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Serotonergic Agonists Inhibit Calcium-Activated Potassium and Voltage-Dependent Sodium Currents in Rat Taste Receptor Cells

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Abstract. Recently we reported that rat taste receptor cells respond to the neurotransmitter serotonin with an inhibition of a calcium-activated potassium current [17]. In the present study, this observation is confirmed and extended by studying the effects of an array of serotonergic agonists on membrane properties, calcium-activated potassium current, and voltage-dependent sodium current in taste receptor cells using the patch-clamp recording technique in the whole-cell configuration. Serotonergic inhibition of calcium-activated potassium current was mimicked by the agonists N-(3-trifluoromethylphenyl)piperazine and by (±)-2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene. Both produced reversible inhibition of K_{Ca} as well as significantly increasing the input resistance of the cell. The agonists 1-(1-naphthyl)piperazine and buspirone (both serotonin receptor 1A agonists) were similarly effective in reducing K_{Ca}. Outward current was unaffected by application of phenylbiguanide, a serotonin receptor 3 agonist, though current was affected by subsequent application of (±)-2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene. Two agonists—N-(3-trifluoromethylphenyl)piperazine and (±)-2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene—were also tested on voltage-dependent sodium currents; both were effective and reversible in reducing its magnitude at a variety of applied potentials.

These data are consistent with the notion that serotonin effects in rat taste receptor cells are mediated by serotonin 1A receptors, though other receptor subtypes may be additionally expressed. Serotonin may affect the taste cell electrical properties during active stimulation in a paracrine fashion.

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Key words: Gustation — Signal transduction — 8OH-DPAT — TFMPP — Piperazine — $5HT_{1A}$ receptor

Introduction

Gustatory information is conveyed to the brain via two peripheral cell types—taste receptor cells of the oral cavity and primary afferent nerve fibers projecting to the medulla. These two cell types are synaptically connected within the taste bud; to date, the transmitter(s) at these synapses is not known with certainty. Experimental approaches towards establishing the identity of neurotransmitters within taste buds have taken two broad approaches—anatomical localization studies (e.g., with immunocytochemistry) and physiological studies (e.g., using electrophysiology and pharmacological manipulation). In mammalian taste systems, supportive evidence for multiple candidates, including norepinephrine, GABA, and serotonin, has emerged (reviewed in 27, 45). This evidence suggests the assumption that a single transmitter is operative in the taste bud may be an oversimplification.

Of the emerging candidates, the strongest evidence to date has accumulated for serotonin. In mammals, serotonin has been localized to taste receptor cells in a variety of species including mouse [37, 38, 41], rat [23], rabbit [12] and monkey [12] taste cells. These immunopositive cells display cytoplasmically localized reaction product and tend to be located at the periphery of the taste bud. In rabbit and monkey taste cells, serotonergic immunopositive cells are reported to occur in type III taste cells [12], the cell type visualized in these species to form synapses with the afferent nerve fiber [26]. In lower vertebrates, such as frog [33, 40] and mudpuppy [9, 22, 23], localization of serotonin has been reported in a unique type of basal cell, the Merkle-like basal cell,

rather than in taste cells, which lack serotonergic immunoreactivity. Additionally, in *Necturus,* Merkle-like basal cells have also been demonstrated to uptake serotonin [27].

Although immunocytochemistry has yielded strong evidence for the localization of serotonin in mammalian taste buds, fewer physiological studies are available. Recently, it was reported that calcium-activated potassium current in rat posterior taste receptor cells was inhibited by serotonin [17]. The observation that taste receptor cells, as opposed to neural elements, are responsive to transmitter suggests that in addition to afferent transmission, serotonin could play a role in information processing within the taste bud in either an autocrine or paracrine manner. Similar physiological studies have been performed on amphibian taste buds. *Necturus* taste receptor cells have been demonstrated to be responsive to serotonin [10] and multiple effects have been observed on calcium currents [8]. These studies have led to the notion of serotonin's role in information processing in the amphibian taste bud where Merkle-like basal cells may influence the electrical properties of taste receptor cells and consequently the neural output in response to taste stimuli.

Many questions remain to be resolved concerning the mechanism of serotonin inhibition of calcium-activated potassium currents in rat taste receptor cells. In particular, the subtype of serotonergic receptor expressed in taste cells and the signal transduction mechanism underlying their activation are of keen interest. Serotonin receptors are among the most complex and diverse of all neurotransmitters receptors. They are classified into seven major subtypes of receptor families, related by amino acid sequence, pharmacology, and intracellular mechanisms and include both metabotropic and ionotropic receptors (for reviews *see* 2, 21, 39, 42, 47). A variety of transduction mechanisms are utilized by these receptors. As well, the same receptor subtype may utilize different signal transduction mechanisms in different cell types. Finally, the effect of serotonin on calcium-activated potassium channels may occur via a transduction mechanism that terminates on these channels or could act by diminishing calcium currents that secondarily inhibit calcium-activated potassium currents.

The purpose of this investigation was to further study the inhibition of calcium-activated potassium channels using an array of serotonergic agonists, to provide some initial insight into receptor subtype, and to further explore the breadth of action of serotonin by including sodium currents in the analysis.

Materials and Methods

Preparation

All experiments were performed on isolated taste receptor cells dissociated from circumvallate and foliate papillae of the rat tongue using standard patch-clamp procedures in the whole-cell recording mode. All procedures were approved by the University's Laboratory Animal Care and Use Committee and adhered to the NIH "Guide for the Care and Use of Laboratory Animals." Taste receptor cells were dissociated from excised papillae of the posterior rat tongue as previously described [16]. Animals were anesthetized with an intramuscular injection of 0.09 ml/100 gm body weight Ketamine/Acepromazine mixture (91 mg/ml Ketamine, Fort Dodge Laboratories; 0.09 mg/ml Acepromazine, Butler Laboratories). Papillae were incubated in a cysteine-activated (1 mg/ml) Papain/divalent-free bicarbonate-buffered solution (14 U/ml) for several hours at 32°C in 5% CO2/95% Air. After incubation, cells were dissociated in a pseudo-extracellular fluid (ECF) by mild agitation. Some papillae were maintained in ice-cold ECF solution for later dissociation. Dissociated taste receptor cells were easily identified by their characteristic morphology.

