The Effect of Oestradiol-17β and Progesterone on the Pituitary Response to Gonadotrophin-Releasting Hormone (Gn-RH) in Anoestrous Ewes

A. M. SHAREHA[1]

ABSTRACT

The effect of progesterone (P) injection (2 × 12.5 mg/day) for 4 days and/or estradiol–17β (E_2–17β) infusion at a rate of 3 μg/hr for 4 hours on the concentration of luteinizing hormone (LH) released by Gn–RH infusion (3 μg/hr for 8 hours) was investigated in anoestrous ewes. The concentration of the LH released by Gn–RH was similar to that observed at oestrus. Sequential (P) and (E_2–17β) administration had no effect on LH release by Gn–RH, however, (E_2–17β) alone increased and (P) alone decreased the response.

INTRODUCTION

Peripheral plasma concentrations of luteinizing hormone (LH) in the ewe can be increased by oestrogen administration (4,9,30). Although progesterone has been shown to suppress LH release by either oestrogen or Gn–RH (14,20,27). Other investigators have reported no effect of Gn–RH in ewes (5,6). It has been suggested by Hooley *et al.* (14) that a progesterone stimulus for more than 24 hours may be required to suppress LH release. Inhibition of the induction of ovulation by Gn-RH was overcome in the rabbit either by increasing the dose of Gn–HR or by treatment with oestrogen (12). Thus the duration of stimulus and dose may both be important factor regulating anterior-pituitary response.

The purpose of this study was to observe the effect of exogenous steroids, in doses producing plasma concentrations similar to those found in normal oestrous cycle, on LH release by Gn–RH in Scottish Blackface ewes during the anoestrous season. Normal values for concentrations of steroids and LH during the oestrous cycle, which have been used for comparative purposes in this study, are taken from previous investigations (28,29).

[1] *Department of Animal Production, Faculty of Agriculture, University of Alfateh, Tripoli, S.P.L.A.J.*

MATERIALS AND METHODS

Twenty nine anoestrous ewes were randomly assigned to seven groups:

I. Gn–RH
II. Oestradiol–17 β B, (E_2), Gn–RH
III. Progesterone (P), Gn–RH
IV. P, E_2, Gn–RH,
V. E_2,
VI. P, E_2 and
VII. P

Treatment was as follows:

Progesterone (Intervet), 12.5 mg in oil, was injected interamuscularly twice daily for 4 days. Eighty four hours after the last progesterone injection, intracarotid infusions were started at a rate of 3 μg/hr of either oestradiol–17 β in 5% ethanolic saline (for 4 hours), or Gn–RH (synthetic decapeptide ICI 82, 286, obtained from ICI Alderley Park, Macclesfield Cheshire U.K.) made up in saline immediately before use (for 8 hours). When oestradioi–17 β and Gn–RH treatments were preceded by progesterone (Group IV), infusion of Gn–RH was started at 88 hours after the progesterone treatment. Jugular blood samples were obtained once daily from the beginnig of progesterone treatment until the infusion, then every 2 hours (with an additional sample at 1 hr after the start of Gn–RH) up to 36 hr. Total oestrogens, progesterone and LH were measured in plasma, using radioimmunoassa (Shareha *et al.*, 1976). Results are expressed as mean $\pm$ standard deviation with N = number of determinations and were analyzed using student's 't' test or analysis of variance.

RESULTS AND DISCUSSION

In Groups I, II, III, and IV (Fig. 1), there was no significant difference between the maximum concentration of LH at oestrus (138.8 $\pm$ 83.4 N = 8) and that following Gn–RH infusion in the anoestrous season (138.8 $\pm$ 22.5 ng/ml N = 4. Fig. 1 I). When ewes were infused with oestradiol–17β (Fig. 1 II) peak concentrations of total-oestrogens (66.9 $\pm$ 14.7 pg/ml, N = 5) were three times those at oestrus (21.1 $\pm$ 3.1 pg/ml, N = 11). A similar infusion used by Pant (19) in anoestrous Clun Forest ewes gave *plasma concentrations* of LH within the upper range of those observed at oestrus. Since total oestrogen concentration was the same when assayed with the two different antisera used in these studies, it is suggested that the two breeds may metabolized oestrogen at different rates. A difference in the number of ovulations induced by Gn–RH between Clun Forest and Scottish Blackface ewes during the anoestrous season has recently been reported (11).

