Relaxant Effect of the Flavonoid Pulicarin

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Abstract It was established that the relaxant effect of flavonoid pulicarin in conditions of phenylephrine and KCl-induced contraction was related to the inhibition of the influx of Ca2+ ions through the receptor-operated and potential-dependent Ca2+-channels of smooth muscle cell (SMC). The relaxant effect of pulicarin was revealed to be endothelium dependent and caused by the activation of NO/guanylate cyclase system.

Keywords Flavonoid; Smooth muscle cells; Contraction activity; Membrane ion channels; Receptor

Introduction
Elucidation of the mechanisms of modulation of calcium homeostasis and cell transport systems involved in its provision remains one of the most urgent problems of modern physiology and biophysics. This is explained by the fact that calcium ions play an important role in the provision and regulation of diverse cellular processes (Cheng et al., 2006). In particular, in the smooth muscle cells (SMC) of blood vessels Ca2+ plays a leading role in the provision and regulation of contractile and functional activity in general (Karakia et al., 1997; Nilius et al., 1997; Sanders, 2001; Berridge, 2008). In these uncontrolled changes of concentration of Ca2+ in the cytoplasm of SMC in a breach of the Ca2+-transporting systems lead to significant changes in their electrical properties, the violation of their excitability and contractile activity. As a result of these deviations disorders regulation of vascular tone and the cardiovascular system as a whole, which ultimately is the main reason for pathologies such as heart disease and hypertension (Niemeyer et al., 2001; Jenitsch et al., 2004; Cheng et al., 2006)?

In this regard, the study of the mechanisms of modulation of calcium homeostasis SMC and especially pharmacological mechanisms of regulation of transport systems involved in its maintenance, is now being given special attention (Cheng et al., 2006). To successfully solution these issues particularly relevant search and characterization of new compounds that specifically modulate the different Ca2+-transporting systems of SMC. With a focus on natural compounds, such as plant flavonoids, which have a wide range of biological effects (Narayana et al., 2001; Gross, 2004; Xiaowu Dong et al., 2009), and a number of them show a pronounced hypotensive effect (Villar et al., 2004; Xiaowu Dong et al., 2009). In this context the study of flavonoids with sophisticated biophysical and electrophysiological techniques will not only study the characteristics of their action at the cellular, sub-cellular and molecular levels, but also to establish the possible mechanisms underlying their biological effects.

Objective: In view of the above mentioned, the aim of this work was to study the mechanism of the hypotensive effect of flavonoid pulicarin (Figure 1) isolated by the Institute of Plant Chemistry of plant Pulicaria gnaphalodes (Eshbakova, 2011) on the contractile activity of isolated vascular smooth muscle of rat aorta induced hyperpotassium solution and phenylephrine.
1 Materials and Methods

The experiments were performed in preparations, which are 3–4 mm wide rings isolated from aortic albino rats (200–250 g) and placed in a special chamber (5 mL) was perfused with solution Krebs Henseleit. In were work with experimental animals completely observed international principles of the Helsinki Declaration and the human treatment of animals.

In this work we used Krebs Henseleit following composition (mmol/L): NaCl 118.6 mmol/L; KCl 4.8 mmol/L; CaCl₂ 2.5 mmol/L; MgSO₄ 1.2 mmol/L; KH₂PO₄ 1.2 mmol/L; NaHCO₃ 20 mmol/L, glucose 10 mmol/L, pH 7.4. In some experiments also used the calcium free solution, which ruled from Krebs calcium ions, and linked them to be added Ethylene glycol-bis (beta-aminoethyl ether)-N, N, N', N'-tetraacetic acid (EGTA) (1 mmol/L). Karbogen oxygenated solutions (95% O₂, 5% CO₂), the temperature of the solution was maintained at 37±0.5°C with ultrathermostat U-8.

To register, the contractile activity of aortic rings suspended from one side to the fixed silver hook of the cell, and on the other – to the transducer FT–03 (Grass Instrument Co., USA), designed to measure isometric tension. Each drug was applied primary voltage corresponding to 10 mN. After a period of stabilization (60 min) induced clonus muscles with KCl (50 mmol/L) and phenylephrine (1 μmol/L) and in these conditions, all experiments performed. In the study of the role of endothelium used drugs aortic endothelial layer removed. Endothelial layer of preparation was removed mechanically of finy the help with swab. The degree of removal of the endothelium was assessed by the lack of effect of acetylcholine (1 μmol/L) on the muscle tension drugs (Gonzales et al., 2000).

