Inhibition of Voltage-Gated Potassium Currents by Gambierol in Mouse Taste Cells

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Ciguatera is a food poisoning caused by toxins of Gambierdiscus toxicus, a marine dinoflagellate. The neurological features of this intoxication include sensory abnormalities, such as paraesthesia, heightened nociception, and also taste alterations. Here, we have evaluated the effect of gambierol, one of the possible ciguatera toxins, on the voltage-gated ion currents in taste cells. Taste cells are excitable cells endowed with voltage-gated Na⁺, K⁺, and Cl⁻ currents (I_{Na}, I_{K}, and I_{Cl}, respectively). By applying the patch-clamp technique to single cells in isolated taste buds obtained from the mouse vallate papilla, we have recorded such currents and determined the effect of bath-applied gambierol. We found that this toxin markedly inhibited I_{K} in the nanomolar range (IC_{50} of 1.8 nM), whereas it showed no significant effect on I_{Na} or I_{Cl} even at high concentration (1 μM). The block of I_{K} was irreversible even after a 50-min wash. In addition to affecting the current amplitude, we found that gambierol significantly altered both the activation and inactivation processes of I_{K}. In conclusion, unlike other toxins involved in ciguatera, such as ciguatoxins, which affect the functioning of voltage-gated sodium channels, the preferred molecular target of gambierol is the voltage-gated potassium channel, at least in taste cells. Voltage-gated potassium currents play an important role in the generation of the firing pattern during chemotransduction. Thus, gambierol may alter action potential discharge in taste cells and this could be associated with the taste alterations reported in the clinical literature.

Key Words: gambierol; ion currents; taste cell; patch clamp; ciguatera.

Ciguatera is a worldwide-spread food poisoning caused by the consumption of fish containing toxins produced by Gambierdiscus toxicus, a marine dinoflagellate (Yasumoto and Murata, 1993; reviewed in Lewis, 2001; Yasumoto, 2001). Symptoms of ciguatera comprise both gastrointestinal disturbances (vomiting, diarrhea, nausea) as well as neurological alterations. The neurological features of this intoxication include sensory abnormalities, such as paraesthesia, heightened nociception, unusual temperature perception, as well as taste alterations (Lehane, 1999; Lewis, 2001; Pearn, 2001).

Ciguatera toxins are highly lipophilic, polycyclic ether compounds (Yasumoto, 2001). Ciguatoxins (CTXs) are considered the principal causative agents of ciguatera (e.g., Yasumoto and Murata, 1993). Several observations suggest that CTXs induce neurological disturbances by acting on voltage-gated Na⁺ channels in excitable tissues. CTXs cause the opening of these channels at resting potential (Hogg et al., 1998), an effect that leads to spontaneous firing of neurons (Hogg et al., 2002) and swelling of axons (Benoit et al., 1996; Mattei et al., 1999).

Unlike ciguatoxins, little is known about the mode of action of gambierol, one of the possible toxins involved in ciguatera (Morohashi et al., 1998; Satake et al., 1993). Gambierol shows toxicity in mice (LD_{50} of 50 μg/kg) and the symptoms resemble those caused by ciguatoxins. Gambierol has been recently synthesized (Fuwa et al., 2002) and this has made it possible to begin detailed biological and pharmacological analysis of its effects on living tissue (Fuwa et al., 2004; Ito et al., 2003). The molecular target of gambierol, however, is still unknown. Given its chemical similarity with ciguatoxins (Yasumoto, 2001), it is likely that also gambierol might affect voltage-gated Na⁺ channels, but no direct information is available.

