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Review

The Taste of Caffeine

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Many people avidly consume foods and drinks containing caffeine, despite its bitter taste. Here, we review what is known about caffeine as a bitter taste stimulus. Topics include caffeine's action on the canonical bitter taste receptor pathway and caffeine's action on noncanonical receptor-dependent and -independent pathways in taste cells. Two conclusions are that (1) caffeine is a poor prototypical bitter taste stimulus because it acts on bitter taste receptor-independent pathways, and (2) caffeinated products most likely stimulate ''taste'' receptors in nongustatory cells. This review is relevant for taste researchers, manufacturers of caffeinated products, and caffeine consumers.

Keywords: caffeine, bitterness, taste, taste receptors, TAS2R43

Introduction

The sense of taste helps to determine whether or not a food or beverage will be ingested. Typically, sweet tastes motivate intake and bitter tastes discourage intake. However, two of the most widely ingested beverages—coffee and tea[1]—are bitter, which contradicts this general rule. One factor that likely contributes to the popularity of coffee and tea is that they contain the psychoactive alkaloid, trimethylxanthine (caffeine).

Many reviews describe the mechanisms supporting the behavioral, cognitive, and emotional effects of caffeine.[2–10] However, none have evaluated caffeine as a bitter taste, or covered what is known about caffeine action in cells between the tongue and the brain. These topics deserve attention because (1) caffeine is sometimes considered a prototypical bitter;[11–17] (2) there are currently more caffeinated products on the market than there have been in the past and many contain higher concentrations of caffeine than do coffee and tea[18]; (3) new mechanisms may be exposed by recent discoveries of bitter taste receptors lining the digestive tract, which must come into contact with caffeine; and (4) new mechanisms may be exposed by recent discoveries that caffeine targets (e.g., adenosine receptors [ARs], GABA receptors, intracellular receptors, and so on) in nontaste cells (e.g., neurons) can modulate taste in the mouth. Thus, this review will place a particular emphasis on caffeine's effects on taste cells and other caffeine-responsive cells that reside outside of the central nervous system.

What Is a Bitter Taste?

Before discussing caffeine's bitterness, we will briefly review bitter taste in general. Bitter chemicals are structurally diverse and include alkaloids (e.g., caffeine, quinine, nicotine, and morphine),[19] some L-amino acids,[20–23] urea,[24] phenylthiocarbamide,[24] 6-n-propylthiouracil,[24] and some divalent salts[25] (for a more comprehensive list of bitter chemicals see Beckett *et al.* [26]). It has been suggested that the more bitter a compound, the more toxic it is, although there are many exceptions.[27] Like sweet tastes, most bitter tastes, regardless of their structure, are detected by G-protein-coupled receptors (GPCRs) in type 2 taste cells (taste receptor cells [TRCs]).

When a taste (i.e., a chemical that elicits a taste percept) binds to a GPCR expressed by a TRC, it activates an intracellular signaling cascade that can result in the release of adenosine triphosphate (ATP) and stimulation of peripheral nerve fibers. Whether or not a TRC is activated by a taste depends on the receptor it expresses. TRCs that express T1Rs are activated by sweet or umami tastes, and cells that express taste 2 receptors (T2Rs) are activated by bitter tastes.

In the canonical bitter taste transduction cascade (Fig. 1), intracellular signaling starts with activation of G-proteins such as α-gustducin. This results in the dissociation of $\beta\gamma$ subunits, which activate phospholipase Cβ2 (PLCβ2). PLCβ2 then cleaves phosphatidylinositol 4,5-biphosphate (PIP2) into inositol (1,4,5) triphosphate (IP_3). The IP_3 triggers release of calcium from the

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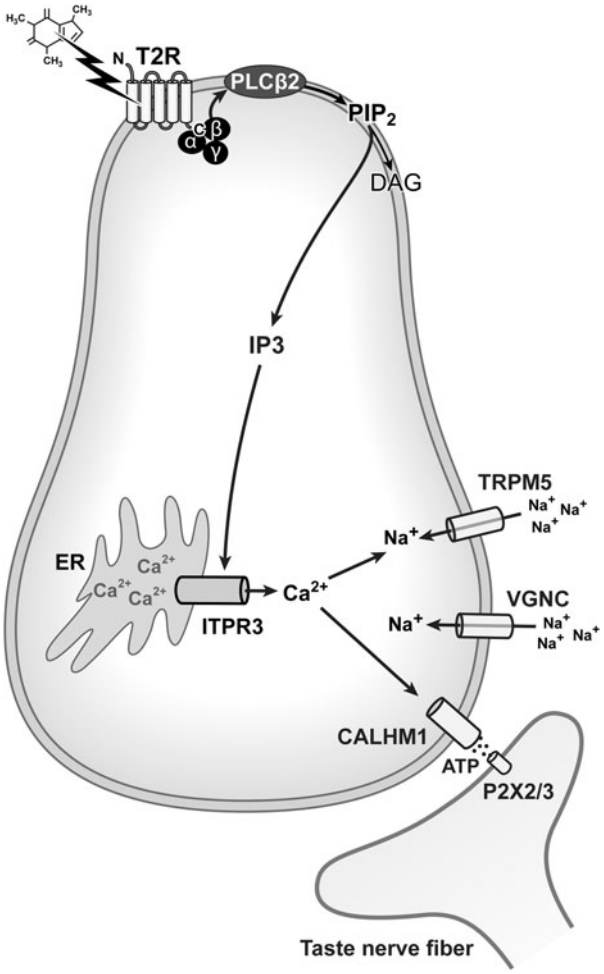

