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Modified Responses of Circulating Cortisol, Thyroid Hormones, and Glucose to Exogenous Corticotropin and Thyrotropin-Releasing Hormone in Food-Deprived Sheep

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WRONSKA-FORTUNA, D., A. SECHMAN, J. NIEZGODA AND S. BOBEK. *Modified responses of circulating cortisol, thyroid hormones, and glucose to exogenous corticotropin and thyrotropin-releasing hormone in food-deprived sheep.* PHARMACOL BIOCHEM BEHAV 45(3) 601–606, 1993.—In a previous experiment, food deprivation was found to suppress the increase of plasma cortisol and thyroid hormones in stressed animals. Because both the hypothalamo-adrenocortical and the thyroid axes are stimulated during stress, we investigated in this study whether a similar pattern of changes occurs in food-deprived sheep following corticotropin (ACTH) or thyrotropin-releasing hormone (TRH) administration. Each hormone was given as a bolus injection on the fifth day of food deprivation. Blood was sampled by venipuncture five times: 0.5 h before and 1, 3, 5, and 9 h after injection of the hormone. The peak of plasma cortisol in food-deprived sheep following ACTH administration exceeded fourfold the corresponding peak in fed animals. This suggests that food deprivation may enhance the sensitivity of the adrenocortical gland to ACTH and/or reduce binding sites for cortisol in target tissues. In fed animals, TRH was without effect on plasma cortisol level, whereas in food-deprived sheep cortisol transiently increased 2.5-fold, suggesting greater permeability of the blood-brain barrier for TRH. In food-deprived animals, plasma T_3 was decreased to 22.6% of basal level, and elevated plasma cortisol after ACTH injection was not able to decrease it further. On the other hand, in fed sheep increased plasma cortisol did decrease plasma T_3 as much as 4.2-fold. Circulating T_4 was not affected by ACTH treatment. The Δ increase of plasma T_3 and T_4 following TRH administration was comparable in fed and fasted animals. In contrast, the maximal plasma levels of both hormones were always lower in fasted animals by about 25% due to lowered basal level, suggesting that food deprivation preserves the sensitivity of the hypothalamo-thyroid axis. Administration of ACTH to fed animals increased plasma glucose by 62% above basal level. Food deprivation lowered plasma glucose. A high level of endogenous cortisol following ACTH injection raised plasma glucose only by 19.5%. The results obtained suggest the lesser effectiveness of cortisol in food-deprived animals.

ACTH TRH Food deprivation Sheep

IT is generally assumed that a food-deprived organism is directed toward minimal energy expenditure. The plasma levels of catabolic hormones are often suppressed. Thyroid hormones are regarded as the main catabolic hormones because they are involved in calorigenesis characterized by an enhanced protein, lipid, and glucose catabolism (6,22). Glucocorticoids are also regarded as catabolic hormones because they participate in stress-induced muscle proteolysis and elevate urine nitrogen loss (16). Unlike in thyroid hormones, the plasma level of glucocorticoids is in most cases not affected by food deprivation (2,15,27,30), although sometimes it may increase (1,26,31). An opposite endocrine picture is obtained in stress manifested by the increased plasma level of catabolic hormones. In the previous experiment (34), it was shown that food deprivation suppresses the increase in plasma cortisol and thyroid hormones in stressed animals. In the present experiment, we wanted to know whether food deprivation alters in a similar fashion the response of these hormones and glucose to exogenous corticotropin (ACTH) and thyrotropin-releasing hormone (TRH). Both hormones are commonly used as diagnostic tests of adrenocortical or hypophysis-thyroid axis function. Variation in nutritional state among human beings or animals at the time of endocrine evaluation could account both for variation in basal concentrations of hormones as well as in concentrations after provocative testing of adrenocortical or hypophysis-thyroidal function with ACTH or TRH. The data obtained revealed that a food-deprived organism modifies its response to exogenous and endogenous

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hormones. Such results may have practical implication because stress in mammals (illness, fear, emotion) is associated with retention of food consumed (25).

