Effect of Newly Synthesized Compounds 44Bu and 444 on QRS-Complex Width and Fast Sodium Current: Differences between Isomers

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Abstract

Two newly synthesized short-acting agents 44Bu and 444 were observed to suppress the aconitine-induced arrhythmias and block the fast sodium current I_{Na} in the rat heart. No data about their effect on the electrocardiographic parameters are available. In this study, we explored the effect of both racemates and particular isomers of 44Bu and 444 on the QRS-complex width *in vivo* in rats and on I_{Na} in isolated rat ventricular myocytes. All variants of 44Bu and 444 (1.5 mg/kg) caused a significant QRS-widening reaching the peak effect at the 1st or 2nd min after their intravenous administration. 44Bu racemate widened the QRS-complex from 16.8 ± 0.4 to 26.3 ± 0.5 ms (by 57%), significantly more than R- (33%-widening) and S-isomer (36%-widening). 444 racemate widened the QRS-complex from 20.8 ± 1.0 to 34.1 ± 0.9 ms (by 64%), which was comparable to S-isomer (63%-widening), however, substantially more than R-isomer (40%-widening). Regarding the effect on I_{Na}, 44Bu caused a significantly deeper I_{Na}-block compared to 444 when applied at the same concentration of 3 μmol/l (~0.1 mg/kg). 44Bu racemate and R-isomer blocked I_{Na} similarly (91.7 ± 0.8 and 91.8 ± 1.6%-block, respectively) and significantly more than S-isomer (82.4 ± 2.3%-block). 444 R-isomer blocked I_{Na} less than racemate and S-isomer (by 31.7 ± 3.9% vs. 48.3 ± 4.7 and 50.2 ± 4.1%, respectively). We conclude that both racemates and particular isomers of 44Bu and 444 induce a QRS-widening and block I_{Na} in the rat heart, however, their effects notably differed. The relative widening of the QRS-complex after application of 44Bu did not conform to the level of I_{Na}-block observed in isolated cardiomyocytes which stresses the importance of *in vivo* experiments in the pre-clinical testing of new drugs.

444; 44Bu; isomer; QRS-complex; sodium current; rat

Despite progressive development and successful use of invasive techniques, the pharmacological treatment is still considered to be the preferred method in many patients suffering from arrhythmias (e.g. Callans 2008) or, in some cases, a combined treatment is desirable (e.g. Singh and Murawski 2007). Unfortunately, beside the beneficial effects, a risk of cardiac adverse effects including life-threatening arrhythmias is related to the use of a majority of the available antiarrhythmic drugs. Therefore, a development of novel agents with antiarrhythmic potency is needed (Roden and Anderson 2006). In the case of acute intravenous treatment, agents with a short period of action are beneficial because they allow to precisely adjust the effective concentration and to readily interrupt the administration if adverse effects appear (e.g. Barbier et al. 1995; Yoshida et al. 2008).

Several new short-acting agents with a prominent antiarrhythmic effect have been synthesized at the Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic. The compounds with draft names 44Bu and 444, which were tested in this study, are derivates of aryl-carbonyl-oxy-amino-propanols varying in the substituent in the aliphatic chain; 44Bu contains *n*-butyl whereas 444 *terc*-butyl (Fig. 1; Mokrý et al. 2003). 44Bu was reported to suppress the aconitine-induced

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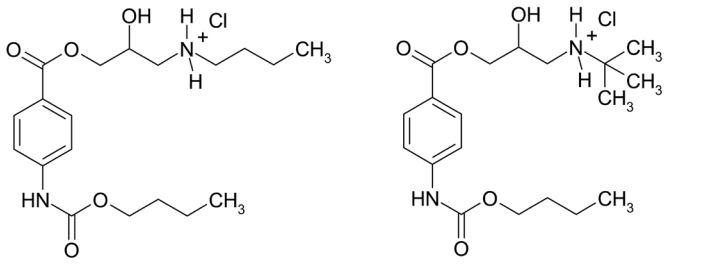

Fig. 1. Chemical structure of 44Bu and 444. These compounds are functional analogues of aryloxyaminopropanols modified by the *n*-butyl and *terc*-butyl in the case of 44Bu and 444, respectively.

arrhythmias more efficiently than lidocaine, a class-Ib antiarrhythmic drug (Bartosova et al. 2007). The antiarrhythmic effect of 44Bu persisted for about 15 min. In recent experiments, 444 has been observed to suppress the aconitine-induced arrhythmias similarly to 44Bu, however, its effect lasted a little longer (unpublished data). Unfortunately, no data related to changes in basic electrocardiographic (ECG) indicators in presence of 44Bu and 444 are available. Both these agents were documented to considerably block the fast sodium current I_{Na} (Bartosova et al. 2006) which might, at least partly, explain their antiarrhythmic potency.

