EFFECTS OF PERHEXILINE ON MYOCARDIAL PHOSPHORYLASE
ACTIVITY, MYOCARDIAL CATECHOLAMINE
CONTENT AND HEART RATE

Young W. Cho, M.D.*, and Haruo Ito, M.D.

A study was conducted on the effects of perhexiline on myocardial phosphorylase activity, myocardial catecholamine content and heart rate. Phosphorylase $a$ activity and heart rate were investigated as an indicator of sympathetic nerve tone in order to clarify the characteristic of perhexiline with regard to the effects on myocardial metabolism in hyperthyroid rats and catecholamine deficient rats. Myocardial catecholamine and phosphorylase $a$ activity were measured by von Euler's method and Cori's method respectively. Conclusion of this study are summarized as follows:
1) Perhexiline reduces the heart rate, and its effect is not always dependent upon the changes in myocardial norepinephrine content.
2) Perhexiline reduces myocardial phosphorylase $a$ activity. It cannot be always said that the reduction is dependent upon the changes in myocardial norepinephrine.
3) Though slightly different from propranolol and dichloroisoproterenol, perhexiline possesses cardiac effects resembling $\beta$-blockers.

It is said that nervous control of the coronary vessel is very weak and that its autonomous adjustment is fairly large! There is a linear positive correlation between $O_2$ consumption and coronary sinus flow. Catecholamine increases coronary sinus flow by increasing $O_2$ consumption in myocardium. On the contrary, in the previous papers on the effects of perhexiline on the cardiovascular system, we reported that perhexiline produced a decrease in $O_2$ consumption of the myocardium, an increase in coronary sinus flow and cardiac slowing, while it reduced cardiac work. Accordingly, it was suggested that the increase in coronary sinus flow induced by perhexiline was not dependent on $O_2$ consumption. It was further pointed out that perhexiline enhanced reduction of $O_2$ consumption of the myocardium and increase of coronary sinus flow in the heart with reduced catecholamine in gangliosympathectomized dogs.

In this study, phosphorylase $a$ activity and heart rate were investigated as an indicator of sympathetic nerve tone in order to clarify the

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**Key Words:**
- Perhexiline
- Myocardial phosphorylase activity
- Myocardial norepinephrine content
- Heart rate

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characteristics of perhexiline with regard to the effects on myocardial metabolism. Studies were also made on hyperthyroid rats and on low-catecholamine rats.

MATERIALS AND METHODS
Male wistar rats weighing 200–250g were used in the experiment.

1. Preparation of myocardial catecholamine deficient rats
   a) Rats were stellectomized on both sides and used after one week of recovery from the surgical operation.
   b) For preparation of reserpinized rats, reserpine, 0.5 mg/kg, was given subcutaneously for a period of 4 days before the experiment.
   c) For preparation of immunosympathectomized (IMS) rats, antiserum of NGF (nerve growth factor), 0.001 ml/g (25 μg/g), was given subcutaneously to newborn rats for 7 days, and these rats were used for the experiment after they attained a body weight of 150g or more. NGF was a gift from Teikoku Zoki Co., Kawasaki.

2. Preparation of hyperthyroid rats
   L-thyroxine (Sigma Chemical Co.), 500 μg, dissolved in 0.2 ml of 0.01N NaOH was given to rats subcutaneously, for 6 consecutive days.

3. Measurement of myocardial norepinephrine
   Myocardial norepinephrine was measured by von Euler’s method. Rats were frozen to death in dry-ice-alcohol solution, and thereafter the myocardium was excised immediately and homogenized by adding 0.4N perchloric acid in the amount 10 times of the myocardium. The homogenized myocardium was then centrifuged, and the supernatant thus obtained was adjusted to pH 8.3 with 5 or 0.5N NaOH, absorbed with aluminum oxide by the Batch method and isolated with 10 ml of 0.35N acetic acid, to which 1 ml of 1N acetic acid buffer solution of pH 6.22 was added and 0.1 ml of 0.25% potassium ferricyanide was further added to produce fluorophore. This fluorescence was determined by the method of von Euler and Lishajko.

4. Measurement of phosphorylase activity
   The measurement was made by the method of Cori and Illingworth. 500 mg of the myocardium were homogenized in 10 ml of a solution containing of 20mM NaF-1mM ethylene diamine tetra-acetic acid (EDTA) (pH 6.8) at 4°C. This diluted enzyme solution was taken in two test tubes and 4% glycogen solution was added and incubated for 20 min at 30°C. Thereafter, 0.64M glucose-1-phosphate (G-1-P) was added to one of the two test tubes and 64mM G-1-P and 4mM AMP was added to the other and the reaction was stopped with 0.025 ml of 0.05N sulfuric acid after 5 min and the inorganic phosphate thus produced was measured by the method of King.

