過激な運動によって起こるラットの糖代謝異常に関する研究

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Studies on Glycometabolic Disorder of Rats in Excessive Fatigue Caused by Heavy Exercise

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ABSTRACT

Severe hyperglycemia was observed in rats during swimming which were subjected to swimming repeatedly every day for a long period of time and showed excessive fatigue. However, the mechanism of such specific hyperglycemia is still not clear. In these studies, the authors tried to clarify the mechanism through investigations on hematological properties, urinary corticosterone, the effects of adrenergic blockers and influence of hyperadrenocorticism on blood glucose levels.

The results were summarized as follows:

1. Incresed urinary corticosteron content and decreased blood insulin level were observed in exhausted rats during forced swimming.
2. Hyperglycemia was inhibited by the administration of α-or β-adrenergic blocking agents.
3. Severe hyperglycemia was observed in ACTH treated rats by single forced swimming.

From these results, the mechanism of hyperglycemia in rats during swimming under excessive fatigue is considered to involve the actions of epinephrine and glucocorticoid on glucose metabolism.

Key words: hyperglycemia, excessive fatigue, adrenergic blockers, rats.

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When rats are subjected repeatedly every day to forced swimming for a long period of time, they are exhausted so much that they cannot continue swimming. Ichiki and Usui showed that bones of rats under such extreme fatigue developed severe bone metabolic disorders such as osteoporosis and suggested that hyperadaptation of the adrenal cortex might be one of the causes of such degeneration[1]. By carrying out the same experiment, Nakatsu and Ichiki also demonstrated that specific changes such as marked hyperglycemia, increased hepatic glycogen, and a rise in hepatic glucose-6-phosphatase activity occured in the rats[6]. However the mechanism of such glucose metabolic disorders is still not clear.

The present studies were carried out to clarify the mechanism of hyperglycemia, one of the above-mentioned glucose metabolic disorders.

Materials and Methods

1. Experiment 1

Male rats of Wistar-Imamichi strain 50 days of age were used throughout the experiment. The animals were divided into intact control rats and exercised rats. During the experiment, they were kept twos to a cage and given a pellet diet (F-2, Funabashi Nohjo) and fresh water ad libitum. The animal room was lighted throughout the day by lamps, and the room temperature was controlled about 25°C.

Forced swimming was applied to the exercised rats in a pool with the water temperature controlled at 29 ± 1°C. The pool was 50×100 cm in perimeter and 70 cm in depth, and filled with water to 30 cm below the rim to prevent the rats from jumping out of the pool. To train these rats in swimming, one hour of swimming was performed on the first day and

was extended gradually at the rate of 30 minutes a day until six hours of swimming was reached on the 11th day of the experiment. This six hours of swimming was continued until the 63rd day, and thereafter extended at a rate of 20 minutes a day until eight hours of swimming was achieved on the 69th day from the beginning of the exercise. The swimming exercise was devided into two parts with 2 hours resting interval.

Body weight was mesured for the intact control rats every mornig and for the exercised rats before swimming.

When the exercised rats were considered in a state of resistance from their body weight increases and swimming conditions, blood samples were drawn from the carotid artery of one half of the swimming rats under general anesthesia as the R-swimming group. At the same time, one half of the intact control rats was designated as the R-control group, and blood samples of them were drawn in the same manner.

When the remaining half of the exercised rats was considered in a state of exhaustion from observations of body weight decreases and inability to continue swimming, blood samples were drawn in the same manner as before. These rats were designated as the E-swimming group. At the same time, the another half of the intact control rats was provided as the E-control group.

Blood samples of these 4 groups were taken after 15 hours of fasting, and those of one half of E-swimming group were drawn before swimming and those of the other half of this group and R-swimming group were drawn 3 hours after the start of swimming.

Using sera separated from these blood samples, total protein, albumin, glucose, non-esterified fatty acid (NEFA), alanine, urea nitrogen, creatinine, Ca, inorganic phosphrus (P), Na^+ and K^+ were determined biochemically, and insulin and glucagon were mesured by radioimmunoassay.

