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Original article

Imidazole-induced contractility of vascular smooth muscle cells in the presence of U-73122, ODQ, indomethacin and 7-nitroindazole

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Abstract

The aim of the study was to assess the impact of modulating factors on vascular smooth muscle cells reactivity. Vascular resistance was induced by the administration of increasing concentrations of imidazole.

The experiments were performed on isolated and perfused tail artery of Wistar rats (weight 250 g – 350 g). Rats were been narcotized by urethane (intraperitoneal injection) at a dose of 120 mg/kg, stunned and then sacrificed by cervical dislocation. In the following investigation classical pharmacometric methods were used. Relationships between concentration-response curves (CRCs) for imidazole observed in the presence of ODQ [(1H-(1,2,4)oxadiazolo-[4,3-a]quinoxalin-1-one)], 7-nitroindazole and indomethacin were analyzed.

Imidazole-induced contractility of vascular smooth muscle cells was independent from alpha-adrenergic receptors and PLC activity. Reactivity of VSMCsinduced by imidazole, was significantly changed in the presence of ODQ and 7-nitroindazole.

Key words: imidazole, rat, tail artery, EC50, ODQ, indomethacin, U-73122

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Introduction

There are many biologically active substances containing the heterocyclic imidazole ring, such as hemoglobin, myoglobin or histamine, and many pharmacological substances with different molecular mechanisms of action. There are many drugs used in pharmacotherapy which contain heterocyclic imidazole ring, such as: moksonidine or clonidine (alpha-adrenergic receptors agonists), metronidazole and tinidazole (for treatment of contagions of anaerobes and protozoans). Antifungal drugs, for example azoles, inhibit synthesis of ergosterol through the 14α-demethylation of lanosterol blockade.

Both exogenic and endogenic imidazole derivatives may exert modulating effect on the activity of the autonomic nervous system.

Imidazoline receptors were identified in the pancreas, central nervous system (CNS), kidneys and heart (El-Ayoubi et al. 2002, 2004, Narahashi 2000). Two subtypes of imidazoline receptors were found: I_1 (controls the central regulation of blood pressure) and I_2 (has neuroprotective function for cerebral ischemia (Greney et al. 2000, Nicolic et al. 2009).

Agmatine, expressed in brain, is a natural agonist of these receptors (Kozaeva and Korobov 2003). The agmatine binds two types of imidazoline receptors in the same place as clonidine. Recently published results suggest that agmatine may be an endogenic substrate for NO synthesis.

The concentration of receptors (I_2) can be a useful diagnostic marker in differentiation gliomas from differentneoplasmic brain tumors. Recent studies support the hypothesis that the increased expression of glycoprotein subtype I_2 in glial tumors is 1.4 times higher than that found in anaplastic astrocytomas (Callado et al. 2004, 2006).

Mechanisms of signal transmission through imidazoline receptors (I_1) are closely related to presynaptic alpha-2-adrenergic receptors, but molecular interaction between glycoproteins has not been explained so far. Both types are related to the cascade of signals transmission dependent on cAMP, however the imidazoline receptor can possess more than one activation pathway (Chen et al. 2003). Imidazoline receptors (I_1) and alpha-2-adrenergic receptors coexist in many types of cells and they bind the same numerous imidazoline ligands (Greney et al. 2000, Chen et al. 2003). It has been found that imidazole has an impact on the contractility of bronchi smooth muscle cells and enhances alpha-adrenomimetic action. Imidazole applied in vitro, contracts smooth muscle cells and inhibits cyclic nucleotides in the active transport of natrium ions (Na^+) through cellular membranes. Imidazole also possesses antithrombotic properties.

The imidazole mechanism of action on the nervous-muscular transmission has not been explained so far. In the proposed mechanism, the impact on the acetylocholinesterase (AChE) activity shoud be taken into consideration. However, it is known that only higher concetrations of imidazole (>10-4M/l) may trigger the AChE activation. Moreover, imidazoline derivatives inhibit synthesis of guanosine 5'-monophosphate (IMP dehydrogenase inhibition), (Nielsen et al. 2000). Thus imidazoline derivatives should be taken into consideration as antyproliferation factors in neoplasmic therapy.

The aim of the presented study was to assess the impact of modulating factors on vascular smooth muscle cells reactivity. Vascular resistance was induced by the administration of increasing concentrations of imidazole.

Our examination has been divided into two phases. In the first part of the investigation, the impact of imidazole on vascular smooth muscle cells (VSMC) contractility and nitric oxide (NO) synthesis were examined. In the second part of the experiment, the impact of guanylate cyclase (CG), phospholipase C (PLC), prostaglandins and adrenergic receptors were examined.

Material and Methods

Animals

Experiments were performed on the isolated and perfused tail artery of Wistar rats (weight 250 g to 350 g). The animals were housed under a 12h light/12h dark cycle and had unlimited access to food and water. The rats were narcotized by urethane (intraperitoneal injection) at a dose of 120 mg/kg, stunned and then sacrificed by cervical dislocation. The study protocol was approved by the Local Ethics Committee (No. 27/2010). All investigations were carried out in accordance with the United States NIH guidelines [Guide for the Care and Use of Laboratory Animals (1985), DHEW Publication No. (NIH) 85-23: Office of Science and Health Reports, DRR/NIH, Bethesda, MD, U.S.A.] (Grześk et al. 2012).

