RNA Content in the Heart Muscle Cells Following Adrenalectomy and Additional Overload in the Rat

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RNA content in the heart muscle cells of the adrenalectomized rats and those with aortic constriction was measured by microspectrophotometry. In the adrenalectomized rats, the RNA content decreased about 32% below that of the intact control rats by 7 days after operation. On the other hand, it decreased more abruptly in the adrenalectomized rats with aortic constriction. These results suggested that in the adrenalectomized rats, hypoplasia of the heart was caused by reduction of RNA content and myocardial compensatory hypertrophy was never induced even by overloading to the heart. The fact that the reduction of RNA content preceded the decrease of the heart weight per 100 g body weight suggests that in the cases of sudden unexpected death by unknown cause, the measurement of RNA content of heart muscle cells may by a clue to judge whether the cause of death is concerned with the heart or not.

Since Bartel (1908) had reported that hypoplasia of the heart, aorta, adrenals and gonads in a group of children with the so-called lymphatic constitution, a certain relationship had been suggested between the adrenal insufficiency and the lymphatic constitution. Later, Jaffe (1924), Marine et al. (1924) and Simpson et al. (1934) observed enlargement of thymus and lymphatic noduls in the adrenalectomized rats. Recently, Murotsu et al. (1972) reported enlargement of the thymus and hypoplasia of the heart and aorta in the adrenalectomized rats. These works suggest that hypoplasia of the heart in such constitution is based on adrenal insufficiency and a cause of sudden death.

Previously, the present author (1967) reported the relationship between acute heart failure and adrenal insufficiency from view point of ultrastructural changes in the heart muscle cells. The present study was made to investigate the changes of RNA content in the heart muscle cells following adrenalectomy and additional overloading to the heart to elucidate the cause of hypoplasia of the heart in adrenal insufficiency, which often results in sudden death.

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MATERIALS AND METHODS

Young adult Wistar breed rats, weighing 70–100 g, were used. They were divided into three groups. Five rats of the first group were used as the intact control and the hearts were excised without any treatment. In the second group, 100 rats were adrenalectomized by the usual dorsal approach, and other 20 rats received the same incision as in the adrenalectomized rats without adrenalectomy and were used as the sham-operated control rats. At 2 and 5 days after adrenalectomy, every 10 rats were sacrificed under ether anesthesia. From 5 to 10 days after adrenalectomy, many rats died before sacrifice and they were excluded from the experiment. Therefore, respective sums of only 6 and 5 rats were sacrificed at 7 and 10 days after adrenalectomy. While in the sham-operated control rats, no rat died before sacrifice, and every 5 rats were sacrificed at 2, 5, 7 and 10 days after sham-operation. From the sacrificed rats, the hearts were excised. In the third group, 110 rats were adrenalectomized by the same method as in the second group, and then their abdominal aortae were constricted as follows: A midline incision of the abdominal region was made and the intestine was separated. Then the abdominal aorta was freed and raised into the incision. A needle with the external diameter of about 1.0 mm was pressed against the aorta and a thread was tied tightly around the aorta just proximal to the renal arteries to occlude the aorta. The needle was quickly withdrawn, thus permitting the rapid re-establishment of circulation through the aorta. Eight or seven rats were killed every day for the first 3 days after operation. From 3 to 5 days after operation, many rats died before sacrifice and they were excluded from the experiment. Respective sums of only 5 and 4 rats could be sacrificed at 4 and 5 days after operation. Other 15 rats received the same incision as in the adrenalectomized rats without adrenalectomy, and their abdominal aortae were also constricted in the same way as in the adrenalectomized rats with aortic constriction. These rats were used as the sham-operated rats with aortic constriction and only 1 rat died in 5 days after operation. For the first 4 days after operation, 3 rats each were sacrificed every day, and at 5 days after operation, 2 rats were sacrificed. From the sacrificed rats, the hearts were excised. All the experimental rats were weighed before sacrifice, and the excised hearts were also weighed. The excised hearts were fixed in freshly prepared Carnoy (alcohol: chloroform: acetic acid, 6:3:1) for 48 hr. The fixed hearts were dehydrated with alcohol, embedded in paraffin and sectioned at 6μ. Immediately after deparaffinization, tissue sections were incubated for 24 hr at 37°C in a deoxyribonuclease solution, containing 0.1 mg cristallized deoxyribonuclease per ml of 0.1 M Gomori’s Tris buffer (pH 5.7) with 0.2 M MgSO₄. Gomori’s Tris buffer was better than phosphate buffer because the addition of 0.2 M Mg²⁺ produced a white precipitate in the latter but not in the former (Amano 1962). Then the slides were washed in three changes of distilled water and stained with aqueous Azur B containing 1 mg Azur B per ml of McIlvain buffer for 24 hr at 37°C, then dehydrated rapidly in three changes of tertiary butanol, cleared in xylol and mounted in Eukitt.

