



Thiamine and Quinine Differently Inhibit the Early Phase of Acetylcholine-Dependent Contraction of Mouse Ileum *in vitro*

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Abstract: Thiamine and quinine are popular bitter substances and their physiological effects have been studied; however, their impact on digestion remains unknown. Here, the physiological effects of thiamine and quinine was investigated for *in vitro* contraction of mouse ileum. Acetylcholine stimulates autonomous contraction of mouse ileum in a dose-dependent manner. The effect of Acetylcholine for contraction of ileum was partly suppressed by the adrenaline administration. Upon simultaneous treatment of the ileum by acetylcholine, thiamine and quinine decreased the maximum contraction. The period till half maximum contraction was prolonged by the presence of thiamine and quinine but not by adrenaline. Because a physiological effect of thiamine and quinine was observed on acetylcholine-induced contraction of the ileum, the repertoire of human bitter taste receptors, TAS2R-1, -4, -7, -10, -14, -31, -39, -40, -43, and -46, were investigated to which thiamine and quinine may bind. These human bitter taste receptors were further analyzed among the database for mouse homologs using evolutionally conserved amino acid sequences. The only bitter receptor for both thiamine and quinine was TAS2R-39, the homology of TAS2R-139 to human TAS2R-39 was 74%. Importantly, the homology of mouse TAS2R-119 to human TAS2R-1 which interact with thiamine was 91%, and that of TAS2R-130 to human TAS2R-7 that interact with quinine was 81%. The present study indicated that thiamine and quinine changed the early phase of contraction of ileum in mice and suggested that TAS2R119 and TAS2R130 expressed in mouse enteroendocrine cells to modify the physiological effects of thiamine and quinine on the acetylcholine-induced contraction of the ileum.

Keywords: Thiamine, Quinine, Movement, Small Intestine, Mouse

1. Introduction

Taste is one of the main chemosensory systems required for survival; it is essential to identify chemical substances in food so as to avoid toxic substances or to intake favorable nutritional sources [1-3]. Compounds with a sweet, bitter, or umami taste bind to specific receptors and modify certain cell functions via taste receptors coupled with calcium-signaling molecules (TRCSMs), such as phospholipase C- β 2, gustducin,

and inositol 1,4,5-triphosphate [3-9]. Recently, taste receptors and their TRCSMs have been histologically identified not only in taste cells but also in extraoral tissues, such as airway smooth muscle cells, enteroendocrine cells of the gastrointestinal tract, pancreatic β cells, sperm, and cells of the urogenital tract [6-14]. In extraoral regions, substances having certain taste cause physiological effects other than taste; for instance, sweet substances promote insulin secretion from pancreatic β cells, whereas bitter substances have been shown to be involved in asthma, pulmonary vasculature, upper

airway immune responses to microbial infections, and bladder reflexes [7-18].

Gastrointestinal contraction is regulated by the autonomous enteric-endocrine nervous system and the autonomic nervous system. Multiple levels of regulatory cells and mechanisms, including interstitial cells of Cajal (ICCs), motor neurons, hormones, paracrine substances, and inflammatory mediators, affect gastrointestinal contraction *in vivo* [19]. Previous studies using isolated intestinal tract have reported that intrinsic pacemaker activity originates from ICCs of the Auerbach plexus, which are electrically coupled to smooth muscle cells [20, 22]. ICCs play a major role in intestinal contraction without the involvement of motor neurons, hormones, or inflammatory mediators, and have been suggested to be modulated by several nutrients [23-26]. Another previous study reported an inhibitory action of caffeine on the smooth muscle contraction [27]. Electrophysiological analysis of cultured ICCs from the mouse jejunum indicated that networks of ICC generated slow waves, and these events were blocked by caffeine in a concentration-dependent manner [28-30]. It has previously been reported that caffeine binds to several type 2 taste receptors (TAS2Rs), which are known to detect bitter taste, and caffeine-binding TAS2Rs have also been shown to be reactive to other bitter substances [30]. Therefore, the effects of several bitter substances and TAS2Rs were investigated in the contraction of the mouse ileum.

