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Effects Of F-18 On KCL And Phenylephrine - Induced Contractions Of Rat Aorta

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ABSTRACT

The mechanism of action of the alkaloid 1-(2´-bromine-4´,5´-dimethoxyphenyl) - 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (F-18) on the functional activity of smooth muscle cells of the rat aorta was studied. Isometric contraction activity was recorded using a Grass FT – 03 (Grass Instrument, USA) mechanotron. The relaxant effect of the F-18 alkaloid was found to be associated with blockade of Ca^{2+} (IP_3R) channels in the SR, along with voltage- dependent and receptor-operated Ca^{2+}channels in the aorta smooth muscle cell plasmalemma.

KEYWORDS

Rat aorta, vascular smooth muscle cells, vasorelaxation, Ca2+-channel.

INTRODUCTION

Today, cardiovascular diseases, which occupy a leading position in overall background of morbidity and mortality in the world population, is one of the most pressing problems of modern cardiology [1]. According to the data, the development of cardiovascular disease is based on a complex of common pathophysiological factors, among which arterial hypertension plays a leading role [7,17]. Several pathophysiological processes are involved in the development of arterial hypertension, which leads to an increase in their tone as a result of impaired contractile function of smooth muscle cells of

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blood vessels [3,10]. Many drugs have now been developed to prevent and treat hypertension, but most of these drugs are ineffective in terms of blood pressure management and have toxic effects on patients [3,13]. Therefore, the creation of a new generation of effective drugs for the prevention and treatment of diseases of the cardiovascular system remains one of the most important scientific tasks of modern pharmacology and medicine [2,5].

MATERIALS AND METHODS

Animals and aortic rings preparation. Male albino rats weighing 200-250 g were anesthetized with sodium pentobarbital and killed by decapitation. All animal care and experimental procedures were approved by the Committee for Animal Experiments of Institute of Biophysics and Biochemistry at the National University of Uzbekistan. The thoracic aorta was quickly removed, cleaned of adherent connective tissues and cut into rings 3–4 mm in length. The aortic rings were mounted in a 5 ml organ bath contained Krebs solution of the following composition (mM): NaCl 118, KCl 4.75, $NaHCO_3$ 25, $MgSO_4$ 1.2, $CaCl_2$ 1.8, KH_2PO_4 1.2 and glucose 11.1, pH 7.4. Krebs solution was maintained at 37°C and bubbled with 95% O_2-5% CO_2 gas mixture. Aortic rings were mounted by means of two stainless steel wire hooks inserted through the lumen of the ring.

Vascular tension recording. The changes in isometric tension were recorded (under a resting tension of 1g) using force-displacement transducer FT03 (Grass Instrument Co. MA, USA) connected to a force transducer amplifier and stored in a computer.

All experiments were performed on endothelium intact aortic rings. Endothelial integrity was evaluated from a degree of relaxation using acetylcholine (10 μM) while under the contractive activity effect induced by phenylephrine (1 μM). Each preparation was allowed to equilibrate for at least 60 min prior to initiation of experimental procedures and during this period the incubation media were changed every 15 min.

*Experimental procedures***.** After equilibrations, the following experimental procedures were performed: At first, to evaluate the vasorelaxant activity of F-18 aortic rings were precontracted by KCl (50 mM) or phenylephrine (PE 1 μM) and then alkaloid were added cumulatively to obtain a concentration-response curve.

To investigate the roles of extracellular Ca^{2+} influx in the F-18 -induced vasorelaxation the aortic rings were exposed to Ca^{2+}-free Krebs solution for 20 min prior to pre-contraction with KCl (50 mM) and then Ca^{2+} was added cumulatively to obtain a concentration-response curve in the presence of alkaloid F-18. To clarify the role of voltage-dependent Ca^{2+} channels in the F-18 - induced vasorelaxation the aortic rings were incubated in normal Krebs solution containing verapamil (0,1 μM) for 30 min prior to precontraction with KCl (50 mM) and then alkaloid F-18 were added to evoke relaxation.

Drugs and solutions

The following drugs were used: phenylephrine, verapamil, all obtained from Sigma Ltd Co., (St. Louis, MO, USA). 1- (2′-

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bromine-4′,5′-dimethoxyphenyl) - 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (F-18) was the generous gift of Dr. Sh. Zhurakulov (Institute of the Chemistry of Plant Substances, Tashkent, Uzbekistan). All drugs were dissolved in distilled deionized water to prepare stock solutions, and verapamil was dissolved in ethanol.

