

Pharmacological evaluation of the effects of phenylcarbamic acid derivatives on cardiovascular functions in rats

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Four phenylcarbamic acid derivatives, (1-(4-fluorophenyl)-4-[3-(4-methoxyphenylcarbamoxy)-2-hydroxypropyl]piperazinium chloride) (1), (1-(2-methylphenyl)-4-[3-(4-methoxyphenylcarbamoxy)-2-hydroxypropyl]piperazinium chloride) (2), (1-(2-methylphenyl)-4-[3-(4-ethoxyphenylcarbamoxy)-2-hydroxypropyl]piperazinium chloride) (3) and (1-(3-trifluoromethylphenyl)-4-[3-(4-methoxyphenylcarbamoxy)-2-hydroxypropyl]piperazinium chloride) (4) were investigated for their ability to affect various cardiovascular functions and to establish their chemical structure-biological activity relationship. The compounds were evaluated for their antiarrhythmic efficacy using ouabain-induced rhythm disturbances and the ability to inhibit the positive chronotropic effect of isoproterenol in isolated atria of Wistar rats. Electrocardiogram (ECG) parameters in isolated hearts of spontaneously hypertensive rats (SHR) perfused according to the Langendorff method and ability to decrease phenylephrine-induced contraction of the aortic strips after repeated administration of the compounds were also analyzed. Only compound 3 delayed significantly the evaluated parameter of arrhythmogenicity and was able to antagonize the isoproterenol-induced positive chronotropic effect in normotensive rats' atria. Similarly, in SHR rats, only compound 3 was able to decrease heart frequency significantly without influencing the duration of QT (time between the start of the Q wave and the end of the T wave) and QTc (frequency corrected QT) intervals. The evaluated endothelial function was improved after administration of compound 2. Fluorine-containing structures (1 and 4) were less effective compared to 2'-methylphenylpiperazine derivatives (2 and 3). The latter two compounds showed suitable efficacy, which supported their use for further pharmacological research.

Keywords: phenylcarbamic acid derivatives, antiarrhythmic activity, isolated atria, electrical activity, vascular contractility, rat

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β -blockers (BBs) are an essential component of medical therapy, mainly in patients with various cardiovascular diseases of any genesis. The condition for a proper β -blocking effect is the aryloxyaminopropanol skeleton and the activity maintained even in a broader spectrum of substituted aromatic nuclei, as well as unsubstituted ones, and also in different substituents on the basic nitrogen of these compounds. Molecular studies with phenoxyaminopropanols confirmed increased cardioselectivity for *para*-substituted derivatives (1). Other studies described changes in the quality of biological activity due to modification in the connecting chain and in the salt forming fragment of *N*-methylpiperazine-derivatives (2). Compounds that contain substituted *N*-phenylpiperazine moiety markedly antagonize the α -adrenergic receptors, which allows design of therapeutically active molecules able to block both types of adrenoceptors. Substitution of the phenyl nucleus in the lipophilic part of the molecule in *ortho*- or *meta*-position enhanced the β -blocking effect while the substitution in *para*-position caused a decrease in activity. Cardioselectivity or additional α -blocking properties could be achieved by modifying the salt forming part of the molecule, when the substituent attached to nitrogen becomes a part of the aminopropanol linking chain (3). In addition, lipo-hydrophilic properties and other pharmacokinetic differences also contribute significantly to various effects of BBs (4).

The rationale of the tested compounds design was the assumed affinity for both β - and particularly α_1 -adrenoceptors because of therapeutically effective phenylpiperazine-containing selective α -adrenolytics (5). Modification of the primary aryloxyaminopropanol structure of BBs by incorporation of the carbamoyl (-NH-CO-) group on the bridge linking the phenyl nucleus to the *N*-phenylpiperazine moiety was made in an attempt to confirm and/or influence the β -blocking efficacy of the compounds. Although the basic β -adrenolytic effects were also noticed in the modified structures, they were found to be approximately 10-times weaker than in the original aryloxyaminopropanols (6).

