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Determination of the role of na+/ca2+ exchanger system in the relaxant effect of motherwort (Leonurus turkestanicus) extract

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Abstract
The Na+/Ca^{2+} exchanger (NCX1) is critical for the regulation of contraction and relaxation in vascular smooth muscle cells, particularly in the rat aorta. Under standard conditions in Krebs physiological solution, the concentration of Na^+ ions is typically maintained at 118 mM. However, when Na^+ concentration is reduced to 25 mM or replaced with choline chloride, a marked contraction occurs, likely due to an increase in intracellular Ca^{2+} concentration facilitated by the Na^+/Ca^{2+} exchange system. In these experiments, Na+ in the Krebs solution was replaced with choline chloride to investigate this response. After removing the endothelium, an initial contraction was induced in normal Krebs solution, followed by a wash and a 30-minute incubation in Krebs solution with 1.18 mM choline chloride replacing NaCl. This modification led to a progressively increasing contraction, hypothesized to result from Ca^{2+} ion entry through the Na^+/Ca^{2+} exchanger. These findings underscore the role of Na^+/Ca^{2+} exchange in smooth muscle cell contraction by illustrating how variations in Na+ concentration can influence Ca^{2+} influx, providing insights into cellular ion exchange mechanisms and their influence on vascular tone.

Keywords: Progressively, exchange, mechanisms

Introduction

Literature indicates that the Na+/Ca^{2+} exchanger (NCX1) plays a significant role in the contraction and relaxation processes of vascular smooth muscle cells [1]. In these experiments, the concentration of Na+ ions in Krebs physiological solution is typically 118 mM in standard conditions when working with rat aorta preparations. When the Na+ concentration in the solution is reduced to 25 mM or replaced with choline chloride (or sucrose, LiCl), a contraction occurs. It is assumed that this contraction is due to an increase in the $[Ca^{2+}]$in concentration facilitated by the stoichiometric movement of Na+ ions out of the cell and Ca^{2+} ions into the cytosol via the Na+/Ca^{2+} exchange system [2].

From this perspective, experiments were conducted by replacing Na+ ions in Krebs physiological solution with choline chloride. For this purpose, the endothelium of the aorta was removed, and after an initial contraction in normal Krebs solution and a subsequent wash, the aorta preparation was incubated for 30 minutes in Krebs solution with NaCl replaced by 1.18 mM choline chloride. A gradually increasing contraction was observed (this contraction is hypothesized to be due to Ca^{2+} ion entry via the Na+/Ca^{2+} exchange system [3-4].

The research object included an extract from the local plant motherwort (Leonurus turkestanicus) and rat aorta preparations.

Materials and Methods

Experiments were conducted on aorta preparations from male white non-bred rats (200–250 g). The rats were euthanized using cervical dislocation, and the aorta was surgically extracted, placed in a specialized chamber (5 ml), and perfused with Krebs-Henseleit physiological solution (mM): NaCl 120.4; KCl 5; NaHCO3 15.5; NaH_2PO_4 1.2; MgCl2 1.2; CaCl2 2.5; C6H12O6 11.5, HEPES pH 7.4. For specific experiments, Ca2+-free Krebs solutions were used, with 1 mM EGTA added. The physiological solutions were oxygenated with carbogen (95% O2, 5% CO_2) and maintained at +37 °C with a U-8 ultrathermostat. After removing connective tissue and fat from the aorta, it was cut into 3–4 mm ring segments.

The aorta rings were attached to a Radnoti (Isometric Transducer, USA) sensor using platinum wire hooks. The rings were allowed to equilibrate for 60 minutes before an initial preload of 1 g (10 mN) was applied. The contraction force was recorded with a mechanotron, and data was processed using OriginLab OriginPro v. 8.5 SR1 (EULA, Northampton, MA 01060–4401, USA). The isometric contraction force (mN) of the rat aorta in vitro was statistically recalculated as a percentage (%) [4].