2. Materials and Methods

2.1. Mice

ICR mice were used for all experiments in the current study according to the guidelines of the Animal Care and Use Committee of Japan Women's University and Tokyo Medical and Dental University. Mice were housed in polycarbonate cages under a 12 h/12 h light/dark cycle with *ad libitum* access to food and water.

2.2. Movement Analysis of the Ileum

For the preparation of *in vitro* studies, a 1 cm-long portion of mouse ileum was isolated while keeping the mouse under barbiturate anesthesia (Nembutal, 20 mg/kg, intraperitoneal injection). The inner contents of the isolated ileum were washed using pre-warmed Tyrode's solution (37°C), and subsequently the isolated ileum was subsequently fixed in a Magnus-type chamber filled with O₂-saturated Tyrode's solution (37°C), as previously reported using rat [31]. The proximal end of the preparation was set upward and connected to a strain gage through a cotton thread (Daruma#30, Yokoi, Osaka, Japan). The distal end of the preparation to the bottom of the chamber. Movement of the ileum was amplified using a strain gage amplifier ($\times 100$) and continuously recorded using a pen recorder (SS259F2, SEKONIK, Tokyo, Japan).

The movement of the ileum (spontaneous peristalsis) was recorded for 60 s following the administration of acetylcholine. Acetylcholine solution (300 μ l total volume, at the

concentration of 10 or 100 ng/ml) was topically injected onto the preparation of ileum using an injection syringe. When the recovery time after wash-out was too long to perform further experiments, acetylcholine and bitter substances were not tested at other concentrations.

Baseline was defined as the bottom line of the phasic deflection immediately prior to the administration of acetylcholine with or without several other substances. The maximum amplitude (MA) of the contraction of ileum was estimated as the baseline-to-peak phasic amplitudes within 20 seconds of administration.

2.3. Bitter Taste Substances

In this experiment, 0.1 M thiamin hydrochloride (Thi) and 0.1 M quinine hydrochloride (Qui) (Wako Pure Chemical Industries, Japan) were used [32]. The two-bottle test was performed using these bitter substances to confirm that there were no abnormalities in bitter receptors or intracellular signaling mechanisms in these mice.

2.4. Homology Analysis

Homology analysis was performed using the UniRef and NCBI databases.

2.5. Statistical Analysis

Data are expressed as the mean \pm standard error. We used the student's *t*-test to compare single values under control and experimental conditions or ANOVA followed by Dunnett's *post-hoc* analysis to compare groups of data. In all statistical analyses, $p < 0.05$ was considered statistically significant. The *n* values reported in the text refer to the number of cells used.

3. Results

3.1. Acetylcholine-Induced Contraction of the Ileum in a Dose-Dependent Manner

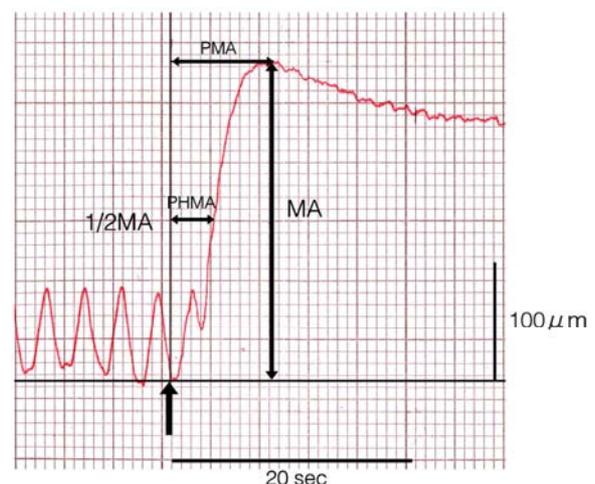


Figure 1. Typical example of contraction of the ileum induced by 10 ng/ml acetylcholine (Ach).

