Anesthesiology
1997; 86:1104-11

Calcium Concentration-dependent Mechanisms through which Ketamine Relaxes Canine Airway Smooth Muscle

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Background: **Ketamine is a potent bronchodilator that, in clinically used concentrations, relaxes airway smooth muscle in part by a direct effect. This study explored the role of calcium concentration (Ca^{2+}) in this relaxation.**

Methods: **Canine trachea smooth muscle strips were loaded with the fluorescent probe fura-2 and mounted in a spectrophotometric system to measure force and intracellular calcium concentration ($[Ca^{2+}]_i$) simultaneously. Calcium influx was estimated using a manganese quenching technique. Cyclic nucleotides in the airway smooth muscle strips were measured by radioimmunoassay.**

Results: **In smooth muscle strips stimulated with submaximal (0.1 μM) and maximal (10 μM) concentrations of acetylcholine, ketamine caused a concentration-dependent decrease in force and $[Ca^{2+}]_i$. The sensitivity of the force response to ketamine significantly decreased as the intensity of muscarinic receptor stimulation increased; the median effective concentration for relaxation induced by ketamine was 59 μM and 850 μM for tissue contracted by 0.1 μM or 10 μM acetylcholine, respectively ($P < 0.05$). In contrast, the sensitivity of the $[Ca^{2+}]_i$ response did not depend on the intensity of muscarinic receptor stimulation. Ketamine at 1 mM significantly inhibited calcium influx. Ketamine did not significantly increase cyclic nucleotide concentrations.**

Conclusions: **Ketamine-induced relaxation of canine airway smooth muscle is associated with a decrease in $[Ca^{2+}]_i$ and calcium influx, effects that are not mediated by an increase in cyclic nucleotides; and the sensitivity of the force response to ketamine decreases as the level of preexisting muscle tone increases, an effect that is not explained by differential effects on $[Ca^{2+}]_i$. (Key words: Measurement techniques: Calcium concentration–fluorescent probe fura-2; cyclic nucleotides; manganese quenching. Anesthetic drug: ketamine. Lung, bronchus: bronchoconstriction. Trachea: canine. Muscle, smooth: airway; trachea.)**

KETAMINE is a potent bronchodilator[1] that relaxes airway smooth muscle in part by a direct effect on airway smooth muscle cells.[2-5] Several issues regarding the mechanism of this direct effect remain unclear.

The concentration of intracellular calcium ($[Ca^{2+}]_i$) is an important determinant of tone in airway smooth muscle.[6,7] In rabbit femoral arteries, ketamine-induced relaxation is associated with a decrease in $[Ca^{2+}]_i$,[8] a finding consistent with studies of various types of smooth muscle that show that ketamine inhibits the function of L-type calcium channels.[9-11] However, decreases in $[Ca^{2+}]_i$ can also be caused by other actions on Ca^{2+} homeostasis, such as increased Ca^{2+} efflux or reduced Ca^{2+} release from intracellular stores.

Adenosine cyclic 3',5'-monophosphate (cAMP) and guanosine cyclic 3',5'-monophosphate (cGMP) are second messengers that can relax airway smooth muscle by decreasing $[Ca^{2+}]_i$.[12,13] The possible role of increases in cyclic nucleotides in causing decreases in $[Ca^{2+}]_i$ during ketamine-induced relaxation has not been evaluated.

The level of airway smooth muscle tone is an important determinant of the efficacy of many bronchodilators. This behavior, called "functional antagonism," may be related to differing mechanisms producing force at different levels of tone. In addition to an increase in $[Ca^{2+}]_i$, agonist-induced contraction is mediated by membrane receptor-coupled mechanisms that increase the amount of force produced for a given $[Ca^{2+}]_i$ (*i.e.*, the Ca^{2+} sensitivity).[14-19] We previously reported that during submaximal muscarinic receptor stimulation, halothane relaxed airway smooth muscle by decreasing $[Ca^{2+}]_i$, but that during maximal muscarinic stimulation halothane relaxed airway smooth muscle by

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Received from the Departments of Anesthesiology and Physiology and Biophysics, Mayo Clinic and Mayo Foundation, Rochester, Minnesota. Submitted for publication September 23, 1996. Accepted for publication January 17, 1997.

