Responses induced by arginine-vasopressin injection in the plasma concentrations of adrenocorticotrophic hormone, cortisol, growth hormone and metabolites around weaning time in goats

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Abstract

In order to assess the biological significance of weaning and water deprivation on the control of plasma concentrations of adrenocorticotrophic hormone (ACTH), cortisol, growth hormone (GH) and metabolites in response to stimulation with arginine-vasopressin (AVP) and corticotropin-releasing hormone (CRH), we carried out three experiments in which male goats before and after weaning were intravenously injected with AVP or CRH alone, or in combination with each other. In experiment 1, 17-week-old (post-weaning) goats were intravenously injected with AVP or CRH alone at the doses of 0.1, 0.3 and 1.0 nmol/kg body weight (BW). The AVP injection significantly and dose dependently increased plasma levels of ACTH, cortisol, GH and metabolites, whereas the injection with CRH did not cause significant increases in the levels of these parameters. In experiment 2, 4-week-old (pre-weaning) and 13-week-old (post-weaning) goats were injected with either AVP or CRH alone, followed by a combined injection of both secretagogues at a dose of 0.3 nmol/kg BW. Although the basal levels of the hormones and metabolites, with the exception of glucose, were greater in the 4-week-old goats, the hormone

responses induced by stimulation with AVP were weaker than those induced in 13-week-old goats. Additionally, there were no responses in any hormone patterns to CRH stimulation in 4-week-old goats. In experiment 3, 13-week-old goats were injected with CRH alone followed by injection with AVP for two consecutive days of water deprivation. The animals were subjected to withdrawal of up to 20% of the total blood volume and water deprivation for up to 28 h. However, no significant differences in plasma ACTH, cortisol or GH levels were observed between days 1 and 2. Based on these results, we concluded that: (1) AVP is a more potent stimulant than CRH in terms of its ability to induce increases in plasma levels of ACTH, cortisol and GH; (2) the role of AVP as a secretagogue of hypothalamus–pituitary–adrenal hormones is strengthened, whereas the ineffective role of CRH remains unaltered, by weaning; (3) acute stress such as massive withdrawal of blood volume and subjection to water deprivation may not be sufficient burdens to alter stress-related hormone levels in young goats.

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Introduction

The release of adrenocorticotrophic hormone (ACTH) is stimulated by two hypothalamic neuropeptides: corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP). ACTH release in response to CRH stimulation is raised in a synergistic manner by AVP, oxytocin, angiotensin II, norepinephrine and epinephrine (Rivier *et al.* 1984, Watanabe & Orth 1987, Engler *et al.* 1999). Although CRH is the most potent ACTH secretagogue in rats and humans, AVP is a more potent secretagogue than CRH in sheep and cattle (Liu *et al.* 1990, Senn *et al.* 1995, Katoh *et al.* 2004a).

With increases in audiovisual stress, the concentrations of AVP have been shown to increase more than those of

CRH in the pituitary portal circulation of sheep (Engler *et al.* 1999). Accordingly, the role of AVP as an activator of the hypothalamus–pituitary–adrenal gland (HPA) axis must be greater than that of CRH. ACTH is known to stimulate cortisol secretion from the adrenal cortex, which exerts lipolytic effects in combination with catecholamines released from the medulla in various mammalian species (Bentley 1998). Growth hormone (GH) as well as ACTH is a lipolytic hormone (Bauman 2000). In addition, AVP may stimulate GH secretion from the anterior pituitary, because a stress such as restricted feeding or low feeding frequency is known to raise plasma GH levels and pulse amplitude in sheep (Thomas *et al.* 1990, 1991). Furthermore, increased GH levels are involved in decreases in SRIF release from the hypothalamus, as chronic food

intake restriction increases synthesis of GH-releasing hormone (GHRH), but reduces that of somatotropin release-inhibitory factor (SRIF) in the hypothalamus of sheep (Henry *et al.* 2001, Thomas *et al.* 1991).

After birth, during the first weeks of life, ruminant neonates are obliged to take milk for several weeks until weaning, and then their intake changes to roughage, whereas this is a more gradual process for other mammals. We recently reported changes in the expression of nutrient transporters and related enzymes around weaning time in the ruminant alimentary tract. We demonstrated that weaning reduced the expression of sodium-dependent glucose transporter (SGLT1) and CD36 (a fatty acid transporter) (Hayashi *et al.* 2004), as well as leptin (Yonekura *et al.* 2002) in the gastrointestinal tract, but increased the activity of carbonic anhydrase (Kitade *et al.* 2002) in the parotid gland of calves and goats.

