A Qualitative Distinction between the Beta-Receptor-Blocking and Local Anesthetic Actions of Antiarrhythmic Agents

By Elliot Shinebourne, Roger White, and John Hamer

ABSTRACT

Fragmented cardiac sarcoplasmic reticulum was isolated by differential centrifugation from canine myocardium. The effects of propranolol, quinidine, lidocaine (lignocaine) and tetracaine (amethocaine) on calcium uptake in sarcoplasmic reticulum in vitro were then measured. All the drugs are antiarrhythmic agents with local anesthetic activity, but propranolol and quinidine are known also to have beta-receptor-blocking actions in that they can prevent the cardiac effects of isoprenaline and depress the myocardium. Lidocaine and tetracaine do not generally depress the heart and had no effect on the calcium uptake, but propranolol and quinidine both significantly inhibited it. It was concluded that the antiarrhythmic actions of propranolol (and quinidine) may be independent of beta-receptor-blocking activity, and that the safety of lidocaine as an antiarrhythmic agent may be related to a lack of effect on the sarcoplasmic reticulum.

ADDITIONAL KEY WORDS excitation-contraction coupling calcium uptake sarcoplasmic reticulum myocardial contractility lidocaine propranolol tetracaine beta-receptor activity dog

The ability of propranolol and other sympathetic blocking drugs to decrease calcium uptake by cardiac sarcoplasmic reticulum and hence to interfere with excitation-contraction coupling has been suggested as an important cellular mechanism underlying sympathetic blockade (1-3). This and other work (4, 5) indicated that many drugs with a negative inotropic effect inhibit sarcotubular calcium uptake.

As well as depressing myocardial contractility, propranolol, the most potent beta-receptor-blocking agent, has local anesthetic and antiarrhythmic actions, and the question arose whether the antiarrhythmic effects were dependent on beta-receptor blockade or on some other nonspecific effect. It has been shown from measurements of intracellular potentials (6-8) that beta-receptor blockade and antiarrhythmic activity were not necessarily linked. It was concluded that changes produced in transmembrane potentials thought to be related to arrhythmia suppression, such as a decrease in maximum rate of depolarization and a smaller overshoot, were independent of any action on beta-receptors.

Lidocaine (lignocaine) is an effective antiarrhythmic agent but, unlike propranolol, is generally devoid of cardiodepressant activity (9-13), as is tetracaine (amethocaine), another potent local anesthetic. Quinidine, widely used to suppress arrhythmias, has local anesthetic and beta-receptor-blocking activity (14) and may depress the myocardium.

We have attempted to clarify the relationship between antiarrhythmic activity and beta-receptor blockade by measurements of the effects of these agents on the calcium uptake of the isolated sarcoplasmic reticulum.

Methods

Sarcoplasmic reticulum was extracted by differential centrifugation from dog myocardium using a method previously described (15) based on a modification (16) of Carsten's technique (17). Dogs of either sex weighing from 11 to 20 kg were anesthetized with sodium pentobarbital, 30 mg/kg body weight. After 3-minute ventilation...
with 50% oxygen through an intratracheal cannula, the chest was opened and the beating heart removed. After rinsing in ice-cold tap water the heart was placed in ice for 15 minutes. The ventricles, trimmed of fat, were chopped into small pieces and were homogenized in a Waring Blender for 60 seconds in 4 volumes of an extraction solution of 0.3M sucrose containing 0.2 mM ascorbic acid at pH 7.0. The homogenate was centrifuged at 2,520g for 15 minutes and the supernatant fluid strained through muslin. Further centrifugation at 18,000g for 20 minutes deposited mitochondria. The supernatant fluid was recentrifuged at 60,000g for 90 minutes to deposit a light-colored pellet. This material was resuspended in extraction solution using a Teflon and glass homogenizer and centrifuged at 198,000g for 30 minutes as a washing procedure. The pellet was again suspended in extraction solution, and a protein determination was carried out (18). The preparation of fragmented sarcoplasmic reticulum prepared in this way is shown in Figure 1.