Measurement of the amount of Azur B combined with RNA were carried out in the length of 5 μ of a heart muscle fiber at the wave length of 530 nm on the UMSP-I (Carl Zeiss). Because the width of the heart muscle fiber of the rats was 10 to 15μ, the amount of RNA contained in a heart muscle fiber was presented by correcting the measured data to the amount of RNA contained in per unit of the fiber, 6μ thick, 5μ long and 1μ wide, and shown in arbitrary unit.

RESULTS

Effects of adrenalectomy and additional aortic constriction on the heart

Changes in heart weight per 100 g body weight after adrenalectomy with or without aortic constriction are illustrated in Fig. 1. In the adrenalectomized rats without aortic constriction, the heart weight per 100 g body weight increased.
Fig. 1. Changes in weight of the heart of the adrenalectomized and the sham-operated rats without aortic constriction (A) and with aortic constriction (B).
Solid circles, adrenalectomized rats; open circles, sham-operated rats.

a little according to reduction in body weight during the first 2 or 3 days after operation. Then in both adrenalectomized and sham-operated rats, the ratio decreased slowly, and the degree of decrease was greater in the adrenalectomized rats than in the sham-operated rats. In the adrenalectomized rats with aortic constriction, the heart weight per 100 g body weight began to decrease from 2 or 3 days after operation. On the other hand, in the sham-operated rats with aortic constriction, there was a considerable increase in this ratio from 3 to 5 days after operation.

**Effects of adrenalectomy on the heart RNA**

The mean RNA content of the heart muscle cells in the intact control rats was 2.38 in arbitrary unit. In the heart muscle cells of the adrenalectomized rats, it decreased slowly from 2 to 7 days after operation. The mean RNA contents of the heart muscle cells at 2, 5, and 7 days after adrenalectomy were 1.92, 1.75 and 1.62, respectively, and the mean RNA content at 7 days after operation was about 32% below that of the intact control rats. At 10 days after operation, the mean RNA content of the heart muscle cells was 1.60 and almost the same as that at 7 days after operation. In the sham-operated control rats, the mean RNA contents in the heart muscle cells of 2, 5, 7 and 10 days after operation were 2.53 to 2.29 and they were almost the same as that in the intact control rats (Table 1 and Fig. 2).

**Effect of aortic constriction on the heart RNA of the adrenalectomized rats**

The RNA content of the heart of the adrenalectomized rats with aortic constriction decreased abruptly by 3 days after operation. The mean RNA contents at 1, 2 and 3 days after operation were 2.01, 1.79 and 1.61, respectively, and the
TABLE 1. RNA content in the heart muscle cells from the intact control, the adrenalectomized and the sham-operated rats (arbitrary units)

<table>
<thead>
<tr>
<th>Day after operation</th>
<th>Intact control</th>
<th>Adrenalectomized</th>
<th>Sham-operated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of rat</td>
<td>RNA</td>
<td>Number of rat</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>2.38±0.35*</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean±s.d.

Fig. 2. Changes in RNA content of the heart muscle cells of rats after adrenalectomy. ■, sham-operated rats; ■■, adrenalectomized rats.

RNA content at 3 days after operation was about 33% below that of the intact control rats. Then, by 5 days after operation, they were almost the same as that at 3 days after operation, while in the sham-operated rats with aortic constriction, they increased slowly by 3 days after operation and the mean RNA content at 3 days after operation reached about 18% above that of the intact control rats. Then by 5 days after operation, they were almost the same or apt to decrease a little (Table 2 and Fig. 3).

