Inhibition of Incorporation of Leucine into Myocardial Proteins of the Rat by Antiarrhythmic Agents

By Barry M. Beller, M.D., and Stephen Mangillo, M.S.

ABSTRACT
Incorporation of tritiated leucine into myocardial proteins was inhibited at low concentration by quinidine, procaine amide, and diphenylhydantoin, but not by lidocaine or propranolol. This inhibition could be demonstrated in vitro and when the animals were treated with these agents. The relationship between this finding and myocardial depression produced by these antiarrhythmic agents is not known but may reside in general depression of cellular metabolism and protein synthesis or in inhibition of synthesis of a specific membrane carrier protein.

ADDITIONAL KEY WORDS quinidine procaine amide propranolol diphenylhydantoin protein synthesis lidocaine actomyosin tritium

In an earlier communication, we described the inhibition by emetine hydrochloride of the incorporation of tritiated leucine into myocardial proteins (1). Emetine was studied because of reported cardiac toxicity with myocardial depression and because evidence for inhibition of protein synthesis by the alkaloid in cell cultures, plants, and yeast had previously been presented by Grollman (2). In our work with emetine, we were able to develop an in-vitro assay system for quantifying the incorporation of isotopic leucine into the soluble proteins and actomyosin of rat heart. With this system we have now been able to test the effects of other pharmacologic agents useful in the therapy of cardiac arrhythmias and also reported to depress myocardial function and the conduction system.

The agents selected for this study were quinidine sulfate, procaine amide hydrochloride, lidocaine hydrochloride, diphenylhydantoin sodium, and propranolol hydrochloride. Results of this study indicate that of these, quinidine, procaine amide, and diphenylhydantoin inhibit the incorporation of isotopically labeled leucine into cardiac proteins both in vitro and in vivo, whereas lidocaine and propranolol do not.

Methods and Materials
A detailed description of the methods employed in this study has been published (1). Rat hearts were exposed in vitro to each agent by incubating increasing concentrations of each drug in 0.1 ml of deionized water or the appropriate solvent with uniform particles of rat myocardium prepared from Sprague-Dawley rats weighing 100 to 150 g. These particles were suspended in 9.8 ml of minimal essential tissue culture medium (3) prepared without leucine and incubated at 39°C for 15 minutes. Fifty μg of [3H-L-leucine in 0.1 ml of 0.025N HCl (specific activity 5 c/cmM) was then added to each flask with the same pipette, and the incubation was continued for 2 hours. The myocardial tissue was then homogenized, and proteins soluble in low ionic strength were extracted in phosphate buffer pH 7.4, containing 0.1M KCl. From the tissue residues, actomyosin was extracted, with constant stirring overnight in phosphate buffer pH 7.0 containing 0.6M KCl and adenosine triphosphate 0.1 mg/ml.
Both protein extracts were purified by methods previously described (1, 4, 5), and aliquots were taken for determination of protein content by the Lowry method (6). Radioactivity was determined in a Packard liquid scintillation counter, using a thixotropic gel for suspension of the proteins (1, 7). The results of protein and radioactivity determinations expressed as specific activity (counts/min/mg protein) were then interpreted as an index of myocardial protein leucine incorporated.

For control studies, similarly prepared rat myocardium was incubated in the same system with 0.1 ml of 0.9% NaCl added. For the control diphenylhydantoin experiments, 0.1 ml of the commercially prepared solvent (40% propylene glycol and 10% ethanol in water, buffered to pH 12 with NaOH) was used. Since this solvent inhibited leucine incorporation into soluble proteins and actomyosin, the diphenylhydantoin data are presented with solvent controls expressed as 100% activity. The exact concentrations of the agents producing 50% inhibition of leucine incorporation into both protein fractions was determined by plotting specific activity versus molar concentration of drug on log-probit paper as described by Miller and Tainter (8).

In-vivo experiments were performed by injecting 150-g male Sprague-Dawley rats with a daily intraperitoneal dose of each drug. Saline injections were given to the controls except in the diphenylhydantoin experiments, in which equivalent amounts of the commercial solvent were used.

Results

The results of in-vitro experiments are summarized in Table 1. Quinidine sulfate and procaine amide produced 50% inhibition of 3H-L-leucine incorporation at the low concentrations of 3 \times 10^{-6} M and 5 \times 10^{-6} M, respectively, whereas diphenylhydantoin was found to be a less potent inhibitor, requiring a concentration

<table>
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<tr>
<th>Drug</th>
<th>50% Inhibition soluble proteins</th>
<th>50% Inhibition actomyosin</th>
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<tr>
<td>Quinidine</td>
<td>(3 \times 10^{-6} M)</td>
<td>(3 \times 10^{-6} M)</td>
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<tr>
<td>Procaine amide</td>
<td>(5 \times 10^{-6} M)</td>
<td>(5 \times 10^{-6} M)</td>
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<tr>
<td>Diphenylhydantoin</td>
<td>(1 \times 10^{-5} M)</td>
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<td>Lidocaine</td>
<td>(1 \times 10^{-5} M)</td>
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<tr>
<td>Propranolol</td>
<td>(1 \times 10^{-5} M)</td>
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Specific activity of soluble proteins and actomyosin from rat heart incubated with increasing concentrations of quinidine, procaine amide, and diphenylhydantoin. An inverse linear relationship exists between the logarithm of the concentration of drug added and the amount of incorporation achieved in all cases.
of $1 \times 10^{-3}M$ for 50% inhibition of control incorporation. Lidocaine was effective as an inhibitor only at $10^{-3}M$ concentration, and propranolol was essentially without effect in vitro. In all cases, the concentration effective for inhibition of labeled leucine into the soluble proteins was equally effective in preventing incorporation into actomyosin. For diphenylhydantoin, quinidine, and procaine amide, a relationship between the degree of inhibition and the concentration of the drug added could be demonstrated (Figure 1), but for propranolol and lidocaine, a sharp decline
in incorporation was achieved only at very high concentrations, while lower concentrations were found to be almost totally ineffective (Figure 2).

