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Priming effect of luteinizing hormone releasing hormone in the hypogonadal mouse

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SUMMARY

We have investigated the LH response to LH releasing hormone (LH-RH) in female hypogonadal (*hpg*) mice in which the hypothalamus contains no LH-RH and the pituitary gland contains significantly less LH than in normal mice. Both the releasing action and the priming effect of LH-RH were not significantly different in *hpg* compared with normal mice. Raised plasma concentrations of oestradiol-17β reduced pituitary responsiveness to LH-RH in normal but not in *hpg* mice. These results show that in the mouse neither long-term exposure to normal levels of LH-RH nor a normal pituitary content of LH are necessary for either the releasing or the priming action of LH-RH.

INTRODUCTION

The mechanism of the priming effect of luteinizing hormone releasing hormone (LH-RH), the capacity of the decapeptide to increase pituitary responsiveness to itself (Aiyer, Chiappa & Fink, 1974), is not known. The priming effect depends upon the synthesis of new protein, but several studies have suggested that this new protein is not gonadotrophin (Fink & Pickering, 1980; Speight & Fink, 1981). Conceivably, the effect may depend upon an increase in specific LH-RH receptors; however, the density of LH-RH receptors on anterior pituitary cells decreases at the time of the spontaneous surge of luteinizing hormone (LH) (Clayton, Solano, Garcia-Vela, Dufau & Catt, 1980) and once the anterior pituitary gland has been exposed to LH-RH a massive amount of LH, equivalent to that released by a second exposure to LH-RH, can be released by exposure to either high extracellular K^+ (Pickering & Fink, 1976) or Ca^{2+} ionophores (Pickering & Fink, 1979). Although steroids do not mediate the priming effect of LH-RH, the magnitude of the effect is increased by oestradiol-17β (Aiyer *et al.* 1974; Meidan, Fink & Koch, 1981). Here we have investigated further the priming effect of LH-RH and the possible effects of oestradiol-17β using the hypogonadal mouse (*hpg*), a mutant in which the hypothalamic content of LH-RH is exceedingly low or undetectable as estimated by radioimmunoassay (Cattanach, Iddon, Charlton, Chiappa & Fink, 1977), bioassay (A. Speight, G. Fink & H. M. Charlton, unpublished data) and high-performance liquid chromatography (A. J. Harmar & G. Fink, unpublished data). The anterior pituitary gland of the mutant contains significantly less gonadotrophin than that of normal mice and the ovaries and uteri are completely atrophic (Cattanach *et al.* 1977). By studying the *hpg* mouse we hoped to determine whether previous exposure of the pituitary gland to normal levels of LH-RH and a normal pituitary content of LH are necessary for the LH releasing action and the priming effect of LH-RH and for the facilitation of pituitary responsiveness by oestradiol-17β.

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MATERIALS AND METHODS

The animals used were adult *hpg* and normal female mice bred in the colony at the Department of Human Anatomy, Oxford. They were maintained under controlled lighting conditions (14 h light : 10 h darkness) and allowed free access to diet FFGM (Dixon & Sons Ltd, Ware, Herts) and tap water.

