J. steroid Biochem. Vol. 22, No. 5, pp. 677–679, 1985
Printed in Great Britain. 

0022-4731/85 $3.00 + 0.00


# CAN NEONATAL BETA-ADRENERGIC STIMULATION PREVENT THE EFFECTS OF ANDROGENIZATION IN FEMALE RATS?

M. J. VIDAL and E. AGUILAR[1]
Departments of Pharmacology and *Physiology, Faculty of Medicine, University of Córdoba, Córdoba, Spain

(*Received* 31 *July* 1984)

**Summary**—A 25 μg dose of testosterone propionate injected at 4 days of age induced 90% anovulation at 100 days of age. The systemic administration of orciprenaline (8 or 16 μg) or yohimbine (100 μg) did not prevent androgenization. Twenty-five or fifty μg of orciprenaline injected intraventricularly reduced only partially (to 54 and 67% respectively) the effectiveness of androgenization. We concluded that β-adrenergic receptor stimulation had a very limited ability to prevent androgenization, since the β-stimulation obtained directly with orciprenaline prevented androgenization to a very limited extent, while the possible indirect stimulation through an increase in norepinephrine endogenous release by alpha-2 receptor blocker yohimbine was ineffective.

## INTRODUCTION

Differentiation of the neonatal brain into a "tonic" or "cyclic" structure is a process controlled by gonadal secretion. A single neonatal injection of androgen [1] or estrogen [2] induced an anovulatory syndrome (AS) due to the absence of cyclic gonadotropin secretion.

The action of neonatal androgen or estrogen was exerted at the hypothalamic level through a complex and not well-understood mechanism that could be blocked by simultaneous administration of progesterone, desoxycorticosterone and pregnanedione [3], reserpine and chlorpromazine [4], tyramine and alpha adrenergic blockers such as phentolamine and phenoxybenzamine [5]. Several of these drugs may protect against androgenization through a beta-adrenergic stimulation of hypothalamic neurons [5, 6].

In the present work, we analyze the effects on the reproductive function of female rats of orciprenaline, a beta agonist traversing the blood–brain barrier which has a long half-life and the ability of yohimbine, a selective blocker of alpha-2 adrenergic receptors [7], and orciprenaline to prevent the effects of neonatal androgenization.

## EXPERIMENTAL

Female Wistar rats were raised in our laboratory under controlled light (12 h light–12 h darkness) and temperature (23°C). The day of birth was considered as day 1. At 21 days of age, the pups were weaned and placed in groups of 4–5 per cage and fed Sanders diet and tap water *ad libitum*.

Testosterone propionate (Sigma) was dissolved in olive oil (250 μg/ml), yohimbine hydrochloride (Sigma) in saline (250 μg/ml) and orciprenaline (Boehringer) in saline (20–40 μg/ml for systemic injection and 3, 5 or 10 mg/ml for intraventricular injection). Systemic injections were given in a volume of 100 μl and the intraventricular injections (Technique of Noble *et al.*)[9] were all in a volume of 5 μl.

This study comprised of three experiments summarized in Table 1. In all the animals, the days of vaginal opening and first estrus were recorded. After vaginal opening occurred, vaginal smears were monitored daily. The rats were decapitated at 100 days and the ovaries removed, cleaned and weighed. Some of them were fixed in Bouin solution and analyzed for histological confirmation of corpora lutea absence.

Table 1. Treatment schedules of the experimental groups

| | Drug | Dose | Day | Route |
|---|---|---|---|---|
| *Expt. 1* | —Saline | 100 μl | 4 | s.c. |
| | —Orciprenaline | 8 μg | 4,5[a] | s.c. |
| | —Orciprenaline | 16 μg | 4,5[a] | s.c. |
| *Expt. 2* | —Oil | 100 μl | 4 | s.c. |
| | —TP | 25 μg | 4 | s.c. |
| | —TP + | 25 μg | 4 | s.c. |
| | Orciprenaline | 8 μg | 4,5[a] | s.c. |
| | —TP + | 25 μg | 4 | s.c. |
| | Yohimbine | 100 μg | 4,5[a] | i.p. |
| *Expt. 3* | —Oil | 100 μl | 4 | s.c. |
| | —TP | 25 μg | 4 | s.c. |
| | —TP + | 25 μg | 4 | s.c. |
| | Saline | 5 μl | 4 | i.v. |
| | —TP + | 25 μg | 4 | s.c. |
| | Orciprenaline | 15 μg | 4 | i.v. |
| | —TP + | 25 μg | 4 | s.c. |
| | Orciprenaline | 25 μg | 4 | i.v. |
| | —TP + | 25 μg | 4 | s.c. |
| | Orciprenaline | 50 μg | 4 | i.v. |

s.c., subcutaneously; i.p., intraperitoneally; i.v., intraventricularly
[a]The total dose was administered in four injections (10 and 22 h of days 4 and 5).

[1]To whom correspondence should be addressed.

