THE EFFECT OF MODIFYING POTASSIUM CONCENTRATION ON THE INHIBITION OF MYOCARDIAL Na⁺–K⁺-ATPASE BY TWO CLASS IB ANTIARRHYTHMIC DRUGS: LIDOCAINE AND TOCAINIDE

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Abstract—1. The inhibitory actions of two class IB antiarrhythmics, lidocaine and tocainide, on Mg²⁺-dependent ATP hydrolysis by myocardial Na⁺–K⁺-ATPase (EC 3.6.1.3), were tested in guinea-pig heart preparations incubated in media containing 2.5, 5.0 and 10 mM K⁺.

2. The IC₅₀ values for lidocaine were 2.4 ± 0.4 mM at 2.5, 4.1 ± 0.8 mM at 5.0 and 5.3 ± 0.5 mM at 10 mM K⁺. The corresponding IC₂₀ values were 0.82 ± 0.12 mM at 2.5, 1.3 ± 0.2 mM at 5.0 and 1.7 ± 0.4 mM at 10.0 mM K⁺ respectively. Tocainide exerted similar action with IC₅₀ values of 3.1 ± 0.9 mM at 2.5, 7.6 ± 1.4 mM at 5.0 and 15.5 ± 1.6 mM at 10.0 mM K⁺ and IC₂₀ values of 0.71 ± 0.19 at 2.5, 2.7 ± 0.5 mM at 5.0 and 12.3 ± 1.2 mM at 10.0 mM K⁺ respectively.

3. Thus, the inhibitory potencies of the drugs on myocardial Na⁺–K⁺-ATPase activity increased significantly with reduction in the K⁺ concentration. These results demonstrate therefore that the inhibitory actions of both lidocaine and tocainide depend on the K⁺ concentration of the incubation medium.

4. These findings may be indicative of the importance of K⁺ in some of the cardiac effects of the antiarrhythmic agents, particularly their tendency to induce or enhance already existing cardiac arrhythmias.

INTRODUCTION

Recent studies have drawn attention to the clinical importance of the proarrhythmic and arrhythmogenic effects of several antiarrhythmic drugs in current use (Morganroth, 1987; Rosen and Wit, 1987; Wooley and Roden, 1987; Zipes, 1987). One way by which these drugs may cause arrhythmias is by impeding the electrogenic pump activity of the myocardial Na⁺–K⁺-ATPase, in a fashion similar to the mechanism ascribed to digitalis-induced arrhythmias (Ferrier, 1977; Kass et al., 1978). Although antiarrhythmic drugs may also potentially impede the electrogenic pump activity of this enzyme (Rosen and Wit, 1987), hardly any attention has been paid yet to the possibility that antiarrhythmic drug-induced arrhythmias may also be a result of their interactions with myocardial Na⁺–K⁺-ATPase. On the other hand, it is now known that several of the antiarrhythmic drugs in current use are potent inhibitors of the digitalis receptor (Kennedy and Neyler, 1965; Goldstein et al., 1973; Cook et al., 1983; Prasada Rao et al., 1986; Aomine, 1989; Dzimiri and Almotrefi, 1991a,b). In particular, we have recently established that several class I agents, including lidocaine and tocainide, also inhibit this receptor in a fashion similar to that of cardiac glycosides (Almotrefi and Dzimiri, 1990; Dzimiri and Almotrefi, 1991c). Furthermore, the involvement of potassium in the genesis and termination of cardiac arrhythmias, especially those resulting from clinical use of cardiac glycosides or antiarrhythmic agents is well established. Thus, while potassium has proved to be a useful remedy against some cardiac arrhythmias (Vassalle and Greenspan, 1963; Watanabe et al., 1983), the loss of this electrolyte can be detrimental, especially if it occurred during treatment with cardiac glycosides (Klieger et al., 1966; Aronson and Nordin, 1988) or antiarrhythmic drugs (Obayani et al., 1975; Levine et al., 1989). Such a disturbance in K⁺ balance is regarded as a high risk factor for the manifestation of proarrhythmic effects of some class I and III antiarrhythmic drugs (Velebit et al., 1982; Levine et al., 1989). In the light of these observations, we investigate in the present study the influence of varying K⁺ concentration on the inhibitory actions of two class IB antiarrhythmic agents, lidocaine and tocainide, on myocardial Na⁺–K⁺-ATPase and attempt to assess its implication on some of the cardiac effects of these drugs.

