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Short communication

Philanthotoxin inhibits Ca^{2+} currents in rat hippocampal CA1 neurons

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Abstract

The wasp venom philanthotoxin-4.3.3 (PhTX-4.3.3) is an antagonist of glutamate transmission in the insect as well as in the mammalian brain. It was recently shown that PhTX-4.3.3 inhibits the *N*-methyl-D-aspartate (NMDA) transmission in rat hippocampus. In this study we show that dideaza-philanthotoxin-12 (dideaza-PhTX-12), an analogue of PhTX-4.3.3, is a potent antagonist of voltage-dependent Ca^{2+} currents in rat hippocampal CA1 neurons. At a concentration of 10 μM it reduces the Ca^{2+} current to 40%. Two voltage-dependent potassium currents, the A current and the delayed rectifier, were hardly affected by dideaza-PhTX-12, indicating selectivity of the drug for Ca^{2+} currents. As a consequence the philanthotoxins will inhibit the calcium influx via voltage dependent as well as NMDA mediated calcium channels and thus reduce excitability in the hippocampus.

Key words: Philanthotoxin; Ca^{2+} current; Hippocampus; Glutamate antagonist; Polyamine; Patch clamp, in situ

1. Introduction

The venom of the digger wasp *Philanthus triangulum* contains at least two fractions that interact with glutamatergic neuromuscular transmission in insects mediated by quisqualate and kainate receptors (Piek, 1986; Karst et al., 1990, 1991). δ -philanthotoxin is the fraction of the philanthus venom, that inhibits the glutamatergic transmission most potently. This fraction (PhTX-4.3.3) is a polyamine, with a molecular structure comparable to the spider toxins of the Joro spider (JSTX), the *Nephila maculata* spider (NSTX) and the spider toxin Argiotoxin636 (see review, Usherwood and Blagborough, 1992), which also block the glutamatergic transmission.

PhTX-4.3.3 not only inhibits the neuromuscular transmission in insect, it also blocks synaptic transmission in mammalian neurons. Synaptic responses in hippocampal CA1 neurons evoked by Schaffer collateral stimulation were blocked by the synthetic PhTX-

4.3.3 and the analogue dideaza-PhTX-12 (for molecular structure see Fig. 1A), which in this preparation was even more potent than PhTX-4.3.3 (Schluter et al., 1992). In a recent study in the slice preparation Federov et al. (1992) showed that another analogue (PhTX-3.4.3) depresses the NMDA receptor in rat hippocampal CA1 neurons. In addition, Ragsdale et al. (1989) demonstrated that PhTX-4.3.3 inhibits NMDA-induced currents in oocytes injected with rat brain mRNA. In contrast to these studies, Jones and Lodge (1991) reported that PhTX-4.3.3 blocks non-NMDA, rather than NMDA-induced excitation in brainstem neurons.

Polyamines are also known to block voltage dependent Ca^{2+} currents. Thus, a polyamine fraction isolated from the funnel web spider, *Agelenopsis aperta*, called FTX, selectively blocks a voltage-dependent Ca^{2+} current in rat cerebellar Purkinje neurons (P current) (Llinás et al., 1992). The other voltage dependent Ca^{2+} currents, i.e., the low threshold T type current and the high threshold N and L type Ca^{2+} currents, were not affected by FTX (Regan et al., 1991). Another synthetic polyamine, *N*-(4,8-diazoctyl)-L-arginine amide tetrachloride, was reported to block the T type Ca^{2+}

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currents in cultured rat dorsal root ganglion neurons (Scott et al., 1991).

In this study we investigated whether the philanthotoxins, PhTX-4.3.3 and dideaza-PhTX-12 in addition to their effect on synaptic transmission also affect Ca^{2+} currents, recorded with patch clamp electrodes in hippocampal slices.