In this study, we have addressed the issue of the effect of gambierol on taste cells. As reported above, sensory disturbances in ciguatera include dysgeusiae, that is, taste alterations (e.g., Pearn, 2001). Taste cells are excitable cells similar to neurons and use a vast array of receptors and ion channels during their activity (Bigiani et al., 2003; Lindemann, 1996). In particular, taste cells are endowed with voltage-gated ion channels, including Na⁺ and K⁺ channels that mediate the generation of action potentials (Chen et al., 1996; Herness and Sun, 1995). Firing in these sensory cells is thought to play a role in several processes, including signal transduction and processing (e.g., Varkevisser et al., 2001). Therefore, we reasoned that voltage-gated channels in taste cells could represent a possible target for the action of gambierol. To test our hypothesis, we applied the patch-clamp technique to single cells...
taste cells of the mouse vallate papilla to record the ionic currents through voltage-gated Na\(^+\) and K\(^+\) channels, and evaluated the effect of gambierol on such currents. We have shown previously that in the mouse, there are three main different subsets of taste cells (Bigiani et al., 2002): “Na/OUT cells” (−60%), which are endowed with voltage-gated Na\(^+\), K\(^+\), and Cl\(^-\) channels; “OUT cells” (−10%) possessing only voltage-gated K\(^+\) channels; and “Leaky cells” (−30%), characterized by the presence of leakage K\(^+\) channels. Here, we present findings on Na/OUT cells because they, or at least part of them, are believed to be sensory cells, that is, cells capable to detect food chemicals and to transmit the relevant information to afferent nerve fibers (Bigiani et al., 2002).

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 ID was pressed. The glass slide was precoated with Cell-Tak (−3 μg/cm²; 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, Union City, CA). 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 two-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 (LJP: −4 mV for KCl or CsCl 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 outward (K\(^+\) or Cl\(^-\)) 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 non-inactivated 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 −90 mV to −15 mV) were applied prior to the test pulse to −20 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 (−20 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 (in mM): 140 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 10 HEPES, 10 glucose, 10 Na pyruvate, pH 7.4 adjusted with NaOH. Drugs were dissolved in modified Tyrode solution to maintain osmolarity. As channel blockers, we used the following chemicals: tetrodotoxin (TTX) for voltage-gated Na\(^+\) channels; tetraethylammonium (TEA) for voltage-gated K\(^+\) channels; 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) for voltage-gated Cl\(^-\) channels (Bigiani et al., 2002).

Synthesized gambierol (Fuwa et al., 2002) was dissolved into dimethyl sulfoxide (DMSO) at a concentration of 1 mM and stored at −20°C. Gambierol solutions (1 nM to 1 μM) were made up in normal Tyrode’s the day of experiments. The final DMSO concentration in these solutions never exceeded 0.1%, which has no effect on taste cells (Doolin and Gilbertson, 1996). Gravity-fed test solutions were controlled by multisolenoide 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 (in mM): 120 KCl, 1 CaCl₂, 2 MgCl₂, 10 HEPES, 11 EGTA, 2 ATP, pH 7.3 adjusted with KOH. In some experiments, KCl was replaced by an equal concentration of CsCl to block potassium currents and to unmask chloride currents. To study voltage-gated sodium currents in isolation, KCl was replaced by Cs gluconate.

All chemicals were from Sigma (Milan, Italy), except TTX (Alomone, Jerusalem, Israel) and gambierol.

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, CA). Results are presented as means ± standard error of the means (SEM). Data comparisons were made with a two-tailed paired t-test.

Dose-response curves for the blocking effect of gambierol on voltage-gated potassium currents were obtained by adding increasing concentrations of the drug into the bath (Tyrode) solution and by measuring the corresponding change in ion current magnitude. Data were fitted to the logistic equation according to a single-binding isotherm (Hill coefficient = 1.0):

\[
\frac{\%I}{100} = 100 \times \left( \frac{1}{1 + \left( \frac{C}{IC_{50}} \right)^{1.0}} \right)
\]

(1)

where \(\%I\) is the percent fraction of the ion current blocked by gambierol, C is the drug concentration, and IC_{50} is the blocker concentration that produces 50% inhibition of the voltage-gated current.

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

\[
I/I_{\text{max}} = \frac{1}{1 + \exp((V - V_{1/2})/k)}
\]

(2)

where \(I/I_{\text{max}}\) is the current elicited during a test pulse and normalized to the maximal current, V is the voltage at which the membrane was held for 300 ms before the test pulse, \(V_{1/2}\) is the membrane potential at which the current is 50% inactivated, and k is the slope.