Solutions

The divalent-free solution for enzymatic incubation was composed of (in mM): 80 NaCl, 5 KCl, 26 $NaHCO_3$, 2.5 $NaH_2PO_4 \cdot H_2O$, 20 D-glucose, and 1 EDTA. The standard ECF solution included 126 NaCl, 1.25 $NaH_2PO_4 \cdot H_2O$, 5 KCl, 5 NaHEPES, 2 $MgCl_2$, 2 $CaCl_2$, and 10 glucose; it was pH adjusted to 7.4 with NaOH. The composition of psuedo-intracellular fluid (ICF) used for filling the recording pipette consisted of 140 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 11 ethylenebis(oxonitrilo)tetraacetate (EGTA), 10 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES), and 4 ATP (disodium salt). Sodium currents were recorded with an ICF that additionally contained 10 mM 4-aminopyridine and with an ECF that contained 20 mM tetraethylammonium. Pharmacological agents were added directly to ECF solution. The source of all serotonergic agonists was RBI (Natick, MA). Our experience that many of these drugs needed to be freshly prepared immediately before testing concurred with reports that most were sensitive to photodegradation.

Whole-Cell Electrophysiological Recording

Microelectrode pipettes were pulled on a gas-cooled multi-stage puller from 1.5 mm (O.D.) borosilicate glass (World Precision Instruments, Sarasota, FL) and were fire polished. Resistances were typically 2–4 MΩ when filled with ICF and measured in ECF. Junction potentials were corrected before the electrode contacted the cell. The pipette tip was positioned to contact the cell membrane and negative pressure was applied to its interior to facilitate gigaseal formation. Seal resistances were on the order of several decades of gigaohms. Further negative pressure was applied to enter whole-cell recording mode.

Fast and slow capacitance compensation was employed as necessary with amplifier controls. Cell membrane capacitance and uncompensated series resistance were adjusted to produce optimal transient balancing. Membrane capacitance was 3–6 pF; series resistance averaged 10 MΩ. Low-pass filtering due to resistance-capacitance coupling was considered minimal. The product of these factors produces a time constant of 30–60 μsec or a cutoff frequency ($1/2\pi RC$) of 2.6–5.3 kHz.

Data were acquired with a high-impedance amplifier and a high resistance feedback headstage (Axopatch 1*B;* Axon Instruments, Foster City, CA), a 486 computer equipped with a 125 kHz A/D converter (LabMaster DMA; Scientific Solutions), and a commercially available software program (pCLAMP, version 6.0.3; Axon Instruments). Membrane currents were acquired after low-pass filtering with a cutoff frequency of 5 kHz (at –3 dB). A software-driven digital-to-analog converter generated the voltage protocols. A P/4 leak subtraction protocol was employed.

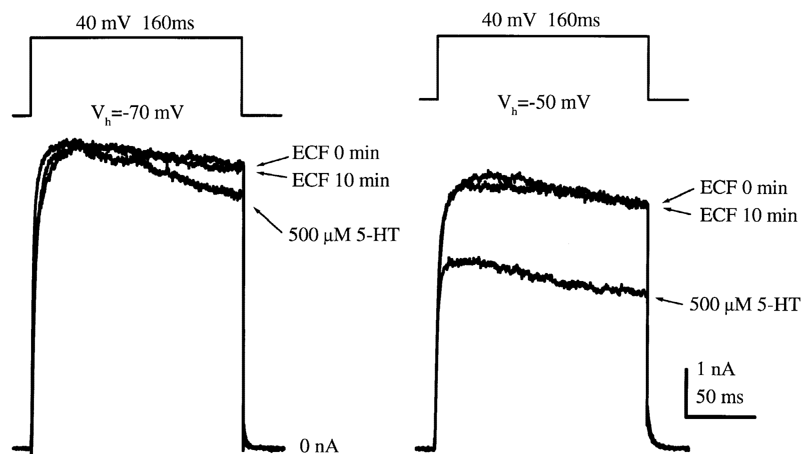


Fig. 1. Serotonin inhibits voltage-dependent outward current in dissociated rat taste receptor cells. Current evoked from the same cell at two different holding potentials (V_{hold}; –70 and –50 mV) is illustrated prior to and during application of 500 μM 5-HT. The voltage protocol used to evoke the current is illustrated at the top of the figure. In both cases, current magnitude was stable prior to application of 5-HT (0 and 10 min) but was diminished when 5-HT was added to the bathing solution. However, note that the inhibition was more profound at a holding potential of –50 mV. This more depolarized holding potential produces outward current that is highly enriched in calcium-activated potassium current.

Recordings were made at room temperature. Data are presented as mean ± SE.

Isolation of Calcium-Activated Potassium Current

We have previously established that voltage-clamped outward currents recorded from posterior taste receptor cells are an admixture of at least three distinct types of potassium current and, to a much smaller degree, an outwardly rectifying chloride current [3, 19]. The three types of outward potassium current include a 4-aminopyridine sensitive slow transient potassium current with decay time constants ranging from 1 to 8 sec, a tetraethylammonium-sensitive delayed-rectifier potassium current, and a calcium-activated potassium current. Inwardly rectifying potassium currents contribute a negligible amount to the outward current [35]. Both the transient and delayed-rectifier potassium currents are largely inactivated at more depolarized holding potentials whereas the calcium-activated potassium current is not. Hence by holding at –50 mV, rather than –70 mV, the total outward potassium current becomes highly enriched in the calcium-activated component. This is evidenced by increased sensitivity to reduced calcium ECF solutions and to apamin, a peptide inhibitor of small-conductance calcium-activated potassium current, at the more depolarized holding potentials. For example, using a test pulse to +40 mV, 200 nM apamin inhibited the current by 22 ± 1.5% when evoked from a holding potential of –70 mV but inhibited the current by 48 ± 2.2% if evoked from –50 mV (n = 8 cells). These currents were also sensitive to charybdotoxin (10^{-8} M), a blocker of large-conductance calcium-activated potassium channels. Charybdotoxin produced an inhibition of about 30% from a holding potentials of –50 mV. We conclude that outward potassium currents in posterior taste cells evoked from a holding potential of –50 mV are highly enriched in calcium-activated current that likely contains both small- and large-conductance calcium-activated potassium channels. Hence we use a test pulse from –50 to +40 mV for the remainder of the experiments as an assay of K_{Ca}. This test pulse is consistent with our previous analysis of serotonin on K_{Ca} on rat taste receptor cells [17].

Abbreviations

Serotonin, 5-HT; serotonin receptor subtype 1A, $5HT_{1A}$; serotonin receptor subtype 3, $5HT_3$; (±)-2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene, 8OH-DPAT; afterhyperpolarization, AHP; extracellular fluid, ECF; 11 ethylenebis(oxonitrilo)tetraacetate, EGTA; 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid, HEPES; intracellular fluid, ICF; calcium-activated potassium current, K_{Ca}; N-(3-trifluoromethyl-phenyl)piperazine, TFMPP.

