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# Capsaicin-induced desensitization is prevented by capsazepine but not by ruthenium red in guinea pig bronchi

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**Abstract**

In isolated guinea pig bronchi, the influence of ruthenium red, capsazepine and extracellular $Ca^{2+}$ on capsaicin-induced desensitization was examined to investigate whether this desensitization was mediated via a specific receptor coupled with an ion channel. Pre-exposure of tissues to capsaicin (1, 3 or 10 μM) caused a dose-dependent desensitization to the second application of capsaicin. However, the contractile responses to exogenous tachykinins were not changed after pre-exposure of tissues to capsaicin. This capsaicin-induced desensitization was prevented by capsazepine (30 μM), but not by ruthenium red added to tissues 20 min before pretreatment with capsaicin (3 μM). While the excitatory contractile response to capsaicin was markedly reduced in the absence of extracellular $Ca^{2+}$, the desensitization induced by capsaicin was not changed by the removal of extracellular $Ca^{2+}$. In summary, the results from the present study suggest that in vitro functional desensitization induced by capsaicin in guinea pig bronchi may involve changes in the vanilloid receptor and occur through a ruthenium red-insensitive pathway. 

*Keywords:* Capsaicin; Desensitization; Capsazepine; Ruthenium red; Bronchus; (Guinea pig)

## 1. Introduction

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), a pungent component of red peppers, is known to exert a selective action on a population of neuropeptide-containing primary afferent sensory neurones (C-fibers) (Holzer, 1988; Chahl, 1989; Amann et al., 1989). In guinea pig bronchi, capsaicin stimulates C-fibers to produce a contractile response by releasing sensory neuropeptides such as substance P and neurokinin A (Lundberg et al., 1983; Ellis and Undem, 1994). At high doses of capsaicin, this excitatory effect is followed by desensitization, a phenomenon defined as reduction or abolition of contractile responses to the second application of chemical stimuli of the same type (Ellis and Undem, 1994). To date, however, the mechanism of this desensitization induced by capsaicin is poorly understood.

The existence of a highly specific receptive site ('vanilloid receptor') for the capsaicin-induced excitation has been demonstrated in sensory nerve terminals (Szallasi, 1995; Rinder et al., 1996) innervating various tissues including guinea pig bronchi (Ellis and Undem, 1994; Szallasi et al., 1995). Furthermore, capsazepine, the first competitive antagonist for the vanilloid receptor, has been reported to antagonize the excitatory effect of capsaicin in peripheral sensory nerves (Fox et al., 1995; Griffiths et al., 1996; Paulino et al., 1996). However, there is little information about the receptive mechanism underlying the desensitizing action of capsaicin in guinea pig bronchi.

Ruthenium red, another functional antagonist for capsaicin with the property of inhibiting transmembrane and mitochondrial $Ca^{2+}$ transport, has been demonstrated to antagonize the desensitizing effect of capsaicin (Maggi et al., 1988b,c; Amann, 1990) as well as its excitatory effect (Dray et al., 1990b) in peripheral tissues. Recently, however, it has been reported that ruthenium red did not prevent capsaicin-induced desensitization in the dorsal root

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ganglion and spinal cord (Geppetti et al., 1991), suggesting differential action mechanisms of capsaicin-induced desensitization in different tissues.

Previously, we confirmed that pre-exposure of guinea pig bronchi to capsaicin produced dose-dependent desensitization to subsequently added capsaicin (Jung et al., 1997). In the present study, to investigate the possible mechanism of capsaicin-induced desensitization in isolated guinea pig bronchi, we examined whether capsazepine and ruthenium red could prevent the capsaicin-induced functional desensitization. In addition, we examined the influence of extracellular $Ca^{2+}$ on the desensitizing as well as excitatory action of capsaicin.

## 2. Materials and methods

### 2.1. Animals

The animals used were male Hartley-outbred guinea pigs (Samyuk Laboratory Animal, Osan, Korea) weighing 400 to 500 g and randomized into groups of five to eight. They were housed in a room with controlled temperature ($22 \pm 3$°C) and a 12 h light/dark cycle, and were given a solid diet and tap water ad libitum.

