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# Effect of Ciguatoxin 3C on Voltage-Gated $Na^+$ and $K^+$ Currents in Mouse Taste Cells

**Valeria Ghiaroni[1], Haruhiko Fuwa[2], Masayuki Inoue[3], Makoto Sasaki[2], Keisuke Miyazaki[3], Masahiro Hirama[3], Takeshi Yasumoto[4], Gian Paolo Rossini[1], Giuseppe Scalera[1] and Albertino Bigiani[1]**

[1]Dipartimento di Scienze Biomediche, Università di Modena e Reggio Emilia, Via Campi 287, 41100 Modena, Italy, [2]Graduate School of Life Sciences, Tohoku University, Sendai 981-8555, Japan, [3]Department of Chemistry, Graduate School of Science, Tohoku University, Sendai 980-8578, Japan and [4]Japan Food Research Laboratory, Tama Laboratories, Tokyo 206-0025, Japan

*Correspondence to be sent to: Albertino Bigiani, Dipartimento di Scienze Biomediche, Università di Modena e Reggio Emilia, Via Campi 287, 41100 Modena, Italy. e-mail: bigiani@unimore.it*



## Abstract

The marine dinoflagellate *Gambierdiscus toxicus* produces highly lipophilic, polycyclic ether toxins that cause a seafood poisoning called ciguatera. Ciguatoxins (CTXs) and gambierol represent the two major causative agents of ciguatera intoxication, which include taste alterations (dysgeusiae). However, information on the mode of action of ciguatera toxins in taste cells is scarce. Here, we have studied the effect of synthetic CTX3C (a CTX congener) on mouse taste cells. By using the patch-clamp technique to monitor membrane ion currents, we found that CTX3C markedly affected the operation of voltage-gated $Na^+$ channels but was ineffective on voltage-gated $K^+$ channels. This result was the exact opposite of what we obtained earlier with gambierol, which inhibits $K^+$ channels but not $Na^+$ channels. Thus, CTXs and gambierol affect with high potency the operation of separate classes of voltage-gated ion channels in taste cells. Our data suggest that taste disturbances reported in ciguatera poisoning might be due to the ability of ciguatera toxins to interfere with ion channels in taste buds.

**Key words:** ciguatera toxins, gustation, patch clamp, taste alteration, voltage-gated ion currents

## Introduction

Ciguatera is a food poisoning caused by the consumption of fish containing toxins produced by *Gambierdiscus toxicus*, a marine dinoflagellate (Watters, 1995; Lewis, 2001; Pearn, 2001; Yasumoto, 2001). Ciguatera toxins accumulate throughout the food chain and eventually affect people eating contaminated fish. The most common symptoms of ciguatera comprise both gastrointestinal disturbances, such as vomiting, diarrhea, and nausea, as well as neurological alterations, in particular sensory abnormalities. These include tingling, unusual temperature perception, heightened nociception, and taste alterations (Lehane, 1999; Lewis, 2001; Pearn, 2001).

Ciguatera toxins are highly lipophilic compounds characterized by several ether rings (Yasumoto, 2001). Two main classes of ciguatera toxins are known: ciguatoxins (CTXs), which include several members, and gambierol (Yasumoto, 2001). The wide diffusion of ciguatera in tropical and subtropical areas has prompted many laboratories to study the mechanism of action of causative toxins and to determine their molecular targets in mammalian cells. We have begun to study the effects of ciguatera toxins on taste cells with the aim of obtaining some insights on the possible molecular basis of dysgeusiae often reported by intoxicated people. We have recently provided evidence that in mouse taste cells, gambierol selectively inhibits voltage-gated $K^+$ channels, whereas it has virtually no effect on voltage-gated $Na^+$ channels (Ghiaroni *et al.*, 2005).

Here, we have taken advantage of the availability of synthetic CTX3C (a CTX congener: Hirama *et al.*, 2001; Inoue *et al.*, 2004) to further address the issue of the action of ciguatera toxins on taste cells. Specifically, we wanted to know whether the effect of CTX3C on voltage-gated $Na^+$ and $K^+$ channels in taste cells was similar to the one described earlier for gambierol (Ghiaroni *et al.*, 2005). Accordingly, we have applied the patch-clamp technique to single taste cells of the mouse vallate papilla to record the ionic currents mediated by voltage-gated $Na^+$ and $K^+$ channels, to evaluate the

action of CTX3C on such currents, and to compare it with that of synthetic gambierol (Fuwa *et al.*, 2002). We have shown previously that mouse vallate papillae contain different electrophysiological types of taste cells (Bigiani *et al.*, 2002). In this study, we analyzed the effect of CTX3C on the so-called ''Na/OUT'' cells, which express functional voltage-gated $Na^+$ and $K^+$, and are thought to be sensory in function.

