Title
Fetal and maternal levels of corticosterone and ACTH after pharmacological adrenalectomy

Journal
Life Sciences, 47(6)

ISSN
0024-3205

Authors
Baram, Tallie Z
Schultz, Linda

Publication Date
1990

DOI
10.1016/0024-3205(90)90607-s

Life Sciences, Vol. 47, pp. 485-489 Pergamon Press
Printed in the U.S.A.

FETAL AND MATERNAL LEVELS OF CORTICOSTERONE AND ACTH AFTER PHARMACOLOGICAL ADRENALECTOMY

Tallie Z. Baram* and Linda Schultz

Dept. Neurology, University of Southern California; CHLA;
P. O. Box 54700; Los Angeles; CA 90054-0700.

(Received in final form June 1, 1990)

Summary

A paradigm of pharmacological adrenalectomy of pregnant rats and fetuses in utero is described. A regimen of twice daily metyrapone injections (10mg/100gm body weight), results in marked depression of serum corticosterone in pregnant and in fetal rats without surgical trauma and stress. The technique should be useful in a wide variety of studies involving the developing brain-adrenal axis.

The mechanisms controlling corticotropin releasing-hormone (CRH) gene expression in the developing rat brain have not been defined. The onset of messenger RNA (CRH-mRNA) synthesis occurs on the 17th fetal day (1,2), and progresses through the late fetal period. CRH-mRNA decreases markedly around the time of birth (1,2), and remains comparatively low during the first postnatal week. Whether the onset and the hiatus in CRH gene expression are intrinsic, constitutively regulated phenomena, or are modulated via negative feedback by circulating ACTH or corticosterone (presumably acting via their respective receptors,) is unknown. Changes in corticosteroid receptor number or type at the level of the pituitary have been invoked as a possible underlying mechanism (3).

This study was designed to examine for the effects of eliminating the negative corticosterone feedback on the brain-adrenal axis in the fetal rat. Our goal was to achieve functional fetal adrenalectomy without the accompanying stress and trauma associated with surgical ablation in utero. The model should be useful in the study of the regulation of CRH gene expression in the developing rat brain.

Materials and Methods

Timed-pregnancy Sprague-Dawley derived rats, weighing less than 350 grams, were obtained from Zivic-Miller (Zelienople, PA). Rats were kept on a 12 hour light/ dark cycle and fed and watered ad

*To whom correspondence should be addressed

0024-3205/90 $3.00 +.00

libitum. Pregnancy was dated by the presence of a vaginal plug which was considered to be day 0. Pharmacological adrenalectomy schedule was started on the 14th, 15th, or 16th day of pregnancy, and continued for 24, 48 or 72 hours: Animals were injected subcutaneously with DMSO vehicle or metyrapone (Sigma, St. Louis, MO) on a 12 hour schedule at 0800 h and 2000 h. Metyrapone, 10 mg/kg dose, was injected up to the morning of sacrifice, when it was followed by a 10 mg/kg dose of aminogluthetimide (a blocker of cholesterol conversion to 20- alpha cholesterol), in vehicle, at 0915h. An additonal group was not injected to control for the effects of handling and DMSO.

Rats were decapitated at 1000h and uteri harvested immediately. Maternal and fetal (fetal day 18 and 19 only) trunk blood was obtained on days 15-18. Fetuses' blood was pooled from within a litter. Litter size and pup weight were assessed for experimental and control groups. Blood was centrifuged immediately for 30 minutes at 4000 G and serum kept frozen at -80^{0}C until assayed.

Hormone Assays:

Corticosterone was assayed in fetal and maternal blood using a commercial radioimmunoassay kit (ICN, Irvine, CA). ACTH was determined in both extracted and nonextracted serum using a radioimmunoassay developed by Pennisula, (Belmont, CA). As no significant differences between extracted and nonextracted plasma were found in preliminary studies, samples were not extracted in subsequent assays. Sensitivity ranges for corticosterone and ACTH were 0.025 and 0.008 ng per tube (0.05 and 0.08 ng/ml), respectively. Coefficient of variation for the corticosterone assay was <15%, and that for the ACTH assay was <10%.

Statistical Analyses: Samples were subjected to analyses of mean and standard deviation, and the significance of differences between group means was assessed by student's t-test. Probability levels are indicated in parentheses.

