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Capsaicin Modifies Responses of Rat Chorda Tympani Nerve Fibers to NaCl

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Abstract

Single-fiber preparations of the rat chorda tympani (CT) nerve were used to study the mechanism of action of capsaicin on salt-taste transduction. Capsaicin selectively suppressed the responses to NaCl of the CT nerve fibers (N-fibers) that are sodium-specific (insensitive or poorly sensitive to potassium). Among the more broadly responsive, cation-sensitive fibers (E-fibers) there are two subtypes, both of which responded to capsaicin but in different ways ('enhanced' type and 'suppressed' type). In both N- and E-fibers, 5% ethanol (the vehicle for capsaicin) slightly reduced the response to 100 mM NaCl. The suppressive effect of capsaicin on the response of the N-type fibers to 100 mM NaCl was significantly stronger than the effect of 5% ethanol. The suppression lasted for at least 20 s after the simultaneous application of 100 p.p.m. capsaicin–100 mM NaCl. These results indicate that 100 p.p.m. capsaicin can modify the response of CT fibers to NaCl. The observed effect of capsaicin on gustatory fibers could be the net result of opposite suppressive and enhancing processes in the taste buds cells and excited intra- or extragemmal trigeminal nerve endings. **Chem. Senses 22: 249–255, 1997.**

Introduction

According to previous reports, some substances can modify salt-taste sensation. For example, amiloride, a diuretic drug, has been shown to inhibit the taste response to NaCl without affecting the responses to KCl in the dog and in the rodents (Heck *et al.*, 1984; Brand *et al.*, 1985; Hill and Bour, 1985; Ninomiya and Funakoshi, 1988; Hettinger and Frank, 1990). On the basis of these reports, it appears likely that amiloride inhibits nerve responses by rapidly and reversibly inhibiting sodium-specific channels. Moreover, the antibiotic, novobiocin (Hinman *et al.*, 1957), appears to activate only amiloride-sensitive sodium transport (Rick *et al.*, 1988). In agreement with this result, novobiocin has been found to enhance the response to 100 mM NaCl (Feigin *et al.*, 1994) of the sodium-specific N-fiber found in the chorda tympani (CT) (Ninomiya *et al.*, 1984, 1987; Ninomiya and Funakoshi, 1988; Frank *et al.*, 1988).

Capsaicin, a pungent compound derived from the red pepper, is in widespread use as a tool to study the role of a

certain subpopulation of thinly myelinated or unmyelinated sensory neurons in irritation. In human studies, application of capsaicin can desensitize the tongue to subsequent exposure to capsaicin. However, Green (1991) demonstrated, while confirming that repeated application of capsaicin can indeed sensitize the human tongue to subsequent exposure to the same drug, that if stimulation were interrupted for a period of minutes, the opposite phenomenon— desensitization—would occur. Karrer and Bartoshuk (1991) demonstrated that the application of 10–100 p.p.m. capsaicin was sufficient to diminish the response to subsequently applied capsaicin for several days. They had earlier observed that capsaicin decreased the taste intensity evoked by NaCl, sucrose, citric acid and quinine (Karrer and Bartoshuk, 1990).

On the other hand, Cowart (1987) found only a very limited effect of capsaicin (1–2 p.p.m.) on taste sensation induced by solutions of NaCl, citric acid and an NaCl–citric acid mixture. This is consistent with the finding of Prescott *et al.* (1993) that an application of capsaicin (2–8 p.p.m.) to the human tongue decreased the intensity of sweetness but had little or no effect on the sensation of saltiness. From the results reported in these papers, it seemed unlikely that a low concentration of capsaicin would modify the CT nerve response to NaCl. However, much higher concentrations of capsaicin are encountered in everyday life. For example, it is known that a concentration of 140 p.p.m. (428 μM) is often ingested by rural Thai people (Kawada *et al.*, 1986). In addition, the concentration of capsaicin in tabasco sauce, a popular seasoning in Western cuisine, can reach 400 p.p.m. (Karrer and Bartoshuk. 1991). Such high doses might well have effects not seen with low doses. Indeed, capsaicin inhibits sodium, potassium and other cation channels at high concentrations (30–300 μM = 9.2–92 p.p.m.) in a broad range of cell types (James *et al.*, 1993). This is presumably the mechanism by which capsaicin blocks conduction when it is applied directly to axons (Holzer, 1991).