Drugs and solutions

The preparates were placed in a 20 ml container filled with oxygenated Krebs solution: NaCl 118, KCl 5.9, $CaCl_2$ 1.5, $MgSO_4$ 0.72, $NaHCO_3$ 25, glucose 11.7 mM (95% O_2, 5% CO_2, pH 7.4). In the experiment imidazole, ODQ – 1H-(1,2,4) oxadiazolo-[4,3-a] quinoxalin-1-one;U-73122-1-(6-[([17β]-3-methoxyestr

Table 1. The influence of alpha-adrenergic receptor antagonists on the blood pressure caused by imidazole.

	N	EC_{50} (M/L)	BP [mmHg]
Imidazole	14	$2.10x10^{-6}$ (±0.95) –	49.2 (±7.1)
Imidazole + prazosin 10^{-7} M/l	12	$2.05x10^{-6}$ (±1.26) (ns)	53.1 (±6.2)
Imidazole + phentolamine 10^{-6}M/l	10	$1.90x10^{-6}$ (±1,15) (ns)	51.4 (±5,8)
Imidazole + yohimbine 10^{-7} M/l	14	$1,84x10^{-6}$ (±1.22) (ns)	49 (±6.2)
Imidazole + idazoxan 10^{-7} M/l	12	$1.56x10^{-6}$ (±1.12) (ns)	50 (±5.3)

a-1,3,5[10]-trien-17-yl)-amino]hexyl)-1H-pyrrole-2,5-dione; nitroindazole; indomethacin; phentolamine; idazoxan; yohimbine (Sigma Aldrich, Germany), prazosin (Tocris) were used.

Isometric responses were monitored on Narcotrace R-40 polygraph via a force displacement transducer (Myograph F-60 Narco Bio-System). Contractile activities were calibrated with 1.0 g standard weight. Isolated rat tail arteries were stabilised for 1 h before analysis.Concentration response curves (CRCs) were calculated according to the method described by van Rossum (1963). Using classical pharmacometric methods the relationships between concentration-response curves (CRCs) for imidazole administered with ODQ, 7-nitroindazole, indomethacin and ODQ were analyzed. The perfusion pressure was continuously measured. The perfusion solution flow was gradually increased using a peristaltic pump up to 0.25-1 ml/min.

The results were presented as means ± standard deviations. The Shapiro-Wilk test was used to determine the normal distribution of the variables investigated. The statistical analysis was performed using the Newman-Keuls test for multiple comparison of means. A two-sided difference was considered significant at $p<0.05$.

Results

Imidazole in a range of concentrations from 10^{-7} to 10^{-5} M/L increased vascular smooth muscle cells contractility. Mean value of perfusion pressure amounted to 49.2 (±7.1) mmHg. The mean value of EC_{50} for imidazole amounted to 2.10 (±0.95) $x10^{-6}$ M/L. In the presence of prazosine at the concentration of 10^{-7} M/L (n=12) the EC_{50} value amounted to 2.05 (±1.26) x 10^{-6} M/L and mean value of perfusion pressure amounted to 53.1 (±6.2) mmHg. The application of phentolamine (n=10) at the concentration of 10^{-6} M/L did not affect action of imidazole in comparison to the control value – EC_{50} 1.90 (±1.15) $x10^{-6}$ M/L, arithmetic mean of perfusion pressure was 51.4 (±5.8) mmHg. Similar results were obtained after the application of yohimbine (n=14) at the concentration of 10^{-7} M/L and idazoxan (n=12) at the concentration of 10^{-7} M/L. The obtained EC_{50} values amounted to 1.84 (±1.22) $x10^{-6}$ M/L (for yohimbine) and 1.56 (±1.12) $x10^{-6}$ M/L (for idazoxan). Mean perfusion pressure amounted to 49 (±6.2) mmHg (for yohimbine) and 50 (±5.3) mmHg (for idazoxan). The results are presented in Table 1.

In the presence of ODQ (n=14) – inhibitor of guanylate cyclase at the concentration of

10^{-6} M/L, the EC_{50} value amounted to 5.30 (±1.35) x 10^{-7} M/L. Maximum effect was increased by 7% ($p<0.05$), accordingly to the control values. Similar results were obtained after application of 7-nitroindazol (n=10) – nitric oxide synthesis inhibitor in concentration 10^{-6}M/L, the value of EC_{50} amounted to 4.38 (±1,21) $x10^{-7}$ M/L and was higher approximately 18% ($p<0.05$), accordingly to the control. Application of indomethacin (n=12) at a dose of 10^{-5} M/L and U-73122 (n=12) – inhibitor of PLC in concentration 10^{-6} M/L resulted in an increase in EC_{50} – 2.70 (±1.12) x 10^{-6} M/L (indomethacin) and 2.84 (±1.34) x 10^{-6}M/L (U-73122). Maximum effect for indomethacin and U-73122 were lower by about 5% and 3%, respectively (p=ns). The results are presented in Table 2.

