Br. J. Pharmacol. (1988), **93**, 811–816

Single ionic channels induced by palytoxin in guinea-pig ventricular myocytes

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1 Mechanisms of palytoxin-induced ion permeability were examined in isolated single ventricular cells of guinea-pig under whole-cell-attached patch clamp conditions.

2 Palytoxin ($1–2 \times 10^{-11}$ M, dissolved in Tyrode solution and put in the patch electrode) induced an elementary current flowing through single channels. Direction of the current was inward and the amplitude was 0.65 ± 0.03 pA (mean ± s.e. mean) at the resting membrane potential. The amplitude increased linearly with membrane hyperpolarization and decreased with depolarization; the single channel conductance was 9.5 ± 0.5 pS.

3 Palytoxin-induced single channel current was resistant to tetrodotoxin (5×10^{-5} M) or cobalt ions (2×10^{-3} M) and was observed under Ca-free conditions. However, no channel current was induced by palytoxin ($10^{-11}–10^{-9}$ M) dissolved in Na^+-free, choline-Tyrode solution.

4 Palytoxin also induced single channel currents in Na^+-free, NH_4^+-, Li^+- or Cs^+-Tyrode solution, and the slope conductances were 16.5 ± 1.6 pS, 9.2 ± 0.7 pS and 11.0 ± 0.7 pS, respectively.

5 These results indicate that palytoxin forms a new type of ionic channel with unique ion selectivity and gating behaviour.

Introduction

Marine toxins alter ion permeability of cell membranes (Narahashi, 1974; Catterall, 1980; Fuhrman, 1980; Honerjäger, 1982; Muramatsu *et al.*, 1985). The related mechanisms are discerned on the basis of the presence or absence of actions on existing ionic channels. For example, guanidinium toxins reduce the maximum Na conductance and polypeptide toxins from marine organisms modify the gating kinetics of the Na channel. However, the effects of several marine toxins unrelated to existing channels are poorly understood. Palytoxin, isolated from the zoanthid *Palythoa* species, is a potent toxic substance (Moore & Scheuer, 1971; Uemura *et al.*, 1981) and depolarizes the membrane in a Na^+-dependent but tetrodotoxin-resistant manner (Dubois & Cohen, 1977; Muramatsu *et al.*, 1984; Ito *et al.*, 1985). In the present study we examined mechanisms of palytoxin-induced ion permeability with the use of a patch clamp technique and found that the toxin forms a new type of ionic channel.

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Methods

Single ventricular myocytes were obtained by dispersion of guinea-pig hearts with collagenase (Nishio *et al.*, 1986) and were perfused externally with Tyrode solution. Cell-attached gigaseals were attained by use of fire-polished microelectrodes of about 10 MΩ (Hamill *et al.*, 1981). To reduce the capacity transient, Sylgard was applied as close to the pipette tip as possible. Currents were filtered at 3 kHz and recorded with a patch clamp amplifier (Nihon Kohden, CEZ-2100) on a video cassette (Victor, BR 6400), using a PCM converter system (NF Circuit Design Block, RP-880). The current was simultaneously recorded on a strip chart.

Palytoxin was isolated from *Palythoa tuberculosa* (Hirata *et al.*, 1979). The toxin was dissolved in ethanol at 10^{-3} M, kept at −20°C as stock solution and diluted with the Tyrode solution immediately before use. Unless mentioned elsewhere, the pipette with tetrodotoxin (5×10^{-5} M) was filled with palytoxin at a concentration of 1 or 2×10^{-11} M. To examine the ion selectivity of the palytoxin-induced single channel, the NaCl of the Tyrode solution in

the pipette was substituted with LiCl, CsCl or NH_4Cl. The composition of normal Tyrode solution was as follows (mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, glucose 10, HEPES 5 (pH 7.4).

Experiments were conducted at room temperature (20–23°C). Experimental values are given as a mean ± standard error of mean (s.e. mean).

