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Short communication

Capsaicin treatment induces muscarinic hyperreactivity in guinea pig trachea: A warning

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Abstract

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) is a widely used tool for the depletion of neuropeptides from sensory C-fibres. Upon capsaicin treatment tachykinins are released, resulting in a variety of responses in the airways. We showed that after capsaicin (0.3 μM; 30 min) treatment of guinea pig tracheal smooth muscle preparations, the maximal contraction of the trachea after methacholine stimulation was strongly increased (capsaicin: 1.147 ± 0.050 g vs. control: 0.717 ± 0.047 g). This effect was completely nullified after pretreatment with capsazepine (2-[2-(4-chlorophenyl)ethyl-amino-thiocarbonyl]-7,8-dihydroxy-2,3,4,5-tetrahydro-1*H*-2benzazepine; a vanilloid receptor antagonist) and YM38336 (a dual tachykinin NK_1 and tachykinin NK_2 receptor antagonist). Our results serve as a warning against using capsaicin as a putatively clean pharmacological tool to deplete the neuropeptides from pools on the C-fibres because we showed that capsaicin also strongly influences basal mechanisms in tracheal smooth muscle control. © 1998 Elsevier Science B.V.

Keywords: Guinea pig; Smooth muscle tracheal; Methacholine stimulation; Vanilloid receptor; Tachykinin receptor

1. Introduction

The tachykinins constitute a group of biologically active peptides which are released from C-fibres upon stimulation by a wide range of chemical and physical stimuli. In the airways the sensory C-fibres innervate the trachea, bronchi and lower respiratory tract, occasionally extending into the alveoli (Joos and Pauwels, 1991; Lundberg et al., 1984). The release of the tachykinins, like substance P, neurokinin A and neurokinin B, causes the activation of neurokinin receptors which are concentrated beneath the epithelium, in airway smooth muscle, in small vessels in the lamina propria and, in humans, in deep submucosal glands (Solway and Leff, 1991). Activation of these receptors leads to a variety of responses in the airways like modulation of bronchomotor tone, bronchial vasodilation and permeability, mucus hypersecretion, recruitment and activation of some types of inflammatory cells, and bronchoconstriction (Barnes et al., 1991; Lundberg and Saria, 1987).

The role of these neuropeptides in the bronchoconstrictive effects of numerous irritants like cigarette smoke, toluene diisocyanate and ozone has been studied by the use of capsaicin (Jimba et al., 1995; Koto et al., 1995; Kuo and Lu, 1995; Marek et al., 1996; Murlas et al., 1993; Tepper et al., 1993). Capsaicin (8-methyl-*N*-vanillyl-6-noneamide) is thought to interact with vanilloid receptors (Bevan et al., 1991) which are present on the C-fibres, causing a depolarisation of the cell membrane; this leads to the depletion of the neuropeptides from these sensitive C-fibres as was shown by immunohistochemical and radioimmunoassay (RIA) studies (Forsberg et al., 1988; Lundberg et al., 1983; Saria et al., 1988). It has also been shown that a capsaicin-operated cation-specific ion channel exists which is closely associated with the capsaicin receptor system in

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the membrane of sensory neurones (Bevan and Docherty, 1993).

In our laboratory we conducted studies in order to investigate the role of signal transmission processes in ozone-induced changes in airway functionality focused on the muscarinic receptor system. In vivo exposure to 3 ppm ozone for 2 h resulted in a significant increase in the maximal attainable contraction (a hyperreactivity) of guinea pig tracheal smooth muscle strips in vitro by using methacholine as a muscarinic agonist (Van Hoof et al., 1997). In order to study the role of tachykinins in this process capsaicin was used. During these studies we unexpectedly observed that capsaicin strongly influences the basal contractile mechanisms of the guinea pig trachea. The present study emphasizes that capsaicin cannot be regarded as a clean pharmacological tool.

