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▶ **To cite this version:**

Jordi Molgó, Roland Bournaud, Sébastien Schlumberger, Makoto Sasaki, Haruhiko Fuwa, et al.. Gambierol blocks a K(+) current fraction and affects action potential duration and the firing rate in rat fetal chromaffin cells. Band Schmidt C.J., Rodríguez Gómez C.F. (Ed.), International Society for the Study of Harmful Algae (ISSHA). Harmful Algae 2021, , pp.199-204, 2022. hal-03787469

HAL Id: hal-03787469
https://hal.science/hal-03787469

Submitted on 25 Sep 2022

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Gambierol blocks a K^+ current fraction and affects action potential duration and the firing rate in rat fetal chromaffin cells

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Abstract

The dinoflagellate genus *Gambierdiscus*, particularly *Gambierdiscus toxicus* is known to produce numerous ladder polycyclic ether compounds, including gambierol, characterized by a transfused octacyclic polyether core, as well as the identified ciguatoxins responsible for ciguatera poisoning. Gambierol inhibits voltage-gated K^+ (K_V) channels in various mammalian excitable and non-excitable cells, and motor nerve terminals of the neuromuscular junction. In the present study, we investigated the action of gambierol on outward K^+ currents of cultured rat fetal adrenal medulla chromaffin cells using the whole-cell configuration voltage-clamp method. The pharmacological dissection of the outward K^+ current, using selective K^+ channel inhibitors, revealed that gambierol reduced just a small fraction of the total outward current without affecting calcium-activated K^+ currents that were sensitive to apamin and iberiotoxin, and ATP-sensitive K^+ currents sensitive to glibenclamide. Cultured fetal chromaffin cells were excitable and generated either evoked (upon direct electric stimulation) or spontaneous action potentials. These action potentials were sensitive to tetrodotoxin that blocks voltage-gated Na^+ (Na_V) channels. Gambierol neither affected the rising phase nor the overshoot of action potentials but significantly prolonged their repolarizing phase and increased the firing rate of action potentials during sustained current depolarization, as determined using current-clamp recordings. Our results highlight the effects of gambierol on K_V channels and on the electrical properties of rat fetal chromaffin cells. It is likely that gambierol may cross the placental barrier as ciguatoxins do. Further studies are needed to determine whether such actions may have deleterious effects on fetuses.

Keywords: gambierol, marine biotoxin, potassium channels

https://doi.org/10.5281/zenodo.7035142

Introduction

Marine dinoflagellates produce bioactive secondary metabolites with unique chemical structures (reviewed in Yasumoto, 2001; Shmukler and Nikishin, 2017). The *Gambierdiscus* genus is known to manufacture a number of ladder cyclic compounds, including the ciguatoxins, responsible for ciguatera poisoning, and gambierol, which is also suspected to be involved in ciguatera fish poisoning. The chemical syntheses of gambierol (Fuwa *et al.*, 2002; Johnson *et al.*, 2005) and analog compounds (Alonso *et al.*, 2012) have been performed. Consequently, the availability of the polyether toxin allowed the study of its mode of action on ion channels from excitable (i.e. those that can be electrically stimulated) and non-excitable cells. Thus, gambierol inhibits voltage-gated K^+ (K_V) channel subtypes in a range of cells, including mouse taste cells (Ghiaroni *et al.*, 2005), *Xenopus* skeletal myocytes (Schlumberger *et al.*, 2010), rodent cerebellar neurons (Pérez *et al.*, 2012), *Xenopus* oocytes or Chinese hamster ovary (CHO) cells expressing mammalian $K_V1.1$-$K_V1.5$ subtypes (Cuypers *et al.*, 2008; Konoki *et al.*, 2015), mouse fibroblasts expressing the $K_V3.1$ subtype (Kopljar *et al.*, 2009), human T lymphocytes expressing the $K_V3.1$ subtype (Rubiolo *et al.*, 2015), and motor nerve terminals of the vertebrate neuromuscular junction (Molgó *et al.*, 2020). The information that some lipid-soluble polyether toxins, like brevetoxin-3, actually cross the rodent maternofetal barrier (Benson *et al.*, 2006), and the fact that fetal chromaffin cells express a diversity of K_V channels (reviewed in Lingle *et al.*, 2018) motivated us to study the effect of gambierol on rat fetal chromaffin cells. In the present work, we performed a pharmacological dissection of the current components present in the outward K^+ current of cultured rat fetal adrenomedullary chromaffin cells and investigated the action of gambierol on such currents. In addition, we studied whether the time course of spontaneous action potentials was sensitive to the action of gambierol and to the combined action of selective blockers of calcium-activated potassium (K_{Ca}) channels.

