Studies on the specificity of adrenocortical function in ruminant nutrition

V. Influence of physical and psychological stresses on plasma adrenocorticoids and blood glucose level in ruminants and rodents

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(Received for Publication on March 18, 1971)

When an animal is exposed to a stress, the body responds towards adaptation^{1a)}. Increase of plasma adrenocorticoids (11-OHCS) is one of the signs of such responses.

Seasonal variations in plasma 11-OHCS level were investigated in the previous study^{b)}, where it was suggested the level in cattle and sheep might be more stable than in rats as described by KNIGGE^{b)}. It was reported in sheep that the elevation of blood glucose is another sign of response to meteorological stresses^{b)}.

In the present study, relative importance of these two parameters as the indices of the responses was investigated by exposing the selected species of animals to meteorological stresses, i.e. cold and hot temperatures.

The author also intended to study on the responses of cattle, sheep and rabbits to psychological stresses. Transportation of the animal was selected as the stressor following the opinion of REID^{7,8)}.

Finally, effects of exogenous ACTH were tested to evaluate the effect of stressors through hypothalamo-hypophyseal level.

Materials and Methods

Experimental animals, cattle (Japanese Black), sheep (Japanese Corriedale), rabbits (Japanese native) and rats (Wistar strain) were fed at the experimental farm of Kyôto University. Cattle were young steers and about 16 months of age. Sheep were adult wethers. Rabbits and rats were adult males.

This study consisted of four experiments. The treatment of each experiment was as follows. Exp. I ("hot" stress): sheep and rats were moved from outdoor environment into a hot room of phytotron at about 6 A.M. The rise of temperature associated with this move was 16-21°C (Table 1). Exp. II ("cold" stress): Sheep and rats were maintained in the hot room for five days, then were moved back to the outdoor environment. The fall of the temperature was 15-20°C, when sheep and rats were moved at 1 and 6-7 A.M., respectively. These two experiments were performed in the period from February 6 to March 18, in 1970 in Kyôto. Blood samples of sheep (4 heads) were taken from each animal at 0, 1, 2, 4, and 8 hours following the move. In rats (8 heads), the samples were taken from different animals allotted to each time, i.e. 0, 1/2, 1, and 2 hours.

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Table 1. Conditions of Experiments I and II

Animal	Experiment I ("hot" stress)			Experiment II ("cold" stress)		
	Date ^{a)}	Time at the start of treatment	Difference of the temperature (°C)	Date ^{a)}	Time at the start of treatment	Difference of the temperature (°C)
Sheep	2. 6	a.m. 5:30	21 (-1-20) ^{b)}	2.12	a.m. 1:00	15 (20-5) ^{b)}
	3. 2	a.m. 6:30	21 (-2-19)	3. 7	a.m. 1:00	19 (20-1)
Rat	2.16	a.m. 6:30	16 (4-20)	2.26	a.m. 7:00	20 (20-0)
	3. 7	a.m. 6:00	19 (1-20)	3.18	a.m. 6:00	18 (20-2)

a) These experiments were carried out in Kyōto in 1970

b) The numbers in parentheses show the temperatures of outdoors and the room of phytotron

Exp. III (ACTH): Either ACTH* or saline (0.9%) was injected intraperitoneally, into cattle (34.0 I.U.), sheep (6.5 I.U.) and rabbits (0.8 I.U.). Blood samples were taken at 0, 2, and 6 hours after the injection in cattle and sheep, and 1 hour in rabbits.

Exp. IV (psychological stress): Cattle, sheep and rabbits were transported by a truck for 120, 90, and 10 minutes, respectively. Blood samples were taken from the same animal at 0, 1, 2, 4, and 8 hours following the initiation of transportation in sheep (4 heads). In rabbits, blood was taken from all animals (12 heads) 10 days before the exposure to the stress to determine the initial control of plasma 11-OHCS level. After the exposure, samples were taken from different animals allotted to each predetermined time, i.e. 1/2, 1, 2, and 4 hours. In cattle (4 heads) blood samples were taken from each animal at one day before, and 2, and 24 hours after the initiation of transportation.

The site from which blood samples were taken and the method of determination of 11-OHCS were the same as in the previous reports^{10,11)}. Blood glucose was determined by Somogyi method¹⁴⁾.

