Mechanism of capsaicin-induced relaxation in equine tracheal smooth muscle

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Mechanism of capsaicin-induced relaxation in equine tracheal smooth muscle. Am. J. Physiol. 273 (Lung Cell. Mol. Physiol. 17): L997–L1001, 1997.—The effects of capsaicin and neuropeptides were examined in equine tracheal smooth muscle (TSM). Neither capsaicin nor substance P (SP) contracted TSM. Capsaicin (100 μM) elicited relaxation in TSM contracted with methacholine. This relaxation was not mimicked by SP or calcitonin gene-related peptide (CGRP). Relaxation was not attenuated by removal of the epithelium or by pretreatment of tissue with medofenamate or the nitric oxide (NO) synthase inhibitor Nω-nitro-L-arginine. Previous exposure of TSM to capsaicin did not eliminate the relaxation responses to subsequent capsaicin. Although vasoactive intestinal peptide (VIP) elicited marked relaxation that was attenuated by α-chymotrypsin, α-chymotrypsin did not affect the capsaicin-induced relaxation. Capsaicin-induced relaxation was abolished by charybotoxin, a blocker of large-conductance Ca2+-activated K+ channels. These results indicate that capsaicin-induced equine TSM relaxation is not mediated either by neuropeptides such as SP or CGRP released from capsaicin-sensitive sensory nerves or by prostanooids, NO, or VIP. Relaxation is due to the effect of capsaicin on large-conductance Ca2+-activated K+ channels. The peptidergic nerves play no important role in the regulation of TSM tone in horse airways.

THE NEURAL CONTROL of airways is complex. Together with the cholinergic and adrenergic mechanisms, there are sensory nerve fibers that are thought to play an important role by release of neuropeptides (20). Capsaicin is used widely to activate sensory nerve fibers and cause release of sensory neuropeptides such as substance P (SP) and calcitonin gene-related peptide (CGRP) from these nerve endings. These neuropeptides are thought to play a role in the pathogenesis of asthma (14) by contracting airway smooth muscle (10), modulating epithelial cell function (1, 8), causing vasodilatation (17), and increasing vascular permeability (18). In addition to these excitatory and proinflammatory effects of neuropeptides, a few studies have revealed apparent inhibitory effects of sensory nerves in airways. These inhibitory effects of capsaicin have been observed in human lung (11, 12) and in isolated human (3), rat (23), and mouse (16) airways. In rat and mouse airways, the relaxation response to capsaicin seems to be due to the release of sensory neuropeptides from capsaicin-sensitive sensory nerves. These neuropeptides activate neurokinin type 1 (NK1) receptors on epithelium and subsequently release the inhibitory prostaglandins that cause relaxation (16, 23). In human airways, Chitano et al. (3) reported that the inhibitory effect of capsaicin may also involve the NK1 receptor, but the mechanism was not clear. However, in a whole cell patch-clamp study on human bronchial smooth muscle cells, Ellis et al. (7) recently demonstrated that capsaicin can enhance outward K+ currents due to activation of large-conductance Ca2+-activated K+ channels. This study indicates that there are tachykinin-independent effects of capsaicin on human smooth muscle cells. We have previously demonstrated that SP does not contract equine tracheal smooth muscle (TSM). In the present studies, we discovered an inhibitory effect of capsaicin on equine TSM precontracted with methacholine (MCh) and investigated both the mechanism of capsaicin-induced relaxation and the role of peptidergic nerves in TSM regulation in the horse.

MATERIALS AND METHODS

Animals. Horses (body wt, 863–997 lb; age, 6–15 yr) were used in this study, which was approved by the All-University Committee on Animal Use and Care of Michigan State University. Horses that had no clinical signs of respiratory disease for several weeks were killed by injection of an overdose of pentobarbital sodium through the jugular vein. Other investigators also used tissues from the same animals for a variety of studies. Postmortem examination revealed that the lungs and airways were normal in gross appearance. A segment of trachea between the 6th and 15th cartilaginous ring above the carina was quickly collected, immersed in Krebs-Henseleit (KH) solution (in mM: 118.4 NaCl, 25.0 NaHCO3, 11.7 dextrose, 4.7 KCl, 2.6 CaCl2·2H2O, 1.19 MgSO4·7H2O, and 1.16 KH2PO4), and gassed with 95% O2-5% CO2 during the whole experiment.

