Quantitative assessment of TRPM5-dependent oral aversiveness of pharmaceuticals using a mouse brief-access taste aversion assay

Heather R. Devantier, Daniel J. Long, Francis X. Brennan, Stacy A. Carlucci, Cynthia Hendrix, Robert W. Bryant, F. Raymond Salemme and R. Kyle Palmer

Many orally administered pharmaceuticals are regarded by humans as aversive, most often described as 'bitter'. Taste aversiveness often leads to patient noncompliance and reduced treatment effectiveness. 'Bitter' taste is mediated by T2R G-protein coupled receptors through a peripheral signaling pathway critically dependent upon function of the TRPM5 ion channel. The brief-access taste aversion (BATA) assay operationally defines aversive taste as suppression of the rate at which a rodent licks from sipper tubes that deliver tastant solutions or suspensions. We have used a mouse BATA assay for rapid quantification of oral aversiveness from a set of 20 active pharmaceutical ingredients (APIs). Robust lick-rate dose–response functions were obtained from both C57BL/6J wild type (WT) and C57BL/6J/TRPM5−/− (TRPM5 knockout) mouse strains, generating reliable determinations of potency and relative maximal oral aversiveness for each API. A subset of APIs was also evaluated in a human bitterness assessment test; effective concentrations for half-maximum responses (EC_{50s}) from both the human test and WT mouse BATA were equivalent. Relative to WT potencies, EC_{50s} from TRPM5 knockout mice were right-shifted more than10-fold for most APIs. However, APIs were identified for which EC_{50s} were essentially identical in both mouse strains, indicating a TRPM5-independent alternative aversive pathway. Our results suggest the BATA assay will facilitate formulation strategies and taste assessment of late development-phase APIs.
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Discovery Research, RedPoint Bio Corporation, Ewing, New Jersey, USA

Correspondence to Dr R. Kyle Palmer, PhD, Discovery Research, RedPoint Bio Corporation, Ewing, NJ 08628, USA
E-mail: kpalmer@redpointbio.com

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Introduction

Many pharmaceuticals are regarded by humans as orally aversive, most frequently described as bitter (Sohi *et al.*, 2004). The unpalatable taste is often cited as the primary cause of failure to adhere to therapeutic regimens, particularly among pediatric patients (Demers *et al.*, 1994; Bauchner *et al.*, 1996; Bauchner and Klein, 1997; Steele *et al.*, 2001, 2006). Formulation strategies designed to ameliorate aversive taste, with the ultimate goal of improving patient compliance, therefore, could benefit from pharmacological approaches to the study of taste and taste aversion.

The sensation of taste begins with the activation of specific taste receptors in the tongue. For at least three taste modalities, sweet, umami, and bitter, the receptors involved are G-protein coupled receptors localized in highly specialized cells within the taste bud (Chandrashekar *et al.*, 2000; Nelson *et al.*, 2001, 2002; Zhao *et al.*, 2003). Stimulation of these G-protein coupled receptors results in the activation of an intracellular signaling pathway that depends on the G-protein gustducin (McLaughlin *et al.*, 1992; Wong *et al.*, 1996) and $PLC_{\beta 2}$ (Zhang *et al.*, 2003). Disruption of the genes for either of these proteins results in severe taste deficits specific to sweet, umami, and bitter sensation (Wong *et al.*, 1996; Ruiz-Avila *et al.*, 2001; Zhang *et al.*, 2003; Dotson *et al.*, 2005; Glendinning *et al.*, 2005). Within the same taste cells (Perez *et al.*, 2002; Kim *et al.*, 2006), and further along the signaling pathway, another significant modulator is present. TRPM5, a member of the transient receptor potential ion channel family of signaling proteins, also plays a critical role in the transduction of taste information. Accordingly, knockout (KO) mice lacking the TRPM5 channel display similar deficits for sweet, umami, and bitter taste (Zhang *et al.*, 2003; Damak *et al.*, 2006).

The interplay between a tastant molecule and its taste receptor appears to be no different in principle from any ligand/receptor interaction in pharmacology. Taste signaling should therefore be amenable to investigation by standard pharmacological techniques. To address basic questions regarding tastant molecule potency, efficacy, selectivity, and structure-activity relationships, in-vitro assays have been developed using recombinant cells that

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express heterologous taste receptors (Bufe *et al.*, 2002; Behrens *et al.*, 2004; Jiang *et al.*, 2004, 2005; Xu *et al.*, 2004). Animal models for studying taste exist (reviewed by Spector, 2002), but only recently they have acquired the semblance of pharmacologic experimental design (Eylam and Spector, 2002, 2003; Bhat *et al.*, 2005).

One such model is the brief-access taste assay, where the licks of water-restricted animals are electronically recorded during brief (usually 5–10 s) exposures to taste solutions. Decreases and increases in lick rate, relative to water, are taken as a measure of oral aversiveness and appetitiveness, respectively. Although not a direct measurement of taste, taste is inferred from changes in the lick rate as the animal samples a standard solution recognized by humans to represent an essential taste modality. For example, quinine is bitter to humans, and suppresses rodent licking in a concentration-dependent manner (Brasser *et al.*, 2005; Glendinning *et al.*, 2005).

In addition to providing a simple and objective measure, the brief-access taste assay can generate robust concentration–effect data, typically within brief 30-min sessions (Boughter *et al.*, 2002; Glendinning *et al.*, 2002; Spector, 2002). The concentration–effect functions generated are amenable to classic pharmacologic analysis, providing a powerful approach to the investigation of taste signaling mechanisms. Furthermore, the brief-access taste assay offers the potential for rapidly predicting the aversive taste of orally administered pharmaceutical drugs in the late phases of preclinical drug development.

