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The Study on Ionic Current of Single Taste Cell with Whole Cell Patch Clamp Technique

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During the last decade, much efforts on the study of identification of various ion channels in taste receptor cells were done based on electrophysiology. However, it's still unknown the electrophysiological response of these cells by various sapid substances, which is important to elucidate the gustatatory transduction mechanism. In the present study, we identified membrane currents of the taste receptor cells in circumvallate papilla in rat and investigated the direct effect of sweet or pungent stimuli on these cells. Epithelial sheets rich in taste buds and free of muscle tissue were isolated from the circumvallate papilla of the rat tongue by enzyme treatment following microdissection. We could get taste buds or receptor cells from the epithelial sheets after exposure to calcium free Tyrode solution. Two types of voltage dependent outward currents were shown in these cells. The first type was a delayed rectifier K^+ currents and it's inhibited by 5 mM TEA. The second type displayed voltage dependent inactivating components, which suggest it might be an A-like current or the summation of A currents and delayed rectifier properties. We observed the response of taste receptor cells by 2 kinds of chemical stimuli; 20 mM saccharin and 500 nM capsaicin which evoke hot sensation as pungent chemical. When 20 mM Saccharin was added to the perfusion solution, as we expected, voltage dependent outward currents were blocked. The effects of capsaicin on the ionic currents were slightly different, time dependently. A continuous increase of inward currents, which was only slightly activated in control Tyrode solution, was observed. Outward currrets also increased, but it was transient. Our study suggest that membrane currents of taste receptor cells could be modulated by both sweet stimuli and pungent chemical, capsaicin.

Key Words : *Electrophysiology, Taste Buds, Taste receptor cells, Saccharin, Capsaicin*

Introduction

Taste is one of the most important sensations in animal and human. In animal, taste perception is closely related to their survival. It could play a role to decide whether intake or rejection of foods. For man and many other vertebrates, the hedonic taste "sweet" signals high-calorie carbohydrates, Meanwhile, "bitter" sensation may be related to poison. Although the taste sensation in human is not directly related to survival, It may be one of the special senses to improve the quality of living. However, gustatatory intracellular transduction mechanism is still unclear.

During the last decade, the ionic basis for gustatory transduction mechanisms were reported in amphibian and mammals (Akabas *et al.*, 1988: Avenet and Lindemman, 1987a ,1988: Kinnamon and Roper, 1987). The taste receptor cell has diverse ionic channels as well as neurons. Voltage dependent Na^+, K^+ current and Ca^{2+} current were reported in amphibian using patch clamp technique (Kinnamon and Roper, 1988: Sugimoto and Teeter, 1990). In Necturus taste cells, there are several voltage dependent K^+ currents in apical regions and they were blocked by sour and bitter taste stimuli, citric acid and quinine (Cummings and Kinnamon, 1992). Voltage-dependent inward and outward currents were also observed in mouse. In this cell, delayed rectifier currents were blocked by denatonium and benzoate (Spielman *et al*, 1989). Akabas *et al* (1990) reported two populations of cells within the taste bud in rat circumvallate taste cells: one expressing voltage dependent K^+ currents and the second containing both voltage dependent Na^+ and K^+ currents. In rat fungiform papilla, there were voltage dependent Na^+, Ca^{2+} and K^+ currents. In these cells, 20 mM saccharin inhibited outward K^+ currents (Behe *et al.*, 1990). Although much efforts on the study of identification of various ion channels

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were done based on electrophysiology, it's still unknown the response of taste receptor cells by various sapid substances, which could verify the true taste receptor cells by function, especially in mammals.

In the present study, we tested the effect of pungent stimuli on the taste receptor cells directly as well as sweet stimuli. The principal component in hot peppers is known as capsaicin, so we used it as a representative chemical for pungent stimuli and saccharin for sweet stimuli respectively in this experiment. It's believed that hot sensation is not belong to one of 4 (sweet, sour, salty and bitter) or 5 (sweet, sour, salty, bitter and umami taste) basic taste modalities and it's rather categorized as one of the pain sensations, a burning sensation, caused by irritation of free nerve endings in mouth. Contrary to the well known mechanism of capsaicin on sensory nerve endings, that of capsaicin on the oral, gustatatory aspects remains unknown.

