Journal of Steroid Biochemistry, 1972, Vol. 3, pp. 871–876. Pergamon Press. Printed in Great Britain

PRIMING EFFECT OF NORETHYNODREL ON THE UPTAKE OF [6,7-^{3}H]-ESTRADIOL IN THE MOUSE UTERUS

VIMLA LAUMAS, A. FAROOQ and K. R. LAUMAS
Department of Reproductive Biology, All India Institute of Medical Sciences, New Delhi-16, India

(*Received* 21 *March* 1972)

SUMMARY

The priming effect of 0·01, 0·1, 1·0, 5·0, 10 and 50 μg of norethynodrel for different intervals of time on the uptake of [6, 7-^{3}H]-estradiol by the mouse uterus has been studied and compared with the priming effect of 0·01 μg estradiol on this uptake. Norethynodrel in doses up to 5·0 μg produced stimulation of [^{3}H]-estradiol uptake while pre-treatment with 50·0 μg norethynodrel caused an inhibition in the uptake of [^{3}H]-estradiol. Maximal stimulation of the [^{3}H]-estradiol uptake was observed after 4 h of the priming dose of 1·0 μg of norethynodrel which is comparable to the priming effect of 0·01 μg estradiol. The dose-dependent stimulatory and inhibitory effects of norethynodrel on [^{3}H]-estradiol uptake by the uterus may have important implications in the mode of action of this oral progestin.

INTRODUCTION

NORETHYNODREL (17αethynyl-17β-hydroxy-5(10)-estren-3-one) is the progestational component of the oral contraceptive 'Enovid' which is used for fertility control in women. Eisenfeld and Axelrod [1] showed that norethynodrel inhibits the uptake of estradiol in the rat uterus. This contention was questioned by Watanabe *et al.*[2] who did not find any receptors for norethynodrel in the rat uterus and thus suggested that inhibition of estradiol uptake observed by Eisenfeld and Axelrod may not be due to norethynodrel but due to the estrogenic impurities present in the norethynodrel. Saucier *et al.*[3] have proposed that norethynodrel may act as a competitive inhibitor for estradiol in the rat uterus. The variation in the above reports required a detailed investigation into the priming effect of norethynodrel on the uptake of estradiol in the uterus. The present communication describes the effect of pre-treatment with different doses of norethynodrel for varying periods of time on the uptake of [6, 7-^{3}H]-estradiol in the mouse uterus. Preliminary findings have been presented [4].

MATERIALS AND METHODS

Animals. Immature female mice, 22–23 days old, of the All India Institute of Medical Sciences Colony were used in this study. The animals were housed in air-conditioned quarters. Mothers of the pups were maintained on pellet diet manufactured by Hindustan Lever Ltd., Bombay.

Injection solutions. [6, 7-^{3}H]-estradiol (s.a. 44 Ci/m mol) was obtained from New England Nuclear Corpn., U.S.A. It was purified by celite column chromatography before use. Non-radioactive estradiol was obtained from Sigma Chemical Co., and norethynodrel (Lot No. MA-Q-924), free of estrogenic contamination, was a gift from Dr. Victor A. Drill of G. D. Searle & Co., Chicago, U.S.A.

All the steroids were dissolved in 5% ethanolic saline for injection. When

higher doses of norethynodrel were administered, ethanol was proportionately increased to achieve solubilization of the steroid. The injections were given subcutaneously in a constant volume of 0·1 ml per mouse. The control mice received at the appropriate time 0·1 ml of the injection vehicle, 5% or stronger ethanolic saline, as used in the corresponding treatment groups.

Procedure. Pre-treatment with estradiol (0·01 μg) or different doses of norethynodrel was given for varying periods of time, i.e. 2, 4, 6, 8 or 18 h before the animals were killed. The uptake of [6, 7-^{3}H]-estradiol was studied by injecting 2 μc of the labelled steroid to each animal 1 h before the time of killing. Six to ten immature female mice (22–23 day old) were used in each treatment or control group. At sacrifice the uteri were quickly removed, dissected free of extraneous tissue and weighed on a torsion balance after uniform blotting to remove excess fluid. The uteri were transferred directly to counting vials and dissolved in 0·3 ml Hyamine hydroxide, 15·0 ml of diotol scintillation fluid was added to each sample and radioactivity determined using Packard model No. 3214 liquid scintillation counter. Quenching corrections were made with the help of an internal standard. The counting efficiency for [^{3}H] was about 33%. The results are calculated as disintegrations per min/mg wet weight of the uterus and expressed as given under statistical analysis.

