Vasopressin Modulates Membrane Properties of Taste Cells Isolated from Bullfrogs

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Abstract

The effect of arginine vasopressin (AVP) on the membrane properties was analyzed in isolated bullfrog taste cells using a perforated whole-cell patch-clamp technique. AVP (100 nM) induced three kinds of responses in rod-type taste cells: appearance of inward current, inhibition of voltage ramp-induced outward current and enhancement of the outward current. The Ca^{2+}-ionophore ionomycin (3 μM) also induced inward current in taste cells. A membrane-permeable cAMP analog, 8-CPT-cAMP (0.3 mM) inhibited voltage ramp-induced outward current in some rod cells, but enhanced the current in other rod cells. The results suggest that AVP may increase either intracellular Ca^{2+} level or cAMP level in taste cells, modulating the membrane excitability. **Chem. Senses 21: 739 –745,1996.**

Introduction

Modulation of taste cell function has been suggested by studies on the effects of hormones and neurotransmitters on taste response (Gilbertson, 1993). It has been demonstrated that aldosterone and vasopressin regulate the gustatory neural response elicited by NaCl in rat and frog (Kosten and Contreras, 1990; Okada *et al.*, 1990, 1991; Herness, 1992). Furthermore, we showed that vasopressin also enhances the gustatory response to an acid stimulus (Okada *et al.*, 1991), consistent with the experiment that vasopressin enhances Na^+ and H^+ currents in isolated hamster taste cells (Gilbertson *et al.*, 1993). These results suggest that taste cells possess vasopressin-like receptors.

However, the target of vasopressin in frog taste response remains unclear. In the present experiments, we show that vasopressin elicits three types of responses in isolated frog taste cells, suggesting that frog taste cells consist of different functional subtypes.

Materials and methods

Cell preparation

Twenty-one bullfrogs (*Rana catesbeiana*) weighing 250–550 g were used for the experiments over the course of a year. Taste cells were isolated from the tongue of decapitated and pithed animals as described before (Miyamoto *et al.*, 1991). Briefly, the fungiform papillae were dissected out from the tongue in Ca^{2+}-free saline. The papillae were bathed in Ca^{2+}-free saline containing 10 mM L-cysteine and 10 U/ml papain (Sigma P3125, St Louis, MO). The papillae were then rinsed with normal saline, and the individual cells were dissociated by gentle trituration in normal saline. Isolated taste cells showing characteristic morphology were readily distinguished from other cells, and were classified in two types of rod and forked cells (Figure 1). Rod cells had one dendrite-like process and forked cells had two or three dendrite-like processes.