Statistical analysis

Significant effects were evaluated using the Student's *t-test* and the results are presented as mean ± standard error of the mean.

RESULTS AND DISCUSSION

The dynamic changes in intracellular calcium is a critical mechanism regulating vascular smooth muscle contractility and it has been confirmed that the contraction force generated by KCl in smooth muscle cell is directly related to voltage-dependent Ca^{2+}-channel activation in the plasmalemma [4,12,14].

As preliminary studies have shown, alkaloid 1-(2′-bromine-4′,5′-dimethoxyphenyl) - 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (F-18) under normal conditions does not themselves affect the basal tone of rat aortic preparations. These data indicate that at rest, F-18 does not cause activation of the contractile apparatus of the rat aortic preparation.

However, in the experiments, rat aortic strips precontracted with KCl (50 mM) were relaxed dose-dependently by the alkaloid F-18 (Figure 1).

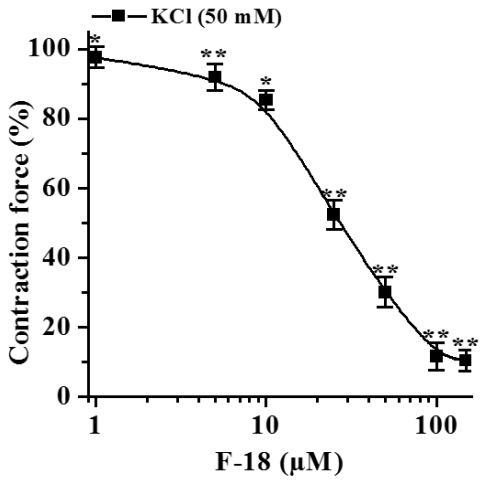

Figure 1. The concentration-dependent vasorelaxant effect of F-18 alkaloid on the contractile activity of the rat aortic rings. The force of contraction caused by the influence of KCl (50 mM) is taken as 100% (control) (reliability index in all cases * – p<0.05; ** – p<0.01; n=6)

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In particular, the alkaloid F-18 at a concentration of 5 μM was found to reduce the amplitude of aortic rings contraction activity by 8.1±3.8% compared to control, and at a concentration of 150 μM this value was 89.5±3.1%. The IC_{50} value, the concentration at which F-18 suppressed reductions by 50 from the maximum level, obtained on the basis of these results was 26.4 μM.

Considering that the development of contractile responses induced by KCl is mainly provided by the entry of Ca^{2+} ions into smooth muscle cells through voltage-dependent Ca^{2+}_{L}-type channels [5,14], these results may indicate that the observed effects of F-18 are due to its interaction with these channels.

To verify this hypothesis, the effects of F-18 on aortic contractions induced by the addition of Ca^{2+}ions to Krebs Ca^{2+}-free solutions containing KCl (50 mM) were studied.

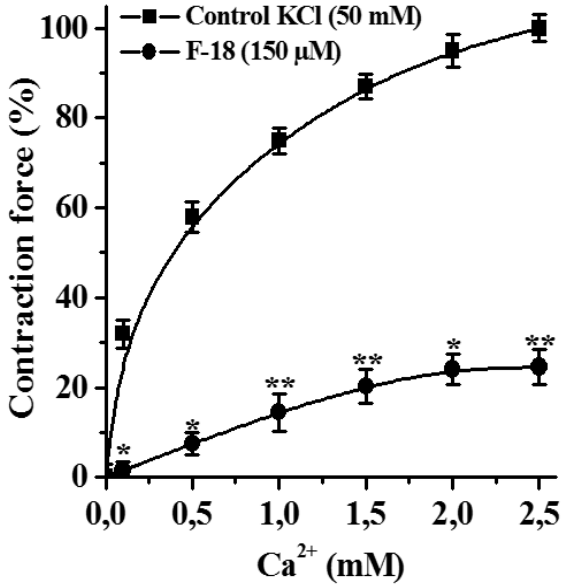

Figure 2. Effects of F-18 on the contraction of a high K^+-depolarized preparation of rat aorta. Aorta was preincubated with F-18 (150 μM) for 15 min, and then cumulative concentrations of Ca^{2+}(0.5–2.5 mM) were used to induce the contraction. The force of contraction caused by the influence of KCl (50 mM) is taken as 100% (control) (reliability index in all cases * – p<0.05; ** – p<0.01; n=5-6)

