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Epithelium-dependent effect of L-glutamate on airways: involvement of prostaglandins

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We investigated the effect of the excitatory amino acid (EAA) receptor agonists L-glutamate, *N*-methyl-D-aspartate (NMDA), (***RS***)-a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainic acid on KCl-induced contractions of rabbit tracheal smooth muscle, as well as the role of epithelium and endogenously produced nitric oxide and prostaglandins on these responses. L-Glutamate decreased KCl-induced contractions up to 30%. This effect was attenuated by epithelium removal, tetrodotoxin, methylene blue and indomethacin but not by N^G-nitro-L-arginine methyl ester. While NMDA, AMPA and kainic acid had no effect, the combination of NMDA + kainic acid decreased KCl-induced contractions. These results suggest that, in rabbit trachea, L-glutamate has, at least in part, an epithelium-dependent effect mediated via prostaglandin formation and that the EAA receptors involved are non-classical.

Key words: Airway smooth muscle, Rabbit, L-Glutamate, Prostaglandins, EAA receptors

Introduction

In humans, glutamate (in the form of monosodium) could be a provoking factor involved in asthma attacks, although the existence of monosodium glutamate-induced asthma has not been established.[1] Data obtained from animal studies support the hypothesis that the intense exposure to glutamate can be toxic for lungs because the over-activation of excitatory amino acid (EAA) receptors, mainly of the *N*-methyl-D-aspartate (NMDA) type, is involved in the pathogenesis of a variety of lung lesions like acute injury,[2] pulmonary edema,[3] oxidant tissue injury,[4] airway hyper-responsiveness and inflammation.[5,6] In animal lungs, accumulating data suggest that the localization of EAA receptors are presumably neuronal and L-glutamate exerts its effect via NMDA[2,3] or via non-classical EAA receptors.[7]

The purpose of the present study was first to investigate the effect of main ionotropic EAA receptors agonists such as L-glutamate, NMDA, (*RS*)-a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainic acid on KCl-induced contractions of rabbit tracheal smooth muscle. Second, we examined the involvement of epithelium and the endogenous formation of nitric oxide (NO) and prostaglandins in the effect of L-glutamate.

Materials and methods

Adult rabbits (1–2 kg body weight) of either sex were sacrificed by an overdose of intravenously administered sodium thiopentone (Abbot, Italy). Exothoracic tracheal tissue was removed and placed in Krebs solution (pH 7.4 at 37°C) with the following composition: Na^+, 137 mM; Mg^{2+}, 1.1 mM; K^+, 5.9 mM; Cl^-, 123.0 mM; Ca^{2+}, 2 mM; $H_2PO_4^-$, 1.2 mM; HCO_3^-, 24.9 mM; and glucose, 9.6 mM. The solution was gassed with 95% O_2 and 5% CO_2.

After the removal of connective tissue, tracheal rings were dissected from tracheas under an SZ30 Olympus stereoscope (Japan). The tracheal strips were 2 mm in width and were cut longitudinally through the cartilage opposite the smooth muscle layer. In experiments with epithelium-denuded tracheal rings, the epithelial layer was removed with a cotton-tipped applicator. Each strip was placed with the superfused luminal side up in a water-jacketed organ bath. One end of the tracheal strip was fixed on the bottom of the organ bath and the other to the transducer tip. The entire strip was continuously perfused with oxygenated Krebs solution at 37°C. The volume of the organ bath was approximately 3 ml and the perfusion rate was 5 ml/min. Tracheal strips were stretched manually to 1 g resting tension and were allowed to equilibrate in the organ bath for at least 60 min. Before starting the experiments, the rings were contracted by elevating the extracellular $[K^+]_o$ concentration to 80 mM or by 10^{-5} M acetylcholine (ACh) until a constant and reproducible contraction was achieved. Elevating potassium solutions were made by isosmotic substitution with $[Na^+]_o$. The concentration of 10^{-5} M ACh was chosen

