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Scorpion venom inhibits carbamylcholine-induced ^{86}rubidium efflux from rat parotid acini

Jean-Paul Dehaye, Jacques Winand and Jean Christophe

Department of Biochemistry and Nutrition, School of Medicine, Université Libre de Bruxelles, rue Evers 2, B-1000 Brussels, Belgium

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The muscarinic agonist carbamylcholine stimulated 5-fold ^{86}Rb efflux from preloaded rat parotid acini. Apamin was without effect on this carbamylcholine-induced ^{86}Rb efflux. By contrast, the venom from *Leiurus quinquestriatus* (a scorpion from Israel) inhibited non-competitively this efflux while being without effect on the carbamylcholine-stimulated ^{45}Ca efflux and amylase release. This heat-resistant inhibitory effect of the venom was destroyed by boiling in the presence of dithiothreitol. These results suggest that the venom from *L. quinquestriatus* contains a toxin capable to block apamin-insensitive calcium-activated potassium channels in rat parotid acini.

Carbamylcholine; Apamin; Ca^{2+}-activated potassium channel; Rb efflux; (Rat parotid gland, Scorpion venom)

1. INTRODUCTION

The venoms from the North African scorpions *Androctonus australis* and *Leiurus quinquestriatus* contain neurotoxin(s) capable to delay the closure of sodium channels [1,2]. These toxins interact with veratridine activation of sodium channels. Tetrodotoxin exerts its blocking effect on another site of sodium channels [3]. These channels have been solubilized, isolated and their physicochemical properties characterized after cross-linking with iodinated scorpion toxins [4].

Calcium-activated potassium channels are more difficult to examine as the peptide ligand apamin (from bee venom) binds to such channels in neuroblastoma and liver but not to similar channels in thymocytes, erythrocytes, bovine chromaffin cells, bullfrog ganglion cells, and muscle [5]. Recently, however, the venom of the scorpion *L. quinquestriatus* [6-9] was shown to contain an inhibitor of apamin-insensitive potassium channels [6] in addition to a peptide immunologically unrelated to apamin but capable of inhibiting apamin-sensitive potassium channels [7].

Parotid glands possess calcium-activated potassium channels [10] through which they respond to muscarinic agents, α-adrenergic agents and substance P by a massive loss of potassium [11]. These secretagogues act by increasing the turnover of polyphosphoinositides and then cytosolic calcium concentration. The purpose of this work was to investigate the effect of the venom of *L. quinquestriatus* on the efflux of ^{86}Rb from rat parotid acini in response to carbamylcholine. These experiments were performed in the presence of a saturating (2 μM) concentration of tetrodotoxin, in order to block any interference with the venom component acting on sodium channels. We demonstrate that the calcium-activated potassium channels of rat parotids are apamin insensitive but are inhibited by the venom of *L. quinquestriatus*.

Correspondence address: J. Christophe, Department of Biochemistry and Nutrition, School of Medicine, Université Libre de Bruxelles, rue Evers 2, B-1000 Brussels, Belgium

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2. MATERIALS AND METHODS

Female Wistar albino rats (200–250 g) fed ad libitum were used. The venom from *L. quinquestriatus quinquestriatus* was purchased from Sigma (St. Louis, MO) as a lyophilized powder. A 2 mg/ml stock solution in distilled water was prepared by sonication. $^{45}CaCl_2$ (25 mCi/mg Ca) was supplied by Amersham (Amersham, England) and $^{86}RbCl$ (3.66 mCi/mg Rb) by New England Nuclear (Brussels, Belgium). Carbamylcholine was from Merck (Darmstadt, FRG). The origin of the other products has already been reported [12].

2.1. *Measurement of amylase release*

After decapitation, the parotid glands were excised and acini were dispersed as described [12]. The acini were resuspended in fresh medium and 140 μl aliquots were incubated at 37°C in the presence of various agents (in a final 200 μl volume). After 20 min, the acini were centrifuged in a Beckman 152 microfuge. The supernatant was collected and assayed for amylase by the method of Noelting and Bernfeld [13]. Non-incubated supernatant controls were taken at the beginning of each incubation period, for determining medium amylase at time 0 (allowing appropriate corrections). Samples of suspended acini were diluted, sonicated and total amylase in the suspension was assayed. Results were expressed as percent total amylase content released in the medium during the 20 min incubation (a period during which linear rates of secretion were always observed).

