https://doi.org/ 10.33472/AFJBS.6.8.2024.72-78

African Journal of Biological Sciences

DESCRIPTION OF THE INFLUENCE OF 1-O-GALLOYL-4,6-HEXAHYDROXYDIPHENOYL-*β*-D-GLUCOSE AND 1,2,6-TRI-O-GALLOY-*β*-D-GLUCOSE HYDROLYZABLE TANNINS ON THE TRANSPORT OF Ca^{2+}-IONS IN THE SARCOPLASMATIC RETICULUM

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Article History

Volume 6,Issue 8, 2024

Received:17 Feb 2024

Accepted : 28 Mar 2024

doi: 10.33472/AFJBS.6.8.2024.72-78

Abstract:

In this work, 1-O-galloyl-4,6-hexahydroxydiphenoyl-β-D-glucose (1-GT) and 1,2,6-tri-O-galloyl- β-D-glucose (2-GT) hydrolyzable tannins in rat the role of sarcoplasmic reticulum (CR) Ca^{2+}-carrying systems in the vasorelaxant effect on aortic smooth muscle was studied. The contraction activity of aortic muscle preparations was measured in vitro using a Grass FT-03 mechanotron (Grass Instrument, USA) and a signal amplifier device that record the force of muscle contraction using the mechanographic method. Endym 621.02 (Russia) was studied. In our experiments, sarcoplasmic receptors such as inositol 1,4,5-triphosphate receptor (IP_3R), ryanodine receptor (RyR) and Ca^{2+}-ATPase (SERCA) were involved in the vasorelaxant effect of 1-GT and 2-GT tannins in smooth muscle cells. It was found that it is provided by reducing the activity of the reticulum Ca^{2+}-carrying systems.

Keywords: *aortic smooth muscle, hydrolyzable tannins, vasorelaxant, ion channels, caffeine, cyclopiosic acid, receptor, concentration.*

Introduction. *Currently, in the world's leading scientific centers, extensive research is being conducted aimed at identifying and characterizing the pharmacological effects of biologically active substances extracted from plants. A large number of different alkaloids and flavonoids with pronounced relaxant effects have been isolated and characterized from various plants [1]. Many of these compounds have hypotensive potential, which is provided by complex mechanisms as a result of their pronounced relaxant activity. The mechanism of action of pharmacological drugs used in the treatment of diseases of the vascular system is directed to the modulation of the components involved in the study of the functional activity of smooth muscle cells - receptors, enzymes and ion transport systems [2]. The function of smooth muscles is a central component in the regulation of the tone of the vascular inner cavity, and in turn, arterial blood pressure, therefore, the study of the modulation of the functional activity of vascular smooth muscle cells is important for the identification of the mechanisms underlying the pathogenesis of hypertension, ischemia, stroke and many other diseases and their pharmacological correction, is important from the point of view [3].*

Materials and methods. Isometric contraction activity of rat aortic vascular smooth muscle was recorded using a standard method (mechanography) [4].

The experimental animals were euthanized by dislocation method, the chest was surgically opened, and the aorta was dissected and cleaned of fatty tissue in a Petri dish with Krebs-Henseleit saline solution. The aorta preparation was cut into rings ~3–4 mm in length and attached to a special hook attached to an experimental cell (5 ml) and on the other end to a Grass FT–03 (Grass Instrument, USA) isometric force measuring device. Isometric contraction activity of the vascular smooth muscle preparation was automatically recorded on a mechanical recording device Endim 621.02 (Czech Republic).

Krebs-Henseleit physiological solution with the following chemical composition was continuously circulated in the experimental cell (in mM): NaCl – 118; KCl - 4.8; $MgSO_4$ – 1.2; KH_2PO_3 – 1.2; $CaCl_2$– 2.5; $NaHCO_3$ – 25; glucose – 11 (pH=7.4). Also, the composition of the physiological solution was aerated with carbogen (O_2–95% and CO_2–5%), temperature stability (t=+37±0,5°C) was ensured using a thermostat. Initially, the rat aorta preparation was incubated for ~45–60 min until normal electromechanical activity was recorded as a result of applying a voltage of 1 g=9,8 mN. Incubation of the aortic blood vessel smooth muscle preparation with the contraction activity of KCl 50 mM, as well as α_1-adrenoceptor agonist - phenylephrine 1 μM or noradrenaline 1 μM was carried out and the control was defined as 100% [5]. Caffeine, cyclopiosic acid, verapamil hydrochloride, phenylephrine (Sigma-Aldrich, Germany), $NaHCO_3$, $CaCl_2$, $MgSO_4$, Glucose, NaCl, KCl, NaH_2PO_4 (Russia) reagents were used in the experiments.