Pre-treatment with oestradiol–17 β resulted in a significant increase ($P > 0.01$) in LH released by Gn–RH (200.0 $\pm$ 15.8 ng/ml N = 5) when compared with that released by Gn–RH alone (138.0 $\pm$ 22.5 ng/ml, N = 4). These findings are in agreement with previous reports on an increase in the sensitivity of the pitutary to synthetic Gn–RH during oestrus and following treatment with oestradiol benzoate (13,24,25).

Investigations of events culminating in the LH surge at oestrus in rats indicate that first, as a result of the increase in oestrogen, there is augmentation of pituitary sensitivity (1) and this is then followed by a self-potentiating effect of Gn–RH on LH release (2). However, one hour after the start of Gn–RH infusion, in ewes pretreated with oestradiol–17 β, the LH concentration was significantly lower ($P > 0.001$) than in ewes

receiving Gn–RH alone (13.2 ± 5.0 ng/ml, N = 5; vs. 46.8 ± 16.1 ng/ml, N = 4). Evidence for a negative feedback effect of oestrogen on LH release has been obtained in the ovariectomized ewe (9,23,27), as well as, in other ovariectomized animals (13,17,18), also in intact rat (13) and in prepuberal intact gilt (21). In ovariectomized and intact rats, maximal inhibitory effect, of oestrogen on LH release has been observed at 2 and 3 hr respectively after the start of treatment, but in ovariectomized rats this effect had disappeared by 4 hr (7). Similarly, in the heifer and ewe, the inhibitory effect has been observed at 2 or 3 hr and in the heifer a positive response could not be elicated by Gn–RH until 4–6 hr after oestrogen. Thus, in the present investigations it is suggested that the Gn–RH infusion was started at a time when the inhibitory effect of oestrogen on LH release was still prevailing, therefore, the system was unable to respond fully to the Gn–RH stimulus. Peak concentrations of LH were observed 4 hr later. Since maximal concentrations of LH are attained 2–4 hr after the start of an infusion of Gn–RH without previous treatment with oestrogen (18), it is assumed that the pituitary would be capable of responding to the Gn–RH after 6–8 hr from the start of oestrogen infusion. However, in Group V there was no change in LH concentration for 16 hrs, at which time only 2/4 ewes responded to the cestradiol–17 β infusion with LH peaks of 36 and 80 ng/ml. The time lapse between oestrogen treatment and LH release is similar to that reported by Beck and Reeves (3), who also observed that there was no dose response relationship between oestradiol–17 β and LH and that 33% anoestrous ewes failed to respond to administration of 12.5 ug oestradiol–17 β. In the present study, 1 of the 2 ewes which did not respond had a relatively high plasma concentration of progesterone (2.3 ng/ml).

