# Influence of New Ultrashort-Acting Beta-Adrenergic Blockers on Systolic Blood Pressure in Rats

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**Abstract**

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Three newly synthesized potential ultrashort-acting beta adrenergic blockers containing metabolically unstable ester functional groups that are easily cleft by plasma esterases were tested. In the experiment, 30 laboratory rats divided into 4 subgroups were used. Agent 42Bu was administered to Group 1 (n = 8), agent 43Bu to Group 2 (n = 8), agent 44Bu to Group 3 (n = 8) and placebo to Group 4 (n = 6). Under general anesthesia the arteria carotis and vena jugularis were exteriorized. The arteria carotis was connected by a cannula to the machine HSE UNIPER UP – 100, a universal perfusion system for isolated organs recording and converting actual values of blood pressure into graphical representation in a computer. Subsequently, 42Bu, 43Bu, 44Bu and placebo were administered into v. jugularis, and systolic blood pressure was recorded within the period of 18 minutes of their administration. All agents were administered at 2.5 mg/kg doses. The systolic blood pressure values after 42Bu, 43Bu, 44Bu administration were compared to those of the placebo Group. The 42Bu caused a statistically significant decrease ($p < 0.05$) in systolic blood pressure 1 minute after administration, 43Bu 1.5 minute after administration, and 44Bu 9 minutes thereafter. Statistically significant decrease of systolic blood pressure began immediately after administration of all three tested substances.

The results of our *in vivo* testing show that agent 44Bu was the most effective of all three tested agents. Its onset of action was rapid and the hypothesis of ultrashort action was confirmed.

*Pharmacology, plasma esterases, ester functional group, universal perfusion system, hypotensive effect*

Beta blockers are one of the main pharmacotherapeutical group in the treatment of cardiovascular diseases (Bartošíková et al. 1998; Nečas et al. 1997). They are indicated in the treatment of hypertension, portal hypertension, angina pectoris, some types of arrhythmias, idiopathic cardiac myopathy and in some non-cardiac disturbances, e.g. hyperthyroidism, glaucoma and some neurological indications (Hynie 1997). In patients dependent on the sympathetic tonus adverse reactions can appear typical of classical beta blockers, e.g. hypotension, bradycardia, heart failure, bronchospasm or peripheral vasoconstriction, whose effect can be manifested for several hours after intravenous administration (Mc Devitt 1979). For that reason, researchers have developed ultrashort-acting beta blockers, whose advantage is in the immediate onset of action after administration, possibility of dose titration, very short duration of action and rapid offset of the action (Barbier et al. 1995). The ultrashort-acting beta blockers are parenteral agents that can be rapidly titrated in clinical situations where immediate beta adrenergic blockade is warranted (Frishman et al. 1998). Their efficacy has been shown in specific clinical settings, e.g. in patients with unstable angina, myocardial infarction, atrial fibrillation or flutter and supraventricular tachycardia (Barbier et al. 1995). Esmolol, the prototype drug of this class, has been approved for treatment of supraventricular

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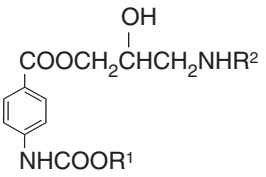
tachyarrhythmias, but has also potential use in the treatment of patients with perioperative hypertension and acute myocardial ischemia (Frishman et al. 1998). Landiolol and flestolol are other agents in the group of ultrashort-acting beta blockers.

Three potential ultrashort-acting beta blockers with working names 42Bu, 43Bu and 44Bu have been synthesised at the Department of Chemical Drugs of the Faculty of Pharmacy of the University of Veterinary and Pharmaceutical Sciences in Brno, see Fig. 1 (Mokrý et al. 2002).




| Agent | $R^1$ | $R^2$ | MW (g/mol) |
|---|---|---|---|
| 42Bu | $C_2H_5$ | $CH_2(CH_2)_2CH_3$ | 374.87 |
| 43Bu | $C_3H_7$ | $CH_2(CH_2)_2CH_3$ | 388.90 |
| 44Bu | $C_4H_9$ | $CH_2(CH_2)_2CH_3$ | 402.92 |

Fig. 1. Chemical structures of the three tested agents

The tested agents were synthesised by 5-step synthesis, and their structure has been verified by elementary analysis, infrared $^1$H-NMR and $^{13}$C-NMR spectroscopy (Bartošová et al. 2002). The tested agents are chemically similar to esmolol, the prototype drug of ultrashort-acting beta blockers. The extremely short duration of action of the agents was secured by the incorporation of a metabolically unstable ester functional group into their structure. The presence on the aromatic ring of a metabolically unstable ester functional group, which is very easily cleft by plasma esterases, leads to the ultrashort duration of action of the tested agents.

