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MOLECULAR PHARMACOLOGICAL ASPECTS OF ANTIARRHYTHMIC ACTIVITY I

CLASS I AND CLASS III COMPOUNDS AND LIPID PEROXIDATION

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Abstract—The effect of nineteen antiarrhythmic agents on nonenzymatic lipid peroxidation, using rat hepatic microsomes, was studied. Lipid peroxidation was induced by Fe^{2+}–ascorbic acid and assayed spectrophotometrically by measuring the 2-thiobarbituric acid reactive material. The compounds tested have various structural characteristics and represent class I and III of antiarrhythmics as classified by Vaughan Williams. The R_M values, derived from reversed-phase thin-layer chromatography, were determined, and Σf values calculated in order to correlate lipophilicity and antioxidant activity. The antiarrhythmics studied inhibited lipid peroxidation to various degrees. No apparent structural factor could definitely be attributed to this effect and antioxidants are found among both class I and class III compounds. There is a trend toward a parabolic relationship between antioxidant potency and lipophilicity. Three of the tested antiarrhythmics, namely the lipophilic amiodarone, aprindine and asocainol, were very potent antioxidants, and a further investigation of concentration and time dependency of lipid peroxidation was performed. It is suggested that, at least for some antiarrhythmic drugs, antioxidant activity may be part of their mode of action, and that it may form an additional beneficial feature for the treatment of cardiac failure.

The peroxidation of cellular lipids and the free radical induced damage of cell membranes have been associated with a variety of pathologic conditions [1, 2].

The sarcoplasmic reticulum and the sarcolemma of the heart are highly susceptible to free radical attack during myocardial injury [3]. Free radical mediated destruction of sarcoplasmatic membranes is evidently one of the cellular processes involved in arrhythmias generated during myocardial ischemia and reperfusion [4]. Furthermore, the protection of chemically different antioxidants against arrythmias [5, 6] indicates the involvement of free radicals in these conditions and suggests that treatment with antioxidants may be part of an antiarrhythmic pharmacotherapy.

We found it of interest, therefore, to investigate some representatives of various classes of the currently used antiarrhythmic agents for a possible antioxidant activity. We used heat inactivated rat hepatic microsomal fraction, and induced lipid peroxidation by the Fe^{2+}–ascorbic acid system in an *in vitro* study of the influence of these drugs on the peroxidation of membrane lipids.

Since the binding affinity of cationic amphiphilic drugs (like many antiarrhythmics and local anaesthetics) to phospholipid membranes [7, 8] increases with increasing hydrophobicity [9], we also determined the R_M and Σf values of the test compounds, as lipophilicity index, in an attempt to correlate antioxidant activity and lipophilicity.

MATERIALS AND METHODS

The antiarrhythmic compounds used (Table 1) were gifts from Albert-Roussel Pharma GmbH, F.R.G. (Disopyramide), Hoechst AG., F.R.G. (Procainamide), Laboratoires Pharmuka, France (Quinacainol), Dr Madaus & Co., F.R.G. (Aprindine), Goedecke AG, F.R.G. (Asocainol), Academy of Medical Sciences of the U.S.S.R. (Ethmozine), Astra Chemicals GmbH, F.R.G. (Lidocaine), Boehringer Ingelheim KG., F.R.G. (Mexiletine), Cassella AG., F.R.G. (Nicainoprol), Kettelhack Riker Pharma GmbH, F.R.G. (Flecainide), Eli Lilly & Co., U.S.A. (Indecainide), Janssen Pharmaceutica, Belgium (Lorcainide), Knoll AG., F.R.G. (Propafenone), LABAZ GmbH, F.R.G. (Amiodarone), Deutsche Wellcome, F.R.G. (Bretylium), Eli Lilly GmbH, F.R.G. (Clofilium), Bristol Arzneimittel, F.R.G. (Sotalol), Laboratoires Delalande SA, France (Carocainide) and a pharmacy (Quinidine).

For the lipid peroxidation experiments, L-(+)-ascorbic acid, ferrous sulphate and 2-thiobarbituric acid were purchased from J. T. Baker B. V. (Deventer, The Netherlands), Merck (Darmstadt, F.R.G.) and Sigma Chemical Co. (St Louis, MO) respectively. All other chemicals used were of analytical purity.

