First Department of Medicine[1], University Medical School of Debrecen, Hungary, and Center for Drug Discovery[2], University of Florida, Gainesville, USA

Cardiovascular studies on different classes of soft drugs

A. JUHÁSZ[1] and N. BODOR[2]

Based on the inactive metabolite approach, three different classes of soft drugs were designed and synthesized. Their cardiovascular effects and duration of actions were studied in anesthetized male Sprague-Dawley rats compared to the traditional drugs. During the experiments ECG (leads II, aVF) and beat-to-beat blood pressure (BP) from the left carotid artery were recorded (except during the anticholinergic studies). The soft anticholinergic methoxycarbonylphenylcyclopentyl-*N,N*-dimethyltropinium methyl sulfate was as potent as atropine in the prevention of carbachol induced bradycardia; however, its action only lasted up to 15–30 min, compared to 2 h of that of atropine. In the isoproterenol-induced tachycardia model, while bufuralol at an i.v. dose of 3.8 μmol/kg (1 mg/kg) diminished heart rate (HR) for at least 2 h, the effects of the soft drugs lasted for only 30–40 min at equimolar doses. The methyl-, ethyl-, isopropyl-, and *tert*-butyl ester-analogs of the carboxylic acid metabolite of bufuralol showed the highest beta-blocking potencies (i.e., 30–50% of that of bufuralol). When these compounds were infused for 10 min at doses ranging from 2–4 μmol/kg/min, they caused a 20–40% decrease in HR and a 30–40% reduction in mean arterial pressure (MAP). These effects were similar to those elicited by esmolol at a dose of 20 μmol/kg/min in respect of the kinetics and in the extent of the reductions in heart rate and MAP. The isopropyl-, the *sec*-butyl-, and the neopentyl-esters of the acidic metabolite of amiodarone, with plasma hydrolytic half-lives of 60, 240 and 300 min, were tested in the benzene/adrenaline induced ventricular tachycardia (VT) model of the rat. All drugs were administered at a dose of 5 μmol/kg i.v. bolus immediately followed by an infusion at 15 μmol/kg/h for 2 h. It was found, that amiodarone resulted a complete suppression of VTs at 30 min after the start of drug administration, but its effect lasted up to the total course of the experiment (up to 180 min). On the contrary, both the *sec*-butyl and the isopropyl-analog resulted in complete suppression of VTs already during the first benzene/adrenaline challenge after drug administration (i.e., at 5 min). However, their effects disappeared between 15 and 30 min after discontinuation of the drug infusions in accordance with the enzymatic inactivation (ester hydrolysis) of these soft drugs. All these three classes of soft cardioactive drugs are good examples for highly potent but short acting drugs whose side effects might also be reduced via the retrometabolism based drug design.

1. Introduction

To improve the therapeutic index of drugs, the retrometabolic drug design concept was developed by Bodor [1–3], which consists of two different design strategies: soft drugs and chemical delivery systems. Based on the soft drug design approach, many compounds were synthesized in several groups of drugs, e.g. anticholinergics [4–7], beta-blockers [8, 9] and steroids, just to mention a few of the classes. One of the soft steroids, loteprednol etabonate [10–13], which is the active ingredient of Alrex® and Lotemax® reached the US market after FDA approval in 1998.

During the development of newly designed drugs, the demonstration of the intended pharmacodynamic effects and the characterization of the kinetics of the biological activities, are among the first steps of evaluation. Cardiovascular effects are most important to investigate, not only in connection to truly cardioactive drugs like beta-blockers or antiarrhythmics, but also in the case of other drugs like anticholinergics administered locally when applied as mydriatics or antiperspirants, because even systemic toxic effects can develop after the topical administration of certain drugs, like scopolamine [14, 15].

The soft drug design concept is especially suitable for designing safer drugs for topical use, e.g., in the anticholinergic class. Based on the inactive metabolite approach, different ester-conjugates of a hypothetical carboxylic acid containing inactive metabolite of the lead compound (e.g., methatropine) were designed (Fig. 1). The esterification of the inactive metabolite lends the desired biological activity to the new compounds, which is comparable to the activity of the lead. However, these ester groups are sensitive to hydrolysis by esterases ubiquitously present in blood and tissues. After the soft anticholinergics exert their desired mydriatic or antiperspirant activities in the eye or on the skin, respectively, upon entering the systemic circulation after drainage or absorption, they rapidly lose their biological activities through enzymatic inactivation (ester-hydrolysis). The purpose of the first series of experiments detailed in the following sections was to demonstrate the short duration of antagonistic activity of a newly designed soft anticholinergic agent, methoxycarbonylphenylcyclopentylacetyl-*N,N*-dimethyltropinium methyl sulfate

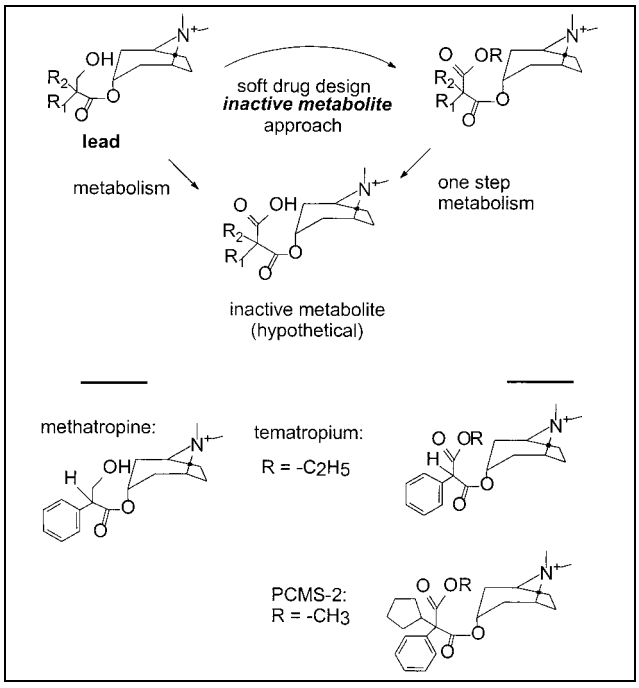

Fig. 1: The retrometabolic design and predicted metabolism of PCMS-2

(PCMS-2), against the carbachol-induced bradycardia in the rat after systemic (i.v.) administration in comparison to that of atropine [7].

Beta-blockers have an established role in the treatment of various cardiovascular diseases, including angina pectoris, hypertension and cardiac arrhythmias [16–18]. Their efficacy and safety have also been proved in reducing the risk of mortality and non-fatal reinfarction in survivors of acute myocardial infarction [16]. However, the use of beta-blockers in seriously ill patients is limited, because of potentially serious adverse effects (i.e., bradycardia, hypotension, aggravation of heart failure, and bronchospasm). The ultrashort acting β-antagonist esmolol is often used to control acute supraventricular arrhythmias, myocardial ischaemia (acute myocardial infarction and unstable angina), and perioperative and postoperative hypertension in critically ill patients [19]. In the structure of esmolol, an ethylene-extended methyl ester group is included, that makes the molecule susceptible to rapid hydrolysis by esterases [20]. On the occurrence of unwanted side effects during esmolol treatment, the discontinuation of the infusion will result in the rapid disappearance of adverse reactions, as the terminal half-life of esmolol is short, only 9.2 min [19].

The oxidative metabolic transformation of several beta-blockers resulting metabolites with significant β-receptor blocking activities, but different biological half-lifes [21] is also a reason for their limited use. For example, bufuralol [22, 23], which has been proved to be very effective in lowering blood pressure and heart rate as a potent, non-selective β-blocker [24–26], produced the complex metabolic transformation to alcohol and ketone metabolites demonstrating significant β-receptor blocking activities and longer half-life [27–32]. Hepatic cytochrome P450 isozymes are responsible for the oxidative transformation of bufuralol, which is under genetic control and falls into the debrisoquine/spartein phenotype [33–36]. A genetically determined defect of the hydroxylation occurs in up to 10% of the Caucasian population (poor metabolizers) [36], and can cause a complex pharmacokinetic picture with increased drug bioavailability, prolongation of elimination half-life, and more intense and sustained beta-blockade [37–39], which can lead to severe hypotension and bradycardia [40]. In the case of bufuralol, registered earlier in Europe under the name Angium®, the occurrence of unpredictable and unwanted side effects (bradycardia, hypotension), especially in persons with the poor metabolizer phenotype, led to the withdrawal of drug from the market.

As the soft drug concept is particularly suitable for addressing the above mentioned therapeutic problems, the design and evaluation of a series of soft β-blockers with an ultrashort duration of action and predictable metabolism was reported previously [8–9]. For the experiments reported here, an acidic inactive metabolite of bufuralol (**1**) was selected as the lead compound to demonstrate that a design strategy, which considers the deactivation of the drugs not by hepatic transformation but by esterases ubiquitously present in blood and tissues as the primary metabolic route, can provide a solution to avoid the formation of active metabolites. Based on the "inactive metabolite" approach, seven different sized alkyl moieties (**2**–**8**) were designed to serve as the ester functionalities to reactivate the inactive metabolite of bufuralol to possess again beta-antagonistic action (Fig. 2). The beta-antagonistic effects of the drugs, both in respect to their potencies and duration of actions, were evaluated in the isoproterenol-induced tachycardia model of the rat in comparison to bufuralol. As the new compounds were expected to possess an ultrashort activity, the kinetics of their effects on resting heart rate and blood pressure were compared to those of esmolol, a currently available ultra-short acting beta-blocker.