It was observed two movement phases of the ileum; the first

was the phasic contraction prior to the administration of acetylcholine and/or other substances, and the second was the tonic amplitudes induced by the substances (Figure 1). Acetylcholine (10 ng/ml) induced phasic or rhythmic contractions of the ileum *in vitro* (Figure 1).

We applied Ach to the tissue fragment of ileum (arrow head), and they induced tonic contraction (red line) within 20 s (black bar). The distance from the baseline (horizontal black line) to the inflection point of contraction (vertical double arrow) was taken as the MA. The period to maximum amplitude (PMA) (upper horizontal double-headed arrow) is defined as the duration from the time of Ach administration with or without other substances (vertical black line) to the MA, and the period to the half maximal MA (PHMA) is

defined as the duration from the time of Ach administration with or without other substances (vertical black line) to the half maximal MA (lower horizontal double-headed arrow). The effects of early phase contraction of the ileum were estimated as the MA, PHMA, and PMA.

Since the MA of the tonic contraction of the ileum indicated the maximum force of smooth muscle contraction, the MA was measured under each treatment condition (Fig. 1). In addition, the periods of MA (PMA) and half MA (PHMA) were also measured because these periods indicate whether the contraction of intestinal smooth muscle is induced directly through the intestinal plexus or indirectly through the intestinal endocrine system.

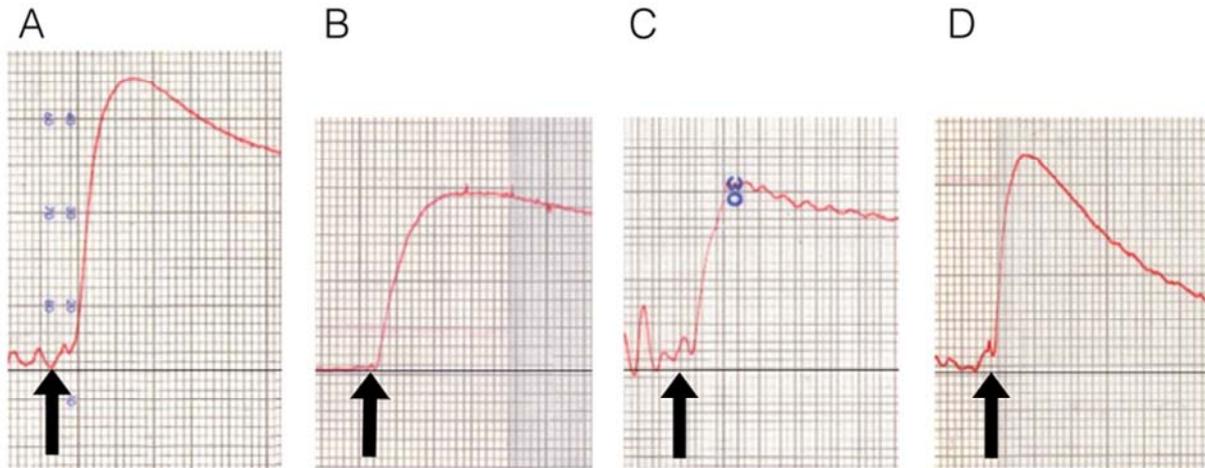


Figure 2. The typical pattern of the effects of acetylcholine (Ach) application with or without other substances on the contraction of the ileum.

Application of 100 ng/ml Ach solution (A) abolished the generation of phasic contraction. Administration of 100 ng/ml Ach solution with 100 ng/ml adrenaline (Ad) solution (B), 100 ng/ml Ach solution with 0.1 mM thiamine solution (C), and 100 ng/ml Ach solution with 0.1 mM quinine (D) was performed. The movement patterns (red line) were estimated using baseline (horizontal black line) and the time of Ach administration into the tissue fragment of ileum with or without other substances (arrow head).

As shown in Figure 2A, the administration of 100 ng/ml acetylcholine induced a clear tonic contraction of the ileum, and phasic or rhythmic contractions were absent because tonic

contractions were strong and long-lasting (data not shown). The MA of the acetylcholine-induced contraction of the ileum increased in a dose-dependent manner (Figures 1, 2A, 3A).

