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Capsaicin de-sensitization of the human nasal mucosa reduces pain and vascular effects of lactic acid and hypertonic saline*

Johan Rinder[1,2], Par Stjame[2], Jan M. Lundberg[1]

[1] Department of Pharmacology, Karolinska Institute, Stockholm, Sweden
[2] Department of Otorhioolaryngology, Karolinska Hospital, Stockholm, Sweden

SUMMARY

The present study was initiated to investigate the effects of hypertonic saline (15%) or low pH (1 M lactic acid, pH 2) applied to the human nasal mucosa. Patients suffering from birch-pollen allergy, which had been de-sensitized with capsaicin, were compared to non-treated, healthy controls. Five patients were pre-treated with an intranasal, unilateral application of 30 µM capsaicin for 15 min during three consecutive days. Six weeks later we applied 50 µl of hypertonic saline (15%) to the inferior turbinate on the capsaicin-pre-treated side of the patients as well as to the controls. Symptom score, using a visual analogue scale (VAS), and the cross-sectional area of the nasal cavity were measured bilaterally using acoustic rhinometry at different intervals. The same procedure was repeated one week later with lactic acid. Provocation with lactic acid and hypertonic saline caused a significantly higher symptom score in controls as compared to capsaicin-pre-treated patients. Furthermore, application of lactic acid caused a significant reduction in cross-sectional area of the nasal cavity suggesting vasodilatation in controls compared to capsaicin-pre-treated patients. The reactions to hypertonic saline were generally lower but the differences in symptom score between capsaicin-pre-treated and non-treated persons remained. The results implies that capsaicin-sensitive afferents are involved in low pH- and hypertonicity-mediated reactions in the human nasal mucosa. Furthermore, local capsaicin de-sensitization causes a very long-lasting loss of sensoly reactivity to these agents.

Key words: capsaicin-sensitive afferents, nasal mucosa, lactic acid, hypertonic saline, allergic rhinitis

INTRODUCTION

In experimental animals capsaicin de-sensitization of the airway mucosa reduces the protective reflexes and vascular effects of certain irritants, such as cigarette smoke and ether, while the response to mechanical stimulation remains (Lundberg and Saria 1983; Lundblad et al., 1983). Furthermore, the protein extravasation response to low pH solutions (Martling and Lundberg, 1988) or hypertonic saline (Umeno et al., 1990) is dependent on stimulation of capsaicin-sensitive nerves. Recent data have shown that ruthenium red, which is a transmembrane calcium-flux inhibitor, significantly reduces both citric acid-induced coughing (Lou et al., 1991), nasal irritation and low pH-induced bronchoconstriction in the guinea pig. Capsazepine, a specific competitive antagonist to capsaicin (Bevan et al., 1992) has also been found to inhibit low pH-evoked bronchoconstriction and local sensory neuropeptide release (Lou and Lundberg, 1992). Lactic acid accumulation is a characteristic of anaerobic metabolism both during inflammation and ischaemia and capsazepine inhibits calcitonin gene-related peptide (CGRP) release by lactic acid in the guinea-pig heart (Franco-Cereceda and Lundberg, 1992). Patients with vasomotor rhinitis have an enhanced reflexogr.mic secretory response to the irritant capsaicin (Stjarne et al.,. 1989). Local capsaicin pre-treatment has, therefore, recently been used to de-sensitize the human nasal mucosa resulting in long-lasting improvement of symptoms of congestion and rhinorrhoea in patients with vasomotor rhinitis (Stjarne et al., 1991; Lacroix et al., 1991) as well as in cluster headache (Sicuteri et al., 1990). Presumably, like in experimental animals (cf., Lundblad, 1984) capsaicin treatment in human nasal mucosa results in functional impairment of C-fibre

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afferents as revealed by the reduced tissue content of CGRP (Lacroix et al., 1992). Interestingly, it was reported that the mildly painful response produced by locally applied kallidin in the human nasal mucosa was not influenced by capsaicin desensitization suggesting a selective action (Gepetti et al., 1991). It was, therefore, of interest to study in humans whether the sensation and possible vascular effects of intranasally applied lactic acid and hypertonic saline were affected by local capsaicin treatment.