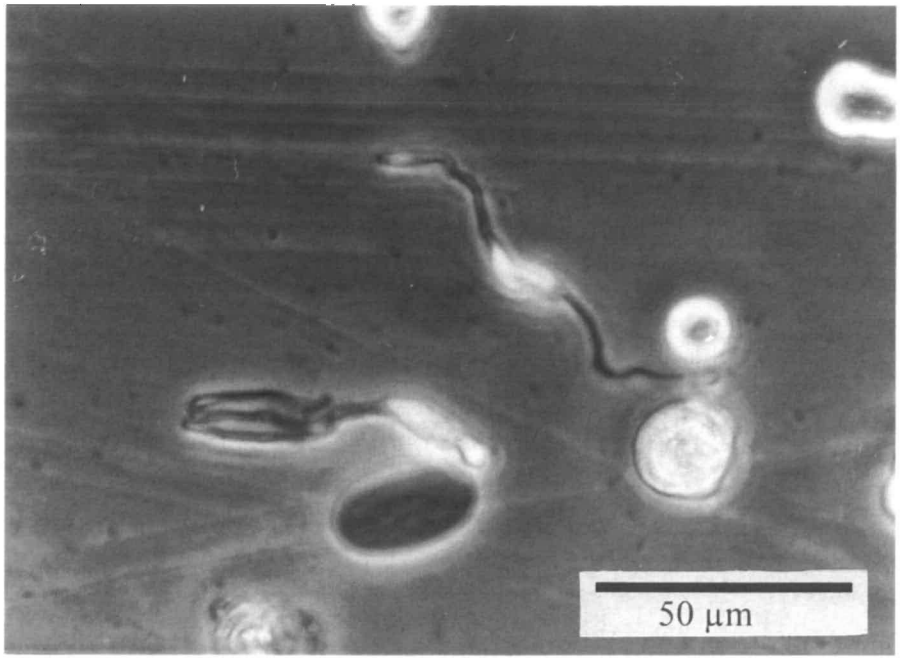

Figure 1 Photograph of taste cells isolated from fungiform papillae of bullfrog tongue taken through an inverted microscope with phase contrast. The taste cell on the right has one dendrite-like process (rod cell) and that on the left has three dendrite-like processes (forked cell).

Recording

Voltage clamp recording was performed in the whole-cell configuration (Hamil *et al.*, 1981) using a CEZ 2300 patch-clamp amplifier (Nihon Kohden, Tokyo, Japan). The patch pipets were pulled from Pyrex glass capillaries containing a fine filament with a two-stage puller (Narishige PD-5, Tokyo). The tips of the electrodes were heat-polished with a microforge (Narishige MF-83). The resistance of the resulting patch electrodes was 5–10 MΩ when filled with internal solution. Amphotericin B (133 μg/ml, Sigma A4888) was added to the pipet solution for the perforated method (Rae *et al.*, 1991). The formation of 5–20 GΩ seals between the pipet and the cell surface was facilitated by applying weak suction to the interior of the pipet. Recordings were made from taste cells settled on the bottom of a chamber placed on the stage of an inverted microscope (Olympus IMT-2, Tokyo). The recording pipet was positioned with a hydraulic micromanipulator (Narishige WR-88). The current signal was low-pass filtered at 5 kHz, digitized at 125 kHz, acquired at a sampling rate of 0.25 kHz and stored on an IBM-PC-compatible personal computer running pCLAMP 5.5 (Axon Instruments, Foster City, CA), which was also used to control the digital/analog converter for generation of the clamp protocols. The indifferent electrode was a chlorided silver wire. Capacitance and series resistance (3–5 MΩ) were properly compensated. The whole-cell current–voltage (I–V) relation- ship was obtained from the current generated by a 167 mV/s voltage ramp from −100 to 100 mV. Input resistance was calculated from the current generated by the voltage ramp from −100 to −50 mV.

Solutions and drugs

Normal saline solution consisted of (in mM): 115 NaCl, 2.5 KCl, 1.8 $CaCl_2$, 10 HEPES–Tris, 20 glucose, pH 7.2. For stock solution, ionomycin (1 mM, Calbiochem, La Jolla, CA) was dissolved in dimethyl sulfoxide. Aliquots of the stock solution were added to normal saline solution to give the desired final concentration. Arginine vasopressin (100 nM, Sigma V5501) and 8-(4-chlorophenylthio)-adenosine 3′:5′-cyclic monophosphate (8-CPT-cAMP, 0.3 mM, Sigma C3912) were dissolved in normal saline solution. The normal pipet solution contained (in mM): 100 KCl, 0.1 $CaCl_2$, 2 $MgCl_2$, 1 EGTA, 10 HEPES–Tris, pH 7.2.

All experiments were carried out at room temperature (20–25°C).