For the R_M determination, experiments were performed on TLC plates silica gel $60F_{254}$ (Merck).

Male Wistar rats (C.P.B. Harlan Olec, Zeist, The

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Table 1. Antiarrhythmic drugs tested, classification according to Vaughan Williams, their effect on lipid peroxidation, their R_M values and their Σf values

No.	Compound	Class*	Per cent inhibition of control absorbance†	R_M‡	Σf‖
1	Disopyramide	Ia	NS§	0.45 ± 0.04	2.51
2	Procainamide	Ia	NS	0.09 ± 0.04	1.21
3	Quinacainol	Ia	20	1.06 ± 0.07	4.37
4	Quinidine	Ia	15	0.19 ± 0.02	3.36
5	Aprindine	Ib	100	0.95 ± 0.03	5.86
6	Asocainol	Ib	100	0.65 ± 0.04	6.66
7	Ethmozine	Ib	32	−0.20 ± 0.04	3.06
8	Lidocaine	Ib	NS	−0.36 ± 0.08	3.04
9	Mexiletine	Ib	6	0.08 ± 0.03	3.15
10	Nicainoprol	Ib	5	0.14 ± 0.04	2.05
11	Flecainide	Ic	5	0.17 ± 0.03	4.41
12	Indecainide	Ic	NS	0.53 ± 0.04	3.33
13	Lorcainide	Ic	10	0.22 ± 0.03	5.25
14	Propafenone	Ic	25	0.46 ± 0.04	4.31
15	Amiodarone	III	50	—	9.46
16	Bretylium (tosylate)	III	22	—	—
17	Clofilium (tosylate)	III	NS	—	—
18	Sotalol	III	NS	−0.32 ± 0.07	—
19	Carocainide	—	NS	0.19 ± 0.04	2.57

* According to Ref. 14.
† After 45 min of incubation, concentration of the compounds 1 mM.
‡ Derived from reversed phase TLC, for details see text.
§ NS: not significant (<5%) compared to controls.
‖ Calculated as indicated in Materials and Methods.

Netherlands) weighing 200–220 g were used. Hepatic microsomes were prepared as described in Ref. 10.

Heat inactivated microsomes (90° for 90 sec) from untreated rats (0.125 g liver/ml) were incubated at 37° for 45 min with shaking (air being freely admitted) in Tris–HCl/KCl buffer (50 mM/150 mM, pH 7.4). Ascorbic acid (0.2 mM), previously neutralized with a KOH solution, was added to the microsomes. The test compounds were dissolved in dimethyl sulfoxide and added (100 μl) at various concentrations (5–1000 μM), and an equal volume of the solvent was added to the control incubate. In all cases the reaction was started with the addition of 10 μM $FeSO_4$ freshly prepared, after a preincubation of 5 min. Aliquots (0.3 ml) of the incubation mixture (final volume 4 ml) were taken at various time intervals.

Lipid peroxidation was assayed by determining the 2-thiobarbituric acid-reactive material, as described previously [10, 11]. Each experiment was performed at least in duplicate, and data in the figures are indicated with ±SE.

For the R_M determination, TLC plates were impregnated by immersion in a 5% (v/v) solution of liquid paraffin in light petroleum (40–60°), and dried, according to a standard method [12]. The plates were used immediately after drying. The test compounds were dissolved in methanol. The plates were developed in closed chromatographic tanks at 20°. The eluent was a mixture of methanol:water:ammonium hydroxide solution (27%) [65:33:2 (v/v/v)]. After development, the plates were dried at 80° for 20 min and the spots were detected under UV light. R_F values were averaged from at least ten determinations, and values with a standard deviation higher than 10% were rejected. They were converted to R_M values via the relationship: $R_M = \log (1/R_F - 1)$.

The Σf values were calculated according to Rekker and de Kort [13].

RESULTS

The antiarrhythmic agents tested, their Vaughan Williams classification [14], their effect on lipid peroxidation after 45 min of incubation (expressed as per cent inhibition of the control absorbance) and their R_M values are given in Table 1.