Recently they suggested that capsaicin and other pungent compounds might directly stimulate taste cells rather than the free nerve endings of the sensory neuron in bullfrog (Yoshii and Matui, 1994). However, there has been no attempt to investigate the direct effect of pungent chemical on the taste receptor cells. In the present study, we identified two types of voltage dependent K^+ currents in taste receptor cells of rat and investigated the response of these cells by sweet substance, saccharin and fungent stimuli, capsaicin.

Materials and Methods

Taste cell preparation

Sprague Dawley rats weighing around 200g were killed by cervical dislocation and the tongues were removed. Removal of the circumvallate (CV) papilla and dissection of CV tissue were done according to the method of Striem et all (Striem *et al*, 1991). The brief description of the procedure is as follow. The enzyme mixture containing collagenase 5 mg/ml and trypsin inhibitor 2 mg/ml was injected submucosally at 3-4 locations around and under the single CV papilla found back section of the tongue. The enzyme treated tongue was incubated for 30 minutes in 37℃ oxygenated Tyrode solution. The CV was identified under a dissection microscope (10-12× magnification). Iris scissors were used to make a circular or square cut around the CV. Then epithelium containing the CV was peeled off from the underlying muscle. The peeled epithelium was treated again with enzyme containing collagenase and trypsin inhibitor to dissolve the remaining basal membrane for 30 minutes. After exposure to calcium free Tyrode solution for about 10 minutes, taste buds from the papilla could be separated. Taste buds or dissociated taste receptor cells could be attached to the bottom of the tissue chamber which was previously coated with Cell Tak.

Patch clamp recording

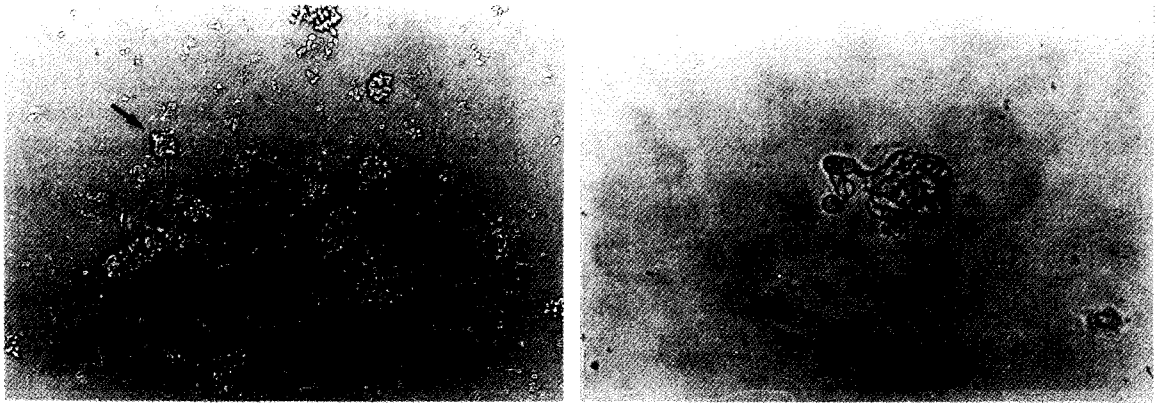
Taste buds or taste receptor cells were well attached to the bottom of the recording chamber (volume 400 μl), and viewed with an Olympus IMT-2 inverted microscope. The Tyrode solution contained (in mM): 140 NaCl, 5 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, pH 7.4 (pH adjusted with NaOH), 5 glucose and 5 pyruvate. Electrodes were manufactured from conventional haematocrit capillaries. These were filled with a K^+-rich solution containing (mM): 140 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 11 EGTA, 10 HEPES, pH 7.2 (pH adjusted with KOH), and had tip resistances of between 3 to 5 MΩ.

