Voltage-gated Inward Currents of Morphologically Identified Cells of the Frog Taste Disc

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Abstract

We used the patch clamp technique to record from taste cells in vertical slices of the bullfrog (Rana catesbeiana) taste disc. Cell types were identified by staining with Lucifer yellow in a pipette after recording their electrophysiological properties. Cells could be divided into the following three groups: type Ib (wing) cells with sheet-like apical processes, type II (rod) cells with single thick rod-like apical processes and type III (rod) cells with thin rod-like apical processes. No dye-coupling was seen either between cells of the same type or between cells of different types. We focused on the voltage-gated inward currents of the three types of cells. Type Ib and type II cells exhibited tetrodotoxin (TTX)-sensitive voltage-gated Na+ currents. Surprisingly, type III cells showed TTX-resistant voltage-gated Na+ currents and exhibited a lack of TTX-sensitive Na+ currents. TTX-resistant voltage-gated Na+ currents in taste cells are reported for the first time here. The time constant for the inactivating portion of the voltage-gated inward Na+ currents of type III cells was much larger than that of type Ib and type II cells. Therefore, slow inactivation of inward Na+ currents characterizes type III cells. Amplitudes of the maximum peak inward currents of type III cells were smaller than those of type Ib and type II cells. However, the density (pA/pF) of the maximum peak inward currents of type III cells was much higher than that of type Ib cells and close to that of type II cells. No evidence of the presence of voltage-gated Ca2+ channels in frog taste cells has been presented up to now. In this study, voltage-gated Ba2+ currents were observed in type III cells but not in type Ib and type II cells when the bath solution was a standard Ba2+ solution containing 25 mM Ba2+. Voltage-gated Ba2+ currents were blocked by addition of 2 mM CoCl2 to the standard Ba2+ solution, suggesting that type III cells possess the voltage-gated Ca2+ channels and they do classical (calcium-influx) synaptic transmission. It appears that type III cells are taste receptor cells.

Key words: chemical transmission, frog, taste cell, TTX-resistant Na+ current, type III cell, voltage-gated Ca2+ current

Introduction

In vertebrates, taste cells are found in clusters, known as taste buds, in the lingual epithelium. Taste cells can be divided into two major classes: elongated (type I, type II, type III) cells, which have processes extending into the taste pore, and ovoid basal (type IV) cells, which lie at the bottom of the taste bud without extending processes to the pore (for reviews, see Roper, 1989; Lindemann, 1996). Unlike mammalian taste buds with small apical pores, the frog taste organ (fungiform papilla) lacks a true pore and is disc-shaped. The frog taste discs contain diverse types of cells with different morphologies, but they contain all of the main morphotypes of taste cells that exist in vertebrates. On the basis of the structural features of taste cells, Osculati and Sbarbati (Osculati and Sbarbati, 1995) suggested that there were four major types (type I, type II, type III and type IV) of cells in the frog taste disc. Mucous (type Ia) cells have large cuboidal cell bodies in the surface layer of the taste disc and correspond to type I cells in the mammalian taste bud. Wing (type Ib) cells possess sheet-like processes that protrude between the mucous cells onto the surface. Rod cells are spindle-shaped cells with rod-like apical processes. They are subdivided into two groups: cells with thick apical processes (type II cells) and cells with thin apical processes (type III cells). Merkel-like basal (type IV) cells are located at the periphery of the taste disc and radiate with long processes into the center of the bottom of the taste disc. The above morphological observation was made using an electron microscope and partial serial reconstruction. Very recently, the morphology of viable taste discs of the frog was investigated using multi-photon microscopy (Li and
Transient TTX-sensitive inward Na\(^+\) currents and sustainedable and that type Ib, type II and type III cells displayedLucifer yellow. Thus, we use the terms type Ia, type Ib, type
III) of cells in slices of the frog taste disc by staining with
et al. (2001) identified four types (type Ia, type Ib, type
II and type III) of cells in slices of the frog taste disc without the use of proteolytic
enzymes and cell isolation (Takeuchi et al., 2001). In the
preparation used by Takeuchi et al. (2001), the cellular
organization of the taste disc was preserved. Takeuchi et al.
(2001) identified four types (type Ia, type Ib, type II and type
III) of cells in slices of the frog taste disc by staining with
Lucifer yellow. Thus, we use the terms type Ia, type Ib, type
II and type III cells for cells identified in a slice preparation.
They showed that type Ia cells were electrically non-excit-able and that type Ib, type II and type III cells displayed
transient TTX-sensitive inward Na\(^+\) currents and sustained
outward K\(^+\) currents in response to depolarization. Although
Takeuchi et al. (Takeuchi et al., 2001) reported the presence
of voltage-gated currents in type III cells, little is known
about the membrane properties of type III cells. It is im-
portant to investigate the membrane properties of type III cells,
because type III cells have synaptic-like junctions with nerve
terminals and are thought to be taste receptor cells (Oscu-lati
and Sbarbati, 1995). In the present study, we used a slice
preparation of the frog taste disc. The use of whole cell patch
clamp recording combined with cell identification using
Lucifer yellow enabled us to identify distinct subpopulations
of taste cells (type Ib, type II and type III cells) based on
their electrophysiological properties. The present study
focused on voltage-gated inward currents of type Ib, type II
and type III cells, and the properties of these cell types were
compared. We confirmed that type Ib and type II cells
possess voltage-gated Na\(^+\) currents that are sensitive to TTX
and lack voltage-gated Ca\(^{2+}\) currents, as was reported for
isolated wing cells and rod cells (Avenet and Lindemann,
1987a; Miyamoto et al., 1991; Bigiani et al., 1998). However,
we report here that voltage-gated Na\(^+\) currents that are
resistant to TTX and voltage-gated Ca\(^{2+}\) currents are
present in type III cells. This is the first report of the pres-
tence of TTX-resistant voltage-gated Na\(^+\) currents in verte-
brate taste cells and voltage-gated Ca\(^{2+}\) currents in frog taste
cells.

