Modification of pituitary-adrenal feedback sensitivity in young rats by neonatal treatment with cortisol

M. S. Erskine1, Edward Geller and Arthur Yuwiler

Neurobiochemistry Laboratory, Veterans Administration, Brentwood Hospital, and
Department of Psychiatry, Center for the Health Sciences,
University of California, Los Angeles, California 90024, USA

Abstract. Neonatal exposure of rats to cortisol acetate was found to alter pituitary-adrenal feedback regulation at 20–25 days of age. Plasma levels of adrenocorticotropic hormone (ACTH) after ether stress were reduced in cortisol-treated rats pre-treated with 100 μg corticosterone/100 g body weight, while rats given vehicle neonatally did not show suppression of the ACTH response below levels in animals given saline only or not injected as pre-treatments. Neonatal cortisol increased sensitivity to dexamethasone in inhibition of the stress response; cortisol-treated animals had a reduced plasma corticosterone response to stress 3 h after pre-treatment with 1.25, 2.5, 25, or 250 μg dexamethasone/100 g body weight, while the stress response in animals given vehicle neonatally was not inhibited by the lowest dosage of dexamethasone. Neonatal cortisol treatment did not affect corticosteroid-binding globulin (CBG) binding capacity in plasma of 25-day-old animals. Thus, neonatal treatment with cortisol appears to increase feedback sensitivity to circulating corticosteroids at 20–25 days of age.

Treatment of rats with cortisol acetate within the first several days of life modifies the developmental patterns of pituitary-adrenal activity as well as several neurophysiological, biochemical, and behavioural processes (Schapiro 1968; Vernadakis & Woodbury 1971; Yuwiler et al. 1978). In rats treated neonatally with corticosteroids, the onset of the diurnal rhythm of plasma corticosterone is delayed from the several days around weaning (Ader 1969) until at least 30 days of age (Krieger 1974; Miyabo & Hisada 1975; Ulrich et al. 1976). Although the adrenocortical response to stress is present at about 15 days of age in untreated animals, both plasma ACTH and corticosterone are suppressed following stress in 20 to 25-day-old rats given neonatal treatment with cortisol (Ulrich et al. 1976; Erskine et al. 1979), and the full adult pattern of stress responsiveness is not present even at 45–48 days of age (Erskine et al. 1979). The present studies explored further the effects of neonatal cortisol treatment on pituitary-adrenal regulation by examining the functional state of the negative feedback system in these animals.

Physiological levels of exogenously administered corticosterone have been shown to inhibit the subsequent pituitary-adrenal response to stress in adult rats (Zimmerman & Critchlow 1969; Jones & Tiptaft 1977) and feedback inhibition of ACTH by corticosteroids also appears to occur in the perinatal period (Paul & D'Angelo 1972; Milkovic et al. 1976). However, studies of feedback inhibition during the period between weaning and puberty have yielded conflicting results. Both decreased (Goldman et al. 1973) and increased (Ramaley 1975) feedback sensitivity have been reported in weanling as opposed to post-pubertal rats, as measured by the dose of exogenous corticosteroid needed to inhibit a subsequent stress response. An increased sensitivity to feedback inhibition by corticosterone in the pre-pubertal period would be consistent with the increased sensitivity to gonadal

1 Present address:
Department of Nutrition and Food Science, Room 37-315, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139, USA.
steroid inhibition of gonadotrophin release seen at this age (Ramirez & McCann 1963; Negro-Vilar et al. 1973).

The present experiments explored the effects of neonatal cortisol treatment on the negative feedback inhibition of plasma ACTH and corticosterone responses to stress by exogenously administered corticosteroids at 23 to 25 days of age. Because previous studies demonstrated decreased immunological capacity and resting levels of plasma globulin in cortisol-treated rats (Schapiro & Huppert 1967; Ulrich et al. 1977), a possibility that a deficit in the plasma binding of corticosterone resulted in an effective increase in available corticosteroids was tested by measuring the binding capacity of plasma corticosteroid-binding globulin (CBG).

Materials and Methods

Animals were offspring of Sprague-Dawley CD rats obtained from Charles-River Labs, Wilmington, Massachusetts, USA. They were born and reared in 24 × 24 × 28 cm stainless steel cages. In most experiments, animals were weaned by removal to 20 × 17 × 23 cm suspended wire cages on days 22–25 of life (day of birth, day 1). When specified, animals remained with the lactating female rat until the end of the experiment. Food and water were available ad libitum and lights were on between 05.00–17.00 h. All experiments were carried out in the 8 h after light onset.

Neonatal treatment

Animals were injected sc within 24 h of birth with 1.0 or 0.5 mg cortisol acetate (Merck, Sharp and Dohme, West Point, Pennsylvania, USA) or its polysorbate-carboxymethyl-cellulose vehicle (Sterile Vehicle 100; Upjohn, Newport Beach, California, USA). Litters were culled at the time of injection to 10 pups. All pups within a single litter received the same treatment and animals from a litter were distributed as evenly as possible across experimental groups.