## Materials and methods

### Tissue preparation

CD-1 mice were used. Taste buds were isolated from the vallate papilla with an enzymatic-mechanical procedure as previously described (Bigiani *et al.*, 2002). Single taste buds were plated on the bottom of a chamber that consisted of a standard glass slide onto which a silicone ring 1–2 mm thick and 15 mm inner diameter was pressed. The glass slide was precoated with Cell-Tak (~3 μg/cm$^2$; Collaborative Research, Bedford, MA) to improve adherence of isolated taste buds to the bottom of the chamber. The chamber was placed onto the stage of an inverted microscope (model IX70, Olympus, Tokyo, Japan), and taste buds were viewed with Nomarski optics at 750×. During the experiments, isolated taste buds were continuously perfused with Tyrode solution (flow rate: 2–3 ml/min) by means of a gravity-driven system.

### Recording techniques

Membrane currents of single cells in isolated taste buds were studied at room temperature (22–25°C) by whole-cell patch clamp (Hamill *et al.*, 1981), using an Axopatch 1D amplifier (Axon Instruments, Union City, CA). Signals were recorded and analyzed using a Pentium computer equipped with a Digidata 1320 data acquisition system and pClamp8 software (Axon Instruments). pClamp8 was used to generate voltage-clamp commands and to record the resulting data. Signals were prefiltered at 5 kHz and digitized at 50-μs intervals.

Patch pipettes were made from soda lime glass capillaries (Baxter Scientific Products, McGaw Park, IL) on a 2-stage vertical puller (model PP-830, Narishige, Tokyo, Japan). Typical pipette resistances were 2–4 MΩ when filled with intracellular solutions. The access resistance of the patch pipette tip was estimated by dividing the amplitude of the voltage steps by the peak of the capacitive transients (from which stray capacitance had been subtracted). Values ranged from about 8 to 15 MΩ. Leakage and capacitive currents were not subtracted from currents under voltage clamp, and all voltages were corrected for liquid junction potential (~4 mV for KCl pipette solution and ~10 mV for Cs gluconate pipette solution) measured between pipette solution and Tyrode (bath) solution (Neher, 1992).

Voltage-gated ion currents were elicited in taste cells by applying a series of 40-ms depolarizing pulses (voltage steps), in 10 mV increments, from a holding potential of −80 mV. Current–voltage (*I–V*) relationship for transient, voltage-gated sodium current was obtained by measuring the peak amplitude of the current for each given membrane potential during the voltage step. For voltage-gated potassium currents, *I–V* plots were obtained by measuring the current amplitude at the end of the 40-ms voltage steps.

To study the voltage dependence of the steady-state inactivation of voltage-gated $Na^+$ currents, we used a typical two-pulse voltage protocol (prepulse and test pulse) that allowed the evaluation of the noninactivated fraction of the sodium current as a function of a prepulse membrane potential (Hille, 2001). Prepulses 300 ms in duration and of variable amplitude (from –100 to −10 mV) were applied prior to the test pulse to −10 mV. During the prepulse, part of the sodium channels became inactivated; the remaining channels were then activated by the test pulse. Cells were held at −80 mV between trials. The magnitude of the current elicited by the test pulse (−10 mV) was normalized to its maximal value and plotted against the prepulse potential.

### Solutions and drugs

Our standard extracellular medium was a Tyrode solution containing the following (in mM): 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 10 glucose, 10 sodium pyruvate, pH 7.4 adjusted with NaOH. Drugs were dissolved in modified Tyrode solution to maintain osmolarity.

Synthetic CTX3C (Hirama *et al.*, 2001; Inoue *et al.*, 2004) and gambierol (Fuwa *et al.*, 2002) were dissolved into dimethyl sulfoxide (DMSO) at a concentration of 1 mM and stored at −20°C. Toxin solutions were made up in normal Tyrode's the day of the experiments. The final DMSO concentration in these solutions never exceeded 0.01%, which has no effect on taste cells (Doolin and Gilbertson, 1996). Gravity-fed test solutions were controlled by multisolenoid manifold valves (Parker Hannifin Corp., Fairfield, NJ) and introduced through a common inlet into the recording chamber.

For patch-clamp recording, the standard pipette solution contained the following (in mM): 120 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, 11 ethyleneglycol-bis(aminoethylether)-tetraacetic acid, 2 ATP, pH 7.3 adjusted with KOH.