Results

Several minutes after the formation of a cell-attached gigaseal with a palytoxin(10^{-11} M)-containing electrode, the membrane current suddenly jumped. Figure 1 shows a continuous recording of the current jump, in which the patch membrane was held at the resting membrane potential (−84 ± 1 mV, 4 experiments). A discrete inward jump was elicited 5 min and 43 s after the formation of a gagaseal in this experiment; subsequently, the current fluctuated between the two levels for approximately 3 min. Thereafter, an additional jump of the same amplitude was produced and overlapped the first one. If high concentrations of palytoxin (10^{-10} or 10^{-9} M) were present in the pipette, staircase-inward current jumps rapidly developed. On the other hand, no jump was observed in the absence of palytoxin. These results suggest that the current flows through ionic channels formed by palytoxin and that the fluctuation reflects gating behaviour of the single channel. The amplitude of the inward single channel current at resting membrane potential was 0.65 ± 0.3 pA (5 experiments).

The palytoxin-induced channel appeared to flicker between two levels: closed and open states. Further inspection revealed that the short duration-closed state frequently occurred during the open state (Figure 1). Figure 2 shows histograms of current durations at the resting potential. Distribution of the open times roughly fitted an exponential component (decay time constant: 235 ms), while the closed state was composed of short and long duration-components (decay time constants: 3.9 ms and 2.65 s), suggesting that the current behaviour may reflect three states of the palytoxin-induced channel (two closed and one open state).

A palytoxin-induced single channel current was observed in the presence of 5×10^{-5} M tetrodotoxin and 2×10^{-3} M Co^{2+} (Figure 3) or in Ca^{2+}-free Tyrode solution. However, when Na^+ in the Tyrode

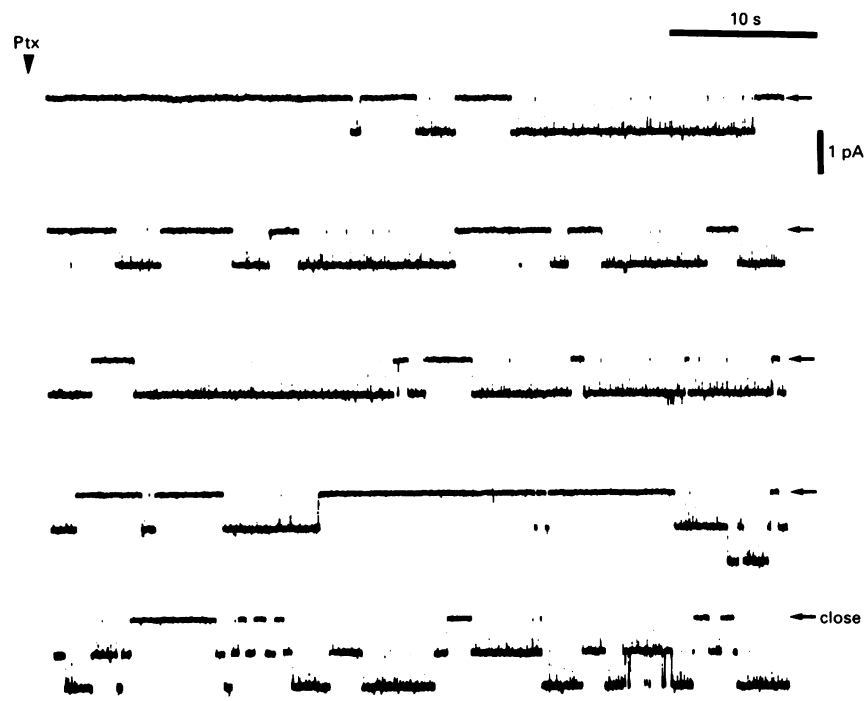

Figure 1 Palytoxin-induced single channel currents recorded from a guinea-pig ventricular myocyte in the cell-attached configuration. Continuous recording from 5 min 20 s after the formation of a gigaseal (from top left to bottom right). The pipette was filled with Tyrode solution which contained palytoxin (Ptx), 1×10^{-11} M and tetrodotoxin 5×10^{-5} M. Arrows represent the holding current level.

