

Original Research Article

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## Vasorelaxant Effect of Heteratisine and 6-O-Benzoyl Heteratisine on Functional Activity of Rat Aorta Smooth Muscle Cells

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### ABSTRACT

#### Keywords

Heteratisine, 6-O-benzoyl heteratisine, Isolated rat aorta, Vasorelaxant effect, Ca<sup>2+</sup>L-channels, NO-synthase, NO/GC/cGMP/PKG

#### Article Info

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The effect of heteratisine (3–200 micromol/L) and 6-O-benzoylheteratisine (10–250 micromol/L) on the contractile activity of the rat aorta smooth muscle cells (SMC) was studied. Isometric tension forces were recorded using a force transducer FT-03 (Grass Instrument Co., USA). In the experiments it was revealed that vasorelaxant effect of heteratisine is mainly associated with the blockade of Ca<sup>2+</sup>L-channel, receptor-operated Ca<sup>2+</sup>-channels (ROCC), and store-operated Ca<sup>2+</sup>-channels (SOCC). Vasorelaxant effect of 6-O-benzoyl heteratisine, which differs from heteratisine with the location of the benzoyl group in C(6)-position, is associated not only with the Ca<sup>2+</sup>L-channel blockade and SOCC/ROCC and the activation of the NO/cGC/cGMP/PKG cascade reaction.

### Introduction

Biological active substances isolated from medicinal plants are considered promising therapeutic agents in official pharmaceuticals (Abbott, 2005). And currently ~25% of pharmacological drugs used in medical

practice are directly/indirectly related to plant-derived substances (Shu, 1998).

From this point of view, one of the most promising plants is *Aconitum* L. Currently, over ~300 species of *Aconitum* L. (Ranunculaceae) are identified in the global

flora, ~76 species are used for therapeutic purposes in traditional medicine and official pharmaceuticals of the East, China and the Far East countries (Nyirimigabo *et al.*, 2014).

In particular, *Aconitum* L. is used to treat cardiovascular diseases and diabetes in traditional medicine (Xiao *et al.*, 2006).

Diterpenoid alkaloids are cyclic organic compounds with N, O atoms in the chemical structure and were isolated from *Aconitum* L., originally in 1833 (Glasby, 1975).

According to the International classification, diterpenoid alkaloids isolated from such as *Aconitum* L – hypaconitin, heteratesine, heterophyllin, heterophyllidine, atidine, isotizin, hetidine, getnsinon, and benzoyl–heteratisine are allocated to groups with a skeleton (Pelletier *et al.*, 1968; Wang *et al.*, 2006).

Some researchers have described in detail the chemical classification, the wide range of pharmacological properties associated with the structure, the toxic properties of the diterpenoid alkaloids of *Aconitum* L. (Nyirimigabo *et al.*, 2014).

The purpose of this research is to study the vasorelaxant effect of the diterpenoid alkaloids heteratisine and 6-*O*-benzoyl heteratisine, isolated from the plant *Aconitum zeravshanicum* on the contractile activity of the rat aortic smooth muscle preparation (Fig. 1).

## Materials and Methods

### Preparation of the aortic vessel muscle preparation, and recording the force of contraction

In the experiments, white, outbred rats (150–200 g) were immobilized with diethyl ether,

anesthetized using cervical dislocation, and a preparation of the aortic vessel was prepared ( $l \approx 3-4$  mm) and placed in an experimental mesh (5 ml) with saline Krebs–Henseleit. The experiments fully complied with the Internationally Accepted "Rules for the Use of Animals in Biomedical Experiments" (CIOMS, 1985). The physiological solution was aerated with carbogen ( $O_2-95\%$ ,  $CO_2-5\%$ ), the temperature ( $+37 \pm 0,5^\circ C$ ) was provided with the help of the U–8 ultra-thermostat (made in Bulgaria). The force of muscle contraction in the isometric condition was registered using the FT–03 sensor (Grass Instrument Co., USA) by the standard method (Vandier *et al.*, 2002).

