Extracellular calcium ions modify the effects of *Anemonia sulcata* toxin (ATX II) in guinea-pig papillary muscles

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Summary. In isolated guinea-pig papillary muscle ATX II prolonged the action potential duration to a lesser extent at high extracellular Ca^{++}-concentrations. This is interpreted as an interference of Ca^{++} with ATX II-binding sites.

The *Anemonia sulcata* toxin ATX II belongs to a group of cardiotonic polypeptides of marine origin, which prolong the action potential duration[1-3]. These compounds are thought to interfere with the inactivation process of the sodium channels so that some sodium current persists during the plateau phase of the action potential[1,4,5]. The increased sodium influx has been regarded as the underlying mechanism for the positive inotropism[6,7].

The influence of the extracellular calcium concentration ($[Ca^{++}]_e$) on the effects of the polypeptide toxins is controversial[3,8]. If arrhythmias and elevation in resting tension are taken as toxic symptoms[1-3,8,9], ATX II is more toxic in atrial preparations at high $[Ca^{++}]_e$[8], whereas goniopora toxin increases the incidence of arrhythmias at low $[Ca^{++}]_e$[3]. The extracellular calcium concentration itself is a major determinant of force of contraction. Therefore, it is difficult to judge the efficacy of ATX II at various $[Ca^{++}]_e$ only by measuring the extent of contractile augmentation. If calcium ions influence the action of ATX II directly they should also modify the effect on the action potential duration.

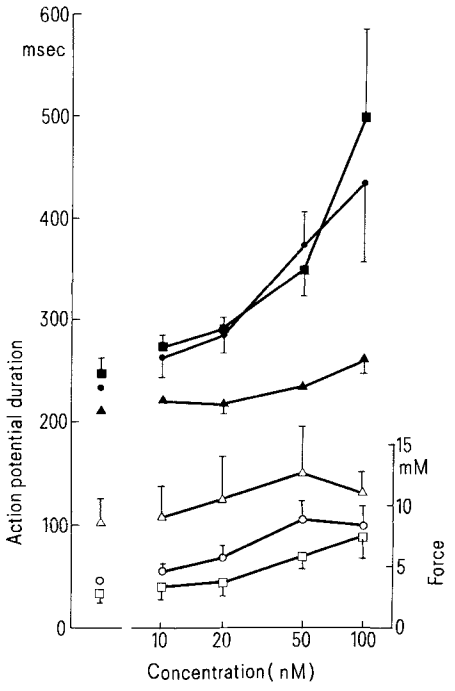

Figure 1. Cumulative dose-response-curves for ATX II-effects in guinea-pig papillary muscles at various calcium concentrations (3.6, 1.8 and 0.9 mM $[Ca^{++}]_e$: triangles, circles and squares, respectively).
Left ordinate: action potential duration at 90% of repolarization in msec (closed symbols); right ordinate: force of contraction in mN (open symbols); abscissa: concentration of ATX II in nM. Duration of exposure at each concentration was 30 min. The control values before ATX II exposure are not connected with the other values. The symbols are means ± SEM from 6 experiments at each level of $[Ca^{++}]_e$. Stimulation frequency was 1 Hz.

Materials and methods. Thin papillary muscles (0.7-1.0 mm in diameter) from the right ventricles of guinea-pigs were superfused continuously in a small muscle chamber. Tyrode solution of the following composition was used (mM): NaCl 137.0; KCl 2.7; $CaCl_2$ 0.9-3.6; $MgCl_2$ 1.0; $NaHCO_3$ 12.0; NaH_2PO_4 0.21; glucose 5. The pH of the solution was maintained at 7.35-7.4 by gassing with 3% CO_2 and 97% O_2. The temperature was 32.0 ± 0.5 °C. The preparations were stimulated via platinum wires with supramaximal impulses at a frequency of 0.1 or 1 Hz. Intracellular potential recordings were obtained with conventional glass microelectrodes filled with 3 M KCl solution. The tension was recorded isometrically via a force transducer (Statham UC-2). Transmembrane potentials and tension were displayed on an oscilloscope (Tektronix 502 A) and were photographed for later analysis. ATX II was dissolved in distilled water as a concentrated stock solution (10^{-4} M) which was stored in the deep freezer until final use.

Results and discussion. In guinea-pig papillary muscles, cumulative dose-response curves for the ATX II effects on the action potential duration and on force of contraction were obtained for 3 different Ca^{++} concentrations (fig. 1). The control values depended on $[Ca^{++}]_e$ as expected[10]. Over the whole concentration range studied, ATX II was more effective in prolonging the action potential duration at 0.9 and 1.8 mM than at 3.6 mM $[Ca^{++}]_e$. Thus calcium ions seem to inhibit the electrical response to ATX II. The maximal attainable contractile augmentation, however, was similar at all levels of $[Ca^{++}]_e$ studied, but resting tension

