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Topical Review

Perspectives of Taste Reception

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Introduction

Chemical probing of the environment by smell and taste is essential for the survival of lower and higher animals. At the level of taste receptor cells (TRCs), this "externally oriented" chemo-reception may have much in common with "internally oriented" reception, well known from cells responding to hormones and transmitters. Here we review recent progress in the understanding of gustatory receptor cell function of vertebrates in terms of signal chains. These are sequences of events triggered by the interaction of a tastant with the receptor molecule and terminated by transfer of the signal to another cell.

How Does It Work?

TRCs, like other epithelial cells, are "polarized" in that the apical and basolateral membranes, which face different compartments, have different functions. The small patch of apical membrane is in contact with the mucosal space and carries receptors for tastants. The larger basolateral membrane fires action potentials by means of voltage-gated channels and releases transmitter at the synapse with a sensory axon or a "basal" cell. Surprisingly, basolateral membranes of TRCs also contain some receptors for tastants and may, in addition, carry receptors for neurocrine agents which have trophic effects and modify reception. In this review the emphasis will be on main sensory signal chains, of TRCs *in situ* or isolated from the tongue, investigated with electrophysiological and biochemical techniques.

Key Words taste reception · chemoreception · gustatory senses · sensory physiology · receptor cells · sweet taste · bitter taste · sour taste · salt taste

The TRCs act as transducers, as amplifiers and pulse shapers as they convert environmental signals into the coded messages of synapses. A main issue is the performance of TRCs as discriminators and/or signal mixers. One view holds that the responses to the so-called primaries—"sweet," "bitter," "sour," "salty"—represent different *modalities* [56], as distinct as are other skin senses like "warm" and "vibration".[1] Yet, *individual TRCs* are usually found to respond to more than one, sometimes to all, primary tastes. How, then, do taste reception and taste discrimination work? Clearly it is necessary to first establish the main signal pathways within the receptor cells. Several groups are concentrating on this problem. Significant findings, which have appeared within the last two years, are summarized in Table 1. Once the main pathways are known, their distribution among cells, and intracellular cross-effects between pathways, can be studied. Then it may become possible to understand taste discrimination at the TRC level.

[1] Bartoshuk pointed out that sensory *qualities* were defined by H. von Helmholtz as sensations with transitions between them, as in color vision or in tone perception [10]. *Modalities* have no transitions, and some claim that this is the case for basic tastes in man, sucrose plus NaCl giving no other impression than sweet plus salty, i.e., there are primaries but no secondaries (*see* [10, 56, 61]). These, however, are psychophysical considerations, which may not be helpful for understanding receptor function. When sensory phenomena are divided into categories (like "primaries," "basic tastes," etc.) in order to obtain schemes of minimal complexity, then different categorization may be necessary for the stage of receptor cells and the various stages of neuronal processing. In fact, the processing may often be described as a change of such categories. (Obviously, the categories may, furthermore, differ among animal species.) We shall use the term "qualities" but keep in mind that there are no transitions between them. It would be awkward to view "sweet" and "salty" as different sensory modalities which are, however, processed by one receptor cell.

Table. Suggested signal pathways in vertebrate taste receptor cells[a]

Taste	Initial binding to	First effector	Second messenger	Next amplifier	Depolarization by	Animal [ref.]
Sweet	Apical receptor	Adenylate cyclase	cAMP	cA-kinase	K-channel closure	Frog [4] rat [92]
Amino acids	Apical receptor	Adenylate cyclase	cAMP	?	?	Fish [39]
Bitter	Apical receptor	?	?	Ca release	?	Rat [1]
Sour	Apical K-channel	→			Apical K-channel closure	*Necturus* [43]
Salty	Apical cation channel	→			Flow of apical inw. current	Frog [7] rat [12]

[a] The electrophysiological data quoted were obtained with patch-clamp experiments [27]. Additional or alternative chains are mentioned in the text.