At the start of the experiment 4–5 mm silicone elastomer capsules (601–321, Dow Corning Corporation, Michigan, U.S.A.) packed with oestradiol-17β were prepared and preincubated as described by Karsch, Dierschke, Weick, Yamaji, Hotchkiss & Knobil (1973) and were implanted subcutaneously into recipient *hpg* or normal mice. In the rat capsules of this size produced plasma concentrations of oestradiol-17β of about 30–50 pg/ml and when left implanted for 12–48 h produced a marked and sustained increase in pituitary responsiveness to LH-RH (Henderson, Baker & Fink, 1977). Insufficient blood remained after the LH assay for estimation of plasma oestradiol-17β in the present study, but in both normal and *hpg* mice implanted with oestradiol-containing capsules the weights of the uteri were significantly increased (Table 1). Control groups of animals received similarly constructed capsules without the oestradiol. In normal mice most of the capsules were implanted 2 days and a few 3 days after the last cornified vaginal smear. At the time of capsule implantation the smear was leucocytic. Pituitary responsiveness to LH-RH was tested 48 h later at which time the smear was comprised of cornified cells in all animals bearing oestradiol-containing capsules and of either cornified or cornified plus nucleated epithelial cells in animals bearing empty capsules. In the *hpg* mice the vagina was opened and the vaginal smear cornified at 48 h after implantation of an oestradiol-containing capsule in most animals. In *hpg* mice implanted with an empty capsule the vagina was closed in about 50% and the smear leucocytic when the vagina was open. To test pituitary responsiveness to LH-RH the animals were anaesthetized with urethane (120 mg/100 g body weight) administered i.p. as a 10% solution in 0·9% NaCl (w/v) solution and, after withdrawal of an initial blood sample (0·2 ml) from the external jugular vein, they were injected with 400 ng synthetic LH-RH (ICI Ltd, Pharmaceuticals Division, Macclesfield, Cheshire) (about 1·2 μg/100 g body weight) i.v. in 0·2 ml 0·9% NaCl. A second injection of the same dose of LH-RH was administered 1 h later. This relatively high dose of LH-RH was used because at the time that these studies were begun preliminary experiments showed that 400 ng LH-RH per mouse were required to produce clear-cut and reproducible LH release. Venous blood samples were taken 30, 59, 90 and 120 min after the first injection of LH-RH. At the end of the experiment animals were killed and the anterior pituitary gland rapidly removed, weighed and homogenized in ice-cold 0·9% NaCl (w/v) solution. The uterus was dissected out, freed from all adhering tissue, blotted and weighed. Pituitary and plasma samples were stored at −20 °C until assayed for LH by the method of Niswender, Midgley, Monroe & Reichert (1968) using NIH-LH-S18 as standard. Previous studies (Cattanach *et al.* 1977) showed that in this assay saline extracts of mouse pituitary gland inhibited binding in parallel with LH standard. The plasma samples were assayed in duplicate usually in volumes of 50 or 20 μl. For a 50 μl volume the lower limit of the sensitivity of the assay was usually 0·8 ng LH/ml but in one assay the sensitivity was 2 ng/ml. The inter- and intra-assay coefficients of variation were 7 and 9% respectively. The significance of differences between groups was assessed by the Mann–Whitney U test.

RESULTS AND DISCUSSION

The plasma concentrations of LH are shown in Fig. 1 and the mean maximal increments, pituitary gland LH contents and concentrations and uterine weights are shown in Table 1. The maximal increments were taken as the differences between the plasma LH concentrations before and the maximal LH concentrations after the injection of LH-RH. In all

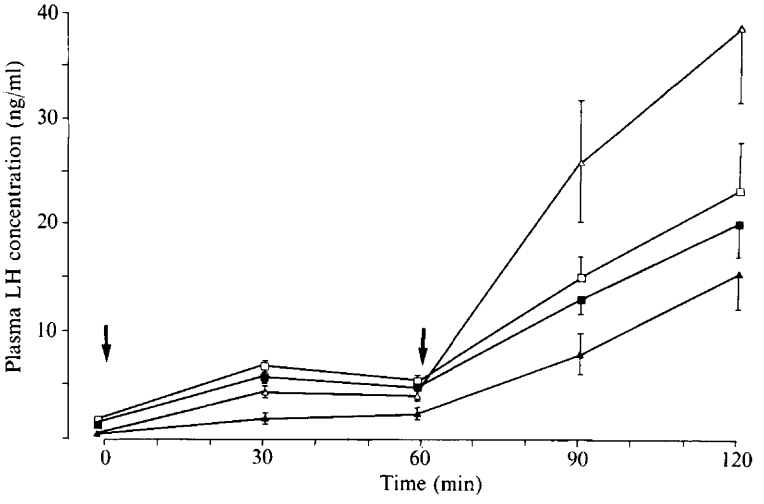

Fig. 1. Mean plasma concentrations of LH before and after the i.v. injection of LH releasing hormone (LH-RH; arrows) in normal mice bearing either empty (△) or oestradiol-containing silicone elastomer capsules (▲) or in hypogonadal (*hpg*) mice bearing either empty (□) or oestradiol-containing capsules (■). The vertical lines indicate S.E.M. These are not shown for the concentrations of LH in some samples taken before the first injection of LH-RH because some of these values were below the limits of detection of the assay. For the purpose of calculating the mean LH concentrations in the preinjection samples of plasma, the undetectable samples were mostly assigned values of 0·8 ng/ml, the lower limit of detection of most of the assays for 50 μl plasma samples. A few samples were assigned a value of 2·0 ng/ml, the lower limit of detection in one assay. Nine to eleven animals were included in each group.

