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Main Articles

Effects of capsaicin pre-treatment in experimentally-induced secretory otitis media

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Abstract

Neurogenic inflammation may play a role in the aetiology of secretory otitis media (SOM). The strongest candidate that initiates the characteristic symptoms of neurogenic inflammation is supposed to be substance P. Capsaicin is a specific antagonist of substance P. The effects of capsaicin on middle ear mucosa have not been studied yet. In an attempt to investigate the effect of pre-treatment with capsaicin on the development of SOM an experimental study was performed. Fourteen Wistar rats were divided into two groups. Seven rats were pre-treated with capsaicin (Group 1) and the others were administered isotonic saline solution (Group 2). Seven days after the third injection rats were operated on and the right tympanal orifice of the Eustachian tube was obstructed. Animals were sacrificed seven days after the operation. Their bullas were excised bilaterally and were studied by light microscopic technique. In Group 1 there was no effusion except for one case. The subepithelial layer was thickened by fibroblast proliferation. Capillary proliferation and some glandular atrophy were observed. In Group 2 the middle ear lumens were filled with effusion. Oedema with dilatation in capillaries and medium-sized vessels of lamina propria was observed as a common feature of the group. Subepithelial fibrosis was found in one case. Capsaicin pre-treatment prevented the formation of effusion in the middle ear lumen in spite of tuba occlusion. The results of this preliminary study lead us to consider that an imbalance in the autonomic innervation of the mucosa of the middle ear may play a role in the aetiology of SOM as in vasomotor rhinitis, and capsaicin may be an alternative in the treatment.

Key words: Substance P; Capsaicin, Ear, middle; Otitis media, serous; Aetiology

Introduction

Stimulation of sensory nerves causes two major reactions of inflammation: vasodilatation and increase in vascular permeability. This response is called neurogenic inflammation (Hellström and Goldie, 1991). It has been reported that neurogenic inflammation may play a role in the aetiology of secretory otitis media (SOM) in addition to Eustachian tube obstruction, infectious agents and immunological factors (Widemar *et al.*, 1985; Goldie and Hellström, 1988; Goldie *et al.*, 1989; Hellström and Goldie, 1991). In a previous study performed on rats, it has been observed that middle-ear fluid is produced by only animals with an intact vagal nerve (Goldie and Hellström, 1988). Based on these findings it has been suggested that transmitters and neuropeptides of the vagal nerve such as substance P, acetyl choline, vasoactive intestinal polypeptide, encephalin, play a role in the appearance of the inflammatory changes in the middle-ear mucosa (Goldie *et al.*, 1989; Hellström and Goldie, 1991).

Neuropeptides are considered increasingly to be important neurotransmitters in the central and peripheral nervous system. Previous studies have demonstrated that peptidergic neurons contribute to the autonomic innervation of the upper respiratory tract (Uddman *et al.*, 1983; Widemar *et al.*, 1995; Oyagi *et al.*, 1987; Ito *et al.*, 1993; Wolf *et al.*, 1995). Peptidergic neurons probably play a key role in the innervation of blood vessels and glands in the upper respiratory tract (Uddman *et al.*, 1985; Ito *et al.*, 1993). The presence of neuropeptide substance P, vasoactive intestinal polypeptide, calcitonin gene-related peptide and neuropeptide Y in the human respiratory tract have previously been demonstrated (Uddman *et al.*, 1983; Uddman *et al.*, 1984). Non-specific mechanical, thermal and chemical irritations cause release of these neuropeptides from blood vessels, glands, smooth muscles and epithelium (Wolf *et al.*, 1995).

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The strongest candidate for initiating the characteristic symptoms of neurogenic inflammation is thought to be substance P, first isolated by von Euler and Gaddum in 1931 (Goldie *et al.*, 1989; Györfi *et al.*, 1993; Wolf *et al.*, 1995). Capsaicin (trans-8-methyl-N-vanillyl-6-noneamide), which is a specific antagonist of substance P, is the hot spicy substance found in red peppers (Györfi *et al.*, 1993; Wolf *et al.*, 1995). Capsaicin releases substance P and causes neural depolarization by stimulation of a specific neural cation channel in its first use, whereas its extended use produces degeneration in peptidergic C fibres. Repeated capsaicin applications are assumed to cause desensitization of the nasal mucosa and a decreased secretory response. This effect of capsaicin is important in the treatment of hyper-reactive changes of the nasal mucosa as it has been stated that C fibres are affected selectively and thus other parts of mucosa remained intact (Wolf *et al.*, 1995).

