Research report

Capsaicin modulates K^+ currents from dissociated rat taste receptor cellsKyungpyo Park^{a,*}, Peter D. Brown^b, Yoon Bae Kim^a, Joong-Soo Kim^a^aDepartment of Physiology, College of Dentistry, Seoul National University and Dental Research Institute, Yeongeondong 28, Chongnoku, Seoul 110-749, Korea^bSchool of Biological Sciences, University of Manchester, Manchester, UK

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Abstract

Chili pepper is one of most widely used spices. The main active component of chili pepper is the capsaicin. The effects of capsaicin on sensory nerve endings are well known; however, little is known regarding the direct effect of capsaicin on taste receptor cells (TRCs). In this study, patch clamp methods were used to study the effects of capsaicin on the K^+ currents in TRCs isolated from the rat circumvallate papilla. Fura-2 microspectrofluorimetry was also used to determine the effects of capsaicin on the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). In the resting state, whole-cell experiments identified outward-rectifying K^+ currents, which were inhibited by 5 mM tetraethylammonium (TEA^+) chloride. Voltage-dependent K^+ channels with a conductance of 55 ± 4 pS (mean $\pm$ S.E.M.; $n=3$), were observed in cell-attached patches. Capsaicin (500 nM) completely inhibited the outward-rectifying K^+ current in the whole-cell recordings. In cell-attached patches 500 nM capsaicin significantly reduced the open probability (P_o) of the K^+ channels from 0.401 ± 0.052 ($n=3$) in the resting state, to 0.018 ± 0.002 ($n=3$, $P < 0.05$ by unpaired t -test). In the fura-2-loaded TRCs, micromolar concentrations of capsaicin increased $[Ca^{2+}]_i$ in a dose-dependent manner, e.g., 100 μ M capsaicin consistently increased the 340:380 fluorescence ratio from 1.04 ± 0.05 in the resting state to 1.40 ± 0.05 ($n=28$). These results suggest that capsaicin can enhance or modify the gustatory sensation by inhibiting the K^+ currents of the TRCs directly.

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1. Introduction

There are at least five basic taste modalities in humans, these include: sweet, salty, sour, bitter and the umami sensation [18]. To improve or modify the quality of the taste of food, mankind has used a variety of seasonings. Spices, such as chili sauce are popular seasonings which are widely used in the cuisine of many cultures. It has long been known empirically that the addition of a pungent compound can elicit pleasant warm sensations in the mouth and prolong the shelf-life of foods.

Capsaicin is the principal active component of chili peppers. The pharmacological actions of capsaicin have

been extensively investigated in sensory neurons. These studies have shown that it stimulates a subpopulation of sensory neurons, evoking nociception mediated by substance P, neurokinin A and the calcitonin gene-related peptide [9,12,20]. The burning or desensitization effects of capsaicin in the mouth have been explained by the stimulation of the capsaicin receptors in free nerve endings. Recently, the capsaicin receptor, which is also activated by an increase in temperature, was successfully cloned from rodent dorsal root ganglion [5].

Capsaicin, however, also elicits a pleasant warm sensation in the mouth at lower concentrations. This response may be due to interactions of capsaicin with taste receptors cells [27], rather than sensory neurons. In contrast to the well known mechanism of capsaicin on the sensory nerve endings [3,19,25,26], the effect of capsaicin on the gus-

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tatatory sensation has not been properly investigated. In the present study the effects of capsaicin on TRCs isolated from the rat circumvallate papilla was investigated. Patch clamp methods and microspectrofluorimetry were used to determine changes in ionic conductances and $[Ca^{2+}]_i$, respectively.

