The Effect of 17 α -Ethinylestradiol-17 β on the Metabolism of Polypeptides in the Hypothalamus

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A very considerable amount of work has been done during recent years by many groups of workers on the effects of steroids as ovulation inhibitors (for references see Pincus, 1965). However, there still remains some uncertainty about the mode of action of ovulation inhibitors, and it is possible that some may act differently from others. Brown, Fotherby & Loraine (1962) and Loraine, Bell Harkness, Mears & Jackson (1963) suggest that certain ovulation inhibitors may have a direct inhibitory action on the ovary; Bucholz, Nocke & Nocke (1964), Starup & Ostergaard (1966) and others suggest, however, that the inhibitors modify gonadotrophic-hormone secretion. The effect of ovulation inhibitors on the secretion of gonadotrophic hormones has been widely studied (for references see Pincus, 1965), and one opinion on the primary action of inhibitors is that they interfere with some undefined biochemical mechanism in the hypothalamus in which the polypeptide gonadotrophic-hormone-releasing factors are involved.

Little information is available on the effect of steroids on the metabolism of polypeptides in the hypothalamus. Hooper (1962, 1963, 1964, 1966*a,b,c*) showed that the hypothalamus contained peptidases capable of inactivating polypeptide hormones, and indirect evidence suggests that some of the peptidases may be implicated in the metabolism of gonadotrophic-hormone-releasing factors. Peptidase activity in the hypothalamus was found to increase under physiological conditions known to suppress ovulation in most animals, i.e. during pregnancy, and in suckled lactating animals (Hooper, 1966*a,b*). It seemed probable that an increase in the concentration of circulating ovarian steroids may be one of the factors controlling peptidase activity in the hypothalamus, since ovariectomy and the injection of oestradiol into intact animals altered enzyme activity (Hooper, 1966*c*). These results may be interpreted as indicating that ovarian steroids are among the factors that control the metabolism of polypeptides in the hypothalamus. If this assumption is correct, antifertility agents might be expected to modify peptidase activity in the hypothalamus.

This communication describes an effect of one

of the commonly employed ovulation inhibitors, 17 α -ethinylestradiol-17 β , on peptidase activity in the hypothalamus.

Materials and methods. Adult female New Zealand white rabbits were employed. Ethinylestradiol was dissolved in arachis oil and injected into the gluteal muscle. The doses of steroid used were calculated on a weight-to-weight basis from doses known to prevent ovulation in human beings (Greenblatt, 1963). One group of animals was injected for 3 days with 0.5 μ g. of ethinylestradiol/day and killed on the fourth day. A second group of animals was injected once with 0.5 μ g. of steroid and killed 18 hr. later; a third group was injected once with 0.5 μ g. of steroid and killed 7 hr. later. A fourth group was used as control. Animals were killed by a blow on the neck and bled out; the brain was removed and the hypothalamus dissected out after removal of the optic chiasma and corpora mammillaria. The hypothalamus was homogenized in water and the nitrogen content measured by a micro-Kjeldahl method. The ovaries and uteri were removed, weighed and kept for future histological examination.

Peptidase activity is expressed as a function of the inactivation of the substrate oxytocin on incubation with homogenate of hypothalamus. Oxytocin (0.5 i.u./ml.) was incubated with various amounts of homogenate at 37° for 3 hr. at pH 7.3 (0.2 vol. of 0.4 M-NaHPO₄-NaH₂PO₄ buffer). Enzyme action was stopped at the end of the incubation period by heating the medium in a boiling-water bath for 15 min. Control experiments with homogenate heated in a boiling-water bath for 15 min. before incubation with oxytocin were used to measure any possible loss of substrate due to factors other than enzyme action. The residual oxytocin was measured on the isolated rat uterus by using a bracketing type of assay, and the recovery of oxytocin is expressed in terms of the hormone recovered from the controls, i.e. as $\log(A_0/A)$ where A_0 and A are the residual concentrations of oxytocin in media containing boiled and non-boiled homogenate respectively. The fiducial limits at a probability of 0.95 are calculated as described by Burn (1952).

Ethinylestradiol was obtained from British

Fig. 1. Inactivation of oxytocin by homogenate of hypothalamus obtained from female rabbits: Δ , normal rabbits; $\circ$, rabbits treated with $0.5\mu\text{g.}$ of ethinyloestradiol for 3 days; $\blacktriangle$, rabbits injected with one dose of $0.5\mu\text{g.}$ of ethinyloestradiol and killed 18 hr. later; $\bullet$, rabbits treated with one dose of $0.5\mu\text{g.}$ and killed 7 hr. later. Oxytocin was incubated with homogenate for 3 hr. at 37° and the residual hormone was measured on the isolated rat uterus. A_0 and A are control and residual polypeptide concentrations respectively. The lines drawn are calculated regression lines, and fiducial limits at $P0.95$ are indicated.

Drug Houses Ltd. (Poole, Dorset) and Pitocin, a synthetic preparation of oxytocin, from Parke, Davis and Co. Ltd. (Hounslow, Middx.).

Results and discussion. The results obtained from the four groups of animals are shown in Fig. 1. The injection of ethinyloestradiol for 3 days

resulted in a considerable increase in enzyme activity, and consequently efforts were directed towards finding the minimum time-interval needed for the steroid to act. Rabbits were injected with one dose of the drug and killed after 18 hr. and after 7 hr. After the 18 hr. injection period, the enzyme activity was still considerably elevated, but was significantly less than that obtained after the 3-day injection period. Even after a 7 hr. injection period an increase in enzyme activity occurred. Time-intervals may well be dependent on the route of administration.

The increase in hypothalamic peptidase activity after injection of ethinyloestradiol parallels previous results (Hooper, 1966c) obtained with oestradiol- 17β . Starup & Ostergaard (1966) suggest that certain inhibitors modify the secretion of pituitary gonadotrophins. If this is so, then some change in the metabolism of hypothalamic releasing factors might be expected. It is possible that there may be some connexion between the increase in the activity of peptidases in the hypothalamus and the availability of releasing factors, but there is no direct biochemical evidence to support this at present. Even so, it is apparent that small amounts of ethinyloestradiol do have a considerable influence on peptidase activity in at least one region of the central nervous system.

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