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Effects of Androgen on Plasma Levels of Adrenocorticotropic Hormone and Cortisol during Transportation in Goats

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ABSTRACT. Previously, we demonstrated that plasma cortisol (Cor) levels were increased by road transportation in castrated male goats, but the extent of the increase was significantly reduced by 5α-dihydrotestosterone (DHT) implantation. This study aims to clarify whether the reduction of Cor secretion by androgen during transportation results from reduced plasma adrenocorticotropic hormone (ACTH). Castrated goats were implanted separately with cholesterol (Cho), testosterone (T) or DHT, followed by transportation. Plasma Cor levels increased during transportation regardless of hormone treatment, but the levels in T and DHT treated animals were lower than those in animals treated with Cho. Plasma ACTH levels also increased during transportation, and those in T treated animals were significantly lower than in those treated with Cho. However, plasma ACTH levels in DHT treated animals varied among the animals and did not differ from those in Cho treated animals. Significant and highly positive correlations between the logarithm of plasma ACTH levels and plasma Cor levels were found in every treatment group. The areas under the regression curves between plasma ACTH levels and plasma Cor levels associated with T and DHT treatments were significantly lower than those with Cho treatment. In conclusion, T was shown to reduce ACTH secretion in response to transportation in castrated goats. However, this suppression of the increase in Cor secretion during transportation by androgen is suggested to be mainly a result of suppression of the responsiveness of the adrenal cortex to ACTH.

KEY WORDS: ACTH, androgen, cortisol, goat, transportation stress.

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Gonadal hormones can influence the stress responses of the hypothalamo-pituitary-adrenal (HPA) axis in rodents [6, 7, 11], humans [10, 18, 19] and domestic animals [1, 4]. For example, our previous work indicates that androgen affects the increase in plasma cortisol (Cor) level induced by road transportation stress [1]. Although plasma Cor levels increase during road transportation in both male and female mature goats, those in males are significantly lower than those in females. We further demonstrated that the increase in plasma Cor levels during transportation was significantly reduced by 5α-dihydrotestosterone (DHT) in castrated male goats [1]. In addition, we have found that ovariectomized female goats show a similar response to DHT (unpublished data).

The secretion of glucocorticoid is stimulated by adrenocorticotropic hormone (ACTH) secreted from the anterior pituitary gland. ACTH secretion is regulated by corticotropin-releasing hormone (CRH) secreted from the hypothalamus [20]. In rodents [6, 7] and humans [18], the physiological mechanisms underlying the involvement of gonadal hormones in the HPA responses to stress are becoming clearer. For example, Handa and co-workers reported that castration of male rats enhances the increase in c-fos mRNA expression in the hypothalamic paraventricular nucleus (PVN) induced by the open field test, and androgen treatment restores this effect of castration [6]. Since the PVN includes the CRH neurons and plays a significant role in the regulation of the HPA axis [20], Handa's report [6] suggests that the PVN is involved in the suppression of the HPA response to stress by androgen.

The physiological mechanism(s) of the suppressive effect of androgen on plasma Cor level during transportation in goats [1] is not clear. Two possible mechanisms of this effect of androgen are a reduction in the ACTH secretion in response to stress, or a reduction in the responsiveness of the adrenal cortex to ACTH.

Transportation can be a strong stress for domestic animals [12, 13] and it causes weight loss in goats [9] or dark cutting beef (DCB), which is an undesirable, dark, firm, and dry lean surface of beef in cattle [16]. Furthermore, the occurrence of DCB in steers is modulated by implantation of gonadal hormones [16]. In order to determine the effects of androgen on stress-induced physical damage in domestic animals and their products, it is necessary to discover which level of the HPA axis is responsible for the androgen-induced reduction in the increase in plasma Cor level during transportation. In addition, to elucidate the physiological significance of androgen-induced suppression of the response of the HPA axis to stress, the physiological effects of androgens in this phenomenon have to be revealed.