2. Materials and methods

2.1. Animals

Male Dunkin–Hartley guinea pigs, weighing 300–350 g, obtained from Harlan CPB (Zeist, Netherlands) were kept in a light-controlled room, and were fed a standard diet (Hope Farms, Woerden, Netherlands). The animals were allowed tap water ad libitum.

2.2. Methacholine dose response curves

Guinea pigs were killed by a blow on the head and bled. The tracheas were removed, put in saline and dissected free from surrounding tissue whereafter they were cut in longitudinal direction opposite the smooth muscle layer. Tracheal strips (two cartilage rings in succession (Constantine, 1965)) were mounted in a 20-ml water-jacketed organ bath containing Krebs-buffer solution of pH 7.4 at 37°C and with the following composition (mM): NaCl, 117.5; KCl, 5.6; $MgSO_4$, 1.2; $CaCl_2$, 2.5; NaH_2PO_4, 1.28; $NaHCO_3$, 25.0 and glucose, 5.5, and gassed continuously with 5% CO_2–95% O_2. After an equilibrium-period of 60 min with six intermediate changes of buffer solution, a steady baseline was established and cumulative concentrations of methacholine were added to the buffer solution while changes in smooth muscle tension were recorded isometrically after applying a passive force of 1.0 ± 0.05 g to each preparation using a force displacement transducer type FT 03-C and a chart drive model 7H 25-50 (Grass Instruments, Quincy, MA, USA). The amplifier and the power supply used were developed at the Vrije Universiteit, Amsterdam, Netherlands. The 1.0 g applied tension was considered the baseline, from which all further tension changes were recorded. Data were recorded and analysed using MacLab® and corresponding Chart software (AD Instruments, Castle Hill, Australia).

2.3. Capsaicin pretreatment

In order to study the role of tachykinins in general, tracheal preparations were pretreated with capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) after the first methacholine dose response curve. After a washing period of 30 min capsaicin was added to the organ baths for 30 min to final concentrations of 0.3 μM. After a wash-out period of 30 min, a second methacholine dose–response curve was recorded as previously described.

2.4. Capsazepine and YM38336 pretreatment

Fifteen min before capsaicin was added, some tracheal preparations were incubated with capsazepine (2-[2-(4-chlorophenyl)ethyl-amino-thiocarbonyl]-7,8-dihydroxy-2,3,4,5-tetrahydro-1*H*-2benzazepine, a vanilloid receptor antagonist (Bevan et al., 1992) that selectively and competitively antagonizes the various capsaicin-induced responses in vivo and in vitro (Bevan et al., 1991) or YM38336 (a dual tachykinin NK_1/tachykinin NK_2 receptor antagonist), both at a final concentration of 10 μM. After capsaicin treatment (as described above) a second methacholine dose response curve was recorded.

2.5. Chemicals

Methacholine (acetyl-β-methylcholine chloride), capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) and capsazepine (2-[2-(4-chlorophenyl)ethyl-amino-thiocarbonyl]-7,8-dihydroxy-2,3,4,5-tetrahydro-1*H*-2benzazepine) were obtained from Sigma, St. Louis, MO, USA. YM38336 was a kind gift of Yamanouchi Europe, Leiderdorp, Netherlands. Capsaicin, capsazepine and YM38336 were all dissolved in ethanol. All other chemicals used were of reagent grade.

2.6. Statistical analysis

All data were expressed as mean $\pm$ S.E.M. Each experiment was performed in duplicate and results were statistically evaluated using Student's *t*-test. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Capsaicin-induced effects on tracheal smooth muscle reactivity after methacholine stimulation

Fig. 1 shows the effect of capsaicin treatment on methacholine-induced contractions of guinea pig tracheal smooth muscle. Capsaicin treatment and subsequent wash out resulted in a strong increase of the highest attainable contraction (i.e., hyperreactivity) after stimulation of the muscarinic receptor system by using methacholine. Pretreat-