In this study we tried to clarify the effect of a high concentration of capsaicin (100 p.p.m.) on salt taste by measuring its effect in the response of single-fiber preparations of the rat CT nerve.

Materials and methods

Animals, maintenance and surgery

Adult female Sprague–Dawley rats weighing 270–320 g were housed in stainless-steel cages and had free access to a commercial rat diet (F-2; Funabashi Farms Co., Funabashi, Japan) and to tap water.

In preparation for recording from the CT nerve, each rat was deeply anesthetized with an initial dose of sodium pentobarbital (50 mg/kg) and urethane (150 mg/kg), with supplemental doses throughout the experiment as needed. Following tracheotomy, the rat was positioned on its right side with the head held stationary by a non-traumatic head-holder. Surgical exposure of the CT nerve was achieved via an incision in the cheek below the level of the zygomatic arch, overlying muscles being dissected away to expose the mandible. After disarticulation of the temporo-mandibular joint, the condyloid and coronoid mandibular processes and the zygomatic arch were removed with rongeurs. A group of nerves can then be seen beneath the pterygoid muscles, the smallest of which is the CT nerve.

Neural recording

The CT nerve was cut as close to the maxilla as possible. Following the removal of the perineural sheath, the nerve was teased apart and individual bundles placed on a Pt–Ir electrode under fluorohydrocarbon oil. Extracellular action potentials were recorded differentially with one electrode placed at the edge of the wound.

Single units were discriminated and counted digitally off-line. The activity of single neurons was also counted in some pauci-fiber bundles using spike-discrimination software (Unit-Discrimination Program, β-version) devised by Dr Hitoshi Suzuki. When the responses of several kinds of fibers were recorded simultaneously, the software permitted discrimination between the action potentials on the basis of waveform. Nerve impulse rates were determined for 27 fibers isolated from 12 animals. Responses are expressed in term of the total number of action potentials in the first 10 s following the onset of stimulation.

Stimuli

Deionized water and analytical grade chemicals were used to make stimulus solutions. Capsaicin was purchased from Sigma Chemical Company (St Louis, MO). Rinse (deionized water) at 28°C was allowed to flow over the anterior part of the tongue. Stimulus solutions were delivered by stopping this background flow and injecting 8 ml of stimulus solution onto the tongue through the flow system, with a stimulus application duration of 6 s. The stimuli used for pauci-fiber preparations were 100 mM NaCl aqueous solution, 100 mM NaCl in 5% ethanol

