

Effect of 1-acyl-5,6-dimethoxy / diethoxy-2-methylthio-benzimidazoles on APD₉₀ and isometric contraction in guinea pig atrium and aortic preparations activated by carbachol and phenylephrine

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Studies have shown that 1-acyl-5,6-diethoxy-2-methylthio-benzimidazole derivatives 3-pyridyl- (1); 2-pyridyl- (2); 4-thiazolyl- (3); 4-pyridyl- (4); methyl- (5); 2-thienyl- (6) and 1-acyl-5,6-dimethoxy-2-methylthio-benzimidazole (7) exhibit positive inotropic properties.

The goal of the study was to investigate the effects of the above-mentioned compounds in restoring a decreased isometric contraction and APD₉₀ induced by carbachol in an isolated guinea pig atrium, and to relax the aortic rings precontracted by phenylephrine.

Isometric contraction and APD₉₀ were recorded by using a force transducer and standard microelectrode technique (stimulation rate 1 Hz).

Carbachol (1 μM) caused a decrease of the isometric contraction and APD₉₀ on an average by 63.2% and 49.6%, respectively. Compounds 1–5 used in a dose-dependent fashion (10–500 μM) abolished the action of carbachol and restored the contraction force and APD₉₀ in the electrically driven atrium significantly; compound 2 reversed the APD₉₀ to the baseline. Compounds 2 and 7 antagonized the contraction of aortic rings evoked by phenylephrine (10⁻⁴ M) and caused their relaxation by 24.4% and 17% at a dose of 10⁻⁴ M and by 76.2% and 72.5% at a dose of 5 × 10⁻⁴ M in groups 2 and 7, respectively.

Conclusion. The results presented in the study have shown that some 1-acyl-5,6-dimethoxy / diethoxy-2-methylthio-benzimidazoles are able to abolish the effects of carbachol and phenylephrine on the AP duration and isometric contraction in guinea pig atrium and blood vessels. These data will contribute to the synthesis of targeted compounds with positive inotropic and blood-vessel relaxing characteristics among benzimidazole derivatives, which could be useful in clinical practice.

Key words: 1-acyl-2-methylthio-5,6-dimethoxy / diethoxybenzimidazoles, guinea pig atrium, blood vessel, phenylephrine hydrochloride, carbamylcholine chloride

INTRODUCTION

Our decision to synthesize derivatives of 1-acyl-2-alkylthio-benzimidazole as potent positive inotropic agents was induced by Bristol JA et al. (1) who summarized the basic principles of the novel agents as phosphodiesterase inhibitors. The synthesis and investigations of benzimidazole derivatives in

pursuance of those principles were fulfilled and the obtained results as a purposeful choice of such basic structure were approved.

Investigations were carried out on guinea pig papillary muscles, atrium and blood vessels of more than 45 1-acyl-2-alkylthio-benzimidazole derivatives the majority of which showed a positive inotropic effect in a dose-dependent manner, and on the basis of the obtained data the following conclusions were made: (i) derivatives of 5,6-dialkoxybenzimidazole are more active than derivatives without alkoxy substitutions; (ii) 5,6-dimethoxy / diethoxy derivatives have

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shown a higher cardiotoxic activity than their cyclic analogues – 5,6-methylenedioxy / ethylenedioxy. Besides, the positive inotropic effect of the cyclic analogues decreases by increasing the size of a cyclic compound; (iii) the inotropic activity of 1-acyl-5,6-dimethoxy-2-methylthiobenzimidazoles is equal to or greater than that of milrinone, a well-known inhibitor of the enzyme phosphodiesterase fraction III, when 1-acyl substitutes are acetyl-, propionyl-, methoxycarbonyl-, izonicotinoyl- and 2-furanoyl-. All these compounds showed the major efficiency in the papillary muscles than in the atrium; (iv) 1-acetyl-2-methylthio-5, 6-diethoxybenzimidazole is more active than its 5- or 6-monoethoxy analogues (2–5).

