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AUTONOMIC MECHANISMS UNDERLYING CAPSAICIN INDUCED ORAL SENSATIONS AND SALIVATION IN MAN

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SUMMARY

1. The effects of capsaicin, citric acid and nicotine applied to the apex or radix of the tongue on taste sensations and salivation were studied in relation to the presence of substance P immunoreactive neurones in man.

2. Application of capsaicin (30 μm) to the apex of the tongue or to the palatinal mucosa, but not to the radix of the tongue, caused a reproducible burning sensation and salivation from the submandibular–sublingual and parotid glands. The salivation response to capsaicin was reduced by methylscopolamine pretreatment.

3. Similar levels of substance P immunoreactivity were present in the lingual apex and radix area (including vallate papillae) of man, while in the cat about 4 times higher levels of substance P immunoreactivity were present in the vallate papillae than in the lingual apex. Immunohistochemistry showed that in the cat many substance P immunoreactive nerves were associated with the taste buds of the vallate papillae, while in man substance P immunoreactive fibres were only seen penetrating into the epithelium of the lingual apex. In addition some subepithelial blood vessels in all regions were surrounded by substance P immunoreactive nerves in both cat and man.

4. Citric acid application to the tongue apex caused both submandibular–sublingual and parotid salivary secretion concomitant with a burning sensation. Salivary secretion was also seen after citric acid application to the radix of the tongue. This response was associated with a sour taste. The salivation response to citric acid was not significantly reduced by methylscopolamine pretreatment.

5. Lingual apex application of nicotine was associated with a sweet taste and a small rise in salivary secretion rate. This response was not significantly reduced by methylscopolamine.

6. In conclusion, the sensitivity to capsaicin of the human tongue is restricted to the apex portion. This is in parallel with the occurrence of intraepithelial substance P immunoreactive nerve fibres. Capsaicin induced salivary secretion seems mainly to be mediated via parasympathetic, cholinergic reflex mechanisms. Citric acid and nicotine induced salivation responses are comparatively more resistant to methylscopolamine pretreatment.

INTRODUCTION

Intake of spiced food containing hot peppers is associated with an oral burning sensation in addition to facial sweat secretion (Lee, 1954). Much interest in sensory physiology has recently been focused on capsaicin, the pungent principle of hot peppers (Fitzgerald, 1983). Capsaicin is considered to rather selectively activate chemosensitive C fibre afferents, some of which contain the peptide substance P. Repeated administration or exposure to a high dose of capsaicin induces functional desensitization of these chemosensitive afferents and a subsequent depletion of substance P-immunoreactivity (Jessell, Iversen & Cuello, 1978). In high local or systemic doses, capsaicin causes a degeneration of peripheral branches of sensory neurones (Hoyes & Barber, 1981; Papka, Furness, Della, Murphy & Costa, 1984). It is also well known that repeated exposure to spiced food causes a desensitization of this burning sensation. Szolcsányi (1977) reported that local capsaicin desensitization of the human tongue did not impair taste sensations to stimulants such as quinine sulphate, NaCl, glucose, ascorbic acid and menthol, and suggested that capsaicin-sensitive nerves represent a population of neurones which are associated with taste buds but are separate from primary taste neurones. A high density of substance P immunoreactive nerves has been observed in the vallate papillae of the cat (Lundberg, Hökfelt, Änggård, Pernow & Emson, 1979) and in various taste bud regions of the rat tongue (Nagy, Goedert, Hunt & Bond, 1982). Ultrastructural analysis has revealed that the substance P immunoreactive nerves in rat vallate papillae do not innervate taste cells directly (Yamasaki, Kubota, Takagi & Tohyama, 1984). Capsaicin pretreatment has been found to induce a loss of substance P immunoreactivity in the tongue epithelium of the rat without seemingly affecting the integrity of taste buds which occurs after degeneration of taste afferents (Nagy *et al.* 1982).

Nicotine is another principle known to activate capsaicin sensitive afferents. Thus, it has been shown that nicotine increases protein extravasation in the trachea via stimulation of capsaicin-sensitive nerves (Lundberg, Saria, & Martling, 1983). Since capsaicin and nicotine both activate chemosensitive afferents, we were interested in finding out how these substances affect taste afferents in man in relation to the occurrence of substance P immunoreactive neurones. Furthermore, we have studied the salivary secretion rate and the composition of the saliva after exposure to these agents, with and without pretreatment with the anticholinergic agent methylscopolamine. For a comparison, the effects of citric acid, a classical stimulant of salivary secretion, were also investigated.

