STIMULATION OF UTERINE RNA SYNTHESIS IN MICE BY THE POST-COITAL ANTIFERTILITY AGENT, ORF 3858

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Oestrogens, according to Emmens & Miller (1969), may be pharmacologically categorized into three classes: (1) compounds which occupy receptor sites and thereby elicit immediate physiological responses, (2) compounds which weakly or briefly occupy receptors and therefore result in impeded responses, and (3) pro-oestrogens, compounds which must undergo metabolic transformation in order to elicit oestrogenic responses.

The compound 2-methyl-3-ethyl-4-phenyl-4-cyclohexene carboxylic acid (ORF 3858) is non-steroidal and has post-coital antifertility and oestrogenic properties (Blye & Himm, 1967; Blye, 1970). During studies of the mechanism of the antifertility activity of this compound, two interesting experimental observations were made. First, when metabolic transformation of ORF 3858 to a *p*-hydroxylated form was inhibited, the antifertility activity of the compound was lost (Yard *et al.*, 1969). Second, ORF 3858 was found not to bind to uterine cytosol oestrogen receptors, whereas the *p*-hydroxylated analogue bound with high affinity (McGuire *et al.*, 1971). These findings suggested that the antifertility activity of the compound is related to the oestrogenicity of a metabolite and that ORF 3858 is a pro-oestrogen according to the scheme of Emmens & Miller (1969). The present study examines this hypothesis.

One of the most immediate cellular responses to oestrogens is the acceleration of uterine (or vaginal) RNA synthesis (O'Malley & Means, 1974). This sensitive end-point allows the measurement of the onset (Hahn *et al.*, 1971) and the duration (Miller & Emmens, 1967) of target level responses after a single administration of oestrogenic drugs. In this study, 0.1 µg of ORF 3858 or its *p*-hydroxylated congener, ORF 5531, was injected subcutaneously in 0.1 ml of 5% EtOH in 0.9% NaCl into female mice which weighed 25 to 30 g and had been ovariectomized 14 to 18 days previously. At various times after injection and 2 hr before autopsy, the mice received 6 µCi [³H]uridine in 0.2 ml of 0.9% NaCl via the lateral tail vein. The uteri were removed, pooled according to the treatment group, and immediately frozen in dry ice. Total RNA synthesis was measured by a modification of the method of Miller & Emmens (1967). Samples were homogenized in distilled water, and homogenates were made 0.2 N with respect to perchloric acid (PCA). The PCA precipitates were allowed to form overnight and were washed in 0.2 N-PCA. Washed precipitates were collected by filtration on Whatman No. 2 filter paper. The precipitates were

then air dried, combusted in a Packard sample oxidizer, and measured for radioactivity using an Intertechnique Model SL-30 spectrometer. The experiment was done in duplicate, each sample consisting of both uteri from a single mouse.

TEXT-FIG. 1. Incorporation of tritiated uridine into uterine RNA after injection of 0.1 μ g ORF 3858 ($\bullet$) or ORF 5531 (Δ). The open circle ($\circ$) is the incorporation rate in animals before the administration of the drug. Each point is the mean of duplicate samples.

The baseline rate of [3 H]uridine incorporation into uterine RNA in this experiment was 40 d/min/mg wet wt/hr. The injection of ORF 5531 resulted in an immediate increase in the rate of RNA synthesis to a 4.7-fold peak within 8 hr (Text-fig. 1). Virtually identical time courses for RNA synthesis have been found for oestradiol-17 β (F. C. Greenslade, unpublished data), ethinyl oestradiol (Hahn *et al.*, 1971) and a variety of other steroidal and non-steroidal oestrogens (Miller & Emmens, 1967). Injection of ORF 3858 did not lead to an immediate increase in uterine RNA synthesis; the rate remained virtually without change at background levels for several hours before it increased to a maximum rate ($\times 2.5$ the background rate by 16 hr after injection). When measured at 32 hr after injection, the synthesis rates induced by both com-

pounds had returned to levels slightly below those found before administration of the drug.

An interpretation of this delay in the uterine RNA synthetic response to ORF 3858 is that a period of time is required for metabolic conversion to the *p*-hydroxylated form, which is recognizable by the uterine oestrogen receptor. These and previous data from our laboratory lead us to conclude, therefore, that the post-coital antifertility action of ORF 3858 lies in its activity as a pro-oestrogen.

REFERENCES

- BLYE, R.P. (1970) The effect of estrogens and related substances on embryonic viability. *Adv. Biosci.* **4**, 326–343.
- BLYE, R.P. & HOMM, R. (1967) Antizygotic activity of 2-methyl-3-ethyl-4-phenyl-4-cyclohexene carboxylic acid in the rat. *Fedn Proc. Fedn Am. Socs exp. Biol.* **26**, 486.
- EMMENS, C.W. & MILLER, B.G. (1969) Estrogens, proestrogens and antiestrogens. *Steroids* **13**, 725–730.
- HAHN, D.W., MCGUIRE, J.L., GREENSLADE, F.C. & TURNER, G.D. (1971) Molecular parameters involved in the estrogenicity of mestranol and ethinylestradiol. *Proc. Soc. exp. Biol. Med.* **137**, 1180–1185.
- MCGUIRE, J.L., TURNER, G.D. & GREENSLADE, F.C. (1971) Studies on the effect of 2-methyl-3-ethyl-4-phenyl- Δ^4 -cyclohexene carboxylic acid (ORF 3858) and its *p*-hydroxylated metabolites on uptake of ^3H -estradiol-17 β by the uterus. *Proc. Soc. exp. Biol. Med.* **136**, 146–149.
- MILLER, B.G. & EMMENS, C.W. (1967) The incorporation of tritiated uridine in the genital tract of the oestrogen treated mouse. *J. Endocr.* **39**, 473–484.
- O'MALLEY, B.W. & MEANS, A.R. (1974) Female steroid hormones and target cell nuclei. *Science, N.Y.* **183**, 610–620.
- YARD, A.S., JUHASZ, L. & GRIMES, R.M. (1969) Studies on the anti-fertility effects and metabolism of a new, postcoital oral contraceptive, 2-methyl-3-ethyl-4-phenyl- Δ^4 -cyclohexene carboxylic acid, sodium salt (ORF 3858). *J. Pharmac. exp. Ther.* **167**, 105–116.