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decreasing Ca^{2+} sensitivity.[20] It is not known if ketamine had similar properties.

Our overall aim was to explore the mechanisms by which ketamine affects Ca^{2+} homeostasis and force in canine tracheal smooth muscle. We tested the following three hypotheses: 1) ketamine decreases calcium influx and $[Ca^{2+}]_i$ in airway smooth muscle when applied during sustained contraction; 2) ketamine reduces $[Ca^{2+}]_i$ in part by increasing the intracellular concentration of cyclic nucleotides; and 3) the effect of ketamine on force and $[Ca^{2+}]_i$ depends on the intensity of muscarinic stimulation.

Materials and Methods

Tissue Preparation

Mongrel dogs of either sex were anesthetized with an intravenous injection of pentobarbital (30 mg/kg) and then exsanguinated. A 5- to 10-cm portion of extrathoracic trachea was excised and immersed in chilled physiologic salt solution (PSS) composed of 110.5 mM NaCl, 25.7 mM $NaHCO_3$, 5.6 mM dextrose, 3.4 mM KCl, 2.4 mM $CaCl_2$, 1.2 mM KH_2PO_4, and 0.8 mM $MgSO_4$. Fat, connective tissue, and the epithelium were removed with tissue forceps and scissors. For technical reasons, $[Ca^{2+}]_i$ and cyclic nucleotides were measured in separate sets of experiments.

For studies estimating $[Ca^{2+}]_i$, all muscle strips (width, 0.8–1.0 mm; length, 4–5 mm) were mounted in a 0.1-ml quartz cuvette and were superfused continuously at 2 ml/min with PSS (37° C) aerated with 96% oxygen and 4% carbon dioxide. One end of the strip was anchored with stainless steel microforceps to a stationary metal rod; the other end was attached with a stainless steel microforceps to a calibrated force transducer (model AE801; Aksjeselkapet Mikro Elektronik, Heidelberg, Germany). During a 2-h equilibration period, the strips were stretched after repeated isometric contractions (lasting 2–3 min) produced by 1 μM acetylcholine until the optimal length for force development was obtained. Each strip was maintained at this optimal length for the rest of the experiment.

For studies measuring intracellular cyclic nucleotide concentrations, all muscle strips (width, 3–5 mm; length, 1.0–1.6 cm; wet weight, 30–98 mg) were suspended in 25-ml water-jacketed tissue baths filled with PSS (37° C) aerated with 96% oxygen and 4% carbon dioxide (producing a *p*H of about 7.4; oxygen pressure of about 550 mmHg, and carbon dioxide pressure of approximately 36 mmHg in the PSS). All strips were incubated with 10 μM indomethacin to prevent formation of prostanoids that could affect cyclic nucleotide measurements.[7,21] Previously we showed that indomethacin does not affect responses to acetylcholine or to bronchodilators.[7] One end of each strip was anchored to a metal hook at the bottom of the tissue bath; the other end was attached to a calibrated force transducer (model FT-03D; Grass Instruments, Quincy, MA). During a 2-h equilibration period, the strips were contracted isometrically for 30 s every 5 min by supramaximal electrical field stimulation (model S88D; Grass Instruments). The strips were stretched after each stimulation until optimal length was achieved.[13,22,23]

Fura-2 Loading

Muscle strips were incubated in PSS (22°C) containing 5 μM of the acetoxymethyl ester of fura-2 (fura-2/AM) aerated with 96% oxygen and 4% carbon dioxide for 3 h.[6,20] The fura-2/AM was dissolved in dimethylsufoxide and 0.02% cremophor. After fura-2 loading, the strips were washed with normal PSS (37° C) for 30 to 50 min to remove extracellular fura-2/AM and dimethylsufoxide and to allow de-esterification of any remaining cytosolic fura-2/AM. All further maneuvers were performed at 37° C.

