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Influence of new ultra-short-acting β-blockers on selected physiological indicators in laboratory rats

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ABSTRACT: High rates of cardiovascular mortality have long been a serious problem in all European countries. Despite advancements in health care the situation is not improving fast enough. In the last decades, no new ultra-short-acting β-blockers have been registered in the European Union except for esmolol and landionol. In this study, eight newly-synthesised ultra-short-acting β-blockers were tested. These β-blockers contain an ester functional group which can be easily cleaved by plasma or cytoplasmic esterases. The substances were prepared in the Department of Chemical Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Brno. Systolic blood pressure, heart rate and the interval of the QRS complex were evaluated using normotensive laboratory Wistar rats. The tested compounds were administered intravenously into the *vena jugularis* during general anaesthesia. The *arteria carotis* was exposed and cannulated to a Universal Perfusion System Basic Unit (UPSBU) of type Uniper UP-100. The universal perfusion system for isolated organs was capable of measuring and transducing actual values of blood pressure. ECG records were made using the ECG SEIVA – Praktik Veterinary. A series of substances named 2FC2a, 2FC2b, 2FC2c, 2FC2d, and another series with substances named 2FT2a, 2FT2b, 2FT2c, 2FT2d were tested at a dose of 3 mg/kg. Results were statistically compared to placebo. The best results were obtained for propyl and butyl derivatives with the highest lipophilicity. These acted as the best blood pressure reducers immediately after their administration. None of the compounds notably affected the heart rate. Statistical data show that carbamate substitution considerably prolongated the duration of the QRS complex as compared to placebo or etheric substitution. The carbamate substitution caused a pronounced arrhythmogenic effect. Thus, we could confirm the short-term hypotensive effect of the compounds. We observed an effect on the electrical conduction system of the heart while no effects were observed on heart rate. Our study contributes to better describing potential new ultra-short-acting β-blockers and facilitates selection for further testing.

Keywords: ultra-short-acting β-blocker; laboratory rat; systolic blood pressure; heart rate; QRS complex

In spite of the fact that appropriate lifestyle changes have recently been adopted in the general population, cardiovascular diseases (CVDs) remain the number one cause of mortality worldwide. While developed western European countries as well as states of southern and northern Europe run successful programs aimed at reducing incidence and the mortality associated with these disorders, these are lacking in post-communist countries where the situation is even worsening in some instances. States with low or intermediate incomes report CVD mortality rates in the range of 70–80%. Differences among the EU countries are declining but expectations point towards a general increase in CVDs due to increasing rates of obesity, diabetes and metabolic syndrome (Kamensky and Murin 2009).

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The increasing occurrence of these disorders, which is reaching almost epidemic proportions, has a number of reasons. The most important factors are smoking, alcohol abuse, overweight and obesity, lack of physical activity, low consumption of fruits and vegetables, high blood pressure and high concentrations of blood cholesterol (Rovny 2015). Together with the increasing average life expectancy, the number of CVD patients is increasing and the condition is now spreading to younger age groups, and even within the child population (Graham et al. 2007). According to the National Cardiovascular Program of the Czech Republic and a Population Health Report published in 2014, mortality due to CVDs is about 30% higher in men as compared to women. This difference is, however, getting smaller. This sex-dependent difference in mortality caused by CVDs resulted from the fact that men died twice as frequently as women in the investigated period of 40 years. On the other hand, differences in death rate related to stroke were insignificant in the considered time period. The collected data show that, in the year 2012, the number of deaths due to CVDs was almost 53 000 in the Czech Republic. This number represents 44% of total deaths in men and 54% in the female population. It is also known that, among CVDs, coronary heart disease is the most frequent cause of mortality in the Czech Republic (Kodl et al. 2014).

The situation in Slovakia is similar: data collected in 2012 and presented in the Report on Public Health in the Years 2009–2011 revealed that the number of deaths in 2008 in the male population in the 25–64 years age group was three times higher than in this respective age group in women. These premature deaths in the male population result in an apparent higher mortality rate of women in the older sections of the age scale. In the investigated period, CVDs were responsible for 45.9% of deaths in men and 59.8% of deaths in the female population (Kamensky and Murin 2009; Rovny 2012).

These data clearly show that there is an urgent need to develop new, safer and more efficient drugs for the treatment of critical conditions related to CVDs. It has become clear that disorders like coronary artery disease (CAD), chronical heart failure and high blood pressure are closely related to the dysregulation of the autonomic nervous system. Above all, the sympathetic nervous system and its enhanced activity are expected to contribute significantly to the individual mentioned disorders. As a result of hectic lifestyles, essential hypertension, CAD, disorders of heart rate, heart insufficiency and cardiomyopathy are being diagnosed in ever younger patients. The sympathetic nervous system is placed under a high amount of strain by intensive working styles and stressful situations in day-to-day life. The long-term administration of blocking agents suppresses the enhanced activation of the heart by the sympathetic nervous system and helps to prevent mortality caused by CVDs.

Competitive antagonists of β-adrenergic receptors, or β-blockers, have received enormous clinical attention because of their efficacy in the treatment of ischaemic heart disease, acute myocardial infarction, hypertension, congestive heart failure, and certain arrhythmias. At the molecular level, the β-receptor is part of the adenylyl cyclase system linked by G protein-coupled receptors. The binding of a molecule of epinephrine or norepinephrine to a β-receptor coupled with G protein results in the activation of adenylyl cyclase, which forms the second messenger cAMP from ATP. Increased levels of cAMP activate a cAMP-dependent protein kinase that phosphorylates L-type calcium channels, which causes increased calcium entry into the cell and increased reuptake of cytosolic calcium into the sarcoplasmic reticulum (Lefkowitz and Shenoy 2005).

Since catecholamines accelerate the heart rate and increase myocardial contractility, β-receptor antagonists have negative chronotropic and negative inotropic effects. However, the antihypertensive action of β-blockers is not only due to decreases in the heart rate and cardiac output. Multiple modes of action are involved in the lowering of blood pressure: inhibitory effects on the central and peripheral nervous system, decreased activity of the renin-angiotensin system, reductions in venous return and plasma volume, altered sensitivity of baroreceptors, reduction of norepinephrine release and decrease of peripheral vascular resistance, etc. (Varon 2008).

Clinically used β-adrenergic receptor antagonists are either non-selective, "first generation" blockers with similar affinity for the β_1- and the β_2-subtypes (propranolol, timolol) or β_1-selective, "second generation" blockers (bisoprolol, metoprolol). Some compounds possess a partial agonist activity called intrinsic sympathomimetic activity. These compounds contain a hydroxy group attached to an aromatic ring structure such as a natural catecho-

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lamine. A newer group of β-blockers or the "third generation" of β-blockers exert various vasodilatory effects. These additional cardiovascular effects are produced through a variety of mechanisms: α_1-adrenergic receptor blockade (labetalol), increased production of endothelial nitric oxide (nebivolol), β_2-receptor agonist properties (celiprolol), Ca^{2+} channel blockade (betaxolol) or antioxidant effects (carvedilol; Frishman 2008; Duricova and Grundmann 2009).

