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Cold-Induced Increment in Rat Adrenal Gland Type II Deiodinase Is Corticosterone Dependent

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In this study we analyzed whether corticosterone synthesis is involved in the regulation of adrenal gland type II deiodinase (AG-D2) activity during acute cold exposure. Two well-known inhibitors of steroidogenesis, aminoglutethimide (AGT) and metyrapone (MTP), were administered to male Wistar rats maintained either at room temperature or acutely exposed to cold (1 h at 4°C). AG-D2 activity was measured by the radioiodide release method, and corticosterone circulating levels were measured by competitive protein binding assay. Results show that resting corticosterone levels and AG-D2 activity were lower in both AGT- and MTP-treated rats. Furthermore, the phasic increase normally exhibited by AG-D2 activity in response to acute cold stress was blunted in AGT- and MTP-treated animals. Therefore, we conclude that corticosterone synthesis is necessary in preserving the physiologic response of AG-D2 activity to cold exposure.

Key Words: Type II deiodinase activity; adrenal gland; corticosterone; cold stress; aminoglutethimide; metyrapone.

Introduction

In addition to their well-known participation in supporting the long-term energy expenditure associated with sustained metabolic demands, a growing body of experimental evidence indicates that circulating and intracellular levels of thyroid hormones are modified during acute and chronic stress responses, such as the so-called sick-euthyroid syndrome and surgery *(1–3)*. The mechanisms implied in these adaptive regulatory process are extremely complex and involve changes at different levels of the hypothalamic-pituitary-thyroid axis, as well as on its peripheral fine-tuning branch—the enzymatic deiodinase system *(2–4)*. Peripheral thyronine organ-specific deiodination plays a key role in determining the intracellular levels of active or inactive thyroid hormones through the action of a family of membrane-bound selenoenzymes *(5)*. Among the members of this enzyme family, type II deiodinase (D2) activity is responsible for the intracellular conversion of thyroxine (T_4) to its most active molecular form, triiodothyronine T_3. Studies aimed at understanding the mechanisms involved in regulating D2 activity have disclosed that its activity becomes significantly increased in those neuroendocrine structures involved in the installment of acute response to stress *(6–8)*. Thus, adrenal gland D2 (AG-D2) activity is stimulated during cold exposure, and this enzymatic response requires the intactness of the splanchnic neural flow impinging the gland, as well as the presence of neurohumoral factors *(6,7)***.** In vitro studies have shown that glucocorticoids increase D2 activity in astroglial cells *(9)*, and both D2 mRNA and enzyme activity in pituitary tumor cell lines *(10)*. Accordingly, in intact rats, a single dose of dexamethasone, but not adrenocorticotropic hormone (ACTH), selectively stimulated AG-D2 activity while the hypothalamic enzyme remain unchanged. However, unexpectedly, both hormones suppressed the high AG-D2 activity that accompanies severe stress (sham hypophysectomy), and dexamethasone, but not ACTH, also suppressed AG-D2 hyperactivity in hypophysectomized animals *(7)*.

In light of these findings, the participation of glucocorticoids on AG-D2 regulation remained unresolved. Therefore, the present study was designed to analyze further the role played by endogenous glucocorticoid (corticosterone) in modulating the immediate increment of AG-D2 activity that accompanies acute cold stress. For that purpose, two well-known inhibitors of steroidogenesis, aminoglutethimide (AGT) and metyrapone (MTP), were administered to rats maintained at room temperature (22°C) and to animals acutely exposed to cold (4°C). Our results show that corticosterone is required to modulate the phasic increase normally exhibited by AG-D2 activity in response to acute cold stress.