### 2.2. Preparation of isolated guinea pig bronchi

The guinea pigs were stunned and exsanguinated. The tracheas were quickly removed with two extrapulmonal main bronchi, and placed in oxygenated Krebs solution for cleaning and dissection. Four bronchial rings were prepared from each animal and transferred to 20- ml organ baths filled with Krebs solution (composition in mM: 118.3 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 $KH_2PO_4$, 25.0 $NaHCO_3$, 2.5 $CaCl_2$ and 11.1 glucose) at 37°C, gassed with a mixture of 5% $CO_2$/95% $O_2$ to produce a constant pH of 7.4 (Dickenson and Dray, 1991). Bronchial tissues were suspended between stainless steel hooks and placed in a 20-ml organ bath under a resting tension of 0.5 g, which was found to be optimal for measuring changes in tension. Tissues were allowed to equilibrate for 60 min before the experiments were commenced. During equilibration, the tissues were washed with fresh buffer at 15-min intervals, and then the maximal active tension in response to acetylcholine (1 mM) was obtained as an internal standard for expressing all the responses in each experiment as percentages.

### 2.3. Desensitization studies with capsaicin

To induce in vitro desensitization, bronchial rings were pre-exposed to vehicle (for control) or capsaicin (1, 3 and 10 μM) for 30 min, and then washed every 15 min for 60 min, at which time the tissues returned to their baseline tension. The contractile response to the subsequently added capsaicin (10 μM) was then examined in these tissues to determine the extent to which pretreatment with capsaicin at various concentrations induced a reduction in functional response as a result of the development of desensitization. To study a possible non-specific desensitizing effect of capsaicin mediated via sensory neuropeptides released from C-fibers, the ability of pretreatment with capsaicin (1, 3 and 10 μM) to desensitize tissues to subsequently added [$Sar^9$, $Met(O_2)^{11}$]substance P (tachykinin $NK_1$ receptor agonist, 1 μM) and [β-$Ala^8$]neurokinin A-(4–10) (tachykinin $NK_2$ receptor agonist, 3 μM) was examined. In preliminary experiments, concentration–response curves for capsaicin and tachykinins were obtained to determine the submaximal concentration needed for obtaining a contractile response following initial desensitizing doses of capsaicin.

In order to investigate the possible mechanisms underlying the capsaicin-induced desensitization, separate series of experiments with different tissues were conducted, where two potential antagonists, capsazepine (10 and 30 μM) and ruthenium red (30, 100 μM), were added to the bath 20 min before pre-exposure of tissues to the submaximal desensitizing concentration of capsaicin (3 μM) for 30 min. After washout and re-equilibration for 60 min, cumulative concentration–response curves for capsaicin ($10^{-8}$–$10^{-5}$ M, cumulatively in 0.5-log increments) were obtained to evaluate the influence of capsazepine and ruthenium red.

### 2.4. The role of extracellular $Ca^{2+}$ in capsaicin-induced excitation and desensitization

Two series of experiments were conducted. In the first series to examine the role of extracellular $Ca^{2+}$ in excitatory contractile effects of capsaicin, cumulative concentration–response curves for capsaicin ($10^{-8}$–$10^{-5}$ M, in 0.5-log increments) were obtained in the presence and absence of extracellular $Ca^{2+}$. To make $Ca^{2+}$-free medium, $CaCl_2$ was replaced isotonically by NaCl in the Krebs solution with 1 mM EGTA added. In the second series, with different tissues, to assess the effect of the extracellular $Ca^{2+}$ on the capsaicin-induced desensitization, tissues were pre-exposed to capsaicin (3 μM) for 30 min in the $Ca^{2+}$-free medium. After washout with normal Krebs solution until re-equilibration, concentration–response curves for cumulative capsaicin were obtained in normal Krebs solution for comparison with those from tissues pre-exposed to vehicle.

### 2.5. Statistics

All data were expressed as means ± S.E.M. The significance of drug effect was assessed by one-way analysis of variance and Student's *t*-test as appropriate. The level of significance was taken as $P < 0.05$ or $P < 0.01$.