2. Material and methods

2.1. Taste cell preparation

Sprague–Dawley rats, weighing approximately 200 g, were sacrificed by a cervical dislocation and the tongues removed. TRCs were isolated from the circumvallate papilla according to the method of Striem et al. [22]. Briefly an enzyme mixture, consisting of Tyrode solution (140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 10 mM Hepes, 5 mM glucose and 5 mM pyruvate; pH 7.4 with NaOH), plus 5 mg/ml collagenase (Type A; Boehringer-Mannheim, Germany) and 2 mg/ml of a trypsin inhibitor (Type A; Sigma, St. Louis, MO, USA), was injected submucosally at three to four locations around and under the single circumvallate papilla found in the back section of the tongue. The enzyme treated tongue was incubated for 30 min at 37 °C in oxygenated Tyrode solution. The circumvallate papilla was identified under a dissection microscope ($\times 10$ – 12 magnification), and iris scissors used to make a square cut around the circumvallate papilla. The epithelium containing the circumvallate papilla was then peeled away from the underlying muscle. The epithelium was further treated with the enzyme-mixture for 30 min to digest the remaining basement membrane. After exposure to calcium-free Tyrode solution ($CaCl_2$ was omitted and 0.5 mM EGTA added) for 10 min, the taste buds from the papilla could be separated from the epithelium. The TRCs were seeded on the bottom of the tissue chamber, which was pre-coated with Cell Tak (BD Biosciences, USA). Capsaicin (Sigma) was dissolved and stored as a 5 mM stock solution in 100% ethanol (EtOH), and diluted to the appropriate concentration in Tyrode solution.

2.2. Patch clamp recording

The TRCs, were viewed at the bottom of the recording chamber (volume=400 μ l) with an Olympus IMT-2 inverted microscope. The cells were superfused during experiments with Tyrode solution. Patch pipettes were manufactured from conventional haematocrit capillaries. They were filled with a K^+ -rich solution containing (mM): 140 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 11EGTA, 10 Hepes at pH 7.2 (adjusted with KOH), and had tip resistances ranging from 3 to 8 M Ω . Channel currents were measured in the isolated cells using the cell-attached or whole-cell configuration of the patch clamp method, with an Axopatch 1C amplifier (Axon Instruments, Foster City, CA). The whole-cell

recording configuration was obtained by applying additional suction and/or voltage pulses to rupture the membrane. Currents were filtered at 1 kHz, and digitized at 10–20 KHz. Linear leak currents were subtracted from some of the whole-cell records.

2.3. Measurement of $[Ca^{2+}]_i$

TRCs were allowed to adhere to the bottom of the experimental chamber. They were then washed twice with Tyrode solution, and incubated with a 2 μ M fura-2 acetoxymethyl ester in a Tyrode solution for 30 min at room temperature (25 °C). After loading, the cells were washed and kept on ice until use. Cells were superfused with the Tyrode solution, and viewed with a Zeiss inverted microscope (Axiovert 10, Zeiss, Germany) with a $\times 40$ oil immersion objective lens. Fura-2 fluorescence was excited by a collimated beam of light from a 200-W Hg arc lamp, fed to a dual spectrophotometer system (PTI, USA). This alternated the wavelength between 340 and 380 nm by means of a spinning chopper (60 Hz). The emitted fluorescence was measured at 510 nm with a photomultiplier tube. Felix software (Version 1.1) from PTI was used for the recording and analysis. Data are expressed as the ratio of the light emitted at the two excitatory wavelengths ($F_{340}:F_{380}$).

2.4. Data analysis

Whole-cell currents were recorded, stored and analyzed using pClamp software (Version 8.0, Axon Instruments). Single channel current amplitudes and open probabilities (P_o ; measured for 10-s intervals or in control experiments just before capsaicin application), were also determined using the pClamp software. The usual conventions of current flow were observed throughout, i.e., inward currents are negative and outward currents are positive. The potential applied to the electrode in cell-attached patches ($-V_p$), does not include the contribution of the membrane potential (V_m) of the cell. Data are presented as mean $\pm$ S.E.M., and statistical analysis was performed using Student's *t*-test for unpaired data.

3. Results

3.1. Identification of TRCs from circumvallate papilla

A mixture of intact taste buds and isolated TRCs were obtained by enzyme digestion. Fig. 1A shows several taste buds (indicated by arrows), surrounded by many single dispersed TRCs. A more highly magnified image of a single taste bud and some spindle-shaped TRCs (s) is shown in Fig. 1B. All patch clamp recordings and measurements of the intracellular Ca^{2+} were made on single TRCs.