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because, in pilot studies, 10^{-5} M ACh produced 50% of the maximal response (data not shown). Changes in tension were recorded on a Grass FT03C force displacement transducer (Astro Med Inc., USA) and displayed via a Universal oscillograph (Harvard Apparatus, England) recorder. To investigate the effect of L-glutamate on ACh-induced or KCl-induced contractions, increasing concentrations of L-glutamate (range, 10^{-9} to 10^{-3} M) were added in the perfusion medium and, after 15 min, contractions were induced by ACh or KCl. The effect of the main ionotropic EAA receptors agonists NMDA, AMPA and kainic acid (range, 10^{-9} to 10^{-4} M) on KCl-induced contractions was studied in the same way. We also studied the effect of kainic acid (range, 10^{-9} M to 10^{-4} M), in the presence of 10^{-6} M NMDA and the effect of the NMDA (range, 10^{-9} M to 10^{-4} M) in the presence of 10^{-6} M kainic acid. The experiments with NMDA were performed in the presence of Mg^{2+} and also in Mg^{2+}-free Krebs solution.

Experiments with epithelium-intact and epithelium-denuded preparations were carried out in parallel. In experiments in which tetrodotoxin (TTX), methylene blue, N^G-nitro-L-arginine methyl ester (L-NAME) and indomethacin were used, the rings were incubated with the inhibitor for 45 min before the exposure to L-glutamate. Contractions were evoked by 80 mM KCl.

Values are expressed as a percentage of reference contraction induced by 80 mM KCl or 10^{-5} M ACh. All data are presented as mean ± standard error (SE), and N refers to the number of animals. Statistical differences between all the responses were assessed using paired or unpaired t-tests, and $p < 0.05$ was considered to be significant.

ACh, L-glutamate, NMDA, AMPA, kainic acid, TTX, methylene blue, L-NAME and indomethacin were all obtained from Sigma (Germany).

Results

L-Glutamate evoked a significant concentration-dependent decrease of KCl-induced contractions (Fig. 1). The maximal decrease (30%) was observed in the presence of 10^{-3} M L-glutamate ($p < 0.001$, paired t-test; Fig. 1). The removal of epithelium, on the contrary, reduced significantly the effect of L-glutamate ($p < 0.05$, paired t-test; Fig. 1). L-Glutamate had no effect on ACh-induced contractions (Fig. 2). The presence of TTX (3×10^{-6} M) in the perfusion medium significantly attenuated the effect of L-glutamate on KCl-induced contractions ($p < 0.01$, paired t-test, for concentrations of L-glutamate between 10^{-8} and 10^{-3} M; Fig. 3). The treatment of preparations with methylene blue (10^{-5} M) attenuated significantly the effect of L-glutamate on KCl-induced contractions ($p < 0.01$, unpaired t-test; Fig. 4) and had

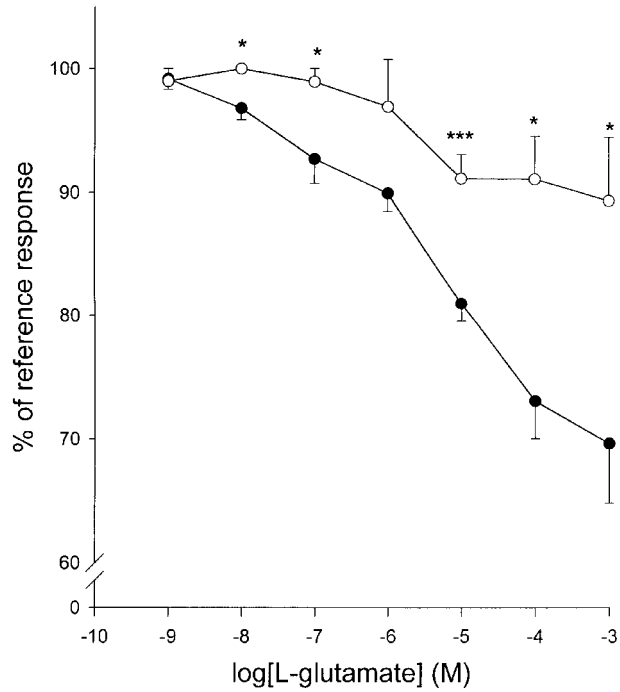

FIG. 1. L-Glutamate effect on epithelium-intact (●) and epithelium-denuded preparations (○). The responses are mean ±SE (n = 5) and are plotted as a percentage of the reference response before the exposure to L-glutamate. *p < 0.05, ***p < 0.001, paired t-test.

