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COMPARISON BETWEEN THE ACTIVITIES OF CATIONIC AMPHIPHILIC DRUGS TO AFFECT PHOSPHOLIPID-MEMBRANES AND TO DEPRESS CARDIAC FUNCTION

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Abstract—The activity of cationic amphiphilic compounds to affect artificial phospholipid-membranes was compared with the membrane-stabilizing cardiodepressant potency of the drugs. The twenty-one investigated catamphiphilic compounds belonged to various pharmacological groups including antiarrhythmic, local anaesthetic, β-blocking, antimalarial, and psychoactive drugs. The perturbing action of the drugs on phospholipid-membranes was evaluated by determining the drug-effect on the temperature of the phase-transition from the gel to the liquid crystalline state in liposomes of dipalmitoylphosphatidic acid by means of differential scanning calorimetry. The ability to interact with the polar headgroups of phospholipid-membranes was measured by recording the effects of the cationic compounds on the binding of $^{45}Ca^{2+}$ to monomolecular layers of phosphatidylserine. The cardiodepressant action was observed in Langendorff-preparations of guinea-pig hearts. The drug-effect on excitability was indicated by the elevation of the threshold-intensity of 50 Hz alternating current to induce ventricular arrhythmia. For the sake of comparison, the negative chronotropic and inotropic effects were evaluated. There was only a moderate correlation found between the activities of the drugs to reduce the transition temperature and to inhibit $^{45}Ca^{2+}$-binding ($r = 0.69$). This result probably reflects that both methods look upon different consequences of the drug–phospholipid interaction. The membrane-stabilizing, anti-excitatory potency corresponded favourably with the ability of the drugs to affect the phospholipids. Almost 80% of the variance between the anti-excitatory potencies could be accounted for by the drug–phospholipid interactions. The negative chronotropic and inotropic effects accompanying the anti-excitatory actions were similar for most of the drugs. The results of the study are compatible with the hypothesis that the interaction with phospholipid-membranes is a major determinant of the membrane-stabilizing cardiodepressant potency of cationic amphiphilic drugs.

Drugs from various groups share the physicochemical property of cationic amphiphilia. An aromatic ring system provides lipophilia and a side chain with an amino-group being predominantly protonized at neutral pH contributes cationic hydrophilia. Catamphiphilic molecules tend to bind to phospholipid-membranes, the cationic part lying within the polar region of the phospholipid-headgroups, the aromatic ring directing to the apolar region of the fatty acyl chains [1–5].

A cardiodepressant action can be elicited by a variety of catamphiphilic drugs, e.g. antiarrhythmic and local anaesthetic drugs [6], β-blockers [7–10], or phenothiazine neuroleptics [9]. The cardiodepression includes a reduction of excitability, heart rate, and force of contraction. It is often attributed to a so-called membrane-stabilizing action [11]. Originally, however, the term membrane-stabilization means a stabilization of the resting membrane potential, i.e. an inhibition of membrane depolarization [12, 13]. The reduced excitability indicates an impaired sodium-channel function (for reviews e.g. Refs. 6, 14, 15).

Yet, the term membrane-stabilization does not elucidate the molecular mode of action. To account for the voltage- and stimulation-dependence of antiarrhythmic drug-action, the models of Hondeghem and Katzung [16, 17] and Starmer *et al.* [18] assume a receptor to be present on the channel protein. On the other hand, it is tempting to relate membrane-stabilization to an unspecific intercalation of drug-molecules into the lipid matrix of heart cell membranes. Catamphiphilic drugs accumulate in intact cardiac tissue depending on their hydrophobicity [19]. In the case of β-blocking agents various investigators found good correlations between the cardiodepressant potency and the hydrophobicity of the drugs (e.g. Refs 10, 20, 21). Lee [22, 23] reported that the capability of drugs to reduce the phase-transition temperature of artificial phospholipid-membranes corresponded with their local anaesthetic potency. Schlieper and coworkers (e.g. Ref 24) found that tetracaine, propranolol, lidocaine, and procaine affected the surface charge of phospholipid-membranes with the same rank order of potency as they acted cardiodepressant. Mechanisms by which drug-incorporation in the phospholipid-bilayer might affect an integral membrane protein have been proposed by Seeman [25], Trudell [26], and Lee [22, 23].

In the present study twenty-one catamphiphilic drugs from various pharmacological groups were compared with respect to their ability to interact with phospholipid-membranes and to depress cardiac function. If the interaction with phospholipid-bilayers were a major determinant of the cardiodepressant activity, a good correlation should emerge between

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the potency to interact with phospholipid-membranes and the membrane-stabilizing activity. For the sake of comparison, three further drugs were included. The uncharged local anaesthetic benzocaine ($pK_a = 2.5$ [27]) should also affect phospholipid-membranes and depress cardiac function. The specifically acting catamphiphilic calcium-antagonists verapamil and diltiazem should deviate from the correlation.

Two aspects of the interaction of the cationic drug molecules with phospholipid-membranes were looked upon separately. In order to evaluate the perturbing activity on the membranes, the drug-effect on the temperature of the transition from the gel to the liquid crystalline state was determined in liposomes of dipalmitoylphosphatidic acid (DPPA) by means of differential scanning calorimetry. Previous studies [28, 29] had indicated that the intrinsic activity of drugs to perturb the bilayer is easier screened in DPPA- than in DPPC-liposomes. The interaction with the phospholipid-headgroups was evaluated by measuring the drug-effects on $^{45}Ca^{2+}$-binding to phosphatidylserine-monolayers. Several catamphiphilic drugs have already been investigated [30] so that the list had only to be completed.

The cardiodepressant potency was screened in isolated guinea-pig hearts perfused according to Langendorff [31]. In the spontaneously beating hearts, it was possible to determine not only the membrane-stabilizing (negative bathmotropic) potency, but also the negative chronotropic, and the negative inotropic drug-actions. In order to evaluate the membrane-stabilizing drug-effect, i.e. the reduction of excitability, alternating current was delivered to the ventricular walls and the threshold current for arrhythmia was determined. The application of alternating current to investigate drug-effects on the excitability of guinea-pig cardiac preparations has been described previously by Borchard and coworkers [32, 33].