Six rats each in the R-control and R-swimming groups, and 5 rats each in the E-control and E-swimming groups were kept in individual metabolic cages 3 days before the blood sampling, and 24 hour urine was collected. Using this urine, corticosterone was determined by radioimmunoassay.

2. Experiment 2

Using the animals in Experiment 1, observations of the effect of adrenergic blocking agents on blood glucose levels were performed.

Four days before the blood analysis, the fasting animals in the R-swimming and E-swimming groups were divided into 4 groups. The first group was given with 0.9% saline solution, the second one was given phentolamine mesylate (α-blocker), the third one was given propranolol chloride(β-blocker), and the fourth one was given both α and β-blockers. The dose of α and β-blockers were 0.5 mg and 1.5 mg per 100 g of body weight respectively. These blockers and saline solution were injected intraperitonealy 15 minutes before swimming in the R-swimming group, and 60 minutes after the start of swimming in the E-swimming group. Blood samples were drawn from the opthalamic vein plexus before swimming and 1, 2 and 3 hours after the start of swimming. Using these samples, the blood glucose level was determined.

3. Experiment 3

Male rats of the Wister-Imamichi strain 16 weeks of age were used. They were divided into two groups, i.e. intact control rats (control group) and ACTH treated rats (ACTH group). Rats of the latter group were injected subcutaneusly with 0.1 mg of synthetic ACTH daily for 14 days. When treatment with ACTH was finished, forced swimming was applied to all rats in the control and ACTH groups prior 15 hours fasting until they could not continue swimming : both groups were deprived of solid food for 15 hours. Blood samples were drawn in the same manner as in Experiment 2 before swimming, 60 minutes after the start of swimming and just before death. Using these blood samples, the glucose level was determined. Adrenal glands were taken from the carcasses and weighed.

Results

1. Experiment 1

1) Body weight: The control rats grew constantly, while the swimming rats showed remarkably rest-

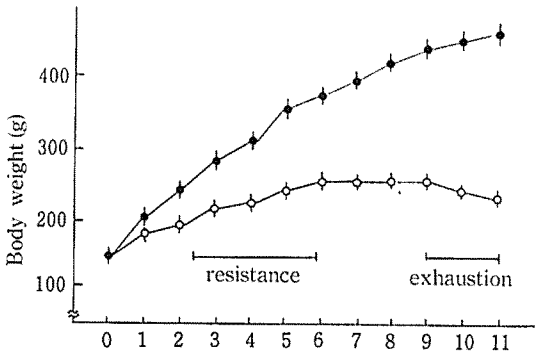

Fig. 1. Changes in body weights of control group and swimming group.
● control group (n=13)
○ swimming group (n=15)

rained growth in the end. The body weight of the latter increassed in the period from 3 to 6 weeks at a low rate but steadily, and thereafter it was kept plateau until 9 weeks. Then it started to decrease gradually and the rats could not continue swimming (Fig. 1).

2) Clinical conditions: The control rats showed no changes in their conditions and kept good health until the end of experiment. The swimming rats were active swimmer in the period of from 3 to 6 weeks and could continue swimming until 9 weeks. After these periods, the swimming rats became difficult to continue swimming, and some ones of them were drowned.

3) Blood chemical constituents: The R-swimming group showed a signficant decrease of blood glucose and slight decrease of Na^+, creatinine, and alanine, while the values of NEFA and urea nitrogen were elevated significantly (Table. 1).

The E-swimming group showed a low level of Ca compared with the E-control group. The level of K^+ was elevated before swimming, but lowered significantly after swimming. The values of creatinine and alanine decreased after swimming. This group showed a markedly low level of blood glucose before swimming, but it was elevated remarkably after swimming. In spite of the remarkably high level of NEFA observed in the R-swimming group, no change was found in the E-swimming group.

4) Blood insulin, glucagon, and urinary corticosterone: The blood level of immunoreactive insulin was low in both the R-swimming and E-swimming groups after swimming. The level of immunoreactive glucagon tended to decrease slightly in both the R-swimming and E-swimming groups after swimming. The total value of urinary corticosterone showed no change in

Table 1. Values of blood biochemical analysis in the control and swimming groups.