Results

Figure 1 shows the effects of AGT on adrenal weight and steroidogenesis. Whereas a single administration of AGT had no effect, multiple doses of the drug resulted in a 30% increase in the wet wt of adrenal gland (Fig. 1A). In parallel and as expected (Fig. 1B), AGT-treated animals consistently

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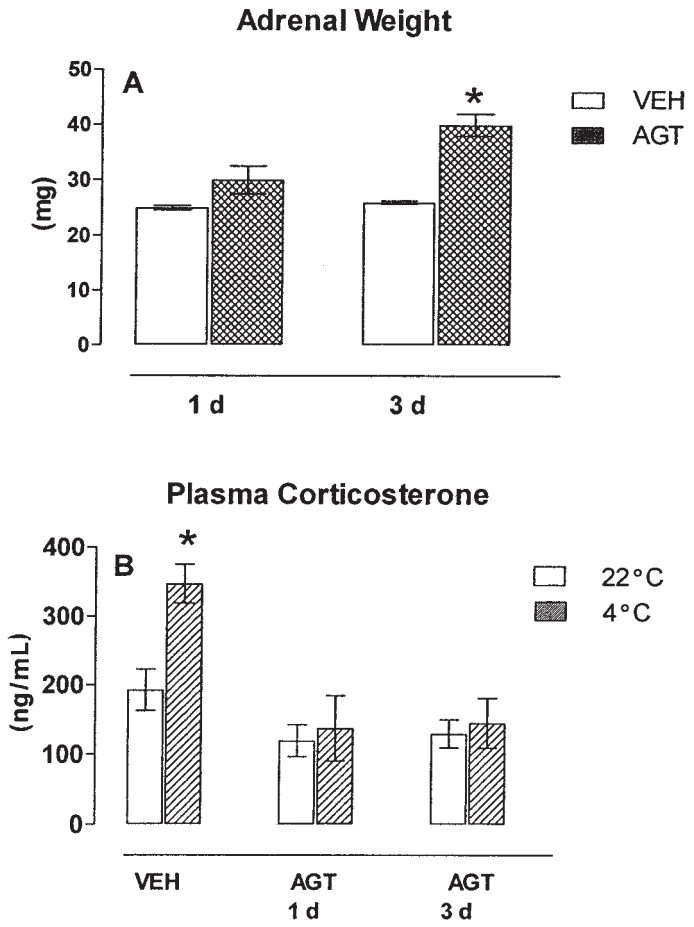

Fig. 1. Effect of AGT on adrenal weight and circulating levels of corticosterone during acute cold exposure. (**A**) Only multiple doses of AGT (3 d) increased the adrenal weight. (**B**) By contrast, both single and multiple doses of AGT inhibited the acute cold-evoked increase in corticosterone circulating levels. Results are expressed as the mean ± SEM of five rats/group. *$p \leq 0.05$. VEH, vehicle.

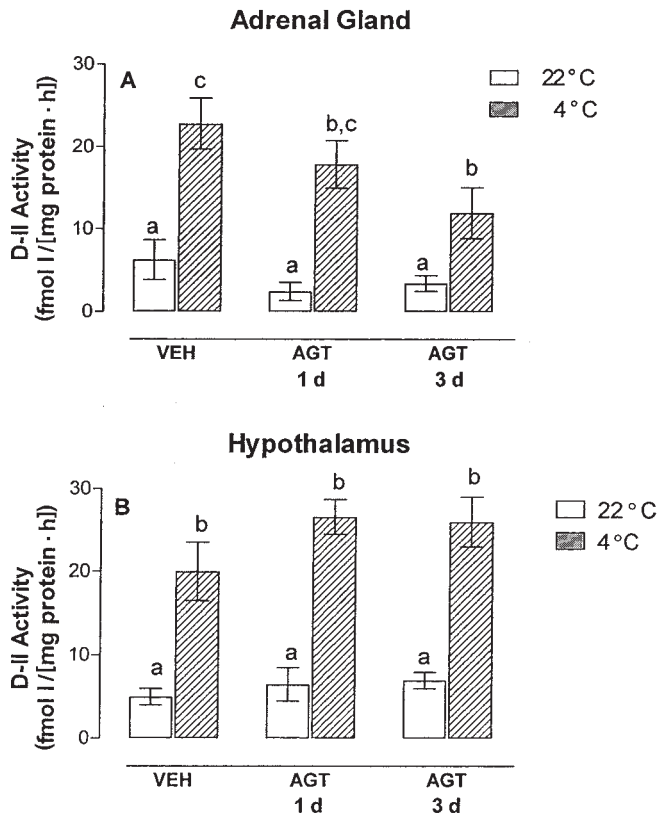

Fig. 2. Comparison of adrenal gland and hypothalamic D2 activity responses to acute cold exposure in rats treated with vehicle (VEH) or AGT. Rats were maintained in a cold room (4ºC), for 1 h. Note that AGT selectively blunts the acute cold-evoked increase in AG-D2 activity (**A**), without affecting the hypothalamic enzyme response that normally accompanies cold stress in the rat (**B**). Means bearing different superscript letters differ significantly ($p \leq 0.05$). Further details are as in Fig. 1.