Fig. 1. (A) Several circumvallate taste buds (indicated by arrows) with many dispersed single TRCs (magnification, $\times 100$). (B) A single circumvallate taste bud at high magnification ($\times 400$). Note the convergence of the taste cells at the apical pole (indicated by arrow). Taste cells had a spindle-shape (indicated by ∇) with one or two elongated process.

3.2. Outward-rectifying K^+ currents of TRCs

Outward-rectifying K^+ currents were observed in seven from a total of 12 TRCs (58%), the remaining five cells exhibited linear, time-independent currents. Fig. 2A shows typical whole-cell currents in one of these cells at potentials from -80 to $+60$ mV (for 50 ms). The outward currents were observed at potentials more positive than -40 mV. At depolarizing potentials of greater than $+40$ mV, time-dependent inactivation of the currents was also observed. The capacitances of these cells were between 4 and 8 pF ($n=7$). The mean current–voltage (I – V) relationship of the steady-state currents in seven cells is shown in Fig. 2C (open circles). The reversal potential for the conductance was of -53 ± 4 mV ($n=7$, mean $\pm$ S.E.M.), which is close to E_K . Fig. 2B shows current profiles observed in the presence of 5 mM TEA $^+$, currents were inhibited at all potentials. The I – V relationship obtained in

Fig. 2. Whole-cell recordings from TRCs. (A) Typical current profile from a single TRC, the holding potential was -80 mV and step potential (50 ms) from -70 to $+60$ mV were applied in 10 mV increments. (B) Inhibition of the whole-cell currents by 5 mM TEA $^+$. (C) I – V relationship of the steady state currents in the absence (open circles, $n=7$) or presence of 5 mM TEA $^+$ (solid circles, $n=3$).

the presence of 5 mM TEA $^+$ is shown in Fig. 2C (solid circles, $n=3$). Current inhibition by TEA $^+$ was evident at all supra-threshold potentials, but was less marked at the less depolarized potentials, e.g., it was $78 \pm 3\%$ of control ($n=3$) at $+30$ mV, but only $54 \pm 4\%$ at -30 mV, suggesting that the inhibition is voltage-dependent. The reversal potential of the residual currents in the presence of TEA $^+$ was -15.8 ± 10.8 mV ($n=3$).

Single channel K^+ currents were recorded from the basolateral membrane of the TRCs. Fig. 3A shows representative currents at various holding potentials in the cell-attached mode. The dotted line on each trace indicates that the channel is closed. Channel openings appear as downward deflections, representing are negative currents. The number of channel openings increased with membrane depolarization (i.e., as $-V_p$ was made more positive). Fig. 3B shows an I – V relationship for channel in three cell-attached patches. The I – V relationship was linear with a

Fig. 3. Single channel recording from TRCs. (A) Currents from a single K⁺ channel in a cell-attached patch on the basolateral membrane of a TRC. The numbers on the right of each trace indicate the applied potential ($-V_p$). (B) I - V relationship for single channel currents from three cell-attached patches. The conductance was estimated as 55 ± 4 pS ($n=3$) by linear regression analysis. (C) The relationship between the channel open probability (P_o) and $-V_p$. The curve was fitted by the Boltzman equation.

conductance of 55 ± 4 pS (determined by linear regression analysis). The resting membrane potential of the cells, estimated from the reversal potential, was -60 ± 4 mV ($n=3$). Fig. 3C shows the relationship between the channel open probability (P_o) and $-V_p$. P_o increased with depolarization of the cell membrane.