Materials and methods

Preparation

Bullfrogs (\textit{Rana catesbeiana}), weighing 200–400 g, were
anesthetized with urethane (3 g/kg body wt). The experi-
ments were performed in accordance with the Guidelines for
Animal Experiments at Iwate Medical University. The
tongues were removed from the animals. The fungiform
papillae were dissected out from isolated tongues. Each
fungiform papilla was cut vertically into \(\sim 100 \mu m\) slices with a
fine scalpel under a binocular dissecting microscope. Slices
were transferred into a recording chamber and fixed to the
bottom of the recording chamber with the cut surface facing
upward. The bottom of the recording chamber was previ-
ously treated with Cell-Tak (BD Biosciences, Bedford, MA)
to improve adhesion of the slices to the glass slide.

Recording

The recording chamber was placed on the stage of a micro-
scope (BX50WI, Olympus, Tokyo, Japan) equipped with
Nomarsky optics and a \(\times40\) water-immersion objective. Lucifer
yellow CH (Sigma, St Louis, MO) was added to the
whole-cell recording, and they could be identified at the end
of experiments. Fluorescent images of cells filled with
Lucifer yellow were obtained using a fluorescence system
(BX-FLA, Olympus) and photographed with a cooled CCD
camera (DP-50, Olympus). Data of the fluorescent images
were saved on a computer. Patch pipettes were made from
borosilicate glass capillaries (WPI, Sarasota, FL) using a
horizontal puller (P-97, Sutter, Novato, CA). When filled
with the intracellular solution, the patch pipette resistance
was 7–12 M\(\Omega\). Whole cell currents were measured at room
temperature using a patch-clamp amplifier (EPC 8, HEKA
electronik, Lambrecht, Germany). Signals were low-pass-
filtered at 3 kHz, recorded, and analyzed using a computer
equipped with an ITC-16 data acquisition interface
(INSTRUTECH, Port Washington, NY) and PULSE and
PULSE FIT software (HEKA electronik). IGOR software
(WaveMetrics, Lake Oswego, OR) was also used for analysis of the experimental data.

Solutions

Our standard bath solution was an amphibian physiological solution (APS) containing (concentrations in mM) 110 NaCl, 2 KCl, 2 CaCl2, 1 MgCl2, 20 glucose, 10 HEPES, buffered to pH 7.2 with NaOH. In some experiments, TTX (SANKYO Co., Tokyo, Japan) was dissolved in standard APS. The bath solution used for investigation of voltage-gated Ca2+ currents (standard BaCl2 solution) contained (in mM) 25 BaCl2, 80 tetraethylammonium chloride (TEACL), 0.001 TTX, 20 glucose, 10 HEPES, buffered to pH 7.2 with TEAOH. Two pipette solutions were used: one solution (standard KCl solution) contained (in mM) 105 KCl, 2 MgCl2, 5 EGTA, 10 HEPES, buffered to pH 7.2 with KOH, and the other solution (standard CsCl solution) contained (in mM) 105 CsCl, 2 MgCl2, 5 EGTA, 10 HEPES, buffered to pH 7.2 with CsOH.