The progesterone treatment schedule in Group III, IV, VI and VII resulted in maximum concentrations (5.8 ± 2.0, N = 4; 5.0 ± 0.9, N = 4; 56.1 ± 2.2, N = 3 and 3.8 ± 1.2 ng/ml, N = 5 respectively), which were not significantly different from each other or from those found in a normal luteal phase (5.1 ± 1.4 ng/ml, N = 11). In a preliminary experiment, ewes were treated with progesterone and the plasma concentrations were measured before, during and up to 96 hr after the treatment. The concentrations returned to pre-treatment values 72–84 hr after the last progesterone injection; therefore an interval of 84 hr was scheduled before starting infusions. Nevertheless, the progestorene concentration for Group III at the start of the infusion was 1.1 ± 0.3 ng/ml, N = 4, which was significantly higher ($P > 0.05$) than that found in the normal cycle prior to oestrus (0.6 ± 0.4, N = 10), while that for each of Groups IV, VI, and VII was significantly lower ($P > 0.25$) being (0.4 ± 0.4, N = 0.5 ± 0.2, N = 3 and 0.4 ± 0.4, N = 5 respectively at 84 hr. The maximum concentration of LH following I treatment with Gn–RH in ewes pretreated with progesterone (Fig. 1 III) being 95.0 ± 19.2 mg/ml, N = 4 significantly less ($P > 0.5$) than that released by Gn–RH alone and concurs with the findings of Pant & Ward (20) and Hooley *et al.* (14). Since Jaume (16) and Pretorius (22) have reported an increase in pituitary content of LH during the luteal phase, also in a previous work Roche *et al.* (26) had shown in ewes, that the pituitary content of LH was high during anoestrus. The inhibitory effect of progesterone is apparently not due to a lack of LH, but may be a result of the stored acting at the pituitary to cause either a reduction in the pool of LH available for release (14) or an impairment in the mechanisms of release. There was no change in the LH concentration in 5 ewes following P pretreatment with progesterone alone (Group VII) or 2/3 ewes receiving progesterone and oestrogen (Group VI). A small amount of LH (20 ng/ml maximum concentration) was released in the third ewe in Group VI, 16 hr after the start of oestrogen infusion.

