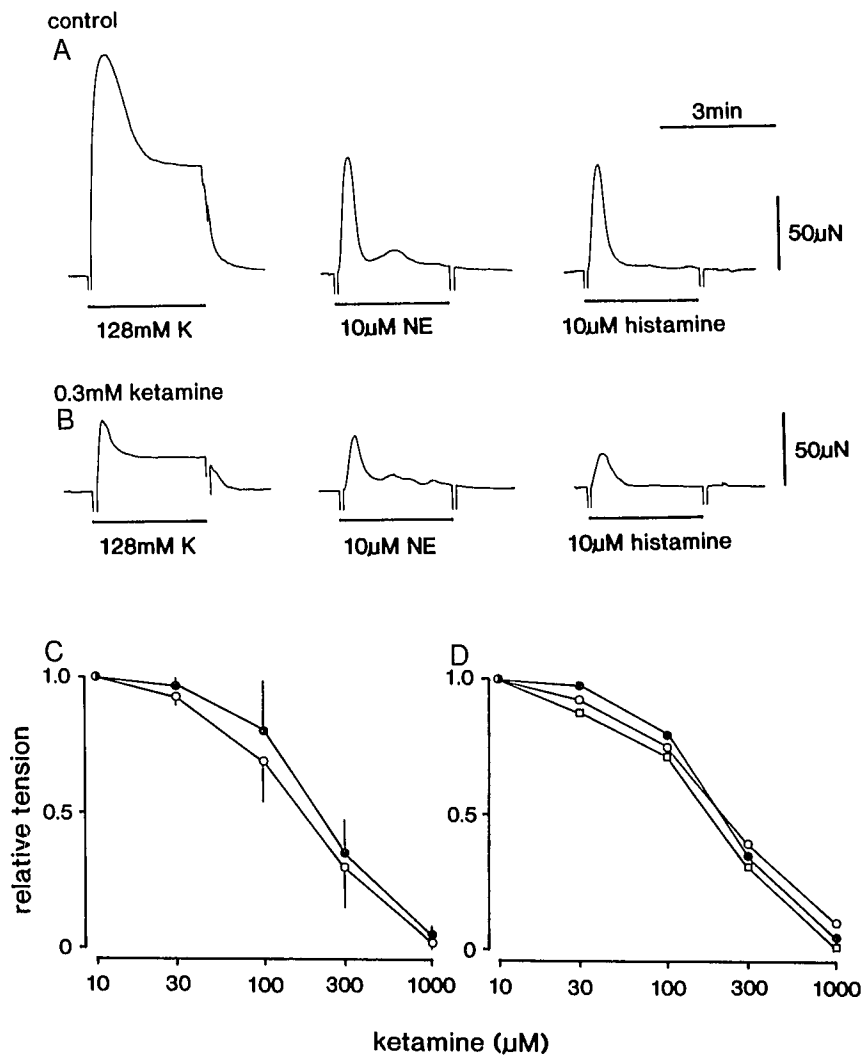

Fig. 2. Effects of ketamine on the contractions evoked by 128 mM K^+, 10 μM NE, and 10 μM histamine in Krebs solution. (*A*) Control contractions evoked by 128 mM K^+, 10 μM NE, and 10 μM histamine. (*B*) Typical example of the effects of 0.3 mM ketamine on the above contractions. (*C*) Concentration-response curves for effects of ketamine on the phasic (●) and subsequently generated tonic (○) contractions induced by 128 mM K^+. The amplitude of phasic or tonic responses evoked in the absence of ketamine was normalized as 1.0. Vertical bars indicate SD, n = 5. (*D*) Concentration-response curves for effects of ketamine on contraction evoked by 128 mM K^+ (●), 10 μM NE (○), or 10 μM histamine (□). Amplitude of the phasic contraction evoked by each stimulant in the absence of ketamine was normalized as 1.0. Vertical bars representing SD have been omitted for clarity. For each data point, n = 5.

The amplitude of the 20 mM caffeine-induced contraction was larger than that induced by either 10 μM NE or 10 μM histamine, being 0.51 ± 0.16 times the amplitude of the 128 mM K^+-induced contraction in Krebs solution.

