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Capsaicin-induced, capsazepine-insensitive relaxation of the guinea-pig ileum

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Abstract

The mechanisms underlying transient receptor potential vanilloid receptor type 1 (TRPV1)-independent relaxation elicited by capsaicin were studied by measuring isometric force and phosphorylation of 20-kDa regulatory light chain subunit of myosin (MLC_{20}) in ileum longitudinal smooth muscles of guinea-pigs. In acetylcholine-stimulated tissues, capsaicin (1–100 μM) and resiniferatoxin (10 nM–1 μM) produced a concentration-dependent relaxation. The relaxant response was attenuated by 4-aminopyridine and high-KCl solution, but not by capsazepine, tetraethylammonium, Ba^{2+}, glibenclamide, charybdotoxin plus apamin nor antagonists of cannabinoid receptor type 1 and calcitonin-gene related peptide. A RhoA kinase inhibitor reduced the relaxant effect of capsaicin at 30 μM. Capsaicin and resiniferatoxin reduced acetylcholine- and caffeine-induced transient contractions in a Ca^{2+}-free, EGTA solution. Capsaicin at 30 μM for 20 min did not alter basal levels of MLC_{20} phosphorylation, but abolished an increase by acetylcholine in MLC_{20} phosphorylation. It is suggested that the relaxant effect of capsaicin at concentrations used is not mediated by TRPV1, but by 4-aminopyridine-sensitive K^+ channels, and that capsaicin inhibits contractile mechanisms involving Ca^{2+} release from intracellular storage sites. The relaxation could be explained by a decrease in phosphorylation of MLC_{20}.

Keywords: Capsaicin; Capsazepine; Intestine; Myosin light chain; K^+ channel

1. Introduction

Capsaicin, the vanilloid responsible for the pungent taste of hot peppers, activates peripheral terminals of a specific subset of primary afferent sensory neurons, and a functional capsaicin's receptor has been cloned and termed the transient receptor potential vanilloid receptor type 1 (TRPV1) (Caterina et al., 1997; Gunthorpe et al., 2002; Montell et al., 2002). TRPV1 is a nonselective cation channel with relatively high permeability to Ca^{2+} in the cell membrane (Szallasi and Blumberg, 1999; Gunthorpe et al., 2002; Holzer, 2004; Nagy et al., 2004). The excitation by capsaicin of peripheral terminals of primary sensory neurons is due to influxes of mono- and divalent cations, resulting in depolarization of the nerve fibers and an increase in intracellular Ca^{2+} concentrations with a subsequent release of neuropeptides from their terminals, which include calcitonin gene-related peptide (CGRP) and substance P (Szallasi and Blumberg, 1999; Geppetti and Trevisani, 2004). CGRP released activates K^+ channels, resulting in hyperpolarization of membrane and relaxation in smooth muscle (Santicioli and Maggi, 1994; Dunn et al., 2003). Thus, capsaicin-sensitive sensory neurons have a role in the regulation of smooth muscle function. Resiniferatoxin is an extremely potent capsaicin analogue (Szallasi and Blumberg, 1999), and capsazepine is a competitive TRPV1 antagonist (Caterina et al., 1997).

The contractile and relaxant responses of smooth muscle to capsaicin may or may not be mediated by histamine, acetylcholine, prostanoids and nitric oxide (Manzini, 1992; Szarek et al., 1998; Barthó et al., 2002; Szallasi and Blumberg, 1999). Cannabinoid receptors also contribute to effects of capsaicin (Calignano et al., 2000).

There is evidence suggesting that capsaicin can produce a smooth muscle relaxation which is not mediated by TRPV1. The relaxation involves an inhibition of voltage-dependent L-type Ca^{2+} channels (Lo et al., 1995; Sim et al., 2001), an

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inhibition of store-operated Ca^{2+} entry (Choi and Kim, 1999; Flemming et al., 2003; Fujimoto and Mori, 2004), an activation of K^+ channels (Ellis et al., 1997; Zhu et al., 1997; Yeon et al., 2001), and a stimulation of arachidonic acid metabolism (Someya et al., 2002). The biological relevance of nonspecific (TRPV1-independent) relaxation is unclear, and mechanisms of this relaxation in intestinal smooth muscle remain to be determined.

Smooth muscle contraction is regulated by intracellular Ca^{2+} and involves phosphorylation of the 20-kDa regulatory light chain subunit of myosin (MLC_{20}), the myosin light chain kinase is activated by a Ca^{2+}-dependent pathway, and MLC_{20} phosphorylation allows activation of myosin adenosine triphosphatase by actin, resulting in contraction (Somlyo and Somlyo, 1994). However, there are smooth muscle contractions independent of phosphorylation of MLC_{20} (Adam et al., 1989; Rembold et al., 2000) and intracellular Ca^{2+} concentrations, suggesting an increase in Ca^{2+} sensitivity of contractile proteins (Pelaez et al., 2000). It is generally accepted that Ca^{2+} sensitization is due to an increase in the sensitivity of MLC_{20} phosphorylation to Ca^{2+}, principally through inhibition of myosin light chain phosphatase by RhoA kinase and subsequent increase in MLC_{20} phosphorylation (Somlyo and Somlyo, 2000).