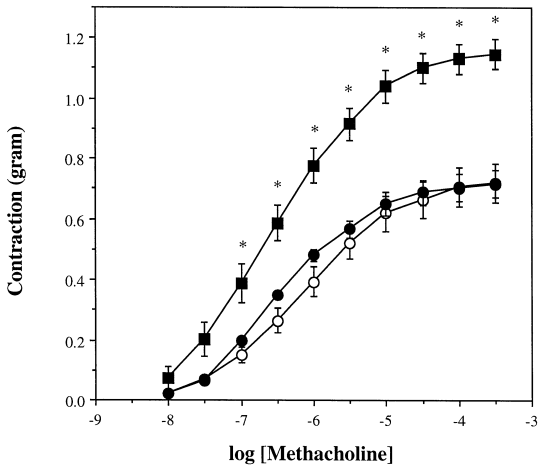

Fig. 1. The effect of capsaicin (0.3 μM; black squares; $n = 4$) and ethanol (black circles; $n = 4$) on methacholine-induced contractions (blanks: open circles; $n = 8$) of guinea pig tracheal smooth muscle strips. Values represent mean ± S.E.M.; $* P < 0.05$.

ment with capsaicin resulted in a maximal methacholine-induced contraction of 1.147 ± 0.050 g as compared to 0.717 ± 0.047 g measured in the situation where methacholine dose–response curves were recorded after pretreatment with only the solvent ethanol. Fig. 1 also shows that treatment with ethanol does not influence the maximal methacholine-induced contraction (0.717 ± 0.047 g with ethanol vs. 0.720 ± 0.065 g blank). Pretreatment with the capsaicin vehicle did not show any contractile response of the guinea pig tracheal strips.

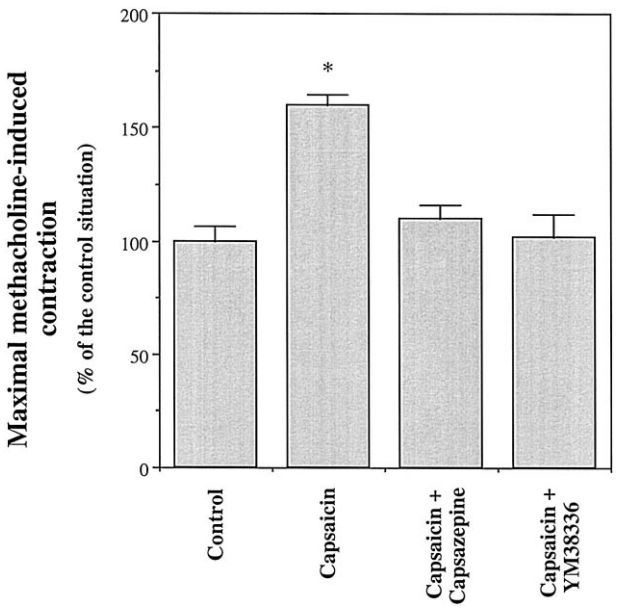

Fig. 2. The effect of capsaicin (0.3 μM) treatment alone and capsaicin treatment in combination with capsazepine- (10 μM) and YM38336- (10 μM) pretreatment on methacholine-induced contractions of guinea pig tracheal smooth muscle strips. Values are represented as a percentage of the maximal methacholine-induced contraction in the control situation (ethanol pretreated). Values represent mean ± S.E.M. ($n = 4$). $* P < 0.05$.

A time-matched experiment with two successive dose response curves of methacholine alone was performed to confirm the reproducibility of the experiment.

3.2. Effect of capsazepine and YM38336 on the capsaicin-induced effects

In another set of experiments we showed that pretreatment with the vanilloid receptor antagonist capsazepine and the neurokinin receptor antagonist YM38336 completely nullified the increase of methacholine-induced contractions after capsaicin treatment, as is shown in Fig. 2.

