
β-Adrenergic Receptor in Heart of Starved Rats

KOJI NAKAGAWA, MIYAO MATSUBARA, KAZUMASA AKIKAWA AND MITSUMASA KUBO

Second Department of Medicine, Hokkaido University School of Medicine, Sapporo 060, Japan

Abstract

Female Wistar-strain rats were starved for 14–19 days by feeding approximately 1/4 of the amount consumed by ad libitum fed controls. The body weight was reduced by 41% and the heart weight by 38% in these starving periods. The 125I-iodocyanopindrol (ICYP) binding capacity of heart preparations from the starved rats was 35.3±11.1 (mean±SD) fmol/mg protein in comparison with 69.3±14.9 for the controls. Serum 3, 5, 3'-triiodothyronine (T3), thyroxine and TSH levels as well as pituitary TSH contents were markedly lower in the starved rats. One group of them further received 20 ng of T3 daily after the 8th day of the experiment. The body weight decreased by 47% of the controls but the ICYP binding capacity recovered to 56.3±10.9 fmol/mg protein. There was no difference in association constants of the receptors in these three groups. It was concluded that quasi-chronic starvation in rats caused a remarkable decrease in the number of β-adrenergic receptors in heart and this was partly offset by the substitution of T3.

Bradycardia is generally observed in patients with anorexia nervosa (Warren and Wiele, 1973). Moreover, in these patients, insulin-induced hypoglycemia causes minimal clinical manifestations such as palpitation, tachycardia, or sweating. This is partly due to decreased release of epinephrine in response to this stimulus in these patients (Nakagawa et al., 1985a). The response of pulse rate or blood pressure to infused exogenous epinephrine was also weakened in patients with anorexia nervosa (Nakagawa et al., 1985b), suggesting decreased function of the β-adrenergic receptor mechanism.

To determine whether this is specific to patients with anorexia nervosa or only a consequence of emaciation in these patients, we studied the effect of quasi-chronic starvation, as a model of simple weight loss, on β-adrenergic receptors in rat. Moreover, we examined the change in β-adrenergic receptors following partial substitution with 3, 5, 3'-triiodothyronine (T3), because the serum thyroid hormones, especially T3, are decreased in the starved state and thyroid hormones have been reported to regulate β-adrenergic receptors (Bilezikian and Loeb, 1983).
Materials and Methods

Twenty Wistar strain female rats in identical sexual stages, confirmed by vaginal smear, were divided into three groups and individually caged. Six control rats were fed ad libitum and consumed an average 15.6 g per day of solid food (MF, Oriental Yeast Co.). The other 14 rats were given 4.0 g per day of the same food. Seven of them received 20 ng of T₃, soaked into the solid food, daily on the 8th day and thereafter. After 14 days, three each from the three groups, and after 19 days, the remainder were sacrificed by decapitation.

The hearts were excised and washed in ice-cold physiological saline. They were weighed and homogenized with Polytron for 30 sec with 20 times the heart weight of 50 mM tris-HCl buffer, pH 7.4, containing 10 mM MgCl₂. The homogenate was filtered through two layers of nylon sheet and centrifuged at 50,000×g for 20 min. The precipitates were washed with 10 ml of the buffer and centrifuged again. The pellets were suspended in the same buffer at a concentration of 1 ml per 100 mg heart weight. A portion of these suspension was subjected to determination of the protein content by the method of Lowrey. Then the concentrations of protein in all samples were adjusted to the same level by adding the buffer. To 200 μl of the suspension containing 500 μg of protein, 750 μl of the buffer and 50 μl of 125I-iodocyanopindrol (ICYP; Amersham) solution in increasing doses were added and incubated for 20 min at 25°C. The incubates were filtered through glass micro-fibre (Whatman GF/C). The filters were washed thoroughly with the buffer and the radioactivity was counted in a gammacounter. The specific binding was calculated by reducing the non-specific binding for each sample and Scatchard plot analyses (Scatchard, 1949) were performed.

Serum concentrations of T₃ and thyroxine (T₄) were determined by radioimmunoassay with kits purchased from Eiken Immunochemical Laboratories, Tokyo. As these kits were considered to have been adjusted for the determination of these hormones in the presence of human plasma or serum, it was confirmed before the assays that the dilution curves for rat serum were parallel to the standard curves even in the presence of rat serum. Serum TSH was determined by radioimmunoassay with materials generously supplied by the Rat Pituitary Hormone Distribution Program, NIH (NIAMDD Rat TSH I-4, NIAMDD Rat TSH S-4 and NIAMDD Rat TSH RP-1).

The results on the 14th and 19th days were in similar ranges, and were therefore combined for each group for statistical analyses.

Results

Body weight

The body weight of rats at the beginning of the experiment was 225.0±11.8 (mean±SD) g in control, 224.3±9.8 in the starved group and 227.1±20.8 in the starved-T₃ group. On the day of sacrifice, they were 254.8±9.1, 151.1±14.8 and 136.2±12.6, respectively. Thus the body weight of the starved rats was on the average 40.7%, and the starved-T₃ rats 46.6%, lower than that of the controls.