FIG. 1. The canonical taste transduction pathway. Caffeine, and other chemicals that elicit bitter taste sensations, activate T2R-type GPCRs. GPCRs have seven domains that span the plasma membrane. When bitter tasting chemicals bind to T2Rs, this elicits an intracellular signaling cascade that starts with activation of G-proteins (e.g., α-gustducin). Activation of G_α causes the dissociation of βγ subunits, which then activate the enzyme PLCβ2. PLCβ2 then cleaves PIP2 into IP_3. The IP_3 triggers release of calcium from the endoplasmic reticulum by binding to ITPR3s. This calcium release activates and opens TRPM5 leading to sodium influx and depolarization of the taste cell. This depolarization activates voltage-gated sodium channels (VGNC)[182] boosting the depolarization triggered by TRPM5, which triggers the release of ATP through CALHM1 channels. The signal, transmitted by ATP release, is then conveyed to the brain through peripheral nerve fibers that express purinergic receptors. ATP, adenosine triphosphate; T2R, taste 2 receptor; TRPM5, transient receptor potential cation channel subfamily M member 5.

endoplasmic reticulum by binding to type 3 IP_3 receptors (ITPR3s). This calcium release activates and opens the nonselective cation channel, transient receptor potential cation channel subfamily M member 5 (TRPM5), leading to cation influx and depolarization of the taste cell (see Kinnamon[28] for a review). This depolarization activates voltage-gated sodium channels, which trigger the release of ATP through CALHM1 channels.[29–31] The signal, transmitted by ATP release, is then conveyed to the brain through peripheral nerve fibers that express purinergic receptors.[32]

Taste information from the peripheral nerve fibers is first sent to a relay station in the brainstem, the nucleus of the solitary tract, which controls swallowing, salivation, and automatic behaviors related to taste rejection or acceptance.[33–35] Thereafter, taste information is sent through multiple synaptic connections to higher brain areas where taste quality perceptions are generated. Although there is some debate about the exact route that taste information follows (see de Brito Sanchez and Giurfa[36] for a discussion), qualities such as bitterness and sweetness can be generated by stimulating bitter and sweet cortical fields in the gustatory cortex alone,[37] which provides strong support that the gustatory cortex harbors taste percepts.

Taste compounds activate taste receptors found not only in the tongue[38,39] but also in the alimentary tract,[38–44] pancreas,[45] other endocrine glands,[46,47] the airway epithelium,[38,48–52] and brain.[38,53–57] The ubiquitous expression of taste receptors raises the possibility that chemicals that are tasted also exert a variety of other functional consequences. There is already evidence that taste stimuli influence the function of cells involved in nutrient absorption,[40,53,58–61] satiety,[62] fertility,[47,63] respiration,[64] and metabolism.[65] Bitter taste receptors specifically have been implicated in several nontaste functions ranging from innate immunity[52] to neurite outgrowth.[54]

Caffeine's Action on the Canonical Bitter Taste Pathway

Caffeine has a bitter taste, and bitter tasting chemicals are detected by a large family of GPCRs (T2Rs). Humans have at least 25 functional T2R subtypes, and rodents have at least 29. Meyerhof *et al.* demonstrated that caffeine is a ligand of five human bitter taste receptors as follows: hTAS2R7, TAS2R10, TAS2R14, TAS2R43, and TAS2R46[66] (see Fig. 2 for tissue-specific expression of caffeine-responsive T2Rs). In their study, the level of intracellular calcium induced by caffeine was analyzed in HEK293T cells transfected with *TAS2R* genes.[66] Although the effects of caffeine on taste cells that endogenously express T2Rs need to be confirmed, there is good reason to believe that the receptors identified by Meyerhof *et al.* are important targets: Two studies show that polymorphisms in *TAS2R43* (a gene encoding one of the receptors that Meyerhof *et al.* found to be responsive to caffeine) are associated with coffee liking[67] and the perceived bitterness of caffeine.[68] This suggests that TAS2R43 might be particularly important for caffeine taste detection in humans. Interestingly, ratings of caffeine bitterness in humans are also positively correlated with levels of TAS2R43 mRNA.[69] Thus, polymorphisms

