EFFECTS OF CLASS I ANTIARRHYTHMIC DRUGS ON MITOCHONDRIAL ATPase ACTIVITY IN GUINEA PIG HEART PREPARATIONS

ABDULRAHMAN A. ALMOTREFI
Department of Pharmacology, King Saud University, and Biological and Medical Research Department, King Faisal Specialist Hospital, Riyadh, Saudi Arabia [Fax 442-7854]

(Received 19 August 1992)

Abstract—1. The effects of three class I antiarrhythmic drugs quinidine, lidocaine and lorcainide on undamaged myocardial mitochondrial ATPase [ATP: phosphohydrolase, EC 3.6.1.3] activity were evaluated in guinea pig heart preparations.
2. All three drugs inhibited the enzyme activity in a concentration-dependent fashion.
3. Lorcainide was the most potent, exerting inhibitory effects in the range of less than 1.0 nM-2.0 mM, with IC_{20} and IC_{50} values of 9.4±0.6 nM and 87.2±5.5 µM. However, in the range of approx. 10 nM-10 µM, the enzyme response decreased only slightly with increasing lorcainide concentrations.
4. Quinidine and lidocaine, on the other hand, inhibited the enzyme activity in the range of 1.0 µM-100 mM.
5. The IC_{20} and IC_{50} values for quinidine were 0.92±0.04 mM and 4.8±0.6 mM and for lidocaine were 115±6 µM and 2.3±0.3 µM.
6. The results show that all three drugs inhibit mitochondrial ATPase activity and that lorcainide is the most potent.
7. These inhibitory effects may be related to the lipophilicity and membrane stabilizing activity of this class of antiarrhythmic drugs.

INTRODUCTION

There is some recent evidence that class II antiarrhythmic drugs, which comprise β-adrenoceptor blockers, interact invariably with myocardial mitochondrial ATPase activity. Thus, lipophilic drugs such as propranolol, which have a strong membrane stabilizing activity exert inhibitory effects on the enzyme activity, while on the other hand, the much less lipophilic drugs which are devoid of membrane stabilizing activity, such as atenolol, stimulate it (Almotrefi and Dzimiri, 1992). Accordingly, the ability of the β-adrenoceptor blockers to inhibit mitochondrial ATPase activity seems to be embedded, in part at least, in both their lipophilicity and membrane stabilizing properties. The same physicochemical properties have also been implicated, to varying degrees, in the interactions of local anesthetic type of drugs (class I) and β-adrenoceptor blockers with the fast Na^{+} channels, the putative receptor for their antiarrhythmic actions (Ijzerman et al., 1987; Ijzerman and Soudjin, 1989). However, although this action on the Na^{+} channels is ultimately responsible for their therapeutic electrophysiological effects (Hondeghem and Katzung, 1984; Catterall, 1987; Sheldon et al., 1989), the biochemical basis of this action remains evasive. Of the several hypotheses advanced so far, the one purporting interference with the Na^{+} channel function as a result of direct inhibition or indirectly by acting on some other receptors or membrane-bound proteins appears to be the most plausible (Postma et al., 1983). The inhibition of enzyme functions, such as those of mitochondrial enzyme activities (Sakurada et al., 1972, 1975; Vieira Lopes et al., 1977; Klüppel et al., 1978, 1986; Fortes et al., 1983a, b, c; Trotz et al., 1987) and ability to induce changes in membrane structures (Surewicz and Leyko 1981; Pappu et al., 1985; Ondrias et al., 1987; Trotz et al., 1987; Kihara et al., 1989; Weitman et al., 1989) have also attracted considerable attention in this regard. All these actions are presumably influenced by both lipophilicity and membrane stabilizing activity. The objective of this study was therefore to compare the effect of class IA (quinidine), class IB (lidocaine) and class IC (lorcainide) drugs on mitochondrial ATPase in guinea pig heart preparations, in order to evaluate the role of lipophilicity and membrane stabilizing activity in their interactions with membrane bound enzymes, and their possible relevance to the pharmacological effects of these drugs.

MATERIALS AND METHODS

Mitochondrial enzyme preparations
Guinea pigs weighing 300–600 g were killed by a blow on the head and bled. The heart was removed rapidly and placed in ice-cooled normal saline. Four guinea pig hearts were trimmed of connective tissue, fat and coagulated blood
and minced into small strips. The unbroken mitochondrial particles were prepared using a modified method of Voss et al. (1961). Accordingly, the strips were homogenized three times for 5 min each using a Stedfast stirrer (Fisher Scientific, U.S.A.) to give 20% suspension in a mannitol-sucrose medium containing (in mM): mannitol 210, sucrose 75, Tris-Cl 10, Na2-EDTA 0.1 (pH 7.4), and the suspension was centrifuged at 1000 g for 10 min. The supernatant was then recentrifuged at 10,000 g for another 10 min, the sediment washed twice by resuspending it in the same amount of medium, homogenizing for 5 min and centrifuging it at 10,000 g. All steps were carried out at temperatures below 4°C and the pellets stored in the mannitol-sucrose medium at the same temperature until use. The mitochondrial protein concentration of the enzyme preparation was determined using Coomassie reagent (Pierce Chemicals, U.S.A.).

