

Possible role of the adrenergic mechanism in experimental hyperketonemia caused by thyrotoxicosis and starvation in rats

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Catecholamine is well known to have an important role to metabolize ketone bodies. Practically, hyperketonemia is frequently observed both in hyperthyroidism and starvation of various species¹⁻⁷⁾. Besides, several investigators have reported an increase of catecholamine in serum and urine during starvation of animals⁸⁻¹⁰⁾. Beylot et al.⁵⁾ have reported an implication that catecholamine affects some symptoms of hyperthyroidism through β -receptors. However, the role of the adrenergic mechanism in hyperketonemia involved in hyperthyroidism and starvation remains to be fully elucidated. In this study, we investigated the possible role of the adrenergic mechanism in experimental hyperketonemia in thyrotoxic or starved rats.

Materials and Methods

Animals and chemicals

Male Wistar albino rats were used in this study. L-Thyroxine was purchased from Sigma Chemical, St Louis. Phentolamine from Ciba Geigy, Takarazuka, Japan, and propranolol from ICI Pharma., Osaka, Japan. L-Thyroxine was dissolved in a small amount of 0.01N NaOH, then brought to a concentration of 100 $\mu\text{g}/\text{ml}$ with saline.

Adrenergic blockers administration into thyrotoxic rats

Into an experimental group of rats, thyroxine (T_4) was first administered subcutaneously at a dose of 100 $\mu\text{g}/\text{kg}/\text{day}$ for 7 days. While into a control group, sole saline was first injected under the same schedule. Into some of both groups,

either a 2 mg/kg dose of phentolamine or a 0.1 mg/kg dose of propranolol was secondly administered subcutaneously twice a day for 7 days. Consequently, the rats were left to fast overnight to consume the drugs, then drawn blood from femoral vein, after being anesthetized with a 30 mg/kg dose of intraperitoneal pentobarbital sodium solution.

Adrenergic blockers administration into starved rats

One group of rats was forced to fast 96 hr, except with a dilute electrolyte solution consisted of 78mEq/l sodium salts and 15mEq/l potassium salts to prevent rats from the depletion of sodium ions. The other group of fed rats was used as control. Into some of both groups, either phentolamine or propranolol was administered at the same dose as above, this time, every 12 hr for 96 hr.

Measurement

Blood glucose was measured by a glucose oxidase method. Plasma insulin, glucagon, triiodothyronine (T_3), and thyroxine (T_4) were measured by respective radioimmunoassay. Amount of plasma free fatty acid (FFA) was measured enzymatically with a commercial kit (NEFA C-Test, Wako, Osaka, Japan). Plasma acetoacetate (AcAc), and β -hydroxybutyrate (BOHB) were measured enzymatically¹¹

Statistical analysis

Analysis of variance and two tailed Student's non-paired t test was applied to verify the values obtained with the measurements, presenting them as means \pm SD

Results

Relation among body weight, amount of blood glucose, and of plasma insulin, glucagon, T_3 , T_4 , FFA, AcAc, and BOHB in thyrotoxic rats

Table 1 shows that the mean value of body weight in thyrotoxic rats is lower than that of control rats. On the contrary, the mean values of blood glucose, plasma insulin, T_3 , T_4 , FFA, AcAc, and BOHB in thyrotoxic rats are significantly higher than those in control. Plasma glucagon level in thyrotoxic rats was similar to it in control. In both groups of rats, phentolamine administered induced to increase the level of plasma insulin, but not of blood glucose, plasma FFA, AcAc, and BOHB. Propranolol could not change the levels of these parameters at all.

Relation among body weight, amount of blood glucose, and of plasma insulin, glucagon, FFA, AcAc, and BOHB in starved rats

Table 2 shows that body weight, blood glucose and plasma insulin in starved rats are significantly lower than those in fed rats. Table 2 also shows that, on the contrary, plasma FFA, AcAc, BOHB in starved rats are significantly higher than those in fed rats. Plasma glucagon level was not different between starved and fed

ats. Phentolamine administered induced to increase the levels of plasma insulin in ny rat. Besides, it lifted up significantly the levels of plasma FFA, AcAc, and BOHB only in starved rats. Propranolol could not change the levels of these parameters at all.

Table 1 Amounts of various parameters after operating adrenergic blockers to experimentally thyrotoxic rats

Adrenergic blockers administration	BW (g)	BG (mg/dl)	IRI (μ U/ml)	IRG (pg/ml)	T ₃ (ng/dl)	T ₄ (μ g/dl)	FFA (mmol/l)	AcAc (mmol/l)	BOHB (mmol/l)
[Control (n=6)]	162 ± 11	74 ± 4	12 ± 5	71 ± 23	80 ± 10	3.1 ± 0.5	1.0 ± 0.3	1.10 ± 0.26	0.46 ± 0.11
[Thyrotoxic (n=6)]	130 ± 10*	82 ± 5*	25 ± 9*	70 ± 22	148 ± 44*	10.9 ± 0.7*	1.7 ± 0.3*	1.85 ± 0.47*	1.06 ± 0.24*
propranolol (n=6)	125 ± 12*	82 ± 6*	24 ± 9*	69 ± 25	139 ± 51*	10.8 ± 0.8*	1.6 ± 0.3*	1.87 ± 0.51*	1.17 ± 0.23*
phentolamine (n=6)	129 ± 11*	83 ± 7*	47 ± 15*	73 ± 24	140 ± 42*	11.0 ± 0.9*	1.7 ± 0.3*	1.84 ± 0.55*	1.09 ± 0.27*

After overnight fast, thyrotoxic rats were induced with either propranolol or phentolamine according to the schedule shown in Materials and Methods. Then, amounts of various parameters were measured with respective procedures shown in Materials and Methods. Symbols show; BW, body weight of rats; BG, blood glucose; IRI, plasma insulin; IRG, plasma glucagon; T₃, triiodothyronine; T₄, thyroxine; FFA, free fatty acid; AcAc, acetoacetata; BOHB, β -hydroxybutyrate; *p < 0.05 in being significantly different from value in control.

