EFFECT OF INTRAMUSCULAR NORETHISTERONE ENANTHATE ON COMPENSATORY OVARIAN HYPERTROPHY IN THE RAT

UMESH K. SRIVASTAVA

Division of Endocrinology,
Central Drug Research Institute, Lucknow-226 001

(Received on May 5, 1980)

Summary : Norethisterone enanthate (N.E.) administered in repeated intramuscular doses was found to bring about by and large a dose-related inhibition of compensatory ovarian hypertrophy (COH) which follows after the surgical removal of one ovary in adult rats. The relative inhibition in the responsiveness of the remaining ovary to compensatory gonadotrophic stimulation leading to the COH was of the order of 14.2, 19.0, 26.3, 25.0 and 42.0% with 0.25, 0.50, 1.0, 2.0 and 4.0 *mg* doses of N.E., respectively. The ED_{50} was calculated to be of the order of 7.0 *mg*. There was no effect of the treatment on the weight of the pituitary at any dose, whatsoever. However, the treatment was found to induce a significant increase in the weight of the uterus and the adrenals at higher doses (2.0 and 4.0 *mg*). The possibility of delineating the antigonadotrophic activity of 'N.E.' from its contraceptive potential, vis-a-vis the dose employed, is discussed.

Key words : norethisterone enanthate, intramuscular, ED_{50}, compensatory ovarian hypertrophy (COH), anti-gonadotrophic

INTRODUCTION

Long-term contraception in women by depot intramuscular Norethisterone enanthate (N.E.), a haptanoic acid ester of 19-nor-testosterone (norethisterone), has been found to be fairly effective (1,2) with its contraceptive effects being mediated via the inhibition of pituitary gonadotrophic secretion (11,13,25). However, the incidence and intensity of drug-induced side-effects attributed to the rather massive dosage schedule have limited its mass scale application, and there have been almost no attempts to investigate the possibility of delineating the contraceptive dose from the strong antigonadotrophic property of this long-acting synthetic progestin. The present study was an effort in that direction.

Although esterification and enol-etherification has been demonstrated to make the 19-nor-testosterone progestins many times more potent in their biological attributes as compared to their corresponding parent ketones (7,9), the structural manipulation of the

steroid molecule also abolished any predictable parallelism between antibonadotrophic, anti-estrual and contraceptive activities of the parent progestin (8,12,22). In view of the fact that overall contraceptive effectiveness of any synthetic progestin was a function of its partial (inherent) estrogenicity/progestational ratio, the dose employed by implication becomes the most important single determinant of the nature and magnitude of its biological effect. Since in the case of N.E. the partial estrogenic property has been demonstrated to dominate over its progestational attributes (16,17) it was deemed of interest to investigate the relationship between its antigonadotrophic and uterine growth stimulating properties in relation to the dose administered, and to determine if there was any parallelism between the two biological attributes.

MATERIALS AND METHODS

Adult female rats, body weight 110-165 g, of the Institute colony were used in the study. Only such animals which showed regular 4-5 day cycles were included in the study. Right ovary of each animal was surgically removed under light ether anaesthesia and the treatment started immediately after the surgery according to the schedule of treatment : Intact control (Gr. I), Hemicastrated (H.C.) control (Gr. II), Sham-Control receiving plain olive oil (Gr. III), H.C.+0.25 *mg* 'N.E.'. (Gr. IV), H.C.+0.50 *mg*. 'N.E' (Gr V), H.C.+2.0 *mg* 'N E.' (Gr. V), and H.C.+4.0 *mg* 'N.E' (Gr. VII), and a subsequent addition of another group of five hemicastrated rats which were treated with 1.0 *mg* 'N.E.')/ d. Lest there was any variation due to the amount of vehicle injected, all animals received 0.5 *ml* olive oil either with or without 'N.E.' Each group had five rats. Animals were autopsied 24 hours after the last injection on day 10 along with a group of five intact animals which were not given any treatment (Gr. I). The remaining ovary was quickly removed, cleaned of adhering fat and tissue debris and weighed on a torsion balance to nearest 0.2 *mg*. Weights of uterus, adrenals and pituitary were also similarly recorded. All statistical analyses were done by employing the student 't' test.

Determination of potency (ED_{50}) :

A dose-response curve was plotted on a semilogarithmic scale for the percent inhibition of COH against the corresponding dose of 'N.E.' employed. The effective dose at 50% level (ED_{50}) was calculated using the method of Litchfield and Wilcoxon (15).

RESULTS

Effect on compensatory ovarian hypertrophy (COH) :

A significant inhibition of COH was observed at all the doses of 'N.E.' ($P<0.05$ and 0.01). The relative inhibition of COH was of the order of 14.2, 19.0, 26.3, 25.0 and 42%

with 0.25, 0.50, 1.0, 2.0 and 4.0 mg doses of 'N.E.' respectively. The ED_{50} was calculated to be of the order of 7.0 *mg*.