Results

Serotonin Inhibits Calcium-Activated Potassium Current

The bath application of serotonin to dissociated taste receptor cells produced inhibitions of evoked outward potassium current that was dependent upon the membrane holding potential. Figure 1 illustrates representative current traces from a single taste cell at two holding potentials, –70 and –50 mV. After establishing a 10-min baseline to ensure stability of the current magnitude, application of 500 μM serotonin in this cell resulted in a 10% inhibition of the current if held at –70 mV and 36% inhibition if held at –50 mV. In all tested cells that were responsive to serotonin, inhibition of the evoked outward current was always greater at –50 mV than –70 mV indicating that serotonin is acting on the calcium-activated portion of this outward current. As previously reported (17), serotonin (at 100 μM) inhibited outward current by 12.9 ± 3% at –70 mV and by 48 ± 2% at –50 mV (n = 6 cells). Approximately 60% of the tested cells were sensitive to serotonin. Serotonin was effective as low as 20 μM and inhibitions at 500 and 1,000 μM were only slightly larger than 100 μM suggesting that the response-concentration function plateau occurs somewhat above 100 μM. In general, inhibitions produced by serotonin were of limited reversibility. Protracted rinse of the bathing solution did not return the test pulse response magnitude back to its original level. For several reasons we do not consider this lack of recovery to be due to the phenomenon known as rundown. First, current magnitudes in these and all cells were stable prior to application of drug. Rundown would have demonstrated a downward tendency in the response profiles prior to

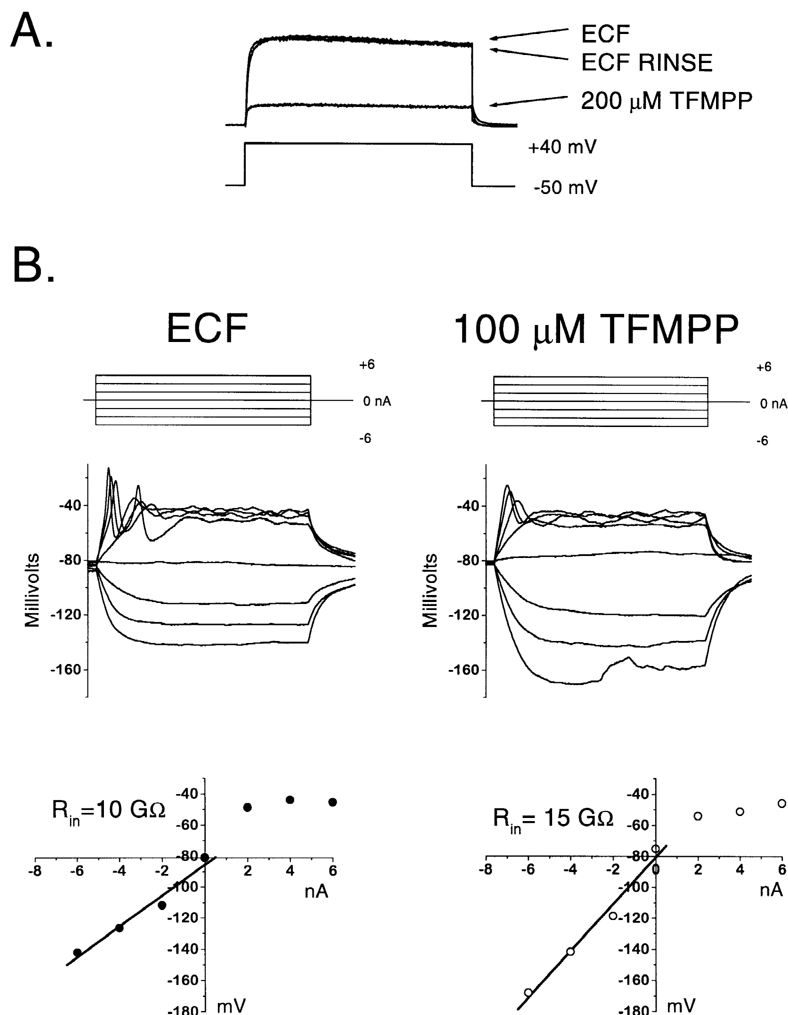


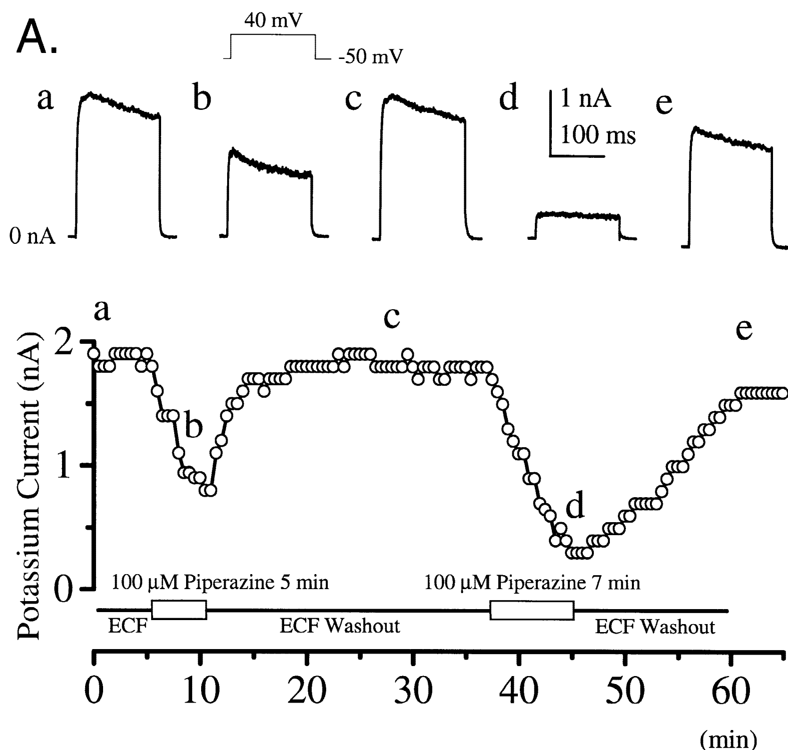
Fig. 2. The serotonin agonist TFMPP (N-(3-Trifluoromethylphenyl)piperazine) mimics the effect of serotonin inhibition on outward currents. Sample current traces are presented in *A* for a test pulse from −50 to +40 mV evoked prior to, during, and after the addition of 200 μM TFMPP to the bathing solution. TFMPP produced a strong inhibition of the current that was reversed when rinsed from the bath. When tested under current-clamp conditions, TFMPP was noted to increase the input membrane resistance of the cell. Data from a representative cell are illustrated in *B*. The cell was held at the zero current potential and a series of positive and negative current pulses were injected to ±6 nA. Input resistance was calculated from the linear portion of the negative quadrant of the current voltage plot. TFMPP increased the input resistance of this cell from 10 to 15 *G*Ω. In cumulated data TFMPP increased the input resistance by 30 ± 1.8% ($n = 4$ cells).