3.3. Effects of capsaicin on the K⁺ currents in TRCs

The effects of 500 mM capsaicin on K⁺ channel activity were examined in whole-cell and single channel experi-

ments. Fig. 4A shows a typical whole-cell current profile recorded in control conditions at $V_m = -80$ to $+60$ mV (leak currents subtracted). Fig. 4B shows the current profile in the same cell 2 min after adding 500 nM capsaicin to the superfusate. The outward currents were blocked under these conditions, leaving very small residual inward currents. The effect of capsaicin on the delayed rectifying K⁺ currents was irreversible. Fig. 4C shows the I - V relationship before (open circles) and after the addition of capsaicin (filled circles). Similar inhibitory effects of capsaicin were observed in a total of three TRCs. In

Fig. 4. Capsaicin inhibits whole-cell K^+ currents in TRCs. (A) Current profile in control conditions. Potentials were evoked by 50-ms step potentials from -80 to $+60$ mV (linear leak currents have been subtracted from the records). (B) Current profile obtained 2 min after the addition of 500 nM capsaicin to the superfusate. All outward currents blocked and only very small inward currents were observed. (C) The I - V relationship before (open circles) and during (filled circles) capsaicin application (data taken from the same cell as in (A) and (B)).

vehicle control experiments, 0.01% EtOH (used with 500 nM capsaicin) had no effect on current amplitude (data not shown).

Fig. 5 shows single channel K^+ currents in a cell-attached, before and after the addition of 500 nM capsaicin. Throughout the experiment (a total of 180s), $-V_p$ was maintained at $+20$ mV. In the control period, frequent channel openings (downward deflections) were observed. After the addition of 500 nM capsaicin (indicated by the arrow), channel openings were gradually reduced so that at the end of the trace (150 s after capsaicin application), the channel was mainly closed. In three experiments, channel P_o was reduced from 0.401 ± 0.052 in control conditions, to 0.039 ± 0.001 60s after capsaicin addition and 0.018 ± 0.002 ($P < 0.05$) after 120 s. P_o did not recover on washing out capsaicin. In time-matched controls, there was no reduc-

tion of P_o over 180 s ($n = 3$). Single channel conductance was unaffected by capsaicin. However, the I - V relationships were shifted to the left so the current amplitude at $-V_p = +20$ mV were reduced slightly from -2.52 ± 0.18 to -2.25 ± 0.16 pA ($n = 3$). The new reversal potential was -55 ± 3 mV ($n = 3$), which is consistent with a depolarization of the cell membrane potential.

3.4. Effects of capsaicin on $[Ca^{2+}]_i$ in fura-2-loaded TRCs

Inhibition of the K^+ channels by capsaicin should result in a depolarization of V_m , and this in turn may cause Ca^{2+} entry via the voltage-dependent Ca^{2+} channels that have previously been identified in peripheral endings of capsaicin sensitive afferents [9]. To test this hypothesis, the effects of membrane depolarization and of capsaicin on $[Ca^{2+}]_i$ were examined. In the first series of experiments, the effects of depolarizing cells with a superfusate containing 60 mM K^+ (replacing 60 mM Na^+) were investigated. Fig. 6A shows typical $[Ca^{2+}]_i$ transients from the single TRCs induced by the high K^+ bath solution. Exposure to the 60 mM K^+ increased intracellular $[Ca^{2+}]_i$ ($F_{340}:F_{380}$) from 1.12 ± 0.03 in the resting state to 2.24 ± 0.15 ($n = 14$). The high K^+ bath solution did not evoke an increase in $[Ca^{2+}]_i$ in Ca^{2+} -free bath solution ($n = 4$). These data suggest that the high K^+ superfusate causes an increase in $[Ca^{2+}]_i$ by opening the voltage-gated Ca^{2+} channels.

Capsaicin caused an increase in $[Ca^{2+}]_i$ in 28 of 91 cells (31%). Fig. 6B shows that $[Ca^{2+}]_i$ increased in a single TRC in response to three different capsaicin concentrations: 10, 50 and 100 μ M. The magnitude of the transient elevation of $[Ca^{2+}]_i$, increased with the increasing capsaicin concentrations. 100 μ M capsaicin caused a significant increase in $[Ca^{2+}]_i$ from 1.04 ± 0.05 in the resting state to 1.40 ± 0.05 ($P < 0.05$; $n = 28$). An increase in $[Ca^{2+}]_i$ was also observed with 50 μ M capsaicin (Fig. 6B), but not at lower concentrations (data not shown). An increase in $[Ca^{2+}]_i$ was induced by 2% EtOH (i.e., the vehicle for 100 μ M capsaicin) five out of eight cells. The magnitude of the $[Ca^{2+}]_i$ increase was 0.06 ± 0.01 ($n = 5$, $P < 0.05$ compared to control). This increase in $[Ca^{2+}]_i$ was, however, significantly less than that observed with 100 μ M capsaicin (0.36 ± 0.05 , $P < 0.05$, $n = 28$). A much smaller increase (0.02) was observed with 1% EtOH in one out of five cells.