In control experiments, vesicles were incubated at 37°C in a KCl-imidazole-oxalate medium; after 3 minutes, 45Ca, calcium chloride, ATP, and magnesium chloride were added to the reaction flask. When the effects of drugs were studied, they were added at the start of the incubation procedure. The final incubation consisted of 0.15 mg sarcoplasmic reticulum protein/ml, 120 mM KCl, 18 mM imidazole, 5 mM MgCl₂, 5 mM ATP, 1.8 mM sodium oxalate, 0.09 or 0.18 mM CaCl₂, and 0.05 μc/ml 45Ca at pH 7. To measure calcium uptake, aliquots of the incubation mixture were removed at timed intervals and filtered through Millipore filters (0.22μm-diameter, pore size). Radioactivity was measured on a liquid scintillation counter (Beckman LS-100) using a 1,4-dioxan, naphthalene, PPO (2,5-diphenyloxazole) counting medium. Calcium uptake was calculated from the difference in radioactivity between the filtrate and the original incubation mixture. A paired comparison of rates of calcium uptake before and after the drug was made by Student’s t-test to see whether the mean
## ANTIARRHYTHMIC AGENTS

**Table 1**

Effect of Various Antiarrhythmic Agents on Uptake of Calcium by Sarcoplasmic Reticulum

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Before drug</th>
<th>After drug</th>
<th>Δ</th>
<th>Before drug</th>
<th>After drug</th>
<th>Δ</th>
<th>Before drug</th>
<th>After drug</th>
<th>Δ</th>
<th>Before drug</th>
<th>After drug</th>
<th>Δ</th>
<th>Before drug</th>
<th>After drug</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.633</td>
<td>0.107</td>
<td>0.526</td>
<td>0.878</td>
<td>0.174</td>
<td>0.794</td>
<td>1.240</td>
<td>0.173</td>
<td>0.067</td>
<td>1.315</td>
<td>0.172</td>
<td>1.143</td>
<td>1.320</td>
<td>0.213</td>
<td>1.107</td>
</tr>
<tr>
<td>3</td>
<td>0.855</td>
<td>0.170</td>
<td>0.688</td>
<td>0.994</td>
<td>0.410</td>
<td>0.584</td>
<td>1.250</td>
<td>0.482</td>
<td>0.768</td>
<td>1.290</td>
<td>0.544</td>
<td>0.738</td>
<td>1.290</td>
<td>0.437</td>
<td>0.853</td>
</tr>
<tr>
<td>5</td>
<td>0.507</td>
<td>0.039</td>
<td>0.468</td>
<td>0.718</td>
<td>0.107</td>
<td>0.611</td>
<td>1.050</td>
<td>0.133</td>
<td>0.917</td>
<td>1.271</td>
<td>0.133</td>
<td>1.138</td>
<td>1.310</td>
<td>0.187</td>
<td>1.123</td>
</tr>
<tr>
<td>7</td>
<td>0.068</td>
<td>0.061</td>
<td>0.007</td>
<td>0.068</td>
<td>0.040</td>
<td>0.028</td>
<td>0.176</td>
<td>0.020</td>
<td>0.156</td>
<td>0.347</td>
<td>0.020</td>
<td>0.327</td>
<td>0.420</td>
<td>0.311</td>
<td>0.860</td>
</tr>
<tr>
<td>10</td>
<td>0.350</td>
<td>0.350</td>
<td>0.000</td>
<td>0.760</td>
<td>0.490</td>
<td>0.270</td>
<td>0.711</td>
<td>0.371</td>
<td>0.340</td>
<td>0.923</td>
<td>0.313</td>
<td>0.610</td>
<td>1.201</td>
<td>0.341</td>
<td>0.860</td>
</tr>
</tbody>
</table>