Results

When the perforated whole-cell configuration was obtained with a pipet filled with K^+-internal solution containing

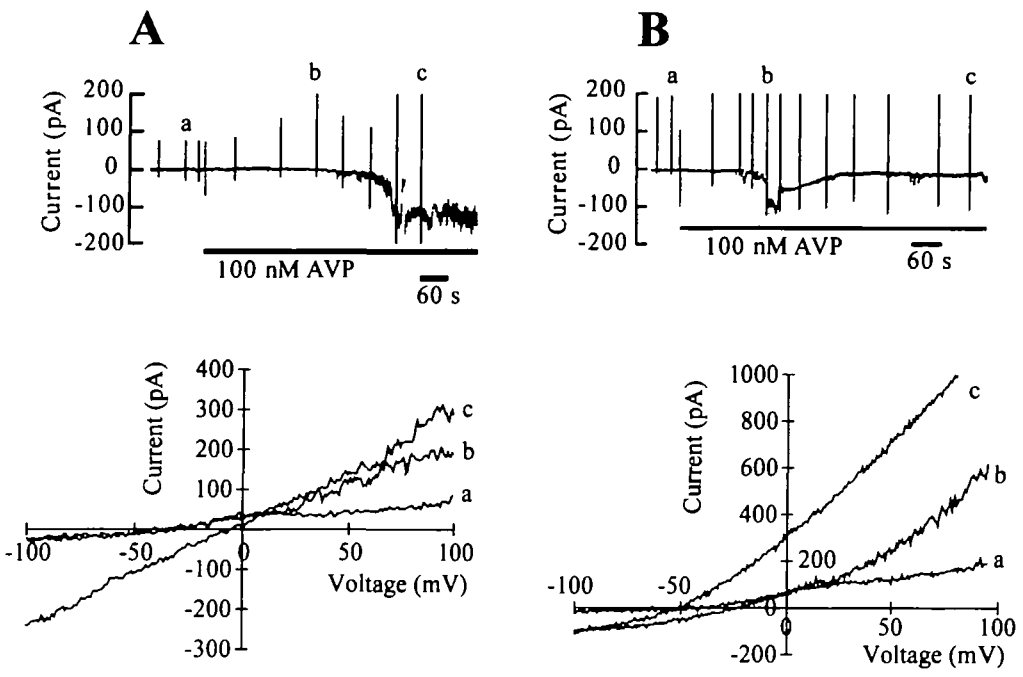

Figure 2 Sustained **(A)** and transient **(B)** inward currents induced by arginine vasopressin (AVP) in rod cells. In this and other figures, pen recordings of the current signals at a holding potential of −50 mV are displayed in upper side. Bottom plots are whole-cell current–voltage (I–V) relationships produced by a voltage ramp (167mV/s) from −100 to 100 mV. I–V relationships labeled small letters (a, b and c) were obtained at the times indicated by same letters in pen recordings. Some transient current deflections (c in A, b and c in B) induced by voltage ramp on pen recordings are out of scale. The pipet contained K^+-internal solution with amphotericin B (133 μg/ml).