To date, it is still unknown whether or not the more potent effect of AVP is innate and/or if it changes around the time of weaning. The aim of the present study, therefore, was to compare the potency of the intravenous injection of AVP and CRH in terms of increases in plasma ACTH, cortisol and GH levels, as well as to compare their respective effects on glucose and non-esterified fatty acid (NEFA) levels. Thus, the responses of these factors before and after weaning were compared; in addition, the acute effects of a 2-day period of blood draining and water deprivation on the levels of these hormones were assessed. The overall aim of the study was therefore to determine the significance of AVP as a hormone that regulates ACTH, cortisol and GH release in young goats.

Materials and Methods

The experiment was carried out according to the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences (The Physiological Society of Japan), and the present experiment was approved by The Animal Care Committee of Tohoku University.

Animals and milk intake

Newborn Shiba goats were fed colostrum from their dams for a week after birth, and then were given a commercial milk replacer for 6 weeks until weaning. The milk replacer contained skim milk, whey protein, casein, corn products, minerals and vitamins (24% crude protein, 20% crude fat, 10% mineral) (Meiji Feed Co., Tokyo, Japan). The animals were fed a milk replacer diet twice a day – i.e. once in the morning (0830 h) and once in the afternoon (1530 h) – until they reached 4 weeks of age, and then they were given a calf starter ration and lucerne hay cubes. On the day of the experiment, animals were not provided with the morning feed until the blood sampling was complete.

Experimental design

Animals were provided with free access to water and mineral salts during the entire study period, with the exception that they only had free access to water during the blood sampling period and except for the experimental goats in experiment 3.

In experiment 1, eight male weaned goats (17 weeks old, 13.2 ± 1.0 kg body weight (BW)) were injected with AVP ($n=4$) or CRH ($n=4$) alone at increasing doses of 0.1, 0.3 and 1.0 nmol/kg BW at 0, 60 and 150 min respectively, after the injection of the first dose of each secretagogue. Blood samples (5 ml each) were collected every 15 min from 30 min before until 270 min after the first injection. For the control experiment, another three animals (17 weeks old, 13.8 kg BW) were injected with physiological saline (5 ml) at the same point in time when AVP or CRH was injected into the AVP- or CRH-treated goats.

In experiment 2, 4-week-old (pre-weaned, 3.3 ± 0.5 kg BW, $n=3$) and 13-week-old (post-weaning, 8.3 ± 0.7 kg BW, $n=4$) goats were injected with AVP alone at a dose of 0.3 nmol/kg BW. This injection was followed by a combined injection of AVP+CRH (0.3 nmol/kg BW of each secretagogue). The injections were administered at 0 and 60 min respectively. Blood samples were collected every 15 min from 30 min before until 270 min after the first injection. In an additional experiment, 4-week-old animals ($n=3$) were injected with CRH, and then AVP+CRH (0.3 nmol/kg BW of each secretagogue) at 0 and 60 min respectively.

In experiment 3, 13-week-old goats (7.8 ± 0.2 kg BW, $n=4$) were injected with CRH followed by an AVP injection (1.0 nmol/kg BW of each secretagogue) on day 1, and the same dose and type of hormones and sampling procedure were repeated on the following day (day 2) under continuous water deprivation. Water deprivation began at the beginning of the blood sampling on day 1 (0930 h) until the blood sampling on day 2 was complete (1300 h). Blood sampling was carried out every 15 min from 30 min before until 210 min after the first injection.

Blood sampling

On the morning of the experimental day, 2 h prior to blood sampling, the animals were fitted with a catheter (Venula S, 20 G, Top, Tokyo, Japan) in the right jugular vein under local anaesthesia. The catheter was filled with sterile iso-osmotic sodium citrate (3.8% w/v) solution before and after each blood sampling. Blood samples (5 ml each) were taken every 15 min, and injection of AVP, CRH or saline was carried out via the catheter. The total volume of blood sampled in experiments 1 and 2 was 95 ml, and 150 ml of blood were sampled during the 2 days of experiment 3. The red blood cells were not re-injected after sampling.