When ketamine was applied before and during an application of agonist, this drug dose-dependently inhibited the NE- and histamine-induced contractions in Ca^{2+}-free solution ($P < 0.01$ in both agonists-induced contractions by regression analysis with replication, $P < 0.01$ by Spearman rank correlation coefficient; fig. 3B). However, the contraction induced by 20 mM caffeine in Ca^{2+}-free solution was little affected by ketamine. To see whether the inhibitory effect of ketamine on the contractions induced by NE and histamine is caused by an inhibition of Ca^{2+} release from the stores or to an enhancement of Ca^{2+} extrusion through the plasma membrane, 10 μM NE and 20 mM caffeine were successively applied in Ca^{2+}-free solution. As shown in figure 4, even after an application of 10 μM NE for 3 min, 20 mM caffeine induced a contraction somewhat larger than that induced by the preapplied 10 μM NE. Ketamine (0.3 mM) significantly inhibited the NE-induced contraction (0.71 ± 0.07 times control, $P < 0.01$, n = 6), but slightly enhanced the amplitude of the contraction induced by subsequently applied 20 mM caffeine (1.18 ± 0.12 times control, $P < 0.05$, n = 6). These results indicate that the inhibitory effects of ketamine on NE-induced contractions in Ca^{2+}-free solution are probably caused by an inhibition of Ca^{2+}

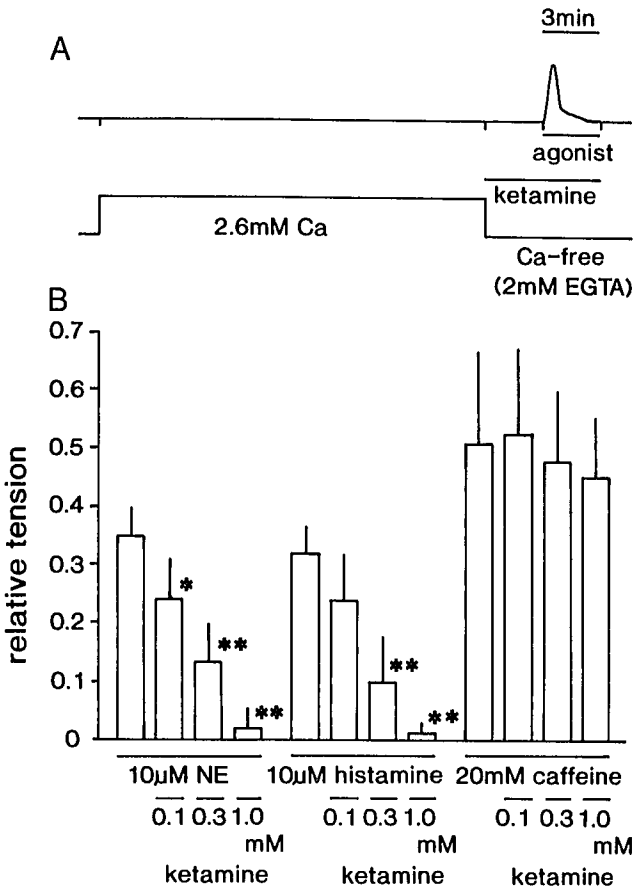

Fig. 3. Effects of ketamine on the contractions induced by 10 μM NE, 10 μM histamine, and 20 mM caffeine in Ca^{2+}-free solution containing 2 mM EGTA. (*A*) The experimental protocol. After repetitive applications of 20 mM caffeine in Ca^{2+}-free solution, 2.6 mM Ca^{2+} (Krebs solution) was applied for 20 min. Norepinephrine (10 μM), histamine (10 μM), or caffeine (20 mM) was then applied in Ca^{2+}-free solution containing 2 mM EGTA after 3 min resuperfusion with Ca^{2+}-free solution containing 2 mM EGTA. (*B*) Concentration-response relationship for effects of ketamine on NE-, histamine-, and caffeine-induced contractions in Ca^{2+}-free solution. The amplitude of contraction was normalized to the maximum evoked by 128 mM K^+ in Krebs solution in the absence of ketamine. Vertical bars indicate SD, n = 5. Asterisks indicate significant difference from the response to stimulant alone (*$P < 0.05$, **$P < 0.01$).

release from the intracellular Ca^{2+} stores, rather than by an enhancement of Ca^{2+} extrusion through the plasma membrane.