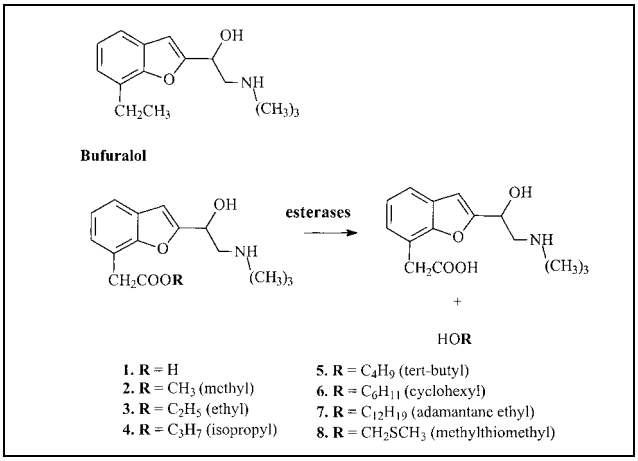

Fig. 2: Design and predicted metabolism of soft bufuralol analogs

Amiodarone (Fig. 3) is an antiarrhythmic agent with complex electrophysiological activity including Class-I (sodium channel), Class-II (beta-receptor), Class-III (potassium channel), and even Class-IV (calcium channel) properties, thus acting on both cardiac conduction and cardiac repolarization parameters [45–48]. These combined electrophysiological properties manifest in reduction of the heart rate (HR) and prolongation of the PR, QRS and QT intervals of the surface ECG [49]. During chronic treatment, amiodarone is effective against ventricular and supraventricular arrhythmias, including atrial fibrillation and flutter, paroxysmal supraventricular tachycardia, ventricular premature beats (VPB), sustained and non-sustained ventricular tachycardia (VT), and ventricular fibrillation (VF) [49, 50]. Randomized controlled trials have shown that amiodarone is one of the very few drugs that actually reduce mortality rates in high-risk patients (post-MI patients and patients with congestive heart failure) [51–53]. Unfortunately, because of side-effects, some of which might be fatal, and substantial management difficulties associated with its use (weeks are needed for the development of full antiarrhythmic protection), amiodarone

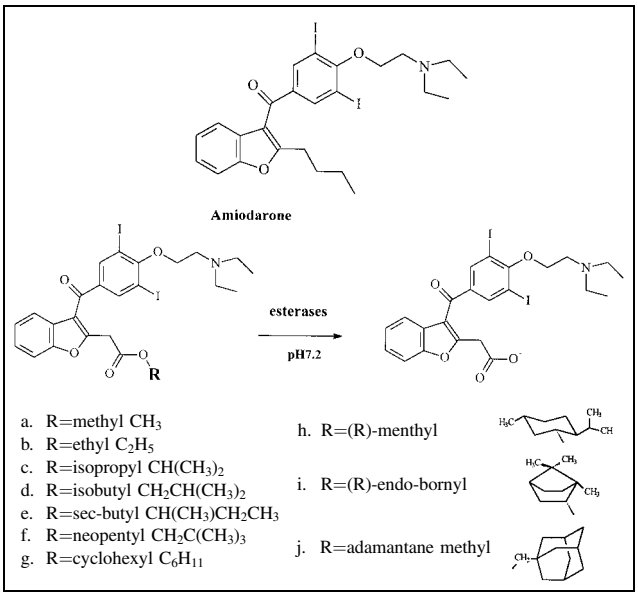

Fig. 3: Design and predicted metabolism of soft amiodarone analogs

is indicated only for life-threatening recurrent ventricular arrhythmias when these have not responded to documented adequate doses of other available antiarrhythmics or when alternative agents are not tolerated [50, 54]. The pharmacokinetics properties of amiodarone are characterized by slow absorption, moderate bioavailability, high lipophilicity, and a very large volume of distribution (60 l/kg on average). Its elimination is almost exclusively hepatic and its clearance rate is very slow. Its terminal elimination half-life is 53 d [49, 50]. As a consequence, upon long-term administration, amiodarone accumulates in virtually every organ including poorly perfused tissues such as the lens. The onset of its antiarrhythmic activity may take days, or even weeks to appear. This therapeutic latency can be shortened with the administration of intravenous loading doses, but is still too long [55]. Amiodarone has several potentially lethal toxicities, the most important of which is pulmonary toxicity (hypersensitivity pneumonitis or interstitial/alveolar pneumonitis). If the progression of symptoms is recognized on time, pulmonary toxicity might be reversible but is still fatal in 10% [50, 54]. Mild liver injury is common but usually mild, although liver disease can occur and had led to death in some cases. Even if toxicity is usually reversible upon cessation of drug administration, the real danger with amiodarone comes from its kinetics, especially slow elimination. For example, although the frequency of pro-arrhythmic events associated with amiodarone appears to be less than with other antiarrhythmic agents (2 to 5%), the effects are prolonged when they occur. As amiodarone poses major management problems and might result in adverse reactions that could be life-threatening, every effort should be made to use alternative agents first, even in patients with high risk for sudden death, in whom the toxicity of amiodarone is an acceptable risk.

New antiarrhythmic drugs with electrophysiological properties similar to that of amiodarone, but with a short onset of action and rapid elimination kinetics would therefore be extremely useful. In an attempt to create shorter-acting amiodarone analogs, the retrometabolic drug design principles [2] were used to create drugs that are primarily metabolized by esterase. A homologous series (**a**–**j**) of compounds were synthesized, in which the 2-butyl chain of the parent drug amiodarone was functionalized to include an ester moiety. Due to the ubiquitous nature of esterases, these compounds are expected to be rapidly metabolized not only in the liver, but also in every tissue to a putative primary metabolite having a carboxylic acid moiety (Fig. 3). Because of its water solubility at physiological pH, this metabolite is expected to have a small volume of distribution and a high clearance rate not only by the liver, but also by the kidneys. The projected long-term clinical consequences of this approach are amiodarone analogs having a smaller volume of distribution and, therefore, a shorter onset of activity, a faster elimination rate, and a safer long-term toxicity profile. Due to the rapid clearance and lack of accumulation, these compounds are also expected to have a rapid offset of antiarrhythmic activity. The benzene/adrenaline induced ventricular tachycardia model of the rat [56] was applied to compare the antiarrhythmic potencies and the durations of actions of three of the amiodarone-analog aseries (**c**, **e**, and **f**), to those of the parent drug amiodarone.

Here, we only review the cardiovascular studies performed on three classes of soft drugs, i.e., PCMS-2, the bufuralol analogs **2**–**8**, and the amiodarone analogs **c**, **e**, and **f**. The syntheses of the compounds and several other data on their *in vitro*, and *in vivo* characterization were published earlier [7] or are submitted for publication [57, 58].

2. Investigations and results

2.1. Effects on carbachol induced bradycardia

The extent and duration of action of the bradycardia protective effect of PCMS-2 was evaluated in comparison with atropineMeBr. With i.v. administration of carbachol at a dose of 5–8 μg/kg to male Sprague-Dawley rats, the temporary development of sinus bradycardia and Mobitz II atrio/ventricular (A–V) block can be evoked safely and repeatedly. This effect can be antagonized by previous administration of an anticholinergic agent, e.g., atropine. The full protection against carbachol induced bradycardia by the anticholinergics was regarded as their ability to protect against both the lengthening of PP cycle (sinus bradycardia) and the development of Mobitz II type A–V block. Bradycardia protective effects of different anticholinergic differ in respect of their potency and duration of action. AtropineMeBr, scopolamineMeBr, propanthelineBr, and glycopyrrolate all showed long duration of action against carbachol induced bradycardia in the rat, which was found to be longer than 2 h for atropineMeBr in preliminary experiments. These compounds were all effective at a dose of 0.2 μmol/kg. The potency of action of PCMS-2 is about the same order of magnitude as that of atropine and the other traditional anticholinergics, as it was effective against carbachol induced bradycardia at the same dose range (0.2 and 2 μmol/kg, Fig. 4). 30 min after the injection of PCMS-2, the repeated doses of carbachol elicit

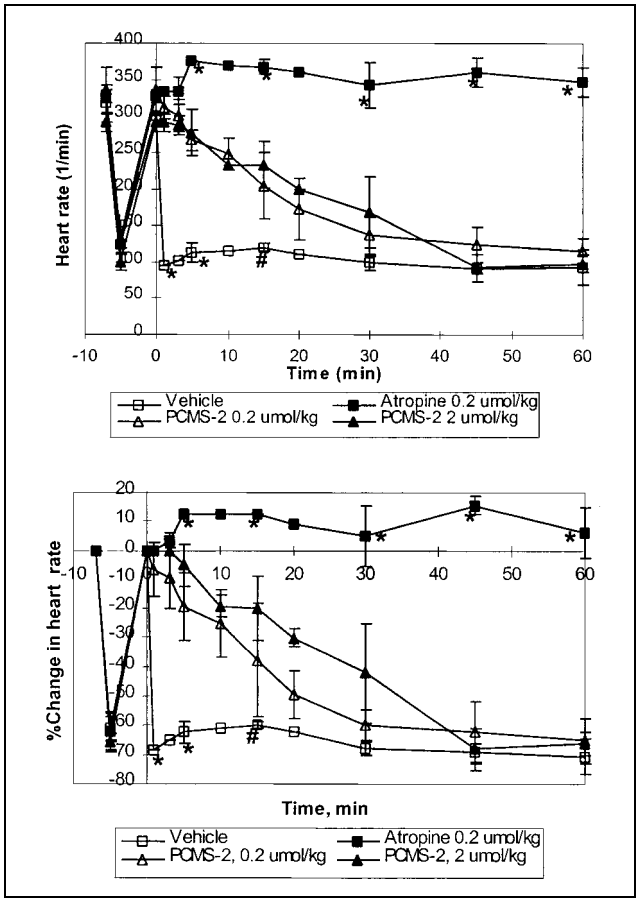

Fig. 4: Effects of PCMS-2 and atropineMeBr on carbachol induced bradycardia. * Denotes significant difference ($p < 0.05$) for both 0.2 and 2 μmol/kg, while # denotes significant difference only for 2 μmol/kg. Vehicle is 0.9% NaCl

again sinus bradycardia and the development of Mobitz II type A–V block, and heart rate values and percent changes in heart rates are not different significantly any more to the values registered after the injection of vehicle. After this period of time, the potency of the drug against the carbachol induced bradycardia is lost, which is in accordance with the enzymatic inactivation and the soft nature of PCMS-2 [7].

2.2. Effects on isoproterenol induced tachycardia

There were no statistically significant differences in the baseline heart rate and blood pressure parameters between the different groups of rats receiving the different compounds, i.e., bufuralol, the soft bufuralol analogs **2**–**8**, the "inactive metabolite" **1**, or vehicle. The average heart rate increased by 23.68% (from 355.26 ± 23.49/min to 439.4 ± 20.59/min) after the s.c. injection of 50 μg/kg isoproterenol. Relative to this isoproterenol induced tachycardia, the i.v. bolus injection of bufuralol at a dose of 1 mg/kg (3.8 μmol/kg) resulted a 35–40% decrease in heart rate (Fig. 5). The maximal bradycardic action of the drug developed after 30 min and remained stable for the total course of the experiment (i.e. up to 120 min) after the injection. The injection of the soft bufuralol analogs **2**–**8** at equimolar doses (i.e., 3.8 μmol/kg) resulted a significant, but temporary decrease (10–20%) in heart rate. The most active compounds in this model were **4**, **5**, **2**, and **3**, while **6**, **7**, and **8** – the compounds with the bulkier ester groups – showed weaker bradycardic action against the isoproterenol induced tachycardia. In accordance with the soft nature of these compounds and the enzymatic inactivation by esterases present in blood and tissues, the bradycardic action lasted only for 10–30 min. After 30 min, as shown by cluster analysis, the effects of the soft drugs did not differ significantly any longer from those of either the vehicle or the inactive metabolite **1** (Fig. 5), which even at a dose of 10 μmol/kg did not decrease heart rate significantly.