To study voltage-gated sodium currents ($I_{Na}$) in isolation, KCl was replaced by Cs gluconate. Note that for the isolation of sodium currents we did not use any maneuvers to avoid potential contributions of voltage-gated calcium currents, which occur in some taste cells. The reason is that to unmask such currents in mouse taste cells, specific ionic conditions are required, including the use of barium instead of calcium in the extracellular solution to augment the currents, which are otherwise negligible (e.g., Furue and Yoshii, 1997; Medler *et al.*, 2003).

Unlike sodium currents, voltage-gated potassium currents ($I_K$) were not studied in isolation, that is, after blocking the

voltage-gated, inward $Na^+$ current. This was done for two main reasons: first of all, $I_{Na}$ provided a functional monitor of the recording conditions because it is very sensitive to variations in the series resistance associated with the patch electrode. An increase in series resistance can produce an artificial change in the amplitude of $I_K$. Second, $I_{Na}$ did not interfere with the analysis of $I_K$ amplitude, which was measured at the end of the 40-ms voltage steps (see Recording Techniques). As shown in Figure 1A, $I_{Na}$ inactivated completely in less than 10 ms after imposing the depolarizing steps to the membrane.

All chemicals were from Sigma (Milan, Italy) except for CTX3C and gambierol, which were synthesized by the laboratory of Dr Hirama and Dr Sasaki, respectively.

### Data analysis

Most data analysis was performed using pClamp8. Additional analysis and plotting were performed using Prism 3.03 software (Graph Pad Software, San Diego, USA). Results are presented as means ± standard error of the means. Data comparisons were made with a two-tailed paired *t*-test.

Steady-state inactivation curves for sodium currents were obtained by fitting the data with a Boltzmann equation:

$$\frac{I}{I_{max}} = \frac{1}{1+\exp[(V - V_{0.5})/k]}, \quad (1)$$

where $I/I_{max}$ is the current elicited during a test pulse and normalized to the maximal current, $V$ is the voltage at which