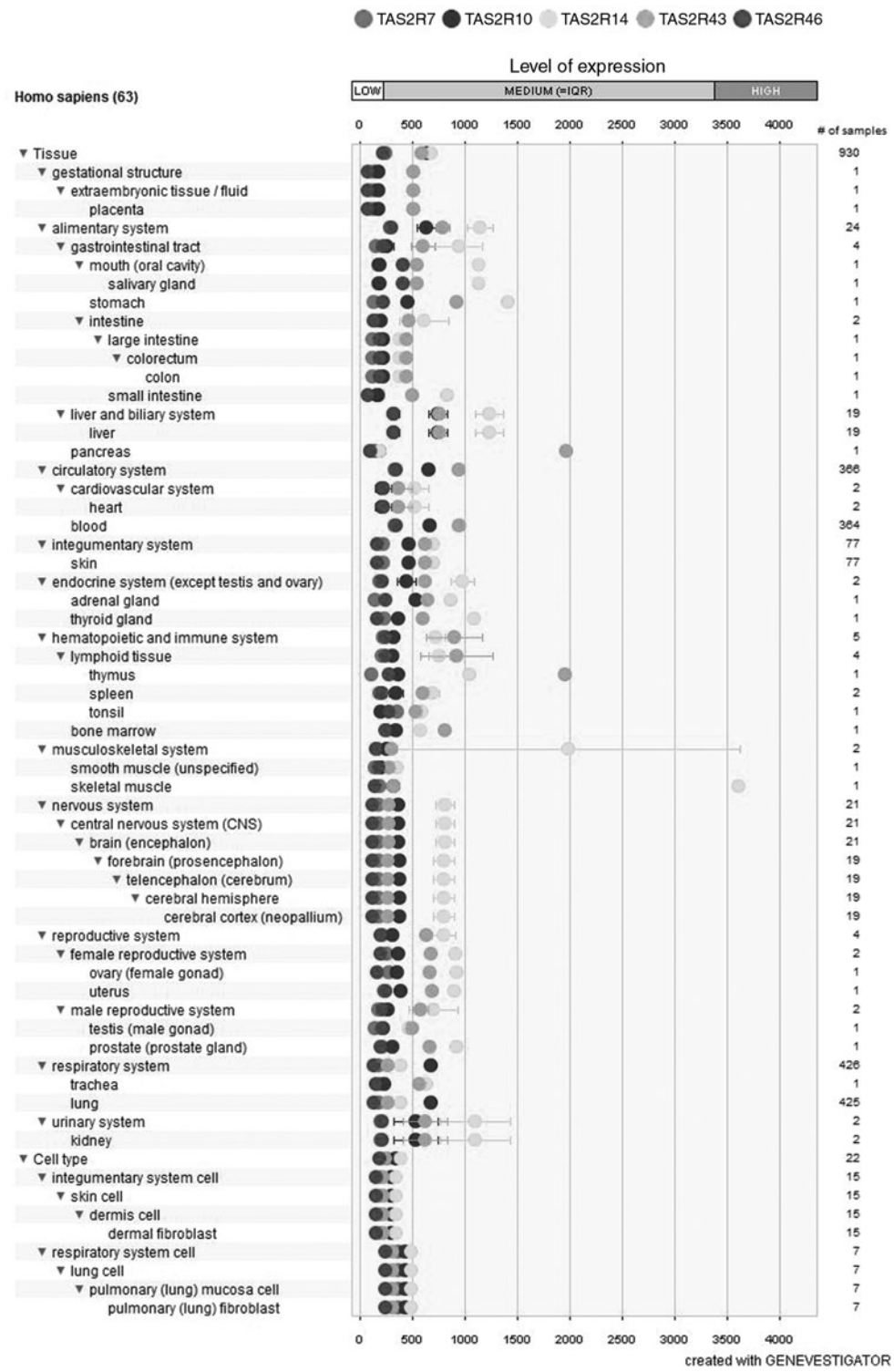

FIG. 2. Tissue-specific expression of caffeine-responsive T2Rs. The gene IDs for TAS2R7, TAS2R10, TAS2R14, TAS2R43, and TAS2R46 were entered in GENEVESTIGATOR® and the Agilent Human Gene Expression 8x60K Microarray dataset was selected. In this dataset, TAS2R14 showed the highest expression in most tissues, including the oral cavity. However, TAS2R43 expression was high relative to TAS2R7, 10, 14, and 46 in the pancreas and thymus. Whether or not caffeine modulates the function of tissues in the alimentary, circulatory, integumentary, endocrine, immune, musculoskeletal, nervous, reproductive, respiratory, and urinary systems by acting on caffeine-responsive T2Rs is a relatively unexplored area of research.

in *TAS2R43* associated with the perceived bitterness of caffeine may indirectly control the expression level of the T2R43 receptor. Alternatively, caffeine drinking could increase expression of T2R43. However, given that the *TAS2R43* gene is absent from the genome of ~18–30% of people,[69–72] yet subjects with this deletion report they can taste caffeine's bitterness,[69] non-T2R43 mechanisms must be partly responsible for the bitter taste of caffeine. A genome-wide meta-analysis identified polymorphisms in *TAS2R7* and *TAS2R14* as being associated with coffee liking,[73] again supporting the conclusion that Meyerhof *et al.* identified important targets for caffeine detection in their *in vitro* study. Although coffee contains multiple bitter chemicals, and caffeine is responsible for only a fraction of coffee's bitterness,[74] the fact that *TAS2R7* and *TAS2R14* were identified as caffeine-responsive in a heterologous expression system suggests that these receptors might be especially important for caffeine taste. However, there are no reports that *TAS2R46* is associated with coffee liking or the perceived bitterness of caffeine. With that said, a recently published study found that the odorant citronellal attenuates caffeine bitterness by blocking T2R43 and T2R46, further evidence that these receptors are important for caffeine taste.[75]

Caffeine taste is aversive to both vertebrates and invertebrates, and bitter-responsive GPCRs are at least partly responsible for aversive responses to caffeine.[76] However, bitter taste receptors are not conserved across species[77] and, therefore, neither are the mechanisms responsible for caffeine taste. For example, a recent study found that caffeine activated only one mouse T2R: Tas2R121, which is encoded by the gene, *Tas2r121*,[14] an ortholog of human *TAS2r13*. Polymorphisms in *hTAS2R13* have been associated with ethanol preference[78] and intake in humans,[79] but hTAS2R13 is not responsive to caffeine.[66]

Caffeine's action on endogenously expressed T2Rs has not yet been confirmed in taste cells (the ability to knockdown or block T2Rs expressed by taste cells in culture or to conditionally knockout [KO] T2Rs in type II TRCs would be required for confirmation), but there is evidence that caffeine is a T2R ligand in other cells that endogenously express T2Rs. For example, Xu et al. demonstrated that caffeine increases calcium release in haploid germ cells (e.g., spermatids and spermatozoa) from mouse seminiferous tubules. Caffeine-induced calcium release was most likely mediated by T2Rs because a T2R antagonist blocked the effect.[63] Although the T2R antagonist used by Xu *et al.*, probenecid, has other pharmacological targets, we[30] (and others[31]) found that knocking out one of the main alternative targets (*Panx1*)[80] has no effect on taste responses. Moreover, α-gustducin (also known as *Gnat-3*) KO mice do not avoid caffeine in brief-access taste tests,[81] which suggest that α-gustducin, another protein important for signaling through T2Rs,[82–87] is necessary for detecting the aversive (presumably bitter) taste of caffeine. It is noteworthy that in a previously published study we found no difference between *Itpr3* WT and KO mice in intake of caffeine during 48-hour tests[88] (Itpr3 is necessary for T2R-mediated signaling). Because caffeine is a psychoactive drug with postingestive effects, it is possible that the Itpr3 KO mice used cues other than bitterness to avoid drinking the caffeine solutions in the study.