The presence of substance P in the middle-ear mucosa and its effects on it have been investigated previously (Uddman *et al.*, 1983; Uddman *et al.*, 1984; Goldie *et al.*, 1989). The effects of capsaicin, which is an antagonist of substance P, on nasal and sinus mucosa have also been investigated before, but, have not been studied on the mucosa of middle ear yet. This experimental study investigated the effect of pre-treatment with capsaicin on the development of SOM.

Materials and methods

Fourteen healthy, male Wistar rats weighing 180–250 g. were used. Specific pathogen free rats were three months old. The animals were divided into two groups: Group 1 – were pre-treated with capsaicin. Seven rats were given 20, 30 and 40 mg/kg capsaicin (10 per cent ethanol, 0.9 per cent NaCl, 10 per cent Tween) sub-cutaneously (totally 90 mg/kg) on three successive days. Group 2 formed the controls. Seven rats were given an isotonic saline solution of the same volume on three successive days. Seven days after the third injection animals were anaesthetized with intra-peritoneal injections of ketamine (90 mg/kg, Ketalar, Parke-Davis) and xylazine (10 mg/kg, Rompun, Bayer) and were operated. The right tympanic bulla was found via retroauricular incision. A small hole was drilled in the floor of the tympanic bulla and through this hole a guttapercha plug (De Trey, Switzerland) was inserted hermetically into the tympanal orifice of the Eustachian tube. After surgery the animals were kept under normal laboratory conditions until they were sacrificed and their bullas were excised bilaterally on the seventh day.

Bullas which were put into EDTA solution (10 per cent formalin, 5.5 per cent EDTA) for decalcification for six weeks were dehydrated in graded alcohols and cleared and blocked in paraffin. Sections of 5–7 mm thickness were taken from the paraffin blocks and examined under the light microscope after staining with haematoxylin-eosin.

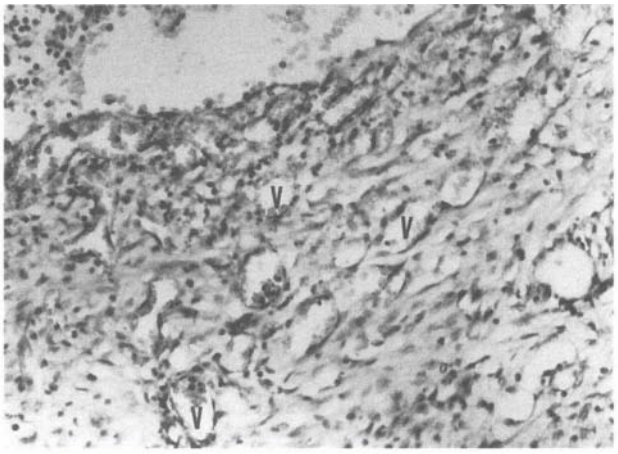

FIG. 1
Group 1. Dense fibrosis and capillary proliferation within submucosa (v = vessels) (H & E; × 220).

Results

Group 1: Pretreated with capsaicin. No effusion was observed except in one animal. Cell collections in the right middle ear lumens of animals were composed of a small number of neutrophil leucocytes, macrophages and erythrocytes. Cell collections were also found in the left ear lumen in two cases. The subepithelial layer was thickened by fibroblast proliferation. This increase reached four to five fold of normal thickness in some areas. Capillary proliferation (Figure 1), some scattered plasma cells and in one case haemosiderin accumulation were also observed within the same area. Atrophy and necrobiotic changes were found in the glands. Pycnosis and cystic dilatations (Figure 2) were observed in the epithelium of the glands of two cases.