METHODS

Patients with allergic rhinitis (n=5) were randomly picked from the Karolinska Hospital (mean age: 26 years; range 24-32; male/female ratio: 1/4). They all suffered from sneezing and rhinorrhoea and/or nasal congestion during birch-pollen season and had positive skin prick test for birch *(Betula verrucosa)* pollen allergens. The study was performed in the pollen-free season, and all patients had been free from any medication at least four weeks prior to the study. Nor did anyone smoke or suffer from any on-going infection. Healthy subjects (n=5) with no history of smoking or atopy were selected as controls (mean age: 31 years; range 23-39; male/female ratio: 4/1). The patients gave their informed consent and the study was approved by the Local Ethics Committee at the Karolinska Hospital. Prior to our study the allergic patients were pre-treated with local application of capsaicin (Serva; Heidelberg, Germany) in the right nostril. Capsaicin was initially dissolved in 70% ethanol and further diluted in 0.9% saline (Stjiirne et al., 1991). Cotton strips was soaked in naphazoline chloride (0.02 mg/ml) mixed with 3.4% lignocaine chloride. The strips were packed into the nasal cavity and left there for 15 min. A new set of cotton strips was soaked in capsaicin (30×10^{-6} M) and then applied to the inferior turbinate at its full length, during 15 min, in order to establish de-sensitization of C-fibre afferents. The procedure was repeated during three consecutive days. Although well anaesthetized, the patients experienced an immediate moderate burning and painful sensation in the treated cavity combined with tear secretion and hyperaemia in the ipsilateral conjunctiva. Furthermore, we noticed a profuse secretion from the nose, mainly from the treated side but also, to some extent, on the contralateral side. The initial response decreased within a few minutes after application and was virtually absent 30 min after removal of the cotton strip. Finally, we noticed a general reduction of these initial symptoms to capsaicin over the three consecutive treatment days. Six weeks later we applied 50 µl of hypertonic (i.e., 15%) saline with a pipette onto the foremost one-third of the inferior turbinate in the right nostril of the pretreated patients as well as to the controls. The cross-sectional area of the nasal cavity at 3.3 cm from the vestibulum (further on referred to as CA 3.3) was measured, on both sides, before and at 30 s, 2, 5, 15, and 30 min after application of drugs, using acoustic rhinometry (G-J Elektronik and Finmekanik, Aarhus, Denmark; cf., Hilberg et al., 1989; Lenders and Pirsig, 1990; Grymer et al., 1991). Each measurement with the acoustic rhinometer consisted in itself of five consecutive signals at 1-s intervals. The mean value of the CA 3.3 was automatically calculated and used for statistical analysis. Furthermore, nasal symptom score was measured, for both nostrils, using a visual analogue scale (VAS). The VAS is a self-reporting device extensively used to measure subjective phenomena such as patient symptoms (eg., pain, nausea). It belongs to a group of measurement and scaling techniques known as "graphic methods" (Freyd, 1923). The scale is composed of a 100-mm long horizontal line with a perpendicular line at each end, ranging from 0 mm (no pain) to 100 mm (worst imaginable pain). There are no gradations since this has been shown to reduce sensitivity (Gift, 1989). The patients are asked to enter a slash mark somewhere along the line to indicate the strength of the pain experience. Responses are scored measuring the distance from the left-most point to the subject's mark across the line. The method has been found to be reliable and valid in several studies (Miller, 1993) and is considered a useful tool when measuring pain response. After measurements mean values were calculated and used for statistical analyses.