Material and Methods

Gambierol (purity >97%) was chemically synthesized, as described previously by Fuwa *et al.* (2002). Stock solutions of gambierol were made in dimethyl sulfoxide (DMSO) and diluted with an external physiological solution. The DMSO concentration was less than 0.1% v/v. Tetrodotoxin, apamin, iberiotoxin, and glibenclamide were obtained from Alomone Labs (Jerusalem, Israel) and Latoxan (Portes-lès-Valence, France). The cell-culture media and all other chemicals were acquired from Sigma-Aldrich (Saint-Quentin-Fallavier, France).

Timed-pregnant Wistar rats were purchased from Elevage Janvier (Le Genest-Saint-Isle, France). Animal care and surgery were performed according to the Directive 305 2010/63/EU of the European Parliament, approved by the French Ministry of Agriculture. The project was submitted to the French Ethics Committee CEEA and obtained the authorization APAFIS #4111-2016021613253432 v5. On day 19-20 of gestation (F19-F20), pregnant rats were euthanized by carbon dioxide gas inhalation, and fetuses were quickly collected and

decapitated with scissors according to the guidance of the European Committee DGXI, regarding animal experimentation.

Adrenal medulla chromaffin cells, removed from adrenal glands of fetuses collected at F19-F20, were used for cell cultures, employing protocols previously described (Bournaud *et al.*, 2007). Membrane currents and potentials were recorded from fetal chromaffin cells using the perforated whole-cell voltage-clamp technique. Patch-glass microelectrodes had a resistance of 2-5 MΩ when filled with the internal solution and were coated with Sticky wax (S.S. White, Gloucester, UK). Under voltage-clamp conditions, the seal resistance was usually 2-10 GΩ, and 75-80% of the series resistance was compensated. Membrane currents (under voltage-clamp conditions) and potentials (under current-clamp conditions in the zero current mode) were monitored with an RK-400 amplifier (Biologic, Claix, France), filtered at 1-3 kHz (Frequency device, MA, U.S.A.), digitized with a DigiData-1200 interface (Axon Instruments, Union City, CA, U.S.A.), and stored on a hard disk. Data acquisition and analyses were performed with the pCLAMP-8.0 software (Axon Instruments). All experiments were performed at constant room temperature (21°C).

The external solution contained (in mM): 135 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 glucose, 10 HEPES [4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid] (adjusted to pH 7.4, with osmolarity of 300 mOsm). The micropipette solution contained (in mM): 105 K^+ gluconate, 30 KCl, 0.1 $CaCl_2$, and 10 HEPES (adjusted to pH 7.2, osmolarity of 290 mOsm) to which amphotericin-B (24 µg mL^{-1}) was added. Tetrodotoxin, apamin, iberiotoxin, glibenclamide, tetraethylammonium chloride (TEA), and 3,4-diamino-pyridine (3,4-DAP) were added to the external solution. The solutions containing these drugs were perfused by a custom-made gravity-fed micro-flow perfusion system positioned close to the recorded cell.

Cells were continuously bathed in a standard physiological solution containing tetrodotoxin (1 µM) to block voltage-gated Na^+ channels. The involvement of different current components in the total outward K^+ current was studied using the selective peptide toxins apamin and iberiotoxin, which block respectively the small-conductance and the large-conductance Ca^{2+}-activated K^+ channels (K_{Ca} channels), and glibenclamide that blocks ATP-sensitive K^+ (K_{ATP}) channels.

Results and Discussion

As shown in a representative recording (Fig. 1A), the perfusion of apamin (400 nM), iberiotoxin (100 nM), and glibenclamide (200 µM) markedly reduced the total outward K^+ current. The remaining K^+ current was further reduced by adding gambierol (100 nM) and was completely inhibited by further perfusion of TEA (10 mM) and 3,4-DAP (500 µM) which are well-known organic K^+ channel inhibitors. Thus, gambierol inhibited only a small fraction of the total K^+ current when added either before or after K_{Ca} and K_{ATP} blockers. Intriguingly, the percentage of K^+ current inhibition by apamin, iberiotoxin, and glibenclamide was not significantly different when determined before or after gambierol action, as shown in figure 1B.