β-receptor antagonists can be divided into two broad groups according to their solubility. Lipid-soluble agents easily penetrate membranes and are eliminated primarily by hepatic metabolism with high first pass effect. Half-lives are relatively short with wide variations in plasma concentrations. Water-soluble agents are rapidly washed out of tissues and eliminated almost unchanged by the kidney. Less lipophilic agents have longer half-lives with stable plasma concentrations. At high concentrations, some β-blockers (lipid-soluble) have local anaesthetic or membrane-stabilising activity on the heart muscle. β_1-selective compounds have less adverse effects, because β_1-receptors are mostly found in the heart, kidney and lungs. Receptors of the β_2-subfamily are more common in other tissues (Fowler 2008; Frishman 2011; Wenquan and Yue 2016; Wolf et al. 2016).

The ordinary β-blockers provide their full curative effects in a time period spanning several days and, therefore, are less suitable for the treatment of life-threatening conditions when an immediate effect is needed. The proper treatment of patients in a critical condition requires the application of ultra-short-acting β-blockers, the effect of which exhibits a fast decay. The fast decay effect can be achieved by adding an ester bond into the respective molecular chain, which is immediately cleaved by blood serum esterases (Potter and Wadkins 2006). In practice, only three substances are considered to exhibit these favourable properties, namely, esmolol, flestolol and landiolol (Gorczynski 1985).

Esmolol is registered in the Czech Republic under the market name Esmocard. This drug is administered to patients suffering from supraventricular tachycardia, atrial fibrillation and atrial flutter before and/or after surgery, or in situations when a short-term control of ventricular rate is required (Bakker et al. 2011). The portfolio of applications also includes cases of tachycardia and hypertension indicated prior to an operation or uncompensated sinus tachycardia when the fast heart pace needs to be controlled according to the surgeon's opinion (Spinar and Vitovec 2006; Marik and Varon 2009; Janota 2012).

Flestolol is applied only experimentally and, compared to esmolol, is a non-selective β-blocker. Landiolol is still not registered in Europe, although it is fully recognised in Japan where it has been extensively studied. The state-of-the-art summarised above leads to the conclusion that further work is required to develop new, safer and more efficient β-blockers that will provide an effect in even shorter time spans (Atarashi et al. 2000).

MATERIAL AND METHODS

Tested substances. Tested substances were synthesised by Pharm.D. Jan Tengler Ph.D. of the Department of Chemical Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Palackeho 1-3, 612 42 Brno, Czech Republic. The research team was led by Prof. RNDr. Jozef Csollei, CSc. The new tested compounds, which exhibit potential as β-antagonists, can be thought of as functional analogues of aryloxyaminopropanol (arylcarbonyloxyaminopropanol). In their structure, the original ether group has been replaced by the ester functional group, the aromatic part in the para-position has been substituted by either methyl or butyl esters of the carbamic acid or by methoxy or butoxy groups (Tengler et al. 2013). The ester bond inserted into the connecting chain guarantees a faster metabolism of the compounds after intravenous administration. The basic part of the molecule is represented by 2-phenoxyethylamine which contains a fluorine atom in the ortho-position. A bulky 2-fluorophenyloxy substituent in the aliphatic part of the molecule serves to increases the affinity towards β-adrenergic receptors.

It is expected that, due to its potential α_1-adrenolytic activity, this substitution lead to similar effects as those of carvedilol (Groszek et al. 2010). At the same time, the chemical structure contains a centre of optical activity that enables R and S isomer forms for all functional analogues. The final products in the form of racemates were synthesised as hydrochlorides in a four-stage reaction starting with 4-aminobenzoic or 4-alcoxybenzoic acid and 2-fluorophenol (Figures 1 and 2), see Tengler et al. (2013).

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Table 1. List of tested compounds and their physical and chemical properties

Substance	R_1	R_2	Formula	Molecular weight	Purity (%)	Dissociation constant	Melting point (°C)
2FT2a	CH_3	2-F	$C_{19}H_{23}ClFNO_5$	399.85	99.24	7.69	137–138
2FT2b	C_2H_5	2-F	$C_{20}H_{25}ClFNO_5$	413.88	99.06	7.68	121–122
2FT2c	C_3H_7	2-F	$C_{21}H_{27}ClFNO_5$	427.91	98.79	7.68	113–115
2FT2d	C_4H_9	2-F	$C_{22}H_{29}ClFNO_5$	441.94	98.95	7.69	118–120
2FC2a	CH_3	2-F	$C_{20}H_{24}ClFN_2O_6$	442.87	98.91	7.98	195–197
2FC2b	C_2H_5	2-F	$C_{21}H_{26}ClFN_2O_6$	456.90	99.34	7.99	209–211
2FC2c	C_3H_7	2-F	$C_{22}H_{28}ClFN_2O_6$	470.93	98.99	7.97	201–203
2FC2d	C_4H_9	2-F	$C_{23}H_{30}ClFN_2O_6$	484.95	99.15	7.96	191–192

Animals. The experiments were performed *in vivo* using 55 normotensive laboratory male rats of the Wistar strain. The rats were 60 days of age and their average weight was 304.3 ± 27.8 g. The animals came from a breeding facility located at AnLab, s.r.o., Videnska 1083, 142 20 Prague 4, Czech Republic, and their housing conditions strictly adhered to the protocol specified in Regulation No. 311/1997 Coll. (temperature – 20–24 °C, humidity 40–60%, 12 : 12 L : D cycles with a maximum lighting of 200 lux). The rats were accommodated in plastic PVC boxes (three rats in each) and fed a standard M1 diet for small laboratory animals and provided with water *ad libitum*. The experiment started after 10 days of acclimatisation. The experimental program closely followed the agenda of a project from the year 2013 which was approved and monitored by the local University Ethical Committee of the University of Veterinary and Pharmaceutical Sciences in Brno.

Experimental procedures. The animals were divided into nine groups. Each rat from Groups 1–4 (n = 5 animals in each group) was given the substance 2FT2a–d at a dose of 3.0 mg/kg. The dose was carefully chosen based on previous experiments with similar compounds. In the second part of the experiment, substances 2FC2a–d at a dose of 3.0 mg/kg were administered to Groups 5–8 (n = 5 animals in each group). Group 9 (n = 5) was given a placebo which consisted of physiological solution and dimethyl sulfoxide as co-solvent.