MATERIALS AND METHODS

Enzyme preparation

Myocardial Na⁺–K⁺-ATPase was prepared as described previously (Dzimiri et al., 1987). Guinea-pigs of either sex weighing 500–800 g were killed by a blow on the head and
bled. The heart was removed rapidly and placed on ice-cooled isotonic sucrose buffer (180 mM). Four hearts were minced using a biohomogenizer (Biospec Products Inc., U.S.A.) and homogenized (Stedfast Stirrer, Fisher Scientific) for 10 min in the sucrose buffer to give a 15% (w/w) suspension. The homogenate was centrifuged for 15 min at 14,000 g using Sorvall RCSC Centrifuge (Sorvall Instruments, U.S.A.), the supernatant filtered using SC 8.0 μm filter (Millipore Corporation, U.S.A.) and then separated on TSK Toyopearl Gel HW-55F column (Pierce Chemicals, U.S.A.) equilibrated with KCl buffer (KCl 500 mM, imidazole 10 mM, Na2-EDTA 1 mM, pH 7.4). Ascending elution of the enzyme was carried out at a flow of 0.7 ml min−1 maintained by means of a peristaltic pump (LKB Bromma). The fractions were collected on LKB-2211 Superrac and dialysed in 20/32 Visking dialysis membrane (Serva Finebiochemica, U.S.A.) three times, each time for at least 6 hr using first 10 mM twice, and then 100 mM imidazole buffer, pH 7.4. All steps were carried out at 4°C. The enzyme was stored at the same temperature and could be used for up to 5 days without appreciable loss of activity. Such enzyme preparations were found to contain mitochondria-free particles.

**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. Series of assays were also run in incubation medium containing 2.5 and 10.0 mM in place of the standard 5.0 mM K+ . 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. The concentrations for half maximal inhibition (IC50) of the Na+-K+-ATPase, and those required to inhibit the enzyme activity by 20% (IC30) were calculated from individual concentration-effect curves as proposed by Hafner et al. (1977). Protein concentration was determined using Coomassie protein assay reagent (Pierce Chemicals, U.S.A.).

Drugs used were lidocaine HCl and tocainide HCl (Haeasle). All the other reagents were of analytical grade. Values are given as mean ± SD. Statistical significance was calculated by Student's t-test using the Statgraphics software package version 3.0 (Graphic Software Systems, Inc., 1988). Probability levels of less than 0.05 were taken as indicating significant differences.

**RESULTS**

Both lidocaine and tocainide exerted concentration-dependent inhibitory effects on the Na+-K+-ATPase activity at all the tested K+ concentration of 2.5, 5.0 and 10.0 mM (Figs 1 and 2). At the “standard” 5.0 mM K+ concentration, lidocaine showed inhibitory effects in the range of 10.0–1200 μM, exhibiting an IC50 value of 1.3 ± 0.2 mM and IC30 value of 4.1 ± 0.8 mM. Reducing the potassium concentration to 2.5 mM shifted the effective inhibition concentration range (5–95% inhibition) to 1.0–800 μM, showing an IC50 value of 0.82 ± 0.12 mM and IC30 value of 2.4 ± 0.4 mM, respectively. Increasing it to 10.0 mM on the other hand shifted the range to 90–1300 μM. Thereby, the IC50 and IC30 values increased to 1.7 ± 0.4 mM and 5.3 ± 0.5 mM, respectively. The effective inhibitory concentration ranges for tocainide were approx. 1.0–10,000 μM at 2.5 mM K+, 0.1–12.4 mM at 5.0 mM and 1.6–16 mM at 10.0 mM K+. At the “standard” 5.0 mM K+ concentration, its IC20 and IC50 values were 2.7 ± 0.5 and 7.6 ± 1.4 mM. These values were reduced to 0.71 ± 0.19 and 3.1 ± 0.9 mM at 2.5 mM K+ and increased to 12.3 ± 12.0 and 15.5 ± 1.6 mM at 10.0 mM K+ respectively (Table 1). Figures 3 and 4 summarize the results of a series of experiments to compare the changes in enzyme activity at different drug concentrations relative to its