exhibited decreased circulating corticosterone levels (40%). Furthermore, AGT-treated animals did not exhibit the well-documented increase in corticosterone secretion accompanying acute cold exposure. Concomitantly and as shown in Fig. 2, a trend to low AG-D2 activity was observed in AGT-treated animals kept at room temperature, and when exposed to cold stress, the physiologic acute rise in AG-D2 activity was blunted (Fig. 2A). AGT selectively impaired AG-D2 activity and had no effect on the normal hypothalamic enzyme response to acute cold exposure (Fig. 2B).

Moreover, to confirm that the observed effects were specifically associated with the adrenostatic actions of the drug and not to its extraadrenal effects, a separate group of rats was treated with MTP. Whereas AGT inhibits cholesterol desmolase (CYP11A1), the first enzymatic step in steroidogenesis, MTP is a selective inhibitor of 11-β-hydroxylase (CYP11B1), the enzyme that catalyzes the final step in the biosynthesis of cortisol and corticosterone *(11–15)*. Results of these experiments (Fig. 3) showed that a 3-d MTP treatment provoked a significant rise in adrenal weight (14%), as well as a clear-cut drop (60%) in circulating corticosterone levels (Fig. 3B). Furthermore, and in contrast to vehicle-treated rats, MTP-treated animals did not exhibit the increase in corticosterone secretion accompanying acute cold exposure (Fig. 3B). As shown in Fig. 4, at room temperature, AG-D2 activity was similar between vehicle- and MTP-treated animals. However, the physiologic acute rise in AG-D2 activity elicited by cold exposure was blunted in MTP-treated rats.

Discussion

The present results show that blockade of corticosterone synthesis by the administration of two well-known adrenostatic agents selectively blunts the acute cold-evoked increase in AG-D2 activity without affecting the hypothalamic enzyme response that normally accompanies cold stress in the rat *(6,7)*. Adrenal steroidogenesis blockade results in

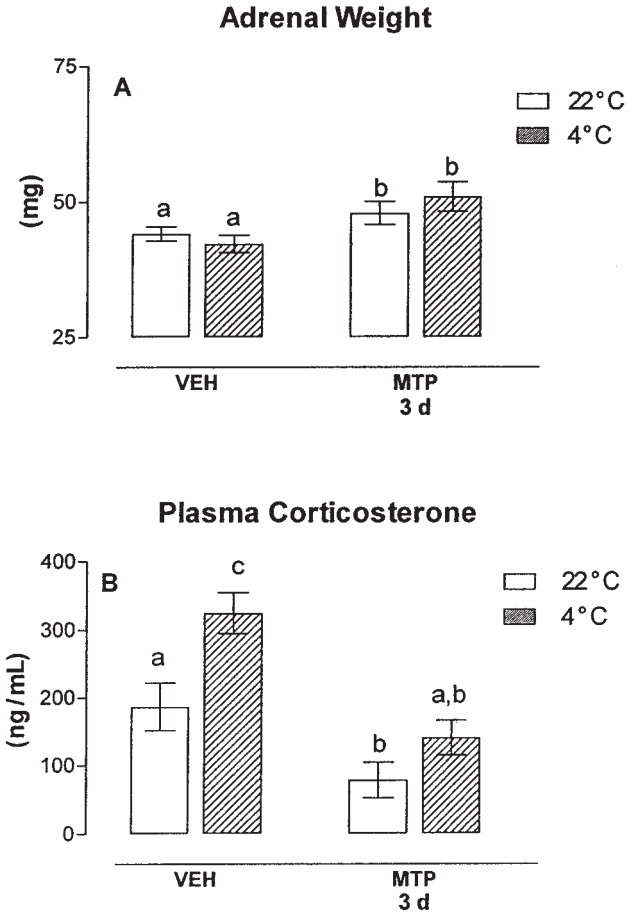

Fig. 3. Effect of MTP on adrenal weight and circulating levels of corticosterone during acute cold exposure. MTP-treated rats exhibited compensatory adrenal hypertrophy (**A**), as well as decreased basal levels and a blunted corticosterone response to acute cold stress (**B**). Results are expressed as the mean ± SEM of six rats/group. Means bearing different superscript letters differ significantly ($p \leq 0.05$). VEH, vehicle.