Group 2: Controls. Effusion was observed in the middle-ear lumens of all animals which was unilateral in three and bilateral in four cases (Figure 3). The middle-ear lumen was filled by dense cell collections that consisted of neutrophil leucocytes

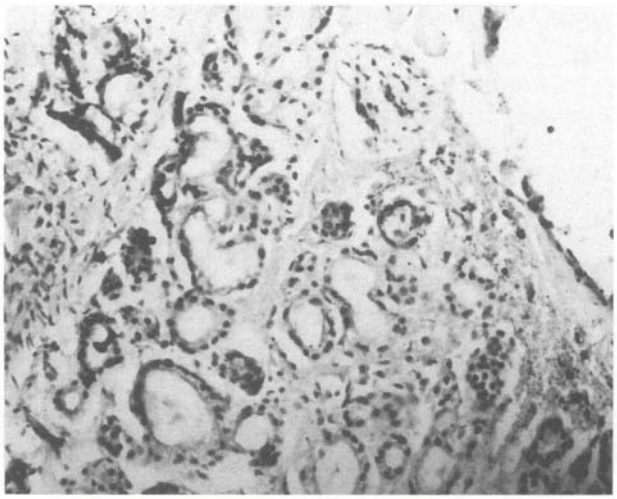

FIG. 2
Group 1. Degeneration within serous glands (H & E; × 220).

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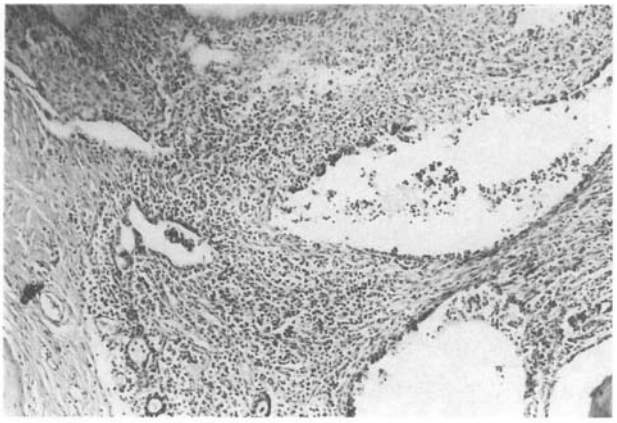

FIG. 3
Group 2. Acute inflammatory granulation tissue (H & E; × 70).

some of which had undergone degeneration, macrophages, erythrocytes and eosinophils. In two animals goblet cell hyperplasia was detected in the tympanal orifice of the Eustachian tube (Figure 4). Oedema with dilatation in capillaries and medium-sized veins of lamina propria was observed as a common feature of the group (Figure 5). The majority of intensive mononuclear cell infiltration in the subepithelial layer was produced by plasmacytes (Figure 6). Subepithelial fibrosis was determined in one case.

Discussion

Because the structure of the rat mucosa shows striking similarities to that of humans, the rat is known to be the most suitable model for experimental research on the morphological characteristics of tympanic mucosa (Albiin *et al.*, 1986). By choosing the rat as the experimental animal we aimed to study the effects of capsaicin on the development of SOM in humans.

Our study revealed macrophage infiltration of the middle-ear mucosa and subepithelial vasodilatation, oedema and eosinophilic effusion in the bulla, on the

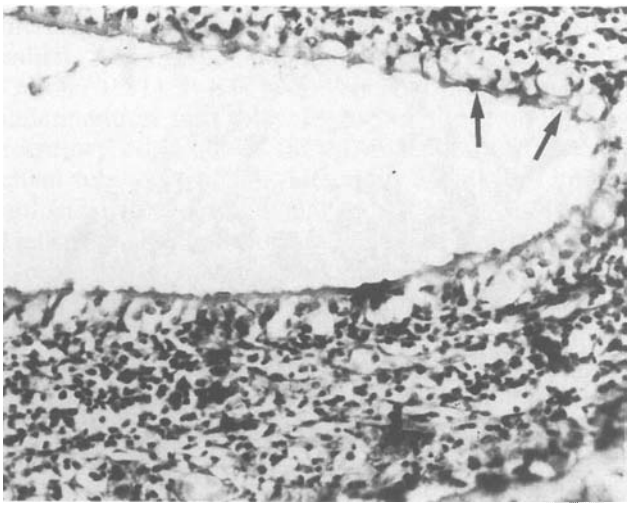

FIG. 4
Group 2. Hyperplasia of goblet cells at the tympanal orifice of Eustachian tube (arrows) (H & E; × 220).