Pharmacological investigations showed that the positive inotropic effect of benzimidazole derivatives is sensitive to carbachol (CCh), an agonist of acetylcholine M-type receptors. Some of them accelerate the relaxation of the isometric contraction in the papillary muscles and relax the blood vessels precontracted by the K^+ -rich physiological solution, and reserpine-induced sympathectomy does not suppress this effect. The positive inotropic effect of derivatives having aliphatic substitutes in the 1st position of the benzimidazole ring was reduced by dl-propranolol, an antagonist of β -adrenergic receptors (6). So, on the basis of these investigations we have hypothesized that the positive inotropic effect of the benzimidazole derivatives depends on the Ca^{2+} getting into the myocardium via slow Ca^{2+} channels in a cyclic adenosine monophosphate (cAMP)-dependent way. The cAMP amount for the inhibition of the enzyme phosphodiesterase fraction III (PDE-III) and / or for the stimulation of the β -adrenoceptors can increase.

A specific inhibition of the PDE-III results in a stimulation of myocardial contractility and relaxation of vascular smooth muscles. Thereby, such compounds could be potentially useful in the treatment of heart failure, including weaning from the cardio-pulmonary bypass pump (7) or after prolonged atrial fibrillation when the contractile function of the atrium is temporarily impaired (8). In addition, the current experimental studies have shown that the cAMP signal transduction plays a key role in the regulation of the endothelial nitric oxide (NO) production and has a significant effect on NO-dependent coronary vasodilatation (9–11).

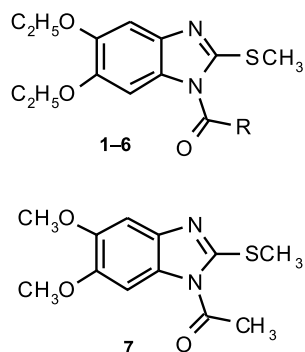


Figure. R = 3-pyridyl (1), 2-pyridyl (2), 4-thiazolyl (3), 4-pyridyl (4), methyl (5), 2-thienyl (6)

In this study, we continue the research of some 1-acyl-2-alkylthio-5,6-dimethoxy / diethoxybenzimidazoles (Figure) as possible inhibitors of the PDE-III enzymes by the same token that after a decade of the disappointment the PDE-III inhibitors are once again in demand (12).

MATERIALS AND METHODS

The experiments were carried out on the left atrium and blood vessels of 38 adult guinea pigs. Male guinea pigs were killed by a blow to the head, and their hearts with the aortic arch were rapidly removed and placed in oxygenated Tyrode's solution at room temperature. The solution had the following composition (in mM): NaCl, 144; KCl, 4; $CaCl_2 + 2H_2O$, 1.8; $MgCl_2 + 6H_2O$, 1; glucose, 5; Tris HCl, 10. The solution was gassed with 100% oxygen. This procedure was reviewed and approved by the Lithuanian State Food and Veterinary Office (Permission to use laboratory animals in research, 29-10-2005, No. 0139, personal permission No. B1-584, 12-07-2007, Vilnius). The animals were obtained from the Lithuanian Veterinary Academy, license No. B-76, 06-06-2005.

Atrium preparations. Immediately after dissection from the whole heart, the left atrium was rinsed in a 1-mL organ chamber and continuously with Tyrode's solution (pH = 7.4) at the rate 4.5 mL/min and superfused at a temperature of 37 °C. One end of the atrium was connected to a force transducer (6MX2B, Moscow, Russia) and the other was fixed to a stationary hook. The preparations were electrically stimulated at a frequency of 1.0 Hz (stimulator ES-50-1, Kursk, Russia) with mild steel electrodes using 2-ms square pulses at a voltage of 20% above the threshold. After a 1-h equilibration period, each atrium was affected by carbachol (CCh) (10^{-6} M). When both signals (contraction force and AP duration) reached their steady state in response to CCh, the above-mentioned agents in a cumulative concentration fashion were added to the solution. The antagonizing time course was recorded continuously.

Transmembrane action potential (AP) recordings were obtained with standard microelectrodes made from borosilicate glass capillaries (GC150F-10, Harvard Part No. 30-0057, England) filled with 2.5 M KCl and connected to a high-input impedance amplification system. The AP duration at a 90% repolarization (APD_{90}) was measured.

