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MODULATION OF L-TYPE CA^{2+} CHANNELS IN RAT AORTIC SMOOTH MUSCLE BY N-3 POLYPHENOL

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ABSTRACT

The present study investigated the vasorelaxant activity and underlying mechanisms of N-3 polyphenol on isolated rat aortic rings. Smooth muscle contraction is largely dependent on intracellular Ca^{2+} concentration, regulated through multiple pathways including voltage-dependent Ca^{2+} channels (VDCCs). Depolarization with 50 mM KCl induced a sustained contraction mediated mainly by activation of L-type Ca^{2+} channels. N-3 polyphenol (10–50 µM) significantly inhibited KCl-induced contraction in a dose-dependent manner, producing maximal relaxation of 79.3 ± 2.6% at 50 µM with an IC_{50} value of 34 µM. In Ca^{2+}-free Krebs solution, cumulative addition of $CaCl_2$ elicited concentration-dependent contraction, which was markedly attenuated by pre-incubation with N-3 polyphenol (50 µM), indicating blockade of extracellular Ca^{2+} influx. Furthermore, pretreatment with verapamil (0.1 µM), a selective L-type Ca^{2+} channel blocker, reduced contraction by 50 ± 2.5%, and the subsequent addition of N-3 polyphenol (34 µM) further decreased tension by 18.3 ± 2.7%. These findings demonstrate that the vasorelaxant effect of N-3 polyphenol is mediated primarily through functional inhibition of L-type Ca^{2+} channels, suggesting its potential as a membrane-targeting vasodilator with possible antihypertensive properties.

KEYWORDS: N-3 polyphenol; aortic smooth muscle; vasorelaxation; KCl-induced contraction; L-type Ca^{2+} channels; verapamil; IC_{50}; antihypertensive effect.

INTRODUCTION

Arterial hypertension is one of the major global public health problems today and represents a leading cause of mortality from cardiovascular diseases.[1] Dysregulation of calcium ions (Ca^{2+}) in vascular smooth muscle cells plays a key role in the development of this pathology. In particular, voltage-dependent L-type Ca^{2+} channels are among the main determinants of vascular tone and contractile activity. Therefore, pharmacological modulation of these channels is considered one of the effective approaches against hypertension.[2]

In recent years, intriguing evidence has emerged that naturally occurring polyphenols may influence ion channel activity. Several experimental studies have demonstrated that flavonoids and other polyphenolic compounds can limit Ca^{2+} influx across smooth muscle membranes, thereby exerting vasorelaxant and antihypertensive effects.[3] However, these findings are often fragmentary, focused on certain compounds, and the underlying mechanisms remain insufficiently explained. For instance, while the effects of classic polyphenols such as quercetin and resveratrol on Ca^{2+} channels have been extensively studied, the role of other novel polyphenolic compounds is not adequately represented in the scientific literature. Moreover, while some studies suggest that polyphenols may directly block Ca^{2+} channels, others report their effects via indirect signaling pathways (NO, ROS, or GPCR-mediated mechanisms). This highlights a significant gap in current knowledge.[4]

From this perspective, investigating the effects of N-3 polyphenol on Ca^{2+} channels is important not only for elucidating the pharmacological properties of this class of compounds but also for providing a scientific basis for the development of new potential antihypertensive strategies derived from natural substances.[5]

MATERIALS AND METHODS

N-3 Polyphenol

N-3 polyphenol ($C_{11}H_{10}O_8$, Mm = 270) is a phenolcarboxylic acid derivative containing an aromatic ring, two methoxy ($-OCH_3$), and two carboxyl (-COOH) groups. Based on its chemical structure and functional groups, it can be classified as a bioactive compound belonging to the polyphenol family. Its molecule is composed of a benzene nucleus substituted with two methoxy ($-OCH_3$) and two carboxyl (-COOH) groups, which positions it within the class of polyphenolic acids (**Figure 1)**. The simultaneous presence of hydrophilic (carboxyl) and hydrophobic (aromatic and methoxy) moieties within the molecule enables its interaction with biological membranes.