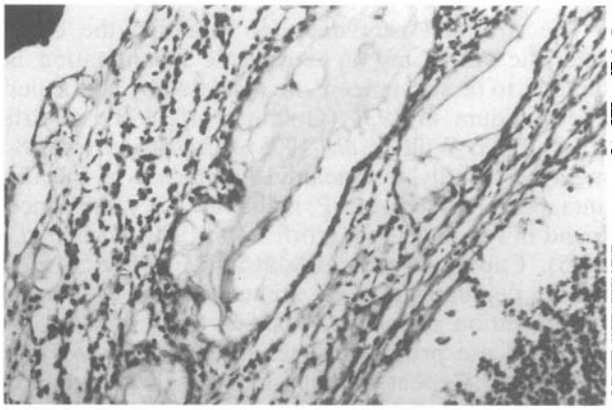

FIG. 5
Group 2. Oedema and vascular dilatation within submucosa (H & E; × 220).

occluded side in the control group. This agrees well with other investigations in rats (Kuijpers *et al.*, 1979; Tos, 1981). On the other hand, transformation of squamous epithelium into the cuboidal epithelium demonstrated in the other studies was not seen in the present study (Kuijpers *et al.*, 1979; Tos, 1981). Tos (1981) has reported his findings of transformation of the originally simple squamous epithelium into a pseudostratified epithelium, polypoid development and hyperplasia of the basal cells within the third week of tubal obstruction. In this study, as the rats were sacrificed at the end of the first week of tubal obstruction, the subsequent events could not be evaluated.

Middle ear effusions observed in the control group animals have also been demonstrated by other investigators and it has been suggested that this is because of an increased permeability in various regions of the middle ear. The major route for increasing vessel permeability is the opening of interendothelial junctions (Hellström *et al.*, 1982; Udmann *et al.*, 1985; Widemar *et al.*, 1985; Goldie *et al.*, 1989; Goldie and Hellström, 1990). It has been reported that vasoactive neuropeptides and particularly substance P, allow gaps the transport of fluid into the middle ear cavity by forming of interendothelial gaps (Hellström *et al.*, 1982; Goldie and

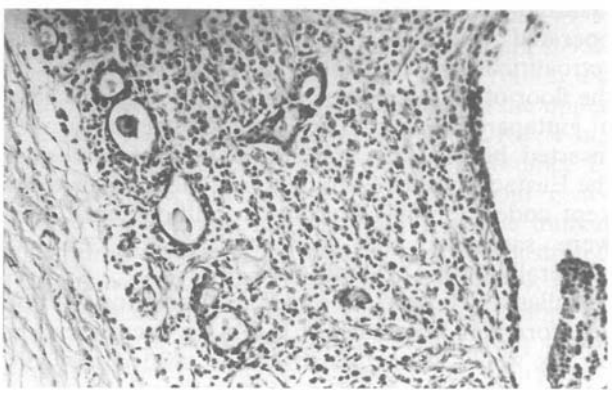

FIG. 6
Group 2. Mononuclear cell infiltration mainly with plasmacytes in submocosa. (H & E; × 230).

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Hellström, 1988; Goldie and Hellström, 1990). In the present study no significant difference between the two groups in respect of vasodilatation was found whereas the presence of effusion in only one case of the group pre-treated with capsaicin supports the views of the authors suggesting that capsaicin can prevent the increase in vascular permeability by decreasing the opening of the interendothelial junctions (Hellström *et al.*, 1982; Györfi *et al.*, 1993; Norlander *et al.*, 1996).

Kuijpers *et al.* (1979) have reported that they could not detect any change in goblet cells during the first week after tubal obstruction. Tos (1981) has reported that it is difficult to determine the goblet cells even in the sections of series 2–4 weeks after tubal obstruction. In this study, however, we found hyperplastic goblet cells at the entrance of the tympanal orifice of the Eustachian tube. In a study of the effects of topical capsaicin treatment on sinus mucosa, Norlander *et al.* (1996) have found degeneration and atrophy in the serous glands of the sinus mucosa. We observed similar changes of the serous glands in the middle ear mucosa of rats pre-treated with capsaicin.

Conclusion

In conclusion, capsaicin pre-treatment prevents the accumulation of fluid in the middle-ear lumen in spite of tubal obstruction. The results of this preliminary study lead us to consider that an imbalance in the mucosal autonomic innervation of the middle-ear may have an important contribution to the aetiology of SOM as in vasomotor rhinitis and capsaicin may be an alternative for the treatment of SOM. Immunohistochemical staining will provide more valuable evidence on the role of neuropeptides and capsaicin, a substance P antagonist. Further studies are necessary to clarify this issue.

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