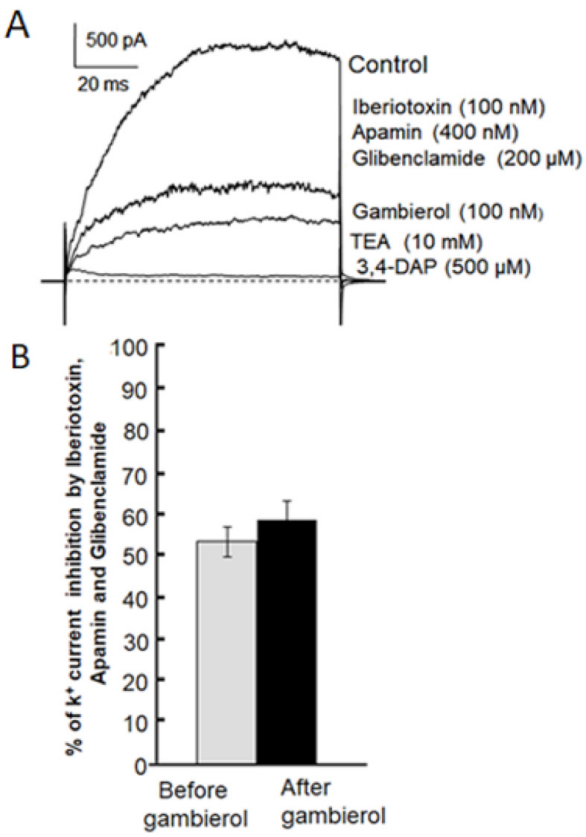


Fig. 1. (A) Superimposed traces of outward K^+ currents recorded under control conditions and after the simultaneous perfusion of apamin, iberiotoxin, and glibenclamide, and the cumulative perfusion of gambierol, TEA, and 3,4-DAP at the concentrations indicated. The K^+ current was recorded during 90 ms depolarizing steps from a holding potential of -70 mV to +40 mV. Note that gambierol blocked only a small K^+ current component. (B) Percentage of K^+ current inhibition by iberiotoxin, apamin, and glibenclamide before (gray column) and after gambierol addition to the medium (black column) Data in columns represent mean ± SEM, n = 6 different experiments (p = 0.848).

To the best of our knowledge, this is the first time that gambierol has been reported to block K_V channels in rat fetal chromaffin cells. These cells express various types of channels that contribute to the total outward K^+ current. Gambierol inhibited a small component of the total K^+ current (i.e. 15.86 ± 4.82%, n = 6) when added before or after selective channel blockers, suggesting that the polyether affects neither K_{Ca} nor K_{ATP} channels.

The cultured rat fetal chromaffin cells studied were excitable and generated either induced (in response to electrical stimulation) or spontaneous all-or-none action potentials. Using the current-clamp technique, we investigated the effects of gambierol on the action potentials of fetal chromaffin cells. Under control conditions, the mean membrane resting potential of fetal chromaffin cells was -51.8 ± 3.1 mV (n = 32), with a mean coefficient of variation of 0.32 (standard deviation/mean). The spontaneous spike activity was present in about 2/3 of the cells studied. As shown in a typical recording in figure 2, gambierol (50 nM) had no significant effect on the depolarizing phase and peak amplitude of spontaneous action potentials. Our results strongly suggest that the polyether toxin does not affect Na_V channels in fetal chromaffin cells.

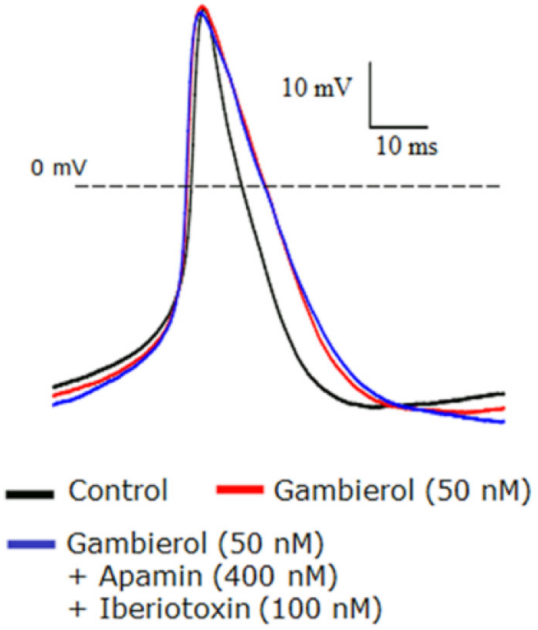


Fig. 2. Superimposed traces of spontaneous action potentials recorded from fetal chromaffin cells under control conditions in standard physiological solution (black trace), during the action of 50 nM gambierol (red trace), and after addition of apamin (400 nM) and iberiotoxin (100 nM) to the extracellular medium (blue trace).

Gambierol specifically and significantly prolonged the repolarizing phase of action potentials, measured as the time elapsed from the peak of action potentials to 0 mV membrane potential, by 31.95 ± 1.62 % (n = 3; $p < 0.05$; Fig. 2). Interestingly, the subsequent perfusion of apamin (400 nM) and iberiotoxin (100 nM) to the physiological solution had no significant effect on the action potential repolarizing phase ($p > 0.05$), indicating that K_{Ca} channels do not participate in the modulation of action potential duration in fetal chromaffin cells. In addition, as shown in Fig. 3, gambierol increased the firing rate of action potentials triggered by depolarizing current pulses of long duration (13 s), when compared to the control, which exhibited during the sustained depolarizing current a decline in action potential frequency or accommodation. These results suggest that the K^+ current blocked by the polyether is involved in controlling the firing rate of fetal chromaffin cells.

