FULL PAPER *Physiology*

Androgen Suppresses Corticotropin-Induced Increase in Plasma Cortisol Level but Enhances The Increase in Plasma Aldosterone Level in Goats

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ABSTRACT. Previously we reported that androgen treatment reduced the extent of the increase in plasma cortisol (Cor) levels induced by adrenocorticotropic hormone (ACTH) administration in goats. In this study, we investigated the effect of androgen on the plasma levels of androstenedione and aldosterone. Four castrated male goats, which were treated with either 5α-dihydrotestosterone (DHT) or cholesterol (cho), were injected intravenously with 0.005, 0.02 or 0.1 mg of ACTH(1–24). Plasma Cor levels were increased significantly by all doses of ACTH injection, and these extents were lower in DHT-treated goats. Plasma androstenedione levels were also increased by ACTH injection, but DHT treatment seemed to little affect. Plasma aldosterone levels were also increased by ACTH injection, and there were no differences between cho- and DHT treated goats at 15 and 30 min after the ACTH injection. However, when goats were given the lower doses of ACTH (0.02 and 0.005 mg), plasma aldosterone levels were restored rapidly only in cho-treated goats, whereas those in DHT-treated goats were maintained throughout the 60 min experimental period. Consequently, plasma aldosterone levels in DHT-treated goats were higher than those in cho-treated goats at 45 and 60 min. One possible mechanism of the effect of DHT on the ACTH-induced increase in aldosterone synthesis may be the reduction of the activity of P450-17α, that is the enzyme to convert pregnenolone to 17α-OH-pregnenolone, and this mechanism may also be responsible to the suppressive effect of DHT on the ACTH-induced Cor synthesis.

KEY WORDS: ACTH, aldosterone, androgen, cortisol, goat.

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Our previous works reported that the increase in plasma cortisol (Cor) levels during road transportation, which can be a severe stress to domestic animals including goats [3, 12, 14], were significantly reduced by testosterone (T) or 5α-dihydrotestosterone (DHT) treatments in castrated male goats [1, 2]. We further demonstrated that androgen treatment reduced the transportation-induced increase in plasma Cor levels even when increase in plasma ACTH levels were not affected [1], and that androgen treatment reduced the increase in plasma Cor levels induced by adrenocorticotropic hormone (ACTH) administration in goats [13]. This report indicates that the suppression of transportation-induced increase in Cor secretion by androgen in goats is mainly a result of the suppression of the response of the adrenal cortex to ACTH, although androgen can affect Cor secretion by acting in the hypothalamus and/or the pituitary levels in some part [7].

The similar suppressions of the ACTH-induced secretion of glucocorticoid by androgen were also reported in experimental rodents [16], sheep [19] and cattle [4]. In rats and mice, the biological mechanisms of the effects of androgen on the response of adrenal cortex to ACTH have been investigated. The expression levels of adrenal ACTH receptor mRNA is decreased by T treatment in castrated rats [21]. While in mice, T decreased the immunoreactive protein, mRNA and activity of 3β-hydroxysteroid dehydrogenase-isomerase, that is one of the enzymes relates the production of adrenal steroid, in the adrenal gland [18]. These reports indicate that androgen may act on the various process of adrenal steroid production. It is need to examine the mechanisms of the suppressive effects of androgen on the caprine adrenal gland.

Three types of steroid hormones, those are glucocorticoids, adrenal androgen and mineralocorticoids, are secreted from the adrenal cortex. Similar to glucocorticoids, the secretion of adrenal androgen, such as dehydroepiandrosterone (DHEA) and androstenedione, are stimulated by ACTH [9]. Mineralocorticoids, such as aldosterone, play the significant roles in the regulation of water and sodium contents in the body [17]. The major stimulating hormone for aldosterone is angiotensin II, but ACTH also stimulates its secretion in some degree [5, 11, 15].