The same procedure was performed one week later with application of lactic acid. Lactic acid was dissolved in saline at a concentration of 1 M, giving a pH of 2. Statistical analyses were performed using Mann-Whitney calculations for non-parametric values.

RESULTS

All capsaicin-pre-treated patients and controls were initially challenged with hypertonic saline. The average symptom score and percentual change in CA 3.3, for the right nostril, are shown in Figures 1a and 2a, respectively. The symptom score, according to VAS for the controls was maximal after 30 s and then declined with time. As shown in Figure 1a the symptom score for the capsaicin-pre-treated group, upon hypertonic saline, was significantly lower at 30 s to 5 min after application. The capsaicin-pre-treated patients did in fact not react significantly as compared to baseline. Whereas in controls there was an initial reduction in CA 3.3, suggesting nasal congestion, no such response was observed in capsaicin-pre-treated patients (Figure 2a). Application of lactic acid in controls caused largely similar time course in symptom score (VAS) as for hypertonic saline, although a slightly larger response to lactic acid was seen (Figure 3a). The symptom score for the capsaicin-pre-treated group between 30 s and 5 min after application of lactic acid was significantly lower as compared to controls (Figure 1b). Lactic acid caused a more prolonged congestive activity, in controls than hypertonic saline as revealed by reduction in CA 3.3 (Figure 3b). Furthermore, a significant ($p < 0.05$) difference for CA 3.3 between the controls and capsaicin-pre-treated groups was present at 30 s after lactic acid application (Figure 2b). Overall, the results with lactic acid indicated a long-lasting reduction in CA 3.3 in the control group, which was more prolonged than the symptom score according to VAS.

Furthermore, there was an early increase in CA 3.3, after lactic acid application followed by a final decrease in the capsaicin-pre-treated group. Finally, when comparing VAS to hypertonic saline and lactic acid in the group of capsaicin-pre-treated patients, we found no significant difference in symptom score

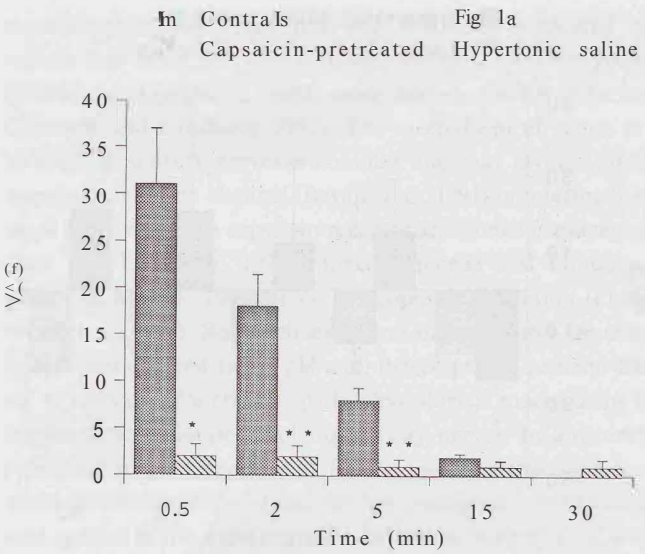

Figure 1a. Average symptom score on a visual analogue scale (VAS) (mean±SEM) for the right nostril after application of hypertonic saline (15%) in controls or six weeks after local capsaicin pre-treatment. *: p <0.05; **: p <0.01.