Preparation of trachealis strips. The trachea was opened longitudinally by dissection of cartilage in its anterior aspect and was pegged flat on a paraffin block submerged by KH solution. TSM strips with epithelium intact were cut with a template along the fiber direction. Strips (2 × 10 mm), tied with 3–0 surgical silk thread on both ends, were suspended between platinum ring electrodes in 15-ml tissue baths that contained KH solution bubbled with 95% O2-5% CO2 during the whole experiment.

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After equilibration, the maximal response to 127 mM KCl-substituted KH solution (KClmax) was recorded, and this response was used to normalize the forces developed by the muscle. The tissues were then repeatedly rinsed with KH solution until the muscle tension returned to baseline.

Protocol 1: Effects of capsaicin and neuropeptides on TSM contracted with MCh. Trachealis was contracted with MCh to ~50–75% of KClmax and, after the tension was stable, except for time-matched MCh control, the tissues were treated with 10^{-4} M capsaicin, 10^{-5}–10^{-4} M SP, or 10^{-6} M CGRP. If CGRP elicited relaxation, the effect of the CGRP antagonist human CGRP fragment 8–37 (10^{-6} M) on CGRP-induced relaxation was also examined.

Protocol 2: Role of epithelium and endogenous prostanoids on capsaicin-induced relaxation. Epithelium-denuded trachealis strips were prepared by carefully rubbing their luminal side with a cotton-tipped applicator. In some strips, the epithelium was peeled away by grasping the epithelium and lamina propria with Allis tissue forceps and gently easing the mucosa from the underlying tissues. These preparations were used to study the effect of epithelium on capsaicin-induced relaxation. To examine the effect of endogenous prostanoids, tissues were incubated for 60 min with 10^{-6} M medofenate, a cyclooxygenase inhibitor, before the administration of MCh. This concentration of medofenate was chosen because it dramatically inhibits the TSM endogenous prostanoid synthesis (24).

Protocol 3: Effects of sensory neuropeptide depletion on capsaicin-induced relaxation. It previously was demonstrated that 10^{-6}–10^{-5} M capsaicin treatment causes neuropeptide depletion from sensory nerves, rendering them insensitive to further exposure of capsaicin (2, 23). Therefore, to examine the effects of sensory nerve desensitization on inhibitory responses evoked by capsaicin or neuropeptides, the trachealis strips were incubated for 30 min with 10^{-5} M capsaicin before being contracted with MCh. Then 10^{-4} M capsaicin-induced relaxation was elicited.

Protocol 4: Role of endogenous nitric oxide and vasoactive intestinal peptide on capsaicin-induced relaxation. To examine the effects of endogenous nitric oxide (NO) and 10^{-6} M vasoactive intestinal peptide (VIP) on capsaicin-induced relaxation, we pretreated trachealis strips with either the NO synthase inhibitor N\textsubscript{G}-nitro-L-arginine (L-NNA; 3 × 10^{-5} M; see Ref. 27) or 2 U/ml a-chymotrypsin. Yu et al. (27) from our laboratory have demonstrated that 3 × 10^{-5} M L-NNA abolishes nitrovasodilator inhibitory nonadrenergic noncholinergic (NANC)-mediated relaxation of equine TSM.

Protocol 5: Effect of charybdotoxin on capsaicin-induced relaxation. We investigated the role of large-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels in capsaicin-induced relaxation. Trachealis strips were incubated for 20 min with 5 × 10^{-7} M charybdotoxin before an attempt to elicit the capsaicin-induced relaxation was made.

Drugs. Acetyl-L-methacholine chloride, SP, CGRP, CGRP-(8–37), VIP, a-chymotrypsin, charybdotoxin, L-NNA, and medofenate sodium monohydrate (Sigma Chemical, St. Louis, MO) were dissolved in distilled water and were diluted in KH solution. Stock solutions of capsaicin (Sigma Chemical) were prepared in ethanol and were diluted with KH as appropriate. All the drugs were prepared on the day of the experiment. Drug solution was pipetted into the tissue bath at 1% of the bath volume. The final concentration of the drugs was expressed as their bath molar concentration.