A solution of 10% ketamine (Narketan®, inj. Spofa) and 2% xylazine (Xylapan®, inj. Spofa) was used to induce general anaesthesia. The anaesthesia solution was administered intramuscularly into the femoral muscle area using a dose of 0.1 ml per 100 g of body weight. The Uniper UP-100 device was connected to the *arteria carotis* by means of a cannula. The Uniper UP-100 is a universal perfusion system for isolated organs which enables the measuring and transducing of actual blood pressure values into a graphical record by means of the HSE-BDAS program. The animals were monitored continuously during the entire duration of the experiment using a Seiva Praktik ECG machine (SEIVA, CR) (standard sensors I, II, III, aVR, aVL a aVF, v1 = 25 mm/s, v2 = 100 mm/s). The tested compounds dissolved in dimethyl sulfoxide were complemented by a physiological solution (sterile

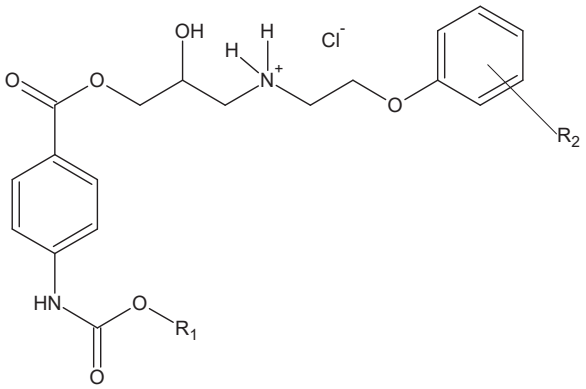

Figure 1. A hydrochloride of the tested substances (2FC2a–d)

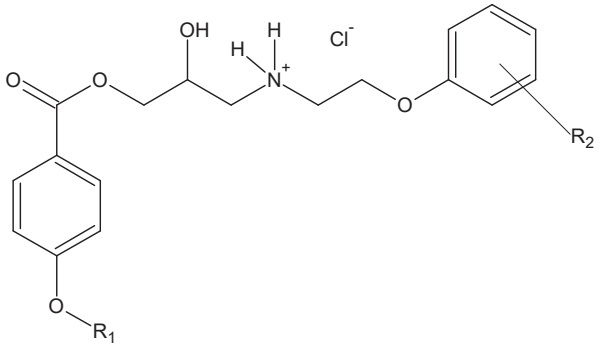

Figure 2. A hydrochloride of the tested substances (2FT2a–d)

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isotonic solution of 0.9% NaCl) in a total volume of 1 ml. The same procedure was applied in the case of placebo solutions. The tested substances and the placebo were injected into the extracted *vena jugularis* at a dose of 1 ml. This bolus injection took no more than 15 seconds. The subsequent measurement procedure ran for 20 minutes during which the device recorded systolic blood pressure, heart rate and other ECG parameters.

All data were statistically assessed using the Microsoft Excel application. The obtained systolic pressure data were related to the initial pressure value and presented in the form of the percentage changes. The same was done for changes in heart rate and the duration of the QRS complex. Here, the initial values for systolic blood pressure, heart rate and QRS complex measured before the application of the tested substance were set to 100%. The averages and standard deviations of the values for systolic blood pressure, heart rate and QRS complex obtained for different substances were calculated. Their dispersion was described using the *F*-test and Student's *t*-test was used to assess the statistical significance of the individual pressure, heart rate and QRS complex duration changes with respect to the placebo experiments.

RESULTS

The experimental data obtained for the tested substances and placebo in all nine tested groups are summarised in the form of tables in which the systolic blood pressure changes are presented as a function of time. The tables also summarise percentage changes of heart rate and the duration of the QRS complex evaluated from the ECG records after the injections. The standard deviations are also presented in the tables.

Experimental results are presented in Tables 2–7. This form of overview is preferred since a graphical representation was insufficient and did not allow clear visualisation of the data obtained for the four substances and the placebo. Average physiological values of rats in the minute before administration were the following: systolic blood pressure, 125 mmHg; heart rate, 355 bpm; and QRS complex duration, 22 milliseconds. Table 2 compares changes in the systolic blood pressure as recorded after the administration of the compounds 2FT2abcd and placebo. The substance 2FT2a showed only a limited effect shortly after administration which peaked in the time period between 6.5 and 7.5 minutes. On the other hand, substance 2FT2b reduced blood pressure in a statistically significant manner and its effect lasted throughout the entire investigated time period. Only a few individual measurements failed to provide fully convincing data, probably because of an insufficient number of investigated animals. The effect of 2FT2c was apparent immediately after the injection and was statistically significant up to 4.5 minutes. Systolic blood pressure did not fully return to the original value and a statistically significant reduction was observed for measurements performed between 12.5–14.5 and 17–19 minutes, respectively. The substance 2FT2d reduced blood pressure immediately after administration and its effect was statistically significant up to 5.5 minutes. Decay back to the original physiological values was apparent after 8 minutes, at which point pressure was slightly higher than at the start of the experiment, remaining at this higher level for the remainder of the investigated timeframe.

Table 3 summarises heart rate data obtained after the administration of compounds 2FT2abcd. The substance 2FT2a showed a statistically significant effect only for up to 30 seconds after the injection. Similar results were obtained for the compound 2FT2b. The effect of 2FT2c was statistically significant for measurements from the time period 7–8 minutes onwards only. The substance 2FT2d reduced the heart rate only immediately after administration. Overall, the data show that after the administration of the investigated compounds, heart rate changes were only minor as compared to the placebo. Thus, we can conclude that the studied compounds do not exert a significant effect on heart rate in the rat.

The effects on the QRS complex interval are presented in Table 4. The substance 2FT2a exhibited a statistically significant influence on QRS duration during the first minute after the injection. During this time, the width of the QRS complex increased. The QRS duration was also prolonged in a statistically significant manner after the administration of 2FT2b; in this case for the period of 1–3 minutes and in the fifth minute. Similarly, the compound 2FT2c prolonged the duration of the QRS complex immediately after administration and the effect lasted up to the end of the third minute. The compound 2FT2d increased QRS complex width immediately after administration, while the aver-

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Table 2. Values of systolic blood pressure (%) after the administration of tested agents and placebo at the dose of 3 mg/kg

