MECHANISM OF ACCUMULATION OF TAURINE IN THE HEART OF STARVED RATS

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Abstract—The mechanism of increase of taurine concentration in the heart of starved rats was investigated. Starvation for 48 to 72 hr markedly increased the taurine level in heart muscle and serum. Adrenalectomy completely prevented the increase of taurine concentration in the heart during starvation. Incorporation of radioactive taurine into the heart tissue was increased in starved rats. Administration of phenoxybenzamine or propranolol prevented the increase of taurine concentration in heart and the enhanced transport of radioactive taurine during starvation. These observations indicate that adrenal factor, especially epinephrine, may be involved in regulation of cardiac taurine level.

There is much evidence that taurine may have various functions in the hearts of dog, rat and guinea pig (1, 2). Its possible functions are membrane stabilizing effects, inotropic actions, anti-arrhythmic actions and neurotransmitter or modulator effects. Taurine is present in high concentrations in the heart and the concentration remains remarkably constant under a wide range of conditions. The taurine content of the heart was found to be increased in some pathological states (3–5) and also with administration of reserpine (6) or glucocorticoids (7) and during starvation, (8) in rats. Moreover, Huxtable and Chubb (9) found that taurine transport into the rat heart is modulated by $\beta$-adrenergic stimulation. However, little is known of the physiological regulation of the cardiac taurine level. We found herein that the adrenal function may be involved in regulation of the cardiac taurine concentration.

MATERIALS AND METHODS

Male Sprague-Dawley rats, 4 weeks old and weighing 60 to 70 g, were housed for 3 weeks in an air-conditioned room under controlled lighting (light on from 06.00 to 18.00 hr) and temperature (21±1°C). Bilateral adrenalectomy and sham-operation were performed under ether anesthesia and the adrenalectomized rats were maintained on the usual chow diet with 0.9% sodium chloride solution in place of drinking water. At the point of experiments, body weights of normal, sham-operated and adrenalectomized rats were 210–240, 180–205 and 185–200 g, respectively. The rats were decapitated between 10.00 and 11.00 hr, unless otherwise indicated. The whole heart was quickly removed and washed, and taurine levels were determined as described previously (6, 10). Epinephrine in the adrenal glands was measured fluorometrically by the conventional...
trihydroxyindole method (11). Serum corticosterone was estimated by the method of Zenker and Bernstein (12). Incorporation of \[\text{\textsuperscript{[35}S\text{]}-taurine}\] into the heart tissue was examined as follows: \[\text{\textsuperscript{[35}S\text{]}-taurine (25 \mu\text{Ci/}

kg, specific activity: 46 \text{mCi/mmole, Amersham/Searle Corp., Arlington Heights, IL})\] was given i.p., and 1 hr later the animals were decapitated. The whole heart was quickly removed, cut into small pieces and washed several times with cold saline. The pieces were weighed and solubilized by incubation with 1 ml of 0.2\% Triton X-100-0.2N NaOH at 50\(^\circ\)C. The solution was neutralized with hydrochloric acid, and the radioactivity counted in a liquid scintillation counter, using Triton X-100-toluene as a cocktail. One hour after the injection of radioactive taurine, more than 95\% of the radioactivity in the heart and the serum was recovered as taurine. Incorporation of \[\text{\textsuperscript{[3}H\text{]}-inulin}\] (specific activity: 1.7 Ci/mmole, Amersham/Searle Corp., Arlington Heights, IL) into the heart tissue was examined in the same manner.

**Drugs:** d,l-propranolol; Sigma Chem. Co., St. Louis, MO, Phenoxybenzamine; Nakarai Chem. Co., Kyoto, Japan.

**RESULTS**

**Effect of starvation on taurine concentration in rat tissues:** Changes in taurine concentration in the heart muscle were markedly increased by starvation for 48 to 72 hr. As may be expected, the weight of heart tissue gradually decreased during starvation. However, the rise in cardiac taurine level was significant even when expressed as \(\mu\text{mole/heart}\). Refeeding rats that had been deprived of food for 48 hr did not affect the cardiac taurine level within 24 hr. The starvation-induced increase of the cardiac taurine level was not observed in adrenalectomized rats (Table 2).

Taurine concentration in skeletal muscle of the hind limb did not change during starvation. However, the taurine level in the serum was significantly increased after 48-hr or 72-hr starvation, and returned to normal on refeeding the starved rats (Fig. 1).