2.3. Effects on resting heart rate and blood pressure

Esmolol (Brevibloc®) is an ultra-short acting beta-blocker, which can be dissolved in water or physiologic salt solution and only available in an i.v. injection formulation. It was expected, that the effects of the soft bufuralol analogs applied in infusion should be similar in respect of the kinetics of their actions to that of esmolol administered in the same way. As esmolol is to be diluted in normal saline, the reason why the free base forms of the soft bufuralol analogs were converted into their corresponding HCl salts was to turn them into a water soluble form. The effects of the most active compounds **2**, **3**, **4**, and **5** were investigated on resting heart rate and blood pressure in comparison to esmolol-HCl and vehicle, which was 0.9% NaCl solution. In preliminary experiments, the necessary doses to obtain similar heart rate and blood pressure reductions were titrated, and it was found that compounds **2**, **4**, and **5** at a dose of 2 μmol/kg/min and compounds **3** at 4 μmol/kg/min were approximately equipotent to esmolol at a dose of 20 μmol/kg/min. All the soft bufuralol analogs and esmolol decreased heart rate (by 20–40% on average) during the course of the infusion (i.e., 10 min). After the discontinuation of drug administration, heart rates gradually returned to baseline values, mostly within 60 min and were not different significantly any more from the values registered during the infusion of normal saline (Fig. 6). Blood pressure changes were similar, but the effect of esmolol was more pronounced, resulting a 60% decrease in MAP, while **2**, **3**, and **4** only decreased MAP by 30–40% on average, and **5** resulted a more than 50% decrease in MAP. Again, after the cessation of the infusions, blood pressure values returned to baseline values (Fig. 7). The kinetics of the heart rate and blood pressure changes were superimposable on the changes evoked by esmolol, as the cluster analysis demonstrated that only the effects of vehicle (0.9% NaCl) differed significantly ($p < 0.05$) from the active compounds. As the soft bufuralol analogs resulted a comparable heart rate and blood pressure decrease at doses of 1/10 to 1/5 of that of esmolol, they probably posses a more potent beta-blocking action than esmolol, at least in this model, but due to the rapid hydrolysis by esterases present in blood, they rapidly lose their beta-blocking activities after the cessation of infusion.

2.4. Antiarrhythmic activity in anesthetized rats

Three members of the soft amiodarone analog series, the isopropyl(**c**)-, the *sec*-butyl(**e**)- and neopentyl(**f**)-esters with enzymatic hydrolytic half-lives of 60, 240 and 300 min, respectively, were tested in an *in vivo* ventricular tachycardia model of anesthetized rats [56]. This test gave data on onset, amplitude, and offset of antiarrhythmic effects. In addition, ECG parameters (RR, PR, QRS, QT durations) were recorded and evaluated, so that information on the

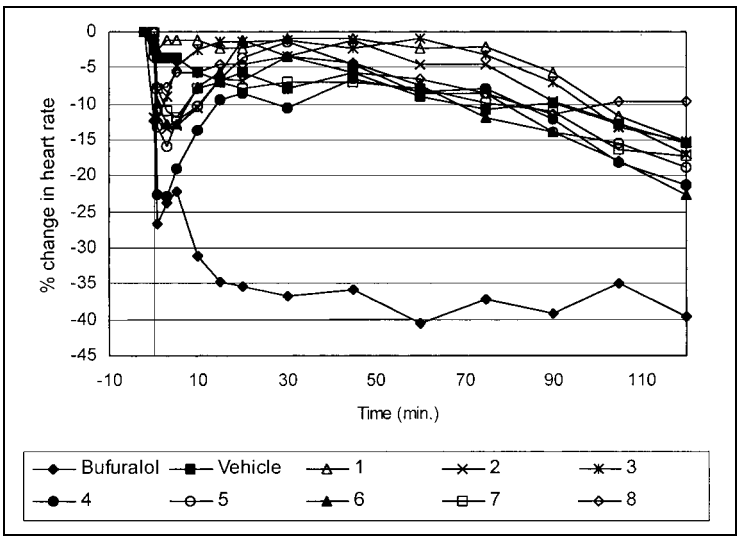

Fig. 5:
The effects of bufuralol (1 mg/kg = 3.8 μmol/kg), vehicle (10% DMSO in 30% HPBCD), and compounds **1**–**8** (3.8 μmol/kg) on isoproterenol induced tachycardia. The symbols represent the mean values of at least 3 animals. Cluster analysis of heart rate data established three different clusters. Between 0 and 5 min the cluster for vehicle and **1** was significantly different ($p < 0.05$) from the clusters of bufuralol and compounds **2**–**8**, while the cluster for bufuralol was different significantly ($p < 0.05$) from those of vehicle and **2**–**8** from 10 to 120 min

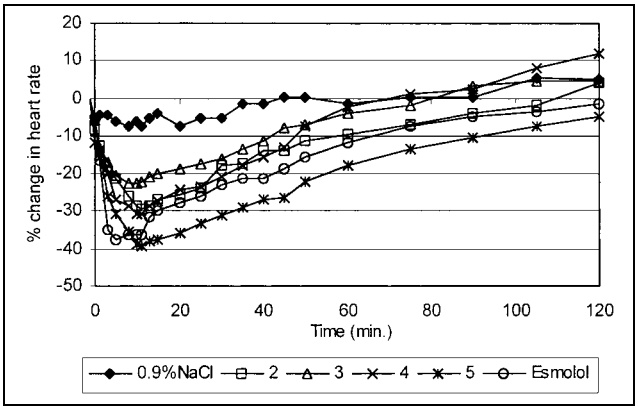

Fig. 6: The effects of normal saline, compounds **2**–**5**, and esmolol on resting heart rate. Infusion time was 10 min. Infusion rates were 2 μmol/kg/min for **2**, **4**, and **5**, 4 μmol/kg/min for **3**, and 20 μmol/kg/min for esmolol. The symbols represent the mean values of at least 3 animals. Cluster analysis demonstrated that there was a significant difference ($p < 0.05$) between the active drugs and 0.9% NaCl for up to 60 min while there was no difference between esmolol and compounds **2**–**5**

electrophysiologic effects of the new compounds could be obtained, too. Cordarone®, the intravenous amiodarone formulation, was chosen as control. In this model of chemically induced arrhythmia, a combination of anoxic conditions and high catecholamine levels resulted in the onset of repetitive ventricular tachycardias (VTs) and ventricular premature beats (VPBs). The antiarrhythmic efficacy of the compounds was evaluated by comparing the number of ventricular complexes during VTs and VPBs that occurred before drug administrations (average of three control benzene/adrenaline challenges) and after the bolus injection (5 μmol/kg), and during the infusion of the drugs (15 μmol/kg/h). Proceeding with the benzene/adrenaline provocations through another 60 min after the discontinuation of drug infusions, made it possible to follow the kinetics of diminution of the antiarrhythmic actions of the drugs. This way of drug administration, i.e., an i.v. bolus injection immediately followed by a slow infusion, is in accordance with current recommendations for i.v. amiodarone therapy [59], and it was applied to provide an initial loading dose, and then to maintain a kind of "steady-state" plasma level of the drugs. These doses were titrated in preliminary experiments to ensure complete VT protection

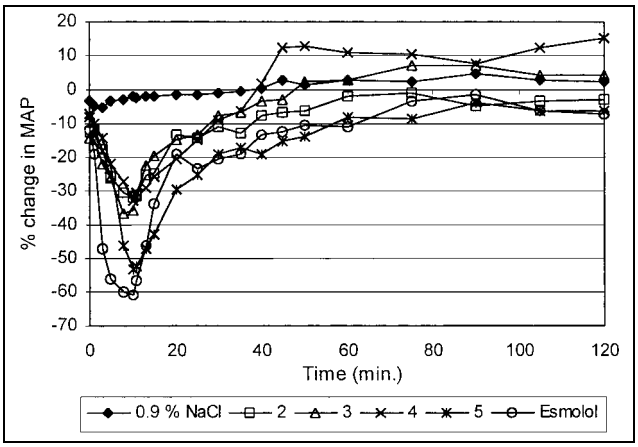

Fig. 7: The effects of normal saline, compounds **2**–**5**, and esmolol on resting mean arterial pressure (MAP). Drug administration was performed as described for Fig. 6. The symbols represent the mean values of at least 3 animals. Cluster analysis demonstrated that there was a significant difference ($p < 0.05$) between the active drugs and vehicle for up to 40 min while there was no difference between esmolol and compounds **2**–**5**