Results and Analysis

In experiments where NaCl in Krebs solution was replaced with equimolar choline chloride, the gradually increasing contraction force was 69.7±3.2%. Under conditions with verapamil (1 µM) in the incubation medium, it was 52.3±3.4% (Fig.). This contraction was considered 100%, and the observed contraction force was attributed primarily to Ca^{2+} ions entering the cytosol via the Na^+/Ca^{2+} exchange system. In these experiments, contraction was first induced with phenylephrine (1 µM), and after washing with normal Krebs solution, the muscle was exposed to choline chloride solution. During a 45-minute wash with $[Na^+]_{out}$ solution, a gradually increasing contraction developed. Upon reaching maximum contraction, the extract's relaxant effect at concentrations from 5 µg/ml to 50 µg/ml was studied, where motherwort extract at the highest dose decreased contraction force to 78±3.6% relative to control (Fig. 1).

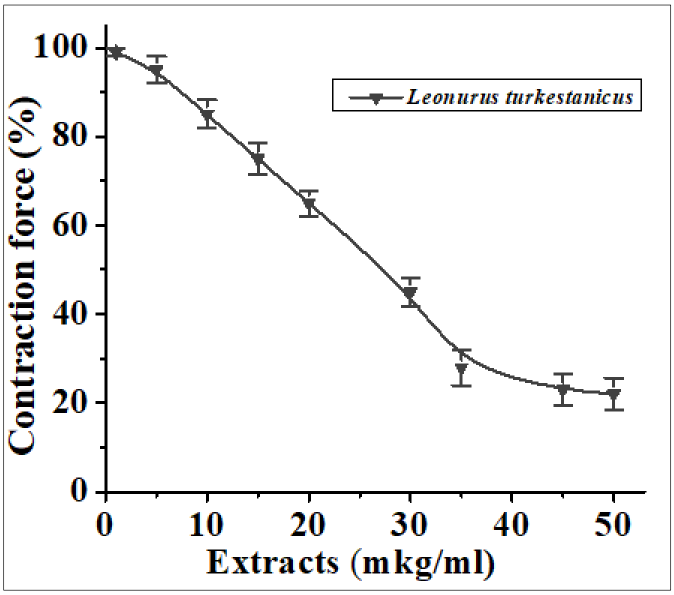

Fig 1: Effect of motherwort extract on rat aorta contraction induced by modification with Na^+-free Krebs solution

The dose-dependent effect of motherwort extract on the contraction force of rat aorta induced by modification with a Na^+-free Krebs solution. On the ordinate axis, the contraction force of the aorta induced in a Na^+-free solution is taken as 100%. On the abscissa axis, the extract concentration is shown (all cases are statistically significant with $^*p<0.05$, $^{**}p<0.01$; n=5-6).

Based on the obtained results, it can be assumed that the muscle relaxation caused by the tested extract occurs due to the blockade of Ca^{2+} transport via the Na^+/Ca^{2+} exchange system. To further clarify the role of the Na^+/Ca^{2+} exchange system, the involvement of Na^+/K^+-ATPase was studied. Literature indicates that Na^+/K^+-ATPase, an enzyme in animal tissue cell membranes, selectively removes sodium ions from cells and accumulates potassium ions inside the cell, utilizing ATP energy [5-6]. Thus, pathological conditions like ischemia may arise under conditions of reduced Na^+/K^+-ATPase activity, responsible for transporting Na^+ ions from the cell interior to the exterior [7]. Modulation of Na^+/K^+-ATPase activity is hypothesized to play a crucial role in the pathogenesis of hypertension [8]. Studies have noted that inhibition of Na^+/K^+-ATPase activity increases contraction strength in smooth muscle cells [9]. According to literature, ouabain binds to the α-subunit of Na^+/K^+-ATPase, inhibiting its activity [10], leading to an increase in $[Na^+]_{in}$ in vascular smooth muscle cells and, through the reversal of the Na^+/Ca^{2+} exchange system, an increase in $[Ca^{2+}]_{in}$ that induces stronger contraction [12]. When the Na^+ ion concentration in the physiological solution is reduced or replaced with choline chloride, ouabain-induced contraction in smooth muscle preparations is either not observed or is very weak. Studies show that ouabain (20 µM) inhibits Na^+/K^+-ATPase activity in the sarcolemma of smooth muscle cells, causing an increase in $[Na^+]_{in}$ in the cell cytosol. During Na^+ ion extrusion through the Na^+/Ca^{2+} exchange system, the resulting increase in $[Ca^{2+}]_{in}$ induces contraction [13-14].