### Solvents and chemicals

All reagents, which were used in experiments, were of analytic-grade (NaCl, KCl,  $CaCl_2$ ,  $MgSO_4$ ,  $KH_2PO_4$ , glucose,  $NaHCO_3$ ), were obtained from "Sigma–Chemical" (St. Louis, Missouri, USA).  $Ca^{2+}_L$ -channels was inhibited by 0.01 micromol/L verapamil hydrochloride ("Sigma Aldrich"; Germany). L-NAME ( $N^0$ -nitro-L-arginine methyl ester, "Sigma–Aldrich"; Germany), a eNOS antagonist (Martinsen *et al.*, 2010), and verapamil hydrochloride, a selective  $Ca^{2+}_L$ -channels antagonist dissolved in distilled water. In the experiments, modified the physiological Krebs–Henseleit solution containing (in mM): NaCl – 118.6; KCl – 4.8;  $CaCl_2$  – 2.5;  $MgSO_4$  – 1.2;  $KH_2PO_4$  – 1.2;  $NaHCO_3$  – 20, glucose – 10 (pH=7.4) were used. This Krebs–Henseleit solution which was continuously bubbled with 95%  $O_2$  and 5%  $CO_2$  and kept at a temperature of  $+36 \pm 0.5^\circ C$  by means of water heating system controlled by temperature controller U1 (Russia), and flowed in and out of the organ bath at a rate of 3–5 ml/min with the peristaltic pump LKB Bromma (Sweden). A smooth aortic muscle contraction was caused by KCl (50 mM) and phenylephrine (1

micromol/L), an  $\alpha_1$ -adrenoreceptor agonist (Vandier *et al.*, 2002).

### Data analysis

The results were statistically processed by a special software package OriginPro v. 8.5 SR1 (EULA, USA). The results of experiments processed mathematically—statistically using standard biometric methods (Lakin, 1990).

The effects of alkaloids and verapamil hydrochloride, a selective  $\text{Ca}^{2+}_L$ -channels antagonist were compared by determining the effective concentration of the preparations ( $EC_{50}$ ) which caused a 50% relaxation (vs. the maximum KCl (50 mM)-induced contraction) of rat aorta segments, by the method of cumulative curves.

The results are given in the  $M \pm m$  form of the values of the experiments carried out in  $n$  replicates,  $M$  is the arithmetic average value and  $m$  is the standard error value. In addition, the results of the experiments, a statistically significant level of values between the groups were calculated using the Student's  $t$ -test and were evaluated as statistically reliable at  $p$  values  $<0.05$ ,  $p < 0.01$ .

## Results and Discussion

### Blockade $\text{Ca}^{2+}_L$ -channels

In the experiments, it was found that alkaloids heteratisine and 6-*O*-benzoyl heteratisine, depending on the concentration, have a vasorelaxant effect on the contraction force of the rat aortic smooth muscle preparation, caused by KCl (50 mM). At the same time, it was found that the  $EC_{50}$  (concentration reducing the reduction by 50%) for heteratisine and 6-*O*-benzoyl heteratisine was respectively 36.9 micromol/L or  $pD_2$  ( $-\log EC_{50}$ )=4.433 and 50.47 micromol/L or

$pD_2(-\log EC_{50})=4.297$  (Fig. 2A). It is known that, under the influence of KCl (50 mM), the depolarization of the plasma membrane of the muscle and, in turn, the activation of  $\text{Ca}^{2+}_L$ -channels induces a contraction (Karaki, 1997; Berridge, 2008). Some researchers suggest that the vasorelaxant effect of diterpenoid alkaloids is associated with the blockade of  $\text{Ca}^{2+}_L$ -channels (Martinsen *et al.*, 2010).

Similarly, in experiments with the  $\text{Ca}^{2+}_L$ -channel blocker – verapamil ( $EC_{50}=0.01$  micromol/L), the vasorelaxant effect is heteratisine ( $EC_{50}=36.9$  micromol/L) and 6-*O*-benzoyl heteratisine ( $EC_{50}=50.47$  micromol/L) with respect to control was respectively –  $52.6 \pm 3.5\%$  and  $62.5 \pm 4.2$  (Fig. 2B).

Based on the results of the experiments and the analysis of literature data, it was suggested that the vasorelaxant effect of the alkaloids heteratisine and 6-*O*-benzoyl heteratisine was associated with the blockade of  $\text{Ca}^{2+}_L$ -channels.