METHODS

Healthy volunteers (aged 17–53, fourteen females and thirteen males) who were non-smokers and not on drug therapy were tested. The study was approved by the regional Ethics Committee (Dnr. 84:07).

The subjects were seated in a quiet environment approximately 10 min before the experiment. One preweighed cotton swab ($4{\cdot}5 \times 3{\cdot}5 \times 0{\cdot}5$ cm: $0{\cdot}670 \pm 0{\cdot}009$ g) was placed in each buccal cavity close to the parotid duct entrance. Another preweighed cotton swab ($7{\cdot}0 \times 2{\cdot}5 \times 0{\cdot}5$ cm: $1{\cdot}323 \pm 0{\cdot}031$ g) was simultaneously placed under the tongue. These three swabs sampled parotid

and submandibular–sublingual saliva, respectively. The swabs were taken out after 7½ min. The procedure was immediately repeated and the results from the two collection periods were taken as a measure of resting salivary secretion. A third set of cotton swabs was then placed in the mouth and 50 μl of capsaicin (6 or 30 μM), citric acid (0·48 mM) or nicotine (62 mM), were applied on the apex or radix linguae. Stimulated saliva was then collected during one five-minute period or during three consecutive five-minute periods. On three of the subjects the effects of capsaicin on apex linguae were tested with two repeated applications. The second capsaicin application was performed 15 min after the first and saliva was again collected during three five-minute periods as previously described. The effects on salivary secretion rate, 30 min after an injection of methylscopolamine (0·15 mg) in the submucosa of the lower jaw vestibulum, following lingual apex application of capsaicin, citric acid or nicotine were tested on four individuals each. A rise in heart-rate (28 ± 4 beats/min) and a feeling of dryness in the mouth was observed 30 min following administration of this anticholinergic agent. Methylscopolamine was chosen since this drug should have a preferentially peripheral antimuscarinic effect (Weiner, 1980).

After each experiment, the cotton swabs were weighed on a Mettler AC 100 balance and, when possible, saliva was pressed out for subsequent analysis of amylase and potassium content. Amylase content was measured as described by Dahlqvist (1962). The potassium content in the saliva was analysed by atomic absorption using a Zeiss Atomic Absorption spectrometer.

Samples, for radioimmunoassay and immunohistochemistry, from the apex and radix (including the vallate papillae) regions of the human tongue were obtained at autopsy within 20 h after death. The patients (aged 36–53) were non-smokers and had not reported any symptoms from the oral cavity, nor were they on anticholinergic drug therapy. Tissues were also taken from four cats under Nembutal (40 mg/kg) anaesthesia. Prior to radioimmunoassay, the superficial layers of the lingual apex, vallate papillae and soft palate (1–2 mm thickness) were dissected and weighed, frozen and extracted in boiling acetic acid. Radioimmunoassay substance P-immunoreactivity was then performed as described by Gamse, Wax, Zigmond & Leeman (1981). Alternatively, tissue pieces were fixed by immersion in a formalin–parabenzoquinone mixture and processed for immunohistochemistry as described by Lundberg, Hökfelt, Änggård, Terenius, Elde, Markey & Goldstein (1982) using a monoclonal substance P antiserum (Cuello, Galfree & Milstein, 1982).

Results are expressed as means ± S.E. of mean. Statistical analysis was performed using the Wilcoxon test for paired differences, Mann–Whitney U test and modified t test according to the Bonferroni method.

Drugs were obtained from the following sources: capsaicin (Merck), citric acid (Merck), methylscopolamine (Pharmacia), nicotine-tartrate (Swedish Tobacco Company).

RESULTS

Capsaicin, 30 μM, applied on the lingual apex caused a burning sensation and a significant transient rise in salivary secretion from both parotid and submandibular–sublingual glands. The increase in the rate of secretion was more marked from the latter glands (Fig. 1*A*). The effect was reproducible, since in three subjects the first dose of capsaicin induced an average maximal secretion of 2·67 g/min and the second a secretion of 2·36 g/min. A five times lower concentration of capsaicin produced a smaller increase in salivary secretion rate, indicating that the response was dose dependent (results not shown). Methylscopolamine pretreatment markedly reduced the basal (by 70–80%) as well as the capsaicin induced stimulation of salivary secretion (Fig. 2). Capsaicin, applied on the lingual radix did not cause any burning or taste sensation nor a significant rise in salivary secretion rate from any of the glands (Fig. 1*A*). Some of the subjects experienced a burning sensation from the palatinal mucosa, however, indicating that the capsaicin solution was not kept on the tongue only.