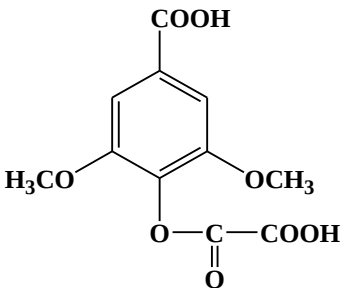

Figure 1: 2D chemical structure of N-3 polyphenol ($C_{11}H_{10}O_8$).

Chemicals

In this study, the N-3 polyphenol compound was isolated from local medicinal plants. Other chemical reagents used in the experiments — including KCl, verapamil, and others — were purchased from Sigma-Aldrich Chemie (Sigma-Aldrich, St. Louis, Missouri, USA).

Animal Ethics

All pre-surgical and experimental protocols within the framework of this study were thoroughly reviewed and approved by the Institutional Animal Ethics Committee. The experimental animals were housed under controlled vivarium conditions, with relative humidity maintained at 55–65% and temperature at 22 ± 2 °C. They had free access to drinking water and standard laboratory chow. Animal handling and care procedures were conducted in full compliance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes. Ethical approval for the study was granted by the Animal Ethics Committee of the Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan (Protocol No. 133/1a/h, August 4, 2016).

Tissue Preparation

All experimental protocols intended for this study were approved by the Institutional Committee for the Care and Use of Laboratory Animals and conducted in accordance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes [6]. All surgical procedures were performed under sodium pentobarbital anesthesia to minimize pain perception. Euthanasia was carried out by cervical dislocation, after which the thoracic aorta was excised and placed in a 5 mL organ bath containing Krebs–Henseleit solution.[7]

The composition of the Krebs–Henseleit solution was as follows: NaCl (120.4 mM), KCl (5 mM), $NaHCO_3$ (15.5 mM), NaH_2PO_4 (1.2 mM), $MgCl_2$ (1.2 mM), $CaCl_2$ (2.5 mM), glucose (11.5 mM), and HEPES (adjusted to pH 7.4). In certain experiments, 1 mM EGTA was added to the solution to prepare a Ca^{2+}-free Krebs solution.[8] The solution was maintained at a constant temperature of 37 °C and continuously aerated with carbogen gas (95% O_2 and 5% CO_2).[9] The aorta was carefully cleaned of connective tissue and fat, cut into rings of 3–4 mm in length, and prepared for physiological experiments.

Measurement of Aortic Ring Contraction

Aortic rings were mounted on platinum wire hooks and attached to a Radnoti isometric transducer, then allowed to equilibrate for 60 minutes prior to the start of measurements. Each ring was given an initial resting tension of 1 g (10 mN) (Figure 2). Contractile responses were recorded via a signal amplifier, converted into digital format, and displayed in real time on a computer screen using a Go-Link data acquisition interface.[10]