The aim of this *in vivo* testing was to monitor the influence of three newly synthesised compounds with the working names 42Bu, 43Bu and 44Bu at doses of 2.5 mg/kg of body weight on the values of systolic blood pressure of laboratory rats.

## Materials and Methods

In the experiment, 30 Wistar male rats of the same age (60 days) and comparable body mass (327 ± 25 g) were used. The animals came from a conventional breeding colony (Faculty of Medicine, Masaryk University, Brno). They were placed in PVC cages (n = 3), fed a standard diet (Diet for small laboratory animals SPF M1) and given water *ad libitum*. After 12 days of acclimatization, the animals were randomly divided into 4 groups: Group 1 (8 rats) was given agent 42Bu, Group 2 (8 rats) was given agent 43Bu, Group 3 (8 rats) was given agent 44Bu and Group 4 was given placebo. In general anesthesia the arteria carotis and vena jugularis were exposed and exteriorized. A. carotis was connected by a cannula to the HSE UNIPER UP – 100 device, which is a universal perfusion system for isolated organs recording and converting blood pressure values into graphical representation in a computer. Subsequently, the tested agents were administered into v. jugularis (approximately 15 min from a. carotis and v. jugularis exteriorization) as a bolus and the values of systolic blood pressure were recorded within the period of 18 min of their administration.

The solution for anaesthesia was administered i.m. in the femur area at a dose of 0.5 ml/100 g of body mass and was composed of: xylazinum (Rometar® 2% inj.) and ketamini hydrochloridum (Narkamon® Spofa 1% inj.) in ratio 1:20. All tested agents were administered at doses of 2.5 mg/kg of body mass as a bolus at a 1 ml dose. As a vehicle for tested agents, a 5% solution of dimethylsulfoxide in saline (Infusio natrii chlorati isotonica Infusia®) was used. The placebo group was administered only 1 ml of 5% solution of dimethylsulfoxide in saline. The experiment was always performed in the morning. First, experiment with placebo group was performed followed by the experiments with Group 1, Group 2 and Group 3, respectively.

The project was approved and monitored by the local University Ethical Committee.

## Statistics

Statistical evaluation was performed using the Microsoft Excel spreadsheet. The statistical significance of differences between the three tested groups and the placebo group was evaluated using Student's *t*-test where $p < 0.05$ was considered as significant and $p < 0.01$ was considered as highly significant. The obtained values of systolic blood pressure were converted to percentage changes of systolic blood pressure in relation to initial values, where initial values of 100% were assessed before administration of the tested agents (placebo).

Table 1
Values of systolic blood pressure (%) after the administration of tested agents and placebo