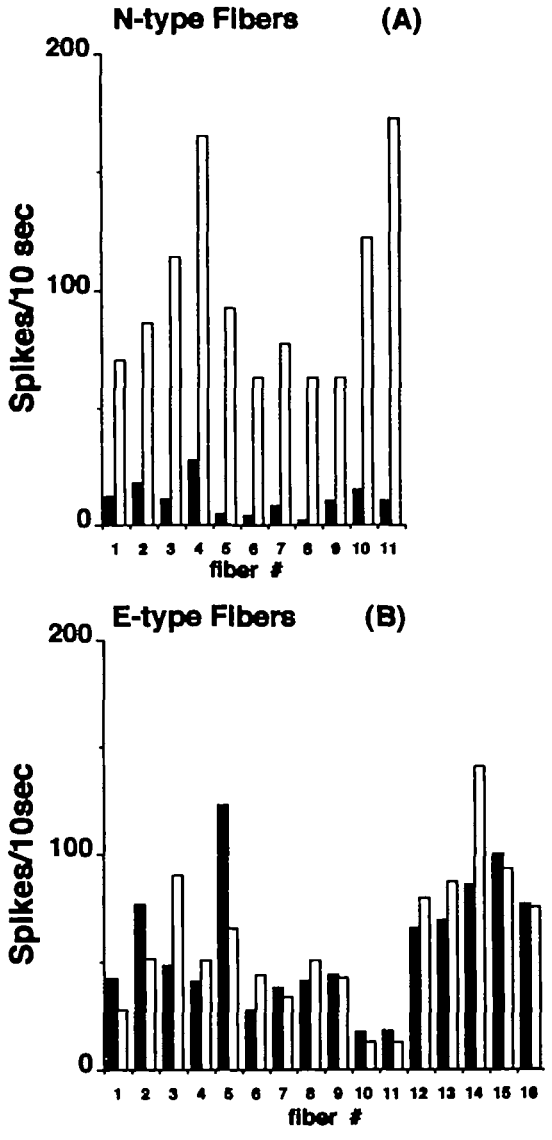

Figure 1 Responses of N- and E-fibers to 100 mM NaCl and to 100 mM KCl, expressed as the number of spikes in the first 10 s for each fiber. Filled columns: responses of fibers to 100 mM KCl before any application of capsaicin. Open columns: responses of fibers to 100 mM NaCl before application of capsaicin.

solution, 100 mM NaCl–100 p.p.m. capsaicin in 5% ethanol solution and 100 mM KCl aqueous solution. Once a particular fiber had been characterized as either an E- or N-fiber, the effect of capsaicin on the response characteristics of that fiber to NaCl was assessed.

Results

Classification of N- and E-fibers

The initial responses of each fiber to 100 mM NaCl and 100 mM KCl are presented in Figure 1A, B. In this experiment, 11 fibers (in seven animals) were classified as N-type and 16 fibers (in eight animals) as E-type (a total of 12 animals were used). Figure 2 indicates the two subpopulations of taste fibers. Capsaicin selectively suppressed the responses to NaCl of the CT nerve fibers (N-fibers) that are sodium-specific (insensitive or poorly sensitive to potassium). Among the more broadly responsive, cation-sensitive fibers (E-fibers), there are two subtypes, both of which responded to capsaicin but in different ways ('enhanced' type and 'suppressed' type). In the N-fibers, the average response to 100 mM KCl was only 8.9% of that to 100 mM NaCl. In contrast, the average response of E-fibers to 100 mM KCl was 95.1% of that to 100 mM NaCl.

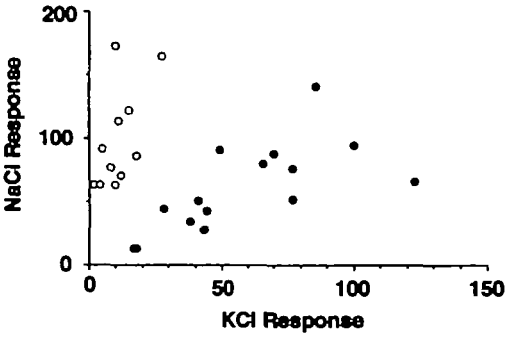

Figure 2 Plot of single neural response to NaCl (100 mM) as a function of the response to KCl (100 mM). Axes represent the number of spikes/10 s. N-fibers are plotted as open circles and E-fibers as filled circles.

Effect of ethanol on the response of N- and E-fibers to NaCl

The response of both N- and E-fibers to 100 mM NaCl aquatic solution was smaller when it was applied in 5% ethanol solution, the solvent used for capsaicin (Figure 3A, N-fibers: $P = 0.021$, Wilcoxon test; and Figure 3B, E-fibers: $P = 0.016$, Wilcoxon test).

