Relaxant effect of ketamine and its isomers on histamine-induced contraction of tracheal smooth muscle

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Summary

The mechanism by which racemic (R (±)) ketamine relaxes airway smooth muscle is unclear and there is no information on the differential effects of ketamine and its isomers. In this study, we have examined the spasmolytic effect of R (±) ketamine and its isomers S (+) and R (−) ketamine and the role of intracellular calcium and opioid receptors in R (±) ketamine-induced relaxation. The tension of isolated guinea pig tracheal strips was measured isometrically with a force displacement transducer and contraction elicited with histamine 10−5 mol litre−1. In histamine-precontracted strips, the two ketamine isomers (4.5–18.0 × 10−5 mol litre−1) produced equipotent relaxation. A subthreshold dose of each isomer of ketamine (10−4 mol litre−1) which alone did not relax histamine-induced contraction (S (+), P < 0.01; R (±), P < 0.01; R (−), P < 0.05) significantly potentiated adrenaline 1.25–5.0 × 10−8 mol litre−1-induced relaxation (potency: S (+) > R (±) > R (−)). Increase in extracellular Ca2+ (1.8–14.4 × 10−3 mol litre−1) significantly reduced R (±) ketamine-induced relaxation. S (−) Bay K 8644, at concentrations up to 2.0 × 10−6 mol litre−1, partially antagonized R (±) ketamine-induced relaxation whereas at 10−5 mol litre−1 or higher it potentiated the response. Naloxone 1.5−6.0 × 10−6 mol litre−1 did not affect the relaxation caused by R (±) ketamine. We conclude that although both ketamine isomers produced equipotent spasmolytic effects on airway smooth muscle precontracted with histamine, they differed in their ability to potentiate the relaxing effect of adrenaline. S (+) ketamine produced the greatest potentiation. Changes in intracellular Ca2+ level secondary to a reduction in the L-type Ca2+ current may partially mediate the spasmolytic effect of R (±) ketamine. (Br. J. Anaesth. 1996; 76: 266–270)

Key words

Racemic (R (±)) ketamine is a potent bronchodilator and has been used for the anaesthetic management of asthmatic patients [1–4]. Although previous reports suggested that the bronchodilator action of R (±) ketamine may be caused by increased catecholamine concentrations [5], or by β receptor activation [6], Ca2+ channel block [7–9], or both, the precise mechanism of action remains to be determined.

There are many pharmacological differences between optical isomers of ketamine [10–13]. Lundy and colleagues [12] reported that S (+) ketamine can potentiate responses to catecholamines in vascular and anococcygeus muscles to a greater extent than R (−) ketamine. However, the differential effects of ketamine isomers on the response of airway smooth muscle to adrenaline have not yet been reported.

In this study we have determined if ketamine isomers have differential spasmolytic effects on tracheal strips precontracted with histamine and differential potentiating effects on the relaxation produced by adrenaline. In addition, we have studied the role of increased extracellular calcium and opioid receptors on these responses.

Materials and methods

The study was approved by the Animal Care and Use Committee of the University of Illinois at Chicago.

GUINEA PIG TRACHEAL STRIP

Female guinea pigs (300–450 g) were killed with an overdose of sodium pentobarbitone 75 mg kg−1 i.p. and section of the abdominal aorta. The trachea was removed, dissected from surrounding tissue and then cut spirally into strips (3 mm × 15 mm) which were mounted in a 10–ml organ bath filled with oxygenated (95 % oxygen–5 % carbon dioxide) Tyrode’s solution at 37 °C. The composition of Tyrode’s solution was (mmol litre−1): NaCl 138, KCl 2.7, MgCl2 1.05, NaHPO4 0.42, NaHCO3 11.9, glucose 5.5 and CaCl2 1.8. Each strip was attached between a fixed point and a force displacement
transducer (FT03, Grass Instrument Co., Quincy, MA, USA). After obtaining reproducible contractions to histamine 10^{-5} \text{mol litre}^{-1}, experiments were begun. \( R(\pm) \), \( S(\pm) \) and \( R(\pm) \) ketamine were tested in random order. Strips were washed for 10 min before each contraction was induced by histamine.

The first experiment was designed to determine the effect of ketamine and its isomers on the tone of tracheal strips precontracted with histamine \((n = 6)\). After eliciting maximal contraction to histamine, \( R(\pm) \), \( S(\pm) \) or \( R(\pm) \) ketamine was cumulatively added to the organ bath \((0.45–1.8 \times 10^{-3} \text{mol litre}^{-1})\). Relaxation was expressed as a percentage (peak contraction, 0 %, fully relaxation, 100 %). The ED_{50} of ketamine (the dose of ketamine that reversed histamine-induced contraction by 50 %) was calculated from the ketamine dose–response curves.

In the second study, we examined the effect of a subthreshold dose of \( R(\pm) \) ketamine and its isomers on relaxation induced by adrenaline \((n = 6)\). The subthreshold dose of \( R(\pm) \) ketamine and its isomers, which alone did not produce relaxation, was determined from the first study. After maximal contraction of each strip was elicited with histamine, 0.9 % saline or \( R(\pm) \), \( S(\pm) \) or \( R(\pm) \) ketamine 10^{-4} \text{mol litre}^{-1} was administered into the organ bath. Five minutes later, adrenaline was given cumulatively \((1.25–5.0 \times 10^{-3} \text{mol litre}^{-1})\) to obtain a dose–response curve to calculate its ED_{50}.