In-vivo experiments in which animals were given the drugs for 5 days and incorporation of leucine was then studied are summarized in Table 2. The data from diphenylhydantoin-treated animals are compared to controls given the same amount of the commercial solvent. At the dosages chosen (which are comparable to those used clinically on a mg/kg basis), only quinidine sulfate, procaine amide hydrochloride, and diphenylhydantoin sodium inhibited leucine incorporation into myocardial proteins.

To determine the effects of higher doses and prolonged administration and the duration of the inhibition produced by each of the agents found to be effective in vivo, three groups of 32 animals were treated daily for 10 days with quinidine sulfate, 35 mg/kg; procaine amide, 40 mg/kg; or diphenylhydantoin, 10 mg/kg. Four animals from each group were killed on days 8, 10-14, 17, and 19. The specific activity of the actomyosin expressed as percent of the control data in this experiment are summarized in Figure 3; for comparison, data from animals treated with emetine, 1.3 mg/kg are included. In the emetine-treated group, data from only three animals per day of death are included, because at this dose level, two animals died on the seventh and ninth days, and one more on the eleventh day.

As shown in Figure 3, although quinidine sulfate, procaine amide hydrochloride, and diphenylhydantoin were less potent inhibitors of leucine incorporation into myocardial proteins than emetine, inhibition did occur when the higher doses of each were being given daily, and for 3 or 4 days after the injections were stopped. By day 17 (7 days after the last injection), specific activities of the proteins isolated from the quinidine, procaine amide, and diphenylhydantoin groups approached those of the controls, whereas in the emetine-treated group isotope incorporation was still severely depressed.

**Discussion**

These experiments show that quinidine, procaine amide, and diphenylhydantoin are inhibitors of leucine incorporation into both the soluble proteins and actomyosin of rat myocardium. This effect occurs both in vitro and in combined in-vivo and in-vitro experiments. In the latter, it appears to persist for several days after drug administration is discontinued. Although the effect of these three agents in vitro is considerably less and briefer than that of emetine, their ability to inhibit incorporation of leucine into rat heart proteins is considered important, since inhibition occurred not only when these agents were incubated directly with the tissue, but also when the animals were treated with daily doses before study.

These three agents, all useful in the suppression of cardiac arrhythmias, depress both myocardial contractility and the impulse generation and conduction system; the adverse effects of diphenylhydantoin are considered to be less than those of quinidine and procaine amide.

Quinidine has been shown by Lee (9) to diminish twitch tension in isolated papillary...
muscles at $3 \times 10^{-4} \text{M}$ and procaine amide is considered by Conn (10) to exert similar effects on myocardial contractility. Boyd and Williams have recently shown that diphenylhydantoin depresses myocardial contractility and interferes with the inotropic effects of ouabain in the dog (11). They consider this effect to be a direct action on the myocardium. Depression of the conduction system by quinidine and procaine amide is frequent enough in clinical practice to require warnings regarding their administration (12, 13). Diphenylhydantoin is contraindicated in the treatment of arrhythmias when bradycardia or preexisting heart block is present (14). Lidocaine, although a potent depressant of the conduction system, causes little myocardial depression (15), and the depressant effects of propranolol are attributable to beta-receptor blockade (16), although a mild quinidine-like action on the conduction system has been demonstrated (17).

Our experiments do not explain the manner by which quinidine, procaine amide and diphenylhydantoin depress the myocardium, but inhibition of amino acid incorporation by these agents suggests several possible mechanisms. The cellular metabolism of quinidine is better understood than that of the other two agents producing inhibition. Radioautographic studies of heart slices have shown that quinidine localizes in the mitochondrial and cell membranes of myocardial tissue (18). General depression of cellular metabolism, including depression of electrical activity and contraction, is associated with this binding. Potassium is lost from the resting cell, whereas sodium and calcium influx and potassium efflux are decreased during contraction (10). Most studies have also shown inhibition of glucose and fructose uptake by muscle cells in the presence of quinidine (10). Thus quinidine and agents such as procaine amide and diphenylhydantoin may produce myocardial depression by generalized depression of myocardial cell metabolism. Inhibition of isotopic leucine incorporation into cardiac proteins may simply be a manifestation of altered membrane permeability and transport induced by cellular intoxication.

Alternatively, Kennedy et al. (19) have described a rapid-turnover enzyme system in cells which functions in the transport of carbohydrate molecules across the cell membrane. Although these enzymes are not well characterized, it is believed that they are constituents of the cell membrane. Elsas et al. (20) have shown that cycloheximide and puromycin, known inhibitors of protein synthesis, acutely interfere with the transport of amino acids into rat skeletal muscle. This suggests the possibility that cells depend on the continuing synthesis of rapid-turnover enzymes to maintain the transport necessary for continuing cell function. Quinidine, procaine amide, and diphenylhydantoin, by interfering with the synthesis of such carrier proteins, might interfere with cardiac function by changing the permeability of the myocardial cell membrane.

A third explanation for myocardial depression induced by these agents is suggested by our data. Although the experiments have not measured net synthesis of protein in the presence of inhibitors, it is likely that decreased incorporation of isotopic leucine is a manifestation of decreased myocardial protein synthesis. Since inhibition occurred in both the soluble proteins and actomyosin of myocardium, inhibition of myocardial protein synthesis by these agents may be a generalized phenomenon that over a period of time leads to decreased synthesis of many cellular components and eventual cellular depression and loss of function.

References

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