### Modulation SOCC/ROCC

In subsequent experiments, it was found that heteratisine and 6-*O*-benzoyl heteratisine have a significant vasorelaxant effect on the contraction force caused by the  $\alpha_1$ -adrenoreceptor agonist phenylephrine (1 micromol/L). It was found that the  $EC_{50}$  for heteratisine and 6-*O*-benzoyl heteratisine was, respectively, 33.1 micromol/L or  $pD_2(-\log EC_{50})=4.48$  and 33.4 micromol/L or  $pD_2(-\log EC_{50})=4.476$  (Fig. 3).

It is known that on the smooth muscle in the contractile process caused by phenylephrine (1 micromol/L) the sarcoplasmic reticulum functions  $\text{IP}_3$  receptor, receptor-operated  $\text{Ca}^{2+}$ -channels (ROCC), store-operated  $\text{Ca}^{2+}$ -channels (SOCC) (Karaki *et al.*, 1997; Buus *et al.*, 1998; Sanders, 2001; Webb, 2003;

Berridge, 2008). Based on the analysis of the data and the results of the experiment, it is assumed that ROCC and SOCC modulation is involved in the vasorelaxant effect of hetaratisine and 6-*O*-benzoyl heteratisine.

### Activation of the cascade of reaction NO/GC/cGMP/PKG

In the following experiments, the hypothetical involvement of the cascade activation of NO/GC/cGMP/PKG reactions in vasorelaxant effects of hetaratisine and 6-*O*-benzoyl heteratisine was studied.

The experiments revealed that when the endothelial layer was removed and also under conditions of incubation with the participation of the NO synthase blocker – L-NAME (100

micromol/L) vasorelaxant, the influence of the alkaloid heteratisine (200 μM) did not change significantly, but the effect of 6-*O*-benzoyl heteratisine (10–250 micromol/L) is reduced (Table 1).

Interaction of NO with the heme group of the soluble fraction of guanylate cyclase (GC) leads to activation of the enzyme and accumulation of (cGMP)<sub>in</sub>, this being followed by a decrease in the (Ca<sup>2+</sup>)<sub>in</sub> due to the blocking of Ca<sup>2+</sup><sub>L</sub>-channels SMC (Dukhanin *et al.*, 1994).

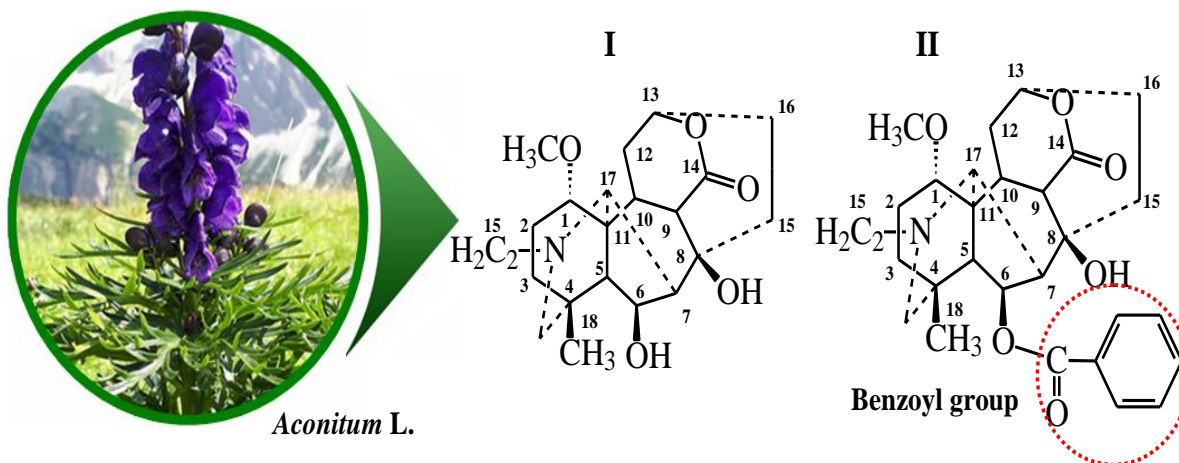
Analysis of the obtained results and literature data showed that vasorelaxant effects of hetaratisine and 6-*O*-benzoyl heteratisine are associated with the activation of the cascade reactions NO/GC/cGMP/PKG (Fig. 4).