Effect of capsaicin on the response of N- and E-type fibers to NaCl

The responses of N- and E-type fibers to the first application of 100 mM NaCl–5% ethanol solution and to 100 mM NaCl–100 p.p.m. capsaicin are presented in Figure 4A, B. Capsaicin significantly suppressed the response to 100 mM NaCl of all (11/11) N-type fibers ($P = 0.003$, Wilcoxon test, Figure 4A). The suppression varied in magnitude in individual fibers: with capsaicin, the response was between 79.1 and 21.0% of the control response. This

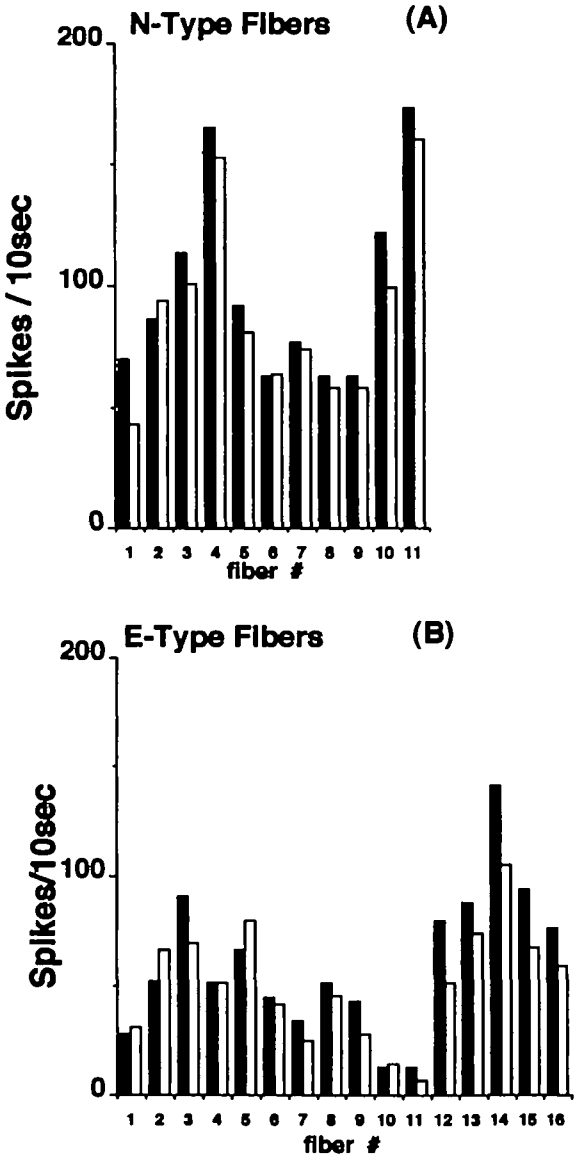

Figure 3 Responses of N- and E-fibers to 100 mM NaCl and to 100 mM NaCl in 5% ethanol solution, expressed as the number of spikes in the first 10 s for each fiber. Filled columns: responses of fibers to 100 mM NaCl aqueous solution. Open columns: responses of fibers to 100 mM NaCl in 5% ethanol solution.