METHOD

Animals and Procedure

An experiment was carried out on 15 ewes of the Polish Mountain breed, aged 1 year. All animals were in the anoestrous period. They were kept in groups of five sheep each under natural light conditions at 15–20°C over the experimental period (May–July). Food (hay and concentrates) and water were available ad lib. Food-deprived animals received water only. The experiment consisted of two trials: In the first, the effect of ACTH and TRH was studied in fed sheep. In the second (1 week later), animals were food deprived for 5 days. The hormones were administered at the beginning of the fifth day of fasting. ACTH (Polfa, Poland) was injected IM as a bolus injection at the dosage of 1 IU/kg b.w. Synthetic TRH (provided by the Chemical Institute, University of Gdańsk, Poland) was dissolved in physiological saline and given in a single jugular injection at a level of 2 μg/kg b.w. The volume of single injection fluctuated about 0.5 ml. Control animals received 0.5 ml saline IV.

Blood was taken by venipuncture five times: 0.5 h before, then at 1, 3, 5, and 9 h after administration of the hormone. The time of the first blood collection was constant, always starting at 9:00 a.m. To avoid a startle response in animals to unfamiliar people, all injections and blood sampling were carried out by the operating staff. The blood was placed into heparinized tubes and immediately centrifuged. Plasma was stored at −16°C, and assays were done within 3 months.

Plasma Hormone Level

All hormones were assayed by radioimmunoassay (RIA) methods. Plasma cortisol was measured according to Stupnicki's procedure (32). The cross-reactivity of antiserum (a-F/R63) is given by the author (32). The intraassay coefficient of variation was 5.72% at 20 nmol/l; the recovery rate was 95% at 40 nmol/1. Total plasma T_3 and T_4 were measured by a double-antibody RIA method based upon those used by Nauman (23). For T_3, the intraassay coefficient of variation was 5.92% at 2.29 nmol/l and the recovery rate was 105.6% at 2.4 nmol/l. For T_4, the corresponding results were 6.38% at 32.4 nmol/l and 106.7% at 73.2 nmol/l. Glucose was measured by the *o*-toluidine method using a Biotest kit produced by Lachemia (Czechoslovakia).

Statistical Analysis

The results of individual blood collections from each group are presented as mean values ± SEM. Differences between and within animal groups were evaluated by two-way analysis of variance in a completely randomized design, followed by the multiple-range Duncan test (13). The Δ increase in serum T_3 and T_4 of individual sheep at the third or fifth hour after administration of TRH was defined as the maximal increment above the respective level at 0.5 h, before administration of the hormone.

RESULTS

Cortisol

The maximal increase of cortisol occurred at 3 and 1 h after ACTH administration in fed and fasted sheep, respectively (Figs. 1A and 1B). The peak of plasma cortisol in food-deprived sheep exceeded fourfold the corresponding peak in fed animals. TRH had no effect on cortisol levels in fed sheep; however, in food-deprived sheep a transient increase of cortisol from 43.7 ± 3.9 (1 h after TRH) to 139.0 ± 5.9 nmol/l was noted (a 3.2-fold increase) at the third hour after TRH injection (Fig. 1B).