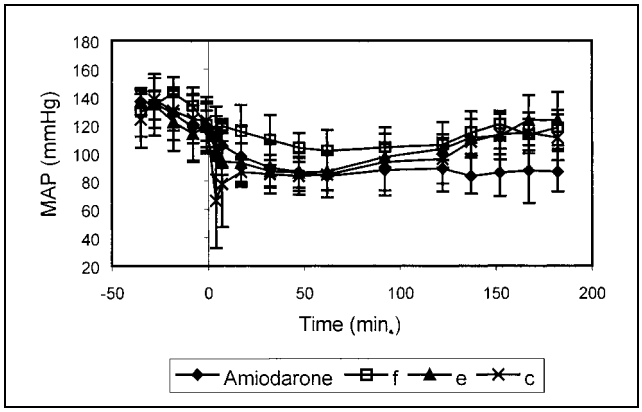

Fig. 8: Changes in mean arterial pressure (MAP). Three control benzene/adrenaline provocations were performed at −30, −20, and −10 min. Drugs (5 μmol/kg/h) were injected i.v. bolus at 0 min, that was followed by infusion (15 μmol/kg/h) for 2 h. Benzene/adrenaline challenges were performed again at 5, 15, 30, 45, 60, 90, 120 (end of infusion), 135, 150, 165, and 180 min. The symbols represent the mean of at least 4–5 animals

during drug administration. There was a significant but temporary blood pressure decrease following the administration of test compounds. The blood pressure drop was highest with compound **c** at 4 min after injection, down to $83 \pm 38.24/58 \pm 30.9$ mm Hg, while at 15 min it was already back to normal ($126.25 \pm 24.55/90.75 \pm 23.54$ mm Hg, Fig. 8). The effects on heart rate were recorded, too Fig. 9. Heart rate was monitored during benzene/adrenaline challenges but also every 5 min between challenges. Control values (−30, −20, and −10 min before drug) show intense tachycardia associated with the onset of VTs. Compounds **c** and **e**, but not **f** or amiodarone, inhibited totally the observed tachycardia as early as 5 min after bolus injection. These effects gradually disappeared upon cessation of drug infusion with **c**, **e**, and **f**, whereas the effects of amiodarone persisted until the end of the experiment. Compound **f** was not as potent as **c** or **e**. During the repeated benzene/adrenaline provocations, all four drugs showed suppressant activity against the development of ventricular tachycardia; however, their potencies and the kinetics of their antiarrhythmic actions were different. While the onset of antiarrhythmic activity of Cordarone® was about at 30 min, which is consistent with its slow onset of activity in humans, the onset of activity of compounds **c** and **e** was immediate, completely preventing the development of VTs at the first benzene/adrenaline challenge at 5 min after the i.v. bolus administration. Compound **f** had a longer onset and was not as potent as the other two test compounds or as amiodarone against the development of VTs/VPBs; however, it did decrease the number of ventricular complexes. There was also a difference regarding the offset of pharmacological activity fol-

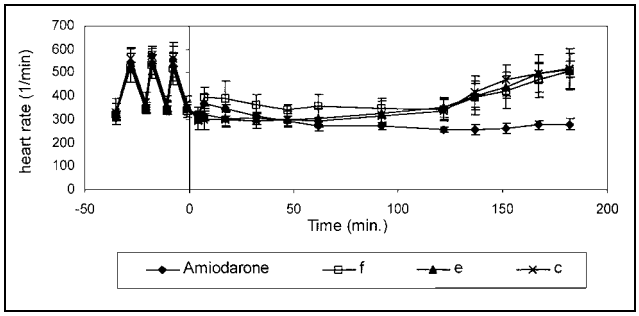

Fig. 9: Changes of heart rate. Experimental conditions were the same as described for Fig. 8

lowing cessation of drug infusions. While amiodarone retained its complete VT-suppressing activity for at least a full hour after the end of infusion, the analogs **c**, **e**, and **f** gradually lost their antiarrhythmic activity starting at between 15 and 30 min after the cessation of infusion (Fig. 10). Interestingly, the rate of disappearance of pharmacological activity seemed to correlate with previously measured half-lives in human plasma, i.e., **c** had the fastest offset of activity, whereas the effects of **f** were the slowest to disappear. In order to demonstrate that the test compounds were truly amiodarone analogs, their effects on certain ECG parameters were evaluated, and a profound prolongation of PR, QRS, QT, and RR intervals was found. The onset of action on all parameters was immediate with the new compounds **c**, **e**, and **f**. PR increased from a control value of 50 ms to 60 and even 65 ms, a 20 to 25% increase (Fig. 11). By comparison, the action of amiodarone on PR required at least 1 h to reach its peak. The offset of activity on PR followed the same pattern as for antiarrhythmic activity, i.e., the effects of amiodarone were maintained for the whole duration of the experiment following cessation of drug infusion, whereas the effects of the test compounds wore off. The effects of **c**, **e**, and **f** on QRS (Fig. 12), were also immediate and stronger than amiodarone, showing a 25–30% decrease in ventricular conduction velocity. These effects were rapidly reversible upon cessation of drug infusion. The effects of the test compounds on QT (Fig. 13) followed the same pattern as for QRS and PR, showing a rapid onset and a rapid offset of action, whereas the effects of amiodarone were slow to appear and were sustained even after the end of the infusion period. The QT values in Fig. 13 were not corrected for heart rate. A significant negative correlation was found between the prolongation of these ECG parameters (PR, QRS, QT) and the number of ventricular complexes during VTs/VPBs (Figs. 14–16). As the Figs. indicate, beyond a certain level of PR, QRS, and QT interval prolongation, the number of ventricular complexes is 0, i.e., no ventricular tachycardia or ventricular premature beat can be elicited by the benzene/adrenaline provocations. A significant positive correlation was found between heart rates and the number of ventricular complexes during VTs/VPBs, i.e., below a certain level of heart rate reduction caused by the drugs, no VPB or VT could be elicited by the benzene/adrenaline provocations (Fig. 17).

3. Discussion

All three classes of drugs evaluated in the above detailed experiments were designed and synthesized following the principles of the inactive metabolite approach of the soft drug design concept. The aim of these experiments was to evaluate the cardiovascular effects of the test compounds and, also, to demonstrate their soft nature in comparison to traditional drugs of their classes. The evaluation of these new compounds provided good examples on certain advantages of the soft drugs over their traditional counterparts.

The muscarinic receptor antagonist compound PCMS-2 was developed for topical use to be applied either as a mydriatic agent or as an antiperspirant. Major limitations in the clinical use of traditional antimuscarinic drugs are their side effects, such as dry mouth, photophobia, difficulty in urination, restlessness, irritability, disorientation, hallucinations, delirium, tachycardia, and cardiac arrhythmias. There were reports on the development of toxic effects, like psychosis, with the use of scopolamine eye drops [14, 15], which proves, that even topical application of traditional anticholinergics can lead to systemic side effects, because of absorption or drainage into the systemic circulation of these drugs. On the contrary, soft anticholinergic are expected to be much safer. The design of their structure starts from an inactive metabolite of the lead compound, e.g., methatropine, and an easily metabo-

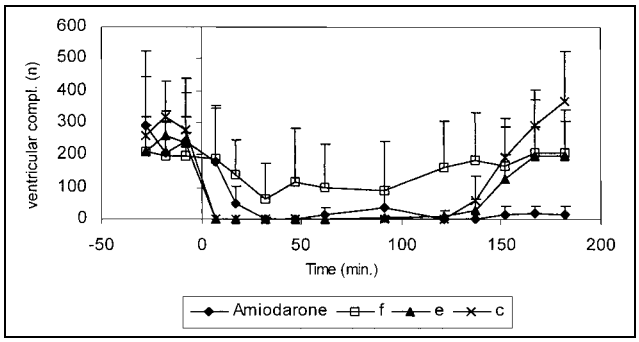

Fig. 10: The changes in the number of ventricular complexes during VT/VPB. Experimental conditions were the same as described for Fig. 8

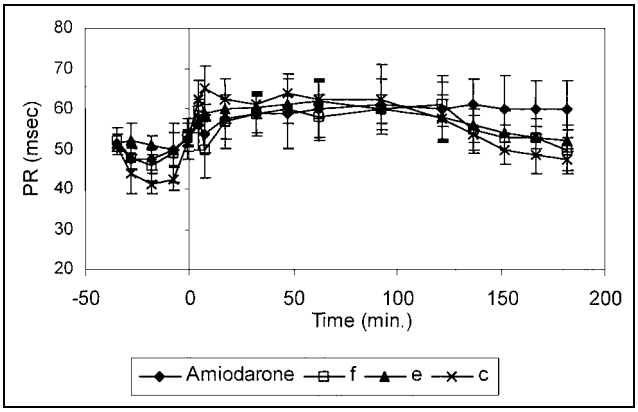

Fig. 11: Changes of PR interval. Experimental conditions were the same as described for Fig. 8

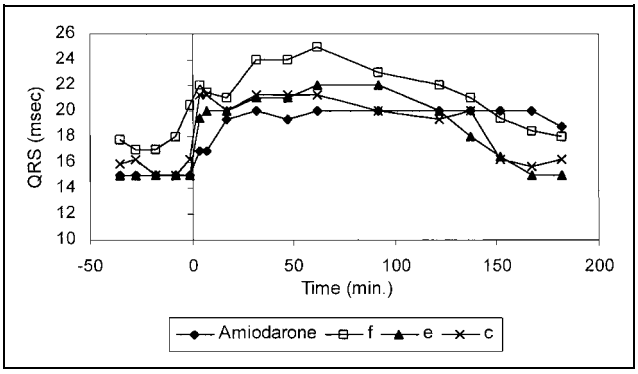

Fig. 12: Changes of QRS interval. Experimental conditions were the same as described for Fig. 8. Error bars are omitted for better visibility

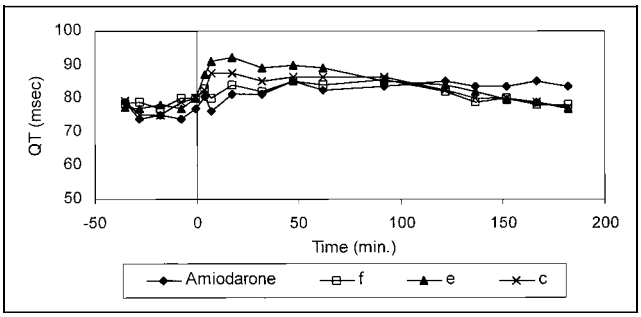

Fig. 13: Changes of QT interval. Experimental conditions were the same as described for Fig. 8. Error bars are omitted for better visibility