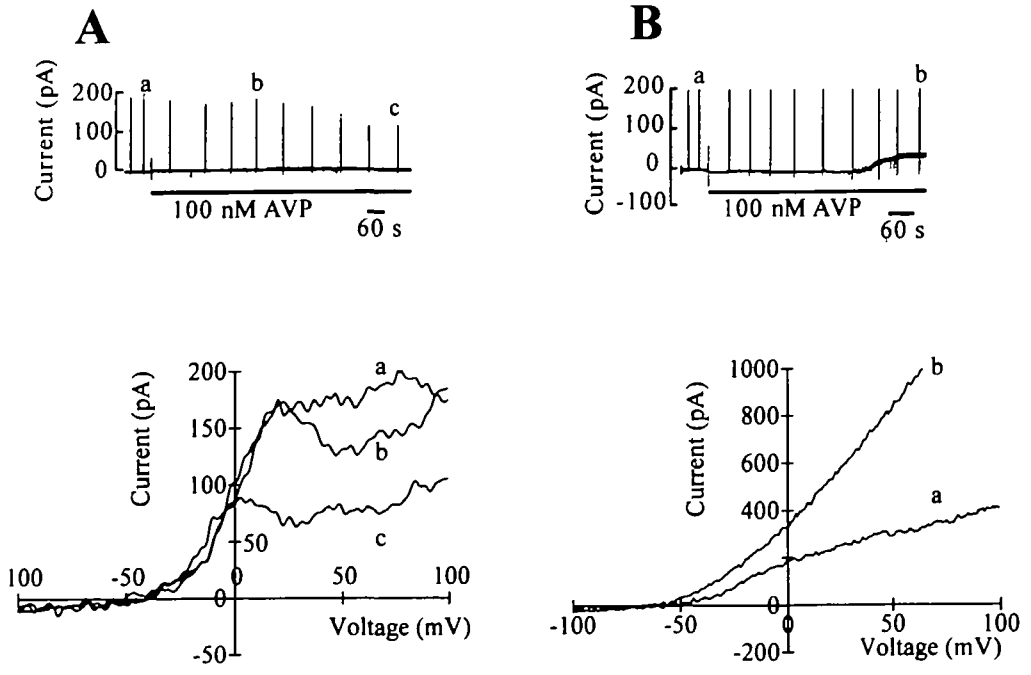

Figure 3 Inhibition **(A)** and enhancement **(B)** of voltage ramp-induced outward current elicited by AVP in rod cells. Some transient current deflections (a and b in B) on pen recordings are out of scale.

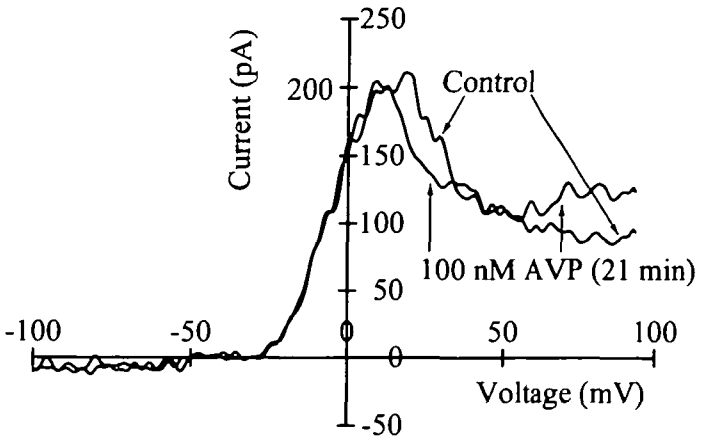

Figure 4 No effect of AVP on membrane properties of a forked cell.

amphotericin B, rod cells had resting potentials (0 current potentials) from –33 to –74 mV (–47 ± 1 mV, mean ± SE, *n* = 44). The input resistances ranged from 1.3 to 13.3 GΩ (5.9 ± 0.5 GΩ, n = 44) and the membrane capacitances ranged from 3.1 to 10.1 pF (5.6 ± 0.2 pF, *n* = 44). Forked taste cells displayed resting potentials from –33 to –71 mV (–46 ± 2 mV, *n* = 14), input resistances from 1.3 to 12.0 GΩ (7.9 ± 1.0 GΩ, *n* = 14) and membrane capacitances from 8.0 to 18.7 pF (12.9 ± 0.8 pF, *n* = 14). All rod and forked cells displayed transient inward currents followed by sustained outward currents in response to depolarizing voltage steps from a holding potential of –80 mV. Almost no rod cells displayed an inactivation of outward currents in the 100 ms step, but forked cells showed a gradual inactivation. The current–voltage relationship obtained by a voltage ramp was S-shaped or nearly linear in rod cells (e.g. see Figures 2Aa and 3Aa), but bell-shaped in forked cells (e.g. see Figure 4). Some rod and forked cells displayed an inward current in response to salt and acid stimuli.