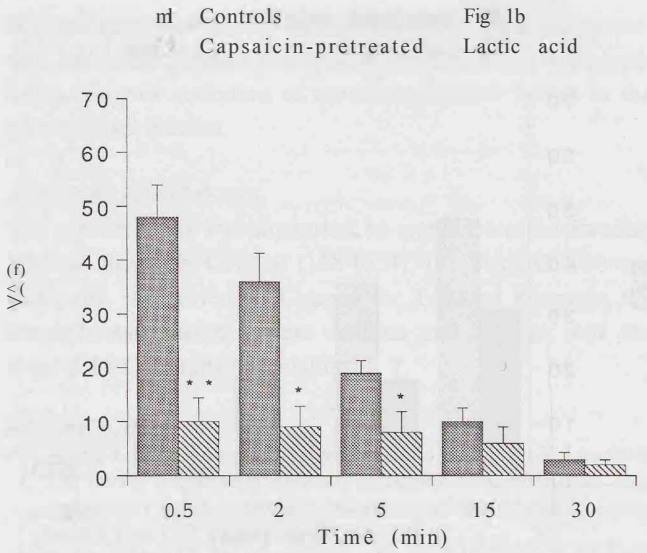

Figure 1b. Average symptom score on a visual analogue scale (VAS) (mean±SEM) for the right nostril after application oflactic acid (1 M) in controls compared to six weeks after capsaicin pre-treatment.*: p <0.05; **: p <0.01.

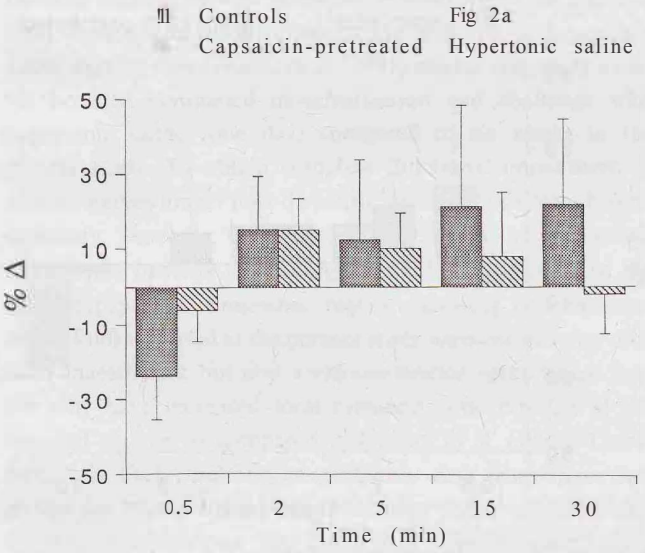

Figure 2a. Average change (mean±SEM) in cross-sectional area of the nasal cavity 3.3 .:m from the vestibulum (CA 3.3) in the right nostril after application of hypertonic saline (15%) in controls compared to six weeks after capsaicin pre-treatment.

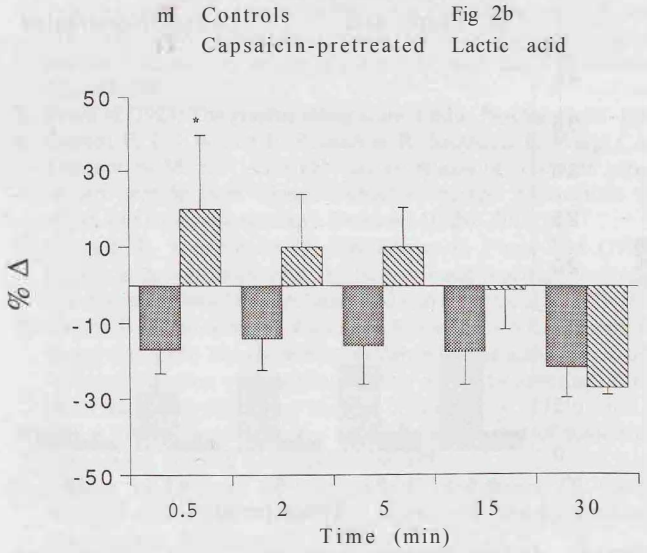

Figure 2b. Average change (mean±SEM) in CA 3.3 in the right nostril after application oflactic acid (1 M) in controls and capsaicin-pre-treated patients. *: p <0.05.

between the two drugs, although there was an indication for higher symptom score with lactic acid (Figure 4a). There was no difference between the agents in the CA 3.3 response except for lactic acid at the 30-min level (p <0.05; cf. Figure 4b). There were neither symptoms nor changes in CA 3.3 in the left nostril for controls or patients after application of hypertonic saline or lactic acid in the right nostril (data not shown).