### 2.6. Chemicals

Drugs used were capsaicin (Sigma, St. Louis, MO, USA), [Sar$^9$, Met($O_2$)$^{11}$] substance P (Research Biochemicals, Natick, USA), [β-Ala$^8$]neurokinin A-(4–10) (Research Biochemicals), ruthenium red (Sigma) and capsazepine (Research Biochemicals). Stock solutions of capsaicin (10 mM) and capsazepine (100 mM) were made up in 100% dimethyl sulfoxide (DMSO), and then diluted with warm saline to give a final concentration of $10^{-8}$–$10^{-5}$ M and $10^{-5}$–$3 \times 10^{-5}$ M, respectively. Ruthenium red was dissolved in distilled water (D.W.). DMSO used to dissolve capsaicin or capsazepine had no effect on the tissue preparations at its final concentration (0.1% DMSO).

## 3. Results

### 3.1. Desensitizing effect of capsaicin

Capsaicin (1, 3 and 10 μM) followed by washout was able to desensitize the isolated guinea pig bronchi to subsequent additions of capsaicin (10 μM) in a dose-dependent manner (Fig. 1). After a 30-min pre-exposure of tissues to capsaicin at concentrations of 1, 3 and 10 μM, the contractile responses to a second application of capsaicin (10 μM) were 64.13 ± 8.58% ($P < 0.05$), 11.11 ± 6.32% ($P < 0.01$) and 0% ($P < 0.01$ vs. vehicle-pretreated group) of the maximal response to acetylcholine (1 mM), respectively ($ED_{50}$ for desensitization: 1.12 μM). Pretreatment with capsaicin at a lower concentration (0.1 μM) had little effect on the development of desensitization (data not shown). The contractile responses to exogenous tachykinins, [Sar$^9$, Met($O_2$)$^{11}$]substance P and [β-Ala$^8$]neurokinin A-(4–10), were not changed after pretreatment of tissues with capsaicin either at 1, 3 or 10 μM (Fig. 1).

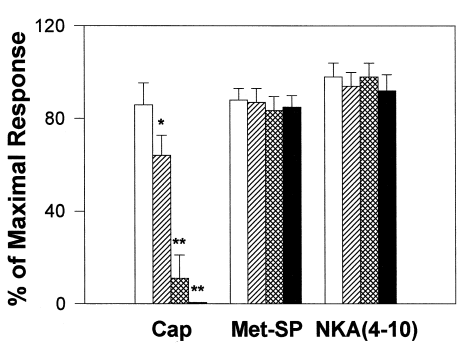


Fig. 1. Effect of capsaicin pretreatment on subsequent challenges with capsaicin (10 μM), [Sar$^9$, Met($O_2$)$^{11}$]substance P (Met-SP, 1 μM), [β-Ala$^8$]neurokinin A-(4–10) [NKA(4–10), 3 μM], in isolated guinea pig bronchi. Tissues were pre-exposed to vehicle (open column) or capsaicin (Cap., 1 μM: hatched column; 3 μM: grid column; 10 μM: black column) for 30 min. Values are means ± S.E.M. of five to seven experiments in each group. * $P < 0.05$, ** $P < 0.01$ vs. vehicle group.

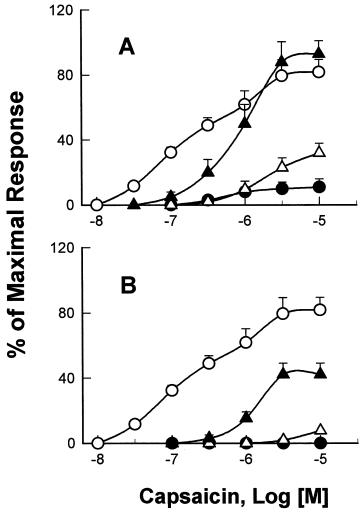


Fig. 2. Effect of capsazepine on capsaicin-induced in vitro desensitization in guinea pig bronchi. Isolated bronchi were pre-exposed to vehicle (control) or capsaicin (panel A: 3 μM, panel B: 10 μM) for 30 min in the presence of vehicle or capsazepine added 20 min before pre-exposure to capsaicin. Values are means ± S.E.M. of six to nine different experiments. ○: control, ●: vehicle; △: capsazepine 10 μM; ▲: capsazepine 30 μM.