5. Measurement of heart rate of rats
   Heart rate of rats anesthetized with pentobarbital sodium (30 mg/kg, i.p.) was measured with a polygraph (Nihon Kohden, RM-45) through a tachometer (Nihon Kohden, RT-5) in lead II of ECG.
   Perhexiline (a gift from Richardson-Merrell Inc., Cincinnati)-carboxy cellulose (Wako Pure Chemical Ltd., Osaka) emulsion was given p.o. in a volume of 0.5 ml/100g body weight. Dichloroisoproterenol hydrochloride (Aldrich Chemical Co., Milwaukee) or propranolol (Sumitomo Kagaku Co., Osaka) was diluted with 0.9% saline solution. These drugs were prepared on each experimental day.

RESULTS

1. Myocardial norepinephrine content in hyperthyroid, reserpinized, sympathetecetmized (ST) and IMS rats and effects of perhexline, propranolol and dichloroisoproterenol (DCI)
   As shown in Table I, in the control group the content of norepinephrine in the left ventricle of the intact rats was 1.04 ± 0.37 μg/g, and that in the myocardium of the hyperthyroid rats was 1.15 ± 0.41 μg/g, showing no significant difference. Norepinephrine contents, however, in the myocardium from reserpinized, ST and IMS rats were decreased (p < 0.001). No difference was observed between the contents of norepinephrine in the control group and those of perhexline, or propranolol, or DCI-pretreated group.

2. Effects of perhexiline, propranolol and DCI on myocardial phosphorylase α activity
   a) Intact rats (given physiological saline solution)
   As shown in Table II, phosphorylase α activity 20 sec after the administration of norepinephrine (1.0 μg/kg, i.v.) was increased as compared with

### TABLE I

**EFFECTS OF PERHEXILINE, PROPRANOLOL AND DICHLOROISOPROTERENOL (DCI) ON MYOCARDIAL NOREPINEPHRINE CONTENTS IN HYPERTHYROID, RESERPINED, SYMPATHECTOMIZED (ST) AND IMMUNOSYPATHECTOMIZED (IMS) RATS**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Intact rats</th>
<th>Hyperthyroid rats</th>
<th>Reserpinized rats</th>
<th>ST rats</th>
<th>IMS rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>1.04 ± 0.37</td>
<td>1.15 ± 0.41</td>
<td>0.26 ± 0.05**</td>
<td>0.41 ± 0.04***</td>
<td>0.54 ± 0.08***</td>
</tr>
<tr>
<td>Perhexiline</td>
<td>0.72 ± 0.30</td>
<td>0.68 ± 0.24</td>
<td>0.24 ± 0.04**</td>
<td>0.40 ± 0.05*</td>
<td>0.44 ± 0.09*</td>
</tr>
<tr>
<td>Propranolol</td>
<td>1.00 ± 0.31</td>
<td>0.92 ± 0.15</td>
<td>0.27 ± 0.03**</td>
<td>0.45 ± 0.06***</td>
<td>0.57 ± 0.06***</td>
</tr>
<tr>
<td>DCI</td>
<td>0.71 ± 0.20</td>
<td>0.66 ± 0.22</td>
<td>0.25 ± 0.05**</td>
<td>0.40 ± 0.06*</td>
<td>0.46 ± 0.04*</td>
</tr>
</tbody>
</table>

Perhexiline, propranolol and DCI: 100 mg/kg orally. Each value represents the mean ± S.E. from 9 experiments. Significance of difference from value of intact rats *p < 0.05; **p < 0.01; ***p < 0.001.

### TABLE II

**THE EFFECT OF PERHEXILINE, PROPRANOLOL AND DCI ON CARDIAC PHOSPHORYLASE α ACTIVITY IN RESERPINED, IMMUNOSYPATHECTOMIZED (IMS) AND SYMPATHECTOMIZED (ST) RATS**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cardiac Phosphorylase α% of Total*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact rats</td>
</tr>
<tr>
<td>Control (saline)</td>
<td>39.8 ± 1.4</td>
</tr>
<tr>
<td>NE</td>
<td>58.3 ± 3.5a</td>
</tr>
<tr>
<td>Perhexiline</td>
<td>40.5 ± 1.2</td>
</tr>
<tr>
<td>Perhexiline + NE</td>
<td>41.9 ± 2.1</td>
</tr>
<tr>
<td>Propranolol</td>
<td>37.0 ± 2.7</td>
</tr>
<tr>
<td>Propranolol + NE</td>
<td>38.6 ± 1.8</td>
</tr>
<tr>
<td>DCI</td>
<td>41.8 ± 2.5</td>
</tr>
<tr>
<td>DCI + NE</td>
<td>43.9 ± 3.4</td>
</tr>
</tbody>
</table>

NE = norepinephrine 1.0 µg/kg, i.v. Perhexiline, propranolol and DCI: 100 mg/kg orally. Each value represents the mean ± S.E. from 7 experiments. Significance of difference from value of intact rats *p < 0.05; **p < 0.01. a = p < 0.05 (vs line 1) b = p < 0.001 (vs line 1) *: Ratio of units without adenylylate units with adenylylate × 100