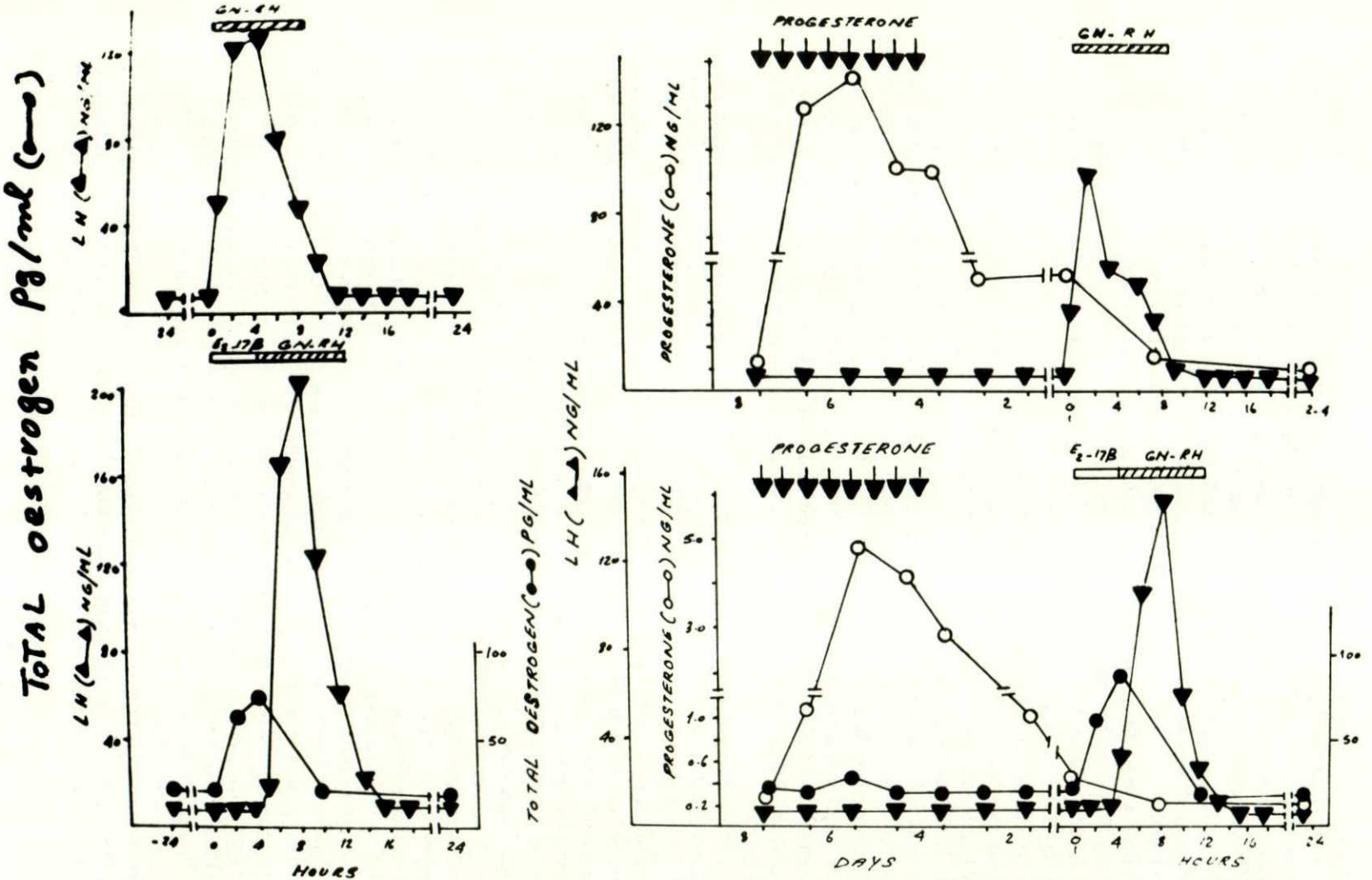

Fig. 1. Plasma concentrations of LH (▲——▲), total oestrogen (●——●) and progesterone (o) following different treatment schedules. Arrows indicate progesterone injections. Horizontal bars indicate infusion open–E_2; crosshatched–Gn–RH. (I) Gn–RH. (II) Gn–RH, (II) P, Gn–RH, (VI) P, E_2, Gn–RH (see text).

Thus, despite treatments simulating a normal cycle, the pituitary response in terms of LH released was inadequate. Since Gn–RH activity of the hypothalamus is greater during anoestrus than in the breeding season (15), it is possible that in anoestrus there are alterations in the potency of the neurohumoral signal required to trigger a possible 'activation' or release of endogenous Gn–RH.

The concentration of LH after progesterone and oestrogen treatment (Group IV), being 148.8 ± 41.7 ng/ml, N = 4, (Fig. 1 IV) was not significantly different from that released by Gn–RH alone, but was more than that released in Group III ($P > 0.05$) and less than that released in Group II ($P > 0.05$). The different results of Debeljuk (7), who reduced the LH release by Gn–RH by pre-treatment with a combination of progesterone and oestrogen can be related to differences in route of administration, timing of treatments and amounts of steroids and Gn–RH administered. Inhibition of the positive feedback effect of oestrogen on LH release by progesterone has been previously demonstrated in the intact ewes, in the present investigation. Also, has been reported in the intact Rhesus Monkey (8).

ACKNOWLEDGEMENT

This work was supported by the Libyan Government. We thank Dr. Wlpole, A. L. and H. Gregory of ICI, Alderly Park, Macclesfield, Cheshire, for a gift of synthetic Gn–RH, (ICI 82–286).