2. Materials and methods

The experiments were carried out in hippocampal slices of male Wistar rats weighing 100–170 g. The brain was quickly removed after decapitation under ether anesthesia, and dipped in ice-cold carbonated (95% O_2 , 5% CO_2) artificial cerebrospinal fluid (ACSF

in mM: 124 NaCl, 3.5 KCl, 1.25 NaH_2PO_4 , 1.5 MgSO_4 , 2 CaCl_2 , 25 NaHCO_3 , 10 glucose, pH 7.4). The hippocampus was dissected and thin slices (120–150 μm) were cut on a tissue chopper (McIlwain). The slices were stored at room temperature in a holding chamber. After an equilibration period of at least one hour, one slice at a time was transferred to the recording chamber, submerged and continuously perfused (2 ml/min) with warm (32°C) carbonated ACSF. Sodium currents were blocked with 0.5 μM tetrodotoxin (TTX). 10 mM tetraethylammonium chloride (TEACl), 5 mM 4-aminopyridine (4AP) and 5 mM CsCl were added to block potassium currents. In cases where we studied potassium currents only 0.5 μM TTX was added to the ACSF.

Fig. 1. A: structure of PhTX-4.3.3 and the analogue dideaza-PhTX-12. B: example of voltage-dependent Ca^{2+} currents in a rat hippocampal CA1 neuron. Voltage protocol is given in the lower panel, the asterisks mark the time period for which current is shown in the upper panel. C: PhTX-4.3.3 (upper panel) and dideaza-PhTX-12 (lower panel) at 10 μM reduce the Ca^{2+} current evoked by a depolarizing command step to -25 mV. See inset for voltage protocol. D: to test the specificity of the toxin for Ca^{2+} currents, we also studied its effect on two voltage dependent potassium currents, the A current and delayed rectifier. Dideaza-PhTX-12 (10 μM) only slightly reduced the A current (8%) and did not affect the delayed rectifier. The voltage protocol shown, activates both the transient A current and sustained delayed rectifier. Without the hyperpolarizing prepulse to -100 mV only the delayed rectifier is activated. Subtracting currents obtained from both voltage protocols, enabled us to study the A current and delayed rectifier separately (as described in Numann et al., 1987). The calibration bars for current traces represent: 0.5 nA for amplitude in B, C and D, 50 ms for timescale in B and C and 100 ms in D.

Hippocampal pyramidal CA1 neurons could be distinguished and selected for recording under an upright microscope (Nikon Optiphot), using a $40\times$ water immersion objective. With a patch pipette (pulled on a Mecnex BB CH micropipette puller from 1.5 mm outer diameter borosilicate glass, 1.5–3.0 M Ω) the selected cell was approached. Positive pressure ensured that the tip of the electrode was kept clean and that the surface of the membrane was freed from surrounding neuropil (Edwards et al., 1989). When the tip of the electrode was placed on the membrane, a giga seal could be established by application of light suction. Subsequently the whole cell configuration at recording was established by additional suction. The pipette solution consisted of (in mM): 100 CsF, 0.5 CaCl₂, 2 MgCl₂, 2 MgATP, 0.1 NaGTP, 10 Hepes, 10 EGTA, 20 creatine phosphate, 50 U/ml creatine phosphokinase, 0.1 albumin, 20 TEACl, pH 7.4. The solution was stored frozen and kept on ice during the day of the experiment. In cases where we studied potassium currents we used a pipette solution containing (in mM): 140 KF, 2 MgCl₂, 1 CaCl₂, 10 Hepes, 10 EGTA, pH 7.4.

Whole cell currents were measured under voltage-clamp conditions with a Biologic RK-300 amplifier. Data were collected with an Atari computer, at 5 kHz sampling rate. Each cell was subjected to the same sequence of timed voltage protocols generated by the acquisition program. Correction for linear leak current was performed off-line.

The philanthotoxins (DOW chemical industries, Walnut Creek, CA, USA) were dissolved in distilled water and stored frozen in a stock concentration of 5×10^{-3} M. About 15 min after the whole cell configuration was established when the Ca²⁺ currents were

constant in amplitude, the toxins were diluted to the intended concentration in ACSF and bath applied.