METHODS

Phase-transition temperature of DPPA-liposomes. The preparation of the liposomes and the measurement of the transition temperature (T_t) by means of differential scanning calorimetry was performed as described previously [28, 29]. DPPA (1,2-dipalmitoyl-*sn*-glycero-3-phosphate monosodium salt, purity 99%; Sigma Chemical Co., München, F.R.G.) was weighed in portions of 5 mg into glass vials. The drugs were dissolved in chloroform or chloroform/methanol (v/v 15/2–3/1) at an ambient temperature of 6° and the solutions were added to the phospholipid in the appropriate volumes to obtain the indicated drug/DPPA-ratios. At a temperature of 20° the samples were first dried under a stream of nitrogen for 2 hr and then evaporated overnight in a vacuum-exsiccator. The next day, 100 µl of a buffer composed of 14 mM TES*/14 mM histidine adjusted with HCl to pH 6 were added to the drug/DPPA mixture. The pH of 6 lies in between the pK_a values of phosphatidic acid of $pK_a = 3.5$ and $pK_a = 9$ [34]. Thus, at pH = 6 DPPA is mono-anionic, the curve for the dependency of T_t from the pH reveals a plateau [34], and the eventual small variations of the sample pH do not affect T_t. At pH 6, the drugs were almost completely protonized, i.e. in the cationic amphiphilic form. The liposome suspension was prepared by incubating the samples in a water bath at 70°, i.e. above T_t, for 2 hr. Every 30 min the sample vials were vigorously shaken on a bench vibrator. Ten µl of the resulting milky suspensions were encapsulated in aluminium pans (Perkin-Elmer, Überlingen, F.R.G.) for determination of the phase-transition temperature by differential scanning calorimetry. The measurement was performed with a "DSC-2C/intracooler II"-equipment (Perkin–Elmer). An aluminium pan containing 10 µl distilled water served as the reference. The pans were heated from 12° to 72° at a rate of 5°/min and a sensitivity range setting of 0.5 mcal/sec. The temperature scale was calibrated using cyclohexane and indium as the standards. The evaluation of T_t from the thermograms is described in Results.

$^{45}Ca^{2+}$-binding to phosphatidylserine-films. The method to measure the binding of $^{45}Ca^{2+}$ to monomolecular films of phosphatidylserine (PS) spread on the surface of an aqueous buffer has been described previously [30, 35]. The buffer was composed of $CaCl_2$, 0.01 mM; NaOH, 5 mM; TES, 2 mM; histidine, 2 mM adjusted with HCl to pH 7.5, and the drugs at the indicated concentrations. Trace amounts of $^{45}CaCl_2$ (New England Nuclear, Dreieich, F.R.G.) were added in order to yield about 2500 counts per minute (cpm) in the absence of the phospholipid-film. Measurements were performed at room temperature (about 20°). 5 ml of the buffer were filled into a Teflon-planchette with 4.5 cm diameter, which was then positioned under the end-window of a Geiger–Müller counting tube (Frieseke & Hoepfner, Erlangen, F.R.G.) in order to determine the radioactivity (electronic equipment by Berthold, Wildbad, F.R.G.) The background impulse rate detected in the absence of $^{45}Ca^{2+}$ amounted to about 300 cpm. Phosphatidylserine (from bovine brain, purity 98–99%, Sigma) dissolved in 1 µl chloroform was applied in an amount of 3 nmol on the surface of the buffer. The counting rate almost doubled indicating $^{45}Ca^{2+}$-adsorption to the film. In the presence of the drug, the adsorption of $^{45}Ca^{2+}$ was reduced. The drug-effect was evaluated by expressing the $^{45}Ca^{2+}$-absorption to the film measured in the presence of the drug as a percentage of the absorption recorded in the absence of the drug.

Functions of the isolated guinea-pig heart. Guinea-pigs of either sex (weight 200 – 400 g) were killed by a blow on the neck and exsanguinated by cutting a carotid artery. Immediately after opening the chest, Tyrode solution at 7° was poured over the heart in order to induce bradycardia and reduce oxygen demand. The heart was isolated and fixed with its aortic stump to the outlet pipe of the Langendorff-apparatus. Coronary perfusion was

* TES = *N*-tris(hydroxmethyl)-methyl-2-aminoethane sulfonic acid.

Table 1. Investigated drugs

Compounds	log*p*	ΔT_t [°]	IC_{50} [μM]	AC_{50} [μM]	Δh.r./Δc.f. [%]
Antiarrhythmic drugs					
Aprindine	5‖	36	40	0.5	9/12
Disopyramide	1.8‖	22	7000	85	20/25
Lidocaine	2.5§	28†	3250‡	20	8/8
Mexiletine	1.3‖	32	300	17	11/11
Procainamide	0.8‖	19	3000	340	19/18
Propafenone	—	27	17	1.2	4/4
Quinidine	1.8§	30	45‡	22	33/23
β-Blockers					
Acebutolol	1.7¶	19	2600	110	19/29
Atenolol	0.2§	15	6300‡	2000	7/10
Diacetolol	—	12	5100	64	10/39
Pindolol	1.6¶	25	580	45	6/21
Propranolol	3.1§	37*	22‡	4.4	12/15
Various	§		§		
2-Aminopyridine	0.2	13	1200∅	11000	40/32
Benzocaine	2.3**	10	∅	240	14/18
Chloroquine	4.6	15	30	32	30/71
Chlorphentermine	2.6	34†	80	43	12/32
Chlorpromazine	5.3	36†	7	0.8	12/18
Dibucaine	4.3	48*	35	0.2	12/10
Diltiazem	—	30	70	∅	∅
Phentermine	1.9	28*	700	160	7/18
Procaine	1.9	21	4000	170	20/16
Quinine	1.8	29	45	20	22/32
Tetracaine	3.7	42†	100	0.5	9/8
Verapamil	2.5	34	150‡	∅	∅

log*p*: octanol–water partition coefficient; ΔT_t: reduction of the transition temperature of DPPA; IC_{50}: concentration to inhibit $^{45}Ca^{2+}$-binding to phosphatidylserine monolayers by 50%; AC_{50}: concentration to elevate the threshold of alternating current to induce arrhythmia in isolated guinea-pig hearts by 50%; Δh.r.: %-reduction of the heart rate at AC_{50}, Δc.f.: %-reduction of the contraction amplitude at AC_{50}.

* : mean values from [29] and the present study.
† : from [29].
‡: mean values from [30] and the present study.
§: from [30].
‖: from [54].
¶: calculated from [55].
**: from [56].
—: not available.
∅: not measurable.

done with oxygenated (95% O_2/5% CO_2) Tyrode solution modified according to von Muralt (NaCl, 136.8 mM; KCl, 2.7 mM; $CaCl_2$ 1.8 mM; $NaHCO_3$ 11.9 mM; $MgCl_2$ 1.05 mM; NaH_2PO_4 0.21 mM; glucose, 5.5 mM; pH 7.3; 35°) at a pressure of 50 mmHg. For the measurement of the contractions, a fine hook was attached to the apex of the heart and connected with a cotton thread to a force transducer. The mechanogram was written with a pen-recorder (R511A, Beckman). As electrodes to deliver the alternating current served two fine, flexible brushes with a diameter of about 5 mm, which were embraced at their basis with platinum tin-plates connected via an amperemeter to a 50 Hz alternating current transformer. The brushes were wetted with Tyrode solution and attached at opposite sites to the ventricular walls well below the auricles. At the end of the preparation, the spontaneously beating heart was enclosed in a waterjacketed chamber which maintained an indoor temperature of 32–33°. During the subsequent equilibration phase of 1 hr, the tension exerted via the hook on the heart was gradually increased to 50 mN. As described in more detail in Results, recordings were made of the threshold current for arrhythmia, of the heart rate and of the force of contraction. Drugs were applied dissolved in the perfusion fluid over a period of 30 min for each concentration with readings after 15 min and 30 min. The drug concentrations were increased cumulatively.