In our experiments, we used the β -adrenoceptor antagonists carvedilol and metoprolol as positive controls. Carvedilol is the third generation of nonselective β -blockers with moderate α_1 -receptor blocking ancillary vasodilator and cardioprotective properties (7). Metoprolol is currently used in clinical practice for the treatment of arrhythmia as well as for improving cardiac autonomic function (8).

In this paper, we present the basic data on four phenylcarbamoxyloxy-2-hydroxy-*N*-phenylpiperazine derivatives, bearing in their molecule the potential β -adrenoceptor blocking structural configuration combined with vasodilatory and cardioprotective components. The basic pharmacological properties such as specific antiisoproterenol and antiarrhythmic activities were first screened in Wistar rats. In the following phase of the study, some electrical parameters of the heart and the potential antihypertensive effect of the chosen compounds on spontaneously hypertensive rats (SHR) were also evaluated.

EXPERIMENTAL

Chemistry

The evaluated compounds (1-(4-fluorophenyl)-4-[3-(4-methoxyphenylcarbamoxyloxy)-2-hydroxypropyl]piperazinium chloride) (1) (1-(2-methylphenyl)-4-[3-(4-methoxyphenylcarbamoxyloxy)-2-hydroxypropyl]piperazinium chloride) (2) (1-(2-methylphenyl)-4-[3-(4-ethoxyphenylcarbamoxyloxy)-2-hydroxypropyl]piperazinium chloride) (3) and (1-(3-trifluoromethylphenyl)-4-[3-(4-methoxyphenylcarbamoxyloxy)-2-hydroxypropyl]piper-

zinium chloride) (4) (Fig. 1) were synthesized by the reaction of 1-(4-fluorophenyl)-, 2-methyl- or 3-trifluoromethyl)phenylpiperazin-1-yl- moiety with the corresponding 2,3-epoxypropan-1-yl esters of 4-alkoxyphenylcarbamic (alkoxy = methoxy or ethoxy) acid according to the literature (9). Derivatives were synthesized as racemates containing one stereogenic center. The obtained basic esters were not crystalline substances. The final compounds were isolated as salts of hydrochloric acid. Physicochemical properties of the compounds were evaluated by standard analytical methods and are reported in the relevant literature (10).

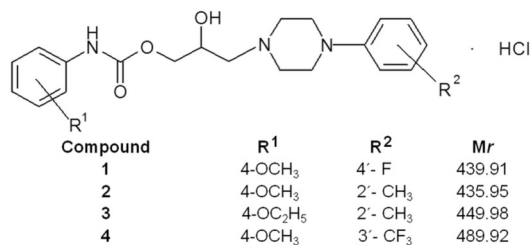


Fig. 1. Chemical structures and molecular masses of the test compounds 1–4.

Pharmacological evaluation

Animals. – All three-month-old rats used in the experiments were obtained from the breeding station Dobra Voda (Slovak Republic). Animals were allowed to acclimatize to the housing conditions with free access to food and tap water for at least seven days. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published in the Collection of Laws of the Slovak Republic and was approved by the Ethics Committee of the Faculty of Pharmacy, Comenius University, and by the State Veterinary and Food Administration of the Slovak Republic.

Antiisoproterenol activity on isolated atria. – Male Wistar rats were anesthetized with thiopental sodium (45 mg kg⁻¹ bm, 5 % solution, *i.p.*, Biochemie GmbH, Austria). The hearts were separated, right atria were then isolated and connected to an isometric transducer in tyrode solution (composition in mmol L⁻¹: NaCl 137.0, KCl 2.7, NaHCO₃ 25, MgCl₂ 1.0, NaH₂PO₄ 0.42, CaCl₂ 1.9, glucose 11.0) at 30 °C under resting tension of 1 g and saturated with pneumoxide (O₂ and 5 % CO₂). The preparation was allowed to stabilize for at least 30 min. Then, isoproterenol chloride was added in increased concentrations (10⁻¹¹–10⁻⁵ mol L⁻¹) and the concentration-response curve (CRC) was plotted. After the atria were washed and allowed to re-equilibrate, test compounds (1 × 10⁻⁶ mol L⁻¹ and/or standard carvedilol 1 × 10⁻⁷ mol L⁻¹) (carvedilol substance was a kind gift from Zentiva, Slovak Republic) were added to the bath 20 min before the second CRC was obtained and changes in the heart rate were registered. The affinity for isoproterenol was expressed as EC₅₀ (agonist concentration producing 50 % of maximum response). The antagonist potency of the compounds was calculated from the shift in CRC of isoproterenol and expressed as dissociation constants (pA₂ values), *i.e.*, negative logarithm of the antagonist molar concentration that caused twofold inhibition in isoproterenol response curves (11).

