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Comparison of the Effect of Eleven β-Adrenoceptor Blocking Drugs in Perturbing Lipid Membrane: An ESR Spectroscopy Study

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SUMMARY

The perturbation effect of the β-adrenoceptor blocking drugs atenolol, propranolol, practolol, oxprenolol, doberol, pronethanol, metipranolol, alprenolol, Kö-1124, pindolol, and exaprolol on rat brain lipid membrane was investigated by ESR spectroscopy using the spin probe method. Using stearic acids spin labeled at the 5th, 12th, and 16th positions, it was found that lipophilic drugs disorder the membrane and their effect is about 5–10 times higher at the 16th carbon membrane depth than at the 5th depth. Exaprolol induced nonlamellar phases in the bovine brain lipid membrane as detected by ^{31}P NMR spectroscopy. The relative potencies of the drugs at 10 mmol/liter concentration to disorder the lipid membrane at the 16th carbon depth were in the order: exaprolol > alprenolol ≈ propranolol > metipranolol ≈ doberol > control sample > pindolol ≈ practolol ≈ atenolol. This order qualitatively corresponds with some of their nonspecific biological membrane activities but is not related to their β-adrenoceptor blocking potencies. The inequality of the membrane perturbation propensities of the drugs indicates that they perturb the lipid membrane in a structure-dependent manner, i.e., that each induces a specific rather than a nonspecific membrane perturbation.

There are important unsolved problems concerning the understanding of the biological effects of drug molecules at the molecular level. BAB drugs, in addition to their specific drug-receptor interaction, which results in β-adrenergic receptor blockade (1), possess a wide range of nonspecific membrane activities, such as calcium entry blockade (2), local anesthetic activity (3), serotonin antagonism (4), antihistamine activity (5), several enzymatic activities (6), and so on.

The mode of these effects is not fully understood. Information regarding possible interactions of BAB drugs with the lipid component of biological membranes has been obtained in studies with lipid membrane systems. It was found that BAB drugs influence physical properties of model lipid membranes. For example, propranolol and timolol influence the phase transition temperature of model lipid membranes (7–9).

The aim of our present work was to compare the propensities of 11 BAB drugs to perturb model lipid membrane at different depths.

Materials and Methods

Producers of the BAB drugs are as follows. Alprenolol was from Hässle (Hälsingborg, Sweden). Atenolol, practolol, pronethalol, and propranolol were from ICI (Alderley Park, Cheshire, England). Doberol and Kö-1124 were from Boehringer (Ingelheim, FRG), Pindolol was from Sandoz (Basel, Switzerland), Exaprolol was from the Institute for Drug Research (Modra, Czechoslovakia), Metipranolol was from Spofa Works (Prague, Czechoslovakia). Oxprenolol was from Ciba-Geigy (Basel, Switzerland).

Stearic acid spin labels with the dimethyloxazolidinyl group at the 5th, 12th, and 16th carbon were purchased from Syva (Palo Alto, CA). These will be referred to as I(12,3), I(5,10), and I(1,14), respectively. All other chemicals were of analytical grade from commercial sources.

Total lipids were extracted from rat brain, rat platelets, or bovine brain according to the method of Folch *et al.* (10). The samples for ESR measurements were prepared using two procedures. In procedure A, spin labeled stearic acid (spin probe) and total lipids (molar ratio of 1:100) were dissolved in chloroform/methanol and the solvent was evaporated in a stream of nitrogen. Residual traces of the solvent were removed by a prolonged evacuation. The total lipids were hydrated with buffer (NaCl, 136.9 mmol/liter; KCl, 2.7 mmol/liter; $NaHCO_3$, 11.9 mmol/liter; NaH_2PO_4, 0.36 mmol/liter; $MgCl_2$, 1 mmol/liter, pH 7.4). BAB drugs were added in the same buffer. For reasons of limited solubility of some drugs, the samples with practolol contained 0.002% (v/v) of 3 mol/liter HCl, the samples with pindolol contained 0.004% (v/v) of 3 mol/liter HCl, those with metipranolol contained 0.002% (w/

ABBREVIATIONS: BAB, β-adrenoceptor blocking; I(12,3), 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxyl; I(5,10), 2-(10-carboxydecyl)-2-hexyl-4,4-dimethyl-3-oxazolidinyloxyl; I(1,14), 2-(14-carboxytetradecyl)-2-ethyl-4,4-dimethyl-3-oxazolidinyloxyl. HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

v) tartaric acid, and the samples with exaprolol contained 8.5% (v/v) ethanol. The final lipid/buffer ratio in the samples with brain or platelet lipids was 1:40 or 1:20 (w/w), respectively. The concentrations of the drugs reported in procedure A refer to those in the aqueous phase immediately after drug addition and not to final concentrations in the aqueous phase after equilibration with membranes.

In procedure B, the BAB drug and spin probe in ethanol and rat brain total lipids in chloroform/methanol were mixed and the solvent was evaporated in a stream of nitrogen followed by evacuation ($\leq 10^{-2}$ Pa, 2–3 hr). The samples were hydrated with buffer (145 mmol/liter NaCl, 5 mmol/liter KCl, 1.4 mmol/liter $MgCl_2$, 1 mmol/liter $CaCl_2$, 5 mmol/liter HEPES·HCl, pH 7.4). The drug/lipid molar ratios in the samples reported in procedure B refer to those in the lipid phase before hydrating the samples with buffer.