The results obtained from OriginLab OriginPro v. Statistical processing was performed using a custom software package 8.5 SR1 (EULA, Northampton, MA 01060–4401, USA). The isometric contraction force (mN) of the rat aorta blood vessel preparation in vitro was calculated as a percentage (%) in statistical recalculation. The results are presented in the form of M±m of the results of experiments carried out in n=6-10 repetitions, where M is the arithmetic mean value and m is the standard error value. Also, the level of statistical reliability of the values between the results of the experiment and the control group was calculated based on the Student's t-test and was considered statistically reliable at $p<0.05$, $p<0.01$.

The obtained results and their analysis. Sarcoplasmic reticulum inositol 1,4,5-triphosphate receptor (IP_3R), ryanodine receptor (RyR) and Ca^{2+}-ATPase (SERCA) play an important role in the activity of vascular smooth muscles in normal physiological and pathological conditions. It is known that the origin or progress of many diseases is related to the disturbance of transport of Ca^{2+} ions. Ca^{2+} ions are in low concentration in the cytosol of the cellular environment, but their high concentration is additionally stored in extracellular and intracellular subcellular organelle reserves, mainly in the sarcoplasmic reticulum (CR), Golgi apparatus, lysosome, nucleus, and mitochondria [6]. Movement of calcium along concentration gradients plays an important role in various cellular processes, including muscle contraction and relaxation [7], metabolism, apoptosis, autophagy, and proliferation. The CR is the main intracellular Ca^{2+} storage organelle, with a steady-state Ca^{2+} concentration close to the extracellular concentration of approximately 1 mM, and is characterized by varying Ca^{2+} concentrations depending on the junction [8]. The release (release) of Ca^{2+} from the SR occurs mainly through IP_3R and ryanodine receptors (RyR). SERCA also plays an important role in the transport of Ca^{2+} ions in the CR. SERCA is the only active transporter that transports Ca^{2+} from the cytosol to the CR [9].

1-O-galloyl-4,6-hexahydroxydiphenoyl-β-D-glucose (1-GT) and 1,2,6-tri-O-galloyl-β-D-glucose (2-GT) in order to investigate the effect of hydrolyzable tannins on aortic muscle contractions induced by phenylephrine (FE). Under the conditions of Ca^{2+}=0, FE contraction occurs in the aortic muscle, this condition is mainly supported by Ca^{2+} ions released from CR through IP_3R [10]. FE experiment in the environment, in the state of Ca^{2+} = 0, it creates a phasic contraction, that is, in the aortic muscle, there is an initial increase in the force of contraction, and then a decrease in contraction.

In our experiments, under the condition of Ca^{2+}=0 mM, FE 1μM caused a reduction of 70,1±2,6% compared to the condition of Ca^{2+}=2,5 mM (100%). In this case, aortic muscle contraction is supported by the release of Ca^{2+} ions into the cytosol via CR IP_3R. Under these conditions, it was found that 1-GT at a concentration of 200 μM dose-dependently reduced by 28,3±1,7% compared to the control, and 2-GT at a concentration of 120 μM by 17,4±3,1% (Fig. 1 A, B).