Fura-2 Fluorescence Measurements

Fura-2 fluorescence intensity was measured using a photometric system (model ph2; Scientific Instruments, Heidelberg, Germany) that measures optical and mechanical parameters of isolated tissue simultaneously. This system was previously described in detail.[24] Light from a xenon high-pressure lamp was monochromatically filtered to restrict excitation light to 340-nm, 360-nm, and the 380-nm wavelengths. Excitation light at these three wavelengths was alternated every 2 ms and focused by a high-aperture objective onto the muscle strips. Surface fluorescence emitted from the strips was filtered at 500 ± 5 nm and detected using a photomultiplier assembly. Illumination intensity of the excitation light passing through the cuvette was detected by an absorbance monitor. The photomultiplier signal was normalized by this absorbance to minimize the influence of fluctuations in the intensity of the excitation lights. The emission fluorescence intensities due to excitation at 340-nm (F_{340}), 360-nm (F_{360}), and 380-nm (F_{380}) wavelengths were measured and stored on a personal computer. The ratio of intensities at 340 nm and 380 nm excitation (F_{340}/F_{380}) was used as an index of

$[Ca^{2+}]_i$.[6] Isometric force was measured simultaneously using a force transducer (model AE801; Aksjeselkapet Mikro Elektronik).

Measurement of Manganese Influx

The quenching of fura-2 fluorescence by Mn^{2+} has been used to estimate Ca^{2+} influx in smooth muscle and other cell types.[25-28] Mn^{2+} rapidly binds to fura-2 and quenches its fluorescence. Mn^{2+} can be admitted to the cell *via* voltage-operated calcium channels. The rate of calcium influx can be estimated by the rate of Mn^{2+} influx[25-28] under the following conditions. When Mn^{2+} is added to the extracellular fluid bathing muscle cells loaded with fura-2, fluorescence declines. The rate of decline in fura-2 fluorescence, as measured by F_{360} (the isobestic wavelength for fura-2), is an index of Mn^{2+}, and thus calcium influx.[25-28]

To measure Mn^{2+} influx, 0.5 mM $MnCl_2$ was added to the perfusate solutions, and fluorescence signals were measured during the next 10 min. Each strip was lysed at the end of the experiment by adding water, and background fluorescence was measured. The F_{360} signal was normalized by considering the signal 1 min before the addition of Mn^{2+} to be 1.0 and the background fluorescence after lysis to be 0. The Mn^{2+} influx rate was calculated by measuring the slope of the normalized F_{360} signal between 15 and 45 s after the onset of quenching, a period when the signal was most linear.[28]

Cyclic Nucleotide Measurements

Muscle strips were weighed and homogenized in 4 ml cold (2°C) 95% ethanol using a ground-glass pestle and homogenizing tube. The precipitated pellet was separated from the soluble extract by centrifugation at 4,000*g* for 10 min. The soluble extract was evaporated to dryness at approximately 55°C under a stream of nitrogen and was then suspended in 0.3 ml 4 mM ethylenediamine tetraacetic acid (*p*H 7.5). [^{3}H]cAMP (1.25 μCi) or [^{3}H]cGMP (0.4 μCi) was added as a tracer for cAMP or cGMP recovery determinations, respectively. Commercially available radioimmunoassay kits were used to determine the concentrations of cAMP and cGMP in the soluble extract.[29] The protein content of the precipitated pellet was determined by the method described by Lowry *et al.*[30] using bovine serum albumin dissolved in 1 N NaOH as the standard. The intracellular concentrations of cAMP ($[cAMP]_i$) and cGMP ($[cGMP]_i$) were expressed as picomoles per milligram of protein.

Materials

Ketamine was purchased from Sigma Chemical Company (St. Louis, MO). Fura-2/AM was purchased from Molecular Probes, Inc. (Eugene, OR). All other drugs and chemicals were purchased from Sigma Chemical Company. Stock solutions of fura-2/AM were prepared in dimethylsufoxide; all other solutions and drugs were prepared in distilled water.