To explore the mechanisms of the capsaicin-induced, capsazepine-insensitive relaxation in guinea-pig ileum, we determined effects of capsaicin on force development and MLC_{20} phosphorylation, and on acetylcholine- and caffeine-induced contractions in a Ca^{2+}-free solution.

2. Materials and methods

2.1. Ileal longitudinal muscle strips and tension measurement

The animal care and research protocols were in accordance with the guidelines of The Japanese Pharmacological Society. Male Hartley guinea-pigs (250–350 g) were anesthetized with pentobarbital (40 mg/kg, i.p.) and then exsanguinated. To obtain the myenteric plexus-longitudinal strip, the method described by Paton and Zar (1968) was used. Briefly, segments (10–15 cm long) of the intestine proximal to the ileocecal valve were removed, the contents expelled by flushing the lumen with a Krebs–Henseleit bicarbonate (KHB) solution containing (in mM) NaCl 114, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and dextrose 10. The ileum was stretched on a glass rod, the mesentery was cut away, and a longitudinal incision was made along the mesenteric border in 5-cm-long segments. A longitudinal muscle strip was prepared by peeling the outer layer of the segment along lines of cleavage with a cotton stick in the KHB solution. The preparation (length 1.5–2 cm, width 2–2.5 mm) was suspended between two metal pins in 10 ml of warmed (35 °C) and oxygenated (95% O_2 and 5% CO_2) KHB solution for isometric recordings, and challenged with repeated exposures to 3 μM acetylcholine and 30 mM KCl and progressive increases in basal tension to determine the optimal resting tension, which was found to be 400 mg. After an equilibration period of 90 min, the preparation was contracted three to four times with 1–10 μM acetylcholine and 65.9 mM KCl for 5–10 min at 40-min intervals. This procedure was found to produce optimal conditions for reproducible isometric force development.

2.2. Relaxant responses to capsaicin, resiniferatoxin and CGRP

The preparation was contracted with acetylcholine and KCl at submaximum concentrations of 10 μM and 65.9 mM, respectively. After the contraction had reached steady state, capsaicin (1–100 μM), resiniferatoxin (10 nM–1 μM) and CGRP (10 nM) were added to the organ bath. When the relaxation became maximum, papaverine (100 μM) was added to obtain the maximum relaxation; papaverine relaxed the preparation back to almost pre-acetylcholine or pre-KCl tensions. The relaxant effects of capsaicin, resiniferatoxin or CGRP were also evaluated 30 min after and during treatment with capsazepine, CGRP(8-37), guanethidine, K^+ channel blockers, a CB1 cannabinoid receptor antagonist and a RhoA kinase inhibitor, and 60 min after and during treatment with tetrodotoxin. A number of experiments performed previously in our laboratory showed that 1 μM tetrodotoxin did not affect the vanilloid-induced relaxation in the preparation, we, therefore, did not always add the drug to the organ bath.

2.3. Acetylcholine- and caffeine-induced contractions in Ca^{2+}-free solution containing 0.6 mM EGTA

The preparation was contracted for 5 min with 10 μM acetylcholine, three times at 40-min intervals in the normal KHB solution. Thereafter, the tissue was treated for 20 min with 10 and 30 μM capsaicin, 300 nM resiniferatoxin, 100 nM nifedipine or 0.1% dimethyl sulfoxide (DMSO, a vehicle) in the KHB solution and contracted with 10 μM acetylcholine before and 2 min after the preparation was exposed to a Ca^{2+}-free solution containing 0.6 mM EGTA with the same concentrations of capsaicin, resiniferatoxin or nifedipine. In another series of experiments, the preparation was contracted with 10 mM caffeine, using the same protocol as that used for acetylcholine. The results are expressed as a percentage of the contractions elicited by acetylcholine and caffeine in the KHB solution containing capsaicin, resiniferatoxin, nifedipine or DMSO.

2.4. Contractions induced by successively applied acetylcholine and caffeine in Ca^{2+}-free solution containing 0.6 mM EGTA

Experiments were carried out using protocols similar to those used previously (Fujimoto et al., 1998). Acetylcholine (10 μM) and caffeine (10 mM) were successively applied in normal KHB solution to the ileal strip for 2 min at 5-min intervals, followed by a 50-min application of the KHB solution. These procedures were repeated twice more, so that reproducible responses were obtained. Thirty minutes after the last contraction with caffeine, the ileum was exposed to 30 μM capsaicin in the normal KHB solution for 20 min and then in Ca^{2+}-free solution containing 0.6 mM EGTA for 2 min before