The effects of prostaglandin and mating on release of LH in the female rabbit

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Summary. The release of LH in response to prostaglandin (PG) treatment of female rabbits in various reproductive states was compared with the surge following mating. Intracarotid infusion of PGE-2 or PGF-2 α (0.3–900 μ g/h) into non-receptive and pseudopregnant does resulted in small, 2–4-fold elevations in jugular vein LH concentration. Similar doses of PGF-2 α in oestrogen-pretreated, oestrous does stimulated a 13-fold increase in plasma LH levels. Mating resulted in a much larger release of LH, as plasma levels increased approximately 60-fold from 1.1 ± 0.2 (S.E.M.) ng/ml to 67.8 ± 10.5 ng/ml. These results indicate that PG can stimulate the hypothalamic–hypophyseal axis to release LH in non-receptive, pseudopregnant and oestrogen-pretreated, oestrous rabbits.

Introduction

Many studies have shown that prostaglandin (PG) treatment causes secretion of LH in a number of species, but most have been conducted with the rat. They suggest that PG stimulates LH release by acting on the hypothalamus (Ojeda, Wheaton & McCann, 1975). For example, Eskay, Warberg, Mical & Porter (1975) reported that doses of PGE-2 causing LH release also produce a 2–3-fold increase in concentrations of LH-RH in hypophyseal portal blood, while Chobsieng, Naor, Koch, Zor & Lindner (1975) found that antiserum to LH-RH prevented PGE-2 from stimulating LH release in rats.

LH release is stimulated by PGF-2 α treatment in sheep (Carlson, Barcikowski & McCracken, 1973) and rhesus monkeys (Carlson, Wong & Perrin, 1977), but not in man (Coudert & Faiman, 1973; Craig, 1975). Although the rabbit is used frequently for endocrinological experiments, little information is available on the effect of PG on gonadotrophin release in this species. Recently, however, Agmo (1975) reported that treatment with PGE-1 resulted in a significant rise in plasma LH concentration in male rabbits whereas similar exposure to PGF-2 α was unsuccessful. Because PGs may play an important role in the hypothalamic control of pituitary secretion, we have examined the ability of PGE-2 and PGF-2 α to stimulate LH release in female rabbits at various stages of the reproductive cycle and have compared this with the reflex release following mating.

Materials and Methods

The 31 adult New Zealand rabbits weighed 3–4 kg each and were housed individually in a room with 14 h light/24 h. Each animal had free access to food and water. All the present experiments were carried out between September and May.

Group 1 consisted of 6 non-receptive rabbits (i.e. does not permitting mating on the day of treatment), Group 2 contained 8 pseudopregnant animals and Group 3, 4 oestrous rabbits. The animals in Group 2 were pretreated with 50 μ g oestradiol benzoate/day for 2 consecutive days as described by Kanematsu, Scaramuzzi, Hilliard & Sawyer (1974) and mated on the 3rd day to a vasectomized male (Day 0). PG was administered on Day 7 or 14 of pseudopregnancy. The oestrous animals in Group 3 received a similar pretreatment with oestradiol benzoate and were examined on the 3rd day for oestrous behaviour with a male, although mating was prevented.

PG administration into the conscious, unanesthetized animal was achieved by placing the right common carotid artery into a skin loop along the neck at least 7 days before treatment. At the same time, a polyethylene cannula (PE50) was inserted into the jugular vein, filled with heparin and sealed.

On the day of infusion, a small incision was made in the skin loop and a PE50 cannula was inserted into the carotid artery towards the head. Each animal received a control infusion of saline for 1 h into this artery followed by PG infusions starting at the lowest dose and increasing at each hour. Total infusion time was 5 h (except for Group 3 which received PGF-2 α for 4 h) during which blood samples (2 ml) were withdrawn every 15 min. Although the red blood cells were not restored, no decline in the haematocrit appeared during collection. After removal, the samples were mixed with heparin, centrifuged and the plasma frozen until assay.

The effects of mating on LH release were studied in 12 normal rabbits (Group 4). Several days before coitus a PE50 cannula for blood collection was inserted into the jugular vein of each rabbit. Eight of the animals were mated to a vasectomized male whenever normal oestrus appeared; the other 4 were given oestradiol benzoate treatment for 2 days (see Group 2) and were mated on the 3rd day. Starting approximately 30 min before mating, blood samples were withdrawn from the jugular vein at frequent intervals for 5 h.