The amylase content of the saliva was significantly increased following capsaicin

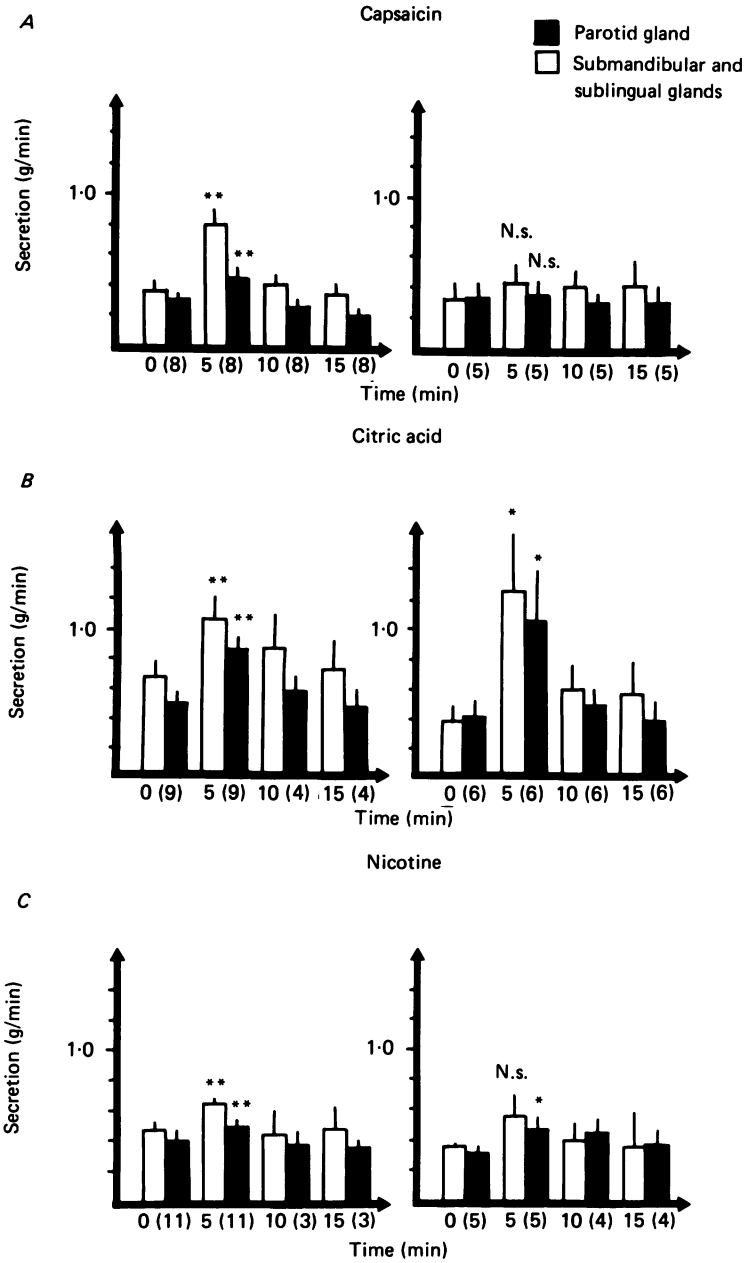

Fig. 1. Salivary secretion rate (means ± S.E. of mean) at various intervals following application of 30 μM-capsaicin, 0·48 mM-citric acid and 30 mM-nicotine on the apex (left) or radix (right) of the human tongue. 0 min = basal secretion. The number of individuals are given within brackets. Statistical significance was calculated according to Wilcoxon's test for paired differences. *, $P < 0.05$; **, $P < 0.01$; n.s., not significant salivation from control period.