Statistical analysis. The mean of the [^{3}H]-estradiol uptake in the control group of animals, expressed as d.p.m./mg uterus, was taken as 100. The uptake in the treated group was expressed as per cent of the control value. The mean ± S.E.M. of treated group was calculated and compared with the mean value of the control group expressed as 100 ± S.E.M. using the student 't' test.

RESULTS

Effect of estradiol or norethynodrel pre-treatment on the uptake of [^{3}H]*-estradiol in the mouse uterus:*

It may be seen (Fig. 1) that 0·01 μg of estradiol pre-treatment for 2, 4 and 6 h to immature mice produced a statistically significant increase in the uptake of [^{3}H]-estradiol. When pre-treatment with the same dose of estradiol was given for 8 h, it did not produce any effect on the uptake of [^{3}H]-estradiol in the uterus. Pre-treatment with 0·01 μg norethynodrel for 2, 6 or 8 h did not produce any significant increase in the uptake of [^{3}H]-estradiol. The pattern of uptake of [^{3}H]-estradiol after pre-treatment with 0·01 μg of estradiol and norethynodrel show similarities in that the maximum stimulation is attained in both cases when pre-treatment is given for 4 h.

Effect of pre-treatment with different doses of norethynodrel on the uptake of [^{3}H]*-estradiol in the mouse uterus*

The effect of pre-treatment with 0·1, 1·0 and 5·0 μg of norethynodrel for 4, 8 and 18 h on the uptake of [^{3}H]-estradiol is given in Fig. 2. Pre-treatment for 4 h with any of the above doses of norethynodrel resulted in a statistically significant increase in the uptake of [^{3}H]-estradiol in the uterus, however by 8 and 18 h a decline in the uptake of [^{3}H]-estradiol took place. The [^{3}H]-estradiol uptake at 8 and 18 h after pre-treatment with 0·1 and 5·0 μg of norethynodrel is almost in the range of the control, however, the pre-treatment with 1·0 μg of norethynodrel for 8 and 18 h still caused a stimulation in the uptake of [^{3}H]-estradiol which was significantly higher than the control level.

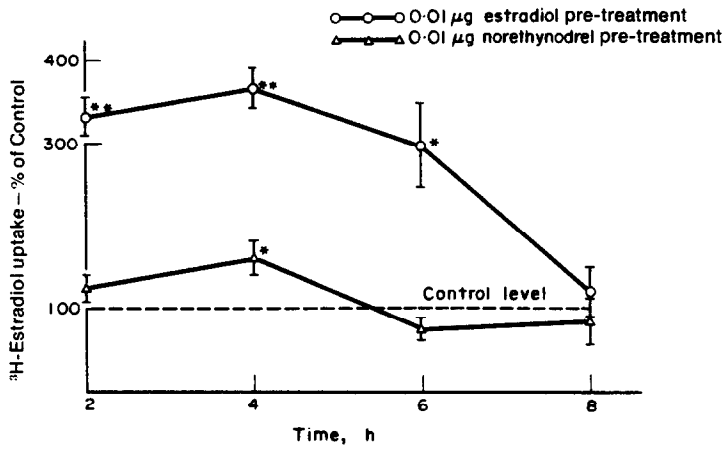

Fig. 1. Uptake of [³H]-estradiol in the mouse uterus after pre-treatment with 0·01 μg estradiol or 0·01 μg norethynodrel for 2, 4, 6 and 8 h.

*P < 0·05; **P < 0·001.

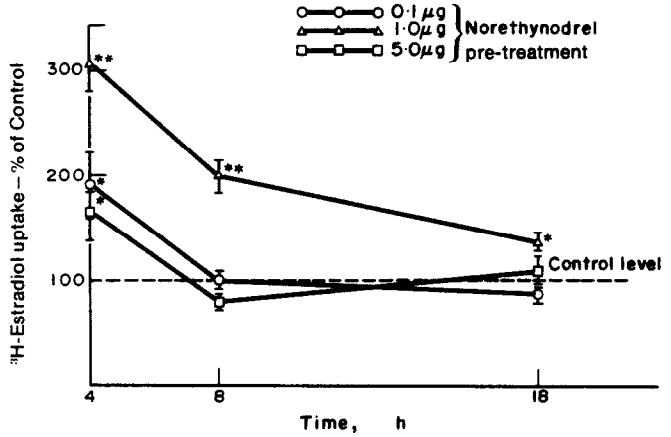

Fig. 2. Uptake of [³H]-estradiol in the mouse uterus after pre-treatment with 0·1, 1·0 and 5·0 μg of norethynodrel for 4, 8 and 18 h.