Assay

LH was measured in triplicate in each sample by a heterologous radioimmunoassay which uses a guinea-pig antiserum (7FGP) raised to rabbit LH. This antiserum has been checked previously for specificity and found to be free from cross-reaction with other pituitary hormones except TSH (~70%) (Moore & YoungLai, 1975). Purified ovine LH (LER 1056 C2) was used as the tracer hormone and labelled with ¹²⁵I (Greenwood, Hunter & Glover, 1963). The LH standard was a preparation of rabbit LH (EX 130 GP) which has a biological potency of 0.89 units NIH-LH-S1. With this standard, the lowest detectable level of LH was 200 pg/ml plasma. This assay was checked for parallelism by comparing the regression line of the rabbit hormone standard with that calculated from results with various dilutions of rabbit plasma. The standard *t* test was used to determine if the slopes of the 2 regression lines were parallel (Steel & Torrie, 1960). Between-assay coefficient of variation was 11.4% and within-assay coefficient of variation was 7.9%.

Results

Mating and LH release

The effects of mating on plasma LH concentrations (Group 4) are shown in Text-fig. 1. In the 8 rabbits mated at normal oestrus, LH levels rose immediately from a mean ($\pm$ S.E.M.) basal concentration of 1.1 ± 0.2 ng/ml to a peak of 67.8 ± 10.5 ng/ml by 1.5 h and declined gradually thereafter. The 4 animals mated after oestradiol priming had a similar release of LH except that the peak occurred sooner (Text-fig. 1).

PG and LH release

The effects of PG infusion on plasma LH concentration in Groups 1, 2 and 3 are shown in Table 1. Small but significant increases in jugular vein LH levels occurred following PGE-2 treatment of non-receptive animals (Group 1) and PGF-2 α treatment during pseudopregnancy (Group 2). In general, the higher doses of PG produced the greatest release. Since the response to PG during pseudopregnancy (Group 2) was similar on Days 7 and 14, the results for these 2 days were combined.

The greatest response to PG administration appeared in the oestrogen-pretreated, oestrous rabbits (Group 3). During PGE-2 infusion, LH concentration rose significantly ($P < 0.01$) above control levels when doses of 30 and 300 μ g/h were given (Text-fig. 2a). PGF-2 α in oestrous rabbits was more effective (Text-fig. 2b): at the highest dose (300 μ g/h), the mean plasma LH concentration

Text-fig. 1. LH concentrations in the jugular venous blood of 8 normal, oestrous rabbits allowed to mate (○) and in 4 oestrous rabbits pretreated with oestradiol benzoate (●). Each point represents the mean $\pm$ 1 S.E.

Text-fig. 2. The effect on jugular LH concentrations of a stepwise intracarotid infusion of (a) PGE-2 and (b) PGF-2 α into conscious, oestrogen-pretreated oestrous rabbits. Each point represents the mean $\pm$ 1 S.E. The peak LH response in (b) is about 8 % of that after mating (Text-fig. 1).

Table 1. Jugular plasma LH concentrations (ng/ml) after intracarotid infusion of prostaglandins E-2 and F-2 α into rabbits during dioestrus, and during pseudopregnancy and oestrus after pretreatment with oestradiol

Reproductive state	PG	No. of rabbits	Saline	Dose of PG (μ g/h)			
				0.3	30	300	900
Dioestrous	E-2	3	0.33 $\pm$ 0.03	0.79 $\pm$ 0.23*	0.44 $\pm$ 0.06	0.30 $\pm$ 0.03	1.18 $\pm$ 0.41*
Dioestrous	F-2 α	3	0.36 $\pm$ 0.02	0.41 $\pm$ 0.04	0.37 $\pm$ 0.04	1.20 $\pm$ 0.63	0.48 $\pm$ 0.06
Pseudopregnant	E-2	4	0.58 $\pm$ 0.06	0.79 $\pm$ 0.13	0.70 $\pm$ 0.11	0.72 $\pm$ 0.11	0.62 $\pm$ 0.10
Pseudopregnant	F-2 α	4	0.47 $\pm$ 0.06	0.52 $\pm$ 0.08	0.71 $\pm$ 0.13	1.46 $\pm$ 0.48*	0.98 $\pm$ 0.09**
Oestrous	E-2	2	0.52 $\pm$ 0.03	0.77 $\pm$ 0.16	1.23 $\pm$ 0.14**	0.84 $\pm$ 0.11**	0.60 $\pm$ 0.06
Oestrous	F-2 α	2	0.44 $\pm$ 0.03	0.66 $\pm$ 0.08*	2.47 $\pm$ 0.48**	5.65 $\pm$ 0.70**	—

Each value represents the mean ($\pm$ S.E.M.) of all samples collected at each dose level from all animals in that group.