Results

Toxicity of the Regimen: Litter size and mean pup weight of rats injected with vehicle alone and of those injected with metyrapone are shown in Table I. There was no significant change in litter size, suggesting no resorption of pups even after 72 hours of pharmacological adrenalectomy. Likewise, no significant change in mean pup weight was observed. The mean weight of pups of rats injected with DMSO was not different from that of pups of untreated pregnant rats. The difference between plasma corticosterone, on the 18th day, of uninjected controls (34.6 ± 8.4 mcg/dl) and DMSO-injected rats (52.5 ± 12.5 mcg/dl), was not significant.

Effect of pharmacological adrenalectomy on maternal and fetal corticosterone levels: Corticosterone levels in DMSO and metyrapone-injected rats during the third week of pregnancy are compared in figure 1. Pharmacological adrenalectomy decreases serum corticosterone levels 85 to 95%. This effect of pharmacological adrenalectomy is seen after 24 hours and does not diminish by 72 hours (figure 2). Table 1 shows the effect of pharmacological

adrenalectomy on serum corticosterone of fetuses on the 18th day. On the 19th fetal day plasma corticosterone was 47.5 ± 3 mcg/dl for controls, and 7 ± 2 mcg/dl for fetuses of metyrapone treated rats.

TABLE I

Effects of Pharmacological Adrenalectomy on Fetal Litter Size, Weight and Serum Corticosterone.

Parameter	Control	Metyrapone-treated
Fetal weight 18th day (gm)	1.44 ± 0.2 n=3 litters	1.44 ± 0.3 n=6 litters
Litter size 18th day (n)	11 ± 2.1 n=3 litters	12 ± 2.4 n=6 litters
Corticosterone 18th day (mcg/dl)	30.2 ± 3.7 n=7 pools	5.9 ± 1.6 n=5 pools

Values given indicate mean ± standard deviation.

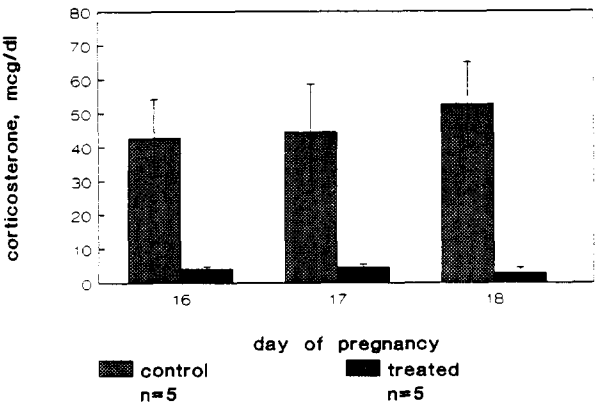

Fig. 1

Serum corticosterone in pregnant rats, effects of pharmacological adrenalectomy. Pharmacological adrenalectomy was achieved by metyrapone injections as described in the text. All plasma corticosterone levels of treated animals were significantly different than those of controls (p = 0.001-0.0035).

Effect of pharmacological adrenalectomy on ACTH levels in fetal and pregnant rats: Plasma levels of ACTH in pregnant rats injected with DMSO or with metyrapone are shown in Table II. A 3-fold increase is evident following metyrapone administration. A parallel, metyrapone-induced increase of serum ACTH levels in rat fetuses is also seen.

TABLE II

Effect of Pharmacological Adrenalectomy on Serum ACTH

Pregnancy/gestation day	Vehicle injected ng/ml	Metyrapone injected ng/ml
16th day, adult	505 ± 63 (n=3)	1,220 ± 665 (n=3)
17th day, adult	506 ± 127 (n=3)	1,466 ± 102 (n=2)
18th day, adult	488 ± 57 (n=7)	2,007 ± 666 (n=3)
18th day, fetus	325 ± 184 (n=4)	1,139 ± 680 (n=3)

($p<0.05$, except for fetus, where $p = 0.19$).