Ouabain-induced arrhythmia in rats. – Male Wistar rats were anesthetized with tribromoethanol (Avertin, Generics, UK, 375 mg kg⁻¹, *i.p.*). Cardiac rhythm disturbances were

induced by infusing ouabain (Sigma- Aldrich, USA) solution into the femoral vein ($82 \mu\text{g min}^{-1}$) and were recorded by an electrocardiograph. Evaluated compounds and/or metoprolol, both at a concentration of $1 \times 10^{-6} \text{ mol kg}^{-1}$, were administered *i.v.* (metoprolol succinate, Sigma-Aldrich) five minutes before ouabain infusion. Increase in the threshold dose of ouabain which induced ventricular extrasystoles, fibrillation and cardiac arrest indicated the antiarrhythmic effect. The threshold dose of ouabain needed to induce different cardiac disturbances was determined and expressed in $\mu\text{g kg}^{-1} \text{ bm}$ (6).

Measurement of vascular contractility in vitro. – Control male SHR rats ($n = 8$) and control male Wistar rats ($n = 6$) received subcutaneously saline as vehicle for seven days, making two negative control groups. The other three SHR groups (marked 1, 2, 3, $n = 8$) received the test compounds subcutaneously in a dose of $1 \text{ mg kg}^{-1} \text{ bm}$ (dissolved in saline) for seven consecutive days. The last group of hypertensive animals received carvedilol orally (Carvedilol Teva, Teva B.V., The Netherlands, $n = 6$) in a dose of 12.5 mg kg^{-1} for seven consecutive days.

Determination of vascular contractility was carried out as described by Racanska *et al.* (6). Briefly, after rats were sacrificed (5 % thiopental, $80 \text{ mg kg}^{-1} \text{ bm}$, *i.p.*, Biochemie GmbH, Austria), the thoracic aorta was excised from the diaphragm to the arch and placed into an isolated tissue bath containing Krebs-Henseleit (K-H) solution. Three-millimeter-long segments were cut from the aorta and placed between two stainless steel hooks inserted into the lumen and placed into the apparatus for isolated organs (TSZ-04 Multi Chamber Tissue Bath, Experimetria, Hungary) for isometric tension recordings. The endothelial function was measured as contraction responses induced by phenylephrine ($10^{-5} \text{ mol L}^{-1}$, Sigma-Aldrich) and relaxation responses induced by acetylcholine ($10^{-5} \text{ mol L}^{-1}$, Sigma-Aldrich). The responses were transferred as digital signals (FSG-01 Force/displacement transducer, Experimetria) and recorded with the S.P.E.L. Advanced ISOSYS software (Experimetria). The presented data are expressed as average values of the animals in the group; three recordings were taken from each animal.

Measurement of cardiac electrical parameters. – The isolating procedure was carried out as described by Kralova *et al.* (12). Briefly, the rats were anesthetized with thiopental sodium ($45 \text{ mg kg}^{-1} \text{ bm}$, *i.p.*, 5 % solution, Biochemie GmbH). After chest opening, the heart was separated and immersed into a cold ($4 \text{ }^\circ\text{C}$) K-H solution where a cannula was inserted into the aorta and fixed with a silk ligature. The heart was placed in the organ chamber of a Langendorff apparatus and retrogradely perfused with K-H solution in constant pressure mode ($90\text{--}100 \text{ cm H}_2\text{O}$). On isolated spontaneously beating hearts, a one-lead ventricular ECG was recorded *in vitro* using a pair of wire electrodes (ECG MLA 1213 Needle Electrodes for FE 136, ADInstruments, Germany) impaled into the LV free wall. Frequency corrected QT intervals (QTc) were derived using modified Bazett's formula (13):

$$\text{QTc} = \text{measured QT} / \sqrt{(\text{RR}/200)}$$

where, QT is the time between the start of the Q wave and the end of the T wave, RR is the time between beats, the heart rate.