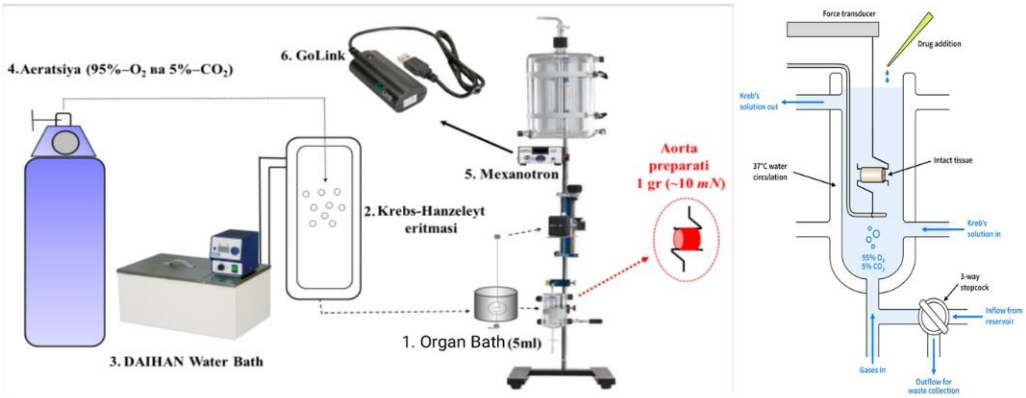

Figure 2: Schematic representation of the apparatus designed for controlling and measuring isometric contraction of isolated rat aortic smooth muscle. 1) The organ bath (5 mL) circulates solution via a specialized reservoir, 2) Krebs-Henseleit solution maintains physiological conditions, 3) A thermostat ensures constant physiological temperature, 4) The system is oxygenated with a gas mixture of 95% O_2 and 5% CO_2. The

contractile activity of the aortic preparation is maintained within the experimental chamber, 5) An isometric transducer (Grass Instrument, USA) records the contractions, 6) GoLink devices amplify and support signal acquisition.

Data Analysis

Collected data were processed and analyzed using OriginPro v9.0 SR1 software (OriginLab Corp., Northampton, MA, USA). The isometric tension values recorded under in vitro conditions were normalized and expressed as a percentage relative to the maximum contractile force (mN).[11]

RESULTS AND DISCUSSION

Contraction of smooth muscle (SM) is dependent on the intracellular concentration of Ca^{2+} ions. The intracellular Ca^{2+} level is regulated through various mechanisms. According to the literature, exposure to 50 mM KCl induces contraction of aortic smooth muscle mainly via the activation of voltage-dependent Ca^{2+} channels. Under such conditions, an increase in extracellular K^+ ions alters the membrane potential, leading to depolarization. As a result, voltage-dependent Ca^{2+} channels open, enhancing the influx of calcium ions into the cells. The subsequent rise in cytoplasmic Ca^{2+} concentration increases the contractile force of smooth muscle tissue.[12]

In the initial in vitro experiments, the vasorelaxant activity of N-3 polyphenol was evaluated in a depolarization-induced contraction model, using 50 mM KCl to elicit smooth muscle contraction. The compound was applied at concentrations ranging from 10 to 50 µM, and its dose-dependent relaxant effect was assessed. The results demonstrated that N-3 polyphenol inhibited KCl-induced smooth muscle contractility in a concentration-dependent manner. Specifically, at 10 µM it reduced contractile force by 3.2 ± 2.5% compared to the control, while at 50 µM it produced maximal relaxation, decreasing contractile force by 79.3 ± 2.6%. Based on these findings, the IC_{50} value of N-3 polyphenol was calculated as 34 µM (Figure 3).

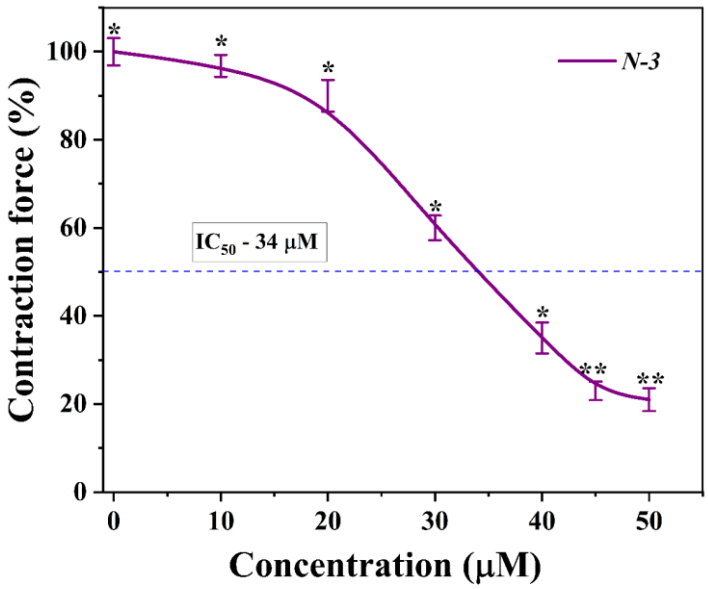

Figure 3: Effect of N-3 polyphenol on the contraction of rat aortic smooth muscle preparations induced by KCl (50 mM). The ordinate axis shows the contraction force of the aortic preparations induced by KCl (50 mM), taken as 100%, while the abscissa axis represents the concentration of N-3 (µM). In all cases, *p<0.05, p<0.01; n = 5–6.