It is possible that androgen affect not only the ACTH-induced secretion of Cor but also adrenal androgen and/or aldosterone because all steroid hormones are synthesized from cholesterol through a certain biosynthetic pathway [6]. In rats, similar to corticosterone, ACTH-induced secretion of aldosterone is also reduced by androgen [8]. However, in ruminants, the effects of androgen on the secretion of adrenal androgen and/or mineralocorticoids are not known. The mechanisms of the suppressive effect of androgen on

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ACTH-induced secretion of Cor in goats might be inferred by investigating the effects on other hormones from the adrenal cortex. In this study, we investigated the effects of DHT treatment on the ACTH-stimulated secretion of androstenedione and aldosterone, as well as Cor, in castrated male goats.

MATERIALS AND METHODS

Animals: Four castrated male Shiba goats (25–33 kg, 4–6 years old) were obtained from the experimental station of the University of Tokyo. They were castrated at least six months prior to the experiment. Usually, they were housed in the research farm of the Faculty of Agriculture, Utsunomiya University. At least seven days prior to the experiment, the animals were kept in the experimental room. Animals were loosely tied to individual stanchions and kept under a constant daylight cycle (light on 7:00–19:00 hr) and temperature (22–23°C). They were fed daily with *ad libtum* lucerne hay and 100 g of pelleted diet (ZC: Oriental Yeast Co., Ltd., Tokyo, Japan), and water was always available. The lucerne hay and water were changed to the new one, and the cage was cleaned every day at 13:00.

Androgen treatment: Administration of androgens was carried out as described by Aoyama *et al.* [1, 2]. Each goat received hormone treatment 9 to 14 days prior to the experiment. Three silicon capsules (made from silicon sheets 70 × 50 mm, 0.5 mm thickness: Tigers Polymer, Osaka, Japan), containing 0.8 g of 5α-dihydrotestosterone (DHT) (Wako Pure Chemical Industries, Osaka, Japan), were implanted in subcutaneous into each animal. Capsules containing cholesterol (cho) (Wako) were used as a control. One capsules containing cho were implanted into each animal as a same manner with DHT. At the end of a series of the ACTH administration that is described below, the implanted hormone was changed and the procedure was repeated. The order of the hormone treatments in two goats was cho-DHT but it was DHT-cho in other two. The interval between hormone treatments was three weeks.

ACTH administration: One or two days before the start of the experiment, a catheter was fitted to the jugular vein for blood sampling and intravenous injection of ACTH. On the day of the experiment, 800 μL of sterile saline containing 0.005, 0.02 or 0.1 mg of ACTH (1–24) (tetracosactide acetate) (Cortrosyn; Daiichi Pharmaceuticals, Tokyo, Japan) were injected into the jugular vein through the catheter at 16:00 hr. Three ACTH doses that were described above were challenged to each goat, and the interval among the injection of each dose was more than three days. The order of the injected dose was randomised.

Blood sampling: Five mL of blood samples were collected at 15:30, 16:00 (just before the ACTH injection), 16:15, 16:30, 16:45 and 17:00 hr. The plasma samples were separated by centrifugation (3000 rpm, 4°C, 10 min).

Assays: The concentrations of the adrenal hormones were measured by radioimmunoassay.

For assays for Cor and androstenedione, these hormones were extracted from the plasma with the following procedure. Two mL of diethyl ether was added to 500 μL of plasma, and mixed vigorously for 30 sec, and then the plasma layer was frozen at –80°C. The ether layer was moved to another tube by decantation and was evaporated with centrifuge (3,000 rpm) under the vacuum condition. This procedure was repeated twice. The extracted substances were reconstituted with 500 μL of 0.01 M phosphate buffer (pH 7.5) containing 0.14 M NaCl, 0.1% gelatin and 0.1% sodium aside (Gel-PBS). These extracted samples were stored at –30°C.