In order to attain equilibration of the BAB drug in the membrane, the samples were sonicated in a bath and subjected to freeze-thaw-vortex cycles several times. For ESR measurements, 100 μl of the sample suspension were filled into a glass capillary (i.d. 1 mm). ESR spectra were recorded by an X-band ERS-230 or BRUKER ER 200 D-SRC spectrometer. Typical instrument settings were: 5 mW microwave power, modulation amplitude of 0.2 mT, or 0.05 mT when using probe I(1,14).

To assess the relative efficiency of the BAB drugs in perturbation of the lipid membrane, the order parameter S was calculated from the outer ($A_\parallel$) and inner splittings ($A_\perp$) of the ESR spectra of spin probes I(12,3) and I(5,10), which were incorporated into the lipid membrane, after $A_\perp$ and polarity corrections according to the method of Gaffney

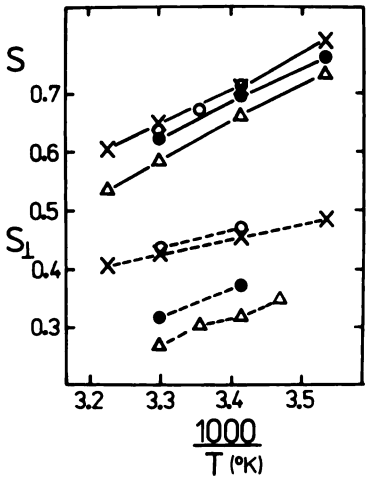

Fig. 1. Temperature dependence of the parameter S and $S_\perp$ of the spin probes I(12,3) (—) and I(1,14) (– – –), respectively, in rat brain lipid liposomes. ×, control sample. The BAB drugs tested at 10 mmol/liter concentration were: atenolol (○), Kö-1124 (●), and exaprolol (△). Samples were prepared by procedure A.

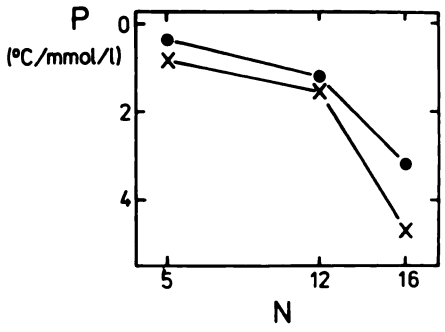

Fig. 2. Dependence of the parameter P on the carbon depth, N, of rat brain lipid lipsomes. ●, Kö-1124; ×, exaprolol. The $\Delta S/\Delta C$ values at 10 mmol/liter BAB drug concentration at 25° were used for calculation of the parameter P. Samples were prepared by procedure A.

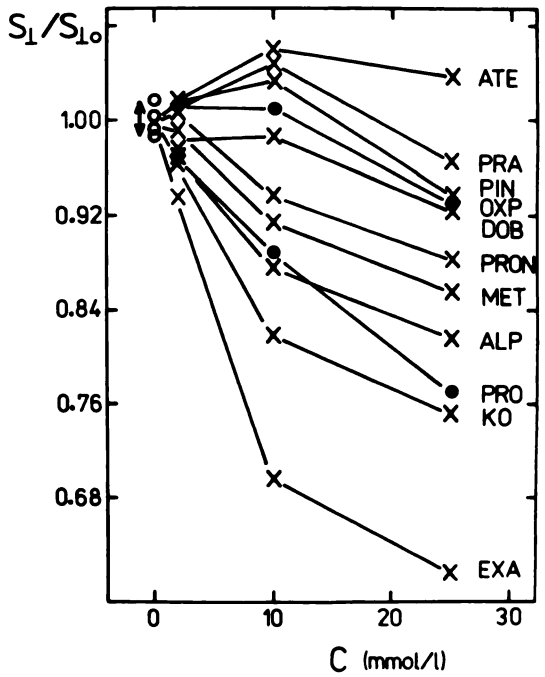

Fig. 3. Dependence of the parameter, $S_\perp/S_{\perp_o}$, of spin probe I(1,14) on the concentration of BAB drugs in the sample. $S_{\perp_o}$ and $S_\perp$ are order parameters of sample without and with BAB drug, respectively. ↕, standard deviation of $S_{\perp_o}$ in control sample from four experiments. The temperature was 25°. Samples were prepared by procedure A. *ATE*, atenolol; *PRA*, practolol; *PIN*, pindolol; *OXP*, oxprenolol; *DOB*, doberol; *PRON*, pronethalol; *MET*, metipranolol; *ALP*, alprenolol; *PRO*, propranolol; *KO*, Kö-1124; *EXA*, exaprolol.