![Fig. 1. Influence of different concentrations of lidocaine in presence of 2.5 (●), 5.0 mM (▲) and 10.0 mM (●) K+ in guinea-pig myocardial Na+-K+-ATPase. The average baseline enzyme activities were 21.4 ± 6.7, 24.2 ± 5.3 and 25.1 ± 7.4 μM P, mg−1 protein hr−1 at 2.5, 5.0 and 10.0 mM K+, respectively. Plotted are the values ± SD of 7–9 individual determinations.](image)

![Fig. 2. Influence of different concentrations of tocainide in presence of 2.5 (●), 5.0 mM (▲) and 10.0 mM (●) K+ in guinea-pig myocardial Na+-K+-ATPase. The average baseline enzyme activities were 23.1 ± 8.1, 22.7 ± 7.7 and 24.5 ± 5.7 μM P, mg−1 protein hr−1 at 2.5, 5.0 and 10.0 mM K+, respectively. Plotted are the values ± SD of 8–10 individual determinations.](image)

<table>
<thead>
<tr>
<th>K+ Concentration (mM)</th>
<th>Lidocaine IC50 (mM)</th>
<th>Lidocaine IC30 (mM)</th>
<th>Tocainide IC50 (mM)</th>
<th>Tocainide IC30 (mM)</th>
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<tr>
<td>2.5</td>
<td>0.82 ± 0.12</td>
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<td>5.0</td>
<td>1.3 ± 0.2</td>
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<td>2.7 ± 0.5</td>
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<td>10.0</td>
<td>1.7 ± 0.4</td>
<td>5.3 ± 0.5</td>
<td>12.3 ± 1.2</td>
<td>15.5 ± 1.6</td>
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*The median inhibitory concentration, defined as the concentration required to inhibit the enzyme activity by 50%.
†Concentration required to inhibit the enzyme activity by 20%.
IC values are expressed as means ± SD of 8–10 individual determinations.
activity at "standard" 5 mM K⁺ level. The results demonstrate that both the differences in the inhibitory potencies and the effective inhibitory concentration ranges of the drugs also increased with decreasing drug concentrations, thereby shifting these actions significantly further towards much lower concentration.

**DISCUSSION**

In the present study, it was demonstrated that the inhibitory actions of both lidocaine and tocainide depend to a great extent on the K⁺ concentration of the incubation medium. Thus, reducing the "standard" 5 mM K⁺ concentration by half caused a significant right-to-left shift in their inhibitory effective concentration ranges. As indicated by the trends of the IC₅₀ and IC₉₀ values, although the actions of both drugs occurred at relatively high concentrations under standard conditions, the reduction in K⁺ concentration shifted these activities towards much lower concentrations. On the other hand, increasing the K⁺ concentration resulted in a left-to-right shift in the effective inhibitory concentration ranges, thereby drastically reducing the inhibitory potencies. It is not surprising that the inhibitory actions of the drugs occur at relatively high concentration under normal "standard" conditions. This argument is based on the observation that other interactions associated with the antiarrhythmic drug action mechanism(s), such as the inhibition of the fast Na⁺ channels for example, are in general also observed at relatively high concentrations (McNeal et al., 1985; Catterall, 1987). Moreover, it is also believed that one of the major factors contributing to the high risk of drug-induced arrhythmias is the use of high doses of antiarrhythmic agents (Morganroth, 1987). Thus, it appears that high concentrations or doses are usually required for the local anesthetic type of antiarrhythmic drugs to attain a wide majority of their effects. Of special interest however, is the observation in the present study that reduction in K⁺ concentrations shifts the inhibitory potencies towards lower drug concentration ranges. This trend can be interpreted as indicating that the interactions between the antiarrhythmic agents and myocardial Na⁺–K⁺-ATPase become increasingly more significant with decreasing K⁺ concentrations. It is therefore quite tempting to associate this type of observation with the disorders that involve changes in potassium concentrations, such as drug-induced arrhythmias.