In the third study, we tested if increases in extracellular Ca^{2+} concentration by addition of CaCl_{2} modified the relaxation produced by \( R(\pm) \) ketamine \((n = 6)\). The relaxation produced by \( R(\pm) \) ketamine 1.8 \times 10^{-3} \text{mol litre}^{-1} was measured when Ca^{2+} concentrations in the perfusate were increased from 1.8 to 14.4 \times 10^{-3} \text{mol litre}^{-1}.

In the fourth study \((n = 8)\), we tested if the L-type Ca^{2+} channel opener \( S(\pm) \) Bay K 8644 reversed the spasmytic effect of \( R(\pm) \) ketamine. When reproducible contractions to histamine were established, \( R(\pm) \) ketamine 9.0 \times 10^{-3} \text{mol litre}^{-1} was added to the bath followed by cumulative administration of \( S(\pm) \) Bay K 8644 from 2 \times 10^{-7} to 10^{-3} \text{mol litre}^{-1}.

In the fifth study \((n = 6)\), we examined the effect of nalozone on \( R(\pm) \) ketamine-induced relaxation. Tracheal strips were precontracted with histamine and the relaxation produced by \( R(\pm) \) ketamine 9.0 \times 10^{-3} \text{mol litre}^{-1} was studied alone and in the presence of nalozone 1.5 \times 10^{-4} \text{mol litre}^{-1}.

DATA ANALYSIS
All data are expressed as mean (SEM). Statistical analysis was by one-way and repeated measures ANOVA followed by Scheffé’s F test. \( P < 0.05 \) was considered significant.

DRUGS AND SOLUTIONS
\( R(\pm) \) ketamine hydrochloride and histamine hydrochloride were obtained from Sigma Chemical Co. (St Louis, MO, USA), sodium pentobarbitone from Abbott Laboratories (North Chicago, IL, USA) and nalozone from DuPont Pharmaceuticals (Manati, Puerto Rico). \( S(\pm) \), \( R(\pm) \) ketamine hydrochloride was generously donated by Parke-Davis GmbH (Munich, Germany). \( S(\pm) \) Bay K 8644 was obtained from Research Biochemicals Inc. (Natick, MA, USA).

Stock solutions of nalozone and ketamine were prepared in distilled water; \( S(\pm) \) Bay K 8644 was dissolved in methanol \((2.8 \times 10^{-3} \text{mol litre}^{-1})\). All stock solutions were stored as aliquots at \(-20^\circ\text{C}\).

Results
EFFECT OF KETAMINE AND ITS ISOMERS ON HISTAMINE-INDUCED CONTRACTIONS
Histamine 10^{-5} \text{mol litre}^{-1} increased basal tension by 1.33 (SEM 0.29) \(g\) \((n = 6)\). After precontraction with histamine, \( R(\pm) \) ketamine and its isomers relaxed the tracheal strips in a concentration-dependent manner (fig. 1). There were no significant differences
between the ED$_{50}$ values of $R(-)$, $R(\pm)$ and $S(+)$ ketamine: 1.14 (0.11), 1.13 (0.09) and 1.09 (0.05) x 10$^{-3}$ mol litre$^{-1}$, respectively ($n=6$). This relaxant effect of $R(\pm)$ ketamine and its isomers was fully reversible after washout.

**EFFECT OF A SUBTHRESHOLD DOSE OF KETAMINE AND ITS ISOMERS ON ADRENALINE-INDUCED RELAXATION**

Adrenaline relaxed histamine precontracted tracheal strips ($n=6$) in a concentration-dependent manner (fig. 2). A subthreshold dose (10$^{-4}$ mol litre$^{-1}$) of $R(-)$, $R(\pm)$ and $S(+)$ ketamine did not change the tension of strips precontracted with histamine (fig. 3). However, the same subthreshold dose significantly reduced the ED$_{50}$ value of adrenaline from 9.4 (1.4) to 5.7 (0.9) x 10$^{-3}$ mol litre$^{-1}$ after $R(-)$ ketamine ($P<0.05$); to 4.9 (0.8) x 10$^{-3}$ mol litre$^{-1}$ after $R(\pm)$ ketamine and to 4.0 (0.8) x 10$^{-3}$ mol litre$^{-1}$ after $S(\pm)$ ketamine ($P<0.01$). $S(\pm)$ ketamine was more potent than $R(-)$ ketamine at adrenaline 2.5 and 5.0 x 10$^{-3}$ mol litre$^{-1}$ ($P<0.05$, fig. 2). The relaxant effect of adrenaline was also fully reversible after washout.