Sweet

For man and many other vertebrates, the hedonic taste "sweet" signals high-calorie carbohydrates, typically originating from plants. Sugars, saccharin and other compounds, notably also the sweet-tasting protein monellin, attach to binding sites of homogenized mucosal material from the tongue [15, 16]. A large fraction of these sites will be the surface receptors of the apical membranes of taste receptor cells[2]. Sweet compounds were shown to cause the generation of cyclic adenosine monophosphate (cAMP), in the presence of GTP and ATP, in homogenates of the rat tongue mucosa. In this assay, the potency of 1 molar saccharide stimulating the adenylate cyclase matched the potency sequence (sucrose > D-glucose > maltose) known from the intact taste organ of the rat [49, 73, 92]. Therefore, does input to the signal chain of "sweet" utilize the Sutherland-cascade, well known from the reception of hormonal messages [94]?

If so, cAMP would be the second messenger of the sweet chain. Indeed, in isolated taste receptor cells (TRCs) of the frog[3] cAMP caused a pronounced, reversible decrease of the membrane potential, provided ATP was present in the cell. The depolarization was accompanied by a decrease in membrane conductance and outward K current [6]. Taste cells of frog and mouse, while maintained in the epithelium, responded to intracellular injections of cGMP or cAMP with depolarizations [64, 102]. In mouse TRCs (type H) the conductance decreased and the response resembled that induced by apical exposure to sucrose in the same cell [102]. (However, the type D TRCs of the mouse depolarized in response to sucrose *without* a conductance change [99].)

In inside-out membrane patches [27] excised from frog TRCs, cAMP alone had no effect on membrane currents, but the catalytic subunit of cAMP-dependent protein kinase caused closure of a set of K channels of 44 pS conductance, provided ATP was present [4]. Thus, the depolarization induced by cAMP appears to involve protein phosphorylation, which causes closure of K channels. Together these results suggested the sweet-signal chain of the Table. The pathway has remarkable similarity to that used by *Aplysia* neurones in their response to serotonin [83]. It remains to be shown that all elements of this pathway are involved in the sweet response of *one* TRC.

In isolated TRCs from the rat it was recently found that the well-known sweetener saccharin causes membrane depolarization which elicits repetitive action potentials [11]. Thus the sweet-signal chain can involve action potentials generated by the receptor cell. The possible significance of action potentials arising in these small cells is discussed below.

Umami

Because the English language has no simple term for the peculiar taste of amino acids such as glutamate, the literature refers to it with the Japanese noun *umami* ("good taste" based on stocks from seaweed, fish and mushrooms [103, 107]). For many species, this taste will indicate nutritive material of animal origin. L-glutamate was shown to bind to membrane fractions of the bovine lingual epithelium. Interestingly, the binding was intensified when the taste enhancer guanosine monophosphate

[2] Receptors for saccharin are also expected at basolateral membranes of TRCs, as indicated by the "intravascular taste" [13], and were found on membranes of cells which are not TRCs [91]. The physiological function of "sweet" receptors at these locations is not known.

[3] *See* Appendix for Methods. TRC potential recordings and nerve recordings showed that frogs taste sugars [74]. Furthermore, the frog intestine has a powerful Na-glucose co-transport system [17], indicating that carbohydrates are of relevance in the diet.

was present. The nucleotide somehow increased the availability of glutamate binding sites [103]. A similar "flavor potentiation" was found in gustatory nerve recordings in the rat [107].

For the extra-oral taste buds of the catfish,[4] a GTP dependent adenylate cyclase, stimulated by micromolar concentrations of L-alanine, was described [39]. The enzyme was inhibited by sub-millimolar concentrations of Ca ions. Thus cAMP may be the second messenger of one amino acid-signal chain. However, the target of this messenger in catfish TRCs is not yet known. The same tissue was found to contain a phosphatidylinositol-4,5 bisphosphate phosphodiesterase, whose activity was also enhanced by alanine [37].

Gating of cation channels (40 pS) directly by L-arginine was also described for the catfish [96]. In response to L-alanine both hyperpolarization, accompanied by an increase in conductance, and depolarization of catfish TRCs was observed [97, 98], suggesting that more than one signal pathway exists. A complete signal chain for reception of amino acids has not yet been proposed (Table). It seems desirable to extend the electrophysiological studies to TRCs of mammals, particularly carnivores.