Time (min)	2FT2a	2FT2b	2FT2c	2FT2d	Placebo
–0.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0.5	56.6 ± 6.8***	52.3 ± 5.6***	49.3 ± 5.6***	47.1 ± 7.1***	90.2 ± 5.0
1	83.7 ± 9.7	72.4 ± 2.5	63.6 ± 10.2*	63.7 ± 12.8*	100.5 ± 21.7
1.5	100.1 ± 9.8	84.0 ± 7.2**	77.4 ± 11.8**	74.2 ± 11.1**	117.4 ± 18.4
2	106.1 ± 10.6	93.1 ± 10.7*	83.0 ± 12.3**	84.7 ± 10.9**	125.2 ± 19.4
2.5	105.5 ± 9.5	95.8 ± 11.1*	85.4 ± 9.1**	87.0 ± 9.7**	124.9 ± 19.2
3	102.2 ± 10.2	96.1 ± 11.6*	89.0 ± 5.9*	91.2 ± 10.5*	125.3 ± 22.0
3.5	99.7 ± 11.4	95.0 ± 11.5*	89.1 ± 6.1*	89.0 ± 10.4*	125.1 ± 21.2
4	98.3 ± 12.0	94.1 ± 11.4	91.0 ± 7.7*	90.9 ± 10.3*	121.3 ± 22.3
4.5	96.0 ± 11.5	93.1 ± 11.1*	91.8 ± 9.1*	93.3 ± 9.7*	120.8 ± 21.0
5	94.1 ± 13.4	92.0 ± 11.2*	93.7 ± 11.0	93.4 ± 9.4*	120.2 ± 20.3
5.5	94.6 ± 12.2	91.8 ± 10.5*	94.3 ± 11.5	94.8 ± 8.5*	120.9 ± 20.5
6	92.5 ± 12.2	90.5 ± 10.0*	96.0 ± 13.6	96.3 ± 7.0	119.7 ± 20.9
6.5	94.3 ± 12.1*	90.7 ± 9.7*	96.4 ± 14.0	97.5 ± 6.5*	122.9 ± 18.7
7	94.3 ± 12.3*	91.5 ± 9.9*	96.9 ± 15.2	98.3 ± 5.9	123.0 ± 20.8
7.5	95.6 ± 12.1*	90.9 ± 9.0*	96.0 ± 15.2	99.3 ± 5.8	123.4 ± 20.8
8	96.2 ± 12.5	90.6 ± 9.1*	95.9 ± 15.7	100.3 ± 5.9	123.8 ± 20.9
8.5	97.1 ± 13.3	90.3 ± 8.2*	96.8 ± 16.3	101.1 ± 5.2	121.3 ± 22.7
9	99.1 ± 14.1	91.5 ± 8.7*	96.3 ± 16.8	101.6 ± 4.7	121.8 ± 22.7
9.5	100.5 ± 15.7	91.4 ± 7.8*	95.5 ± 16.6	102.2 ± 4.4	121.0 ± 22.8
10	101.7 ± 16.0	91.1 ± 7.9*	95.8 ± 16.9	102.6 ± 4.1	122.2 ± 21.4
10.5	102.0 ± 17.0	91.4 ± 7.0*	95.1 ± 16.3	103.3 ± 4.0	122.5 ± 20.9
11	103.8 ± 18.7	92.0 ± 6.2*	94.5 ± 16.3	104.1 ± 4.5	123.5 ± 20.9
11.5	104.4 ± 19.8	91.7 ± 5.5	94.4 ± 15.7	103.7 ± 4.8	121.8 ± 22.1
12	105.0 ± 21.0	91.7 ± 4.9*	93.8 ± 14.6	104.9 ± 5.1	122.2 ± 20.8
12.5	103.9 ± 21.0	92.9 ± 5.6*	93.1 ± 14.5*	105.1 ± 5.2	121.7 ± 20.0
13	102.7 ± 18.1	92.7 ± 5.4**	92.2 ± 14.5*	105.5 ± 5.7	122.5 ± 16.5
13.5	104.5 ± 21.1	92.3 ± 4.3*	92.4 ± 14.2*	106.0 ± 6.3	120.3 ± 14.8
14	103.8 ± 20.0	92.6 ± 4.8**	92.9 ± 16.3*	106.3 ± 7.0	118.8 ± 12.2
14.5	105.6 ± 20.6	93.2 ± 4.3**	92.5 ± 15.7*	105.6 ± 6.1	116.9 ± 12.5
15	106.0 ± 19.5	94.4 ± 4.8*	93.0 ± 17.1	106.3 ± 7.5	112.7 ± 10.6
15.5	105.6 ± 19.0	93.7 ± 4.3*	92.9 ± 16.9	106.5 ± 7.5	112.2 ± 10.8
16	104.1 ± 19.5	93.7 ± 4.1**	93.9 ± 17.9	105.5 ± 7.6	112.4 ± 10.3
16.5	104.9 ± 18.7	93.0 ± 3.1*	92.5 ± 17.2	106.2 ± 6.8	112.2 ± 10.8
17	105.3 ± 18.5	94.8 ± 3.8*	90.9 ± 15.1*	106.4 ± 6.3	113.0 ± 10.4
17.5	104.6 ± 18.3	93.0 ± 3.3**	91.0 ± 14.9*	105.8 ± 5.9	113.4 ± 10.0
18	103.2 ± 17.4	93.6 ± 4.5**	90.8 ± 15.1*	106.8 ± 5.6	115.0 ± 8.9
18.5	104.4 ± 18.7	94.4 ± 4.1***	91.1 ± 15.6*	106.8 ± 6.2*	117.9 ± 7.1
19	105.7 ± 20.4	93.4 ± 3.4**	90.4 ± 15.7*	107.2 ± 6.8	114.0 ± 9.1
19.5	106.2 ± 20.0	94.4 ± 4.6**	91.9 ± 17.5	107.1 ± 6.1	114.9 ± 9.6
20	105.4 ± 19.2	93.9 ± 3.9**	91.9 ± 16.9	106.9 ± 5.7	115.0 ± 10.8

100% = initial value in time of intravenous administration – time 0

*Value is statistically significant at the significance level $P < 0.05$

**Value is statistically significant at the significance level $P < 0.01$

***Value is statistically significant at the significance level $P < 0.001$

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Table 3. Values of heart rate after the administration of tested agents and placebo at the dose of 3mg/kg

Time (min)	2FT2a	2FT2b	2FT2c	2FT2d	Placebo
–1	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0.5	84.1 ± 7.1**	89.5 ± 5.4*	68.7 ± 26.6	72.5 ± 15.6*	100.7 ± 1.4
1	93.7 ± 7.2	97.2 ± 4.8	87.0 ± 17.5	85.5 ± 20.4	101.4 ± 1.7
2	96.4 ± 5.7	99.4 ± 4.7	88.4 ± 16.4	97.9 ± 5.9	101.4 ± 1.7
3	97.7 ± 6.8	98.9 ± 3.7	87.4 ± 13.2	98.6 ± 8.2	101.4 ± 1.7
4	99.2 ± 5.5	98.4 ± 3.6	92.3 ± 8.0	98.9 ± 9.8	101.4 ± 1.7
5	102.0 ± 4.7	99.6 ± 4.8	92.9 ± 6.8	100.0 ± 9.4	100.7 ± 1.3
6	107.4 ± 8.0	99.6 ± 4.8	93.1 ± 6.7	101.7 ± 10.0	102.0 ± 1.7
7	108.0 ± 12.0	99.9 ± 5.2	93.2 ± 6.6*	102.3 ± 9.8	102.7 ± 1.4
8	109.3 ± 14.4	100.6 ± 5.0	93.8 ± 6.4*	102.2 ± 9.8	102.7 ± 1.3
9	109.0 ± 15.8	99.9 ± 5.2	93.4 ± 8.5	100.8 ± 10.4	102.7 ± 1.4
10	111.2 ± 18.1	99.9 ± 5.2	93.9 ± 8.5	101.0 ± 10.4	102.7 ± 1.3
12	112.2 ± 20.1	100.3 ± 4.5	94.1 ± 6.3	100.5 ± 10.4	102.7 ± 1.4
14	112.8 ± 21.2	100.9 ± 3.8	95.3 ± 6.7	100.2 ± 10.5	102.1 ± 1.7
16	113.7 ± 24.8	99.6 ± 3.4	94.6 ± 5.9	100.0 ± 11.0	101.4 ± 1.7
18	114.9 ± 25.4	99.0 ± 3.6	95.5 ± 6.8	99.1 ± 11.3	102.1 ± 1.7
20	115.7 ± 26.0	99.0 ± 3.6	96.6 ± 5.2	97.2 ± 12.8	102.0 ± 1.7

100% = initial value at the time of intravenous administration – time 0

*Value is statistically significant at the significance level $P < 0.05$

**Value is statistically significant at the significance level $P < 0.01$

age value was characterised by a large standard deviation.