Fig. 6C shows that the increase in $[Ca^{2+}]_i$ was not observed when 100 μ M capsaicin was applied in the presence of Ca^{2+} -free bath solution. This suggests that the effect of capsaicin stimulates Ca^{2+} influx into TRCs.

4. Discussion

Capsaicin is one of the most widely used of culinary spices. The effects of capsaicin on sensory nerves endings

Fig. 5. Continuous recording of single channel K^+ activity, before and during superfusion with 500 nM capsaicin (addition indicated by the arrow). The dotted lines represent the channel-closed current level. $-V_p$ was +20 mV throughout the experiment.

are well known; however, little is known about how it affects TRCs. In this study the effects of capsaicin on the electrical properties of TRCs isolated from the rat circumvallate papilla were examined.

4.1. TRC channel activity in the absence of capsaicin

In the absence of capsaicin, large outward whole-cell currents were observed at depolarizing potentials in over 50% of TRCs. The I - V relationship for these currents exhibited outward-rectification, and the reversal potential

(−53 mV) was close to E_K . The conductance was almost completely inhibited by 5 mM TEA^+ , and the reversal potential of the TEA^+ -insensitive current was shifted towards zero (−16 mV). These data suggest that the majority of the whole-cell conductance in rat TRCs is carried by delayed-rectifier K^+ channels. Such channels have been identified in previous studies of mammalian TRCs [1,2,10,15]. The reversal potential observed in the presence of TEA^+ , indicates that the remainder of the current was either leak or Cl^- current. In our experiments there was no evidence for the presence of A-type K^+

Fig. 6. Capsaicin increases $[Ca^{2+}]_i$ in TRCs. (A) Transient increase in $[Ca^{2+}]_i$ induced by a high K^+ (60 mM) bath solution. The high K^+ bath solution rapidly increased $[Ca^{2+}]_i$ from 1.25 in the resting state to 3.25 in 30 s. K^+ did not stimulate an increase in $[Ca^{2+}]_i$ in a Ca^{2+} free bath solution. (B) $[Ca^{2+}]_i$ response in a single TRC to 10, 50 and 100 μM capsaicin. (C) The capsaicin-induced increase in $[Ca^{2+}]_i$ was not observed in a Ca^{2+} -free bath solution.

currents, which have been observed in some studies of TRCs, e.g., larval tiger salamander and *Necturus* [4,23].

Single channel openings were observed over a range of applied potentials in single channel recordings from TRCs in the absence capsaicin. The I - V relationships for the channels were linear and gave a conductance of 55 pS. Currents reversed at an applied potential of -60 mV, which under the recording conditions employed, indicates that the channel is selective for K^+ . The P_o of the channel increased at depolarizing potentials. Previous single channel studies have identified K^+ channels with conductances from 30 to 175 pS, including one of 57 pS in the apical membrane in *Necturus* TRCs [7]. The properties of the 55-pS channel (i.e., K^+ selectivity and voltage-dependence) are consistent with those of the delayed rectifier conductance observed in the whole-cell experiments. Fur-

thermore, the increase of single channel P_o at depolarizing potentials may help explain the voltage-dependence of the TEA^+ block in the whole-cell experiments. Thus, it seems very probable that the 55-pS channels are the main contributor to the delayed-rectifier K^+ conductance.