### 3.2. Effect of capsazepine on capsaicin-induced desensitization

Separate experiments were carried out to determine whether capsazepine, added 20 min before the pre-exposure to capsaicin, was able to block the development of desensitization to the subsequently applied capsaicin in guinea pig bronchial rings. Pre-exposure of the tissues to capsaicin (3 and 10 μM) markedly reduced the maximum contractile responses to subsequently added capsaicin ($10^{-8}$–$10^{-5}$ M), confirming the establishment of desensitization (Fig. 2). At the submaximal desensitizing concen-

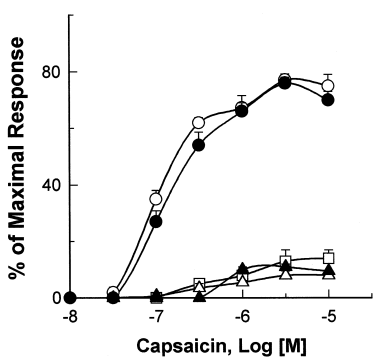


Fig. 3. Effect of ruthenium red on capsaicin-induced in vitro desensitization in guinea pig bronchi. Isolated bronchi were treated with ruthenium red or its vehicle (distilled water, D.W.) 20 min before pre-exposure of tissues to 0.1% DMSO or capsaicin for 30 min: ○, D.W. + 0.1% DMSO; ●, ruthenium red (30 μM) + 0.1% DMSO; □, D.W. + capsaicin (3 μM); △, ruthenium red (30 μM) + capsaicin (3 μM); ▲, ruthenium red (100 μM) + capsaicin (3 μM). Values are means ± S.E.M. of 6–10 different experiments.

tration of capsaicin (3 μM), the maximum response to the second application of capsaicin ($10^{-8}$–$10^{-5}$ M, cumulatively) was completely restored by the pretreatment with 30 μM capsazepine, up to the level of that in the control (panel A of Fig. 2). In the tissues pretreated with 10 μM capsazepine, the maximum response to subsequent capsaicin was approximately 50% of that in the control. As shown in panel B of Fig. 2, when desensitization was induced by capsaicin at a higher concentration (10 μM), the antagonistic effect of capsazepine was smaller than that against the desensitization induced by a lower concentration of capsaicin (3 μM). The pretreatment with capsazepine at 10 μM had little effect on the response to the subsequently added capsaicin (10 μM), whereas 30 μM capsazepine slightly restored the response to the subsequently added capsaicin (maximal capsaicin response: approximately 40% compared to that in tissues pretreated with vehicle).

### 3.3. Effect of ruthenium red on capsaicin-induced desensitization

In order to test whether ruthenium red could inhibit the capsaicin-induced desensitization in isolated guinea pig bronchi, we investigated different groups of tissues, where ruthenium red at two concentrations (30 and 100 μM) was added 20 min prior to pretreatment with capsaicin. As shown in Fig. 3, pre-exposure of tissues to ruthenium red (30 μM) without pretreatment with capsaicin (3 μM) had no residual effect on the contractile responses to the subsequently added capsaicin ($10^{-8}$–$10^{-5}$ M). In addition, ruthenium red (30 and 100 μM) applied 20 min before tissues were pre-exposed to capsaicin (3 μM), did not restore the desensitized responses, seen in tissues pretreated with capsaicin (3 μM) alone, to the subsequently added capsaicin ($10^{-8}$–$10^{-5}$ M).