The currently used drugs are usually available as the racemic mixture (or racemate), i.e. as the equimolar mixture of R- and S-isomer. The above mentioned effects of 44Bu and 444 on the cardiac electrophysiology were also studied only in presence of their racemates. However, the particular isomers of an optically active compound are often characterized by notably different pharmacokinetic and pharmacodynamic properties (Kulig et al. 2004). Nowadays, a tendency to develop and test new drugs in the form of pure isomers is obvious (e.g. Agrawal et al. 2007). In the case of 44Bu, the S-isomer has been recently observed to suppress the aconitine-induced arrhythmias more efficiently compared to the R-isomer whereas the racemate exerted the best effect in suppressing ventricular fibrillation (Bartosova et al. 2008). As mentioned above, the antiarrhythmic effect of 44Bu and 444 might result from their potency to block I_{Na}. Therefore, we decided to explore the level of I_{Na}-block under the effect of the racemates and also the particular R- and S-isomers of 44Bu and 444 at the same concentration of 3 μmol/l (for details see 2.1.). We also analyzed the QRS-complex width, a measure of the excitation velocity through the cardiac tissue which should correlate with I_{Na}-block, in absence and presence of all the 44Bu and 444 variants at a dose of 1.5 mg/kg.

Materials and Methods

Tested substances

The chemical structure of 44Bu and 444 was verified by the elementary analysis, IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy (Mokrý et al. 2001), purity was verified chromatographically (Mokrý et al. 2003), and elementary physical characteristics were determined (Opatrilova et al. 2005).

The tested substances were dissolved in sterile isotonic 0.9% NaCl solution for infusion in *in vivo* experiments. In the case of *in vitro* measurements of their effect on I_{Na}, 44Bu and 444 were prepared as 1 mM stock solution in deionized water and diluted to the final concentration of 3 μmol/l before each experiment. The concentration of 3 μmol/l corresponds to ~0.1 mg/kg, thus, it is 15 × lower than the concentration used in *in vivo* experiments. It was selected with the purpose to reveal prospective differences among the level of I_{Na}-block in presence of all the optical variants of 44Bu and 444 because their racemates at the concentration of 10 μmol/l were previously shown to block I_{Na} completely (Bartosova et al. 2006).

ECG monitoring and evaluation of changes in QRS-complex width

Experiments were performed *in vivo* on 43 male Wistar laboratory rats (290 ± 25 g). The animals came from a conventional breeding colony (Faculty of Medicine, Masaryk University, Brno, Czech Republic). They were housed in agreement with the conditions as per Regulation No. 311/1997 Coll. (temperature - 20-24 °C, humidity 40-60%, 12:12 L:D cycles with lighting maximum up to 200 lux). The animals were fed a standard diet (Diet for small laboratory animals M_1) and given water *ad libitum*. Experimental protocol was approved and monitored by the local University Ethics Committee of the University of Veterinary and Pharmaceutical Sciences in Brno. The animals were anaesthetized by intramuscular administration of a mixture of 1% ketamine (Narkamon® inj. Spofa, Czech Republic) and 2% xylazine (Rometar® inj. Spofa, Czech Republic) at a dose of 0.5 ml/100 g.

ECG was monitored continuously on a Seiva Praktik ECG machine (Seiva Praktik Veterinary, Czech Republic). The tested substances were administered intravenously into the exposed vena jugularis at a dose of 1.5 mg/kg (corresponding to 3.72 µmol/kg in the case of both 44Bu and 444).

The ECG records (ECG Seiva Praktik Veterinary) were made at predetermined time intervals (after induction of anaesthesia - the initial value, at the time of administration of the tested substance, at 0.5 and 1 min, at every 1 min until the 6th min and, subsequently, at every 2nd min until the 20th min). The most significant changes in ECG indicators were observed during the first 5 min. Thus, only this time interval was used for the evaluation. Measurement of the QRS-complex width was done using the ECG-SEIVA Praktik software and the automatic measurement was verified manually if necessary.

