Pituitary uptake of ^{125}I -D-Leu⁶,Des-Gly NH₂¹⁰ LH-RH-ethylamide in ovariectomized rats pretreated with oestradiol-17 β

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Summary. The objective of this study was to determine if pretreatment of ovariectomized rats with oestradiol-17 β affects the anterior pituitary uptake of ^{125}I -D-Leu⁶,Des-Gly NH₂¹⁰-LH-RH-ethylamide (^{125}I -D-Leu⁶-LH-RH). Oestradiol-17 β (0.5 $\mu\text{g}/0.5$ ml oil) or oil was administered to ovariectomized rats at 2, 4, 8, 12, 16, 20 or 24 h before death, and at 30 min before death, 5 ng ^{125}I -D-Leu⁶-LH-RH were injected intravenously. The serum LH response to analogue administration in oil-treated rats did not change over time, but that in oestradiol-treated rats was depressed for 4 h and restored 8–24 h after oestradiol treatment, with the greatest response being at 16 h. However, the pituitary (adrenal, CNS cortex and thyroid) uptake of ^{125}I -D-Leu⁶-LH-RH in oestradiol-treated and control rats did not change over the 24-h time period. These data suggest that oestradiol-17 β does not affect pituitary responsiveness to ^{125}I -D-Leu⁶-LH-RH by inhibiting or facilitating the uptake of this analogue by the anterior pituitary.

Introduction

It is well established that the ovulatory LH surge which occurs on the afternoon of pro-oestrus in the rat is preceded by a rise in oestrogen levels (Brown-Grant, Exley & Naftolin, 1970; Kalra & Kalra, 1974). The bulk of evidence from studies to date supports the theory that this rise in oestrogen following follicular maturation is an essential signal in the course of events leading to the ovulatory LH surge. The precise mechanisms by which oestrogen exerts its effects, however, are not yet fully defined. Past findings suggest that oestrogen mediates some of its effects by modulating the pituitary responsiveness to luteinizing hormone-releasing hormone/follicle-stimulating hormone-releasing hormone (LH-RH). It has been reported that the pituitary response to LH-RH is greatest during pro-oestrus (Ripple, Johnson & White, 1973; Vilchez-Martinez, Arimura, Debeljuk & Schally, 1974a; Legan & Karsch, 1975; Greeley, Muldoon & Mahesh, 1975) when circulating levels of oestradiol are greatest. Treatment of ovariectomized rats (Kulkarni, Simpson & Macleod, 1974; Libertun, Cooper, Fawcett & McCann, 1974) and monkeys (Spies & Norman, 1975) with oestradiol enhanced pituitary responsiveness to LH-RH. In-vitro work has also demonstrated enhanced pituitary sensitivity to LH-RH after pretreatment with oestrogen *in vivo* (Apfelbaum & Taleisnik, 1976; Liu & Jackson, 1977) or *in vitro* (Drouin, Lagacé & Labrie, 1976).

Oestrogen could alter pituitary sensitivity by several mechanisms: by increasing pituitary stores of LH; by altering pituitary permeability to LH-RH; by altering receptor populations for LH-RH; or by increasing pituitary binding of LH-RH. Recent studies have shown that LH-RH binds to rat anterior pituitary cells in culture (Grant, Vale & Rivier, 1973) and that sex steroids alter receptor affinity for LH-RH (Spona, 1973). In addition, binding of iodinated LH-RH to rat

anterior pituitary homogenates has been found to be maximal in pituitaries collected during pro-oestrus (Park, Saxena & Gandy, 1976). The above findings suggest that oestrogen could affect the release of LH by modulating the anterior pituitary uptake of LH-RH.

The present study was undertaken to investigate the oestrogen-mediated changes in pituitary uptake of LH-RH *in vivo*. This effect was measured in ovariectomized rats pretreated with oestradiol-17 β by determining the pituitary uptake of ^{125}I -D-Leu⁶,Des-Gly-NH₂¹⁰-LH-RH-ethylamide. This LH-RH analogue possesses greater biological potency (Fujino *et al.*, 1974; Vilchez-Martinez *et al.*, 1974b) and binding affinity (Reeves, Tarnavsky, Becker, Coy & Schally, 1977) than the natural decapeptide.

Materials and Methods

Sprague-Dawley rats (300–400 g), ovariectomized for 5 months, were housed individually in a temperature-controlled environment in a 14 h light/10 h dark cycle. Food and water were supplied *ad libitum*.