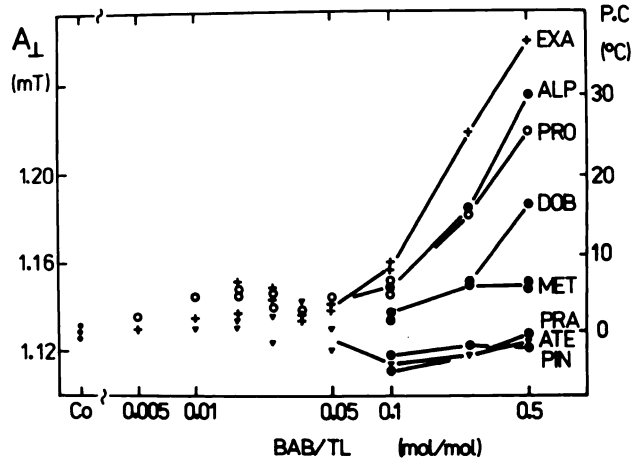

Fig. 4. Dependence of the parameter $A_\perp$ of spin probe I(1,14) on the BAB drug:lipid molar ratio in the sample (*BAB/TL*). The effect of BAB drug concentrations (C) on parameter $A_\perp$ expressed in the temperature scale (*P.C*) is depicted on the *right*. The values of $(\Delta A_\perp/\Delta T)_o = 3.5\ \mu T \cdot °C^{-1}$ were used. Lipid:buffer ratio was 1:10 (w/w). Temperature was 25°. Samples were prepared by procedure B. *Co*, control sample. Abbreviations as in Fig. 3.

(11). In the case of spin probe I(1,14), where the parameter $A_\perp$ was evaluated directly from the inner splitting of the ESR spectrum only, a similar formula was applied for the order parameter $S_\perp$ as used by Sauerheber *et al.* (12):

$$S_\perp = (A_{11} + A_{22} + A_{33} - 3A_\perp)/[A_{33} - 0.5(A_{11} + A_{22})]$$

The values of A_{ii} (diagonal elements of the hyperfine splitting tensor) were taken from the literature (11). The parameters S and $S_\perp$ assume values between 0 and 1; these extreme order parameters indicate that

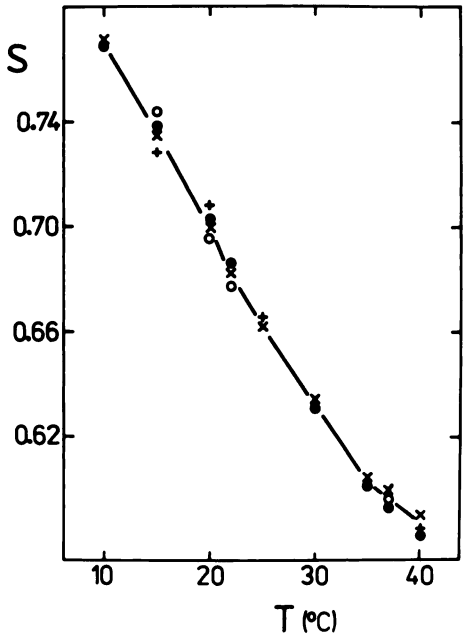

Fig. 5. Temperature dependence of the parameter S of I(12,3) spin probe in platelet lipid liposomes. ×, control sample. The BAB drugs at 10 mmol/liter concentration were: atenolol (○), Kö-1124 (●), and exaprolol (+). Samples were prepared by procedure A.

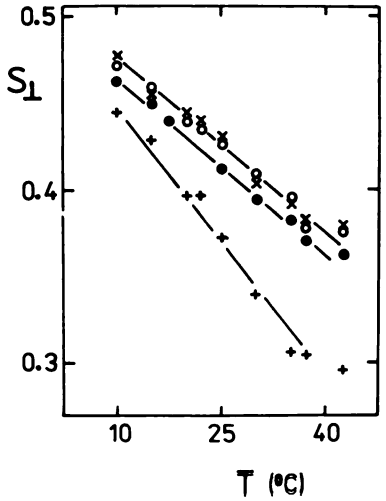

Fig. 6. Temperature dependence of the parameter $S_\perp$ of I(1,14) spin probe in platelet lipid liposomes. ×, control sample. The BAB drugs at 10 mmol/liter concentration were: atenolol (○), Kö-1124 (●), and exaprolol (+).

the spin probe samples have fluid and immobilized environments, respectively. Decrease of the parameters S or $S_\perp$, or increase of the parameter $A_\perp$ indicates higher disorder ("fluidity") of the hydrophobic part of the membrane. It must be emphasized that, in this study, the parameter S, $A_\perp$, and particularly the parameter $S_\perp$, where values of $A_\perp$ were used without correction, were applied to estimate the relative propensities of the BAB drugs to perturb the membrane at the given depth. To compare the efficiency with which the drugs perturb the membrane at different depths, a parameter, P, defined as

$$P \equiv (\Delta S/\Delta C)/(\Delta S/\Delta T)_s$$

was used (13), where C is the drug concentration in the sample, T is temperature, and $(\Delta S/\Delta T)_s$ is a temperature gradient of S or $S_\perp$ in the control sample without BAB drug. The parameter P expresses the temperature effect necessary to reach in the control sample the same