The whole-cell recording configuration was obtained by applying additional suction and/or voltages pulses to rupture the membrane with the pipette held at -80 mV. Membrane currents were measured in isolated cells using the whole cell mode (Hamil *et al*, 1981) with an Axopatch 1C amplifier (Axon Instruments, Foster City, CA). The current signal was filtered at 1 KHz, digitized at 10-20 KHz. Membrane patches were clamped at a series of potentials to +60 mV potential at holding potential -80 mV by 10 mV step. The resultant current records were stored and analysed using pClamp soft ware (Version 6.0, Axon Inst.). Both membrane capacitance and series resistance were compensated electrotonically. Saccharin (Sigma) or capsaicin (Sigma) was directly added to the tissue chamber at final concentration 20 mM and 500 nM, respectively:

Results

Identification of taste receptor cells

After enzyme treatment, several CV taste buds (indicated by arrow) with many dispersed single



(A) (B)

Fig. 1. A. Several CV taste buds (indicated by arrow) and single cells under the inverted microscope. B. A single CV taste bud at the high magnification (×400), which shows a typical onion shape. Taste pore is clearly seen in this bud. (indicated by arrow). One taste receptor cell is now lysed from the bud (indicated by ▽).

cells were observed under the inverted microscope (Fig. 1A). Fig. 1B shows one taste bud under the high magnification(×400), taste pore (indicated by arrow) was clearly seen in this bud. By controlling the exposure time of calcium free Tyrode solution, we could get more taste buds than single cells or more single taste cells than taste buds ,alternatively. Taste receptor cells are quite different from the epithelial cells in shape, so they were chosen by cell morphology in our experiment; Taste cells looked as spindle-shaped ones with one or two elongated process contrary to the spherical epithelial cells. The pipettes which we used were fire polished and the resistance of pipettes were 3-5 MΩ. The capacitances of taste receptor cells were variable between 1.5 and 4 pF.

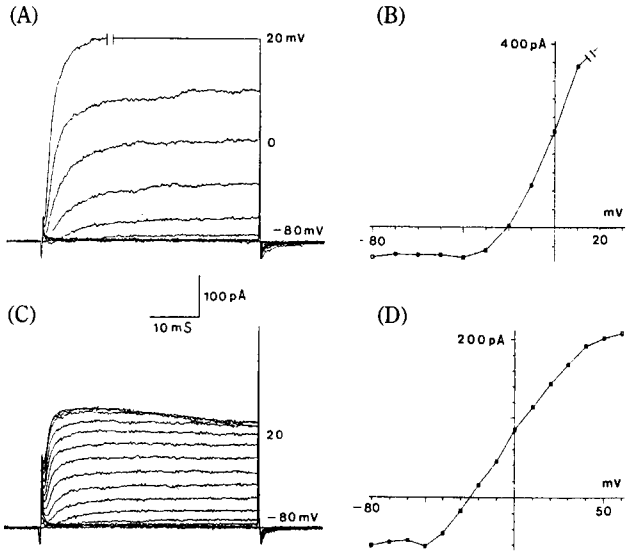


Fig. 2. Two types of outward current profiles recorded from CV taste cells. Holding potential was -80 mV and step potentials were increased to 20 or 60 mV by 10 mV unit. A. Typical delayed recetifier currents. B. Corresponding stedy state outward currents from A were plotted against the membrane potential C. Voltage dependent inactivating currents, which are saturated around at +40 mV command potential. D. Corresponding stedy state outward currents from B were plotted against the membrane potential. The slope of conductance is more steeper in B than D.

Membrane currents

Time and voltage-dependent inward and outward membrane currents were observed after step depolarization of voltage-clamped taste cells (n=11). The other taste cells could show only passive membrane properties with linear leak currents (n=8). Typical currents records from a single taste cell held at -80 mV and depolarized in 10 mV steps to 20 or 60 mV are presented in Fig. 2. Only two taste cells displayed a small transient inward currents that activated and inactivated rapidly with depolarizing pulses (Fig. 5A). The inward currents peaked at +30 mV(Fig. 5E). We tried to record pure inward currents using CsCl rich pipette solution instead of KCl, but we could not see voltage dependent inward currents in any other taste receptor cells (n=5,data not shown).