Measurement and evaluation of changes in fast sodium current

Ventricular myocytes were isolated from right ventricular free walls of adult male Wistar rats (250 ± 50 g) anaesthetised by intramuscular administration of a mixture of 1% ketamine (Narkamon® inj., Spofa) and 2% xylazine (Rometar® inj., Spofa) at a dose of 0.8 ml/100 g. The experiments were carried out with respect to recommendations of the European Community Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the Local Committee for Animal Treatment at Masaryk University, Faculty of Medicine (permission No. 12175/2001-1020(A)).

The dissociation procedure has been previously described in detail (Bébarová et al. 2005). In brief, the heart was retrogradely perfused via aorta with 0.9 mM $CaCl_2$ Tyrode solution and then with nominally Ca-free Tyrode solution. During the first digestion step, the perfusion continued with nominally Ca-free Tyrode solution containing collagenase (type S, Yakult Pharmaceuticals; 0.2 mg/ml), protease (type XIV, Sigma-Aldrich; 0.053 mg/ml), albumin (bovine, fraction V, Sigma-Aldrich; 2 mg/ml), and EGTA (Sigma-Aldrich; 34 mM). In the second digestion step, protease and albumin in the enzyme solution were omitted. The enzyme solution was then washed out in two steps by perfusion with low calcium Tyrode solutions (0.09 and 0.18 mM $CaCl_2$). All solutions were oxygenated with 100% O_2 at 37 °C.

Composition of the Tyrode solution used (mM): NaCl 135, KCl 5.4, $CaCl_2$ 0.9, $MgCl_2$ 0.9, HEPES 10, NaH_2PO_4 0.33, glucose 10 (pH adjusted to 7.4 with NaOH). $CaCl_2$ (0.9 mM) and the calcium channel blocker $CoCl_2$ (2 mM; Sigma-Aldrich; prepared as 1 M stock solution in deionized water) were administered in the course of the experiments. The patch electrode filling solution contained (mM): L-aspartic acid 130, KCl 25, $MgCl_2$ 1, K_2ATP 5, EGTA 1, HEPES 5, GTP 0.1, Na_2-phosphocreatine 3 (pH 7.25 adjusted with KOH).

Single rod-shaped cells with well visible striations were used for I_{Na}-measurements applying the whole-cell patch-clamp technique at room temperature (22 ± 2 ºC). The patch pipettes were pulled from borosilicate glass capillary tubes and heat polished on a programmable horizontal puller (Zeitz-Instrumente). The resistance of the filled glass electrodes was below 1.5 MΩ to keep the access resistance as low as possible. For generation of experimental protocols and data acquisition, the Axopatch 200B equipment and pCLAMP 9.2 software (Axon Instruments, Inc.) were used. The series resistance was compensated up to 80%. The measured current was filtrated with a four-pole Bessel filter at 5 kHz, digitally sampled at 20 kHz and stored on the hard disc. Experimental protocol and evaluation of the data are described in Results. The stimulation frequency of 0.2 Hz was used to avoid an accumulation of I_{Na}-block. The tested substances were applied into the close vicinity of the measured cell through a rapid perfusion system.

Statistical analysis

The results are presented as means ± SEM from *n* animals/cells. The one-way ANOVA with Bonferroni post test (used to assess the significance of differences between the tested substances both in the case of changes in the QRS-complex width and in I_{Na}-block) was performed using GraphPad Prism, version 4.0 (GraphPad Software, Inc.); $P < 0.05$ was considered significant.

Results

Changes in QRS-complex width after administration of 44Bu and 444

Table 1 summarizes averaged values of the QRS-complex width before and during the first 5 min after administration of the tested substances at a dose of 1.5 mg/kg. All the substances caused a significant QRS-widening which usually culminated 1 to 2 min after their administration. In the case of 444 variants, the QRS-widening started 30 s after their

Table 1. Changes in QRS-complex width after administration of 44Bu and 444.