Statistics

In all experiments using three different concentrations of ketamine and comparing the difference between submaximal and maximal muscarinic stimulation, the data were compared by nonlinear regression analysis as described by Meddings.[31] In this method, force (F) at any concentration of drug (C) was given by the equation:

$$F = F_m C/(EC_{50} + C)$$

where F_m represents the maximal (or minimal) isometric force or $[Ca^{2+}]_i$ and EC_{50} represents the concentration that produces half-maximal (or minimal) isometric force or $[Ca^{2+}]_i$ for that drug. Nonlinear regression analysis was used to fit values of F_m and EC_{50} to data for F and C for each condition studied. This method allows comparison of curves to determine whether they are significantly different and whether this overall difference can be attributed to differences in F_m, EC_{50}, or both parameters.

Multiple comparisons were performed with one-way analysis of variance as appropriate. Single comparisons were made using paired or unpaired *t* tests. A probability value of 0.05 or less was considered significant. In all studies, *n* refers to the number of dogs studied. Values are reported as means ± SD.

Results

Effects of Ketamine on Force and Intracellular Calcium Concentration.

After contraction with acetylcholine for 10 min, the strips were superfused with increasing concentrations of ketamine, which produced concentration-dependent decreases in both force and $[Ca^{2+}]_i$ (figs. 1 and 2). The sensitivity of the force response to ketamine significantly decreased as the intensity of muscarinic stimulation increased; the EC_{50} for relaxation induced by ketamine was 59 μM and 850 μM for stimulation with 0.1 μM and 850 μM for stimulation with 10 μM acetylcholine,

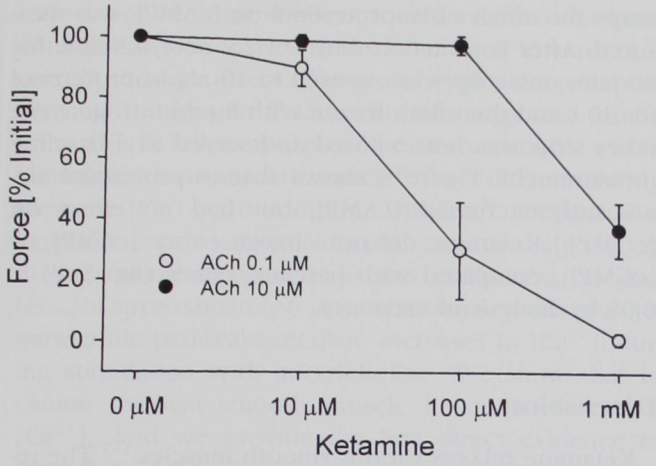

Fig. 1. Effect of ketamine (10 μM to 1 mM) on canine smooth muscle strips contracted with two different concentrations of the physiologic agonist acetylcholine. In strips contracted with 0.1 μM acetylcholine (open circles, n = 6), ketamine caused a dose-dependent decrease in force. In strips contracted with 10 μM acetylcholine (filled circles, n = 6), relaxation occurred only in response to 1 mM ketamine. Values are means ± standard deviations.

respectively ($P < 0.05$). With maximal stimulation (10 μM acetylcholine), relaxation occurred only with 1 mM ketamine. In contrast, the sensitivity of the response of F340/F380 to ketamine did not depend on the intensity