Here, we describe the use of C57BL/6J mice in the brief-access taste assay to establish the oral aversive potency of a number of pharmaceuticals that are reported as bitter by humans. Some of the pharmaceuticals were also tested using C57BL/6J mice deficient in the gene for the TRPM5 ion channel, to determine the dependence of oral aversiveness on the function of TRPM5. Finally, a subset of the compounds tested in mice was evaluated by a human taste panel for bitter potency, across a range of concentrations. We have found the brief-access taste assay to be an effective method of generating reliable dose–response functions suitable for pharmacological investigation of mechanisms underlying taste signaling, and note a striking correspondence in oral aversive potency between humans and mice.

Methods

Subjects

Mouse subjects

C57BL/6J TRPM5 + / + (WT) and C57BL/6J/TRPM5 − / − (TRPM5 KO) mice were procured from Taconic Laboratories (Rennsalear, New York, USA). TRPM5 KO mice were rederived from breeding pairs originally obtained from Dr Robert Margolskee at Mt. Sinai School of Medicine (New York, New York, USA). Each group consisted of roughly equal numbers of males and females between the ages of 8 and 24 weeks. A 12-h light/dark cycle was maintained in the colony room (lights on at 07.00 h) and all animals had free access to food and water, until water restriction began for experimental purposes (see below). All procedures were approved by the Institutional Animal Care and Use Committee of Albert Einstein Medical Center (Philadelphia, Pennsylvania, USA).

Human participants

A trained taste panel consisting of 10 healthy, nonpregnant, Caucasian women between the ages of 24 and 52 years (mode = 41) participated in the human bitter taste assessment. The test was administered by ABIC International (Fairfield, New Jersey, USA), and NIH guidelines for use of human participants were followed. Participants were compensated for their participation in the study.

Apparatus

A Davis MS-160 lickometer chamber obtained from DiLog Instruments (Tallahassee, Florida, USA) was used and has been fully described elsewhere (Boughter *et al.*, 2002). Briefly, the apparatus consisted of a Plexiglas-walled cage with a wire mesh floor. The front wall was stainless steel with a port, 2.5 cm from the floor, for the mouse to insert its snout to access a sipper tube attached to a 10 ml glass bottle. Sixteen such bottles containing different doses of the compounds of interest were mounted on a rack that moved laterally via a computer-controlled linear actuator. The sipper tubes were positioned one at a time in front of the port. The order of presentation (and thus the dose) of the sipper tubes was randomized. A computer-programed shutter over the port controlled access to the sipper tube.

Procedure

Brief-access taste aversion assay

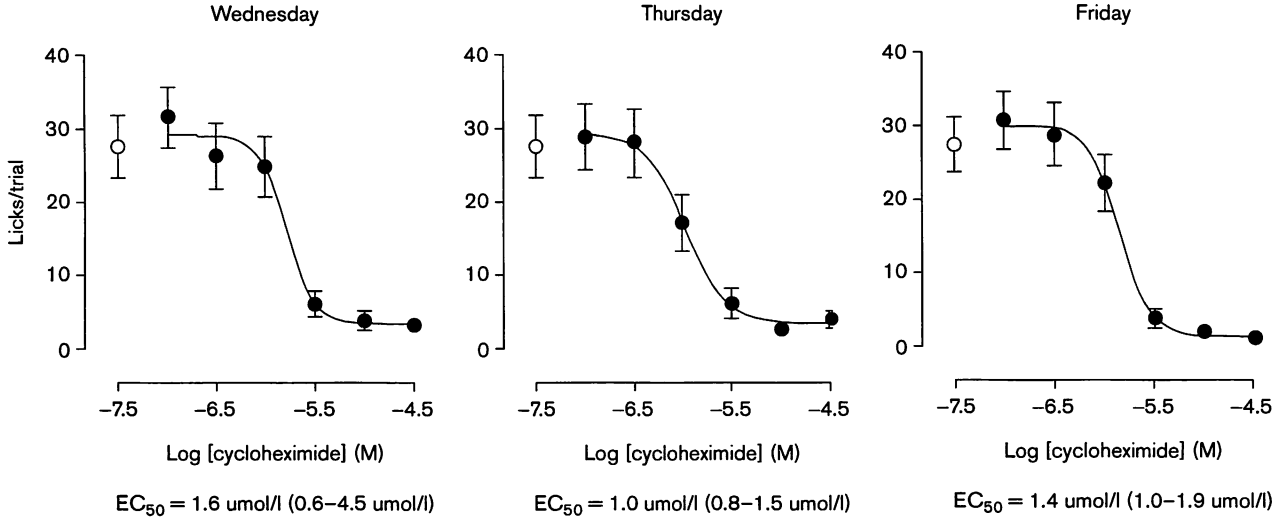
Water deprivation schedule

Mice were mildly water deprived according to a weekly deprivation schedule so that they were sufficiently motivated to lick from sipper tubes delivering either water or varying concentrations of pharmaceuticals in aqueous solution or suspension. Water bottles were removed from the home cages on Sunday evenings, 16 h before the Monday session. From Monday through Thursday, daily water consumption was comprised of intake during training and test sessions (typically about 1 and 0.3 ml, respectively) and 1 h of free access to supplementary water after sessions. Upon completion of the Friday session, water bottles were returned to the home cages so that water was given freely throughout the weekend.

Water training

Water training was conducted on Monday and Tuesday of each week. On Mondays, the shutter port was left

Fig. 1



Stability and reliability of the BATA assay across days. Seven concentrations of cycloheximide or water were presented in a randomized order to WT mice ($n = 18$ per group) during 30-min test sessions. Sample duration for each presentation was 5 s. The number of licks per trial was recorded and averaged across all animals for each concentration and analyzed by nonlinear regression. EC_{50s} and $CI_{95\%}$ (in parentheses) were obtained by curve fitting (Prism). The figure shows data obtained from the same mice on three consecutive test days. Similar results were obtained from at least five additional experiments. Open symbols represent number of licks to the vehicle control (water), collected during the same session. BATA, brief-access taste aversion; CI, confidence interval; EC_{50}, effector concentration for half-maximum response.

open for the entire training session (30 min) to allow free access to a single water-delivering sipper spout. On Tuesdays, two water bottles were placed in the rack of the Davis rig. Each sipper tube was presented one at a time and mice were allowed access when the port shutter opened at the beginning of each sampling trial. Mice initiated a 5-s trial by licking from the sipper tube; that is, the shutter remained open for 5 s after the first lick was detected. The intertrial interval was also 5 s. All sessions were of 30 min in duration. Consistent with previously reported results (Dotson and Spector, 2005), each mouse consumed approximately 1 ml of water under these conditions.