	44Bu			444		
	Racemate	R-isomer	S-isomer	Racemate	R-isomer	S-isomer
IV	16.8 ± 0.4	16.7 ± 0.5	16.0 ± 0.8	20.8 ± 1.0	21.4 ± 0.9	20.8 ± 1.3
0	17.7 ± 0.5	17.4 ± 0.4	16.5 ± 0.9	21.2 ± 1.3	24.1 ± 0.6	23.4 ± 1.7
0.5	22.7 ± 0.6 ***	20.2 ± 0.7	20.2 ± 0.9 *	27.5 ± 0.4 *	26.7 ± 0.5 *	28.8 ± 0.5 **
1	26.3 ± 0.5 ***	21.8 ± 0.9 **	20.9 ± 1.1 **	34.1 ± 0.9 ***	29.8 ± 0.8 ***	33.8 ± 1.0 ***
2	26.3 ± 0.6 ***	22.2 ± 1.1 ***	21.7 ± 0.9 **	33.5 ± 1.6 ***	30.0 ± 1.3 ***	33.8 ± 1.3 ***
3	25.1 ± 0.6 ***	21.8 ± 1.0 **	20.7 ± 1.0 *	31.5 ± 1.5 ***	31.7 ± 1.2 ***	33.0 ± 1.4 ***
4	24.0 ± 0.5 ***	21.8 ± 1.0 **	20.1 ± 0.9	30.8 ± 1.4 ***	29.3 ± 1.5 ***	32.0 ± 1.6 ***
5	23.2 ± 0.5 ***	21.1 ± 0.7 **	19.9 ± 0.8	29.4 ± 2.0 **	28.5 ± 1.1 ***	30.4 ± 1.5 ***

The absolute value of the QRS-complex width [ms] stated as mean ± SEM; IV – the initial value; the following records were made at the time point when the tested substance was administrated (0 min) and 0.5, 1, 2, 3, 4 and 5 min after its administration; * - $P < 0.05$, ** - $P < 0.01$, *** - $P < 0.001$ – statistical significance of the increase in the QRS-complex width vs. the initial value in the particular variants of 44Bu and 444 evaluated by the one-way ANOVA with Bonferroni post test.

administration and was highly significant. On the contrary, only the 44Bu racemate exerted highly significant effect which started 30 s after its administration. In the case of the R- and, especially, of S-isomer of 44Bu, the QRS-widening was considerably smaller and transient compared to the 44Bu racemate and to all the 444 variants.

For better comparison, we decided to also count the relative percentage changes of the QRS-complex width (100% ~ the initial value before administration of the tested substance). Figure 2 clearly shows a significantly higher potency of the 44Bu racemate to cause a QRS-widening in comparison with both its pure isomers (Fig. 2A). In the case of 444, a smaller QRS-widening after administration of the R-isomer compared to its racemate and S-isomer was observed (Fig. 2B). Regarding differences between 44Bu and 444, their peak effect (observed in presence of the racemates of 44Bu and 444, and under the effect of the S-isomer of 444) resulting in an increase of the initial QRS-complex width to about 160% did not significantly differ. Similarly, the effect of the less effective variants, namely the both particular isomers of 44Bu and the R-isomer of 444, caused a comparable QRS-widening to values between 132 and 142%.