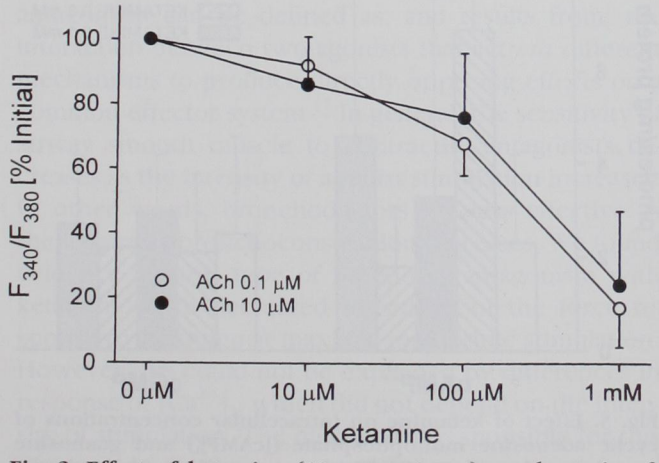

Fig. 2. Effect of ketamine (10 μM to 1 mM) on the ratio of fluorescence intensities due to excitation at 340 and 380 nm (F_{340}/F_{380}, an index of intracellular calcium concentration). At submaximal (0.1 μM acetylcholine; open circles; n = 6) and maximal (10 μM acetylcholine; filled circles; n = 6) doses, ketamine caused a dose-dependent decrease in F_{340}/F_{380} and thus in the intracellular calcium concentration. There was no difference in response between the two different acetylcholine concentrations. Values are means ± standard deviations.

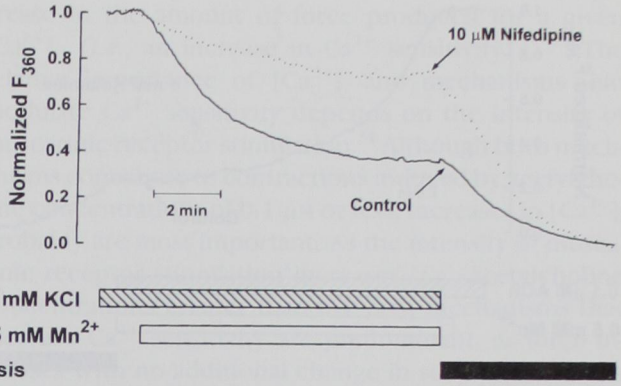

Fig. 3. Fluorescence intensity due to excitation at 360 nm (F_{360}) in two strips of canine tracheal smooth muscle during stimulation with 45 mM KCl. The rate of decline of F_{360} produced by the addition of 0.5 mM Mn^{2+} was less in the strips exposed to nifedepine (10 μM). F_{360} was normalized to values before Mn^{2+} addition and after lysis.

of muscarinic stimulation (fig. 2); the EC_{50} for the response was 0.20 mM and 0.32 mM for stimulation with 0.1 μM and 10 μM acetylcholine, respectively. When ketamine was washed from the tissues, isometric force and F340/F380 fully recovered (data not shown).

Characterization of Manganese Influx Technique

In strips contracted with 45 mM isotonic KCl, the sustained increases in force and $[Ca^{2+}]_i$ are due to calcium influx through voltage-dependent calcium channels sensitive to dihydroperidines.[32] To validate the Mn^{2+} influx technique in canine tracheal smooth muscle, initial experiments were performed to look for the effect of nifedipine, a dihydroperidine-sensitive Ca^{2+} channel antagonist, on strips contracted with 45 mM isotonic KCl. Figure 3 shows the effect of nifedepine (10 μM) on Mn^{2+}-induced quenching of fura-2. In control strips, the addition of 0.5 mM Mn^{2+} 10 min after contraction with KCl produced a rapid decrease in F_{360}, indicating that Mn^{2+} entered the cells and quenched fura-2. Ten minutes after the addition of Mn^{2+}, the strips were lysed with water, producing a further decrease in F_{360} to the level of background fluorescence. The addition of 10 μM nifedipine 5 min after contraction (5 min before Mn^{2+}-induced quenching) decreased the rate of decline of F_{360} compared with control (0.051 ± 0.017 min^{-1} and 0.075 ± 0.018 min^{-1}; $P < 0.05$ by paired t test; n = 5), indicating that nifedipine decreased the rate of Mn^{2+} influx (and thus calcium influx) during KCl-mediated contractions (fig. 3). We conclude from these experiments that changes in Mn^{2+} influx as mea-

