EFFECT OF PHORATOXIN B ON ELECTRICAL AND MECHANICAL ACTIVITIES OF RAT PAPILLARY MUSCLE

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ABSTRACT The effect of phoratoxin B (PHTX), a small basic protein isolated from mistletoe, on transmembrane potentials and contraction of papillary muscles was studied using conventional intracellular microelectrodes. PHTX depolarized the resting potential, decreased the initial depolarizing phase of the action potential and the corresponding V_{max} and lengthened the action potential duration. PHTX increased the amplitude of the contraction. The data suggest that PHTX effect is similar to that of cytotoxic or cardiotoxin molecules and may be related to a reversible blockade of Na, K-ATPase.

KEY WORDS papillary muscles; membrane potentials; muscle contraction; phoratoxin B

Phoratoxin B (PHTX) has recently been isolated from mistletoe[1,2]. Mistletoe toxins are small basic proteins of 46 amino acid residues tightly bound together by 3 disulfites bridges. Their pharmacological properties are similar to those of cardiotoxins or membrane toxins from elapid snake venom. They are hemolytic and they depolarize cell membranes. Generally, membrane toxins are believed to work in 2 steps. First they attach electrostatically to the membrane via positively charged basic amino acids and second they either penetrate membrane structures through their lipophilic residues or interact with S-H groups in the membrane through their S-S bridges, changing the structure of the membrane and causing leakage of ions[3,4]. In this study the effect of PHTX was studied on transmembrane action potential (AP), resting potential (RP) and contraction of isolated rat papillary muscles.

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MATERIAL AND METHODS

Rats(♂, 250 g) were anesthetized with ether. The heart was excised and perfused using Langendorff technique. Papillary muscles (5-7 mm) isolated from both ventricles were mounted in a fast flow chamber. RP and AP were recorded on 12 papillary muscles using conventional intracellular microelectrode technique (resistance 20-30 MΩ; tip potential <3 mV). Contraction of 4 papillary muscles was recorded by a transducer attached to the tendon end with a thin silk thread. Tyrode solution was maintained at pH 7.3 with Hepes buffer (5 m*M*) and oxygenated at 35℃. PHTX was readily soluble in water. Muscles were stimulated with square pulses at 0.5 Hz.

RESULTS

Fig 1 A shows that PHTX 2 μg/ml markedly depolarized the RP recorded in the absence of stimulation. The depolarization induced by PHTX was not immediately abolished after PHTX removal from the control solution; the recovery occurred very slowly. PHTX 1 μg/ml produced a depolarization of small amplitude; PHTX <1 μg/ml did not produce any detectable decrease of RP. The depolarizing effect of PHTX on RP was not suppressed after total Na replacement by choline in the Tyrode solution.

In the presence of stimulation, PHTX1 μg/ml moderately depolarized the RP, decreased the amplitude of the initial depolarizing phase of the AP and the corresponding V_{max} and lengthened the AP duration (Fig 1B).

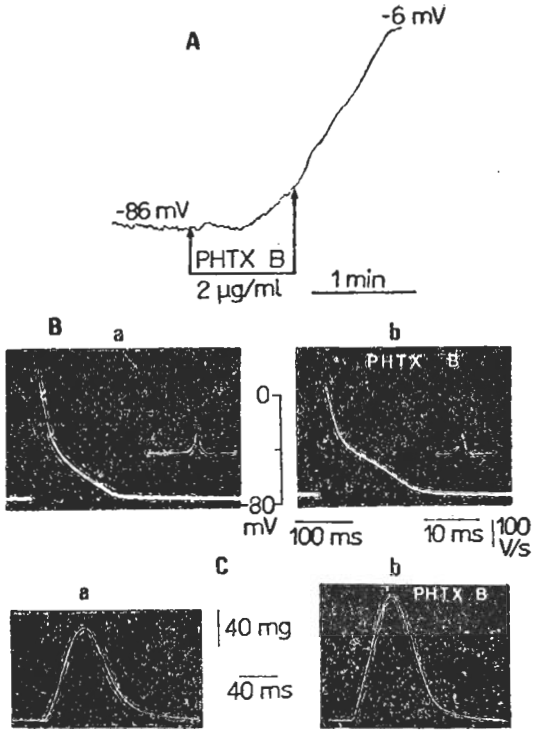

Fig 1. **Fffects of phoratoxin B on isolated rat papillary muscles. A) Effect of 2 µg/ml on RP in the absence of stimulation. B) AP(lower tracing) and its V_{max} (upper tracing) before (a) and 2 min after 1 µg/ml (b). C) Contraction before (a) and 4 min after 1 µg/ml (b).**

Fig 1 C shows that PHTX 1 µg/ml strongly increased the contraction without alteration of the time constant of the relaxation phase.

DISCUSSION

This study provides some clues to the possible mode of action of PHTX on mammalian cardiac cells. The main effect was to depolarize the RP. This effect takes a comparatively long time to develop and to disappear. This means at least: 1) PHTX has to cross the membrane and acts at an intracellular site; 2) PHTX has a membrane effect which does not become apparent until intracellular concentration of ions had time to change.

Our results show that PHTX effect on RP are not suppressed by Na removal from the control solution,suggesting that PHTX does not generate an inward movement of Na throughout the membrane. The reduction of the fast Na conductance seen in our experiments may be the consequence of an inactivation of Na channels promoted by the depolarizing effect rather than an alteration of the kinetic parameters governing the Na conductance. Similarly, the lengthening of AP duration may also be the consequence of development of the depolarizing effect.

The observation that PHTX increases the amplitude of contraction without alteration of the kinetics of relaxation phase suggests that PHTX increases the entry of Ca^{2+} into the cells and does not alter the Na/Ca exchange. These results are similar to those reported for cardiotoxins or cytotoxic substances which generally depolarized the cell membrane.

Cardiotoxins apparently bind to lipid-type receptors and then trigger a structural rearrangement in the membrane that inactivates Na, K-ATPase[5]. They interact with lipids in natural and synthetic membranes[6].The present results concerning the effect of PHTX may be interpreted in terms of a reversible blockade of Na, K-ATPase. A depolarizing effect associated to a decrease in V_{max} and a lengthening of AP duration are generally expected as a result of Na, K-ATPase blockade.

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Phoratoxin B 对大鼠乳头状肌的电和机械活动的作用

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提要　Phoratoxin B (PHTX)是从槲寄生中分离出一小的碱性蛋白。用细胞内微电极技术观察 PHTX 对大鼠乳头状肌的跨膜电位和机械活动的作用。发现 PHTX 除极静息膜电位，降低动作电位(AP)初去极化相和 V_{max} 以及延长 AP 的时程。PHTX 增加收缩的幅度。结果提示 PHTX 的作用相似于细胞毒或心脏毒分子的作用，可能与 Na, K-ATP 酶可逆性阻滞有关。

关键词　穗花毒素 B；　乳头状肌；　膜电位；　肌肉收缩

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