**Table.1** Vasorelaxant effect of 6-*O*-benzoyl heteratisine under conditions of incubation of the NO-synthase blocker – L-NAME (M±m)

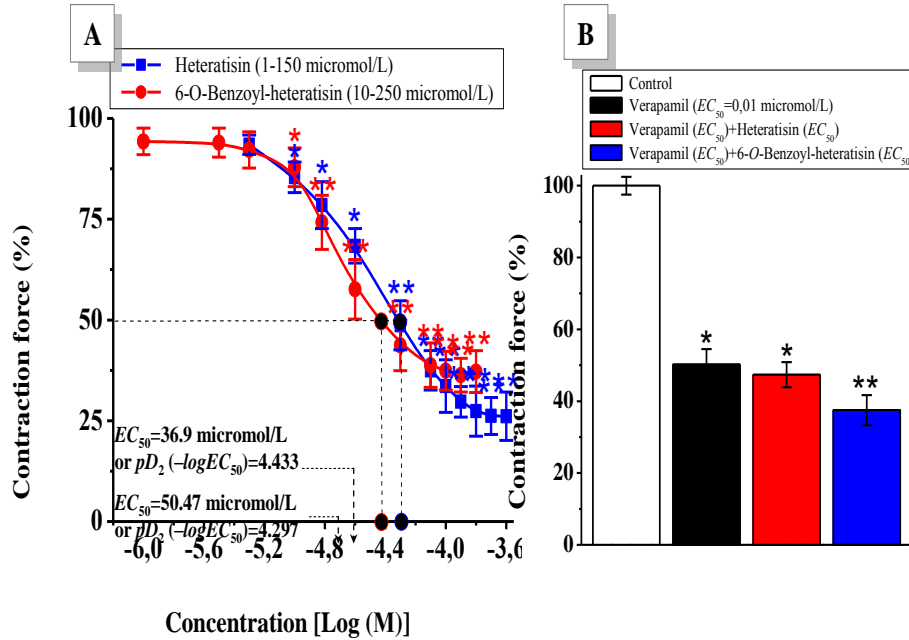
6- <i>O</i> -Benzoyl heteratisine	Experimental group	Concentration (micromol/L)				
		10	50	100	200	250
	Endothelium-intact (+E) (Control)	73,6±6,3	26,4±5,5	18,7±3,7	14,3±4,6	14,1±3,5
	Endothelium-denuded (-E)	88,5±7,3	70,2±7,6	67,3±4,7	65,9±3,8	64,8±4,2

**Note:** The force of contraction caused by the influence of phenylephrine (50 micromol/L) is taken as 100% (control) (\* – *p*<0,05 and \*\* – *p*<0,01 indicates value compared to control (*n*=4–6)).

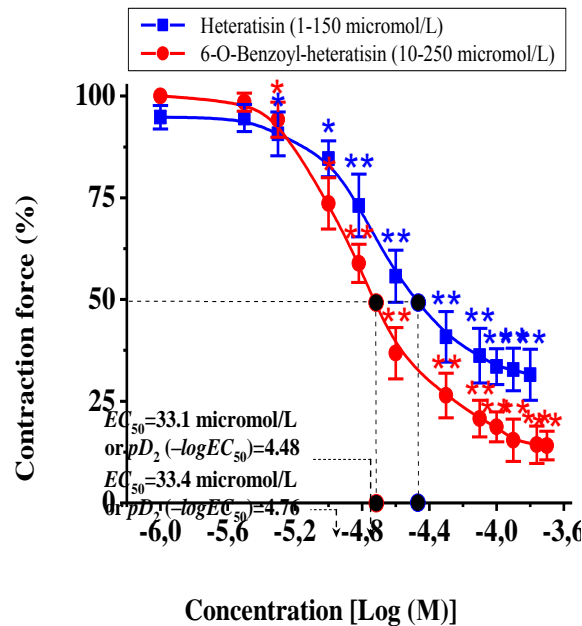
**Fig.1** Chemical structure of diterpenoid alkaloids heteratisine (I) and 6-*O*-benzoyl heteratisine (II) [Fu *et al.*, 2006; Salimov, 2017]