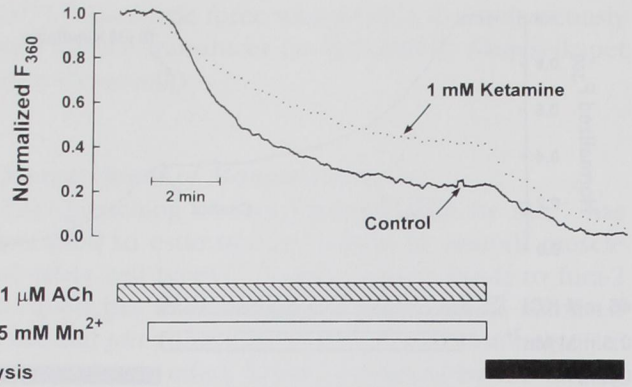

Fig. 4. Fluorescence intensity due to excitation at 360 nm (F_{360}) in two strips of canine tracheal smooth muscle during stimulation with 0.1 μM acetylcholine. The rate of decline of F_{360} produced by the addition of 0.5 mM Mn^{2+} was less in the strip exposed to ketamine (1 mM). F_{360} was normalized to values before Mn^{2+} addition and after lysis.

sured by this technique can be used as an index of changes in calcium influx in this tissue.

Effects of Ketamine on Manganese Influx

Ketamine (1 mM) was added to the strips 10 min after contraction with 0.1 μM acetylcholine. For each experiment, another strip from the same dog did not receive ketamine and served as a control.

Figure 4 shows the results of Mn^{2+}-induced quenching of fura-2 performed 20 min after contraction with 0.1 μM acetylcholine. In control strips, the addition of 0.5 mM Mn^{2+} produced a rapid decrease in F_{360}, indicating that Mn^{2+} entered the cell and quenched fura-2. Ten minutes after the addition of Mn^{2+}, the strips were lysed with water, producing a further decrease in F_{360} to the level of background fluorescence. The addition of 1 mM ketamine 10 min after contraction (10 min before Mn^{2+}-induced quenching), decreased the rate of decline of F_{360} compared with control (0.08 ± 0.02 min^{-1} and 0.17 ± 0.03 min^{-1}; $P < 0.03$, unpaired t test; n = 6), indicating that ketamine inhibited the rate of Mn^{2+} influx (and thus calcium influx) during acetylcholine-mediated contractions (fig. 4).

Effect of Ketamine on Cyclic Nucleotides

A set of four strips were contracted with 0.1 μM acetylcholine for 10 min. The strips were exposed to 0 (baseline), 0.1, 0.3, or 1 mM ketamine for 1 min (the time required to produce maximal relaxation) and flash frozen in liquid nitrogen. To provide a positive control for increases in $[cAMP]_i$, in an additional set of two muscle strips the effect of isoproterenol on $[cAMP]_i$ was measured. After contraction with 0.1 μM acetylcholine for 10 min, one strip was exposed to 10 μM isoproterenol for 30 s and then flash frozen with liquid nitrogen; the other strip was not exposed and served as a baseline measurement. Figure 5 shows that isoproterenol significantly increased $[cAMP]_i$ but had no effect on $[cGMP]_i$. Ketamine did not change either $[cAMP]_i$ or $[cGMP]_i$, compared with baseline values (fig. 5; $P > 0.05$ by analysis of variance).

Discussion

Ketamine relaxes various smooth muscles.[1-5] The relaxation has been associated with a decrease in $[Ca^{2+}]_i$ in vascular smooth muscle.[8] This decrease in $[Ca^{2+}]_i$ could result from decreased influx, increased sequestration of calcium from the cytosol, or decreased calcium release from intracellular stores.

Data from Kanmura *et al.*[33] obtained in a skinned rabbit artery preparation suggests that ketamine does not affect sarcoplasmatic reticulum calcium stores.