These compounds, as well as dimethyl sulfoxide, did not interfere with the spectrophotometric determination of lipid peroxidation, expressed as the 2-thiobarbituric acid-reactive material, at the concentrations used.

From the 19 antiarrhythmic compounds, seven have negligible effect (<5%) on lipid peroxidation up to a concentration of 1 mM: i.e. disopyramide, procainamide, lidocaine, indecainide, clofilium, sotalol and carocainide. The order of increasing protection against lipid peroxidation offered by the remaining 12 compounds at a concentration of 1 mM is: flecainide, nicainoprol < mexiletine < lorcainide < quinidine < quinacainol < bretylium < propafenone < ethmozine < amiodarone < aprindine, asocainol.

Figures 1A and B illustrate the time course of lipid peroxidation as influenced by the antiarrhythmic drugs.

Since the two quaternary compounds, bretylium and clofilium were tosylates, we tested the possible interference of this anion with lipid peroxidation. *Para* toluene-sulfonic acid had no effect on lipid peroxidation up to a concentration of 1 mM.

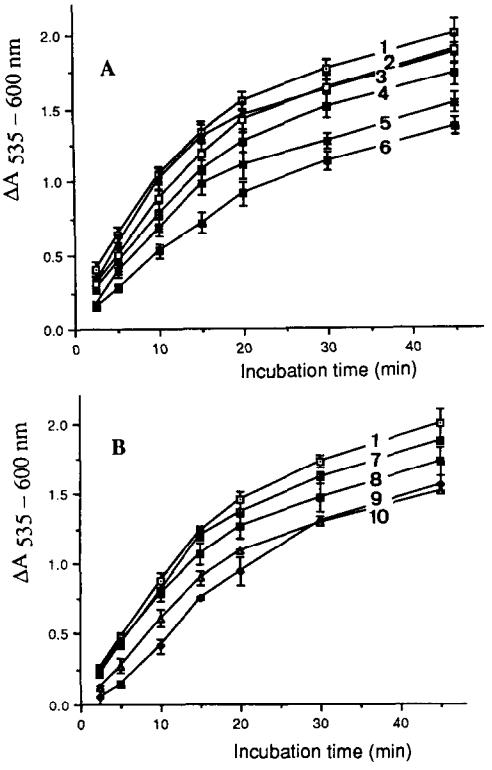

Fig. 1. Effect of some antiarrhythmics (1 mM) on time course of lipid peroxidation. 1A: Controls (1), flecainide (2), nicainoprol (3), lorcainide (4), propafenone (5), ethmozine (6). 1B: Controls (1), mexiletine (7), quinidine (8), bretylium (tosylate) (9), quinacainol (10).

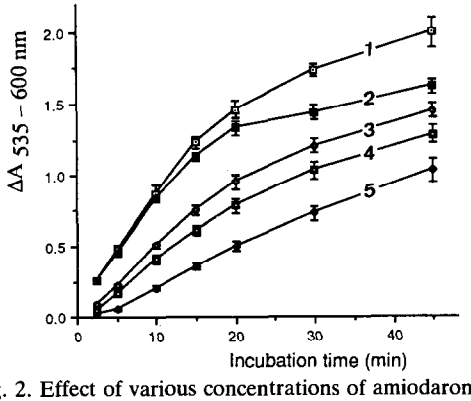

Fig. 2. Effect of various concentrations of amiodarone on lipid peroxidation. Controls (1), amiodarone concentrations: 0.10 mM (2), 0.25 mM (3), 0.50 mM (4), 1 mM (5).

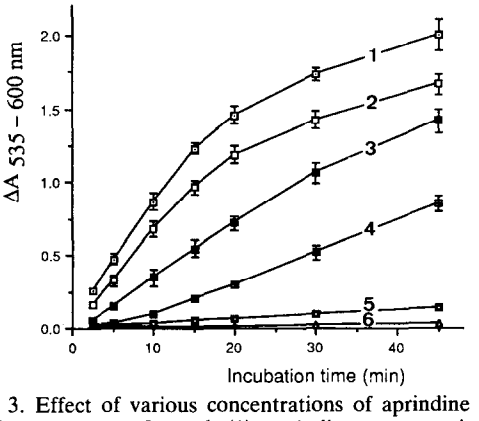

Fig. 3. Effect of various concentrations of aprindine on lipid peroxidation. Controls (1), aprindine concentrations: 25 μM (2), 50 μM (3), 100 μM (4), 250 μM (5), 500 μM (6).