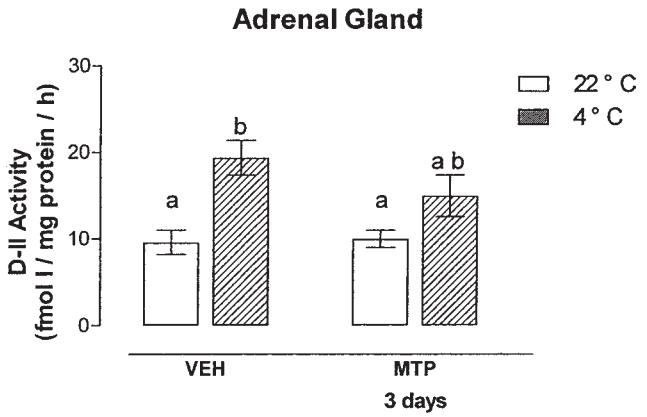

Fig. 4. Effect of MTP on AG-D2 activity during acute cold exposure. Note that the physiologic response of AG-D2 activity to acute cold stress was blunted in MTP-treated rats. Further details are in Fig. 3. VEH, vehicle.

decreased corticosterone circulating levels and a compensatory increase in pituitary ACTH secretion *(11–15)*. As our results demonstrate, administration of either AGT or MTP effectively decreased circulating corticosterone levels and provoked a compensatory adrenal hypertrophy that was more pronounced in those animals that received AGT repeatedly. The adrenostatic effectiveness of both drugs became more evident during the thermoregulatory demand imposed by cold exposure, a situation in which the physiologic corticosterone secretory response observed in control rats was blunted in AGT- or MTP-treated animals. Furthermore, this partial interruption in adrenal steroidogenesis was associated with reduced AG-D2 activity in rats maintained at room temperature, as well as with a significant decrease in the cold-evoked rise in the enzyme in drug-treated animals. These data indicate that glucocorticoids exert an organ-specific regulatory effect on AG-D2 activity and add further support to our proposal that AG-D2 is under a dual neuroendocrine regulatory influence in which the sympathetic nervous system plays a major role *(7)*. The steroid is locally required to maintain a tonic or resting level of AG-D2 activity, as well as to modulate the immediate enzyme activation that accompanies acute cold stress. This activation of D2 by glucocorticoids in the adrenal gland could be an important component of the acute stress response, in which the resultant T_3 may metabolically contribute to the biosynthesis or release of both catecholamines and glucocorticoids.

Our experimental design still does not allow us to offer an explanation regarding the mechanism of action of corticosterone. However, since dexamethasone has a synergistic effect with cyclic adenosine monophosphate (cAMP) on D2 activation in glial cells *(9)*, and human and rat *dio2* gene contain a cAMP response element *(16)*, it seems reasonable to suggest that regulation of AG-D2 by the glucocorticoid might involve specific gene transcriptional mechanisms.

In conclusion, the present results demonstrate that corticosterone synthesis is necessary in preserving the physiologic increase normally exhibited by AG-D2 activity in response to acute cold stress. Thus, the adrenal gland seems to belong to that group of specialized organs, such as the nervous system and the anterior pituitary gland *(9,10)*, in which glucocorticoids exert a tissue-specific regulatory influence on D2 activity.

Materials and Methods

Reagents

Nonradioactive T_4 of the highest available purity was obtained from Henning (Berlin, Germany). Corticosterone, 6-*n*-propyl-2-thiouracil (PTU), polyethylene glycol, AGT, and MTP were from Sigma (St. Louis, MO). ^{125}I-Labeled thyroxine and 1,2,6,7-[^{3}H]-hydrocortisone were purchased from New England Nuclear (Boston, MA). The specific activities of T_4 and corticosterone were 1250 and 220 Ci/g, respectively. Dithiothreitol (DTT) was obtained from Calbio-

chem (La Jolla, CA), Sep Pack C18 cartridges from Waters (Milford, MA), and Bradford's Reagent and Dowex 50 W-X2 from Bio-Rad (Richmond, CA). All other chemicals were of analytical grade.