Table 5 presents data on the changes in systolic heart pressure which were recorded over time after the administration of the 2FC2abcd substances. Fluorine substitution is common to this group of compounds. The administration of the compound 2FC2a resulted in a reduction in blood pressure lasting throughout the measuring period. The statistical significance of the reduction varied with time. Statistical assessment of the data suggests that within the interval of the first 8 minutes, blood pressure decreased by 20% and this reduction was highly significant with respect to the physiological value. A similarly highly significant decrease was observed within the first 7 minutes after the injection of the 2FC2b. In this case, the reduction extended for up to 15 minutes after the administration and was statistically significant up to the last 2 minutes of the measurement, which were characterised by slow decay towards the physiological value and high experimental variation. In contrast, the compound 2FC2c resulted elicited a reduction in blood pressure for only 2.5 minutes after administration. The most promising results were obtained for the compound 2FC2d, which resulted in a statistically significant reduction in pressure during for the first three minutes after injection. Subsequently, blood pressure increased to above the physiological value, as after the administration of placebo, before stabilising at the pre-injection level.

Table 6 summarises data on heart rate obtained after the administration of compounds 2FC2abcd. The substance 2FC2a reduced the heart rate in the first and second minute after administration while the subsequent values remained within the physiological limits throughout the investigated time interval. The effect of 2FC2b on heart rate was statistically insignificant throughout the experiment. Similar results were obtained for substance 2FC2c. The butyl derivative of 2FC2d reduced the heart rate during the first minute and later during the 10–12-minute timeframe, while at the remaining time points it did not elicit any statistically significant changes.

The effects of the 2FC2abcd group on the QRS complex interval are presented in Table 7. The sub-

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Table 4. Values of the QRS complex after the administration of tested agents and placebo at the dose of 3 mg/kg

Time (min)	2FT2a	2FT2b	2FT2c	2FT2d	Placebo
–1	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0.5	121.0 ± 7.9***	113.5 ± 15.9	123.3 ± 15.8*	135.2 ± 23.2*	96.9 ± 3.9
1	122.0 ± 10.7**	119.4 ± 10.3*	126.5 ± 10.2**	121.9 ± 30.7	97.8 ± 2.7
2	110.1 ± 11.6	114.0 ± 7.7*	118.0 ± 8.8*	120.3 ± 24.9	99.2 ± 8.4
3	105.6 ± 6.9	108.4 ± 6.9*	109.8 ± 10.2*	107.9 ± 20.8	96.0 ± 5.0
4	103.0 ± 6.0	103.7 ± 4.6	105.5 ± 9.1	106.0 ± 17.7	97.7 ± 11.7
5	102.0 ± 6.8	106.8 ± 6.3*	103.5 ± 12.5	104.3 ± 17.4	96.2 ± 4.7
6	102.0 ± 8.1	102.9 ± 11.3	103.4 ± 11.6	103.3 ± 17.0	97.8 ± 2.7
7	105.7 ± 5.7	106.7 ± 8.0	102.1 ± 14.2	104.8 ± 16.3	101.7 ± 7.1
8	102.0 ± 8.1	104.0 ± 10.5	100.2 ± 13.7	100.9 ± 15.2	96.2 ± 4.7
9	101.0 ± 8.0	102.2 ± 10.3	100.2 ± 13.7	100.1 ± 15.8	95.1 ± 9.0
10	100.0 ± 5.5	102.9 ± 11.3	100.2 ± 13.7	98.9 ± 13.1	93.4 ± 4.2
12	102.0 ± 8.1	104.8 ± 8.8	99.2 ± 12.9	100.1 ± 15.8	100.9 ± 9.6
14	102.0 ± 8.1	103.0 ± 8.7	99.9 ± 16.3	102.1 ± 13.6	92.4 ± 7.0
16	99.0 ± 9.2	102.4 ± 7.4	98.0 ± 15.4	97.4 ± 13.0	96.2 ± 4.7
18	99.0 ± 9.2	99.1 ± 7.8	99.9 ± 16.3	94.3 ± 14.6	94.1 ± 7.5
20	99.0 ± 9.2	102.2 ± 10.3	98.3 ± 17.4	93.3 ± 11.3	95.6 ± 7.8

100% = initial value at the time of intravenous administration – time 0

*Value is statistically significant at the significance level $P < 0.05$

**Value is statistically significant at the significance level $P < 0.01$

***Value is statistically significant at the significance level $P < 0.001$

stance 2FC2a exhibited a statistically significant broadening of the QRS interval from the start up to the 18th minute after the injection. The QRS duration was also prolonged in a statistically significant way after the administration of 2FC2b; in this case, up to the third minute, and its effect was observed up to the 14th minute. Similarly, the compound 2FC2c prolonged the duration of the QRS complex during the first 6 minutes, with the effect evident up to the 10th minute after the application. The strongest effect was obtained for the compound 2FC2d which extended QRS complex duration up to the 18th minute. The impact was statistically highly significant and the QRS was broadened to 150–200% of the physiological level.

DISCUSSION

The compounds investigated in this study belong to a class of drugs called β-blockers. This promising group of substances has been extensively studied in the Faculty of Pharmacy at the University of Veterinary and Pharmaceutical Sciences in Brno. Research has focused on ultra-short-acting substances modified by an ester bond on the side-chain. Esmolol, the drug used in clinical practice, exhibits its maximum effect 5 minutes after administration and has a biological half-life of 9 minutes. This drug has an ability to effectively block β-receptors for 10–30 minutes (Tengler and Stropnicky 2014). The aim of the present study was to find new substances with a faster impact that would be quickly decomposed in blood plasma and thus exert only short-term effects on biological systems (Marik and Varon 2009).

To this end, we selected two groups of compounds with similar molecular structures. The first group of 2FC2abcd substances is characterised by carbamate substitution on the main benzene nucleus. The substances differ from each other in their lipophilicity and alkyl chain length (-methyl, -ethyl, -propyl, -butyl) at the mentioned substitution (Keckesova et al. 2008a).