RESULTS

In this study, we have analyzed the action of gambierol on a well-defined group of taste cells, the so-called Na/OUT cells, which are thought to be responsible for sensory function (Bigiani et al., 2002). These cells express three types of voltage-gated ion currents: TTX-sensitive sodium currents (I_{Na}), TEA-sensitive potassium currents (I_K), and DIDS-sensitive chloride currents (I_Cl). These currents underlie the generation of gustatory action potentials (Chen et al., 1996;
Current-voltage relationships for INa. Current values for each membrane potential (Vm) were recorded from four taste cells. INa activated at approximately hyperpolarizing the membrane to \(-80\, \text{mV}\) and peaked at about \(-130\, \text{mV}\) (Gambierol). (C) Voltage-dependence of the steady-state inactivation of INa in modified Tyrode solution (control) and during application of 0.1 M gambierol. A standard two-pulse voltage protocol was used for this analysis. The magnitude of the current elicited by the test pulse (\(-20\, \text{mV}\)) was normalized to its maximal value and plotted against the prepulse potential. Each point represents the mean ± SEM of four measurements. Data were fitted to a Boltzmann equation. In control conditions, the half-maximal voltage (\(V_{0.5}\)) was \(-44\, \text{mV}\) and the slope (\(k\)) was 11 mV. During application of gambierol, \(V_{0.5}\) was \(-45\, \text{mV}\) and \(k\) was 7 mV.

Herness and Sun, 1999). The effect of gambierol on voltage-gated ion currents was studied in 36 Na/OUT cells, hereafter referred simply as taste cells.

Gambierol Does Not Affect Voltage-Gated Sodium Currents

Previous studies on the effect of classical ciguatoxins, showed that they alter the activity of voltage-gated sodium channels. Therefore, in a first series of experiments we evaluated whether gambierol, which is lipid-soluble, also affected these channels in taste cells. To this aim, we recorded voltage-gated sodium currents (I\(_{\text{Na}}\)) in voltage-clamped taste cells and studied the effect of bath-applied gambierol. I\(_{\text{Na}}\) was studied in isolation by using a pipette solution containing Cs gluconate and a modified Tyrode solution devoid of K\(^+\) and Cl\(^-\) (replaced by Na gluconate). Unexpectedly, we found that gambierol was unable to alter significantly the biophysical properties of I\(_{\text{Na}}\). Specifically, amplitude, activation threshold and steady-state inactivation of these currents were insensitive to gambierol (Fig. 1). For these experiments, we used a gambierol concentration of 0.1 μM, that is, the same concentration adopted for testing the toxin effect on potassium currents (see next section). However, we also found that 1 μM gambierol was unable to significantly affect sodium currents in taste cells (data not shown). Finally, we did not observe any apparent effect of gambierol on these currents even during long application (5 min) of the toxin to the recorded cell.

It has been reported that ciguatoxins can induce an increase in the background, leakage current through the opening of voltage-gated sodium channels (Strachan et al., 1999). We found no evidence of such an effect with gambierol applied to mouse taste cells (data not shown).

In conclusion, voltage-gated sodium channels appear not to be the main molecular target of gambierol in taste cells.

Gambierol Inhibits Voltage-Gated Potassium Currents

In addition to voltage-gated Na\(^+\) channels, membrane excitability depends also on voltage-gated potassium channels. Indeed, these channels mediate currents that permit excitation because they do not interfere with the rise to the threshold for action potential firing, and they actively promote recovery and rapid refiring. We then evaluated the effect of bath-applied gambierol on voltage-gated potassium currents (I\(_{\text{K}}\)) in taste cells, and typical recordings of I\(_{\text{K}}\) are shown in Figure 2. Under control conditions (Tyrode solution bathing the cell; Fig. 2A, Control), potassium currents appeared as sustained, upward deflections in the current trace (outward currents). Application of TEA completely blocked these currents (Fig. 2A, TEA), indicating that they were mediated by the opening of potassium channels. The effect of TEA was totally reversible (Fig. 2A, Wash). After removing TEA, application of gambierol to the same cell induced a reduction in the TEA-sensitive outward currents, indicating an effect on I\(_{\text{K}}\) (Fig. 2A, Gambierol). The inhibitory action of gambierol on I\(_{\text{K}}\) was observed in all tested cells expressing these currents (\(n = 32\)).