The most common physiological mechanisms ascribed to arrhythmias initiated by antiarrhythmic drugs are either conduction block (reentry) or abnormal impulse initiation which may be triggered by afterdepolarizations (Rosen and Wit, 1987; Levine et al., 1989). The latter usually results from secondary depolarizations arising during the cardiac action potential, defined as early or delayed afterpotentials (Rosen, 1971; Bailie et al., 1988). Thus, if the administration of the cardiac glycosides or antiarrhythmic drugs is known to cause early afterdepolarizations for example (Levine et al., 1989). The loss of potassium, or other disturbance in electrolyte balance such as hypokalemia, is, on the other hand, often accompanied by arrhythmias resulting from either early or delayed afterpotentials (Rosen, 1971; Bailie et al., 1988). Thus, if the administration of the cardiac glycosides or antiarrhythmic drugs is associated with conditions leading to loss of intracellular potassium, the chances of drug-induced arrhythmias would be greatly enhanced.

In order to evaluate the possible role of potassium in the interactions of the antiarrhythmic drugs with Na⁺–K⁺-ATPase, one should perhaps first consider the mechanism ascribed to digitalis-induced cardiac arrhythmias. To begin with, it is known that by inhibiting the ATP-hydrolytic action of Na⁺–K⁺-ATPase, the steroids induce a decrease in the inward movement of K⁺ and outward movement of Na⁺, thereby decreasing the intracellular concentration of the former and increasing that of the latter (Schatzman, 1953). This usually upsets the electrogenic...
pump action of the enzyme, thereby causing delayed afterdepolarizations, which may in turn trigger cardiac arrhythmias (Ferrier, 1977; Kass et al., 1978). Accordingly, it seems reasonable to suggest that such interactions may lay the biochemical basis underlying the type of arrhythmias encountered during treatment with antiarrhythmic agents. This supposition implies that by inhibiting the ATP-hydrolytic activity of the enzyme, the antiarrhythmic drugs may impede its electrogenic pump activity, thereby causing secondary depolarizations which in turn may result in arrhythmias. However, our knowledge with regards to the ability of, or extent to which, some of the antiarrhythmic drugs may cause afterpotentials in general is incomplete. Also, the lack of data pertaining to the ability of these drugs to cause disturbances of the electrogenic pump activity of the Na⁺-K⁺-ATPase makes any direct interpretation of these findings difficult with regard to their relevance of mechanism of the arrhythmias induced by antiarrhythmic drugs. Nonetheless, it seems noteworthy that there exists a number of similarities in the mode of interaction of the tested drugs and those of the cardiac glycosides with Na⁺-K⁺-ATPase activity. These include the aforementioned inhibition of the ATP-hydrolytic action of the Na⁺-K⁺-ATPase, the enhancement of such effects by the decrease in K⁺ concentrations, as well as their ability to cause afterdepolarizations (Levine et al., 1989), all of which appear to suggest some common basis for some of those actions shared by these two groups of cardiac drugs, such as in particular, their tendency to induce or enhance cardiac arrhythmias.

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REFERENCES