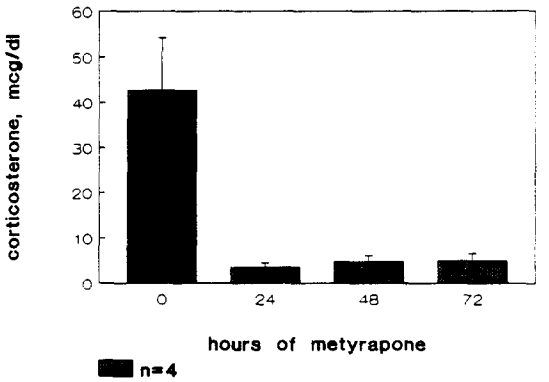

Fig. 2
Time course of corticosterone response to pharmacological adrenalectomy. Metyrapone injection regimen is described in the text. Plasma corticosterone levels of treated rats at all time points differ significantly from those of controls ($p<0.01$).

Discussion

We present a paradigm of pharmacological adrenalectomy of fetal rat pups in utero. Metyrapone (which crosses the placenta) is injected into pregnant rats for 1-3 days, resulting in functional adrenalectomy in both mothers and pups.

The efficacy of this regimen is evident from the dramatic alterations in both maternal and fetal serum corticosterone and ACTH. Corticosterone levels decrease to 5 mcg/dl, levels that have been reported in surgically adrenalectomized rats (6). As corticosterone crosses the placenta, it could be argued that only maternal adrenalectomy is achieved, and fetal levels reflect decreased maternal synthesis. It is well established, however, that

the fetal adrenal is relatively large and can secrete corticosterone during late fetal life (4), and that the majority of fetal plasma corticosterone is fetal-derived (5). Furthermore, surgical maternal adrenalectomy results in marked increase in fetal plasma corticosterone, in an attempt to "compensate" for lack of maternal hormone (6).

Consequent to adrenalectomy of the pregnant rat alone, fetal serum ACTH (a peptide which does not cross the placenta) should be unchanged (6), or decreased, due to negative feedback effect of hyperactive fetal adrenal. The observed elevation in fetal serum ACTH suggests elimination of steroid feedback, or stimulation of pituitary synthesis and release of this hormone at the brain or pituitary level. The fetal pituitary has been shown to produce ACTH in response to CRF during the late fetal period (7), and serum ACTH levels reported in this study are comparable to previous reports (5,6).

This model of pharmacological adrenalectomy is derived from the regimen proposed by Plotsky et al. (8). As in their adult model, it circumvents surgery-induced trauma and significant stress which, by themselves, affect the CRH-ACTH-adrenal axis. Moreover, metyrapone crosses the placenta and can thus block corticosterone synthesis in the fetal adrenal gland in a relatively specific manner: Since metyrapone is a 11-beta-hydroxylase blocker, the synthesis of rat mineralocorticoids and testosterone is not affected.

We have modified Plotsky's pharmacological adrenalectomy regimen extensively to eliminate the increased toxicity in pregnant female rats. Using Plotsky's original doses of 20mg of metyrapone per 100gm body weight every 8 hours resulted in maternal weight loss, and in fetal wasting or death. Our version does not affect fetal weight, litter size, or the apparent well-being of fetuses, and can thus allow direct manipulation of the fetal brain-adrenal axis. The model of a pharmacologically adrenalectomized fetal rat may thus serve as a tool to investigate mechanisms controlling CRH gene expression.

Acknowledgments

Supported in part by NINDS KO8-NS010307 (to TZB).

References

1. M.W. GRINO, W.S. YOUNG, III, and J.M. BURGUNDIER, Endocrinology 124 60-68 (1989).
2. T.Z. BARAM and S.P. LERNER, Neurosci. Lett. (in press).
3. R.M. SAPOLSKY RM and M.J. MEANEY, Brain Res. Rev. 11 65-76 (1986).
4. A JOST, Recent Prog. Horm. Res. 22 541-573 (1966).
5. K. ARISHIMA, S. NAKAMA, Y. MORIKAWA, Y. HASHIMOTO and Y. EGUCHI, J. Endocrinol. 72 239-240 (1977).
6. A. CHATELAIN, J.P. DUPOUY and P. ALLAUME, Endocrinology 106 1297-1303 (1980).
7. J.P. DUPOUY and A. CHATELAIN, J. Endocrinol. 101 339-344 (1984).
8. P.M. PLOTSKY and P.E. SAWCHENKO, Endocrinology 120 1361-1369 (1987).