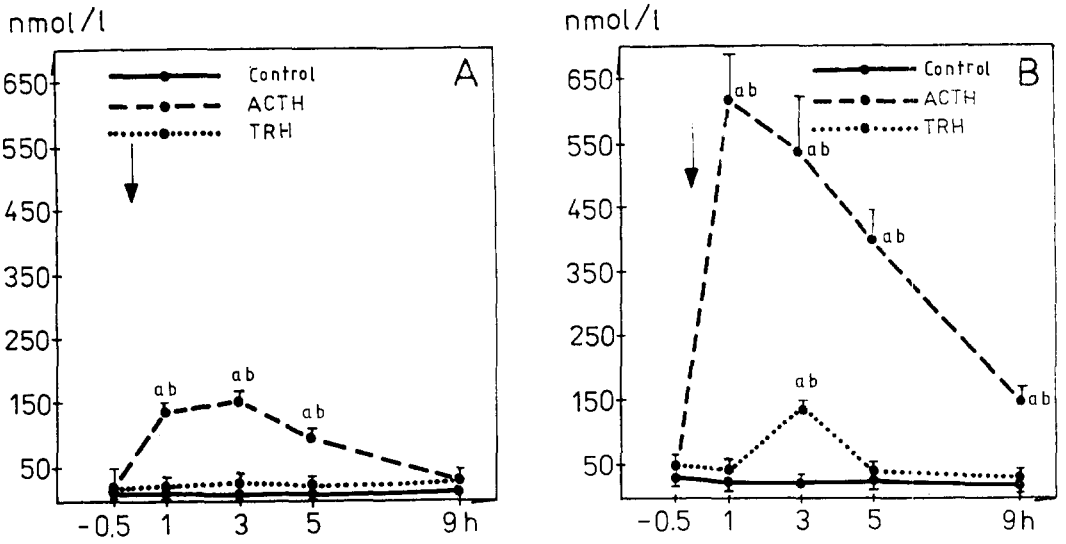

FIG. 1. Plasma cortisol level in fed (A) and food-deprived (B) sheep after corticotropin (ACTH) or thyrotropin-releasing hormone (TRH) injection; time of hormone administration is marked by an arrow. Values given are the mean ± SEM; in all cases, $n = 5$. a, statistically different from control values; b, statistical difference between two experimental groups; $F(40, 216) = 30.54$, $p < 0.01$.

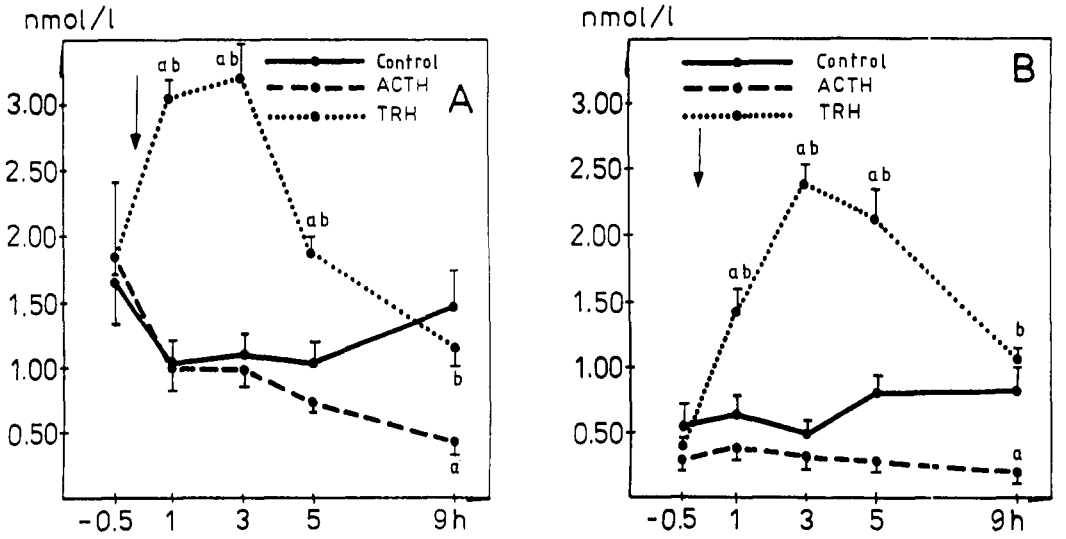

FIG. 2. Plasma T_3 level in fed (A) and food-deprived (B) sheep after corticotropin (ACTH) or thyrotropin-releasing hormone (TRH) injection; time of hormone administration is marked by an arrow. Values given are the mean ± SEM; in all cases, $n = 5$. a, statistically different from control values; b, statistical difference between two experimental groups; $F(40, 216) = 8.56, p < 0.01$.