Results

The levels of plasma 11-OHCS in Exps. I and II are shown in Figs. 1 and 2. By the "hot" stress, plasma 11-OHCS level in sheep did not change for the first two hours, but somewhat increased later and decreased beneath the original value in 8 hours after the exposure to the stress. All these changes were not statistically significant. In rats, the level increased rapidly and significantly, and returned to the original value within 1 hour after the exposure.

By the "cold" stress, sheep and rats responded as shown in Fig. 2. Changes of plasma 11-OHCS level from the original level in sheep were not significant. In rats, the level rose rapidly and significantly after the exposure to the stress, and remained high for more than two hours.

Figs. 3 and 4, indicate the changes of blood glucose in Exps. I and II. By the "hot" stress, blood glucose increased significantly as the time proceeded in sheep ($p < 0.05$), but did not change in rats. By the "cold" stress, the blood glucose did not change in sheep, while there was a slight increase in rats.

ACTH injection had no effect on blood glucose in any animal tested at the dose employed here, as shown in Table 2.

The effects of transportation on plasma 11-OHCS level are shown in Fig. 5. The level

* ACTH "Daiichi", a commercial preparation of ACTH by Daiichi Seiyaku Co. Ltd., in Japan

Influence of stresses on plasma 11-OHCS and blood glucose level

Fig. 1. The influence of "hot" stress on plasma 11-OHCS level in sheep and rats (Experiment I)

Each mark shows mean plasma 11-OHCS level and standard deviation (cross (×)-sheep, solid circle (●)-rats)

Fig. 2. The influence of "cold" stress on plasma 11-OHCS level in sheep and rats (Experiment II)

Each mark shows mean plasma 11-OHCS level and standard deviation (cross (×)-sheep, solid circle (●)-rats)

Table 2. The influence of ACTH on blood glucose level (mg%) in cattle, sheep, and rabbits

Animal	Treatment	Number	Pre-injection	Post-injection (hours)	
				2	6
Cattle	0.9% NaCl	4	57.2±10.3	56.1± 9.2	54.5±13.8
	ACTH	5	63.5± 5.0	63.3± 5.2	63.8± 9.4
Sheep	0.9% NaCl	6	50.9± 5.9	51.4± 4.8	50.5± 3.7
	ACTH	6	50.0± 7.2	52.0± 6.6	60.0± 8.3
Rabbits	0.9% NaCl	5	—	102.8± 5.4 ^{a)}	—
	ACTH	5	—	102.9±12.4	—

Results expressed as mean±standard deviation

a): The means are the values at one hour after injection of ACTH

in cattle and sheep increased twice the initial value, after having been transported for 2 hours in cattle and 1 hour in sheep. The rise in rabbits was three times of the control, though the time of transportation was only for 10 minutes.

Fig. 3. The influence of "hot" stress on blood glucose level (mg%) in sheep and rats

Each mark shows mean blood glucose level of four and two individuals in sheep and rats, respectively (cross (×)-sheep, solid circle (●)-rats)

Fig. 4. The influence of "cold" stress on blood glucose level (mg%) in sheep and rats

Each mark shows mean blood glucose level of four and two individuals in sheep and rats, respectively (cross (×)-sheep, solid circle (●)-rats)

Fig. 5. The influence of transportation on plasma 11-OHCS level in cattle, sheep and rabbits (Experiment IV)

Each mark shows mean plasma 11-OHCS level and standard deviation (cross (×)-sheep, solid circle (●)-rabbits, open circle (○)-cattle)

Discussion

In general, the action of glucocorticoids may be divided into two main classes: one is to help or protect the animal body from the stress by increasing the resisting ability, and the other is to regulate the metabolic pattern³⁾. In the present study, the plasma 11-OHCS and blood glucose levels were determined to know whether there is any difference in adaptability to stresses between ruminants and rodents. The results suggested that sheep are less responsive to the "hot" and "cold" stresses than rats. REID⁸⁾ mentioned that plasma cortisol level did not alter when ewes were exposed to cold, even in combination with other types of stressors, undernutrition and pregnancy. On the other hand, acute exposure to cold causes a transient rise of adrenocortical activity in rats¹²⁾. These data seem to be in agreement with the results of present experiments.