* $P < 0.05$; ** $P < 0.01$, denotes significant difference from control level during saline infusion.

was approximately 13-fold greater than that recorded during the control infusion of these animals. No changes in LH levels were observed in an oestrous rabbit infused with saline for 4 h. The maximum LH concentration following PGF-2 α treatment of oestradiol-pretreated rabbits was approximately 8% of that released after mating.

Discussion

Mating produced an immediate surge of LH in the untreated and the oestradiol-primed does. Although the amount released was comparable in both groups, oestradiol pretreatment resulted in the LH peak appearing earlier. Similar observations have been reported previously (Dufy-Barbe, Franchimont & Faure, 1973; Kanematsu *et al.*, 1974; Hilliard, Pang, Penardi & Sawyer, 1975).

Administration of PGE-2 or PGF-2 α to rabbits in various reproductive states also stimulated LH release in the present study, as has been reported for the effects of PG treatment of female rats (Harms, Ojeda & McCann, 1974; Sato, Taya, Jyujyo, Hirono & Igarashi, 1974; Chobsieng *et al.*, 1975; Eskay *et al.*, 1975), sheep (Carlson *et al.*, 1973) and monkeys (Carlson *et al.*, 1977). In these reports, the PGs have been administered by various routes and over a wide range of doses. PGE-2 is more effective than PGF-2 α in stimulating LH release in rats (Ojeda *et al.*, 1975; Harms *et al.*, 1974), but our results with oestrogen-pretreated, oestrous animals show that PGF-2 α is more potent than PGE-2 in this respect in rabbits.

As in rats (Harms *et al.*, 1974), prostaglandin treatment was most effective when administered to oestrogen-pretreated, oestrous rabbits. Higher titres of oestrogen in these animals as a result of prior exposure to oestradiol benzoate appear to increase the sensitivity of the hypothalamic-hypophyseal axis to PG. Pretreatment of rats with oestrogen also results in greater LH release in response to LH-RH (Arimura & Schally, 1971; Libertun, Cooper, Fawcett & McCann, 1974), and Dufy-Barbe, Franchimont, Dufy & Vincent (1975) have observed a greater LH release following LH-RH treatment in oestrogen-primed female rabbits. These results therefore suggest that PG may be stimulating LH release in both species by a similar mechanism.

Our study does not demonstrate whether PGs are required for LH release in the rabbit as reported for the sheep (Carlson, Barcikowski, Cargill & McCracken, 1974). In the rabbit the greatest response to PG treatment occurred in oestrogen-pretreated, oestrous animals during the highest infusion dose of PGF-2 α —a level that appears to exceed physiological concentrations in the blood (<0.5 ng/ml: Challis, Davies & Ryan, 1973). However, it is difficult to determine the amount of this PG actually reaching the hypothalamus because of dilution in the carotid artery before reaching the central nervous system. In addition, we observed a small but significant increase in plasma LH at the lowest dose of PGF-2 α (0.30 μ g/h). By comparison, a PGF-2 α intracarotid infusion rate of 6 μ g/h was found to be the minimal effective dose for stimulating LH release in sheep (Carlson *et al.*, 1973). Although more studies are necessary before definite conclusions can be drawn, the current observations may

reflect a physiological response of the hypothalamus and/or pituitary in the rabbit to PG administration. Clearly, however, the amount of LH released by PG administration is much less than that following mating.

Although the increase in plasma LH after mating and PG treatment in this study are being interpreted as LH release from the pituitary gland, it is possible that we are measuring some TSH because of the antiserum cross-reaction with this hormone. However, it seems unlikely that there would be significant amounts of the latter released at mating in the rabbit, and the LH values during the surge following coitus are similar to those obtained by use of a more specific antiserum (Hilliard *et al.*, 1975). It is not known whether PG administration can stimulate TSH secretion in the rabbit, but this treatment does not cause release of TSH in the rat (Brown & Hedge, 1974; Eskay *et al.*, 1975; Warberg, Eskay & Porter, 1976). It therefore appears that the elevation of plasma LH levels observed after exposure to PG is primarily due to LH secretion.

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