Caffeine is a toxic deterrent to honeybees[89] and other insects at higher concentrations (e.g., the concentration found in vegetative and seed tissues[90]), but at lower concentrations (e.g., the concentration found in pollen, which is below the bitter detection threshold for bees) it can enhance a pollinator's memory of reward, which could increase the likelihood of the plant's reproductive success.[91] Caffeine appears to be the product of selective pressure on plants to protect their leaves and seeds, while encouraging pollination. Like in mammals, caffeine activates canonical bitter taste receptors in insects. In drosophila, at least Gr33, Gr66, and Gr93 are important for aversive responses to caffeine.[92] Knocking out Gr66 diminishes aversive responses to the drug[93,94] and activation of neurons that are responsive to bitter receptor cells causes insensitivity to caffeine.[95] Interestingly, inducing apoptosis in gustatory receptor neurons that express Gr93 completely abolishes caffeine sensing in drosophila.[13] Either Gr93 is sufficient for caffeine sensing or the cells that express Gr93 are necessary for caffeine sensing. Caterpillars also display aversive behavioral responses to caffeine, which are mediated by their epipharyngeal and lateral styloconi taste sensillum—sensilla that are also sensitive to other chemicals that humans describe as bitter.[96,97] However, it is not clear if bitter taste receptors mediate these responses.

Because bitter taste receptors are responsible for rejection of bitter food across (nearly) all species, it is likely that caffeine binds to these receptors because the taste of caffeine is universally avoided. Goldfish reject caffeine.[98] Guinea pigs, hamsters, and mice also avoid the taste of caffeine, suggesting that it is bitter (or elicits a negative taste quality) to these species as well.[17,99] Rhesus macaques generalize between quinine and caffeine (i.e., they do not discriminate between the taste of quinine and the taste of caffeine), again demonstrating its similarity to other bitter tastes, at least in primates.[100] In rodents, discrimination between caffeine and other chemicals that taste bitter to humans has not received enough attention. As would be expected based on their aversive responses to the taste of caffeine, rats easily discriminate between sweet taste (which elicits appetitive responses) and caffeine taste.[101] However, somewhat surprisingly, one study found that golden hamsters do not cross-generalize a conditioned taste aversion to bitter tastes and caffeine, suggesting that caffeine possesses qualities beyond bitterness that this species can detect.[17]

The authors proposed that caffeine may elicit aversive reflexes in hamsters using a nontaste route. This seems possible given that caffeine elicits little to no chorda tympani[17] or glossopharyngeal nerve responses[102] in rodents. With that said, a separate study using single neuron recordings found that caffeine both inhibits and activates chorda tympani and glossopharyngeal neurons in rats.[103] Although whole-nerve recordings show small responses to caffeine, individual gustatory nerve fibers in rodents likely respond to caffeine. Overall, responses to the aversive properties of caffeine are well-conserved, but whether or not these properties always include bitterness as humans perceive it or some other quality remains to be determined.

In summary, bitter taste receptors are likely the main mechanism responsible for caffeine's bitterness across species. However, there is good reason to believe that caffeine acts on pathways that do not involve bitter taste receptors. There are several lines of evidence that support this claim: First, even after total ablation of bitter responsive neurons in drosophila larvae, some avoidance to caffeine remains intact.[13] Second, some species do not generalize between caffeine and other bitter tastes, suggesting that caffeine may not elicit a purely bitter taste. Finally, as will be discussed in the following section, caffeine is known to act on a number of molecular pathways that do not involve bitter taste receptors.

T2R-Independent Mechanisms of Caffeine Taste

T2R-independent mechanisms of caffeine action on taste cells are mediated by three types of mechanism: (1) non-T2R receptors on TRCs (Fig. 3A), (2) plasma membrane ion channels on nerve fibers (Fig. 3B), and (3) intracellular (both receptor-dependent and independent) (Fig. 4). We discuss each in turn.

Non-T2R receptors on TRCs

Adenosine receptors. Caffeine is a nonspecific AR antagonist.[4] In fact, its action as an AR antagonist in the brain is the primary mechanism responsible for its psychoactive effects.[2] Like T2Rs, ARs are GPCRs that modulate activity of effector molecules such as adenylate cyclase and PLC.[104] However, in contrast to T2Rs, which are always linked to inhibitory G proteins (i.e., G proteins that decrease levels of cAMP), AR antagonism can both increase and decrease cAMP levels depending on whether or not the AR is linked to a stimulatory G