As we have described above, Cor secretion is stimulated by ACTH secreted from the anterior pituitary. The aim of this study is to examine the effects of androgen on the increase in ACTH secretion induced by road transportation, and to clarify whether the reduction of Cor secretion during transportation is a result of the reduction in plasma ACTH level by androgen.

MATERIALS AND METHODS

This experiment was conducted between April-June and between October-December in the year 2002 at temperatures ranging between 14–28°C.

Animals: Five male Shiba goats (*Capra hircus*) (24–30 kg, 2 years old) were obtained from the experimental station of the University of Tokyo, and were placed in the research farm of the faculty of agriculture, Utsunomiya University. Animals were castrated at least two months prior to the experiments.

Transportation: The five animals were divided into two groups containing two and three animals, and animals in one group were simultaneously studied. The first or the second group was used for the experiment between April-June or October-November, respectively. The detailed transportation procedure is described in our previous work [1]. Briefly, the experiment was performed as follows: On the day of the basal session (BS), in which animals were housed and fed as usual, 2 m*l* blood samples were collected at 9:00, 9:15, 9:30, 9:45, 10:00, 10:30, 11:00 and 13:00. Two to three days after BS the transportation sessions (TS) were conducted using the same animals. Animals were driven in a truck around the research farm for one hour (9:00–10:00). The average and maximum speed of the truck was 26 and 60 Km/hr, respectively. While on the truck, animals were kept in individual cages (W60 × D120 × H120 cm for each animal) made of steel pipe (Yazaki, Shizuoka, Japan), and the cages were covered to prevent them from looking outside. The floor of the truck was covered with a rubber mat to prevent goats from slipping. Fodder and water were not given during transportation. After transportation, the animals were housed and fed as usual. During the TS, blood samples were collected in the same manner as in the BS. The same driver drove the truck throughout the study.

Administration of gonadal hormones: At least 12 days prior to the BS, each animal received testosterone (T: Wako, Osaka, Japan) or 5α-dihydrotestosterone (DHT: Wako) replacement by the implantation of silicon capsules containing crystalline steroid. As a control for gonadal hormones, capsules containing cholesterol (Cho: Wako) were implanted. Three capsules made of silicon sheets (70 × 50 mm, 0.5 mm thickness: Tigers Polymer, Osaka, Japan) each containing 0.8–1.0 g of T or DHT, were implanted into each animal. As a control, one same sized capsule containing 0.8–1.0 g of Cho was implanted into each animal. Using DHT, this method is sufficient to reduce the plasma Cor level during road transportation in castrated male goats [1]. After one experiment was completed, the implanted hormone was changed and the procedure was repeated. The order of hormone treatments was based on the Latin square design. The interval from one experiment to the next was a minimum of 21 days.

To examine the titer of this androgen treatment, plasma T levels of intact males and castrated males treated with T were compared. Another 1 m*l* of blood samples was collected from each of three goats in the second group at the end (13:00) of each experiment. In addition, blood samples were collected from four intact male Shiba goats (20–40 kg, 2–4 years old) placed in the research farm on December.

Assays: Plasma samples were separated by centrifugation (3,000 rpm, 4°C, 15 min) immediately after blood collection, and stored at –30°C. Concentrations of Cor, ACTH and T were measured by radioimmunoassay. Cor was measured using a commercial kit (DPC cortisol kit; Diagnostic Products Co., Los Angeles, CA). ACTH was measured by double antibody radioimmunoassay using IgG-ACTH-1 (IgG Corporation, Nashville, TN) as the primary antibody and (3-[^{125}I] iodotyrosyl-23) adrenocorticotropic hormone (1–39) (Amersham Bioscience, Buckinghamshire, UK) as the labeled hormone. Previous work indicates that this method of ACTH assay is appropriate for several mammalian species including goats [17]. Plasma T level was measured using a commercial kit (DPC total testosterone kit; Diagnostic Products Co.).

