The Effects of Simulated Transportation on Plasma Levels of Catecholamines, Cortisol and Glucose and Eosinophil Count in the Goat

Tin Myo NWE, Emiko HORI, Yoshitaka NAKANISHI,
Masaharu MANDA and Shozo WATANABE*

Faculty of Agriculture, Kagoshima University, Kagoshima-shi 890

* The Leasing Corporation for Livestock Industry's Environmental
Improvement, Minato-ku, Tokyo 105

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Abstract The effects of simulated transportation on plasma levels of catecholamines, cortisol, glucose and number of eosinophils were studied in adult male Tokara goats. The swing model experiment (simulated transportation) composed of two types of movement; irregular and intense movements for 10 min, and regular movement for 5 min and then these were repeated 6 times for 90 min. Blood was obtained via indwelling catheters in jugular or femoral veins. During the swing movement, the behaviors apparently indicated nervousness or excitement. At 30 min from the beginning of the swing, plasma concentrations of epinephrine, norepinephrine, cortisol and glucose were all significantly elevated about 2 times of the base values, showing the peaks at the end of the swing movement except norepinephrine and they all returned to the base values after the termination of the swing movement. The number of eosinophils were started to decrease immediately after the beginning of the swing and showed the minimum level 1.5 h and then returned to the base value by 10.5 h after the termination of the swing movement. The present results clearly show that the swing movement activates the sympathetic nervous system and then both the adrenal medulla and cortex are simultaneously activated followed by immediate physiological changes such as glucose metabolism and the number of eosinophils. The results of the present study are in general agreement with those observed during the real transportation in our previous experiments. Thus, our swing model is thought to be able to simulate the real transportation stress.

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The hypothalamo-pituitary-adrenal axis is activated by stressors, resulting in increased concentrations of plasma cortisol and catecholamines^{10,22,24}. Cortisol and catecholamines are essential components of adaptation to stress. Catecholamines assist not only the progress of the stress response but also accelerates the cortisol actions. Epinephrine and norepinephrine secretions may be increased

by transportation, and glycogenolysis and lipolysis are stimulated and thus meat composition may be changed in the stressed animals^{6,25}. It is also well known that stress affects the immune system of farm animals because immunity is reduced by both cortisol and catecholamines^{5,8,14,17}.

Simulated transportation has been proposed to study the effects of physical and emotional

stress of a real transportation by Locatelli *et al.*¹⁵⁾ and Sartorelli *et al.*²⁰⁾. But, there were no reports comparing a simulated transportation with a real transportation. Thus, in the previous study, we devised a swing model as a simulated transportation and we compared it with a real transportation. In the previous study¹⁹⁾, it was shown that, the effect of transportation on plasma cortisol, glucose and the total number of white blood cell and eosinophils were quite similar to that of the swing model.

To establish the mechanism of the initiation of the transportation stress, determination of catecholamines is necessary, but no comprehensive analyses of the changes in plasma cortisol and catecholamines concentrations undergoing transportation or simulated transportation have been reported.

Thus, in the present study, a simulated transportation experiment was conducted to show the effect of transportation on plasma levels of catecholamines, cortisol, glucose and the number of eosinophils simultaneously.

Materials and Methods

The experiment was conducted in the period from October 1993 to March 1994.

Animals :

Three male adult Tokara goats, aged 1 to 3 years and approximately 25 kg body weight, were used in this study.

Housing and feeding :

The experimental animals were housed in wooden cages from 1 month prior to adapt for the new environment and were fed a mixture of beet pulp and hay cube, formula feed and

barley twice daily at 8:30 h and 16:00 h as reported previously¹⁹⁾.

Catheterization :

To minimize stress resulting from frequent venipuncture and animal manipulation, blood was withdrawn from an indwelling jugular vein catheter. The catheter was inserted into a left jugular vein by surgical operation as reported previously¹⁹⁾. After the catheterization, the goats were allowed to recover from surgical trauma for at least 5 days before the start of the experiment. To prevent clogging the both catheters were flushed twice daily with sterile saline containing 10,000 IU unit heparin per ml.