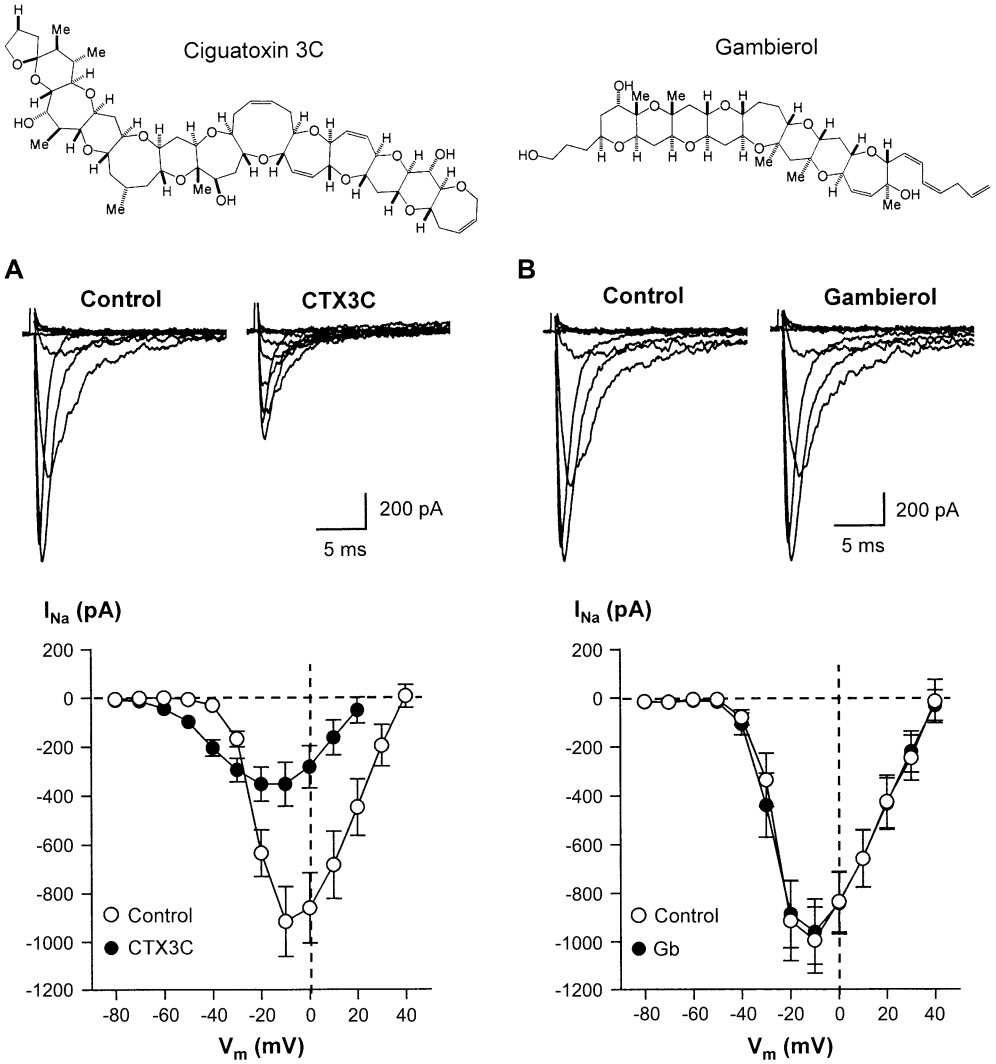


**Figure 1** Effect of ciguatera toxins (CTX3C and gambierol) on voltage-gated sodium currents ($I_{Na}$) in mouse taste cells. **(A)** CTX3C inhibits $I_{Na}$. Patch-clamp recording from a single taste cell held at −80 mV and stepped in 10 mV increments between −70 and 0 mV (top). Bath application of 100 nM "CTX3C" induced a marked reduction of the currents previously recorded in regular Tyrode (Control). *I–V* relationships for $I_{Na}$ (bottom) revealed that CTX3C strongly affected also the activation threshold of $I_{Na}$, which was shifted toward a more negative value. Current values for each membrane potential ($V_m$) were recorded from 8 taste cells. **(B)** Gambierol is ineffective on $I_{Na}$. Same protocols and analysis as in A.

the membrane was held for 300 ms before the test pulse, $V_{0.5}$ is the membrane potential at which the current is 50% inactivated, and $k$ is the slope.

## Results

We have analyzed the action of synthetic CTX3C on a well-defined group of taste cells, the so-called Na/OUT cells, which are thought to be sensory in function (Bigiani *et al.*, 2002). These cells possess voltage-gated ion currents that underlie the generation of gustatory action potentials (Herness and Sun, 1995; Chen *et al.*, 1996): namely, inward, tetrodotoxin-sensitive sodium currents ($I_{Na}$) and outward, tetraethylammonium-sensitive potassium currents ($I_K$). In addition to $I_{Na}$ and $I_K$, however, some Na/OUT cells possess also voltage-gated chloride currents that, in our standard ionic conditions, appear as outward currents in the records (Bigiani *et al.*, 2002). For this study, we have selected those Na/OUT cells in which the chloride component of the outward currents was negligible. This was established quickly during whole-cell recordings by observing the absence of tail currents at the end of voltage pulses, which are due to the activity of voltage-gated chloride channels (Ghiaroni *et al.*, 2005).

Previous studies on other excitable tissues have shown that CTXs exert a vigorous action on voltage-gated currents when used at concentrations in the nanomolar range ($IC_{50}$ of a few nanomoles: e.g., Strachan *et al.*, 1999). We therefore reasoned that a concentration of 100 nM, which elicits maximal effect in other preparations, would be adequate to reveal any effect of CTX3C on $I_{Na}$ and $I_K$ in taste cells. As a reference, we monitored also the effect of synthetic gambierol, which is known to block $I_K$, but not $I_{Na}$, in Na/OUT cells (Ghiaroni *et al.*, 2005). To compare the effects of the two toxins, we adopted a concentration of 100 nM also for gambierol.

The effect of CTX3C or gambierol on voltage-gated ion currents was studied in 75 Na/OUT cells, hereafter referred simply as taste cells.

### CTX3C affects voltage-gated sodium currents

CTXs, including CTX3C, are neurotoxins that alter specifically the activity of voltage-gated sodium channels. Therefore, we tested whether CTX3C affected these channels also in taste cells. This experiment was relevant as in a previous study we showed that gambierol, another ciguatera toxin related to the CTXs, had no effect on voltage-gated sodium channels in taste cells (Ghiaroni *et al.*, 2005). We recorded $I_{Na}$ in voltage-clamped taste cells and evaluated the effect of bath-applied 100 nM CTX3C. $I_{Na}$ was studied in isolation by using a pipette solution containing Cs gluconate and a modified Tyrode solution devoid of $K^+$ and $Cl^-$ (replaced by sodium gluconate). According to the published reports, we found that CTX3C affected markedly the biophysical properties of $I_{Na}$ in taste cells. Figure 1A (top) shows typical recordings of $I_{Na}$ in control conditions (normal Tyrode solution bathing the cell) and during perfusion with CTX3C for 4–5 min. Although it is clear from the records that the toxin reduced the amplitude of $I_{Na}$, the analysis of the $I$–$V$ relationships revealed that CTX3C affected also the activation threshold of the current (Figure 1A, bottom), which was shifted toward more negative voltages (around −40 mV in control condition and −60 and −70 mV in the presence of CTX3C). The complexity of the action of CTX3C on sodium channels was further underscored by changes in the inactivation properties of $I_{Na}$. To study the voltage dependence of the steady-state inactivation, we used a typical two-pulse voltage protocol (prepulse and test pulse) that allowed the evaluation of the noninactivated fraction of the sodium current as a function of a prepulse membrane potential. Steady-state inactivation curves showed that in the presence of CTX3C, inactivation was shifted by about 30 mV in the hyperpolarizing direction (Figure 2A). This meant that at any given membrane potential the fraction of inactivated channels was larger when CTX3C was added to the bath solution. These findings indicated that CTXs, specifically CTX3C, affected markedly voltage-gated sodium channels also in taste cells, as it has been shown for other cell types, including neurons.