DISCUSSION

RNA in the heart muscle cell, which is stained by Azur B, is composed of messenger, ribosomal and transfer RNA, and the microspectrophotometrically measured RNA is sum of these kinds of RNA. It is known that under the normal condition the ribosomal RNA constitutes more than 90% of the total RNA content in the differentiated cells (Meerson et al. 1968). Therefore, it is naturally understood that the majority of the reduced RNA is the ribosomal RNA. It is highly polymerized and relatively stable, and is synthesized in the specialized...
nucleolar apparatus, transported to cytoplasm and concerned in the synthesis of protein with which the cellular structures are formed (Meerson et al. 1968). From the results of the present experiment, the reduction of ribosomal RNA per unit of muscle cell seems to play a role in the disturbance of protein synthesis in myocardial cells, and greater decrease of the heart weight per 100 g body weight in the adrenalectomized rats seems to be caused by reduction of the ribosomal RNA.

Comparing the RNA content of the heart muscle cells in the adrenalectomized rats with that in the sham-operated rats, it was clear that this reduction of RNA was caused by adrenalectomy. Greenman et al. (1965) demonstrated that RNA synthesis in the liver of adrenalectomized animals was generally stimulated several hr after administration of cortisol, and showed that adrenal cortical hormones have an important role in RNA synthesis. According to their work, reduction of RNA

### Table 2. RNA content in the heart muscle cells from the adrenalectomized and the sham-operated rats with aortic constriction (arbitrary units)

<table>
<thead>
<tr>
<th>Day after operation</th>
<th>Adrenalectomized rat with aortic constriction</th>
<th>Sham-operated rat with aortic constriction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of rat</td>
<td>RNA</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>2.01±0.46*</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>1.79±0.43</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>1.61±0.54</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>1.58±0.39</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>1.59±0.35</td>
</tr>
</tbody>
</table>

* Mean±s.d.
in the heart muscle cells of the adrenalectomized rats resulted from disturbance of RNA synthesis caused by deficiency of the adrenal cortical hormones.

Fauburg and Ponser (1968) stated that the hearts of rats with aortic constriction increased in weight about 50% above those of the control rats by 7 days after operation, while RNA content increased within 2 days after constriction, then returned to the control level. Other several works (Meerson 1962, 1965; Gluck et al. 1964) also showed that constriction of the aorta resulted in increase in heart weight and nucleic acid content. In the sham-operated rats with aortic constriction in the present study, the heart weight per 100 g body weight increased and the RNA content in the heart muscle cells increased about 18% above that of the intact control rats by 3 days after operation. In adrenalectomized rats with aortic constriction, the heart weight per 100 g body weight decreased, and the RNA content in the heart muscle cells decreased more abruptly than that of the adrenalectomized rats without aortic constriction, and by 3 days after operation it was 33% below that of the control rats. These results suggested that in the acute overloading to the adrenalectomized rats, RNA content in the heart muscle cells decreased suddenly, and it could be scarcely supplied because of disturbance of RNA synthesis due to adrenal insufficiency. As a consequence of disturbance of protein synthesis due to reduction of RNA, compensatory hypertrophy of the heart muscle did not occur and the heart became unbearable to overload in a few days. This seemed to be the reason why the majority of the adrenalectomized rats with aortic constriction died in 2 to 5 days after operation, while in the sham-operated rats with aortic constriction, only 1 rat died at 5 days after operation. In the sham-operated rats with aortic constriction, RNA synthesis in the heart muscle cells was activated. In a short period after beginning of cardiac hyperfunction, the RNA content increased and the heart weight per 100 g body weight increased. Hyperfunction of the heart as a consequence of acute overloading rapidly induced a myocardial compensatory hypertrophy and the heart was adapted to overloading. In the adrenalectomized rats, acute overloading to the heart did not induce hypertrophy but hypoplasia of the heart due to reduction of RNA content in the heart muscle cells and the heart was apt to become insufficient in overloading by diminution of adaptability to overwork. Thus, reduction of ribosomal RNA content in the heart muscle cells seemed to play a role in the development of wear and tear of the heart.

The reduction of RNA content in the heart muscle cells preceded the decrease of the heart weight per 100 g body weight. This fact suggests that the reduction of RNA content in the heart muscle cells can be proved before development of hypoplasia of the heart, and the measurement of RNA content in the heart muscle cells of man, who had an apparently normal heart and died suddenly by unknown cause, may be a clue to judge whether the cause of death is concerned with the heart or not.
References