Bitter

In man, and in vertebrates with a similar diet, the predictive value of the "bitter" sensation may be related to poison, i.e., the taste of alkaloids [56], bitter almonds and the like. A large diversity of chemicals has a bitter taste. More than one receptor or pathway for this taste quality would therefore be expected. For those bitter-tasting agents which are membrane permeant, *cellular* phosphodiesterase was suggested to act as the receptor [47, 48]. Blockage of this enzyme is expected to increase the cellular concentration of cAMP. As yet, the effect of this messenger on surface membrane events, as part of the bitter-signal chain, remains to be described.

Bitter agents like quinine were often found to induce a depolarizing receptor potential accompanied by an increase in membrane resistance of TRCs impaled *in situ* with microelectrodes. In the case of 20 mM quinine applied to the rat tongue, this response was attributed to a blockage of K channels [66]. In retrospect, the deduction seems reasonable also because quinine does by itself block TRC K channels at the lower concentration of 100 μM [6].

In the frog, the quinine-evoked receptor potential was increased following injection of KCl into the TRCs, while injection of K acetate had no such effect, indicating that Cl rather than K ions were involved. Basolateral exposure to furosemide decreased the receptor potential, presumably by inhibiting Cl accumulation via Na-Cl cotransport [65]. It was proposed that chloride is accumulated in TRCs by basolateral cotransport with Na, while, in response to quinine, depolarization develops by secretion of Cl through the apical membrane via primary active transport [65].

In contrast to permeant bitter tastants, the non-permeant bitter agent denatonium most likely requires an apical surface receptor [1]. In the rat, denatonium was shown to cause release of Ca from intracellular stores of some of the TRCs, presumably those cells concerned with the denatonium taste. Inositol tris-phosphate or cAMP, both able to cause Ca release in other systems, were mentioned as possible second messengers of this bitter pathway [1].

Changes in membrane potential or conductance of rat TRCs, preceding or following release of intracellular Ca, have not been described [1], and in a mucosal preparation bitter substances did not elicit visible electrical events [87]. Indeed, Ca-dependent exocytotic secretion may [70], but need not in all cases [52, 108], require membrane depolarization. Thus it will be interesting to learn, whether the response to denatonium involves changes in membrane conductance. Does the "bitter" signal *bypass* the integrative action of the membrane potential? This is an interesting possibility, particularly for "bitter" as a warning signal. It will also be interesting to see whether release of Ca from intracellular stores of TRCs is induced not only by denatonium, but also by the permeant bitter tastants, particularly since in the sarcoplasmic reticulum the bitter tastants caffeine and quinidine interfere with Ca sequestration [*see* 25].

Sour

An increase in the mucosal proton concentration was shown to depolarize TRCs while the small-signal resistance remained constant or decreased [2, 78, 104]. The "sour"-evoked depolarization elicited action potentials in amphibian TRCs maintained in the epithelium [45]. In the frog, two mechanisms were held responsible for the depolarization, one of which appeared to be an H^+-gated Ca channel which would also conduct small ions like Na and K [60]. The activation of this channel by mucosal protons would account for the observed 6–10% *decrease* in resistance. In the dog, a change-over from cation-conducting to anion-conducting apical pathways was held responsible for "sour" transduction [84].

[4] The extra-oral (cutaneous) buds are more sensitive to tastants than the oral buds. They serve to find food rather than to probe the food already found [40].

Patch-clamp experiments with isolated TRCs of *Necturus* revealed the remarkable fact that K channels are concentrated apically rather than basolaterally [18, 43]. At neutral pH, large outward currents passed through the apical membrane, while the basolateral membrane had small outward currents. The apical K currents, which were voltage-sensitive and blocked by tetraethylammonium, decreased when the mucosal pH was lowered. The TRC then depolarized. In outside-out excised patches a 100-pS K channel was blocked by protons [18]. Thus the direct block of apical K channels by protons was suggested to be the input step of the sour-signal chain [18, 43]. The channel would thus be at once receptor and depolarizing effector, and a resistance *increase* would be expected to accompany the depolarization, as found in *Necturus* [45]. The seemingly remote possibility of a separate proton receptor modulating some of the apical K channels has not yet been excluded [42]. TRC-patch clamp results concerning the sour-response in higher vertebrates are not available to date.