Blood samples were mixed with heparin sodium (10 U/ml; Wako Pure Chem., Osaka, Japan) and were centrifuged at 8000 *g* for 15 min. The blood plasma was divided into five portions and stored at -30°C until used for the hormone and metabolite analyses.

Analyses

Plasma concentrations of ACTH and GH were determined by RIA as described previously in reports by our group (Kato *et al.* 2004a,b). Cortisol levels were determined using an RIA kit (Eiken, Tokyo, Japan). The glucose and NEFA concentrations were determined using commercial kits (Glucose CII-Test and NEFA C-Test respectively, Wako Pure Chem., Osaka, Japan).

Statistics

The data were expressed as means $\pm$ S.E.M. An unpaired Student's *t*-test (Zar 1984) was employed for analysis of the data between two groups, and Bonferroni's multiple range test following one-way ANOVA (Wallenstein *et al.* 1980) for the analysis of data obtained during the pre- (at 0 min) and post-injection period. Statistical significance was set at $P < 0.05$.

Results

Effects of CRH and AVP alone on ACTH release

The changes in plasma ACTH, cortisol and GH levels are depicted in Fig. 1 in response to each secretagogue intravenously injected at the increasing doses of 0.1, 0.3 and 1.0 nmol/kg BW. The AVP injection significantly and dose dependently increased the levels of ACTH (Fig. 1A), cortisol (Fig. 1B), and GH (Fig. 1C), whereas the CRH injection did not increase the levels of these hormones. The responses induced by AVP injection were significantly greater than those induced by the CRH injection.

The plasma glucose and NEFA levels are represented in Fig. 2. Although the AVP injection significantly and dose dependently increased the concentrations of these metabolites, CRH injection did not induce any significant responses in these two metabolites. The responses induced by AVP injection were also significantly greater than those induced by CRH injection. No significant differences were found between the data for hormone (except ACTH concentrations) and metabolite concentrations in control and CRH-treated animals.

Effects of weaning on ACTH response

In the next experiment, we assessed the responses induced by AVP (0.3 nmol/kg BW) alone and AVP+CRH

Figure 1 Effects of intravenous injection of AVP (●) or CRH (▲) at increasing doses (0.1, 0.3 and 1.0 nmol/kg BW) administered via a jugular vein catheter on plasma concentrations of ACTH (A), cortisol (B) and GH (C) in 17-week-old goats ($n=4$). The values are represented as the mean $\pm$ S.E.M. Open symbols indicate a significant difference from the pre-injection level (at 0 min) (Bonferroni's multiple range test). The continuous line (control, represented as the mean value) indicates the effects of an intravenous injection of physiological saline (5 ml) administered to control goats at the same time as AVP or CRH injection. * $P < 0.05$; ** $P < 0.01$ (AVP vs CRH, unpaired Student's *t*-test).

Figure 2 Effects of intravenous injection of AVP (●) or CRH (▲) at increasing doses (0.1, 0.3 and 1.0 nmol/kg BW) administered via a jugular vein catheter on plasma concentrations of glucose (A) and NEFA (B) in 17-week-old goats ($n=4$). The values are represented as the mean $\pm$ S.E.M. Open symbols indicate a significant difference from the pre-injection level (at 0 min) (Bonferroni's multiple range test). The continuous line (control, represented as the mean value) indicates the effects of an intravenous injection of physiological saline (5 ml) administered to control goats at the same time as AVP or CRH injection. * $P<0.05$; ** $P<0.01$ (AVP vs CRH, unpaired Student's t -test).

(0.3 nmol/kg BW of each secretagogue) in 4-week-old (pre-weaning) and 13-week-old (post-weaning) animals (Fig. 3). The AVP injection significantly increased plasma ACTH levels in the pre-weaning animals, whereas it did not cause a significant increase in either cortisol or GH. Although the basal levels of these hormones were lower than those in the pre-weaning animals, in the post-weaning animals AVP injection was found to increase the levels of all of these hormones significantly.

The combined AVP+CRH injection following AVP injection only slightly altered the levels of ACTH and cortisol in the pre-weaning goats, whereas it caused a significant increase in both hormone levels in the

Figure 3 Comparison of the responses between 4- (●) and 13-week-old (▲) goats in terms of the levels of ACTH (A), cortisol (B) and GH (C) induced by the intravenous injection of AVP alone and AVP+CRH administered via a jugular vein catheter (0.3 nmol/kg BW of each secretagogue) ($n=3$ or 4). Open symbols indicate a significant difference from the pre-injection level (at 0 min) (Bonferroni's multiple range test). * $P<0.05$ (4- vs 13-week-old goats, unpaired Student's t -test).