As shown in Figure 2A, we did not study the potassium currents in isolation, that is, after blocking the voltage-gated, inward Na\(^+\) current (I\(_{\text{Na}}\)). This was done for two main reasons: first of all, I\(_{\text{Na}}\) provided a functional monitor of the recording conditions, since 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\(_{\text{K}}\). Secondly, I\(_{\text{Na}}\) did not interfere with the analysis of I\(_{\text{K}}\) amplitude, which was measured at the end of

**FIG. 1.** Gambierol does not affect voltage-gated sodium currents (I\(_{\text{Na}}\)) in mouse taste cells. (A) Typical recording of I\(_{\text{Na}}\) elicited by a test pulse to \(-20\, \text{mV}\) after hyperpolarizing the membrane to \(-130\, \text{mV}\) for 300 ms from a holding potential of \(-80\, \text{mV}\). Current trace was unaffected by the presence of 0.1 μM gambierol. (B) Current-voltage relationships for I\(_{\text{Na}}\). Current values for each membrane potential (V\(_{\text{m}}\)) were recorded from four taste cells. I\(_{\text{Na}}\) activated at approximately \(-50\, \text{mV}\) and peaked at about \(-10\, \text{mV}\) when recorded either in modified Tyrode solution devoid of K\(^+\) and Cl\(^-\) (Control) or in the presence of 0.1 μM gambierol (Gambierol). (C) Voltage-dependence of the steady-state inactivation of I\(_{\text{Na}}\) in modified Tyrode solution (control) and during application of 0.1 μM gambierol. A standard two-pulse voltage protocol was used for this analysis. The magnitude of the current elicited by the test pulse (\(-20\, \text{mV}\)) was normalized to its maximal value and plotted against the prepulse potential. Each point represents the mean ± SEM of four measurements. Data were fitted to a Boltzmann equation. In control conditions, the half-maximal voltage (V\(_{0.5}\)) was \(-44\, \text{mV}\) and the slope (\(k\)) was 11 mV. During application of gambierol, V\(_{0.5}\) was \(-45\, \text{mV}\) and \(k\) was 7 mV.
the 40-ms voltage steps (see Materials and Methods). As shown in Figure 2A during TEA application, I_{Na} inactivated completely in less than 10 ms after imposing the depolarizing steps to the membrane.

It has been reported that voltage-gated K^+ currents can include a Ca^{2+}-dependent component in rat taste cells (e.g., Chen et al., 1996). To establish the contribution of this component to the recorded I_{K}, we applied 1 mM Cd^{2+}, an inorganic blocker of Ca^{2+} channels (Bean, 1992). Under this condition there was a reduction of potassium currents at a given membrane potential, but the overall shape of the I–V curve did not change (Fig. 3). This suggests that in the presence of cadmium, the threshold for outward current activation was shifted in a positive direction, presumably because of the effect of the high concentration of divalent cations on the external surface charge of the membrane (Hille, 2001). Therefore, Ca^{2+}-dependent potassium currents were negligible in vallate taste cells of the mouse, consistent with previous findings (Bigiani et al., 2002).

Gambierol (0.1 μM) in the presence of cadmium was still able to induce a reduction of the outward current, indicating that its action was direct towards pure, voltage-gated potassium current (Fig. 3A). Similar results were obtained in five other taste cells: at a reference potential of +50 mV, gambierol caused a 58.7 ± 12% reduction of the potassium current in the presence of cadmium.

Compared to the blocking effect of TEA, the inhibition of I_{K} by gambierol showed some distinctive features. First of all, gambierol never abolished I_{K} completely, as it was the case for TEA (for example, see Fig. 2). On average, 0.1 μM gambierol induced a ~60% reduction of I_{K}. Secondly, gambierol effect was virtually irreversible under our conditions (Fig. 4).
whereas TEA could be rinsed easily (Fig. 2). Thus, gambierol affected voltage-gated potassium channels in taste cells in a specific and unique fashion.

Sensitivity of $I_K$ to the blocking effect of gambierol was established by evaluating $IC_{50}$ from the analysis of the dose-response curve. As indicated in the sample recordings of Fig. 5A, gambierol inhibited considerably $I_K$ already at a concentration as low as 1 nM. Percent reductions of the current amplitude in the presence of different gambierol concentrations are reported in the plot shown in Figure 5B. The sigmoidal curve is the best fit for the experimental data, and represents the single-binding isotherm expressed by Equation 1 for an $IC_{50}$ of 1.8 nM. This finding indicated that $I_K$ was extremely sensitive to gambierol in mouse taste cells.