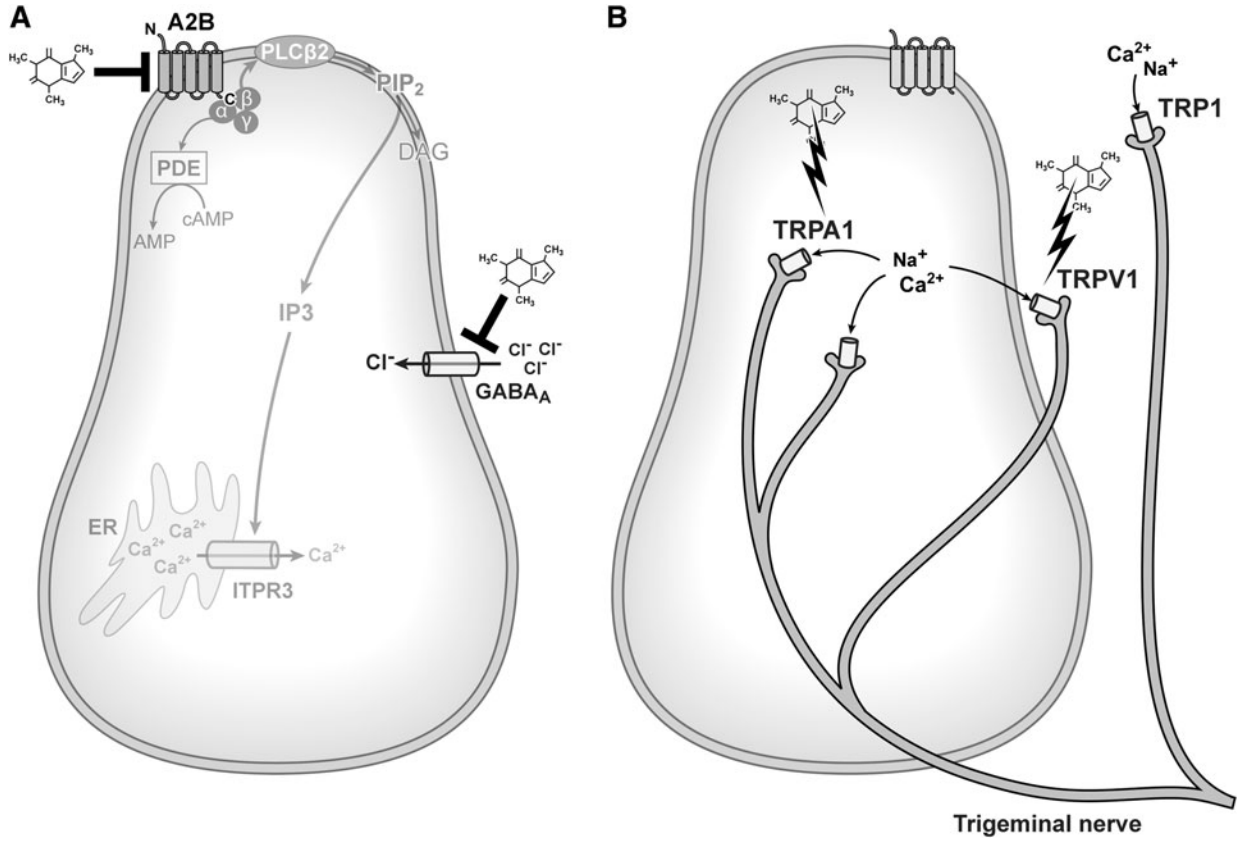

FIG. 3. T2R-independent mechanisms of caffeine taste. Caffeine can inhibit the A2B GPCR and the $GABA_A$ channel on TRCs **(A)**. Caffeine can activate Trpa1 and TRPV1 channels, which are expressed by trigeminal nerve fibers **(B)**. GPCRs, G-protein-coupled receptors; TRCs, taste receptor cells.

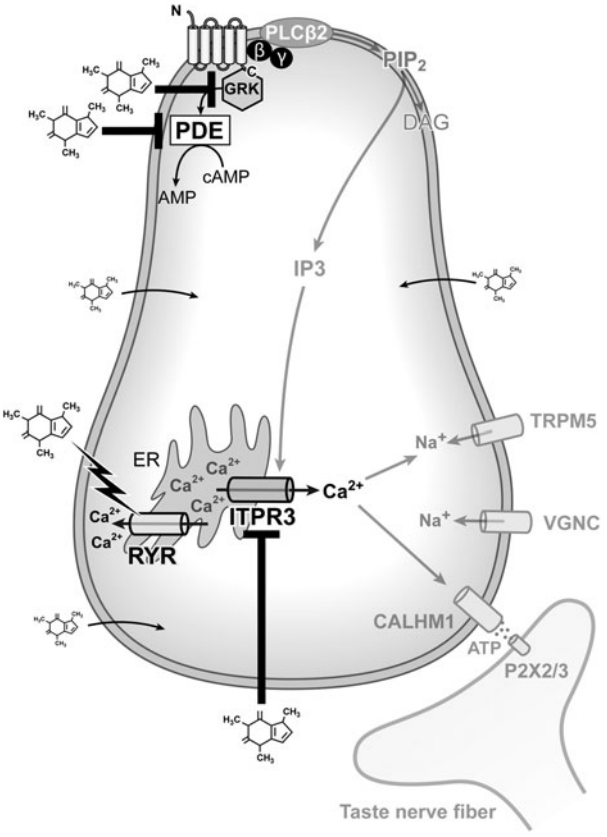

FIG. 4. Intracellular mechanisms of caffeine taste. Caffeine can directly block PDEs, GRKS, and ITPR3 by entering TRCs and other cells. Caffeine can activate RYRs. GRKs, GPCR kinases; PDE, phosphodiesterase; RYRs, ryanodine receptors.

protein or an inhibitory G protein. The ultimate effect of an AR antagonist such as caffeine depends on the relative expression of the AR subtype in the tissue. Four ARs have been cloned as follows: A1, A2A, A2B, and A3.[105] A1 and A3 are coupled to inhibitory G proteins. This means that their activation inhibits adenylate cyclase and decreases the production of cAMP. In contrast, A2A and A2B are coupled to stimulatory G proteins.[106] Caffeine displays similar micromolar (μM) affinity at A1, A2A, and A2B and lower affinity for A3.[107]