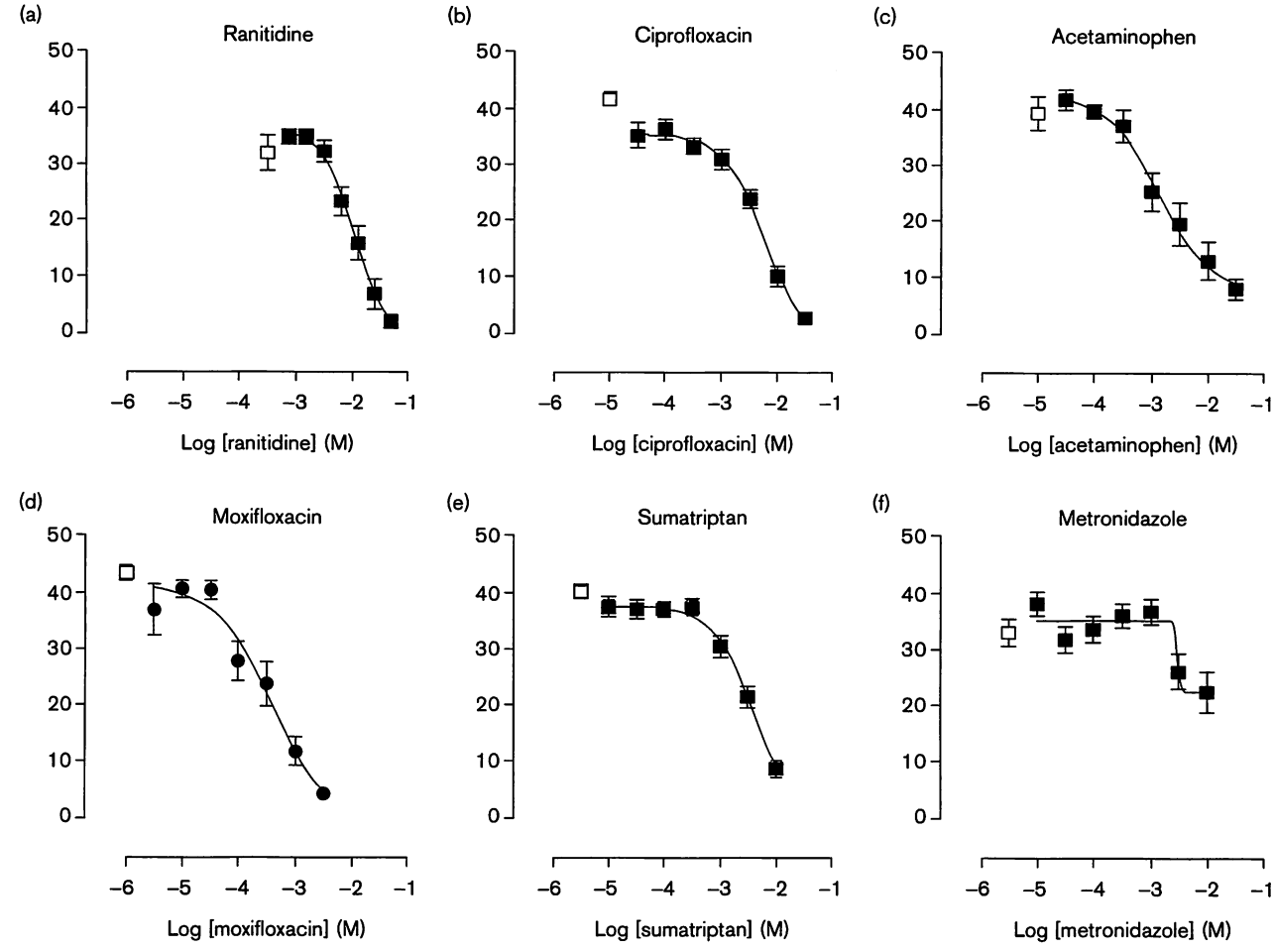
Taste aversion testing

Wednesday through Friday were test days on which mice were randomly presented sipper tubes that delivered seven different concentrations of pharmaceutical solutions or suspensions, as well as the vehicle. Each sipper tube was presented one at a time for a 5-s sampling trial. There was no 'rinse' in between trials. As with training sessions, intertrial intervals were of 5 s and test sessions were of 30 min in duration. As each trial was initiated by the subject, the number of trials could vary for each subject. Relative to water consumption on Tuesdays, substantially less aversive tastant solution was consumed during test sessions. Considering quinine HCl as a representative aversive tastant, WT mice consumed an average of 301 ± 25 (SEM) µl of solution during a 30-min test session. Thus, with 10 mmol/l as the high end of the concentration range, mice consumed approximately 30 µg of quinine during a test session. Active pharmaceutical ingredients (APIs) that were presented in a higher concentration range, such as ranitidine, therefore, are likely to have achieved greater cumulative dosage. Nevertheless, no evidence of post-ingestive effects of consumed API was indicated when vehicle lick rate during test sessions was compared with lick rates obtained on Tuesday water-training sessions.