Effects of Ketamine on $InsP_3$ Production Induced by NE

Inositol 1,4,5-trisphosphate acts as a second messenger of agonists to release Ca^{2+} from the intracellular Ca^{2+} stores in smooth muscle cells of the rabbit mesenteric artery.[13] To further investigate the mechanism of the inhibitory action of ketamine on agonist-induced contractions, we assessed the effects of ketamine on the $InsP_3$ production induced by NE in Ca^{2+}-free solution containing 2 mM EGTA. The amount of $InsP_3$ reached a peak 10 s after the application of 10 μM NE, and then declined to a steady level in the presence of NE. Ketamine (0.3 mM) lowered the concentration of $InsP_3$ under resting conditions and inhibited the increase in $InsP_3$ induced by 10 μM NE at any given time (fig. 5A). The inhibitory action of ketamine on the maximum increase in the synthesis of $InsP_3$ induced by 10 μM NE, measured at 10 s after the application of NE, was concentration dependent ($P < 0.05$ by regression analysis with replication, $P < 0.01$ by Spearman rank correlation coefficient; n = 15, fig. 5B).

Discussion

The state of contraction or relaxation of vascular smooth muscle is mainly dependent on the concentration of free Ca^{2+} in the myoplasm. Increased intracellular free Ca^{2+} binds to calmodulin and the Ca^{2+}-calmodulin complex activates myosin light chain kinase (MLCK). Activated MLCK phosphorylates myosin light chain (MLC), and contraction then occurs.[19,20] Thus, the intracellular free Ca^{2+} plays an essential role in the contraction–relaxation cycle of vascular smooth muscle (fig. 6). The amount of Ca^{2+} in the myoplasm can be increased by an influx of Ca^{2+} and by the release of Ca^{2+} from the intracellular Ca^{2+} stores (mainly SR). Influx of Ca^{2+} occurs through a voltage-dependent Ca^{2+} channel. The voltage-dependent Ca^{2+} channel opens with membrane depolarization induced by action potentials, by excess concentrations of extracellular K^+, and by exogenously applied agonists, such as NE. Activation of a receptor by its agonist produces contraction, with or without membrane depolarization. The amplitude of contractions evoked by agonists without membrane depolarization are reduced in Ca^{2+}-free solution containing EGTA. Therefore, activation of some receptors is thought to modulate voltage-dependent Ca^{2+} channels.[7] In addition, agonists can induce release of Ca^{2+} from the intracellular Ca^{2+} stores and, thus, increase the intracellular free Ca^{2+} concentration.

A reduction in the free Ca^{2+} concentration results mainly from: 1) reuptake of Ca^{2+} into the Ca^{2+} stores (SR) *via* activation of Ca^{2+}-Mg^{2+} ATPase on the SR membrane; and 2) pumping out of Ca^{2+} to the extra-

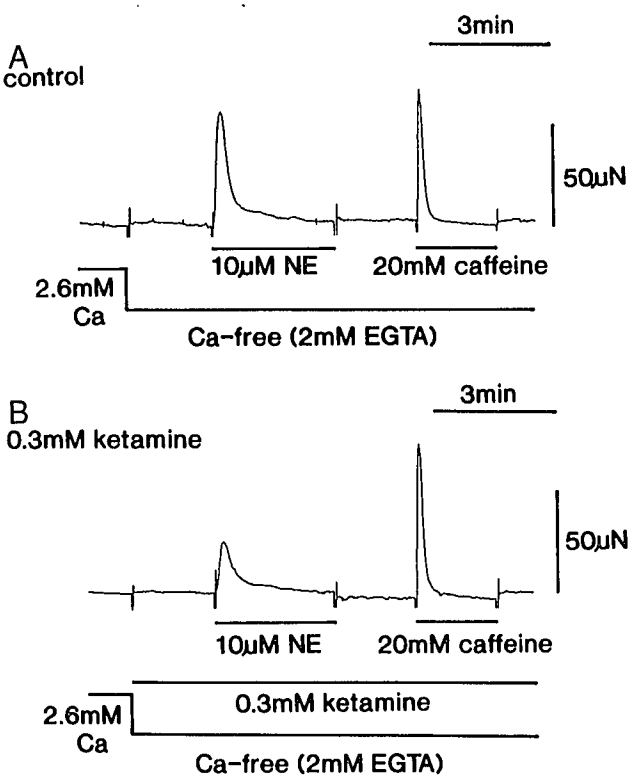

Fig. 4. Effects of ketamine on the contractions induced by successively applied NE and caffeine in Ca^{2+}-free solution containing 2 mM EGTA. After 2.6 mM Ca^{2+} was applied for 20 min, 10 μM NE and 20 mM caffeine were successively applied with a 2-min interval. (*A*) Control. (*B*) Ketamine, 0.3 mM, was applied in Ca^{2+}-free solution. These results were typical of those obtained in five experiments.