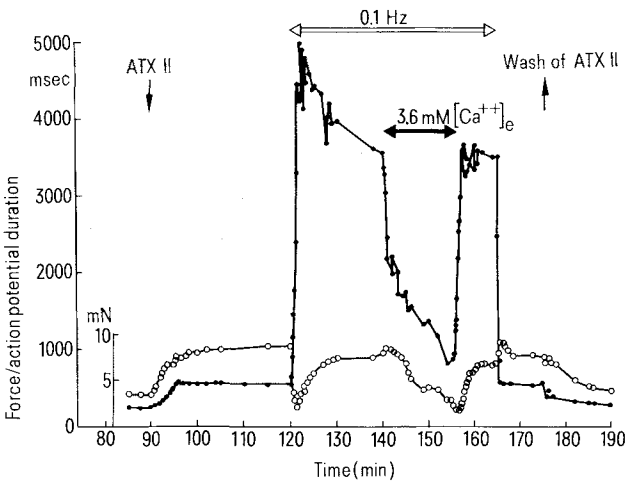

Figure 2. The influence of the elevation of the extracellular calcium concentration on the ATX II effects in a guinea-pig papillary muscle (constant microelectrode impalement throughout the experiment). Left ordinate: action potential duration at 90% of repolarization in msec (closed symbols); right ordinate: force of contraction in mN (open symbols); abscissa: time in min after preparation. The left downward arrow marks the beginning of exposure to 5×10^{-8} M ATX II at 1.8 mM $[Ca^{++}]_e$ and with 1 Hz stimulation frequency. The open horizontal arrow indicates the time period of stimulation at 0.1 Hz; the closed horizontal arrow shows the period of exposure to elevated $[Ca^{++}]_e$ (3.6 mM). The right upward arrow marks the end of exposure to ATX II.

increased with ATX II concentrations above 5×10^{-8} M at 1.8 and 3.6 mM $[Ca^{++}]_e$. The reason for this lack of $[Ca^{++}]_e$ influence on the positive inotropic effect could simply be that the direct enhancement of force of contraction by elevated $[Ca^{++}]_e$ may just balance the smaller effect of ATX II at high $[Ca^{++}]_e$. The incidence of contracture does not necessarily prove a direct increase of toxicity of ATX II at high $[Ca^{++}]_e$[8] but may indicate that the calcium sequestering processes of the muscle are closer to decompensation.

The influence of Ca^{++} on the ATX II-induced prolongation in action potential duration was also tested after the full effect had already been established (fig. 2). The stimulation frequency was lowered from 1 to 0.1 Hz because the ATX II effect on the action potential duration is more prominent at low frequencies[11], although contractile augmentation is also obscured by the direct effect of stimulation frequency on force of contraction[12]. When $[Ca^{++}]_e$ was elevated from 1.8 to 3.6 mM, the lengthened action potential shortened rapidly. The contraction amplitude showed a small transient increase which probably reflected the direct effect of raised $[Ca^{++}]_e$, but this was soon overcome by the diminished efficacy of ATX II. The reverse effects were observed when lowering $[Ca^{++}]_e$ back to 1.8 mM. Similar results were obtained in 4 additional experiments.

The Ca^{++}-dependence of the ATX II effect demonstrated here for heart muscle has also been observed in myelinated nerve fibers[13]. The mechanism of interaction between Ca^{++} and ATX II is not clear. Calcium ions have been shown to delay inactivation of the sodium current in nerve membranes, and this may involve negative surface charges associated with the inactivating gate[14]. It could be speculated that the strongly basic polypeptide ATX II requires the same negative surface charges in order to become effective in delaying sodium current inactivation and thus prolong the action potential duration.

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Is prostacyclin subserving a vasodilator effect of methoxamine involving alpha adrenoreceptors?[1]

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Summary. The influence of methoxamine on the contractile tension of isolated rat abdominal aorta, and on its capacity to produce a platelet antiaggregating substance, were explored. Methoxamine stimulated platelet antiaggregation and diminished arterial tone. The last action was blocked by phentolamine as well as by inhibitiors of cyclo-oxygenase and prostacyclinsynthetase.

Catecholamines are known to stimulate prostaglandin (PG) biosynthesis and release in a variety of animal species and tissues[3]. However, the mechanism by which catecholamines stimulate the biosynthesis of PGs is unclear. They may participate as a phenolic cofactor required for the cyclo-oxygenation of arachidonic acid[4] or alternatively could act by influencing the mechanical activity of the tissue via the stimulation of adrenoreceptors[5]. Although it is difficult to differentiate between these two possibilities, we decided to explore whether an atypical mechanical response of rat aorta to methoxamine is evoked by the activation of alpha adrenoreceptors accompanied by the stimulation of the synthesis and/or release of platelet antiaggregating material.

Methods. Abdominal aortae were obtained from male albino rats of the Wistar strain weighing between 200 and 250 g. The animals were stunned by a blow on the neck and their vessels were removed, dissected en bloc and immersed in Krebs-Ringer-Bicarbonate solution (KRB) composed as reported elsewhere[6]. Arterial strips were mounted vertically in a muscle bath containing 20 ml of KRB solution gassed with 5% of CO_2 in oxygen and kept at 37 °C and pH 7.4. The preparations, clamped between a stationary holder and an isometric force transducer, were 2 cm long. The contractile activity was recorded as previously reported[7]. After an initial preload of 1 g, the arterial strips underwent a stress relaxation and the tension stabilized after 90 min around 600 mg. The tonic changes of the vessels elicited by experimental additions were expressed in mg of tension above or under the basal resting tension or initial preload (0 mg in the figs). Cumulative dose-response curves for methoxamine (Burroughs Wellcome Co.) were constructed