**Effects of phenoxybenzamine and propranolol on starvation-induced changes in taurine level of the rat tissues:** Previously, we reported that repeated injection of hydrocortisone acetate also markedly increased the cardiac taurine level (7). In a separate experiment, we confirmed that this effect of glucocorticoid was not observed in adrenalectomized rats. The fact that starvation or glucocorticoid did not affect the cardiac taurine level in adrenalectomized rats strongly indicates that some adrenal

| Tissue concentration in rat heart | Tissue weight (g) | Taurine concentration (\(\mu\text{mole/g wet weight}\)) | Taurine concentration (\(\mu\text{mole/heart}\)) |
|----------------------------------|------------------|--------------------------------------------------|-----------------------------------------------|
| Control                          | 0.762±0.011      | 20.9±1.4                                         | 15.9±1.1                                      |
| Starvation 24 hr                 | 0.769±0.013      | 25.9±2.0                                         | 19.4±1.3                                      |
| 48 hr                            | 0.677±0.013*     | 32.2±2.4*                                        | 21.7±1.9*                                     |
| 72 hr                            | 0.618±0.014**    | 34.9±0.8**                                       | 21.6±0.5*                                     |
| Refed after 48-hr starvation     | 0.680±0.014*     | 34.2±2.8*                                        | 23.2±1.8*                                     |

Taurine concentration in a heart of rats starved for 24 to 72 hr was determined as described in the Methods. In the refed group, food was given for 24 hr after 48-hr starvation. Values are mean±S.E. of 6 separate experiments. *\(P<0.05\), **\(P<0.01\), ***\(P<0.001\), compared with control.
factor(s) is involved in regulation of the cardiac taurine level. Therefore, we examined the serum corticosterone level and epinephrine content in the adrenal glands of rat that had been starved for 48 hr.

Table 2. Effect of adrenalectomy on starvation-induced increase of cardiac taurine

|                     | Tissue weight (g) | Taurine concentration (µmole/g wet weight) | (µmole/heart) |
|---------------------|-------------------|------------------------------------------|---------------|
| Sham operated       |                   |                                          |               |
| Control             | 0.640±0.016       | 20.6±0.5                                 | 13.2±0.3      |
| Starvation (48 hr)  | 0.678±0.020       | 28.5±0.8*                                | 16.5±0.7*     |
| Adrenalectomized    |                   |                                          |               |
| Control             | 0.687±0.023       | 19.4±0.7                                 | 13.2±0.4      |
| Starvation (48 hr)  | 0.654±0.011*      | 18.5±0.7                                 | 12.2±0.4      |

Adrenalectomy was performed 2 weeks before the experiments. Values are means±S.E. of 5 separate experiments. *P<0.01, compared with control.

We found that starvation had no effect on the serum steroid level, but did markedly reduce the total epinephrine content of the adrenal glands (Fig. 2).

We found that starvation had no effect on the serum steroid level, but did markedly reduce the total epinephrine content of the adrenal glands (Fig. 2).

A previous study indicated the involvement of adrenergic mechanisms in the transport of taurine into the heart (9). We therefore examined the effects of adrenergic blocking agents on the increase in cardiac taurine level during starvation (Fig. 3). Administration of phenoxybenzamine or propranolol (10 mg/kg×2, i.p.) prevented increases of the level during starvation, without affecting the basal level. These agents also prevented the increase in the serum taurine level during starvation (Table 3).

Accumulation of [35S]-taurine in rat heart:
To determine the mechanism of the increase of the taurine level in the heart during starvation, we compared the accumulations of radioactive taurine in the heart tissue of normal rats and rats that had been starved for 48 hr. In preliminary experiments, we
found that incorporation of radioactive taurine into heart tissue increased linearly for 2 hr after the injection of $[^{35}\text{S}]$-taurine. Therefore, we examined the amounts of $[^{35}\text{S}]$-taurine and $[^{3}\text{H}]$-inulin in the heart tissue and serum at 1 hr after the injection (Fig. 4). There was no significant difference between the amounts of inulin in the heart tissues of normal and starved rats, indicating that the extracellular space (inulin space) did not change during starvation. On the contrary, accumulation of $[^{35}\text{S}]$-taurine into heart tissue expressed as c.p.m./g wet weight was significantly greater in starved rats, though the amount of $[^{35}\text{S}]$-taurine in the serum was not significantly different from that in normal rats. Figure 1 shows that the taurine concentration in the serum was markedly increased after 48 hr starvation. Therefore, the specific