Plasma 11-OHCS level increased rapidly in the animals subjected to transportation in this study (Fig. 5). REID *et al.*⁷⁾ reported that serum cortisol level increased by transportation. MESCHAKS⁹⁾ reported that urinary neutral steroids increased in the bull following 15-40 km transportation. It is apparent that transportation of long duration might be stressful in physical means, but effects of transportation of short duration should be investigated from psychological viewpoints. In the present experiment (Exp. IV), plasma 11-OHCS level promptly returned to the initial value after a transient but significant rise, though the time course and the degree of elevation were different depending upon the species tested. REID *et al.*⁷⁾, and REID⁸⁾ listed the transportation as a psychological stress. So far as plasma 11-OHCS is concerned, we suggest that sheep may be less sensitive to physical and psychological stresses than the rodents. The results also showed that psychological stresses would be the major cause of trouble rather than the physical ones in the practical husbandry of the sheep.

The responses of blood glucose by cold exposure differ among species^{4,8)}. In our present

study, blood glucose level was elevated in rats by exposing to "cold", but in sheep it was elevated by "hot" stress. The mode of response of blood glucose was similar to that of 11-OHCS in the same species of animal and under the same experimental condition, though the metabolic pattern of the species was different^{1,2,3}).

The injection of ACTH gave no clear result on blood glucose (Table 2). The doses of ACTH used here were reported to elevate plasma 11-OHCS level significantly¹⁰). The reason of ineffectiveness was not clear. It can be suggested, however, the elevations of blood glucose often encountered in this study might not be due to adrenocorticoids, since endogenous hormones other than adrenocorticoids could cause similar changes of blood glucose as suggested by REID⁸).

Summary

The effects of meteorological and psychological stressors on plasma 11-OHCS and blood glucose were investigated in cattle, sheep, rabbits and rats. The results are as follows;

- 1) Plasma 11-OHCS increased in sheep and rats by "hot" stress. This response was remarkable and evident in rats.
- 2) Plasma 11-OHCS level increased significantly in rats by "cold" stress, but the level somewhat decreased in sheep.
- 3) In general, the responses of plasma 11-OHCS level to physical stresses in sheep were less marked than those in rats.
- 4) Blood glucose level increased by "hot" stress in sheep, and by "cold" stress in rats.
- 5) ACTH had no effect on the blood glucose level when administered to cattle, sheep and rabbits.
- 6) Plasma 11-OHCS level was increased by transportation, especially in rabbits.

Acknowledgement

The author wishes to express the deepest appreciation to Dr. S. UESAKA, Dr. R. KAWASHIMA and Dr. K. KUMAZAKI for their valuable advices and encouragements. Thanks are due to Mr. T. Dôgo who cooperated during this experiment.

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副腎皮質機能の反芻動物栄養における特異性に関する研究

V. 各種動物におけるストレスが血漿中副腎皮質ホルモン含量および血糖量に及ぼす影響

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ウシ、ヒツジ、ウサギ、ラットを用いて、環境（とくに温度）の急激な変化（約 20°C）および輸送が血漿中副腎皮質ホルモン（以下 11-OHCS と略す）含量および血糖量に及ぼす影響を調べ、動物間の比較を行なった。

（1）環境温度を急に高くすると、ヒツジおよびラットの血漿中 11-OHCS 含量は上昇した。ことにこれはラットにおいて著しかった。

（2）環境温度を急に低くすると、ラットでは血漿中 11-OHCS 含量の急速かつ有意な上昇をみたが、ヒツジではいくぶん減少する程度であった。

（3）概して、環境温度の急激な変化に対する血漿中

11-OHCS 含量の反応はヒツジの方がラットより低いようであった。

（4）血糖量はヒツジでは高温ストレスにより、有意に上昇し、またラットでは低温ストレスによりいくぶん上昇する傾向にあった。

（5）ウシ、ヒツジ、ウサギにおいて、ACTH 注射により血糖量は増加しなかった。

（6）ウシ、ヒツジ、ウサギにおいて、輸送により血漿中 11-OHCS 含量の増加がみられた。ことにウサギで、この現象は著しかった。

（7）ここに得られた結果から、反芻動物の生理的ストレスや心理的ストレスに対する感受性は非反芻動物のそれよりも低いように思われる。

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