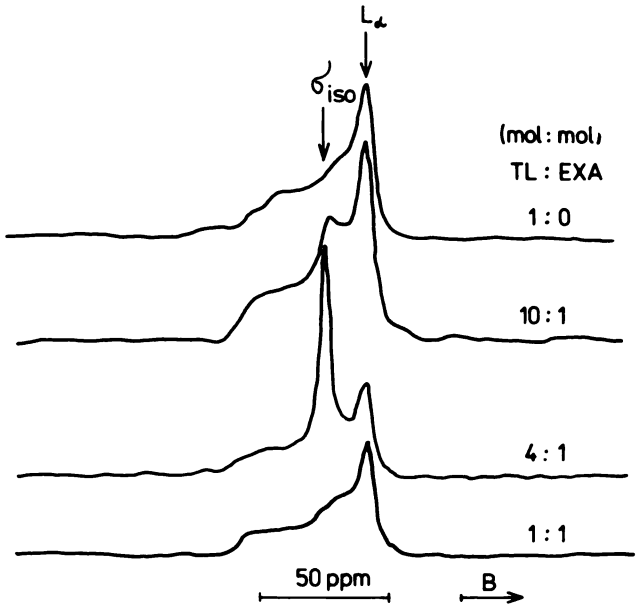

Fig. 7. The 36.4-MHz ^{31}P NMR proton-decoupled spectra of bovine brain lipids (TL):exaprolol (EXA) dispersion in ^2H_2O at different TL:EXA ratios.

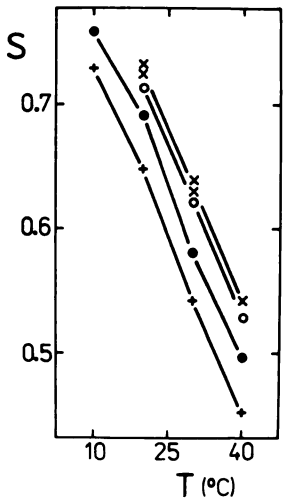

Fig. 8. Temperature dependence of the parameter S of I(12,3) spin probe in bovine brain lipid liposomes. ×, control sample. Lipid:exaprolol molar ratios are 10:1 (○), 4:1 (●), and 1:1 (+).

value of the order parameter as in the sample with fixed temperature but with the BAB drug. The ESR parameters were estimated to be within a relative error of ±3.5% and ±1% using procedures A and B, respectively.

Samples for ^{31}P NMR measurements were prepared as follows. Bovine brain lipids and exaprolol were dissolved in chloroform/methanol and the solvent was evaporated in a stream of nitrogen followed by evacuation. The lipids were hydrated with 100 mmol/liter NaCl in D_2O. The final (lipid + exaprolol):buffer weight ratio in the sample was 1:4. In order to attain equilibration of exaprolol in the lipids, the samples were subjected to freeze-thaw-vortex cycles several times and were homogenized by centrifugation. The lipid/exaprolol molar ratio in the sample was calculated assuming the lipid molecular weight to be 700. The samples were transferred into glass tubes and equilibrated at room temperature for 24 hr before measurement. ^{31}P NMR spectra were recorded on a BRUKER HX 90 spectrometer with a deuterium lock and a home-built proton 600-W decoupling unit at 25°. For the Fourier transform spectra up to 1000 free induction decays were accumulated, employing 7.5-μsec radiofrequency pulse, 25-μsec dwell time,

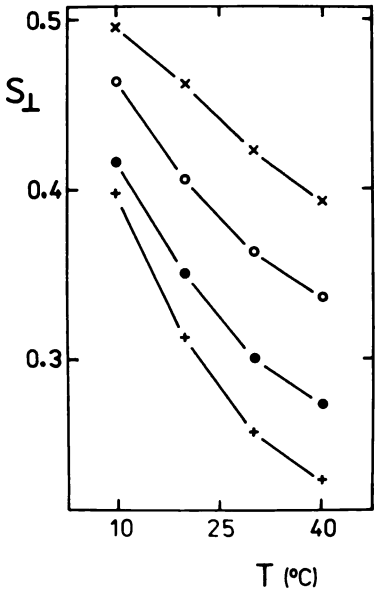

Fig. 9. Temperature dependence of the parameter $S_\perp$ of I(1,14) spin probe in bovine brain lipid liposomes. ×, control sample. Lipid:exaprolol molar ratios are 10:1 (○), 4:1 (●), and 1:1 (+).

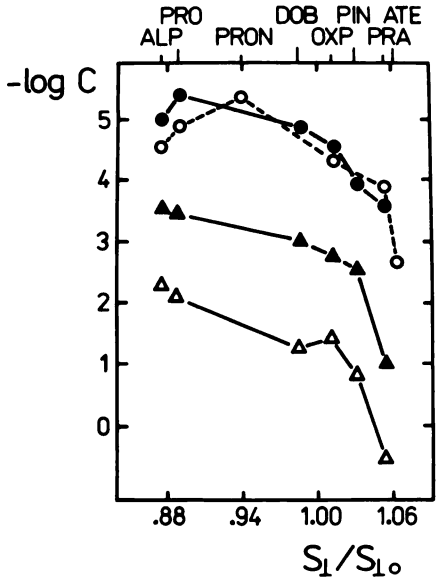

Fig. 10. Comparison of half-maximal doses, IC_{50} values, and concentration (C) values of the BAB drugs which affect some nonspecific membrane activities, with their $S_\perp/S_{\perp_o}$ values taken from Fig. 3 at 10 mmol/liter drug concentration. The IC_{50} values of BAB drugs showed a decrease of the time-dependent active uptake of serotonin by human platelets (●), inhibition of noradrenaline uptake in crude synaptosomal fractions from rat brain (○), depression of the 10-min cumulative dose response curves of transmission velocity of the extracellularly recorded action potential of muscle strips of the frog heart (▲), and depression of the extracellular action potential of the isolated frog sciatic nerve after 10 min of incubation (△). Concentration (C) is given in mol/liter. IC_{50} values are from other studies (4, 14, 15). Abbreviations as in Fig. 3.