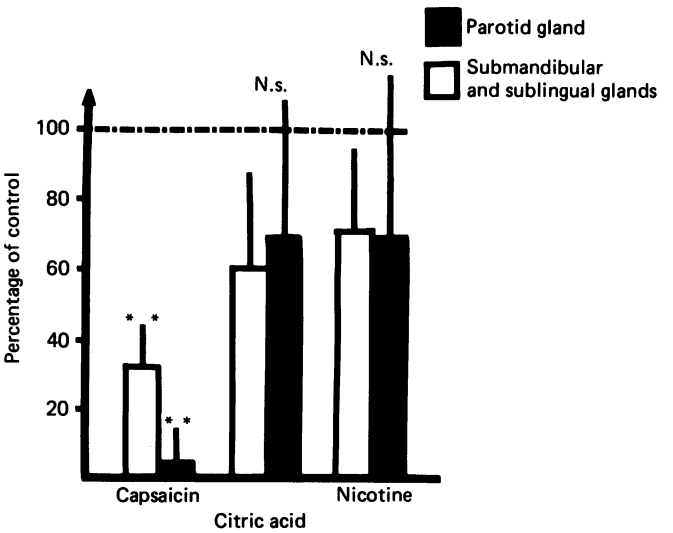

Fig. 2. Salivary secretion rate following 30 μM-capsaicin, 0·48 mM-citric acid or 62 mM-nicotine applied on the lingual apex, 30 min after methylscopolamine pretreatment. Values are given as means ± S.E. of mean and have been calculated as a percentage of the responses in untreated individuals. Statistical significance was calculated according to the Mann–Whitney U test. **, $P < 0{\cdot}01$, n.s. = not significant from salivation response in untreated individuals.

application on the lingual apex ($P < 0{\cdot}05$), but not following application on the lingual radix. The mean increase in amylase activity per minute in the submandibular–sublingual saliva was 241 %, while the corresponding rise in the parotid saliva was 58 % (Fig. 3). The potassium concentration in the saliva showed a slight, but statistically insignificant, decrease following capsaicin application to the lingual apex.

Citric acid (0·48 mM) stimulated salivary secretion both from submandibular–sublingual and parotid glands, when applied either on the lingual apex or on the radix (Fig. 1*B*). However, the sensations were different. Application of citric acid to the apex of the tongue caused a burning sensation similar to that of capsaicin. Application to the radix, in contrast, elicited a distinct sour taste. Pretreatment with methylscopolamine did not significantly reduce the citric acid response (Fig. 2). The mean increase in amylase content following citric acid application was 94 % in the submandibular–sublingual glands and 124 % in the parotid glands (Fig. 3).

Nicotine (62 mM) applied to the lingual apex induced a small rise in salivary secretion rate concomitant with a sweet flavour (Fig. 1*C*). Pretreatment with methylscopolamine did not significantly reduce the nicotine induced rise in salivation (Fig. 2). Lingual radix application of nicotine caused salivation and was associated with a very unpleasant taste especially from the pharynx, again indicating that it was difficult to keep the substance on the tongue only. Nicotine did not cause any change in amylase activity (Fig. 3).

There was a difference in the ability of the three different substances to evoke salivation in the parotid gland *versus* the submandibular–sublingual glands. Thus, nicotine and capsaicin had moderate effects on the parotid gland secretion, whereas

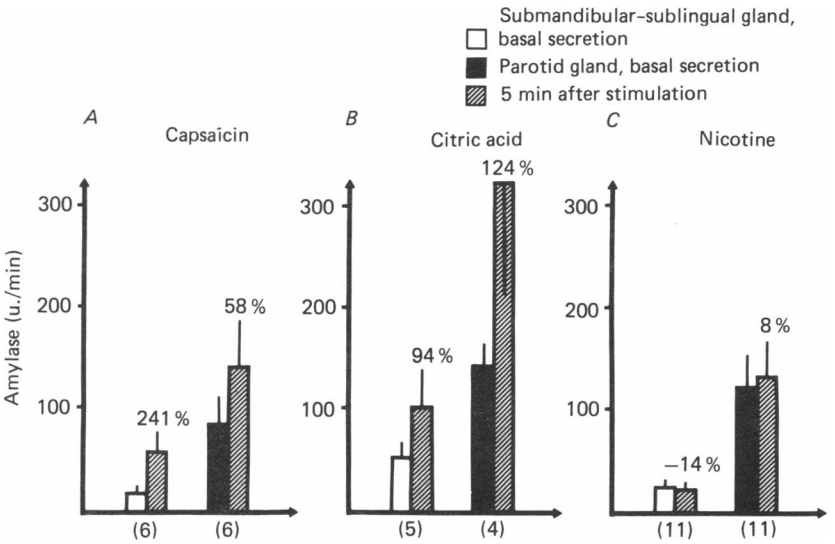

Fig. 3. Amylase secretion expressed as u./min following 30 μM-capsaicin. 0·48 mM-citric acid and 62 mM-nicotine applied on the lingual apex. The number of individuals have been indicated within brackets. Values have been expressed as a percentage of the basal secretion and given as means ± S.E. of mean.