Data analysis: To examine the effects of transportation, differences between BS and TS were tested using repeated measures analysis of variance (ANOVA) for each hormone treatment. To examine the effects of the hormone treatments, repeated measures ANOVA followed by Tukey's Studentized range test were used. Because the levels of plasma ACTH in DHT treated goats during the TS varied, the plasma ACTH levels with T treatment were compared independently to those with Cho treatment. Further, the correlations and the height of the regression curves between plasma ACTH levels and plasma Cor levels in TS were examined. P values below 0.05 were considered significant.

Plasma T levels were compared between the castrated males given T and intact males using one-way ANOVA.

RESULTS

Plasma cortisol: In Fig. 1, two typical examples (Fig.1A, B) and the average ± SE (Fig. 1C) of plasma Cor levels are represented. During BS, there was little change in plasma Cor levels throughout the session with any hormonal treatment. Plasma Cor levels during TS were significantly higher than those in BS throughout the transportation and remained so up to 30 min afterwards ($P<0.01$) in every treatment. During transportation, the plasma Cor levels of T and DHT treated goats were significantly lower than those of Cho treated goats ($P<0.05$–0.01). There were no differences between T and DHT treatments.

Plasma ACTH: Two examples (Fig. 1D, E) and the average ± SE (Fig. 1F) of plasma ACTH levels are represented. Plasma ACTH levels increased during transportation and those in TS were significantly higher than those in BS with every treatment (Fig. 1F). In both goat #1 (Fig. 1D) and #2 (Fig. 1E), plasma ACTH levels with T treatment were lower than those with Cho treatment during TS. Similar to goat #1 and #2, when the other three goats were implanted with T, their plasma ACTH levels during TS were lower than those