Taste cells show prominent expression of A2B (stimulatory), moderate expression of A1 (inhibitory), and no expression of A2A and A3.[108] Thus, because caffeine is a nonspecific AR antagonist with micromolar affinities for all ARs, caffeine most likely antagonizes A2B (Fig. 3A) and A1, the receptors expressed by taste cells, at the concentration typically found in coffee (coffee contains $\sim$0.75 mg/mL [$\sim$4 mM] caffeine); a typical 8-oz cup and provides a dose of 2.5 mg/kg and a peak plasma concentration of 10 μM.[4] Because salivary concentrations are 65–85% of plasma concentrations if caffeine is taken in pill form, the concentration of caffeine in the oral cavity likely ranges between 8.5 μM and 4 mM (depending on whether or not coffee is in the mouth). Although no studies have explored whether or not caffeine antagonism of A2B or A1 is responsible for aspects of caffeine taste, several studies have indirectly examined the interplay between adenosine and caffeine on taste with contradictory results. One study found that adenosine reverses caffeine-induced enhancement of NaCl and quinine taste in humans[109] (the ability of caffeine to enhance flavors, at least the flavor of soda, remains controversial[110–112]). In contrast, a later study found that neither caffeine nor caffeine paired with adenosine influenced taste responsivity or taste intensity ratings in humans.[113] Adenosine can enhance sweet taste through A2B receptors in mouse taste bud cells that coexpress the sweet sensing receptor subunit Tas1r2.[28,108] Therefore, it is possible that adenosine has a general bitter-masking effect, at least in rodents (and maybe in humans, too[109]). However, this seems unlikely because adenosine does not mask responses to other bitter stimuli in mice.[108] Therefore, caffeine may elicit bitterness or reduce sweetness by blocking the action of endogenous adenosine at A2B. Interestingly, one study found that preadministration of an adenosine agonist increases caffeine consumption[114] suggesting that adenosine decreases caffeine's aversive taste qualities. Furthermore, a genome-wide meta-analysis identified 15 polymorphisms in *ADORA2B*, the gene that encodes A2B, as being associated with coffee drinking.[73] Whether or not caffeine's bitterness can be partly attributed to its ability to antagonize A2B receptors remains unknown.

GABA receptors. Caffeine (and other methylxanthine stimulants) is thought to be an antagonist at the benzodiazepine-positive modulatory site on $GABA_A$ receptors (Fig. 3A). The first evidence for this was provided by Marangos et al.,[115] who found that caffeine competitively inhibited H^3 diazepam binding. Although it remains somewhat unclear if caffeine directly binds to $GABA_A$ receptors, or indirectly decreases binding of $GABA_A$ ligands (i.e., if caffeine's effect on $GABA_A$ ligand binding or currents are primary or secondary),[4] the effects of caffeine on $GABA_A$ signaling have been demonstrated using several different methods and cell types (mainly neuronal subtypes[116–119] and caffeine seem to either effect $GABA_A$ ligand binding directly or to affect the stability or function of $GABA_A$ receptors). Interestingly, GABA (the endogenous amino acid ligand for $GABA_A$ receptors) masks the bitter taste of caffeine (in addition to the bitter taste of quinine, coca, and chocolate).[120] It was reported that GABA is a bitter taste receptor antagonist, but it is also possible that GABA prevents caffeine blockade of $GABA_A$ receptors. In taste buds, like in the mature brain, GABA is an inhibitory transmitter. In TRCs during taste stimulation, GABA acts on both $GABA_A$ and $GABA_B$ receptors to suppress ATP secretion.[121] It has been postulated that GABA not only suppresses ATP secretion in taste cells but also regulates

the growth and differentiation of taste buds.[121] In support of the role of GABA in development of oral tissue, $GABA_A$ receptor KO mice show abnormal palate development.[122] Because $GABA_A$ receptors are also expressed in other cells in the gastrointestinal tract, including the duodenum where they appear to regulate motility,[123] it is possible that caffeine can also modulate gastric motility by acting on GABA transmission in duodenal cells. In fact, increased gut motility is a well-documented side effect of caffeine. Although this effect of caffeine can be partly attributed to its blockade of ARs,[124] caffeine action at GABA receptors is a possibility as well.

Plasma membrane ion channels

TRP channels. There is evidence that caffeine is not only a bitter taste but also has other chemosensory qualities such as oral irritation (e.g., burning and tingling). However, the mechanisms supporting these sensations are not yet clear. Published anecdotal evidence led to recommendations that patients who have facial pain caused by trigeminal neuralgia should reduce or avoid food and drink that contains caffeine.[125] There is experimental evidence to support this. For example, cultured rat trigeminal neurons respond to caffeine as measured by changes in intracellular calcium.[126] However, other alkaloids, such as nicotine, seem to have a much stronger effect on trigeminal neurons than does caffeine. The principal machinery responsible for trigeminal neuron activation in response to oral stimulation with taste stimuli is TRP channels. TRP channels are expressed in taste buds and trigeminal nerve fibers, and are important for transducing signals that give rise to the sensations of taste, irritation, warmth, coolness, and pungency.[127] Several TRP channels could be responsible for aspects of caffeine taste (e.g., TRPM5, TRPV1, and TRPA1). TRPM5 is downstream of all signaling through taste receptors and triggers ATP release necessary for the detection of taste qualities, including sweetness and bitterness (Fig. 1). Caffeine does not appear to act directly on Trpm5.[128] In contrast, caffeine does act on TRP channels expressed by sensory fibers (Fig. 3B). For example, Trpv1, a receptor for irritants such as capsaicin—the chemical responsible for the spiciness and burning sensation caused by hot chili peppers—is present in sensory fibers, but not TRCs. Trpv1 is activated by acidic (and basic) pH.[129] Caffeine solutions are neutral ($\sim$6.9) and there are no published reports of caffeine activating Trpv1 channels expressed by trigeminal fibers. However, caffeine activates Trpv1 channels in rat nodose ganglion neurons.[130] Furthermore, other bitter alkaloids (with the exception of quinine) activate Trpv1.[127,131,132] Therefore, it is reasonable to conclude that caffeine activates Trpv1 channels in trigeminal fibers as well. Caffeine activates mouse Trpa1 channels in a heterologous expression system, but inhibits human TRPA1 channels.[133] Why caffeine would have different effects on Trpa1 signaling in rodents and humans is not clear (however, the aversive quality of caffeine does seem to be more than bitterness in rodents. As mentioned previously, rodents do not generalize between caffeine and other bitter tastes, but primates do). Caffeine does not act on Trpa1 channels in moths either, again highlighting the species-specific responses to caffeine.[134]

