NOTE

Effect of AA560 (A Nonsteroidal Antiandrogen) Implantation In the Hypothalamus on Gonadotropin Secretion in Male Rats

HIDETOSHI YAMANAKA¹, ATSUSHI KOYA¹, KYOICHI IMAI¹,
KATSUYUKI NAKAI¹, HISAKO YUASA¹, YUKO SUGIYAMA¹,
KEIZO SHIDA¹ AND KATSUMI WAKABAYASHI²

¹Department of Urology, School of Medicine, Gunma University,
Maebashi, 371, ²Hormone Assay Center, Institute of Endocrinology,
Gunma University, Maebashi, 371

Abstract

AA 560, one of potent nonsteroidal antiandrogens, was implanted in the arcuate nucleus of the hypothalamus of intact male rats through a stainless double cannula. The prostate weight and serum LH level significantly increased on the 5th day after the implantation of AA 560 when compared with those in the sham-operated animals. There existed a definite correlation between the weight of the ventral prostate and the serum LH level in AA 560 implanted animals.

Many reports have indicated that the presence of a 5 α -dihydro-testosterone receptor in the cytosol prepared from male rat brain (Kato and Onouchi, 1973a; Kato and Onouchi, 1973b; Naess *et al.*, 1975; Mercier *et al.*, 1976; Lieberburg *et al.*, 1977). A novel nonsteroidal antiandrogen, [N-(2-chloromethyl-2-hydroxypropionyl)-3,4,5-trichloroaniline](AA560) is a derivative of anilide (Fig. 1) and a comparative study on castrated and intact male rats revealed that the inhibitory effect of AA560 on rat prostate weight was stronger than that of SCHI3521 (Shida, 1980). Shida *et al.* (1980) have shown that the oral administration of AA560 to intact male rats cause a decrease in prostate weight and a rise in the serum LH and FSH levels. We have also indicated that AA560 prevents the binding of 5 α -dihydrotestosterone at the receptor in the brain as well as in the prostate (Yamanaka *et al.*, 1979).

The present study was aimed at finding whether the intrahypothalamic implantation of AA560 in male rats might exert any effect on gonadotropin secretion and consequently on prostate weight.

Fig. 1. Chemical structure of AA 560

Materials and Methods

Chemicals

AA 560 [N-(2-chloromethyl-2-hydroxypropionyl)-3, 4, 5-trichloroaniline] was obtained from Teikoku Hormone Mfg. Co., Kawasaki, Japan.

Animals

Male Wister strain rats (200–220 g.) were maintained on laboratory chow from Japan Clea Co. and water *ad libitum*. The animals were housed in metabolic cages in a temperature controlled room under a 12 h light 12 h dark lighting schedule.

AA 560 implantation was performed through a double cannule. The stainless 0.7 mm outside diameter tube was stereotactically implanted into the arcuate nucleus of the hypothalamus as the outer cannula under ether anesthesia according to the atlas by Sherwood and Timiras (1970). On the 10th day after the outer cannula implantation, the 0.35 mm outer diameter inner cannula bearing crystalline AA 560 was inserted into the outer cannula and extended approximately 0.5 mm beyond the tip of the outer cannula.

The animals were autopsied on the 5th day after insertion of the inner cannula. At autopsy, the prostate was weighed and brain was fixed in formalin, sectioned, and stained for verification of implant locations. All blood samples were taken from the vena cava and sera were stored at -80°C until assayed.

Serum LH, FSH measurement

Serum LH or FSH was measured as described previously (Wakabayashi, 1977) by radioimmunoassay employing RIA kits supplied by Dr. A. F. Parlow of Rat Pituitary Hormone Program, NIAMDD, NIH. The results were expressed as the equivalent of NIH-LH-SI for LH and NIH-FSH-SI for FSH.

Results

Table 1 shows the changes in ventral prostate weight, serum LH and FSH concentration 5 days after AA560 implantation into the arcuate nucleus of the hypothalamus of mature male rats. The ventral prostate weight was significantly higher in AA560 implanted animals than in sham-operated controls. It is also apparent from Table 1 that AA560 in the arcuate nucleus significantly raised the serum LH level. On the other hand, serum FSH titers were not significantly altered by AA560 implant in the arcuate nucleus. There existed a definite correlation between the weight of the ventral prostate and the serum LH level in AA560 implanted animals ($r: +0.8621$), but not in sham-operated animals ($r: -0.0206$). There was a significant difference between AA560 implanted and sham-operated animals in the r value ($p < 0.05$).

Table 1. Effect of AA 560 implantation in the arcuate nucleus of the hypothalamus of male rats.

