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Effects of ketamine on neostigmine-induced contractile and phosphatidylinositol responses of the rat trachea

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Abstract

Purpose. Neostigmine causes airway smooth muscle contraction through the direct stimulation of muscarinic receptors and the activation of phosphatidylinositol (PI) responses. Ketamine attenuates airway smooth muscle contraction. It is not clear whether ketamine attenuates neostigmine-induced airway smooth muscle contraction by inhibiting the PI response. This study was designed to examine the effects of ketamine on neostigmine-induced contractile and PI responses of the rat trachea.

Methods. Thirty male Wistar rats weighing 250–350 g were used. In the experiment on the contractile response, active contraction was induced with 1 μM neostigmine in the presence or absence of ketamine. In the experiment on the phosphatidylinositol response, the trachea slices were incubated with [^{3}H]*myo*-inositol, 1 μM neostigmine, or 100 μM aluminum fluoride, and ketamine. The formation of [^{3}H]inositol monophosphate (IP_1), a degradation product of the phosphatidylinositol response, was measured with a liquid scintillation counter. Statistical significance ($P < 0.05$) was determined by analysis of variance.

Results. Neostigmine 1 μM caused tracheal ring contraction. This contraction was attenuated by ketamine dose-dependently and reached resting tension at 100 μM. Neostigmine- and aluminum fluoride-induced IP_1 accumulation was also attenuated by ketamine.

Conclusion. The results suggest that ketamine attenuates neostigmine-induced contractile responses, at least in part, through the inhibition of phospholipase C coupled with G protein in the PI response.

Key words Ketamine · Neostigmine · Intravenous anesthetics · Phosphatidylinositol response

Introduction

Anticholinesterase (anti-ChE) drugs are used to reverse the action of nondepolarizing neuromuscular blocking drugs after operation, and are also used to stimulate peristalsis of the intestinal tract, in the symptomatic treatment of myasthenia gravis, and in the treatment of Alzheimer's disease [1–3]. Anti-ChE drugs are currently the most established treatment strategy in Alzheimer's disease, although the effect of anti-ChE drugs appears to be mainly symptomatic. The number of patients treated with anti-ChE drugs before surgery will increase, because the treatment of Alzheimer's disease is of increasing importance as the population ages and the number of people with the disease increases. Because anti-ChE drugs stimulate M_3-muscarinic receptors in airway smooth muscle cell membranes [4], as well as nicotinic receptors in neuromuscular junctions, the airway smooth muscle of patients with Alzheimer's disease treated with anti-ChE drugs may contract.

Ketamine is frequently used to alleviate attacks in patients with active asthma. Although ketamine attenuates airway smooth muscle contraction, it is not clear whether it affects airway smooth muscle tension in patients treated with anti-ChE drugs. The present study was carried out to clarify the effects of ketamine on anti-ChE drug-induced contractile and phosphatidylinositol (PI) responses of the rat trachea, since there is a direct relationship between PI response and airway smooth muscle contraction.

Methods

The studies were approved by our animal care committee. Thirty male Wistar rats weighing 250–350 g were used for the experiments. The rats were anesthetized with pentobarbital (50 mg·kg^{-1} intraperitoneal), and

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their tracheas were rapidly isolated. We used neostigmine as a typical anti-ChE drug.

Isometric tensions were measured as described previously [4,5]. The organ chamber used in this study was small enough that the equilibrium concentration could be reached immediately after the addition of drugs. In the present study, neostigmine was used at a concentration of 1 μM, which was the appropriate ED_{50} for the contractile response [4]. It was observed that neostigmine-induced contraction was sustained constantly over 2 h if no drugs were used.

Thirty minutes after application of neostigmine (at a final concentration of 1 μM) ketamine was added in a stepwise cumulative manner (0–1000 μM final concentration). The interval between doses of ketamine was set at 5 min, because its effect reached a maximum within 3 min.

[^{3}H]Inositol monophosphate (IP_1) in tracheal slices incubated with [^{3}H]*myo*-inositol was measured as described previously [4,5]. Varying doses of ketamine (0–100 μM final concentration) were added, and 15 min later neostigmine (1 μM final concentration) was added. The tubes were reincubated for an additional 60 min. The reaction was stopped with chloroform : methanol (1 : 2 v/v). Chloroform and water were then added, and the phases were separated by centrifugation at 90 *g* over a period of 5 min. The [^{3}H]IP_1 was separated from [^{3}H]*myo*-inositol by column chromatography. The [^{3}H]IP_1 formed in the tracheal slices was counted with a liquid scintillation counter and measured in becquerels (Bq). The scintillation counts of the blank values (no slices present) were subtracted to obtain the experimental data.

