identify experimental animals as Sprague-Dawley rats or Hartley guinea pigs or some other similar designation. It is also necessary to identify the supplier of the animals.

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Cardiac myofibrillar ATPase activity in hypophysectomized or thyroidectomized rats*

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RECENTLY it was shown that there is a significant reduction in adenosinetriphosphatase (EC 3.6.1.3, ATPase) activity of cardiac myofibrils prepared from hypophysectomized or thyroidectomized rats.[1] The hydrolysis of ATP by the contractile protein of muscle might be the force-generating reaction that is ultimately linked to the velocity of shortening or the rate of isometric tension development. Thus, there is at least a plausible explanation for the marked reduction in cardiac output in hypophysectomized or thyroidectomized animals.[2, 3] The experiments described herein were designed to test whether thyroxine and growth hormone are effective in restoring the cardiac myofibrillar ATPase activity to normal. The results have important implications with regard to recent experiments on the physiological effects of these hormones.

MATERIALS AND METHODS

Female rats, Sprague-Dawley strain, were purchased from Hormone Assay Laboratories, Chicago, Ill. They were given Purina laboratory chow and water *ad libitum*. Two series of experiments were done.

Series I. Thyroidectomized rats, two days post-operative, weighed 102–134 g on arrival. They were kept for 2 months and then divided at random into two groups, one of which was started on thyroxine treatment. The dose of thyroxine (Na, L-thyroxine dissolved in 0·9% NaCl–0·001 N NaOH) was 8 μg/rat per day. Subcutaneous injections were given 6 days a week. ATPase assays were done between days 22 and 35 of treatment. Normal rats were received about 2 weeks before the ATPase assays were begun. They weighed 136–150 g, and at the time of sacrifice their final weight was approximately the same as the untreated, thyroidectomized rats. Therefore, the control rats in this series were slightly younger than the experimental rats.

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Series II. Normal and hypophysectomized rats (two days post-operative) weighed 84–115 g on arrival. After one week of observation, the hypophysectomized rats were divided at random into four groups. Three treatments were started: (1) growth hormone, (2) thyroxine, and (3) growth hormone + thyroxine. The dose of growth hormone (bovine, NIH-GH-B9) was 120 μg/rat; the dose of thyroxine was 16 μg/rat. Subcutaneous injections were given every other day. ATPase assays were done between days 11 and 30 of treatment.

In Series I, three rats were used each day—one from each group. In Series II, five rats were used. Myofibrils were prepared each day according to the method of Perry and Gray.[4] The rats were decapitated in random order, the hearts quickly excised, blotted, and weighed, and pieces of left ventricle (200–285 mg) were removed. After homogenization in 0·025 M KCl–0·039 M borate buffer (pH 7·1), the myofibrils were resuspended and centrifuged five times to remove sarcoplasmic proteins and granules. The protein content was estimated by the biuret reaction standardized against bovine plasma albumin.

ATPase assays were carried out in a shaking water bath at 37°. The mixture consisted of 0·2 ml myofibrils, 1·7 ml Tris–$MgCl_2$ buffer (pH 7·4), and 0·1 ml ATP. The final concentrations were 0·5 mg protein/ml, 0·05 M Tris buffer, 2·0 mM $MgCl_2$, and 2·5 mM ATP. After a 5-min temperature equilibration, the reaction was started by the addition of ATP; it was stopped 15 min later by addition of cold 12% (w/v) trichloroacetic acid. Control determinations also were made by addition of trichloroacetic acid immediately after the ATP addition. After centrifugation at 0°, the inorganic phosphate was determined by the method of Fiske and SubbaRow.[5] All assays were done in triplicate, and the average of the three values was used.

TABLE 1. RAT CARDIAC MYOFIBRILLAR ATPASE ACTIVITY

Group	No. of rats	Final body wt. ± S.D. (g)	Heart wt. / Body wt. ± S.D. (mg/g)	ATPase (μmoles Pi/mg protein × 15 min)
Series I				
Normal	9	189 ± 9	3·32 ± 0·12	1·65*
Thyroidectomy	9	187 ± 20	2·48 ± 0·25	1·41
Thyroidectomy + thyroxine	9	216 ± 20	3·16 ± 0·14	1·70
Series II				
Normal	8	192 ± 17	3·65 ± 0·24	1·59
Hypophysectomy	8	104 ± 4	3·40 ± 0·25	1·35
Hypophysectomy + growth hormone	8	128 ± 9	3·25 ± 0·22	1·39
Hypophysectomy + thyroxine	8	107 ± 9	4·69 ± 0·50	1·54
Hypophysectomy + growth hormone + thyroxine	8	130 ± 7	4·45 ± 0·34	1·55

* The data were analyzed by analysis of variance for a randomized complete block design where the several rats done on a day constitute a block. For the difference among treatments in Series I, $F_{[2\ \&16df]} = 6·52$, $P < 0·01$. Tukey's test[6] for all possible comparison among treatment means gave a critical difference of 0·22 at the 5% level of significance. In Series II, $F_{[4\ \&28df]} = 3·62$, $P < 0·05$. The critical difference at the 5% level was 0·23.

RESULTS AND DISCUSSION

The results are summarized in Table 1. Mean values are given for body weight at the time of sacrifice, heart weight:body weight ratio, and ATPase activity. In Series I, the ATPase activity, which is reduced by thyroidectomy, is restored to normal after treatment with 8 μg thyroxine. Between 22 and 35 days of treatment there is no correlation between length of treatment and enzyme activity.

In Series II, the effect of hypophysectomy on heart weight:body weight ratio is not particularly evident, because the control and experimental rats were of the same age and not of the same weight; however, the heart weight:body weight ratio is characteristic of hypophysectomized rats weighing 100–120 g.[7] From preliminary experiments on the measurement of ATPase activity, it was concluded that, within the range of age and body weight employed, it does not matter whether the control rats are either the same age (Series II) or the same weight (Series I) as the experimental rats.

Growth hormone, in a dose sufficient to promote body growth of hypophysectomized rats, does not alter the heart weight:body weight ratio. This agrees very well with the experiments of Whitehorn *et al.*[8] They reported that growth hormone-treated, hypophysectomized rats gained about 27 g in 21–24 days, with no change in ventricle weight:body weight ratio. The low ATPase activity as a result of hypophysectomy is restored either by thyroxine or thyroxine plus growth hormone, but it is not restored by growth hormone alone.

Thyroxine treatment in hypophysectomized rats has a marked effect on heart weight and enzyme activity, although it does not stimulate general body growth. This latter effect agrees with the results of other investigations.[9–12]

It is known that hypophysectomized rats have low cardiac output and blood pressure. Beznak[9] has shown that thyroxine treatment in hypophysectomized rats restores the cardiac output and blood pressure to normal. However, during loading by infusion of polyvinylpyrrolidone into the right side of the heart, the maximal cardiac output and work are still low in these animals. In addition, Beznak[10] has demonstrated that cardiac index, stroke-work index, and minute-work index are returned to normal in hypophysectomized, thyroxine-treated rats, and the response of these variables to acute loading is normal after treatment with thyroxine plus growth hormone. Growth hormone itself has comparatively little effect.

Our results on myofibrillar ATPase activity are in accord with these physiological studies, although we have no evidence for the role of growth hormone in the responses to acute stress. If one attributes a casual role in muscle contraction to the hydrolysis of ATP by the contractile protein (for review see Perry[13]), then these results are an additional step in the attempt to explain the effects of thyroxine and growth hormone on cardiac muscle function.

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