For Cor assay, 10 μL of each extracted sample was adjusted to a total volume of 300 μL by Gel-PBS. Then 100 μL of antibody against cortisol (FKA-404; Cosmo Bio Co., Ltd., Tokyo, Japan) that was diluted (1:5,000) with 0.01 M phosphate buffer containing 0.14 M NaCl, 0.05 M EDTA and 0.1% sodium aside (EDTA-PBS), and 100 μL of [3H]-labeled hydrocortisone (NET-396; Perkin Elmer Inc, Waltham, MA) that was adjusted to 20,000 dpm per tube with Gel-PBS, were added to each sample and incubated for 36–48 hr at 4°C. After the incubation, 250 μL of dextran-coated charcoal solution (DCC), that was the 0.01 M phosphate buffer containing 0.14 M NaCl, 0.05% dextran (Dextran T-70; Pharmacia Corporation, Peapack, NJ) and 0.5 % charcoal (Norit sx-3; Wako), was added to per tube and further incubated for 20 min at 4°C. Then DCC was separated by centrifuge (3,000 rpm, 4°C, 15 min), and supernatant was moved to assay tube and mixed with 2 mL of scintillation fluid (Clearsol I; Nacalai Tesque, Kyoto, Japan), and then the radioactivity was measured with a scintillation counter (LSC-6100; Aloka Co., Ltd., Tokyo, Japan).

For androstenedione assay, 200 μL of each extracted-sample was adjusted to a total volume of 300 μL by Gel-PBS. Then each sample was incubated with 100 μL of antibody against androstenedione (FKA-138; Cosmo Bio) (dilution 1:10,000) and 100 μL of [3H]-labeled androstenedione (NET-469; Perkin Elmer) (10,000 dpm per tube) for 24 hr at 4°C. After the incubation, the plasma androstenedione level was measured with the same manner as Cor assay.

Plasma aldosterone levels were measured using a commercially available radioimmunoassay kit (DPC aldosterone kit; Diagnostic Product Co., Los Angeles, CA). Normal plasma was used for the aldosterone assay.

Data analysis: In order to examine the effects of ACTH injection on the plasma levels of each adrenal hormones, the difference among the sampling points within the same ACTH dose and the same hormone treatment were compared using repeated measures analysis of variance (ANOVA) (animals × sampling point) and Tukey's test.

In order to examine the effects of DHT treatment, the differences between cho and DHT treatment within the same sampling point and the same ACTH dose were compared with repeated measures ANOVA (animals × hormone treatment).

P values less than 0.05 were considered as significant.

RESULTS

Cortisol: Regardless of the presence or absence of DHT treatment, plasma Cor levels were increased by all doses ACTH injection, and the high levels of plasma Cor were maintained for 60 min (Fig. 1). Plasma Cor levels between 15 and 60 min point (after ACTH injection) were significantly higher than the data at 0 min (just before the ACTH injection) in all ACTH doses in both treatments. There were no differences in the basal levels of plasma Cor (at –30 and 0 min point) between cho- and DHT-treated goats, but after the ACTH injection, those in DHT-treated goats were significantly lower than those in cho-treated goats throughout the 60 min experiment when goats were given 0.1 and 0.02 mg of ACTH (Fig. 1B, C). When goats were given 0.005 mg of ACTH, plasma Cor levels in DHT-treated goats tended to be lower than those in cho-treated goats although it was not statistically significant (Fig. 1A).

Androstenedione: Plasma levels of androstenedione in DHT-treated goats were higher than those in cho-treated goats at –30 and 0 min, before the ACTH injection (Fig.2 A, B, C). The anti-androstenedione antibody used in this study cross-react with 1.23% of DHT, thus the plasma androstenedione level might be overestimated in DHT-treated goats. Therefore, the averaged value at –30 and 0 min was regarded as the basal level, and the difference from the basal level within each test was regarded as the effect of ACTH (Fig.2 D, E, F). Plasma androstenedione levels between 15 and 60 min point were significantly higher than the data at 0 min excepting in DHT-treated goats given 0.005 mg ACTH, in which plasma androstenedione levels tended to be increased by ACTH but it didn't reach to the statistical sig-