Inhibition of the stress response

The inhibition of the pituitary-adrenal response to ether stress by prior exposure to corticosterone was measured in two experiments. In the first, 1.0 mg cortisol acetate or vehicle was administered on day 1. On day 25, animals received sc injections of 100 μg corticosterone/100 g body weight or the saline vehicle, or were not injected. They were then returned to their home cage. Ten min or 3 h later, animals were exposed to ether for 1 min and were killed 2.5 min after the onset of exposure to ether. Following decapitation, trunk blood was collected in cold polystyrene tubes containing approximately 1 mg EDTA. The samples were placed on ice until centrifuged at 4°C for 20 min at 5300 g. Plasma was placed into cold polystyrene collection vials and stored at −18°C until assayed for ACTH.

In the second experiment, rats were injected on day 1 with either 0.5 mg cortisol acetate or vehicle. On day 23, animals were injected with 1.25, 2.5, 25, or 250 μg dexamethasone phosphate (Decadron; Merck, Sharp and Dohme)/100 g body weight and were returned to their home cages. Control animals were un.injected. Three h later, animals were given an ether stress and killed 20 min after the onset of ether exposure. Trunk blood for later determination of plasma corticosterone was collected in heparinized tubes. Samples were centrifuged at room temperature at 5300 g for 20 min and plasma samples were stored at −15°C until assayed.

Plasma corticosteroid-binding globulin binding capacity

At 25 days of age, male and female rats from cortisol-treated (0.5 mg) and vehicle-treated litters were removed from the cage containing the lactating female rat, decapitated and trunk blood was collected in heparinized tubes. Samples were centrifuged at room temperature at 5300 g for 20 min and plasma was frozen at −70°C until assayed for plasma CBG binding capacity (μg/100 ml).

Assays

Adrenocorticotrophin. Plasma ACTH was estimated using a radioimmunoassay kit obtained from CIS Radiopharmaceuticals, Bedford, Massachusetts, USA. Values, obtained against a human ACTH standard, are expressed as pg/ml. The coefficients of variation (CV) for intra- and inter-assay variability, as previously reported (Erskine et al. 1979) were 8.83 and 12.75%, respectively. All samples were run within one assay.

Corticosterone. Plasma corticosterone was measured by the microfluorometric method of Glick et al. (1964).

Corticosteroid-binding globulin. The binding capacity of CBG (μg/100 ml) was measured using a radiolabelling technique established for cortisol which involves charcoal separation of the free and CBG-bound fractions of the tritiated steroid (Moore et al. 1978). Using this assay system, [6,7-3H](N)-dexamethasone (New England Nuclear, Boston, Massachusetts, USA) showed background binding levels, and non-specific binding of [1,2-3H]corticosterone (New England Nuclear) following heat denaturation of the protein (60°C) was approximately 15% of the total binding. For each sample, a non-specific binding level was measured and subtracted from the final value obtained. The CV for within- and between-assay variability were 13.7 and 7.3%, respectively. The mean levels of
Mean plasma ACTH response to ether stress following pre-treatment with corticosterone (hatched bars), saline (lined bars), or no injection (empty bars) in 25-day-old cortisol- and vehicle-treated rats. * Significantly \( P < 0.05 \) lower levels than saline-injected cortisol-treated rats. ** Significant \( P < 0.001 \) lower levels than rats given vehicle neonatally.

Vertical lines denote SEM and figures above each bar show number of animals.

Among animals neonatally treated with cortisol, overall significant differences occurred between the three pre-stress treatment groups \( P < 0.025 \). Plasma ACTH levels were significantly lower in corticosterone-injected animals than in saline-injected animals \( P < 0.05 \). Prior injection of corticosterone did not suppress the ACTH response below levels obtained in non-injected control animals. In rats treated neonatally with vehicle, no significant effects of pre-treatment with either corticosterone or saline were observed \( F(1) < 1 \).

**Corticosterone response to stress after pretreatment with dexamethasone**

Plasma corticosterone values obtained from dexamethasone-injected animals 20 min after ether stress are presented in Fig. 2. Male and female rats did not differ in their plasma corticosterone response (not shown) and these values were combined. As expected from previous data (Erskine et al. 1979) levels of corticosterone in cortisol-treated and vehicle-treated animals not receiving a pre-stress injection did not differ 20 min after ether stress.

Among rats receiving cortisol neonatally, all four doses of dexamethasone were effective in suppressing levels of corticosterone below those obtained in the uninjected animals \( P < 0.02 \) for 1.25 and CBG from 6 normal adult female and 6 adult male rats were 63.00 ± 10.54 (SEM) and 29.52 ± 4.65 μg/100 ml, respectively.

**Statistics**

Analysis of variance was used to test significance before between-group comparisons using Student’s t-test.