Unlike CTX3C, and consistent with our previously published results (Ghiaroni *et al.*, 2005), 100 nM gambierol was unable to alter significantly the biophysical properties of $I_{Na}$ in taste cells in the same experimental conditions. As shown in Figures 1B and 2B, amplitude, activation threshold, and steady-state inactivation of sodium currents were completely insensitive to gambierol applied for 4–5 min. Even by prolonging the application of gambierol for several tens of minutes, we could not detect any significant effect on $I_{Na}$. In conclusion, voltage-gated sodium channels appeared to be a molecular target for CTX3C, but not gambierol, in taste cells.

### CTX3C does not affect voltage-gated potassium currents

Next, we evaluated the effects of bath-applied CTX3C on voltage-gated potassium currents ($I_K$) in taste cells. These experiments were relevant in that we have shown previously that $I_K$ exhibits high sensitivity to gambierol (Ghiaroni *et al.*, 2005) and we wondered whether CTX3C could affect potassium currents as well. Also for these tests, we used a toxin concentration of 100 nM, that is, the same concentration adopted for evaluating the toxin effect on sodium currents (see CTX3C Affects Voltage-Gated Sodium Currents) and which has proven to induce maximal blocking effect (~60–70% current reduction) on $I_K$ (Ghiaroni *et al.*, 2005). Figure 3 shows typical recordings of whole-cell ion currents recorded from a taste cell. Under control conditions (Tyrode solution bathing the cell; Figure 3A, "Control"), potassium currents appeared as sustained, upward deflections in the current trace (outward currents). Note that we did not study the potassium currents in isolation, that is, after blocking the

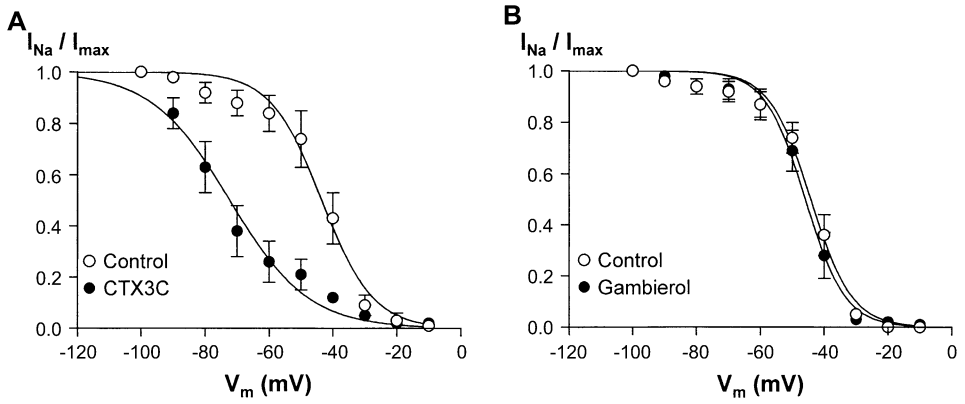


**Figure 2** Effect of ciguatera toxins (CTX3C and gambierol) on the voltage dependence of the steady-state inactivation of $I_{Na}$ in mouse taste cells. A standard two-pulse voltage protocol was used for this analysis. The magnitude of the current elicited by the test pulse (−10 mV) was normalized to its maximal value and plotted against the prepulse potential. Each point represents the mean ± SEM of 4–5 measurements. Data were fitted to a Boltzmann equation. **(A)** CTX3C alters conspicuously the steady-state inactivation. In control conditions, the half-maximal voltage ($V_{0.5}$) was −43 mV and the slope ($k$) was 8 mV. During application of 100 nM CTX3C, $V_{0.5}$ was −72 mV and $k$ was 12 mV. **(B)** Gambierol does not affect the steady-state inactivation of $I_{Na}$. In control conditions, $V_{0.5}$ was −44 mV and $k$ was 6 mV. During application of 100 nM gambierol, $V_{0.5}$ was −46 mV and $k$ was 6 mV.