The prominent delayed outward currents observed in taste receptor cells. Two types of outward currents were observed in this experiment. The first type currents were delayed rectifiers, which were activated at -40 mV command potential (Fig. 2A and 2B) and the conductance slop of the first type currents was more steeper than the second type currents (Fig. 2C and 2D). The second type currents usually displayed voltage de-

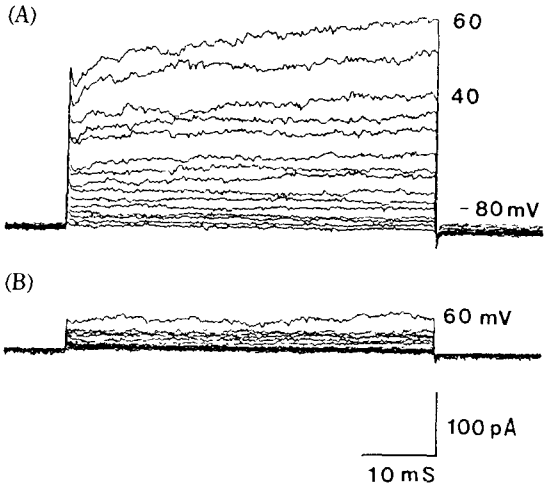


Fig. 3. Block of outward rectifying currents by 5 mM TEA in bath. Holding potential was -80 mV and step potentials were increased 60 mV by 10 mV unit. A. Outward rectifying currents before the TEA. B. Currents profile after 5 mM TEA in bath in the same cell. Most of outward currents were inhibited, which indicate that they were carried by K^+.

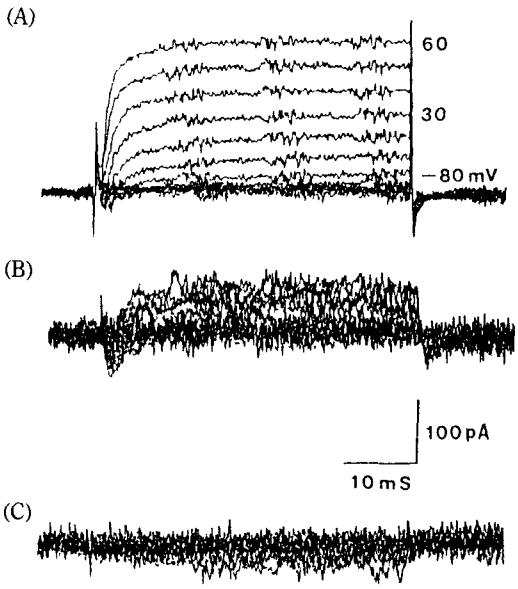


Fig. 4. The effect of 20 mM saccharin on voltage dependent outward currents. Holding potential was -80 mV and step potentials were increased to 60 mV by 10 mV unit. A. Current profile in which leakage currents are not subtracted in unstimulated cell B. Leakage currents are subtracted in the same cell in A. Outward currents clearly could be seen. C. Outward currents are blocked by saccharine in bath (subtracted currents).

pendent inactivating components and currents were saturated around at +40 mV command potential (Fig. 2C), but we couldn't see saturation phenomena in the first type. The outward currents, in this experiment, were blocked by addition of 5mM TEA to bath, indicating that they were carried by K^+. 5 mM TEA reduced peak outward K^+ currrents by about 80% without shifting the voltage dependence (Fig. 3).

Effects of taste stimuli

We observed the response of taste receptor cells by 2 kinds of chemical stimuli. One is saccharin for sweet sensation and the other is capsaicin which evoke hot sensation as pungent chemical. When 20 mM saccharin was added to the bath, as we expected, voltage depent outward currents were blocked (Fig. 4). We could also record the reduced K^+ currrents by 20 mM saccharin from another cell.

We also examined the direct effect of capsaicin on the taste receptor cells (Fig. 5). The effects of 500 nM capsaicin on the ionic currents were slightly different, time dependently. There was only a small voltage activated inward currents in control Tyrode solution, but increase of inward currents was clearly observed 30 seconds after capsaicin application (Fig. 5B). The small peak inward current in resting state was -100 pA (Fig. 5A). By capsaicin application, peak inward currents was increased to -180 pA in 90 seconds (Fig. 5D). Meanwhile, capsaicin also transiently increased stedy state outward K^+ currrents by 30-40% in 60 seconds (Fig. 5C) but the increased outward current was reduced to control level within next 30 seconds. I-V relationships of peak inward currents (from Fig. 5A and 5D) and stedy state outward currents (from Fig. 5A and 5C) were plotted in Fig. 5E and 5F, respectively.