These results indicate that N-3 polyphenol possesses the ability to attenuate KCl-induced depolarization-mediated contraction, suggesting that the underlying mechanism may involve functional blockade of L-type Ca^{2+} channels. To further investigate the vasorelaxant mechanism of N-3 polyphenol, its effect on L-type Ca^{2+} channels was evaluated using a functional assay under Ca^{2+}-free conditions [13]. In this experiment, aortic smooth muscle preparations were first incubated in Ca^{2+}-free Krebs solution containing 50 mM KCl. Subsequently, to induce muscle contraction, $CaCl_2$ was cumulatively added to the bath solution (from 0 to 2.5 mM) [14]. In the control group, the stepwise increase in $CaCl_2$ concentration led to a gradual enhancement of contraction force in the aortic smooth muscle, attributable to Ca^{2+} influx through depolarization-activated L-type Ca^{2+} channels. However, in the presence of N-3 polyphenol, this contractile response was markedly reduced (Figure 4). Specifically, in preparations preincubated with N-3 polyphenol (50 µM), the contraction amplitude in response to $CaCl_2$ was significantly lower compared with the control, indicating its Ca^{2+} influx-limiting effect, most likely through blockade of L-type Ca^{2+} channels.

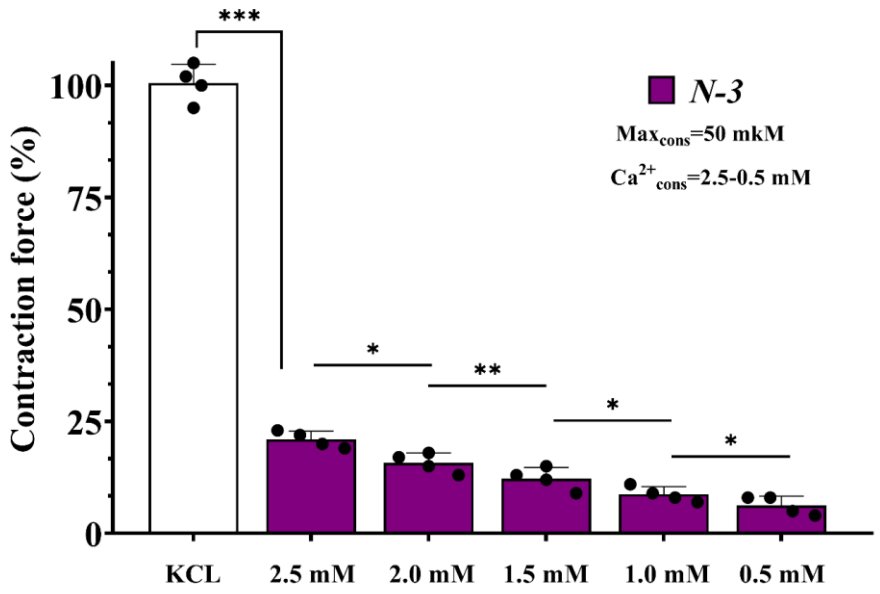

Figure 4: Effect of extracellular [Ca^{2+}] concentration on the relaxant activity of quercetin flavonoid. The ordinate axis shows the contraction force of aortic preparations induced by KCl (50 mM), taken as 100%, while the abscissa axis represents Ca^{2+} concentration (0–2.5 mM). In all cases, *p<0.05, p<0.01; n = 5–6.