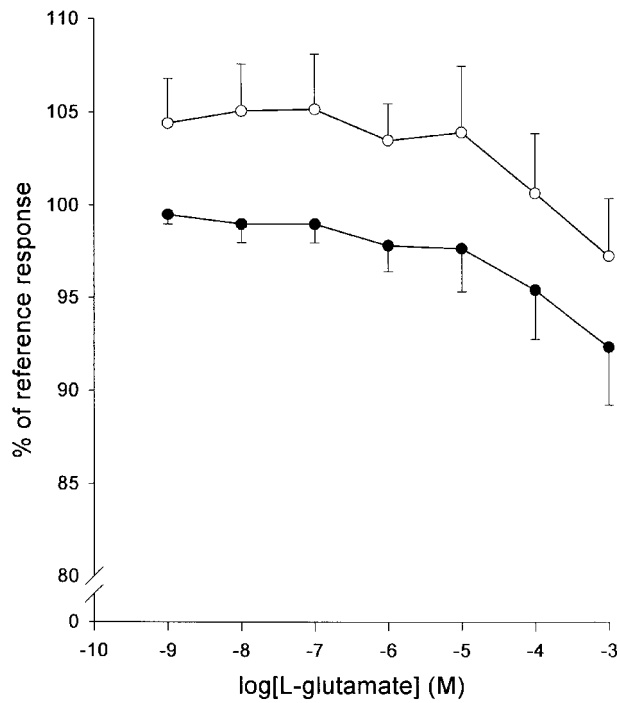

FIG. 2. L-Glutamate effect on ACh-induced contractions (●), and in the presence of methylene blue (○). The responses are mean ±SE (n = 6) and are plotted as a percentage of the reference response before the exposure to L-glutamate.

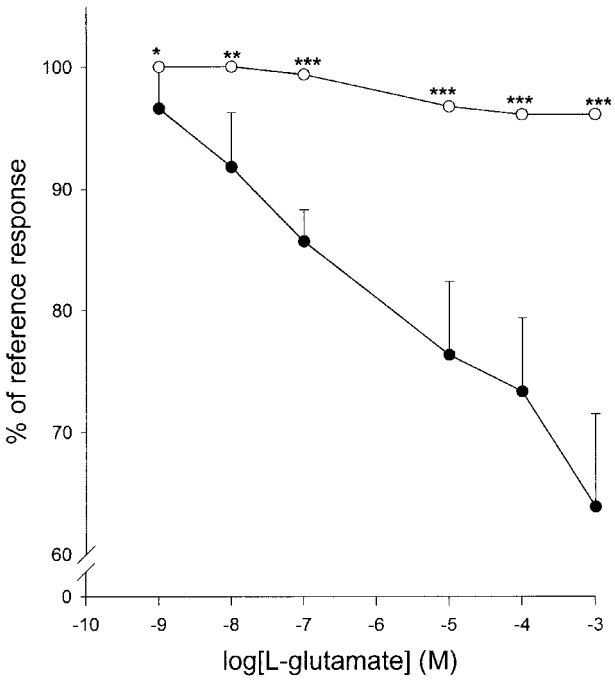

FIG. 3. The effect of L-glutamate on KCl-induced contractions (●), and in the presence of TTX (○). The responses are mean ±SE (n = 6) and are plotted as a percentage of reference response before the exposure to L-glutamate. $p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, paired t-test.