The drugs were gifts from the manufacturers

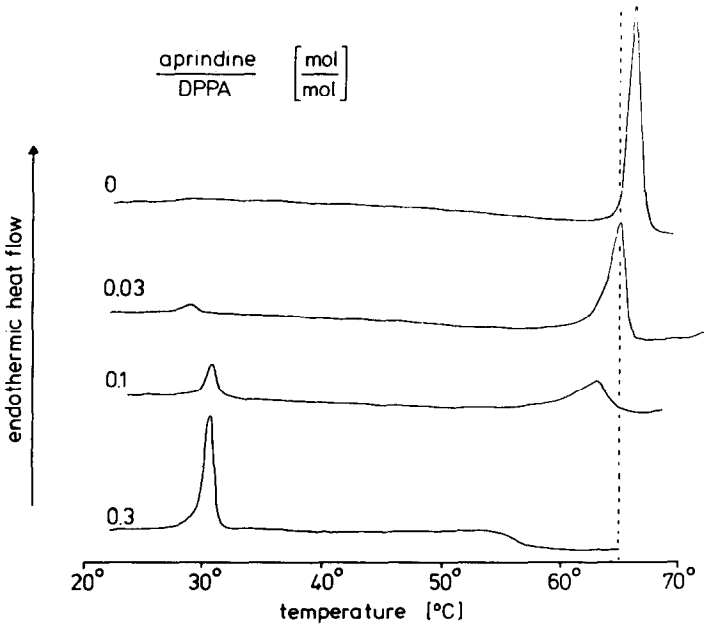

Fig. 1. Effect of aprindine on the transition-temperature of liposomes made of dipalmitoylphosphatidic acid (DPPA). The thermograms were recorded by means of differential scanning calorimetry. Upward deflection: endothermic heat flow into the liposome containing sample. Abscissa: temperature of the sample. The amount of drug added is indicated relative to the amount of DPPA present.

or purchased from Sigma. In the case of chiral compounds the drugs were applied as the racemates, except quinidine, quinine, and *cis*-(+)-diltiazem.

RESULTS

Interaction of the catamphiphilic drugs with phospholipid-membranes.

The effects of the catamphiphilic drugs on the transition temperature of DPPA-membranes are illustrated by differential scanning calorimetry recordings obtained with the antiarrhythmic drug aprindine (Fig. 1). Under control conditions a narrow transition signal occurred at about 65°. The onset temperature of the phase-transition was evaluated by extrapolating the baseline of the thermogram, fitting a straight line to the upward deflection of the signal, and then connecting the intersection of these lines with a line perpendicular to the temperature axis of the thermogram. In 28 liposome suspensions the transition temperature (T_t) was found to lie in the range of 63.5 – 67.7°, which is in correspondence with the T_t-values reported in the literature [36, 37]. In the presence of aprindine (Fig. 1) an additional signal emerged at about 29°. With an increasing amount of drug added this drug-induced signal rose at the expense of the control signal. The separate drug-induced signal probably reflects the formation of drug-containing DPPA-domains coexisting with drug-free domains. Whereas the majority of the investigated drugs elicited drug-induced transition signals at the same molar ratios as did aprindine, some had to be applied in a higher dosage (2-aminopyridine, atenolol, chloroquine, procaine, and procainamide). The temperatures at which the drug-induced signals occurred differed considerably for the various compounds. The difference between the control T_t and the drug-induced T_t-value (ΔT_t) was taken as a measure for the perturbing activity of the drugs. The ΔT_t-values found for a drug at the various molar ratios were averaged. The ΔT_t-values thus obtained for the investigated drugs in the present and in the previous study [29] are compiled in Table 1. Also the neutral benzocaine lowered the transition temperature with $\Delta T_t = 10°$. In order to check the reproducibility of the results, the previously investigated drugs dibucaine, phentermine, and propranolol were also included in the present study. The difference between the actual and the previous Δt_t-values was smaller than 10%. Table 1 indicates the mean ΔT_t-values for these drugs.

The effects of some antiarrhythmic drugs on the $^{45}Ca^{2+}$-binding to the phosphatidylserine-monolayers is illustrated by the respective concentration–response curves in Fig. 2. As reported previously [30], the catamphiphilic drugs inhibited $^{45}Ca^{2+}$-binding concentration-dependently with different potency. The reduction of $^{45}Ca^{2+}$-binding reflected that the cationic drugs intercalated in between the phosphatidylserine-headgroups, thus neutralizing negative charges and occupying Ca^{2+}-binding sites [30]. As a measure of potency served the drug-concentration at which $^{45}Ca^{2+}$-binding was depressed down to 50% of the control-value obtained in the absence of drug. The IC_{50}-values found in the present and in the previous investigation [30] are listed in Table 1. Again the reproducibility was checked by including some drugs in both studies; Table 1 indicates the mean IC_{50}-values in these cases. Since the

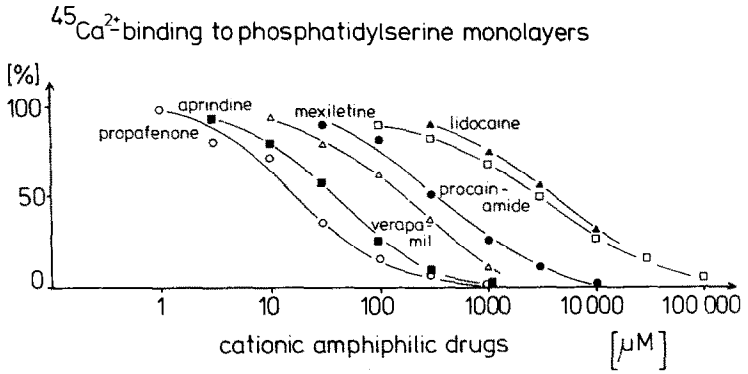

Fig. 2. Inhibition of $^{45}Ca^{2+}$-binding to phosphatidylserine-monolayers by the indicated drugs. Ordinate: $^{45}Ca^{2+}$-binding as a percentage of the binding obtained in the absence of drug. Abscissa: drug-concentration in the aqueous subphase on which the monolayer was spread. Points represent mean-values of two to three determinations. The deviation of the individual values from the respective mean-value was usually less than 10%-points and amounted at maximum to 14%-points.