cellular space *via* activation of Ca^{2+}-Mg^{2+} ATPase on the plasma membrane. In addition, Na^+(influx)-Ca^{2+}(efflux) exchange diffusion may play some role in decreasing the intracellular free Ca^{2+} concentration.

In vascular smooth muscle, it has been reported that ketamine inhibits high K^+- or agonist-induced contraction through an inhibition of transmembrane Ca^{2+} influx.[9,10] Recently, using the patch clamp technique, Yamazaki *et al.* demonstrated that ketamine directly inhibits a voltage-dependent Ca^{2+} channel (L-type) in single smooth muscle cells of the rabbit portal vein.[21] In the current experiments, ketamine dose-dependently inhibited high K^+-induced contractions, which are possibly provoked by Ca^{2+} influx through the voltage-dependent Ca^{2+} channel. However, the manner of the ketamine-induced inhibition was different from that of organic Ca^{2+} antagonists, which typically inhibit the voltage-dependent L-type Ca^{2+} channel. Organic Ca^{2+} antagonists inhibit the tonic component of high K^+-induced contractions more than the phasic component,[22] whereas the inhibitory effects of ketamine were similar for both the phasic and tonic components, as we reported previously.[11] Therefore, the inhibitory actions of ketamine on the high K^+-induced contraction may not be exerted only on the voltage-dependent Ca^{2+} channel.

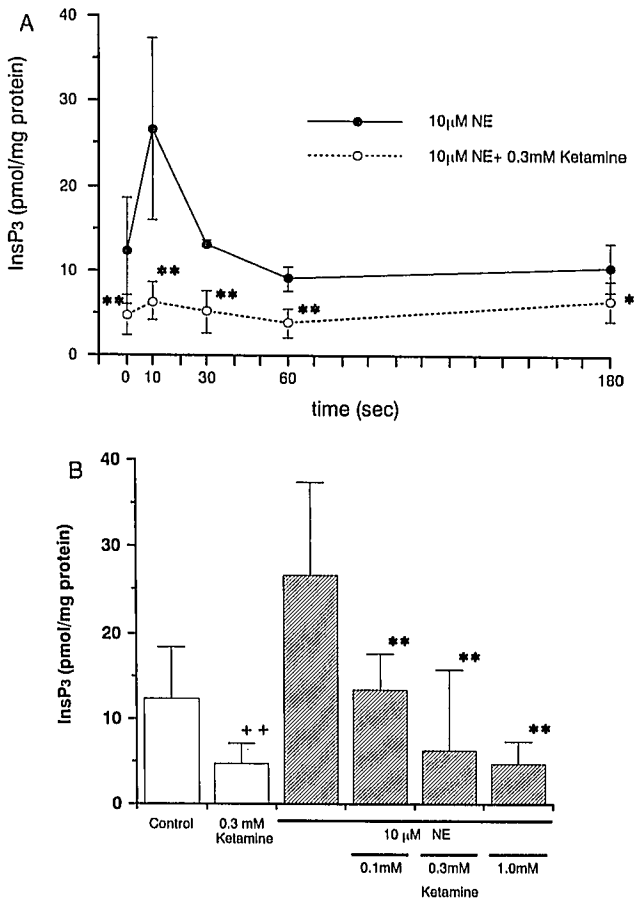

Fig. 5. Effects of ketamine on $InsP_3$ synthesis induced by 10 μM norepinephrine (NE) in Ca^{2+}-free solution containing 2 mM EGTA. (*A*) Time-dependent changes in the synthesis of $InsP_3$ after application of 10 μM NE in the presence (○) or absence (●) of 0.3 mM ketamine. Norepinephrine (10 μM) was applied at time zero. Results shown are each the mean of 15 observations, with SD shown by vertical bar. Asterisks indicate significant difference from response to NE alone (*$P < 0.05$, **$P < 0.01$). (*B*) Dose-dependent effects of ketamine on $InsP_3$ synthesis induced by 10 μM NE in Ca^{2+}-free solution containing 2 mM EGTA. Norepinephrine (10 μM) was applied for 10 s after a 3-min removal of Ca^{2+} (see fig. 1). Results shown are each the mean of 15 observations, with SD shown by the vertical bar. ††Significant difference ($P < 0.01$) from the control (strips not stimulated by NE); **Significant difference ($P < 0.01$) from response to NE alone.