*P < 0·05; **P < 0·001.

The pattern of stimulation of [³H]-estradiol uptake after priming with 0·1, 1·0 and 5·0 μg of norethynodrel was similar to that presented for the pre-treatment with 0·01 μg estradiol and 0·01 μg norethynodrel. The only noteworthy difference is that the pre-treatment with 1·0 μg norethynodrel had lasting stimulatory effect on [³H]-estradiol uptake and this could be observed even up to 18 h.

Stimulation of [³H]*-estradiol uptake after pre-treatment with different doses of norethynodrel for 4 h*

The data for the effect of pre-treatment for 4 h with different doses of norethynodrel on the uptake of [³H]-estradiol is given in Fig. 3. It shows that the maximal stimulation of [³H]-estradiol uptake was obtained with 1·0 μg of norethynodrel and doses higher than 1·0 μg produced lesser stimulation in the uptake of estradiol. Pre-treatment with 10 μg norethynodrel showed neither increase nor decrease of

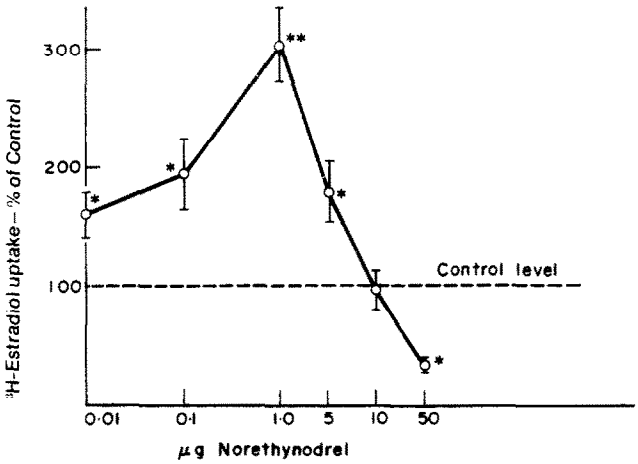

Fig. 3. Stimulation of [^{3}H]-estradiol uptake after pre-treatment with 0·01, 0·1, 1·0, 5·0, 10·0 and 50·0 μg norethynodrel.

*$P < 0.05$; **$P < 0.001$.

[^{3}H]-estradiol uptake. However, 50 μg of norethynodrel produced a statistically significant decrease in the uptake of [^{3}H]-estradiol compared with the control.

DISCUSSION

A careful study of the effect of pre-treatment of mice with norethynodrel has shown that it can produce a stimulatory or an inhibitory effect on the uptake of [^{3}H]-estradiol by the uterus and this depends upon the dosage of norethynodrel used and the period of pre-treatment. The maximum stimulatory effect was exerted by a priming dose of 1·0 μg of norethynodrel after 4 h. Prolongation of the time of pre-treatment with norethynodrel for 8 or 18 h did not stimulate the uptake of estradiol with other doses except the 1·0 μg dose. This dose continued to produce a stimulatory effect on the uptake of [^{3}H]-estradiol when pre-treatment was given for 8 and 18 h. However, the extent of [^{3}H]-estradiol uptake was lower at 8 and 18 h as compared with the uptake seen at 4 h. A dose of 50·0 μg of norethynodrel produced an inhibition in the uptake of [^{3}H]-estradiol by the mouse uterus.

The results show for the first time that a progestational steroid, like norethynodrel, not only inhibits the uptake of [^{3}H]-estradiol, possibly by competitive inhibition, but also stimulates its uptake. This finding of the interaction of norethynodrel with estrogen at the endometrial level either by augmenting or reducing the uptake of this hormone may have important implications in the mode of action of this oral progestin. Eisenfeld and Axelrod[1] using a high dose of norethynodrel (220 μg/100 g body weight) reported that norethynodrel acted as a competitive inhibitor of estradiol uptake. Saucier *et al.*[3] showed that administration of 100 μg norethynodrel per 100 g body weight to rats 15 minutes after the administration of [^{3}H]-estradiol produced a reduction in the uptake of [^{3}H]-estradiol. These authors concluded that norethynodrel produces a competitive inhibition in the uptake of [^{3}H]-estradiol. The present results are in agreement with the above studies, when a relatively high dose of norethynodrel (50 μg) is used for the pre-treatment. The findings of stimulation of [^{3}H]-estradiol uptake by rela-

tively small doses of norethynodrel have a similarity to some of the earlier reports [1, 5–8] in which small doses of estradiol were shown to cause stimulation in the uptake of [^{3}H]-estradiol. Kraay and Black [7] made a detailed study of the effect of estrogen priming on the uptake of [^{3}H]-estradiol by the mouse uterus. It was seen that 0·03 μg estradiol pre-treatment for 3–6 h produced a maximal stimulation of [^{3}H]-estradiol uptake.