Human taste testing

A double-blind procedure was used for preparing and administering the tastant solutions. Participants orally self-administered a total of six different concentrations of each pharmaceutical agent and vehicle (0.1% Tween 20/2% ethyl alcohol) in a random order. The mode of administration was 2 ml of solution or suspension in a paper cup. Each participant tested two replicates of each concentration (or vehicle) for a total of 12 samples, within a single session. Participants were directed to swirl the 2 ml sample in their mouth for 3–5 s, then expectorate the entire sample into a discarded cup. Sampling was followed immediately with a water rinse (2 ml), which was also spit out. Participants were then required to rate the intensity of bitterness for the sample on a scorecard by marking a numerical value along a scale from 0 to 8 (0 = none, 2 = slight, 4 = definite, 8 = very strong). A period of 20 min was allowed to elapse between presentations of each sample to reduce the potential influence of residual taste effects.

Fig. 2



Dose–response function for six APIs from different structural and therapeutic classes. Data were obtained as described in Methods and Fig. 1. Data shown in each panel are representative of at least three independent experiments (n=16–18 per group). Open symbols represent the number of licks to the vehicle control (panels a–e: water; panel f: 0.5% DMSO/0.1%) collected during the same session. APIs, active pharmaceutical ingredients; DMSO, dimethyl sulfoxide.

Chemicals

Cycloheximide (bitter standard), quinine HCl (bitter standard), capsaicin (TRPM5-independent aversive standard, TRPV1 agonist), ciprofloxacin (antibiotic), nystatin (antifungal), ranitidine (antihistamine, H2 receptor antagonist), metronidazole (antibiotic), cimetidine (antihistamine, H2 receptor antagonist), levofloxacin (fluoroquinolone antibiotic), nicotine (nicotinic acetylcholine receptor agonist), diphenhydramine (antihistamine, H1 receptor antagonist), topiramate (anticonvulsant), dextromethorphan (cough suppressant), sodium valproate (anticonvulsant), vardenafil (erectile dysfunction, PDE5 inhibitor), acetaminophen (analgesic), dimethyl sulfoxide (DMSO), polysorbate (Tween) 20, and ethanol (EtOH) were purchased from Sigma/Aldrich (St. Louis, Missouri, USA). Clarithromycin (macrolide antibiotic), sumatriptan succinate (antimigraine, 5HT1 receptor agonist), moxifloxacin (fluoroquinolone antibiotic), cetirizine (antihistamine, H1 receptor antagonist), and desloratidine (antihistamine, H1 receptor antagonist) were obtained from API Services (Hong Kong). Finally, transcutol was obtained from Gattefossé (Paramus, New Jersey, USA).

Data analysis

For the mouse brief-access taste assay, the number of licks per trial for each concentration of solution/suspension or vehicle was collected and averaged across animals in each experimental group (n = 16–18). Lick rates for TRPM5 KO mice tended to be slightly lower than those of WT. Thus, for comparisons between genotypes, data were normalized by dividing the number of licks emitted by a mouse for each trial by licks for the vehicle control trials, to yield a 'lick ratio'. Curve fitting (Prism; GraphPad Software, San Diego, California, USA) was performed on the data and effector concentration for half-maximum responses (EC_{50s}) and 95% confidence inter-

vals ($CI_{95\%}$) were derived from the curve fit. Statistical determination of differences in dose–response functions between genotypes was achieved by extra sum-of-squares F tests (Prism; GraphPad Software). For the human taste test, bitterness scores were averaged across participants for each concentration of tastant. EC_{50s} and $CI_{95\%}$ were calculated from the Prism curve fit as described above. For statistical comparison of EC_{50s} between mouse brief-access taste aversion and human bitterness assessment, data for both assays were transformed to percent maximal response and analyzed by nonlinear regression followed by extra sum-of-squares F test as above.

Results

Stability and reliability of the brief-access taste assay

Quinine and cycloheximide are well known to be orally aversive to both mice and rats (e.g. Ishii *et al.*, 2003). Accordingly, we used them as standard bitter compounds. Full concentration–effect functions for suppression of licking were obtained for both quinine and cycloheximide and were stable across daily sessions. The EC_{50s} varied little from values of approximately 300 and 1 μmol/l, for both compounds. The cycloheximide data across three separate days are presented in Fig. 1.

Effect of solvents in the brief-access taste assay

As some of the compounds tested required using organic solvent vehicles, we tested whether the vehicles themselves were orally aversive to mice. Ethanol, DMSO, transcutol, and Tween 20 were tested over a range of concentrations. Ethanol and DMSO up to concentrations of 1% (v/v) had no measurable effect on lick rate compared with water alone. The mice tolerated higher concentrations of transcutol, with concentrations up to 4% having little or no impact on licking. The maximum concentration of Tween 20 without effect was 0.2%. The combination of 0.5% DMSO/0.1% Tween 20 did not affect lick rates and was used as an excipient for several of the insoluble compounds tested.

Effect of pharmaceuticals in the brief-access taste assay

Concentration–response functions were obtained for each drug tested. The results with the quinolone antibiotics ciprofloxacin and moxifloxacin, the H2 histamine receptor antagonist ranitidine, the serotonin receptor agonist sumatriptan, the analgesic acetaminophen, and the antiinfective drug metronidazole are presented in Fig. 2. The results from all compounds tested are summarized in Table 1. The potencies of this widely diverse group of drug compounds were remarkably similar; most EC_{50s} ranged narrowly around 1 mmol/l.

The maximal effect on lick suppression varied somewhat, but complete suppression of licking was achieved with the majority of pharmaceuticals tested. Two notable exceptions were metronidazole (Fig. 2f) and nystatin (Fig. 3g). Formulation issues may have interfered with the capacity to test high concentrations of these two drugs. The compounds were administered as suspensions and tended to settle at the bottom of the water bottles during the 30-min session. Even with incomplete suppression of licking, oral aversiveness potencies could still be estimated. For example, visual inspection of the dose–response data for metronidazole and nystatin suggest oral aversiveness potencies of approximately 3–10 mmol/l for both.