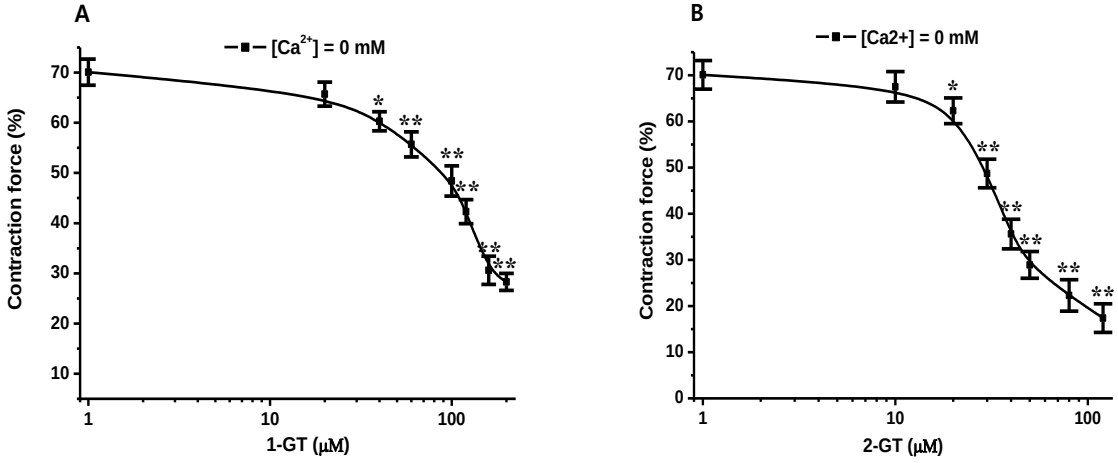

Figure 1. Dose-dependent vasorelaxant effect of hydrolyzable tannins 1-GT (A) and 2-GT (B) on FE-evoked rat aortic contraction in Krebs solution without Ca^{2+} ions. Aortic

contraction evoked by FE (1 μM) in Ca^{2+}-free Krebs solution was taken as 100% as control (in all cases confidence index *p<0.05; **p<0.01; n=8).

The obtained results indicate that the relaxant effect of 1-GT and 2-GT hydolyzable tannins is related to the blockade of CR IP_3R and is ensured by the reduction of release of Ca^{2+} ions into the cytosol through IP_3R.

In our next experiments, the effect of hydrolyzable tannins on the release of Ca^{2+} ions from the SR on aortic muscle contractions induced by caffeine was investigated. It is known from the literature that caffeine acts as an activator of RyR, causing a transient contraction, which is associated with an increase in the amount of $[Ca^{2+}]i$ in smooth muscle cells. [11;12]. Also, the contraction amplitude of smooth muscle cells induced by caffeine is an indicator of the amount of Ca^{2+} ions present in the CR.

In our experiments, caffeine at a dose of 10 mM caused a reduction of 72,1 ± 3,1% compared to the control (100%) under the conditions of Ca^{2+}=2,5 mM. Under these conditions, i.e., the dose-dependent vasorelaxant effect of 1-GT and 2-GT hydrolyzable tannins on aortic muscle contraction induced by caffeine (10 mM) was investigated. It was found that 1-GT (2-200 μM) and 2-GT (20-120 μM) doses reduced aortic contraction by 28,2 ± 2,9% and 21,4 ± 3,4% compared to the control (Fig. 2 A and B).

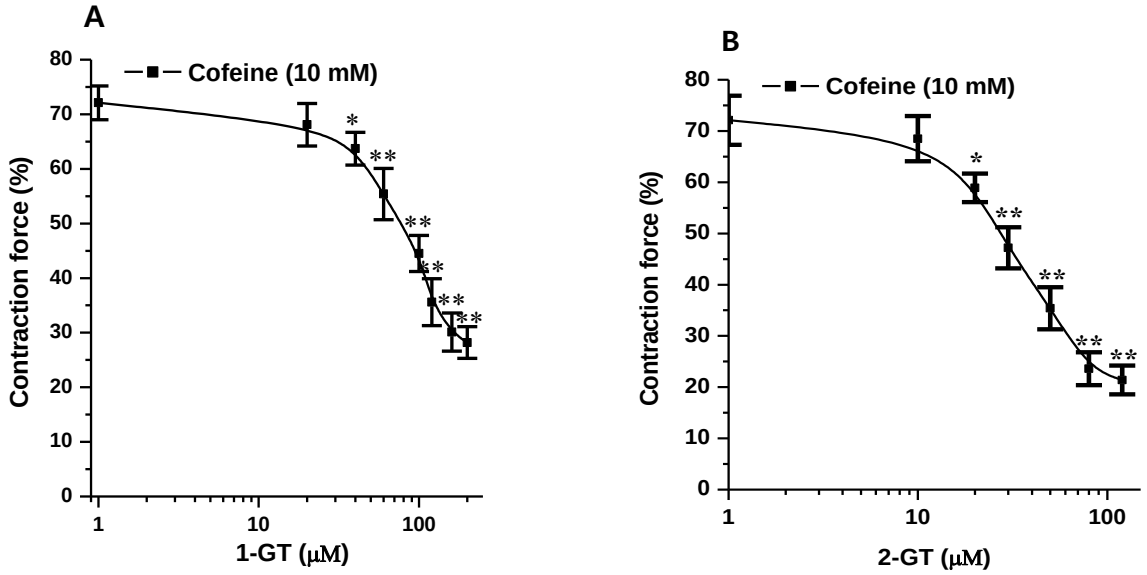

Figure 2. Dose-dependent vasorelaxant effects of 1-GT (A) and 2-GT (B) hydrolyzable tannins on caffeine-induced rat aortic muscle contraction in normal Krebs solution. Aortic contraction induced by FE (1 μM) was taken as 100% as control (in all cases confidence index *p<0.05, **p<0.01; n=7).