Results

Cell types

Figure 1 shows a photomicrograph of the frog taste disc in vertical cross section. Mucous (type Ia) cells occupy the superficial layer (a in Figure 1) of the taste disc. Cell bodies of type Ib, type II and type III cells are all located in the intermediate layer (b in Figure 1) of the taste disc, but the location of cell bodies of type Ib cells in the intermediate layer is above the locations of type II and type III cells (Osculati and Sbarbati, 1995). Patch pipettes can be positioned in the intermediate layer under a microscope. Injection of Lucifer yellow enabled identification of taste cell types in a slice preparation after recording their electrophysiological properties.

Figure 2 shows examples of three cell types filled with Lucifer yellow. A type Ib cell had one sheet-like apical process reaching the free surface of the taste disc and one basal process that reached the base of the taste disc (Figure 2A). A type II cell had a spindle- or ovoid-shaped cell body with one rod-like apical process (Figure 2B). A type III cell had an ovoid- or spherical-shaped cell body with thinner rod-like apical processes (Figure 2C) than the apical processes of a type II cell. The single apical process of type III cell was tortuous, sometimes bifurcated (Figure 2C) or trifurcated. Some type III cells had furcated basal processes or several basal processes. Since the basal processes of type III cells ramified and have a variable shape in relation to the position of the cell body, the shape of basal processes of type III cell could not be shown in photographs satisfactorily.

In the present study, 34 type Ib cells, 40 type II cells and 48 type III cells were identified by Lucifer yellow staining combined with electrophysiological recording. We could not observe any dye-coupling between cells of the same type or between cells of different types.

Passive membrane properties

Resting potentials were measured under a current clamp condition. APS was used as a bath solution and standard KCl solution was used as a pipette solution. The values of resting potential in type Ib, type II and type III cells were $-59.1 \pm 2.5 \text{ mV (mean} \pm \text{SEM,} \ n = 6), -56.3 \pm 2.3 \text{ mV (} \ n = 6)$ and $-63.8 \pm 4.0 \text{ mV (} \ n = 8)$, respectively. The resting potentials in these cells were not significantly different ($t$-test, $P > 0.05$). Input resistances were measured by applying a 20 mV hyperpolarizing step pulse followed by a 20 mV depolarizing step pulse from a holding potential of $-80 \text{ mV}$. The values of input resistance in type Ib, type II and type III cells were $3.3 \pm 0.8 \text{ G}\Omega (\text{mean} \pm \text{SEM; } \ n = 10), 9.9 \pm 2.9 \text{ G}\Omega (\ n = 10)$ and $7.4 \pm 1.8 \text{ G}\Omega (\ n = 13)$, respectively. Input resistance of type Ib cells was significantly smaller than those of type II and type III cells ($t$-test, $P < 0.01$). Cell membrane capacitance was measured at a holding potential of $-80 \text{ mV}$. The values of cell membrane capacitance in type Ib, type II and

![Figure 1](http://chemse.oxfordjournals.org/)

Figure 1 Photomicrograph of a slice preparation viewed with Nomarsky optics. Scale bar, 20 µm. a, the superficial layer; b, the intermediate layer; c, the basal layer.
type III cells were 20.6 ± 1.5 pF (mean ± SEM; n = 10), 10.6 ± 1.5 pF (n = 12) and 4.3 ± 0.3 pF (n = 16), respectively. Cell membrane capacitance was significantly larger in type Ib cells than in type II and type III cells (t-test, P < 0.01).

Whole cell currents under voltage clamp
Voltage-gated ion currents in type Ib, type II and type III cells were recorded by application of depolarizing pulses from a holding potential of –80 mV. With APS in the bath and standard KCl solution in the pipette, the application of depolarizing pulses elicited transient inward currents and sustained outward currents in the three types of cells (Figure 2, bottom). Although Bigiani et al. (1998) reported that some isolated rod cells did not show any detectable transient inward currents, all three types of cells tested in slice preparations displayed transient inward currents.

Voltage-gated Na⁺ currents
It has been reported that substitution of CsCl for KCl in the pipette eliminated the outward currents from the three types of cells (Takeuchi et al., 2001). This implies that outward currents from the three types of cells are K⁺ currents. In the present study, we focused on the inward currents. In subsequent experiments, the properties of inward currents were investigated with the pipette containing CsCl.