**Results**

**ACTH response to stress after corticosterone pretreatment**

Within each neonatal treatment group, similar ACTH values were obtained, whether animals were pre-treated 10 min or 3 h before the ether stress (not shown, \( F < 1 \)). This suggested that at this age, the rate-sensitive (fast feedback) and level-sensitive (delayed feedback) components of the feedback system (Dallman & Yates 1969; Jones et al. 1974) were not distinguishable. The data combined across the two sampling times are presented in Fig. 1. Rats treated neonatally with cortisol displayed an overall suppression of the ACTH response to ether stress below vehicle control levels \( P < 0.001 \); this result confirms previous findings of suppressed pituitary responsiveness to stress in steroid-treated animals at this age (Erskine et al. 1979).
Mean plasma corticosterone response to ether stress in 23-day-old rats neonatally treated with cortisol (hatched bars) or vehicle (empty bars) 3 h after injection of various doses of dexamethasone. Asterisks indicate significantly (* $P < 0.02$; ** $P < 0.01$; *** $P < 0.001$) lower levels than control values. Vertical bars indicate SEM and the figures above each bar show number of animals.

Vehicle-treated rats given dexamethasone only in doses of 2.5, 25, and 250 µg/100 g body weight showed plasma corticosterone levels after stress that were significantly suppressed below uninjected control levels ($P < 0.001$ for all comparisons); no suppression was seen after administration of the 1.25 µg dose in these animals (Student's $t = 1.19$). At the 1.25 µg dosage, rats treated neonatally with cortisol showed a significantly ($P < 0.01$) lower plasma corticosterone response than did vehicle-injected control rats. No significant differences between neonatal treatment groups were seen at any other dosage.

**Corticosteroid-binding globulin binding capacity**

Levels of CBG binding capacity (µg/100 ml) measured in plasma from 25-day-old cortisol-treated animals were not significantly different from levels seen among vehicle-treated control rats (Table 1). Confirming previous findings (Gala & Westphal 1965; Keller et al. 1966), relative binding capacity was less at this age than in adulthood (see Methods), and no sex difference was seen in either group at this age.

**Discussion**

Data from the present studies have demonstrated that administration of cortisol acetate to newborn rats alters the sensitivity of the hypothalamo-pitui-
tary axis to feedback inhibition by corticosteroids. In general, this neonatal treatment resulted in an increased responsiveness of the feedback system as measured at 20–25 days of age. After pre-treatment with 100 μg corticosterone/100 g body weight, cortisol-treated but not vehicle-treated rats showed a significant inhibition of plasma ACTH levels 2.5 min after ether stress. In addition, the plasma corticosterone response to stress was inhibited in steroid-treated animals by a dose of dexamethasone too low to inhibit the stress response in control animals.

These experiments did not clarify whether neonatal cortisol treatment alters the normal developmental pattern of feedback responsiveness, or whether it produces some more direct and perhaps enduring effect. If neonatal treatment with cortisol acts by retarding development of the feedback system as it does with other components of pituitary-adrenal function (Krieger 1974; Ulrich et al. 1976; Erskine et al. 1979), it might be expected, from the present results, that the immature feedback system of normal infant rats would be more responsive than the mature system of adults. Evidence for this is equivocal. One study demonstrated an increased sensitivity to suppression by dexamethasone of a subsequent stress in pre- as opposed to post-pubertal rats (Ramaley 1975), while another has shown a decreased sensitivity pre-pubertally (Goldman et al. 1973).

The results of these studies suggest that neonatal treatment with cortisol may affect central or pituitary, rather than peripheral components of the feedback system. Since neonatal treatment did not affect circulating levels of CBG, a decrease in corticosterone binding with consequent enhanced negative feedback inhibition of ACTH secretion (Kawai & Yates 1966) seems unlikely to account for our results. Furthermore, there is no indication that basal or resting levels of corticosterone are elevated in cortisol-treated rats, which might result in more rapid or efficient inhibition of ACTH following stress. Basal levels of plasma corticosterone do not differ between cortisol-treated and control rats at this age (Erskine et al. 1979), and the adrenocortical response to ether stress is sluggish in these animals (Erskine et al. 1979). In addition, adrenal weights of cortisol-treated rats at 20 days are significantly smaller than control weights (unpublished results), suggesting that basal ACTH levels are lower than in control rats. Thus, in these experiments circulating levels of corticosterone would not have been expected to affect appreciably the inhibition of ACTH by exogenously administered steroids.

The apparent increase in feedback sensitivity in cortisol-treated rats could result from a greater efficacy of the available corticosteroid at the central or pituitary feedback site, since it is presumably acting to inhibit a system which already shows decreased activation after stress (Erskine et al. 1979). If the reduced ACTH response to stress is reflected in a lowered number of steroid receptors, then smaller concentrations of steroid might be more effective in dampening subsequent stress responsiveness. However, it is not known whether feedback sensitivity itself is linked directly to the activity of the stress responsive system. Use of neonatal cortisol treatment to alter the developmental patterns of stress responsivity and/or feedback sensitivity may shed some light on the nature of the joint action of the two systems.

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