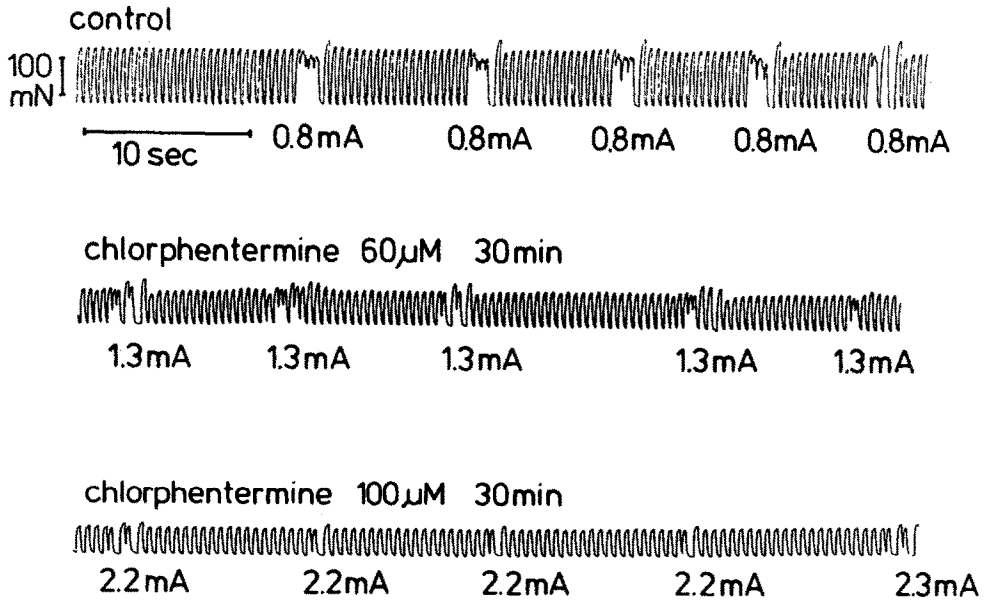

Fig. 3. Effect of chlorphentermine on the threshold-intensity of alternating current to induce arrhythmia in an isolated guinea-pig heart perfused in the Langendorff-mode. Original tracings of the contractions of the heart. Indicated is the intensity of the alternating current (in mA) required to induce arrhythmia. Upper tracing: control after the 1 hr equilibration period. Lower tracings: registration after the drug had been present in the perfusion fluid at the indicated concentrations for 30 min. Note also the drug effects on heart rate and contraction amplitude.

neutral benzocaine does not affect the surface charge of PS, an IC_{50}-value cannot be given for this drug.

Effects of the catamphiphilic drugs on cardiac function.

Original tracings of the contractions of an isolated spontaneously beating guinea-pig heart recorded in an experiment with chlorphentermine are depicted in Fig. 3. At the end of the 1 hr equilibration period the contraction amplitude amounted to 142 mN and the heart rate was 140 beats per min (bpm). The control values obtained with all isolated hearts ranged between 80–200 mN and 100–200 bpm, respectively. In order to evaluate the threshold current for arrhythmia, the alternating current was switched on and slowly increased with a rate of about 0.05–0.1 mA/sec. The mechanogram of Fig. 3 revealed arrhythmia at a current intensity of 0.8 mA. When arrhythmia had been elicited, the current intensity was immediately reduced; the heart fell into the normal sinus rhythm and attained the previous contraction amplitude after a few contractions. The determination of the alternating current (AC) threshold was repeated four times with the same result. The control values for the AC-threshold found in all experiments ranged between 0.3 and

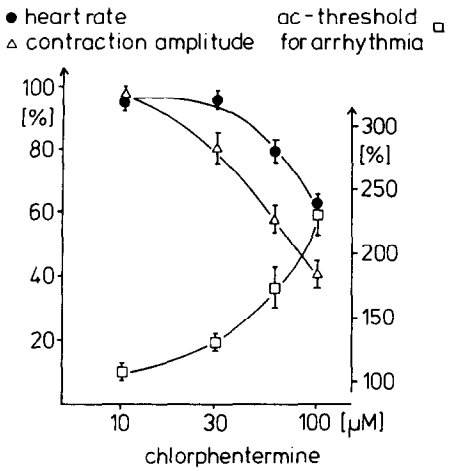

Fig. 4. Concentration–response curves for the chlorphentermine effects in isolated guinea-pig hearts on arrhythmia-threshold (right ordinate), and on heart rate and contraction amplitude (left ordinate). The data were expressed as a percentage of the values obtained at the end of the equilibration period. Indicated are mean ± SE for N = 6.

1.5 mA. With increasing concentrations of chlorphentermine present in the perfusion solution, higher current intensities were required for arrhythmia (Fig. 3). The values recorded after 15 min perfusion were similar to the 30 min values, indicating that the effect had attained its steady state. Heart rate and contraction amplitude fell with increasing drug concentrations. At 150 μM, chlorphentermine induced an elevation of the diastolic tension, and up to a current intensity of 9 mA arrhythmia did not occur; the experiment was terminated. The concentration–response curve as evaluated from a total of six experiments is depicted in Fig. 4. The figure also reveals the concentration–response curves for the drug-effects on the heart rate and on the contraction amplitude. In order to define the spontaneous alteration of cardiac function, control experiments were performed with a drug-free perfusion fluid. When the values obtained at the end of the equilibration period were set 100%, the following values resulted after a 3 hr period (mean ± SE, N = 6): AC-threshold: 118 ± 4%, heart rate: 96 ± 4%, contraction amplitude: 84 ± 4%. Because the shifts were rather slight, the dose–response curves were not corrected for spontaneous alterations of the parameters.

As a measure of the antiarrhythmic, membrane-stabilizing potency was taken the drug-concentration at which the AC-threshold was increased by 50%. In the case of chlorphentermine, the AC_{50}-value amounted to 43 μM. At this concentration, heart rate was reduced by 12% and contraction amplitude by 32%.

Figure 5 displays the dose–response curves for the antiarrhythmic effects of various drugs. Reasons for the termination of the experiments were a strong depression of contractile force, an increment of diastolic tension, an atrioventricular blockade or other forms of spontaneous arrhythmia. In the case of the Ca-antagonists diltiazem (Fig. 5) and verapamil, only marginal effects on the AC-threshold could be observed, before the effects on force of contraction and atrioventricular conduction prevented the continuation of the cumulative drug-exposure. The neutral drug benzocaine elevated the AC-threshold with an AC_{50} of 240 μM. The AC_{50}-values of all drugs investigated are compiled in Table 1. The table also indicates the alterations of heart rate and contraction amplitude as read from the individual dose–response curves (not shown) at the AC_{50}-concentration. Whereas the AC_{50}-concentrations varied by a factor of 100,000, the majority of the drugs reduced heart rate and force of contraction to a similar extent at this concentration. However, the di-cationic chloroquine (pK_a: 8.1, 10.1 [30]) especially exerted a more pronounced effect on contractile force.