Figure 4 Comparison of the responses between 4- (●) and 13-week-old (▲) goats in terms of the glucose (A) and NEFA (B) levels induced by the intravenous injection of AVP alone and AVP+CRH administered via a jugular vein catheter (0.3 nmol/kg BW of each secretagogue) ($n=3$ or 4). Open symbols indicate a significant difference from the pre-injection level (at 0 min) (Bonferroni's multiple range test). * $P<0.05$; ** $P<0.01$ (4- vs 13-week-old goats, unpaired Student's t -test).

post-weaning goats. However, there were no significant changes in GH levels in response to the AVP+CRH injection following the AVP injection.

There was neither significant difference in the basal glucose levels nor significant increases induced by AVP and AVP+CRH injection in pre- and post-weaning animals (Fig. 4A). The basal NEFA levels in the pre-weaning animals were significantly greater than those in the post-weaning animals. In the post-weaning animals, the injection of AVP+CRH led to significantly increased levels of NEFA, whereas in the pre-weaning animals,

Figure 5 Effects of the intravenous injection of CRH and AVP+CRH administered via a jugular vein catheter (0.3 nmol/kg BW for each secretagogue) on the plasma concentrations of ACTH (A) and cortisol (B) in 4-week-old goats ($n=3$). The values are represented as the mean $\pm$ S.E.M. Open symbols indicate a significant difference from the pre-injection level (at 0 min) (Bonferroni's multiple range test).

AVP alone and the combined injection did not cause significant changes in NEFA levels (Fig. 4B).

Figure 5 depicts the ACTH and cortisol responses to the injection of CRH alone and AVP+CRH (0.3 nmol/kg BW of each secretagogue) in pre-weaning animals. The CRH injection did not lead to a significant increase in either ACTH or cortisol levels, whereas the combined

injection was associated with a significant increase in the levels of both hormones.

Effects of water deprivation on ACTH release

The changes in plasma ACTH, cortisol and GH in response to CRH and then AVP injection (1.0 nmol/kg BW of each secretagogue) were assessed during water deprivation, to which the goats were subjected for 2 days, i.e. from the onset of the blood sampling on day 1 to the end of the sampling period on day 2 (Fig. 6). Despite the total volume of blood drawn (150 ml over the 2-day period), and the duration of water deprivation (up to 28 h), there were no significant changes between days 1 and 2 in these hormone levels in response to injection with both CRH and AVP.

Discussion

In this study, we demonstrated that the intravenous injection of AVP (0.1, 0.3 and 1.0 nmol/kg BW) significantly and dose dependently increased the levels of ACTH, cortisol and GH in post-weaning goats. The CRH injection did not evoke any significant changes in ACTH, cortisol or GH levels. Previous findings have indicated that CRH is the most potent ACTH secretagogue in rats and humans, whereas AVP is the most potent in sheep and cattle (Engler *et al.* 1999). The effect of AVP as a potent ACTH secretagogue may be a common feature among ruminants because, as shown in the present study, AVP was a potent ACTH secretagogue in goats as well as in sheep and cattle. At present, it remains unknown why AVP is such a potent ACTH secretagogue in ruminants; however, it is possible that these animals exhibit unique water metabolism and/or water retention ability. Here, it was observed that AVP injection under water-deprivation conditions did not alter the basal or AVP-induced responses of goats, as shown in Fig. 6. The blood volume drawn from each animal was 150 ml over the course of 2 days, and the duration of water deprivation was for a period of up to 28 h, over two successive days. If the total blood volume is estimated at 8% of the BW, the volume of blood drawn in this study (150 ml) should have amounted to more than 20% of the total blood volume. Although the biological significance of AVP in the regulation of ACTH secretion remains to be clarified, it is possible that the volume of blood withdrawn has different effects on HPA axis activity in pre-weaned and post-weaned goats, depending on the weight of the animal. Another explanation for the observed lack of an effect of water deprivation on the HPA axis responsiveness to CRH and AVP might be a specific effect of the types of stress applied in this study.