From the antiarrhythmics demonstrating antioxidant activity, the most potent appeared to be aprindine and asocainol, which caused 100% inhibition of lipid peroxidation at 1 mM concentration, and amiodarone, which offered a 50% protection at the same concentration.

For these three compounds a more detailed study was performed: the time and concentration dependence of the inhibition of lipid peroxidation was examined by testing various concentrations of these agents at various time intervals. The course of lipid peroxidation, as affected by amiodarone, aprindine and asocainol is shown in Figs 2, 3 and 4 respectively.

At 0.1 mM concentration, amiodarone and aprindine offered 22 and 57% inhibition of lipid peroxidation respectively, while asocainol inhibited lipid peroxidation completely at 30 μM concentration, and by 20% at a concentration of 10 μM.

The two optical isomers, *d*- and *l*-asocainol, were tested at a concentration of 25 μM, and found to have an identical effect on lipid peroxidation compared to the racemate, under the same experimental conditions. Since the phenolic moiety of asocainol could be largely responsible for the observed inhibition of lipid peroxidation (*vide infra*), we tested (freshly distilled) *ortho*-methoxy-phenol, at a concentration

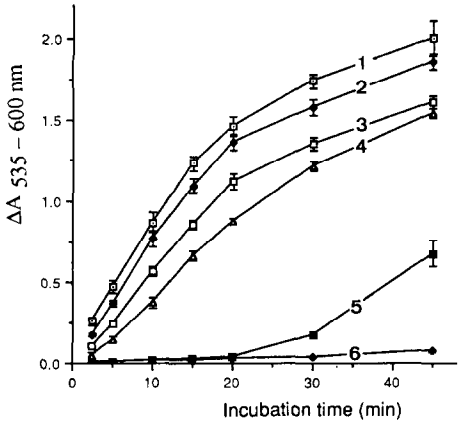

Fig. 4. Effect of various concentrations of *dl*-asocainol on lipid peroxidation. Controls (1), *dl*-asocainol concentrations: 5 μM (2), 10 μM (3), 20 μM (4), 25 μM (5), 30 μM (6).

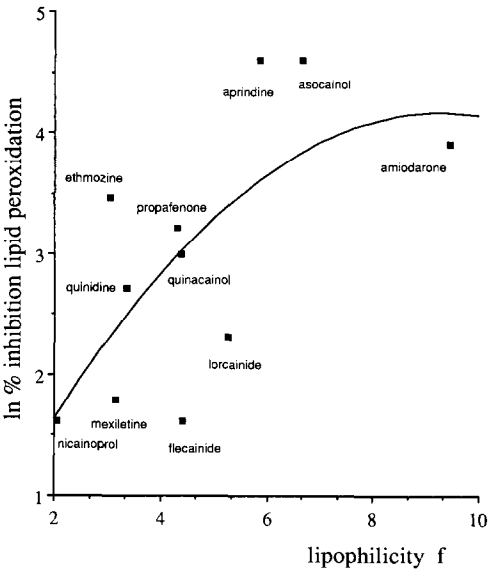

Fig. 5. Plot of the lipophilicity, Σf of the antiarrhythmics against the natural logarithm of the percentage inhibition of lipid peroxidation as indicated in Table 1. The graph is described by Eqn 3 (see text).

of 25 μM; at this concentration, no inhibition of lipid peroxidation was produced, compared to controls.

The R_M values were derived from R_F values between 0.10 (for aprindine) and 0.71 (for lidocaine), with the exception of quinacainol (R_F = 0.08). No R_F values could be obtained for amiodarone using different methanol:water:aqueous ammonia mixtures (65:33:2, 70:28:2, 80:18:2), and for the two quaternary compounds, bretylium and clofilium tosylate. The Σf values for bretylium, clofilium and sotalol could not be calculated because of missing fragmental constants in Ref. 13. The order of increasing lipophilicity as expressed by the R_M values runs from lidocaine to quinacainol; if expressed as Σf it runs from procainamide to amiodarone.