Sensor signal was applied to the transducer amplifier and recorded with a recorder Endim 621.02 (Czech Republic) and the data are processed by a computer program OriginPro 7.5 (OriginLab Corporation, USA). The values of the contractile responses were expressed as percentage of the maximal response induced by phenylephrine (1 μmol/L) or KCl (50 mmol/L) and were calculated as the mean values for 4–8 different experiments (n=4–8). The significance of differences was determined deviation Student (t) for the coefficients of variation, the value of P<0.05 indicate statistically significant differences.

2 Result and Discussion

In preliminary studies, flavonoid pulicarin under normal conditions in a wide range of concentrations (3–50 mmol/L) had no effect on the tone of rat aorta preparations. These data suggest that at rest pulicarin not cause the activation of the contractile apparatus of the preparation of rat aorta. However, in further experiments, we found that pulicarin effectively relaxes rat aorta preparations, pre-cut hyperpotassium solution (50 mmol/L KCl), i.e. have a pronounced effect relaxation. In particular, it was found that the effects are dose-dependent nature pulicarin, and from a concentration of 3 mmol/L, it caused inhibition of force reductions (to 17.6% ± 4.4%, relative to controls) induced hyperpotassium environment, the extent of which increased with increasing concentration and reached a maximum at 30 mmol/L (to 97.4% ± 2.4%, relative to controls (Figure 2). Original recording of the contractile responses of aortic preparations, the arrow indicates the time of addition of KCl and pulicarin (mmol/L). Force of contraction induced by 50 mmol/L KCl, taken as 100% (P <0.01; n = 6–8).

In these conditions, the EC₅₀ (concentration causing inhibition of strength reduction of 50%) for pulicarin was 8.71 mmol/L or pD₂ (-log EC₅₀) = 5.06.

However, we have found that pre-incubation with drugs pulicarin (30 mmol/L) also results in a significant inhibition of contractile responses induced hyperpotassium solutions.
It is known that KCl-induced reduction of SMC aorta is associated with activation of potential-dependent Ca\(^{2+}\)-channels of plasma membranes of SMC. At the same time, the increase (K\(^+\)), changes the membrane potential and causes depolarization, due to it activates potential-dependent Ca\(^{2+}\)-channels, which leads to an increase (Ca\(^{2+}\)), which in turn causes a reduction of SMC (Vandier et al., 2002).

Taking this into account and analyzing the data obtained, it can be assumed that the mechanisms pulicarin effect may be due to inhibition of Ca\(^{2+}\) inflow in the cytosol of SMC, by blocking potential-dependent Ca\(^{2+}\)-channels sarcolemma.

To test this hypothesis, we performed a special series of experiments using a calcium free Krebs solution. As the results of these experiments, and in the absence of Ca\(^{2+}\) in the incubation medium pulicarin retain the ability to inhibit the contractile responses induced by KCl (50 mmol/L).

The results of these experiments show that the implementation of the relaxation effect pulicarin is important extracellular Ca\(^{2+}\), which may indicate the interaction of flavonoid with this potential-dependent Ca\(^{2+}\)-channels of plasma membranes of SMC.

Additional confirmation of this was obtained in experiments with verapamil (0.01 µmol/L), a specific blocker of potential-dependent Ca\(^{2+}\) channels, in whose presence relaxant efficiency of pulicarin significantly increased (Figure 3).

The results obtained in these experiments suggest that the relaxant effect of pulicarin in KCl-induced contraction, due to their interaction with the potential-dependent Ca\(^{2+}\)-channels of plasma membranes of SMC. As a result of this interaction, apparently, is blocking these channels, which leads to suppression of Ca\(^{2+}\) entry and reducing their concentration in the cytoplasm of SMC. In turn, the decrease in the concentration...
of Ca$^{2+}$ in the SMC is known to be a cascade of reactions leading to the inhibition of the contractile apparatus and relaxation.

Also, we investigated the effect of pulicarin on receptor-operated and store-operated Ca$^{2+}$ channels of plasma membrane, which are functionally related to the Ca$^{2+}$-transporting systems of the sarcoplasmic reticulum (SR). It is known that the contractile responses induced by phenylephrine caused SMC activated receptor IP$_3$ (Buus et al., 1998).