Table 2. Effects of neonatal orciprenaline administration on the female rat reproductive function

| Treatment | Vaginal opening (days) | First estrus (days) | Ovarian weight (mg % g) | Incidence of AS |
|---|---|---|---|---|
| Saline | 34.7 ± 1.1 | 38.1 ± 2.1 | 32.7 ± 1.7 | 0/7 |
| Orciprenaline | | | | |
| (8 μg) | 33.9 ± 0.7 | 36.7 ± 0.9 | 30.1 ± 1.2 | 0/13 |
| (16 μg) | 37.1 ± 0.7 | 38.3 ± 1.0 | 30.7 ± 1.3 | 0/12 |

Table 3. Effects of neonatal orciprenaline or yohimbine administration on the androgenized female rat

| Treatment | Vaginal opening (days) | First estrus (days) | Ovarian weight (mg % g) | Incidence of AS |
|---|---|---|---|---|
| Oil | 36.4 ± 1.0 | 39.7 ± 0.9 | 32.3 ± 1.5 | 0/10 |
| TP | 31.1 ± 0.7[a] | 33.6 ± 0.8[a] | 22.5 ± 1.9[a] | 18/22 |
| TP + OP | 30.0 ± 0.6[a] | 32.0 ± 1.0[a] | 20.9 ± 3.8[a] | 6/6 |
| TP + YOH | 31.6 ± 1.4[a] | 32.8 ± 1.2[a] | 22.0 ± 2.3[a] | 12/14 |

TP = Testosterone propionate; OP = Orciprenaline; YOH = Yohimbine.
[a] $P < 0.01$ vs oil-injected.

Table 4. Effects of neonatal intraventricular orciprenaline administration on the androgenized female rats

| Treatment | Vaginal opening (days) | First estrus (days) | Ovarian weight (mg % g) | Incidence of AS |
|---|---|---|---|---|
| Oil | 39.8 ± 1.2 | 43.2 ± 1.1 | 35.6 ± 3.7 | 0/7 |
| TP | 35.4 ± 1.2[a] | 38.1 ± 0.5[a] | 15.9 ± 1.3[a] | 26/27 |
| TP + Sal. | 34.8 ± 1.1[a] | 36.8 ± 1.3[a] | 20.5 ± 1.5[a] | 18/22 |
| TP + OP (15) | 32.9 ± 1.1[a] | 37.6 ± 1.1[a] | 17.3 ± 2.4[a] | 11/13 |
| TP + OP (25) | 33.6 ± 1.5[a] | 40.0 ± 1.2 | 25.0 ± 3.2[a,b] | 7/13[b] |
| TP + OP (50) | 29.0 ± 0.7[a,b] | 35.0 ± 1.7[a] | 19.1 ± 2.5[a] | 6/9[c] |

TP = Testosterone propionate; OP = Orciprenaline. 15, 25 or 50 was the dose injected (μg).
[a] $P < 0.01$ vs oil-injected female rats; [b] $P < 0.01$ vs TP-injected female rats.
[c] $P < 0.05$ vs TP injected female rats.

Results were expressed as means ± SEM and the differences between groups were analyzed using the analysis of variance and the pairwise test [10]. Differences in the incidence of AS were analysed by $X^2$.

## RESULTS

### *Experiment 1*

The systemic administration of orciprenaline (8 or 16 μg) did not induce any change in vaginal opening, first estrus occurrence, vaginal cycles or ovarian weights (Table 2).

### *Experiment 2*

Neonatal androgen treatment produced an advancement in the day of vaginal opening ($P < 0.01$), and the first estrus ($P < 0.01$). Of the 22 androgenized females 18 showed anovulatory syndrome, and a significant reduction ($P < 0.01$) in the ovarian weight of the group was recorded. These effects were not modified by simultaneous orciprenaline or yohimbine administration (Table 3).

### *Experiment 3*

In this experiment in order to obtain a higher degree of beta stimulation than in expt 2, orciprenaline was intraventricularly administered in a large pharmacological dose. Of the 27 androgenized females 26 showed anovulatory syndrome and a reduction in ovarian weight ($P < 0.01$) of the group was recorded. With the higher doses (25 and 50 μg) of orciprenaline, a reduction in the incidence of AS was obtained, the lower dosage (15 μg) being ineffective (Table 4).

The incidence of AS in all groups was summarized in Table 5.

## DISCUSSION

Neonatal administration of testosterone propionate advanced the day of vaginal opening and first estrus and induced a high incidence (90%) of anovulatory syndromes, in agreement with previous reports [10,11]. In this work the anovulatory syndrome was defined as ten or more consecutive days of smears showing cornified cells accompanied by the absence of fresh corpora lutea in the ovaries.