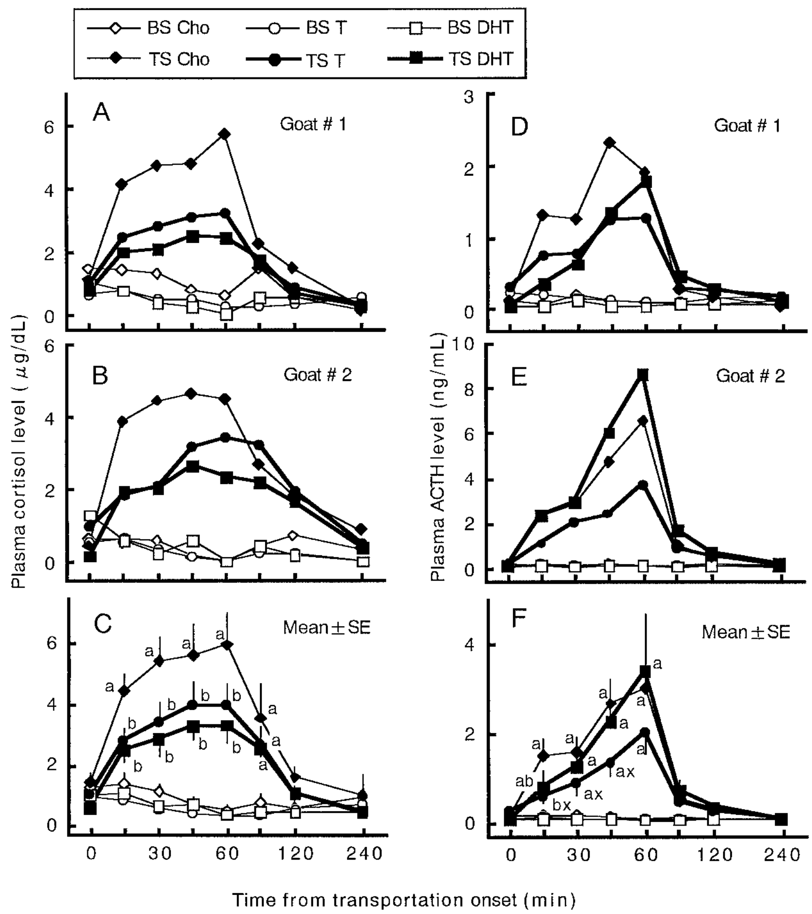

Fig. 1. Effects of androgen on the increases in plasma levels of cortisol (A, B, C) and ACTH (D, E, F) induced by road transportation in castrated goats. Each goat was given Cho, T or DHT implantation. The results of goat #1 (A, D) and #2 (B, E) are represented as typical examples, and each data point in panels C and F represents the mean ± SE of five goats. Animals were transported for 60 min in TS, but they were housed and fed as usual in BS. BS: the basal session, TS: the transport session, ACTH: adrenocorticotropic hormone, Cho: cholesterol, T: testosterone, DHT: 5α-dihydrotestosterone. a, b: Significant differences from BS within each hormone treatment are indicated by superscript letters ($P<0.05$; repeated measures analysis of variance), significant differences between hormone treatments within the session are indicated by absence of the same superscript letters ($P<0.05$; repeated measures analysis of variance and Tukey's-test). "x" indicates significant differences from Cho treatment within TS when statistical analysis is performed without including DHT treatment data.

given Cho treatment (data not shown). In goat #1 treated with DHT, plasma ACTH levels were similar to those with T treatment (Fig. 1D). In three goats (including #1), the plasma ACTH levels with T and DHT treatments during TS were similar. However, in two goats (including #2; represented in Fig. 1E), plasma ACTH levels with DHT treatment were rather higher than those with Cho treatment during transportation. The averaged plasma ACTH levels with DHT treatment did not differ from those with Cho or T treatment (Fig. 1F). Comparing among the three hormone treatments, plasma ACTH levels in T treated goats were significantly lower ($P<0.05$) than those in Cho treated goats only at 15 min after transportation onset. However, when plasma ACTH levels of Cho and T treated goats were compared, without including data from DHT treated goats, those in T treated goats were significantly lower than those with Cho treatment at 15, 30 and 45 min ($P<0.05$) after transportation onset. Although it was not statistically significant, a similar tendency was also observed at 60 min ($P=0.069$).

Correlation of the plasma ACTH and cortisol level: Figure 2A shows a typical example of the correlation of plasma levels of ACTH and Cor during TS. There was a significant highly positive correlation between the logarithm for the plasma ACTH levels and the plasma Cor levels in all goats