drug application. Second, rundown is more commonly attributed to calcium currents. We have previously established that sodium and potassium currents are stable for protracted recording periods. However, rundown of calcium current could secondarily diminish calcium-activated potassium current. If this were true, then current magnitudes should be less stable at −50 mV (enriched in calcium-activated potassium current) than when holding at −70 mV. This was not observed. Finally, application of agonists was reversible and usually accompanied by complete recovery of response magnitude demonstrating that recordings were stable for the recording session. It thus appears that inhibition produced by serotonin, rather than serotonergic agonist, is of more protracted time course, though at present the explanation of this phenomenon remains obscure.

TFMPP Is An Effective Inhibitor and Alters Membrane Properties

N-(3-Trifluoromethylphenyl)piperazine hydrochloride (TFMPP) is a serotonin receptor agonist with general specificity towards serotonergic receptor subtype and hence should be expected to mimic serotonin effects. We previously reported [17] that 100 μM TFMPP reversibly inhibits a test pulse from −50 to +40 mV by 73 ± 3.1% ($n = 9$ cells). We have additionally tested TFMPP at 50 μM and recorded inhibition from a −50 mV holding potential of 44 ± 2.2% ($n = 4$ cells). Unlike serotonin application, inhibitions produced by TFMPP were completely reversible within a 10 min rinse period. Often two applications of TFMPP were achievable during a recording period, both demonstrating reversibility. Higher concentration (500 μM) was also reversible. An example is illustrated in Fig. 2*A* for a test pulse presented before, during, and after application of 200 μM TFMPP.

Effects of TFMPP on the membrane properties of taste cells were also examined. TFMPP increased the input resistance of tested cells. Input resistance was measured by injection of a series of hyperpolarizing and depolarizing current injections during current clamp conditions. A typical result is presented in Fig. 2*B*. This cell was held at a zero-current potential and a series of ±6 nA currents of 160 msec duration were injected (upper



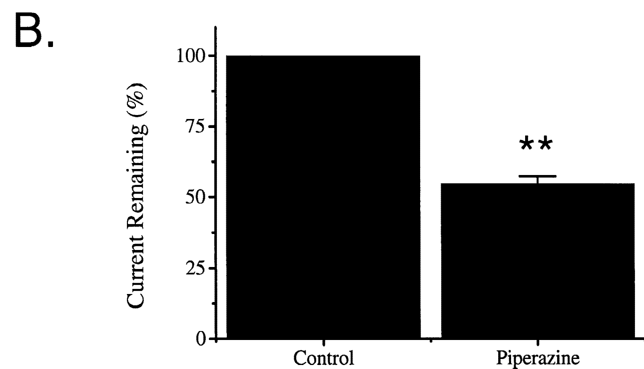


Fig. 3. Piperazine (1-(1-napthyl)piperazine) was an effective inhibitor of the outward current evoked from a holding potential of –50 mV. Data from a representative cell are illustrated in *A*. The response magnitude to the test pulse was recorded over a period of 60 min prior to, during, and after two applications to 100 µM piperazine. Individual current traces for the points labeled a–e are presented above the summary data. Note that piperazine application was reversible for both applications. In *B* summarized data of seven cells are presented. Piperazine inhibited the magnitude of the test pulse by 48.4 ± 2.6%. Asterisks indicate statistical significance ($P = 2.5 \times 10^{-6}$).

traces). Input resistance was calculated from the linear portion of the hyperpolarizing region where the contribution of active currents would be expected to be minimal. In the illustrated cell the input resistance increased from 10 to 15 MΩ. A mean increase of 30 ± 1.8% (n = 4 cells; $P = 0.00045$) in the resting membrane input resistance was observed. At present ionic conductances that contribute to the resting potential are not known with certainty though personal observations suggest that potassium, chloride, and likely some small sodium conductance are active at zero-current potentials in these cells.

1-(1-Naphthyl)piperazine Is An Effective Inhibitor of Calcium-Activated Potassium Current

1-(1-Naphthyl)piperazine hydrochloride has agonist properties at 5-HT_1 receptors with antagonistic properties at 5-HT_2 receptors. The application of exogenous 100 µM 1-(1-Naphthyl)piperazine to a +40 mV test pulse delivered from a holding potential of –50 mV resulted in marked inhibition of the evoked outward current. Representative data from a single cell are presented in Fig. 3. Two applications of piperazine were applied during a 60 min recording session. Current traces at points marked a–e are illustrated at top. Both applications were reversible. Mean data of seven tested cells produced an inhibition of 48.4 ± 2.6% (Fig. 3*B;* $P = 2.5 \times 10^{-6}$).

Serotonin Inhibition Is Mimicked by the $5HT_{1A}$ Agonist 8-OH-DPAT and It Alters Membrane Properties

8-OH-DPAT ((±)-2-Dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene hydro-bromide) is a selective 5-HT_{1A} serotonin receptor agonist. We have previously reported [17] that the 5-HT_{1A} agonist 8-OH-DPAT (100