In these experiments, it was found that in the presence of alkaloid F-18, the cumulative addition of Ca^{2+} ions (0.5–2.5 mM) was accompanied by the development of contractile responses, the amplitude of which was significantly lower compared to the control obtained in a normal Krebs solution containing Ca^{2+} ions. In these conditions in the presence of F-18 (150 μM), the maximal response to $CaCl_2$ was reduced by 75.4±3.9% (Figure 2).

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Suggesting that this effect of the alkaloid is possibly mediated through the inhibition of L-type Ca^{2+}channels

To clarify this further, the effect of F-18 on the concentration-response curve to $CaCl_2$ was compared with those of verapamil, an L-type voltage-gated Ca^{2+} channel blocker.

In these experiments, it was found that in the presence of verapamil (0,1 μM) (concentration corresponding to its IC_{50} value), the addition of F-18 (IC_{50} = 26.4 μM) led to further relaxation of rat aortic preparations by 18.3±4.1% (Figure 3).

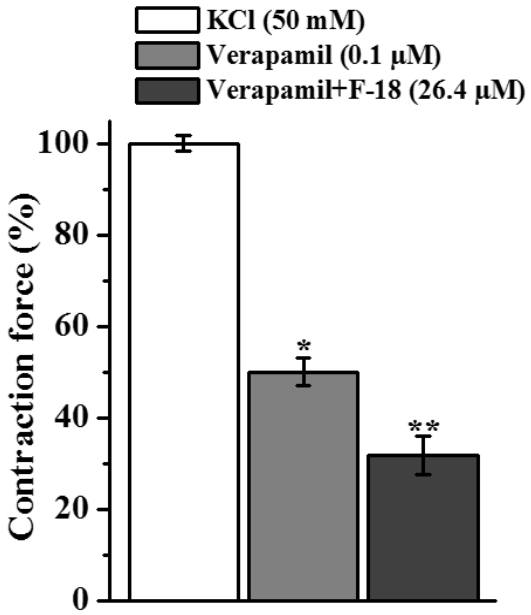

Figure 3. Relaxant effect of the blocker of voltage -dependent Ca^{2+} channels of verapamil (0.1 μM) on the relaxing effect of F-18 alkaloid. The force of contraction caused by the influence of KCl (50 mM) is taken as 100% (control) (reliability index in all cases * – p <0.05; **– p <0.01; n = 5)

In this position , to further characterize the mechanism of the relaxant action of F-18, its effects on contractions of rat aortic preparations induced by phenylephrine, an α_1-adrenergic agonist, which are mainly provided by Ca^{2+} ions entering through plasmalemmal receptor–operated Ca^{2+} channels and released from the sarcoplasmic reticulum (SR), were studied [8,9].

Vasorelaxant effect of F-18 - after 1 μM phenylephrine induced steady contraction, F-18 induced a significant ($p<0.05$) concentration-dependent (5-150 μM) relaxation in phenylephrine pre-contracted

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aortic rings. The maximal relaxation responses in phenylephrine contracted rings were 78.2±3.2% and the IC_{50} was 39.4 μM (Figure 4).