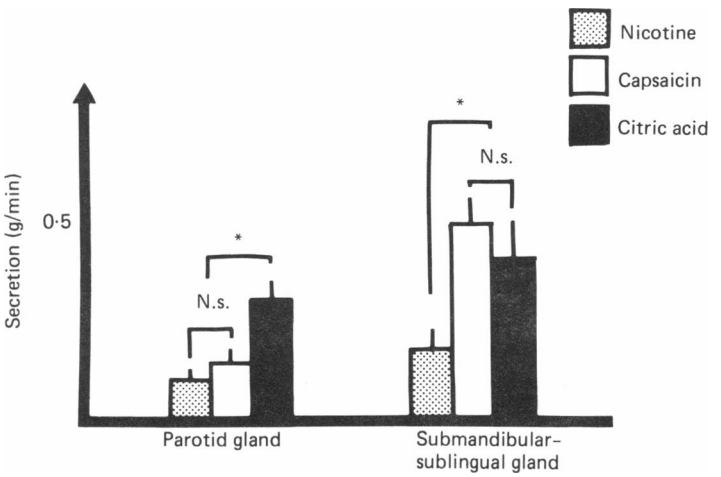

Fig. 4. Salivary secretion rate (means ± S.E. of mean) above basal salivation following 30 μM-capsaicin, 0·48 mM-citric acid or 62 mM-nicotine applied on the lingual apex. Statistical significance was tested according to the Bonferri method of modified t test. *, $P < 0·05$; n.s. = not statistically different.

citric acid induced a large parotid salivation. In the submandibular–sublingual glands, citric acid and capsaicin were equally active while nicotine gave a significantly lower response (Fig. 4).

The levels of substance P immunoreactivity in man were similar in the apex and radix regions of the tongue (including the vallate papillae) and the soft palate (Table 1). In the cat, about 4 times higher levels of substance P immunoreactivity

were observed in the vallate papillae area compared to the apex region (Table 1). Immunohistochemical analysis revealed that thin, weakly fluorescent varicose substance P immunoreactive nerve fibres were seen close to and partly within the lining epithelium of the tongue apex, while no substance P immunoreactive nerves were observed in the vicinity of the epithelial taste buds of the vallate papillae in man. In both regions substance P immunoreactive nerves were found close to subepithelial blood vessels. In the cat, many substance P immunoreactive nerves were found within the vallate papillae and some fibres also within the epithelium of the lingual apex (not shown).

TABLE 1. Content of substance P immunoreactivity (pmol/g) in the tongue apex and vallate papillae of man and cat as well as soft palate of man. Values are given as means ±S.E. of mean with the number of individuals within parenthesis

	Substance P immunoreactivity (pmol/g)		
	Tongue apex	Vallate papillae	Soft palate
Man	1·27±0·25 (5)	1·49±0·37 (5)	0·85±0·14 (5)
Cat	5 1±0·6 (4)	20·3±0·4 (4)	

DISCUSSION

Capsaicin produced a burning sensation when applied to the apex but not when applied to the radix of the human tongue. The ability to elicit this sensation was parallelled by the presence of intraepithelial substance P immunoreactive nerves. In contrast to cat and rat which have many substance P immunoreactive nerves in the region of the vallate papillae (Lundberg *et al.* 1979; Nagy *et al.* 1982; Yamasaki *et al.* 1984) no such fibres were observed within the vallate papillae of man. It should be emphasized that intraepithelial substance P immunoreactive nerves were only weakly fluorescent in the apex of the human tongue. Furthermore, the human tongue epithelium showed a considerable background fluorescence. For these reasons the contrast was not sufficient to allow photography for publication. Therefore these negative immunohistochemical findings of substance P immunoreactivity in the epithelium of the vallate papillae region should be interpreted with caution. Especially since tongue specimens were obtained on post-mortem tissue. Two other findings, however, support the immunohistochemical observations. First, in the human tongue similar levels of substance P immunoreactivity, as revealed by radioimmunoassay, were obtained in the apex and radix regions whereas in the cat, where many substance P immunoreactive nerves were identified in the vallate papillae epithelium, 4 times higher levels of substance P immunoreactivity were present in the radix compared to the apex of the tongue. This suggests that in man most of the substance P immunoreactivity revealed by radioimmunoassay was present in vascular substance P neurones which were equally abundant in the apex and vallate papillae regions. Secondly, there was no burning sensation or reflex salivary secretion upon capsaicin application to the radix area, providing a functional correlate to the absence of capsaicin-sensitive, substance P immunoreactive nerves in the epithelium of the human vallate papillae.