	State of resistance		State of exhaustion		
	R-control group (n = 8)	R-swimming group after swim. (n = 8)	E-control group (n = 10)	E-swimming group	
				before swim. (n = 4)	after swim. (n = 5)
Total Protein (g/dl)	4.8±0.04	4.8±0.06	5.6±0.08	5.5±0.08	5.5±0.04
Albmin (g/dl)	2.0±0.02	2.0±0.06	2.1±0.03	2.0±0.06	2.0±0.04
A/G	0.70±0.02	0.71±0.03	0.59±0.02	0.59±0.03	0.58±0.02
Na (mEq/l)	146.7±0.47	144.2±0.56*	146.7±0.27	144.4±0.67**	144.6±1.15*
K (mEq/l)	5.30±0.14	5.44±0.17	5.46±0.19	6.33±0.31**	5.23±0.39
Ca (mg/dl)	9.40±0.08	9.11±0.12	9.48±0.10	8.52±0.08**	8.53±0.07**
Inorganic P (mg/dl)	9.8±0.23	9.8±0.14	7.4±0.40	7.6±0.76	7.2±0.90
Urea-N (mg/dl)	17.1±0.91	25.3±2.87**	21.9±0.56	26.5±1.45	25.0±2.50
Creatinine (mg/dl)	0.61±0.02	0.54±0.02*	0.69±0.02	0.61±0.03	0.53±0.39**
Glucose (mg/dl)	144.3±9.26	102.3±4.24**	147.0±5.57	39.0±17.32**	176.1±18.38
NEFA (mEq/l)	0.61±0.05	1.22±0.09**	0.61±0.03	0.64±0.06	0.66±0.03
Alanine (μM/dl)	42.7±4.20	19.5±2.60	36.0±4.05	32.0±3.10	20.1±1.30**

* Significantly different from the control ($P<0.05$)
** Significantly different from the control ($P<0.01$)

Table 2. Plasma concentration of immunoreactive insulin, immunoreactive glucagon and total urinary corticosterone.

	State of resistance		State of exhaution		
	R-control group	R-swimming group after swim.	E-control group	E-swimming group	
				beforo swim.	after swim.
Insulin (μU/ml)	55.4±15.80 (n=5)	24.5±4.08 (n=6)	83.8±27.61 (n=5)	53.8±17.60 (n=4)	13.2±5.49 (n=5)
Glucagon (pg/ml)	50.8± 5.80 (n=6)	37.2±5.48 (n=3)	24.8± 1.70 (n=4)	38.3±11.25 (n=4)	21.6±1.38 (n=5)
Corticosterone (ng/day)	85.9±12.90 (n=6)	83.3±8.08 (n=6)	114.6±16.19 (n=5)	354.2±115.90 (n=5)	

mean±S.D. n: Number of rats.

the R-swimming group, while it increased excessively in the E-swimming group (Table 2).

2. Experiment 2

An increase in the blood glucose level was observed 60 minutes after the start of swimming in the saline treated rats of the R-swimming group, and it dropped gradually, while this elevation of the blood glucose level was inhibited by the injection of an α or β-blocker. When rats were treated with both blockers, the blood glucose was kept at a constantly low level (Fig. 2).

The blood glucose level of the saline treated rats in the E-swimming group increased continuously after the start of swimming. The constant elevation of blood glucose level was lowered by injection of either the α or β-blocker, but the lowered rate caused by the β-blocker was slower than that by the α-blocker. When rats were treated with both together 60 minutes after the start of swimming, the elevated value of blood glucose was lowered to the intial low level (Fig. 3).

3. Experiment 3

The blood glucose level of the control and ACTH groups before swimming was about 100 mg/dl, and no difference was observed between these two groups.