	(No. rats)	Ventral prostate (mg/100g. B.W)	LH ng $\times$ SI/ml	FSH ng $\times$ SI/ml
Sham-operated	(11)	66.19 $\pm$ 8.97	1.01 $\pm$ 0.19	663.27 $\pm$ 24.82
AA 560 treated	(10)	97.45 $\pm$ 7.89**	2.64 $\pm$ 0.76*	746.80 $\pm$ 88.42

Legend to Table 1.

Data are shown as mean $\pm$ standard error.

*: Significant difference from the mean for the sham-operated group. ($p < 0.05$)

**: Significant difference from the mean for the sham-operated group. ($p < 0.02$)

Fig. 2. The relationship between the serum LH level and the ventral prostate weight. The r s represent the coefficient correlation.

Discussion

The negative feedback effect of androgens on LH or FSH secretion in the rat has been reported by some investigators (Verjans *et al.*, 1974; Danguy *et al.*, 1977). Whether steroid hormone receptors participate or a steroid hormone metabolizing enzyme system participates in the regulation of gonadotropin secretion in male is not clear. The present study showed that non-steroidal antiandrogen, AA560, implanted in the hypothalamus resulted in an increase in the serum LH concentration and ventral prostate weight. Chronic intrahypothalamic implantation of cyproterone, an antiandrogen which has no progestational activity, resulted in the stimulation of the testes, seminal vesicles and prostates (Block and Davidson, 1967). Noguchi *et al.* (1980) reported that the implantation of chlor-

madinone acetate, one of the progestins, in the hypothalamic median eminence-arcuate nucleus region resulted in a decrease in the serum LH level on day 3 after implantation. Chlormadinone acetate has a progestational activity that might exceed its antiandrogenic effect and cause decreased gonadotropin output. Progestins such as chlormadinone acetate and cyproterone acetate might interact with 5α -dihydrotestosterone receptor, in addition to acting upon the progesterone receptor.

AA560 has antiandrogenic properties and possesses a potent affinity for the hypothalamic 5α -dihydrotestosterone cytosol receptor and inhibits the formation of the 5α -dihydrotestosterone receptor complex (Yamanaka *et al.*, 1979). The results of this study suggested that AA560 might stimulate gonadotropin secretion, especially LH secretion, acting on the 5α -dihydrotestosterone receptor in the arcuate nucleus of the hypothalamus. However, the possibility still remains that AA560 might reach the pituitary gland by diffusion or via the portal vein and act on the 5α -dihydrotestosterone receptor in this gland. The present study also suggested that AA560 could be a useful tool for research on the precise mechanism of feedback control of gonadotropin secretion in male animals.

Acknowledgements

We wish to thank Dr. Mori and Dr. K. Shibata for kindly supplying AA 560. This work was partly supported by a Grant from the Ministry of Education, Science and Culture, Japan and Grant-in-Aid Cancer Research from the Ministry of Health and Welfare, Japan.

References

- Bloch, G. J. and J. M. Davidson (1967). *Science* 155, 593.
- Danguy, A., F. Ectors and J. L. Pasteels (1977).
- Haruki, Y., K. Seiki and Y. Imanishi (1980). *Endocrinol. Japon.* 27, 439.
- Kato, J. and T. Onouchi (1973a). *Endocrinol. Japon.* 20, 641.
- Kato, J. and T. Onouchi (1973b). *Endocrinol. Japon.* 20, 429.
- Lieberburg, I., N. J. Maclausky and B. S. Macwen (1977). *Endocrinology* 100, 598.
- Mercier, L., C. Le Guellic, M. L. Thieulant, S. Samperez and P. Jonan (1976). *J. Steroid Biochem.*
- Naess, O., A. Attramadal and A. Aakvaak (1975). *Endocrinology* 91, 1.
- Noguchi, K., R. Nishimura, S. Takai, J. Arita, T. Higuchi and M. Kawakami (1980). *Endocrinol. Japon.* 27, 285.
- Sherwood, N. M. and P. S. Timiras (1970). A stereotaxic atlas of the developing rat brain. University of California Press, Berkeley, Los Angeles, London.
- Shida, K., H. Yamanaka, A. Koya, K. Wakabayashi, H. Mori, K. Shibata and E. Shimazawa (1980). *Endocrinol. Japon.* 27, 69.
- Shida, K. (1980). *Clin. Endocrinol.* 28, 899 (in Japanese).
- Verjans, H. L., K. B. Eik-nes, J. H. Aafjes, F. J. Vels and H. J. Molen (1974). *Acta Endocrinol.* 77, 673.
- Wakabayashi, K. (1977). *Endocrinol. Japon.* 24, 473.
- Yamanaka, H., M. Matsuoka, H. Yuasa, A. Koya, K. Imai, K. Ebihara, K. Nakai and K. Shida (1979). *Enocrinol. Japan.* 26, 97.