Also, in our next experiments, in order to clarify the mechanisms of action of hydrolyzable tannins on the aorta muscle contractions induced by caffeine, their effect was studied in the incubation medium under the conditions of Ca^{2+}=0. In this case, the aortic contraction induced by caffeine was 35,2±2,3% compared to the control without Ca^{2+}. A significant attenuation of caffeine-induced aortic muscle contractions was observed under conditions of pre-incubation of the tested hydrolyzable tannins. In the presence of 100 μM 1-

GT and 60 μM 2-GT in the medium, it was found that caffeine-induced aortic contraction was reduced by 16,1±1,9% and 10,2±1,6% compared to the control (Fig. 3 A and B).

This situation is explained by the fact that under the influence of caffeine, the outgoing Ca^{2+}-ions of CR are expelled through the function of the Ca^{2+}-transport systems located in the plasma membrane of smooth muscle cells, and the reduction of Ca^{2+}-ions in the cytosol.

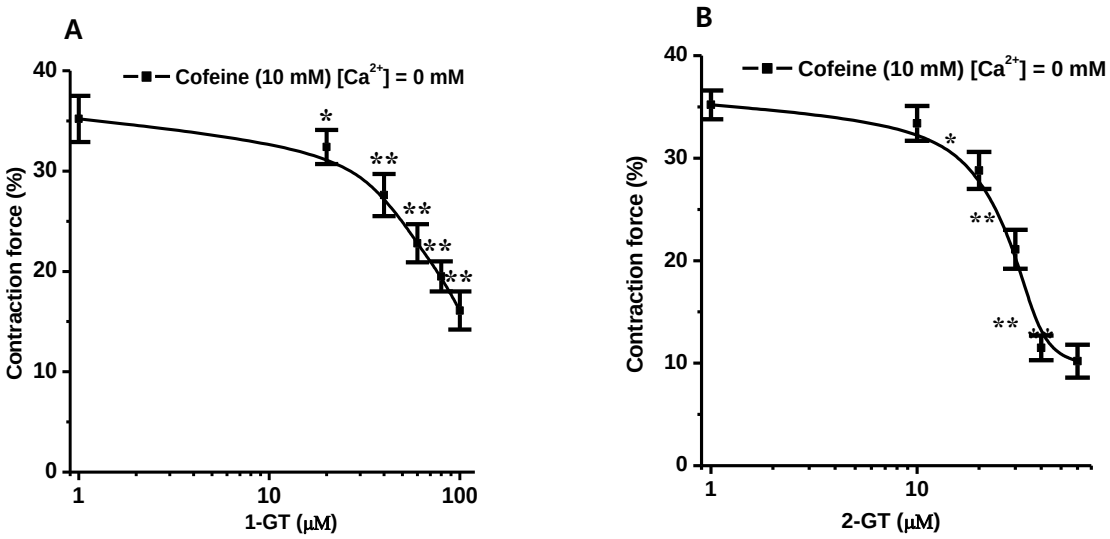

Figure 3. Dose-dependent vasorelaxant effects of 1-GT (A) and 2-GT (B) hydrolyzable tannins on caffeine-evoked rat aortic contractions in Ca^{2+}=0 Krebs solution. Aortic contraction induced by FE (1 μM) in Ca^{2+}=0 Krebs solution was taken as 100% as control (in all cases confidence index *p<0.05; **p<0.01; n=7).