### Propranolol, 10⁻³ M

| Mean         | 0.337       | 0.337      | 0.439 | 0.439       | 0.560      | 0.560 | 0.907       | 0.907      | 0.854 |
| ± SE         | 0.141       | 0.129      | 0.126 | 0.198       | 0.137      | 0.137 | 0.144       | 0.144      | 1.000 |
| P            | <0.05       | <0.05      | <0.05 | <0.01       | <0.01      | <0.01 | <0.01       | <0.01      | 1.000 |

### Quinidine, 10⁻³ M

| Mean         | 0.346       | 0.574      | 0.803 | 0.803       | 0.818      | 0.818 | 0.808       | 0.808      | 1.000 |
| ± SE         | 0.093       | 0.102      | 0.122 | 0.120       | 0.009      | 0.009 | 0.002       | 0.002      | 1.000 |
| P            | <0.02       | <0.02      | <0.01 | <0.01       | <0.01      | <0.01 | <0.01       | <0.01      | 1.000 |

### Lidocaine, 10⁻³ M

| Mean         | 0.042       | 0.038      | 0.067 | 0.077       | 0.018      | 0.018 | 0.044       | 0.044      | 0.000 |
| ± SE         | 0.005       | 0.007      | 0.018 | 0.018       | 0.037      | 0.037 | 0.000       | 0.000      | 1.000 |
| P            | <0.02       | <0.02      | <0.01 | <0.01       | <0.01      | <0.01 | <0.01       | <0.01      | 1.000 |

### Tetracaine, 10⁻³ M

| Mean         | 0.031       | 0.026      | 0.071 | 0.071       | 0.168      | 0.234 | 0.084       | 0.084      | 1.000 |
| ± SE         | 0.051       | 0.007      | 0.003 | 0.003       | 0.094      | 0.094 | 0.000       | 0.000      | 1.000 |
| P            | <0.05       | <0.05      | <0.05 | <0.05       | <0.05      | <0.05 | <0.05       | <0.05      | 1.000 |

### Lidocaine, 10⁻¹ M

| Mean         | 0.157       | 0.102      | 0.082 | 0.079       | 0.000      | 0.003 | 0.064       | 0.106      | 0.042 |
| ± SE         | 0.083       | 0.048      | 0.004 | 0.012       | 0.017      | 0.017 | 0.125       | 0.174      | 0.049 |
| P            | 0.109       | 0.244      | 0.202 | 0.224       | 0.224      | 0.224 | 0.275       | 0.275      | 0.275 |

### Lido, 10⁻¹ M

| Mean         | 0.003       | 0.027      | 0.144 | 0.223      | 0.000      | 0.000 | 0.000       | 0.000      | 0.000 |
| ± SE         | 0.046       | 0.064      | 0.091 | 0.100      | 0.000      | 0.000 | 0.000       | 0.000      | 0.000 |
| P            | <0.05       | <0.05      | <0.05 | <0.05      | <0.05      | <0.05 | <0.05       | <0.05      | 1.000 |

All values are μmoles Ca²⁺/mg sarcoplasmic reticulum protein. Δ = Fall in calcium uptake after drug.
Effect of propranolol $10^{-5}M$ on the rate of uptake of calcium by the sarcoplasmic reticulum. Symbols show mean and standard deviation before (open circle) and after (solid circle) drug was given.

All drugs were dissolved in the KCl-imidazole-oxalate mixture and were prepared to give concentrations during the final incubation as follows: dl-propranolol, $10^{-5}M$; lidocaine, $10^{-5}M$ and $10^{-2}M$; quinidine, $10^{-3}M$; and tetracaine, $10^{-3}M$.

**Results**

As previously (1, 2), $10^{-5}M$ propranolol (Table 1, Fig. 2) caused a marked and significant fall in calcium uptake. An even greater depression in calcium uptake was produced by the same concentration of quinidine (Table 2, Fig. 3). When lidocaine (Table 3, Fig. 4) or tetracaine (Table 4, Fig. 5) in this concentration was added to the incubation mixture, the calcium uptake rates showed no significant change from the controls.

