Capsaicin-desensitization to the human nasal mucosa selectively reduces pain evoked by citric acid

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- 1 Kallidin (5–500 nmol), hypertonic saline (0.9–20% NaCl) or low pH medium (citric acid: pH 2.5–1) applied (50 μ l) to the human nasal mucosa produced a pain response (evaluated by a visual analogue scale) that was related to the concentration of the peptide, NaCl or hydrogen ions, respectively.
- 2 Application (50 μ l) of capsaicin (50 nmol) to the human nasal mucosa produced overt pain. After repeated administrations (once a day for 5–7 days) to one nostril this effect underwent almost complete desensitization, while in the contralateral nostril, treated with the vehicle, the response to capsaicin was unaffected.
- 3 The pain response produced in the human nasal mucosa by topical application (50 μ l) or kallidin (50–500 nmol), NaCl (10–20%) or citric acid (pH 1.5–1) solutions was then studied before and after local capsaicin desensitization.
- 4 The pain response to pH 1.5 or 1 citric acid was markedly reduced (by 60% and 75%, respectively) in the capsaicin-treated nostril. However, the pain response to 10% or 20% NaCl or the mild pain response to 50 or 500 nmol kallidin were unaffected by capsaicin pre-treatment.
- 5 The present results suggest that prolonged topical capsaicin treatment to the human nasal mucosa may lead to selective desensitization to certain algescic stimuli such as capsaicin itself and hydrogen ions.

Keywords capsaicin human nasal mucosa bradykinin hypertonic saline citric acid

Introduction

Capsaicin, the pungent ingredient of red peppers, selectively affects the function of a subset of primary afferents (Holzer, 1991; Maggi, 1991). Recently, it has been proven as a useful agent for the treatment of several painful human diseases (Bernstein *et al.*, 1987; Fuller, 1990; Sicuteri *et al.*, 1990; Watson *et al.*, 1988). The drug was given by topical application to the skin or mucosal surfaces, repeatedly with the aim of 'desensitizing' the peripheral endings of these nerves. However, it is far from clear which kind of impairment of the function of sensory nerves is associated with such therapeutic regimens.

High doses of systemic capsaicin induce a permanent or long-lasting desensitization of capsaicin-sensitive afferents in newborn rats. In adult rats the same doses provoke a long lasting but temporaneous block of these fibres (see Holzer, 1991). In both cases nociceptive responses to various noxious stimuli were inhibited or abolished in capsaicin-treated animals. This effect is

presumed to arise from the toxic action of capsaicin and the consequent neurodegeneration of C-fibre nociceptors (Jancsó *et al.*, 1977). However, more recent reports have suggested that dissociation between the neurotoxic effect and other capsaicin actions may be obtained by the use of low doses of capsaicin (Dray *et al.*, 1989). Using electrophysiological recording of ventral root potentials in neonatal spinal cord, it was found that exposure to high concentrations of capsaicin produced a complete loss of responsiveness to capsaicin and to other agents, including bradykinin or noxious heat. In contrast, low doses of capsaicin produced a selective loss of sensitivity to capsaicin, without altering the response to the other agents (Dray *et al.*, 1989).

Capsaicin application to the human nasal mucosa produces a painful sensation and copious secretion of nasal fluid (Geppetti *et al.*, 1988). These effects undergo desensitization after repeated applications, thus offering a suitable model for studying in an intact human tissue

in vivo the action of pain producing agents after desensitization to capsaicin. We have recently observed that the mildly painful response produced by kallidin in the human nasal mucosa was not affected by this type of capsaicin desensitization (Geppetti *et al.*, 1991b). In the present study we have confirmed this finding and we have explored whether the response to other noxious stimuli in the nasal mucosa is affected by capsaicin desensitization. For this purpose we have used acidic and hypertonic solutions, because they are known to evoke nociceptive response in animals (Kumazawa *et al.*, 1987), to be painful in humans (Lindhal, 1961) and they have been proven to activate capsaicin-sensitive sensory nerves (Arvier *et al.*, 1977; Geppetti *et al.*, 1991; Tramontana *et al.*, 1991; Umeno *et al.*, 1990).