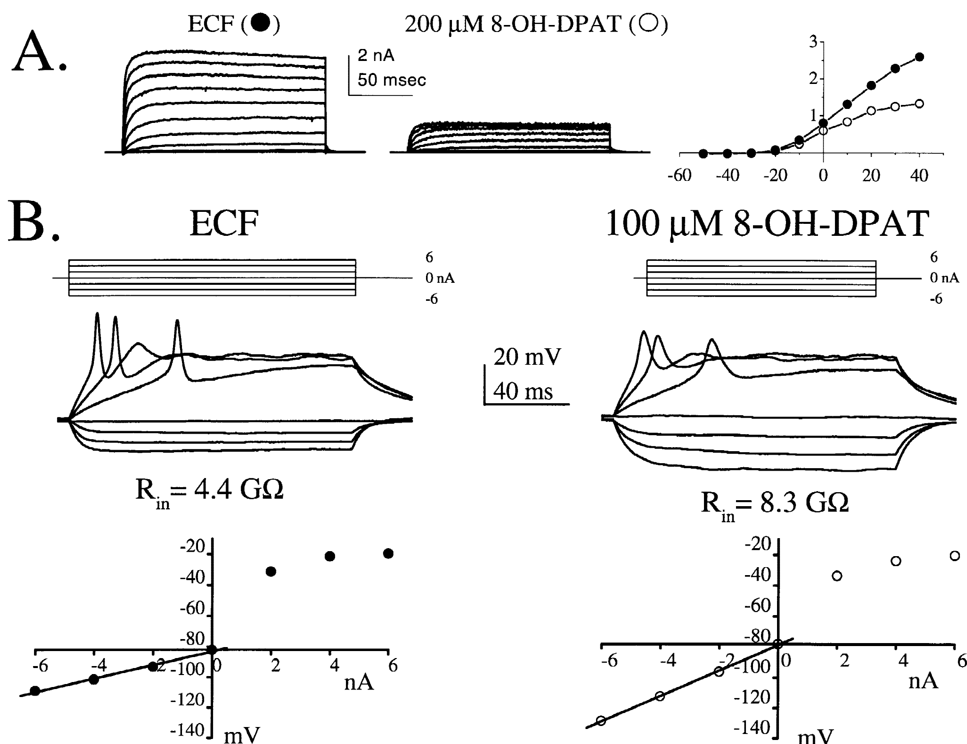


Fig. 4. The serotonin agonist 8-OH-DPAT, specific for $5\text{-}HT_{1A}$ receptors, was effective in inhibiting outward currents. A family of such currents, evoked from a holding potential of –50 mV, is presented in *A*. Sample currents prior to (●) and during (○) application of 200 μM 8-OH-DPAT are illustrated with the corresponding current-voltage relationship. 8-OH-DPAT produced a strong inhibition of the current that was completely reversible when rinsed from the bath and occurred over all suprathreshold potentials. When tested under current-clamp conditions, 8-OH-DPAT was noted to increase the input membrane resistance of the cell. Data from a representative cell are illustrated in *B*. The cell was held at the zero current potential and a series of positive and negative current pulses were injected to ±6 nA. Input resistance was calculated from the linear portion of the negative quadrant of the current voltage plot. In this cell 8-OH-DPAT increased the input resistance from 4.4 to 8.3 *G*Ω. In summarized data the input resistance was increased 40.8 ± 2.3% ($n = 4$ cells).

μM) is an effective and reversible inhibitor producing about a 70% inhibition at a holding potential of –50 mV [68.4 ± 2.6 ($n = 10$ cells)] but only about a 50% inhibition at –70 mV [49.8 ± 2.7 ($n = 6$ cells)]. A family of voltage-dependent outward currents, evoked using 10 mV command potentials to a final potential of +40 mV from a holding potential of –70 mV, are illustrated in Fig. 4*A* prior to and during application of 200 μM 8-OH-DPAT. The current-voltage plot for this cell is illustrated at the right. Note that inhibitions were produced at all membrane potentials that elicited outward current suggesting minimal voltage-dependence of the inhibition produced by 8OH-DPAT.

The effect of 8OH-DPAT on the input membrane resistance was also tested (Fig. 4*B*). Input resistance was measured as described in Fig. 2. A representative cell is illustrated in Fig. 4*B*. In this cell, application of 100 μM 8-OH-DPAT increased the membrane resistance from 4.4 GΩ to 8.3 GΩ. A mean increase of 40.7 ± 2.28% in the input membrane resistance was observed ($n = 4$ cells; $P = 0.00039$). These data are consistent with the results of TFMPP application suggesting that a calcium-activated potassium conductance is open at negative membrane potentials and is reduced by these agents.

The $5HT_{1A}$ Agonist Buspirone Was Effective in Reducing Outward Current

Buspirone (8-[4-[4-(2-Pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-7,9-dione HCl) is a $5\text{-}HT_{1A}$ receptor agonist used as a non-benzodiazepine anxiolytic. Buspirone, tested at 100 μM, was an effective inhibitor of the outward current evoked from a holding potential of –50 mV (Fig. 5). The top panel represents test pulses indicated at points *A, B,* and *C* on the middle panel. Inhibition developed within the first 2 min and was reversible with rinse of the drug from the bath. Recovery was complete within 10 min. Summarized data (bottom panel) produced a mean inhibition of 39.6 ± 1.6% ($n = 6$ cells; $P = 2.4 \times 10^{-6}$).

The $5HT_3$ Agonist Phenylbiguanide Was Ineffective

Phenylbiguanide (N-Phenyl-imidocarbonimidic diamide) is a $5HT_3$ receptor agonist. $5HT_3$ receptors are unique among serotonin receptor subclasses since they are ionotropic rather than metabotropic receptors.

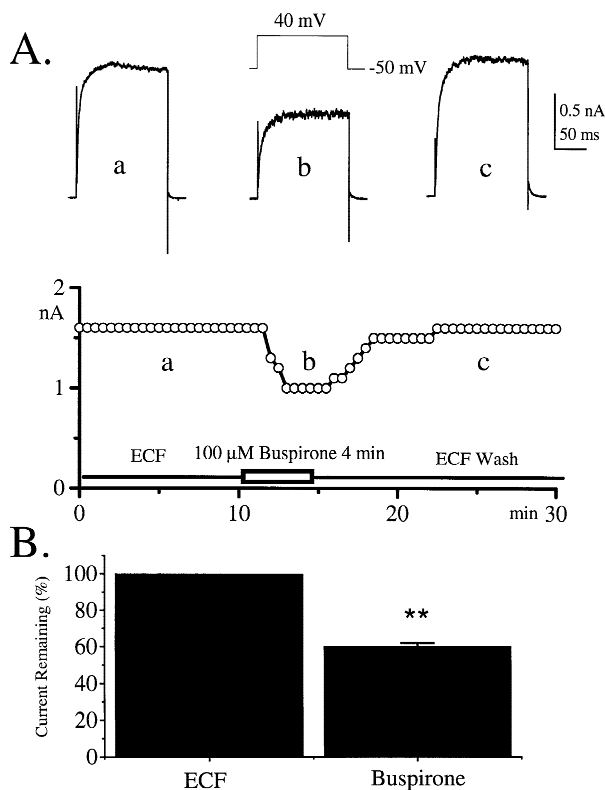


Fig. 5. Buspirone, a $5HT_{1A}$ receptor agonist, reduced the magnitude of outward current evoked by a test pulse from −50 to +40 mV. Data from a representative cell are illustrated in *A*. The current magnitude in response to the test pulse is illustrated in the bottom panel with sample test pulses from points marked *a, b,* and *c* illustrated at top. Buspirone, applied at 100 µM, reversibly inhibited the outward current with a time course that matched the delivery and removal of the drug from the bathing solution. Summarized data from six cells are illustrated in *B*. Buspirone inhibited the current by 39.6 ± 1.6%. Asterisks indicate statistical significance ($P = 2.4 \times 10^{-6}$).