Data analysis. In vitro relaxation responses are represented as percentage of relaxation from the level of contraction induced by MCh. All values are expressed as means ± SE. The effect of treatments was evaluated by an unpaired Student’s t-test by Statview II (Abacus Concepts, Calabasas, CA) for the Macintosh computer. P < 0.05 was considered significant; n represents the number of horses studied.
Protocol 2: Role of epithelium and endogenous prostanoids on capsaicin-induced relaxation. Neither denuding nor peeling off of epithelium affected capsaicin-induced relaxation. Capsaicin-induced inhibitions were 32.9 ± 6.0 and 30.9 ± 5.0% before and after denuding the epithelium, respectively, and 33.7 ± 6.0 and 31.1 ± 6.0% before and after peeling off of the epithelium, respectively. Treatment of the tissues with the cyclooxygenase inhibitor meclofenamate (10⁻⁶ M) had no effect on relaxation responses to capsaicin. Capsaicin-induced inhibitions were 32.9 ± 6.4 and 33.0 ± 7.4% before and after meclofenamate, respectively (n = 4).

Protocol 3: Effects of sensory neuropeptide depletion on capsaicin-induced relaxation. Depletion of sensory neuropeptides with 10⁻⁵ M capsaicin did not eliminate relaxation responses upon subsequent addition of 10⁻⁴ M capsaicin. Capsaicin (10⁻⁴ M)-induced inhibition was 39.5 ± 4.9 and 34.1 ± 1.9% with or without 10⁻⁵ M capsaicin pretreatment, respectively (n = 6). In addition, after 10⁻⁴ M capsaicin-induced relaxation, the second administration of 10⁻⁴ M capsaicin to the same trachealis strip still elicited the same magnitude of relaxation (Fig. 3).

Protocol 4: Role of endogenous NO and VIP on capsaicin-induced relaxation. VIP (10⁻⁶ M) elicited a rapid and large-magnitude relaxation that was inhibited by pretreatment of the tissues with the peptidase enzyme α-chymotrypsin (2 U/ml; Fig. 4; n = 5). However, the capsaicin-induced relaxation was not affected by 2 U/ml α-chymotrypsin (Fig. 4; n = 5). Pretreatment of tissues with the NO synthase inhibitor L-NNA (3 × 10⁻⁵ M) also did not alter capsaicin-induced relaxation. The capsaicin-induced relaxations were 32.8 ± 6.3 and 33.3 ± 6.0% before and after L-NNA pretreatment, respectively (n = 4).

Protocol 5: Effect of charybdotoxin on capsaicin-induced relaxation. Pretreatment of tissue with 5 × 10⁻⁷ M charybdotoxin, the blocker of the large-conductance Ca²⁺-activated K⁺ channels, almost totally abolished the capsaicin-induced relaxation (Fig. 5; n = 5).

DISCUSSION

Neuropeptide-containing sensory nerves have been described in airways of many species, including the horse. Activation of these sensory nerve fibers results in the release of NKs such as SP and CGRP. These peptides induce a number of biological effects within the lungs, such as airway smooth muscle contraction, mucus secretion, and enhanced vascular permeability. However, in airways of some species, such as the Sprague-Dawley rat, mouse, and human, an inhibitory effect of these neuropeptides has also been reported. Therefore, it seems likely that the peptidergic nerves may play different physiological and pathophysiological roles in airway regulation in different species. In horse TSM, our present study demonstrated that SP, CGRP, and capsaicin (< 10⁻⁴ M) do not produce either contraction or relaxation. These results indicate that neuropeptides released from capsaicin-sensitive nerves play no direct role in the regulation of horse TSM tone. This functional observation agrees with the patterns of...
distribution of SP- and CGRP-like immunoreactive nerves in horse airway. Nerve fibers immunoreactive for SP or CGRP are most prominently associated with the epithelium and the vasculature of the airways, where they may play an important role in the modulation of epithelial and bronchial vascular function (21). Only a small number of immunoreactive nerve fibers are closely associated with smooth muscle, suggesting that these neuropeptides have no direct effect on equine airway smooth muscle. It remains possible that tachykinins or CGRP may regulate TSM tone indirectly, either by causing the release of inflammatory mediators from mast cells (22) or by modulation of cholinergic neurotransmission at nerve terminals (4) or at parasympathetic ganglia (19, 21).

