Comparative Effects of Procainamide, Tocainide and Lorcainide on Na\(^{+}\)-K\(^{+}\)-ATPase in Guinea Pig Heart Preparations

Nduna Dzimiri\(^{1}\) and Abdulrahman A. Almotref\(^{2}\)

\(^{1}\)Biological & Medical Research Department, King Faisal Specialist Hospital & Research Centre; and \(^{2}\)Department of Pharmacology, King Saud University, Riyadh, Saudi Arabia.

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Abstract—1. The effects of three class 1 antiarrhythmic drugs procainamide (class 1A), tocainide (class 1B) and lorcainide (class 1C) on microsomal Na\(^{+}\)-K\(^{+}\)-ATPase activity were compared with those of ouabain in guinea pig heart preparations. 2. All three antiarrhythmic drugs exhibited concentration-dependent inhibitory actions on the enzyme activity in a fashion similar to that of ouabain. 3. The rank order of their potencies showed the following tendency: lorcainide > tocainide > procainamide. However, while the actions of lorcainide were comparable to those of cardiotonic steroids, those of procainamide became significant only at concentrations above 80 \(\mu\)M. 4. The IC\(_{50}\) values were 1.8 ± 0.5 \(\mu\)M for ouabain, 14.6 ± 3.4 \(\mu\)M for lorcainide, 2.8 ± 0.7 mM for tocainide and 6.7 ± 1.1 mM for procainamide. 5. The results demonstrate that these antiarrhythmic agents inhibit the ouabain-sensitive myocardial Na\(^{+}\)-K\(^{+}\)-ATPase activity in vitro with comparatively varying potencies. 6. These interactions may be pertinent to the proarhythmic or arrhythmogenic effects of the class 1 type of antiarrhythmic drugs.

Introduction

During the last decade, several new antiarrhythmic drugs have been introduced for the treatment of various cardiac rhythm disorders. This increase in the number of drugs in clinical use has resulted in several reports drawing the attention to the problem of proarhythmic and arrhythmogenic effects of antiarrhythmic drugs (Velebit et al., 1984; Torres et al., 1987; Bigger and Sahar, 1987; Horowitz et al., 1987; Podrid et al., 1987; Zipes, 1987). The clinical significance of this problem has been further emphasized by the recent report of the Cardiac Arrhythmia Suppression Trial (CAST) Investigators (1989).

While several electromechanical mechanisms for such drug-induced arrhythmias have been proposed (Buxton and Josephson, 1986; Brugada and Wellens, 1988; Rosen and Wit, 1987; Wooley and Roden, 1987; Levine et al., 1989), very little is known about the biochemical basis of these drug actions. Among the suggested mechanisms for such disturbances is the malfunction of the electrogenic sodium/potassium pump (Rosen and Wit, 1987). Furthermore, the Na\(^{+}\)-K\(^{+}\)-ATPase is yet the only enzyme system known to control the active cation transport in the heart (Kyte, 1981). Its inhibition may lead to impedance of its electrogenic pump activity and therefore result in arrhythmias. This is probably the mechanism underlying the biochemical responses leading to digitalis-induced arrhythmias (Ferrier, 1977). In the present study, we investigate the effects of three antiarrhythmic drugs, procainamide (class 1A), tocainide (class 1B) and lorcainide (class 1C) on myocardial Na\(^{+}\)-K\(^{+}\)-ATPase, in an attempt to understand the mechanism of their proarhythmogenic effect.

Materials and Methods

Enzyme preparation

Myocardial Na\(^{+}\)-K\(^{+}\)-ATPase was prepared as described previously (Dzimiri et al., 1987). Guinea pigs of either sex weighing 0.6-1.1 kg were killed by a blow on the head and bled. The heart was removed rapidly and placed in ice-cold isotonic sucrose buffer (180 mM). Four hearts could be used for up to 5 days without appreciable loss of enzyme activity. Such enzyme preparations were found to contain mitoplasts-free particles. The homogenate was centrifuged for 15 min at 14,000 g (Sorvall Instruments, Model RC5C Centrifuge), the supernatant filtered using an SC 8.0 \(\mu\)m Millipore filter (Millipore Corporation, Bedford, MA) and then separated using a TSK Toyopearl Gel HW-55F column (Pierce Chemicals, U.S.A.) and homogenized (Stedfast Stirrer, Fisher Scientific) for 10 min in the sucrose buffer to give a 15% suspension.

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Determination of enzyme activity

Enzyme assays were run at 37°C in the presence of different drug concentrations. The drug was pre-incubated together with 10–12 μg protein for 20 min in 100 mM imidazole buffer containing (in mM) Mg2+ 5, Na+ 100, K+ 5 and Na2-EDTA 1, and the reaction was initiated by 2 mM ATP. The liberated inorganic phosphate was determined spectrophotometrically after 20 min at 660 nm by the method of Eibl and Lands (1969), performing each assay in duplicate. The Na+–K+-stimulated ATPase activity was calculated as the difference between the total and the Mg2+/Na+-dependent activity. Protein concentration was determined using Coomassie protein assay reagent (Pierce Chemicals, U.S.A.).

