COMPARISON OF CARDIAC LESIONS INDUCED IN RATS BY ISOPROTERENOL AND BY REPEATED STRESS OF RESTRAINT AND WATER IMMERSION WITH SPECIAL REFERENCE TO ETIOLOGY OF CARDIOMYOPATHY

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Cardiac lesions induced in rats by isoproterenol, a potent β-agonist, and by repeated stress of restraint and water immersion, in which sensitization of β-adrenergic receptors would be expected to be induced, were investigated morphologically and following facts were revealed.

1) Cardiac lesions induced by isoproterenol, characteristic findings of which were myocardial hypertrophy, myocardial degeneration and myocardial necrosis replaced by interstitial fibrosis, were more analogous to cardiomyopathy than myocardial infarction or cardiac hypertrophy.

2) Cardiac lesions induced by repeated stress of restraint and water immersion, characteristic findings of which were myocardial hypertrophy, myocardial degeneration and myocardial necrosis replaced by interstitial fibrosis, were similar to those induced by isoproterenol.

These results suggest that the endogenously induced dominant β-adrenergic stimulating action during stress may play an important role in the pathogenesis of cardiomyopathy, the specific etiology of which is not yet known.

THE role of isoproterenol (ISO), a potent β-agonist, as a cause of cardiac lesions has been considered mainly in association with myocardial infarction in large doses1-3 or cardiac hypertrophy in small doses4-5. As previously reported, coronary arterial lesions4, volume overload and pressure overload6,7 are not involved in these cardiac lesions. Myocardial necrosis and myocardial hypertrophy seem to occur primarily in the heart itself as a result of the direct stimulation of myocardial β-adrenergic receptors.

Therefore, we first examined the cardiac lesions induced by ISO administration under different conditions (different doses and different survival periods) and tried to show that ISO-induced cardiac lesions are more analogous to

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Key Words:
Isoproterenol (ISO)
Stress
Cardiomyopathy
Sensitization of β-adrenergic receptors
Light microscopy

(Received on January 7, 1980; Accepted on July 10, 1980)
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cardiomyopathy; in which myocardial necrosis replaced by interstitial fibrosis and myocardial hypertrophy occur primarily in the heart itself without the participation of coronary arterial lesions volume overload and pressure overload, than to myocardial infarction or cardiac hypertrophy.

Meanwhile, β-adrenergic stimulation can also be expected to be induced endogenously by stress, during which catecholamines (adrenaline and noradrenaline) possessing both α- and β-adrenergic stimulating actions are liberated. However, if β-adrenergic stimulation is accompanied by α-adrenergic stimulation, as usually occurs in simple stress, cardiomyopathy-like cardiac lesions may not be always induced since the α-adrenergic stimulation opposes the β-adrenergic hypertrophic effect and also leads to hypertension. Therefore, it becomes a problem whether a dominant β-adrenergic stimulating state can be induced or not.

Such a state might be induced in prolonged or repeated stress. In prolonged stress, that is, during the “stage of resistance” in which the adrenal cortex is highly activated, β-adrenergic receptors are extraordinarily sensitized, while the sensitivity of α-adrenergic receptors remains unchanged or even suppressed. In repeated stress, in which repeated catecholamine liberation may occur, β-adrenergic receptors would be expected to be sensitized since chronic administration of catecholamines which possess β-adrenergic stimulating action is known to sensitize β-adrenergic receptors selectively. In such situations, endogenous catecholamines liberated by newly added stress may show a dominant β-adrenergic stimulating action, as though ISO were being administered.

Therefore, we examined the cardiac lesions induced by repeated stress due to restraint and water immersion and compared them with those induced by the administration of ISO.

**MATERIALS AND METHODS**

*Experiment I: Cardiac lesions induced by ISO*

1) Acute experiment: Comparison of three different dose schedules

Eighteen Wistar rats weighing 220–260g were
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Fig. 2. Photomicrograph of ISO-induced cardiac lesion. Left ventricular anterior wall from a rat in Group-B (5 mg/kg x 2 x 10). Myocardial necrotic area with stromal reaction (large arrow), degenerated myocardium showing positive PAS reaction (middle-sized arrow), perinuclear halo giving cytoplasm a vacuolated appearance (small arrow) and thickened endocardium (arrow head) are observed. Surviving myocardium is hypertrophic (see Table I). Stain. PAS and hematoxylin, x 320.

