Uptake of Ketone Bodies in Perfused Hindquarter and Kidney of Starved, Thyrotoxic, and Diabetic Rats (43572)

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Abstract. **To elucidate the peripheral disposal of ketone bodies in hyperketonemia accompanied with starvation, hyperthyroidism, or diabetes mellitus, the uptake of acetoacetic acid (AcAc) and β-hydroxybutyrate (BOHB) was investigated in the perfused hindquarter and kidney of starved, thyrotoxic, and diabetic rats. Thyrotoxicity was induced by daily subcutaneous injection of thyroxine (100 μg/kg/day) for 7 days, and diabetes mellitus was induced by intraperitoneal streptozotocin (50 mg/kg) injection. Plasma concentration of AcAc and BOHB was significantly higher in starved, thyrotoxic, and diabetic rats than in controls. The hindquarter or kidney of rats was perfused with synthetic medium containing 0.2 or 1.5 m*M* AcAc or BOHB for 30 min at a flow rate of 0.5 ml/g muscle wt/min or 3.2–3.5 ml/kidney/min, respectively. In perfused hindquarter, the uptake of AcAc and BOHB was significantly reduced in starved and diabetic rats, but not in thyrotoxic rats. In perfused kidney, the uptake of AcAc and BOHB was not significantly different between the experimental and control rats. These results suggest that in starvation or diabetes mellitus, disposal of AcAc and BOHB was impaired in the hindquarter but not in the kidney, and that disposal of AcAc and BOHB by hindquarter and kidney was not impaired in thyrotoxic rats.** [P.S.E.B.M. 1993, Vol 203]

Increased lipolysis, ketogenesis, and hyperketonemia are frequently observed in starvation, hyperthyroidism, and diabetes mellitus (1–8). Plasma ketone levels are regulated both by hepatic ketogenesis and by peripheral clearance of ketone bodies. Thus, peripheral clearance of ketone bodies may have an important role in regulating plasma levels of ketone bodies. Many studies have reported an increased ketogenesis in starvation, hyperthyroidism, and diabetes mellitus (2, 3, 6, 9–12). Several investigators (6, 8, 12–14) have suggested that the decreased metabolic clearance rate of ketone bodies in starvation and diabetes mellitus, and we have shown that β-hydroxybutyrate (BOHB) disposal by the perfused hindquarter, was significantly decreased in starved and diabetic rats (15). However, the precise role of the peripheral clearance of ketone bodies in hyperketonemic states remains to be fully elucidated. In the present study, the uptake of acetoacetic acid (AcAc) and BOHB, the main ketone bodies in hyperketonemia, by the perfused hindquarter and kidney was investigated in starved, thyrotoxic, and diabetic rats.

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Received January 17, 1992. [P.S.E.B.M. 1993, Vol 203]
Accepted December 10, 1992.

0037-9727/93/2031-0055$3.00/0

Materials and Methods

Animals. Male Wistar albino rats weighing approximately 120 g were used in the present study.

Reagents. Dextran T-70 was purchased from Green Cross Co., Osaka, Japan. Bovine serum albumin (fraction V), acetoacetic acid, D-β-hydroxybutyrate, palmitic acid, L-thyroxine, and streptozotocin were obtained from Sigma Chemical Co., St Louis, MO. DL-α-Alanine was purchased from Wako Chemical, Osaka, Japan. L-Thyroxine was dissolved in a small volume of 0.01 *N* NaOH and brought to a concentration of 100 μg/ml with saline. The experimental rats were administered thyroxine (100 μg/kg/day) subcutaneously for 7 days, or administered intraperitoneal streptozotocin (50 mg/kg) 7 days before the experiment. The control rats were injected with subcutaneous or intraperitoneal ve-

hicle alone. After an overnight fast, control, thyrotoxic, and diabetic rats were anesthetized with pentobarbital sodium (40 mg/kg, ip) and the blood was drawn from the femoral vein, and then used for the perfusion study. After fasting for 48 hr with free access to a dilute electrolyte solution (78 mEq/liter of sodium and 15 mEq/liter of potassium), starved rats were treated in the same manner.

Perfusion Medium. Palmitic acid was dissolved in ethanol and bovine serum albumin on a hot plate (60°C). The perfusion medium consisted of a Krebs-Ringer bicarbonate buffer containing 0.5% bovine serum albumin, 4.6% dextran T-70, 0.5 m*M* alanine, 0.5 m*M* palmitic acid, and 8.3 m*M* glucose. To prevent glucose metabolism by erythrocytes and possible influence on AcAc and BOHB metabolism by erythrocytes, erythrocyte-free medium was used in the present study.