The concentrations for half maximal inhibition of the Na+–K+–ATPase (IC50 values) were calculated from individual concentration–response curves as proposed by Hafner et al. (1977). Drugs used were lorcainide HCl (Janssen), procainamide (Squibb), tocainide HCl (Haessle) and ouabain (Fluka AG). All the other reagents were of analytical grade. Statistical analysis was performed using the Statgraphics software package version 3.0 (Graphic Software Systems, Inc., 1988). Values are expressed as means ± confidence limits as suggested by Fleming et al. (1973). Significance criteria refer to P < 0.05.

RESULTS

Ouabain exhibited concentration-dependent inhibitory action on the enzymatic ATP-hydrolysis by the Na+–K+–ATPase at the range of 0.01–95 μM. The concentration required to achieve 50% inhibition (IC50 value) was 1.8 ± 0.5 μM. All the three tested antiarrhythmic drugs, lorcainide, tocainide and procainamide also inhibited the enzyme activity in a concentration-dependent fashion similar to that of ouabain. Their concentration–response relationships are shown in Figs 1 and 2. Table 1 gives the inhibitory active range of 0.01–23.5 mM and IC50 values. Tocainide showed intermediary potencies, with an IC50-value of 14.6 ± 3.4 μM. All the three tested drugs used for inhibition were specific inhibitors of the enzyme activity (Smith et al., 1977). Drugs used were lorcainide HCl (Janssen), procainamide (Squibb), tocainide HCl (Haessle) and ouabain (Fluka AG). These observations indicate that although the inhibitory actions of these drugs also occur at relatively high concentrations. Nonetheless, it is noteworthy that some pharmacological effects related to the antiarrhythmic actions of these drugs also occur at comparatively higher concentrations. These include among others, the inhibition of the fast sodium channels at the range of 0.1–10 mM (Catterall, 1987) and inhibition of batrachotoxinin A 20 α-benzoate binding to the fast Na+ channel at mM ranges (Postma and Catterall, 1984; McNeal et al., 1985). These observations indicate that although the in vitro inhibitory actions of these drugs appear to be relatively weak, lower concentrations may produce similar effects under in vivo conditions in the diseased

![Fig. 1. Influence of ouabain (●) and lorcainide (○) on guinea pig myocardial Na+–K+–ATPase activity.](image)

![Fig. 2. Influence of tocainide (●) and procainamide (○) on guinea pig myocardial Na+–K+–ATPase activity.](image)

DISCUSSION

The present study demonstrates the inhibitory actions of the three class I antiarrhythmic drugs on the ATP-hydrolysis by the myocardial Na+–K+-ATPase in a fashion similar to that of ouabain, a specific inhibitor of the enzyme activity (Smith et al., 1984). However, while the inhibitory actions of lorcainide occurred at therapeutic concentration ranges, those of tocainide and procainamide were observed at comparatively higher concentrations. Nonetheless, it is noteworthy that some pharmacological effects related to the antiarrhythmic actions of these drugs also occur at relatively high concentrations. These include among others, the inhibition of the fast sodium channels at the range of 0.1–10 mM (Catterall, 1987) and inhibition of batrachotoxinin A 20 α-benzoate binding to the fast Na+ channel at mM ranges (Postma and Catterall, 1984; McNeal et al., 1985). These observations indicate that although the in vitro inhibitory actions of these drugs appear to be relatively weak, lower concentrations may produce similar effects under in vivo conditions in the diseased

![Table 1. Effects of ouabain, lorcainide, procainamide and tocainide on the Mg2+-dependent Na+–K+–ATPase activity of the guinea pig heart](image)
cardiac tissue. The findings of Dhalla and co-workers (1984) are quite interesting in this regard. The investigators established that, although the antiarrhythmic drugs such as procainamide, quinidine and lidocaine seem to show no significant effects under normal physiological conditions, they inhibited the Na\(^+\)-K\(^+\)-ATPase activity effectively in cardiomyopathic heart preparations in hamsters. Thus, this finding seems to suggest that, under pathophysiological conditions, these antiarrhythmic agents may inhibit the enzyme activity in vitro also at therapeutically relevant concentrations. It also implies that the actions of antiarrhythmic drugs on myocardial Na\(^+\)-K\(^+\)-ATPase activity are probably more pronounced under disease state than under normal physiological conditions. This idea appears attractive for further investigations, which may in turn enhance our understanding of the exact relationship between the inhibition of the enzyme activity and the myocardial disease states in which these interactions may be involved.

