(Vd/f) and total clearance (CL/f) for celecoxib were obtained for each subject using a computer program KINETICA (1999 Inna Phase Corporation) intended for calculation of model independent parameters. In the present study, $AUC_{(0-t)}$ refers to the AUC from 0 to 48 h and $AUC_{(0-\infty)}$ refers to the AUC from 0 to infinite time. C_{max} and t_{max} were determined as the highest observed concentrations and the time to reach the maximum concentrations, respectively. Elimination half life ($t_{1/2}$), area under the serum concentration curve (AUC), volume of distribution (Vd/f) and clearance (Cl/f) were calculated using non-compartmental model.

5. Statistical analysis

The resulting means of various pharmacokinetic parameters obtained in different subjects were compared using Student's t-test (paired data). A value of $p < 0.05$ was considered to be statistically significant.

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Changes in heart rate after application of newly developed ultrashort acting beta-adrenergic blockers

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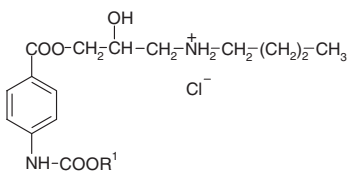
Antagonists of adrenergic β-receptors are widely used ischemic heart disease, hypertension, arrhythmias and hypertrophic cardiomyopathy [1–5]. Adverse effects of classical beta-blockers include hypotension, bradycardia, heart failure, bronchospasm, or peripheral vasoconstrictions, which can last up to several hours after intravenous application [6]. For this reason, beta-adrenoreceptor antagonists with an ultrashort duration of action are being developed [7].

The objective of our pilot study was to test the effect of three newly synthesised compounds on the physiological level (baseline) of a laboratory rat's heart rate. The compounds (derivates of arylcarbonyloxyaminopropanols) are substituted by a linear alkyl chain in the aliphatic part of the molecule – by ethyl in the substance 42 Bu, by propyl in 43 Bu and by butyl in 44 Bu [8, 9] (Table 1). Solubility and lipophility of the corresponding compounds also correspond with the chain length. In all three tested substances, a statistically proven short-acting bradycardic effect was detected. The onset of action of the substances tested was very fast. Inmediately in the first minute following application, a statistically highly significant bradycardic effect was noted in all three substances. Heart rate changes are given in Table 2.

The biggest significant decrease in heart rate ($13.00 \pm 5.53\%$) in comparison with the other two compounds showed the substance 44 Bu. The duration of the bradycardic effect was demonstrably longer in this sub-

Table 1: Chemical structures of the substances tested



Tested substance	R^1	T.t. (°C)	Solvent	R_F*
42 Bu	C_2H_5	120–123	Propan-2-ol	0.61
43 Bu	C_3H_7	116–119	Propan-2-ol	0.65
44 Bu	C_4H_9	109–112	Propan-2-ol	0.69

Table 2: Change of heart rate (%) after the application of the tested substances and placebo

Time (min)	Average value ± standard deviation (in%)			
	42 Bu	43 Bu	44 Bu	Placebo
0	93.32 ± 4.11**	95.03 ± 3.96*	95.43 ± 4.21*	102.63 ± 4.55
1	91.40 ± 4.09**	91.99 ± 3.9**	90.82 ± 5.18**	102.45 ± 7.23
2	92.41 ± 4.42*	95.77 ± 9.42	87.95 ± 5.15**	102.25 ± 8.44
3	93.15 ± 4.17*	96.16 ± 9.18	87.75 ± 5.07**	102.35 ± 8.44
4	93.89 ± 4.08	96.74 ± 8.77	87.25 ± 5.36**	102.16 ± 8.59
5	94.59 ± 4.56	96.85 ± 8.41	87.36 ± 5.53**	102.36 ± 9.69
6	95.16 ± 5.01	94.31 ± 5.58	87.00 ± 5.53**	102.16 ± 9.69
8	95.20 ± 5.23	95.52 ± 7.59	88.55 ± 6.09*	102.7 ± 11.46
10	94.95 ± 7.27	96.35 ± 8.93	92.03 ± 6.52*	103.91 ± 9.82
12	94.16 ± 8.5	97.44 ± 10.9	93.98 ± 7.67*	104.93 ± 11.8
14	94.90 ± 9.91	99.05 ± 13.08	94.64 ± 7.31*	106.43 ± 9.69
16	96.08 ± 10.91	99.86 ± 15.33	96.42 ± 7.97	103.01 ± 11.08
18	99.51 ± 11.5	102.19 ± 17.86	98.97 ± 7.07	106.91 ± 11.73
20	99.10 ± 11.06	102.87 ± 17.86	100.36 ± 7.24	107.77 ± 12.12