### Table 3. Effects of phenoxybenzamine and propranolol on starvation-induced changes in taurine level of the serum and skeletal muscle

|                   | Serum (μmole/ml serum) | Muscle (μmole/g wet weight) |
|-------------------|------------------------|-----------------------------|
| Control           |                        |                             |
| None              | 0.46±0.01              | 15.3±1.1                    |
| Phenoxybenzamine  | 0.41±0.02              | 16.9±1.5                    |
| Propranolol       | 0.46±0.03              | 15.6±0.9                    |
| Starvation (48 hr)|                        |                             |
| None              | 0.59±0.03$^*$          | 14.7±0.9                    |
| Phenoxybenzamine  | 0.47±0.03$^*$          | 17.6±0.8                    |
| Propranolol       | 0.52±0.03              | 16.3±2.2                    |

Drug treatment was as described in Fig. 2. Samples were obtained as described for Fig. 1. Values are means±S.E. of 5 to 8 separate experiments. $^*P<0.05$, $^*P<0.01$, compared with control.
activity of radioactive taurine in the serum was significantly less in the starved rats. The amount of taurine, transported to the heart tissue, was therefore calculated from the specific activities in the serum of the normal and starved rats. As shown in Fig. 5, starvation markedly enhanced the uptake of taurine by the heart tissue. While injection of phenoxybenzamine or propranolol had no effect on the uptake of taurine in normal rats, these blockers completely blocked the enhanced uptake of taurine in starved rats.

**DISCUSSION**

In the present study, we found that starvation for 48 to 72 hr markedly increased the taurine level in rat heart muscle. This increase was not due to decrease in weight of the heart tissue, because the total content of taurine in a whole heart was significantly increased. Cardiac and skeletal muscles usually have higher concentrations of taurine than do other tissues. It is interesting that starvation increased the taurine concentration in the heart, but not in the skeletal muscle. Starvation also increased the taurine concentration in the serum, and thus it must accelerate the release of taurine from some tissues, since, obviously, taurine in the serum is not obtained from food during starvation. Previously, Fujihira et al. (8) reported that taurine was the only amino acid that increased in the plasma and heart during starvation for 72 hr. However, Awapara (13) reported that taurine levels in the heart did not change during starvation for 16 hr or 7 days. The difference between these two reports may be due to the duration of starvation.

The increased level of cardiac taurine observed even after refeeding may be explained by the slow turnover rate of taurine in this tissue (14). Our results on adrenalectomized rats indicated that some adrenal factor(s) was involved in the increase of cardiac taurine level in the starved rats. Starvation may act as a stress via the pituitary-adrenal system, and in fact, starvation reduced adrenal epinephrine, although it had no effect on the glucocorticoid level in the serum. Moreover, administration of phenoxybenzamine or propranolol prevented the increase of cardiac taurine concentration induced by starvation. These results suggest that some adrenergic mechanism is involved in regulation of the cardiac taurine concentration.

Recently, Huxtable and Chubb (9) reported that a system for taurine transport in the heart is modulated by the level of β-adrenergic activation. They also found that isoproterenol stimulated taurine influx into the heart, which is mediated by a high affinity transport system, and that propranolol blocked this stimulation (15, 16). In the present study, we examined the effect of the starvation on taurine transport into the heart tissue and we found that accumulation of radioactive taurine
in the heart tissue was markedly increased whereas the extent of accumulation of inulin was not changed. In order to clarify the involvement of adrenergic receptors in starvation-induced increase of taurine transport, we examined the effect of adrenergic blockers. This increased accumulation of taurine in starved rats was partially prevented by administration of adrenergic blockers. These blockers also prevented the increase in the serum taurine level in starvation. In our previous study, we found that propranolol prevented the increase of cardiac taurine level by glucocorticoid (7) at doses of 10 mg/kg but not at 5 mg/kg (Matsuda, not published). From these results, we used 10 mg/kg of propranolol and phenoxybenzamine in the present study. It seems reasonable that these blockers function through their receptor-blocking actions. However, other factors also should be considered in the effect of these blockers, since there are blockers which have various actions on adrenergic mechanisms when given in high doses (17, 18).

The rat heart can synthesize taurine, but the quantitative importance of this synthesis is uncertain (19, 20). Conversion of taurine to isethionic acid, the only metabolic pathway for taurine in the heart, is possibly negligible (21). Increase in the concentration of cardiac norepinephrine and some changes in the cardiac adrenergic mechanism were observed in starved rats, but change in the adrenergic mechanism in the heart was not prevented by adrenalectomy (22, 23). Recently, Young and Landsberg (24) showed that cardiac sympathetic nerve discharge is diminished in the rat starved for 48 hr. Therefore, cardiac norepinephrine does not seem to be involved in starvation-induced increase of taurine transport in the heart.

From these results it is possible that during starvation, epinephrine released from the adrenals may interact with heart tissue causing an influx of taurine into the heart.

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