Perfusion of Hindquarter. The modified method (15) of Ruderman *et al.* (16) was used for the isolation and perfusion of the rat hindquarter. In brief, after anesthesia, the abdomen was opened. After injection of heparin (200 units), the abdominal aorta was ligated and incised between the left renal and iliolumbar vessels. An inflow cannula was inserted and passed to a point midway between the iliolumbar vessels and the aortic bifurcation. The cannula was then fixed in place. Then the perfusion pump was started. All viscera except the urinary bladder, testes, prostate, and seminal vesicle were removed, and some of the major abdominal branch of great vessels were ligated. Since it was not possible to collect the perfusate quantitatively from the inferior vena cava because of anastomotic connections with the vertebral veins, the operated animal was bisected just above the aortic cannulation. The effluent was allowed to drip into the chilled tube. The hindquarter was perfused without recirculation with a synthetic medium at a flow rate of 0.5 ml/g muscle wt/min.

Perfusion of Kidney. The technique for the isolated, perfused rat kidney was described elsewhere (17). After the abdomen was opened, a loose ligature was placed around the inferior vena cava above and below the right renal vein and the right renal artery. The polyethylene cannula (1 French scale) was inserted into the right ureter to collect the urine, and an inflow cannula was inserted through the superior mesenteric artery into the right renal artery. The ligature around the vena cava above the right renal vein was tied, and an outflow cannula was inserted into the vena cava. Finally, the ligature around the vena cava below the right renal vein was tied. The mean weight of right kidney of six rats was 1.0 g in control and 0.9 g in starved, thyrotoxic, and diabetic rats. Therefore, the right kidney was perfused without recirculation with a synthetic medium at a flow rate of 3.5 ml/min in control and 3.2 ml/min in starved, thyrotoxic, and diabetic rats.

Perfusion Method. After a 15-min basal period, the hindquarter or kidney was perfused with the medium containing AcAc or BOHB (0.2 m*M* or 1.5 m*M*) after 30 min. The venous effluent was collected every 5 min, and stored at −70°C until the time of assay. During perfusion, the medium and the chamber were warmed and kept at 37°C and the medium was bubbled with a mixture of 95% O_2 and 5% CO_2. The pH was maintained at 7.4.

Calculations

The oxygen consumption in the hindquarter was calculated from the difference between influent and effluent oxygen concentrations. The uptake of glucose, AcAc, or BOHB by the hindquarter and kidney for 30 min was calculated by the formula: glucose, AcAc, or BOHB infused for 30 min and effluent glucose, AcAc, or BOHB for 30 min, respectively. The concentration of effluent AcAc during the BOHB perfusion and effluent BOHB during the AcAc perfusion was measured, and the effluent BOHB or AcAc concentration was calculated by subtracting these interconversions of ketone bodies.

Measurements. The oxygen content of the medium was determined according to VanSlyke and Neill (18). Effluent glucose concentration was measured by the glucoside oxidase method (19). Lactate (20), AcAc, and BOHB concentrations were measured enzymatically (21). ATP was determined by the method of Lamprecht and Trautschold (22). Insulin was measured by radioimmunoassay (23). Triiodothyronine and thyroxine were assayed by commercial radioimmunoassay kit. Intra- and interassay coefficients of variation were 4% and 8% in AcAc and 3% and 7% in BOHB.

Statistical Analysis. The data are expressed as means ± SD. Analysis of variance and two-tailed Student's *t* test were used for statistical evaluation.

Results

Oxygen Consumption, Lactate Formation, and ATP Concentration. Oxygen consumption was 0.3–0.4 μmol O_2/min/g, and lactate formation was 0.08–0.12 μmol/min/g in perfused hindquarter. ATP concentration in perfused hindquarter (4.6 ± 0.4 μmol/g) was not significantly different from that in hindquarter *in vivo* (5.1 ± 0.5 μmol/g).

Urinary Excretion of Ketone Bodies. Urine volume was 600–1000 μl/30 min. There was no significant difference in urine volume between the experimental and control rats. Urinary concentration of AcAc was 0–0.01 mmol/liter or 0.02–0.04 mmol/liter in the kidney perfused with 0.2 or 1.5 m*M* AcAc, and that of BOHB was 0–0.02 mmol/liter or 0.02–0.05 mmol/liter in the kidney perfused with 0.2 or 1.5 m*M* BOHB.