In preliminary experiments, it was shown that pulicarin (30 mmol/L) on the back of the aorta contraction induced by phenylephrine (1 μmol/L) and in the presence of verapamil—a specific blocker of potential-dependent Ca$^{2+}$-channels (0.01 mmol/L), caused a suppression of power cuts SMC rat aorta to (74.8±4.3)%, relative to a control (n=6, P<0.05).

In these conditions, and in blocking potential-dependent Ca$^{2+}$-channels sarcolemma verapamil contractile responses induced by phenylephrine produce revenues of Ca$^{2+}$ on receptor-operated and store-operated Ca$^{2+}$-channels of plasma membrane, which are functionally linked to Ca$^{2+}$-transport systems SR (Buus et al., 1998).

Taking this into account and analyzing the data, we can assume that the relaxant action of flavonoid pulicarin due to its effect on receptor-operated and store-operated Ca$^{2+}$-channels and the plasma membrane Ca$^{2+}$-transporting system SMC rat aorta.

To test this possibility pulicarin effects on contractile responses induced by phenylephrine were studied in media containing no Ca$^{2+}$. As the results of our experiments, pulicarin (30 mmol/L) and calcium free media retains the ability to inhibit the contractile responses induced by phenylephrine (1 μmol/L).

The results of these experiments may indicate that, in the absence of Ca$^{2+}$ ions in the incubation medium pulicarin inhibits phenylephrine-induced responses, mainly by inhibiting the release of Ca$^{2+}$ from SR.

In particular, the effect of a number of compounds relaxant realized through activation processes, providing relaxing factor synthesis in endothelial cells (Nilius and Droogmans, 2001). In this regard, it is interesting to evaluate the role of the endothelium in the implementation of the relaxant effect of pulicarin.

In preliminary experiments it was shown that the efficiency of the relaxant effect of pulicarin significantly depended on the presence of endothelium and markedly decreased in the preparations with a remote endothelium (Table 1).

These results strongly suggest that the implementation of the relaxant effect of pulicarin is important endothelial cells. Saving part relaxant effect of pulicarin on preparations remote endothelium may indicate that, in these conditions, it inhibits the entry of Ca$^{2+}$ in the SMC via localized in their plasma membrane.

In this context, and given that the action relaxant effect of pulicarin significantly depends on the endothelium, we hypothesized that the effects pulicarin can be realized through its effect on NO production by endothelial cells. To test this hypothesis, we performed experiments with an inhibitor of NO-syntase – L-NAME (100 mmol/L) and the blocker of cyclooxygenase – indomethacin (10 μmol/L).

<table>
<thead>
<tr>
<th>Condition of experiment</th>
<th>Concentration of pulicarin (μmol/L)</th>
<th>(Force of reduction in % from the control accepted for 100%)</th>
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<tbody>
<tr>
<td></td>
<td>3</td>
<td>5</td>
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<tr>
<td>In endothelium-intact rat aorta rings</td>
<td>82.4±4.4</td>
<td>74.6±3.4</td>
</tr>
<tr>
<td>In endothelium-denuded rat aorta rings</td>
<td>89.7±2.6</td>
<td>87.3±4.9</td>
</tr>
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Note: Tension was measured and calculated as a percentage of the contraction in response to KCl (50 mmol/L). Each point represents the mean ±SEM of 4-8 experiments (n=4~8, P<0.05)
As the experiments in denudat endothelium conditions and, in front of a medium NO-synthase inhibitor – L-NAME (100 mmol/L) and the blocker of cyclooxygenase – indomethacin (10 mmol/L) in the intact endothelium hypotensive effect of pulicarin much decreased. The results of these experiments suggest that the effect relaxant of pulicarin endothelium-dependent and may be mediated by its interaction with the NO-synthase and its activation.

The amplification of the synthesis of NO and its diffusion in the SMC should lead to activation guanylyl-ciclase system and increase production of cyclic guanosine monophosphate (cGMP). Increasing the concentration of cGMP in the SMC, in turn, triggers a cascade of reactions leading to a decrease in intracellular Ca\(^{2+}\) concentration and relaxation.

3 Conclusions

Overall, on the basis of the data we can conclude that the action relaxant effect of pulicarin mainly sold by activating NO/cGMP cascade (in phenilefrin-induced contractures) and block potential- dependent Ca\(^{2+}\)-channels (in KCl-induced contraction). These data may serve as a basis for further detailed pharmacological mechanism of action of this compound.

References