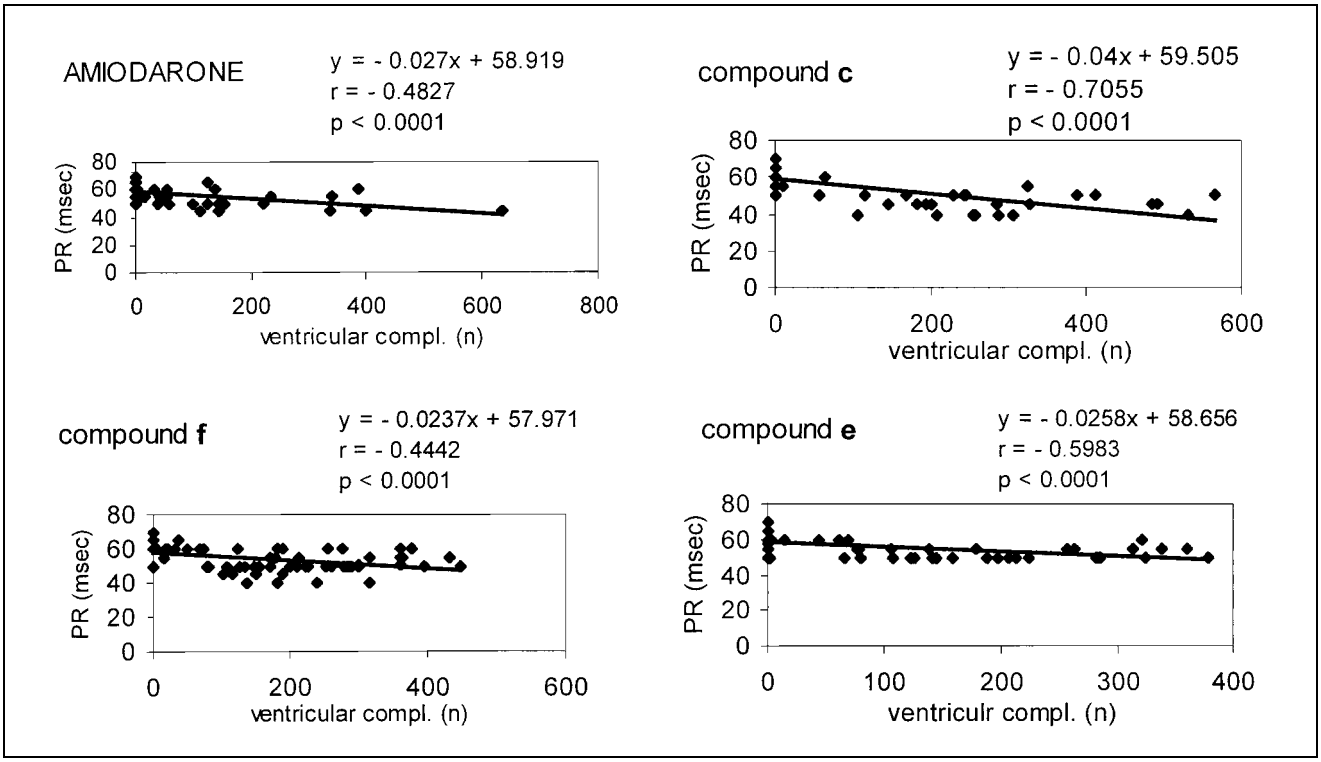

Fig. 14: Correlation of PR interval changes and the number of ventricular complexes during VT/VPB

lizable alkyl ester group is introduced into it, which provides the molecule with the desired antimuscarine activity again. Locally the drug can exert its therapeutic effect (mydriasis or antiperspirant activity); however, upon absorption or drainage into the systemic circulation, a rapid enzymatic deactivation by aspecific esterases present in blood and tissues will occur. The hydrolysis of the ester group results in the formation of the inactive metabolite, which is water soluble and can be eliminated rapidly through the kidneys. In *in vitro* tests, PCMS-2 showed somewhat lower receptor binding affinities to all four muscarinic receptor subtypes and a lower pA_2 value than atropineMeBr [7]. When tested as an eye drop in New-Zealand White rabbits, PCMS-2 caused pupil dilations only in the treated eye, but not in the control, untreated eye. Its mydriatic action only lasted for 6 h, while that of atropine lasted for 40 h. The dilation of the pupil in the untreated eye was observed with atropine and also when tropica-

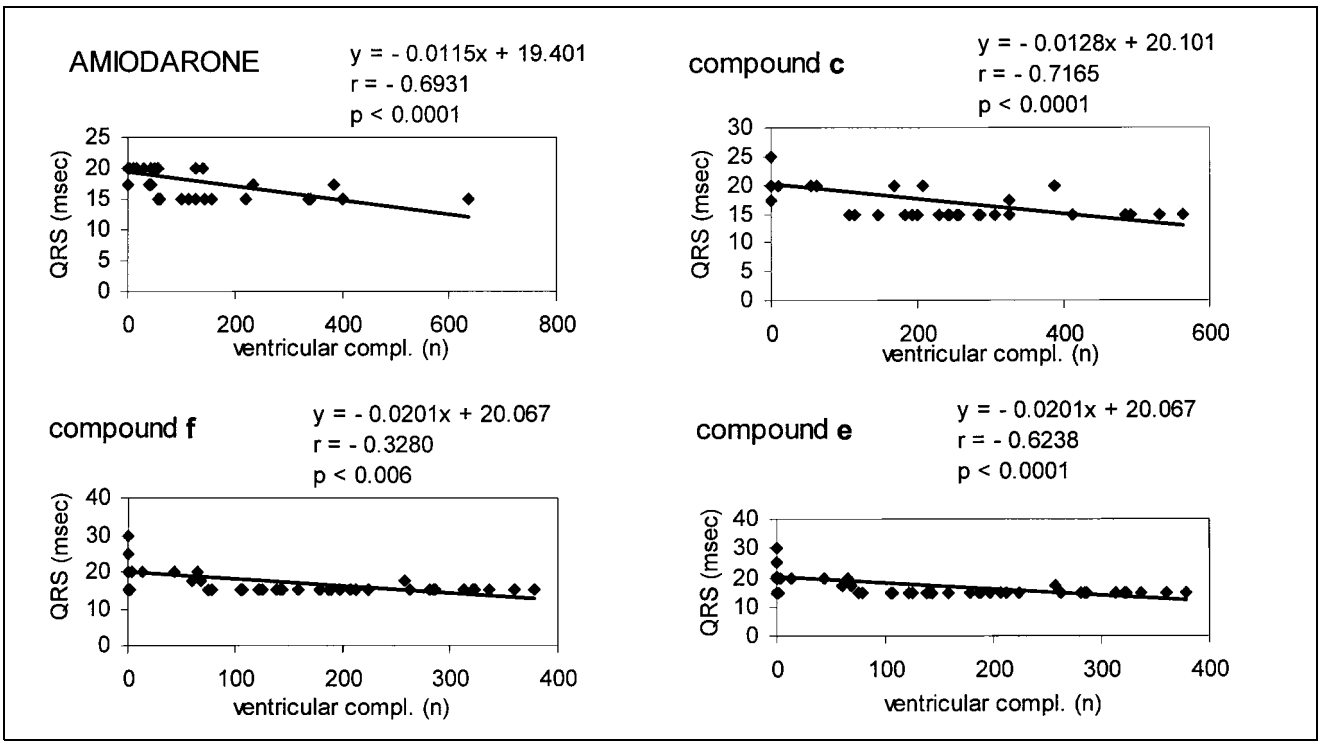

Fig. 15: Correlation of QRS interval changes and the number of ventricular complexes during VT/VPB

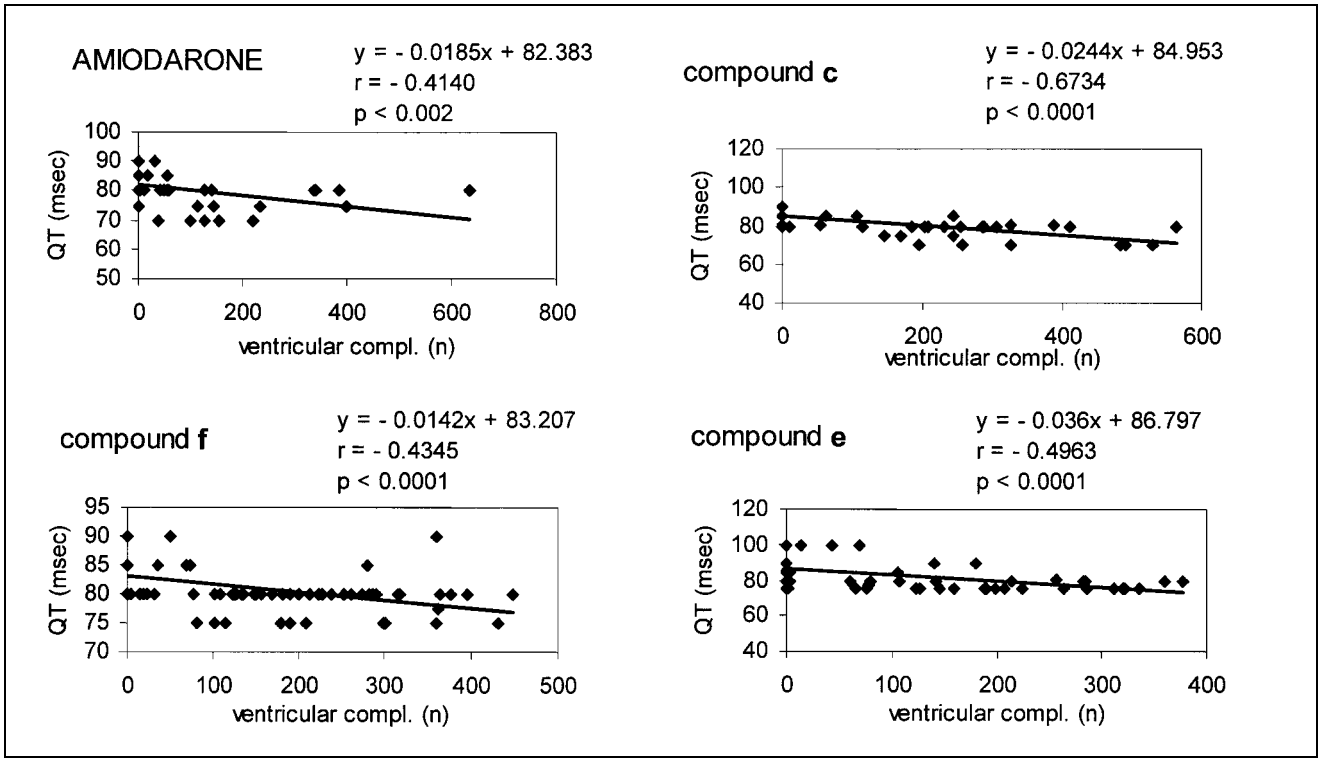

Fig. 16: Correlation of QT interval changes and the number of ventricular complexes during VT/VPB

mide, a short-acting mydriatic agent, was applied. The dilation of the untreated pupil suggests systemic actions of these drugs after their drainage and persistent presence in the systemic circulation [7]. Even if drainage or absorption of PCMS-2 into the circulation actually occurs, the chance for the development of systemic actions or adverse reactions would be minimal, due to the rapid enzymatic deactivation and fast elimination of the drug. This hypothesis was tested in those experiments when PCMS-2 was administered i.v. directly into the systemic circulation and its protection against the development of carbachol induced bradycardia was compared to the effects of atropineMeBr. PCMS-2 exerted its protection against the development of sinus bradycardia and A–V block elicited by repeated carbachol injections only for 15 min at a dose of 0.2 μmol/kg, while the effects of atropine MeBr at the same dose lasted for 2 h. This supports the assumption that when soft anticholinergics, like PCMS-2, are applied locally, the therapeutic index of the drug will be much higher than that of a traditional anti-