and 0.7-sec delay time. After measuring the ^{31}P NMR spectra, the samples of bovine brain lipids with exaprolol were kept at −70°C for 2–10 days. Ethanol containing 40 μg of spin probe I(12,3) or I(1,14) was evaporated in plastic vials. The lipid-drug dispersion (50 μl) was added, samples were vigorously vortexed for 3–5 min, and ESR measurement was performed as described above.

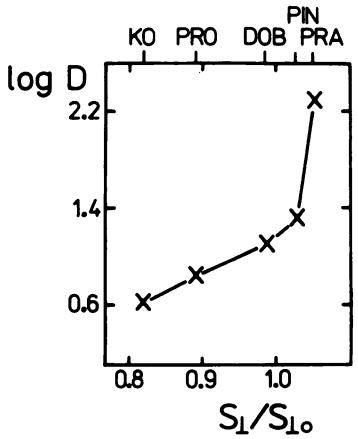

Fig. 11. Half-maximal doses of the BAB drugs (D) which produced nonspecific depression of myocardial contractility (maximum rate of rise of left ventricular pressure − dP/dT) in anesthetized cats (data taken from Ref. 16) versus their $S_\perp/S_{\perp_o}$ values taken from Fig. 3 at 10 mmol/liter drug concentration. D values are given in μmol/kg. Abbreviations as in Fig. 3.

Results

Rat brain lipid liposomes. BAB drugs possess different propensities to perturb lipid membranes. The highest perturbation effect at all three membrane depths was found for exaprolol and Kö-1124. An example is shown in Fig. 1, in which, as detected by probe I(12,3), exaprolol and Kö-1124 decreased the order parameter S, whereas atenolol did not affect it significantly, nor did the other BAB drugs, studied at 10 mmol/liter concentration (data not shown). The perturbation effect on the lipid membrane was different at the hydrocarbon core, as detected by probe I(1,14). Exaprolol and Kö-1124 decreased the order parameter $S_\perp$, whereas atenolol slightly increased it (Fig. 1). Exaprolol and Kö-1124 had a severalfold higher influence at the 16th carbon depth than at the 5th one. Because of different temperature gradients of $(\Delta S/\Delta T)_o$ for the control sample detected by different spin probes, the parameter P was used to compare the effect of the drugs at different membrane depths. The values of $(\Delta S/\Delta T)_o$ for the control sample in the temperature range 20–30° detected by I(12,3), I(5,10), and I(1,14) spin probes were −0.008, −0.013, and $-0.0031°C^{-1}$, respectively. The perturbation effect of exaprolol and Kö-1124 increased toward the hydrocarbon core of the lipid membrane (Fig. 2). These drugs are about 5–10 times more effective in disordering membrane at the 16th carbon depth than at the 5th depth. A similar disordering effect was also displayed by other lipophilic BAB drugs, namely, alprenolol, propranolol, metipranolol, doberol, and pronethalol, which disordered the membrane at the 16th carbon depth but had no pronounced effect at the 5th carbon depth (Fig. 3).

Because of the highest BAB drug disordering efficiency at the 16th carbon depth, the perturbation effect of the 11 BAB drugs was compared at this depth at three concentrations. The results are shown in Fig. 3, where parameter $S_\perp/S_{\perp_o}$ is depicted versus BAB drug concentrations in the sample at 25° ($S_{\perp_o}$ = 0.422 ± 0.015; mean value from four samples), and $S_\perp$ is the order parameter for the sample containing BAB drug at 25° (mean value from measurements at 20° and 30°). Doberol, pronethalol, metipronolol, alprenolol, propranolol, Kö-1124, and exaprolol decreased $S_\perp$ with their increasing concentration in the sample, whereas practolol and pindolol had no significant

effect (within the experimental error) at 2 and 10 mmol/liter concentrations, and decreased the order parameter $S_\perp$ slightly only at the concentration of 25 mmol/liter (Fig. 3). Atenolol seemed to increase the parameter $S_\perp$ at a concentration of 10 mmol/liter.