Statistical analysis

Results are expressed as mean \pm standard error of the mean (SEM). Means were compared using Student's unpaired *t*-test (isolated atria). One-way analysis of variance (ANO-

VA) followed by the LSD (least significant difference) *post-hoc* test was used to assess the presence of significant differences in the remaining experiments. Values were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

In this work, we evaluated the biological efficacy of four original compounds in which the basic aryloxyaminopropanol structural moiety proper for all clinically used β -blockers was modified. In the first part of the experiment (screening), we evaluated how the extension of the connecting chain between lipo- and hydrophilic parts of the molecule contributed to specific antiisoproterenol (potential β -adrenolytic) as well as antiarrhythmic activity of the compounds. In the second part of the study, we excluded compound **4** because of its low biological efficiency observed in previous experiments; thus, compounds **1**, **2** and **3** were measured for some other cardiovascular parameters.

Antiisoproterenol activity on isolated atria

We evaluated how extension of the chain linking, lipo- and hydrophilic part of the molecule, contributed to specific antiisoproterenol efficacy of the compounds. Carvedilol was used as a positive control. Antiisoproterenol activity of the compounds on the heart rate was examined *in vitro* in spontaneously beating right atria of rats and expressed as EC_{50} values, from which pA_2 values were calculated. All the evaluated compounds confirmed the effect when their calculated pA_2 values varied in the range of 5.23–7.41 (Table I). Then, compared to the used β -blocker, the carvedilol, compounds **2** and **3** showed a moderate effect ($p < 0.05$, $p < 0.01$, resp.) and only a slight effect was detected for analogues bearing a fluorine and/or trifluoromethyl group in the piperazine ring (compounds **1** and **4**).

Table I. Effective concentrations (EC_{50}) of isoproterenol for the atria heart rate before and after incubation with the evaluated compounds and their pA_2 values

Compd.	EC_{50} (isoproterenol, mol L ⁻¹)	pA_2
Control	$(5.38 \pm 1.99) \times 10^{-11}$	
1 ^a	$(4.99 \pm 1.99) \times 10^{-10}$	6.83 ± 0.33
Control	$(9.43 \pm 0.90) \times 10^{-11}$	
2 ^a	$(3.66 \pm 1.63) \times 10^{-9*}$	7.41 ± 0.35
Control	$(3.08 \pm 1.30) \times 10^{-11}$	
3 ^a	$(4.12 \pm 1.17) \times 10^{-10**}$	7.22 ± 0.25
Control	$(3.73 \pm 1.55) \times 10^{-10}$	
4 ^a	$(8.55 \pm 2.69) \times 10^{-10}$	5.23 ± 0.38
Control	$(8.05 \pm 1.93) \times 10^{-10}$	
Carvedilol ^b	$(1.88 \pm 0.65) \times 10^{-8**}$	8.15 ± 0.49

Incubation with: ^a 10^{-6} mol L⁻¹, ^b 10^{-7} mol L⁻¹. Control for: evaluated compounds saline, carvedilol: DMSO+saline. Data are expressed as mean ± SEM, $n = 6$. Significant difference *vs.* control: * $p < 0.05$, ** $p < 0.01$. For chemical structures see Fig. 1.