Aluminum fluoride (AF) stimulates G proteins [6,7] and produces IP_3 [8]. Thus, we examined the effects of ketamine on AF-induced IP_1 accumulation. Ketamine (100 μM final concentration) was added to the suspension of tracheal slices, the tubes were flushed with 95% O_2/5% CO_2, and 15 min later AF (100 μM final concentration) was added. After an additional 60 min, the reaction was stopped with 940 μl of chloroform : methanol (1 : 2 v/v), as described above.

The data are expressed as means ± SE. The results of repeated measures and multiple groups were subjected to two-way analysis of variance. Multiple pairwise comparisons between groups were assessed by Scheffé's test. A P value < 0.05 was considered to indicate a statistically significant difference.

Results

Figure 1 shows a typical recording of the effects of ketamine on neostigmine-induced contraction of a rat tracheal ring. Neostigmine caused tracheal ring contraction. This contraction was attenuated by ketamine dose-dependently and reached resting tension at 100 μM (Fig. 2). The dose of ketamine needed to exert 50% inhibition (ID_{50}) of neostigmine-induced contraction was 42 ± 8 μM. Neostigmine- and AF-induced IP_1 accumulations were also attenuated by ketamine (Figs. 3 and 4). The attenuation by ketamine of IP_1 accumulation correlated with the inhibition of contraction (ketamine: R = 0.847, $P < 0.0001$) (Fig. 5).

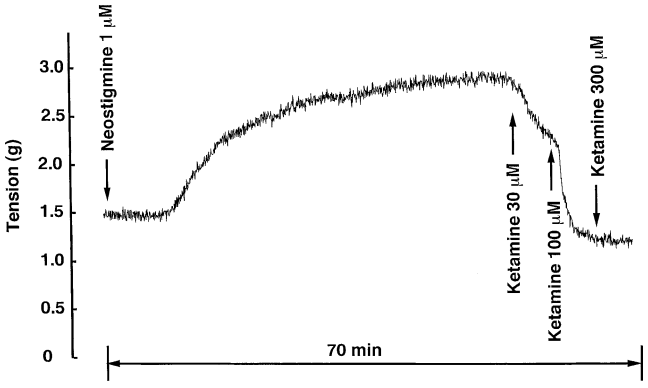

Fig. 1. A typical recording of the effects of ketamine on neostigmine-induced contraction of a rat tracheal ring

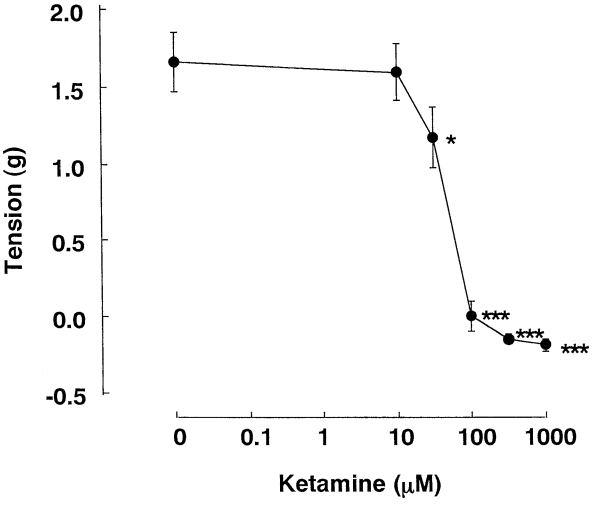

Fig. 2. Effects of ketamine on 1 μM neostigmine-induced contraction of the rat trachea (mean ± SE, n = 6–9). * $P <$ 0.05, *** $P < 0.001$ vs 0

Discussion

Ketamine attenuated the neostigmine-induced contraction and PI responses of the rat trachea. The mechanism involved in the effects of ketamine on neostigmine-induced contraction of airway smooth muscle may be clarified by the following explanations.