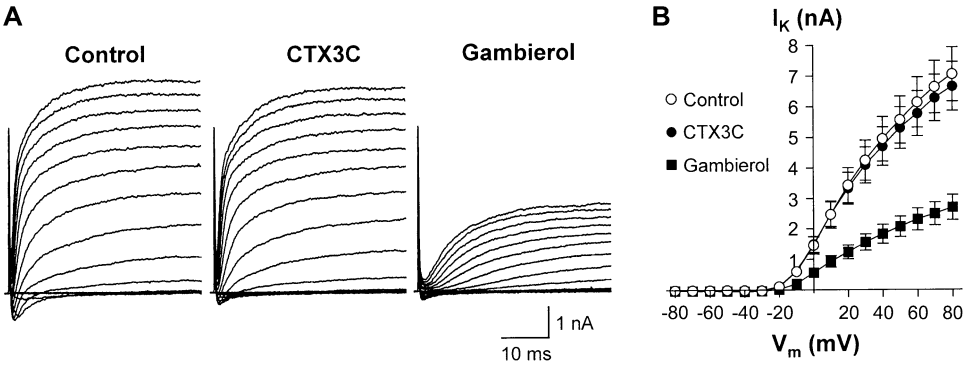


**Figure 3** Gambierol, but not CTX3C, blocks voltage-gated potassium currents ($I_K$) in mouse taste cells. **(A)** Patch-clamp recording from a single taste cell held at −80 mV and stepped in 10 mV increments between −70 and +80 mV. In regular Tyrode (Control), whole-cell currents consisted of voltage-gated sodium currents (downward deflection in the current records) and of voltage-gated potassium currents (upward deflections in the current records). Application of 100 nM CTX3C did not affect significantly $I_K$, although it inhibited considerably $I_{Na}$. Subsequent application of 100 nM gambierol induced a marked reduction of $I_K$. **(B)** *I–V* relationships for $I_K$ recorded from 6 taste cells in normal Tyrode (Control) and during subsequent applications of toxin (CTX3C and Gambierol). In all *I–V* plots, $I_K$ was measured at the end of 40-ms voltage steps. Note that activation threshold of $I_K$ is not changed under any condition.

voltage-gated, inward $Na^+$ current (for details, see Materials and Methods). Application of 100 nM CTX3C for 4–5 min induced a negligible reduction in potassium currents, whereas it strongly inhibited the sodium current (Figure 3A, "CTX3C"). On the contrary, subsequent application of 100 nM gambierol to the same cell elicited a conspicuous reduction in $I_K$ (Figure 3A, "gambierol"). The *I–V* relationship for $I_K$ revealed that CTX3C was ineffective as current blocker as compared with gambierol (Figure 3B). At a reference potential of +50 mV, CTX3C caused less than 5% reduction of the potassium current, whereas block by gambierol was more than 60%, which represents its maximal effect (Ghiaroni *et al.*, 2005). Interestingly, activation threshold for $I_K$ (about −15 mV) was not affected significantly under any conditions, either in the presence of CTX3C or gambierol.

We have previously shown that gambierol slows down activation process of $I_K$ (Ghiaroni *et al.*, 2005). We then evaluated whether CTX3C had some effects on the activation kinetics of these currents. We found that CTX3C did not influence significantly the operation of the potassium channels. Figure 4 shows that activation constant remained virtually unchanged during the application of CTX3C, whereas it increased significantly about 5 times when gambierol was present.

## Discussion

The neurological features of ciguatera intoxication include sensory abnormalities, such as paresthesia, heightened nociception, unusual temperature perception, and taste alterations (Watters, 1995; Lehane, 1999; Lewis, 2001; Pearn,

2001). Sensory "confusion" in the evaluation of taste stimuli (dysgeusiae) suggests that taste cells in the oral cavity are affected by ingested toxins (CTXs and gambierol). The recent availability of synthetic CTX3C (a CTX congener) and gambierol has allowed us to study their mechanism of action in taste cells in order to obtain information on the molecular basis of the taste alterations reported by intoxicated people. In this paper, we provide evidence that CTX3C and gambierol affect two distinct voltage-gated ion channels in mouse taste cells, the sodium channel and the potassium channel, respectively. Their effects are extremely specific, as underscored by the lack of cross-reactivity with the high toxin concentration we used (100 nM). Thus, our data suggest that CTXs and gambierol may induce the taste alterations reported in ciguatera poisoning by affecting with high potency the operation of separate classes of voltage-gated channels in taste cells. Table 1 summarizes our main findings on the biophysical properties of $I_{Na}$ and $I_K$ that are altered by the CTX3C and gambierol in mouse taste cells.