Determination of mitochondrial ATPase activity

The activity of the mitochondrial ATPase preparation was determined as the rate of P i released in the presence of P-enolpyruvate and pyruvate kinase according to a modified method of Klüppel et al. (1986). Accordingly, different drug concentrations were pre-incubated at 30°C for 10 min together with 0.1–0.4 #g protein in 1.0 ml containing (in mM): sucrose 50, Tris-Cl 12 (pH 7.4), MgCl2 5, KCl 5, P-enolpyruvate 0.05, pyruvate kinase 1.67 units. The reaction was then initiated by 3 mM ATP. Aliquots of 750 #l were drawn after 10 min, the reaction was stopped with 6% ice-cooled trichloroacetic acid and the mixture left on ice for 15 min. The mixture was microfuged at 10,000 g using Eppendorf Minifuge (Eppendorf, Hamburg, Germany) for 4 min, then 1.0 ml was drawn to determine the liberated phosphate spectrophotometrically at 660 nm after 20 min by the method of Elbl and Lands (1969), performing each assay in duplicate. The enzyme activity was calculated as the difference between the total activity and that of the ATP autohydrolysis in the absence of the enzyme. All values were corrected for blank values without the enzyme. The concentrations for half maximal inhibition of the ATPase activity (IC50) and IC90 values were calculated from individual concentration-effect curves as proposed by Hafner et al. (1977).

Drugs used were quinidine bisulphate, lidocaine HCl (Haessle) and lorcainide HCl (Janssen). All other reagents were of analytical grade. Values are given as mean ± SEM. Statistical significance was calculated by Student’s (unpaired) t-test using Statgraphics software package version 3.0 (Graphic Software System, 1988). Probability levels of less than 0.05 were taken as indicating a significant difference.

RESULTS

The concentration–response curves in Fig. 1 illustrate that all three tested drugs, quinidine, lidocaine and lorcainide inhibited the pyruvate-driven ATPase activity of undamaged mitochondria in a concentration-dependent fashion. Lorcainide was the most potent of the three drugs, thereby exerting its inhibitory effects in the range of less than 1.0 nM–2.0 mM. However, as depicted in this figure, the ATPase activity decreased only slightly with drug concentrations in the range of 10 nM–10 #M. The concentrations required to inhibit the enzyme activity by 20% (IC20) and by 50% (IC50) were 9.4 ± 0.6 nM and 87.2 ± 5.5 #M (Table 1). Quinidine and lidocaine, on the other hand, inhibited the enzyme activity at relatively higher concentrations. The drugs exhibited weak but significant (P < 0.05) equipotent inhibitory effects in the concentration range of 1.0–10 #M. Above this range, the two drugs exhibited similar characteristics in their inhibitory actions.

DISCUSSION

In the present study, a prototype of each subgroup of class I antiarrhythmic agents was selected for a comparative investigation of their actions on mitochondrial ATPase activity. The results indicate that all three drugs, quinidine (class IA), lidocaine (class IB) and lorcainide (class IC), inhibited mitochondrial ATPase activity. Lorcainide was the most potent inhibitor of enzyme activity. Quinidine and lidocaine exhibited significantly weaker inhibitory potencies with similar profiles. In particular, the effects of lorcainide stretched over a very wide concentration range, while those of the other two drugs exhibited
relatively steeper concentration–response relationships within practically similar ranges. One major factor that often determines drug interactions with membrane-bound enzymes, such as the mitochondrial ATPase, is the lipophilic nature of a pharmacological agent. It appears that the actions of the local anesthetic type of antiarrhythmic drugs on ATPase activity are no exception to this general phenomenon. Comparison of this property reveals that the trend in the inhibitory potencies of the three drugs correlates well with their lipophilicity. Thus, lorcainide \[ N-(4-chlorophenyl)-N-{ 1-(l-methylethyl)-4-piperidinyl}benzeneacetamide \] and lidocaine \[ 2-(diethy lamino)-N-(2,6-dimethylphenyl)acetamide \] are both acetamides which differ in the moieties attached to the acetamido group. Because of the very lipophilic nature of the three moieties, benzyl, \( N' \)-substituted methylethylpiperidinyl and \( p \)-chlorophenyl groups, lorcainide is significantly more lipophilic than its counterpart lidocaine which possesses only one dimethylphenyl and a diethylamino attached to the acetamido nucleus. Quinidine, \( 6' \)-methoxyacinchonan-9-ol, on the other hand, is a quinoline derivative possessing sterically free hydroxyl and nucleophilic vinyl groups, which according to its octanol/water partition coefficient, render the molecule considerably more hydrophilic than would be associated with such a structure. This is indeed supported by the observation that its \( K_p \) value (30) is even lower than that of lidocaine (70) (Drayer, 1984). Accordingly, the comparatively more lipophilic lorcainide is a significantly more potent inhibitor of mitochondrial ATPase than both quinidine and lidocaine which exhibit relatively equipotent inhibitory properties as a result of their similarly low lipophilicity.