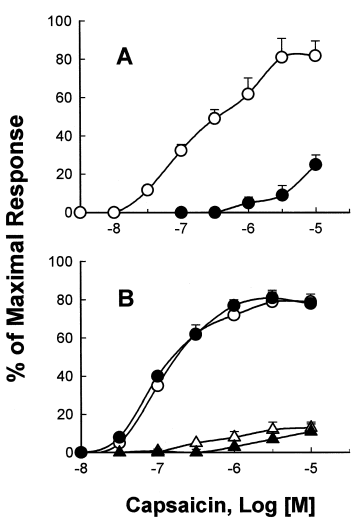


Fig. 4. Influence of $Ca^{2+}$-free medium on excitatory (panel A) and desensitizing (panel B) action of capsaicin in the isolated guinea pig bronchi. Panel A: Concentration–response curves for capsaicin were obtained in the presence (open circle) or absence (closed circle) of extracellular calcium. Panel B: Tissues were pre-exposed to vehicle (circle) or capsaicin (triangle) in the presence (open) or absence (closed) of extracellular calcium. All concentration–response curves for the second addition of capsaicin were obtained in the presence of extracellular calcium. Values are means ± S.E.M. of six to seven different experiments.

### 3.4. Effect of extracellular $Ca^{2+}$ on capsaicin-induced desensitization

Using a $Ca^{2+}$-free, EGTA-containing medium, we examined whether extracellular $Ca^{2+}$ was essential for production of capsaicin-induced excitation and desensitization. The muscle-contracting action of capsaicin was markedly reduced in the absence of extracellular $Ca^{2+}$ (panel A of Fig. 4). The desensitizing action of capsaicin, however, remained unaltered when tissues were pretreated with capsaicin in the absence of extracellular $Ca^{2+}$ (panel B of Fig. 4).

## 4. Discussion

In the present study, we have shown that the capsaicin-induced functional desensitization in guinea pig bronchi could be prevented by capsazepine but not by ruthenium red, in an extracellular $Ca^{2+}$-independent manner.

The specific action of capsaicin is characterized by an initial excitation followed by a long-lasting refractory state, termed ‘desensitization’. This phenomenon of desensitization represents a therapeutic potential to diminish the pain syndrome (Maggi and Meli, 1988; Maggi, 1992), and has been investigated for decades to identify the capsaicin-sensitive neuronal pathways (Holzer, 1991). The mechanism of acute excitatory responses to capsaicin can be explained by its interaction at a specific recognition site (Szallasi and Blumberg, 1990) known as ‘vanilloid receptor’, and the resultant $Ca^{2+}$ and $Na^{+}$ influx through the channel (Bevan et al., 1992) closely coupled to the recognition site. However, the mechanism of the desensitization by capsaicin is still poorly understood because this desensitization appears to involve a very complex process (Holzer, 1991; Dray, 1992). Recently, it has been reported that capsaicin-induced desensitization requires the presence of extracellular $Ca^{2+}$ and that this desensitization is prevented by capsazepine in rat dorsal root ganglion (Cholewinski et al., 1993). Based on this observation, we attempted to test the hypothesis that blockade of either the vanilloid receptor or of $Ca^{2+}$ entry during pretreatment with capsaicin can prevent the development of the desensitization in guinea pig bronchi.

In the present study, we observed that the contractile responses to exogenously added tachykinins were not altered after the pre-exposure of tissue to capsaicin. This

observation is consistent with other reports (Maggi et al., 1988a) that the function of tachykinin receptors, which mediate the contractile response to tachykinin released via a post-receptor mechanism following treatment with capsaicin, is not altered during the process of capsaicin-induced desensitization. The vanilloid receptor, on the other hand, seems to be associated with the desensitization since capsazepine can antagonize the in vitro desensitization induced by capsaicin as shown in this study. This result further supports the hypothesis that in vitro desensitization by capsaicin is mediated via a specific receptor (Cholewinski et al., 1993). Furthermore, there is evidence for a role of the vanilloid receptor in the development of in vivo desensitization (neurotoxicity). Systemic administration of resiniferatoxin, an ultrapotent capsaicin analogue, induces a dose-dependent loss of vanilloid receptors in sensory ganglia (Szallasi and Blumberg, 1992), spinal cord and urinary bladder (Goso et al., 1993) of the rat. In addition, it has been demonstrated that specific resiniferatoxin binding, thought to represent capsaicin receptor binding, decreases 80–90% in membranes from dorsal root ganglia and 50–70% in membranes from Gasserian ganglia of adult rats treated neonatally with resiniferatoxin (Szallasi et al., 1990).