Methods

Subjects

Thirty-four healthy volunteers of either sex (21 males and 13 females, 18–52 years of age) were included in the study. Verbal consent was obtained after full explanation of the study methods and purposes. The study was fully approved by the Supervisory Committee of the Institute of Internal Medicine IV of the University of Florence. Before being tested all subjects were examined in order to exclude any disease of the nose or of the tracheo-bronchial tree. This examination was repeated at the end of the study.

Drug preparation

Capsaicin (Sigma, St Louis, MO) was dissolved in 0.9% saline containing 10% paraffin oil and 10% Tween 80 (Geppetti *et al.*, 1988). Kallidin (Peninsula, Belmont, CA) was diluted in 0.9% saline. Hypertonic media were prepared by adding NaCl to 0.9% saline. Acidic media were prepared by adding citric acid to 0.9% saline. The pH of 0.9% saline varied between 5.9 to 6.8.

Experimental procedure and capsaicin desensitization

We used essentially the same procedure reported previously (Geppetti *et al.*, 1991a,b). The effects of kallidin, hypertonic saline and citric acid were assessed in 20 subjects. All the administrations were given between 09.00 h and 11.00 h. The subject head was laid carefully on one side and 50 µl of the required solution were applied by a micropipette into the nostril, approximately 1.5 cm from the external orifice. Then the external surface of the nose was gently massaged for a few seconds. Each individual was instructed to refer the painful sensation assessed in a visual analogue scale (VAS). Preliminary experiments indicated that capsaicin was the most powerful agent among those used. All subjects were given a pre-determined dose of capsaicin (50 nmol, 50 µl) 4 days before beginning the experiments. The intensity of the sensation experienced at that time was considered the maximum value (i.e. 100) on the analogue scale, and all successive responses were scored in relation to this value.

Intensity of the sensation indicated by the volunteer on the analogue scale was recorded by an investigator, who was unaware of the experimental design, at various times (30 s and each min for 10 min) after nasal administration. The maximal response obtained after each application was used for the statistical comparisons.

Desensitization to capsaicin of one nostril, chosen at random, was obtained by applying 50 µl containing 50 nmol of capsaicin once a day for 5–7 consecutive days following the method already reported with slight modification (Geppetti *et al.*, 1988). The contralateral nasal cavity was treated with capsaicin vehicle, and served as control. One day before the beginning of capsaicin desensitization seven subjects received kallidin in both nostrils, seven subjects were given hypertonic saline and seven subjects received citric acid solution. The same procedure was repeated in the same group of subjects 24 h after the last capsaicin administration. Then, the day after that, capsaicin was again applied in both nostrils of each subject.

Statistical analysis

Data in the text and figures are mean $\pm$ s.e. mean. Data of the different doses of the various agents were statistically evaluated by using an analysis of variance (ANOVA). For the statistical comparisons of the experiments on capsaicin desensitization the significance of the difference between two mean values was assessed by using the Student's *t*-test for paired data.

Results

Effect of kallidin, hypertonic saline and acidic media

Application of 0.9% saline containing kallidin (5–500 nmol, 50 µl) produced a mildly painful response that was related to the dose applied. The maximal painful response was attained 1–2 min after drug administration and faded after 5 min (Figure 1). 0.9% saline (50 µl) did not produce any detectable effect. 5–10% saline induced a mild pain. However, 15%, and even more 20% saline produced a marked pain response (Figure 1). The pain response induced by 20% saline was already maximal 30 s after the instillation and persisted for 4–6 min. The application of hypertonic saline (15–20%) was accompanied by secretion of fluid that however was not collected in the present study.

A solution of 0.9% NaCl (50 µl) containing citric acid to a pH as low as 2.5 did not produce any painful sensation. A pH 2 citric acid solution induced a mild effect. Citric acid solution at a pH as low as 1.5 or 1 evoked a marked painful response. The pain was almost immediately maximal and vanished after 5–6 min (Figure 1). Citric acid (pH 1.5–1) also induced secretion of nasal fluid that however was not investigated.