Animals and Experimental Design

Male Wistar rats of 200 ± 10 g of body wt were used. They were housed in stainless steel cages under controlled temperature (22 ± 1ºC), humidity (67 ± 4%), and lighting conditions (12 h:12 h; lights on 7:00 AM to 7:00 PM).They had free access to rat chow (Purina, Richmond, CA) and tap water. Procedures regarding care, administration of treatment, and euthanasia of animals were reviewed and approved by an ad hoc ethics committee.

Administration of AGT

Two groups of rats were subcutaneously injected with AGT dissolved in acetate buffer (pH 4.0) and saline solution (0.9% NaCl). The first group received a single dose of AGT (50.0 mg/kg), and 1 h later they were transferred to a cold room (4ºC) for 1 h. Rats were decapitated 2 h post-injection. The second group received the same dose of AGT, but the drug was administered twice daily for 3 consecutive days. One hour after the last injection of AGT, the rats were kept at 4ºC for 1 h and then sacrificed as just described. Each group had its own control animals, which were kept at room temperature (22 ± 1.0ºC).

Administration of MTP

Rats were administered 50 mg/kg of MTP each day for 3 d. One hour after the last injection of MTP, the rats were kept at 4°C for 1 h and killed 1 later. MTP was dissolved in a solution of polyethylene glycol (40%) plus physiologic saline solution. Control animals were treated with vehicle.

Handling of Samples

On the day of the experiment, adrenal glands were rapidly removed, freed of pericapsular fat, and immediately processed. The hypothalamus was used as a control tissue in some experiments. Tissue samples were homogenized individually on 10 vol (w/v) of 0.25 *M* sucrose and 20.0 m*M* Tris-HCl (pH 7.6) containing 10 m*M* DTT and 1.0 m*M* EDTA. Crude homogenates were centrifuged at 10,000*g* for 15 min at 4ºC. The supernatant (microsomal crude fraction) was quickly frozen in dry ice–acetone and stored at –70ºC until determination of D2 activity. Trunk blood was allowed to clot at room temperature and centrifuged at 4ºC for 15 min. Serum was stored at –20ºC until determination of corticosterone.

Deiodinase Assay

As described elsewhere *(17)*, adrenal and hypothalamic D2 activity were determined by using a modification of the radiolabeled iodide release method originally described by Leonard and Rosenberg *(18)*. Briefly, D-II activity was assayed by the release of ^{125}I from the phenolic ring of [^{125}I]-T_4 (2.0 n*M* at 200,000 cpm) in the presence of non-radioactive T_4 (28 n*M*), DTT (20 m*M*), and PTU (1.0 m*M*). Reaction time was 3 h at 37ºC. The reaction was stopped by adding 50 µL of a cold solution containing 50% normal bovine serum plus PTU (10 m*M*) and 350 µL of 10% trichloroacetic acid. The samples were centrifuged for 10 min at 1500*g*, and the liberated radioiodide was measured after isolation by ion-exchange chromatography (Dowex 50 W-X2). [^{125}I]-T_4 was purified immediately before use with Sep-Pack C18 cartridges yielding a purity of 98%. The protein contents of the homogenates were determined by Bradford's *(19)* method. Results of enzyme activity are expressed as femtomole iodide released/milligrams of protein·hour. All samples were processed individually, and each sample was determined in duplicate. Coefficient of variation (CV) was 6.0%. Control incubations were performed by omission of the homogenate.

Corticosterone Assay

Circulating serum levels of corticosterone were assessed without previous purification by a competitive protein binding assay, as described elsewhere *(20)*. The limit of sensitivity was 0.25 ng/(assay·tube). Recoveries ranged from 63 to 87%. Intra- and interassay CVs were 8.0 and 8.4%, respectively.

Statistical Analysis

All results are expressed as the mean ± SEM. The data were analyzed by analysis of variance followed by Newman-Keuls Test. A value of $p \leq 0.05$ was considered statistically significant. Results were analyzed with the help of a statistical program (Sigma, Stat. Jandel, 1994).

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