The class I antiarrhythmic drugs inhibit many other aspects of mitochondrial function with characteristics and activity levels generally similar to their inhibition of the mitochondrial ATPase activity observed in the present study (Chazotte and Vanderkooi, 1981; Chazotte et al., 1983; Komai and Berkoff, 1978). Besides their effects on mitochondrial enzymes, the studied drugs are also potential inhibitors of several other enzymes of diverse biochemical functions. For example, they inhibit the electrogenic \( \text{Na}^+/\text{K}^+ \) pump activity of myocardial microsomal \( \text{Na}^+–\text{K}^+ \)-ATPase with IC\(_50\) values of 0.46 mM, 4.1 mM and 14.6 \( \mu \)M for quinidine, lidocaine and lorcainide, respectively (Almotreﬁ and Dzimir, 1990, 1991). Although lidocaine and quinidine show a reversed order of potency on this enzyme, they both remain essentially less effective than lorcainide. On the other hand however, quinidine appears to be as potent as lorcainide in inhibiting \([H] \)batrachotoxinin binding to the \( \text{Na}^+ \) channel, suggesting therefore that this action may not be dependent on lipophilicity alone. For example, binding studies have shown that lorcainide inhibits toxin binding to the \( \text{Na}^+ \) channel with an IC\(_50\) value of 5.84 \( \mu \)M, lidocaine with an IC\(_50\) of 134.9 \( \mu \)M and quinidine with an IC\(_50\) of 3.88 \( \mu \)M (Voigt and Mannhold, 1989). This is in complete agreement with previous studies indicating that quinidine inhibits the same action with an IC\(_50\) value of 3.2 \( \mu \)M, while lidocaine exerts similar effects with an IC\(_50\) value of 240 \( \mu \)M (McNeal et al., 1985). Accordingly, it is therefore questionable whether the effects of the class I antiarrhythmic drugs on the \( \text{Na}^+ \) channels have the same biochemical basis as their inhibition of mitochondrial ATPase. Nonetheless, it appears that the inhibition of membrane bound enzymes by these drugs is a general phenomenon, which is probably related to their lipophilicity and, perhaps also, their ability to exert membrane stabilizing effects. Our recent observation (Almotreﬁ and Dzimir, 1992) that some antiarrhythmic drugs which do not possess this property and are at the same time hydrophilic, such as atenolol, exhibit little or no tendency to inhibit mitochondrial ATPase, strongly supports the notion that these two factors are probably essential for the inhibition of enzyme activity.

However, despite the similarities enumerated above, it is extremely questionable whether their inhibitory actions on these different enzymes or lipids, exhibiting such diverse biochemical functions, could lead to a common pharmacological effect. On the contrary, it can be reasonably argued that the inhibition of the individual enzymes may in fact be contributory to various pharmacological actions of these agents. For example, their inhibition of the electrogenic pump activity of myocardial \( \text{Na}^+–\text{K}^+ \)-ATPase may, at least in part, account for their tendency to cause cardiac arrhythmias (proarrhythmias) rather than antiarrhythmic effects (Almotreﬁ and Dzimir, 1991). With regard to the mechanism of the antiarrhythmic actions of class I agents in particular, it should be pointed out that their subclassification is mainly based on their kinetics of interaction with the \( \text{Na}^+ \) channel, their actions on the repolarization phase of action potential in atrial, ventricular and Purkinje fibers, as well as their effects on sinoatrial and ventricular node action potentials (Campbell, 1989). Accordingly, lidocaine (IB) has fast kinetics (the onset of rate-dependent block on \( V_{\text{max}} \) is quick, and the time constant of recovery from rate-dependent block is small), while lorcainide (IC) has very slow kinetics (rate-dependent block develops slowly, the time constant of recovery is high), and quinidine (IA) is intermediate (Kecskemeti, 1991). Although there are plausible suggestions, indicating that the actions of antiarrhythmic drugs on the fast
Na+ channels is probably an indirect product of their inhibition of some other membrane-bound protein(s), there is hardly any convincing evidence to link their effects on any of these enzymes with this action. On the contrary, as indicated by the trend in their inhibitory potencies on the Na+ channels, it seems that there is no relationship between the inhibition of these enzymes and binding to the Na+ channels. On the other hand, however, the specificity by which the drugs inhibit the mitochondrial ATPase in particular, or mitochondrial enzymes as a whole, could be an indication that this may be related to some yet unknown effects of the drugs. It can therefore be concluded that there exists a well-defined relationship between the inhibitory actions of class I antiarrhythmic drugs on the mitochondrial ATPase activity and their lipophilic properties, as well as membrane stabilizing activity. However, the relevance of these inhibitory actions to their clinical use in the management of cardiac arrhythmias remains to be delineated.

Acknowledgements—I would like to thank the drug manufacturers Janssen for their gift of lorcainide and Haessle for lidocaine. I am also grateful to Dr Nduna Dzimiri for reading the manuscript and for his valuable suggestions. I also thank Ms Shailja Nigdikar for her excellent technical assistance.

REFERENCES