Effect of capsaicin desensitization on the painful response produced by kallidin, hypertonic saline and citric acid

Figure 2 shows the painful response produced by kallidin (50 nmol), hypertonic saline (10%) or citric acid (pH 1.5)

Figure 1 The algogenic response produced by kallidin (upper panel), hypertonic saline (middle panel) and citric acid (lower panel) applied topically to the human nasal mucosa. Each agent was applied diluted in 50 μ l of 0.9% NaCl. Columns are mean $\pm$ s.e. mean of at least 5 experiments. N.D., not detectable.

in both nostrils before monolateral capsaicin desensitization. The response observed in one side was not significantly different from that measured in the contralateral side for each of the agents used. Capsaicin (50 nmol, 50 μ l) produced a remarkable pain response (always considered as 100 in the VAS), sneezing and secretion of a copious flow of nasal fluid. Capsaicin applications were performed every day for 5–7 days until the painful response reported by each subject was reduced by about 80% (20 ± 3 arbitrary units of the VAS, $n = 19$).

The pain responses produced by kallidin (50 nmol), hypertonic NaCl (10%) and citric acid (pH 1.5) administered 24 h after the last capsaicin application are illustrated on the right side of Figure 2. The mildly painful response produced by kallidin in the side treated with capsaicin was not statistically different from the response observed in the vehicle treated nostril. Likewise, we did not find any statistical difference in the pain response to hypertonic saline in the capsaicin-desensitized nostril as compared with the vehicle treated nostril. The response to citric acid was, however, sig-

Figure 2 The algogenic response produced by kallidin (50 nmol, $n = 7$, upper panel), hypertonic saline (10%, $n = 7$, middle panel) and citric acid (pH 1.5, $n = 7$, lower panel) before and after topical capsaicin pretreatment (50 nmol in 50 μ l, every day for 5–7 days). Columns indicate the pain response obtained in nostrils before and after capsaicin vehicle (▨) and before and after capsaicin (■), respectively. *, $P < 0.05$.

nificantly decreased in the side desensitized to capsaicin as compared with the side treated with capsaicin vehicle (Figure 2, lower panel).

Figure 3 illustrates the effect of capsaicin desensitization on the response to doses of kallidin (500 nmol), hypertonic NaCl (20%) and citric acid (pH 1) higher than those shown in Figure 2. Before capsaicin or vehicle treatment the painful responses to the three different stimuli were not significantly different in the two nostrils. After the 5–7 day treatment the painful response to citric acid solution was significantly reduced in the capsaicin-pretreated side, while the responses to hypertonic NaCl or kallidin were not significantly affected by desensitization to capsaicin.

Discussion

Capsaicin application to the human nasal mucosa was found to produce a painful response that is markedly

Figure 3 The algescic response produced by kallidin (500 nmol, $n = 7$, upper panel), hypertonic saline (20%, $n = 7$, middle panel) and citric acid (pH 1, $n = 7$, lower panel) before and after topical capsaicin pretreatment (50 nmol in 50 μ l, every day for 5–7 days). Columns indicate the pain response obtained in nostrils before and after capsaicin vehicle (▨) and before and after capsaicin (■), respectively. *, $P < 0.01$.

attenuated after repeated administrations performed during subsequent days (Geppetti *et al.*, 1988). Treatment of one nostril with capsaicin and the contralateral with its vehicle allowed us to desensitize one side and use the contralateral as its control (Geppetti *et al.*, 1991a). By using this method we observed previously that the mildly painful response to kallidin was not affected by capsaicin desensitization (Geppetti *et al.*, 1991b). In the present study we have confirmed this previous result and we have investigated the effect of capsaicin desensitization on the response produced by other algescic stimuli, such as hypertonic saline and low pH solution. These were chosen, because they are known as nociceptive producing agents in experimental animals (Kumazawa *et al.*, 1987) and algescic stimuli in humans (Lindhal, 1961). Furthermore, both hypertonic saline and acidic media have been found to affect the function of capsaicin-sensitive sensory nerves (Arvier *et al.*, 1977; Geppetti *et al.*, 1991a; Tramontana *et al.*, 1991; Umeno *et al.*, 1990).