4.2. Capsaicin inhibits K^+ channel activity in TRCs

K^+ channels are thought to be the target of at least three of the major taste modalities, i.e., sweet, sour and bitter [6,8,16]. The effects of capsaicin on K^+ channel activity in TRCs were therefore examined. Capsaicin inhibited the whole-cell K^+ currents, and also reduced the P_o of the 55-pS channel. In both the whole-cell and single channel experiments the time course of channel inhibition was similar (i.e., maximum within 2 min), providing further evidence that the 55-pS channels carry the whole-cell K^+ conductance. The mechanism of K^+ channel inhibition by capsaicin is not clear. However, capsaicin may act directly as channel blocker, in the same way as other taste modalities, i.e., sweet, sour, bitter [6,8,16], and fatty acids [10,13].

It is interesting to note that the concentrations of capsaicin (500 nM), which cause channel inhibition, are of the same order of magnitude as those employed in behavioral experiments on humans. These studies showed that when 0.6 μM capsaicin is placed on the human tongue, it induces a warm sensation. By contrast, a burning, stinging sensation is evoked at 3 μM , and a painful sensation at 15 mM [11,14,17]. Thus K^+ channel inhibition in TRCs may contribute to the generation of the 'warm sensation' elicited by capsaicin.

4.3. K^+ channel inhibition and $[Ca^{2+}]_i$

Inhibition of K^+ channels by capsaicin should cause a depolarization of the V_m of the TRCs. In the present study the effects of capsaicin on V_m were not measured directly; however, the reversal potential of the single channel currents does provide an indication of V_m . These experiments showed that the reversal potential in the presence of capsaicin was more depolarized compared control, suggesting that capsaicin does indeed depolarize V_m . K^+ channel block is also thought to cause TRC depolarization for at least three of the major taste modalities, i.e., sweet, sour and bitter [6,8,16].

TRCs are known to express voltage-gated Ca^{2+} channels [1,2,4,21], any depolarization in V_m may therefore result in an influx Ca^{2+} . The present study therefore examined the effects of capsaicin on $[Ca^{2+}]_i$. As expected, exposing TRCs to a K^+ -rich extracellular solution caused a transient $[Ca^{2+}]_i$ (measured by fura-2 microspectrofluorimetry). Furthermore the increase in $[Ca^{2+}]_i$ was dependent on extracellular Ca^{2+} , indicating that it was due to Ca^{2+} influx through the voltage-gated channels. Capsaicin also induced an increase in $[Ca^{2+}]_i$, which was

dependent on Ca^{2+} influx. These data therefore suggest that the depolarization of V_m caused when capsaicin inhibits the outward-rectifying K^+ channels, may cause an increase in $[\text{Ca}^{2+}]_i$ by opening voltage-gated channels. Previous studies have found similar effects of other taste modalities on $[\text{Ca}^{2+}]_i$ [24].

One problem with this explanation, however, is that the concentration of capsaicin required to produce a significant increase in $[\text{Ca}^{2+}]_i$ was several orders of magnitude greater than that required to inhibit the K^+ channels. This may be because the high concentration of EtOH vehicle has a non-specific effect on $[\text{Ca}^{2+}]_i$. Indeed control experiments indicate that 2% EtOH does cause a significant increase in $[\text{Ca}^{2+}]_i$; however, the increase was still significantly less than that observed with 100 μM capsaicin. Furthermore, capsaicin only caused an increase in $[\text{Ca}^{2+}]_i$ in about 30% of TRCs, a sub-population which is not too dissimilar in size to that in which capsaicin inhibits K^+ channel activity. A more likely explanation therefore is that the sub-micromolar concentrations of capsaicin which inhibit K^+ channel activity do cause an increase in $[\text{Ca}^{2+}]_i$, but that these are small and possibly localized so cannot be resolved by fura-2 microspectrofluorimetry. However, we cannot rule out the possibility that high concentrations of capsaicin have effects on other receptors expressed by the TRC. For instance the vanilloid receptor is known to cause Ca^{2+} influx via non-selective cation channels [5].

In conclusion capsaicin inhibits outward-rectifying K^+ channels in TRCs. The concentration of capsaicin which inhibit the channels are similar to those reported to elicit the warm sensation in humans. K^+ channel inhibition may therefore play an important role in capsaicin sensing.

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