DISCUSSION

The aim of the study is to compare the cardiodepressant potency of cationic amphiphilic compounds with their capability to interact with phospholipid-membranes. In artificial phospholipid-membranes, two consequences of the incorporation of catamphiphilic drugs were investigated: the reduction of the phase-transition temperature of DPPA-bilayers and the inhibition of the $^{45}Ca^{2+}$-binding to PS-monolayers. To check whether the two test systems were affected with corresponding or deviating activities, the respective measures of potency for the various drugs were plotted in the correlation depicted in Fig. 6. The linear regression analysis revealed a correlation coefficient of $r = 0.69$. Thus, only 50% of the variation between the drugs with respect to the depression of the phase-transition temperature can be predicted from the different potencies to inhibit $^{45}Ca^{2+}$-binding. The deviation is most prominent with the di-cationic compound chloroquine. The most likely explanation for the deviations seems to be that the two methods look upon separate consequences of the drug-incorporation into the phospholipid-membranes.

The reduction of $^{45}Ca^{2+}$-binding indicated the uptake of the positively charged hydrophilic moiety of the catamphiphilic drugs. $^{45}Ca^{2+}$-binding declined with increasing drug concentration and probably the concentration–response curves reflected the underlying binding curves [30]. The reduction of the transition temperature indicated the perturbing action on the structural arrangement exerted by the catamphiphilic drugs placing their hydrophobic moiety into the hydrophobic interior of the bilayers. The different ability of the various drugs to depress T_t presumably results from a different depth of penetration of the hydrophobic moieties into the fatty acid chain region of the phospholipid bilayer. For instance, the depth of penetration into phospholipid bilayers has been reported to reach to the 6–8th carbon of the fatty acid chains for tetracaine [1, 4], for propranolol to the 2nd–4th [3], and for chlorpromazine to the 2nd–3rd [38] carbon. The rank order corresponds with the rank order of the depression of T_t: tetracaine ($\Delta T_t = 42°$) > propranolol (37°) ~ chlorpromazine (36°). Thus, the different degrees of T_t-reduction may reveal different intrinsic

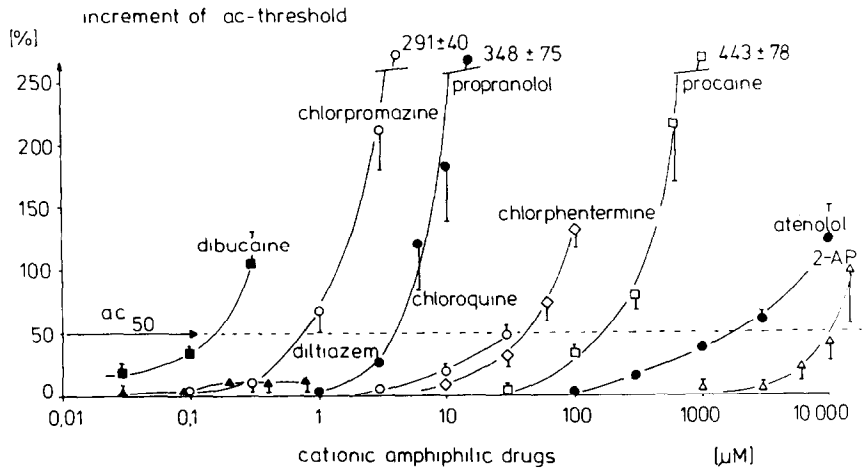

Fig. 5. Concentration–response curves for the effects of various cationic amphiphilic drugs on the threshold of alternating current (AC) to induce arrhythmia. Ordinate: AC-threshold as percentage of the value recorded at the end of the equilibration period before drug-exposure. Abscissa: concentration of the indicated drugs. 2-AP: 2-aminopyridine. Indicated are mean ± SE of N = 3–6, or mean ± deviation of the individual values of N = 2. AC_{50}: drug-concentration at which the AC-threshold for arrhythmia was elevated by 50%.

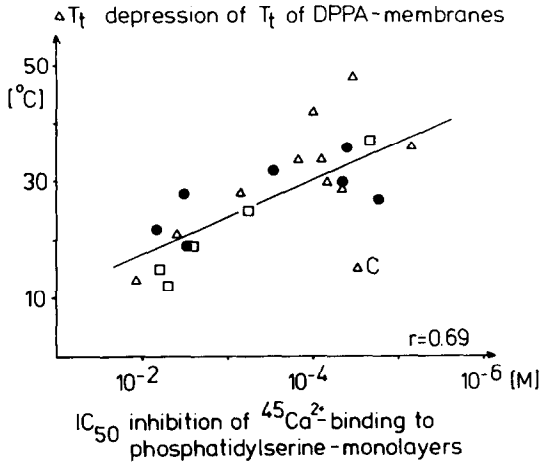

Fig. 6. Plot of the activity of the catamphiphilic drugs to reduce the phase-transition temperature (T_t) of DPPA-membranes vs the potency to inhibit $^{45}Ca^{2+}$-binding to phosphatidylserine monolayers. Ordinate: ΔT_t, difference between the control-T_t and the drug-induced T_t. Abscissa: IC_{50}, drug-concentration to inhibit $^{45}Ca^{2+}$-binding by 50%; logarithmic scale. •: antiarrhythmic drugs; □: β-blockers; △: various; C: chloroquine. The regression line ($P < 0.01$) is given by (95% confidence limits in parentheses): $\Delta T_t = 4.8\ (\pm 5.4) - 6.4\ (\pm 1.5) \times \log IC_{50}$; $r = 0.69$.

potencies of the drugs to perturb the hydrophobic interior of the lipid bilayer.

The ΔT_t- and IC_{50}-values to affect the phospholipids were also compared with the available log*p*-values (Table 1) as a measure of the hydrophobicity. Linear regression analysis revealed a rather weak correlation between ΔT_t and log*p* ($r = 0.62$), whereas the correspondence between IC_{50} and log*p* was good ($r = 0.77$). Since hydrophobicity is a major determinant for drug-uptake into phosphilipid-membranes, these results are in keeping with the view that the affinity of the drugs to bind to phospholipids governs the potency to affect $^{45}Ca^{2+}$-binding but not the activity to reduce the transition temperature.

In conclusion, the notion is supported by the presented results that the various aspects of the interaction of cationic amphiphilic drugs with phospholipid membranes (i.e. binding and effects) cannot be described appropriately by a single physicochemical measure such as the octanol/water partition coefficient but should be analysed separately [39, 40].