Discussion

Hyperketonemia was sure to be observed in the thyrotoxic or starved rats in this study, according to many reports that an increased ketogenesis and subsequent hyperketonemia were observed in hyperthyroidism or starvation in several species¹⁻⁷⁾. Phentolamine or propranolol could not change the amount of plasma FFA, AcAc, and BOHB in thyrotoxic rats, suggesting that the adrenergic mechanism did not operate to eliminate hyperketonemia at least at the hyperthyroid state. Although phentolamine administration increased plasma level of insulin, the amount of plasma FFA, AcAc, and BOHB were not changed, suggesting that the insulin resistance may partly be involved in hyperketonemia of thyrotoxic rats. Beylot et al.⁶⁾ have reported that propranolol does not have a direct, inhibitory effect on ketogenesis as it is, but on that of hyperthyroid patients so far, being probably due to the decrease of the triiodothyronine level. Several investigators¹²⁻¹⁵⁾ have reported that β -adrenergic blockade inhibits the conversion of T₄ to T₃ in peripheral tissues. In this study, the plasma T₃ level was not changed in propranolol-treated thyrotoxic rats. Therefore, propranolol may not inhibit the conversion of exogenous thyroxine into T₃ in rats.

Propranolol could not change the amount of plasma FFA, AcAc, and BOHB in starved rats, suggesting that the β -adrenergic mechanism have no important role in ketogenesis caused by the starvation. On the other hand, phentolamine administered lifted up the levels of plasma FFA and ketone bodies in starved rats. The current results suggest that α -adrenergic mechanism has an inhibitory effect on lipolysis and ketogenesis in the starvation. This is partly supported by the fact that catecholamine exerts not only β -lipolytic action but also α_2 -antilipolytic action¹⁶⁻¹⁸⁾, though Carpéné et al.¹⁹⁾ have reported that the starvation induces a significant decrease of α_2 -adrenoceptors in number and an antilipolytic effect of α_2 -agonists in male golden hamsters. The mechanism, by which phentolamine increases the amount of plasma ketone bodies in starved rats but not in fed rats, is unknown. Phentolamine may reduce the metabolic clearance rate of ketone bodies in the starvation. Further studies are necessary to clarify the current phenomena.

Concisely speaking, the adrenergic mechanism may not contribute to hyperketonemia of thyrotoxic rats, but to the slight inhibition of ketogenesis in starved rats, probably being due to α -antilipolytic action.

Table 2 Amounts of various parameters after operating adrenergic blockers to starved rats

Adrenergic blockers administration	BW (g)	BG (mg/dl)	IRI (μ U/ml)	IRG (pg/ml)	FFA (mmol/l)	AcAc (mmol/l)	BOHB (mmol/l)
[Fed (n=6)]	179 \pm 10	114 \pm 10	49 \pm 9	78 \pm 20	0.5 \pm 0.1	0.05 \pm 0.01	0.07 \pm 0.01
propranolol (n=6)	181 \pm 11	117 \pm 8	44 \pm 8	85 \pm 25	0.5 \pm 0.1	0.05 \pm 0.01	0.08 \pm 0.01
phentolamine (n=6)	182 \pm 11	112 \pm 8	78 \pm 15*	84 \pm 24	0.5 \pm 0.1	0.05 \pm 0.01	0.08 \pm 0.01
[96hr starved (n=6)]	113 \pm 8	72 \pm 5	10 \pm 3	80 \pm 21	1.1 \pm 0.2	1.41 \pm 0.24	2.32 \pm 0.51
propranolol (n=6)	123 \pm 9	80 \pm 6	11 \pm 3	94 \pm 24	1.0 \pm 0.3	1.45 \pm 0.25	2.17 \pm 0.53
phentolamine (n=6)	129 \pm 9	71 \pm 5	19 \pm 4*	90 \pm 24	1.8 \pm 0.4*	2.00 \pm 0.31*	3.49 \pm 1.00*

For legend, see the footnote of Table 1. *p <0.02 in being significantly different from value in corresponding control.

Summary

To elucidate the possible role of the adrenergic mechanism in hyperketonemia observed in hyperthyroidism and starvation, two groups of rats were first forced to a series of thyroxine (T₄)-intoxication for 7 days and of starvation for 96 hr, respec-

vely. Each of the groups was secondly administered subcutaneously with phentolamine or propranolol to make operate the adrenergic mechanism. As several parameters were measured, both of phentolamine and propranolol did not change the amount of plasma FFA, AcAc, and BOHB in thyrotoxic rats. Phentolamine lifted the amount of plasma FFA, AcAc, and BOHB in starved rats, though propranolol did not change the amount of them. These results indicate that the adrenergic mechanism of the hyperketonemia may not contribute to it in case of thyrotoxic rats, but in case of starved rats, probably being due to α -antipolytic action.

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