Vascular preparations. Twelve samples of the aorta arch were used in this series of experiments. The segments were dissected by cleaning them carefully from the connective tissue and cut into 3–4-mm long rings, suspended horizontally and mounted in a 1-mL chamber perfused constantly with warmed (37 °C) and oxygenated (100% O_2) Tyrode's solution. One end of a ring was attached to an isometric force transducer and the other to a tissue holder (stainless hook). The vessel rings were stretched with 1.0 g resting force and equilibrated for 1 h. After equilibration, each ring was contracted by treating it with phenylephrine hydrochloride (10^{-4} M). When the phenylephrine-induced contraction reached its

steady state, one of compounds 2 and 7 in a cumulative concentration manner was added to the solution.

Signals of the isometric tension (of atrium and blood vessels) and APs were digitized at a minimum sampling rate of 10 kHz with a 12-bit A/D converter (Digidata 1200, Keithley Instruments, Cleveland Ohio, USA) and recorded with a computer. They were preserved there and analyzed to obtain the maximal force of contraction and APD in the atrium and the contraction-relaxation amplitude in blood vessels. Each sample of atrium and vessel rings was perfused with either concentration of the test drugs for 15–20 min.

Statistical analysis

All the values are presented as means \pm SE. Statistical analysis by Student's t-test was performed for paired and unpaired observations. The level of $p < 0.05$ was adopted as a critical value of significance. The confidence intervals were considered significant at $p < 0.05$.

Drugs

All benzimidazole derivatives were synthesized at the Department of Chemistry, Vilnius University. Compounds 1–7 were obtained in good yields (58–81%) by acylation of

5,6-diethoxy- or 5,6-dimethoxy-2-methylthiobenzimidazole with corresponding acyl chlorides in anhydrous chloroform in the presence of triethylamine excess. The analytical data on the synthesized compounds are in agreement with those published in the literature (2–5).

Phenylephrine hydrochloride and carbamylcholine chloride (carbachol) were purchased from Sigma-Aldrich Chemie (Taufkirchen, Germany). Phenylephrine and carbachol were dissolved in deionized water to make a stock solution of 0.1 mM and stored at low temperature. The test compounds were dissolved in a minimum amount (0.1–0.4 ml) of dimethylsulfoxide and diluted with Tyrodé's solution. For all experiments, only fresh-made solutions were used.

RESULTS

Effects of the benzimidazole derivatives 1–6 on isometric contraction and APD₉₀ in guinea pig atrium

Application of CCh (1 μ M), an agonist of mAChR resistant to hydrolysis by cholinesterase, resulted in a rapid initial decrease of both the contraction force (on an average to 274.5 mg, range 231.0–322.6 mg, $n = 26$) and AP duration at 90% repolarization (64.7 ms, range 50.1–81.7 ms, $n = 26$) (Table). At a

Table. Effect of cumulative doses of benzimidazole derivatives 1–6 on the CCh-induced contraction force and action potential duration in electrically driven isolated guinea pig left atrium