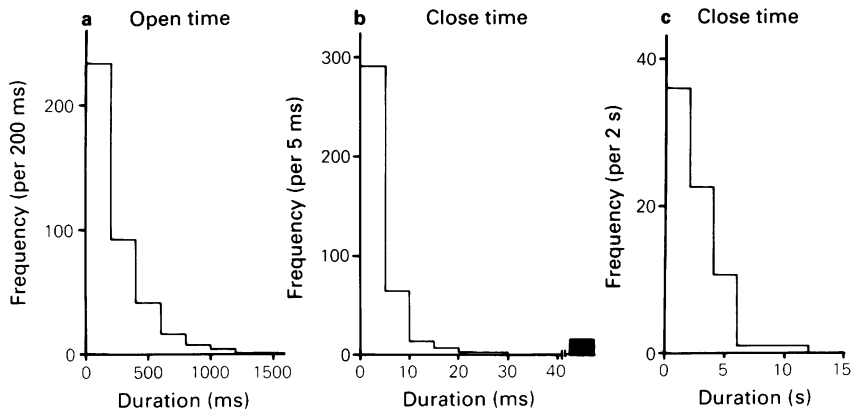

Figure 2 Distribution of durations of palytoxin-induced single channel current. Membrane potential was held at the resting potential. The distribution of open time (a) is fitted by a single exponential; decay time constant, 235 ms. The distribution of close time (b and c) is composed of two components; decay time constant, 3.9 ms and 2.65 s. The last bin (closed column) in (b) shows durations longer than 200 ms. Since such long durations of closed time were infrequent, the events were collected from 4 patches and plotted (c).

solution was completely substituted with choline, no jump was evoked by palytoxin, even with a high concentration (10^{-9} M, Figure 3).

Ion selectivity of the palytoxin-induced channel was further characterized by determining the conductance sequence for monovalent cations. At first, the voltage-dependence of the single channel was examined in normal Na^+-Tyrode solution. As shown in Figure 4, the single channel current was induced in depolarized or hyperpolarized membranes. The amplitude changed linearly between the range of ± 50 mV deviation from the resting potential (Figure

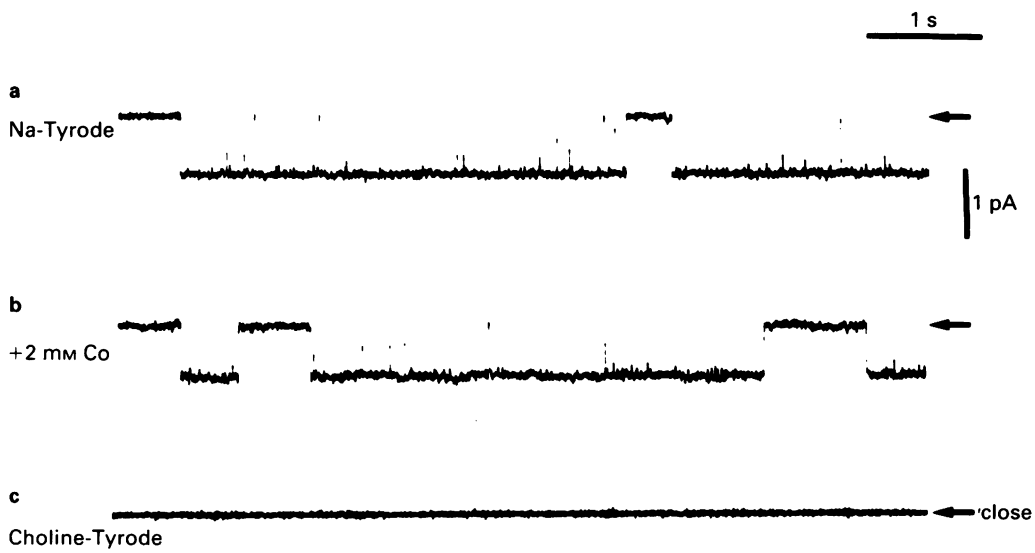

Figure 3 Effects of Co^{2+} and choline substitution on palytoxin-induced single channel current recorded from guinea-pig ventricular myocytes in cell-attached configuration. (a) Control recording: the pipette was filled with normal Na^+-Tyrode solution which contained palytoxin 1×10^{-11} M and tetrodotoxin 5×10^{-5} M. (b) Co^{2+} (2×10^{-3} M) was present in the pipette solution in addition to the substances present in the control recording. (c) The pipette was filled with Na^+-free, choline Tyrode solution which contained palytoxin 1×10^{-9} M and atropine 1×10^{-5} M. Each result was obtained from three different cells. Arrows represent the closed state. Strip chart recordings.