In the present study, the stimulation of [^{3}H]-estradiol uptake with norethynodrel priming may be due to two possibilities. Firstly, it may be due to the inherent estrogenic activity of norethynodrel. Norethynodrel has been reported to possess 3–5% [9] or 7% [10] of the estrogenic potency of estrone. The second possibility may be due to the estrogenic metabolites of norethynodrel which may be exerting a mild estrogenic effect on the uterus as also noted with very small doses of estradiol. Arai *et al.*[11] isolated 17-α-ethynyl-19-norandrost-4-ene-3β,10β,17β-triol as one of the major urinary metabolites of norethynodrel which has been found to possess estrogenic activity [12]. It is possible that the mild estrogenic activity of this metabolite of norethynodrel stimulates the uptake of [^{3}H]-estradill by the uterus. Yadava and Laumas [13] studied the metabolism of [U-^{14}C]-glucose in the rabbit uterus after prolonged administration of norethynodrel and found an increased rate of synthesis of lipid, RNA and protein. This observation further confirmed the estrogenic properties of norethynodrel and its metabolites, since estrogens are known to increase lipid, RNA and protein synthesis in the uterus, Ui and Mueller [14]. However, the prime question as to how the mild estrogenic activity of norethynodrel per se or that of its metabolites can stimulate the uptake of [^{3}H]-estradiol is not completely understood. Its effect on cell permeability, transport, protein synthesis and possibly increase in estradiol binding sites are worth considering.

The results further brought out that priming with 0·01 μg of estradiol for 4 h produced 364% uptake of [^{3}H]-estradiol when the uptake in the control uterus was taken as 100. A similar magnitude of uptake of [^{3}H]-estradiol was observed with the priming dose of 1·0 μg of norethynodrel for the same period of time. It thus became obvious that a priming dose of norethynodrel which is 100 times more than that of estradiol produces an effect similar to estradiol pre-treatment. According to this criterion norethynodrel may be 1·0% in estrogenic activity compared with estradiol, an estimate which is comparable with the earlier reports using bio-assay procedures [9, 10].

ACKNOWLEDGEMENT

This work was supported by a grant from the Ford Foundation. Grateful thanks are due to Dr. Victor Drill for the supply of estrogen-free norethynodrel used in this study.

REFERENCES

1. Eisenfeld A. J. and Axelrod J.: *Endocrinology* **79** (1966) 38.
2. Watanabe H., Saha N. N. and Layne D. S.: *Steroids* **11** (1968) 97.
3. Saucier R., Banerjee R. C., Brazeau, Jr. P. and Husain S. M.: *Steroids* **16** (1970) 463.
4. Laumas K. R.: In discussion in the proceeding of the conference on the regulation of mammalian reproduction, N.I.H. Bethesda. Md. Sept. 27–Oct. 1 1970.
5. Laumas K. R.: *Proc. Third Asia Oceania Cong. Endocrinol.* **1** (1967) 124.
6. Laumas K. R.: Symposium uber Biochemische. Aspekte Der. Steroid forschung. Jena 18, Des 21 Sept. 1967 B. (1969).
7. Kraay R. J. and Black L. J.: *Proc. Soc. Exptl. biol. Med.* **133** (1970) 376.
8. Terenius L.: *Acta Endocr.(Kbh.)* **50** (1965) 584.

9. Drill V. A.: *Oral Contraceptives*. McGraw-Hill, New York (1966) p. 20.
10. Edgren R. A., Jones R. C. and Peterson D. L.: *Fertil. Steril.* **18** (1967) 238.
11. Arai K., Golab T., Layne D. S. and Pincus G.: *Endocrinology* **71** (1962) 639.
12. Bialy G., Layne D. S. and Pincus G.: *Proc. Soc. Exptl. biol. Med.* **118** (1965) 862.
13. Yadava H. S. and Laumas K. R.: *J. Reprod. Fertil.* **20** (1969) 71.
14. Ui H. and Mueller G. C.: *Proc. Natn. Acad. Sci. (U.S.)* **50** (1963) 256.