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An α_1 Adrenergic Mechanism Mediates Estradiol Stimulation of LHRH mRNA Synthesis and Estradiol Inhibition of POMC mRNA Synthesis in the Hypothalamus of the Prepubertal Female Rat

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We showed previously that the surge of luteinizing hormone-releasing hormone (LHRH) induced by estradiol-17β (E_2) in the female rat can be blocked by an α_1 adrenergic antagonist. The aim of the present study was to determine whether this was due to a direct action of E_2 on noradrenergic projections to LHRH neurons or whether it also involved other systems such as the arcuate pro-opiomelanocortin (POMC) neurons which are thought to inhibit LHRH biosynthesis and release. The experimental preparation was the prepubertal female rat in which an LHRH surge is induced by pregnant mare serum gonadotropin. Prazosin was used as a specific α_1 adrenergic antagonist and LHRH and POMC mRNA concentrations and cell numbers, in the medial preoptic area and rostral arcuate nucleus, respectively, were determined by *in situ* hybridization. Prazosin significantly reduced the total number of LHRH mRNA expressing cells, and increased the total number of POMC mRNA expressing cells and the concentration of POMC mRNA per cell. These results suggest that the inhibition of E_2-stimulated LHRH biosynthesis and release by α_1 adrenergic blockade may be mediated by two mechanisms; (i) increased POMC synthesis leading to inhibition of LHRH neurons and (ii) direct inhibition of a stimulatory α_1 adrenergic/LHRH mechanism.

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INTRODUCTION

Estradiol-17β (E_2) in its positive feedback mode increases luteinizing hormone-releasing hormone (LHRH) mRNA in neurons of the medial pre-optic area (MPOA) of the rat hypothalamus [1]. Since few, if any, LHRH neurons contain estrogen receptors [2], this action of E_2 is probably mediated by one or more interneurons and, by way of multisynaptic pathways, could involve disinhibitory as well as stimulatory mechanisms [3]. One possible disinhibitory mechanism is the arcuate pro-opiomelanocortin (POMC) system which projects to LHRH neurons [4, 5] and inhibits LHRH release [3]. Estradiol-17β, in its positive feedback mode, significantly reduces the concentrations of POMC mRNA in the rostral arcuate nucleus [6]. This, together with the fact that E_2 inhibits POMC gene transcription [7], suggests that E_2 stimulation of LHRH synthesis and release could be mediated, in part, by disinhibition of LHRH neurons consequent on the inhibition of POMC biosynthesis.

Starting with the pioneering findings of Sawyer *et al.* [8], many pharmacological data show that an α_1 adrenergic mechanism plays a crucial role in generating the spontaneous ovulatory LHRH and LH surges [9, 10]. Central noradrenergic neurons concentrate E_2 [11] and project to the MPOA [12–15]. Our primary aim, therefore, was to determine whether an α_1 adrenoreceptor mechanism is involved in mediating E_2 stimulation of

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LHRH mRNA synthesis as well as LHRH release. As an experimental preparation we used the prepubertal female rat treated with pregnant mare serum gonadotropin (PMSG) which stimulates an LHRH/LH surge by way of stimulating E_2 release [16]. The apparent similarity between the PMSG-induced and spontaneous LHRH/LH surge and the fact that the timing of the PMSG-induced surge of LHRH and LH is more precise than the spontaneous LHRH/LH surge have led to the extensive use of the PMSG-treated prepubertal female rat as a model for pharmacological studies of the neurotransmitter mechanisms involved in the LHRH surge (see ref. 9 for review). The specific α_1 adrenoreceptor antagonist, prazosin, administered intraperitoneally, intravenously or by intracerebral implant, blocks the spontaneous ovulatory surge of LH in adult female rats [17–19]. Our preliminary studies with prazosin (1 mg kg^{-1}, i.p.) showed that α_1 adrenoreceptor blockade reduced the number of cells expressing LHRH mRNA [20] in PMSG-treated prepubertal female rats. Preliminary observations also suggested that the level of POMC mRNA in arcuate neurons appeared to be increased in PMSG-treated animals injected with prazosin. These changes were associated with reduced LH concentrations in plasma from trunk blood in the prazosin compared with saline-treated, matched control animals. The aim of the present study was to follow up these preliminary observations by a formal study designed to determine whether an α_1 adrenergic mechanism is involved in mediating the effects of E_2 on the hypothalamic levels of LHRH mRNA and POMC mRNA as assessed by quantitative *in situ* hybridization.