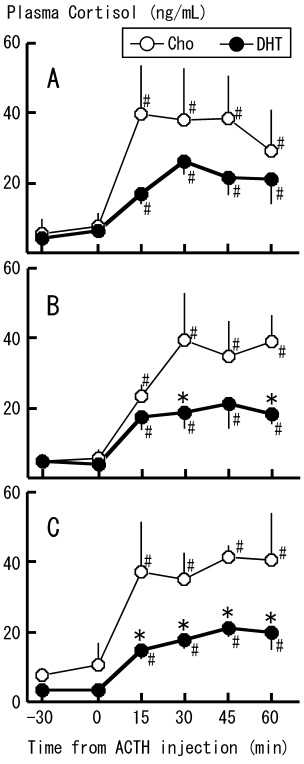

Fig. 1. Effects of androgen on the increases in plasma levels of cortisol induced by different doses (A: 0.005 mg, B: 0.02 mg, C: 0.1 mg per animal) of adrenocorticotropic hormone (1–24) administration in castrated male goats. Data were represented as the mean ± SE of four goats. Cho: cholesterol, DHT: 5α-dihydrotestosterone. #: Significantly different from the data at 0 min within the same hormone treatment (P<0.05; repeated measures analysis of variance and Tukey's test), *: significantly different from cho-treated goats at the same time point (P<0.05; repeated measures analysis of variance).

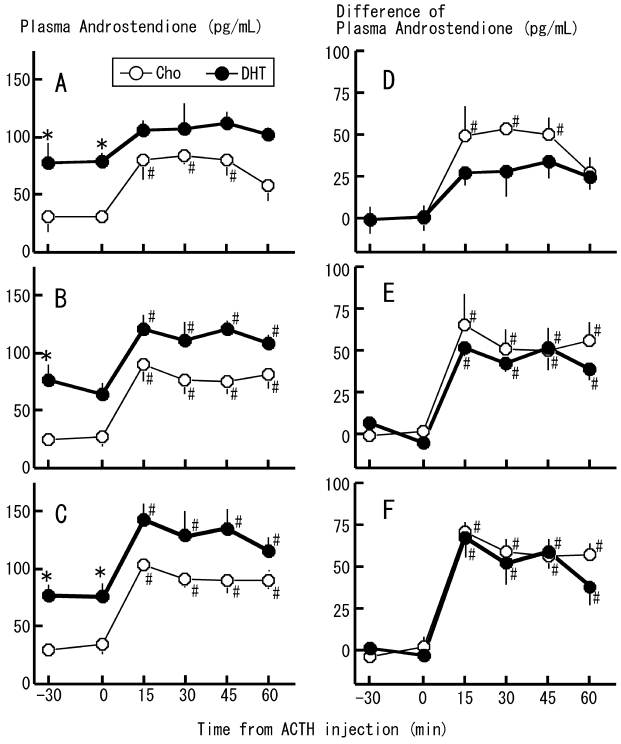

Fig. 2. Effects of androgen on the increases in plasma levels of androstenedione induced by different doses (A and D: 0.005 mg, B and E: 0.02 mg, C and F: 0.1 mg per animal) of adrenocorticotropic hormone (1–24) administration in castrated male goats. Data were represented as the mean ± SE of four goats. Panel A, B and C represent the original data, and panel D, E and F are represented as the difference from the basal level (defined as the averaged value of –30 and 0 min point) in each treatment. Cho: cholesterol, DHT: 5α-dihydrotestosterone. #: Significantly different from the data at 0 min within the same hormone treatment (P<0.05; repeated measures analysis of variance and Tukey's test), *: significantly different from cho-treated goats at the same time point (P<0.05; repeated measures analysis of variance).

nificance (Fig. 2D, E, F). Although the plasma androstenedione levels in DHT-treated goats given 0.005 mg of ACTH tended to be lower than those in cho-treated goats, there was no difference in plasma androstenedione levels between cho- and DHT-treated goats.