So far, voltage-gated Na⁺ currents have been reported to be sensitive to TTX in isolated wing cells (Miyamoto et al., 1991; Bigiani et al., 1998) and rod cells (Avenet and Lindemann, 1987a), and in type Ib, type II and type III cells in slice preparations (Takeuchi et al., 2001). Figure 3 shows the sensitivities of voltage-gated Na⁺ currents to TTX. The transient inward currents recorded from a type Ib cell (Figure 3A) and a type II cell (Figure 3B) were blocked by addition of 1 µM TTX to APS. This effect was completely reversible. Surprisingly, 1 µM TTX in the bath solution did not block the transient inward currents recorded from a type III cell (Figure 3C). When sodium ions were replaced with N-methyl-D-glucamine (NMDG) ions in the bath, the inward currents recorded from a type III cell disappeared (see the third traces in Figure 3C). The results indicate that the inward currents in the three types of cells are carried through the voltage-gated Na⁺ channels. The effects of various concentrations of TTX on the peak inward Na⁺ currents of three types of cells are presented in Figure 4A.
The inward Na⁺ currents of type Ib and type II cells were almost completely eliminated by TTX in concentrations >0.5 µM. In type III cells, however, TTX at 0.1–5 µM did not affect the transient inward currents. Figure 4B shows that voltage-gated Na⁺ currents recorded from a type III cell were not affected even during application of a high concent-
These results indicate that voltage-gated Na+ channels of type Ib and type II cells are TTX-sensitive and that voltage-gated Na+ channels of type III cells are TTX-resistant.

The transient inward currents in the three types of cells were activated by depolarization exceeding –40 mV from a holding potential of –80 mV and peaked at approximately –20 mV (Figure 5). Peak amplitudes of the inward currents in type Ib, type II and type III cells were –1666 ± 124 pA (mean ± SEM; n = 9), –1523 ± 190 pA (n = 10) and –680 ± 50 pA (n = 14), respectively (Figure 6A). The peak amplitude was significantly smaller in type III cells than in type Ib and type II cells (t-test, P < 0.001). However, after normalizing the peak amplitude to the membrane capacitance, statistical analysis revealed that the current density was significantly smaller in type Ib cells than in type II and type III cells (t-test, P < 0.05) (Figure 6B).

As shown in Figure 3, the time course of inactivation of the voltage-gated Na+ current recorded from a type III cell was much slower than those from other types of cells. The time course of an inward Na+ current shown in Figure 7A illustrates the time to peak for the activating portion and the time constant for the inactivating portion in an inward Na+ current. The decay of the current was fitted with a single exponential function. As shown in Figure 7B, the values of the time to peak were significantly longer in type Ib cells than those in other types of cells (t-test, P < 0.001). The decay time constants of inward Na+ currents in individual cells are shown in Figure 7C. The mean values of the time constants in type Ib, type II and type III cells were 1.3 ± 0.1 ms (mean ± SEM; n = 9), 0.8 ± 0.1 ms (n = 10) and 4.4 ± 0.2 ms (n = 14), respectively. The differences among the three types of cells were statistically significant (t-test, P < 0.001).

The order for the slow inactivation of the voltage-gated Na+ channel was type III >> type Ib > type II. It is clear that slow inactivation of the voltage-gated Na+ channel characterizes type III cells. Therefore, we were able to distinguish type III cells from type Ib and type II cells by time courses of
inactivation of inward currents. Cells showing resistance to TTX exhibited slow inactivation of inward currents (Figure 7C).

Voltage dependence of inward Na\(^+\) current inactivation was investigated using a typical two-pulse protocol (prepulse and test pulse). The inward current \(I\), as obtained with a test pulse to \(-10\) mV from different holding potentials (at which cells were held for 1 s), was normalized to \(I_{\text{max}}\) measured at a holding potential of \(-100\) mV. The plot of \(I/I_{\text{max}}\) as a function of holding potential resulted in a sigmoid curve (Figure 8) that could be fitted by the equation

\[
\frac{I}{I_{\text{max}}} = \frac{1}{1 + \exp[(V - V_{0.5})/k]}
\]

where \(V_{0.5}\) is the membrane potential at which the current showed half-inactivation, and \(k\) is the slope. The values for half-inactivation voltage in type Ib, type II and type III cells were \(-41.2, -44.0\) and \(-39.8\) mV, respectively, and the slopes in type Ib, type II and type III cells were \(7.3, 6.7\) and \(5.4\) mV, respectively (pooled data from six cells in each cell type). Bigiani et al. (1998) reported that the value for half-inactivation voltage in isolated wing cells was \(-70.7\) mV, which was more negative than that of type Ib obtained here. The difference in results between the present study and the previous study is due to methodological variables for duration of the prepulse. In their experiments, the prepulse was applied to the cell for long periods of time (10 s). It has been shown that half-inactivation voltage in isolated rat taste cells was more negative in long duration of the prepulse than in short duration (Herness and Sun, 1995).