Experimental animals were divided into 8 time groups with 17 animals per group. Oestradiol-17 β (0.5 μg /0.5 ml oil) was administered intraperitoneally to 9 rats in each time group at 2, 4, 8, 12, 16, 20 or 24 h before death. Control rats received an oil injection at times corresponding to those of the oestradiol-treated rats. All rats were anaesthetized approximately 1 h before death with 25% (w/v) urethane (0.66 ml/100 g body wt) to block random LH surges and, 40 min before the rats were exsanguinated, the jugular veins were exposed and a blood sample taken. Each rat was given an intravenous injection of 5 ng ^{125}I -D-Leu⁶-LH-RH, iodinated according to the lactoperoxidase method of Reeves *et al.* (1977), 30 min before death. Serum, adrenal, cerebral cortex, thyroid and anterior pituitary samples were removed at the time of autopsy and counted for ^{125}I -D-Leu⁶-LH-RH uptake. A ratio of tissue c.p.m./mg to serum c.p.m./ μl was used to express uptake in each tissue and to correct for nonspecific uptake of radioactivity present from the blood in the tissue. Blood samples taken before and after analogue injection were assayed for LH by a validated double-antibody radioimmunoassay (Niswender, Midgley, Monroe & Reichert, 1968), using NIH-LH-S17 as the standard. In our laboratory this assay has a 0.2 ng/ml sensitivity and a between-assay coefficient of variation of 14%.

The tissue:serum data were analysed by least-squares analysis of variance with unequal subclasses, according to a completely randomized design with time and treatment arranged factorially. Both time and treatment were considered to be fixed effects (Steel & Torrie, 1960). Values of LH were analysed according to a completely randomized design in which oestradiol and oil were treatment groups. Unequal numbers within treatment groups were considered by analytical procedures.

Results

Tissue uptake

The uptake of ^{125}I -D-Leu⁶-LH-RH in the anterior pituitary of oestradiol-treated and oil-treated animals, expressed as a radioactive tissue-to-serum (T/S) ratio, is shown in Text-fig. 1(a). The radioactive T/S ratio ranged between 4 and 6 over the 24-h period. There was no significant difference in the T/S ratio between the oestradiol-treated and oil-treated animals at any of the 7 times. There also was no significant change in the T/S ratio within treatment groups over time.

The radioactive uptake of ^{125}I -D-Leu⁶-LH-RH in the adrenal, cerebral cortex and thyroid is shown in Text-figs 1(b), 1(c) and 1(d). There was no significant difference in the radioactive uptake of ^{125}I -D-Leu⁶-LH-RH between treatment groups at any of the 7 times, or within treatment groups over time for the adrenal and CNS cortex. There was no significant difference in the

Text-fig. 1. Uptake of radioactivity, expressed as a tissue/serum ratio, in the (a) anterior pituitary, (b) adrenal, (c) CNS cortex and (d) thyroid of ovariectomized rats, measured at various times after injection of oestradiol-17 β (shaded columns) or oil (open columns). Rats were injected with 5 ng ^{125}I -D-Leu⁶-LH-RH 30 min before tissue collection. Values are mean $\pm$ s.e.m.

radioactive uptake of ^{125}I -D-Leu⁶-LH-RH between the treatment groups at any of the time periods. There was, however, a significant time effect for the thyroid in the oestradiol-treated and oil-treated animals. The T/S ratios were greatest at 2 h and decreased in a logarithmic manner over time.

LH response

The change in serum LH measured before and after ^{125}I -D-Leu⁶-LH-RH injection (ΔLH) is shown in Text-fig. 2. Serum LH increased in both treatment groups after analogue administration at all time periods. The LH response to analogue injection in oil-treated rats did not change over time. The large ΔLH noted at the 4-h time for the oil-treated group was due to high values in one rat. In the oestrogen-treated animals, there was an initial inhibition of LH release at the 2- and 4-h periods: less LH ($P < 0.05$) was released at these times than in the oil-injected animals. There was an increase ($P < 0.05$) in LH response at 8 h compared to that at 4 h in the oestradiol-treated rats. The change in serum LH in response to analogue injection then continued to increase at 12 and 16 h, with a maximum LH response at the 16-h collection period. Thereafter, the LH response declined throughout the remainder of the experiment.

Text-fig. 2. Change in serum LH (ΔLH) measured before and after ^{125}I -D-Leu⁶-LH-RH injection at various times after injection of oestradiol-17 β (shaded columns) or oil (open columns). Rats were injected with 5 ng ^{125}I -D-Leu⁶-LH-RH 30 min before serum collection. Values are mean $\pm$ s.e.m.