On the other hand, the second group of 2FT2abcd substances contains an ether substitution on the main

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Table 5. Values of systolic blood pressure after the administration of tested agents and placebo at the dose of 3 mg/kg

Time (min)	2FC2a	2FC2b	2FC2c	2FC2d	Placebo
–0.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0.5	65.7 ± 10.1**	74.8 ± 9.1*	69.6 ± 5.9***	68.7 ± 15.7*	90.2 ± 5.0
1	65.8 ± 6.1*	77.8 ± 11.2	64.8 ± 3.1*	60.6 ± 16.8*	100.5 ± 21.7
1.5	67.6 ± 7.3**	77.0 ± 12.6**	69.4 ± 4.3**	65.6 ± 12.4**	117.4 ± 18.4
2	69.6 ± 9.1***	80.5 ± 12.0**	78.2 ± 12.7**	70.5 ± 17.4**	125.2 ± 19.4
2.5	73.2 ± 8.6**	80.5 ± 11.0**	86.1 ± 17.8*	78.4 ± 18.1**	124.9 ± 19.2
3	75.6 ± 7.3**	78.6 ± 8.9**	91.0 ± 21.9	86.4 ± 21.2*	125.3 ± 22.0
3.5	76.5 ± 6.6**	78.5 ± 8.5**	93.6 ± 24.0	93.4 ± 18.8	125.1 ± 21.2
4	77.6 ± 6.3*	79.0 ± 8.4**	95.6 ± 24.8	103.5 ± 17.6	121.3 ± 22.3
4.5	78.1 ± 7.5**	78.7 ± 8.2**	98.4 ± 27.3	109.7 ± 19.4	120.8 ± 21.0
5	78.5 ± 7.0**	79.6 ± 8.7**	99.1 ± 28.9	115.3 ± 20.4	120.2 ± 20.3
5.5	79.4 ± 7.9**	80.1 ± 9.4**	99.6 ± 29.6	119.5 ± 22.1	120.9 ± 20.5
6	78.8 ± 10.1**	81.0 ± 10.1*	99.7 ± 29.6	122.5 ± 23.6	119.7 ± 20.9
6.5	79.9 ± 10.0**	82.0 ± 10.1**	98.7 ± 28.9	123.9 ± 24.0	122.9 ± 18.7
7	79.4 ± 10.9**	83.9 ± 9.2**	97.9 ± 29.0	125.2 ± 23.6	123.0 ± 20.8
7.5	80.6 ± 10.9**	86.0 ± 10.0*	96.8 ± 27.7	125.8 ± 24.0	123.4 ± 20.8
8	80.3 ± 11.8**	86.2 ± 11.8*	94.8 ± 27.0	127.0 ± 26.7	123.8 ± 20.9
8.5	81.0 ± 11.8*	86.2 ± 12.0*	94.6 ± 27.3	126.9 ± 27.5	121.3 ± 22.7
9	81.0 ± 12.0*	86.4 ± 13.6*	93.6 ± 26.9	126.2 ± 25.9	121.8 ± 22.7
9.5	82.5 ± 11.5*	87.2 ± 13.1*	93.0 ± 27.3	125.2 ± 25.1	121.0 ± 22.8
10	81.9 ± 13.0*	85.4 ± 15.2*	93.6 ± 27.4	124.3 ± 24.7	122.2 ± 21.4
10.5	81.2 ± 13.5*	86.2 ± 13.8*	93.4 ± 26.0	123.5 ± 24.0	122.5 ± 20.9
11	82.6 ± 11.8**	87.8 ± 14.7*	93.0 ± 28.2	122.3 ± 23.6	123.5 ± 20.9
11.5	83.0 ± 11.3*	89.9 ± 14.7*	92.9 ± 27.4	121.2 ± 22.9	121.8 ± 22.1
12	82.9 ± 10.7*	90.2 ± 14.9*	93.0 ± 26.8	120.7 ± 21.7	122.2 ± 20.8
12.5	85.0 ± 10.6*	91.3 ± 15.3*	93.5 ± 26.3	119.1 ± 20.8	121.7 ± 20.0
13	85.0 ± 10.1**	91.0 ± 14.2*	93.7 ± 25.9	119.7 ± 21.0	122.5 ± 16.5
13.5	87.0 ± 10.0**	91.4 ± 14.1*	94.0 ± 25.1	118.4 ± 20.5	120.3 ± 14.8
14	88.3 ± 11.1**	91.6 ± 15.6*	93.6 ± 24.8	117.0 ± 20.5	118.8 ± 12.2
14.5	88.6 ± 10.8**	91.2 ± 14.6*	92.9 ± 23.3	116.4 ± 19.1	116.9 ± 12.5
15	87.8 ± 11.8*	91.8 ± 14.7*	92.9 ± 22.8	115.8 ± 19.9	112.7 ± 10.6
15.5	89.9 ± 11.4*	92.2 ± 15.4	92.1 ± 22.6	114.0 ± 19.3	112.2 ± 10.8
16	90.8 ± 12.0*	93.7 ± 15.8	93.2 ± 22.1	113.1 ± 19.2	112.4 ± 10.3
16.5	91.7 ± 10.5*	93.9 ± 15.6	93.3 ± 20.4	112.0 ± 19.2	112.2 ± 10.8
17	90.8 ± 11.8*	93.5 ± 16.3	93.0 ± 20.2	111.5 ± 18.8	113.0 ± 10.4
17.5	91.4 ± 11.3*	92.5 ± 15.3	93.9 ± 19.8	109.4 ± 18.8	113.4 ± 10.0
18	89.6 ± 14.9*	92.7 ± 16.3	92.9 ± 19.5	108.3 ± 18.3	115.0 ± 8.9
18.5	91.1 ± 12.0**	92.3 ± 13.3**	93.0 ± 19.7*	106.8 ± 17.9	117.9 ± 7.1
19	90.7 ± 14.6*	93.7 ± 12.4*	93.3 ± 19.5	105.4 ± 17.5	114.0 ± 9.1
19.5	90.9 ± 12.2*	93.5 ± 12.4*	94.1 ± 19.6	104.4 ± 18.5	114.9 ± 9.6
20	88.7 ± 9.4**	94.4 ± 11.7*	94.6 ± 19.0	103.0 ± 18.4	115.0 ± 10.8

100% = initial value at the time of intravenous administration – time 0

*Value is statistically significant at the significance level $P < 0.05$

**Value is statistically significant at the significance level $P < 0.01$

***Value is statistically significant at the significance level $P < 0.001$

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Table 6. Values of heart rate after the administration of tested agents and placebo at the dose of 3 mg/kg