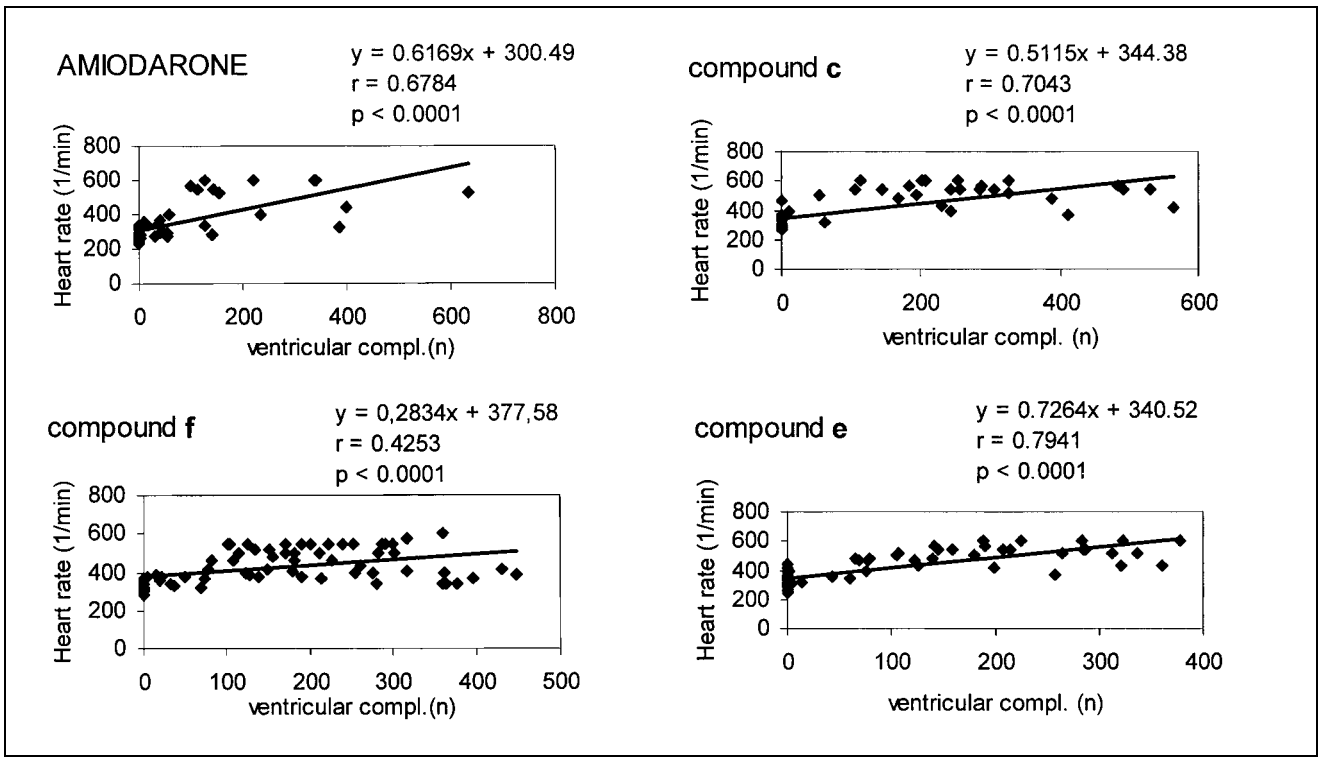

Fig. 17: Correlation of heart rate changes and the number of ventricular complexes during VT/VPB

cholinergic agent. Even higher concentrations of soft anticholinergics can be applied safely, because at a dose 10 times higher then the necessary concentration for preventing carbachol induced bradycardia (i.e., 2 μmol/kg), the duration of action of PCMS-2 only increased to 30 min. This suggests, that the capacity of the enzymes responsible for the hydrolysis of the ester groups is high enough to provide rapid inactivation of PCMS-2.

One of the reasons for the limited use of beta-blockers is their negative inotropic effect, which can be detrimental in critically ill patients with left ventricular dysfunction. Another problem arises when the transformation of these drugs by the liver cytochrom P450 isozymes results in the formation of multiple metabolites with considerable β-antagonist activities, but different half-lives. This is the situation with bufuralol, whose hepatic transformation into active alcohol and ketone metabolites leads to a complex pharmacokinetic picture [28–32, 60] with increased bioavailability, prolongation of elimination half-life, and more intense and sustained beta-blockade, which than can lead to severe hypotension and bradycardia especially in persons with the poor metabolizer phenotype [33, 34, 37–40].

Earlier it was discussed in details by Bodor in connection with the active metabolite approach of the soft drug design that "whenever oxidative metabolic transformations of a drug take place going through pharmacologically active species, if activity and pharmacokinetic considerations permit it, the drug of choice should be the active metabolite that is in the highest oxidized state" [60]. In spite of the possibility to chose an active metabolite of bufuralol as the lead compound and following the active metabolite principle of the soft drug design, the design strategy of the compounds **2**–**8** evaluated here followed the inactive metabolite approach. During the synthesis of these compounds, differently sized ester groups were introduced in the molecule to reactivate the inactive metabolite of bufuralol to posses again beta-antagonist action. This design strategy considered the observation that not only the rate of hydrolytic deactivation can be controlled by the ester structure, but esterases ubiquitously present in blood and tissues should quickly hydrolyze the labile ester functionality to produce the corresponding inactive acetic acid derivative. The soft drug design principle, which utilizes aspecific esterases as the primary enzymes for one-step deactivation into the inactive metabolite instead of hepatic transformation into multiple metabolites, is again a good example for an approach to develop safer drugs and, in this case, also with an ultra-short duration of action. These compounds exerted their beta-blocking actions for a much shorter period of time (30–40 min) than the parent drug bufuralol (at least 2 h) as demonstrated in the isoproterenol induced tachycardia model of the rat. Their beta-antagonist potencies at equimolar doses, i.e., at 3.8 μmol/kg, were somewhat lower than that of bufuralol; however, the most active compounds **4**, **5**, **2**, and **3** still demonstrated an activity of 30–50% of that of bufuralol. These most active compounds were compared to esmolol, the only ultra-short acting beta-blocker currently available for clinical use. Compounds **2**, **3**, **4**, **5**, and esmolol were infused for 10 min, and their effects on resting heart rate and blood pressure were evaluated. The equipotent doses of the soft bufuralol analogs were $1/10^{th}$ to $1/5^{th}$ of those of esmolol, i.e., 2–4 μmol/kg/min opposed to the dose of 20 μmol/kg/min of esmolol. However, they resulted a similar heart rate and blood pressure reduction as esmolol, with a kinetics that was not significantly different from that of esmolol. Based on these results, compounds **2**, **3**, **4**, and **5** can be good candidates for further development in search for more potent but ultra-short acting beta-blockers, which can be applied with a better safety profile than other currently available i.v. beta-blockers like propranolol, metoprolol, or labetalol in the treatment of supraventricular tachycardias or postoperative/perioperative hypertension even in critically ill patients with left ventricular dysfunction.

Based on retrometabolic drug design principles, a homologous series of amiodarone analogs were synthesized in which the 2-butyl chain of the parent drug was functionalized to include an ester function to be rapidly metabolized not only in the liver, but also in blood and tissues by aspecific esterases to a putative primary metabolite having a carboxylic acid moiety. Enzymes such as hydrolases (esterases, peptidases, lipases, glycosidases, phosphatases, etc.) are particularly well suited for the purpose of rapid metabolism of drugs, since this is a metabolism that is not dependent on age, gender, or weight, and which is unaffected by renal or hepatic function, and these enzymes are usually non-specific and ubiquitously distributed. Before the evaluation of the antiarrhythmic actions of these drugs, it was shown that the new compounds are good substrates to human plasma esterases *in vitro*. The purpose of these studies was to determine the half-life ($t_{1/2}$) of the members of the series in fresh human plasma at 37 °C and to determine a relationship between the nature of the individual esters and enzymatic hydrolytic rate, and also to give an idea of the relative stability of the individual homologs toward human plasma esterase; the ultimate goal being to provide a ranking order from which preferred condidates could be chosen for further *in vivo* testings. The time course of plasma concentrations gave a first-order exponential decay curve from which half-lives were calculated. Half-lives ranged from 6 min to more than 10 h. More importantly, a wide range of half-lives (6 min to 5 h) were found for compounds with relatively small ester groups of up to 5 carbons. This observations is important because the electrophysiological properties of these compounds in isolated guinea pig heart preparations seem to decrease when the ester group is bigger than 5 carbons (results to be published). When the test compounds were incubated in the presence of paraoxon, an esterase inhibitor, their concentrations did not change over time. This was taken as convincing evidence that the compounds did not simply hydrolyze due to pH and temperature, but rather because of enzymatic degradation by esterases. There was a clear relationship between the steric properties of the ester function and the observed metabolic rate. Hydrolysis was very rapid for small esters (12 and 6 min for **a** and **b**, respectively), but very slow for bulky groups (more than 10 h for **h**–**j**), with intermediate values for esters of intermediate size (30, 60, 90, 240, and 300 min for **c**–**g**, respectively) [58].

Finally, three members of the series, the isopropyl homolog **c**, the *sec*-butyl homolog **e**, and the neopentyl homolog **f**, with hydrolytic half-lives of 60, 240, and 300 min, respectively, were chosen for further testing in an *in vivo* ventricular tachycardia model of anesthetized rats [56]. The advantages of this model are the high reproducibility of the onset of VTs after the benzene/adrenaline provocations, the relative simplicity and cheapness of the method as it is applicable on small laboratory animals, like rats and guinea pigs. Altogether, it is a useful screening method for evaluating the antiarrhythmic efficacy of newly designed compounds. In our experiments, not only the inducibility of VTs were evaluated, but intraarterial blood

pressure and certain ECG parameters (PR, QRS, QT, and RR) were registered, too. By evaluating the ECG and blood pressure registrations, data on the electrophysiologic and haemodynamic effects of the tested compounds could be obtained, too. Compounds **c**, **e**, **f**, and amiodarone had profound actions on all channels; PR, QRS, and QT segments were prolonged indicating a blocking action on calcium, sodium, and potassium channels, respectively. PR is a combination of both sodium channel – atrial conduction – and calcium channel – AV nodal conduction – activity, but the main component is calcium channel effect, and the effects on QT are a combination of sodium and potassium channel activities, with the effect on potassium channel being equal to QT–QRS. The onset of action on all ion channels was immediate with the soft amiodarone analogs, but delayed with amiodarone. The effects on the ECG parameters with amiodarone were maintained for the whole duration of the experiment following the cessation of drug infusion, whereas the effects of the test compounds diminished within 15–30 min. The prolonging actions on the ECG parameters are in good accordance with the amiodarone-like activities of the new compounds; however, further electrophysiological studies are needed to establish their Class III properties. Due to the rapid onset and the rapid disappearance of their pharmacological activities, these soft analogs of amiodarone are expected to be useful during the treatment of ventricular tachycardias/fibrillation in emergency situations, and they are also expected to have a better safety record, due to their metabolism into a water-soluble inactive metabolite that can be eliminated by the kidneys, by which the chance for profound accumulation and for the development of serious adverse reactions can be minimized.

