

## NEONATAL PRAZOSIN EXPOSURE REDUCES OVARIAN WEIGHT AND ESTROGEN RECEPTOR BINDING IN ADULT FEMALE RATS

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**Abstract**—Exposure of estrogen treated adult female guinea pigs to the alpha-1 antagonist prazosin has been shown to reduce levels of estrogen binding in the hypothalamus and preoptic area. To further investigate this interaction between the noradrenergic and neuroendocrine axes, newborn female rat pups received an s.c. implant of prazosin (0.0125 mg/day for 5 days) or placebo. In adulthood, subjects were sacrificed by perfusion with DMSO on the morning of proestrus. Tissue analysis of the medial preoptic area, corticomedial amygdala, and mediobasal hypothalamus revealed that cytosolic estrogen binding was significantly reduced in all three areas for the prazosin treated group as compared to controls. Ovarian weight was also significantly reduced in the prazosin treated group, although uterine weight was unaffected. Interestingly, prazosin treated females showed a post-pubertal increase in body weight characteristic of ovariectomized females, while controls showed no such increase.

These results support the existence of a significant developmental interaction between the noradrenergic system and the neuroendocrine axis as measured by ovarian weight and estrogen binding in the brain.

*Key words:* noradrenergic, alpha-1 adrenoceptor antagonist, neuroendocrine, development.

Noradrenergic drugs have been shown to modulate the binding of steroid ligands to estrogen,<sup>6</sup> progesterone<sup>15,16,18</sup> and androgen receptors<sup>1,9</sup> in hypothalamic and preoptic areas of adult guinea pigs and rats. Conversely, estradiol manipulations have been shown to affect alpha-2 noradrenergic binding in estradiol concentrating regions of adult guinea pig brain.<sup>10</sup> Exposure to various noradrenergic blockers has also been shown to alter the effects of ovarian hormones on some reproductive behaviors.<sup>17,19</sup> These effects may be modulated via alterations in the binding of steroids to their receptors, given the exposure to noradrenergic blockers and dopamine-hydroxylase-inhibitors results in decreased nuclear estrogen receptor binding in guinea pig<sup>3,5</sup> and rat<sup>4</sup> hypothalamus.

The effects of noradrenergic drugs on steroid receptors and sexual behavior have primarily been examined in adult animals. Because there is evidence that catecholaminergic manipulations in neonatal rats result in permanent changes in gonadotropin secretion and sexual behavior<sup>7,11,15</sup> we decided to investigate possible organizational effects of neonatal noradrenergic manipulations — specifically, exposure to the alpha-1 noradrenergic blocker prazosin — on reproductive development and estrogen-receptor binding in female rats.

### EXPERIMENTAL PROCEDURES

Litters from eight Long-Evans/Sprague-Dawley females were culled to six females and two males within 2 h of delivery. Four of the six females from each litter received a subcutaneous implant of 0.0625 mg prazosin (from IRA Inc.) under cryogenic anaesthesia. These implants were designed to release 0.0125 mg/day at a constant rate for 5 days. The remaining two females received a placebo-carrier implant. Pups were labelled via india ink injections to the foot-pad, and warmed under a lamp before being returned to the nest.

At 28 days the litters were weaned by removal of the males and mother, leaving eight litters of six group-housed females. Starting at 50 days of age, daily vaginal smears were taken from all subjects. Regular estrous cycles ranging from 4 to 6 days were established for all treated and

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*Abbreviations:* AMG, corticomedial amygdala; CTC, temporal cortex; HYP, mediobasal hypothalamus; POA, medial preoptic area.

non-treated females. Subjects were then selected for sacrifice between 08:00 and 11:00 on the morning of proestrus. The age at sacrifice ranged from 70 to 115 days. Subjects were weighed and deeply anaesthetized with pentobarbital (6.5 mg/kg). The uterus and ovaries were removed and subjects were perfused transcardially with 30 cc ice-cold DMSO. The brain was extracted onto powdered dry ice, and stored at  $-90^{\circ}\text{C}$ .