Exchanging the normal saline solution with the solution containing 100 nM AVP induced a sustained inward current at a holding potential of –50 mV in three rod cells of one response group after voltage ramp-induced outward current had been transiently increased (Figure 2A). Three other rod cells of the same group displayed a transient appearance of the inward current at –50 mV by AVP (Figure 2B). On average, AVP increased the inward current at –50 mV by 54 ± 11 pA (*n* = 6), depolarized the resting potential from –42 ± 3 mV to –22 ± 4 mV (*n* = 6), and decreased input resistance from 4.1 ± 0.8 to 0.9 ± 0.2 GΩ (*n* = 6). In five rod cells of the second response group, AVP inhibited voltage ramp-induced outward current (Figure 3A), but did not

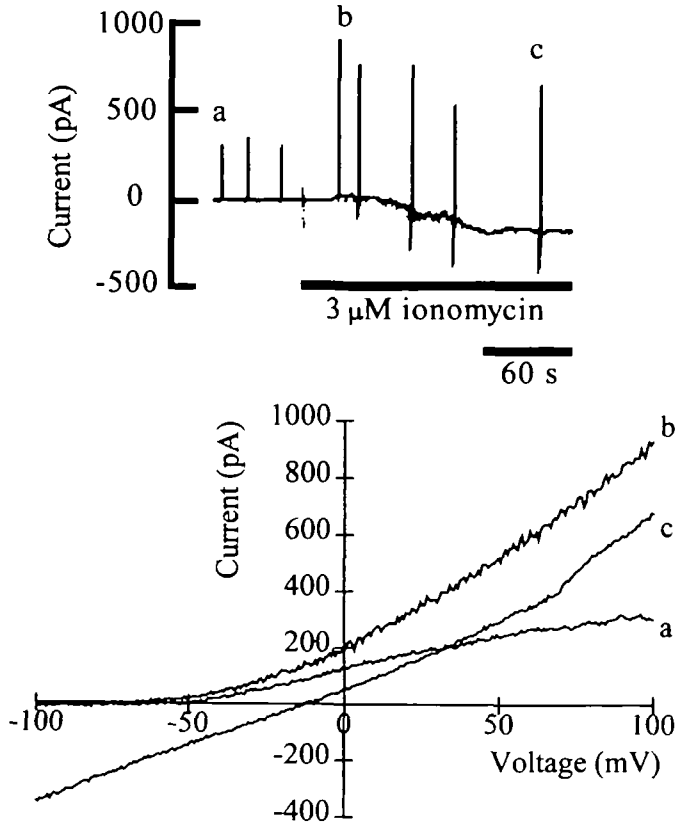

Figure 5 Effect of 3 μM ionomycin on the membrane properties of a rod cell.

change the magnitudes of resting potential and input resistance. AVP decreased the outward current at 50 mV by 161 ± 58 pA (*n* = 5). In four rod cells of third response group, AVP also increased the voltage ramp-induced outward current at 50 mV by 189 ± 118 (*n* = 4) (Figure 3B), but did not change the magnitudes of resting potential and input resistance. Even when the external solution was returned to normal saline solution, the current–voltage relationships in three kinds of responses did not recover to the initial level in 10 min. AVP did not exert any effect in 9 of 24 rod cells sampled. Forked cells (*n* = 10) did not display any response to AVP (Figure 4), except for one whose outward current was enhanced by the hormone.

When rod cells were exposed to a Ca^{2+}-ionophore, 3 μM ionomycin, the cells displayed a sustained inward current accompanied with a conductance increase after voltage ramp-induced outward current had been transiently increased (Figure 5). Ionomycin increased the current at –50 mV by 230 ± 20 pA (*n* = 8), depolarized the resting potential from –49 ± 3 to –7 ± 1 mV (*n* = 8), and decreased the input resistance from 4.8 ± 0.7 to 0.2 ± 0.1 GΩ (*n* = 8). The