Considering these findings, in our experiments, contraction of rat aortic smooth muscle preparations was induced with phenylephrine (1 µM), which was taken as 100%. After washing the aortic preparations with physiological Krebs solution, contraction was induced with ouabain (20 µM), resulting in a gradual increase in contraction, which reached 76.5±4.8% relative to that induced by phenylephrine. In the presence of verapamil (1 µM), the contraction strength was 64.9±4.1%, which was taken as 100%.

To determine the role of Na^+/K^+-ATPase in the effect of the tested extracts, their dose-dependent relaxant effect on the contraction activity of rat aortic preparations induced by ouabain (20 µM) was studied. When tested at concentrations of 5–50 µg/ml, the motherwort extract reduced the contraction force induced by ouabain by 79.8±3% relative to the control (Figure 2).

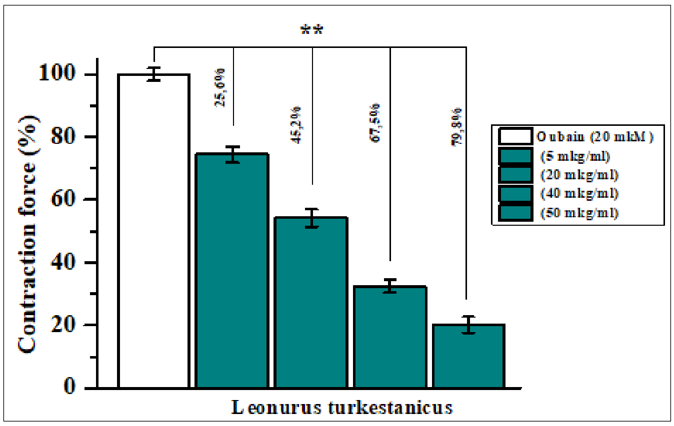

Fig 2: Effect of chamomile, hawthorn, and yarrow extracts on ouabain-induced contraction in rat aorta.

Effect of chamomile, hawthorn, yarrow, and motherwort extracts on ouabain-induced contraction of rat aorta preparations. On the ordinate axis, the contraction force of the aorta induced by 20 µM ouabain is taken as 100%. On the abscissa, the concentration of the extracts is shown (all cases are statistically significant with *p<0.05; **p<0.01; n=4-5).

According to the obtained experimental results, the reduction of the contraction caused by ouabain of the examined extract indicates the possibility of its effect on Na^+/K^{+-}ATPase. This, in turn, can be the basis for assuming that it has a significant effect on the Na^+/Ca^{2+} exchange system.

Conclusion

In conclusion, the findings of this study highlight the potential role of *Leonurus turkestanicus* extract in modulating vascular smooth muscle contraction through their influence on Na+/K+-ATPase and Na+/Ca2+ exchange systems. The dose-dependent relaxation effects observed in response to the extracts, particularly motherwort, suggest that these extracts inhibit calcium influx through the Na+/Ca2+ exchanger by altering intracellular sodium levels. This mechanism, induced under conditions of Na+-free Krebs solution and further amplified by ouabain's Na+/K+-ATPase inhibition, highlights a complex interplay between sodium and calcium homeostasis in vascular smooth muscle contraction. Given the documented inhibition of Na+/K+-ATPase activity by ouabain and its link to increased contraction force, the relaxant effects of these extracts present a promising avenue for further investigation into natural vasorelaxants and their potential therapeutic applications in managing hypertension and vascular dysfunctions.

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