Topical application of either hypertonic saline or citric acid solutions applied to the human nasal mucosa produced a concentration related pain response, thus allowing us to use these agents as suitable stimuli for studying their effect before and after topical desensitization to capsaicin. While the effect of hypertonic saline was unaffected by capsaicin desensitization the response produced by low pH solution was markedly reduced by this procedure. Since both 20% hypertonic saline and pH 1 citric acid produced comparable pain responses, the difference between these two agents observed after capsaicin desensitization cannot be ascribed to a difference in the intensity of the response produced by the two different stimuli. The observation that the response to kallidin, that was even less intense than those produced by hypertonic saline or citric acid, was not affected by this type of capsaicin desensitization further supports this contention. Finally, selectivity of capsaicin desensitization in reducing the algescic response to acidic solution in the nasal mucosa is further supported by the observation that capsaicin pretreatment reduced only the response to citric acid and not those to kallidin or hypertonic NaCl even when lower doses of these stimuli were used (Figure 2).

The possibility that hypertonic saline or kallidin may reach mucosal layers deeper than those containing the nerve fibres desensitized to capsaicin seems unlikely especially for kallidin which is a 10 amino acid residue molecule and is rapidly inactivated by various peptidases. Therefore, two hypotheses may account for the present result. It is possible that in the human nasal mucosa hypertonic saline and kallidin mainly act on sensory afferents different from those sensitive to capsaicin and for this reason after functional impairment or even degeneration of these nerve fibres, the ability of these algescic stimuli to produce a pain response is unaffected. Alternatively, it may be hypothesized that application of a small amount of capsaicin to the nasal mucosa in humans may lead to a 'selective desensitization' for capsaicin itself as defined recently (Holzer, 1991). It is also possible that this kind of desensitization involves certain algescic stimuli such as acidic solution.

A great improvement in the knowledge of the molecular mechanism by which capsaicin acts on sensory afferents has been achieved in these recent years. A putative receptor for vanilloid-like molecules has been described (Szallasi & Blumberg, 1990) and a non-selective channel for cations has been shown to be operated by these drugs (Marsh *et al.*, 1987; Wood *et al.*, 1988). It has also been found that protons may act on sensory neurons by a mechanism that at least in part is similar to that activated by capsaicin, e.g. by the opening of a non-selective channel for cations (Bevan & Yeats, 1991). Furthermore, the ability of acidic media to release neuropeptides from capsaicin-sensitive peripheral nerve endings is blocked by the inorganic dye ruthenium red (Geppetti *et al.*, 1991a), which selectively abolishes the effects produced by capsaicin (Amann & Lembeck, 1989; Maggi *et al.*, 1989) by blocking the channel operated by this drug (Dray *et al.*, 1990). In contrast, neuropeptide release evoked by bradykinin (Amann *et al.*, 1989; Geppetti *et al.*, 1990) or hypertonic saline (Del Bianco *et al.*, 1992) is unaffected by ruthenium red.

The observation that a high capsaicin concentration

made the nerve insensitive to various algesic stimuli, while a small concentration of the drug produced selective desensitization to capsaicin itself (Dray *et al.*, 1989), together with the finding that protons share a common pathway with capsaicin to activate capsaicin-sensitive afferents (Bevan & Yeats, 1991; Geppetti *et al.*, 1991b) suggest the hypothesis that capsaicin desensitization of the human nasal mucosa may affect selectively the ability of sensory nerves to be excited by agents that utilize a common mechanism such as capsaicin and hydrogen ions, without affecting the pain response to other algesic stimuli.

The mechanism by which repeated topical applications of capsaicin exert a therapeutic effect in various human

painful diseases (Bernstein *et al.*, 1987; Sicuteri *et al.*, 1990; Watson *et al.*, 1988, 1989) is not known. The present results may have some relevance for supporting the hypothesis that the therapeutic effectiveness of such capsaicin treatments might not be necessarily linked to degeneration of the nerve fibre due to the neurotoxic effect of this drug, but might rely on 'desensitization' of the mechanism (receptor-channel) activated by capsaicin on the nerve terminal.

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