Effect of intramuscular 'N.E.' on organ weights :

Weights expressed on relative value basis : The results of im 'N.E.' on the uterus, adrenals and pituitary have been presented in Table I. Consequent to surgical removal of one ovary (hemicastration) there was a marked increase in the uterine weight as compared to the weight of the uterus in intact control animals. 'N.E.' treatment further induced a dose-depended stimulation of uterine growth increase both in terms of its relative as well as absolute weight ($P<0.05$ and 0.01). There was, however, just about no effect of the treatment on the weight of the pituitary except for an indication of slight stimulation (as compared with intact control, Gr. I) at the lowest dose of 0.25 *mg*, all the same it was statistically insignificant when compared with H.C. control, Gr. III. Hemicastration resulted in a significant decrease in the weight of adrenals ($P<0.02$), however, except the 0.5 *mg* dose, the progestin treatment was found to restore the adrenal weight to the control levels.

Weights expressed on absolute value basis (*mg*/100 *g*). Calculated on the basis of per 100 *g* body weight, the increase in uterine weight following surgical removal of one ovary was further accentuated by 'N.E.' treatment ($P<0.01$) at 2.0 and 4.0 *mg* doses whereas the 0.25 and 0.50 *mg* doses had just about no effect. The results showed that the slight increase in the weight of pituitary following hemicastration was, by and large, maintained with no significant differences between the weights of pituitary either among treatment groups or the controls. With regard to the adrenals the decrease in weight following removal of one ovary was restored with the 0.25 and 4.0 *mg* doses of 'N.E.' ($P<0.02$, compared with sham-operated controls, Gr. III).

DISCUSSION

Consequent to the surgical removal of one ovary, there was significant hypertrophy of the remaining ovary (COH) in the adult rat, however, treatment with intramuscular (im) 'N.E.' was observed to inhibit COH at all doses investigated showing, by and large, a direct linear relationship. In fact, 19-nortestosterone progestins in general are known to be potent inhibitors of pituitary gonadotrophic secretion with their esters or enol-ethers being many times more effective (7,8,9,12,18). The increasing compensatory ovarian growth in a unilaterally spayed animal has been attributed to a nearly two-fold increase in the synthesis and release of gonadotrophins from the pituitary (5,6,14,19) providing nearly double the stimulation, both in terms of intensity as well as duration, to the remaining

ovary causing it to hypertrophy. However, the identity of the gonadotrophin(s) involved in the COH has not been clearly established, nonetheless, FSH is generally believed to be the principal gonadotrophin responsible for COH (6,14,19,26). On the basis of the available evidences it would seem that 'N.E.' inhibited any de novo synthesis and/or release of FSH thereby depriving the remaining ovary of compensatory gonadotrophic stimulation, as was available in the absence of progestin treatment (Gr. II & III). The generally unaltered weights of the pituitary, by implication, lend reasonable credence to this hypothesis. All the same, effects of 'N.E.' on the ovary rendering it refractory/unresponsive to elevated peripheral gonadotrophic stimulation through a direct interaction at the ovarian site (4,20) and/or via an impairment of its metabolic and biosynthetic integrity (20, 23) can not be ruled out. In the rat 'N.E.' has been demonstrated to exert effects directly at the target tissues without the mediation of gonadotrophins, and the high anti-gonadotrophic effects have been atrributed to its relatively high partial estrogenicity/progestational ratio (17). Because of this ratio only, atleast it would appear so, 'N.E.' behaved like an 'impeded estrogen' (shallow dose-response curve-slope) in so far as its uterine growth stimulating property was concerned. Similar observations have been made with other closely related norethisterone esters such as norethisterone acetate, and quingestanol acetate (12). As far as 'N.E.' is concerned it has been found to be so much estrogenic in rats as to induce implantation, an established estrogen-dependent phenomenon with its progestational property being exhibited through sustainance of subsequent nidation (16). However, it is yet to be established whether this estrogenic activity was a function of the progestin *per se* or its metabolite(s) (3). Further, it was noted that while the antigonadotrophic ED_{50} was comparatively very high (7 *mg*), maximum uterine stimulation was achieved with as little as 0.25 *mg* 'N.E.', there being no significant difference between the increase in uterine weight obtained with 0.25, 2.0 and 4.0 *mg*, respectively. This would indicate that there was apparently no parallelism between the antigonadotrophic and uterine growth stimulating property (dose) of 'N.E.' In fact, such a lack of parallelism has been demonstrated to be an intrinsic property of esters and enolethers of norethisterone attributed to the substitution of a carboxylic acid or an alcohol side-chain at the α or β-unsaturated carbonyl position of 19-norprogestins (8). Further, accentuation of hemicastration-induced increase in uterine weight by 'N.E.'. indicated a synergistic interaction between 'N.E.' and endogenous estradiol and such an effect may not be unlikely in the light of similar additive effects observed with norethisterone acetate (10, 21).