Potassium, sodium, and chloride channels. Caffeine modulates voltage-activated ionic currents in taste cells.[135] Using patch clamp and ratiometric imaging techniques on dissociated rat TRCs, Zhao et al. found that caffeine inhibits outwardly and inwardly rectifying potassium currents.[135] In the same study, caffeine inhibited sodium and calcium currents, yet had no effect on chloride currents.[135] These results are interesting because Breslin et al. found that NaCl suppresses the bitterness of caffeine in humans.[136] Together, these studies raise the possibility that the ability of NaCl to suppress caffeine bitterness is due to NaCl-induced reversal of the inhibitory effect of caffeine on sodium currents.

Intracellular targets

Caffeine is lipophilic (i.e., fat soluble). As a result, it readily crosses all biological membranes and, thus, has the potential to exert direct intracellular effects, independently of cell surface receptors or ion channels (Fig. 4). This is an important point for understanding caffeine taste because taste quality is determined by the cell, not by the receptor expressed by the cell. For example, sweet taste cells that are genetically engineered to express bitter taste receptors confer appetitive qualities to aversive chemicals like caffeine.[86] Therefore, if caffeine unselectively enters all types of taste cells, it could produce an unpredictable taste quality (i.e., a combination of all tastes).

ITPR3 and other intracellular calcium channels. Caffeine elicits distinct effects in cells that express many of the effector proteins in the canonical taste pathway (e.g., ITPR3 and TRPM5), but do not express T2Rs. Caffeine has no effect on TRPM5 currents; however, it can modestly inhibit ITPR3-mediated calcium flux (Fig. 4).[128] Given that Itpr3s have a caffeine binding domain,[137] caffeine could modulate intracellular calcium release independently of T2Rs by directly binding to ITPR3. Much evidence suggests that caffeine also increases intracellular calcium levels by acting on ryanodine receptors (RYRs)[138,139] (Fig. 4). The importance of intracellular receptors in the effect of caffeine on calcium levels is strengthened by the finding that caffeine-induced changes in calcium levels are dependent on intracellular stores, not extracellular calcium levels in TRCs.[135] In addition, our unpublished work showing that *Itpr3* KO mice do not detect the aversive taste of caffeine during brief-access taste tests suggests that a direct interaction between ITPR3 and caffeine could modulate taste.

Intracellular enzymes. Phosphodiesterases: Although much is known about caffeine's action as a

phosphodiesterase (PDE) inhibitor in insects, little is known about how caffeine acts on mammalian PDEs. As was mentioned earlier (What Is a Bitter Taste section), when taste receptors are activated, the $\beta\gamma$ subunit of the GPCR activates PLCβ and IP_3-mediated release of calcium from intracellular stores. Another part of the taste transduction pathway involves the α subunit, α-gustducin, which likely activates PDEs to decrease intracellular cAMP levels[140–144] (Fig. 4). If caffeine is able to directly interfere with PDE activity in taste cells, this could prolong stimulus-induced increases in intracellular cAMP or cGMP. In fact, Rosenzweig et al. found that caffeine induces rapid, transient, and gustatory tissue-specific increases in cGMP levels (cGMP is a second messenger like cAMP; however, cGMP is a more specialized messenger than cAMP) and hypothesized that this rise in cGMP levels was responsible for caffeine's bitter taste.[145] The authors proposed that the increase in cGMP was due to caffeine's action as a PDE inhibitor (PDEs inhibit the degradation of cGMP). However, this hypothesis was not investigated further.[146,147]

GPCR kinases: In intestinal cells, caffeine activates GPCR kinases (GRKs) and increases calcium in a PLC-dependent manner.[148] In contrast, caffeine and other amphipathic taste compounds have been shown to inhibit GRKs in taste cells (Fig. 4).[149] Because GRKs are important for phosphorylation and desensitization of GPCRs (including bitter taste receptors),[150] caffeine may be able to modulate the responsiveness of T2Rs, ARs, and GABARs to their ligands. In other words, caffeine may have the ability to modulate its own receptors by directly activating GRKs.

Other Factors Contributing to Caffeine's Bitterness

Over the past 15 years twin studies, double-blind/placebo-controlled trials, and genome-wide association studies have identified associations between more than 20 genes and caffeine taste, responses to caffeine, and/or drinking of caffeinated beverages.[73,151–162] This work sheds light on mechanisms responsible for caffeine detection and voluntary caffeine consumption. Genes encoding bitter taste receptors, ARs, and intracellular enzymes have all been implicated in responses to caffeine and caffeinated beverages, as described in What Is a Bitter Taste, Caffeine's Action on the Canonical Bitter Taste Pathway, and T2R-Independent Mechanisms of Caffeine Taste sections. However, there are many variables to take into consideration when analyzing these genetic studies, including the caffeine vehicle (e.g., water, coffee, or soda), whether or not the subject or animal has had prior experience with caffeine, and age, to name a few. Next, we will discuss these other factors.