Brain tissue was extracted for assay via the procedure described by Luine *et al.*<sup>12</sup> Brains were cut on a cold plate, and preoptic area (POA), basomedial hypothalamus (HYP), medial amygdala (AMG) and a sample of temporal cortex (CTX) were dissected out and placed into tubes of 1.5 ml TEMS buffer. These samples were pooled from pairs of like-treated littermates.

Cytosolic\* receptor assays were run according to the procedure described by Giordano *et al.*<sup>18</sup> Cytosolic protein was measured according to the method of Bradford.<sup>5</sup> Cytosol data are expressed as femtomoles [3H]estradiol specifically bound per milligram cytosolic protein.

## RESULTS

Some of the prazosin treated females were used in other analyses, resulting in a final *n* of eight control littermate pairs and seven prazosin-treated littermate pairs. Although brain tissue from pairs of females was pooled to obtain assay measures, individual ovarian, uterine, brain weight and body weight measures were used in the analyses.

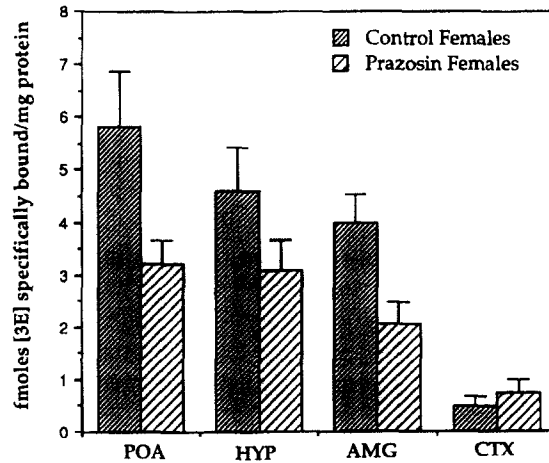


Fig. 1. Cytosolic estrogen binding, expressed in fmoles [3E] specifically bound per mg protein, for each brain region and by treatment. Analyses were run on tissue pooled from littermate pairs ( $n = 8$  control pairs, 7 prazosin-treated pairs).

### Cytosolic estrogen binding

A repeated-measures analysis was performed for measures of estrogen binding in the POA, HYP and AMG, with treatment serving as a between-subject variable. Results showed a main effect of treatment ( $df = 1,13$ ;  $P < 0.02$ ), with binding significantly reduced among prazosin females. Binding levels for the POA, HYP and AMG were reduced by 45, 33 and 48%, respectively (see Fig. 1). No interaction between treatment and region was observed. When treatment effects were analyzed for each region separately, significance was found in the AMG ( $df = 1,13$ ;  $P < 0.02$ ) and a marginal effect was observed in the POA ( $df = 1,13$ ;  $P < 0.054$ ). Binding levels in CTX were low and showed no group differences.

Overall levels of cytosolic binding as measured in controls were slightly lower than has been reported by others.<sup>3,5,13</sup> However, this may be a consequence of two factors. First, intact rather than Ovx-primed females were used, and McGinnis *et al.*<sup>13</sup> reported that cytosolic estrogen receptor levels in intact females are reduced by approximately half in the hypothalamus and preoptic area on diestrus II as compared to proestrus. Hence a minor variation in the assessment

\*The term cytosolic is used to refer to unoccupied receptors in the cytosol cellular fraction after homogenization and centrifugation.

of proestrus may account, in part, for lower binding measures. Secondly, brain tissue was frozen rather than assayed immediately, since female littermates were not always in proestrus on the same day (and hence fresh tissue could not be pooled). Although subjects were perfused with DMSO, it is likely that some receptors were lost as a consequence of freezing. It should be noted that the relative levels of binding in the POA, HYP and AMG were consistent with the reports of others, and that SEs were relatively small.