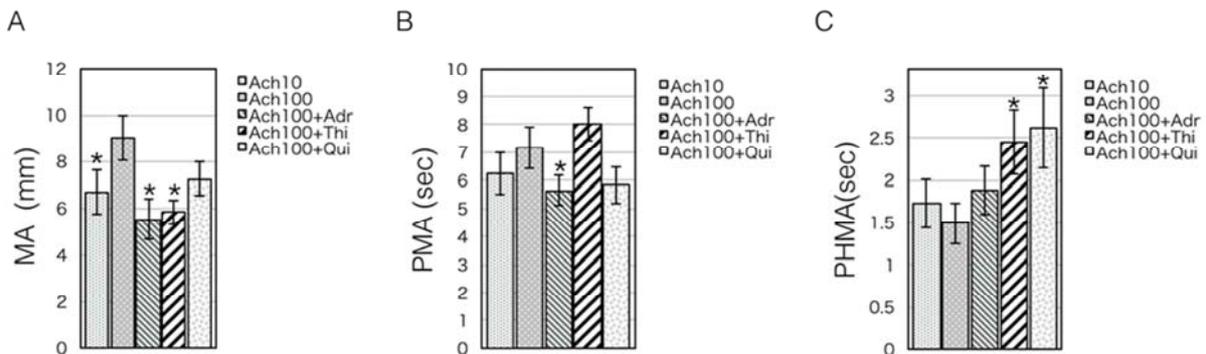


Figure 3. Changes in the MA, PHMA, and PMA of the early phase contraction of the ileum within 20 s after administration of Ach with or without other substances.

Significant differences in the MA ($p < 0.05$) between 100 ng/ml Ach (Ach100) and 10 ng/ml Ach (Ach10), and between Ach100 and 100ng/ml Ach + 0.1 mM thiamine (Ach100+Thi) were observed (A). Significant differences in the PHMA ($p < 0.05$) between Ach100 and Ach100+Thi, and between Ach100 and 100 ng/ml Ach + 0.1 mM quinine (Ach100+Qui) were observed (B). A significant difference in the PMA ($p < 0.05$) between Ach100 and 100 ng/ml Ach + 100 ng/ml adrenaline (Ach100+Ad) was observed (C).

3.2. Adrenaline and Bitter Substances Affected the Acetylcholine-Induced Contraction of the Ileum

Adrenaline (100 ng/ml) when administered with acetylcholine (100 ng/ml) resulted in a decrease in MA by approximately 60% (Figures 2B, 3A), and also the PMA (Figures 2B, 3C), without significantly changing PHMA (Fig. 3B). Thiamine (0.1 M) induced weak rhythmic contractions when administered with 100 ng/ml acetylcholine (Figure 2B). Thiamine (0.1 M) decreased the MA by approximately 65%, similar to 100 ng/ml adrenaline (Figures 2B, 2C, 3A), and increased the PHMA (Figures 2C, 3B) without changing the PMA (Figure 3C). Moreover, 0.1 M quinine decreased the MA by approximately 80% (Figure 2A) and increased the PHMA without changing the PMA (Figure 2B, C). Thiamine and quinine differently affect acetylcholine-induced contraction, but similarly increase the PHMA in the tissue fragments of mouse ileum.

3.3. Bitter Receptors Involved in Acetylcholine-Induced Contraction of the Ileum *in vitro*

Bitter taste receptors that are likely to be relevant to acetylcholine-induced contraction of the ileum were searched in mice. Because quinine and thiamine modified acetylcholine-induced contraction of the ileum in this experiment, TAS2R-1, -4, -7, -10, -14, -31, -39, -40, -43, and -46 were considered to be possibly involved. The only bitter receptor for both these substances was TAS2R-39 (Table 1). Subsequently, homologs of human and mouse TAS2 receptors were searched, and their evolutionary preservation was examined. As a result, the homology of TAS2R-139 to human TAS2R-39 was 74%. Importantly, the homology of mouse TAS2R-119 to human TAS2R-1 was 91%, and that of TAS2R-130 to human TAS2R-7 was 81%. These data suggest that suppression of the contraction of the ileum is independently related with TAS2R-119 for thiamine and TAS2R-130 for quinine.