Aldosterone: Plasma aldosterone levels between 15 and 60 min point were significantly higher than the data at 0 min in all ACTH doses in both treatments (Fig. 3). However, in cho-treated goats given the lower ACTH doses (0.02 and 0.005 mg), plasma aldosterone levels began to decrease within 30 min (Fig. 3A, B), and especially in goats given 0.005 mg of ACTH, plasma aldosterone level at 60 min did not differ from that at 0 min anymore (Fig. 3A). These rapid restores of plasma aldosterone levels were not seen in DHT-treated goats. When goats were given 0.02 and 0.005 mg of ACTH, plasma aldosterone levels in DHT-treated goats were significantly higher than those in cho-treated goats at 45 and 60 min point (Fig. 3A, B). In 0.1 mg of ACTH injection, plasma aldosterone levels in cho- and DHT-treated goats did not differ throughout the 60 min experimental period (Fig. 3C).

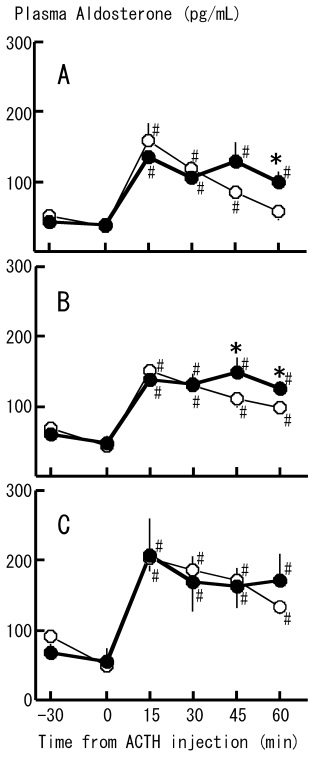

Fig. 3. Effects of androgen on the increases in plasma levels of aldosterone induced by different doses (A: 0.005 mg, B: 0.02 mg, C: 0.1 mg per animal) of adrenocorticotropic hormone (ACTH) (1–24) administration in castrated male goats. Data were represented as the mean ± SE of four goats. Cho: cholesterol, DHT: 5α–dihydrotestosterone. #: Significantly different from the data at 0 min within the same hormone treatment ($P<0.05$; repeated measures analysis of variance and Tukey's test), *: significantly different from cho-treated goats at the same time point ($P<0.05$; repeated measures analysis of variance).

DISCUSSION

In this study, DHT treatment reduced the levels of the ACTH-induced increase in plasma Cor in goats. This result agrees with our previous report [13]. In the lowest ACTH dose test (0.005 mg), one cho-treated goat had showed the smaller increase in plasma Cor level in the lowest ACTH dose, and this is why the difference between cho- and DHT-treated goats failed to be statistical significant. However, other three goats had showed the greater increase in plasma Cor level even when they were given the lowest ACTH dose during cho-treatment. It seems that DHT treatment suppress the increase in plasma Cor level induced by the lowest ACTH dose, as well as other two doses (0.02 and 0.1 mg), although it did not reach to statistical significance.

In this study, plasma Cor levels were increased within 15 min by ACTH injection, and these levels were maintained throughout the 60 min experimental period. DHT treatment decreased these maintained levels of plasma Cor. Since the maximum levels of plasma Cor were decreased by DHT treatment, androgen may reduce the rate of the limiting step for Cor synthesis. Androgen may decrease the reaction rate of a certain enzyme that relates to the step of the synthetic pathway of the steroid hormones; Stalvey reported that the activity of the 3β-hydroxysteroid dehydrogenase-isomerase, that is one of the enzymes related the production of adrenal steroid, was reduced by T in the mouse adrenal gland [18].

Plasma androstenedione levels were also increased by ACTH injection. Although the levels of androstenedione in DHT-treated goats tended to be lower than those of cho-treated goats when they were given 0.005 mg of ACTH, DHT treatment did not affect the ACTH-induced increase in plasma androstenedione levels. However, the effects of DHT on plasma androstenedione levels may not be regarded as the exact value. The antibody we used in this study cross-react with DHT 1.23%, thus the effect of DHT treatment on the androstenedione level might be masked by the higher level of plasma DHT in DHT-treated goats.