Discussion

Identification of taste receptor cells

Taste receptor cells are different from the epithelial cells in shape but we couldn't record voltage dependent currents from all of the taste receptor cells, identified by cell morphology. 58% of these cells could show voltage dependent currents. We conclude that half of cells in a taste bud might be taste receptor cells. Rat CV taste cells were thinner, which explain their small capacitance of 1. 5-4 pF. A membrane capacitance of 10 pF was measured for frog taste receptor cells (Avenet and Lindemann, 1987b) and 50 pF for Necturus (Kinnamon and Roper, 1988).

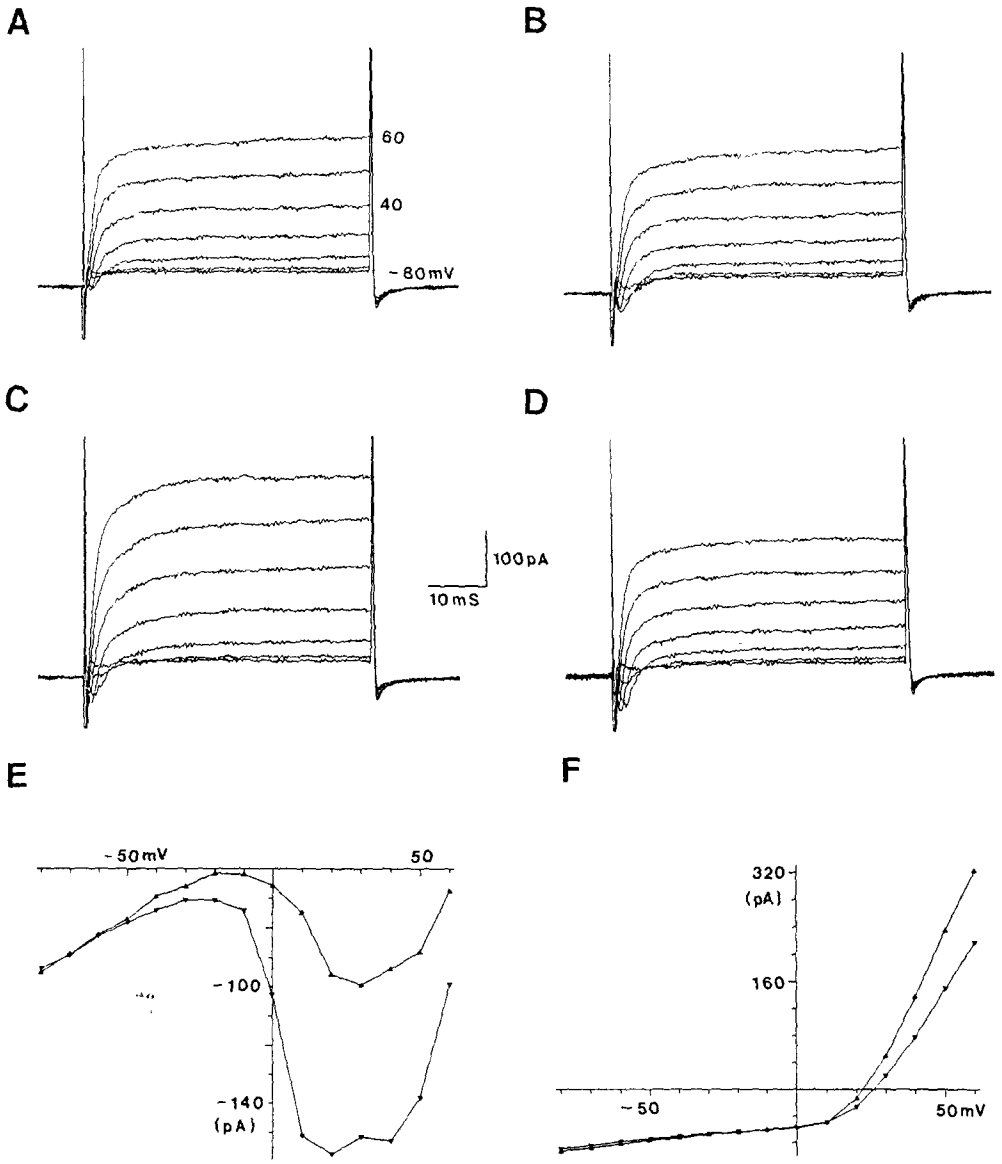


Fig. 5. The effect of 500 nM capsaicin on the membrane currents in taste receptor cells at 30 seconds interval. Holding potential was -80 mV and step potentials were increased to 0,10,20,30,40,50 and 60 mV by 10 mV unit. A. The current profile before capsaicin application. Only a small, transient inward currents could be seen. B. 30 seconds after capsaicin application in bath. Inward currents were activated, but amplitude of outward currents are same as A. C. 60 seconds after capsaicin application. Inward currents were continuously increased and outward currents were also transiently increased. D. 90 seconds after capsaicin application. Inward currents were more increased than in C, but the increased outward currents in C are reduced to control level in A. E. Current voltage relation of peak inward currents, before (△) and after capsaicin application (▽) from A and D respectively. F. Current voltage relation of stedy state outward currents, before (▽) and after capsaicin (△) from A and C respectively.

Membrane currents

In our experiment, there were few currents observed in taste receptor cells that could be attributed to Na^+ channels except 2 cells. Even the voltage dependent inward currents from 2 cells were not prominent. The peak inward current in resting state was only about -100 pA. The failure to observe Na+ currents may be due to different cell preparation technique. Another possibilty is that the Na^+ channel density in rat CV taste cells, in fact, is lower than that of the K^+ channels. The inactivating inward currents were observed only about 10% from the total recordings in the same cell (Akabas *et al*, 1990). We tried to record pure inward currents using CsCl rich pipette solution instead of KCl, but we could not see voltage dependent inward currents in any other taste re-

ceptor cells (n=5, data not shown). So, voltage dependent inward currents were not studied further.