#### *Ovarian and uterine weight*

No significant group effects were observed for absolute or corrected uterine weight. However, absolute ovarian weight was significantly reduced in the prazosin treated group ( $df=1,38$ ;  $P<0.0001$ ), as was ovarian weight corrected as a function of body weight ( $df=1,38$ ;  $P<0.001$ ) (see Table 1). Since subjects were sacrificed across the range of ages, the effects of age on ovarian and uterine weight was examined within both treatment groups. However, no significant effects of age were observed.

Table 1. Weights by group (in grammes);  $n=16$  control females, 14 prazosin-treated females

	Control females	Prazosin-treated females
Body weight (all ages)	312.474	292.182*
[SE]	[4.887]	[8.011]
Body weight (< 90 days)	311.714	274.786**
[SE]	[6.489]	[6.931]
Body weight (> 90 days)	312.917	322.625
[SE]	[6.820]	[12.855]
Brain weight	1.922	1.906
[SE]	[0.034]	[0.035]
Uterine weight	0.408	0.384
[SE]	[0.023]	[0.017]
Ovarian weight	0.204	0.155***
[SE]	[0.007]	[0.007]
Ovarian/body weight	0.00065	0.00054***
[SE]	[0.00002]	[0.00002]

\* $P<0.05$ ; \*\* $P<0.01$ ; \*\*\* $P<0.001$

#### *Body weight*

Prazosin treated females weighed significantly less, overall, than control females ( $df=1,38$ ;  $P<0.05$ ) (see Table 1). However, this group difference was most marked when the body weights of females sacrificed at 90 days of age or less were assessed (see Table 1). When females greater than 90 days of age were examined, the opposite pattern emerged. This reversal is a consequence of the fact that prazosin treated females showed a significant *increase* in body weight with age ( $Rsq=0.388$ ;  $P<0.01$ ), while control females showed no such increase.

#### *Brain weight*

No significant differences in brain weight as a function of treatment were observed (Table 1).

### CONCLUSION

These results demonstrate that exposure of neonatal rats to the specific alpha-1 noradrenergic blocker prazosin significantly affected ovarian weight and binding of estradiol to cytosolic estrogen receptors in combined POA-HYP-AMG of adult female rats. In addition, prazosin treatment had a dual effect on body weight, first by reducing the body weight of treated females at younger ages, and secondly by leading to a post-pubertal rise in body weight which resembles the growth curve of ovariectomized females.<sup>2</sup> The latter finding suggests that prazosin treatment may have contributed to lower levels of plasma estrogen, possibly through direct effects on the development of the ovaries. However, this hypothesis will require further investigation. It is also

possible that observed reductions in cytosolic estrogen receptor concentration in POA–HYP–AMG reflect reduced estrogen levels. Such a hypothesis would be consistent with the fact that prazosin treatment did not appear to exert regionally specific effects. Treatment of adult female rats with dopamine-B-hydroxylase inhibitors, in contrast, reduced cytosolic E receptor levels in the POA and HYP but not in the AMG.<sup>3</sup> It should be noted that normal estrous cycles were observed in prazosin treated females, verifying that the ovaries of treated females were producing enough estrogen and progesterin to sustain estrous cyclicity.

Additional studies examining long term behavioral effects of neonatal prazosin exposure, as well as effects on nuclear receptor levels and plasma estrogen levels, will be necessary to fully interpret the current findings. It will also be of interest to examine the effects of neonatal exposure to alpha-2 blockers or dopamine-hydroxylase inhibitors on these measures.