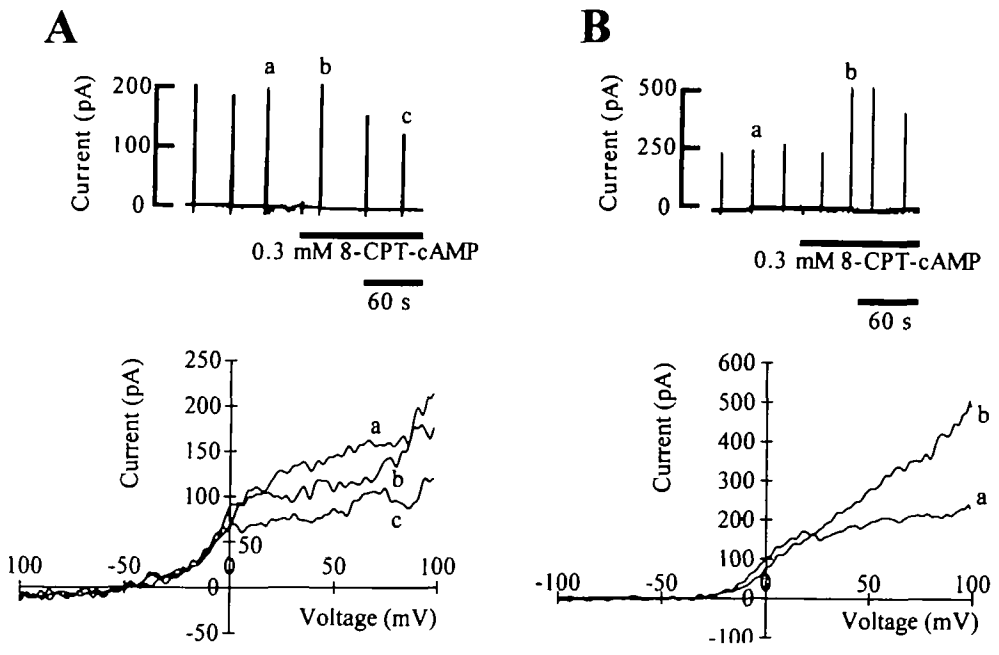

Figure 6 Effect of 0.3 mM 8-CPT-cAMP on the membrane properties of two rod cells. 8-CPT-cAMP decreased voltage ramp-induced outward current in a rod cell **(A)**, while the drug increased the current in another rod cell **(B)**.

Table 1 Number of rod-type taste cells affected by vasopressin, ionomycin and 8-CPT-cAMP

	Appearance of inward current at −50 mV	Inhibition of voltage ramp-induced outward current at 50 mV	Enhancement of voltage ramp-induced outward current at 50 mV	No effect	Total no. of cells examined
Vasopressin (100 nM)	6	5	4	9	24
Ionomycin (3 μM)	8	0	0	0	8
8-CPT-cAMP (0.3 mM)	0	4	4	1	9

Ca^{2+}-induced inward currents also appeared in cells whose voltages were clamped in the conventional whole-cell mode. When 0.3 mM 8-CPT-cAMP, which is a membrane-permeable cAMP analog, was added to the external solution, some rod cells displayed a decrease of voltage ramp-induced outward current at 50 mV from 160 ± 29 to 111 ± 32 pA (n = 4) (Figure 6A), while other rod cells were enhanced the current from 164 ± 14 to 222 ± 24 pA (n = 4) (Figure 6B). The magnitudes of resting potential and input resistance, however, were not affected by 8-CPT-cAMP. The rod cells in the conventional whole-cell clamp (n = 4) did not display the 8-CPT-cAMP-induced responses seen with the perforated whole-cell clamp. The effects of vasopressin, ionomycin and 8-CPT-cAMP on the membrane properties of frog taste cells are summarized in Table 1.