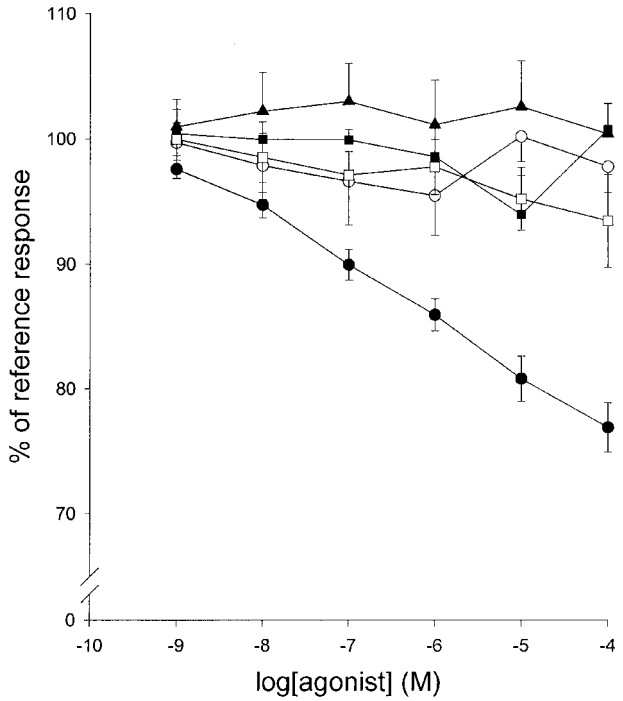

FIG. 5. The effect of L-glutamate (●), AMPA (○), kainic acid (□), NMDA (■) and NMDA in magnesium-free medium (▲). The responses are mean ±SE; n = 11 for L-glutamate, and n = 5 for each other agonist. The responses are plotted as a percentage of the reference response before the exposure to agonist.

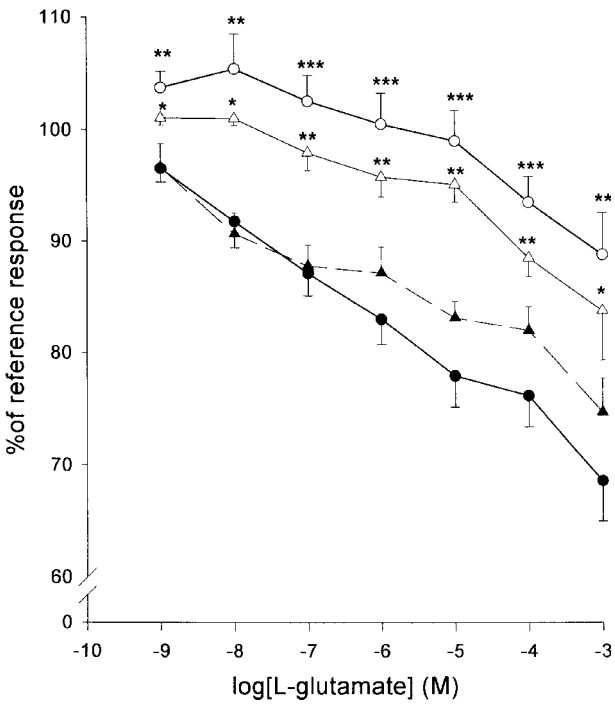

FIG. 4. The effect of L-glutamate on KCl-induced contractions (●), and in the presence of methylene blue (○), L-NAME (▲) and indomethacin (△). The responses are mean ±SE; n = 11 for experiments with L-glutamate, n = 7 for experiments in the presence of methylene blue, n = 5 for experiments in the presence of L-NAME, and n = 6 for experiments in the presence of indomethacin. The responses are plotted as a percentage of the reference response before the exposure to L-glutamate. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$, unpaired t-test.