Blood pressure was measured before sacrifice in every group using the tail cuff method.

Experiment II: Cardiac lesions induced by repeated stress of restraint and water immersion

Ten Wistar rats weighing 200–220g were subjected to repeated stress of restraint and water immersion (5 hours/day, every other day, 30 times) without ISO administration. They were sacrificed two days after the last immersion. Blood pressure was measured before sacrifice using the tail cuff method.

Twelve Wistar rats weighing 230–260g were used as controls.

The chest of each rat was opened under Nembutal anesthesia and 15% KCl solution was injected into the inferior vena cava to stop the heart in diastole. Then the heart and the adrenal glands were removed, fixed with Bouin fixative, weighed and embedded in paraffin in the usual manner. Transverse sections of both ventricles (8μ in thickness) were stained with hematoxylin and eosin, PAS and Azan stains. Cytometry was used to assess the degree of myocardial hypertrophy of the following five areas; the inner layer of the left ventricular anterior wall, the inner

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Fig. 3. Photomicrographs of ISO-induced cardiac lesions. Left ventricular anterior wall from a rat in each group. 3a: Normal myocardial structure in control rat. 3b: Massive myocardial necrosis showing appearance of infarction in Group-A (85 mg/kg × 2). 3c: Moderate myocardial necrosis being replaced by interstitial fibrosis showing myocarditis-like appearance in Group-B (5 mg/kg × 2 × 10). 3d: Cardiac hypertrophy-like appearance with small myocardial necrotic area in Group-C (300 μg/kg × 21). 3d': High power view of small myocardial necrotic area in 3d. Stain: Hematoxylin and eosin, x 140 (3a, 3b, 3c, 3d), x 280 (3d').

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>CYTOMETRIC MEASUREMENT OF MYOCARDIAL THICKNESS (μ)</th>
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<tbody>
<tr>
<td></td>
<td>ILVAW</td>
</tr>
<tr>
<td>Control</td>
<td>15.1 ± 3.7</td>
</tr>
<tr>
<td>Group-A</td>
<td>21.5 ± 6.2*</td>
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<tr>
<td>Group-B</td>
<td>22.6 ± 5.0*</td>
</tr>
<tr>
<td>Group-C</td>
<td>21.5 ± 6.8*</td>
</tr>
<tr>
<td>Group-B (5w)</td>
<td>22.7 ± 6.4*</td>
</tr>
<tr>
<td>Group-B (11w)</td>
<td>23.2 ± 8.4*</td>
</tr>
<tr>
<td>Group-B (18w)</td>
<td>21.8 ± 8.2*</td>
</tr>
<tr>
<td>Group-B (5w + STRESS)</td>
<td>26.5 ± 6.4*</td>
</tr>
<tr>
<td>Group-STRESS</td>
<td>21.0 ± 6.3*</td>
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Values are means ± SEM. *: p < 0.01, **: p < 0.05
Abbreviations: ILVAW = inner layer of left ventricular anterior wall, ILVPW = inner layer of left ventricular posterior wall, MLVPW = middle layer of left ventricular posterior wall, MIVS = middle layer of interventricular septum, MRVW = middle layer of right ventricular wall

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layer of the left ventricular posterior wall, the middle layer of the left ventricular posterior wall, the middle layer of the interventricular septum and the middle layer of the right ventricular wall. Each area was photographed and the myocardial thickness was measured.

RESULTS

Experiment I: Cardiac lesions induced by ISO

1) Acute experiment: Comparison of three different dose schedules

The weight of the heart was greatly increased in all three groups (Fig. 1).