Salty

Of the diversity of tastes induced by inorganic salts, that of NaCl has found primary attention. For man, NaCl is the only substance which has a *pure* salty taste [80], and the sodium ion appears to be more important for this taste than the anion. The transepithelial short-circuit current through the tongue mucosa of many vertebrates has a Na- and K-dependent component and a nerve response related to it. In the frog and the dog [57, 58, 90] this component is less Na-specific than in the rat, hamster and monkey [14, 29, 32, 34, 88]. Typically, the current is in part inhibited by amiloride, a blocker of the common epithelial Na channel [54]. Recordings from the sensory nerve, as well as sensory experiments with humans, supported the conclusions drawn from the transmucosal measurements, i.e., that amiloride-blockable Na channels provide one prominent transducer mechanism for the quality "salty" [29, 32, 34, 35, 81, 88]. However, others suggested "that the taste responses to salts, including NaCl, are not induced by entry of cations via apical membranes of taste cells" [106]. This issue was subsequently investigated at the single-cell level.

Whole-cell patch-clamp recordings from isolated *frog* TRCs, immersed in Na Ringers, showed that in a sub-population of cells a stationary inward current was present, a part of which was blocked by low concentrations of amiloride. The blockable fraction varied among cells, and the inhibition constant was near 0.3 μM [7]. Even TRCs with large stationary inward currents had in addition voltage-activated transient inward currents blocked by TTX.

Recordings from outside-out excised membrane patches of such cells also showed stationary inwardly-directed currents, which were in part blocked by amiloride with an inhibition constant near 0.3 μM. In these experiments, differences to the common epithelial Na channel were noted [8, 9]: (i) in the sensory channel the ion selectivity was $K > Na > Rb > Li > Cs >$ N-methyl-D-glucamine, in the common channel $Li > Na \gg K, Rb$ [68]. Thus the sensory channel is much less selective. (ii) At the sensory channel the blocking potency of the amiloride analogs phenamil and benzamil was *less* than that of amiloride, while at the common Na channel these analogs block with higher efficacy than amiloride [50]. (iii) Power spectra of the inward current, recorded in the presence of 0.3 μM amiloride, indicated that the blocking rate constants of amiloride at the sensory channel were more than 10 times higher than at the common channel [53]. (iv) The single unit conductance, estimated from noise data, was 1–2 pS when the patch was exposed to 110 mM Na, and thus significantly smaller than in the common epithelial Na channel of amphibia [24, 28, 53]. Recent single channel recordings confirmed the small conductance below 2 pS.

Thus, when comparing the sensory channel from *lingual* TRCs with the common apical Na channel from *dermal* epithelial cells of the same species of frogs, pronounced differences are found. However, the channels may well belong to the same family of proteins, since antibodies, raised against amiloride-sensitive Na channels of kidney cells, labeled apical membranes of canine taste pores [85]. The amiloride-*insensitive* inward current of frog TRCs has a slightly different ion selectivity and an even smaller unit conductance [9].

In conclusion, NaCl-salt taste appears to be mediated by cation-conducting channels, in the frog of low selectivity and low conductance, one sub-population of which is blocked by submicromolar concentrations of amiloride. The presence of these channels in the apical membrane will allow flow of inward current, and depolarize the cell, as soon as Na ions appear in the mucosal solution in sufficient concentration. The pre-existing, operative channel would thus at once be Na receptor and depolarizing effector.

Even though a second messenger does not seem to participate in the above salty signal, long-term modulation of Na taste, mediated by an unknown signal chain and operating, perhaps, through

changes in apical Na permeability, takes place.[5] The interesting phenomenon of salt taste adaptation appears to occur at the level of TRCs [35, 76, 77] and may also involve a second messenger and a change in Na permeability.[6]

Cellular Specificity and Common Steps

The signal pathways indicated in Table 1 need to be extended by several steps, up to the release of transmitter at the synapse. It is interesting that in response to sweet, sour and $CaCl_2$*, TRC action potentials were observed; in the case of sweet and $CaCl_2$ they were trains of action potentials [5, 11, 45]. Furthermore, in the TRCs of bullfrog and *Necturus*, voltage-dependent Ca channels were found [41, 44]. Thus a plausible conclusion of the chains would be *depolarization → action potential → Ca influx → release of transmitter*. The action potential would serve to overcome the voltage span between the Na channel threshold (−40 mV [44]) and the less negative Ca channel threshold. The release of transmitter would then be pulsed.