We also demonstrated that the responses of ACTH and other hormones change around the time of weaning,

Figure 6 Comparison of the responses on day 1 (●) and day 2 (▲) induced by the intravenous injection of CRH and AVP administered via a jugular vein catheter (1.0 nmol/kg BW of each secretagogue) with respect to the plasma concentrations of ACTH (A), cortisol (B) and GH (C) in 13-week-old goats ($n=4$). The values are represented as the mean $\pm$ S.E.M. Open symbols indicate a significant difference from the pre-injection level (at 0 min) (Bonferroni's multiple range test).

although AVP was found to be a more potent secretagogue than CRH in terms of HPA hormone secretion. This finding may indicate the possibility that the ACTH response to AVP stimulation is gradually established along with the aging process. In the somatotrophic axis, the postprandial GH response has been shown to differ before and after weaning in neonatal calves (Katoh *et al.* 2004b). That is, postprandial plasma GH levels significantly increased following a feeding with milk but these levels decreased following a concentrate+hay feeding. Thus, the secretion of pituitary hormones such as GH and ACTH may be altered and gradually established after weaning.

Here, we have demonstrated for the first time that AVP injection increased not only HPA hormones such as ACTH and cortisol, but also GH. It is well known that stressors such as restricted feeding or low feeding frequency increase plasma GH levels and, in particular, the pulse amplitude in sheep (Thomas *et al.* 1990, 1991). These responses are due to the inhibition of the release and synthesis of SRIF as well as to an increase in GHRH synthesis in the hypothalamus of sheep (Henry *et al.* 2001, Thomas *et al.* 1991). However, the increased GH levels observed here may not have been due to increases in ACTH and/or cortisol levels, as no significant differences were observed in terms of when these hormones reached peak values (Figs 1, 3 and 6).

The AVP injection induced a significant increase in NEFA levels at the dose of 1 nmol/kg BW. This result may have been due to the increase in ACTH levels, because ACTH, as well as other HPA hormones, possesses lipolytic activity in various mammalian species (Bentley 1998). We recently demonstrated that various fatty acids suppress ACTH release induced by CRH stimulation in rat anterior pituitary cells (Katoh *et al.* 2004a). A significant inhibitory effect of caprylate was found to be concentration dependent, and was even seen at 1 µmol/l. These findings indicate the possibility of a feedback regulation system between ACTH and fatty acids. It has also been reported in the ruminant *in vivo* and *in vitro* that GH release is inhibited by various fatty acids (Hertelendy & Kipnis 1973, Reynaert *et al.* 1975, Sartin *et al.* 1988, Coxam *et al.* 1989, Estienne *et al.* 1989, Matsunaga *et al.* 1998, 1999, Ishiwata *et al.* 2000).

Vasopressin, as well as beta-adrenergic agonists, is known to stimulate glycogen breakdown in a Ca^{2+} -dependent mechanism in hepatocytes (Exton 1981). These agonists also stimulate lactate output in the liver of fed rats (Hue *et al.* 1978, 1981). However, vasopressin is reported to markedly suppress NEFA levels in rats (Caisova *et al.* 1980). These findings indicate the possibility that changes in glucose (but not NEFA) levels induced by the AVP injected in the present study were, at least in part, a direct effect of vasopressin on hepatocytes.

The synergistic action induced by the combination of CRH and AVP has been reported in the rat *in vivo* and *in vitro* (Gillies *et al.* 1982, Katoh *et al.* 2004a). We found

that a synergism in ACTH release was also reproduced in ovine anterior pituitary cells, but this response was not found to occur in parallel with increases in cellular calcium ion concentrations (K Katoh and D Engler, unpublished data). Although these findings suggest the possibility that synergism in ACTH release occurs in a variety of animal species, despite unknown cellular mechanisms, it remains unclear whether or not this synergism occurs in young goats. The present findings may have been due to the prompt decrease in ACTH secretion when AVP was injected in step-wise increasing doses in the present study. This assumption is made because the ACTH peak value induced by the first AVP trial (Fig. 6) was 10 times greater than that observed in the third trial (Fig. 1) at the dose of 1.0 nmol/kg BW, even though similar responses in cortisol and GH levels were observed.

In summary, AVP is a more potent secretagogue than CRH in terms of its ability to induce increased plasma levels of ACTH, cortisol and GH; moreover, the role of AVP as a secretagogue of HPA hormones is strengthened by weaning in young goats. However, the acute stressors applied here – i.e. a massive withdrawal of blood, and water deprivation over the course of two consecutive days – may not have been sufficient to alter stress-related hormone levels in young goats.

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