The most important morphological changes commonly observed in every group were myocardial hypertrophy, myocardial degeneration, including positive PAS reaction, and myocardial necrosis followed by interstitial fibrosis, although differences in degree were noted among the groups. These changes were widely distributed in the heart having had no relation to the coronary arterial distribution, but conspicuous in the inner layer of the ventricular wall. Interstitial edema, mononuclear cell infiltration, endocardial and pericardial thickening were also noted (Fig. 2). No coronary arterial occlusion was seen in any group. These morphological changes were essentially the same as those described in previous reports.1,2

In Group-A, myocardial necrosis was dominant and some massive necrotic areas resembled infarction. In Group-C, myocardial necrosis being replaced by interstitial fibrosis was so slight or almost absent that the whole appearance was similar to that of cardiac hypertrophy. In Group-B which showed features intermediate between Group-A and Group-C, areas of hypertrophic myocardium alternated with moderate myocardial necrosis being replaced by interstitial fibrosis with moderate mononuclear cell infiltration and the overall appearance resembled myocarditis (Fig. 3).

Cytometric measurements revealed that the myocardial hypertrophy was greater in the inner layer than in the middle layer of the ventricular wall in all three groups (Table I).

2) Chronic experiment: Comparison of different survival periods

In rats left at rest after cessation of ISO treatment, the heart weight decreased at first, Group-B (5w) and Group-B (11w), but after that it tended to increase again, Group-B (18w). If repeated stress was applied, Group-B (5w + Stress), the decrease of heart weight was much less than Group-B (5w) (Fig. 4).
The myocardial necrotic debris and stromal inflammatory changes seen in the acute stage diminished and the interstitium was replaced by compact fibrosis during the 5th week, after which the histological appearance changed very little (Fig. 5). Interestingly, myocardial hypertrophy continued to be seen microscopically in spite of the decrease of heart weight and it even increased in Group-B (5w + Stress) (Table I). The overall appearance of all these four groups resembled cardiomyopathy.

No coronary arterial occlusive lesions and valvular lesions could not be seen.

Blood pressure was normal in these four groups, ranging from 92 to 110 mmHg. So the participation of coronary arterial impairment, volume overload and pressure overload seemed to be negligible.

Experiment II: Cardiac lesions induced by repeated stress of restraint and water immersion

The weight of the heart and the adrenal glands was greatly increased, although there were wide individual differences (Fig. 6).

In the heart, the characteristic histological changes were myocardial hypertrophy, myocardial degeneration, including positive PAS reaction, and myocardial necrosis followed by interstitial fibrosis. Myocardial degeneration and myocardial necrosis were rather confined to the inner layer of the ventricular wall, although myocardial hypertrophy was seen not only in the inner layer but also in the middle layer of the ventricular wall (Table I). Myocardial necrosis was generally slight and the overall appearance resembled that seen in Group-C in Experiment I treated with low doses of ISO. Endocardial and pericardial thickening were also seen. In some rats in this group, fatty infiltration from the pericardial side and multinuclear myocardial cells were noticed (Fig. 7).

Blood pressure was normal, ranging from 98 to 120 mmHg, and no coronary arterial occlusive lesions or valvular lesions were seen, so the par-
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Fig. 6. Effect of restraint and watery immersion on the wet weight of the heart (left) and the adrenal glands (right). Values are mean ± S.D.

ticipation of coronary arterial impairment, volume overload and pressure overload seemed to be negligible.

DISCUSSION

The cardiac lesions seen in Group-A in Experiment I have been regarded as analogous to myocardial infarction and those seen in Group-B and Group-C as analogous to cardiac hypertrophy. But no coronary arterial occlusion could be found in Group-A, and volume overload and pressure overload are negligible during ISO treatment, as previously reported. Myocardial necrosis and myocardial hypertrophy are considered to occur primarily in the heart itself as a result of direct stimulation of myocardial β-adrenergic receptors by ISO. We suspected, therefore, that ISO-induced cardiac lesions were essentially different from those of myocardial infarction or cardiac hypertrophy and could be regarded rather as analogous to those of cardiomyopathy in which myocardial necrosis replaced by interstitial fibrosis, myocardial hypertrophy and myocardial degeneration develop in the heart itself without the participation of coronary arterial impairment, volume overload and pressure overload. The distribution of the cardiac lesions, conspicuous in the inner layer of the ventricular wall, also indicates a similarity to cardiomyopathy.

