Mode of Action of New Anti-Arrhythmic Agents

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SUMMARY

Propranolol, diphenylhydantoin and tetrodotoxin showed inhibitory or removal effects on fibrillation induced in isolated rabbit atrial muscle strip. Depending upon methods of induction of fibrillation, effects of these drugs were different. The mode of their action seemed to be due to following electrical natures of each drug in its effective dose.

Diphenylhydantoin decreased rate of rise and amplitude of the action potential of dog ventricular fibers and rabbit atrial fibers, which was also a cause of prolongation of conduction time. Duration of action potential was slightly prolonged in an effective low concentration. In contrast propranolol showed a relatively little effect on these items, but depressed ectopic spontaneous activity. Tetrodotoxin had both effects more markedly.

Additional Indexing Words:
Propranolol Diphenylhydantoin Tetrodotoxin Aconitine-induced-fibrillation Acetylcholine-induced-fibrillation Calcium-induced-fibrillation Microelectrode

VARIOUS new anti-arrhythmic drugs have appeared. The mode of action of some of them seems to be different from that of old quinidine or procaine amide. To study whether it is true or not and, if so, to reveal what it is, would give us an insight in the genesis of cardiac fibrillation and the principle of its treatment. Three new anti-arrhythmic agents, propranolol (Inderal), diphenylhydantoin (Dilantin), and tetrodotoxin, were studied from an electrophysiological point of view to reveal the mode of their action and differences in their effects on fibrillations induced by different methods.

METHODS

The rabbit was killed instantaneously by a blow to the back of the head and the heart was quickly removed. The right atrium was excised, opened and flattened in a muscle chamber containing about 70 ml. of the Tyrode solution aerated with
100% oxygen. Atrial fibrillation was induced by one of following three methods: 1) aconitine was added in the Tyrode solution in the concentration of from 1 to 5 µg./ml. (aconitine-induced-fibrillation), 2) the normal Tyrode solution was changed to another Tyrode solution containing acetylcholine with a concentration from 5 to 10 µg./ml. and KCl at only one-fourth the normal potassium content (acetylcholine-induced-fibrillation), and 3) the normal Tyrode solution was changed to another Tyrode solution containing calcium twice its normal content and potassium one fourth the normal content (calcium-induced-fibrillation). Frequently employed doses of the anti-arrhythmic drugs were: 1, 10 and 50 µg./ml. for propranolol and diphenylhydantoin, and 0.1, 1 and 5 µg./ml. for tetrodotoxin. Such a dose was added in the perfusate and effects were observed by a microelectrode and close direct bipolar lead electrocardiographic electrodes placed at several convenient sites of the preparation.

In order to know the mode of action of these agents, each of them was added in the normal Tyrode solution and their effects on the atrial action potentials and other electrical characteristics were observed without inducing fibrillation. For this purpose preparations were also excised from the dog right ventricle and the microelectrode was inserted in the endocardial surface. Our microelectrode method was an ordinary one, employing a glass microelectrode, filled with 3 M KCl, with an external tip diameter of 0.5 to 1.0 µ, which had a tip resistance of 10 to 30 MΩ.

Results

When any of these drugs was added in the muscle chamber in a relatively large amount, atrial fibrillation was almost always stopped, at least transiently, irrespective of the method of its induction. For instance, 50 µg./ml. of propranolol and diphenylhydantoin and 1 or 5 µg./ml. of tetrodotoxin could stop the majority of fibrillations induced by any of the three methods (Fig. 1 and 2A). The dose size at which the atrial or ventricular action potentials began to show any change in the experiments described later was 10 µg./ml. of propranolol, 10 µg./ml. of diphenylhydantoin and 1 µg./ml. of tetrodotoxin. With this dose the fibrillations were totally stopped in 54.0%, 28.5% and 95.5% of the cases by propranolol, diphenylhydantoin and tetrodotoxin, respectively (Fig. 1 and 2B). Propranolol was found to remove calcium-induced-fibrillations with ease (80%), but aconitine-induced-fibrillations with difficulty (25%), whereas diphenylhydantoin was found to remove acetylcholine-induced-fibrillations rather easily (50%), but aconitine-induced-fibrillations with difficulty (11%). Tetrodotoxin removed any of these fibrillations most effectively with this dose.

With this dose tetrodotoxin stopped fibrillation rather suddenly, but propranolol stopped it gradually and the diphenylhydantoin rate was in between.