Next, we evaluated whether gambierol affected other biophysical properties of $I_K$, such as the activation kinetics and the inactivation process. We found that, indeed, this toxin influenced markedly the operation of the potassium channels. Figure 6A shows that gambierol slowed down the activation process of $I_K$. In the presence of the toxin, the activation time constant underwent an averaged four-fold increase (Fig. 6B), indicating that the opening of potassium channels upon depolarization was further delayed.

$I_K$ typically exhibited a certain degree of inactivation (reduction in current amplitude) during 400 ms depolarization pulses in mouse taste cells (Fig. 7A). In the presence of gambierol, inactivation was reduced. We evaluated inactivation properties of $I_K$ during gambierol application by measuring the ratio between the current amplitude at 400 ms and the current peak. At about 50 mV, this ratio was increased (slower inactivation) by gambierol (Fig. 7B).

**Gambierol Does Not Affect Voltage-Gated Chloride Currents**

Some taste cells used in this study expressed also voltage-gated chloride currents ($ICl$). Like $I_K$, chloride currents mediate action potential repolarization in taste cells where they occur (Herness and Sun, 1999). We then wondered whether gambierol also affected $ICl$ in the same way it did with $I_K$. To study chloride currents in isolation, we used a patch pipette (intracellular) solution containing CsCl to block potassium currents. Under these conditions, $ICl$ appeared as outward currents (Fig. 8, Control). Application of gambierol, however, did not affect these currents at all (Fig. 8, Gambierol). After removing gambierol, application of DIDS to the same cell completely abolished the outward currents, showing that they

![Fig. 4](image-url)  
**Fig. 4.** Gambierol effect on voltage-gated potassium currents ($I_K$) is virtually irreversible. Patch-clamp recording from a single taste cell held at $-80$ mV and stepped in 10 mV increments between $-70$ mV and $+50$ mV. Current traces recorded in regular Tyrode (Control), during application of 0.1 \mu M gambierol (middle), and after washing out the cell for 50 min (right). Note that $I_K$ recovered only slightly after application of gambierol. $I_Na$, voltage-gated sodium currents.

![Fig. 5](image-url)  
**Fig. 5.** Sensitivity of voltage-gated potassium currents ($I_K$) to gambierol. (A) Membrane currents were elicited by a series of depolarizing pulses between $-70$ mV and $+50$ mV, in 10-mV increments, from a holding potential of $-80$ mV. The cell was tested with three subsequent gambierol concentrations ($10^{-9}$ M, $10^{-8}$ M, and $10^{-7}$ M). Control, recordings in regular Tyrode. (B) Dose-response curve for the effect of gambierol on $I_K$. Percentage inhibition of the total potassium current was evaluated for the current elicited by a depolarizing voltage step to $+50$ mV from a holding potential of $-80$ mV. Current magnitude was measured at the end of the 40-ms voltage step. Points represent mean ± SEM (bars) of measurements in four cells. Sigmoidal curve is the best fit ($R^2 = 0.9895$) of the data obtained with the logistic equation (assuming a single binding isotherm) with an $IC_{50}$ of 1.8 nM.
were indeed carried by chloride ions (Fig. 8, DIDS). The insensitivity of $I_{Cl}$ to gambierol action was observed in four other taste cells. These data clearly indicated that among repolarizing currents ($I_K$ and $I_{Cl}$) occurring in taste cells, gambierol affected specifically only $I_K$.