Table 1. *Mean maximal increments of plasma LH after LH releasing hormone (LH-RH) injection, pituitary gland LH content and concentration and uterine weight in hypogonadal* (hpg) *and normal mice implanted with either oestradiol-containing or empty silicone elastomer capsules. Values are means* ± S.E.M.; *nine to eleven animals per group*

Treatment		Maximal increments in plasma LH concentration (ng/ml) after:		Pituitary gland LH		
Mice	Capsule	first injection of LH-RH	second injection of LH-RH	Content (ng/gland)	Concentration (ng/mg)	Uterine weight (mg)
Normal	Empty	4·4 ± 0·6	34·8 ± 6·5**	468·3 ± 55·9	333·3 ± 54·4	89·2 ± 14·0
Normal	Oestradiol	2·1 ± 0·5	13·5 ± 3·1**	340·2 ± 37·1	218·3 ± 32·5	170·7 ± 15·4
hpg	Empty	5·0 ± 0·5	19·4 ± 4·4*	76·0 ± 7·8	63·3 ± 6·0	5·9 ± 1·2
hpg	Oestradiol	4·4 ± 0·2	15·9 ± 3·2*	110·1 ± 16·7	79·3 ± 14·9	31·8 ± 3·0

*$P<0·01$, **$P<0·001$ compared with increment after first LH-RH injection (Mann–Whitney U test).

treatment groups the mean maximal increments after the second injection of LH-RH were greater than after the first injection. In animals implanted with an empty capsule the maximal increments after the second LH-RH injection in the *hpg* mice were lower, but not significantly lower compared with those in normal mice. Implantation of an oestradiol-containing capsule significantly ($P<0·01$) reduced the LH response to both the first and second injection of LH-RH in normal mice but had no significant effect on the responses in the *hpg* mice. Pituitary LH contents in the *hpg* mice were significantly ($P<0·001$) lower than in the normal mice. Implantation of an oestradiol-containing capsule had no significant effect on pituitary LH content or concentration in either *hpg* or normal mice. In order to economize on animals, control groups in which animals were injected with LH-RH

followed by saline and vice versa were not included because previous studies in mice showed that sequential blood sampling either in untreated or saline-injected animals did not significantly alter plasma LH concentrations (Iddon, Charlton & Fink, 1980) and in rats the priming effect of LH-RH could not be elicited by injecting LH-RH followed by saline or vice versa (Aiyer *et al.* 1974; Fink & Pickering, 1980). The reason for the difference in dosage of LH-RH required to produce a clear-cut and reproducible LH release in the present study compared with that used by Iddon *et al.* (1980) is not readily apparent, although Iddon *et al.* (1980) used male mice.

Several conclusions may be drawn from these results. First, neither the releasing nor the priming action of LH-RH depends upon previous long-term exposure of the anterior pituitary gland to LH-RH. In the context of the finding by Clayton *et al.* (1980) and Clayton & Catt (1981) that exposure of the anterior pituitary gland to increased concentrations of LH-RH for longer periods (days) increases the density of LH-RH receptors, the present findings add to the evidence cited in the Introduction that LH-RH receptor density may not play a critical role in the priming effect of LH-RH. The LH-RH receptor density in *hpg* male mice has recently been found to be only 30% of that in normal mice (L. S. Young, H. M. Charlton & R. N. Clayton, unpublished data). Secondly, the priming effect of LH-RH can be elicited in the presence of a low pituitary LH content and the magnitude of the effect is not directly proportional to pituitary LH content. Thirdly, as already reported by Bronson (1981), the mouse differs markedly from the rat and hamster in that continuous exposure to oestradiol-17β reduces rather than facilitates gonadotrophin function. Comparison of the effect of oestradiol in *hpg* mice with that in normal mice suggests that a normal LH-RH mechanism may be necessary for the inhibitory effect of oestradiol.

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