**Voltage-gated Ca\(^{2+}\) currents**

Ba\(^{2+}\) is known to pass through Ca\(^{2+}\) channels as well as or better than Ca\(^{2+}\) (Bean, 1989). To test for the presence of voltage-gated Ca\(^{2+}\) currents in the three types of cells, we used standard BaCl\(_2\) solution as a perfusion solution and standard CsCl solution as a pipette solution. Since the standard BaCl\(_2\) solution contained Ba\(^{2+}\), TTX and TEA\(^+\) in the absence of Na\(^+\), voltage-gated Na\(^+\) and K\(^+\) currents could not appear. Hence, the presence of voltage-gated Ba\(^{2+}\) currents would be obvious. We could not detect voltage-gated Ba\(^{2+}\) currents in type Ib and type II cells even in this experimental condition (Figure 9A,B). In type III cells, however, sustained inward currents were clearly detected in the standard BaCl\(_2\) solution (the third traces in Figure 9C and the middle traces in Figure 10A). No voltage-gated inward currents were elicited when 25 mM BaCl\(_2\) was replaced with 35 mM NMDG (the second traces in Figure

![Figure 8](http://www.chemse.oxfordjournals.org/content/8/1/67/F8.large.jpg)

**Figure 8** Inactivation of voltage-gated Na\(^+\) currents in the three types of cells. (A) Inward Na\(^+\) currents were elicited by application of depolarizing pulse (test pulse) to \(-10\) mV after holding the membrane potential (prepulse) of various amplitudes (\(-100\) to \(-20\) mV) for 1 s. (B) Peak currents \(I\) during a depolarizing pulse to \(-10\) mV were normalized with respect to that obtained at a holding potential of \(-100\) mV \(I_{\text{max}}\) and plotted as a function of holding potential. Bath solution, APS; pipette solution, standard 105 mM CsCl. Each point represents mean ± SEM (bars) for type Ib cells \((n = 6)\), type II cells \((n = 6)\) and type III cells \((n = 6)\). In each type of cell, the data points were fitted by a sigmoid curve. Half-maximal voltages for type Ib, type II and type III cells were \(-41.2, -44.0\) and \(-39.8\) mV, respectively, and the values of the slope were \(7.3, 6.7\) and \(5.4\) mV, respectively.
Addition of 2 mM CoCl₂, a known Ca²⁺ channel blocker, to the standard BaCl₂ solution blocked the voltage-gated Ba²⁺ currents. Current-voltage relationships for the peak inward currents with and without 2 mM CoCl₂ are shown in Figure 10B. The peak inward currents with CoCl₂ were then subtracted from those without CoCl₂ to obtain the Co²⁺-sensitive currents (Ba²⁺ currents). Ba²⁺ currents were activated around −40 mV and reached a peak at 0 mV. We concluded that voltage-gated Ca²⁺ channels are present in type III cells. However, the voltage-gated Ba²⁺ currents that appeared in the standard BaCl₂ solution rapidly waned with time after recording (rundown of Ba²⁺ currents). Furthermore, voltage-gated Ba²⁺ currents appeared when BaCl₂ was replaced with CaCl₂ (data not shown).

It should be noted that sustained inward currents independent of voltage appeared in type III cells when APS was replaced with the standard BaCl₂ solution (the middle traces in Figure 10A). The inward current measured at −80 mV was −17.6 ± 6.5 pA (mean ± SEM, n = 9). Since the inward current disappeared when the bath solution was changed to APS again (the right traces in Figure 10A), the inward component independent of voltage is not due to a leak current. Similar inward currents were also observed in bath solutions containing 25 mM Ca²⁺ (data not shown). The inward component was not reduced by 2 mM CoCl₂. Type III cells did not display rundown of the inward current independent of voltage. Inward currents could not be observed in type Ib and type II cells.

**Contribution of Na⁺ currents to action potentials in response to current injections**

To investigate the contribution of Na⁺ currents to action potential, cells were made to generate action potentials by depolarizing current pulses under current-clamp conditions. Type Ib, type II and type III cells generated action potentials when the bath perfusion was APS (Figure 11). In the presence of 1 μM TTX, type Ib and type II cells did not generate action potentials, but type III cells did generate action potentials. When the sodium ions in the bath solution were replaced with NMDG ions, type III cells no longer generated action potential (Figure 11C). These results indicate that TTX-sensitive Na⁺ currents contribute to the generation of action potentials in type Ib and type II cells and that TTX-resistant Na⁺ currents contribute to the generation of action potentials in type III cells.