To find out whether the measures of potency obtained in the present experiments for the ability to affect phospholipid-membranes corresponded with the AC_{50}-values as the measure of membrane-stabilizing, negative bathmotropic activity, the data were plotted in the correlations depicted in Fig. 7. The AC_{50}-values to reduce the excitability correlated well with the ΔT_t-values for the depression of the transition temperature (Fig. 7A) and the IC_{50}-values for the inhibition of $^{45}Ca^{2+}$-binding (Fig. 7B). Remarkably, in both cases the correspondence was better than found with the comparison of the effects on the phospholipid-membranes (Fig. 6). The neutral drug benzocaine fitted well into the correlation between AC_{50} and ΔT_t. The Ca-antagonists, verapamil and diltiazem, could not be included in the correlations shown in Fig. 7, since an elevation of the alternating current threshold by 50% could not be obtained. This finding reflects that the AC-threshold depends upon the sodium channel function. The correlations allow to predict the AC_{50} of verapamil from its effects on the phospholipid-membranes; the AC_{50} should amount to 6 μM. In fact, Nawrath *et al.* [41] showed a sodium-channel blocking activity of verapamil to occur at this concentration. The other catamphiphilic compounds investigated in the present study fit more or less well

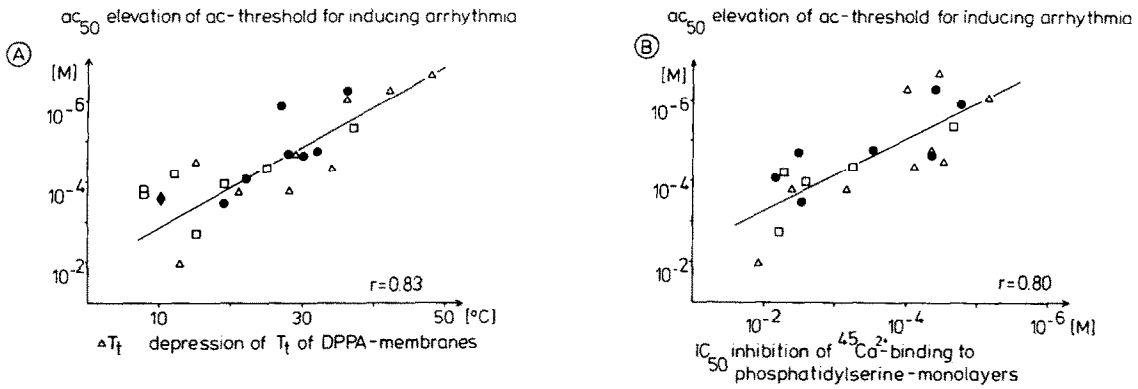

Fig. 7. Plot of the anti-excitatory potency of the catamphiphilic drugs vs the activity to reduce T_t of DPPA-membranes (A) and the potency to inhibit $^{45}Ca^{2+}$-binding to PS-monolayers (B). Ordinates: AC_{50}, drug-concentration to elevate the threshold-intensity of alternating current to induce arrhythmia in isolated guinea-pig hearts by 50%, logarithmic scale. Abscissae: (A): ΔT_t, difference between the control-T_t and the drug-induced T_t; (B): IC_{50}, drug-concentration to inhibit $^{45}Ca^{2+}$-binding by 50%, logarithmic scale. ●: antiarrhythmic drugs; □: β-blockers; △: various. B: benzocaine, not included in the regression analysis. The regression lines ($P < 0.01$) are given by (95% confidence limits in parentheses): (A) $\log AC_{50} = -1.86\ (\pm 0.46) - 0.10\ (\pm 0.02) \times \Delta T_t$; $r = 0.83$; (B) $\log AC_{50} = -1.45\ (\pm 0.59) + 0.90\ (\pm 0.16) \times \log IC_{50}$; $r = 0.80$. Multiple correlation leads to ($P < 0.01$): $\log AC_{50} = -1.14 - 0.07 \times \Delta T_t + 0.48 \times \log IC_{50}$; $r = 0.88$.

into the correlations independent of their membership in a pharmacological class. It should be noted that the antiarrhythmic drugs did not exhibit a stronger membrane-stabilizing activity than expected from their capability to affect phospholipid-membranes on the basis of the present correlations. A multiple regression analysis yielded an even better correspondence between the ability to interact with phospholipid-membranes and the membrane-stabilizing potency: $r = 0.88$. Accordingly, almost 80% of the variation between the AC_{50}-values could be accounted for by the different activities of the drugs to affect phospholipid-membranes.

In order to check the correspondence between the AC_{50}- and the available log*p*-values, a linear regression analysis was made, which yielded a good correlation: $r = 0.84$. Accordingly, as has been described previously for β-blocking agents [10, 20, 21], also the present study proved the membrane-stabilizing potency to parallel the hydrophobicity of catamphiphilic drugs.

One might argue that the AC_{50}-values for the drugs are not comparable, since the anti-excitatory action may depend, in a drug-specific manner, on the heart rate and the heart rate was not kept constant. However, as can be taken from Table 1, the heart rate was affected to a similar extent at the AC_{50}-concentrations of the various drugs. It is unlikely that AC_{50}-values obtained at a constant driving frequency would deviate from the presented AC_{50}-values to such an extent as to alter the presented correlations essentially.

Thus, the good correlation between the activity of the catamphiphilic drugs to affect phospholipid-membranes and the anti-excitatory potency, as well as the fit of the drugs, irrespective of their pharmacological class, both lend support to the hypothesis that the membrane stabilizing effect may result from an interaction with the phospholipid-matrix of cell membranes. With regard to the anti-excitatory action, the phospholipids surrounding the sodium channel protein would represent the site of action. The improvement of the correlation obtained by the combined consideration of the effects on $^{45}Ca^{2+}$-binding and on the transition temperature could be interpreted to show that both the alteration of the polar headgroup region and of the inner structure of the phospholipid-annulus might be involved in the effect on the sodium channel function.

On the other hand, some aspects of sodium-channel block point to a more specific, presumably direct interaction of catamphiphilic drugs with the channel protein. The actions of the catamphiphilic antiarrhythmic agents depend in a drug-specific manner on the membrane potential and the frequency of stimulation [e.g. 16–18, 42]. Furthermore, the enantiomers of some antiarrhythmic drugs have been found to affect sodium channel function with differing potencies [43, 44]. The seeming contradiction between the evidence for a specific interaction with the sodium-channel and the good correlation between the anti-excitatory action of catamphiphilic compounds and their potency to affect phospholipid-membranes might be resolved by one of the following interpretations. Firstly, since the Na-channel protein faces both the hydrophilic pore and the hydrophobic interior of the surrounding phospholipid-matrix, it will also contain an amphiphilic interfacial region. Thus, the common ability of the catamphiphilic drugs to bind to interfaces may underly both the interaction with the phospholipid-membranes and with the sodium-channel. The artificial phospholipid-membranes would thus behave as models for interfacial regions. Nevertheless, cationic amphiphilia would remain the decisive property for the drug action. Secondly, as pointed out by Herbette and coworkers [39, 45], drugs could approach binding sites on integral membrane proteins via the surrounding lipid phase. The

way of interaction of a cationic amphiphilic drug with the phospholipid matrix could determine the access to the sodium channel protein and could thus dominate the effect on the channel function. Thirdly, the interaction of catamphiphilic drugs with phospholipid-membranes depends, apart from the hydrophobicity of the drugs, on their charge, on the charge of the phospholipid-membrane and on its fluidity [3, 46–52]. The electric field of about 100,000 V/cm imposed on cell membranes at a resting potential of about −80 mV [53] may be expected to affect severely the charge and structural arrangement of the phospholipids and the drug–phospholipid interaction. A voltage- and stimulation-dependence of the drug-action could thus be imagined to result from a voltage-dependence of the drug–phospholipid interaction. Since the biological membranes are composed stereoselectively of phospholipids, it might also be assumed that they could discriminate between drug-enantiomers.