Ouabain-induced arrhythmias in rats

In the present study, it was found that only compound 3 with a methyl group in position-2 of the phenyl-*N*-piperazine part of the molecule, delayed significantly ($p < 0.05$, $p < 0.01$ and $p < 0.001$) the evaluated parameters of arrhythmogenicity (Fig. 2). Compound 2 which differs only in alkoxy substituent in the phenylcarbamoil acid counterpart of the molecule (-OCH₃ instead of -OC₂H₅) affected the ouabain cardiotoxicity (fibrillation and cardiac arrest) non-significantly. From the tested compounds, compounds 4 and 1 seem to be the least anti-ouabain active. However, no significant differences among all test compounds were observed. None of the test compounds exceeded the efficacy of the used positive control, metoprolol. The obtained results are in accord with our previous studies where the compounds with 4-fluorophenyl and 2- or 4-alkoxy-substitution decreased slightly the amount of infused cardiotoxic ouabain needed for the first signs of arrhythmogenicity. Comparison of the current results to the values of anti-ouabain activity of structurally related compounds led to the conclusion that the bulky substituent attached to the phenylpiperazine moiety together with the extended carbamoyloxyaminopropanol chain reduced their antiarrhythmic properties (6).

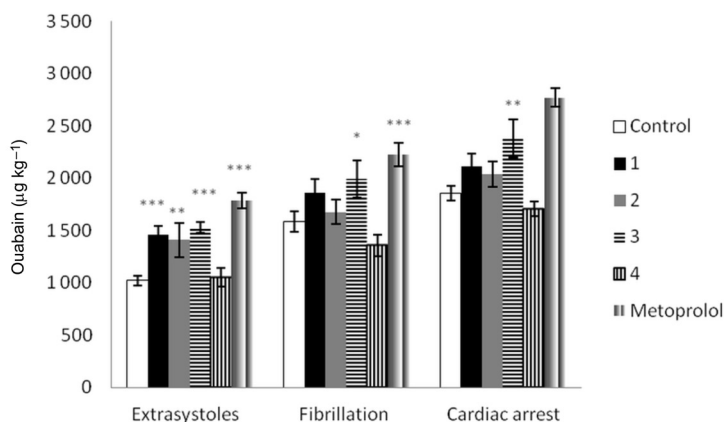


Fig. 2. Protection provided by test compounds 1–4 and metoprolol at the concentration 1×10^{-6} mol kg⁻¹ against ouabain-induced arrhythmia in anesthetized rats. Data are expressed as mean \pm SEM, $n = 7$. Significant difference vs. control: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. For chemical structures see Fig. 1.

Measurement of vascular contractility in vitro

To demonstrate the effects of the compounds on the endothelial function, we measured the contractile and relaxation responses of the thoracic aorta. We assessed an antagonistic effect of the compounds against the constriction induced by the α_1 -adrenergic agonist phenylephrine, as well as the relaxation induced by acetylcholine on isolated aortic rings. SHR aortic rings showed increased contractile responses compared to Wistar rats, which could indicate endothelial damage as a result of hypertension (Fig. 3). The relaxation

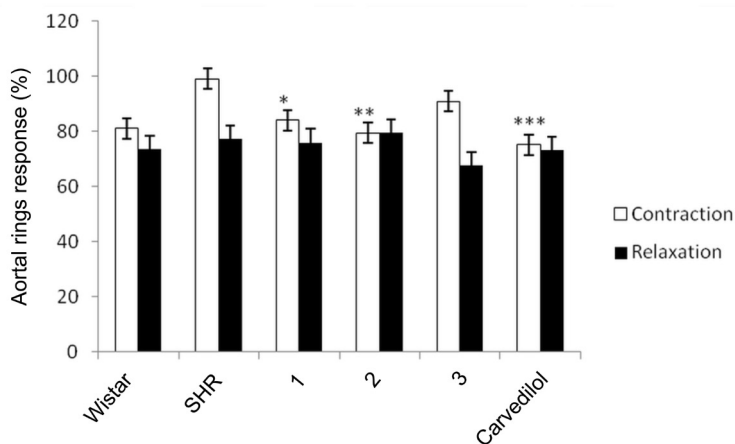


Fig. 3. Contraction and relaxation responses of aortal rings from normotensive Wistar rats ($n = 6$), hypertensive rats (SHR, $n = 8$), SHR rats pretreated with compound **1**, **2** or **3** ($n = 8$) and carvedilol rats ($n = 6$), of phenylephrine-induced contraction and maximum relaxation after administration of acetylcholine. Data are expressed as mean \pm SEM. Significant difference vs. SHR: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. For chemical structures see Fig. 1.