Time (min)	2FC2a	2FC2b	2FC2c	2FC2d	Placebo
–1	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0.5	93.5 ± 5.4	99.9 ± 4.5	93.8 ± 6.6	77.7 ± 19.1	100.7 ± 1.4
1	96.7 ± 2.9*	94.2 ± 12.1	96.1 ± 8.8	74.3 ± 19.4*	101.4 ± 1.7
2	97.4 ± 2.3*	100.8 ± 4.0	101.8 ± 17.5	68.3 ± 25.3	101.4 ± 1.7
3	98.8 ± 4.5	101.6 ± 4.2	100.0 ± 15.0	74.0 ± 31.3	101.4 ± 1.7
4	101.6 ± 5.4	99.3 ± 4.2	98.3 ± 14.8	74.4 ± 31.3	101.4 ± 1.7
5	103.5 ± 5.3	100.6 ± 3.6	97.4 ± 13.3	76.1 ± 33.4	100.7 ± 1.3
6	102.9 ± 5.8	100.6 ± 3.6	99.0 ± 13.9	74.0 ± 34.3	102.0 ± 1.7
7	101.5 ± 5.1	100.6 ± 3.6	100.3 ± 14.2	73.7 ± 34.0	102.7 ± 1.4
8	102.2 ± 6.3	101.4 ± 3.6	101.3 ± 14.3	90.4 ± 8.9	102.7 ± 1.3
9	102.1 ± 6.4	100.9 ± 2.8	98.7 ± 11.7	91.5 ± 9.4	102.7 ± 1.4
10	103.5 ± 7.2	101.5 ± 2.9	98.8 ± 14.7	89.7 ± 6.3*	102.7 ± 1.3
12	101.4 ± 6.4	102.2 ± 3.6	99.9 ± 19.1	92.2 ± 3.5*	102.7 ± 1.4
14	102.1 ± 6.0	102.4 ± 4.5	100.4 ± 23.6	94.9 ± 6.3	102.1 ± 1.7
16	102.8 ± 5.9	101.1 ± 5.9	102.3 ± 28.3	94.1 ± 7.0	101.4 ± 1.7
18	103.5 ± 5.3	101.8 ± 6.3	103.8 ± 28.9	96.2 ± 10.4	102.1 ± 1.7
20	100.8 ± 6.6	102.4 ± 5.5	102.8 ± 29.6	100.1 ± 11.9	102.0 ± 1.7

100% = initial value at the time of intravenous administration – time 0

*Value is statistically significant at the significance level $P < 0.05$

benzene nucleus and a similar alkyl chain as the first group. These substitutions in the para-position enhance the selectivity of β-blockers for β_1-receptors, increase the effect on the myocardium and minimise peripheral adverse reactions such as effects on the respiratory tract. A bulky substituent in an aliphatic part of the molecular chain serves to considerably increase the affinity towards β-adrenergic receptors. This effect is similar to the one provided by the structure of carvedilol and nebivolol (Frydrych et al. 2004; Tengler et al. 2013a; Tengler et al. 2013b).

The substitution by a single fluorine atom in the ortho-position yields an electron-donating group like in modern β-blockers of the third generation (Fumagalli et al. 2005). The tested compounds do not exhibit intrinsic sympathomimetic activity and do not contain any free hydroxyl groups in their main skeleton except the one in the connecting chain of the molecule. However, this hydroxyl group is common to all compounds which exhibit β-adrenergic effects.

It was confirmed that the 2FT2abcd substances administered at a dose of 3 mg/kg decreased the systolic blood pressure. We suggest that the weak effect of the methyl derivative results from an increased difficulty in permeating membranes. In the case of the other investigated parameters, the activity of 2FT2a also decayed immediately after administration (Keckesova et al. 2008b).

An unexpected result was obtained for the compound with ethyl substitution which, just after application, decreased the blood pressure considerably with blood pressure remaining at a level of about 5 to 10% below the starting value for the remainder of the experiment. At the same time, this substance did not show any negative chronotropic effects and any interference with the electrical conduction system of the heart was minimal. The more lipophilic compound containing the propyl substitution had an excellent impact on blood pressure immediately after the application, but the influence lasted into the second half of the experiment. This prolonged effect was probably associated with the retention of the tested compound in tissues. Similar to the compound 2FT2b, the initial drop of blood pressure down to about 50% was followed by a regress and stabilisation at a level of about 5 to 10% below the initial value. The substance did not significantly reduce the heart rate but did elicit the largest decline of heart rate from all the

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Table 7. Values of the QRS complex after the administration of tested agents and placebo at the dose of 3 mg/kg

Time (min)	2FC2a	2FC2b	2FC2c	2FC2d	Placebo
–1	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
0.5	146.2 ± 29.6*	132.5 ± 10.4***	139.4 ± 21.3*	156.7 ± 39.6*	96.9 ± 3.9
1	162.7 ± 30.0*	130.8 ± 13.4**	168.4 ± 29.8**	191.7 ± 37.7**	97.8 ± 2.7
2	146.6 ± 21.7**	127.9 ± 10.7**	145.7 ± 15.7***	214.1 ± 25.8***	99.2 ± 8.4
3	140.6 ± 12.3***	122.3 ± 4.6***	140.3 ± 14.4***	199.0 ± 38.5**	96.0 ± 5.0
4	134.5 ± 11.2**	113.5 ± 8.4	135.1 ± 13.9**	188.6 ± 44.2*	97.7 ± 11.7
5	132.5 ± 17.5*	110.8 ± 8.7*	124.2 ± 6.3***	173.6 ± 25.7**	96.2 ± 4.7
6	123.8 ± 13.0*	107.9 ± 7.4*	115.3 ± 5.5***	159.7 ± 19.9**	97.8 ± 2.7
7	118.3 ± 9.4*	106.8 ± 5.1	115.3 ± 5.5*	150.0 ± 14.0***	101.7 ± 7.1
8	115.2 ± 9.6**	108.8 ± 5.9**	109.7 ± 7.5*	131.3 ± 14.0**	96.2 ± 4.7
9	113.2 ± 8.3*	103.9 ± 4.7	103.9 ± 7.2	130.4 ± 10.1***	95.1 ± 9.0
10	114.5 ± 10.2**	105.9 ± 6.5*	103.9 ± 7.2*	123.6 ± 10.5***	93.4 ± 4.2
12	110.1 ± 8.4	103.9 ± 4.7	103.9 ± 7.2	120.5 ± 11.2*	100.9 ± 9.6
14	109.0 ± 7.7*	103.9 ± 4.7*	103.9 ± 7.2	116.8 ± 10.9**	92.4 ± 7.0
16	109.9 ± 6.8*	102.9 ± 4.9	102.9 ± 7.4	115.6 ± 9.4**	96.2 ± 4.7
18	109.2 ± 8.7*	101.9 ± 4.8	108.2 ± 11.7	111.6 ± 8.8*	94.1 ± 7.5
20	108.3 ± 10.5	103.9 ± 4.7	108.2 ± 11.7	107.9 ± 8.6	95.6 ± 7.8

100% = initial value at the time of intravenous administration – time 0

*Value is statistically significant at the significance level $P < 0.05$

**Value is statistically significant at the significance level $P < 0.01$

***Value is statistically significant at the significance level $P < 0.001$

tested ether derivatives. It also exerted the most pronounced arrhythmogenic effects and the QRS duration was slightly prolonged. We may assume that these phenomena may be caused by a blocking of ion channels, similar to substances tested in the last few years (Brunton et al. 2006; Basgut et al. 2010; Tengler et al. 2013b). Butyl derivatives exhibited the most promising effects in the present study, in that they reduced blood pressure without any interference with other heart parameters investigated. The compound 2FT2d decreased blood pressure markedly up to 5.5 minutes, after which blood pressure returned to the physiological range. It is expected that lipophilic compounds permeate cytoplasmic membranes rather easily and are quickly cleaved in the cytoplasm of erythrocytes (Potter and Wadkins 2006; Zicha et al. 2006).