Phenylbiguanide was ineffective in altering the magnitude of the current elicited by the test pulse. Sample data are illustrated in Fig. 6. This cell was superfused for 15 min with 200 µM phenylbiguanide. Current magnitude was unaltered during this period. To verify that these cells were responsive to serotonin inhibition, cells were subsequently tested with 8-OH DPAT, which proved to be an effective inhibitor. Sample currents are presented for the points corresponding to the letters *a, b, c,* and *d.* In eight tested cells, phenylbiguanide was without any noticeable effect in diminishing the current to the test pulse. All eight cells included in this group were responsive to subsequent administration of 8-OH-DPAT.

Agonist Inhibition of Sodium Currents

In addition to their effects on outward potassium currents, the effects of two agonists—TFMPP and 8OH-DPAT—were also tested on sodium currents. We have previously reported a detailed characterization of sodium currents in these cells [14]. Sodium currents were pharmacologically isolated by use of both extracellular and intracellular potassium channel blockers and were evoked by depolarizing command potentials in 10 mV increments from a holding potential of −70 mV. Currents were leak subtracted.

TFMPP reduced the magnitude of sodium currents. A family of sodium currents was evoked prior to, during, and after exposure to 100 µM TFMPP is presented in Fig. 7*A*. No obvious differences were observed in the activation or inactivation properties of the currents. The IV plot (Fig. 7*B*) shows currents activate somewhat more positive to −40 mV and that maximum current was evoked at 0 mV. These values are typical for rat posterior taste cells [18]. There was not any appreciable voltage-dependence to the inhibition (Fig. 7*B*). Currents were inhibited at all suprathreshold potentials, although there was some tendency for larger inhibitions at more depolarized potentials (>−20 mV). Data from six cells are summarized in Fig. 7*C*. Using the pulse from −70 to 0 mV, a mean inhibition of 31 ± 1.7% was measured ($n = 6$ cells; $P = 1.0 \times 10^{-5}$).

Figure 8 illustrates representative data for 8OH-DPAT. As with TFMPP, 8OH-DPAT also diminished the magnitude of the sodium currents without noticeable effects on activation or inactivation properties. The inhibition was reversible, though for the illustrated cell, the recovery was less than complete. Data from six cells are summarized in Fig. 8*C*. Current evoked from −70 to 0 mV was inhibited by 44.5 ± 2.9% ($n = 6$ cells; $P = 2.2 \times 10^{-5}$).

Discussion

Neurotransmitters in the Mammalian Taste Bud

As the concluding act of gustatory transduction, taste receptor cells encode stimulus information into volleys of action potentials that, in turn, elicit action potentials along sensory afferent nerve fibers. The identity of the transmitter that taste receptor cells use to communicate with the afferent nerve is still not known with certainty. A variety of neurotransmitters and neurotransmitter-related enzymes have been localized to taste buds, including recent reports of metabotropic glutamate receptors [4] and GABA transporters [29]. Physiological actions for glutamate have been reported in mammalian taste buds [1, 15] but physiological studies of GABA await experimentation. Physiological actions of adrenergic agents (e.g., 28) in mammalian taste cells have also been recently reported. Both potassium and chloride currents in rat taste receptor cells are influenced by norepinephrine or its agonists. Outward potassium currents are inhibited by exogenous application of norepinephrine

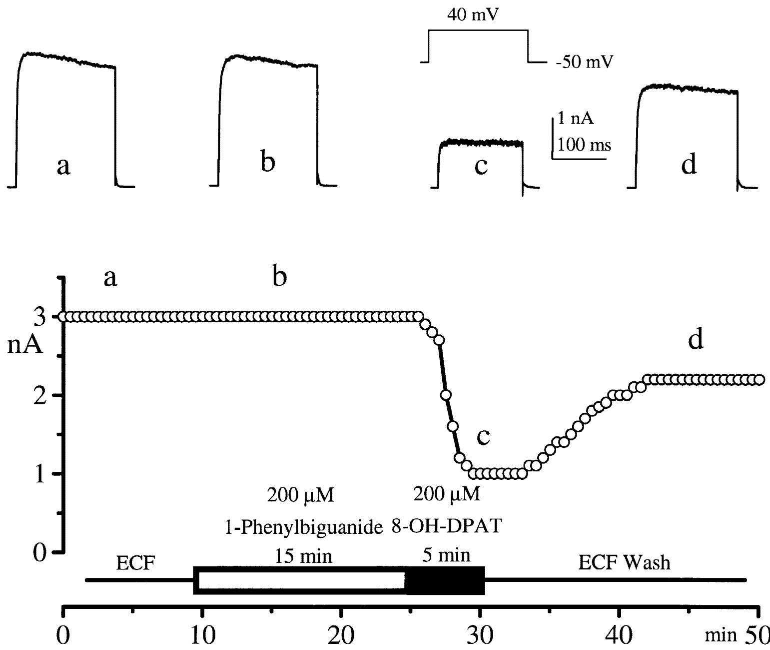


Fig. 6. The serotonin agonist phenylbiguanide, specific for $5HT_3$ receptors, was ineffective in inhibiting the outward current. Sample current traces from a single cell are presented above the measured amplitudes accumulated over a 50-min period. Application of 200 µM phenylbiguanide for a 15-min period did not alter the magnitude of the outward current (*b*) when compared to magnitude prior to drug application (*a*). To ensure that the cell was responsive to serotonin, 8-OH-DPAT was applied subsequent to phenylbiguanide application. 8-OH-DPAT effectively inhibited the outward current (*c*) in a reversible manner (*d*). Phenylbiguanide was similarly ineffective in eight tested cells.