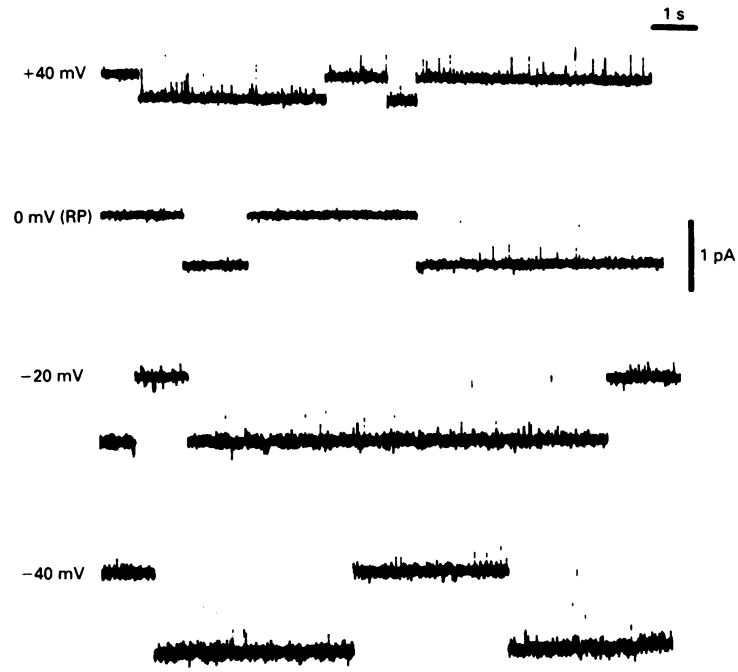

Figure 4 Palytoxin-induced single channel currents recorded at various membrane potentials. Experimental conditions were the same as those in Figure 1. When the patch membrane was depolarized by 40 mV, two single channel currents were observed consecutively.

5); a single channel conductance of 9.5 ± 0.5 pS being estimated (5 experiments). When the reversal potential was extrapolated from the slope, a shift of 68.2 mV in the depolarizing direction (from the resting potential) was estimated. Single channel currents with a linear voltage-dependence were also produced by palytoxin in NH_4^+, Li^+ or Cs^+-Tyrode solution (Figure 5) and the slope conductances were 16.5 ± 1.6 pS, 9.2 ± 0.7 pS and 11.0 ± 0.7 pS, respectively (4 experiments). The reversal potential estimated showed the depolarizing shift of 73.0 mV, 70.0 mV and 66.9 mV from the resting potential in NH_4^+, Li^+ and Cs^+-Tyrode solutions, respectively.

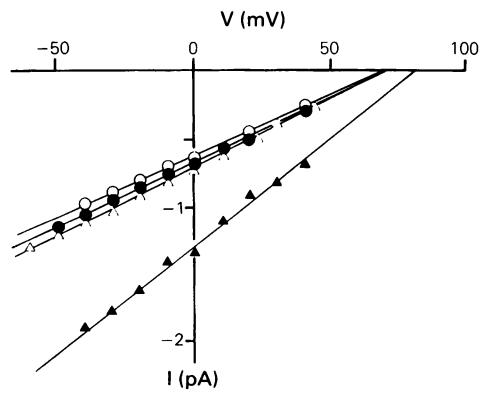

Figure 5 Voltage-dependence of the amplitude of palytoxin-induced single channel current recorded in various ionic environments. Na^+ in the pipette Tyrode solution was completely substituted with NH_4^+, Li^+ or Cs^+: Na^+(●); NH_4^+(▲); Li^+(○); Cs^+(△). Abscissa scale: deviation of patch membrane potential from the resting potential.

Discussion

The present results clearly show that palytoxin induces single channel currents under patch clamp conditions. The channel can open at all the membrane potentials tested, and is highly selective for Na^+.