Surprisingly, microelectrode recordings from single TRCs showed that few cells responded to just one taste quality [67]. Most cells responded to two or more qualities [62, 63, 75, 100, 101], and some authors found that sensitivity to sweet, bitter, sour and salty is distributed *randomly* between taste cells [67, 75]. Many single gustatory nerve fibers also respond to more than one tastant [23, 55, 89]. The fibers obtain input from several TRCs, but the poor specificity of single TRCs may already set an upper bound to the specificity of the single fiber response.

In the "broadly tuned" TRCs more than one of the above signal chains must be operative. Patch-clamp results addressing TRC selectivity are still rare. Among isolated TRCs of the frog, cells were found which had *both* an amiloride-blockable inward current and a depolarizing response to cAMP [7]. Similarly, a TRC of the rat responded under whole-cell patch-clamp conditions to amiloride by hyperpolarization and to saccharin with a depolarization and a train of action potentials [11]. This may mean that one cell can detect two qualities like "salty" and "sweet," and respond to each with a depolarization. Here depolarization would be the first common step of the two chains.

Despite much experimentation, the question of TRC specificity is still a fascinating, in detail unsettled, issue. While it seems clear that most TRCs are broadly tuned, we await future work to show how many signal pathways exist in TRCs, how they are segregated among TRCs, at which steps pathways merge in single TRCs and which functional consequences this might have.

Sweet Again

"Multimodal" receptor cells are likely to show *cross-effects* in that blockage or use of one signal chain will enhance or suppress the reception of another quality. Of the following cases the first may be a cross-effect while the second probably is not.

In psycho-physical experiments with human volunteers amiloride blocked not only the reception of "salty" but also of "sweet" [81]. While the drug may have acted as a competitive inhibitor at the sweet receptor, the possibility exists that enough Na was present at the tongue surface to allow amiloride to cause a hyperpolarization by block of Na inflow. This might inhibit sweet-reception in those cells which respond to both "salty" and "sweet," comparable to a synapse where excitation is blocked by the hyperpolarization of an inhibitory postsynaptic potential. The third possibility is that in man, like in the dog, an amiloride-sensitive current is induced by sweet agents as an essential step in the sweet signal chain (*see below*). Since superfusion of human tongues with small adhering chambers is possible [30], it will be interesting to see whether the human sweet response disappears when small cations are constantly washed out of the mucosal space.

Recent evidence indicates that, in dogs, membrane events other than closure of K channels may cause the depolarization that is part of the sweet signal chain. The mucosa of the dog tongue generates an inward current, apparently through the apical membranes of TRCs, in response to sweet tastants [57, 86]. The current depends on the presence of Na or K ions in the mucosal solution (their

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[5] The common epithelial Na channel is controlled by hormones, particularly by antidiuretic hormone and by aldosterone, which enhance channel density [26, 51, 69]. There are indications that salt taste is also controlled by steroids, but here salt sensitivity is decreased by the hormones [36].

[6] The human "water taste" was related to a change of mucosal NaCl below the level in the saliva, to which adaptation had previously occurred [10]. The change in surface potential of the apical membrane, which is expected to develop when the ionic strength of the medium is decreased, was claimed to be essential for the water response of the frog [93].

* $CaCl_2$ appears to induce one of the "salty" tastes of amphibia [2, 74]. As in fish, it may provide clues for the animal's navigation [38]. In the frog, Ca taste is blocked by mucosal $CoCl_2$, a blocker of Ca channels [33], and abolished by pronase treatment, which leaves the response to NaCl unchanged [46].

concentrations in the saliva being sufficient). Partial blockage of the sugar-evoked current by 100 μM amiloride suppressed the sweet response recorded from the sensory nerve [57]. This dose of amiloride also caused partial blockage of the nerve response to NaCl in the absence of sugar.

The findings indicate a sweet pathway which relies on the presence of monovalent cations in the mucosal compartment as cofactors which are obligatory, but also modulating, such that the sweet signal becomes stronger when the salt concentration is increased. The phenomenon needs investigation at the cellular and single-channel level to decide whether a directly "sweet"-activated channel is involved, or a second messenger, of presently unknown nature, operating on amiloride-sensitive channels. This is in contrast to the case of salt taste, where amiloride-sensitive channels appear to act as pre-existing, conductive receptors, apparently not requiring an opening signal as part of the main signal chain (*see above*).