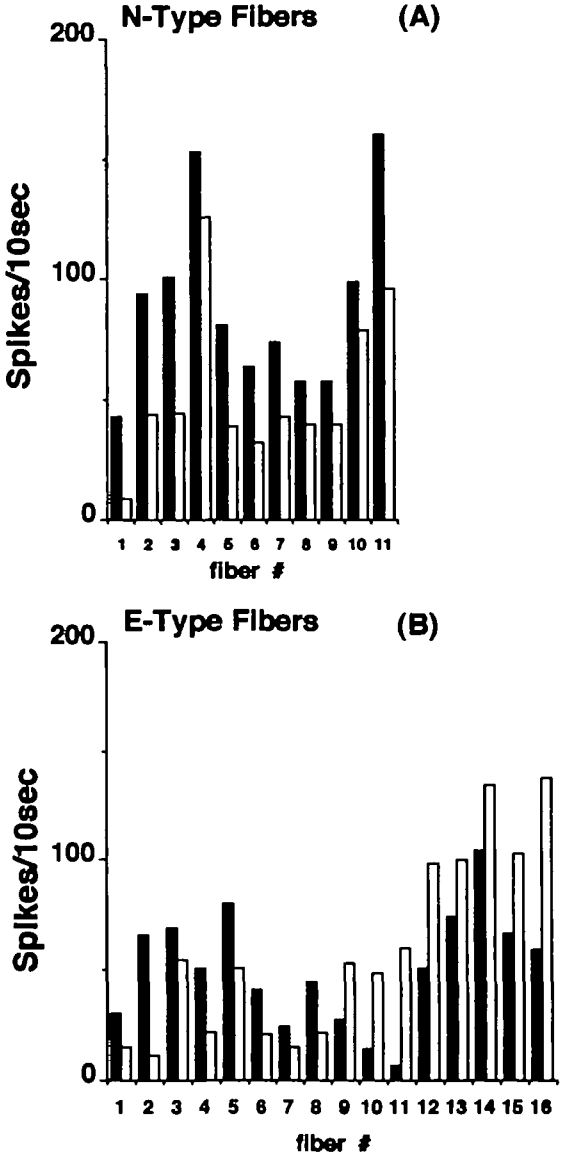

Figure 4 Responses of N- and E-fibers to 100 mM NaCl in 5% ethanol solution and to 100 mM NaCl –100 p.p.m. capsaicin in 5% ethanol solution, expressed as the number of spikes in the first 10 s for each fiber. Filled columns: responses of fibers to 100 mM NaCl in 5% ethanol solution. Open columns: responses of fibers to 100 mM NaCl –100 p.p.m. capsaicin in 5% ethanol solution.

suppression was significantly ($P < 0.001$, paired t-test) stronger than that induced by 5% ethanol.

The effect of capsaicin on the population of E-type fibers was more complex. In 8/16 fibers the response to NaCl was suppressed, whereas in the remaining 8 it was enhanced (Figure 4B).

Figure 5A–C shows the response of N- and E-neurons to 100 mM KCl, 100 mM NaCl, 100 mM NaCl in 5% ethanol solution, and 100 mM NaCl plus 100 p.p.m. capsaicin in 5% ethanol solution. In the case of the N-type fibers, the presence of 100 p.p.m. capsaicin led to a firing rate much slower than that evoked by 100 mM NaCl in its absence (Figure 5A). In the case of those E-fibers whose activity was suppressed by capsaicin, suppression of sensitivity to NaCl by capsaicin persisted for at least 20 s (Figure 5B). On the other hand, in the 'enhanced' E-fibers the initial response to NaCl was weak, but was greatly increased by capsaicin (Figure 5C). This pattern was the opposite of that seen in N-fibers and suppressed E-fibers.