Discussion

The present results show that in rod-type taste cells of frogs three types of responses (appearance of inward current, inhibition of voltage ramp-induced outward current and enhancement of the outward current) were elicited by vasopressin. Vasopressin receptors are classified into two subtypes of V_1- and V_2-receptors (Kinter *et al.*, 1988). Vasopressin binding to V_1-receptors increases intracellular Ca^{2+} concentration via the increase of inositol 1,4,5-trisphosphate (IP_3) level, while the binding to V_2-receptors elicits the increase of intracellular cAMP level by stimulating adenylate cyclase. The characteristics of vasopressin-induced inward current were similar to those of ionomycin-induced current, suggesting that some rod

cells may have V_1-like receptors. On the other hand, inhibition and enhancement of voltage ramp-induced outward currents were elicited by vasopressin as well as by 8-CPT-cAMP, suggesting that other rod-type taste cells may have V_2-like receptors. However, it was confirmed that the V_2 receptor is linked to both adenylate cyclase and calcium mobilization in inner medullary collecting duct of rat kidney (Ecelbarger *et al.*, 1996). Vasopressin is produced in mammalian tissues, but not in amphibian tissues. The vasopressin-like hormones in amphibians are vasotocin and mesotocin. The receptor proteins for the hormones have been cloned (Mahlmann *et al.*, 1994; Akhundova *et al.*, 1996), and further studies should be undertaken using those hormones.

Previous studies have demonstrated that frog taste cells possess cAMP-blockable K^+ channels (Avenet *et al.*, 1988; Fujiyama *et al.*, 1994a). In the present study, we also found that cAMP as well as vasopressin enhanced outward current in some rod-type taste cells. However, the modulation of voltage ramp-induced outward current did not change the resting potential. Either the hormone- or ionomycin-induced depolarization accompanied an increase in conductance in other rod cells. We could not, therefore, relate cAMP to the frog taste transduction mechanism, but the result suggested Ca^{2+}-elicited enhancement of gustatory neural responses. The mechanism of cAMP-induced inhibition on the neural response (Nagahama and Kurihara, 1985; Okada *et al.*, 1991) was unclear in the present experiments.

We have demonstrated that the depolarization of frog taste cells induced by monovalent salts and acid stimuli is due to an amiloride-insensitive cation inflow (Miyamoto *et al.*, 1988, 1989; Okada *et al.*, 1994), and that vasopressin enhanced the neural responses for NaCl and HCl (Okada *et al.*, 1991). Both rod and forked cells could display inward currents in response to salt and acid stimuli, suggesting that both cell types have molecular apparatus for salt and acid transduction mechanisms. It is supposed that rod cells have a receptor site for vasopressin-like peptide, but that forked cells lack the site. The enhancing effect of vasopressin on the neural responses may be related to the vasopressin-induced inward currents in rod cells. It is concluded that vasopressin can modulate the taste response in some part of taste cells which have salt- and acid-transduction systems. Intracellular dialysis of IP_3 from patch pipet also induced inward current similar to ionomycin-induced current in 37% of taste cells sampled (Okada *et al.*, 1996), suggesting that vasopressin may increase intracellular Ca^{2+} level via IP_3 production. We could not find amiloride-sensitive Na^+ channels in bullfrog taste cells using microelectrode (Miyamoto *et al.*, 1989) and patch-clamp techniques (unpublished data), although Avenet and Lindemann (1988) reported the existence of the channels in another species of frog. Since amiloride has a bitter taste, the drug induced depolarization in bullfrog taste cells (Sato *et al.*, 1994) and elicited inward current (Kolesnikov and Margolskee, 1995). On the other hand, Fujiyama *et al.* (1994b) identified a Ca^{2+}-activated non-selective cation channel in bullfrog taste cells using single-channel recording. It was supposed that vasopressin-stimulated electrogenic sodium transport in amphibian epithelial cells might be linked to a Ca^{2+}-mobilizing signal transduction mechanism (Hayslett *et al.*, 1995). Interestingly, hamster taste cells have amiloride-sensitive Na^+ channels activated by cAMP as well as by vasopressin (Gilbertson *et al.*, 1993). Thus, the transduction mechanisms for salt and acid stimuli in frogs is different from those in mammals.

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