**Discussion**

Membrane properties of frog taste organ cells have been extensively studied by using the patch-clamp technique to
isolated wing cells and rod cells (for review, see Lindemann, 1996). Wing cells can be readily identified by the presence of sheet-like apical process, whereas a rod-like apical process characterizes rod cells. On the basis of their structural features (Osculati and Sbarbati, 1995), rod cells have been divided into two subpopulations: cells having a pear-shaped body with one thick straight apical process (type II cells) and cells having a spherical cell body with thin apical processes and with several basal processes stemming directly from the cell body (type III cells). In isolated rod cells, however, it has not been possible to divide into the two subpopulations: type II and type III cells. It is probable that isolated type III cells with thin apical processes might lose their morphological features due to damage inflicted during the isolation procedure. Therefore, it is likely that information on membrane properties of type III cells can not be obtained from isolated rod cells. Takeuchi et al. (2001) used the patch clamp technique to record from cells in vertical slices of the frog taste disc. They morphologically identified type Ia (mucous), type Ib, type II and type III cells by staining the cells with Lucifer yellow after recording their electrophysiological properties and showed that type Ia cells lacked voltage-gated currents but that type Ib, type II and type III cells had voltage-gated inward Na\(^+\) currents and outward K\(^+\) currents. Our results obtained by using slice preparations confirmed and extended their findings by showing characteristics of voltage-gated inward currents of type Ib, type II and type III cells.

**Passive membrane properties**

A number of investigators have shown dye-coupling between neighboring cells in vertebrate taste buds (for reviews, see Lindemann, 1996; Bigiani, 2002), including those in frogs (Sata et al., 1992). Dye-coupled cells have a much higher membrane capacitance than that obtained from non-coupled receptor cells (Bigiani and Roper, 1993). In the present study, we failed to find dye-coupling between neighboring cells in slice preparations of the frog taste disc. This result is in agreement with that reported previously (Takeuchi et al., 2001). Hence, the present data were obtained from single cells.

The values of resting potential, input resistance and cell membrane capacitance in the three types of cells were measured. The resting membrane potential values in the three types of cells were almost identical, around –60 mV. Cell membrane capacitance was much larger in type Ib cells than in type II and type III cells. Input resistance was smaller in type Ib cells than in the other cell types. The findings for cell membrane capacitance and input resistance are consistent with differences between membrane surface areas of the apical processes in type Ib and other types of cells (type II and type III cells). The values of resting potential, input
resistance and cell membrane capacitance in type Ib cells in slice preparations were similar to those obtained from isolated wing cells (Bigiani et al., 1998), suggesting that the membrane electrical properties of isolated wing cells were preserved during the dissociation procedure. For type II and type III cells, the mean values of 9.9 GΩ in type II and of 7.4 GΩ in type III obtained in the present study were higher than the mean value of 4.1 GΩ obtained in isolated rod cells (Bigiani et al., 1998). The use of a proteolytic enzyme during the dissociation procedure may alter the electrophysiological membrane properties of isolated rod cells. Bigiani et al. (1998) reported that some rod cells did not show any detectable voltage-gated Na⁺ currents and possessed K⁺ currents only. We could not find any cells (type Ib, type II and type III cells) lacking voltage-gated Na⁺ currents in our slice preparations. Okada et al. (2001) reported that almost all isolated rod cells of frogs displayed transient inward currents. In that study, the mean value for the input resistance of isolated rod cells was 7.0 GΩ. This value in isolated rod cells was close to the mean value of 9.9 GΩ in type II and of 7.4 GΩ in type III obtained here. It is possible that membrane properties of cells are not always altered by cell dissociation.