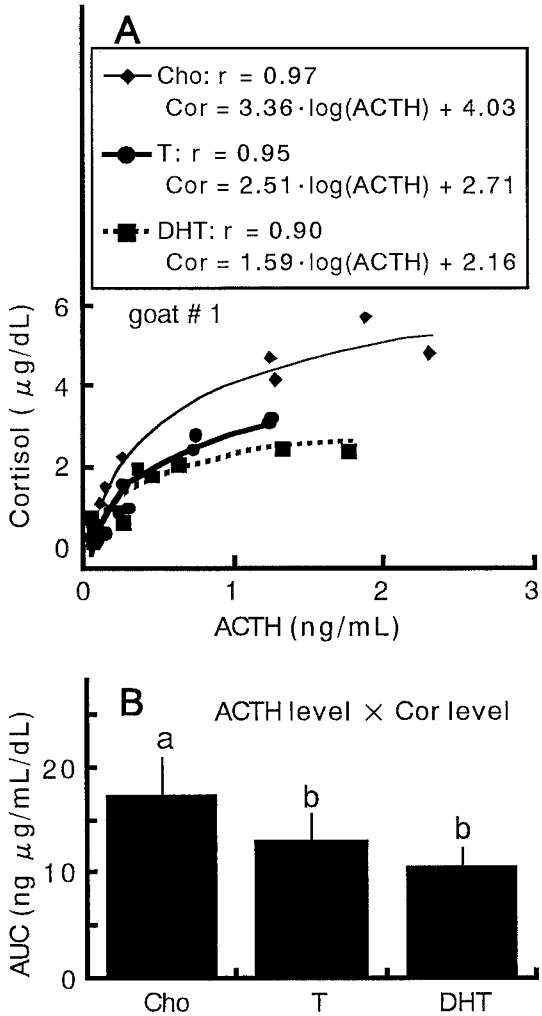

Fig. 2. Effects of androgen on the relationship between plasma levels of ACTH and cortisol during transportation in castrated goats. In panel A, the correlation between plasma levels of ACTH and cortisol in goat #1 with each hormone treatment is represented as the typical example. The correlation coefficient and the formula of the regression curve in each hormone treatment are represented in panel A. In panel B, the areas under the regression curves (see text for the explanation of calculation) are represented as mean ± SE of five animals. Each goat was given Cho, T or DHT implantation. See the legend of Fig. 1 for explanations of the abbreviations. a, b: Significant differences between hormone treatments are indicated by absence of the same superscript letters ($P < 0.05$; repeated measures analysis of variance and Tukey's-test).

with every treatment (Cho: r=0.92–0.99, T: r=0.75–0.95, DHT: r=0.88–0.97). In all five goats, the height of the regression curves with T and DHT treatments were lower than those with Cho treatment. The areas under the regression curves (AUC) of between 0.45 and 3.71 *n*g/m*l* (the averaged plasma ACTH level at the 15 min and 60 min points after transportation onset) are represented in Fig.2B, showing mean ± SE of five animals. The AUC with T and DHT treatments were significantly lower than those with Cho treatment. There was no difference between T and DHT.

Plasma testosterone: The average ± SE of plasma T level in intact males was 275 ± 79.0 (ranging 141–430) *n*g/d*l*. The average ± SE of plasma T level in T treated castrated males was 476 ± 52.9 (ranging 378–560) *n*g/d*l*, and did not significantly differ from that in intact males. The plasma T levels in Cho or DHT castrated males were less than 20 *n*g/d*l*, the minimum available value of this assay.

In this experiment, each animal was trucked three times. Therefore, in order to determine whether repeated transportation had any effects, the data from the first, second and third TS were compared. None of the parameters significantly differed among the three TS (data not shown). In addition, none of the parameters significantly differed between the first and the second group (data not shown).

DISCUSSION

Plasma T level of castrated male goats given T was slightly higher than that in intact males, but it was not statistically significant difference. Furthermore, plasma T levels of intact males ranged from 141 to 430 *n*g/d*l* and their higher levels were comparable with those in castrated males given T. Therefore the T treatment in this study can restore the blood T level of intact males in castrated goats. We did not measure plasma DHT level, but the molecular weights of T and DHT are similar so we assumed that the plasma levels of the DHT of castrated males given DHT was similar in plasma T level of intact males.

Although the plasma Cor levels of castrated male goats given T or DHT increased during transportation, the levels were significantly lower than those of goats given Cho. These results are consistent with our previous report which indicated that castrated goats given DHT show a lesser increase in plasma Cor levels than animals given Cho or estradiol [1]. In this study, we have shown that T, as well as DHT, reduces the transportation-induced increase in plasma Cor levels.