In order to eliminate the effect of the solvents used in procedure A, the samples for ESR measurements were prepared by procedure B. The effect of the BAB drugs on the parameter $A_\perp$ is shown in Fig. 4. Depending on the drug/lipid molar ratio, exaprolol (EXP), alprenolol (ALP), propranolol (PRO), doberol (DOB), and metipranolol (MET) increased the parameter $A_\perp$, whereas pindolol (PIN), practolol (PRA), and atenolol (ATE) decreased it. The results are qualitatively similar to those shown in Fig. 3. To estimate whether the different drug perturbation effects are due to their various lipid/buffer partition coefficients or to their different "intrinsic efficacy," the drug effect at the constant drug/lipid molar ratio of 0.25 was compared at three lipid/buffer (w/w) ratios. It was found that $A_\perp$ did not change by more than ±0.005 mT for any drugs tested for a lipid/buffer ratio from 1 to 0.1, except in the case of doberol ($\Delta A_\perp = -0.03$ mT). The average $A_\perp$ values (in mT) for the BAB drugs at 25° were: ATE = PRA = 1.120; PIN = 1.125; control = 1.130; MET = 1.155; PRO = 1.185; ALP = 1.190; EXA = 1.215 and DOB = 1.185–1.155.

Platelet lipid liposomes. Atenolol, exaprolol, and Kö-1124 at 10 mmol/liter concentration did not influence parameter S of the I(12,3) spin probe in the platelet lipid membranes as shown in Fig. 5, but they exhibited a perturbation effect at the 16th carbon depth as detected by the I(1,14) spin probe. Exaprolol and Kö-1124 decreased the parameter $S_\perp$, whereas atenolol had no significant effect on it (Fig. 6).

Bovine brain lipid liposomes. The influence of exaprolol on polymorphism of the bovine brain lipid membrane is shown in Fig. 7. The control sample exhibits an asymmetric ^{31}P NMR spectrum, characteristic of the lamellar lipid phase L_α. The effective anisotropy of the chemical shift $-\Delta\sigma_{eff}$ was −38.5 ± 0.5 ppm. When the sample contained exaprolol at a lipid/exaprolol molar ratio of 10:1 or 4:1, in addition to the lamellar phase signal, a new isotropic signal appeared in the spectrum (σ_{iso}). The ^{31}P NMR spectra were thus a superposition of lamellar and isotropic signals; i.e., phase separation occurred in the sample. The lamellar NMR signal is seen also from the sample at a lipid/exaprolol molar ratio of 1:1.

To characterize the mobility of the hydrophobic part of the samples without and with exaprolol, the samples were spin labeled and taken for ESR measurement. Exaprolol decreased the parameter S of spin probe I(12,3) in the membrane within the temperature range 20–40° (Fig. 8), and this effect increased with the increasing molar ratio of exaprolol in the sample. A severalfold higher perturbation effect of exaprolol was found at the 16th carbon membrane depth as detected by spin probe I(1,14), where exaprolol decreased parameter $S_\perp$ while increasing its molar ratio in the sample (Fig. 9).

Discussion

It is known that some of the nonspecific membrane activities of BAB drugs, such as inhibition of serotonin (4) and noradrenaline (14) uptake, the ability of the drugs to decrease conduction velocity of the isolated frog heart or to raise an electrically induced ventricular fibrillation threshold in guinea pigs, the efficiency of the drugs to depress ventricular contractility in the cat, or their local anesthetic activities, correlate with their octanol/buffer partition coefficient (4, 14–16). This may indicate that nospecific membrane effects caused by BAB drugs are dependent on their membrane concentration.

Considering only nonspecific drug-membrane interaction, the drug incorporated in the membrane may influence membrane function in three different ways. First, since the pK_a values of the investigated drugs are within the range of 9.5 ± 0.3 (17), they are mostly positively charged at physiological pH. They change the surface membrane potential proportionally to their membrane concentration (18), which may influence membrane function. Second, the drugs may dissolve into membrane proteins and thus perturb their function, as was suggested also for local anesthetics (19). Third, BAB drugs incorporate into the lipid part of biological membrane, influencing its dynamics and/or structure, and, thus, they may secondarily affect membrane proteins (7). Since the perturbation effect of BAB drugs was studied on the lipid membrane only, this paper evaluates the third mode of action, however, the other two cannot be excluded.

The effect of only a limited number of BAB drugs has so far been studied in influencing the dynamics of membranes. Propranolol was found to influence thermally induced structural transitions in the intact human erythrocyte membrane (20). More studies were done on BAB drug-lipid bilayer interaction. It was found that propranolol and timolol reduced the temperature of a gel to liquid-crystalline phase transition in dimyristoyl- or dipalmitoyllecithin bilayers (7–9). In contrast, practolol had no effect on the lipid phase transitions (7).

There is a good discrimination among the BAB drugs in relation to their ability to perturb lipid membrane (Figs. 3 and 4). The drugs were found to possess various propensities to perturb the lipid membrane at different membrane depths (Figs. 2, 5, 6, 8, and 9). In the case of platelet lipid liposomes, it may be supposed that the membrane concentration of the drugs was so low that the significant effect was detected only at the 16th carbon depth and not at the 5th (Figs. 5 and 6). This also indicates that the "fluidizing effect" of BAB drugs does not correspond to fluidization of the membrane by temperature; i.e., in liposomes the effect of the drugs cannot be abolished by cooling. Drugs with higher lipophilicity, such as exaprolol, Kö-1124, alprenolol, propranolol, metipranolol, doberol, and pronethalol, had a severalfold higher perturbation effect at the hydrocarbon membrane core than at the region close to the polar lipid part. This may be explained by spatial molecular orientation of the drug in the lipid membrane. Herbette *et al.* (9) and Govil *et al.* (8) showed by neutron diffraction and by ^{13}C NMR spectroscopy, respectively, that a naphthalene moiety of propranolol partitions into the lipid bilayer, and that a charged amine side chain is positioned in the polar phospholipid head group region. We suppose that such a spatial incorporation of the drug into the membrane induces a "free volume" at the hydrocarbon lipid core resulting in higher molecular freedom of motion of the lipid chains at the 16th carbon depth, as was found also for local anesthetics (13, 21, 22). Creation of free volume in the hydrophobic membrane core may destabilize lamellar membrane structure as was shown for exaprolol (Fig. 7), which induced a nonlamellar membrane phase in bovine brain lipid liposomes. The rigidization effect of atenolol, practolol, and pindolol may be connected with their interaction with the polar membrane region. Recently, the propensities of