The strong antihypertensive effect in combination with almost no effect on the electrical conduction system of the heart can be explained by the above-mentioned α-adrenolytic effect. The mechanism operating on the molecular level may thus involve two processes: the first one involves blocking β-receptors in working myocardium, and the second involves blocking presynaptic alpha receptors in arterioles. Although the 2FT2d molecule is the most lipophilic in the investigated set of compounds, it does not insert into the cytoplasm membrane, does not bind to ion channels and does not show any negative bathmotropic and dromotropic effects at the tested dosage (Bartosova et al. 2004a). The compound 2FT2d is thus the most promising candidate for the managing hypertensive crises with minimal effect on myocardial contractility (Brunton et al. 2006; Tengler et al. 2013a).

We can also confirm that substances 2FC2abcd administered at a dose of 3 mg/kg decreased systolic blood pressure. In comparison to the ether group, this carbamate group exerted a stronger impact on the electrical conduction system of the heart and extended the QRS complex as compared to the placebo.

In contrast to 2FT2a, the methyl derivative 2FC2a had a significantly stronger effect on the systolic blood pressure. Immediately after administration, blood pressure dropped to about 30% of the initial

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value. This drop was followed by regression and stabilisation at about 5 to 10% below the initial level. Interestingly, and in accordance with a previous study (Bartosova et al. 2004b), the tested substance slowed the conduction of the impulse and influenced the duration of QRS complex without changing the heart rate. The ethyl derivative 2FC2b also exhibited prolonged antihypertensive effects.

As compared to the 2FC2a compound, the decrease in blood pressure elicited by 2FC2b was not so pronounced; this compound also did not change heart rate and exerted a similar effect on the QRS complex. As the length of the substituted chain and the lipophilicity increased, the duration of the effect on blood pressure was shortened, while the duration of the QRS interval and the conduction of the impulse in myocardium were extended. Some β-blockers exhibit a stabilising effect on cellular membranes. Especially more lipophilic derivatives have shown an ability to block Na^+ channels and thus influence the propagation of the action potential in myocardium. β-blockers can influence membrane channels either directly or indirectly via the reduced activation of β-adrenergic receptors, resulting in reduced activation of membrane channels. The two mechanisms can operate simultaneously (Bartosova et al. 2004b; Keckesova et al. 2008b; Bado et al. 2014).

The compound 2FC2c decreased blood pressure for 2.5 minutes following injection. This decrement was statistically highly significant. For the remainder of the experiment, blood pressure remained below initial values, at a level which was about 5 to 10% lower than the pre-injection measurement. We consider this difference as statistically insignificant. The propyl-derivative had no influence on heart rate but strongly modified the ECG curve. This derivative caused a statistically highly significant broadening (from 30 to 40%) of the QRS interval. This effect might be explained by bundle branch block. The most probable reason is right bundle branch block leading to deceleration of the depolarisation of the right ventricle, and also the left ventricle when the block is bifascicular. These late activations of the ventricle muscle lead to characteristic changes of the QRS curve termed RR breaks and the T-wave inversion (Styk 2013; Bado et al. 2014).

The butyl derivative 2FC2d elicited a statistically significant decrease in blood pressure up to the third minute only while after the fifth minute blood pressure increased above the initial values. Thereafter, blood pressure gradually returned to the physiological range, similarly to the placebo data.

This reaction of the organism can be explained on a basis of the internal adaptive mechanism (autoregulation) that is sets in motion when the decrease of systolic blood pressure is large and sudden. We expect that the sympathetic nervous system increases its activity and that the level of stress hormones rises due to their faster release. At the same time, this most lipophilic substance could stabilise membranes into which it can insert easily. If this process is fast enough, it could prevent the butyl derivative from swift decomposition in the cytoplasm of erythrocytes (Frydrych et al. 2004).

This hypothesis can be supported by observing the effects over time: the hypotension period induced by a passivation of β-adrenergic receptors or presynaptic α receptors seems to be very short as compared to the period in which the broadening of QRS complex was recorded (Bado et al. 2014).

2FC2d did not exert any statistically significant effects on heart rate, mainly because a large standard deviation did not allow a straightforward interpretation of the data. This substance had the most marked effect on the electrical conduction system of the heart and exhibited negative bathmotropic and dromotropic effects. The width of the QRS complex increased by 50 to 100% instantly after administration, lasting for 7 minutes, while up to the 10^{th} minute it was at a significance level of $P < 0.001$. Arrhythmogenic compounds caused fatal arrhythmia in a couple of instances and these measurements were not included in the assessment. We have closely followed a protocol compliant with Law 246/1992 on the protection of animals against abuse. For this reason, the final results have a high standard deviation caused by the low number of experiments in this group. β-adrenoreceptor antagonists possess high internal variability, which was also confirmed by a number of earlier studies (Cuneo et al. 1994; Bartosova et al. 2004a; Wada et al. 2016).

Due to their arrhythmogenic and also antiarrhythmic potential, these substances could potentially be administered during surgical interventions, in preoperative preparations, in treatments of supraventricular tachycardia and in the management of hypertensive crisis.

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Hypertensive crisis is a typical condition accompanying cardiovascular surgery, surgery of main blood vessels, neurosurgery and head injuries. This condition also occurs after surgical intervention as a consequence of increased sympathetic tone and increased vascular resistance (Varon 2008; Yamakage et al. 2009; Wada et al. 2016).

The different pharmacological and toxicological effects of racemate result from the structures of enantiomers. Since an organism is considered a chiral medium, the interaction of these substances with enzymes or receptors differs and is determined by their differing potentials to undergo stereospecific reactions with biomolecules in the organism (Cizmarikova 2002; Keckesova et al. 2008a).

Differences in interactions lead to diversity in pharmacodynamics and pharmacokinetics that can be further manifested as in different rates of absorption, distribution, biotransformation or elimination. All the tested compounds in this study were applied in the form of racemates. Application of pure enantiomers in follow-up experiments should be considered in order to clarify the contributions to individual effects and the effects of biotransformation on the chemical structure (Bartosova et al. 2008; Mokry et al. 2009). β-adrenoreceptor antagonists from the aryloxyaminopropanol group are more effective in the (S)-(–) form. This form is identical with the configuration of physiological norepinephrine and substances in this configuration exhibit 10 to 100 times higher selectivity for β-receptors (Cizmarikova 2002; Bado 2009; Styk 2013).

In conclusion, in this study, we have compared two groups of new aryloxyaminopropanol compounds which exhibit potential as ultra-short-acting β-blockers. The two groups differed in the carbamate substitution and in the length of the alkyl chain, but the tested molecules were otherwise identical. We have confirmed short-term hypotensive effects of these compounds and effects on the electrical conduction system of the heart, while no effect was observed concerning heart rate. The results justify limiting the selection for follow-up testing to the substances 2FT2c, 2FT2d and 2FC2c, mainly due to their superior performance and short time frames of action. The tables presented in this work may facilitate assessment of the influence of chemical modifications on the pharmacokinetics and pharmacodynamics of the individual molecules and predictions of future developments regarding drugs affecting the cardiovascular system.

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