Compared to the control, capsaicin treatment results in a significant increase of the maximal methacholine-induced contraction ($100.0 \pm 6.6\%$ vs. $160.0 \pm 4.4\%$; see also Fig. 1). After treatment with capsaicin and capsazepine this effect was inhibited and the maximal contraction reached $109.6 \pm 6.7\%$. Comparable results were observed after YM38336 pretreatment. When the tracheal preparations were treated with YM38336 as well as capsaicin the maximal methacholine-induced contraction was $101.7 \pm 10.4\%$.

4. Discussion

The results as presented in this study clearly demonstrated that the release of tachykinins after capsaicin treatment resulted in an increased contraction after stimulation of the muscarinic receptor system by using methacholine. This effect was not due to the presence of capsaicin since the tracheal preparations had been washed extensively. The observed effects could be inhibited by using YM38336 (in a concentration that both the tachykinin NK_1 and tachykinin NK_2 receptor are blocked) and also after capsazepine treatment (vanilloid receptor antagonist) indicating a receptor-mediated effect.

Capsaicin is frequently used as a tool to deplete the tachykinins from the storage pools in studies where the role of these tachykinins in certain processes is under investigation (Jimba et al., 1995; Koto et al., 1995; Kuo and Lu, 1995; Marek et al., 1996; Murlas et al., 1993; Tepper et al., 1993). In this study we observed that capsaicin, through the release of the tachykinins, influences basal mechanisms in tracheal smooth muscle control and contraction.

After having antagonised the vanilloid receptor by capsazepine the capsaicin-induced muscarinic hyperreactivity was not observed anymore. This finding suggests that the effect is mediated via the vanilloid receptor system and is not due to nonspecific interactions of capsaicin with membrane lipids and proteins (Bevan and Docherty, 1993). Due to this vanilloid-receptor-mediated effect tachykinins like substance P and neurokinin A are released. The release of these products seems also necessary in the observed hyperreactivity, since blockade of the neurokinin receptor sys-

tems by using YM38336 also nullified the effect. These results demonstrate that there is no direct coupling between the vanilloid receptor system and the muscarinic receptor system. Stimulation of the neurokinin receptor system seems necessary for the observed capsaicin-induced muscarinic hyperreactivity.

These observations led to our hypothesis that stimulation of the tachykinin NK_1 and/or tachykinin NK_2 receptor system in some way stimulates effects mediated through activation of the muscarinic receptor system. Since experiments in which histamine was used did not show any effect on the maximal contraction after capsaicin treatment (data not shown), the observed effects appeared to be selective for the muscarinic receptor system. Based on this observation it is not likely that the inositoltrisphosphate/diacylglycerol second messenger system is directly involved, since both the muscarinic and histamine receptor system exert the majority of their contractile effects in the airways through this system. Possibly the release of prostanoids (prostaglandins and leukotrienes) is involved in the activation by and coupling to the neurokinin receptor system. It has been shown that these mediators are released from tracheal smooth muscle strips upon stimulation of the muscarinic and also the histamine receptor system, but stimulation with either methacholine or histamine resulted in a completely different profile of release (Van Hoof et al., 1998). We are currently investigating the precise mechanism underlying the capsaicin-induced muscarinic hyperreactivity, focusing on the possible involvement of the prostanoids.

The capsaicin-induced hyperreactivity as described in this study shows great resemblance to the effects that were measured after in vivo exposure of guinea pigs to 3 ppm ozone for 2 h (Van Hoof et al., 1997). The exact role of tachykinins in this ozone-induced effect in our in vitro system needs further investigation. Experiments in order to elucidate this role are currently performed in our laboratories.

This study is a warning for other investigators who use capsaicin as a pharmacological tool to deplete neuropeptides from capsaicin-sensitive pools in C-fibres. It is shown that after capsaicin treatment the maximal methacholine-induced contractions are enhanced. This effect is probably caused by a coupling between the tachykinin NK_1 and/or tachykinin NK_2 receptor system and the muscarinic receptor system, emphasizing that capsaicin also influences basal contractile mechanisms in the guinea pig tracheal smooth muscle preparations.

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