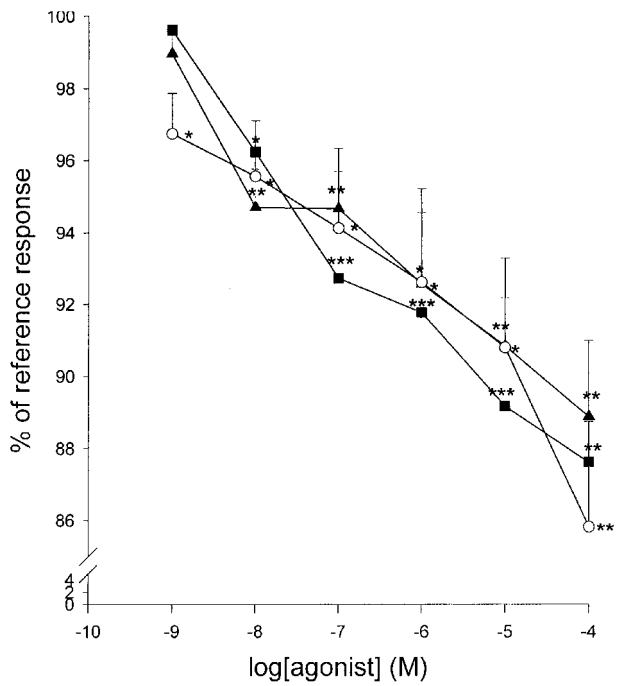

FIG. 6. The effect of NMDA in the presence of 10^{-6} M kainic acid (▲), of kainic acid in the presence of 10^{-6} M NMDA (■), and in magnesium-free medium (○). The responses are mean ±SE (n = 6) and are plotted as a percentage of the reference response before the exposure to agonists. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ paired t-test.

a small, statistically not significant, potentiating effect on contractions evoked by ACh (Fig. 2). The presence of L-NAME (10^{-4}M) in the perfusion medium did not influence the effect of L-glutamate (Fig. 4), while the presence of indomethacin (10^{-5}M) significantly reduced the effect of L-glutamate ($p < 0.05$, unpaired *t*-test; Fig. 4).

Finally, NMDA, in the presence or absence of Mg^{2+} in the perfusion medium, AMPA or kainic acid had no effect on KCl-induced contractions (Fig. 5). However, the combination of NMDA + kainic acid (NMDA in the presence of 10^{-6}M kainic acid or kainic acid in the presence of 10^{-6}M NMDA) evoked a significant decrease of KCl-induced contractions (Fig. 6). The absence of Mg^{2+} from the perfusion medium had no effect on these responses (Fig. 6). The combined effect of the two ionotropic EAA agonists, kainic acid and NMDA, was not as potent as that by L-glutamate alone. L-Glutamate, NMDA, AMPA or kainic acid in concentrations already mentioned had no effect on tracheal strips under resting conditions and in the absence of KCl-induced contractile activity (data not shown).

Discussion

Our results demonstrate that L-glutamate application on rabbit trachea decreases KCl-induced contractions, without affecting ACh-induced contractions of smooth muscle. KCl in high concentration induces contractions in rabbit trachea mainly via the ACh release;[8] therefore, we assume that L-glutamate, at the dose tested, had no post-junctional effect. The effect of L-glutamate on rabbit tracheal smooth muscle was attenuated by the removal of epithelium, as well as by the presence of TTX, methylene blue or indomethacin in the perfusion medium. These data provide evidence, first, for the release of a neuronal agent that mediates the L-glutamate effect and, second, for an epithelium-dependent and prostaglandin-dependent way of action.

In perfused rat lungs, activation of NMDA receptors, in the presence of the NO precursor L-arginine, stimulates cGMP formation and synthesis of NO.[2,3] To investigate the involvement of cGMP and/or NO in the relaxant effect of L-glutamate, we treated our preparations with methylene blue, an inhibitor of cGMP formation. While methylene blue significantly reduced the effect of L-glutamate, the treatment of our preparations with L-NAME, an inhibitor of NO synthase, had no effect. These results suggest that NO was not involved in the L-glutamate effect on rabbit trachea. Previous studies reported that methylene blue might exert its effect via a mechanism independent of the inhibition of cGMP formation, and may inhibit the synthesis and/or the release of prostanoids.[9–11] For this reason, we treated our preparations with indomethacin, an inhibitor of cyclooxygenase. The presence of indomethacin in the perfusion medium attenuated the effect of L-glutamate, thus suggesting that L-glutamate exerts its effect, at least in part, via prostaglandin formation.