2.2. *Measurement of ^{45}Ca and ^{86}Rb efflux*

After isolation, the acini were resuspended in 2.5 ml fresh medium, in the presence of $^{45}CaCl_2$ (0.5 mM, 40 μCi/ml) or $^{86}RbCl$ (70 μM, 20 μCi/ml), then preincubated for 60 min at 37°C, washed twice with fresh medium and centrifuged through 3 ml of similar medium except for a higher concentration of bovine serum albumin (4% instead of 0.5%). After this washing procedure, the acini were resuspended in fresh medium and 140 μl aliquots were incubated for 2 min at 37°C, in the presence of various agents. The acini were then centrifuged and 75 μl of the supernatant were counted in a Beckman 7100 spectrometer, in the presence of 4 ml Aquasolve (New England Nuclear). Non-incubated controls were taken at the beginning of each incubation period for determining ^{45}Ca or ^{86}Rb released into the medium at time 0, allowing appropriate correction. An aliquot of the whole suspension was also counted. Results were expressed as percent intracellular residual ^{45}Ca or ^{86}Rb released after 2 min.

3. RESULTS

Carbamylcholine dose-dependently stimulated ^{86}Rb efflux from preloaded acini. Half-maximal and maximal effects were observed at, respectively, 0.3 μM and 10 μM concentration (fig.1). This dose-response curve was affected neither by 2 μM tetrodotoxin nor by 1 μM apamin. Similarly, the stimulations by carbamylcholine of ^{45}Ca efflux and amylase release were unaffected by these two agents (not shown).

The effects of the venom from *L. quinquestriatus* were tested on ^{86}Rb efflux, ^{45}Ca efflux, and amylase secretion. Venom concentrations as high as 100 μg/ml did not affect the release of ^{86}Rb (fig.2A) and ^{45}Ca (fig.2B) but amylase secretion doubled at venom concentrations higher than 10 μg/ml (fig.2C).

Similar dose-response curves for the venom were performed in the presence of a maximal (100 μM) concentration of carbamylcholine. By itself, the muscarinic agonist stimulated ^{86}Rb efflux 4.5-fold, ^{45}Ca efflux 5-fold and amylase secretion 7-fold. Venom concentrations higher than 10 μg/ml inhibited carbamylcholine-stimulated ^{86}Rb efflux and at 100 μg/ml the effect of carbamylcholine was reduced by 65% (fig.2A). In parallel experiments, the venom affected neither the efflux of ^{45}Ca nor the amylase hypersecretion induced by carbamylcholine (fig.2B and C).

In order to evluate the type of inhibitory effect exerted by the venom on carbamylcholine-stimulated ^{86}Rb efflux, the venom was tested in the presence of increasing concentrations of the muscarinic agonist. As shown in fig.1, the venom did not modify the half-maximal concentration of carbamylcholine on ^{86}Rb efflux, but decreased its maximal effect by 65%. This inhibition was not affected by the removal of tetrodotoxin from the incubation medium. In parallel experiments, the venom exerted no effect on dose-response curves of carbamylcholine on ^{45}Ca efflux and amylase release (not shown).

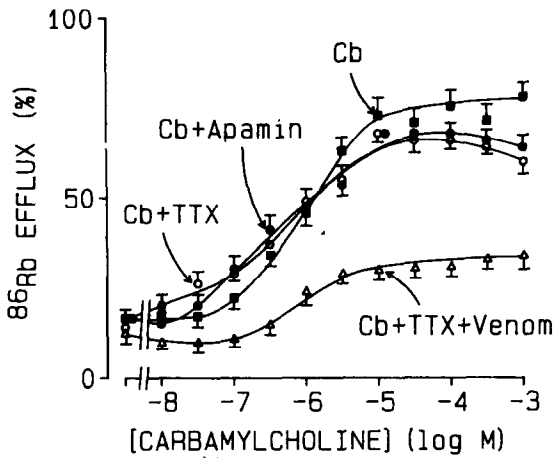

Fig.1. Effect of increasing concentrations of carbamylcholine on ^{86}Rb efflux from rat parotid acini. The acini were preincubated for 60 min in the presence of ^{86}RbCl, washed and subsequently incubated for 2 min in the presence of various concentrations of carbamylcholine (Cb) (■) or in the combined presence of carbamylcholine and 2 μM tetrodotoxin (TTX) (○); carbamylcholine and 1 μM apamin (●); carbamylcholine, 2 μM tetrodotoxin and 100 μg/ml of the venom from *L. quinquestriatus* (△). Results are expressed as % intracellular isotope content released and were the means ± SE of 3 experiments.

To get some information on the nature of the inhibitory component, the crude venom was boiled for 5 min in the absence or presence of 10 mM dithiothreitol. As shown in fig.3, boiling by itself

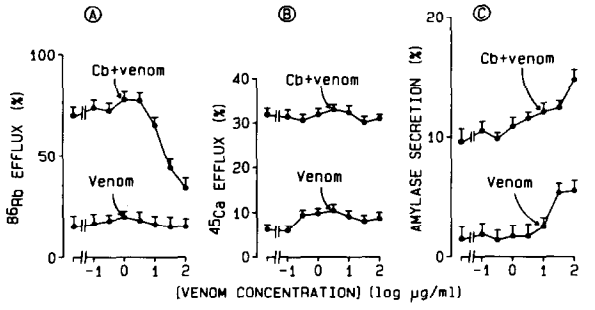

Fig.2. Effect of increasing concentrations of the venom from *L. quinquestriatus* on the release of ^{86}Rb (A), ^{45}Ca (B) and amylase (C) from rat parotid acini. Experiments were performed in the absence (○) or in the presence of 100 μM carbamylcholine (●). 2 μM tetrodotoxin was always present. Results were the means ± SE of 3 experiments.