Taste cells in adult mammals are functionally heterogeneous, and many of them are capable of generating action potentials by voltage-gated $Na^+$ and $K^+$ channels (e.g., Bigiani *et al.*, 2002). Action potential firing appears to be one important step in the transduction and signaling of sensory information by some taste cells. For example, the frequency of spike discharge is related to the concentration of certain stimuli (Gilbertson *et al.*, 1992; Cummings *et al.*, 1993). In addition, membrane depolarization during action potential may be necessary to activate $Ca^{2+}$ channels for neurotransmitter release underlying signal transfer to afferent nerves (Béhé *et al.*, 1990; Furue and Yoshii, 1997). Our previous study (Ghiaroni *et al.*, 2005) and the present findings suggest that both CTXs (CTX3C) and gambierol could modulate taste perception by acting on both sodium and potassium channels in these sensory cells, and this may lead to dysgeusiae. Of course, we cannot exclude that other mechanisms may be involved in determining taste alterations by ciguatera toxins. CTX3C and gambierol are lipophilic substances that could have easy access not only to taste cells but also to nerve endings in the oral mucosa, including taste nerve terminals in the buds. This possibility deserves further studies before concluding that the action of ciguatera toxins

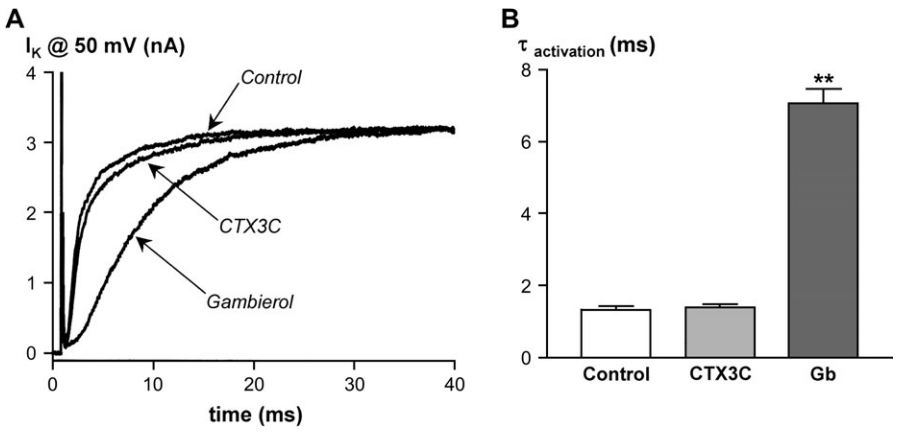


**Figure 4** Gambierol, but not CTX3C, affects activation kinetics of voltage-gated potassium currents in mouse taste cells. **(A)** Overlay of 3 current records obtained from a cell bathed with regular Tyrode (Control), then with 100 nM CTX3C (CTX3C), and finally with 100 mM gambierol (Gambierol). The cell membrane was stepped to +50 mV from a holding potential of −80 mV. The current recorded during application of gambierol was scaled to the same maximum amplitude of the current recorded during application of CTX3C. **(B)** Comparison of the activation constants ($\tau_{activation}$) measured in 6 cells bathed with regular Tyrode (Control), in the presence of 100 nM CTX3C (CTX3C), and of 100 nM gambierol (Gb). On average, CTX3C did not change the activation constant, whereas gambierol induced a 5-fold increase in $\tau_{activation}$, consistent with the slowing down of the activation kinetics. Asterisks indicate significant difference ($P < 0.001$).

**Table 1** Summary of the effects of CTX3C and gambierol (100 nM each) on the biophysical properties of voltage-gated $Na^+$ and $K^+$ currents ($I_{Na}$ and $I_K$, respectively) in mouse taste cells

| | $I_{Na}$ | | | $I_K$ | | |
|---|---|---|---|---|---|---|
| | Activation threshold (mV) | Percent reduction in peak amplitude | Steady-state inactivation $V_{0.5}$ (mV) | Activation threshold (mV) | Percent reduction in amplitude at +50 mV | Activation tau (ms) |
| Control | −40 | — | −44 | −15 | — | 1.33 |
| CTX3C (0.1 μM) | **−70** | **62** | **−72** | −15 | 5 | 1.40 |
| Gambierol (0.1 μM) | −40 | 4 | −46 | −15 | **63** | **7.08** |

For clarity, sample size and standard error of mean have been omitted in this table.

on the ion channels in taste cells is sufficient to produce taste alterations reported by intoxicated people. Nonetheless, the specificity of the action of CTX3C and gambierol on separate classes of voltage-gated ion channels in taste cells suggests the possibility that these toxins may turn useful as pharmacological tools to further explore the role of those channels in taste cell physiology.