The results from the present experiments showed that pretreatment with ruthenium red 20 min before the first application of capsaicin could not prevent the capsaicin-induced desensitization to subsequently added capsaicin. In addition, this study showed that the desensitization by capsaicin was not altered by the removal of extracellular $Ca^{2+}$ during capsaicin pretreatment, whereas the capsaicin-induced contractile response was markedly reduced in the absence of extracellular $Ca^{2+}$. These findings are in line with other observations that systemic administration of ruthenium red fails to attenuate the desensitizing effect of capsaicin at the central endings of primary afferent (Geppetti et al., 1991). Previously, we also found that ruthenium red had no effect on the capsaicin-induced antinociception in mice (Lee et al., 1995). However, there are contradictory reports that ruthenium red or removal of extracellular $Ca^{2+}$ can block the desensitizing action of capsaicin on neurones (Maggi et al., 1988b,c; Koplas et al., 1997). One possible explanation for the discrepancy among these results would be the difference in species and tissues. On the other hand, the results for the influence of ruthenium red on excitatory action of capsaicin, rather consistently demonstrate that ruthenium red can antagonize the capsaicin-induced stimulatory function (Amann et al., 1989; Dray et al., 1990b). Indeed, ruthenium red markedly inhibits the capsaicin-induced release of peptides and contractile function in guinea pig bronchi (Maggi et al., 1989; Jung et al., 1994), although in the present study, it failed to inhibit capsaicin-induced desensitization. This differential effect of ruthenium red supports the assumption that excitatory and desensitizing actions of capsaicin may involve distinct mechanisms (Szallasi, 1994).

Another possible point in favor of this hypothesis is the remarkable difference in the excitatory and the desensitizing potency of capsaicin and its analogues. For example, resiniferatoxin is only marginally more potent or equipotent with capsaicin to produce a contractile response in rat urinary bladder (Maggi et al., 1990), while it is 10,000-fold more potent than capsaicin to inhibit twitch responses to field stimulation in rat vas deferens (Maggi et al., 1990). Another capsaicin analogue, zyngerone, exhibits excitatory activity without desensitizing activity (Hayes et al., 1984), and other capsaicinoids display desensitizing activity in the absence of excitatory activity (Dray et al., 1990a). In accord with these results, it has been reported that in vitro desensitization to capsaicin in the guinea pig ureter occurs at doses considerably higher than those needed to release neuropeptide. Our present observations are also consistent with these results by showing that the maximum dose (10 μM) of capsaicin for desensitization was approximately 10-fold greater than that (1 μM) for the excitatory contractile response, although others (Ellis and Undem, 1994) did not find this to be the case in guinea pig trachea. Further investigations are needed to demonstrate whether the mechanisms underlying capsaicin-induced excitation and desensitization are different. The results could possibly lead to the potential application of the dual effects of capsaicin separately for medical purposes.

In summary, the present results suggest that in vitro functional desensitization to the capsaicin-induced contractile response in guinea pig bronchi may be mediated via a specific receptor and a ruthenium red-insensitive pathway.

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## References

Amann, R., 1990. Desensitization of capsaicin-evoked neuropeptide release—Influence of $Ca^{2+}$ and temperature. Naunyn-Schmiedeberg's Arch. Pharmacol. 342, 671–676.

Amann, R., Donnerer, J., Lembeck, F., 1989. Capsaicin-induced stimulation of polymodal nociceptors is antagonized by ruthenium red independently of extracellular calcium. Neuroscience 32, 255–259.

Bevan, S., Hothi, S., Hughes, G., James, I.F., Rang, H.P., Shah, K., Walpole, C.S.J., Yeats, J.C., 1992. Capsazepine, a competitive antagonist of the sensory neurone excitant capsaicin. Br. J. Pharmacol. 107, 544–552.