However, two problems must be taken into account. The first is that ISO-induced cardiac hypertrophy is to a large extent reversible, while that of cardiomyopathy is usually progressive. The second is that this study was an acute experiment, and myocardial necrotic debris and stromal inflammatory changes were still present. These changes are rarely seen in cardiomyopathy which usually takes a chronic course. That is why we picked up Group-B which resembled myocarditis as a representative and followed it for various lengths of time in the following chronic experiment.

The early decrease of heart weight, Group-B (5w) and Group-B (11w), shows that the primary cardiac hypertrophy induced by ISO treatment is to a large extent reversible, at least in the resting state and at least macroscopically, as previous workers pointed out. However, if the probably dominant stimulation of β-adrenergic receptors is continued, as in Group-B (5w + Stress), the primary cardiac hypertrophy induced by ISO treatment is largely maintained. The recurring increase of heart weight, Group-B (18w), is thought to be due to compensatory cardiac hypertrophy (secondary cardiac hypertrophy) because of heart failure due to cardiac lesions, as is usually seen in various types of heart failure.
It is possible, therefore, that ISO-induced cardiac hypertrophy progresses in the long run as primary or secondary cardiac hypertrophy as in cardiomyopathy. The myocardial necrotic debris and stromal inflammatory changes, in a meanwhile, almost disappeared and the myocarditis-like features in the acute atage came to show a close resemblance to cardiomyopathy. The results of Experiment I, therefore, lead us to the conclusion that ISO-induced cardiac lesions can be regarded as analogous to those of cardiomyopathy.

The increase of the weight of the adrenal glands seen in Experiment II indicates the adrenal hyperfunction which is considered to sensitize $\beta$-adrenergic receptors.\textsuperscript{18} The histological features of the heart, characteristic changes of which were myocardial hypertrophy, myocardial degeneration and myocardial necrosis followed by interstitial fibrosis, resembles ISO-induced cardiac lesions and those of cardiomyopathy. The results of Experiment II suggest that endogenously induced dominant $\beta$-adrenergic stimulation, which is based on the selective sensitization of $\beta$-adrenergic receptors by stress, may play an important role in the pathogenesis of cardiomyopathy.

Although there have been some reports suggesting the participation of catecholamines in the pathogenesis of cardiomyopathy, no definite evidence has been presented. Goodwin suspected some participation of catecholamines in the pathogenesis of cardiomyopathy (hypertrophic type) and pointed out the following facts: 1) some association with hypertension, although this is rare, 2) excessive noradrenosis in the left ventricular outflow tract, although this has not been confirmed, 3) hemodynamic deterioration with $\beta$-adrenergic stimulator and improvement with $\beta$-adrenergic blockade, 4) association with pheochromocytoma, neurofibromatosi and lentiginosis.\textsuperscript{19} On the other hand, he noted in the same paper that the majority of patients with
cardiomyopathy show no overt evidence of catecholamine abnormality since most of them have; 1) normal 3-methoxy-4-hydroxymandelic acid (VMA) excretion, 2) absence of hypertension, and 3) no evidence of excessive circulating catecholamines or adrenal medullary hyperfunction. Haneda et al. reported that catecholamine concentration in the coronary sinus and aorta was even subnormal in patients with cardiomyopathy (hypertrophic type)20. Angelakos et al. described sympathetic hyperactivity in the hearts of hamsters with cardiomyopathy (congestive type)21 but as this cardiomyopathy of hamsters is hereditary, such a finding cannot explain the pathogenesis of cardiomyopathy except of the hereditary type.

In these previous reports, attention was focused only on the stimulator side of the catecholamine effect. Our attention, however, has been focused not only on the stimulator side but also on the receptor side of the catecholamine effect. As mentioned already, sensitization of β-adrenergic receptors can be induced a posterior by the rather undetectable biological phenomenon of stress. Such well known facts in human cardiomyopathy as remarkably high left ventricular contractility, high sensitivity to catecholamines,22 sudden death following documented or undocumented arrhythmia suggest sensitization of β-adrenergic receptors. On the other hand, there have been some reports23–26 in the field of experimental medicine, which indicate that acquired cardiomyopathy may be brought about by reaction to stress without any specific extrinsic etiology, although they do not mention sensitization of β-adrenergic receptors. We think, therefore, that our speculation may be a reasonable explanation of part of the etiology of acquired cardiomyopathy, the specific etiology of which is not yet known.

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