DISCUSSION

The present data show that locally-applied lactic acid and hypertonic saline cause transient pain sensation in the human nasal mucosa. Furthermore, lactic acid especially evokes nasal congestion probably due to dilatation of venous sinusoids, an effect which lasted longer than the subjective pain response as revealed by reduced cross-sectional area of the nasal cavity. Interestingly, the pain response to both lactic acid and hypertonic saline was virtually absent six weeks after local capsaicin de-sensitization. At a similar time interval the secretory and pain responses to capsaicin were also absent in capsaicin-pretreated patients with vasomotor rhinitis (Stjarne et al., 1991) suggesting a long-lasting effect using this de-sensitization procedure. It may be suggested that some of the capsaicin-, low pH- and hyperosmolar effects are due to other non-specific actions on residing cells in the nasal mucosa such as mast cells with subsequent release of cell mediators. Furthermore, it has to be taken into consideration that low pH, hyperosmolar drugs and capsaicin may damage epithelial cells, vessel walls, secretory glands and autonomic nerve endings in the human nasal muco-

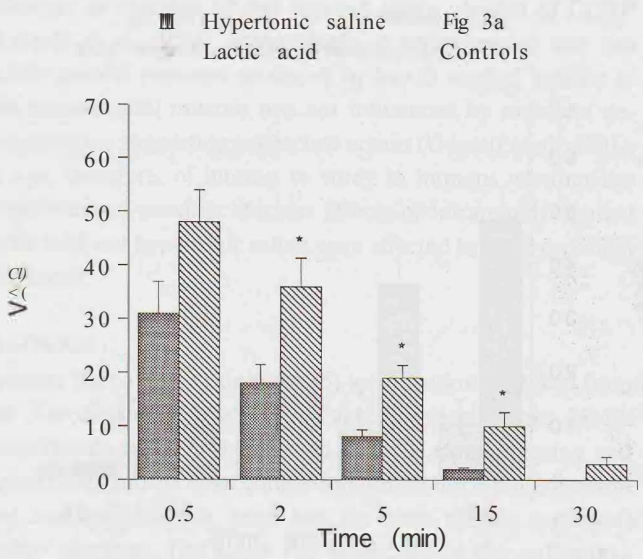

Figure 3a. Average symptom score on a visual analogue scale (VAS) (mean±SEM) for controls after application of hypertonic saline (15%) and lactic acid (IM). *: $p < 0.05$.

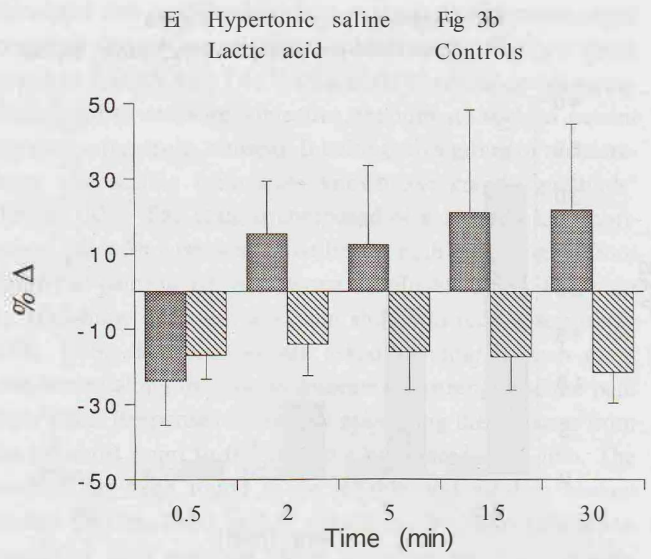

Figure 3b. Average change (mean±SEM) in CA 3.3, right nostril, in controls after application ofhypertonic saline (15%) and lactic acid (1 M).