As equimolar concentration of drugs may not be equipotent in their pharmacologic actions, in a further series of experiments $10^{-2}M$ lidocaine was added to the suspension of sarcoplasmic reticulum (the therapeutic dose of lidocaine for an antiarrhythmic effect is approximately ten times that of propran-
The method of extracting sarcoplasmic reticulum and purity of the preparation have been discussed elsewhere (16). The four drugs used in the experiment are all local anesthetics and, with the exception of tetracaine, are commonly used therapeutically as antiarrhythmic agents. Tetracaine is a more potent local anesthetic than lidocaine and has not been reported to depress cardiac function.

In these experiments propranolol and quinidine, both known to depress the myocardial force-velocity relationship, inhibited the rate of calcium uptake by sarcoplasmic reticulum. In contrast, lidocaine and tetracaine, both with little effect on myocardial force generation but with antiarrhythmic actions, did not appreciably alter calcium uptake.

Quinidine has some beta-receptor-blocking activity (14), although this may not be the only way its cardiodepressant effects are mediated. One possible conclusion to be drawn from our results is that beta-receptor blockade or depression of the force-velocity curve is related to impaired calcium uptake by sarcoplasmic reticulum but that antiarrhythmic activity relates to effects on membrane polarization—the one feature common to lidocaine, tetracaine, quinidine, and propranolol.

The effects of inhibition of calcium uptake by propranolol have been discussed elsewhere (2, 3, 19) and result primarily in a slower rate of relaxation of muscle fibers and a decrease in contractility.

The concentrations of drugs required to produce significant impairment of calcium uptake are much higher than required in vivo. Also, the dose of quinidine required to suppress arrhythmias does not necessarily depress the myocardium, whereas antiarrhythmic doses of propranolol are usually cardiodepressant. This being so, a concentration of quinidine equimolar with the lowest propranolol concentration effective in depressing the sarcotubular calcium pump might not be expected to inhibit calcium uptake. Considerations of the distribution and local concentrations of propranolol and quinidine within the cell may partly explain the situation.

Electron microscopic examination of myocardium from animals given isotopically labeled propranolol or quinidine show that propranolol is concentrated in the vicinity of the Z lines, probably bound to the lateral}

Discussion

The method of extracting sarcoplasmic reticulum and purity of the preparation have been discussed elsewhere (16). The four drugs used in the experiment are all local anesthetics and, with the exception of tetracaine, are commonly used therapeutically as antiarrhythmic agents. Tetracaine is a more
cisternae of the sarcoplasmic reticulum (20). Furthermore, labeled propranolol settles with the microsomal fraction (20), and this also suggests binding to sarcoplasmic reticulum, so that in vivo the local concentration of the drug at the sarcoplasmic reticulum will be higher than in the circulating blood. Quinidine, on the other hand, is predominantly bound to cell membrane and mitochondria (21), and its local concentration at the sarcoplasmic reticulum will be less. The same dose of propranolol or quinidine in vivo therefore gives rise to widely different local concentrations at the sarcoplasmic reticulum. Myocardial depression is a toxic effect of quinidine and it is this action which may be explained by its ability to inhibit calcium uptake by the sarcoplasmic reticulum.

Neither tetracaine nor lidocaine significantly depressed calcium uptake by the sarcoplasmic reticulum, and this may be the reason they do not depress the myocardium. It perhaps also offers an explanation for the safety of lidocaine as an antiarrhythmic agent, especially after myocardial infarction when cardiac function is already impaired.

The general conclusions that can be drawn from our findings are that while depression of the myocardial force-velocity relationship is associated with inhibition of calcium uptake by the sarcoplasmic reticulum, antiarrhythmic activity can be independent of such a mechanism. In previous work (1-3) it was suggested that impaired calcium uptake was the direct result of beta-receptor blockade and that an increase in calcium uptake accompanied sympathetic stimulation. Our results indicate that the antiarrhythmic activity of propranolol (or quinidine) may be independent of beta-receptor blockade.

We have also demonstrated at a cellular level a qualitative distinction between agents with both beta-receptor-blocking and local anesthetic activity, and those with local anesthetic activity alone.

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References


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