### TABLE III

**EFFECTS OF PERHEXILINE, PROPRANOLOL AND DCI ON HEART RATE IN HYPERTHYROID, RESERPINED, SYMPATHECTOMIZED (ST) AND IMMUNOSYPATHECTOMIZED (IMS) RATS**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact rats</td>
</tr>
<tr>
<td>Control (saline)</td>
<td>326 ± 37</td>
</tr>
<tr>
<td>Perhexiline</td>
<td>268 ± 20a</td>
</tr>
<tr>
<td>Propranolol</td>
<td>256 ± 29a</td>
</tr>
<tr>
<td>DCI</td>
<td>320 ± 25</td>
</tr>
</tbody>
</table>

Perhexiline, propranolol and DCI: 100 mg/kg orally. Each value represents the mean ± S.E. from 7 experiments. Significance of difference from value of intact rats *p < 0.05; **p < 0.01; ***p < 0.001. a = p < 0.05 (vs line 1).
that of the group given physiological saline solution. The administration of perhexiline, propranolol or DCI alone did not produce any change in phosphorylase activity, but an increase in phosphorylase activity produced by administration of norepinephrine was inhibited by perhexiline, propranolol or by DCI.

b) Hyperthyroid rats
The phosphorylase activity in the myocardium of the rats treated with 500 μg of thyroxine subcutaneously for 6 consecutive days increased (p < 0.05). This increase was enhanced by the administration of norepinephrine and this effect on the phosphorylase activity of norepinephrine was inhibited by propranolol, DCI or perhexiline. The effect of thyroxine on the myocardial phosphorylase activity was not affected by the administration of perhexiline, propranolol or DCI (Table II).

c) Reserpined rats, ST rats and IMS rats
These groups showed significant decreases in norepinephrine content in the myocardium (Table I). Despite this decrease in the myocardial norepinephrine content, the phosphorylase activity was not changed in the reserpinized rats, whereas the phosphorylase activity decreased in ST and IMS groups (Table II). Furthermore, phosphorylase a activity was enhanced by norepinephrine (1.0 μg/kg, i.v.) (p < 0.001), but this effect of norepinephrine was inhibited by perhexiline, propranolol and DCI.

3. Effects of perhexiline, propranolol and DCI on heart rate
In the group of hyperthyroid, a marked increase (p < 0.001) in the heart rate was observed, while the other three groups showed decrease in the heart rate (p < 0.05) (Table III). Perhexiline produced further decrease in the heart rate in reserpinized and IMS groups of rats (p < 0.05). Propranolol also decreased the heart rate, and this decrease was particularly marked in the intact group and the group given thyroxine (p < 0.05). The effects of DCI on the heart rate was not constant, resulting in an increase in some and a decrease in others.

DISCUSSION
1. Heart rate and myocardial norepinephrine content
Our data of myocardial norepinephrine content in rats agrees well with Montanari et al. and Maitre and Staehelin. In their experiments rats were killed by a blow to the head, while in ours they were killed by freezing to death in a dry-ice-alcohol solution. Myocardial norepinephrine content is 1.12 ± 0.10 μg/g heart tissue according to Montanari et al. and 0.88 ± 0.17 μg/g heart tissue according to Maitre and Staehelin.

The myocardial norepinephrine content was not increased in the group given thyroxine and the lowest content of norepinephrine was observed in the reserpine treated group (Table I). In IMS and ST groups the norepinephrine content was decreased. It was observed that the heart rate showed a decrease in accordance with the degree of decrease of norepinephrine. This finding is in agreement with the previous report that changes in heart rate depend on catecholamine levels in rabbit hearts. But there was an increase in the heart rate despite no increase being produced in myocardial norepinephrine content by thyroxine. From the above, it can be said that the heart rate may not be controlled only by the myocardial catecholamine content, which is also supported by the result that as seen in the perhexiline or propranolol treated group the heart rate was decreased without any change in the myocardial norepinephrine content. The effect of perhexiline on myocardial norepinephrine level, phosphorylase activity and heart rate was almost similar to that of propranolol which is a β-blocker. In the pretreatment with DCI, which is also a β-blocker, heart rate did not change in comparison with that in the control group. This probably results from the fact that DCI is a β-stimulant as well as β-blocker.

2. Myocardial phosphorylase a activity and myocardial norepinephrine content
With regard to the relation between myocardial norepinephrine content and phosphorylase a activity, the phosphorylase activity showed a significant increase despite little change in norepinephrine content, as can be seen in the group given thyroxine, while on the other hand, the phosphorylase activity showed a level close to the control group despite a marked decrease in the norepinephrine content produced by reserpine. Another point is that the phosphorylase activity remained low in the other two groups in proportion to the norepinephrine content. Furthermore, the phosphorylase activity was extremely enhanced by norepinephrine in all of the groups, and this enhanced activity could be inhibited by perhexiline, propranolol or DCI.

Consequently, it can be said that cardiac effects of perhexiline resemble those of β-blockers, since perhexiline inhibits the norepinephrine-induced enhancement of phosphorylase α activity in the myocardium like other β-blockers. These results were also seen in the experiments of heart rate. Phosphorylase α activity may not be dependent on norepinephrine levels in myocardium.

REFERENCES


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