Triiodothyronine

A single injection of ACTH to fed animals was associated with a gradual fall in plasma T_3 to near the basal level of food-deprived sheep (Fig. 2A). In fasted sheep, ACTH was not able to further decrease of plasma T_3 despite several higher levels of plasma cortisol (Fig. 2B). Maximal levels of plasma T_3 after treatment with TRH were significantly higher in fed (3.19 ± 0.32 nmol/l) than in fasted animals (2.38 ± 0.13 nmol/l; $p < 0.05$). However, similar to T_4, differences in T_3 Δ_{max} following TRH treatment were not statistically significant in fed (1.43 ± 0.35 nmol/l) or food-deprived sheep (2.00 ± 0.12 nmol/l).

Thyroxine

ACTH injection followed by elevated circulating cortisol exerted no effect on plasma T_4 levels in fed and food-deprived sheep. As expected, TRH raised T_4 levels in both groups of animals. In fed sheep, the maximal increase of plasma T_4 occurred earlier (peak at 3 h after TRH) and to a higher value (175.6 ± 12.9 nmol/l) than in fasted sheep (peak at 5 h after

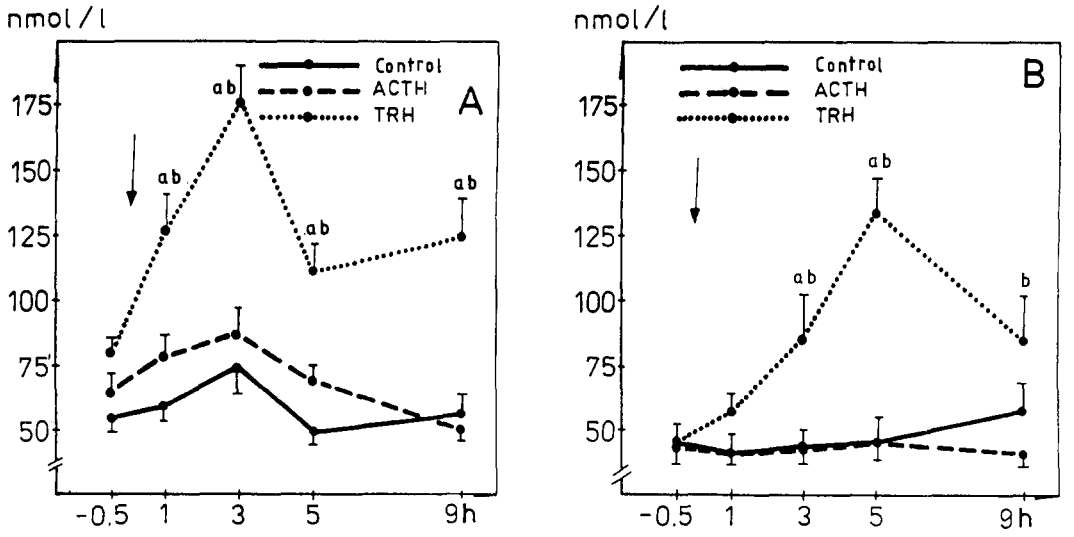

FIG. 3. Plasma T_4 level in fed (A) and food-deprived (B) sheep after corticotropin (ACTH) or thyrotropin-releasing hormone (TRH) injection; time of hormone administration is marked by an arrow. Values given are the mean ± SEM; in all cases, $n = 5$. a, statistically different from control values; b, statistical difference between two experimental groups; $F(40, 216) = 6.06, p < 0.01$.

TRH, at 132.7 ± 13.4 nmol/l; $p < 0.05$) (Figs. 3A and 3B). Nevertheless, the Δ_{max} values do not differ significantly in fed (97.4 ± 14.6 nmol/l) and fasted groups (89.52 ± 11.34 nmol/l). The discrepancies between maximal levels of T_4 and Δ_{max} values are due to the lower basal concentration of T_4 in food-deprived sheep.