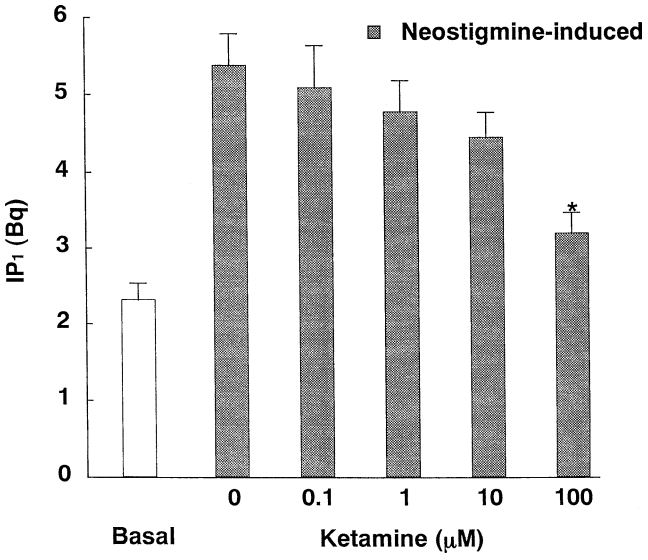

Fig. 3. Effects of ketamine on 1 μM neostigmine-induced inositol monophosphate (IP_1) accumulation in the rat trachea (mean ± SE, $n = 6$–10). * $P < 0.05$, *** $P < 0.001$ vs 0. *Bq*, becquerel

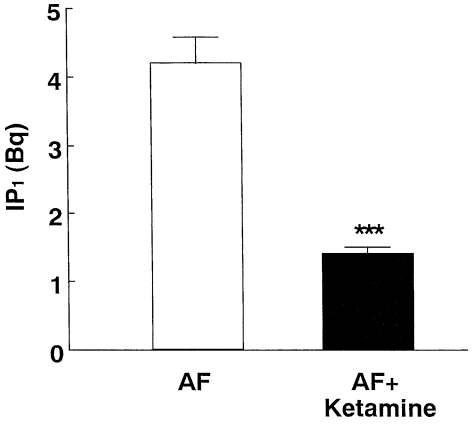

Fig. 4. Effects of ketamine on 100 μM AF-induced inositol monophosphate (IP_1) accumulation in the rat trachea (mean ± SE, $n = 7$–8). * $P < 0.05$, *** $P < 0.001$ vs AF. *AF*, aluminum fluoride; *Bq*, becquerel. Ketamine: 100 μM final concentration

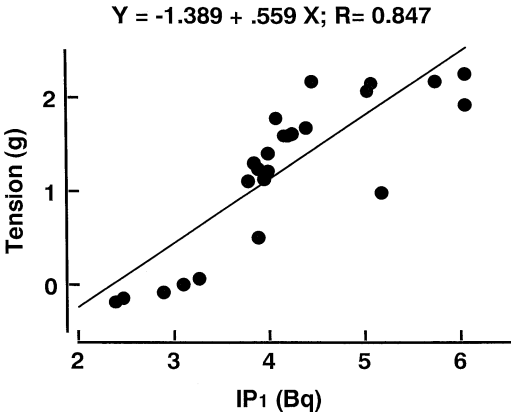

Fig. 5. Concentration-effect relationship between inositol monophosphate (IP_1) accumulation and relaxation in the rat trachea. The attenuation by ketamine of IP_1 accumulation correlated with the inhibition of contraction ($R = 0.847$, $P < 0.0001$)

Ketamine may inhibit muscarinic ACh receptors, resulting in attenuation of airway smooth muscle contraction. Hanazaki et al. [9] reported that ketamine relaxed canine tracheal smooth muscle without affecting Ca^{2+} sensitivity. Yamakage et al. [10] reported that ketamine inhibited voltage-activated Ca^{2+} currents of porcine tracheal smooth muscle cells. Pabelick et al. [11] reported that ketamine-induced relaxation of canine airway smooth muscle was associated with a decrease in calcium influx and intracellular Ca^{2+} concentration. Ketamine relaxes rabbit femoral arteries by reducing intracellular Ca^{2+} and phospholipase C activity, estimated by measuring inositol phosphate production [12]. Ketamine affects M_3-muscarinic receptors, resulting in attenuation of airway smooth muscle contraction.

The stimulation of M_3-muscarinic receptors in airway smooth muscle activates phospholipase C (PLC). Aluminum fluoride induces IP_3 formation through stimulation of G protein coupled with PLC [8]. In the present study, the aluminum fluoride-induced PI response was also attenuated by ketamine. Ketamine inhibited G proteins coupled with PLC in PI responses, resulting in an attenuation of the contractile responses of the rat trachea to neostigmine.

Ketamine completely inhibited neostigmine-induced contraction at doses of 100 μM, whereas ketamine did not completely inhibit neostigmine-induced PI responses at the same doses. Ketamine also inhibited neostigmine-induced contraction in part by different mechanisms from PI responses.