Vehicle

Caffeine is a natural compound found in coffee and tea and is an additive found in sodas and energy drinks. Because of its reinforcing properties, it has been challenging to determine how the taste of caffeine *per se* is influenced by other compounds in coffee and tea or by sugars or other chemicals in sodas and energy drinks (i.e., how caffeine taste is influenced by the vehicle). It also remains unclear how the temperature or pH of caffeinated solutions influences caffeine action and caffeine taste. There is evidence both supporting[109] and refuting[111,112] caffeine's purported role as a flavor enhancer. However, there is evidence that fatty vehicles interfere with caffeine taste. One study demonstrated that caffeine can be sequestered by biophenols in olive oil, which could prevent it from reaching its targets and decrease its bitterness.[163] Another study demonstrated that lipoproteins can inhibit nerve responses to caffeine.[164] Although beverage manufacturers have described caffeine as a ''flavor enhancer,'' evidence does not support this claim. For example, most people cannot distinguish between caffeinated and noncaffeinated beverages.[111] Only after repeated pairings of caffeine and various flavors do preferences begin to shift, suggesting that caffeine's purported flavor enhancing effects are actually the result of its action in the central nervous system.[111,165–167] Similarly, caffeine increases soda liking in adolescents, but only after repeated exposures,[168] suggesting that immediate taste responses are not involved. In contrast, as mentioned previously, Breslin et al. found that NaCl reduces the bitterness of caffeine.[136] Therefore, the salt content of a beverage may affect caffeine taste. More work is needed to understand under what circumstances caffeine modulates taste and flavor perception.

Saliva composition

Saliva composition may also determine the taste of caffeine. Dsamou et al. demonstrated that subjects who are hypersensitive to the bitterness of caffeine had higher levels of amylase fragments, immunoglobulins, and serum albumin in their saliva.[169] The saliva of the same hypersensitive subjects contained lower levels of cystatin SN (a protease inhibitor). Therefore, proteolysis within the oral cavity may, in part, determine sensitivity to the bitter taste of caffeine.

Prior exposure to caffeine

Substances that are perceived as bitter are typically avoided by animals and this may be an adaptation that protects them from consuming foods that will produce adverse physiological effects.[170–173] Consumption of bitter substances such as caffeine may include a learned component. For instance, Newland et al. found that when caffeine-naive rats were presented with caffeine or water, they consumed very little caffeine.[114] However, rats with a history of forced caffeine consumption consumed caffeine more readily. Therefore, repeated exposure to caffeine likely results in positive associations between the bitterness of caffeine and its psychoactive effects.

There may be other effects of prior exposure to caffeine that increase its palatability, but that do not involve learning. For example, Lipchock et al. recently found that caffeine intake is correlated with PAV-TAS2R38 expression.[174] Therefore, caffeine intake may alter expression of taste receptor genes and influence bitter detection as a consequence.

Psychiatric conditions

Differential reactivity to sweet and bitter tastes is associated with psychiatric disorders such as depression,[175] alcoholism,[176,177] and posttraumatic stress disorder.[178] Taste preferences may therefore be a marker for drug abuse vulnerability and other psychiatric disturbances. Interestingly, caffeine-enhanced sensitivity to the bitterness of quinine may be a characteristic of panic disorder.[178]

The focus of this review is on caffeine action in taste cells, not the brain, but the line between taste and nontaste receptors has become blurry. Neurons are able to respond to various taste compounds and caffeine may act directly on bitter taste receptors in the brain to modulate bitter perception. Singh et al. found that T2Rs are expressed in multiple regions of the rat brain. Tas2r4, Tas2r107, and Tas2r38 transcripts were present in the brain stem, cerebellum, cortex, and nucleus accumbens.[54] In the same study the authors demonstrated that quinine could activate these T2Rs. Caffeine likely activates T2Rs in the brain as well because caffeine-responsive T2Rs are expressed there (Fig. 2).[56] Whether or not caffeine taste perception is mediated by both peripheral and central bitter taste receptors remains uncertain.

Age-related responses to caffeine

Children generally dislike bitter tastes more than adults do.[179] Not surprisingly, children also display different responses to bitter-masking compounds. Although sodium gluconate decreases caffeine bitter perception in adults, it has no effect on caffeine taste responses in children.[180] Interestingly, both children and adults respond similarly to the bitter masking effect of sucrose on caffeine.[180] Age-related differences in caffeine taste deserve more attention because caffeinated beverages are marketed to children and adolescents.

Conclusion

Caffeine directly activates T2Rs,[14,66] but there are other taste transduction mechanisms as well, including ARs, GABA receptors, TRP channels, and intracellular receptors and enzymes. These targets, including T2Rs, are expressed not only in taste tissue but also in diverse cell types throughout the digestive, endocrine, and reproductive systems (Fig. 2). In summary, caffeine should not be considered a prototypical bitter taste—not only can it act on many T2R-independent pathways in taste cells but also it can activate the trigeminal system, and it acts directly on the central nervous system.

Public health message: At low concentrations the effects of caffeine are likely benign, which is why it has been labeled a GRAS substance by the FDA. However, exposure to caffeine is increasing due to an increase in the number of products containing caffeine[18,112] and the concentration of caffeine added to them.[181] Furthermore, caffeinated products are being marketed to, and consumed by, children, whose taste systems and preferences are developing. Therefore, it is important to understand how caffeine might influence taste sensation and perception and the function of the digestive system. Specifically, more research is needed to better understand how higher concentrations of caffeine might influence the function and development of the taste system and digestive system.

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