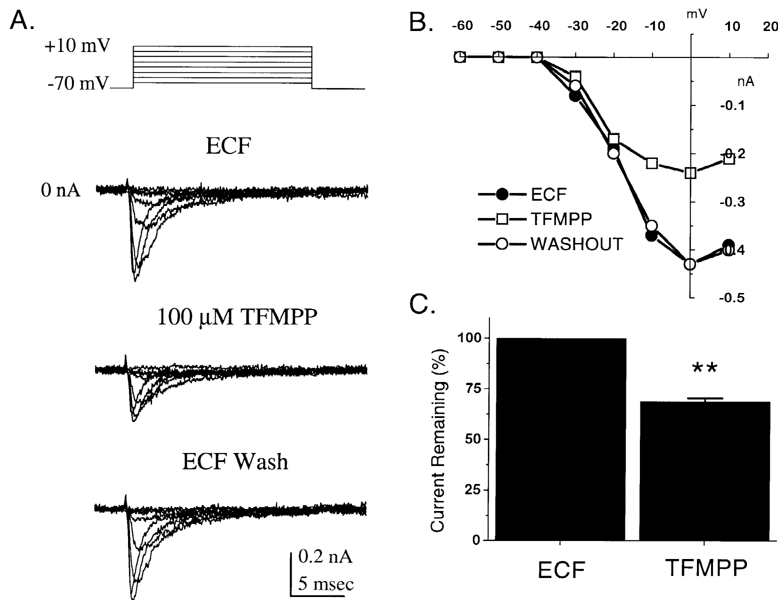


Fig. 7. The serotonergic agonist TFMPP, which demonstrates little selectivity among subtypes of serotonin receptors, inhibited voltage-dependent sodium currents in rat taste receptor cells. Sodium currents, illustrated from a sample cell in *A,* were evoked by a set of voltage steps from a holding potential of −70 mV to a final potential of +10 mV in 10 mV increments. Application of 100 µM TFMPP to the bathing solution reduced the magnitude of the evoked sodium currents in a reversible manner. The current-voltage relationship for the data in *A* is presented graphically in *B.* Currents were inhibited at all suprathreshold voltage pulses. Data from six cells are summarized in *C.* Current magnitude to the maximally effective voltage pulse was inhibited by 31 ± 1.7%. Asterisks indicate statistical significance ($P = 1.0 \times 10^{-5}$).

[36] whereas an outwardly rectifying chloride current is enhanced by the β-agonist isoproterenol and blocked by the β-receptor antagonist propranolol [19]. Additionally, in rat posterior taste cells, serotonin was observed to inhibit a calcium-activated potassium current [17]. Thus, complex physiological roles involving several transmitter candidates may co-exist within the mammalian taste bud.

As well, because taste cells (as well as presumably the afferent nerve fibers) respond to transmitters, these roles may include cell to cell interactions beyond those of strictly afferent transmission. For example, in the *Necturus* taste bud, where serotonin has been localized to basal rather than receptor cells, bidirectional synapses between basal cells and taste receptor cells have been observed suggesting that basal cells could release serotonin onto taste receptor cells, as well as onto the afferent nerve. Exogenous application of serotonin to *Necturus* taste receptor cells produced changes in their membrane properties [10] and alterations of calcium currents [8]. A similar situation may occur in the mammalian taste bud, since mammalian taste receptor cells also respond to

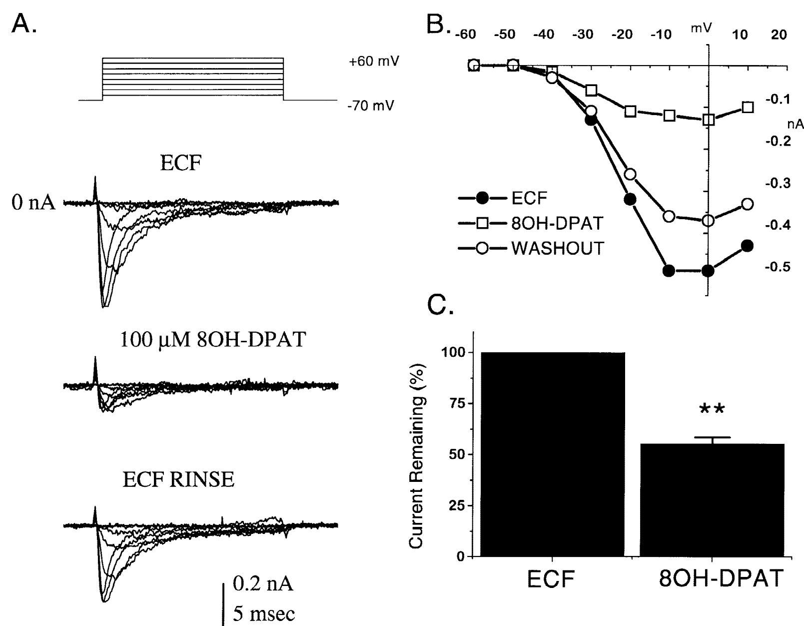


Fig. 8. Voltage-dependent sodium currents were inhibited by the 5-HT_{1A} receptor agonist 8OH-DPAT. Currents from a sample cell are illustrated in *A* prior to, during, and after bath application of 100 μM 8OH-DPAT. Note that 8OH-DPAT inhibited the sodium currents and that recovery occurred with rinse of the drug from the bathing solution. The current-voltage plot for this cell is presented in *B*. Currents were inhibited over all suprathreshold command potentials suggesting there is little voltage-dependence to the inhibition of the current. Summated data from six cells are presented in *C*. Current evoked by a maximal test pulse was inhibited by 44.5 ± 2.9%. Asterisks indicate statistical significance ($P = 2.2 \times 10^{-5}$).

exogenous serotonin. Collectively, these studies suggest that information processing in the form of autocrine, such as pre-synaptic inhibition, or paracrine, where the responsive taste cells react to transmitter released from a neighboring taste cell, roles may occur in taste buds and could serve as a basis for neuromodulation [28].

Receptor Subtype and Signal Transduction Mechanism

Two pressing questions regarding serotonergic effects on membrane currents in taste receptor cells include the identity of serotonergic receptor subtype(s) expressed in taste cells and its underlying signal transduction mechanism. Data in this communication preliminarily support the notion that serotonin may exert its effects on taste receptor cells via a $5HT_{1A}$ receptor subtype (summarized in the Table). Both $5HT_{1A}$-specific agonists (8OH-DPAT and busprione) were effective inhibitors of K_{Ca} whereas the $5HT_3$ agonist, phenylbiguanide, was ineffective. Additionally, this view is supported by data obtained with 1-(1-naphthyl)piperazine with agonist properties at 5 HT_1 and antagonistic properties at $5HT_2$ receptors. $5HT_{1A}$ receptors were also concluded to be operative in *Necturus* taste receptor cells [8]. However, taken together these data are still far from conclusive. Nonspecific effects are always a concern for application of any pharmacological agent but are of unique concern for taste receptor cells. Since taste receptor cells are specialized to respond to the presence of chemical stimuli in their immediate environment, there exists the possibility that some agonists may exert actions via taste receptors as well as serotonin receptors. Many bitter stimuli elicit responses in the range of 10^{-4} M, the concentration of many drugs used in this study. Thus agonists used in this study could exert some action on these cells as tastants, though this concern is mitigated by the negative effect observed with phenylbiguanide. Additionally, these concentrations are well above those of the K_i values for these drugs, which are typically in the nanomolar range. K_i values are presented in the Table. However, K_i values are generally determined by biochemical receptor binding assays and often do not correlate with concentrations required to produce physiological effects either in vivo or in vitro. Since the serotonergic receptor subtype expressed in taste cells is not yet known with certainty, it is possible that these agonists have lower affinity for the receptors in our cell type and hence require higher concentrations. However, our concentrations of these agonists are in line with other in vitro single cell measurements using these agonists (e.g., 30, 31). Pharmacological experiments with a broader array of agonists combined with the application of specific receptor antagonists would help to compliment the present set of data. Additionally, the use of complimentary techniques that address receptor localization or its messenger RNA, will be required for a definitive conclusion to be reached.