Ketamine may inhibit ACh release from parasympathetic postganglionic neurons, resulting in attenuation of airway smooth muscle contraction. Contreras et al. [13] reported that ketamine blocked the toxic effects of neostigmine and physostigmine. Wilson et al. [14] studied the effect of ketamine on the peripheral vagus nerve motor pathway of the isolated porcine trachealis muscle. They concluded that ketamine interacted with the peripheral vagus nerve by decreasing the excitability of the nicotinic ACh receptors of the parasympathetic postganglionic neurons. It seems probable that decreasing the excitability of the postsynaptic nicotinic ACh receptors of the intramural ganglia is the mechanism involved in the effects of ketamine on neostigmine-induced contraction of airway smooth muscle. However, rat tracheal slices do not appear to contain a sufficient number of functional postganglionic

cells, which can be activated by nicotinic agonists [4]. In the present study, attenuation by ketamine of neostigmine-induced contraction and PI response was not due to a reduction in ACh release from parasympathetic postganglionic nerve terminals.

The peak plasma concentrations in humans have been reported to be approximately 60 μM for an intravenous dose of 2 mg·kg^{-1} ketamine [15]. However, Hijazi and Boulieu [16] recently reported that the percentage of ketamine bound to serum protein was 60% and 64% at 30°C and 20°C, respectively, and that the percentage was independent of ketamine concentration. According to these reports, the peak free ketamine concentration was about 24 μM. The ED_{50} of ketamine in the present study was 42 μM. This concentration appears to be higher than the free concentrations observed clinically in serum.

In conclusion, the results suggest that ketamine attenuates the neostigmine-induced contractile response, at least in part through inhibition of phospholipase C coupled with G protein in the PI response.

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References

1. Cummings JL (2000) Cholinesterase inhibitors: a new class of psychotropic compounds. Am J Psychiatry 157:4–15
2. Nordberg A, Svensson AL (1998) Cholinesterase inhibitors in the treatment of Alzheimer's disease: a comparison of tolerability and pharmacology. Drugs Safety 19:465–480
3. VanDenBerg CM, Kazmi Y, Jann MW (2000) Cholinesterase inhibitors for the treatment of Alzheimer's disease in the elderly. Drugs Aging 16:123–138
4. Shibata O, Tsuda A, Makita T, Iwanaga S, Hara T, Shibata S, Sumikawa K (1998) Contractile and phosphatidylinositol responses of rat trachea to anticholinesterase drugs. Can J Anaesth 45:1190–1195
5. Shibata O, Kanairo M, Zhang S, Hasuo H, Morooka H, Fujie T, Sumikawa K (1996) Anticholinesterase drugs stimulate phosphatidylinositol response in rat tracheal slices. Anesth Analg 82:1211–1214
6. Sternweis PC, Gilman AG (1982) Aluminum: a requirement for activation of the regulatory component of adenylate cyclase by fluoride. Proc Natl Acad Sci USA 79:4888–4891
7. Chabre M (1989) Aluminofluoride action on G-proteins of the adenylate cyclase system is not different from that on transducin [letter]. Biochem J 258:931–932
8. Wood SF, Szuts EZ, Fein A (1989) Inositol trisphosphate production in squid photoreceptors. Activation by light, aluminum fluoride, and guanine nucleotides. J Biol Chem 264:12970–12976
9. Hanazaki M, Jones KA, Warner DO (2000) Effects of intravenous anesthetics on Ca^{2+} sensitivity in canine tracheal smooth muscle. Anesthesiology 92:133–139
10. Yamakage M, Hirshman CA, Croxton TL (1995) Inhibitory effects of thiopental, ketamine, and propofol on voltage-dependent Ca^{++} channels in porcine tracheal smooth muscle cells. Anesthesiology 83:1274–1282
11. Pabelick CM, Jones KA, Street K, Lorenz RR, Warner DO (1997) Calcium concentration-dependent mechanisms through which ketamine relaxes canine airway smooth muscle. Anesthesiology 86:1104–1111
12. Ratz PH, Callahan PE, Lattanzio FA Jr (1993) Ketamine relaxes rabbit femoral arteries by reducing $[Ca^{++}]_i$ and phospholipase C activity. Eur J Pharmacol 236:433–441
13. Contreras CM, Marvan ML, Mexicano G, Puente A, Morfin A (1990) Ketamine antagonizes toxic action of anticholinesterase agents. Bol Estud Med Biol Mex 38:10–15
14. Wilson LE, Hatch DJ, Rehder K (1993) Mechanisms of the relaxant action of ketamine on isolated porcine trachealis muscle. Br J Anaesth 71:544–550
15. Idavall J, Ahalgren I, Arosen KR, Stenberg P (1979) Ketamine infusions:pharmacokinetics and clinical effects. Br J Anaesth 51:1167–1173
16. Hijazi Y, Boulieu R (2002) Protein binding of ketamine and its active metabolites to human serum. Eur J Pharmacol 58:37–40