Three main cell groups have been identified in vallate taste buds of the mouse: Na/OUT cells, which are endowed with voltage-gated $Na^+$ and $K^+$ channels; OUT cells, characterized by the presence of voltage-gated $K^+$ channels only; and Leaky cells, which exhibit leakage potassium channels (Bigiani *et al.*, 2002). Na/OUT cells are thought to be sensory in function, and for this reasons, we used them in this study. It is clear, however, that it will be important to extend the analysis of CTX3C and gambierol action also on the other cell types in order to obtain a coherent picture of the mechanism of action of ciguatera toxins in taste cells.

CTXs are known to affect voltage-gated sodium channels in several excitable tissues, including myelinated nerve fibers (Benoit *et al.*, 1996; Mattei *et al.*, 1999), parasympathetic neurons (Hogg *et al.*, 1998, 2002), dorsal root ganglion neurons (Strachan *et al.*, 1999), skeletal muscle myotubes (Hidalgo *et al.*, 2002), and neuroblastoma cells (Bidard *et al.*, 1984). By using the synthetic CTX congener CTX3C, Yamaoka *et al.* (2004) have shown that the toxin dramatically affects the gating properties of 3 different (neuronal, skeletal muscle, and cardiac) isoforms of voltage-gated sodium channels expressed in HEK293 cells. Specifically, they found that activation threshold and half-inactivation voltage for $I_{Na}$ were shifted to hyperpolarized potentials by ~30 and ~20 mV, respectively. Our results with taste cells, which are specialized epithelial cells, are in agreement with Yamaoka's findings. Thus, CTXs can affect the functioning of different cell types (neurons, muscle cells, and taste cells) by acting on the same molecular target, namely, the voltage-gated sodium channels.

Gambierol is considered as one of the possible toxins involved in ciguatera because it shows toxicity in mice, and the symptoms resemble those caused by CTXs (Satake *et al.*, 1993; Morohashi *et al.*, 1998). In the present study, we confirm and extend our earlier observations that, unlike CTXs, gambierol acts on a completely different channel protein, namely, the voltage-gated potassium channel. By showing that CTX3C exerts its canonical effect on $I_{Na}$ in taste cells, we have provided evidence that the lack of gambierol effect on $I_{Na}$ is not related to the cell type we used.

CTXs are thought to bind almost irreversibly to the α-subunit of the voltage-gated $Na^+$ channel at the level of protein transmembrane segments D1:S6 and D4:S5, which participate in the formation of receptor site 5 for these toxins (Ogata and Ohishi, 2002; S.-Y. Wang and G.K. Wang, 2003). It is not yet known how gambierol interacts with the voltage-gated potassium channel. Further studies, for instance, by using other gambierol analogues (Fuwa *et al.*, 2004), may provide useful to get some insights into this issue. Nevertheless, the characteristics and selectivity of the effect exerted by CTX3C and gambierol in taste cells could be the basis for the development of functional assays for their detection in contaminated material, including the possibility to discriminate the two toxins. As a next step in this direction, it will be important to identify the specific isoforms of the voltage-gated potassium channels targeted by gambierol in taste cells.

To our knowledge, this is the first article that shows a direct comparison between the effects of two prototypical ciguatera toxins in the same cell type (the taste cell) and clearly suggests that ciguatera toxins, like CTX3C and gambierol, exert a rather complex action on mammalian cells by targeting distinct types of membrane proteins, the voltage-gated sodium and potassium channels. These proteins underlie the generation of action potentials in excitable cells. It is interesting to note that, by virtue of their specific action on $I_{Na}$ and $I_K$, respectively, CTX influences the depolarizing phase, whereas gambierol the repolarizing phase of action potentials. Thus, it is likely that a concerted action of CTX and gambierol will lead to a strong impairment of the electrical impulses required for cell–cell communication, and this concerted effect can underlie the variety of neurological symptoms described in ciguatera (e.g., extremital paresthesia, itching, taste disturbance). It seems likely, however, that gambierol may affect cell functioning in a more diffuse way than CTXs, given the widespread occurrence of voltage-gated potassium channels even in cell types incapable of generating $Na^+$-based action potentials, such as lymphocytes (Cahalan *et al.*, 2001), alveolar epithelial cells (O'Grady and Lee, 2003), kidney cells (Hebert *et al.*, 2005), and cells of the peripheral microcirculation (Jackson, 2005) to mention a few. On the basis of the specificity and potency with which gambierol affects potassium channels, it will be of interest to evaluate the action of this toxin also in nonexcitable cells in future studies.

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