Similarly to other animals [5, 11, 15], ACTH increased also plasma aldosterone level in goats. Interestingly, unlike plasma Cor level, DHT treatment enhanced, rather than suppressed, the ACTH-induced increase in plasma aldosterone level. The aspects of the effects of ACTH injection on plasma aldosterone levels were different from those in Cor. The lower doses of ACTH injection (0.02 and 0.005 mg) increased plasma aldosterone levels within 15 min, but these levels began to decrease immediately only in cho-treated goats. While in DHT-treated goats, the increased levels of plasma aldosterone were maintained for 60 min. Consequently, plasma aldosterone levels did not differ between cho- and DHT-treated goats at 15 and 30 min point, but those in DHT-treated goats were higher than those in cho-treated goats at later half of the experiment. DHT treatment did not seem to affect the rate of the limiting step for aldos-

terone synthesis because the peak levels, which were seen at 15 min point, did not differ between cho- and DHT treated goats. One possible mechanism of the effect of DHT on the ACTH-induced increase in aldosterone synthesis is the reduction of the activity of P450-17α, that is the enzyme to convert pregnenolone to 17α-OH-pregnenolone (or progesterone to 17α-OH-progesterone). In cho-treated goats, pregnenolone and progesterone, which are the precursor of aldosterone, might be lost rapidly by the higher activation of P450-17α, and consequently, plasma aldosterone level might be decreased. DHT treatment might keep the high level of plasma aldosterone by reducing the activity of P450-17α.

If androgen decreases the activity of P450-17α, the suppressive effect of androgen on the ACTH-induced Cor synthesis might be a result of this effect at least partially. This hypothesis will be confirmed by examining the effect of androgen on the expression of P450-17α or their activity in the cultured adrenal cortical cells, like a previous work [6].

While, the effects of DHT on the ACTH-induced increase in plasma Cor and aldosterone levels may be the results of other biological mechanisms. For example, adrenal steroid hormone(s) can influence the secretion of other adrenal steroid hormones [20]. The enhancement of increase in plasma aldosterone level may be a result of the relatively lower level of plasma Cor instead of the directly effects of DHT. Otherwise, DHT might enhance the ACTH-induced increase in plasma aldosterone level by suppressing the metabolism of aldosterone. Latif *et al.* reported that the metabolism of aldosterone in the liver is influenced by other steroid hormones in rats [10]. The further researches are necessary to confirm the mechanisms of the effect of androgen on the steroid synthesis in the caprine adrenal glands.

In this study, we have found that androgen suppressed the ACTH-induced plasma Cor level but enhanced the ACTH-induced plasma aldosterone level in goats.