Swing model experiment :

The procedure for the swing model experiment has described in the previous paper¹⁹⁾. The swing schedule consisted of the 15 min module of two types of movement as summarized in Table 1. Where, velocity (V) of swing at horizontal position, for example, is $\sqrt{2gh}$, here "h" is the maximum height of vertical swing movement (50 cm) and g the gravity (981 cm/sec²), therefore $V = 313$ cm/sec. The 15 min swing schedule was 20 cycles/min and inclination angle was 45° and repeated 6 times during 90 min of the swing movement. The swing experiment was started at 9:00 h and the blood sample was taken at 8:00 h before the start of the swing experiment. During the swing experiment blood samples were taken at 30, 60, 90 min and then continued at 3 h intervals for 12 h and the next 6 h intervals for 24 h after the start of the swing movement.

Ten ml of blood was withdrawn each time and immediately cooled in an ice container.

Table 1. The time schedule of the swing treatment

	0	10	20	30	40	50	60	70	80	90	min
Irregular and intense movement		↔	↔	↔	↔	↔	↔	↔	↔		
Regular movement			↔	↔	↔	↔	↔	↔	↔	↔	

The swing schedule composed of two types of movement ; irregular and intense movements for 10 min, and regular movement for 5 min and then these were repeated 6 times (↔) for 90 min.

Before centrifugation, a 1 ml aliquot was taken for hematological counting and 9 ml of the heparinized blood was centrifuged at 2,500 rpm ($1,300 \times g$, 20°C) for 10 min. The plasma was stored at -70°C until assayed. The number of eosinophils was counted using a standard hemocytometer. The concentration of plasma glucose was determined by Spectrophotometer RaBA-ACE with Unikit reagents (Chugai, Daiichi, Kyoto, Japan).

The plasma levels of epinephrine (E) and norepinephrine (NE) were determined by high-performance liquid chromatography with fluorescence detection¹⁶⁾. After cation-exchange chromatography on a Toyopak SP cartridge, the catecholamines and isoproterenol (internal standard) in $500\ \mu\text{l}$ of plasma are converted into the corresponding fluorescent compounds by reaction with 1, 2-diphenylethylenediamine in aqueous acetonitrile. These compounds are separated within 8 min on a reversed-phase column, TSK-gel ODS-120 T, with isocratic elution using a mixture of water, methanol and acetonitrile containing a Tris-hydrochloric buffer (pH 7.0). The amounts of catecholamines were calculated based on the internal standard method. Isoproterenol was used as an internal standard.

Plasma cortisol concentration was measured by the method of Scott and Dixon²³⁾ using HPLC as described in the previous paper¹⁹⁾.

Statistical analysis :

The data were subjected to analyses of variance (ANOVA), and the difference of means was tested by Student "t" test.

Results

The behaviors observed during the experiment are summarized in Table 2. The behaviors apparently indicated nervousness or excitement.

Plasma levels of E were increased significantly ($P < 0.05$) 30 min of the start (Fig. 1 a) which was 2 times higher than that before the swing treatment. The highest value was observed at the end of the swing ($P < 0.05$) and then returned to the base value by 9 h after the start of the swing movement. The plasma levels of NE were increased significantly ($P < 0.05$) 30 min of the start (Fig. 1 b) which was 2 times higher than that before the swing treatment. The highest value was observed 4.5 h ($P < 0.05$) and then returned to the base value 7.5 h after the termination of the swing movement. Both plasma levels of E and NE were increased 4.5 h after the termination of the swing movement.

Plasma cortisol concentration was significantly ($P < 0.05$) increased within 30 min of the start (Fig. 2 a). The peak value was observed at the end of the swing which was on the average four times higher than that before the start and the elevated value was maintained throughout the swing movement. Immediate-