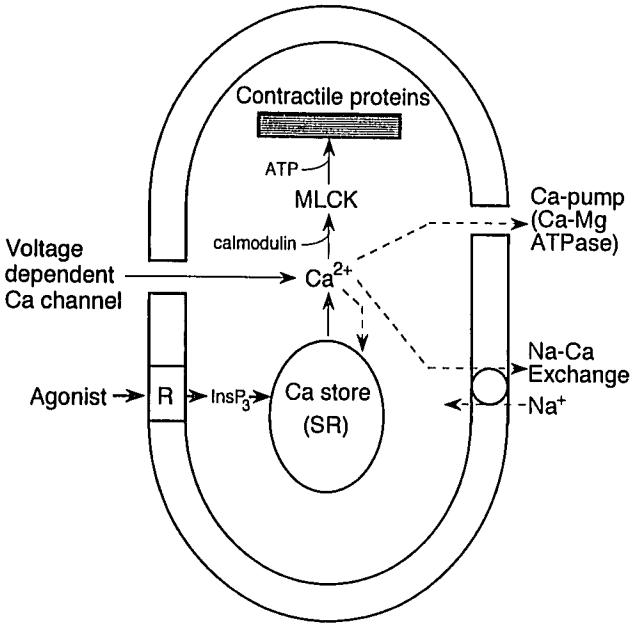

Fig. 6. Excitation-contraction coupling in vascular smooth muscle. Ca^{2+} can enter the cell through voltage-dependent Ca^{2+} channel. Agonist binding to specific receptor (R) results in the production of its intracellular second messenger, $InsP_3$, and modulation of voltage-dependent Ca^{2+} channel. The $InsP_3$ releases Ca^{2+} from the intracellular Ca^{2+} stores (the SR). Increased intracellular free Ca^{2+} binds calmodulin, and Ca^{2+}-calmodulin complex activates MLCK. Activated MLCK phosphorylates contractile proteins, and contraction then occurs. Some portion of the increased intracellular Ca^{2+} is extruded *via* the plasma membrane Ca^{2+} pump (Ca^{2+}-Mg^{2+} ATPase) and Na^{+}-Ca^{2+} exchange mechanism. Ca^{2+} is reaccumulated into the SR *via* another type of Ca^{2+}-Mg^{2+} ATPase.

Ketamine attenuated agonist-induced contractions not only in Krebs solution, but also in Ca^{2+}-free solution containing 2 mM EGTA. However, ketamine did not inhibit caffeine-induced contraction in Ca^{2+}-free solution. In Ca^{2+}-free solution, the contraction induced by agonists or caffeine is thought to be caused by their ability to release Ca^{2+} from the intracellular stores. The agonist-induced Ca^{2+} release is thought to be mediated by the action of $InsP_3$, which is produced after receptor occupancy by the agonist.[13] By contrast, caffeine acts directly on the Ca^{2+} stores and releases Ca^{2+} through an activation of the Ca^{2+}-induced Ca^{2+} release mechanism.[17] Ketamine does not have an effect either on the contractile proteins or on $InsP_3$-induced Ca^{2+} release from the intracellular stores in saponin-treated skinned vascular smooth muscle.[11] These results indicate that ketamine may act somewhere between receptor occupancy by the agonist on the plasma membrane and Ca^{2+} release from the stores, and, thus, inhibit agonist-induced contraction.