Fine differences between the negative chronotropic and inotropic actions of the drugs were found that may well be of therapeutic relevance. Yet, the negative chronotropic and negative inotropic effects accompanying the anti-excitatory action were in a similar range for most of the drugs. Accordingly, appropriate measures of potency would well correlate with the ΔT_t and IC_{50}-values to affect phospholipid-membranes. Since sinus node function and electromechanical coupling depend on various cellular events, a variety of sites could be discussed as points of attack for the catamphiphilic drugs. But again, cationic amphiphilia seems to dominate these other cardiodepressant actions of the drugs.

In conclusion, the cardiodepressant action of cationic amphiphilic drugs was found to parallel the ability of the drugs to affect phospholipid-interfaces. This result indicates that the cationic amphiphilic nature is a main determinant of the cardiodepressant effects, and it may point to a pivotal role of the intercalation of catamphiphilic drugs in the phospholipid-matrix of cardiac cell membranes for the cardiodepressant action.

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REFERENCES

1. Cerbon J, NMR evidence for the hydrophobic interaction of local anaesthetics. Possible relation to their potency. *Biochim Biophys Acta* **290**: 51–57, 1972.
2. Yeagle PL, Hutton WC and Martin RB, Molecular dynamics of the local anesthetic tetracaine in phospholipid vesicles. *Biochim Biophys Acta* **465**: 173–178, 1977.
3. Herbette L, Katz AM and Sturtevant JM, Comparisons of the interaction of propranolol and timolol with model and biological membrane systems. *Mol Pharmacol* **24**: 259–269, 1983.
4. Kelusky EC and Smith ICP, The influence of local anesthetics on molecular organization in phosphatidylethanolamine membranes. *Mol Pharmacol* **26**: 314–321, 1984.
5. Brasseur R, Ruysschaert J-M and Chatelain P, Semi-empirical conformational analysis of propranolol interacting with dipalmitoylphosphatidylcholine. *Biochim Biophys Acta* **815**: 341–350, 1985.
6. Vaughan Williams EM, Classification of antidysrhythmic drugs. *Pharmacol Ther B* **1**: 115–138, 1975.
7. Morales-Aguilera A and Vaughan Williams EM, The effects on cardiac muscle of β-receptor antagonists in relation to their activity as local anaesthetics. *Br J Pharmacol* **24**: 332–338, 1965.
8. Levy JV, Myocardial and local anesthetic actions of β-adrenergic receptor blocking drugs: relationship to physicochemical properties. *Eur J Pharmacol* **2**: 250–257, 1968.
9. Langslet A, Membrane stabilization and cardiac effects of *d,l*-propranolol, *d*-propranolol and chlorpromazine. *Eur J Pharmacol* **13**: 6–14, 1970.
10. Hellenbrecht D, Lemmer B, Wiethold G and Grobecker H, Measurement of hydrophobicity, surface activity, local anaesthesia, and myocardial conduction velocity as quantitative parameters of the non-specific membrane affinity of nine β-adrenergic blocking agents. *Naunyn-Schmiedeberg's Arch Pharmacol* **277**: 211–226, 1973.
11. Smith HJ, The need to redefine membrane stabilizing activity of beta-adrenergic receptor antagonists. *J Mol Cell Cardiol* **14**: 495–500, 1982.
12. Shanes AM, Electrochemical aspects of physiological and pharmacological action in excitable cells. Part I. The resting cell and its alteration by extrinsic factors. *Pharmacol Rev* **10**: 59–164, 1958.
13. Shanes AM, Electrochemical aspects of physiological and pharmacological action in excitable cells. Part II. The action potential and excitation. *Pharmacol Rev* **10**, 165–273, 1958.
14. Szekeres L and Papp GJ, *Experimental Cardiac Arrhythmias and Antiarrhythmic Drugs*. Akadémiai Kiadó, Budapest, 1971.
15. Grant AO, Starmer CF and Strauss HC, Antiarrhythmic drug action – Blockade of the inward sodium current. *Circ Res* **55**: 427–439, 1984.
16. Hondeghem LM and Katzung BG, Time- and voltage-dependent interactions of antiarrhythmic drugs with cardiac sodium channels. *Biochim Biophys Acta* **472**: 373–398, 1977.
17. Hondeghem LM and Katzung BG, Antiarrhythmic agents: the modulated receptor mechanism of action of sodium and calcium channel-blocking drugs. *Ann Rev Pharmacol Toxicol* **24**: 387–423, 1984.
18. Starmer CF, Grant AO and Strauss HC, Mechanisms of use-dependent block of sodium channels in excitable membranes by local anesthetics. *Biophys J* **46**: 15–27, 1984.
19. Lüllmann H, Timmermans PBMWM and Ziegler A, Accumulation of drugs by resting or beating cardiac tissue. *Eur J Pharmacol* **60**: 277–285, 1979.
20. Rauls DO and Baker JK, Relationship of nonspecific antiarrhythmic and negative inotropic activity with physicochemical parameters of propranolol analogues. *J Med Chem* **22**: 81–86, 1979.
21. Sada H, Harada S and Ban T, Effects of beta-adrenoceptor blocking agents of *N*-tertiary butyl derivatives on maximum upstroke velocity of action potential in guinea-pig papillary muscles. *Naunyn-Schmiedeberg's Arch* **324**: 50–57, 1983.
22. Lee AG, Model for action of local anaesthetics. *Nature (Lond)* **262**: 545–548, 1976.
23. Lee AG, Local anesthesia: The interaction between phospholipids and chlorpromazine, propranolol, and practolol. *Mol Pharmacol* **13**: 474–487, 1977.
24. Schlieper P and Tawfik H, Comparative studies of cardiodepressant drugs on contraction dynamics and electrophysiological parameters of cardiac tissues at pH 7.4 and 9. *J Pharm Pharmacol* **39**: 517–521, 1987.