These obtained results indicate the role of IP_3R and RyR receptors located in the aortic smooth muscle CR in the relaxant effect of 1-GT and 2-GT. Processes of release of CR Ca^{2+} ions are controlled by a number of factors. SERCA plays a central role in controlling the amount of Ca^{2+} ions in the sarcoplasmic reticulum [13;14]. CR SERCA plays an important role in the maintenance of Ca^{2+} homeostasis in smooth muscle cells and smooth muscle relaxation, which mainly carries out the accumulation of Ca^{2+} ions in the CR [15;16]. Therefore, in our experiments, to characterize the effect of 1-GT and 2-GT tannins on the transport of Ca^{2+} ions in the CR, the effect of cyclopiazonic acid (SPK)-induced aortic contraction was studied. At the same time, a selective blocker of CR SERCA in smooth muscles is SPK. SPK blocks SERCA and reduces the entry of Ca^{2+} ions into the CR, leading to an increase in $[Ca^{2+}]i$ in smooth muscle cells. This process takes place with the occurrence of contraction in smooth muscles [17;18].

In our experiments, we examined the vasorelaxant effect of the investigated hydrolyzable tannins on the contraction of the aortic preparation in the presence of SPK, a specific blocker of SERCA. In the presence of SPK (1-10 μM), the maximum vasorelaxant effect of 1-GT and 2-GT tannins was observed to decrease compared to the control. That is, in the experiments, it was found that the vasorelaxant effect of tannins decreased by 45,8±3,5% and 61,9±3,2% compared

to the control, along with the increase in the dose of SPK (Fig. 4. A and B)

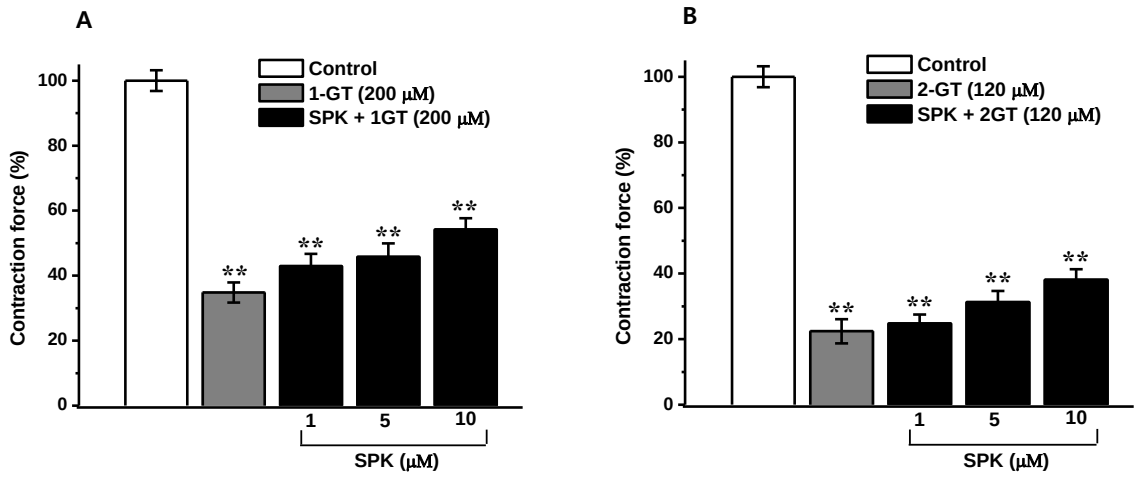

Figure 4. Effect of SPK on the vasorelaxant effect of 1-GT (A) and 2-GT (B) hydrolyzable tannins. Aortic contraction evoked by FE (1 μM) was taken as 100% as control (in all cases confidence index **p<0.01; n=6).

From these obtained results, it became clear that the vasorelaxant effect of tannins decreased as the concentration of SPK (1-10 μM) increased as a result of blocking of SERCA and inhibiting the accumulation of Ca^{2+} ions in the CR. Based on the analysis of the obtained results, it was assumed that the observed vasorelaxant effect of 1-GT and 2-GT may be related to the reduction of Ca^{2+}-ion reserve in CR.

Conclusion. Based on the analysis of the results of the experiment, we can conclude that the vasorelaxant effect of 1-GT and 2-GT is related to the blockade of CR Ca^{2+}-channel IP_3R. The above-mentioned experimental results indicate that the vasorelaxant effect of hydrolyzable tannins is related to the release of Ca^{2+} ions into the cytosol through IP_3R located in the CR membrane. Based on the analysis of the results of the study, taking into account the experiments conducted using FE in the environment Ca^{2+}=0, we can conclude that the vasorelaxant effect of 1-GT and 2-GT is related to the decrease in the release of Ca^{2+} ions from the CR. Therefore, based on the analysis of the obtained results, we concluded that the vasorelaxant effect of 1-GT and 2-GT may be related to the modification of the accumulation of Ca^{2+} ions in the CR.

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