3. Results

Ca²⁺ currents were evoked as described in a previous paper (Karst et al., 1993), by 200 ms depolarizing command potentials to -100 , -75 , -50 , -25 mV or 0 mV, in five consecutive steps (time between steps was 10 s). The depolarization was preceded by a hyperpolarizing prepulse to -130 mV for 3 s which completely removed the inactivation (Fig. 1B). This voltage protocol was repeated every 2 min. As previously shown the threshold for activation of the Ca²⁺ current was around -80 mV, and the peak amplitude was reached around -30 mV (Karst et al., 1993). Dideaza-PhTX-12 (10 μ M), the philanthotoxin analogue that most potently inhibits synaptic transmission in the rat hippocampus (Schluter et al., 1992), caused a reduction of the Ca²⁺ current within 10 min (Fig. 1C). The block was only partly reversible (Fig. 2A). Input resistance was not affected by the compound. The voltage for half maximal removal of steady state inactivation was not changed (before $V_H = -117 \pm 2$ mV, after incubation with 15 μ M dideaza-PhTX-12 $V_H = -114 \pm 1$ mV, $n = 4$) indicating that dideaza-PhTX-12 does not affect the voltage dependency of the Ca²⁺ current inactivation. The inhibition caused by dideaza-PhTX-12 was dose-dependent and approximately half maximal for 5 μ M (Fig. 2B). At a concentration of 10 μ M where dideaza-PhTX-12 had its maximal effect, the peak Ca²⁺ current was reduced to $39 \pm 4\%$ (mean $\pm$ SEM, $n = 5$, $p < 0.05$). PhTX-4.3.3 was less potent at this concentration and reduced the Ca²⁺ current to 72% (Fig. 2B).

Fig. 2. A: in this I-V relationship of the Ca²⁺ current, the inhibiting effect of dideaza-PhTX-12 is shown. Within 10 min 10 μ M dideaza-PhTX-12 reduces the Ca²⁺ current to 40%. After 18 min of wash-out the Ca²⁺ current is only partly restored. B: relative Ca²⁺ current amplitude as a function of dideaza-PhTX-12 concentration (open squares, for each concentration at least $n = 4$). PhTX-4.3.3 is less potent, at a concentration of 10 μ M it reduces the current to $71.9 \pm 0.6\%$ (filled square, $n = 4$).

To study whether the philanthotoxins also affect potassium-currents, we studied their effect on two voltage-dependent potassium currents, i.e., the A current and the delayed rectifier. The A current and delayed rectifier were activated as described by Numann et al. (1987). When we applied 10 μ M dideaza-PhTX-12 to the ACSF, the A current and delayed rectifier were only marginally reduced (to $92 \pm 3\%$ and $88 \pm 4\%$ respectively, $n = 3$, see Fig. 1D).

4. Discussion

In this study we report that the philanthotoxin fraction PhTX-4.3.3 and its analogue dideaza-PhTX-12 partially inhibit a Ca^{2+} current in rat hippocampal CA1 neurons. When we compare the inhibiting capacities of dideaza-PhTX-12 with the natural synthetic compound, PhTX-4.3.3, it is clear that like the effect on synaptic transmission in the hippocampus (Schluter et al., 1992), PhTX-4.3.3 is less potent than dideaza-PhTX-12. The inhibition of the Ca^{2+} current by the most potent toxin, dideaza-PhTX-12, was much more pronounced than the effects observed for voltage-dependent potassium currents, the A current and delayed rectifier, indicating a possible Ca^{2+} current specificity. Ca^{2+} currents in rat hippocampus, measured with the in situ patch clamp technique, can be divided in (1) a low-threshold inactivating Ca^{2+} current and (2) a high-threshold Ca^{2+} current that shows hardly any inactivation. In this study the effect of the toxins on the total Ca^{2+} current was measured. Since dideaza-PhTX-12 already reduced the total Ca current at low voltages (-50 mV), when the high threshold Ca current is not yet activated, we may tentatively conclude that at least the low threshold current is affected. In a future study we have to investigate with more extensive voltage protocols and pharmacological tools which Ca^{2+} currents are specifically affected by the toxins.

In a recent paper Fedorov et al. (1992) reported that PhTX-3.4.3 did not affect the EPSP in rat hippocampal CA1 neurons, but at a concentration of 2 μ M markedly depressed the activity of NMDA-gated channels. Activation of NMDA receptors induces a considerable calcium influx in CA1 neurons (MacDermott et al., 1986). Voltage dependent Ca^{2+} currents could also be responsible for a large influx of calcium in CA1 neurons (Regehr and Tank, 1992). If dideaza-PhTX-12 blocks both the NMDA-gated channels and the voltage dependent Ca^{2+} currents, it should be very effective in reducing calcium influx. If so, dideaza-PhTX-12, may

prove to be a useful compound for preventing calcium intoxication during ischemic events.

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