The interesting observation of this study is that capsaicin at a concentration of 10^{-4} M caused a significant relaxation of horse trachealis TSM. This concentration of capsaicin induced a relaxation of similar magnitude in human bronchi (7). It is widely accepted that capsaicin selectively stimulates sensory nerves leading to a local release of sensory neuropeptides such as tachykinins and CGRP (9, 15). However, in our present study, direct application of SP or CGRP did not mimic the capsaicin-induced relaxation. Furthermore, the CGRP antagonist did not attenuate the capsaicin-induced relaxation. These results indicated that capsaicin-induced relaxation was not mediated via SP or CGRP.

In rat and mouse airways, capsaicin induces relaxation through release of prostanoids from the epithelium (16, 23). In equine airway, both epithelium and prostanoids, such as prostaglandin E_2, have an inhibitory effect on smooth muscle contraction (26). Therefore, it was possible that capsaicin-induced relaxation in equine TSM may be due to release of prostanoids or another epithelium-derived factor. However, this is unlikely because removal of epithelium and inhibition of endogenous prostanoids by use of the cyclooxygenase inhibitor meclofenamate did not affect the relaxation of TSM to capsaicin.

Capsaicin administration causes a chemical desensitization of sensory nerves and renders them insensitive to further exposure of capsaicin (2). In our present studies, pretreatment of the horse trachealis with capsaicin to deplete sensory neuropeptides did not change the relaxation responses to subsequent capsaicin. Furthermore, repeated application of 10^{-4} M capsaicin to the same tissue strips elicited the similar magnitude of relaxation. These results further confirmed that capsaicin-induced relaxation does not involve the sensory neuropeptides.

Yu et al. (27) have previously reported that the iNANC system is a major inhibitory nervous system in equine airway and that neurotransmission involves NO. Because NO synthase and VIP have been colocalized with SP in the same nerve fibers and cell bodies of cat and ferret airways (5, 6), we hypothesized that the capsaicin-induced relaxation response may be due to activation of NO synthase or release of VIP. This, however, does not appear to be the case. The NO synthase inhibitor L-NNA was without effect on the relaxant responses to capsaicin. VIP is a very potent relaxant of airway smooth muscle of many species, and capsaicin can induce release of VIP-like immunoreactivity from guinea pig airways (13). Therefore, we further investigated the effect of VIP on horse trachealis and the role of VIP on capsaicin-induced relaxation response. VIP elicited a marked relaxation in horse trachealis. The relaxation to VIP was different from that of capsaicin, being more rapid and of greater magnitude. Even though the peptidase enzyme α-chymotrypsin eliminated the VIP-induced relaxation response, capsaicin-induced relaxation was not affected by α-chymotrypsin. These results indicate that VIP has a strong inhibitory effect in equine TSM but does not mediate the relaxation to capsaicin.

The results discussed so far indicate that the capsaicin-induced relaxation does not involve either sensory neuropeptides released from capsaicin-sensitive nerves or endogenous prostanoids, epithelium, NO, or VIP. It is therefore plausible to hypothesize that 10^{-4} M capsaicin may have a direct effect on smooth muscle cells, possibly acting via an inhibitory ion channel. In whole cell patch-clamp studies on human isolated bronchial smooth muscle cells, Ellis et al. (7) have recently reported that capsaicin can activate charybdoxin-sensitive large-conductance Ca^{2+}-activated K^{+} channels. Activation of these channels tends to hyperpolarize the membrane and leads to relaxation. We tested this possibility by examining the effect of charybdoxin, a potent blocker of large-conductance Ca^{2+}-activated K^{+} channels, on capsaicin-induced relaxation in equine TSM. In our experimental conditions, pretreatment of the tissue with charybdoxin virtually abolished the capsaicin-induced relaxation. This observation indicates that relaxation is due to the activation of these large-conductance Ca^{2+}-activated K^{+} channels. Capsaicin therefore appears to have two mechanisms of action in the airways. First, it stimulates sensory nerves, causing release of neuropeptides; and second, at a high concentration, it activates the charybdoxin-sensitive large-conductance Ca^{2+}-activated K^{+} channels of the smooth muscle. When capsaicin is used in pharmacological studies, both effects need to be considered in data interpretation.

In summary, our present study has demonstrated that in equine TSM 1) sensory neuropeptides released from capsaicin-sensitive nerves have no direct effect on smooth muscle tone, 2) VIP is a potent relaxant, and 3) capsaicin at high concentrations can activate charybdoxin-sensitive large-conductance Ca^{2+}-activated K^{+} channels and cause relaxation of TSM.

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