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## REFERENCES

1. Ahdieh H. B. and Feder H. H. (1988) Sex differences in nuclear androgen receptors in guinea pig brain and the effects of an  $\alpha_2$  noradrenergic blocker on androgen receptors. *Brain Res.* **456**, 275–279.
2. Bell D. D. and Zucker I. (1971) Sex differences in body weight and eating: organization and activation by gonadal hormones in the rat. *Physiol. Behav.* **7**, 27–34.
3. Blaustein J. D., Brown T. J. and Sweargen E. S. (1986) Dopamine-B-hydroxylase inhibitors modulate the concentration of functional estrogen receptors in female rat hypothalamus and pituitary gland. *Neuroendocrinology* **43**, 150–158.
4. Blaustein J. D. and Letcher B. (1987) Noradrenergic regulation of cytosol estrogen receptors in female rat hypothalamus: possible role of alpha-2 noradrenergic receptors. *Brain Res.* **404**, 51–57.
5. Bradford M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principal of protein-dye binding. *Analyt. Biochem.* **72**, 248–254.
6. Clark A. S., Nock B., Feder H. H. and Roy J. (1985)  $\alpha_1$ -Noradrenergic receptor blockade decreases nuclear estrogen receptor binding in guinea pig hypothalamus and preoptic area. *Brain Res.* **330**, 197–199.
7. Dornier G. (1980) Sexual differentiation of the brain. *Vitam. Horm.* **38**, 325–381.
8. Giordano A. L., Ahdieh H. B., Mayer A. D., Siegel H. I. and Rosenblatt J. S. (1991) Cytosol and nuclear estrogen receptor binding in the preoptic area and hypothalamus of female rats during pregnancy and ovariectomized, nulliparous rats after steroid priming: correlation with maternal behavior. *Horm. Behav.* **24**, 232–255.
9. Handa R. J. and Resko J. A. (1989)  $\alpha$ -adrenergic regulation of androgen receptor concentration in the preoptic area of the rat. *Brain Res.* **483**, 312–320.
10. Johnson A. E., Nock B., McEwen B. S. and Feder H. H. (1985) Estradiol modulation of  $\alpha_2$ -noradrenergic receptors in guinea pig brain assessed by tritium-sensitive film autoradiography. *Brain Res.* **336**, 153–157.
11. Kikuyama S. (1962) Inhibition of induction of persistent estrous by chlorpromazine in the rat. *Annoumes zool. jap.* **35**, 6–11.
12. Luine V. N., Khylichevskaya R. I. and McEwen B. S. (1974) Oestrogen effects on brain and pituitary enzyme activities. *J. Neurochem.* **23**, 925–934.
13. McGinnis M. Y., Krey L. C., MacLusky N. J. and McEwen B. S. (1981) Steroid receptor levels in intact and ovariectomized estrogen-treated rats: an examination of quantitative, temporal, and endocrine factors influencing the efficacy of estradiol stimulus. *Neuroendocrinology* **33**, 158–165.
14. Nock B. and Feder H. H. (1984)  $\alpha_1$ -Noradrenergic regulation of hypothalamic progesterin receptors and guinea pig lordosis behavior. *Brain Res.* **310**, 77–85.
15. Stumpf W. E., Sar M., Reisert I. and Pilgrim C. (1983) Estrogen receptor sites in the developing central nervous system and their relationship to catecholamine systems. *Monographs Neural. Sci.* **9**, 205–212.
16. Thornton J. E., Nock B., McEwen B. S. and Feder H. H. (1986) Noradrenergic modulation of hypothalamic progesterin receptors in female guinea pigs is specific to the ventromedial nucleus. *Brain Res.* **377**, 155–159.
17. Vincent P. A. and Feder H. H. (1988) Alpha-1 and alpha-2-noradrenergic receptors modulate lordosis behavior in female guinea pigs. *Neuroendocrinology* **48**, 477–481.
18. Vincent P. A. and Feder H. H. (1990) Progesterin receptors in the ventromedial nucleus of the hypothalamus and arcuate nucleus-median eminence are decreased by idazoxan. *Brain Res.* **528**, 95–98.
19. Vincent P. A., Thornton J. E., Peterson C. S. and Feder H. H. (1989) Different roles of alpha-noradrenergic receptor subtypes in regulating lordosis. *Pharmacol. Biochem. Behav.* **34**, 89–93.