Chahl, L.A., 1989. The effect of ruthenium red on the response of guinea pig ileum to capsaicin. Eur. J. Pharmacol. 169, 241–247.

Cholewinski, A., Burgess, G.M., Bevan, S., 1993. The role of calcium in capsaicin-induced desensitization in rat cultured dorsal root ganglion neurones. Neuroscience 55, 1015–1023.

Dickenson, A.H., Dray, A., 1991. Selective antagonism of capsaicin by capsazepine: evidence for a spinal receptor site in capsaicin-induced antinociception. Br. J. Pharmacol. 104, 1045–1049.

Dray, A., 1992. Neuropharmacological mechanisms of capsaicin and related substances: commentary. Biochem. Pharmacol. 44, 611–615.

Dray, A., Bettaney, J., Rueff, A., Walpole, C., Wrigglesworth, R., 1990a. NE-19550 and NE-21610, antinociceptive capsaicin analogues: studies on nociceptive fibers of the neonatal rat tail in vitro. Eur. J. Pharmacol. 181, 289–293.

Dray, A., Forbes, C.A., Burgess, G.M., 1990b. Ruthenium red blocks the capsaicin-induced increase in intracellular calcium and activation of membrane currents in sensory neurones as well as the activation of peripheral nociceptors in vitro. Neurosci. Lett. 110, 52–59.

Ellis, J.L., Undem, B.J., 1994. Inhibition by capsazepine of resiniferatoxin- and capsaicin-induced contractions of guinea pig trachea. J. Pharmacol. Exp. Ther. 268, 85–89.

Fox, A.J., Urban, L., Barnes, P.J., Dray, A., 1995. Effects of capsazepine against capsaicin- and proton-evoked excitation of single airway C-fibers and vagus nerve from the guinea-pig. Neuroscience 67, 741–752.

Geppetti, P., Del Bianco, E., Patacchini, R., Santicioli, P., Maggi, C.A., Tramontana, M., 1991. Low pH-induced release of calcitonin gene-related peptide from capsaicin-sensitive sensory nerves: mechanism of action and biological response. Neuroscience 41, 295–301.

Goso, C., Piovacari, G., Szallasi, A., 1993. Resiniferatoxin-induced loss of vanilloid receptors is reversible in the urinary bladder but not in the spinal cord of the rat. Neurosci. Lett. 162, 197–200.

Griffiths, C.D., Eldershaw, T.P., Geraghty, D.P., Hall, J.L., Coloquhoun, E.Q., 1996. Capsaicin-induced biphasic oxygen uptake in rat muscle: antagonism by capsazepine and ruthenium red provides further evidence for peripheral vanilloid receptor subtypes (VN1/VN2). Life Sci. 59, 105–117.

Hayes, A.G., Oxford, A., Reynolds, M., Shingler, A.H., Skingle, M., Smith, C., Tyers, M.B., 1984. The effects of a series of capsaicin analogues on nociception and body temperature in the rat. Life Sci. 34, 1241–1248.

Holzer, P., 1988. Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. Neuroscience 24, 739–768.

Holzer, P., 1991. Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurones. Pharmacol. Rev. 43, 143–201.

Jung, Y.S., Lee, B., Kong, J.Y., Park, N.S., Cho, T.S., Shin, H.S., 1994. Effect of capsaicin and its novel derivative on the isolated guinea pig bronchi. J. Fd. Hyg. Safety 9, 163–168.

Jung, Y.S., Cho, T.S., Moon, C.H., Shin, H.S., 1997. Neurotoxic desensitizing effect of capsaicin on peripheral sensory nerve endings in guinea pig bronchi. Yakhak Hoeji 41, 139–146.

Koplas, P.A., Rosenberg, R.L., Oxford, G.S., 1997. The role of calcium in the desensitization of capsaicin responses in rat dorsal root ganglion neurones. J. Neurosci. 17, 3525–3537.

Lee, B., Jung, Y.S., Choi, J.I., Kong, J.Y., 1995. The effect of ruthenium red on the capsaicin-induced antinociception in vivo. J. Appl. Pharmacol. 3, 54–57.