Although our results provide evidence for a neuronal localization of L-glutamate receptors, their possible site of localization is not clear. L-Glutamate receptor subunits are present on airways.[12,13] According to the available data, the L-glutamate receptors, mainly of NMDA type,[14] are located on parasympathetic nerves.[15] In guinea pigs, indirect evidence also support the existence of L-glutamate receptors on airways sensory neurons, where they mediate the acute response to capsaicin.[5] Thus, in rat bronchi, activation of glutamate receptors enhances cholinergic contractions.[7] This effect may be mediated indirectly by the release of sensory peptides, because sensory peptides may facilitate cholinergic neurotransmission in airways.[16–18]

In contrast to these previous observations, our findings demonstrate a relaxant effect of L-glutamate in rabbit trachea. The discrepancy may be due to species and/or tissue differences. Supporting this, L-glutamate has no effect on the responsiveness of rabbit airways.[19] In addition, guinea pig airways contain significantly greater amounts of substance P[20] and are more sensitive to NK-1 and NK-2 receptors agonists than rabbit.[21] Capsaicin induces plasma extravasation in guinea pig and rat trachea[22–24] but has no effect on rabbit trachea.[25] Concerning tissue differences, the density and the type of tachykinin receptors, as well as the tissue responsiveness to sensory peptides, alter from proximal to distal airways.[26,27] Considering these observations, L-glutamate receptors may exist on the sensory fibers of rabbit but their activation and the subsequent release of sensory peptides neither provoke direct contractions or enhance ACh release. Direct measurements are necessary to test this hypothesis.

The presence of the NMDA receptor subunit has also been demonstrated by immunocytochemistry in neurons of rat larynx and esophagus containing NO and vasoactive intestinal peptide (VIP),[28] from which the inhibitory nerves supplying airway smooth muscle originate.[29] The presence of a functional nonadrenergic non-cholinergic inhibitory (iNANC) system in rabbits has also been demonstrated,[30,31] but this system seems not to use NO as a neurotransmitter.[32] The other neurotransmitter of the iNANC system (VIP) has been detected in rabbit airways.[33,34] Although VIP exerts its effect via cAMP formation,[35] prostaglandins[36] as well as epithelium[37,38] may be involved in its effect. The release of VIP in rabbit trachea stimulated by L-glutamate could be a reasonable explanation, while L-glutamate failed to induce contractions since VIP has also a modulatory effect on ACh release.[39]

Previous observations in mammalian peripheral tissues,[7,40–42] as well as in airways,[7] suggest the existence of non-classical glutamate receptors. Studies in the central nervous system have reported glutamate receptor subtypes composed of both kainic acid or AMPA and NMDA receptor subunits,[43] or non-classical glutamate receptors blocked by both NMDA and non-NMDA antagonists.[44] In this respect, we tested the effect of the main EAA receptor agonists NMDA, AMPA and kainic acid as well as the combination of the NMDA plus kainic acid. Our results show that L-glutamate exerts its effect via a non-classical EAA receptor, since the classical EAA receptor agonists, NMDA, AMPA and kainic acid, were ineffective. However, the combination of NMDA + kainic acid decreased KCl-induced contractions. The role of Mg^{2+} was probably not significant in our system, as the presence or absence of Mg^{2+} did not affect the decrease of contraction evoked by kainic acid in the presence of NMDA in the bath medium. The effect of the combinations of EAA agonists tested in the present study was less potent than L-glutamate. Therefore, we cannot exclude the involvement of metabotropic receptors in this effect.

In conclusion, in rabbit trachea, L-glutamate in part has an epithelium-dependent effect, mediated via prostaglandin formation. The EAA receptors involved in this effect are non-classical EAA receptors of the NMDA/non-NMDA type.

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