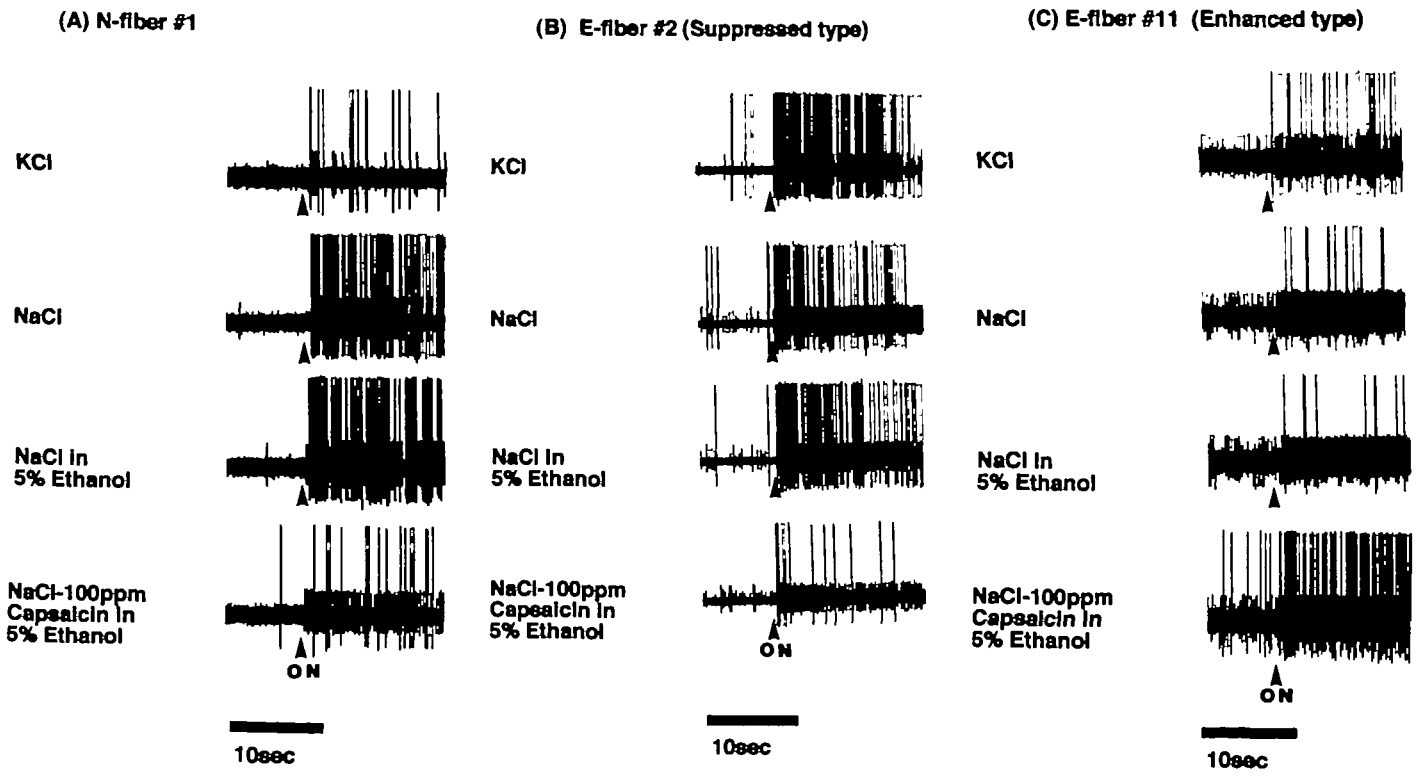

Figure 5 (A–C) Response of three CT single units (N-type, #1; E-type, #2; and E-type, #11) to 100 mM KCl aqueous solution, 100 mM NaCl aqueous solution, 100 mM NaCl in 5% ethanol solution and 100 mM NaCl–100 p.p.m. capsaicin in 5% ethanol solution.

Discussion

The effect of 5% ethanol on the CT response to NaCl

The vehicle for capsaicin, 5% ethanol solution, weakly modified the response of both N-and E-fibers to NaCl. This observation is consistent with a previous report on human taste (Martin and Pangborn, 1970) which showed that an ethanol solution of >1% diminished salty taste. Our result may provide an electrophysiological basis for that earlier observation.

The effect of 100 p.p.m. of capsaicin on the CT response to NaCl

In the present study we observed that capsaicin suppressed 19/27 CT fibers. In the case of the N-fibers, their response to NaCl was selectively depressed by capsaicin. In contrast, capsaicin had either of two effects on E-fibers: enhancement or suppression of the response to NaCl. The suppressed type of E-fiber showed a response pattern similar to that of the N-fibers.

There are several direct and indirect mechanisms by which capsaicin may affect CT response to sodium. Capsaicin is known to inhibit sodium and potassium channels in chicken and guinea-pig neurons (Petersen *et al.*, 1987), and sodium channels in crayfish (Yamanaka *et al.*, 1984) and molluscan neurons (Erdelyi and Such, 1986). These channel-blocking effects occur at relatively high (30–300 μM) capsaicin concentrations in a broad range of cell types. Thus, the neurons innervated by the N- and E-fibers that suffered a decrement in sensitivity to sodium may have been exposed to a sufficiently high concentration of capsaicin to suppress depolarizing Na^+ currents.