**TTX-sensitive and TTX-resistant Na⁺ currents**

Voltage-gated inward Na⁺ currents have been found in many vertebrate taste cells (for review, see Lindemann, 1996; Bigiani, 2002). They are sensitive to TTX. In frogs, TTX-sensitive voltage-gated Na⁺ currents have been reported in isolated wing cells (Miyamoto et al., 1991; Bigiani et al., 1998) and rod cells (Avenet and Lindemann, 1987a) and in type Ib, type II and type III cells of slice preparations (Takeuchi et al., 2001). We also showed that type Ib and type II cells had TTX-sensitive voltage-gated Na⁺ currents. However, it is surprising that all type III cells investigated in the present study had TTX-resistant voltage-gated Na⁺ currents. This may indicate that membrane properties of type III cells are preserved during the dissociation procedure.
Na⁺ currents and lacked TTX-sensitive Na⁺ currents. Although electrical signals of excitable cells in vertebrates are fundamentally dependent on TTX-sensitive voltage-gated Na⁺ channels, TTX-resistant Na⁺ currents have been found in a variety of animals and tissues, such as mammalian dorsal root ganglia, petrosal ganglia, nodose ganglia and small-diameter peripheral neurons (for review, see Yoshida, 1994). It has been shown that the activation and inactivation process of TTX-resistant Na⁺ channels is slower than that of TTX-sensitive Na⁺ channels (for review, see Yoshida, 1994). In the present study, the time constant for the inactivating portion of the TTX-resistant Na⁺ currents was much larger than that of TTX-sensitive Na⁺ currents (Figure 7). Hence, slow inactivation of inward Na⁺ currents characterizes type III cells.

Our data are very different from those obtained by Takeuchi et al. (2001), who reported that voltage-gated Na⁺ currents of type III cells were sensitive to TTX. It is probable that the difference in results between the present study and the previous study is due to methodological variables for identification of cell types. In the study by Takeuchi et al. (2001), cell types were identified only by their morphological features. The numbers of cells used in experiments to examine the sensitivity to TTX were not stated in their paper. In our study, the time course of the inactivating portion of voltage-gated Na⁺ currents, combined with cell type identification using Lucifer yellow staining, enabled us to identify type II and type III cells. All type III cells identified by both their morphological features and slow inactivation of inward Na⁺ currents were insensitive to TTX, and other types of cells were sensitive to TTX (Figure 7).

Although sensitivity to TTX in voltage-gated Na⁺ currents of type III cells differs from that of type Ib and type II cells, some properties of voltage-gated Na⁺ currents in the three types of cells are identical. As shown in Figure 5, in the three types of cells, Na⁺ current was activated at –40 mV and reached a peak at –20 mV (Figure 5). The kinetics of Na⁺ channel inactivation determined by the use of a two-pulse protocol are shown in Figure 8. Half-inactivation potentials for the three types of cells were almost the same, between –40 and –44 mV. However, amplitudes of the maximum peak currents of type III cells were smaller than those of type Ib and type II cells (Figures 5 and 6A). This finding is consistent with that previously reported (Takeuchi et al., 2001). From the characteristics of the small inward current of type III cells, Takeuchi et al. (2001) suggested that type III cells are immature cells, still in the midway stage of development, because expression of voltage-gated inward currents is incomplete in immature cells (Mackay-Sim et al., 1996). However, the density (pA/pF) of the maximum peak inward currents of type III cells was much higher than that of type Ib cells and close to that of type II cells (Figure 6B). Therefore, it is likely that type III cells are mature cells.

Voltage-gated Ca²⁺ currents and chemical transmission

By using the whole-cell voltage clamp technique, voltage-gated Ca²⁺ channels have been found in taste cells of amphibians (for review, see Bigiani, 2002), rats (Behe et al., 1990) and mice (Medler et al., 2003). However, there is no evidence of the presence of voltage-gated Ca²⁺ channels in isolated frog wing cells (Miyamoto et al., 1991; Bigiani et al., 1998) or rod cells (Avenet and Lindemann, 1987a). In the present study, we observed voltage-gated sustained Ba²⁺ inward currents in type III cells in standard BaCl₂ solution. The voltage-gated inward currents were blocked by addition of 2 mM Co²⁺ to the standard BaCl₂ solution. We concluded that voltage-gated Ca²⁺ channels are present in type III cells. Classical chemical synapses are usually associated with voltage-gated Ca²⁺ channels. Since type III cells are able to generate action potentials (Figure 11) and they form synaptic-like contacts with afferent axons (Osculati and Sbarbati, 1995), it appears that type III cells are taste receptor cells. However, it is unclear whether taste stimulation of type III cells in the frog produce depolarization that reach threshold and evoke action potentials. It has been demonstrated that amphibian taste cells are able to generate action potentials in response to a chemical stimulus (CaCl₂) applied to the mucosal surface (Avenet and Lindemann, 1987b).