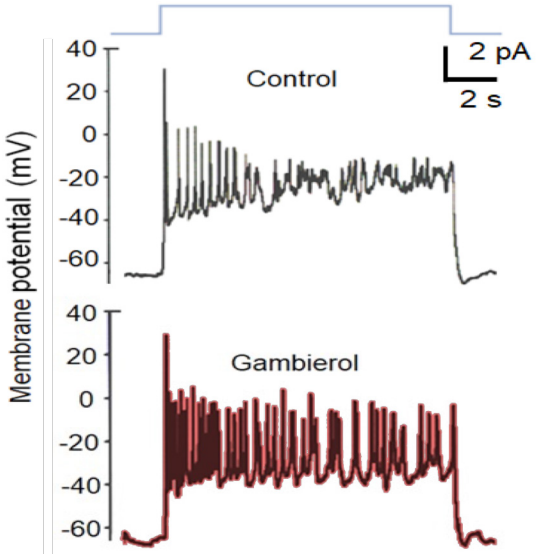


Fig. 3. Current-clamp recordings in a single fetal chromaffin cell under control conditions and during the action of 100 nM gambierol. The upper scheme (in blue) shows the long current pulse depolarization used to trigger the changes in membrane potential (before, in black, and after, in red, gambierol addition).

Overall, our study enhances the knowledge we have on the several types of K^+ channels contributing to the total outward current of rat fetal chromaffin cells and clearly indicates that gambierol affects a small K^+ current component distinct to the K_{Ca} and K_{ATP} currents. In addition, K_V blockade by gambierol prolonged the repolarizing phase and increased the firing rate of action potentials. Further work is needed to determine whether gambierol affects Ca^{2+}-dependent catecholamine secretion, and has harmful effects on fetuses.

Acknowledgments. This study was supported in part by the project ALERTOX-NET (EAPA_317/2016), funded by the Interreg Atlantic program, and in part by the CNRS.

References

Alonso, E., Fuwa, H., Vale, C., Suga, Y., *et al*., (2012). J. Am. Chem. Soc. 134, 7467-7479.

Benson, J.M., Gomez, A.P., Statom, G.L., Tibbetts, B.M., *et al.,* (2006). Toxicon 48, 1018-1026.

Bournaud, R., Hidalgo, J., Yu, H., Girard, E., Shimahara, T. (2007). Pflügers Arch. 454, 83-92.

Cuypers, E., Abdel-Mottaleb, Y., Kopljar, I., Rainier, J.D., *et al*., (2008). Toxicon 51, 974-983.

Fuwa, H., Kainuma, N., Tachibana, K., Sasaki, M. (2002). J. Am. Chem. Soc. 124, 14983-14992.

Ghiaroni, V., Sasaki, M., Fuwa, H., Rossini, G.P., *et al*., (2005). Toxicol. Sci. 85, 657-665.

Johnson, H.W., Majumder, U., Rainier, J.D. (2005). J. Am. Chem. Soc. 127, 848-849.

Konoki, K., Suga, Y., Fuwa, H., Yotsu-Yamashita, M., Sasaki, M. (2015). Bioorg. Med. Chem. Lett. 25, 514-518.

Kopljar, I., Labro, A.J., Cuypers, E., Johnson, H.W., *et al*., (2009). Proc. Natl. Acad. Sci. U.S.A. 106, 9896-9901.

Lingle, C.J., Martinez-Espinosa, P.L., Guarina, L., Carbone, E. (2018). Pflügers Arch. 470, 39-52.

Molgó, J., Schlumberger, S., Sasaki, M., Fuwa, H., *et al*., (2020). Neuroscience 439,106-116.

Pérez, S., Vale, C., Alonso, E., Fuwa, H., *et al*., (2012). Chem. Res. Toxicol. 25, 1929-1937.

Rubiolo, J.A., Vale, C., Martín, V., Fuwa, H., Sasaki, M., Botana, LM. (2015). Arch. Toxicol. 89, 1119-1134.

Schlumberger, S., Ouanounou, G., Girard, E., Sasaki, M., *et al*., (2010). Toxicon 56, 785-791.

Shmukler, Y.B. and Nikishin, D.A. (2017) Mar. Drugs 15, 232.

Yasumoto, T. (2001). Chem. Rec. 1, 228-242.