In addition to the direct inhibition of ion channels, suppression of responses to Na^+ may be due in part to trigeminal excitation and concomitant effects of peripherally released neuropeptides. Wang *et al.* (1995) demonstrated that electrical stimulation of the lingual trigeminal nerve (LN) can modulate the magnitude of the response of some CT fibers to 100 mM NaCl. In fact, the activity evoked by 100 mM NaCl in most CT single-fibers (26/29 fibers) was decreased during electrical stimulation of LN. Moreover, in rats treated postnatally with subcutaneous injections of capsaicin to reduce or eliminate polymodal nociceptors, LN stimulation failed to evoke changes in CT

activity. This result suggested that the decrease in NaCl-evoked CT response during the period of LN stimulation was due to activation of capsaicin-sensitive afferents. The present study is consistent with that report in that the CT response to NaCl was depressed in majority fibers by simultaneous stimulation of capsaicin-sensitive afferents in LN.

A significant number (~30% of all CT fibers in the present study) of E-fibers were not suppressed but rather were unaffected by capsaicin or had enhanced responses to NaCl. This lack of inhibition may be attributed to the differential access of capsaicin to the taste cells. It has been proposed that cells which are innervated by E-fibers are located below the tight junctions (Ye *et al.*, 1993). These cells would therefore be exposed to lower concentrations of capsaicin. Cells innervated by N-fibers, on the other hand, have Na^+ channels exposed on their apical membranes and would therefore be exposed to higher concentrations of capsaicin. They may explain why all N-fibers were suppressed.

Wang *et al.* (1995) found that only 7% of their CT-fibers were slightly enhanced by electrical stimulation of the LN. In contrast, we observed in 8/16 E-fibers that the response to 100 mM NaCl was enhanced by simultaneously applied capsaicin. In total, 30% of the CT-fibers that we surveyed in this study were enhanced by capsaicin. The difference in the proportions of fibers that were enhanced in our study and in that of Wang *et al.* (1995) could be attributed to the different methods used to stimulate LN; namely, electrical versus chemical stimulation.

Finally, it has been reported that the neuropeptides cholecystokinin and substance P (SP) increase rat CT responses to NaCl (Esakov and Serova, 1988). Moreover, intra- and perigemmal nerve endings contain neuropeptides such as SP (Finger, 1986). Capsaicin stimulates the thin afferent neurons (C-fibers and Aδ-fibers) of mammals and can cause local release of neuro peptides (calcitonin gene-related peptide, cholecystokinin and SP) from peripheral endings (Holzer, 1991). Thus, the involvement of peripherally released neuropeptides may underlie the enhancement by capsaicin or electrical stimulation of responses to NaCl in some CT nerve fibers.

REFERENCES

Brand, J.G., Teeter, J.H. and Silver, W.L. (1985) Inhibition by amiloride of chorda tympani responses evoked by monovalent salts. *Brain Res.*, **334**, 207–214.

Cowart, B.J. (1987) Oral chemical irritation. Does it reduce perceived taste intensity? *Chem. Senses*, **12**, 467–479.

Erdelyi, L. and Such, G. (1986) Effects of capsaicin on molluscan neurones: a voltage clamp study. *Comp. Biochem. Physiol.*, **85C**, 319–327.

Esakov, A.E. and Serova, O.N. (1988) Influence of substance P on taste receptor organ and salt intake in rats. *Neurosciences*, **14**, 321–327.

Feigin, A.M., Ninomiya,Y., Bezrukov, S.M., Bryant, B.P., Moore, P.A., Komai, M., Wachowiak, M., Tetter, J.H. and Brand, J.G. (1994) Novobiocin enhances responses of rat chorda tympani nerve fibers to NaCl and forms cation channels in lipid bilayers. *Am. J. Physiol.*, **266**, C1165–C1172.

Finger, T.E. (1986) Peptide immunochemistry demonstrates multiple classes of perigemmal nerve fibers in the circumvallate papilla of the rat. *Chem. Senses*, **11**, 135–143.

Frank, M.E., Bieber, S.L. and Smith, D.V. (1988) The organization of taste sensibilities in hamster chorda tympani nerve fibers. *J. Gen. Physiol.*, **91**, 861–896.