Capsaicin-sensitive nerves in the apex of the human tongue seem to be different

from traditional taste fibres since capsaicin desensitization (performed with much higher concentrations than those used in the present study) did not impair taste sensations to other stimuli such as quinine sulphate, NaCl, glucose, ascorbic acid and menthol (Szolcsányi, 1977). Capsaicin sensitive nerves in man may constitute a defence system which is activated when the apex of the tongue comes in contact with hot peppers in spiced food. It would be of interest to know whether persons that are daily exposed to spiced food, and who are more 'resistant' to hot spices, have degenerated substance P immunoreactive neurones.

The salivation seen upon capsaicin exposure seems to mainly derive from the submandiular–sublingual glands. Methylscopolamine pretreatment significantly reduced the salivary response to capsaicin, which suggests that capsaicin induced salivation was reflexogenic and to a large extent mediated via postganglionic cholinergic neurones, since this muscarinic antagonist should have a preferentially peripheral action (Weiner, 1980). The present observations on salivation are in line with findings on lacrimal secretion (Jancsó, Jancsó-Gábor & Szolcsányi, 1968) as well as bronchial secretion upon capsaicin exposure which have cholinergic effector pathways in experimental animals (Davis, Roberts, Coleridge & Coleridge, 1982). The salivary secretion elicited upon capsaicin exposure may dilute the pungent principles in spices and thereby reduce the oral irritation.

Citric acid applied to the apex of the tongue caused salivation both from the submandibular–sublingual and from the parotid glands. This response was associated with a burning sensation similar to that seen upon capsaicin exposure. Citric acid application on the vallate papillae, however, induced a sour taste from the tongue, which was also associated with salivation from both types of salivary glands. The citric acid-induced salivation was reduced by 30–40 % by methylscopolamine in the dose used, but the variation among individuals was considerable and the reduction was not statistically significant. In contrast, the capsaicin induced salivation, which was of a similar magnitude in the submandibular–sublingual glands, was significantly reduced by methylscopolamine. It should be emphasized that methylscopolamine is a competitive muscarinic receptor antagonist and that the presently used dose probably does not induce a complete cholinergic blockade. Mózsik, Jávor, Dobi, Petrássy & Szabó (1967) found that the citric acid induced salivation was not completely blocked following a single atropine dose of 0·9 mg orally or 1·0 mg parenterally. Furthermore, unilateral section of the chorda tympani in man did not entirely abolish the salivation response, from the submandibular gland of the operated side, induced by lemon juice applied on the tongue (Diamant, Enfors & Holmstedt, 1958) or dropped in the mouth (Laage-Hellman, Strömblad & Charles, 1960). This suggests that non-cholinergic pathways are of importance for citric acid induced salivary secretion. Babkin (1950) and Emmelin (1967, 1981) have pointed out that the sympathetic pathways probably play an important role in the autonomic control of salivation. Gjörstrup (1980) has later provided evidence that citric acid causes a reflex activation of sympathetic nerves when given orally to rabbits.

Nicotine induced a sweet taste when applied to the apex linguae, while an application to the radix evoked an unpleasant taste especially from the pharynx. The nictoine induced salivation appeared to be partly resistant to methylscopolamine. In contrast to capsaicin and citric acid, nicotine did not increase the amylase content

of the saliva. This is probably not due to a lower degree of salivary secretion since capsaicin and nicotine produced a similar parotid secretion response. Since activation of sympathetic fibres is characterized by a particularly rich amylase content of the saliva (Gjörstrup, 1980), it is possible that the noncholinergic pathways activated by nicotine stimulation are different from adrenergic neurones.

In conclusion, capsaicin excites chemosensitive afferents in the apex of the human tongue, a region which also contains epithelial substance P immunoreactive nerves. This initiates a reflexogenic salivation primarily by parasympathetic mechanisms. The pattern of taste sensations and salivary secretion in man following application of compounds which activate lingual sensory neurones differs not only between the agents used, but also between different sites of application.

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