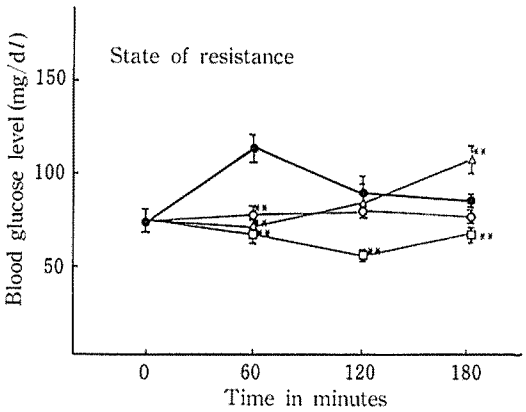

Fig. 2. Effect of adrenergic blockers on blood glucose level in swimming rats.
● given saline (n=6).
○ given α blocker (n=8).
△ given β blocker (n=7).
□ given $\alpha+\beta$ blocker (n=7).
** Significantly different from the saline treated rats. ($P<0.01$)

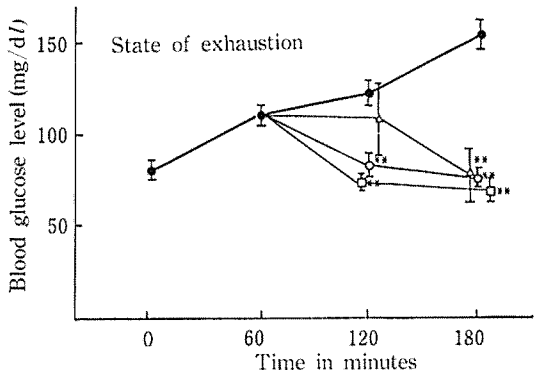

Fig. 3. Effect of adrenergic blockers on blood glucose level in swimming rats.
● given saline (n=7).
○ given α blocker (n=7).
△ given β blocker (n=6).
□ given $\alpha+\beta$ blocker (n=5).
** Significantly different from the saline treated rats. ($P<0.01$)

Table 3. Changes in blood glucose level in the control and ACTH groups in Experiment 3.

Groups	Before swimming	60 min. after start	Just before death
Control group	107.1±2.9 mg/dl	97.9±13.7	42.5± 6.6
ACTH group (n=7)	103.2±4.8	466.2±38.9**	397.6±31.9**

mean±S.D. n: Number of rats.
** Significantly different from the control ($P<0.01$)

The level in the control group 60 minutes after the start of swimming showed slight decreases, but there were no significant changes. The final values decreased markedly. The glucose level in the ACTH group 60 minutes after the start of swimming increased remarkably to about 4.5 times the initial value. The final value in this group remained high, 4 times the initial level (Table 3).

The weight of the adrenal glands of the control group was about 25 mg for the left and right sides. On the other hand, the weight in the ACTH group was about 8 times greater than that of the control group.

Discussion

Marked changes detected by examinations of the blood and urine in exercised rats were seen for NEFA, glucose, alanine, insulin, and urinary corticosterone.

NEFA is an important energy source for muscular motion and is released from adipose tissue by epinephrine when animals exercised[5]. A marked increase in blood concentration of NEFA was observed in the rats in the R-swimming group, while no increase could be found in the rats of the E-swimming group. The latter fact is thought to indicate the lack of body fat stores, and to be one of the factors inducing exhaustion in rats.

The blood glucose level of rats in a state of exhaustion was elevated excessively after only 3 hours of swimming. It is well known that a lack of insulin causes hyperglycemia[3]. The very low level of insulin observed in the rats of the E-swimming group suggests that the appearance of hyperglycemia in this group was caused by this low level. Although the blood insulin level was lowered in the R-swimming group, the blood glucose level dropped to the initial value after an early temporary increase. These phenomena are considered to be due to the low decrease rate of the insulin level and the increase in peripheral glucose utilization. This increase in peripheral utilization was considered to be caused by exercise and to exceed the depression of glucose utilization caused by the lack of insulin.

An excessive amount of urinary corticosterone was observed in the E-swimming group, while like this phenomenon did not appear in the R-swimming group. The former result is considered to be another cause of hyperglycemia in the E-swimming group since an excessive amount of corticosterone has an antagonisitc effect against insulin[7].

Although it is well known that the increase of alanine stimulates the secretion of glucagon under conditions of hypersecretion of glucocorticoid[4,10], a decrease in alanine and a slight lowering of glucagon levels were observed in both the R-swimming and E-swimming groups. Therefore, it is considered that the hyperglycemia observed in the E-swimming group was not affected by glucagon.