One of the characteristics of K^+ channels is the diversity. Two types of outward currents were observed in this experiment. Beside the first type, delayed rectifiers, some membranes have a different K^+ channel type that activates outward currents transiently in the subthreshold range of potential. The first type currents were linearly increased by depolarizing pulses and the magnitude of increase of outward currents was larger than the second type. The second type currents usually displayed voltage dependent inactivating components and currents were saturated at 40 mV command potential. We could see neither inactivation nor saturation phenomena in the first type. Inactivating K^+ currrents such as those we reported here have been first described on molluscan neurons and termed A current by Connor and Stevens (1971). Since then, currents with properties to those of molluscan A currents have been described in a variety of taste cells, including larval tiger salamander, rat and Necturus (Sugimoto and Teeter,1990; Behe *et al.*, 1990; Bigiani and Roper, 1993). There are variations in the stady-state inactivation of A currents and in activation threshold. Although the K^+ currrents we observed in rat taste receptor cells did not seem to match the properties of typical A currents (inactivation thresholds were different but activation threshold was nearly same at -50 mV), we could consider that these currents to be an A-like current or the combined currents mixed with delayed rectifier properties. First type, delayed rectifiers, was inhibited by 5 mM TEA in bath. Although 5mM TEA could not inhibit the outward currents completely, it showed that delayed outward currents were carried by K^+.

Effects of taste stimuli

The effects of sugar or any other artificial sweetner on the taste receptor cells are well known. Saccharin is also a sweet agent in the rat (Sato,1971). In our experiment, 20 mM saccharin nearly blocked outward current. Although we didn't record the action potentials, decreased outward K^+ currrents might have evoked depolarzation of cell. The involvement of cyclic neucleotides as a second messenger, like exposure to sucrose, is well known. Intact taste cells were shown to generate cAMP in response to exposure with sucrose (Striem *et al.*, 1991).

The response of taste receptor cells to 500 nM capsaicin in bath in our experiment was complicated. An activation inward currents, which showed small peak inward currents of -100 pA in control Tyrode solution, was observed 30 seconds after capsaicin application in bath. It suggests that voltage dependent Na^+ channels are inactive or very few in unstimulated taste receptor cells. But, by pungent chemical, 500 nM capsaicin, inward current was continuously increased to -180 pA until 90 seconds. We didn't study further whether increase of inward current is large enough to induce action potentiai in these cells. But, it's probable that increase of inward current would contribute to the depolarization and repetitive firing of the taste receptor cell. Meanwhile, capsaicin also transiently increased the outward currrets after onset of the increase of inward current. It could be partly explained by desensitization effect of capsaicin (Wood, J., 1993).

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