Table 1 Summary of all pharmaceuticals tested, grouped into those sensitive and those insensitive to TRPM5 function

Compound	WT	TRPM5 KO
Oral aversiveness potencies of compounds 'sensitive' to TRPM5 function		
Cycloheximide	0.002 (0.001–0.005)	>0.100
Acetaminophen	1 (0.3–5)	>30
Cimetidine	2 (2–3)	>30
Ciprofloxacin	2 (1–3)	>100
Clarithromycin	0.07 (0.02–0.3)	>1
Diphenhydramine	1 (0.7–2)	>30
Levofloxacin	3 (2–5)	>30
Moxifloxacin	0.4 (0.06–3)	>10
Nicotine	0.2 (0.1–0.4)	5 (1–24)
Quinine HCl	0.3 (0.2–0.5)	>30
Ranitidine	8 (6–11)	>30
Topiramate	1 (0.8–2)	>10
Oral aversiveness potencies of compounds that are 'insensitive' to TRPM5 function		
Capsaicin	0.002 (0.001–0.004)	0.003 (0.001–0.005)
Cetirizine	0.3 (0.2–0.4)	0.3 (0.1–0.9)
Dextromethorphan	1 (1–2)	5.1 (1.5–17)
Ibuprofen	13 (10–17)	8 (5–12)
Oxybutinin	0.5 (0.3–0.8)	0.3 (0.07–1)
Sumatriptan	4 (1–13)	2 (0.4–9)
Valproate	8 (3–12)	7 (2–12)
Vardenafil	0.4 (0.3–0.5)	0.7 (0.4–1)

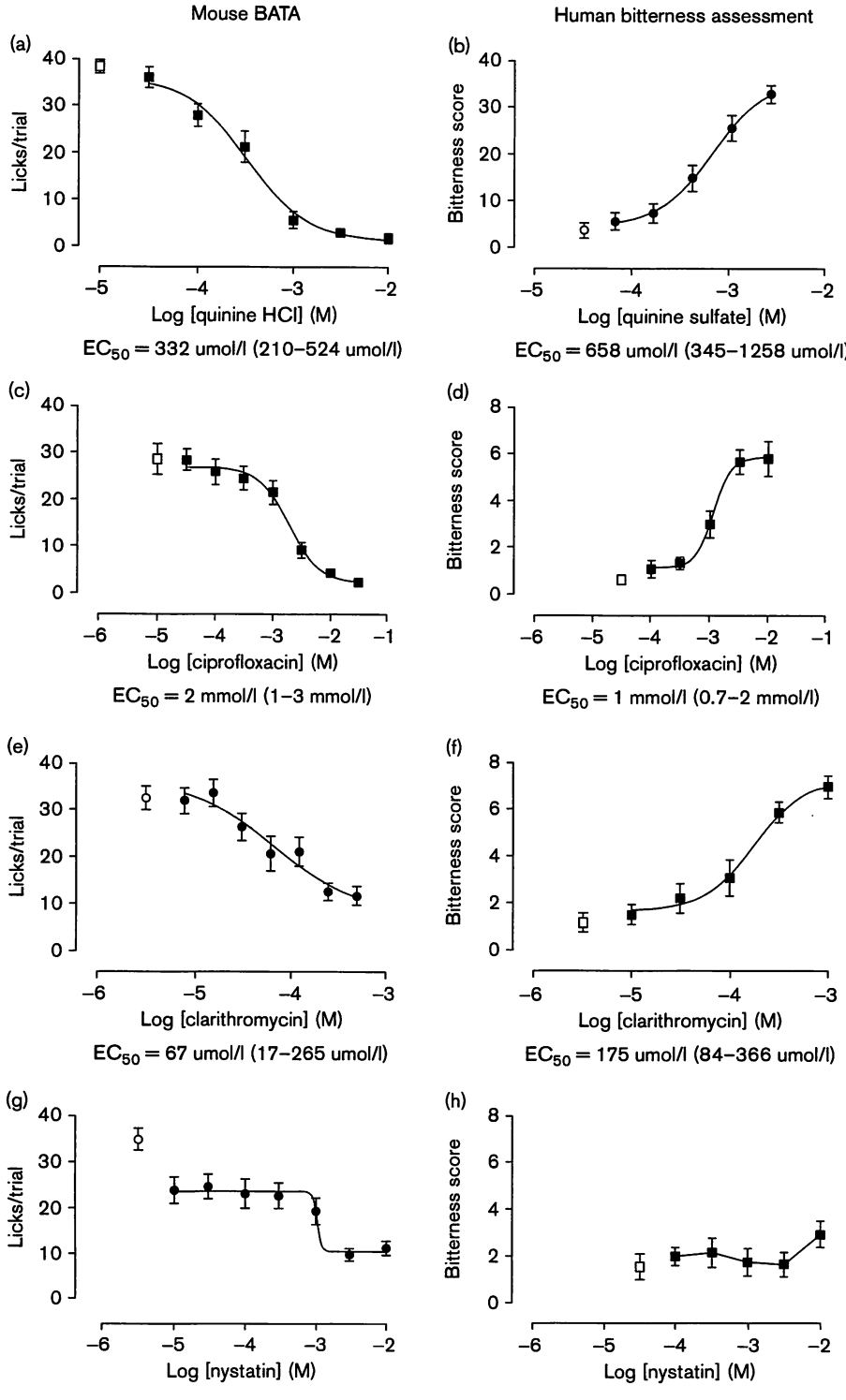
Values given are EC_{50s} expressed in units of mmol/l, with 95% confidence intervals given in parentheses, obtained from WT and TRPM5 knockout (KO) mice. Differences in EC_{50s} between strains sensitive to TRPM5 were determined by an F test ($P<0.0001$; see Methods). Data are representative of at least two similar experiments ($n=16$–18).