Glucose

Food deprivation lowered basal glucose levels. Bolus injection of ACTH to fed animals was accompanied by an increase of plasma glucose to 58% ($p < 0.01$) above basal level (Fig. 4A). The corresponding rise in fasted sheep was only 27% ($p < 0.05$) despite a much higher plasma cortisol level (Fig. 4B). At 9 h after ACTH, the plasma glucose level of fasted sheep returned to the basal level of the fed group.

DISCUSSION

The findings presented in this article indicate that food deprivation modifies the response of circulating cortisol to exogenous ACTH, whereas the responsiveness of the hypophysis-thyroid axis to exogenous TRH remains unchanged. Moreover, the suppressed plasma levels of T_3 and glucose following food deprivation are much less affected by ACTH injection compared to those in fed animals. The hypothalamo-adrenocortical axis of fed animals does not respond to intravenously injected TRH, whereas in food-deprived animals a transient increase of plasma cortisol level is observed.

The much higher increment of plasma cortisol in food-deprived compared with fed sheep may be the result of the higher sensitivity of the adrenocortical gland to ACTH or the suppressed number of binding sites for cortisol in target tissues. It has been observed that stimulation of sympathetic splanchnic nerves increases adrenal cortical sensitivity to ACTH (7). Plasma noradrenaline levels, which reflect the functional activity of the peripheral sympathetic postganglionic nerves, are significantly enhanced after 3 days of fasting in humans (4). Our studies done on the same breed of sheep revealed an about threefold increase of plasma noradrenaline on the fourth day of fasting (unpublished data). Other factors may be the drop in cellular receptor numbers for glucocorticoids and a decreased distribution space. A lack of or a smaller effect of enhanced plasma cortisol on T_3 or plasma glucose levels in the present experiment supports the suggestion of suppressed binding of cortisol in target tissues. Gould and Siegel (17) observed in chicken thymocytes about a 50% decline in the cellular concentration of cytoplasmic corticosteroid receptor sites at 0.5 or 2 h after one or two IM injections of ACTH. In addition, thyroid hormone deficiency decreases the number of glucocorticoid receptor sites in the hippocampus (21) as well as the number of cytosolic glucocorticoid receptors in the livers of rats (19). It is worth adding that no changes were observed in basal levels of cortisol, T_3, and T_4 from 12–36 h in fasted dogs challenged with exogenous ACTH or TSH (28). The basis for the different results with dogs is not known. It may be related to differences in the dose of ACTH, which was higher (2.2 IU/kg), and the time of food deprivation, which was shorter than in our experiment. It has been stated that dogs respond differently to fasting when plasma reverse T_3 was taken into consideration (20). No differences in plasma thyroid hormone responses were obtained in sheep after TRH or TSH injections (24).

Food deprivation makes it possible for TRH given IV to act on the hypothalamo-adrenocortical axis. Normally, TRH acts within the CNS through a corticotropin-releasing factor (CRF)-dependent mechanism (5). TRH given IV was devoid of these activities. We assume, therefore, an increased penetrability of the blood-brain barrier in food-deprived sheep for TRH. The methodological objection should be excluded because all determinations of cortisol were performed in one assay.

Fasting of sheep is accompanied by a drop in plasma T_3 and development of a "low T_3 syndrome," often found in starvation and nonthyroidal illness in humans and animals (8). The decrement in plasma T_3 following ACTH administration has been explained on the basis of reduced 5′-monode-