Discussion

Several possible mechanisms whereby oestrogen affects the preovulatory release of LH have been suggested, with one particular area of concern being the role that oestrogen plays in the modulation of pituitary sensitivity to LH-RH. Data from in-vitro binding studies (Spona, 1973; Park *et al.*, 1976) support the concept that oestrogen increases the pituitary response to LH-RH by affecting the pituitary uptake of LH-RH. Results from the present experiment, however, failed to show any increase or decrease in uptake of ^{125}I -D-Leu⁶-LH-RH by the anterior pituitary following oestradiol-17 β pretreatment under in-vivo conditions. Pituitary uptake of this analogue as measured by a radioactive T/S ratio did not change over the 24-h collection period, and no differences were detected between the oil-injected and oestradiol-treated animals. Oestradiol pretreatment was also shown to have no effect on the uptake of ^{125}I -D-Leu⁶-LH-RH by the adrenal, CNS cortex or thyroid. The T/S ratios for the adrenal and CNS cortex were less than 1, which suggests that there is no active uptake of the analogue by these tissues. The radioactive T/S ratios calculated for the thyroid ranged between 2 and 6, which demonstrated an active uptake

of the analogue, its breakdown products, or free ^{125}I . These results correlate well with those reported by Reeves *et al.* (1977).

Failure of oestradiol-17 β to mediate changes in the T/S ratio of the anterior pituitary over a 24-h time period cannot be accounted for by a possible lack of specific binding of ^{125}I -D-Leu⁶-LH-RH at the level of the anterior pituitary as compared to the natural decapeptide. Reeves *et al.* (1977) have demonstrated the specific binding nature of this analogue in the anterior pituitary and a concomitant lack of specific binding in the uterine horns, liver, kidney, adrenal, spleen, heart, thyroid and CNS cortex.

It has also been demonstrated that there is a characteristic pulsatile pattern of LH release throughout the day in ovariectomized rats, with peaks occurring approximately every 15 min (Blake, 1974), a finding which suggests a possible pulsatile release of LH-RH. Under the present experimental conditions, animals were anaesthetized with urethane, an anaesthetic that has been shown to block ovulation with the proposed method of action being the blockage of LH-RH release from the hypothalamus (Blake & Sawyer, 1972). Since our experimental animals were anaesthetized with urethane before ^{125}I -D-Leu⁶-LH-RH administration, endogenous LH-RH release would have been blocked, thus eliminating competition between the analogue and endogenous LH-RH for binding sites at the level of the pituitary. Such treatment should also rule out the hypothalamus as the site of action of the inhibitory effects of oestrogen on LH secretion (Text-fig. 2).

Several authors have reported a biphasic effect of oestrogen on LH release and pituitary responsiveness to LH-RH. Studies *in vivo* (Negro-Vilar, 1973; Cooper, Fawcett & McCann, 1974) and *in vitro* (Cooper *et al.*, 1974; De Koning, Van Dieten & Van Rees, 1976; Apfelbaum & Taleisnik, 1976) have demonstrated an initial depressed response to LH-RH approximately 3 h after oestrogen treatment followed by an augmented response to LH-RH 6–9 h after treatment. The LH data from the present study confirm previous findings in that an initial inhibition of pituitary response to ^{125}I -D-Leu⁶-LH-RH due to oestrogen treatment was seen, followed by a restoration of pituitary response.

That oestradiol-17 β was effective in changing pituitary responsiveness to ^{125}I -D-Leu⁶-LH-RH treatment in this study was shown by the differences in LH response at various times after oestradiol treatment. The present findings indicate that oestrogen does not exert this action by increasing or decreasing the uptake of ^{125}I -D-Leu⁶-LH-RH by the pituitary. These results do not exclude the possibility that oestrogen mediates pituitary sensitivity by affecting changes in the LH-RH or LH-RH-analogue receptor populations in the anterior pituitary. Since it has been reported that pituitary gonadotroph secretion granules bind LH-RH (Sternberger, Petralli, Joseph, Meyer & Mills, 1978) both cell membrane and secretion granule binding of ^{125}I -D-Leu⁶-LH-RH would have been measured by the present techniques. Although total pituitary uptake of ^{125}I -D-Leu⁶-LH-RH did not change over time, the possibility exists that, at one of these two sites, a change in binding occurred which could not be detected by the present experimental methods.

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