Summarizing our experience with soft drugs in respect to their cardiovascular effects, it can be concluded that the compounds in all three classes of drugs studied here showed their class-specific pharmacological actions, i.e., PCMS-2 prevented the carbachol induced bradycardia, the soft bufuralol analogs **2**–**8** diminished the heart rate increase after isoproterenol and also reduced resting blood pressure and heart rate, and, finally, the amiodarone analogs **c**, **e**, and **f** protected the development of VTs in a stress induced ventricular tachycardia model of the rat in good correlation with their amiodarone-like (Class III) actions on the ECG parameters. In accordance with the soft nature and the rapid enzymatic deactivation of these drugs, their pharmacological actions were temporary compared to their traditional counterparts. By the soft drug concept, where the structure of the drugs determines the metabolic fate of the compounds, which follows predicted one-step transformation into an inactive metabolite, the improvement of the therapeutic index can be expected.

4. Experimental

4.1. Antagonistic effect on the carbachol induced bradycardia (PCMS-2)

Male Sprague-Dawley rats with body weights of 300 ± 30 g (Harlan Sprague Dawley Inc., Indianapolis, IN) were anesthetized with Na pentobarbital (50 mg/kg i.p. – Butler Co., Columbus, OH). The skin from the neck area was removed, and the jugular veins on both sides were cleared of connective tissue. Needle electrodes were inserted s.c. into the limbs of the anesthetized rats and were joined to a GOULD TA 2000 recorder (GOULD Inc., Cleveland, OH). Standard leads I, II, and III were recorded at a paper speed of 25 mm/s. Baseline electrocardiography (ECG) recordings and all drug administrations were performed after a stabilization period of 15 min. PP and RR cycle length (ms) were measured manually, and HR (1/min) was calculated by the equation of 60000/RR cycle length. The presence of Mobitz II type A–V block (2 : 1, 3 : 1, etc.) was registered, too. Cholinomimetics such as carbachol have four primary effects on the cardiovascular system: vasodilation, a decrease in cardiac rate (negative chronotropic effect), a decrease in the rate of conduction in the sinoatrial (SA) and atrioventricular (AV) nodes (negative dromotropic effect), and a decrease in the force of cardiac contraction (negative inotropic effect) [61]. These effects of carbachol were manifested on the surface ECG as sinus bradycardia (lengthening of the PP cycle) and as development of Mobitz II type A–V block. After analyzing the ECG recordings, both heart rate and percent changes of heart rate, as compared to that of baseline, were plotted against time, and the effects of the different drugs on heart rate and on percent changes of heart rate were characterized. All drugs were dissolved in 0.9% NaCl (vehicle), and solutions were administered by direct injections into the jugular veins on either side. Anticholinergic drugs PCMS-2 and atropineMeBr (0.2 and 2 μmol/kg, in ~0.3 ml volume) or vehicle (~0.3 ml volume) were administered at 0 time, while carbachol (5–8 μg/kg in ~0.06–0.1 ml volume according to the initial individual ECG response of each rat) was injected at −5, 1, 3, 5, 10, 15, 20, 30, 45, and 60 min. Each point on the figures represents the mean ±S.D. of three experiments. Student's t-test (two-sample assuming equal variances) was used for statistical evaluation [7].

4.2. Effect on the isoproterenol induced tachycardia (soft bufuralol analogs **2**–**8***)*

The weight of the male Sprague-Dawley rats in these experiments was between 400–460 g and the anesthesia was performed as previously. Both jugular veins and the left carotid artery were isolated, and the latter tied up cranially with a surgical silk (Ethicon 4-0, Ethicon Inc., Australia). A plastic catheter (Intracath 19GA, Becton Dickinson, Sandy, UT) filled up with a solution containing 10% Na-Heparin (Elkins-Sinn Inc., Cherry Hill, NJ) in normal saline (100 U/ml Na-Heparin) was introduced into the artery and fixed with surgical silk. The catheter was connected to a pressure transducer (Ohmeda P23-XL, Ohmeda Medical Devices Division Inc., Madison, WI) filled up with the same heparinized 0.9% NaCl solution to register beat-to-beat arterial pressure. Needle electrodes were inserted s.c. and together with the pressure transducer were joined to the Gould TA 2000 recorder. Leads II, aVF, and intraarterial blood pressure were monitored simultaneously throughout the experiments and recorded at certain intervals at 50 mm/s paper speed. Baseline heart rate and blood pressure parameters were recorded for 25 min, at every 5 min, before any drugs were given. Drug administrations were carried out as bolus injections into the jugular veins. 50 μg/kg isoproterenol (Sigma, St. Louis, MO) was injected s.c. at −5 min. Heart rate and blood pressure were recorded at −5, −4 and −2 min. At 0 min the acidic metabolite of bufuralol (**1**), the soft bufuralol analogs **2**–**8** as free base forms dissolved in 10% DMSO (Fisher Scientific, Fair Lawn, NJ) in 30% hydroxypropyl-beta-cyclodextrin (HPBCD) (Pharmos Inc., Alachua, FL), or bufuralol (alpha-⟨(tert-butylamino)methyl⟩-7-ethyl-2-benzofuranmethanol; Roche Products Ltd., Welwyn Garden City, UK) dissolved in the same vehicle, or vehicle (i.e., 10% DMSO in 30% HPBCD) were injected into the jugular veins as bolus injections. Heart rate and blood pressure were registered at 1, 3, 5, 10, 15, 20, 25, 30, 40, 45, 50, 60, 70, 80, 90, 105, and 120 min. The percent changes in heart rate were calculated as follows: % change in HR = $(HR_t\text{-}HR_{-2})/HR_{-2} \times 100$ relative to the maximal isoproterenol induced heart rate increase registered at −2 min, where t = 0, 1, 3, ... 120 min.

4.3. Effect on resting heart rate and blood pressure (soft bufuralol analogs **2**–**8***)*

The anesthesia, the surgical procedures, the ECG and blood pressure recordings were the same as previously. The methyl-(**2**), ethyl-(**3**), isopropyl-(**4**), and *tert*-butyl-(**5**) analogs of bufuralol were converted to their corresponding HCl salt forms, and were dissolved in 0.9% NaCl. For the conversion to the salt forms a concentrated, methanolic solution of the free base was treated with HCl (1 M) in ether, then the salt form of the compound was obtained by the evaporation of the solvents in vacuum. Baseline heart rate and blood pressure values were registered again at every 5 min for 30 min. At 0 min the four bufuralol analogs, or esmolol-HCl (Brevibloc®, 2.5 g/10 ml Ohmeda Pharmaceutical Products Division Inc., Liberty Corner, NJ) diluted with 0.9% NaCl were infused into the jugular vein for 10 min with a syringe pump (Sage Instruments, Model 341B, Orion Res. Inc., Boston, MA) through a plastic catheter (Terumo 24GA × 3/4″, Terumo Medical Corp., Elkton, MD) fixed in the jugular vein. The doses were 2 μmol/kg/min for **2**, **4**, and **5**, 4 μmol/kg/min for **3**, and 20 μmol/kg/min for esmolol. The percent changes in heart rate and blood pressure values [mean arterial pressure: MAP = (SBP-DBP)/3 + DBP] were calculated relative to the average baseline values recorded between −30 to 0 min., i.e., % change in HR = $(HR_t\text{-}HR_{av})/HR_{av} \times 100$ or % change in MAP = $(MAP_t\text{-}MAP_{av})/Map_{av} \times 100$, where t = 1, 3, ... 120 min. In both series of experiments, each compound and vehicle were administered to at least 3 different animals, the average and the standard deviation of all heart rate and blood pressure data were calculated. All data were subjected to cluster analysis and to multivariate analysis of variance for repeated measures (GLM Repeated Measures of Anova) with the SPSS for Windows 7.5 program. Statistically significant difference between the effects of the different compounds was accepted at $p < 0.05$.

4.4. Antiarrhythmic activity in anesthetized rats (soft amiodarone analogs c, e, and f)

The anesthesia of the male Sprague-Dawley rats (weight: 400–450 g), the preparation of the jugular veins and of the left carotid artery, and the registration of ECG and intraarterial beat-to-beat blood pressure were performed as previously. The surgery was completed with an incision on the trachea where a short plastic tubing was placed and the animal was connected to a rodent-model ventilator (Harvard Model 683, Harvard Apparatus, South Natick, MA). The animals were ventilated in an assisted way depending on their spontaneous breathing frequency (55–65/min). Drug administrations were carried out through i.v. catheters (Terumo 24 GA × 3/4″, Terumo Medical Corp., Elkton, MD) inserted into both jugular veins. The left side was used for drug infusions by means of a syringe pump (Sage Instruments, Model 341B, Orion Res. Inc., Boston, MA), and the right side was used for adrenaline injections. The experiments were started after a minimum of 15 min stabilization period. Benzene/adrenaline challenges were performed as follows: the respirator inlet was connected to a 50-ml bubbler half-full with benzene (Fisher Scientific, Fair Lawn, NJ) so that the inspired air was saturated with benzene vapor. The animal was ventilated with benzene vapor for 2 min. The tidal volume was typically 1 ml/100 g of animal. During the last 30 s of benzene ventilation, 10 μg/kg adrenaline was injected into the right jugular vein [56]. Typically within 30 s ventricular premature beats (VPB) and hemodynamically stable sustained and non-sustained repetitive ventricular tachycardia (VT) occurred. After 30–60 s, the normal sinus rhythm returned, and the animal did not show other abnormal signs. These arrhythmias could be elicited repeatedly. After three control VTs (at −30, −20 and −10 min), amiodarone and the ester analogs (compounds **c**, **e**, and **f**) were injected at a dose of 5 μmol/kg i.v. bolus, immediately followed by a slow infusion of 15 μmol/kg/h for 2 h. There were 4 or 5 rats per test compound. The ability of the different drugs to suppress these arrhythmias was tested against repeated benzene/adrenaline challenges at times 5, 15, 30, 45, 60, 90, 120 (end of drug infusion), 135, 150, 165, and 180 min after i.v. bolus administration. The arterial pressure, heart rate, number of ventricular complexes during the occurrence of VTs and VPBs, and certain ECG parameters (PR, QRS, QT durations and RR cycle lengths in ms) were recorded. Each animal served as its own control. The amplitudes of the systolic arterial pressure and diastolic arterial pressure were registered, and the mean arterial pressure (MAP) was calculated as previously. The results were subjected to Anova and Newman – Keuls test using the SAS for Windows 6.12 program. The changes in the observed parameters were accepted to be significant at $p < 0.05$.