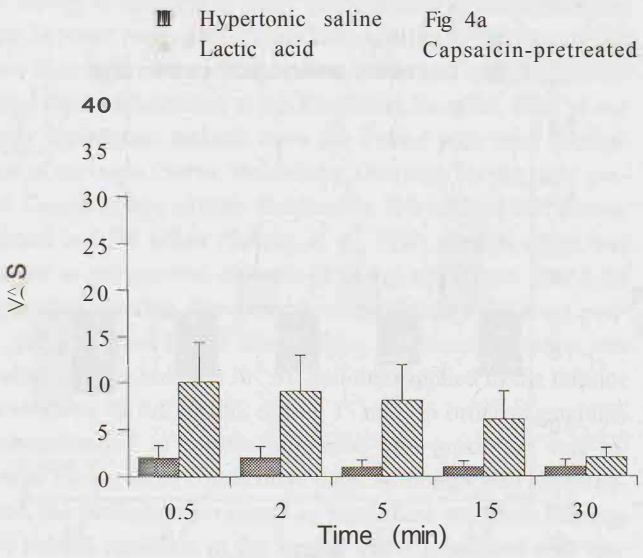

Figure 4a. Average symptom score on a visual analogue scale (mean±SEM) in capsaicin-pre-treated patients after application of hypertonic saline (15%) and lactic acid (1 M), in the right nostril.

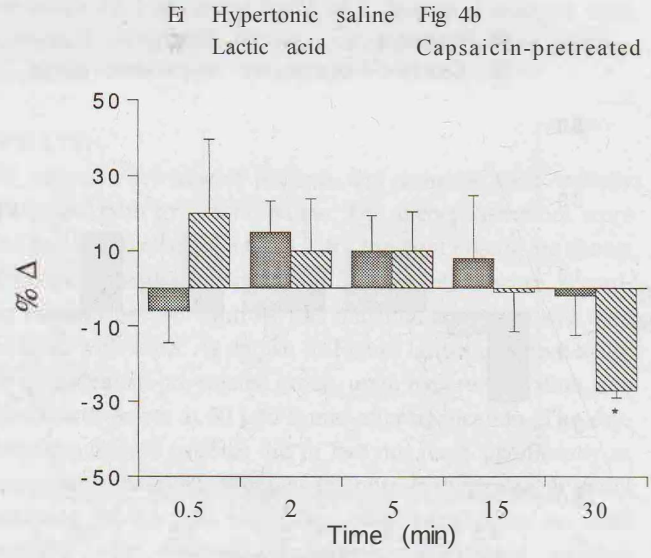

Figure 4b. Average change (mean±SEM) in CA 3.3, in capsaicin-pre-treated patients after application of hypertonic saline (15%) and lactic acid (1 M) in the right nostril. *: $p < 0.05$.

sa. Capsaicin is a well known and experimentally commonly-used substance and yet no reports on significant non-specific, non-neural effects in short-term experiments have been reported. In studies by Lundblad et al. (1983) and Alving et al. (1991) it has been shown that capsaicin pre-treatment of pigs or guinea pigs abolished all CGRP/SP-containing sensory nerve fibres in the airway epithelium, whereas there were no changes in the number or distribution of parasympathetic and sympathetic nerves, tissue mast cells, granulocytes or lymphocytes. Nor was there any change in the number of blood leukocytes. Furthermore, the finding of a specific capsaicin-sensitive vanilloid receptor on sensory nerve endings (Szallasi et al., 1990) suggests a specific action of capsaicin. Tramontana et al., (1991) has shown that hypertonic saline causes CGRP-LI release in rat urinary bladder, which is dependent on extracellular Ca^{2+} and sensitive to *in vitro* capsaicin pre-treatment. Capsaicin pre-treatment depleted the CGRP-LI content which strongly indicates that CGRP is entirely contained in capsaicin-sensitive sensory neurons. Nevertheless, K.rayenbuhl et al. (1989) found increased levels of histamine in nasal lavages of patients with allergic rhinitis after local, intranasal application of hypertonic saline, suggesting activation of mast cells, but whether this finding represents a direct effect on mast cells or a secondary response to sensory neuropeptide release is unclear. Acidic media may damage tissues and extract peptides. However, studies on rat urinary bladder *in vitro* (Gepetti et al., 1990) have revealed that low pH activates sensory nerve endings with subsequent release of CGRP-LI and, furthermore, that this neuropeptide release is completely abolished after pre-exposure to capsaicin. These results therefore suggest a specific action on