Sustained inward currents independent of voltage appeared only in type III cells when APS was replaced with the standard BaCl₂ solution that contained 25 mM BaCl₂ and 80 mM TEACl. It has been shown that various salts applied to the tongue produce neural response in the frog glossopharyngeal nerve (Kusano, 1960; Nomura and Sakada, 1965; Kitada, 1978; Kitada, 1994). In pilot work, we observed that application of 25 mM BaCl₂ alone, 80 mM TEACl alone or the standard BaCl₂ (CaCl₂) solution with and without 2 mM CoCl₂ used in this study to the tongue is effective for producing neural responses. Therefore, it is probable that the standard BaCl₂ solution activates a cationic conductance in type III cells as a taste stimulus and induce an inward current. Additional studies are needed to clarify the characteristics of the inward current.

We show that type Ib and type II cells are able to generate action potentials but they lack voltage-gated Ca²⁺ currents. Anatomical studies reported that type Ib cells lack any obvious contacts with afferent axons (Osculati and Sbarbati, 1995). Based on electrophysiological studies, Bigiani et al. (1998) pointed out that the presence of ion channels in wing (type Ib) cells might be suggestive of a role in controlling the microenvironment inside the taste organs or the functioning of chemosensory cells or both. However, it is unknown how type Ib cells participate in the taste transduction. Since type II cells contact with afferent axons (Osculati and Sbarbati, 1995), it is possible that type II cells serve as taste receptors. The lack of voltage-gated Ca²⁺ currents in type II cells suggests that type II cells may not communicate with nervous system using conventional synapse. If type II cells
participate directly in taste transduction, the transmission from type II cells to afferent axons would be mediated by Ca\textsuperscript{2+} release from intracellular stores or Ca\textsuperscript{2+} influx through Ca\textsuperscript{2+}-permeable non-selective cation channels. In rat taste cells, denatonium, a bitter-tasting compound, induces Ca\textsuperscript{2+} release from intracellular stores (Akabas et al., 1988). It has been proposed that bitter and sweet tastants might activate phosphodiesterase via gustducin, resulting in a reduction of cyclic nucleotide level in taste cells (Wong et al., 1996). The reduction of cyclic nucleotide levels in taste cells resulted in cell depolarization (Kolesnikov and Margolskee, 1995). Saccharin activates cation conductance via inositol 1,4,5-trisphosphate production in a subset of isolated rod cells in the frog (Okada et al., 2001).

**Morpho-functional correlation for taste cells**

The study to correlate electrophysiological properties with taste cell structural features was performed with isolated Necturus taste cells (McPheeters et al., 1994). The taste cells investigated were identified after recording by electron microscope. In that study, dark (type I) cells had voltage-gated Na\textsuperscript{+}, K\textsuperscript{+} and Ca\textsuperscript{2+} currents. Light (type II) cells were divided into two functional populations based upon electrophysiological criteria: cells with voltage-gated Na\textsuperscript{+}, K\textsuperscript{+} and Ca\textsuperscript{2+} currents and cells with only voltage-gated outward K\textsuperscript{+} currents. Very recently, morpho-functional correlation for isolated mammalian taste cells was reported (Medler et al., 2003). Taste cell types isolated from mouse vallate and foliate papillae were identified by using antibodies to external epitopes. Antigen H-immunoreactive (-IR) (type I) cells, many antigen A-IR (type II) cells and all gustducin-expressing (type II) cells had small voltage-gated inward Na\textsuperscript{+} and outward K\textsuperscript{+} currents but no voltage-gated inward Ca\textsuperscript{2+} currents. In contrast, a subset of antigen A-IR (type II) cells and all neural cell adhesion molecules-IR (type III) cells had large voltage-gated Na\textsuperscript{+}, K\textsuperscript{+} as well as voltage-gated inward Ca\textsuperscript{2+} currents. In frog taste cells, we showed that type I and type II cells had TTX-sensitive voltage-gated Na\textsuperscript{+} currents, voltage-gated K\textsuperscript{+} currents but no voltage-gated Ca\textsuperscript{2+} currents and that type III cells had TTX-resistant voltage-gated Na\textsuperscript{+}, voltage-gated K\textsuperscript{+} and voltage-gated Ca\textsuperscript{2+} currents. The previous data and our data suggest that there is a good correlation between electrophysiological properties and cell morphotypes in vertebrate taste organs.

**Acknowledgements**

We thank Drs K. Okuda-Akabane and K. Narita for their critical comments on an earlier draft of the manuscript. We are grateful to Professor M. Kubota, School of Dentistry, Iwate Medical University, for his contributions to this study. This work was supported by a Grand-in-Aid for Scientific Research (no. 14571771) to Y. Kitada and High Performance Biomedical Materials Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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Accepted November 17, 2003