There were no significant differences in urinary ketone bodies between the experimental and control rats.

Blood Glucose, Plasma Insulin, Triiodothyronine, Thyroxine, and BOHB Levels in Starved, Thyrotoxic, and Diabetic Rats. As shown in Table I, blood glucose was significantly higher in thyrotoxic and diabetic rats and significantly lower in starved rats compared with control rats. Plasma insulin, triiodothyronine, and thyroxine levels were significantly higher in thyrotoxic rats and significantly lower in starved and diabetic rats. Plasma AcAc and BOHB concentration was significantly higher in starved, thyrotoxic, and diabetic rats than in controls.

AcAc Concentration in Effluent Perfused with BOHB. AcAc concentration was 0–0.01 and 0–0.02 mmol/liter in the effluent of hindquarter perfused with 0.2 and 1.5 mmol/liter of BOHB, and it was 0–0.01 mmol/liter in the effluent of kidney perfused with 0.2 and 1.5 mmol/liter of BOHB, respectively. There were no significant differences between the experimental and control rats.

BOHB Concentration in Effluent Perfused with AcAc. BOHB concentration was 0–0.01 and 0.02–0.04 mmol/liter in the effluent of hindquarter perfused with 0.2 and 1.5 mmol/liter of AcAc, and it was 0–0.01 and 0.01–0.03 mmol/liter in the effluent of kidney perfused with 0.2 and 1.5 mmol/liter of AcAc, respectively. There were no significant differences between the experimental and control rats.

Glucose Uptake by Perfused Hindquarter and Kidney. As shown in Table II, glucose uptake by perfused hindquarter was significantly decreased in starved and diabetic rats. That in thyrotoxic rats was not significantly different from controls. Glucose uptake by perfused kidney was not significantly different between the experimental and control rats.

AcAc and BOHB Uptake by Perfused Hindquarter and Kidney. As shown in Table III, AcAc and BOHB uptake by perfused hindquarter was significantly decreased in starved and diabetic rats. Uptake in thyrotoxic rats was similar to that in controls. AcAc and BOHB uptake by perfused kidney was similar in all groups.

Discussion

Plasma AcAc and BOHB concentrations were increased to similar plasma levels in starved, thyrotoxic, and diabetic rats, which suggests that the present animal model is available for studying the metabolism of AcAc and BOHB. Glucose uptake by the hindquarter was significantly reduced in starved and diabetic rats, but not in thyrotoxic rats. This result was in agreement with the report that glucose uptake by the muscle was significantly decreased in diabetes mellitus (24–26) and that glucose disposal rate in hyperthyroid patients was not significantly different from that in controls (27). Glucose uptake was not influenced by the concentration of perfused AcAc or BOHB, which suggests that ketone bodies have no direct effect on glucose uptake. This result was consistent with the reports that ketone bodies caused no change in glucose disposal in perfused rat heart (28), perfused rat hindquarter (15), and humans (29), although several reports have shown that ketone infusion decreased glucose disposal in humans (30) and miniature pigs (31).

The uptake of AcAc and BOHB by perfused hindquarter, as well as the glucose uptake, was significantly decreased in starved and diabetic rats. This result suggests that the decreased clearance of ketone bodies in the muscle may be responsible in part for the decrease in metabolic clearance rate of ketones observed in starvation and diabetes (6, 8, 15). Ruderman and Goodman (13, 14) have reported that using perfused rat hindquarter, the uptake of AcAc was not altered by starvation. They used female rats and we used male rats. There may be sex differences in ketone body utilization during starvation. The mechanism by which the uptake of glucose, AcAc, and BOHB in the hindquarter was reduced in starved and diabetic rats but not in thyrotoxic rats is unknown in the present study.

As far as we know, this is the first report regarding the uptake of ketone bodies by perfused kidney. Starvation, thyrotoxicosis, and diabetes mellitus did not influence the uptake of glucose, AcAc, and BOHB in the perfused kidney, which suggests that their metabolism in the kidney may be different from that in the muscle.