Plasma ACTH levels were also increased by road transportation regardless of hormone treatment. However, the effects of androgen on the response of plasma ACTH levels to transportation did not resemble those of plasma Cor levels. During transportation, plasma ACTH levels in T treated goats were significantly lower than those in goats with Cho treatment. On the other hand, plasma ACTH levels in DHT treated goats during transportation varied: three goats showed similar levels to T treatment, but the other two showed rather higher levels than Cho treatment when they were treated with DHT. Consequently, plasma ACTH levels in DHT treated goats during transportation did not statistically differ from those in goats given Cho treatment. It is unclear why the effect of DHT treatment on plasma ACTH levels varied among the animals in this study. Experimental season does not seem to influence the effect of DHT treatment, because goat #2 was included in the first group but another goat (goat #4), whose plasma ACTH level was not reduced by DHT treatment (data not shown), was in the second group. The order of hormone treatment also does not seem to have any relation, because that in goat #2 and #4 was Cho-DHT-T and DHT-T-Cho, respectively. Furthermore, plasma ACTH level of goat #5 that was treated in the same order as #2 was significantly reduced by DHT treatment (data not shown). Previous work indicates that both T and DHT treatments reduce the increase in plasma ACTH levels caused by foot shock stress in castrated male rats and no difference was observed between T and DHT treated rats [7]. In addition, DHT treatment is known to reduce the increase in c-fos mRNA expression in the PVN induced by the open field test in castrated rats [6], as well as plasma ACTH levels. These reports indicate that DHT reduces the secretion of ACTH in response to stimuli such as foot shock stress or novelty in rats. It is not clear why the effect of DHT on the ACTH secretion induced by stress in goats was not the perfectly same as rats. The effective dose of DHT on the ACTH response for goats may not be the same as rats, thus the dose of DHT in this study may not be enough to reduce the response of the ACTH secretion to transportation at least for two goats in spite of the enough dose to reduce plasma Cor response.

T can be converted to estrogen (Es) by aromatization whereas DHT cannot, and previous reports indicate that some behavioral effects of T cannot be induced by DHT treatment [3, 5] but can be induced by Es [5]. We may be able to hypothesize that Es is involved in the suppressive effect of T on the plasma ACTH level during transportation in goats, because DHT treatment failed to suppress plasma ACTH level in two goats but T treatment was effective for them. However, previous reports indicate that Es enhances, rather than suppresses, CRH synthesis in the PVN in rats [6, 7] and humans [10, 18, 19]. Furthermore, Es also enhances the response of ACTH secretion to CRH in rats [14]. In addition, we cannot conclude that DHT has no effect on the ACTH response to stress, because in three of five goats, their plasma ACTH levels in response to DHT treatment were similar to those with T treatment and were lower than those with Cho treatment. Although Es may contribute partially to the effect of T on the response of ACTH secretion to transportation in goats, it is difficult to hypothesize that Es plays a significant role in the suppression of ACTH secretion during transportation at the present time. Further studies are required to reveal the mechanisms of the effects of gonadal hormones on the response of ACTH secretion to transportation in goats.

Although T, and possibly also DHT, suppressed the increase in plasma ACTH levels induced by transportation in goats, this androgen-induced reduction in the increase in plasma Cor levels during transportation seems to be mainly a result of the reduction in the responsiveness of the adrenal cortex to ACTH caused by androgen. There were significant and highly positive correlations between the logarithm of the plasma ACTH levels and the plasma Cor levels in transported goats with every hormonal treatment. However, the heights of the regression curves between plasma ACTH levels and plasma Cor levels with T and DHT treatments were significantly lower than those with Cho treatment. This result suggests that androgen reduces the response of Cor secretion to a certain level of plasma ACTH. Thus, it is thought that androgen reduces the responsiveness of the adrenal cortex to ACTH. A previous study indicates that the increase in Cor level following intramuscular ACTH administration is inhibited by 100 days of prior intramuscular T injections in heifers [4]. A similar androgenic mechanism may exist in goats.

Previous reports indicate that the androgen receptor is present in the adrenal gland in rats [2, 15] and rhesus monkeys [8, 15]. The androgen receptor is distributed on the zonae fasciculata and reticularis of the adrenal cortex, by which glucocorticoids are produced, in rhesus monkeys [8]. These results suggest that androgen may play some roles in the synthesis and secretion of glucocorticoids. Although there are no reports demonstrating the distribution of the androgen receptor in the adrenal glands in goats, androgen may act directly on the adrenal cortex to reduce its response to ACTH in goats.

In conclusion, androgens, especially T, reduce the response of ACTH secretion to transportation to some degree in castrated male goats. However, the suppression of the increase in Cor secretion during transportation by androgen is thought to be mainly a result of the suppression of the responsiveness of the adrenal cortex to ACTH by androgen.

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