Table 1. Homology of the amino acid sequences of mouse to human bitter receptor binding to quinine or thiamine.

humanTas2R		mouse homolog		
Quinine	Thiamine	ID	identities	positives
	TAS2R1	TAS2R119	260/299 (87%)	274/299 (91%)
TAS2R4		TAS2R108	199/299 (67%)	236/299 (78%)
TAS2R7		TAS2R130	213/312 (68%)	255 /312 (81%)
TAS2R10		TAS2R114	166/288 (58%)	208/288 (72%)
TAS2R14		TAS2R140	151/311 (49%)	200/311 (64%)
TAS2R31		TAS2R136	140/291 (48%)	193/ 291 (66%)
TAS2R39	TAS2R39	TAS2R139	171/298 (57%)	221/298 (74%)
TAS2R40		TAS2R144	211/319 (66%)	249/319 (78%)
TAS2R43		TAS2R144	72/293 (25%)	156/293 (53%)
TAS2R46		TAS2R120	148/291 (51%)	204/291 (70%)

Only TAS2R-39 overlaps with respect to binding quinine and thiamine; however, many TAS2Rs are different in humans. Mouse homologs of each human bitter taste receptor are shown in "ID". In each amino acid sequence of the homolog, the ratio at which the sequences are consistent is represented by "identities", and the ratio at which the properties of the amino acids are identical or similar is represented by "positives".

4. Discussion

It was observed that bitter substances differently affect the acetylcholine-induced contraction of the ileum *in vitro*. Inhibitory control of the acetylcholine-induced contraction of the ileum because of the bitter substances, quinine and thiamine, can be related to either enterochromaffin (EC) cells within the epithelial layer of gastrointestinal tract, ICCs within the nerve plexus, or the intestinal smooth muscle itself.

There are reports regarding the levels of TAS2R gene expression in mouse intestine [34, 35]. The gene expression levels of mouse Tas2R genes changes throughout its growing process. Almost no expression has been found in the small intestine during the neonatal period, whereas in adults, the thiamine-binding TAS2R-119 is mostly expressed, but not TAS2R-130 or -139, in small intestine [35]. In the current

study, it was observed that thiamine reduced the MA and extended the PHMA in tissue fragment of ileum. These observations suggest, at least in part, that thiamine interacts with TAS2R-119 in the ileum to reduce its movement.

Although there exists no previous study regarding the expression of the TAS2R family in ICCs or intestinal smooth muscle, it has been previously reported that EC cells have TRCSMs and receptors for sweet, umami, bitter, and fatty acids [35]. Moreover, because enterocendocrine cell-derived STC-1 cells have been reported to express at least TAS2R-119, -123, -103, -107, -130, -108, -105, and -126, thiamine may bind to TAS2R-119 and quinine to TAS2R-130 [35]. Therefore, TAS2 receptors in EC cells may mediate the inhibitory effects of thiamine and quinine on acetylcholine-induced contraction of the ileum. Furthermore, it has also been reported that EC cells release 5-HT to induce gut peristalsis of the mouse ileum [37], and that quinine significantly reduces its contraction, acting as an antagonist of the 5-HT₃ receptors [37]. These studies suggest that EC cells are important for the inhibition of the contraction of the ileum by bitter substances, such as quinine and thiamine. However, the possibility that thiamine functions as an antagonist at 5-HT₃ receptors remains to be elucidated in further studies.

5. Conclusion

In conclusion, the present study indicated that thiamine and quinine changed the early phase of contraction of ileum in mice. The evolutionary conservation in TAS2R receptors that were expressed in enteroendocrine cells partly explain the differences in early phase of contraction of ileum between thiamine and quinine in mice.

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