In the current experiments using the rabbit mesenteric artery, 10 μM NE induced synthesis of $InsP_3$ in Ca^{2+}-free solution. Ketamine (0.1 mM–1.0 mM or 27.4 μg/ml–274 μg/ml) dose-dependently inhibited this NE-induced increase in $InsP_3$ synthesis. Inositol 1,4,5-trisphosphate releases Ca^{2+} from the intracellular stores in this tissue.[13] Therefore, the inhibitory effects of ketamine on agonist-induced contractions can be assumed to be caused by an inhibition of $InsP_3$ synthesis. It has been reported that concentrations of ketamine in human plasma reach a maximum of 94 μM 1 min after, or approximately 60 μM 5 min after, intravenous administration (2 mg/kg) of this drug,[23] and that clinically observed concentrations of ketamine (10–60 μM) did not have a direct effect on the vascular tone.[24,25] These findings indicate that the concentration of ketamine used here may be slightly greater than that used in clinical practice. However, it should be noted that ketamine produced a transient decrease in arterial pressure just after intravenous injection in pithed rabbits.[6] Thus, the inhibitory effects of ketamine on contraction and $InsP_3$ synthesis induced by NE may explain this circulatory change by ketamine in the rabbit.

In vascular smooth muscle, receptor activation by agonists initiates the hydrolysis of the plasma membrane phospholipid PIP_2 through an activation of phospholipase C, and, thus, produces $InsP_3$ and DAG (fig. 7).[26] The coupling between the receptor and phospholipase C is mediated by GTP-binding proteins, which functionally transmit the signal from the surface receptor to the phospholipase C. Therefore, the inhibitory action of ketamine on agonist-induced $InsP_3$ synthesis could be exerted on GTP-binding proteins and/or on phospholipase C. It may be thought that ketamine acts on the receptor as its antagonist and so prevents receptor occupancy by the agonist. However, because ketamine inhibits contraction induced by several different kinds of agonist (NE and histamine in the rabbit mesenteric artery in this experiment and also in the rabbit ear artery[11]; epinephrine, angiotensin2, and vasopressin in the rat aorta and portal vein[9]), it may be unlikely that the main site of action of ketamine is on the agonists' receptors.

Another product of PIP_2 breakdown, DAG, also acts as an intracellular second messenger. 1,2-Diacylglycerol activates a Ca^{2+}- and phospholipid-dependent protein kinase, protein kinase C, which has been shown to phosphorylate myosin light chain purified from

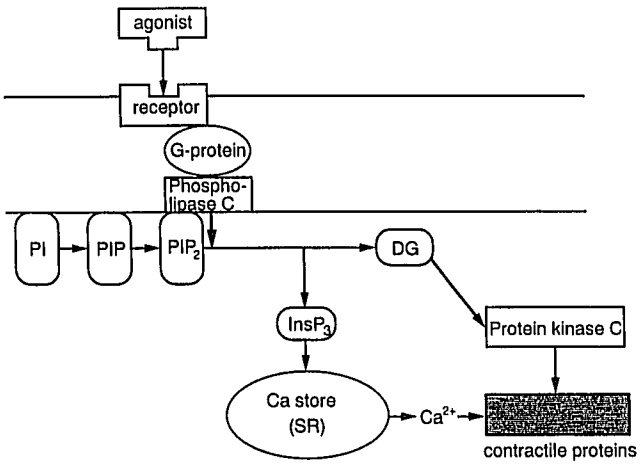

Fig. 7. Pathways for the generation of $InsP_3$ and DAG in vascular smooth muscle. Agonist binding to its specific receptor results in the activation of phospholipase C with the mediation of GTP binding proteins (G-protein). Activated phospholipase C initiates the breakdown of PIP_2, resulting in the production of $InsP_3$ and DAG. Inositol 1,4,5-trisphosphate releases Ca^{2+} from intracellular Ca^{2+} stores (the SR), while DAG activates protein kinase C. PI = phosphatidylinositol; PIP = phosphatidylinositol 4 monophosphate.

smooth muscle,[27] and to modulate contractions in vascular smooth muscle.[28] In addition, protein kinase C has been reported to increase Ca^{2+} channel conductance in a vascular smooth muscle cell line.[29] At this point, the physiologic role of DAG and protein kinase C is not clear, but it is conceivable that ketamine exerts its inhibitory effects on contraction through a reduction in the amount of DAG produced by agonists.

In conclusion, ketamine inhibits high K^+- and agonist-induced contractions in Krebs solution and agonist-induced contraction in Ca^{2+}-free solution. Ketamine inhibits agonist-induced synthesis of the intracellular second messenger, $InsP_3$, possibly as a result of an inhibition of the functions of GTP-binding proteins and/or phospholipase C.

The authors wish to thank Dr. R. J. Timms, for his help in preparing the manuscript; and Prof. S. Akiba, for his advice about statistical analysis.

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