Comparison of human taste test to mouse brief-access taste aversion

Four of the compounds tested in mice were selected and evaluated by a human taste panel. Participants were asked to rate the bitterness of varying concentrations of quinine, ciprofloxacin, clarithromycin, and nystatin (Fig. 3b, d, f, h). All four drugs were rated as bitter. Mean maximal scores obtained were 6.6, 5.8, and 7.0 to the highest doses of quinine, ciprofloxacin, and clarithromycin, respectively (Fig. 3, panels b, d, and f). Nystatin elicited a slight bitterness rating (2.97) only at the highest concentration tested (Fig. 3h).

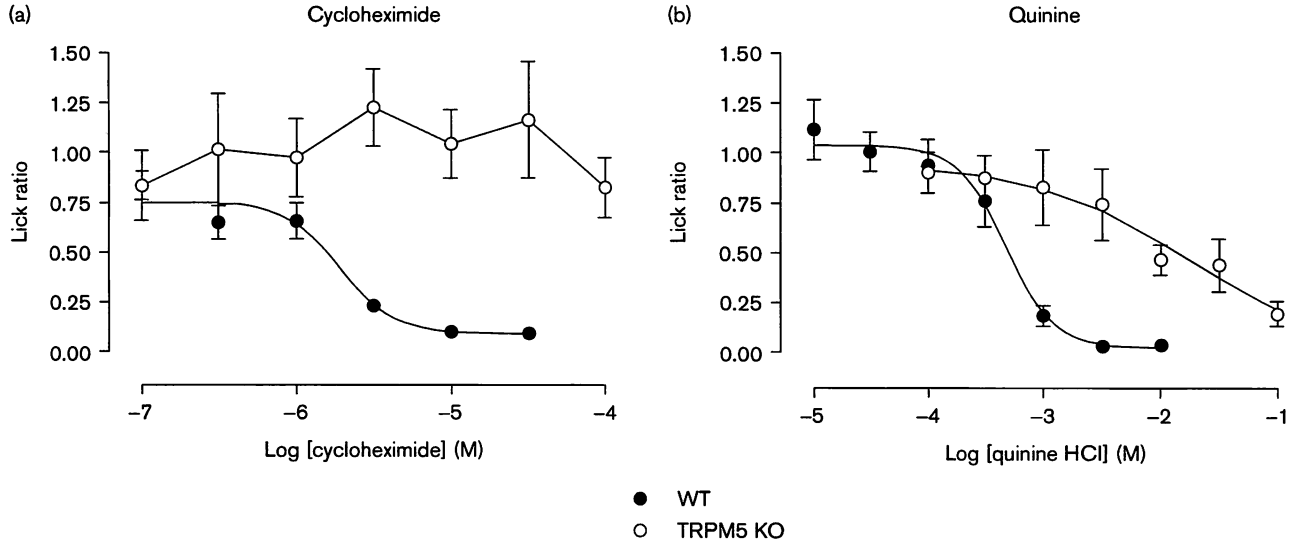
The relative potencies of the three compounds for which a full concentration–effect function was obtained in the human taste test was clarithromycin > quinine > ciprofloxacin, which mirrors the relative potency in the mouse brief-access taste assay. In addition, the absolute potency of the drugs corresponded closely between humans and mice; EC_{50} values obtained from the human taste test

Fig. 3



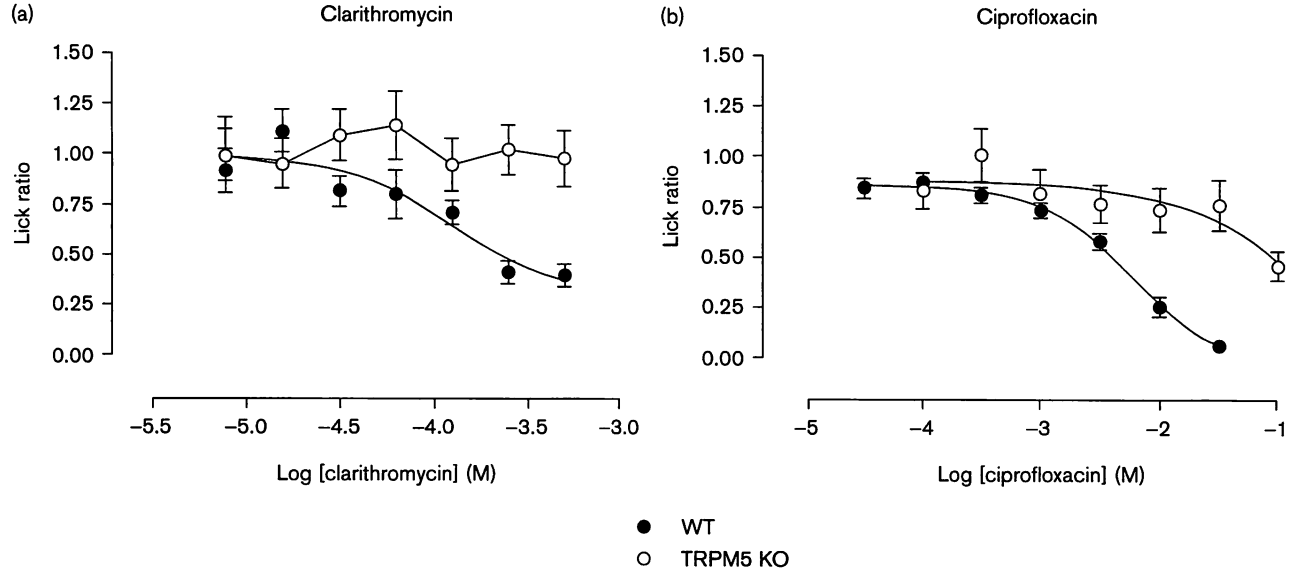
Comparison of lick suppression in mice ($n=16$–18 per group) and human bitterness score ($n=10$) for four compounds. (a, b) Quinine, (c, d) ciprofloxacin, (e, f) clarithromycin, and (g, h) nystatin. Results shown for mice are representative of at least two similar experiments. Human bitterness assessment experiments were performed once for each compound tested. Open symbols represent responses to the vehicle control (panels a–d, g, h: water; panels e, f: 0.5% DMSO/0.1%) collected during the same test session. BATA, brief-access taste aversion; DMSO, dimethyl sulfoxide; EC_{50}, effector concentration for half-maximum response. 95% confidence intervals are given in parentheses.