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REFERENCES

1. Aoyama, M., Maejima, Y., Keyaki, S., Muroi, M., Tohei, A. and Sugita, S. 2005. Effects of androgen on plasma levels of adrenocorticotropic hormone and cortisol during transportation in goats. *J. Vet. Med. Sci.* **67**: 1109–1114.
2. Aoyama, M., Negishi, A., Abe, A., Maejima, Y. and Sugita, S. 2003. Sex differences in stress response to transportation on goats: Effects of gonadal hormones. *Anim. Sci. J.* **74**: 511–519.
3. Aoyama, M., Negishi, A., Abe, A., Maejima, Y. and Sugita, S. 2008. Short-term transportation in a small vehicle affects the physiological state and subsequent water consumption in goats. *Anim. Sci. J.* **79**: 526–533.
4. Boissy, A. and Bouissou, F.M. 1994. Effects of androgen treatment on behavioral and physiological responses of heifers to fear-eliciting situations. *Horm. Behav.* **28**: 66–83.
5. Chavarri, M.R., Yamakita, N., Chiou, S. and Gomez-Sanchez, C.E. 1993. Calf adrenocortical fasciculata cells secrete aldosterone when placed in primary culture. *J. Steroid Biochem. Mol. Biol.* **45**: 493–500.
6. Engelbrecht, Y. and Swart, P. 2000. Adrenal function in angora goats: a comparative study of adrenal steroidogenesis in Angora goats, Boer goats, and Merino sheep. *J. Anim. Sci.* **78**: 1036–1046.
7. Handa, R.J., Nunley, K.M., Lorens, S.A., Louie, J.P., McGivern, R.F. and Bollnow, M.R. 1994. Androgen regulation of adrenocorticotropin and corticosterone secretion in the male rat following novelty and foot shock stressors. *Physiol. Behav.* **55**: 117–124.
8. Kau, M.M., Lo, M.J., Wang, S.W., Tsai, S.C., Chen, J.J., Chiao, Y.C., Yeh, J.Y., Lin, H., Shum, A.Y., Fang, V.S., Ho, L.T. and Wang, P.S. 1999. Inhibition of aldosterone production by testosterone in male rats. *Metabolism* **48**: 1108–1114.
9. Kramer, R.E., Buster, J.E. and Andersen, R.N. 1990. Differential modulation of ACTH-Stimulated cortisol and androstenedione secretion by insulin. *J. Steroid. Biochem.* **36**: 33–42.
10. Latif, S.A., McDermott, M.J. and Morris, D.J. 1983. The effects of adrenal and gonadal steroids and K^+-canrenoate on the metabolism of aldosterone by rat liver microsomes. *Steroids* **42**: 283–297.
11. Lehoux, J.G., Fleury, A. and Ducharme, L. 1998. The acute and chronic effects of adrenocorticotropin on the levels of messenger ribonucleic acid and protein of steroidogenic enzymes in rat adrenal *in Vivo*. *Endocrinology* **139**: 3913–3922.
12. Maejima, Y., Aoyama, A., Abe, A. and Sugita, S. 2005. Induced expression of c-fos in the diencephalon and pituitary gland of goats following transportation. *J. Anim. Sci.* **83**: 1845–1853.
13. Maejima, Y., Aoyama, M., Kobayashi, N. and Sugita, S. 2006a. Adrenocorticotropic hormone-induced secretion of cortisol in goats is inhibited by androgen. *Anim. Sci. J.* **77**: 87–94.
14. Maejima, Y., Aoyama, M. and Sugita, S. 2006b. Expression of c-fos-like immunoreactive cells in the adrenal gland following transportation stress in goats. *Small Rum. Res.* **63**: 162–169.
15. Müller, J.1998. Regulation of aldosterone biosynthesis: the end of the road? *Clin. Exp. Pharmacol. Physiol. (Suppl.)* **25**: S79–S85.
16. Nowak, W.K., Neri, G., Nussdorfer, G.G. and Malendowicz, K.L. 1995. Effect of sex hormones on the steroidogenic activity of dispersed adrenocortical cells of the rat adrenal cortex. *Life Sci.* **57**: 833–837.
17. Pocock, G. and Richards, C.D. 2004. The regulation of body fluid volume. pp. 611–627. *In*: Human Physiology: The Basis of Medicine Second Edition. Oxford University Press, Oxford.
18. Stalvey, D.R.J. 2002. Inhibition of 3β-hydroxysteroid dehydrogenase-isomerase in mouse adrenal cells: a direct effect of testosterone. *Steroids* **67**: 721–731.
19. Turner, A.L., Canny, B.J., Hobbs, R.J., Bond, J.D., Clarke, I.J. and Tilbrook, A.J. 2002. Influence of sex and gonadal status of sheep on cortisol secretion in response to ACTH and on cortisol and LH secretion in response to stress: importance of different stressors. *J. Endocrinol.* **173**: 113–122.
20. Vecseiweisz, P., Farkas, K., Kemeny, V. and Harangozo, M. 1965. Investigations on the mechanisms of reduced aldosterone production caused by the administration of hydrocortisone. *Steroids* **5**: 415–421.
21. Yoshimura, S., Sakamoto, S., Kudo, H., Sassa, S., Kumai, A. and Okamoto, R. 2003. Sex differences in adrenocortical responsiveness during development in rats. *Steroids* **68**: 439–445.