The increase of blood glucose level in the early period of exercise in the R-swimming group and the continuous increase in the E-swimming group were inhibited by the administrations of adrenergic blockers. These results suggest that epinephrine affected the hyperglycemia found in the exercised rats.

The mode of action of epinephrine on glucose metabolism is highly complex. Epinephrine directly stimulates peripheral glucose utilization via α-adrenergic receptors[11] and inhibits the utilization vai the β-receptors[8]. Epinephrine also stimulates the secretion of insulin via the β-receptors and inhibits it via the α-receptors[2]. Thus, there are multiple mechanisms for adrenergic regulation of glucose responses of the body[9]. For example, hypo-

glycemia is observed under certain conditions such as alkalosis[13] or during strenuous exercise[11] because peripheral utilization is accelerated by epinephrine via α-receptors. On the other hand, hyperglycemia is observed under conditions of hypersecretion of glucocorticoid[12], because the susceptibility of β-receptors in peripheral tissue is accelerated by glucocorticoid.

Adrenergic blockers have no effect on hepatic glucogenesis *in vivo*[8]. Therefore, changes in blood glucose level caused by the injection of adrenergic blockers in exercised rats are considered to be due to the effect of blockers on peripheral glucose utilization and insulin secretion.

In brief, the following conclusions have been drawn: 1) the hyperglycemia observed in the E-swimming group during swimming is caused by hypersecretin of epinephrine which inhibits the peripheral glucose utilization; 2) this inhibition is induced by the direct action of epinephrine via the β-receptors of peripheral tissue and its inhibiting effect on insulin secretion via the α-receptors; and 3) the predominant susceptibility of peripheral β-receptors is caused by hypersecretion of glucocorticoid, and the predominance of α-receptors in the pancreas is induced by swimming.

If the above conclusions are correct, hyperglycemia should occur in rats after swimming only once under conditions of hypersecretion of glucocorticoid. Therefore, the authors carried out experiment 3 to confirm this phenomenon.

The results of the experiment showed severe hyperglycemia in the rats treated with ACTH, and hypoglycemia in the control rats when they underwent forced swimming only once. An excessive increase of adrenal weight was detected in the ACTH group. This excessive increase in adrenal weight indicates clearly that the rats had hyperadrenocorticism. The hypoglycemia in the control rats is naturally considered to due to the effect of epinephrine secreted by the stimilation of swimming on peripheral tissue via the α-receptors. These data indicates that hyperglycemia occurs after swimming only once under conditions of hypersecretion of glucocorticoid, as the authors concluded.

From the conclusions stated above and the results of experiment 3, the mechanism of hyperglycemia observed in the E-swimming group during swimming has been clarified to be affected by the actions of epinephrine and glucocorticoid.

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induced by α-adrenergic stimulation during alkalosis. Eur. J. Pharmacol., 41, 93-102.

過激な運動によって起こるラットの糖代謝異常に関する研究

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要約 長時間，反復して遊泳を負荷し，極度の疲労に陥らしめたラットは遊泳中に高血糖を生じた。この高血糖の発症機序を明らかにするため，血清生化学的変化，糖代謝に関与するホルモンの変化，血糖変化に対するアドレナリン遮断薬の影響，および副腎皮質機能亢進時の血糖値の変化を検討した。

その結果以下のことが知られた。1. 高血糖を示したラットでは血中インスリンの低下および尿中コルチコステロンの増加が認められた。2. 血糖上昇は，α，およびβ-アドナリン遮断薬のいずれによっても抑制された。3. ACTH を投与されたラットは唯一回の遊泳負荷によっても高血糖を生じた。

以上の結果から，長期の強制遊泳運動による極度の疲労に陥ったラットの遊泳時に見られた高血糖の発生機序は，遊泳によって分泌されるエピネフリンと，過剰に分泌されるグルココルチコイドによって引き起されるものと考えられた。

キーワード：高血糖，過労，アドレナリン遮断薬，ラット． 日獣畜大研報，35，97～103，1986.