capsaicin-sensitive nerves and this is further supported by reports that lactic acid activates afferents of the C-fibre group (Stahl and Longhurst, 1992) and release CGRP (Franco-Cereceda and Lundberg, 1992). The mechanism of action for protons on sensory nerves is complex and may involve direct opening of a cation channel (Bevan et al., 1991) or intermediate steps sensitive to the capsaicin-receptor antagonist capsazepine (Lou and Lundberg, 1992; Franco-Cereceda and Lundberg, 1992; Satoh et al., 1992) or cyclo-oxygenase inhibition (Longhurst et al., 1991). Both ischaemia and inflammation are associated with reduced tissue pH and, therefore, the present data are of relevance for possible pathophysiological mechanisms in human airway disease involving sensory nerves. In a recently published study, Gepetti et al. (1993) described that local capsaicin de-sensitization reduced the VAS symptom score to citric acid applied in the nasal mucosa, and this is in agreement with the present findings using lactic acid. However, these authors did not find any reduction of the VAS response to hypertonic saline, contrasting the clear-cut results in the present study and earlier data using experimental animals (Umeno et al., 1990). Possible explanations for this difference regarding the effect of local capsaicin de-sensitization on the response to hypertonic saline may be that Gepetti et al. (1993) used a very short interval between completed de-sensitization and challenge with hypertonic saline (one day) compared to six weeks in the present study. To obtain complete functional impairment of sensory nerves longer post-de-sensitization periods may thus be necessary. Secondly, Gepetti et al. (1993) used normal individuals while patients with allergic rhinitis were studied in the present paper. A somewhat higher capsaicin concentration (three-fold) was used in the present study together with not only local anaesthetics, but also a vasoconstrictor agent which further may have increased local capsaicin concentration at the mucosa! surface, as compared to Gepetti et al. (1993). Therefore, it is likely that the contradictory data from these two groups can be explained by the efficiency of the respective capsaicin de-sensitizations. The initial pain response upon locally applied agents onto the nasal mucosa is likely to be associated with sympatho-adrenal activation, which may influence nasal vascular control (cf. Lundblad, 1984). In the present study the duration for the nasal congestive effect as revealed by reduced cross-sectional area of the nasal cavity was much longer than the intense pain sensation to lactic acid. This finding may imply that lactic acid initiated some additional effects which caused prolonged activation of parasympathetic mechanisms (Stjarne et al., 1989). In contrast, the vascular effect of hypertonic saline was very brief, suggesting different mechanisms of action. The increased cross-sectional area of the nasal cavity upon lactic acid application in capsaicin-pre-treated patients may be due to a slight remaining sympatho-adrenal activation caused by the pain sensation. In controls, however, the powerful parasympathetic reflexes evoked by lactic acid seems to counteract and mask such a reaction. The delayed congestive effect of lactic acid after 30 min in the capsaicin-pre-treated group also indicates other actions of lactic acid.

In conclusion, the present data show that both the pain sensation and initial vascular response to lactic acid and hypertonic saline involves activation of capsaicin-sensitive nerves in the human nasal mucosa.

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Dr. J. Rinder
Dept. of Pharmacology
Karolinska Institute
S-10401 Stockholm
Sweden