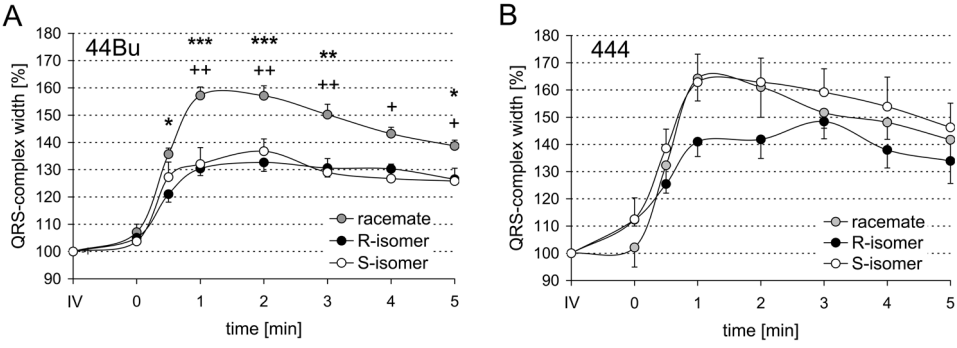

Fig. 2. Percentage changes in the QRS-complex width under the effect of racemate, and R- and S-isomers of 44Bu (A, $n = 8$) and 444 (B, $n = 5$).
100% ~ the initial value (IV) before administration of the tested substance. All the tested substances increased the QRS-complex width. The effect of 44Bu racemate was significantly higher compared to its R- and S-isomer; *, ** and *** - significance at $P < 0.05$, 0.01 and 0.001, respectively, for differences between the racemate vs. R-isomer; $^{+}$ and $^{++}$ - significance at $P < 0.05$ and 0.01, respectively, for differences between the racemate vs. S-isomer. In the case of 444, the R-isomer caused a lower increase in comparison with the racemate and S-isomer which did not differ in their effect; changes were not significant, very likely due to the lower number of animals included in the statistics.

Effect of 44Bu and 444 on I_{Na}

To measure I_{Na}, 25-ms rectangular pulses from the holding potential of -75 mV to -40 mV were applied at the stimulation frequency of 0.2 Hz. The level of I_{Na}-block was evaluated from changes in I_{Na}-amplitude after administration of the agents; the changes were corrected for the spontaneous run down of the current. All variants of 44Bu and 444 caused a reversible block of I_{Na} (illustrated for 44Bu racemate in Fig. 3A and B). In the case of 44Bu (Fig. 3C, left panel), we observed an almost complete block of I_{Na} after application of the racemate and R-isomer (91.7 ± 0.8% and 91.8 ± 1.6%, respectively; $P > 0.05$) whereas the S-isomer exerted a significantly lower effect (82.4 ± 2.3%; $P < 0.01$ compared to both the racemate and R-isomer). In the case of 444 (Fig. 3C, right panel), the racemate and S-isomer blocked I_{Na} comparably (48.3 ± 4.7% and 50.2 ± 4.1%, respectively; $P > 0.05$) but the blocking potency of R-isomer was substantially less (31.7 ± 3.9%; $P < 0.05$ and 0.01 compared to the racemate and S-isomer, respectively). Confronting the effect of various variants of both tested substances, 44Bu caused significantly deeper I_{Na}-block in comparison with 444 ($P < 0.001$).

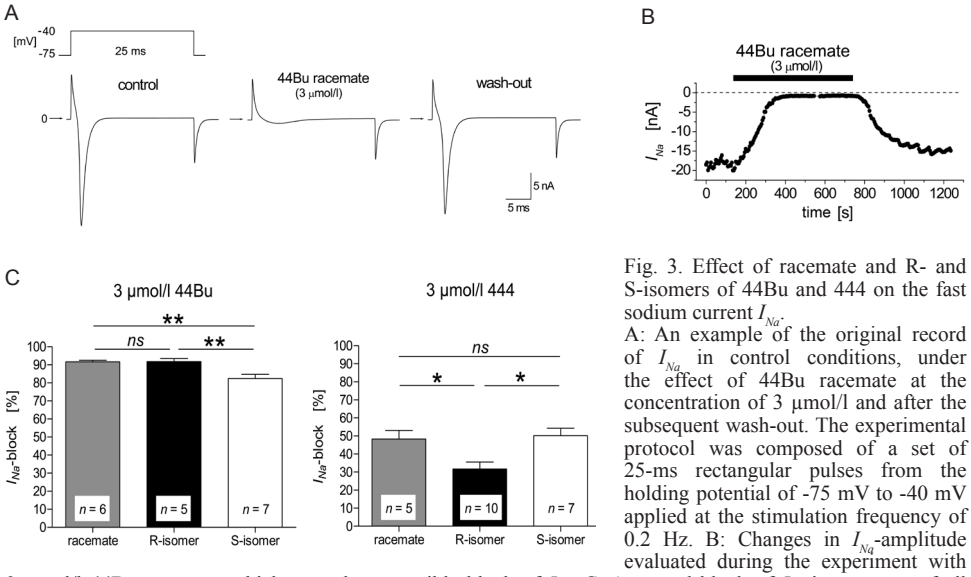

Fig. 3. Effect of racemate and R- and S-isomers of 44Bu and 444 on the fast sodium current I_{Na}.
A: An example of the original record of I_{Na} in control conditions, under the effect of 44Bu racemate at the concentration of 3 μmol/l and after the subsequent wash-out. The experimental protocol was composed of a set of 25-ms rectangular pulses from the holding potential of -75 mV to -40 mV applied at the stimulation frequency of 0.2 Hz. B: Changes in I_{Na}-amplitude evaluated during the experiment with 3 μmol/l 44Bu racemate which caused a reversible block of I_{Na}. C: Averaged block of I_{Na} in presence of all the tested substances at the same concentration of 3 μmol/l; *n* - number of experiments, *ns* - non-significant ($P > 0.05$), * and ** - $P < 0.05$ and 0.01, respectively.