The effects of 'N.E.' in restoring the hemicastration-induced decrease in the adrenal weight were again typical of a weak estrogen. Although the results did not indicate any dose-response relationship either on the relative or absolute weight basis, yet corroborative evidence (12) substantiated the nature of response observed in the present study.

TABLE I : Effect of intramuscular norethisterone enanthate on organ weights of unilaterally ovariectomized adult female rats.

Treatment group	Body weight (gm)	Weight of organs (mg)							
		Uterus		Pituitary		Adrenal		Left ovary (mg)	
		Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute
I Intact	124±3.0†	128.40±† 25·70	104·58± 23·08	6·5± 0·42	5·19± 0·19	36·8± 3·13	29·61± 2·11	21·60± 2·99	17·94± 2·98
II H. C. control	118±6.2	215.00± 29·43	151·03±* 28·13	6·4± 0·40	5·49± 0·52	31·2± 2·72	26·43± 1·83	30·40± 4·9	25·46± 4·8
III H. C. +vehicle	116±4.9	164.40±* 27·90	141·69±* 22·42	8·0± 1·90	6·9± 0·91	32.8±* 1.85	23·24±* 0 88	30·20± 5.62	17·48± 3.14
IV H. C. +0·25 *mg*	121.0±4·3	208·40±* 26·96	170·17±* 19·02	7.4±* 0·24	6·15±* 0·36	35·2±* 3·58	29 39±* 3·48	19·80±* 3.49	16·10± 2·48
V H. C. +0·50 *mg*	119±7·3	183·20± 32·50	157·24± 31·28	6·8± 0·72	5·69± 0·44	29·6± 2·29	24.99± 1·70	21.40±* 2·31	18·34± 2.50
VI H. C. +2·0 *mg*	123±5·8	256·80±** 13·63	209·16±** 8·58	7·2± 0·87	5·85± 0·61	32·4± 2·40	26·72± 2·62	22·80±* 1·20	18·75± 1·49
VII H. C. +4·0 *mg*	116±3·6	246·36±** 28·99	227·66±** 16·26	6·8± 0·5	5·9± 0·28	33·72±* 3·25	28·75±* 2·06	17·60±* 1·07	15·12± 1·70

(†) Values are Mean ± S. E. of 5 animals in each group.
* $P<0.05$
** $P<0.01$

It is, thus, postulated that while im 'N.E.' exerted high antigonadotrophic effects, there was no parallelism between its antigonadotrophic property and uterine growth stimulating attribute and, thus, it may be possible to achieve effective contraception at much lower doses without suppressing the pituitary gonadotrophic secretion.