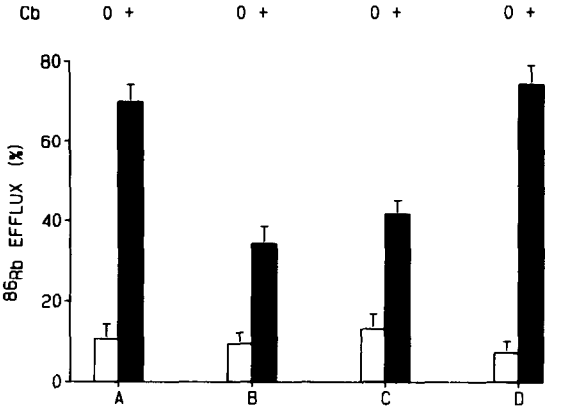

Fig.3. Effect of venom pretreatment on the response of rat parotid acini. The acini were incubated in the absence of venom (A), in the presence of untreated venom (B) or with the venom previously boiled for 5 min in the absence (C) or presence (D) of 10 mM dithiothreitol. The venom was tested alone (○) or in the presence (+) of 100 μM carbamylcholine. Results were the means ± SE of 3 experiments.

did not reduce the effect of the venom on ^{86}Rb efflux. On the other hand, pretreating the venom with the reducing agent completely reversed the effect of the venom on carbamylcholine-induced ^{86}Rb efflux.

4. DISCUSSION

The venom of *L. quinquestriatus* inhibited the increased efflux of ^{86}Rb from rat parotid acini normally observed in response to carbamylcholine. This effect was specific in that the concurrent increases of ^{45}Ca efflux and amylase secretion observed in response to carbamylcholine were unaffected, suggesting that the venom acted at a step distal to calcium mobilization. The inhibitory effect appeared to be non-competitive as the K_a for carbamylcholine was not altered but only the V_{max}, suggesting a reduction in the number of activatable potassium channels. Besides, the inhibitory effect of the venom decreased after its pretreatment with a reducing agent. Altogether these results might reflect the presence, in the venom of *L. quinquestriatus*, of a toxin inhibiting calcium-activated potassium channels. In this respect, two relevant toxins have been characterized in this venom: one inhibits apamine-sensitive potassium channels [6]

while the second, charybdotoxin, inhibits apamin-insensitive potassium channels [7]. Charybdotoxin is a 10 kDa peptide, rich in basic amino acids, resistant to boiling, but sensitive to reducing agents [6]. Considering that parotid potassium channels were apamin-insensitive and that the inhibitory component of the venom was resistant to boiling but totally destroyed by dithiothreitol, one can speculate that charybdotoxin was the inhibitory agent involved. Since charybdotoxin toxin represents 0.1% only of the protein content of crude venom, the data of fig.1 might suggest a K_i for charybdotoxin of 10 nM, in agreement with K_i values observed in muscle and erythrocytes [6–9].

Our data indirectly confirm that potassium efflux (from basolateral membranes) and amylase release are not necessarily associated in parotid glands. This has been demonstrated (in mirror) by Putney et al. [14] who observed that phorbol esters, as activators of protein kinase C, stimulate amylase release without affecting ^{86}Rb movements [13]. The activation of calcium-activated potassium channels by carbamylcholine might explain why, in response to vagal stimulation, parotid glands secrete a saliva rich in ions and fluid but rather poor in protein. This secretion is under the control of the cytosolic Ca^{2+} concentration and of protein kinase C. Ca^{2+} is involved in the activation of calcium-dependent protein kinase(s) and the opening of calcium-activated potassium channels. According to Pedersen [15], the increased open state probability of these channels in rat parotids leads to a large release of potassium from basolateral membranes. Potassium is secondarily pumped by a 3 Na/2 K ATPase pump and a 3 K/3 Na/6 Cl cotransport system [16]. The net result is an uptake of chloride at the basolateral membrane of acini that is balanced by an efflux of chloride at the luminal membrane under the control of the cytosolic calcium concentration [17]. In parallel, sodium ions and water flow towards the lumen via a paracellular pathway. Based on this model, water secretion would be triggered by channels inhibited by one of the toxins present in the venom from *L. quinquestriatus*. This toxin might thus prove to be a useful tool for further biochemical characterization of potassium channels in parotid glands and for studying their physiological role on ion and water movements.

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