Fig. 4



Oral aversiveness of cycloheximide (a) and quinine (b) is mitigated in TRPM5 knockout (KO) mice. Open circles=TRPM5 KO mice; closed circles=WT mice (n=16–18 per group). Data are expressed as lick ratio (see Methods) and are representative of at least three similar experiments.

Fig. 5



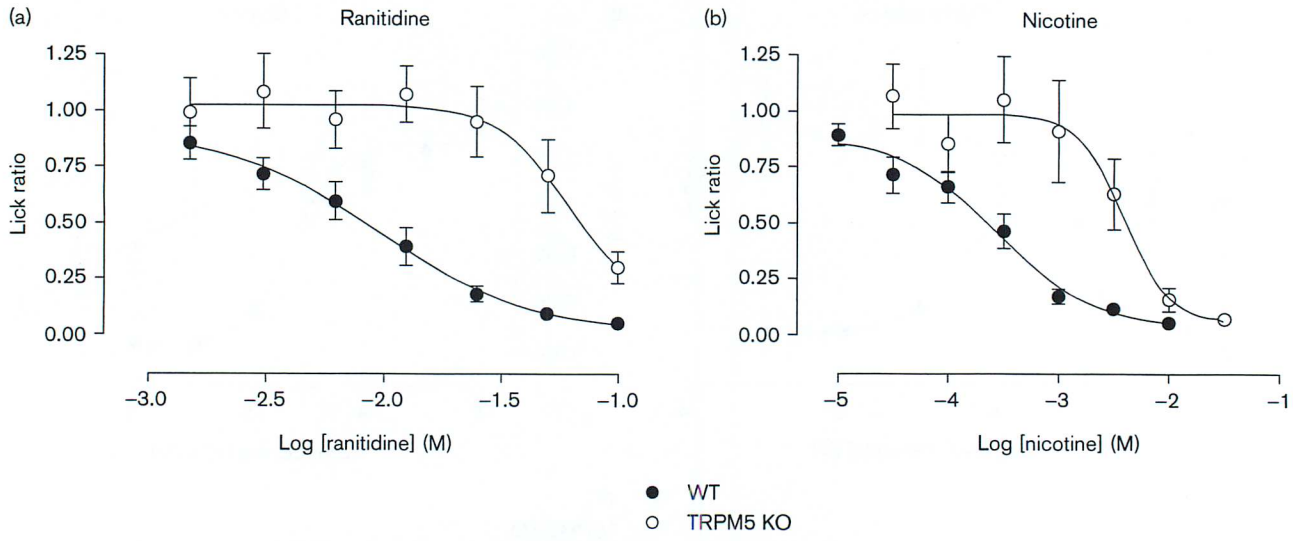
Oral aversiveness of the antibiotic pharmaceuticals clarithromycin (a) and ciprofloxaxin (b) is mitigated in TRPM5 knockout (KO) mice. Open circles=TRPM5 KO mice; closed circles=WT mice (n=16–18 per group). Data are expressed as lick ratio (see Methods) and are representative of at least two experiments.

were within one-half log unit of those derived from the mouse brief-access taste assay and did not differ statistically (by extra sum-of-squares F test; Methods).

Effect of pharmaceuticals in the TRPM5 knockout

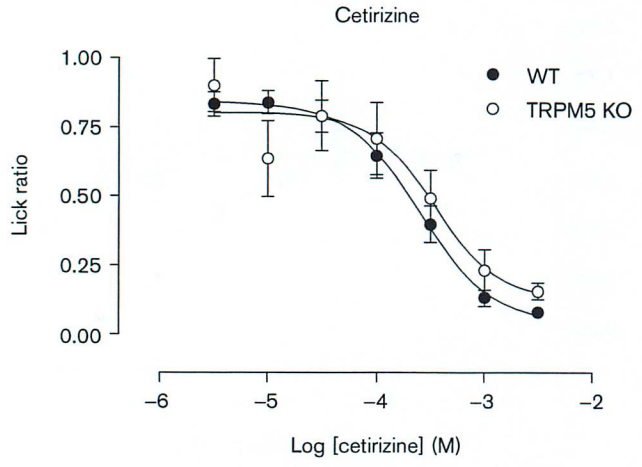
All of the compounds were tested in the brief-access taste assay using TRPM5 KO mice. These data are presented in Figs 4–7, and Table 1. Cycloheximide and quinine were much less effective at suppressing lick rates in the TRPM5 KO mice than in the WT mice (Fig. 4). A similar reduction in potency for clarithromycin, ciprofloxacin, ranitidine, and nicotine in KO mice compared with WT mice was observed (Figs 5 and 6; Table 1). The H1 receptor antagonist cetirizine was representative of

Fig. 6



TRPM5 dependence of oral aversiveness is observed across pharmaceutical classes. Open circles=TRPM5 knockout (KO) mice; closed circles=WT mice (n=16–18 per group). Data are representative of two similar experiments.

Fig. 7



Oral aversiveness of cetirizine is independent of TRPM5. Open circles=TRPM5 knockout (KO) mice; closed circles=WT mice (n=16–18 per group). Data are representative of two similar experiments.

compounds that induced identical effects in both the TRPM5 KO and the WT mice (Fig. 7). Interestingly, diphenhydramine, also an H1 receptor antagonist, was potently aversive only to the WT mice (Table 1).