**DISCUSSION**

Gambierol is a lipophilic toxin, which is believed to be involved in ciguatera food poisoning. Little is known, however, about its mechanism of action and its molecular targets. In this article, we have provided evidence that gambierol inhibits voltage-gated potassium currents ($I_K$) in mouse taste cells. This effect is extremely specific, since gambierol does not affect other voltage-gated ion currents in these cells, such as the sodium and chloride currents. Given the role of $I_K$ in defining the pattern of action potential firing in taste cells during chemotransduction, our findings suggest a mechanism for gustatory alterations reported in ciguatera poisoning. In addition, our

**FIG. 6.** Gambierol affects activation kinetics of voltage-gated potassium currents ($I_K$) in mouse taste cells. (A) Overlay of two current records obtained from a cell bathed with regular Tyrode (Control) and then in the presence of 0.1 µM gambierol (Gambierol). The cell membrane was stepped to $+50$ mV from a holding potential of $-80$ mV. The currents were scaled to the same maximum amplitude. (B) Comparison of the activation constants ($\tau_{activate}$) measured in nine cells bathed with regular Tyrode (Control) and in the presence of 0.1 µM gambierol (Gambierol). On average, gambierol induced a four-fold increase in $\tau_{activate}$, consistent with the slowing down of the activation kinetics. Asterisks indicate significant difference ($p < 0.005$).

**FIG. 7.** Gambierol affects inactivation of voltage-gated potassium currents ($I_K$) in mouse taste cells. (A) Sample records of $I_K$ elicited by stepping the membrane to $+50$ mV from a holding potential of $-80$ mV. When the cell was bathed by Tyrode solution (Control), $I_K$ amplitude decreased over time (inactivation). In the presence of 0.1 µM gambierol (Gambierol), inactivation process was reduced. (B) Ratio between the current amplitude at 400 ms during the depolarizing step (b) and the current peak (a) evaluated in five taste cells. At a reference membrane potential of $+50$ mV, this ratio was significantly increased by gambierol treatment. Note that an inactivation ratio of 1 would correspond to the absence of inactivation. Asterisks indicate significant difference ($p < 0.005$).

**FIG. 8.** Gambierol does not block voltage-gated chloride currents in mouse taste cells. (A) Patch-clamp recording from a single taste cell held at $-80$ mV and stepped in 10-mV increments between $-70$ mV and $+110$ mV. In regular Tyrode (Control), whole-cell currents consisted of outward currents (upward deflections in the current records). The outward currents were not affected by 0.1 µM gambierol (Gambierol). Subsequent application of 0.5 mM DIDS, a chloride channel blocker, completely abolished the outward currents, indicating that they were chloride currents. Note the presence of tail chloride currents (arrows) in the records. In this specific experiment, voltage-gated Na$^+$ currents are missing from the records because they were blocked with 1 µM TTX. Patch pipette solution: CsCl replacing KCl. (B) Current-voltage ($I-V$) relationships for the outward currents shown in (A). In all $I-V$ plots, currents were measured at the end of 40-ms voltage steps.
findings on the sensitivity of taste cell $I_K$ to gambierol ($IC_{50} = 1.8$ nM) indicate that the potency of this toxin is similar to that exhibited by ciguatoxins on voltage-gated sodium channels (e.g., Molgo et al., 1992).

**Inhibition of $I_K$ by Gambierol in Taste Cells**

Although gambierol inhibits $I_K$ in taste cells, the reduction in current amplitude is never complete, as it is typified by TEA block. This difference might be indicative of the mechanism by which gambierol affects $I_K$. Unlike TEA, which is positively charged and remains in solution, gambierol is a highly lipophilic molecule and therefore it is expected to enter into the lipid bilayer of taste cell membranes. An emerging concept in the molecular physiology of voltage-gated potassium channels is that lateral interactions with membrane lipids in the so-called “lipid rafts” may affect their biophysical properties (reviewed in Martens et al., 2004). Thus, gambierol might disrupt the normal lipid environment that surrounds the potassium channels and this would likely have conspicuous effect on the channel operation. The high potency of gambierol ($IC_{50} = 1.8$ nM), however, would rather suggest that the toxin actually binds to some specific transmembrane segments of the channel protein, as it is the case for ciguatoxins (see below).

Alternatively, the incomplete block of $I_K$ by gambierol could be explained as an action of the toxin on a specific subtype of potassium channels. Although Ca$^{2+}$-activated potassium currents have been described in rat taste cells (e.g., Chen et al., 1996), these currents are negligible in Na/OUT taste cells from the mouse vallate papilla (present findings; see also Bigiani et al., 2002). In addition, we have previously provided electrophysiological evidence that the biophysical properties of $I_K$ in these cells are consistent with the presence of a single channel type (see Figure 9 in Bigiani et al., 2002). We cannot exclude, of course, the occurrence of different isoforms of the K channels, which might exhibit distinct sensitivity to gambierol. This will require further investigations.