Compound	Dose, mol/l	Contraction force, mg	Confidence interval	APD ₉₀ , ms	Confidence interval
1 Predrug (n = 4) Carbachol only + compound		390.4 \pm 76	148.5–632.23	129.7 \pm 10.0	97.88–161.52
	1 \times 10 ⁻⁴	125.0 \pm 18.8*	65.2–184.82	48.0 \pm 7.0**	7.11–88.88
	1 \times 10 ⁻⁵	165.4 \pm 33.5*	58.8–272	58.0 \pm 8.0**	11.27–104.7
	1 \times 10 ⁻⁴	192.8 \pm 50.0	33.6–352	77.0 \pm 6.7**	23.4–130.6
	5 \times 10 ⁻⁴	245.4 \pm 31.4	145.4–345	96.6 \pm 1.9*	90.55–102.64
2 Predrug (n = 5) Carbachol only + compound		413.0 \pm 52.8	266.4–559.5	117.0 \pm 8.3	90.6–143.4
	1 \times 10 ⁻⁴	154.8 \pm 45.9**	27.4–282.2	66.9 \pm 6.7***	48.3–86.5
	1 \times 10 ⁻⁵	163.0 \pm 28.4**	84.16–241.8	75.0 \pm 3.3**	65.8–84.16
	1 \times 10 ⁻⁴	195.0 \pm 25.6**	123.9–266	85.0 \pm 6.5	64.32–105.68
	5 \times 10 ⁻⁴	379.7 \pm 66.8	194.3–565.4	* 118.0 \pm 4.5	105.5–130.5
3 Predrug (n = 4) Carbachol only + compound		463.3 \pm 19.0	402.8–523.75	137.7 \pm 13.0	96.34–179.06
	1 \times 10 ⁻⁴	217.0 \pm 32.6***	113.2–320.73	78.6 \pm 9.6**	48.05–109.14
	1 \times 10 ⁻⁵	228.0 \pm 25.6***	146.6–309.4	72.0 \pm 10.0**	40.18–103.82
	1 \times 10 ⁻⁴	246.0 \pm 32.2**	143.5–348.46	85.0 \pm 6.5**	64.3–105.68
	5 \times 10 ⁻⁴	274.3 \pm 34.4**	164.8–383.76	118.0 \pm 4.5	103.6–132.3
4 Predrug (n = 5) Carbachol only + compound		503.4 \pm 43.5	382.6–624.15	155.5 \pm 5.9	139.1–171.87
	1 \times 10 ⁻⁴	178.6 \pm 37.5**	74.5–287.7	89.0 \pm 7.4***	68.45–109.54
	1 \times 10 ⁻⁵	256.4 \pm 47.7**	123.98–388.8	86.9 \pm 7.6***	65.8–108
	1 \times 10 ⁻⁴	281.0 \pm 40.2**	169.4–392.6	107.0 \pm 9.0**	82.0–131.98
	5 \times 10 ⁻⁴	449.0 \pm 51.7	305.48–592.5	137.4 \pm 5.0	123.1–150.88
5 Predrug (n = 4) Carbachol only + compound		453.8 \pm 66.3	242.8–664.7	126.0 \pm 2.8	118.2–133.8
	1 \times 10 ⁻⁴	131.2 \pm 18.0**	73.92–188.5	59.4 \pm 3.5***	49.68–69.11
	1 \times 10 ⁻⁵	144.0 \pm 8.5**	116.95–141	63.0 \pm 5.8***	46.89–47.00
	1 \times 10 ⁻⁴	185.0 \pm 17.0**	130.9–239	75.8 \pm 2.8***	68.02–68.02
	5 \times 10 ⁻⁴	284.6 \pm 56.7	103.5–464	112.9 \pm 3.8*	102.3–123.4
6 Predrug (n = 4) Carbachol only + compound		384.0 \pm 26.7	299.0–468.95	128.0 \pm 3.8	117.4–138.5
	1 \times 10 ⁻⁴	152.9 \pm 21.2***	85.44–220.35	68.0 \pm 2.7***	60.5–75.5
	1 \times 10 ⁻⁵	149.0 \pm 22.3***	78.04–219.95	67.0 \pm 3.1***	58.38–75.6
	1 \times 10 ⁻⁴	132.8 \pm 17.6***	76.79–188.8	70.0 \pm 3.3***	60.83–79.16
	5 \times 10 ⁻⁴	260.9 \pm 15.8**	210.6–311.17	96.5 \pm 2.8***	88.72–104.27

Data are expressed as mean \pm SE. Stimulation rate 1 Hz; the number in parentheses is the number of preparations. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs. the predrug value.

concentration of 10^{-5} M, none of the compounds tested significantly affected the AP duration or isometric contraction, although compounds 1 and 2 showed a slow trend to increase APD_{90} . At a concentration of 10^{-4} M, compound 1, partially but significantly, reversed the CCh-induced APD_{90} shortening (29 ms, $P < 0.05$, vs. CCh only). The other compounds did not have any effect. Meanwhile, compound 4 increased the isometric contraction gradually and more evidently. In the meantime, in response to compound 2 at a dose of 5×10^{-4} M, the CCh-depressed contraction force as well as the APD_{90} were finally restored. The other benzimidazole derivatives, except 6, showed a similar effect. Compound 7 had been studied in this aspect earlier and the data are presented in (13).