Table 2. The behaviors observed during the model swing treatment

Behaviors observed
Kneeling down (Supporting the body with the knee of the forelegs and feet of extended hindlegs)
Sniffing and Flehmen (Gesture directing the nose within 10 cm of a surface and/or object)
Trembling (Shake involuntarily from emotion, weakness)
Scrambling or Struggling (Try to keep their balance and to restore their disturbed postures)
Laying down
Salivation

ly after the end of the swing, cortisol concentration started to decline and returned to the base value 1.5 h after the termination of the swing. Plasma glucose significantly increased ($P < 0.01$) within 30 min after the beginning (Fig. 2 b) and the peak value was observed at the end of the swing, that level was 3 times higher than that before the swing value. The elevated value was maintained throughout the swing movement and then returned to the base value 4.5 h after the termination of the swing. The number of eosinophils started to decrease within 30 min ($P < 0.05$) and reached the minimum level at 3 h from the start, that level showed 2.5 times lower than that before the swing ($P < 0.05$). And then eosinophil level returned to the base value 10.5 h after the ter-

mination of the swing (Fig. 2 c).

Discussion

In the stressful conditions, catecholamines were released from adrenal medulla and nerve endings and present in the blood. Epinephrine and norepinephrine are produced and stored in different cells in the adrenal medulla and other chromaffin cells. Collections of

Fig. 1. (a) Plasma levels of epinephrine (□) and (b) norepinephrine (○) before, during and after swing treatment in the goat. Values are means $\pm$ SE; $n=3$. Bold line in the figure shows period of the swing movement. * $P < 0.05$ compared with the value before start.

Fig. 2. (a) Plasma levels of cortisol (■), (b) glucose (●) and (c) the number of eosinophils (▲) before, during and after swing treatment in the goat. Values are means $\pm$ SE; $n=3$. Bold line in figure shows period of the swing movement. * $P < 0.05$; ** $P < 0.01$ compared with the value before start. Dotted line (.....) in Fig. 2. (a) and (b) shows the concentrations of previous paper²⁵.

these cells are also found in the heart, liver, kidney, gonads, adrenergic neurons of the post-ganglionic sympathetic system, and central nervous system. Most of norepinephrine present in these organs innervated by sympathetic nerve is made in situ (~80% of the total) and most of the rest is made in other nerve endings and reaches the target sites via the circulation¹⁸. These are quickly removed from plasma so that blood levels fluctuate rapidly, reflecting the degree of sympathetic neuronal activity⁴. The present paper is the first reporting the time course changes in plasma catecholamines concentrations in goats during a simulated transportation.

In earlier studies on transportation stress, blood sampling for hormone assay was performed only before and after the transportation and thus, the time course changes of plasma hormones were not reported which is very important to show the stress response². The changes in plasma levels of E and NE observed in this study provide evidence for fluctuations in activity of the sympathetic-adrenal medulla axis during the swing treatment. Plasma E levels increased significantly at 30 min after the beginning of the swing movement and the level was two-fold higher than the base value. Plasma NE levels significantly increased at the same timing as E but not so high. After the termination of the swing movement, plasma E levels returned to the base value, but plasma NE levels did not decline. Itoh¹¹ has reported that the concentration of plasma NE showed the high levels and these levels were maintained up to the 60 days when exposed to the cold environment, although plasma E level once increased and decreased quickly after the exposure. The increased levels of plasma NE after the termination of the swing movement may be connected with increased muscle activity¹. The animal was very nervous and restless during the swing movement and after the termination of the swing, the animal was taking a rest and

after this the animal became active again (eating and moving).

Plasma concentrations of cortisol and glucose were significantly increased after the beginnings of the swing movement. The maintenance of glucose in the blood is one of the most finely regulated of homeostatic mechanisms in which the liver, the extrahepatic tissues, and several hormones play a part. The glucocorticoids are secreted by the adrenal cortex and have an important role in carbohydrate metabolism. Administration of these steroids causes increased gluconeogenesis. This is a results of increased protein catabolism in the tissues, increased hepatic uptake of amino acids, and increased activity of transaminases and other enzymes concerned with gluconeogenesis in the liver. In the stressful conditions, plasma glucocorticoids are increased and glucose synthesis is accelerated^{12,13}. At the same time, catecholamines were secreted from the sympathetic nerve endings and adrenal medulla as a results of stressful stimuli and induced excessive glycogenolysis in liver and muscle owing to a stimulation of phosphorylase. In muscle, as a result of the absence of glucose-6-phosphatase, glycogenolysis ensues with the formation of lactate, whereas in liver, glucose is the main product leading to an increase in blood glucose which is regarded as being fundamental to increase plasma glucose concentrations¹⁸.