Concentration /effect for imidazole, before and after application of 7-nitroindazol, ODQ and indomethacin are presented in Fig 1. 7-nitroindazole in concentration 10^{-5} M/L caused left side shift of CRC for imidazole. Mean value of EC_{50} for imidazole was 5 times lower, in comparison to the control (Fig. 1, Table 2). Increased reactivity for imidazole was observed in the presence of ODQ. ODQ application resulted in left side shift of the CRC for imidazole.

Non-selective inhibitors cyclooxygenase (COX-1 and COX-2) – indomethacin in a dose of 10^{-5}M/L did

Table 2. The influence of CG (ODQ) and NOS (7-Nitroindazol) inhibitors as well as non- selective COX-1 and COX-2 inhibitors on stimulative action of imidazole on arterial pressure in rats.

	N	EC_{50} (M/L)	E_{max} (%)
Imidazole	14	$2.10x10^{-6}$ (±0.95) –	100
Imidazole + ODQ 10^{-6}M/l	14	$5.30x10^{-7}$ (±1.35) (p<0.05)	107
Imidazole + 7-nitroindazole 10^{-6}M/l	10	$4.38x10^{-7}$ (±1.21) (p<0.05)	118
Imidazole + indomethacin 10^{-5}M/l	12	$2.70x10^{-6}$ (±1.12) (ns)	95
Imidazole + U-73122 10^{-6} M/l	12	$2.84x10^{-6}$ (±1.34) (ns)	97

not affect values of perfusion pressure caused by imidazole. U-73122 – in concentration of 10^{-6} M/L, also does not affect changed in perfusion pressure (Fig. 1, Table 2).

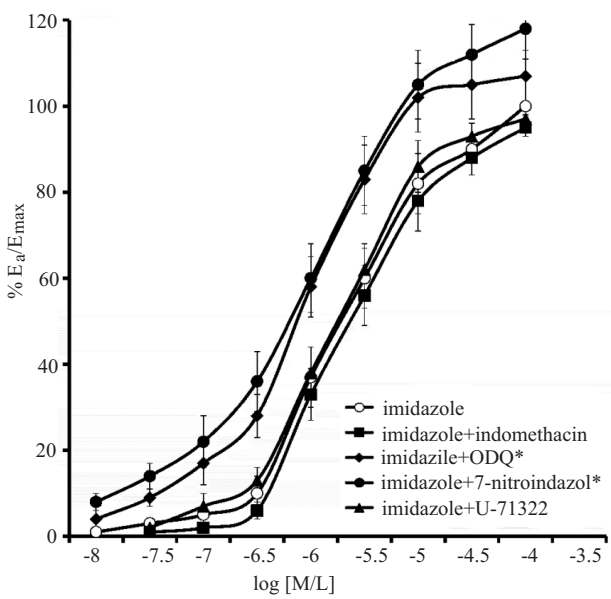

Fig. 1 Concentration Response Curves (CRCs) obtained for imidazole in the absence and presence of 7-nitroindazole, ODQ, U-73122 and indomethacin. Points and represent mean values ± standard deviations.
* – a value of p<0.05 when compared the control curve for points of effect between 20% and 80% of the maximal response.

Discussion

The present study has revealed that an increase in blood pressure triggered by imidazole is independent of the alpha-adrenergic receptors (Munroe and Caulfield 2000, El-Ayoubi et al. 2004) and does not depend on the activity of phospholipase C (PLC), what has been proved by the investigations on U-73122 – PLC inhibitor (Vogt et al. 2002, Grześk et al. 2012). This finding suggests that the mechanism of action of imidazole is independent of IP_3 synthesis.

The derivatives of imidazole and agmatine cause histamine release, but agmatine mechanism of action has not been explained so far. However, the role of imidazoline receptors in CNS, but also in PNS has not been revealed so far (Garcia-Sevilla et al. 1999, El-Mas et al. 2009).

Independently of the impact of imidazole on metabotropic receptors, there is also necessity to analyse imidazole influence on intra- and extracellular Ca^{2+} pool (Suzuki et al. 1982, Grześk and Szadujkis-Szadurski 2001). Examination of biological structures which contain the imidazoline ring becames essential to explain many physiological, neurobiological and neuropharmacological processes. Endogenous and exogenous derivatives of imidazole modulate the serotoninergic transmission, which may be of essential significance to antidepressants and addictive drugs (Watts and Cohen 1992, Ansah et al. 2003).

Finally, synthesis of new agonists for imidazoline receptors require further scientific investigation.

Conclusions

1. Imidazole-induced contractility of vascular smooth muscle cells is alpha-adrenergic receptors and PLC activity independent.
2. Imidazol induced contraction is caused by functioning of the endothelial tissue and NO production.

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