the same BAB drugs to perturb platelet membranes were studied (23). Comparing the results of Nosál *et al.* (23) with these presented in our paper, it is found that BAB drug propensities to perturb lipid or platelet membranes are similar.

We have found that exaprolol had a higher perturbation effect than alprenolol and propranolol at different lipid/buffer ratios, and pindolol, atenolol, and practolol had effects on parameter $A_\perp$ (Figs. 3 and 4) opposite to those of the other studied drugs. The perturbation effect of metipranolol decreased at the high membrane concentrations (Fig. 4). These results indicate that the BAB drugs perturb the membrane in a structure-dependent manner, i.e., that each drug induces a specific rather than a nonspecific membrane perturbation.

To estimate whether the perturbation effects of the BAB drugs were somehow related with their nonspecific biological activities, the values of $S_\perp/S_{\perp_o}$ for the BAB drugs at the 16th membrane carbon depth and at 10 mmol/liter concentration (Fig. 3) were compared with their biological potency as reported in the literature. The order of the BAB drugs according to their $S_\perp/S_{\perp_o}$ parameters corresponds with their IC_{50} values for the inhibition of serotonin (4) or noradrenaline (14) uptake, for depression of the action potential transmission velocity of the heart muscle, and for blocking the action potential on the sciatic nerve (15) (Fig. 10). The data obtained by Hellenbrecht and Gortner (16) for half-maximal doses of the drugs which produced nonspecific depression of myocardial contractility in the anesthetized cat also correspond with the BAB drug $S_\perp/S_{\perp_o}$ values found in our work (Fig. 11). The higher the perturbation effect of the drugs, the lower the drug concentration needed to achieve IC_{50} effects. However, the $S_\perp/S_{\perp_o}$ parameters of the BAB drugs are not related to their β-adrenoceptor blocking potencies (24).

It is noteworthy that the perturbation effect of local anesthetics on lipid membrane at the 16th carbon depth was found to correspond with their ability to block action potential on nerve (13). It is also known that BAB drugs and local anesthetics possess similar nonspecific membrane activities; for example, local anesthetic activity (3, 25), inhibition of calcium current (26), decrease of stimulated platelet aggregation (27, 28), antiarrhytmic actions (29, 30), or inhibition of phospholipase A-induced swelling of mitochondria (6). The close similarity of the disordering effects and the nonspecific biological membrane activities of BAB and local anesthetic drugs suggest that their mode of membrane effects may be similar.

The findings presented in this paper resulting from the comparison of the BAB drug perturbation of the lipid membrane with their nonspecific biological activities suggest that some of the BAB drug nonspecific membrane activities may be mediated, at least in part, through their perturbation effect on biological membranes.