Discussion

In this study, we have documented that two newly synthesized short-acting agents with draft names 44Bu and 444 cause a significant widening of the QRS-complex and, in agreement, block I_{Na} in the rat heart. Substantial differences in the effects of racemates and particular isomers of these agents were observed.

The averaged initial values of the QRS-complex width diverged in the experiments with 44Bu and 444 (Table 1). However, these differences were not significant and all the values varied within the usually reported range of the QRS-complex width in the rat *in vivo* (e.g. 14.3 ms – Akita et al. 1998; 15.1 ms - Králová et al. 2008; 18.6 ms – Brisinda et al. 2006; 22.8 ms - Maatz et al. 2009; about 27 ms – Saitoh et al. 2002). The diversity of the values is probably related to the fact that effects of 44Bu and 444 on the QRS-complex width were recorded within two independent sets of experiments.

The QRS-widening notably differed in the 44Bu and 444 variants. In the case of 44Bu, the effect of the particular isomers seems to potentiate when present together in the racemic mixture (Fig. 2A). In agreement, a study dealing with the antiarrhythmic effect of 44Bu variants has recently showed that the racemate was more effective in suppression of the ventricular fibrillation compared to both its particular isomers (Bartosova et al. 2008). As for 444, a comparable potency of the S-isomer and racemate to widen the QRS-complex was substantially higher than those of the R-isomer (Fig. 2B). It might imply that the S-isomer is mainly responsible for the pharmacological effect of 444 on the rate of cardiac conduction and, thus, also for its antiarrhythmic effect. Unfortunately, the antiarrhythmic effect of 444 variants has not been studied yet. In the past, other pure isomers were documented to exert the majority of the pharmacological effects of an optically active compound. In some cases, the pure isomer was even shown to provide an advantage in the clinical use of a compound administered in the form of racemate so far (e.g. Valenzuela et al. 1995; Stoschitzky et al. 2001; Magyar et al. 2003; Eap et al. 2007; Wang et al. 2008). Thus, it might be beneficial to produce and test the pure S-isomer of 444 if this compound was considered to be used in the clinical practise in the future.

I_{Na} plays a central role in the cardiac excitability and conduction (for review, see e.g. Remme et al. 2008). Thus, changes in the QRS-complex width, which corresponds to the ventricular depolarization, are considered to be a good indicator of the action of I_{Na}-channel blockers (Ranger et al. 1991; Sakai et al. 1995; Shinozaki et al. 1997). In agreement, the QRS-widening recorded *in vivo* in our study corresponded well to the level of I_{Na}-block measured *in vitro* in isolated ventricular myocytes in the case of 444 (Fig. 2B, and Fig. 3C, right panel). Surprisingly, in the case of 44Bu, changes in these two indicators notably differed (Fig. 2A, and Fig. 3C, left panel). Even complete absence of changes in the QRS-complex width under the effect of a drug blocking I_{Na} has been documented, for example under the effect of mexiletine (Shimizu et al. 2000) and cyclo(Trp-Pro) isomers (Jamie et al. 2002). These differences between results of *in vivo* and *in vitro* experiments may be caused, among others (e.g. temperature), by the comprehensive character of the drug effect on a living organism which is primarily influenced by the pharmacodynamic and pharmacokinetic properties of the drug. In the case of 44Bu and 444, a variable accessibility of the ester binding in their particular isomers caused by the distinct three-dimensional structure, and, thus, differences in their degradation by esterases might play a role similarly to esmolol, an ultrashort-acting blocker of the β-adrenergic receptors (Quon et al. 1988). The R-isomer of 444 has been recently documented to be degraded by esterases faster in comparison with its S-isomer and racemate (unpublished data) which might explain its weaker effect on the QRS-complex width (Fig. 2B). No parallel data are available in the case of 44Bu. The above discussed facts support the necessity of *in vivo* experiments within pre-clinical testing of new compounds.