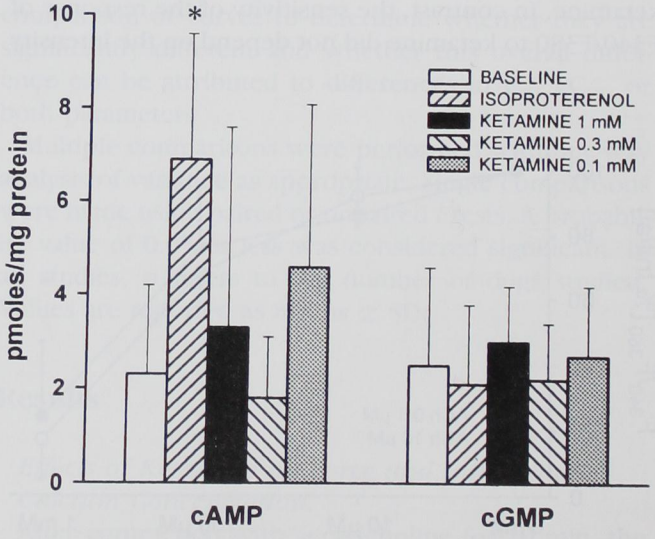

Fig. 5. Effect of ketamine on intracellular concentrations of cyclic adenosine monophosphate ($[cAMP]_i$) and guanosine monophosphate ($[cGMP]_i$). There was no effect of ketamine in canine smooth muscle strips (n = 7) incubated with 10 μM indomethacin and contracted with 0.1 μM acetylcholine on $[cAMP]_i$ and $[cGMP]_i$. In canine smooth muscle strips (n = 6) incubated with 10 μM indomethacin and contracted with 0.1 μM acetylcholine, 10 μM isoproterenol caused a significant increase in $[cAMP]_i$ and served as a positive control. *Significant difference from baseline (t test, n = 6). Values are means ± standard deviations.

Electrophysiologic studies provide evidence for ketamine-induced decreases in the whole-cell inward Ca^{2+} current through direct effects on L-type voltage-dependent Ca^{2+} channels.[9,11] Other studies suggested inhibition of Ca^{2+} influx as a possible mechanism by which ketamine relaxes smooth muscle[10,34] but did not directly measure $[Ca^{2+}]_i$. In addition, Durieux[35] showed that ketamine depressed signaling pathways mediated by rat m1 muscarinic receptors expressed in *Xenopus* oocytes (EC_{50} of approximately 6 μM). In airway smooth muscle, muscarinic pathways mediate increases in $[Ca^{2+}]_i$ during stimulation with acetylcholine. We show that in canine tracheal smooth muscle ketamine decreases $[Ca^{2+}]_i$, and we provide the first direct evidence in smooth muscle cells with intact cytosol that this is associated with a decrease in calcium influx.

Decreases in $[Ca^{2+}]_i$ and calcium influx could be caused by increases in cyclic nucleotides (cAMP and cGMP), which play an important role as second messengers in airway smooth muscle relaxation. They may relax smooth muscle by reducing $[Ca^{2+}]_i$, by directly interfering with the function of contractile proteins, or by both mechanisms.[12,36] However we found no evidence that ketamine works by increasing either $[cAMP]_i$ or $[cGMP]_i$.

A key issue when considering the net result of the effects of various excitatory and inhibitory inputs on the contractile state in airways is the phenomenon of "functional" or "physiologic" antagonism. Functional antagonism can be defined as, and results from, the interaction between two agonists that act *via* different mechanisms to produce directly opposing effects on a common effector system.[37] In general, the sensitivity of airway smooth muscle to contractile antagonists decreases as the intensity of agonist stimulation increases. In other words, bronchodilators are less effective as the degree of bronchoconstriction increases. We found evidence for this type of functional antagonism with ketamine, with decreased sensitivity of the force response to ketamine at maximal muscarinic stimulation. However this could not be explained by differences in response of $[Ca^{2+}]_i$, which did not depend on the intensity of stimulation. The mechanism responsible for this observation may be related to how mechanisms of contraction differ with the intensity of stimulation.