In general microelectrodes remained more easily in the ventricular fibers than in the atrial fibers. In order to pursue changes of action potentials before, during and after application of a drug without causing any dislodgment of the
Fig. 1. The removal effect of tetrodotoxin on an aconitin-induced-fibrillation. The muscle preparation was isolated from a rabbit atrium. Two pairs of close bipolar lead electrodes (A and B) were placed in convenient places in the atrial strip.

After aconitine was added in the Tyrode solution in the concentration of 5 μg./ml., fibrillation occurred. Tetrodotoxin inhibited this remarkably in the concentration of 0.1 μg./ml. already, and removed it in 1.0 μg./ml. completely.

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microelectrode, experiments with ventricular fibers were easier and gave more reliable results than with atrial fibers. Therefore, mainly changes of ventricular action potentials by these drugs are described here. Chief attention was focussed on changes induced by the dose of these drugs, with which fibrillation was removed. Changes of atrial action potentials were also examined and found to be similar to these.

Effects of drugs on normal dog ventricular fibers were as follows: Propranolol did not slow the rate of rise of the action potential remarkably in a dose by which atrial fibrillation was effectively removed, i.e. with 10 μg./ml. (Fig. 3 left, C and Fig. 4C). In a higher concentration the slowing became prominent. The spike of the action potential was diminished even with 10 μg./ml. and so the amplitude of the action potential was decreased (Fig. 4A). The duration of the action potential became even shorter in a higher
Fig. 2. Removal effects of propranolol, diphenylhydantoin and tetrodotoxin on various types of fibrillation.

Ca-induced fibrillation showed an inhibitory effect. Fibrillation occurred still occasionally.

Diphenylhydantoin showed a complete removal of fibrillation, even transient.

A showed rate of removal of fibrillation by 10 μg./ml. of propranolol, 10 μg./ml. of diphenylhydantoin and 1 μg./ml. of tetrodotoxin.

<table>
<thead>
<tr>
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<th>Ca induced F.</th>
<th>ach induced F.</th>
<th>aconitin induced F.</th>
<th>TOTAL</th>
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<tr>
<td>Tetrodotoxin</td>
<td>87%</td>
<td>100%</td>
<td>100%</td>
<td>95.5%</td>
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<tr>
<td>Diphenylhydantoin</td>
<td>25%</td>
<td>50%</td>
<td>11%</td>
<td>28.5%</td>
</tr>
<tr>
<td>Propranolol</td>
<td>80%</td>
<td>57%</td>
<td>25%</td>
<td>54.0%</td>
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</table>
Fig. 3. Changes of action potentials of dog ventricular muscle fibers.
A: Control Tyrode solution. Propranolol and diphenylhydantoin were added in 1 μg./ml. in B, increased to 10 μg./ml. in C, and to 50 μg./ml. in D. Tetrodotoxin was added in 0.1 μg./ml. in B, increased to 1 μg./ml. in C, and to 5 μg./ml. in D. All recoveries were shown in E after changing to a new normal Tyrode solution.

Microelectrodes stayed in the preparation from A to E throughout.

concentration (Fig. 4B). Diphenylhydantoin slowed the rate of rise of the action potential (Fig. 3, middle and Fig. 4C) and often prolonged its duration in a lower concentration (Fig. 4B). But in a higher concentration the duration was slightly shortened. The amplitude of the action potential diminished least (Fig. 3 and Fig. 4A). Tetrodotoxin slowed the rate of rise of the action potential, and diminished its amplitude most markedly even with 1 μg./ml. (Fig. 3, right and Fig. 4). The duration of the action potential was often prolonged prominently because an incisura appeared occasionally between the spike and the plateau of the action potential. In a higher concentration it remained to be prolonged or, when there was no incisura, it was mostly shortened (Fig. 4B).

Since the right atrial preparation included the sinus node, the rate of its spontaneous activity was easily measured. Sinus node rate was depressed by tetrodotoxin, slightly with 0.1 μg./ml. and 1.0 μg./ml., and markedly with
Fig. 4. Changes of amplitude (A), of duration (B) and of rate of rise (C) of
dog ventricular action potential in each representative experiment of effects of
propranolol (open circles), diphenylhydantoin (closed circles) and tetrodotoxin
(cross marks)

Each value is shown in per centage compared with the control value in Tyrode
solution.