Cross-Effects

In the cases of sweet-tasting amino acids or bitter-tasting sugars and amino acids, competitive receptor sharing may be responsible and *cross-adaptation* is to be expected. It is instructive to view the published psychophysical descriptive tables "based on semantic differential judgement" [80]. In contrast to NaCl, KCl tastes bitter and sour as well as salty. Is the bitter and sour component due to depolarization of TRC apical membranes having K channels [43]? $CaCl_2$ tastes bitter. Is Ca uptake involved, bypassing the bitter receptor recently proposed [1]? In many cases cross-effects may be due to signal chains joining at the level of the membrane potential of broadly tuned TRCs. Progress in understanding cross-effects resulting from TRC "multimodality" will depend on the prior elucidation of the main signal chains. Some of these effects may be necessary, if poorly understood, features of the peripheral signal processing.

Apart from cross-effects within receptor cells, further interactions may occur between receptor cells by electrical coupling [104] (gap junctions and dye-coupling were established [3, 95, 105]), between receptor cells and basal cells by chemical coupling [19, 79], at the level of sensory axons [59] and, for experiments involving semantic judgement, higher up in the nervous system.

The Emerging Picture

The recent data suggest that (i) "sweet" and "*umami*," two tastes correlating with high-calorie food, are in part transduced by nucleotide cyclase. The second messengers induce changes in TRC membrane conductance and potential. At the level of membrane potential, interactions with other signal chains are likely to occur. (ii) Bitter taste (one variety of probably several chains) invokes release of intracellular Ca. The possibility exists that modulation of membrane voltage is not an essential step in this chain. (iii) "Sour" and "salty," the two tastes based on detection of small ions, transduce by blockage (sour) or usage (salty) of pre-existing apical channels, thus causing cellular depolarization.

Alternative pathways have been suggested for each of these signals. While the evidence is still sketchy and in part puzzling, methods for further elucidation of TRC function are available. The question of TRC "multimodality," and related peripheral signal processing, deserves the continued attention of investigators. Its solution will help with another, much discussed issue [56, 82]: the origin and nature of the neural code of gustatory reception.

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Appendix

Cells *in situ* are preferable

Great efforts have been made in recent years to isolate TRCs of several species of vertebrates in order to study their membrane functions by patch-clamp techniques. While this remains a useful approach, we should not ignore the loss of functional polarity, which follows the opening of tight junctions and the removal of the sensory cells from the epithelium [22, 71, 72]. The results obtained from isolated cells may not always reflect physiological mechanisms. It is highly desirable, therefore, to record in addition from sensory cells maintained in the epithelium. Since impalement of small cells with microelectrodes usually causes flow of leak current, the cells should be made accessible to patch clamping, but have an undisturbed tight junction and remain fully polarized in that intrinsic proteins of apical and basolateral membrane remain in place and do not mix [22].

This was recently attempted by using strips of isolated tongue epithelium of the rat. The strips were stretched out "on edge" under the microscope, and a large pipette (50 μm diameter) pressed onto a fungiform papilla. Thereby the papilla "popped" and a group of TRCs protruded at the interstitial opening. When the basal poles of the TRCs were patch clamped and the apical membranes of the bud stimulated chemically by perfusion of the large pipette, cellular responses to amiloride and sweeteners could be observed [12].

The patch-clamp experiments with isolated cells were often guided by chamber experiments. Even though the apical membranes of TRCs constitute an only small fraction of the total mucosal area, recordings of short-circuit current from tongue mucosa in Ussing chambers led to rather precise predictions and concepts [20, 21]. Correlation of the short-circuit current changes with nerve responses, which were recorded simultaneously, provided further support for the validity of chamber results [30, 31, 88, 90].

The possibility of recording current or voltage transepithelially from a *small* mucosal area, containing just one taste bud, has not been explored as yet. One advantage such a method might offer, is to record TRC-action currents or potentials, contained in the transepithelial signal, in response to chemical stimuli.