Discussion

In this study, we demonstrated the utility of the brief-access taste assay to study oral taste aversion in mice. Further, we determined the oral aversive potencies of a number of pharmaceutical drugs, including representatives of the following therapeutic classes: drugs to treat allergies, gastrointestinal disorders, migraine, epilepsy, erectile dysfunction, as well as microbial and fungal infections. A total of 20 chemicals were tested in oral solutions, including quinine and cycloheximide, two of the most commonly used substances to study bitter taste. We thereby extended earlier work on oral taste aversion (Bhat *et al.*, 2005) in mice to include pharmaceutical compounds from a wide range of drug classes and chemical structures.

A primary focus of the current work was the pharmacological concept of potency. Frequently overlooked in the taste literature, this measure is critical in advancing knowledge of taste signaling mechanisms. For the most part, data that determine the oral aversive potency of tastants come from animal models and have been limited to a relatively small number of standards, such as quinine (Ishii *et al.*, 2003), cycloheximide (Boughter *et al.*, 2005), and denatonium (Boughter *et al.*, 2005). Although reports in the human literature refer to bitter potency, it is unclear whether potency in these cases was determined from evaluations of full concentration ranges. We generated orderly concentration–effect functions and reliable EC_{50} values for suppression of lick rate for each compound.

One major advantage of the brief-access taste assay is its capacity to assess oral taste aversion in an objective and quantitative manner. Unlike other methods that rely on

predefining bitterness either by qualitative behavioral response or by similarity of response to quinine and cycloheximide, the brief-access taste assay simply characterizes whether consumption rate of a test compound is greater than or less than that of water. This bidirectional response allows both appetitive and aversive responses to be evaluated. Therefore, any agent that results in a reduction or avoidance of consummatory behavior is operationally defined as orally aversive. In addition, by varying the concentration of a chemical compound in solution, concentration–effect relationships can be determined along with their derivative parameters of potency and maximum effect.

Whereas this method of placing pharmaceutical compounds on a continuum of consummatory behavior may be devoid of overt descriptions of flavor, it produces a profile that is highly consistent with more subjective human taste tests. We ascertained bitterness potencies in a human taste test for a subset of pharmaceutical drugs: quinine, clarithromycin, nystatin, and ciprofloxacin. The potencies determined in humans were essentially the same as those determined in mice. This suggests that the aversiveness of these pharmaceuticals is similar across species and that the brief-access taste assay is predictive of human taste assessment as well. Additional testing in humans, using other compounds over a range of concentrations, needs to be done to strengthen this assertion.

The modality of bitterness has long been the focus of efforts to improve the taste of orally administered pharmaceutical drugs. All of the pharmaceutical compounds tested currently have been reported as bitter by human participants. However, although bitterness is the most frequent cause of eliciting an oral aversion, it is not the only one. Capsaicin, for instance, is also orally aversive, apparently by activation of a response through TRPV1 receptors expressed in the tongue (Pingle *et al.*, 2007). A description of bitterness, however, has been included in the verbal responses in human taste tests of capsaicin (e.g. Lim and Green, 2007). Although it is possible that capsaicin can activate bitter taste pathways in addition to the TRPV1 pathway, it is unknown to what degree these qualitative features are conflated in verbal reports of taste. In any case, it is not clear that the bitter taste reported with capsaicin is the same as that reported with quinine. The brief-access taste assay cannot address this possibility; sophisticated taste discrimination analysis can, but it is a time-consuming process. More to the point, such confusion obscures mechanisms that might identify targets for pharmacologic intervention of the bitter signal produced by a known bitter agent.

Studies on the physiology of taste indicate that TRPM5 channels are important in the post-receptor signaling cascade that occurs after bitter molecules interact with their receptors on taste buds. We studied a subset of pharmaceutical compounds in mice lacking the TRPM5 channel and found that the taste aversion was greatly diminished or completely abolished compared with wild-type mice. This sensitivity to intact TRPM5 channels held for the bitter standards quinine and cycloheximide, the antibiotics clarithromycin and ciprofloxacin, as well as the histamine blocker ranitidine and the cholinergic agonist nicotine. In contrast, oral aversiveness to acidic solutions (described by humans as sour; DeSimone *et al.*, 2001), high concentrations of NaCl (Damak *et al.*, 2006), and capsaicin (unpublished data) have been shown to be independent of TRPM5 in a variety of taste assays. Further, even though high concentrations of quinine can act on additional signaling systems to induce oral aversion, within the range experienced as bitter by humans, quinine aversion was dependent on the function of TRPM5.

We propose that TRPM5 sensitivity in the brief-access taste assay be used as an operational definition of bitter taste for two reasons. First and most importantly, it avoids the confusion that can result from use of subjective descriptors of taste modalities. Second, it suggests a drug target with which to lessen oral taste aversion to bitter therapeutic drugs. The bitterness of many medicines is reported to be a significant problem for compliance. Because of this, much effort has been devoted to developing formulations that mitigate unpalatable tastes. At the same time, the elucidation of receptor-mediated taste signaling pathways presents an opportunity to control taste pharmacologically. To test potential taste-altering interventions, a more objective definition of bitterness is required than verbal reports. Quinine has been widely used as a bitter standard across species and is proven to be highly dependent on the presence of an intact TRPM5 channel for the bitter sensation. Thus far, among the taste sensations that are dependent on TRPM5, bitterness (e.g. quinine) is the only one that is aversive.

In summary, the brief-access taste assay is a rapid, in-vivo, reliable, quantitative method for determining oral taste aversion that correlates extremely well with human taste evaluations. Using this method, novel compounds or early-phase pharmaceuticals can be evaluated, including the potency to induce oral taste aversion. Further, compounds can be characterized by TRPM5 sensitivity. This technique may ultimately aid in the development of pharmacologic interventions for oral aversiveness.

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