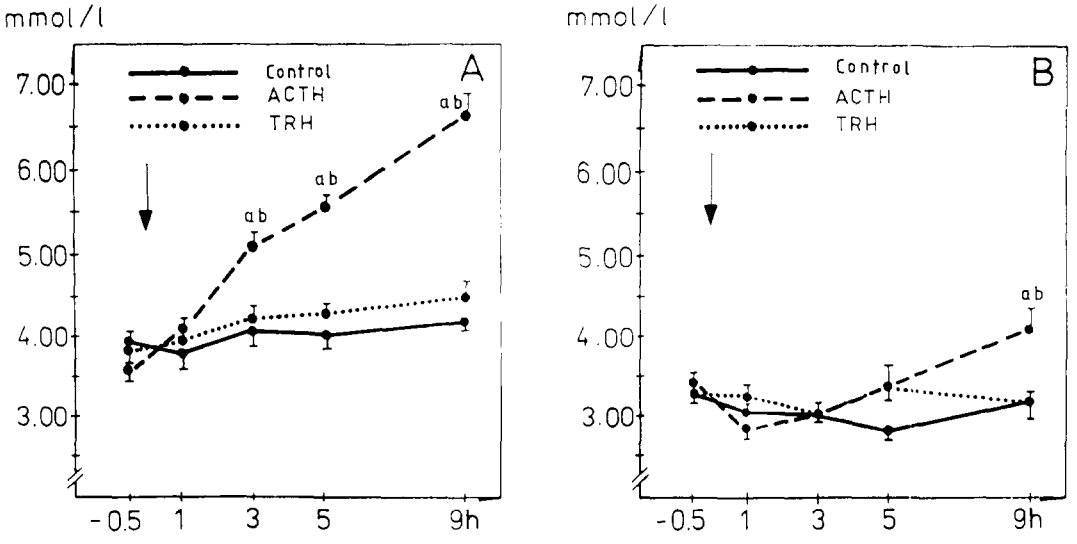

FIG. 4. Plasma glucose level in fed (A) and food-deprived (B) sheep after corticotropin (ACTH) or thyrotropin-releasing hormone (TRH) injection; time of hormone administration is marked by an arrow. Values given are the mean ± SEM; in all cases, $n = 5$. a, statistically different from control values; b, statistical difference between two experimental groups; $F(40, 216) = 10.39$, $p < 0.01$.

iodination of T_4 to T_3 in the tissues (9,29). Glucocorticoids are known to impair the extrathyroidal production of T_3 by decreasing the 5'-deiodinase activity in various tissues (3, 12,33). The suppressed plasma T_3 level obtained during food deprivation was resistant to elevated plasma cortisol. This supports the suggestion of reduced sensitivity of target tissues in food-deprived animals to cortisol (19).

Food deprivation was not associated with a significant fall in serum T_4 levels. It has been reported that serum TSH and T_4 concentrations in fasted animals depend upon gender. Unlike in males, plasma T_4 concentration in females is not consistently affected by food deprivation (11).

Stimulating of the hypophysis–thyroid axis by TRH significantly increased plasma T_4 and T_3 in both fed and food-deprived sheep. The Δ increase of T_4 and T_3 differed insignificantly between the fed and fasted groups and thus is in accordance with our previous results (34). It supports the suggestion (10,18) that fasting can lower plasma thyroid hormone levels without altering the sensitivity of the hypothalamus–hypophysis–thyroid axis to TRH or TSH. Nonetheless, the maximal plasma T_3 level in fed animals after TRH stimulation was significantly higher than the corresponding level in food-deprived sheep.

Prolonged fasting resulted in the expected decrements in baseline plasma glucose. As stated, in humans this is the result of both decrements in glucose production and increments in glucose clearance (4). Along with other hormones, cortisol may be involved in gluconeogenesis (14). The rise in plasma cortisol following ACTH injection resulted in 58 and 27% increases in plasma glucose in fed and food-deprived sheep, respectively. In the latter animals, the reduced glucose elevation was obtained despite the fourfold higher plasma cortisol increment in fasted compared to fed animals. Gelfand et al. (16) stated that in obese humans receiving IV glucose cortisol produces a 20–25% glycemic response. Based upon our experiment, the response in fed sheep is much more accentuated. The difference between fed and food-deprived animals may be partly attributed to decreased plasma T_3 levels in the latter because T_3 stimulates hepatic gluconeogenesis and glycogenolysis (33).

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