Our electrophysiological data indicate that gambierol, in addition to reducing the current magnitude, affects other biophysical properties of $I_K$, such as the activation process. In this regard, it is interesting to note that potassium channels open more slowly in the presence of gambierol. This would further hamper the functioning of these channels in real situations, that is, when the cell is firing action potentials and the voltage-gated potassium channels have limited time (a few milliseconds) to become activated.

Our finding that gambierol’s effect on $I_K$ is virtually irreversible is consistent with the observation that ciguatera symptoms persist over several days/weeks after poisoning (Pearn, 2001). The high lipophilic nature of gambierol suggests that this compound could remain in the lipid bilayer and would be removed at a very slow rate from the cell membrane, as it has been described for ciguatoxins (Pearn, 2001).

**Gambierol and Ciguatoxins: Different Molecular Targets?**

Several studies have shown that the voltage-gated sodium channel represents the primary target for many lipophilic toxins (reviewed in Wang and Wang, 2003). Also ciguatoxins (CTXs) are known to affect these 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). Therefore, our expectation was that gambierol action could be directed towards sodium channels as well. Surprisingly, our data showed that these channels are not significantly affected by the presence of the toxin at concentrations as high as 1 μM. Instead, we found evidence that the molecular target of gambierol is the voltage-gated potassium channel, at least in mouse taste cells. Ciguatoxins are thought to bind quasi-irreversibly to the $\alpha$-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; Wang and Wang, 2003).

A recent study involving competitive inhibition assay on rat brain synaptosomes (Inoue et al., 2003) has shown that gambierol might likely bind to site 5 of voltage-gated sodium channels but with a much lower affinity than ciguatoxins (inhibition constant of 1.4 μM for gambierol and 1.5 × 10$^{-4}$ μM for CTX). This finding could explain our observations that gambierol does not influence $I_Na$ in taste cells, at least in the concentration range we used.

It is also possible that the voltage-gated sodium channel expressed by taste cells is an isoform differing from that found in neurons or other excitable tissues and which is affected by ciguatoxins. At least nine different isoforms of the voltage-gated Na$^+$ channel $\alpha$-subunit have been identified in mammalian excitable tissues (Goldin, 2002; Ogata and Ohishi, 2002). However, there is no information on the channel isoform expressed in taste cells. Further experimentation will be required to solve this issue.

In any case, the characteristics and selectivity of the effect exerted by gambierol in taste cells could be the basis for the development of a functional assay for its detection in contaminated material, including the possibility to discriminate this toxin from canonical ciguatoxins.

**Gambierol and Taste Perception**

Taste cells are located in the oral mucosa and, therefore, are likely to be the first excitable cells to be contacted by ciguatera toxins. In this study, we show that gambierol strongly affects the biophysical properties of $I_K$ in mouse taste cells at concentrations as low as 1 nM. $I_K$ plays a key role in defining the firing pattern of these cells, which is believed to encode taste qualities (Varkevisser et al., 2001). Partial inhibition of $I_K$ might then have a profound effect on quality coding at
peripheral level and could cause sensory “confusion” in the evaluation of taste stimuli (dysgeusiae). It is tempting to speculate, therefore, that taste disturbances reported in clinical literature after eating fish contaminated by ciguatera toxins may derive from the action of gambierol on voltage-gated potassium channels in taste cells. Our data do not exclude, however, the possibility that sodium channels might also contribute to the proper functioning of taste cells, and, hence, that CTX could modulate taste perception by acting on sodium channels in these cells. The recent total synthesis of ciguatoxin CTX3C (Inoue et al., 2004) represents the prerequisite for future studies of its effect on taste cells.

Taste cells are heterogeneous as to the expression of ion channels. In the mouse, three main cell groups have been recently identified: Na/OUT cells, which are endowed with voltage-gated Na⁺, K⁺, and Cl⁻ 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 reason we used them in this study. As a future direction, it will be interesting to extend the analysis of gambierol action also on OUT cells, which are believed to represent maturational stages of taste cells, and on Leaky cells, which likely have a glial role.

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