The decreased number of eosinophil was also observed during the swing movement. It is well known that eosinopenia is induced by physical and emotional stress and this is thought to be attributed to the elevated levels of plasma cortisol. In fact, it has been reported that continuous release of corticosteroids or prolonged steroid therapy causes decreased production of eosinophils³. It is also known that cytokines are important mediators of the response of the haematopoietic system to stress. Cytokines released during stress not only increase blood cell production

but also modulate the types of cells produced. The stress responses thus involves not only acceleration of the normal mechanisms of haematopoiesis but also overriding of some types that are produced²¹⁾. The present results suggest that transportation stress affects immunity of the animals in addition to plasma concentrations of catecholamines, cortisol, glucose and so on.

The present results also clearly show that the swing movement activates the sympathetic nervous system and then both the adrenal medulla and cortex were simultaneously activated followed by immediate physiological changes such as glucose metabolism and the number of eosinophils. The results of the present study are in general agreement with those observed during the real transportation in our previous experiments. Thus, our swing model is thought to be able to simulate the real transportation stress.

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References

- 1) Akerstedt T, Gillberg M, Hjemdahl P, Sigurdson K, Gustavsson I, Daleskog M, Pollare T. Comparison of urinary and plasma catecholamine response to mental stress. *Acta Physiol. Scand.*, 117 : 19-26. 1983.
- 2) Becker BA, Nienaber JA, Deshazer JA, Hahn GL. Effect of transportation on cortisol concentrations and on the circadian rhythm of cortisol in gilts. *Amer. J. Vet. Res.*, 46 : 1457-1459. 1985.
- 3) Beeson PB, Bass DA. The Eosinophil. In *Major Problems in Internal Medicine*. (Smith LH Jr. ed.) Vol. 14. W. B. Saunders. Philadelphia. 1977.
- 4) Charles RL, Michael GZ, Irwin JK. Use of plasma norepinephrine for evaluation of sympathetic neuronal function in man. *Life Sci.*, 18 : 1315-1326. 1976.
- 5) Crary B, Borysenko M, Sutherland DC, Kutz I, Borysenko JZ, Benson H. Decrease in mitogen responsiveness of mononuclear cells from peripheral blood after epinephrine administration in humans. *J. Immunol.*, 130 : 694-697. 1983.
- 6) Dantzer R, Mormede P. Stress in farm animals : A need for reevaluation. *J. Anim. Sci.*, 57 : 6-18. 1983.
- 7) Dalin AM, Magnusson U, Haggendal J, Nyberg L. The effect of transport stress on plasma levels of catecholamines, cortisol, corticosteroid-binding globulin, blood cell count, and lymphocyte proliferation in pigs. *Acta vet. scand.*, 34 : 59-68. 1993.
- 8) Fauci AS. Immunosuppressive and anti-inflammatory effects of glucocorticoids. *Monograph. Endocrinol.*, 12 : 449-465. 1979.
- 9) Freeman BM. Stress and the Domestic Fowl : Physiological Fact or Fantasy ? *World's Poultry Sci.*, 41 : 45-51. 1985.
- 10) Heny JP, Stephens PM. Stress, health and social environment. A sociobiologic approach to medicine. *Topics in environmental physiology and medicine*. Springer-Verlag. Berlin. 1977.
- 11) Itoh S. Hormones. *Scientific American* (Japanese edition), 37 : 204-217. 1986.
- 12) Kent JK, Ewbank R. The effect of road transportation on the blood constituents and behavior of calves. I. Six month old. *Br. Vet. J.*, 139 : 228-235. 1983.
- 13) Kent JK, Ewbank R. The effect of road transportation on the blood constituents and behavior of calves. II. One to three weeks old. *Br. Vet. J.*, 142 : 131-140. 1986.
- 14) Landmann RMA, Miller FB, Perini CH, Wesp M, Erne P, Buhler FR. Changes of immunoregulatory cells induced by psychological and physical stress : relation to plasma catecholamines. *Clin. Exp. Immunol.*, 58 : 127-135. 1984.
- 15) Locatelli A, Sartorelli P, Agnes F, Bondiolotti GP, Picotti GB. Adrenal response in the calf to repeated simulate transport. *Br. Vet. J.*, 145 : 517-522. 1989.