response of SHR aortic rings induced by acetylcholine was unchanged compared to the rings from normotensive rats. One week administration of compounds **1** and **2** significantly ($p < 0.05$, $p < 0.01$, resp.) improved the contractive response of aortic rings in SHR compared to SHR without treatment but not so effectively as carvedilol. We could suppose that 2-methyl-substitution on the phenyl-*N*-piperazine part in compound **2** was responsible for a more desirable effect on vessels. It is obvious that damage of the endothelium caused by hypertension worsened the dilatatory ability of aortic rings. However, none of the evaluated substances improved relaxation of the aorta significantly.

Measurement of electrical activity on isolated rat heart

Bradycardia induced by β -blockers may be beneficial for protecting myocytes through improvement of the relationship between the myocardial blood flow and oxygen consumption (14). Our results showed no significant differences between the Wistar control and SHR control rats (Table II). Administration of compounds **2** and **3** resulted in a significant ($p < 0.05$) decrease of heart frequency compared to SHR without pretreatment. However, all the evaluated substances were less effective than the used standard carvedilol. Administration of the above-mentioned compounds did not change the duration of QT and QTc intervals. On the basis of their chemical structure and our previous experiments of the negative chronotropy of structurally similar compounds, it could be presumed that compounds **2** and **3** are able to act *via* inhibition of cardiac β -adrenoceptors (11). Various experiments with hypertensive SHR animals showed a prolongation of the QT and QTc intervals related to the slowed ventricular conduction compared to conventional Wistar animals (15). In our experiment, however, the tested compounds failed to shorten the duration of QT and QTc intervals.

Table II. Selected electrocardiographic parameters of hearts

Parameter	Wistar control ^a	SHR control ^b	1 ^{b,c}	2 ^{b,c}	3 ^{b,c}	Carvedilol ^{a,c}
Frequency (beat min ⁻¹)	209 ± 21	198 ± 1	178 ± 6	172 ± 9*	170 ± 12*	166 ± 9*
RR (ms)	287 ± 46	303 ± 12	341 ± 12	357 ± 18	369 ± 36*	366 ± 19
QT (ms)	66.98 ± 4.05	71.63 ± 3.79	73.77 ± 1.51	79.24 ± 7.83	68.41 ± 3.64	66.81 ± 3.09
QTc	55.39 ± 4.32	58.18 ± 2.99	55.89 ± 1.01	57.72 ± 5.68	51.22 ± 3.64	49.43 ± 1.79

SHR – hypertensive rats, QT – time between the start of the Q wave and the end of the T wave, QTc – frequency corrected QT intervals, RR – time between beats, heart rate. Data are expressed as mean ± SEM: ^an = 6, ^bn = 8.

^cSHR animals pretreated with compounds 1, 2, 3, carvedilol. For chemical structures see Fig. 1. Significant difference *vs.* SHR control: * *p* < 0.05.

CONCLUSIONS

We may conclude that all the evaluated compounds, designed and synthesized as potential β -blockers, exhibited only moderate cardiovascular effects. Due to antiisoproterenol activity in rats' atria and ability to inhibit ouabain-induced heart disturbances, only compound 3 could be assumed to be a potential β -blocking agent. In SHR, only compound 2 improved significantly the contractile properties of the aorta. It seems that participation of α -adrenoceptor inhibition might be responsible for this effect. Heart frequency measured on isolated hearts from SHR rats was significantly decreased by compounds 3 and 2. According to the obtained results, it is obvious that substitution of phenyl-*N*-piperazine by the 2-methyl group (compounds 2 and 3) was more effective than the fluorine analogues. The presence of fluorophenyl- or trifluoromethylphenyl-moiety on the piperazine ring in compounds 1 and 4, resp., does not seem suitable for the postulated properties of the compounds.

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