Table I. Blood Glucose, Plasma Insulin, Triiodothyronine, Thyroxine, BOHB, and AcAc Concentrations in Starved, Thyrotoxic, and Diabetic Rats[a]

	Blood glucose (mg/dl)	Plasma insulin (μU/ml)	Triiodothyronine (ng/dl)	Thyroxine (μg/dl)	BOHB (mmol/liter)	AcAc (mmol/liter)
Control ($n = 6$)	78 ± 5	10 ± 4	78 ± 12	2.9 ± 0.4	0.42 ± 0.11	0.10 ± 0.01
Thyrotoxic ($n = 6$)	88 ± 6[b]	27 ± 9[b]	173 ± 35[b]	12.1 ± 0.7[b]	1.17 ± 0.25[b]	0.55 ± 0.06[b]
Starved ($n = 6$)	66 ± 5[b]	<5[b]	39 ± 8[b]	1.2 ± 0.2[b]	1.26 ± 0.20[b]	0.70 ± 0.11[b]
Diabetic ($n = 6$)	412 ± 94[b]	<5[b]	39 ± 10[b]	1.2 ± 0.2[b]	1.42 ± 0.18[b]	0.73 ± 0.12[b]

[a] The values are presented as means ± SD.
[b] $P < 0.02$, significantly different from control.

Table II. Glucose Uptake by Perfused Hindquarter and Kidney of Starved, Thyrotoxic, and Diabetic Rats[a]

	Perfusate BOHB 0.2 mmol/liter		Perfusate BOHB 1.5 mmol/liter		Perfusate AcAc 0.2 mmol/liter		Perfusate AcAc 1.5 mmol/liter	
	Hindquarter	Kidney	Hindquarter	Kidney	Hindquarter	Kidney	Hindquarter	Kidney
Glucose uptake (μmol/30 min)								
Control (n = 6)	281 ± 56	38 ± 10	284 ± 53	39 ± 9	280 ± 63	38 ± 10	289 ± 59	39 ± 10
Thyrotoxic (n = 6)	253 ± 60	39 ± 10	243 ± 52	39 ± 9	268 ± 59	40 ± 9	252 ± 58	39 ± 10
Starved (n = 6)	166 ± 42[b]	39 ± 9	189 ± 41[b]	40 ± 10	180 ± 41[b]	39 ± 9	188 ± 46[b]	40 ± 9
Diabetic (n = 6)	106 ± 39[b]	38 ± 10	120 ± 38[b]	38 ± 10	110 ± 41[b]	39 ± 10	128 ± 51[b]	38 ± 9

[a] The values are presented as means ± SD.
[b] $P < 0.02$, significantly different from control.

Table III. AcAc and BOHB Uptake by Perfused Hindquarter and Kidney of Starved, Thyrotoxic, and Diabetic Rats[a]

	Perfusate AcAc or BOHB 0.2 mmol/liter		Perfusate AcAc or BOHB 1.5 mmol/liter	
	Hindquarter	Kidney	Hindquarter	Kidney
AcAc uptake (μmol/30 min)				
Control (n = 6)	7.6 ± 2.0	2.6 ± 1.0	39.8 ± 9.8	16.5 ± 3.1
Thyrotoxic (n = 6)	6.2 ± 1.9	2.6 ± 0.9	37.5 ± 9.1	16.1 ± 3.0
Starved (n = 6)	4.1 ± 0.9[b]	2.3 ± 0.9	28.1 ± 8.0[c]	15.4 ± 3.1
Diabetic (n = 6)	2.8 ± 0.7[b]	2.1 ± 1.0	24.8 ± 6.5[b]	15.7 ± 3.1
BOHB uptake (μmol/30 min)				
Control (n = 6)	5.1 ± 1.0	3.0 ± 1.0	28.2 ± 5.5	16.2 ± 2.6
Thyrotoxic (n = 6)	4.8 ± 0.9	3.0 ± 0.9	27.1 ± 5.2	16.0 ± 2.7
Starved (n = 6)	3.5 ± 0.8[c]	2.9 ± 0.9	16.9 ± 4.8[b]	15.9 ± 2.9
Diabetic (n = 6)	2.5 ± 0.4[b]	2.7 ± 1.0	15.3 ± 3.0[b]	15.7 ± 3.0

[a] The values are presented as means ± SD.
[b] $P < 0.02$, significantly different from control.
[c] $P < 0.05$, significantly different from control.

We conclude that the utilization of ketone bodies is impaired in the muscle, but not in the kidney, in starvation and diabetes, and that peripheral disposal of ketone bodies may have no important role in hyperketonemia in hyperthyroidism.

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