Vasorelaxant effects of benzimidazole derivatives 2 and 7

In this series of experiments, we analyzed the vasorelaxing properties of two (2 and 7) benzimidazole derivatives by using the guinea pig aorta rings precontracted with phenylephrine. Phenylephrine (10^{-4} M) significantly increased smooth muscle contraction in aortic rings. The tension was on an average 240.5 ± 15.5 mg (range: 187–275 mg) and 538.8 ± 80.8 mg (range: 318–804 mg) of the baseline in groups 2 and 7, respectively. Upon achieving the maximum response to phenylephrine, the test compounds, in a concentration-dependent fashion added to the solution at a concentration of 10^{-4} M, triggered a gradual relaxation of the vessel samples to 181.8 ± 21.5 mg (24.4%) and to 447.5 ± 101 mg (17%) in groups 2 and 7, respectively, which approached the background (54.4 ± 4.2 mg, 76.2%, and 148.4 ± 7.1 mg, 72.5 %, in groups 2 and 7, respectively) at a concentration of 5×10^{-4} M.

DISCUSSION

The physiological process of aging is associated with profound changes in the autonomic regulation of the human heart atrium. An imbalance between the sympathetic and parasympathetic nerve systems may contribute to the high incidence of sudden cardiac deaths in patients with the coronary heart disease. By the predominating parasympathetic activity, the release of the neurotransmitter acetylcholine, which binds to the muscarinic M_2 receptors, is increased. Stimulation of these receptors is associated with a shorter APD and an effective refractory period, and a loss of rate-dependent APD adaptation (14). This may be important for the initiation and perpetuation of atrial fibrillation which is associated with alterations in the ion currents activity (15–18). In the present study, we have demonstrated that the benzimidazole derivatives 1–5 are able to restore the decreased isometric contraction and APs duration induced by the muscarinic agonist CCh (1 μ M). Our earlier investigation has shown (6) that the above-mentioned benzimidazole derivatives possess positive inotropic properties as they increase significantly the contraction force of guinea pig papillary muscles in a dose-dependent fashion. The maximum of isometric contraction

in papillary muscles, as in the current case in the atrium, was achieved at a concentration of 5×10^{-4} M. According to the tests on papillary muscles with 10-fold higher doses of CCh (10 μ M) and dl-propranolol (10 μ M), a conclusion was made that in the positive inotropic action of benzimidazole derivatives the cAMP is involved (6). The cAMP amount for the inhibition of PDE-III activity can augment. On the other hand, experimental studies have shown that parasympathetic stimulation triggers the heterotrimeric G protein cascade in cardiac myocytes, resulting in activation of the mAChR gated inward rectifier potassium current $I_{K,ACh}$ which predominates in mammalian atria (19). Promotion of $I_{K,ACh}$ leads to a shortening of the late repolarization phase APD_{90} (20). According to Schorten et al. (8), stimulation of α -subunits of Gi proteins inhibits the adenylyl cyclase as well, resulting in a decreased L-type calcium current which is activated by cAMP or the cAMP-dependent protein kinase A. Taken together, these events contribute to the narrowed APs duration and reduced isometric contraction. Thus, our investigations support the idea that the presented benzimidazole derivatives under study are able to reverse the effects of carbachol in guinea pig atrium of the altering currents. So, the test compounds increase the slow Ca^{2+} current and decrease $I_{K,ACh}$. Such characteristics are typical of the PDE inhibitors which increase the intracellular cAMP content in the myocardium (21, 22). Besides, the data obtained in the second series of the experiments have shown that the test benzimidazole derivatives, whose chemical structure differs by the substitutes at positions 1, 5 and 6 of the benzimidazole ring, possess vasodilatation properties as well. Compounds 2 and 7 eliminate the vasoconstrictor action of phenylephrine at concentrations of 10^{-4} M and 5×10^{-4} M as they cause blood vessel relaxation. The vascular tone is determined by the contractile state of the vascular smooth muscle cells, which is controlled by the level of intracellular Ca^{2+} (23). Vasoconstrictors, such as phenylephrine or potassium ions of higher concentrations, act through increasing Ca^{2+} on the contractile apparatus of the vascular smooth muscle, whereas the relaxing factors have the opposite effect. Although the precise mechanism underlying the relaxation of smooth muscles is not known well enough, the growing evidence indicates that the stimulation of the cAMP signal-transduction pathway can increase the endothelial nitric oxide (NO) production from the blood vessels and cause endothelium-dependent vasodilatation (24–26). According to Zhang et al. (9), cAMP signal transduction stimulates the endothelial NO generation by inducing phosphorylation of the endothelial NO-synthase through the cAMP-dependent protein kinase A. The endothelium-released NO easily diffuses to the cells underlying the smooth muscles and triggers their relaxation by increasing the level of cyclic guanosine monophosphate and subsequently opening the endothelial potassium channels (K_{ATP} , K_{Ca}) which cause sarcolemmal membrane hyperpolarization, APD shortening and a decreasing Ca^{2+} influx through slow calcium channels (27–31). Ultimately, these events lead to vasodilatation.