LITERATURE CITED

1. Aiyer, M. S. and G. Fink. 1974. The role of sex steroid hormones in modulating the responsiveness of the anterior pituitary gland to luteinizing hormone releasing in the female rat. J. Endocr. 62:553–572.
2. Aiyer, M. S., S. A. Chi ppa and G. Fink. 1974. A priming effect of luteinizing hormone releasing factor on the enterior pituitary gland in the female rat. J. Endocr. 62:573–588.
3. Beck, T. W. and J. J. Reeves. 1973. Serum luteinizing hormone (LH) in ewes treated with various dosages of 17–estradiol at three stages of the anoestrous season. J. Anim. Sci. 36:566–570.
4. Bolt. D. J., H. E. Kelley and H. W. Hawk. 1971. Release of LH by estradiol in cycling ewes. Biol. Reprod. 4:35–40.
5. Chakraborty, P. K. and J. J. Reves. 1973. Pituitary responsiveness to infusion with LH/RSH–RH. J. Anim. Sci. 37:304–Abst.
6. Cumming I. A., J. M. Buckmaster, J. C. Cerini, M. E. Cerini, W. A. Chamley, J. K. Findlay and J. R. Goding. 1972. Effect of progesterone on the release of luteinizing hormone induced by a synthetic gonadotrophin-releasing factor in the ewe. Neuroendocrinology, 10:338–348.
7. Debeljuk, L., A. Arimura and A. V. Schally. 1971. Effect of estradiol and progesterone on the LH release induced by LH-releasing hormone (LH–RH) in intact diestrous rats and anoestrous ewes. Proc. Soc. Exp. Biol. Med. 139:774–777.
8. Dierschke. D. J., T. Yamagi, F. J. Karsch, R. R., Weick, G. Weiss and E. Knobil. 1973. Blockade by progesterone of estrogene induced LH and FSH release in Rhesus monkey. Endocrinology 92:1496–1501.

9. Goding J. R., M. A. de D. Blockey, J. M. Brown, K. J. Catt and I. A. Cumming. The role of oestrogen in the control of the oestrous cycle of the ewe. J. Reprod. Fert. 21:368–369.
10. Goding, J. R., K. J. Catt., J. M. Brown, C. C. Kaltenbach., I. A. Cumming and B. J. Mole. 1969. Radioimmunoassay for ovine luteinizing hormone. Secretion of luteinizing hormone during estrus and following estrogen administration in the sheep. Endocrinology 85:133–142.
11. Haresing, W. 1975. Ovarian response to synthetic LH-RH anoestrous ewes. J. Reprod. Fert. 44:127–129.
12. Hilliard J., A. W. Schally and C. H. Sawyer. 1970. Progesterone blockade of the ovulatory response to intra-pituatry infusion of LH-RH in rabbits. Endocrinology, 88:730–736.
13. Hobson, W. and W. Hansel. W. 1974. Increased in vitro pituitary response to LH–RH after in vivo estrogen treatment. Proc. Soc. Exp. Boil. Med. 146:470–474.
14. Hooley, R. D., R. W. Baxter, W. A. Chamley, I. A. Cumming, H. A. Jones and J. K. Findlay. 1974. FSH and LH response to gonadotropin-releasing hormone during the ovine estrous cycle and following progesterone administration. Endocrinology, 95:937–942.
15. Jackson, G. L., J. F. Roche, D. L. Foster and P. J. Dziuk. 1971. Luteinizing hormone releasing activity in the hypothalamus of anestrous and cyclic ewes. Biol. Reprod. 5:5–12.
16. Jaume, C. M. 1974. Ph.D. Thesis, University of Sidney-cited by Hooley *et al.* 1974.
17. Krey, L. C., W. R. Butler and E. Knobil. 1975. Surgical disconnection of the medical basal hypothalamus and pituitary function in the Rhesus monkey. 1. Gonadotropin secretion. Endocrinology 96:1073–1087.
18. Libertun, C., L. Orias and S. M. McCann. 1974. Biphasic effect on estrogen on the sensitivity of the pituitary to luteinizing hormone-releasing factor (LRF). Endocrinology 94:1094–1100.
19. Pant, H. C. 1974. Regulation of gonadotrophin secretion in the ewe. Ph.D. Thesis, University of Liverpool.
20. Pant, H. C. and W. R. Ward. 1973. Effect of progesterone on the pituitary responsiveness to luteinizing hormone releasing hormone (LH–RH) in intact anoestrous ewes. J. Physical. Lond. 232:45–47.
21. Penerantz, D. K., G. R. Foxcroft and A. V. Nalbandov. 1975. Acute and chronic estrodiol–17 inhibition of LH release in prepubertal female pigs : time course and site of action. Endocrinology, 96:558–563.
22. Pretorius, P. S. 1974. Fluctuations in pituitary FSH and LH in the normally cycling and in the anoestrous angora goat. J. S. J. S. Afr. Vet. Assoc. 45:173–176.
23. Radford, H. M. and A. L. C. Wallace. 1974. Central nervous blockade of estradiol stimulated release of luteinizing hormone in the ewe. J. Edocr. 60:246–252.
24. Reeves, J. J., A. Arimura and A. V. Schally. 1971a. Changes in pituitary responsiveness to luteinizing hormone-releasing hormone (LH–RH) in anoestrous ewes pretreated with estradiol benzoate. Biol. Reprod. 4:88–92.
25. Reeves, J. J., A. Arimura and A. V. Schally. 1971b. Pituitary responsiveness to purified luteinizing hormone-releasing hormone (LH–RH) at various stages of the estruous cycle in sheep. J. Amin. Sci. 32:123–126.
26. Roche, J. F., D. L. Foster. F. J. Karsch, B. Cook and P. J. Dziuk. 1970. Levels of