These findings suggest that the vasorelaxant effect of N-3 polyphenol is mainly mediated through the blockade of L-type Ca^{2+} channels. This mechanism can be explained by the reduced influx of extracellular Ca^{2+} into aortic smooth muscle cells, leading to a decrease in intracellular [Ca^{2+}] and, consequently, attenuation of contractility.

To further clarify whether the relaxant effect of N-3 polyphenol is related to the blockade of L-type Ca^{2+} channels, additional experiments were conducted in the presence of verapamil (VP), a selective L-type Ca^{2+} channel blocker.[15] When 0.1 µM verapamil was pre-applied to aortic smooth muscle preparations, the contraction force decreased by 50 ± 2.5%, indicating a marked reduction of muscle tone through L-type Ca^{2+} channel blockade. Under these conditions, subsequent application of N-3 polyphenol at its IC_{50} concentration (34 µM) further reduced the contraction force by 18.3 ± 2.7% (Figure 5). These results demonstrate that N-3 polyphenol, similar to verapamil, induces relaxation most likely via functional inhibition of L-type Ca^{2+} channels.[16]

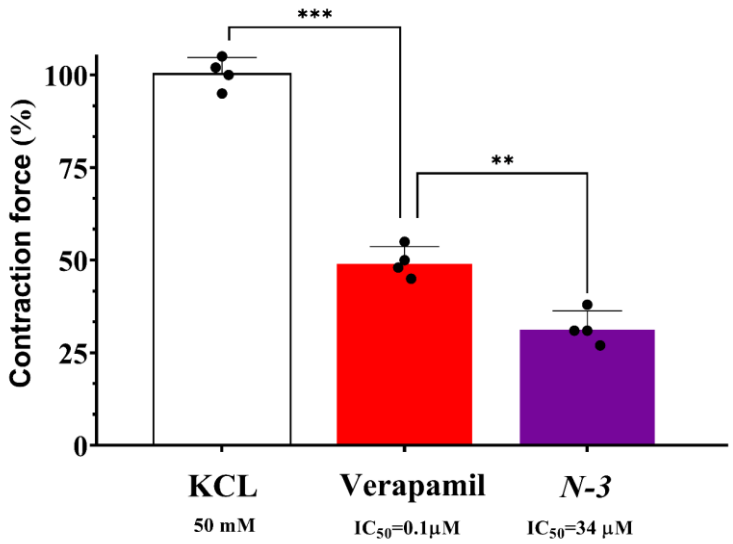

Figure 5: Combined effect of N-3 polyphenol and the Ca^{2+} channel blocker verapamil (IC_{50}) on KCl (50 mM)-induced contraction of aortic preparations. The ordinate axis represents the contraction force of aortic smooth muscle preparations induced by KCl (50 mM), taken as 100% for the control. In all cases, *p<0.05, p<0.01; n = 4–5.

The present study demonstrates that N-3 polyphenol exerts a dose-dependent relaxant effect on aortic smooth muscle. The attenuation of contraction under Ca^{2+}-free conditions with cumulative $CaCl_2$ addition, together with the further reduction of contraction in combination with verapamil, indicates that the relaxant effect of N-3 polyphenol is mediated via functional blockade of L-type Ca^{2+} channels. These findings support the evaluation of N-3 polyphenol as a potential vasodilatory compound acting through a membrane-targeted mechanism.[17]

CONCLUSION

Based on the results of the conducted in vitro studies, N-3 polyphenol exhibited a strong dose-dependent relaxant effect on aortic smooth muscle. The significant reduction in muscle contractility in the KCl-induced depolarization contraction model, along with the attenuation of contraction under Ca^{2+}-free conditions with cumulative $CaCl_2$ addition, indicates that N-3 polyphenol directly affects L-type Ca^{2+} channels. Additional experiments with verapamil further confirmed this mechanism, demonstrating that the relaxant effect of N-3 polyphenol is mediated via functional blockade of L-type Ca^{2+} channels. These findings provide a scientific basis for evaluating N-3 polyphenol as a potential vasodilator and as a bioactive compound that may exhibit antihypertensive effects in future studies.

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