**EFFECT OF CA$^{2+}$ CONCENTRATION ON KETAMINE-INDUCED RELAXATION**

$R(\pm)$ ketamine 1.8 x 10$^{-3}$ mol litre$^{-1}$ caused 89.7 (4.6) % relaxation of histamine-induced strip contractions. Increasing the extracellular Ca$^{2+}$ concentration from 1.8 to 14.4 x 10$^{-3}$ mol litre$^{-1}$ reduced $R(\pm)$ ketamine relaxation by 22.2% (fig. 4, $n=6$).

**EFFECT OF S(-) BAY K 8644, AN L-TYPE CA$^{2+}$ CHANNEL OPENER, ON KETAMINE-INDUCED RELAXATION**

$S(-)$ Bay K 8644 decreased the relaxation of the strips ($n=8$) produced by $R(\pm)$ ketamine. The maximal effect occurred with $S(-)$ Bay K 8644 10$^{-6}$ mol litre$^{-1}$. However, $S(-)$ Bay K 8644 10$^{-5}$ mol litre$^{-1}$ potentiated $R(\pm)$ ketamine-induced relaxation (fig. 5).

**EFFECT OF NALOXONE ON KETAMINE-INDUCED RELAXATION**

$R(\pm)$ ketamine 9.0 x 10$^{-3}$ mol litre$^{-1}$ significantly reduced histamine-induced contractions from 100 to 64.5 (4.4) % ($n=6$, $P<0.01$). However, naloxone
Discussion

R (±) ketamine is known to produce bronchodilatation in asthmatic patients who do not respond to conventional therapy [1–4]. Although the mechanism of this spasmolytic effect of R (±) ketamine is still unclear, previous studies suggested that Ca²⁺ channel blocking effects [7–9], catecholamine release [5] and/or inhibition of catecholamine uptake [12, 13] may contribute. In addition, Fink and Ngai [11] reported that R (±) ketamine inhibited the contractile response of the ileum to electrical stimulation and they suggested that this inhibition by R (±) ketamine was mediated partially via opioid receptors.

In the present study, when extracellular Ca²⁺ was increased, the relaxing action of R (±) ketamine was reduced in a concentration-dependent manner. Moreover, the L-type Ca²⁺ channel opener, S (−) Bay K 8644, but not naloxone, partially antagonized the relaxation induced by R (±) ketamine. Therefore, the spasmolytic effect of R (±) ketamine may be caused partially by reduction in L-type Ca²⁺ channel activity. R (±) ketamine may produce its relaxant action on airway smooth muscle by inhibition of agonist-induced second messenger synthesis such as inositol 1,4,5-triphosphate [14] or by stimulating Ca²⁺ ATPase to cause Ca²⁺ efflux [15], or both. Further investigation of the action of ketamine at the subcellular level is needed to clearly identify the mechanisms of action, as other modulators such as diacylglycerol, protein kinase C or G proteins may be involved in the relaxing effect of ketamine. We found that S (−) Bay K 8644 10⁻⁸ mol litre⁻¹ potentiated the relaxation produced by R (±) ketamine although up to 10⁻⁴ mol litre⁻¹ antagonized the relaxation in a concentration dependent manner. As we dissolved S (−) Bay K 8644 in methanol (0.36 % S (−) Bay K 8644 10⁻⁴ mol litre⁻¹), this may potentiate the effect of ketamine. However, this finding is consistent with previous reports [16–19] that this Ca²⁺ channel opener has a biphasic effect on vascular smooth muscle and cardiac muscle. Wei and colleagues demonstrated that the dual effects of S (−) Bay K 8644 were not caused by involvement of the antagonists isomer, R (−) Bay K 8644 [17]. Another study showed that S (−) Bay K 8644 potentiated or inhibited Ca²⁺ current at polarized and depolarized potentials, respectively [18].

Although the pharmacological differences [10–13] between the ketamine isomers, S (+) and R (−) ketamine, are known, their differential spasmytic effects on contracted airways have not been reported. Our previous report [9] showed that both isomers equipotently inhibited contraction of guinea pig ileum induced by inflammatory mediators, whereas Fink and Ngai [11] demonstrated that S (+) ketamine was 2–3 times more potent than R (−) ketamine in relaxing guinea pig ileum precontracted by electrical stimulation. In the present study we found that ketamine isomers produced equipotent spasmytic effects on tracheal strips precontracted with histamine, a result consistent with our previous study on guinea pig ileum [9].

Using rabbit vascular smooth muscle and rat anococcygeus muscle, Lundy, Gverdzys and Frew [20] found that R (±) ketamine potentiated the catecholamine response by inhibition of extra-neuronal uptake. Using the same muscles [12] they also demonstrated that S (+) ketamine inhibited catecholamine uptake more than R (−) ketamine. In the present study, we found similar results as the subthreshold dose of R (±) ketamine and its isomers potentiated adrenaline-induced relaxation of tracheal strips precontracted by histamine and the potentiating effect of S (+) ketamine was greater than that of R (−) ketamine. We conclude that the spasmytic effect of R (±) ketamine on contracted airway smooth muscle may result partially from a decrease in Ca²⁺ influx through the L-type Ca²⁺ channel and from inhibition of catecholamine uptake. We suggest that ketamine (especially S (+) ketamine) could be a useful therapeutic agent, in combination with a β₂ agonist, for the treatment of status asthmaticus.

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References

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