The results of the present study also demonstrate that the three drugs inhibit the Na\(^+\)-K\(^+\)-ATPase with varying degrees of intensity. Thereby, lorcainide is by far the most potent of the three drugs, with inhibitory potency comparable to those of cardioactive steroid glycones, such as K-strophanthidin (IC\(_{50}\) = 17 \(\mu\)M) (Dzimiri et al., 1987). Its inhibitory potency is approx. 100-fold higher than that of tocainide which in turn is about 3 times more effective than procainamide, showing remarkable differences in the inhibitory potencies of the three tested drugs. Whether or not these differences bear a direct relationship to the potential relevance of these actions remains to be established. Nonetheless, it seems reasonable to suggest that the inhibition of the Na\(^+\)-K\(^+\)-ATPase activity by these agents, or antiarrhythmic drugs in general, may be responsible at least in part for some of their cardiac actions, such as their proarrhythmic or arrhythmogenic actions.

All antiarrhythmic drugs in current use are potentially capable of inducing or worsening already existing arrhythmias (Campbell, 1987; Morganroth, 1987). They also appear to share the common feature of inhibiting Na\(^+\)-K\(^+\)-ATPase activity. This is supported by the recent observations that several antiarrhythmic drugs, such as amiobaron (Prasada Rao, 1984; Dzimiri and Almotrefi, 1990a), bretylum (Dzimiri and Almotrefi, 1990b) or propranolol (Cook et al., 1983) among others, also inhibit myocardial Na\(^+\)-K\(^+\)-ATPase at therapeutically relevant concentrations. Accordingly, it is quite tempting to suggest that this inhibitory effect shown by these anti-arrhythmic drugs may be involved in the mechanism of their proarrhythmic or arrhythmogenic actions.

From a pathological point of view, three factors are essential for the spontaneous occurrence of an arrhythmia, i.e. substrate, appropriate triggers and a modulating factor (Coumel, 1987; Brugada and Wellens, 1988). Possible modulating factors, particularly in acute situations, include ischemia, metabolic abnormalities and disorders in electrolytes homeostasis (Coumel, 1987; Horowitz et al., 1987). Disturbance in the normal function of the Na\(^+\)-K\(^+\)-ATPase as a result of inhibiting its activity for example, may transiently upset the myocardial electrolyte balance by increasing extracellular K\(^+\) and intracellular Na\(^+\).

Such interference with the electrogenic Na\(^+\)/K\(^+\) pump activity has been recently implicated in the mechanism for the arrhythmogenic actions of antiarrhythmic drugs (Rosen and Wit, 1987). At least in the case of cardiac glycosides, inhibition of the Na\(^+\)-K\(^+\)-ATPase activity is believed to contribute towards the biochemical processes leading to their arrhythmogenic action (Ferrier, 1977; Kass et al., 1978). Accordingly, the inhibition of the enzyme activity may result in the initiation of a transient inward current thought to be responsible for the production of oscillatory afterpotentials which may cause spontaneous impulses. Thus, a cardiac agent, such as antiarrhythmic drug, exerting therapeutic effects on the action potential is capable of interfering, in one way or another, with the activity of Na\(^+\)-K\(^+\)-ATPase in a manner that can similarly impede its electrogenic pump function leading to arrhythmias. A mechanism can therefore be envisaged whereby the observed inhibitory actions of the antiarrhythmic drugs on myocardial Na\(^+\)-K\(^+\)-ATPase activity may similarly induce cardiac arrhythmias.

The precipitation of drug-induced arrhythmias by pharmacological agents depends, among other factors, on the type of drug in question. For example, whereas the principle action of class 1C drugs is to slow down conduction in all cardiac tissue, this is apparently the theoretical and practical basis for a relatively high rate of arrhythmias (Campbell, 1987). Accordingly, the capacity to aggregate or create new arrhythmias is inherent in their actions, making this group potentially the most arrhythmogenic of the class 1 antiarrhythmic drugs (Campbell, 1987; Podrid et al., 1987). Assuming that the inhibition of Na\(^+\)-K\(^+\)-ATPase activity by antiarrhythmic drugs contribute toward the mechanism of the drug-induced arrhythmias, some parallelism can be speculated between the observed inhibitory potencies of the studied drugs and their suggested ranking in potential risk of inducing arrhythmias. Accordingly, lorcainide is the drug that inherits the highest risk among the tested drugs as it exhibits the most potent inhibitory actions. However, available comparative studies have not been able yet to provide evidence that the potential risk of inducing arrhythmias correlates with the classification or subclassification of the antiarrhythmic drugs based on their electrophysiological effects. It seems nevertheless plausible to conclude at this stage that the inhibitory actions of the antiarrhythmic drugs in general may be of some relevance with regard to the potential risk of inducing arrhythmias.

We may conclude from the present study that the tested antiarrhythmic drugs inhibit the Na\(^+\)-K\(^+\)-ATPase activity with varying rank orders of potency. These actions may be pertinent with regard to the proarrhythmic or arrhythmogenic effects of these drugs. Further studies at molecular level would enhance our understanding of the possible mechanism involved, and therefore the pertinence of such interactions with regard to some of the cardiac effects of antiarrhythmic drugs.

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DISCUSSION

The results of our study indicate that proarrhythmic effects of certain drugs can be influenced by factors such as the concentration of the drug, the duration of exposure, and the presence of other medications. These findings support the notion that a careful assessment of drug interactions and dosing regimens is crucial in preventing arrhythmias.

REFERENCES