Multiple regression analysis in which we tried to correlate the antioxidant potency (as ln of percentage inhibition of lipid peroxidation) with the lipophilicity we found

$$\ln(\%\text{inhib}) = 1.54\,R_M + 2.3$$
$$N = 10, s = 0.985, r = 0.555, F = 3.6 \quad (1)$$

$$\ln(\%\text{inhib}) = 0.34\,\Sigma f + 1.34$$
$$N = 11, s = 0.895, r = 0.638, F = 6.2 \quad (2)$$

Including Σf in Eqn. 2 gives

$$\ln(\%\text{inhib}) = -0.048\,\Sigma f^2 + 0.893\,\Sigma f + 0.027$$
$$N = 11, s = 0.909, r = 0.675, F = 3.3 \quad (3)$$

Equation 3 has been plotted in Fig. 5, which reveals a possible dependence of antioxidant potency on lipophilicity.

DISCUSSION

There is a growing evidence in recent years that active oxygen metabolites and lipid peroxidation are of major importance in ischemia and reperfusion generated arrhythmias [15–19]. The electrophysiological derangements induced by ischemia have been characterized [20] and alteration of membrane structure has been proposed to mediate adverse electrophysiological responses [20, 21]. It is well documented that, in myocardial ischemia, conditions are generated that favour the production of free radicals (such as decrease in intracellular pH and ATP) [3], and lower the protective capacity of heart tissue [22]. Malondialdehyde, a product of lipid peroxidation, has been found to increase rapidly upon reoxygenation of hypoxic hearts [16], and associated with ECG abnormalities [21]. Lipid peroxidation and free radical production can be stimulated by disturbances in calcium homeostasis [23]. Increased calcium concentrations, able of causing myocardial after-contractions, can also activate phospholipases, and thus enhance free radical formation from arachidonic acid metabolism [24].

Thus, there are strong indications that free radical induced membrane damage might be involved in the generation of rhythm disturbances. This is further supported by the fact that various compounds, that can either inhibit the formation of free radicals or scavenge them, can protect against reperfusion-induced arrhythmias. Antioxidants of various structure, both natural [3, 24] and synthetic [25, 26], are known to possess besides antiarrhythmic activity a cardioprotective function [6]. It has also been shown that combination of different free radical scavengers have additive antiarrhythmic properties, thus suggesting that various radical species may be involved in arrhythmias [27].

In this study, rat hepatic microsomes were used for the *in vitro* investigation of the antioxidant properties of the antiarrhythmic drugs. It is known that liver microsomes serve as a good peroxidizable membrane system [28]. Any enzymatic activity of the microsomes was abolished by heating. Antioxidant activity of the compounds is measured as the inhibition of the iron/ascorbate mediated lipid peroxidation. In this way antioxidant activity is a rather broad term used to indicate any ability of a com-

pound to prevent the formation of, or to scavenge free radicals, thus, more than one mechanism of action could be involved.

Three of the antiarrhythmic drugs present special interest because of their potent antioxidant action: i.e. amiodarone, aprindine and asocainol. Amiodarone is effective in controlling ventricular arrhythmias due to coronary artery disease. It has also been reported to protect against phospholipid depletion in isoproterenol-induced myocardial ischemia [29], and characterized as a phospholipase A_2 inhibitor [30]. It is also known that amiodarone induces generalized phospholipidosis and formation of inclusion bodies, considered as lysosomal accumulation of phospholipids. The same applies to chlorpromazine and chloroquine [30], known for their antioxidant properties [31, 32] and their cardiodepressant effects [9]. Amiodarone is a very lipophilic compound with a Σf value of 9.46. The determination of its lipophilicity by measuring the R_M value was not possible, since it was tightly held by the lipophilic stationary phase, despite the various methanol/water mixtures used as eluents.