In conclusion, the results presented in the study have shown that some 1-acyl-5,6-dimethoxy / diethoxy-2-methylthiobenzimidazoles are able to restore the decreased action potential duration (APD₉₀) and isometric contraction caused by carbachol in guinea pig atrium and to relax the blood vessels precontracted by phenylephrine. These data will contribute to the synthesis of targeted compounds with positive inotropic and blood-vessel relaxing characteristics benzimidazole derivatives.

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1-ACIL-2-METILTIO-5,6-DIMETOKSI / DIETOKSI-BENZIMIDAZOLŲ POVEIKIS JŪRŲ KIAULYČIŲ PRIEŠIRDŽIŲ IR AORTOS ŽIEDŲ, AKTYVUOTŲ KARBACHOLU BEI FENILEFRINU, IZOMETRINIAM SUSITRAUKIMUI IR VEIKIMO POTENCIALO TRUKMEI

S a n t r a u k a

Ankstesni tyrimai rodo, kad 1-acil-5,6-dietoksi-2-metiltiobenzimidazolai: 3-piridil- (1); 2-piridil- (2); 4-tiazolil- (3); 4-piridil- (4); metil- (5); 2-tienil- (6) ir 1-acil-2-metiltio-5,6-dimetoksi / dietoksi-benzimidazolai (7) pasižymi ryškiu teigiamu inotropiniu poveikiu, o jų veikimo mechanizmai panašūs į milrinono, fermento fosfodiesterazės trečios frakcijos inhibitoriaus, veikimo mechanizmus.

Šio darbo tikslas buvo ištirti minėtų darinių kuriamąjį poveikį jūrų kiaulyčių kairiojo prieširdžio susitraukimo jėgai bei jo veikimo potencialo trukmei (VP₉₀) ir kraujagyslių izometriniam susitraukimui, prieš tai paveikus atitinkamai karbacholu ir fenilefrinu.

Karbacholas (1 μ M) mažino 1 Hz dažniu stimuliuojamų prieširdžių susitraukimo jėgą vidutiniškai 63,2 %, veikimo potencialo trukmę – 49,6 %. 1–5 junginiai (10–500 μ M) reikšmingai silpnino karbacholo poveikį, nes susitraukimas bei VP₉₀ trukmė priartėjo, o atskirais atvejais pasiekė bazinį lygį. 2 ir 7 junginiai taip pat ženkliai silpnino fenilefrino (10⁻⁴ M) sutraukiamąjį poveikį aortos žiedeliams: juos veikiant 10⁻⁴ M šių junginių koncentracija, žiedeliai atsipalaidavo 24 % ir 17 %, o veikiant 5 \times 10⁻⁴ M – 76,2 % ir 72,5 %.

Straipsnyje pateikti rezultatai rodo, kad kai kurie 1-acil-2-metiltio-5,6-dimetoksi / dietoksibenzimidazolai naikina karbacholo poveikį jūrų kiaulyčių prieširdžių izometriniam susitraukimui bei jų veikimo potencialo trukmei (VP₉₀) ir fenilefrino hidrochlorido poveikį kraujagyslių kontrakcijai. Gauti duomenys pravers tikslinei sintezei junginių, pasižymintį teigiamomis inotropinėmis ir kraujagysles atpalaiduojančiomis savybėmis.

Raktažodžiai: 1-acil-2-metiltio-5,6-dimetoksi / dietoksibenzimidazolai, jūrų kiaulyčių prieširdis, kraujagyslės, fenilefrino hidrochloridas, karbamilcholino chloridas