In our previous study (Bartosova et al. 2006), we showed that 10 μmol/l racemates of 44Bu and 444 induced a complete I_{Na}-block. In the current study, a reversible block of I_{Na} under the effect of both substances, both in the form of racemates and pure isomers, was documented at the concentration of 3 μmol/l (Fig. 3). These results provide a clear evidence that 44Bu and 444 are efficient blockers of I_{Na} with the blocking potency comparable to class-I antiarrhythmic drugs (e.g. propafenone - Šimurdová et al. 1997; flecainide -Liu et al. 2002; lidocaine - Xiao et al. 2004; ajmaline - Bébarová et al. 2005). As known, the block of I_{Na} results in a slower rate of conduction, increased threshold for excitation and prolongation of the effective refractory period. These changes may lead to suppression of arrhythmias as reviewed by e.g. Carmeliet and Mubagwa (1998). Therefore, the potency of 44Bu and 444 to block I_{Na} very likely plays a role in their antiarrhythmic effects which were observed formerly (44Bu - Bartosova et al. 2007; 444 - unpublished data).

However, further experiments focused on the exploration of other electrophysiological effects of 44Bu and 444 are needed to complete their profiles.

We conclude that the newly synthesized ultra-short acting compounds 44Bu and 444 significantly widen the QRS-complex and block I_{Na} in the rat heart. The observed differences between the effect of racemates and particular isomers support the idea of development and testing of new drugs as pure isomers. Results of the *in vivo* and *in vitro* experiments agreed well in the case of 444, however, not in the case of 44Bu. It implies that *in vivo* experiments play an important role in the pre-clinical testing of new drugs.

Účinek nově syntetizovaných látek 44Bu a 444 na šíři QRS-komplexu a na rychlý sodíkový proud: rozdíly mezi izomery

Nově syntetizované sloučeniny 44Bu a 444 potlačují akonitinem vyvolanou arytmii a blokují rychlý sodíkový proud I_{Na} v srdci laboratorního potkana. O jejich účinku na parametry EKG křivky však dosud nebyla publikována žádná data. V této studii jsme se zaměřili na účinek jak racemátů, tak jednotlivých izomerů látek 44Bu a 444 na změnu šíře QRS-komplexu v pokusech *in vivo* na laboratorních potkanech a na účinek těchto substancí na I_{Na} v izolovaných komorových srdečních buňkách laboratorního potkana (technika *whole cell patch-clamp*, pokojová teplota). Všechny varianty látek 44Bu a 444 (1,5 mg/kg) způsobily signifikantní prodloužení QRS-komplexu s maximem v 1. nebo 2. minutě po jejich intravenózním podání. Racemát látky 44Bu prodloužil QRS-komplex ze 16,8 ± 0,4 na 26,3 ± 0,5 ms (o 57 %), signifikantně více než R- (o 33 %) and S-izomer (o 36 %). Racemát látky 444 prodloužil QRS-komplex z 20,6 ± 1,1 na 34,2 ± 1,0 ms (o 66 %), srovnatelně s S-izomerem (o 63 %), nicméně více než R-izomer (rozšíření o 49 %). Co se týká vlivu na I_{Na}, látka 44Bu způsobila významně hlubší blokádu I_{Na} ve srovnání s látkou 444 při podání ve stejné koncentraci 3 µmol/l (~0,1 mg/kg). Racemát a R-izomer látky 44Bu blokovali I_{Na} stejně (blokáda 91,7 ± 0,8 % respektive 91,8 ± 1,6 %), avšak signifikantně více než S-izomer (82,4 ± 2,3 %). R-izomer látky 444 blokoval I_{Na} méně než racemát a S-izomer (31,7 ± 3,9 % oproti 48, 3± 4,7 a 50,2 ± 4,1 %). Lze tedy říci, že jak racemáty, tak jednotlivé izomery látek 44Bu a 444 prodlužují QRS-komplex a blokují I_{Na}, nicméně jejich účinky se významně liší. Míra poměrného prodloužení QRS-komplexu v *in vivo* pokusech neodpovídala velikosti blokády I_{Na} po aplikaci 44Bu u experimentů na izolovaných buňkách, což vyzdvihuje důležitost *in vivo* pokusů při preklinickém testování nových léčiv.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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