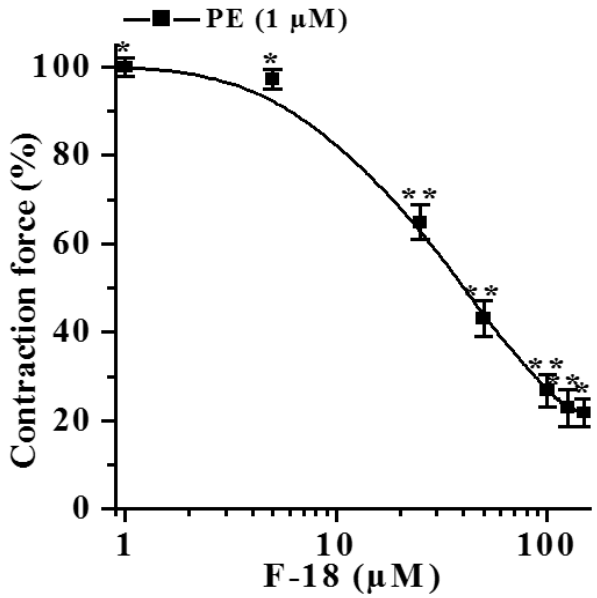

Figure 4. The concentration-dependent vasorelaxant effect of F-18 alkaloid on the contractile activity of the rat aortic rings. The force of contraction caused by the influence of phenylephrine (1 μM) is taken as 100% (control) (reliability index in all cases * – p<0.05; ** – p<0.01; n=7)

Obtained results based on that it can be hypothesized that the relaxant effect of the studied F-18 alkaloid occurs with blockade of receptor-controlled Ca^{2+}-channels. In subsequent experiments, the effect of the F-18 alkaloid on the release of Ca^{2+} ions through inositol trisphosphate receptor (IP_3R) in the sarcoplasmic reticulum was studied.

In these experiments, the reduction force caused by phenylephrine (1 μM) in the absence of Ca^{2+} ions in the incubation medium determines the process of release of Ca^{2+} ions from SR to IP_3R [15].

In our research, the shrinkage strength caused by phenylephrine (1 μM) was 66.7±4.2%

relative to normal Krebs solution conditions, and this was taken as 100% as a control. Under these conditions, when the F-18 isoquinoline alkaloid was studied at a concentration of 150 μM, it was found that the contraction force decreased by 57.2 ± 3.5% compared to the control (Figure 5).

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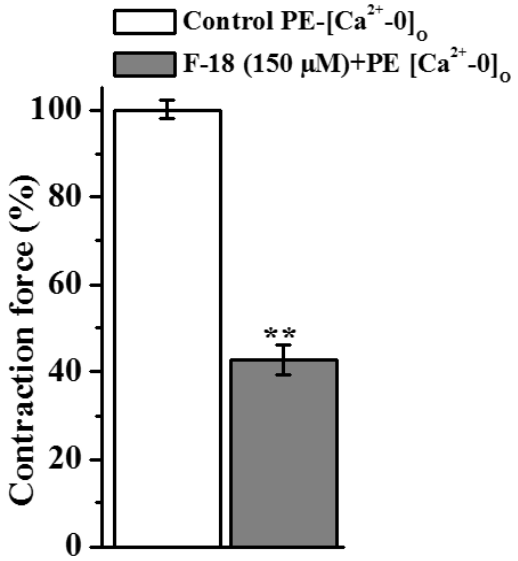

Figure 5. Effect of F-18 alkaloid on aortic smooth muscle contraction caused by phenylephrine in Krebs solution in the absence of Ca^{2+} ions. In a Krebs solution without Ca^{2+} ions, the aortic contraction force induced using PE (1 µM) was taken as control 100% (reliability index in all cases ** – p <0.01; n = 5).

The results confirm that the relaxant effect of this F-18 alkaloid on the contractile activity of aortic vascular drug in a Krebs solution environment free of Ca^{2+} ions is mainly associated with blockade of the release process of Ca^{2+} ions from intracellular stores to IP_3R.

CONCLUSION

The results of the present research clearly show that F-18 isoquinoline alkaloid induced relaxation of rat aortic smooth muscle following precontraction induced by phenylephrine or high K^+ in a concentration-dependent manner. This observation of relaxation effect of F-18 is consistent with the previous report that F-18 possessed a direct vasodilatory effect on vascular smooth muscle.

Contractions induced by phenylephrine are due partly to calcium release by intracellular stores (IP_3 receptor) and partly to the influx of extracellular calcium into the cell via receptor-operated channels following the stimulation of α_1-receptors [11,12,15]. It has also been reported that high K^+ concentrations cause marked contractions in blood vessels by depolarization of smooth muscle fibres, leading to increased influx of calcium through L-type voltage- dependent channels [5,14].

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Based on the results of the experiments and the analysis of literature data, it was suggested that the vasorelaxant effect of the alkaloid F-18 was associated with the blockade of Ca^{2+} (IP_3R) channels in the SR, along with voltage- dependent and receptor-operated Ca^{2+}channels in the aorta smooth muscle cell plasmalemma.

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