25. Seeman P, The membrane actions of anesthetics and tranquilizers. *Pharmacol Rev* **24**: 583–655, 1972.
26. Trudell JR, A unitary theory of anesthesia based on lateral phase separations in nerve membranes. *Anesthesiology* **46**: 5–10, 1977.
27. Reynolds EF, *Martindale, The Extra Pharmacopoeia* 28th Edn. The Pharmaceutical Press, London, 1982.
28. Kursch B, Lüllmann H and Mohr K. Influence of various cationic amphiphilic drugs on the phase-transition temperature of phosphatidylcholine liposomes. *Biochem Pharmacol* **32**: 2589–2594, 1983.
29. Hanpft R and Mohr K, Influence of cationic amphiphilic drugs on the phase-transition temperature of phospholipids with different polar headgroups. *Biochim Biophys Acta* **814**: 156–162, 1985.
30. Lüllmann H, Plösch H and Ziegler A, Ca^{2+}-replacement by cationic amphiphilic drugs from lipid monolayers. *Biochem Pharmacol* **29**: 2969–2974, 1980.
31. Langendorff O, Untersuchungen am lebenden Säugetierherzen. *Pflügers Arch* **66**: 355–400, 1897.
32. Borchard U, Bösken R and Greeff K, Characterization of antiarrhythmic drugs by alternating current induced arrhythmias in isolated heart tissues. *Arch Int Pharmacodyn* **256**: 253–268, 1982.
33. Borchard U, Clemm S and Hirth C, Influence of arrhythmogenic and antiarrhythmic agents on alternating current-induced arrhythmias in guinea-pig heart preparations. *Arch Int Pharmacodyn* **269**: 83–93, 1984.
34. Träuble H and Eibl H, Electrostatic effect on lipid phase transitions: membrane structure and ionic environment. *Proc Natl Acad Sci. USA* **71**: 214–219, 1974.
35. Lüllmann H and Vollmer B, An interaction of aminoglycoside antibiotics with Ca-binding to lipid monolayers. *Biochem Pharmacol* **31**: 3769–3773, 1982.
36. Jacobson K and Papahadjopoulos D, Phase transitions and phase separations in phospholipid membranes induced by changes in temperature, pH, and concentration of bivalent cations. *Biochemistry* **14**: 152–161, 1975.
37. Blume A and Eibl H, A calorimetric study of the thermotropic behaviour of 1,2-dipentadecylmethylidene phospholipids. *Biochim Biophys Acta* **640**: 609–618, 1981.
38. Frenzel J, Arnold K and Nuhn P, Calorimetric, ^{13}C NMR, and ^{31}P NMR studies on the interaction of some phenothiazine derivatives with dipalmitoyl phosphatidylcholine model membranes. *Biochim Biophys Acta* **507**: 185–197, 1978.
39. Herbette LG, Chester DW and Rhodes DG, Structural analysis of drug molecules in biological membranes. *Biophys J* **49**: 91–94, 1986.
40. Ondriaš K, Staško A and Balgavý P, Spin label study of the perturbation effect of the local anaesthetics tetracaine and dibucaine on synaptosomes at pharmacological concentrations. *Biochem Pharmacol* **36**: 3999–4005, 1987.
41. Nawrath H, Blei I, Gegner R, Ludwig C and Zong X-G, No stereospecific effects of the optical isomers of verapamil and D-600 on the heart. In: *Calcium Antagonism in Cardiovascular Therapy: Experience with Verapamil* (Eds. Zanchetti A and Krikler DM), pp. 52–63. Excerpta Medica, Amsterdam-Oxford-Princeton, 1981.
42. Ehring GR, Moyer JW and Hondeghem LM, Quantitative structure activity studies of antiarrhythmic properties in a series of lidocaine and procainamide derivatives. *J Pharmacol Exp Ther* **244**: 479–492, 1988.
43. Späh F, Asocainol, a new antiarrhythmic drug with natrium- and calcium-antagonistic effects on ventricular myocardium. *J Cardiovasc Pharmacol* **6**: 1027–1035, 1984.
44. Sheldon RS, Cannon NJ, Nies AS and Duff HJ, Stereospecific interaction of tocainide with the cardiac sodium channel. *J. Pharmacol Exp Ther* **33**: 327–331, 1988.
45. Rhodes DG, Sarmiento JG and Herbette LG, Kinetics of binding of membrane-active drugs to receptor sites. Diffusion-limited rates for a membrane bilayer approach of 1,4-dihydropyridine calcium channel antagonists to their active site. *Mol Pharmacol* **27**: 612–623, 1985.
46. Singer MA, Interaction of dibucaine and propranolol with phospholipid bilayer membranes – effect of alterations in fatty acyl composition. *Biochem Pharmacol* **26**: 51–57, 1977.
47. Römer J and Bickel MH, Interactions of chlorpromazine and imipramine with artificial membranes investigated by equilibrium dialysis, dual-wavelength photometry, and fluorimetry. *Biochem Pharmacol* **28**: 799–805, 1979.
48. Surewicz WK and Jozwiak Z, Effect of quinidine on membrane properties. Depression of the lipid phase transition temperature and changes in the permeability of the lipid bilayer. *Biochem Pharmacol* **32**: 1467–1471, 1983.
49. Schreier S, Frezzatti WA Jr, Araujo PS, Chaimovich H and Cuccovia IM, Effect of lipid membranes on the apparent pK of the local anesthetic tetracaine. Spin label and titration studies. *Biochim Biophys Acta* **769**: 231–237, 1984.
50. Eftink MR, Puri RK and Ghahramani MD, Local anesthetic–phospholipid interactions. The pH dependence of the binding of dibucaine to dimyristoylphosphatidylcholine vesicles. *Biochim Biophys Acta* **813**: 137–140, 1985.
51. Kelusky EC, Boulanger Y, Schreier S and Smith ICP, A ^{2}H-NMR study on the interactions of the local anesthetic tetracaine with membranes containing phosphatidylserine. *Biochim Biophys Acta* **856**: 85–90, 1986.
52. Luxnat M and Galla H-J, Partition of chlorpromazine into lipid bilayer membranes: the effect of membrane structure and composition. *Biochim Biophys Acta* **856**: 274–282, 1986.
53. Lüllmann H and Peters T, Action of cardiac glycosides on the excitation-contraction coupling in heart muscle. *Prog Pharmacol* **2**: 1–57, 1979.
54. Courtney KR, Interval-dependent effects of small antiarrhythmic drugs on excitability of guinea-pig myocardium. *J Mol Cell Cardiol* **12**: 1273–1286, 1980.
55. Sada H and Ban T, Time-independent effects on cardiac action potential upstroke velocity (resting block) and lipid solubility of beta adrenergic blockers. *Experientia* **37**: 171–172, 1981.
56. Leo A, Hansch C and Elkins D, Partition coefficients and their uses. *Chem Rev* **71**: 525–616, 1971.