- 16) Mitsui A, Nohta H, Okura Y. High-performance liquid chromatography of plasma catecholamines using 1, 2-diphenyl-ethylenediamine as precolumn fluorescence derivatization reagent. *J. Chromatogr.*, 344 : 61-70. 1985.
- 17) Munck AP, Guyre P, Holbrook N. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocr. Rev.*, 5 : 25. 1984.
- 18) Murray RK, Granner DK, Mayes PA, Rodwell VW. Gluconeogenesis and control of the blood glucose ; Hormones of the adrenal cortex and adrenal medulla. *Harper's Biochemistry*. 12 th ed. 186-187, 499-515. Prentice-Hall International Inc. 1990.
- 19) Nwe TM, Tsukahara Y, Manda M, Watanabe S. Initiation of the simulate and real transportation stress in the goat. *Anim. Sci. Technol. (Jpn.)*, 65 : 1008-1017. 1994.
- 20) Sartorelli P, Dominoni S, Agnes F. Influence of duration of simulated transport on plasma stress markers in the calf. *J. Vet. Med.*, A 39 : 401-403. 1992.
- 21) Schrader JW. Interleukin-3. *The Cytokine Hand book*. (Thomsom AW ed.) 103-118. Academic Press. London and New york Inc. 1991.
- 22) Sconberg S, Nockels CF, Bennet BW, Bruyninckx W, Blancouaert Anne-Marie B, Craig AM. Effects of shipping, handling, adrenocorticotrophic hormone, and epinephrine on α -tocopherol content of bovine blood. *Am. J. Vet. Res.*, 54 : 1287-1293. 1993.
- 23) Scott NR, Dixon PF. Determination of cortisol in human plasma by reversed phase high performance liquid chromatography. *J. Chromatogr.*, 164 : 29-34. 1979.
- 24) Stephens DB. Stress and its measurement in domestic animals : a review of behavioral and psychological studies under field and laboratory situations. *Adv. Vet. Sci. Comp. Med.*, 24 : 179-210. 1980.
- 25) Tarrant PV, Sherington J. An investigation of ultimate pH in the muscles of commercial beef carcasses. *Meat Sci.*, 4 : 287-297. 1980.

ヤギにおける輸送シミュレーション負荷が血中カテコールアミン、 コルチゾール、グルコース濃度および好酸球数に及ぼす影響

ティン ミョウ スエ・堀 英美子・中西良孝・萬田正治・渡邊昭三*

鹿児島大学農学部，鹿児島市 890

* 畜産環境整備リース協会，東京都港区 105

成雄トカラヤギを供試して輸送シミュレーション負荷後の血中カテコールアミン，コルチゾール，グルコースおよび好酸球数の変動を明らかにした。ブランコ振動による輸送シミュレーションでは，ケージに入れたまま不規則な振動と規則的な振動を組み合わせた1セット15分間の負荷を6回繰り返した(90分間)。採血はヤギの頸静脈あるいは大腿静脈に装着した残置カテーテルより行なった。ブランコ振動中，ヤギは落ち着きがなく，やや興奮した状態であった。ブランコ振動開始30分後，血漿中エピネフリン，ノルエピネフリン，コルチゾールおよびグルコース濃度は振動開始前の値の2倍に上昇し，ノルエピネフリンを除きそれらの値はブランコ振動終了時にピークを示したが，その後は次第に低下する傾向を示し，振動開始前の値に戻った。好酸球数はブランコ振動開始直後に減少し，振動終了1時間半後に最小値を示したが，振動終了10時間半後には振動開始前の値に戻った。本研究の結果は，輸送シミュレーション負荷が交感神経系を刺激するとともに，副腎皮質および髄質を同時に賦活化し，糖質代謝や免疫機能などの生理的变化をもたらすことを示している。また，上述の生理諸元の変動は実際の輸送実験の結果と定性的に一致しており，ブランコ振動が輸送モデルとなり得るものと考えられる。

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