Fig. 5. Changes of conduction time (A), of rate of spontaneous ventricular
activity (B) and of the sinus node rate (C).

Figure construction is similar as Fig. 4.

5.0 μg./ml. (Fig. 5C). The averaged decrease of sinus rate was 3%, 12% and
54% of the control, respectively. With 5.0 μg./ml. a marked conduction block
usually appeared in atrial fibers, but sinus node still showed spontaneous ac-
tivity. The sinus node rate was depressed equally slightly by propranolol
and diphenylhydantoin. Namely, an averaged decrease of sinus node rate
was 0, 4 and 15% of the control by 1, 10 and 50 μg./ml. of propranolol, respec-
tively. It was 6, 7 and 21% of the control by 1, 10 and 50 μg./ml. of di-
phenylhydantoin, respectively.
A muscle strip isolated from the endocardial side of the ventricle frequently showed spontaneous activity. The effects on this activity can be regarded as effects on the spontaneous activity of an ectopic focus of the heart. Tetrodotoxin depressed this most markedly (Fig. 5B). With 10 µg./ml., namely with the dose most frequently used to remove atrial fibrillation in above experiments, propranolol depressed this more markedly than diphenylhydantoin. With a large dose of 50 µg./ml. both drugs depressed the spontaneous activity equally well.

In dog ventricular muscle strips one microelectrode was inserted into a portion of the false tendon near the free wall and another microelectrode in a peripheral ordinary ventricular muscle fiber. Conduction time was measured with these 2 microelectrodes. Corresponding to the changes of rate of rise of the action potential, tetrodotoxin induced prolongation most markedly. Namely, with 0.1, 1.0 and 5.0 µg./ml. of tetrodotoxin, the conduction time increased 1.6, 2.6 and 3 times the control value, respectively (Fig. 5A). With 5.0 µg./ml. conduction block appeared frequently. Diphenylhydantoin induced prolongation second most markedly. With 1.0, 10 and 50 µg./ml. of diphenylhydantoin, the conduction time increased 1.2, 2.8 and 3.6 times the control value, respectively. Propranolol induced prolongation least markedly. With 1.0, 10 and 50 µg./ml. of propranolol, the conduction time increased 1.1, 1.7 and 3.2 times the control value.

**DISCUSSION**

Looking through the results, the three drugs were found to have a depressing effect on the sodium carrying system, since all of them showed a slowing of the rate of rise of the action potential (Fig. 6). In order to be significantly effective for removing fibrillation, this factor seems to be fairly indispensable. However, since propranolol could remove fibrillation in spite of having small effects on the rate of rise, there must be another mechanism.

<table>
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<tr>
<th>Drug</th>
<th>S-N Rhythm</th>
<th>Spont Activity of Vent</th>
<th>Size of AP</th>
<th>Rate of Rise of AP</th>
<th>Conduction Time</th>
<th>Duration of AP</th>
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<tr>
<td>Tetrodotoxin</td>
<td>↓</td>
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<td>Diphenylhydantoin</td>
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<td>Propranolol</td>
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Fig. 6. Summarized effects of the three drugs.
Presumably the anti-fibrillatory mechanism of propranolol lies comparatively more in depression of ectopic spontaneous activity, and that of diphenylhydantoin lies comparatively more in depression of sodium carrying system. Tetrodotoxin has both effects equally strongly.

This mode of action of propranolol is supported from the results of our previous experiments on dichloroisoproterenol and pronethalol.\(^1\) There we showed directly that the slow diastolic depolarization of dog Purkinje fibers, induced by adding adrenaline or spontaneously, was depressed by these drugs.

There is some doubt whether tetrodotoxin has the same selective inhibitory effect on the sodium carrying system with myocardial fibers and especially with specialized cardiac fibers as was shown by Narahashi, et al.\(^2\), \(^3\) with the nerve or the skeletal muscle fiber, since we showed\(^4\) that the sinus node retained spontaneous activity in a much larger concentration of tetrodotoxin than was reported from the studies with such tissues. For the same reason, clinical application of tetrodotoxin as an anti-fibrillatory agent may not be so effective as was observed in this study: the dose effective for removal of fibrillation may be toxic to other tissues.

Whether the prolongation of the duration of the action potential is essential for anti-fibrillatory effects or not is rather controversial at present. But we found that propranolol caused rather shortening of this duration, while it had such effects.

REFERENCES