Green, B.G. (1991) Topical characteristics of capsaicin sensitization and desensitization on the tongue. *Physiol. Behav.*, **49**, 501–505.

Heck, G.L., Mierson, S. and DeSimone, J.A. (1984) Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway. *Science*, **223**, 403–405.

Hettinger, T.P. and Frank, M. (1990) Specificity of amiloride inhibition of hamster taste responses. *Brain Res.*, **513**, 24–34.

Hill, D.L. and Bour, T.C. (1985) Addition of functional amiloride-sensitive components to the receptor membrane: a positive mechanism for altered taste responses during development. *Dev. Brain Res.*, **20**, 310–313.

Hinman, J.W., Caron, E.L. and Hoeksema, H. (1957) The structure of novobiocin. *J. Am. Chem. Soc.*, **79**, 3789–3800.

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

Jacobs, E.R. and Decoursey, T.E. (1990) Mechanism of potassium channel block in rat alveolar epithelial cells. *J. Pharmacol. Exp. Ther.*, **255**, 459–472.

James, I.F., Ninkina, N.N. and Wood, J.N. (1993) The capsaicin

receptor. In Wood, J.N. (ed.), *Capsaicin in the Study of Pain*. Academic Press, New York, pp. 83–104.

Karrer, T. and Bartoshuk, L. (1991) Capsaicin desensitization and recovery on the human tongue. *Physiol. Behav.*, **49**, 757–764.

Karrer, T. and Bartoshuk, L. (1990) Oral capsaicin desensitization and its effects on taste. *The Twelfth Annual Meeting of The ACHEMS—1990*, p. 87.

Kawada, T., Hagiwara, K. and Iwai, K. (1986) Effects of capsaicin on lipid metabolism in rats fed a high fat diet. *J. Nutr.*, **116**, 1272–1278.

Martin, S. and Pangborn R.N. (1970) Taste interaction of ethyl alcohol with sweet, salty, sour and bitter compounds. *J. Sci. Food Agric.*, **21**, 653–655

Ninomiya, Y. and Funakoshi, M. (1988) Amiloride inhibition of responses of rat single chorda tympani fibers to chemical and electrical tongue stimulations. *Brain Res.*, **451**, 319–325.

Ninomiya, Y., Mizukoshi, T., Higashi, T. and Funakoshi, M. (1984) Differential responsiveness of two groups of rat chorda tympani fibers to ionic chemicals and electrical tongue stimulation. *Proc. Jap. Symp. Taste Smell*, **18**, 145–148.

Ninomiya, Y., Mizukoshi, T., Nishikawa, T. and Funakishi, M. (1987) Ion specificity of rat chorda tympani fibers to chemical and electrical tongue stimulations. *Brain Res.*, **404**, 350–354.

Petersen, M., Pierau, F.-K. and Weyrich, M. (1987) The influence of capsaicin on membrane currents in dorsal root ganglion neurones of guinea pig and chicken. *Pflügers Arch.*, **409**, 403–410.

Prescott, J., Allen, S. and Stephans, L. (1993) Interaction between oral chemical irritation, taste and temperature. *Chem. Senses*, **18**, 389–404.

Rick, R., Dorge, A. and Sesselman, E. (1988) Na transport stimulation by novobiocin: transepithelial parameters and evaluation of E_{Na}. *Pflügers Arch. Eur. J. Physiol.*, **411**, 243–251.

Wang, Y. Erickson, R.P. and Simon S.A. (1995) Modulation of chorda tympani nerve activity by lingual nerve stimulation. *J. Neurophysiol.*, **73**, 1468–1483.

Yamanaka K., Kigoshi, S. and Muramatsu, I. (1984) Condition-block induced by capsaicin in crayfish giant axon. *Brain Res.*, **300**, 113–119.

Ye, Q., Heck, G.L. and Desimone, J.A. (1993) Voltage dependence of the rat chorda tympani response to Na+ salts: implications for the functional organization of taste receptor cells. *J. Neurophysiol.*, **70**, 167–178.

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