**Fig.2** A. Vasorelaxant effect of heteratisine and 6-O-benzoyl heteratisine on the contractile activity of the smooth muscle preparation of rat aorta. B. Effect of verapamil Ca<sup>2+</sup>-L-channel blocker on the vasorelaxant effect of heteratesine and 6-O-benzoyl heteratisine. The force of contraction caused by the influence of KCl (50 mM) is taken as 100% (control) (\* – p<0,05; \*\* – p<0,01; n=4–6)

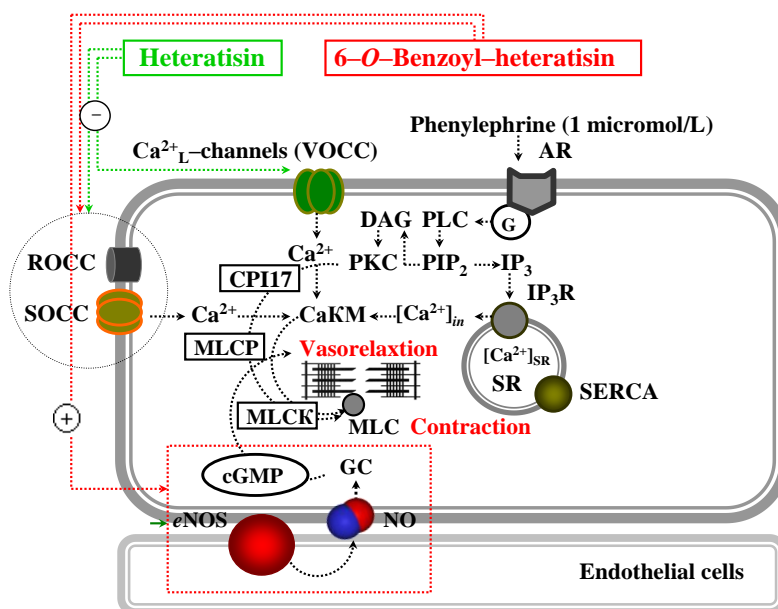


**Fig.3** Vasorelaxant effect of heteratisine and 6-O-benzoyl heteratisine on the contractile activity of the smooth muscle preparation of rat aorta. The force of contraction caused by the influence of phenylephrine (1 micromol/L) is taken as 100% (control) (\* – p<0, 05; \*\* – p<0, 01; n=4–6)





**Fig.4** Schematic illustration of the intracellular signalling pathways involved in the response of heteratisine and 6-O-benzoyl-heteratisine in the isolated rat aorta SMC. SR – sarcoplasmic reticulum; eNOS – endothelial NO-synthase; GC – guanylate cyclase; SERCA – The sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase; G – Guanine nucleotide-binding proteins; cGMP – Cyclic guanosine monophosphate; PKC – protein kinase C; AR – adrenoreceptors; SR = Sarcoplasmic reticulum



In conclusion, accordingly, diterpenoid alkaloids, heteratisine and 6-O-benzoyl heteratisine, isolated from the plant *Aconitum zeravshanicum* have a marked vasorelaxant effect. Vasorelaxant effect of heteratisine is mainly associated with the Ca<sup>2+</sup><sub>L</sub>-channel, SOCC/ROCC blockade. The vasorelaxant effect of 6-O-benzoyl heteratisine, characterized by the location of the benzoyl group in the C(6)-position, is linked by the Ca<sup>2+</sup><sub>L</sub>-channel and the SOCC/ROCC blockade, and the cascade activation by the NO/GC/cGMP/PKG. These data may serve as a basis for further detailed pharmacological mechanism of action of these compounds.

**Abbreviations used in this paper are as follows**

1. Ca<sup>2+</sup><sub>L</sub>-channels = The L-type Ca<sup>2+</sup>-channels;
2. (Ca<sup>2+</sup>)<sub>in</sub> = The intracellular concentration of Ca<sup>2+</sup> ions;

3. L-NAME = N<sup>ω</sup>-nitro-L-arginine methyl ester;
4. EC<sub>50</sub> = The values of concentration for 50% of the maximal effect;
5. SR = Sarcoplasmic reticulum;
6. GC – guanylate cyclase;
7. eNOS – endothelial NOS;
8. cGMP – Cyclic guanosine monophosphate.

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