| Time (min) | 42Bu | 43Bu | 44Bu | Placebo |
|---|---|---|---|---|
| -0.5 | 100.0 ± 0.00 | 100.0 ± 0.00 | 100.0 ± 0.00 | 100.0 ±0.00 |
| 0.0 | 100.0 ± 0.00 | 100.0 ± 0.00 | 100.0 ± 0.00 | 100.0 ± 0.00 |
| 0.5 | 89.7 ± 3.81** | 91.5 ± 2.78** | 87.1 ± 4.11** | 96.2 ± 2.37 |
| 1.0 | 98.0 ± 3.40* | 97.7 ± 3.48* | 86.3 ± 5.95** | 102.5 ± 2.16 |
| 1.5 | 102.7 ± 3.22 | 100.4 ± 4.72* | 85.7 ± 5.60** | 106.5 ± 3.12 |
| 2.0 | 105.1 ± 3.40 | 102.3 ± 5.19 | 86.1 ± 5.69** | 108.0 ± 4.21 |
| 2.5 | 106.1 ± 3.27 | 103.9 ± 5.63 | 86.2 ± 6.47** | 108.3 ± 4.73 |
| 3.0 | 106.7 ± 3.48 | 105.0 ± 5.78 | 86.5 ± 6.83** | 108.1 ± 5.13 |
| 3.5 | 106.7 ± 3.45 | 105.5 ± 6.18 | 87.9 ± 7.08** | 108.3 ± 5.04 |
| 4.0 | 106.8 ± 3.32 | 106.0 ± 6.27 | 88.8 ± 8.23** | 108.3 ± 5.04 |
| 4.5 | 106.6 ± 3.97 | 106.0 ± 6.27 | 88.9 ± 7.75** | 108.0 ± 5.61 |
| 5.0 | 106.4 ± 4.20 | 106.3 ± 6.34 | 90.2 ± 8.89** | 108.0 ± 5.61 |
| 5.5 | 106.2 ± 4.33 | 106.1 ± 6.20 | 91.7 ± 9.19** | 108.0 ± 5.61 |
| 6.0 | 105.7 ± 4.23 | 105.6 ± 5.97 | 92.8 ± 10.01** | 108.0 ± 5.61 |
| 6.5 | 105.1 ± 4.33 | 105.5 ± 5.77 | 91.5 ± 11.77* | 107.9 ± 5.67 |
| 7.0 | 104.4 ± 4.58 | 104.9 ± 5.44 | 91.8 ± 12.93* | 107.4 ± 5.81 |
| 7.5 | 104.1 ± 4.54 | 105.1 ± 5.36 | 93.3 ± 11.17* | 107.2 ± 5.94 |
| 8.0 | 103.9 ± 4.73 | 104.5 ± 5.00 | 95.5 ± 8.87* | 106.9 ± 5.58 |
| 8.5 | 103.7 ± 4.69 | 103.8 ± 4.93 | 97.5 ± 6.85* | 106.2 ± 5.57 |
| 9.0 | 103.5 ± 4.59 | 103.2 ± 4.70 | 98.8 ± 7.03* | 105.7 ± 5.75 |
| 9.5 | 103.5 ± 4.40 | 103.3 ± 4.03 | 99.5 ± 7.55 | 105.3 ± 5.39 |
| 10.0 | 103.1 ± 3.99 | 103.2 ± 4.06 | 100.0 ± 7.88 | 104.5 ± 5.25 |
| 10.5 | 103.2 ± 3.87 | 102.8 ± 3.94 | 99.8 ± 8.01 | 104.3 ± 5.07 |
| 11.0 | 103.2 ± 3.89 | 102.5 ± 3.90 | 99.8 ± 8.20 | 104.5 ± 5.25 |
| 11.5 | 103.1 ± 3.78 | 102.2 ± 3.84 | 99.6 ± 8.19 | 104.3 ± 5.15 |
| 12.0 | 103.0 ± 3.89 | 101.8 ± 3.56 | 98.9 ± 8.16 | 104.3 ± 5.15 |
| 12.5 | 103.1 ± 4.13 | 101.5 ± 3.24 | 100.6 ± 8.42 | 104.3 ± 5.01 |
| 13.0 | 103.2 ± 4.12 | 101.0 ± 2.78 | 100.4 ± 8.71 | 104.3 ± 5.01 |
| 13.5 | 103.3 ± 3.99 | 100.9 ± 2.69 | 100.6 ± 8.45 | 104.3 ± 5.06 |
| 14.0 | 103.3 ± 3.99 | 100.8 ± 2.53 | 100.4 ± 8.54 | 104.3 ± 5.06 |
| 14.5 | 103.2 ± 3.98 | 100.5 ± 2.37 | 100.5 ± 9.15 | 102.4 ± 3.99 |
| 15.0 | 91.9 ± 30.86 | 100.4 ± 2.59 | 101.5 ± 7.46 | 104.0 ± 4.77 |
| 15.5 | 103.1 ± 3.98 | 100.4 ± 2.59 | 101.5 ± 7.37 | 103.7 ± 4.62 |
| 16.0 | 103.0 ± 3.98 | 100.4 ± 2.59 | 101.9 ± 7.45 | 103.6 ± 4.52 |
| 16.5 | 102.9 ± 4.00 | 100.2 ± 2.76 | 102.0 ± 7.46 | 103.5 ± 4.52 |
| 17.0 | 102.6 ± 4.07 | 100.1 ± 2.64 | 102.1 ± 7.49 | 103.2 ± 4.41 |
| 17.5 | 102.6 ± 4.04 | 100.0 ± 2.60 | 102.0 ± 7.43 | 103.0 ± 4.38 |
| 18.0 | 102.6 ± 4.04 | 100.0 ± 2.60 | 102.0 ± 7.43 | 102.8 ± 4.25 |

- 0.5 min = time before tested agents and placebo administration
0 min = time of tested agents and placebo administration
* = the value is significantly different at $p < 0.05$ against placebo
** = the value is significantly different at $p < 0.01$ against placebo

## Results

The results are given in Table 1 (see previous page).

### Systolic blood pressure values after placebo administration

Immediately after placebo administration there was a mild decrease of blood pressure, which was most expressive in the 0.5 min after administration – 96% of the initial value. In the following minutes there was a mild elevation of systolic blood pressure to 108%, which slightly decreased to 102%.