Although palytoxin-induced single channel is highly selective for Na^+ in normal Tyrode solution, it is unlikely that the channel is induced by a modification of the voltage-dependence and ion selectivity of the existing Na channel. Unlike the Na channel (Kunze *et al.*, 1985), the palytoxin-induced channel has the following features: (1) the palytoxin-induced channel is insensitive to tetrodotoxin; (2) the amplitude of single channel conductance differs from that

Table 1 Permeability ratios of various cations in palytoxin-induced channels and normal or modified Na channels

	Palytoxin-channel		*Normal Na channel*		*Na channel modified by* grayanotoxin I	*veratridine*	*batrachotoxin*
Cations	*Guinea-pig ventricle* (I)	*Squid axon* (II)	*Frog node* (III)	*Squid axon* (IV)	*Squid axon* (IV)	*Rat skeletal muscle* (V)	*Rat skeletal muscle* (V)
Na^+	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Li^+	0.93	0.62	0.91	1.12	1.03	0.91	0.91
Cs^+	0.95	0.75	<0.012	0.085	0.067	<0.05	<0.022
NH_4^+	1.21	1.45		0.2			

(I) Present study. The ratio of test cation permeability (P_x) to sodium permeability (P_{Na}) was calculated from the equation: $P_x/P_{Na} = \exp[F(E_x - E_{Na})/RT]$, where E_x and E_{Na} represent the reversal potentials of palytoxin-induced single channel current in test cation and sodium solutions, respectively.
(II) Muramatsu *et al.* (1984); (III) Hille (1972); (IV) Hironaka & Narahashi (1977) and Hagiwara *et al.* (1972); (V) Garber & Miller (1987).

of the normal Na channel; (3) no inactivation is produced when the membrane is depolarized; (4) the ion selectivity is much different from that of the normal Na channel. These features also differ from the single Na channels modified by many toxins (Quandt & Narahashi, 1982; Garber & Miller, 1987). Table 1 is a summary of the relative permeability of various cations in the palytoxin-induced channel and normal or modified Na channels.

Palytoxin is effective on a variety of tissues in increasing cation permeabilities. Sodium-sensitive and tetrodotoxin-insensitive depolarizing actions of the toxin have been observed in squid axons (Pichon, 1982; Muramatsu *et al.*, 1984), cockroach axons (Pichon, 1982), frog nodes of Ranvier (Dubois & Choen, 1977), frog skeletal muscle fibres (Deguchi *et al.*, 1976) and dog, rabbit and guinea-pig heart muscles (Weidmann, 1977; Ito *et al.*, 1985). Palytoxin is also very effective in inducing haemolysis of human and rat erythrocytes (Ahnert-Hilger *et al.*, 1982), contractions of vascular smooth muscle (Ito *et al.*, 1976) and of cardiac tissues (Rayner *et al.*, 1975; Deguchi *et al.*, 1976; Alsen *et al.*, 1982). These observations all led to the notion that palytoxin increases the permeability of various membranes to Na^+, K^+ and/or Ca^{2+}. However, the present results suggest that an increase in Na^+ permeability may be the main effect of palytoxin, because of high selectivity for Na^+ of palytoxin-induced channels.

Palytoxin-induced depolarization and cation permeability are partially inhibited by ouabain (Habermann & Chhatwal, 1982; Chhatwal *et al.*, 1983; Ito *et al.*, 1985). Since single ($Na^+ + K^+$)-ATPase molecules incorporated into planar bilayers have been demonstrated to produce single channel behaviour (Reinhardt *et al.*, 1984), the palytoxin-induced channel may be somehow associated with these enzyme molecules.

Antibiotics are known to create ionic channels or pores in biological membranes and in lipid bilayers, but these channels are permeable to not only many kinds of ions but also non-electrolytes (Ermishkin *et al.*, 1976; 1977; Medoff *et al.*, 1983). In this respect, the palytoxin-induced channel appears to be novel because of its high selectivity for Na^+, under physiological conditions. This may be the first report of single channel formation by a marine toxin, and the possibility that other marine toxins also form or create unique ionic channels unrelated to the existing channels warrants investigation.

The authors thank M. Ohara for reading the manuscript and Naomi Yamada for secretarial assistance. This work was supported in part by a grant from the Ministry of Education in Japan.

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(Received September 8, 1987
Revised November 11, 1987
Accepted November 16, 1987)