luteinizing hormone in sera and pituitaries of ewes during the estrous cycle and anestrus. Endocrinology. 86:568–572.
27. Scaramuzzi, R. J., S. A. Tillson, I. J. Thorneycroft and B. V. Caldwell. 1971. Action of exogenous progesterone and estrogen on behavioural estrus and luteinizing hormone levels in the ovarioctomized ewe. Endocrinology. 88:1184–1189.
28. Shareha, A. M., W. R. Ward and K. Bichall. 1976. Effect of continuous infusion of gonadotrophin releasing hormone (Gn–RH) in ewes at different times of the year. J. Reprod. Fert. Vol. 46, 331–340.
29. Shareha, A. M. 1975. Gonadotrophin releasing hormone action in the ewe during anoestrus and the breeding season. Ph.D. Thesis, University of Liverpool.
30. Symons, A. M., N. F. Cunningham and N. Saba. 1973. Oestrogen induced LH sugars in the anoestrous and cycling ewe. J. Reprod. Fert. 35:569–571.
31. Van Dieten, J. A. M., J. J. Steijsinger, J. Dullaart and G. P. Van Ress. 1974. The effect of estradiol benzoat on the pituitary responsiveness to LH–RH in male and female rats. Neuroendocrinology, 15:182–188.

تأثير هرمون الاستروجين والبروجسترون على حساسية الغـــدة اللمفاوية للهرمون المسبب (Gn–RH)

فى غير موسم الاخصاب عند الاغنام

د · عاشور مسعود شريحه

المستخلـــص

تأثير حقن هرمون البروجسترون (٥ر١٢ مللى جرام مرتين فى اليوم لمدة أربعـــة أيام) و / أو حقن مستمر فى الأوعية الدموية للاستروجين (٣ ميكروجرام / الساعة لمـدة أربعـــة ســـاعات) على تركيـــز هرمون (LH) الـــذى أرتفع فى الـدم بعد حقـن مســـتمر للهرمون المســـبب (Gn-RH) (٣ ميكروجرام / الساعة / لمدة ٨ ساعات) · قد اكتشف فى غير موسم الاخصاب عند الاغنام ·

١ ــ تركيز هرمون المبيض (LH) فى الدم كنتيجة لحقن الهرمـــون المسبب (Gn–RH) كان مماثلا للتركيز الملحوظ عند عملية الشبق ·

٢ ـ تعاقب حقن الهرمونين البروجسترون والاستروجين ليس له تأثير ملحوظ على ارتفاع هرمون المبيض (LH) كنتيجة لحقن الهرمون المسبب (Gn–RH) غير أن حقن مستمر لهرمون الاستروجين لوحده زاد من حساسية لغدة اللمفاوية للهرمون المسبب (Gn–RH) وحقن البروجسترون لوحده سبب فـــى انخفـــاض لهـــذه الحساسية ٠