Heart weight and protein

The heart weighed 773±36 mg in the controls, 476±61 in the starved and 454±40 in the starved-T₃ rats. The latter two were 38.4 and 41.3% lower than the former, nearly corresponding to the decrease in body weight.

The concentrations of protein in myocardial 50,000×g pellets were 64.5±4.4 μg/mg wet heart weight in the controls, 49.7±5.3 in the starved and 52.6±4.5 in the starved-T₃. The latter two values were significantly (p<0.001) lower than those for the controls.

ICYP binding of heart (Figure 1–2)

The ICYP binding capacity was 69.3±14.9 fmol/mg protein in the control rats and was remarkably decreased to 35.3±11.1 in the starved group (p<0.001) and recovered to 56.3±10.9 in the starved-T₃ group (p<
Fig. 1. Binding capacity (left) and affinity constant (right) of $^{125}$I-ICYP binding receptors in heart preparation from the rats of control, simply starved and starved-T$_3$ groups. Horizontal bars represent mean ± 1 SD.

Fig. 2. Scatchard plot analysis of $^{125}$I-ICYP binding receptors in heart preparation of rats, one each from control, simply starved and starved-T$_3$ groups.

Thyroid hormones and TSH (Figure 3)
Serum T$_3$ levels were 110.0 ± 14.1 ng/dl in the control, 40.8 ± 9.9 in the starved (p<0.001) and 41.9 ± 12.7 in the starved-T$_3$ rats (p<0.001). The serum concentrations of T$_4$ were also decreased to 2.89 ± 1.16 µg/dl in the starved, and 1.16 ± 0.35 in the starved-T$_3$ rats, in comparison with 6.50 ± 1.54 in the control rats (p<0.001). Serum TSH levels were 138 ± 58 ng/ml in control, 34 ± 14 in the starved (p<0.001) and 18 ± 9 in the starved-T$_3$ rats (p<0.05 against the starved group). Pituitary TSH content was 227 ± 118, 97 ± 44 (p<0.001) and 35 ± 5 µg (p<0.05 against the second), in the 3 groups, respectively.

Discussion

An increase in the number of $\alpha_2$-adrenergic receptors has been reported in blood platelets of patients with anorexia nervosa (Luck et al., 1983) and in hypothalamus of starved rats (Spyra and Pirke, 1982). However, no report can be found on $\beta$-adrenergic receptors in the starved state.

In the present study, food restriction in rats to approximately 1/4 of the ad libitum
Fed controls resulted in a 41% body weight loss. In these rats, decreased binding of a β-adrenergic antagonist, ICYP, was observed in the heart. The Scatchard plot analysis demonstrated that the decreased binding is the consequence of a decrease in the number of binding sites, not of decreased affinity. This is consistent with our previous observation that patients with anorexia nervosa were less sensitive to exogenously infused epinephrine (Nakagawa et al., 1985b).

The decrease in the number of receptors may result from down regulation by an increased number of ligands. However, starvation was reported to suppress sympathetic activity (Young and Landsberg, 1977; Schweiger et al., 1985).

On the other hand, it has been well demonstrated that β-adrenergic receptors are regulated by thyroid hormones (Bilezikian and Loeb, 1983; Williams et al., 1977) and, in the hypothyroidal state, these receptors were shown to decrease in heart and other tissues, except liver and adipose tissue where they were found to increase in number (Bilezikian and Loeb, 1983). Emaciation causes a decrease in circulating thyroid hormones, especially T₃. The starved rats in the present study also had lower levels of serum T₃ and T₄. Therefore, the decrease in the number of β-adrenergic receptors could be the consequence of thyroid hormone deficiency, though it is controversial whether malnutritional states cause true hypothyroidism or only low T₃ euthyroidism (Larsen, 1985).

To examine the role of thyroid hormones in the regulation of β-adrenergic receptor in the starved state, T₃ was administered to a group of starved rats. Serum T₃ at the time of sacrifice was not elevated in comparison with the simply starved rats, but this seemed to be due to the lapse of 18 hours between the time of the last T₃ intake and sacrifice, as the disappearance of thyroid hormones from the blood in rats is reported to be much faster than in man (Oppenheimer et al., 1970). Actually we observed in rats that serum T₃ levels clearly rose 4 hours after TRH injection, but, 4 or 8 hours thereafter it became rather lower than the untreated controls (Nakagawa, 1975). The decrease in serum T₄ and TSH as well as the decrease in pituitary TSH content would be the reflections of increased T₃. In these starved-T₃ rats, body weight was naturally further decreased to 53% of the controls, but the ICYP binding capacity...
was recovered to 81.2% of the controls in the contrast to 50.9% in simply starved rats. This is consistent with, but does not conclusively prove, the possibility that the decreased number of β-adrenergic receptors in starvation is due to the hypothyroidal state. In any case, the decreased β-adrenergic function in patients with anorexia nervosa can be explained, at least partially and in their emaciated states, by weight loss per se.

References