In smooth muscle, agonist-induced contraction is mediated by an increase in $[Ca^{2+}]_i$, which produces an increase in myosin light chain kinase activity and phosphorylation of the 20-kd regulatory myosin light chain.[38] Agonist-induced contraction may also produce an increase in the amount of force produced for a given $[Ca^{2+}]_i$, (*i.e.*, an increase in Ca^{2+} sensitivity).[14–19] The relative importance of $[Ca^{2+}]_i$ and mechanisms that modulate Ca^{2+} sensitivity depends on the intensity of muscarinic receptor stimulation.[39] Although both mechanisms contribute to contractions induced by acetylcholine concentrations of 0.1 μM or less, increases in $[Ca^{2+}]_i$ probably are most important. As the intensity of muscarinic receptor stimulation increases (*i.e.*, acetylcholine concentrations greater than 0.1 μM), mechanisms that increase Ca^{2+} sensitivity are predominant, as force increases with no additional change in steady-state levels of Ca^{2+}.[39] We found this in an additional six strips. The strips were contracted for 10 min with 0.1 μM acetylcholine or 10 μM acetylcholine sequentially applied (in random order), with applications separated by a 20-min washout and recovery period. Acetylcholine (at 10 μM) produced significantly more force than did 0.1 μM acetylcholine (4.3 $\pm$ 3.0 times greater; $P < 0.03$). In contrast, 10 μM acetylcholine did not produce a significantly greater increase in F_{340}/F_{380} than 0.1 μM acetylcholine (1.2 $\pm$ 0.4 times greater; $P = 0.27$). Thus the increase in force induced by maximal muscarinic receptor stimulation (compared with 0.1 μM acetylcholine) appears to be caused largely by increases in Ca^{2+} sensitivity, with no additional increase in $[Ca^{2+}]_i$.

The decreases in $[Ca^{2+}]_i$ produced by 100 μM ketamine during maximal stimulation (fig. 2) appear to be insufficient to affect force. This occurred probably because increased Ca^{2+} sensitivity is the predominant mechanism producing force during maximal stimulation, so that small changes in $[Ca^{2+}]_i$ have little effect on force under these conditions. The larger decrease in $[Ca^{2+}]_i$ produced by 1 mM ketamine may have been sufficient to reduce force. These results also suggest that ketamine, at least at concentrations less than or equal to 100 μM, does not affect Ca^{2+} sensitivity. It is not possible from these experiments to determine if 1 mM ketamine might affect Ca^{2+} sensitivity. The fact that ketamine in concentrations as much as 100 μM did not appear to affect Ca^{2+} sensitivity during maximal stimulation suggests that ketamine does not inhibit muscarinic signaling pathways during maximal muscarinic stimulation.[35]

It is interesting to contrast these results with those obtained with halothane, another anesthetic with bronchodilating properties.[20] In contrast to ketamine, halothane decreases $[Ca^{2+}]_i$ and Ca^{2+} influx during submaximal, but not maximal, muscarinic stimulation.[20] In addition, the sensitivity of the force response to halothane

does not depend on the level of muscarinic stimulation, so that halothane decreases force while $[Ca^{2+}]_i$ is constant. Thus halothane, unlike ketamine, decreases Ca^{2+} sensitivity.

Results from these *in vitro* measurements should be applied to live animals or humans only with caution. Although comparison between *in vitro* and *in vivo* drug concentrations across species may have limited value, peak plasma concentrations in humans have been reported to be approximately 60 μM for an intravenous dose of 2 mg/kg ketamine,[40] indicating that the range of ketamine concentrations studied may be clinically relevant. Comparisons are facilitated by modest protein binding of ketamine *in vivo*.[41] To the extent that the direct effects of anesthetics are significant *in vivo*, our results suggest that ketamine may have a modest direct effect during submaximal bronchoconstriction but little direct effect on airway smooth muscle during severe bronchoconstriction. In contrast, halothane produces direct relaxation of maximally constricted canine airway smooth muscle.[42]

Ketamine-induced relaxation of canine airway smooth muscle is associated with a decrease in $[Ca^{2+}]_i$ and calcium influx not mediated by cyclic nucleotides. The sensitivity of the force response to ketamine decreases as the level of preexisting airway smooth muscle tone increases, an effect that is not explained by differential effects on $[Ca^{2+}]_i$.

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