Lundberg, J.M., Brodin, E., Saria, A., 1983. Effects and distribution of vagal capsaicin-sensitive substance P neurones with special reference to the trachea and lungs. Acta Physiol. Scand. 119, 243–252.

Maggi, C.A., 1992. Therapeutic potential of capsaicin-like molecules: studies in animals and humans. Life Sci. 51, 1777–1781.

Maggi, C.A., Meli, A., 1988. The sensory efferent function of capsaicin-sensitive sensory neurones. Gen. Pharmacol. 19, 1–43.

Maggi, C.A., Geppetti, P., Santicioli, P., Frilli, S., Giuliani, S., Furio, M., Theodorsson, E., Fusco, B., Meli, A., 1988a. Tachykinin-like immunoreactivity in the mammalian urinary bladder: correlation with the functions of the capsaicin-sensitive sensory nerves. Neuroscience 26, 233–242.

Maggi, C.A., Patacchini, R., Santicioli, P., Giuliani, S., Geppetti, P., Meli, A., 1988b. Protective action of ruthenium red toward capsaicin desensitization of sensory fibers. Neurosci. Lett. 88, 201–205.

Maggi, C.A., Santicioli, P., Geppetti, P., Parlani, M., Astolfi, M., Pradelles, P., Patacchini, R., Meli, A., 1988c. The antagonism induced by ruthenium red of the actions of capsaicin on the peripheral terminals of sensory neurones: further studies. Eur. J. Pharmacol. 154, 1–10.

Maggi, C.A., Patacchini, R., Santicioli, P., Giuliani, S., Del Bianco, E., Geppetti, P., Meli, A., 1989. The efferent function of capsaicin-sensitive nerves—ruthenium red discriminates between different mechanisms of activation. Eur. J. Pharmacol. 170, 167–177.

Maggi, C.A., Patacchini, R., Tramontana, M., Amann, R., Giuliani, S., Santicioli, P., 1990. Similarities and differences in the action of resiniferatoxin and capsaicin on central and peripheral endings of primary sensory neurones. Neuroscience 37, 531–539.

Paulino, N., Cechinel Filho, V., Pizzolatti, M.G., Yunes, R.A., Calixto, J.B., 1996. Mechanisms involved in the contractile responses induced by the hudroalcoholic extract of *Phyllanthus urinaria* on the guinea pig isolated trachea: evidence for participation of tachykinins and influx of extracellular $Ca^{2+}$ sensitive to ruthenium red. Gen. Pharmacol. 27, 795–802.

Rinder, J., Szallasi, A., Lundberg, J.M., 1996. Capsaicin-, resiniferatoxin-, and lactic acid-evoked vascular effects in the pig nasal mucosa in vivo with reference to characterization of the vanilloid receptor. Pharmacol. Toxicol. 78, 327–335.

Szallasi, A., 1994. The vanilloid (capsaicin) receptor: receptor types and species differences. Gen. Pharmacol. 25, 223–243.

Szallasi, A., 1995. Autoradiographic visualization and pharmacological characterization of vanilloid (capsaicin) receptors in several species, including man. Acta Physiol. Scand. Suppl. 629, 1–68.

Szallasi, A., Blumberg, P.M., 1990. Resiniferatoxin and its analogues provide novel insights into the pharmacology of the vanilloid (capsaicin) receptor. Life Sci. 47, 1399–1408.

Szallasi, A., Blumberg, P.M., 1992. Vanilloid receptor loss in rat sensory ganglia associated with long term desensitization to resiniferatoxin. Neurosci. Lett. 140, 51–54.

Szallasi, A., Szallasi, Z., Blumberg, P.M., 1990. Permanent effect of neonatally administered resiniferatoxin in the rat. Brain Res. 537, 182–186.

Szallasi, A., Goso, C., Manzini, S., 1995. Resiniferatoxin binding to vanilloid receptors in guinea pig and human airways. Am. J. Respir. Crit. Care Med. 152, 59–63.