REFERENCES

1. Benagiano, G. Long-acting Systemic Contraceptives. In : Proc. Symposium on 'Advances in Regulation of Human Fertility' ed. E. Diczfalusy, Scriptor, Copenhagen, 323-360, 1976.
2. Benagino, G., E. Diczfalusy, J.W. Goldzeiher, R. Gray, R. Apelo, N. Bergstein, J.C. DeSouza, P.K. Devi, A.N. Gupta, E. Kessuru, S. Kotesawang, A.O. Ojo, A. Pretnar and H.K. Toppazada. Multinational comparative clinical evaluation of two long-acting injectable contraceptive steroids : Norethisterone enanthate and Medroxyprogesterone acetate. I. Use-Effectiveness. *Contraception*, **15** : 513-533, 1977.
3. Brown, J.B. and H.A.F. Blair. Urinary estrogen metabolism of 19-nortestosterone and its esters. *Proc. Roy. Soc. Med.*, **53** : 433-434, 1960.
4. Callentine, M.R., Lee Shiao-Lung and R.R. Humphrey. Action of progestogens on estrogen-induced ovarian growth. *Proc. Soc. Exptl. Biol. Med.*, **124** : 1001-1005, 1967.
5. Damassa, D.A. and J.M. Davidson. Effects of ovariectomy and constant light on responsiveness to estrogen in the rat. *Horm. & Behaviour*, **4** : 269-281, 1973.
6. Edgren, R.A., A.F. Parlow, D.L. Peterson and R.C. Jones. On the mechanism of ovarian hypertrophy following hemicastration. *Endocrinology*, **76** : 97-102, 1965.
7. Ercoli, A., G. Bruni, G. Falconi, R. Gardi and A. Meli. Biological activities of some steroid-ethers : methyltestosterone enol-ethers. *Endocrinology*, **67** : 521-525, 1960.
8. Falconi, G. and A. Ercoli. Effect of enol-etherification on anti-estrual and contraceptive activity of some progestins in rats. *Proc. Soc. Exptl. Biol. Med.*, **108** : 3-6, 1961.
9. Falconi, G. and G. Bruni. Studies on steroidal enol-ethers: Antigonadotrophic activity of cyclopentyl derivatives of some orally active progestins. *J. Endocrin.*, **25** : 169-173, 1962.
10. Folman, Y. and G.S. Pope. Effect of norethisterone acetate, dimethylstilbestrol, genistein and coumestrol on the uptake of (3H)-oestradiol by uterus, vagina and skeletal muscle of immature mice. *J. Endocrin.*, **44** : 213-218, 1969.
11. Fotherby, K., G. Howard, K. Shrimanker, M. Elder and P.G.T. Bye. Occurrence of ovulation in women receiving injectable contraceptive norethisterone enanthate. *Contraception*, **18** : 535-542, 1978.
12. Giannina, T., B.G. Steinetz, C.L. Rassaert, E.A. McDougal and A. Meli, Biological Profile of Quingestanol Acetate *Proc. Soc. Exptl. Biol. Med.*, **131** : 781-789, 1969.
13. Goebelsman, U., Z. Frank, P. Stanczyk, F. Brenner, E. Alicia, E.K. Goebelsman, E. Gentzschein and D.R. Mishell Jr. Serum norethindrone (NET) concentrations following intramuscular NET enanthate injections : effect upon serum LH, FSH, estradiol and progesteone. *Contraception*, **19** : 283-313, 1979.
14. Johnson, D.C. and E. Witschi. Endocrinology of ovarian tumour formation in parabiotic rats. *Can. Res.*, **21** : 783-789, 1960.
15. Litchfield, J.T. Jr. and F. Wilcoxon. A simplified method of evaulating dose-response effect experiments. *J. Pharmacol. & Exptl. Therap.*, **96** : 99-113, 1949.
16. Neumann, F., Berswadt-Wallrabe, R-vow., W. Elger, K.J. Graf, S.H. Hasan, M. Mehring, Y. Nishino and H. Steinbeck. Special problems in toxicity testing of long-acting depot-contraceptives. In : *'Pharmacological Models in Contraceptive Development : Animal Toxicity and Side-effects in Man'* edited by E. Diczfalusy, Scriptor, Copenhagen, 315-354, 1973.
17. Nishino, Y. and F. Neumann. Ostrogens Partialwirkung von Gestagenen, speziell von Norethisterononanthat. *Arzneim.-Forsch./Drug Res.*, **30** : 439-452, 1980.
18. Peterson, D.L., R.A. Edgren and R.C. Jones. Steroid-induced block of ovarian compensatory hypertrophy in hemicastrated rats. *J. Endocrinology*, **29** : 255-262, 1964.
19. Ramirez, V.D. and C.H. Sawyer. The sex difference in the rat pituitary FSH-response to unilateral gonadectomy as revealed in plasma radioimmunoassay. *Endocrinology*, **94** : 475-482, 1974.

20. Smith, B.D. and J.T. Bradburry. Influence of progestins on ovarian response to estrogens and gonadotrophins in rats. *Endorcinology*, **78** : 279-301, 1966.

21. Srivastava, U.K. Mechanism of action of micro-amounts of gestagens administered by subcutaneous silastic implants. Ph.D. Thesis submitted to the All-India Institute of Medical Sciences, New Delhi, 1973.

22. Srivastava, U.K. and K.R. Laumas. Long-term Contraception with Steroid-releasing Implants. IV Biological effectiveness of norethindrone acetate (ENTA) in rats and rabbits. *Contraception*, **12** : 549-568, 1975.

23. Srivastava, U.K. Mechanism of Contraceptive action of micro-dose norethisterone enanthate administered by subdermal silastic implants. *J. Steroid Biochem.*, **9** : Suppl. 9, p. 258, 1979.

24. Srivastava, U.K. Contraceptive efficacy of microdose norethisterone enanthate : A preliminary study on its use in Polydimethylsiloxane implants. *Ind. J. Exp. Biol.*, **19** : 416-418, 1981.

25. Weiner, E. and E.D.B. Johnsson. The influence of norethisterone enanthate on ovarian function. *Acta Endocri. (Kbh)*, **83** : 386-392, 1976.

26. Welschen, R., J. Dullart and F.H. De-Jong. Inter-relationships between circulating levels of estradiol-17β, progesterone, FSH and LH immediately after unilateral ovariectomy in the cycling rat. *Biol. Reprod.*, **18** : 421-427, 1978.