To analyze the effects of beta-stimulation on androgenization, we injected orciprenaline, a beta agonist that crosses the brain–blood barrier with little

Table 5. Incidence of AS in the different experimental groups

| Group | No. of rats with AS | % |
|---|---|---|
| Control[c] | 0/24 | 0 |
| TP | 44/49 | 90 |
| Orciprenaline (8 μg) | 0/13 | 0 |
| Orciprenaline (16 μg) | 0/12 | 0 |
| TP + Orciprenaline | 6/6 | 100 |
| TP + Yohimbine | 12/14 | 86 |
| TP + Saline (i.v.) | 18/22 | 82 |
| TP + Orciprenaline (15 μg i.v.) | 11/13 | 85 |
| TP + Orciprenaline (25 μg i.v.) | 7/13 | 54[a] |
| TP + Orciprenaline (50 μg i.v.) | 6/9 | 67[b] |

[a] $P < 0.01$ vs TP-injected; [b] $P < 0.05$ vs TP injected.
[c] Control group included oil and saline-injected female rats.

difficulty and yohimbine, a specific alpha-2 blocker that increased endogenous norepinephrine release.

Our results showed that the specific alpha-2 blocker yohimbine, systemically administered at a dose sufficient to affect presynaptic adrenergic receptors [12, 13] did not modify the effects of androgenization. On the other hand, phentolamine and phenoxybenzamine were effective in blocking the development of anovulation [6,14]. These blockers have two effects, pre and postsynaptic, on hypothalamic norepinephrine transmission [5]. Since their protective effect could be reversed with the beta-blocker propanolol, Raum and Swerdloff[5, 6] hypothesized that the mechanism of protection was a presynaptical alpha receptor blockade leading to an increase in norepinephrine release and stimulation of postsynaptic beta receptors. This beta receptor stimulation inhibits either the aromatization of testosterone to estrogen or the nuclear uptake of estradiol [6]. Our data contradict Raum and Swerdloff's[5, 6] interpretation of the mechanism of action of phenoxybenzamine and phentolamine. If the protection obtained with these drugs was due to a presynaptic alpha-receptor blockade, yohimbine could produce the same protection.

Higher doses (25 or 50 $\mu$g) of orciprenaline injected intraventricularly only partially blocked the effects of androgenization. Lower doses (15 $\mu$g) or systemic administration were ineffective. These data suggested that the direct stimulation of beta receptors was able to prevent androgenization to a very limited extent.

*Acknowledgements*—This work has been supported by Grant 0243 from Comisión Asesora de Investigación Cientifica y Técnica (Spain). Orciprenaline was generously supplied by Boehringer (Barcelona, Spain).

## REFERENCES

1. Barraclough C. A.: Production of anovulatory sterile rats by single injections of testosterone propionate. *Endocrinology* **68** (1961) 62–67.
2. Gorski R. A.: Modification of ovulatory mechanisms by postnatal administration of estrogen to the rat. *Am. J. Physiol.* **205** (1963) 842–844.
3. Arai Y. and Gorski R. A.: Protection against the neural organizing effects of exogenous androgen in the neonatal female rat. *Endocrinology* **82** (1968) 1005–1009.
4. Kikuyama S.: Inhibition of induction of persistent estrus by chlorpromazine in the rat. *Annotnes zool. jap.* **35** (1962) 6–11.
5. Raum W. J. and Swerdloff R. S.: The role of hypothalamic adrenergic receptors in preventing testosterone-induced androgenization in the female rat brain. *Endocrinology* **109** (1981) 273–278.
6. Raum W. J., Marcano M. and Swerdloff R. S.: Nuclear accumulation of estradiol derived from the aromatization of testosterone is inhibited by hypothalamic beta-receptor stimulation in the neonatal female rat. *Biol. Reprod.* **30** (1984) 388–396.
7. Starke K.: Regulation of noradrenaline release by presynaptic receptor systems. *Rev. Physiol. Biochem. Pharmac.* **77** (1977) 1–124.
8. Noble E. P., Wurtmann R. J. and Axelrod J.: A simple and rapid method for injecting $^3$H-norepinephrine into the lateral ventricle of the rat brain. *Life Sci.* **6** (1967) 281–291.
9. Yamane T.: *Statistics: an Introductory Analysis.* Harper & Row, New York (1970).
10. Barraclough C. A.: Modifications in the CNS regulation of reproduction after exposure of prepubertal rats to steroid hormones. *Recent Prog. Horm. Res.* **22** (1966) 503–539.
11. Kincl F. A., Folch Pi A., Maqueo M., Herrera Laso L., Oriol A. and Dorfmann R. I.: Inhibition of sexual development in male and female rats treated with various steroids at the age of five days. *Acta endocr., Copenh.* **49** (1965) 193–206.
12. Paciorek P. M. and Shepperson N. B.: A study of pre- and post-synaptic alfa-2 adrenoceptors in the pithed rat. *Br. J. Pharmac.* **79** (1983) 225p.
13. Meltzer H. Y., Simonovic M. and Gudelsky G. A.: Effect of yohimbine on rat prolactin secretion. *J. Pharmac. exp. Ther.* **224** (1983) 21–27.
14. Nishizuka M.: Neuropharmacological study on the induction of hypothalamic masculinization in female mice. *Neuroendocrinology* **20** (1976) 152–165.