This research paper was presented during the 2nd Conference on Retrometabolism based Drug Design and Targeting, May 11–14, 1999, Amelia Island, Florida, USA

References

1 Bodor, N.: Chemtech. **Oct.** 22. (1995)
2 Bodor, N.; In: de Vito, S. C.; Garrett; R. L. (eds.): ACS Symposium Series, V. **640**, p. 84 Am. Chem. Soc., Washington D.C: 1996
3 Bodor, N.: Pharmazie **52**, 491 (1997)
4 Bodor, N.; Woods, R.; Raper, C.; Keany, P.; Kaminski, J.: J. Med. Chem. **23**, 474 (1980)
5 Bodor, N.; ElKoussi, A.; Hammer, R. H.: J. Biopharm. Sci. **3**, 215 (1990)
6 Brouilette, G.; Kawamura, M.; Kumar, G. N.; Bodor, N.: Pharm. Sci. **85**, 619 (1996)
7 Juhász, A.; Huang, F.; Ji, F.; Buchwald, P.; Wu, W. M.; Bodor, N.: Drug Dev. Res. **43**, 117 (1998).
8 Bodor, N.; Oshiro, Y.; Loftsson, T.; Katovich, M.; Caldwell, W.: Pharm. Res. **3**, 120 (1984)
9 Yang, H. S.; Wu, W. M.; Bodor, N.: Pharm. Res. **12**, 329 (1995)
10 Bodor, N.; Loftsson, T.; Wu, W. M.: Pharm. Res. **9**, 1275 (1992)
11 Bodor, N.; Wu, W. M.; Murakami, T.; Engel, S.: Pharm. Res. **12**, (1995)
12 Howes, J.; Novack, G. D.: J. Ocul. Pharmacol. Ther. 14, 153 (1998)
13 The Loteprednol Etabonate Postoperative Inflammation Study Group 2: Ophthalmology **105**, 1780 (1998)
14 Birkhimer, L. J.; Jacobson, P. A.; Olson, J.; Goyette, D. M.: J. Fam. Prac. **18**, 464 (1984)
15 Hamborg, P. M.; Neilsen, M. M.; Thordal, C.: Acta Ophthalmol. Scand. **62**, 485 (1984)
16 Frishman, W. H.; Hershman, D.: In: Messerli, F. H. (Ed.): Cardiovascular Drug Therapy, Second Edition, p. 465. W. B. Saunders Co. 1996
17 Gerber, J. G.; Nies, A. S.: Ann. Rev. Med. **36**, 145 (1985)
18 McDevit, D. G.: Drugs **17**, 267 (1979)
19 Frishman, W. H.; Shrinivas Murthy, V.; Strom, J. A.; Hershman, D.: In: Messerli (Ed.): Cardiovascular Drug Therapy, Second Edition, p. 507. W. B. Saunders Co. 1996
20 Erhardt, P. W.; Woo, C. M.; Gorzynski, R. J.; Anderson, W. G.: J. Med. Chem. **25**, 1402 (1982)
21 Bourne, G. R.: Progr. Drug Metab. **6**, 77 (1981)
22 Fothergill, G. A.; Osbond, J. M.; Wickens, J. C.: Arzneim.-Forsch. **27**, 978 (1977)
23 Hamilton, T. C.; Parkes, M. W.: Arzneim.-Forsch. **27**, 1410 (1977)
24 Johnston, G. D.; Finch, M. B.; Shanks, R. G.: Eur. J. Clin. Pharmacol. **30**, 649 (1986)
25 Magometschnigg, D.; Bonelli, J.; Kaik, G.; Rameis, H.: Int. J. Clin. Pharmacol. **17**, 334 (1979)
26 Magometschnigg, D.; Bonelli, J.; Hitzenberger, G.; Kaik, G.; Korn, A.: Int. J. Clin. Pharmacol. **16**, 54 (1978)
27 Dayer, P.; Balant, L.; Courvoisier, F.; Kupfer, A.; Kubli, A.; Gorgia, A.; Fabre, J.: Eur. J. Drug Metab. Pharmacokin. **7**, 73 (1982)
28 Machin, P. J.; Hurst, D. N.; Osbond, J. M.; J. Med. Chem. **28**, 1648 (1985)
29 Weerawarnna, S. A.; Geisshusler, S. M.; Murthy, S. S.; Nelson, W. L.: J. Med. Chem. **34**, 3091 (1991)
30 Francis, R. J.; East, P. B.; Larman, J.: Eur. J. Clin. Pharmacol. **23**, 529 (1982)
31 Eckert, M.; Cocco, G.; Strozzi, C.; Heizmann, P.; Sfrisi, C.: Eur. J. Clin. Pharmacol. **24**, 479 (1983)
32 Pringle, T. H.; Francis, R. J.; East, P. B.; Shanks, R. G.: Br. J. Clin. Pharmacol. **22**, 527 (1986)
33 Dayer, P.; Kronbach, T.; Eichelbaum, M.; Meyer, U. A.: Biochem. Pharmacol. **36**, 4145 (1987)
34 Dayer, P.; Leemann, T.; Kupfer, A.; Kronbach, T.; Meyer, U. A.: Eur. J. Clin. Pharmacol. **31**, 313 (1986)
35 Guengerich, F. P.; Umbenhauer, D. R.; Churchill, P. F.; Beune, P. H.; Bocker, R.; Knodell, R. G.; Martin, M. V.; Lloyd, R. S.: Xenobiotica **17**, 311 (1987)
36 Gut, J.; Gasser, R.; Dayer, P.; Kronbach, T.; Catin, T.; Meyer, U. A.: FEBS Lett. **173**, 287 (1984)
37 Lennard, M. S.; Tucker, G. T.; Woods, H. F.: Clin. Pharmacokin. **11**, 1 (1986)
38 Smith, R. L.: Eur. J. Clin. Pharmacol. **28**, (Suppl) 77 (1985)
39 Lennard, M. S.: Klin. Wschr. **63**, 285 (1985)
40 Dayer, P.; Kubli, A.; Kupfer, A.; Courvoisier, F.; Balant, L.; Fabre, J.: Br. J. Clin. Pharmacol. **12**, 750 (1982)
41 Bodor, N.; Oshiro, Y.; Loftsson, T.; Katovich, M.; Caldwell, W.: Pharm. Res. **3**, 120 (1984)
42 Bodor, N.; ElKoussi, A.: Curr. Eye Res. **7**, 369 (1988)
43 Bodor, N.; ElKoussi, A.; Kano, M.; Khalifa, M.: J. Med. Chem. **28**, 1651 (1988)
44 Yang, H. S.; Wu, W. M.; Bodor, N.: Pharm. Res. **12**, 329 (1995)
45 Charlier, R.; Delaunois, G.; Bauthier, J.: Cardiologia **54**, 82 (1969)
46 Singh, B. N.; Vaughan Williams, E. M.: Br. J. Pharmacol. **39**, 657 (1970)
47 Rosenbaum, M. B.; Chiale, P. A.; Ryba, D.: Am. J. Cardiol. **34**, 215 (1974)
48 Rosenbaum, M. B.; Chiale, P. A.; Halpern, M. S.: Am. J. Cardiol. **38**, 934 (1976)
49 Naccareli, G. V.; Rinkenberger, R. L.; Dougherty, A. H.; Giebel, R. A.: Pharmacotherapy **6**, 298 (1985)
50 Kerr, C. R.; Rosenbaum, M. B.; Chiale, P. A.: In: Messerli, F. H. (Ed.): Cardiovascular Drug Therapy, Second Edition, p. 1247. W. B. Saunders Co. 1996
51 Cairns, J. A.; Connolly, S. J.; Roberts, R. S.; Gent, M. and the Camiat Investigators: Lancet **349**, 675 (1997)
52 Julian, D. G.; Camm, A. J.; Frangin, G.; Janse, M. J.; Munoz, A.; Schwartz, P. J.; Simon, P.; Emiat Investigators: Lancet **349**, 667 (1997)
53 Amiodarone Trials Meta-Analysis Investigators: Lancet **350**, 1417 (1997)
54 Vrobel, T. R.; Miller, P. E.; Mostow, N. D.; Rakita, L.: Progr. In Cardiovasc. Dis. **31**, 393 (1989)
55 Kowey, P. R.; Levine, J. H.; Herre, J. M.; Pacifico, A.; Lindsay, B. D.; Plumb, V. J.; Janosik, D. L.; Kopelman, H. A.; Scheinman, M. M.; for the Intravenous Amiodarone Multicenter Investigators Group: Circulation, **92**, 3255 (1995)
56 Tripathi, R. M.; Thomas, G. P.: J. Pharmacol. Meth. **15**, 279 (1986)
57 Hwang, S. K.; Juhász, A.; Yoon, S. H.; Bodor, N.: submitted for publication
58 Juhász, A.; Bodor, N.; Milner, P. G.; Druzgala, P.: submitted for publication
59 Naccarelli, G. V.; Jalal, S.: Circulation **92**, 3154 (1995)
60 Bodor, N.: In: Dulbecco, R. (Ed.): Encyclopedia of Human Biology Vol. 7, p. 101, Academic Press Inc. 1991
61 Higgins, C. B.; Vatner, S. F.; Braunwald, E.: Pharmacol. Rev. **25**, 119 (1973

Prof. Dr. Nicholas Bodor
Center for Drug Discovery
University of Florida
Box 100497
Gainesville, FL 32610-0497
USA
bodor@cop.ufl.edu