The heart rate value before the application is 100%
0 minute = intravenous application of the substance
* The value is statistically significant with the significance level of $p < 0.05$
** The value is statistically highly significant with the significance level of $p < 0.01$ against placebo

stance also. A statistically significant decrease in the heart rate was registered up to the 14th minute. This corresponds with the fact that the substance 44 Bu has the highest lipophility of all three compounds tested. Therefore, it penetrates cell membranes more easily and remains there for the longest time.

For all three substances tested, marked changes in the ECG record were registered immediately after the intravenous application. There appeared prolongations of the PQ interval (to 228% of their initial values), QT interval (to 135% of their initial values) and QRS complex (to 180% of their initial values), S wave, T wave elevation (to 207% of their initial values), R wave reduction (to 44% of their initial values), as well as a change in the position of QRS axis.

From all the above-described changes in ECG readings we can conclude, that the tested compounds inhibit the transfer of the impulse from the atrium to the ventricle (prolongation of PQ interval), inhibit conduction of the impulse in the musculature of the ventricle (prolongation of QRS complex) and inhibit depolarization and repolarization of the ventricles (prolongation of QT interval). Because PQ interval is prolonged the tested compounds seem to have a negatively dromotropic influence on the myocardium.

Regarding the fact that all the changes occurred within a very short period of time a hypothesis of influence on sodium ion channels in the transmission system of the heart arises [10]. According to Lüllman et al. certain beta-blockers can stabilise cell membranes. Especially more lipophilic derivates have the capacity to inhibit the function of sodium channels and thus the conduction of impulses through heart tissue [11]. The cause of these changes is subject to further research. The testing was performed *in vivo* in sixty laboratory male Wistar rats with a normal blood pressure and an average weight of 360 ± 85 g.

Chemical structures of the tested substances are given in Table 1. The compound 42 Bu was tested in the concentration 4.5 $mg \cdot kg^{-1}$ (12×10^{-6} $mol \cdot kg^{-1}$), 43 Bu in the concentration 3.5 $mg \cdot kg^{-1}$ (8.9×10^{-6} $mol \cdot kg^{-1}$) and 44 Bu in the concentration 2.5 $mg \cdot kg^{-1}$ (6.2×10^{-6} $mol \cdot kg^{-1}$) of the tested animal weight. The concentrations used in the experiment are the results of the *in vivo* screening of each substance tested. The screening criterion used to determine the concentration of the compounds was a significant decrease in the heart rate by at least 5% of three out of five animals.

The tested substance was injected intravenously in the prepared jugular vein of the animals in total anaesthesia (i.m. application of cocktail solution of Narkamon 1% + Rometar 2% in the dose of 0.5 ml/100 g of animal weight), as a bolus in a volume of 1 ml. Saline, used for the preparation of the injected solution, was used as a placebo.

In predetermined time intervals (Table 1), ECG records were taken in all of the animals (Standard leads I, II, III aVR, aVL and aVF, $v_1 = 25$ $mm \cdot s^{-1}$, $v_2 = 100$ $mm \cdot s^{-1}$). The heart rate values were converted into percentages of variation, where heart rates measured immediately before the application of the test substance were taken as 100% values. Standard deviation was calculated for each value. Dispersion variability was determined using F-test; statistical significance of the given change of heart rate against placebo was determined using Student's t-test.

The methodology of the experiment was approved by the expert committee for animal protection at VPU under no. 16356/2002–30

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