Aprindine, an even more potent antioxidant, is also very lipophilic, as shown by one of the highest R_M and Σf values in the series. Aprindine has been reported to be highly effective in reducing the beat frequency of spontaneously contracting guinea-pig atria [9]. This activity is found to correlate well with its potency to inhibit ^{45}Ca-binding to phosphatidylserine-monolayers, which represents its ability to bind to a phospholipid membrane [33]. Thus, it seems possible that lipophilicity favours the incorporation of this drug to the membrane phospholipid, where its action against peroxidation of the polyunsaturated fatty acids is expressed. In fact a correlation between the lipophilicity of antiarrhythmics and their potency to bind to phosphatidylcholine has been reported [34].

Finally the very effective antioxidant asocainol (Fig. 4) is known to possess membrane stabilizing, local anesthetic properties, and mixed Na^+, Ca^{2+}-antagonistic effects in mammalian ventricular myocardium [35]. These characteristics make asocainol a rather efficient antiarrhythmic agent. It has also been suggested that the action of asocainol may be stereospecific, and that its *d*-enantiomer is responsible for the Ca^{2+}-antagonistic activity, while the *l*-form mostly decreases the Na^+-dependent rate of the action potentials rise [36]. In our study the two isomers appeared to be equipotent as lipid peroxidation inhibitors, indicating an additional, independent action. There is a phenolic hydroxyl in the molecule of asocainol, and we investigated the possibility that this part of the molecule may be responsible for its antioxidant activity, since it is known that many phenolic compounds are good antioxidants [37]. For this purpose we used *ortho*-methoxy-phenol, at a concentration at which asocainol is found to inhibit lipid peroxidation by 75% under the same experimental conditions. The lack of any effect on lipid peroxidation observed with *ortho*-methoxy-phenol indicates that the phenolic structure alone is not enough for antioxidant activity. One of the properties involved could possibly be the evident increase of lipophilicity, that may enable the interaction of asocainol with the cell membranes. This steep concentration antioxidant relationship observed with asocainol indicates that binding to phospholipids is not the only determinant in its antioxidant activity, since binding to phospholipids takes place over a larger concentration range [34]. Other possible characteristics, which might be involved are radical scavenging or iron complexation.

Amiodarone, aprindine and asocainol are the most lipophilic compounds of our series and at the same time display the most prominent antioxidant activity, as indicated by their R_M and Σf values. However, bretylium, which as a quaternary ammonium salt, is expected to be rather hydrophilic also possesses some antioxidant activity. Therefore, lipophilicity is apparently not solely responsible for antioxidant activity. This is emphasized by the significant antioxidant effect of the moderately lipophilic compound ethmozine. The antioxidant action of this drug can probably be attributed to its phenothiazine structure, since it is known that phenothiazine derivatives, like chlorpromazine are antioxidants [31, 38]. Moreover, the statistics of Eqn 3, envisioned in Fig. 5, is not good which also indicates that lipophilicity is not the only determinant for the antioxidant action of the antiarrhythmics.

During an ischemic situation the pH is about 6 [39], and under these acidic conditions most of the test compounds, as (tertiary) amines, act as cationic amphiphiles. It is generally accepted that cationic amphiphilic drugs, with a relatively highly lipophilic moiety, can interact with the phospholipid part of the sarcolemma, and thus depress cardiac function [40]. It seems possible, therefore, at least for some antiarrhythmic agents, that an antioxidant activity accompanying their potential interaction with the membranes, may form part of their mechanism of action. Antioxidant activity is certainly not the common descriptor for antiarrhythmic activity. Moreover, antioxidants are found in both the class I and class III series of compounds. Of course, the concentrations shown to be effective in the present study are relatively high, which questions the predictive value for the *in vivo* situation. On the other hand phosphatidylcholine binding occurs in the 10^{-6} to 10^{-4} M range and has been suggested to be of clinical importance [34]. Moreover the process of lipid peroxidation has not been fully elucidated yet with regard to initiation and propagation. The effect of local and perhaps critical interference with drugs is not known. The observed high antioxidant activity by some antiarrhythmic drugs may therefore contribute to their pharmacological profile in the management of cardiac failure.

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