Though it is probable that $5HT_{1A}$ receptors are expressed in taste receptor cells, its signal transduction mechanism remains unknown. Activation of 5-HT_{1A} receptors is generally associated with inhibition of adenylate cyclase via G_i proteins (e.g., 2, 6, 11, 21, 42, 47). Early observations in the hippocampus presented equivocal results of either stimulation or inhibition of cAMP production (e.g., 47). Expression of cloned receptors in a variety of cell types has consistently demonstrated in-

Table Summary of agonist effects on calcium-activated potassium current

Agonist	Concentration	Receptor specificity	Sample Ki (nM) for 5HT1/5HT2	Reference	Percent inhibition of test pulse	*n*
Serotonin	100 μM	Non-specific	2/560	13	48 ± 2	6
TFMPP	100 μM	Non-specific	20/160	13	73 ± 3.1	9
8-OH-DPAT	100 μM	5-HT_{1A}	2–10[a]/5500	13	68.4 ± 2.6	10
1-(1-Naphthyl) piperazine hydrochloride	100 μM	5-HT_1 agonist 5-HT_2 antagonist	5/18 18/88	14 7	54.8 ± 2.6	7
Buspirone	100 μM	5-HT_{1A}	110	43	39.6 ± 1.6	6
Phenylbiguanide	200 μM	5-HT_3	3[b]	25	0	8

[a] For 5HT1A receptor.
[b] For 5HT3 receptor.

hibition of adenylate cyclase as well as other pleotropic roles [24, 32]. Some of the early stimulatory roles of $5HT_{1A}$ observed in the hippocampus may have been due to endogenous co-expression of $5HT_{5A}$, $5HT_{5B}$, or $5HT_7$ receptors (e.g., 2). However, increase of cAMP induced by 8OH-DPAT and antagonized by propranolol has been observed using a microdialysis technique [34] which suggests that in a limited number of cases the $5HT_{1A}$ receptor subtype may couple to stimulatory rather than inhibitory G-protein. Elevation of cAMP in rat taste receptor cells causes a phosphorylation dependent inhibition of outward potassium currents [20]. Thus positive coupling of $5HT_{1A}$ to adenylate cyclase in taste cells could help explain the inhibition of K_{Ca}, though regional localization of the second messenger and/or kinase may be required since serotonin does not seem to affect outward potassium currents as readily as K_{Ca}.

Additionally, the target ion channel of the presumed $5HT_{1A}$ receptor is not yet known with certainty. Two possibilities exist. Serotonin receptors could couple to calcium-activated potassium channels resulting in their inhibition, or receptors could couple to calcium channels, which secondarily result in inhibition of calcium-activated channels, including calcium-activated potassium channel. Some precedence exists for this latter possibility since serotonin has been demonstrated to have bimodal effects on calcium currents in *Necturus* taste receptor cells (8). However only inhibitions of calcium-activated potassium current have been observed in rat taste receptor cells.

Finally, these data do not preclude the possibility that more than one receptor subtype may be present in taste receptor cells. Because different 5HT receptors have varying affinities for 5HT, receptor subtypes provide the capacity to detect and respond differentially to quantitative changes in the amount of neurotransmitter in the synapse. Therefore, the expression of multiple serotonergic receptors would allow an additional level of information processing within the taste bud.

Physiological Consequence of Serotonin Release

It is reasonable to infer that serotonin release within the taste bud would be occurring during active gustatory stimulation. However, at present the consequences of such putative release remains speculative. Considered singularly, the most prominent effect of the inhibition of K_{Ca} would be to decrease the magnitude of the afterhyperpolarization (AHP). As with other excitable cells, K_{Ca} makes strong contribution to the AHP in taste receptor cells [3]. Increase in intracellular calcium, produced by calcium influx during repetitive firing, acts to augment K_{Ca} which, in turn, increases the AHP phase of the action potential. The increased AHP results in a decreased firing rate commonly referred to as adaptation. Inhibition of the AHP, produced by serotonin via its inhibition of K_{Ca}, would act to counter the normal increase of AHP produced during repetitive firing, thus maintaining the firing rate of action potentials and acting as a mitigating force to adaptation.

There are, however, several important complications to this simplistic view. Along with inhibition of K_{Ca}, the present data additionally suggest that serotonin may produce inhibition of voltage-dependent sodium currents. Since taste cells eliciting action potentials express both K_{Ca} and voltage-dependent sodium current [3], these effects may be occurring concurrently. Inhibition of sodium current might be expected to reduce excitability by attenuating spike height. The magnitude of the sodium inhibition does not appear to be sufficient to prevent spike initiation. Along this line, sodium inhibition may require higher concentrations of serotonin—for example it could be mediated by a second set of serotonin receptors with differing affinities for serotonin. Additionally, it is important to consider which subsets of cells within the taste bud are affected—that is, if serotonin sensitive cell is autocrine or paracrine in nature. It is easy to envisage that the serotonin-containing cell releasing transmitter as a result of its electrical excitation may release serotonin onto a neighboring cell and reduce its excit-

ability. Hence the target cell needn't be in an active state when stimulated with serotonin. Finally, possible effects on calcium currents need to be addressed before serious consideration of physiological effect can be considered. If inhibition of K_{Ca} is indirect via inhibition of calcium currents rather than a direct inhibition of K_{Ca}, then inhibition of calcium currents might also be expected to reduce transmitter release. Other calcium-activated currents, such as calcium-activated chloride currents, which similarly contribute to AHP, could also be affected. In contrast to actions of serotonin in the amphibian taste bud, where serotonin was observed to either increase or decrease calcium currents [8], no cells in the mammal were observed with increase of K_{Ca}, suggesting that if the effect is on calcium currents, it is not a twofold effect.

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