### Systolic blood pressure values after a 2.5 mg/kg dose of the 42Bu agent administration

Agent 42Bu caused a significant ($p < 0.01$) decrease in blood systolic pressure compared to the placebo 0.5 min after administration. One minute after administration, 42Bu caused a significant decrease ($p < 0.05$). In the following minutes, 42Bu did not differ from the placebo.

### Systolic blood pressure values after a 2.5 mg/kg dose of the 43Bu agent administration

Agent 43Bu caused a significant decrease ($p < 0.01$) in blood systolic pressure compared to the placebo 0.5 min after administration. One and a half minutes after administration, 43Bu caused a significant decrease ($p < 0.05$). In the following minutes, 43Bu did not differ from the placebo.

### Systolic blood pressure values after a 2.5 mg/kg dose of the 44Bu agent administration

Agent 44Bu caused a significant decrease ($p < 0.01$) in blood systolic pressure compared to the placebo 6 minutes after administration. Nine minutes after administration, 44Bu caused a significant decrease ($p < 0.05$). In the following minutes, 44Bu did not differ from the placebo.

The most marked decrease of systolic blood pressure in all three tested agents appeared 1.5 min after administration (85.7% of initial value).

## Discussion

We tested *in vivo* three new potential ultrashort-acting beta blockers with working names 42Bu, 43Bu and 44Bu. These substances belong to the group of aryloxyaminopropanole analogues. The effects of agents 42Bu and 43Bu did not markedly differ from the placebo. Only in the first minute after administration, they caused a 5-6% stronger decline of systolic blood pressure than the placebo, and then subsequently the values of agents 42Bu and 43Bu did not significantly differ from the placebo. Agent 44Bu caused a significant decrease of systolic blood pressure lasting for 9 minutes after administration. This was the most marked and longest decrease of all the tested agents. We presume that this decrease was due to its highest lipophilicity of all the tested agents. With regard to the high lipophilicity of agent 44Bu and its influence upon ion membrane channels, we also assume that agent 44Bu contributes to a quite pronounced membrane stabilizing activity (MSA).

The longer pharmacodynamic effect of agent 44Bu, in contrast to agents 42Bu and 43Bu, is evidently due to slower washing out from the tissue and higher lipophilicity. In contrast with esmolole (Flaherty et al. 1989), whose effect starts 5 minutes after administration, all the tested agents have shown their hypotensive effect immediately after administration. The effect of esmolole fades away in 30 minutes after administration (Angaran et al. 1986; Blanski et al. 1988; Reilly et al. 1985) but agent 44Bu affected

blood pressure 9 minutes after administration. We consider both of the above mentioned features of agent 44Bu – immediate effect on blood pressure and rapid fading away of its therapeutical effect – as beneficial and very promising for further laboratory *in vivo* testing of other pharmacodynamic and pharmacokinetic parameters of agent 44Bu.

## Vliv nových ultrakrátce působících beta adrenergních blokátorů na systolický krevní tlak laboratorního potkana

V této experimentální práci byly testovány tři nově syntetizované ultrakrátce působící beta adrenergní blokátory obsahující ve své struktuře esterovou funkční skupinu, která je snadno štěpitelná plazmatickými esterázami. Testovaná skupina zvířat obsahovala 30 laboratorních potkanů rozdělených do tří podskupin. Látka 42Bu byla podávána první skupině (8 potkanů), látka 43Bu druhé skupině (8 potkanů), látka 44Bu třetí skupině (8 potkanů) a čtvrté skupině bylo podáváno placebo (6 potkanů). V celkové anestézii byly vypreparovány arteria carotis a vena jugularis. Arteria carotis byla připojena na přístroj HSE UNIPER UP-100, což je univerzální perfúzní systém pro izolované orgány snímající a převádějící aktuální hodnoty krevního tlaku na grafický záznam do PC. Následně byly do vena jugularis jednorázově injikovány roztoky látek 42Bu, 43Bu, 44Bu a placeba a hodnoty krevního tlaku byly snímány po dobu 18 minut od aplikace. Látky 42Bu, 43Bu a 44Bu byly aplikovány v dávce 2,5 mg/kg. Zkoumané látky byly porovnány proti placebu.

Látka 42Bu zaznamenala statisticky významný rozdíl v poklesu systolického krevního tlaku po dobu 1 minuty od aplikace, látka 43Bu 1,5 minuty od aplikace a látka 44Bu 9 minut od aplikace. Statisticky významný pokles krevního tlaku nastal u všech tří testovaných sloučenin bezprostředně po aplikaci. Výsledky našeho *in vivo* testování ukazují, že látka 44Bu vykazuje nejvýraznější efekt ze všech tří testovaných látek. Nástup jejího účinku byl rychlý a byla potvrzena hypotéza ultrakrátkého účinku.

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