References

1. Lefkowitz, R. J. Direct binding studies of adrenergic receptors: biochemical, physiologic and clinical implications. *Ann. Intern. Med.* **91**:450–458 (1979).
2. Scales, B., and D. A. D. McIntosh. Effects of propranolol and its optical isomers on the radio-calcium uptake and the adenosine triphosphatases of skeletal and cardiac sarcoplasmic reticulum function (SRF). *J. Pharmacol. Exp. Ther.* **16**:261–267 (1968).
3. Gill, E. W., and E. M. Vaughan-Williams. Local anaesthetic activity of the β-blocking drug pronethalol. *Nature (Lond.)* **201**:199 (1964).
4. Lemmer, B., G. Wienhold, D. Hellenbrecht, I. J. Bak, and H. Grobecker. Human blood platelets as cellular models for investigation of membrane active drugs: beta-adrenergic blocking agents. *Naunyn-Schmiedebergs Arch. Pharmacol.* **275**:299–313 (1972).
5. Raper, C., and J. Wale, Specificity of β-receptor antagonists. *Eur. J. Pharmacol.* **3**:279–281 (1968).
6. Seppala, A. J., N. E. L. Saris, and M. L. Gauffin. Inhibition of phospholipase-A-induced swelling of mitochondria by local anaesthetics and related agents. *Biochem. Pharmacol.* **20**:305–313 (1971).
7. Lee, A. G. Local anesthesia: the interaction between phospholipids and chlorpromazine, propranolol and practolol. *Mol. Pharmacol.* **13**:474–487 (1977).
8. Govil, G., R. S. Phadke, and S. Srivastava. Magnetic resonance studies on drug-membrane interactions. *Curr. Sci. (India)* **51**:493–499 (1982).
9. Herbette, L., A. M. Katz, and J. M. Sturtevant. Comparisons of the interaction of propranolol and timolol with model and biological membrane systems. *Mol. Pharmacol.* **24**:259–269 (1983).
10. Folch, J. M., M. Lees, and G. H. S. Stanley. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226**:497–509 (1957).
11. Gaffney, B. J. Practical considerations for the calculation of order parameters for fatty acid or phospholipid spin labels in membranes, in *Spin Labeling Theory and Applications* (L. J. Berliner, ed.). Academic Press, New York, 567–571 (1976).
12. Sauerheber, R. D., T. S. Zimmermann, J. A. Esgate, W. P. VanderLaan, and L. M. Gordon. Effect of calcium, lanthanum and temperature on the fluidity of spin-labeled human platelets. *J. Membr. Biol.* **52**:201–219 (1981).
13. Ondriaš, K., P. Balgavý, S. Štolc, and L. I. Horváth. A spin label study of the perturbation effect of tertiary amine anesthetics on brain lipid liposomes and synaptosomes. *Biochim. Biophys. Acta.* **732**:627–635 (1983).
14. Street, J. A., and A. Walsh. Inhibition of synaptosomal (^{3}H) noradrenaline uptake by β-adrenoceptor blocking drugs: influence of lipophilicity. *Eur. J. Pharmacol.* **102**:315–324 (1984).
15. Hellenbrecht, D., B. Lemmer, G. Wiethold, and H. Grobecker. 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-Schmiedebergs Arch. Pharmacol.* **227**:211–226 (1973).
16. Hellenbrecht, D., and L. Gortner. Correlations between hydrophobicity and non-specific pharmacological effects of β-adrenoceptor blocking agents. *Pol. J. Pharmacol. Pharm.* **28**:625–630 (1976).
17. Laxer, M., A. C. Capomacchia, and G. E. Hardee. Intramolecular hydrogen-binding in beta adrenergic blocking agents. *Talanta* **28**:973–976 (1981).
18. Schlieper, P., P. K. Medda, and R. Kaufmann. Drug-induced zeta potential changes in liposomes studied by laser doppler spectroscopy. *Biochim. Biophys. Acta* **644**:273–283 (1981).
19. Richards, C. D., K. Martin, S. Gregory, C. A. Keightley, T. R. Hesketh, G. A. Smith, G. B. Warren, and J. C. Metcalfe. Degenerate perturbations of protein structure as the mechanism of anaesthetic action. *Nature (Lond.)* **276**:775–779 (1978).
20. Janoff, A. S., D. L. Mazorow, R. T. Coughlin, A. J. Bowdler, A. Haug, and E. J. McGroarty. The modification of human erythrocyte membrane structure by membrane stabilizers: an electron spin resonance study. *Am. J. Hematol.* **10**:171–179 (1981).
21. Boulanger, Y., S. Schreier, and I. C. P. Smith. Molecular details of anesthetic-lipid interaction as seen by deuterium and ^{31}P NMR. *Biochemistry* **20**:6824–6830 (1981).
22. Ondriaš, K., S. Štolc, L. Beneš, and P. Balgavý. Perturbation effect of local anaesthetics on synaptosomes: variation with depth of the spin lipid probe. *Gen. Physiol. Biophys.* **3**:327–337 (1984).
23. Nosál, R., V. Jančinová, K. Ondriaš, J. Jakubovský, and P. Balgavý. On the interaction of β-adrenoceptor blocking drugs with platelet aggregation, calcium displacement and fluidization of the membrane. *Biochim. Biophys. Acta* **821**:217–228 (1985).
24. Man in't Veld, A. J., and M. A. D. H. Schalekamp. Effects of 10 different β-adrenoceptor antagonists on hemodynamics, plasma renin activity, and plasma norepinephrine in hypertension: the key role of vascular resistance changes in relation to partial agonist activity. *J. Cardiovasc. Pharmacol.* **5**:S30–S45 (1983).
25. Seeman, P. The membrane actions of anesthetics and tranquilizers. *Pharmacol. Rev.* **24**:583–655 (1972).
26. Akaike, N., H. Ito, K. Nishi, and Y. Oyama. Further analysis of inhibitory effects of propranolol and local anaesthetics on the calcium current in *Helix* neurones. *Br. J. Pharmacol.* **76**:37–43 (1982).
27. Weksler, B. B., M. Gillick, and J. Pink. Effect of propranolol on platelet function. *Blood* **49**:185–196 (1977).
28. Nosál, R., V. Jančinová, and M. Petríková. On the relationship between aggregation and cyclic nucleotides in platelets treated with betaadrenoceptor blocking drugs. *Gen. Physiol. Biophys.* **2**:353–362 (1983).
29. Somani, P., and B. K. B. Lum. The anti-arrhythmic effects of beta-adrenergic blocking agents. *J. Pharmacol. Exp. Ther.* **147**:194–204 (1965).
30. Sasyniuk, B. I., and S. Nattel. Lidocaine—problems in understanding an antiarrhytmic drug. *Trends Pharmacol. Sci.* **3**:297–300 (1982).

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