MATERIALS AND METHODS

Animals

The experimental paradigm was as described previously [9, 16]. Briefly, the animals used were female Wistar rats, bred in the Department of Pharmacology, maintained under controlled lighting (lights on 0500–1900 h) and temperature (22°C) and allowed free access to food (SDS diet RM3: Special Diet Services, Witham, Essex, England) and tap water. The rats were injected with 20 IU PMSG (Intervet U.K. Ltd, Science Park, Milton Road, Cambridge, England) at 1100 h on day 30. On day 32 at 1200 h the animals were injected, i.p., with either 1 mg kg^{-1} prazosin–HCl (RBI-Semat Technical (U.K.) Ltd, Hatfield Road, St Albans, Herts, England) dissolved in 0.9% (w/v) saline or 0.9% (w/v) saline alone. At 1630–1700 h of the same day the animals were anaesthetized with sodium pentobarbitone (Sagatal: RMB Animal Health Ltd, Dagenham, England) and killed by decapitation. To determine the efficacy of the PMSG treatments the uteri were examined and trunk blood was collected into heparinized tubes for LH assay [1, 21].

Pairing and matching of animals and tissues

In order to allow paired comparisons, tissues from pairs of saline- and prazosin-treated animals were collected on the same day and matched and processed together as described before [1, 6].

Tissue sectioning and blood collection

Whole brains were removed, frozen in iso-pentane at −45°C for 5 min and stored at −70°C. Coronal (10 μm) sections were collected at −18°C and thaw mounted onto acid washed, poly-L-lysine coated slides. For LHRH *in situ* hybridization histochemistry serial sections were collected from +8.6 to +8.0 (AP coordinates of Pellegrino *et al.* [22]) and POMC *in situ* hybridization from the rostral arcuate nucleus (+7.0 to +6.2 [22]).

Probe preparation

A 30-base oligonucleotide probe, complementary to the sequence for the LHRH decapeptide [23], was supplied by the Oswel DNA Service (Department of Chemistry, University of Edinburgh, Edinburgh, Scotland). The probe was labelled, using T_4 polynucleotide kinase (Gibco-BRL Ltd, Paisley, Strathclyde, Scotland), at the 5′ end with ^{35}S-γ-ATP (specific activity >1000 Ci/mmol, Du Pont (U.K.) Ltd, Wedgewood Way, Stevenage, Herts, England) [24] and purified through D-25 Nu-Clean columns (IBI Ltd, Cambridge, England).

A 538 bp fragment of the POMC cDNA, which included the β-endorphin coding domain, was cloned into the plasmid vector pGEM4 (Promega, Epsilon House, Chilworth, Research Centre, Southampton, England). The plasmid was digested with EcoRI and the ^{35}S-labelled cRNA was transcribed by utilizing the T7 RNA polymerase promotor [6].

For both the LHRH and POMC probes RNase A and sense strand controls were included [1, 6].

Prehybridization and hybridization

Anatomically and experimentally matched alternate slides were removed from storage at −70°C and allowed to stand at room temperature for 10 min, immediately followed by fixation in 4% (w/v) paraformaldehyde in phosphate buffer (0.1 mol/l; pH 7.4), for 10 min. The prehybridization and hybridization buffers [6] contained 40% and 50% (v/v) deionized formamide for LHRH and POMC, respectively. For LHRH, the hybridization buffer contained 2 × 10^6 cpm/ml ^{35}S-labelled LHRH oligonucleotide probe. The procedures for the LHRH and POMC *in situ* were as described previously [1, 6].

Autoradiography

Slides were dipped in Ilford K5 liquid photographic emulsion (Ilford Ltd, Mobberly, Knutsford, Cheshire, England) diluted 1:2 with distilled water and air dried

for 12–14 h in total darkness followed by exposure, in light-tight boxes containing desiccant, at 4°C for either 21 days (LHRH) or 10 days (POMC). Sections were developed (Ilford Phenisol diluted 1 + 4 with distilled water) for 3 min, fixed (Ilford Hypam Fix diluted 1 + 4 with distilled water) for 5 min, lightly stained with haematoxylin and eosin and mounted with DPX mountant.

Detection of mRNA containing cells

LHRH mRNA and POMC mRNA containing cells were located, under bright field illumination in an optical microscope, in anatomically matched sections from experimentally (i.e. simultaneously processed) matched brains.

Sections (from +8.6 to +8.2 [22]) containing LHRH mRNA were mapped for total positive cell counts using a light microscope under bright field illumination. Silver grain counts were carried out, with the aid of an AMS Optomax V image analysis system, on single cells only where a nucleus was visible. The sample, consisting of equal numbers (85–90) of cells from matched pairs of brains, was spread over 12 sections. Within each section, cells were randomly and bilaterally selected. Background silver grain counts were determined as described previously [25].

Sections containing POMC mRNA (from +6.8 to +6.4 [22]) were counted for total positive cell counts using the AMS Optomax V image analysis system. Positive cells (130–150) were silver grain counted as described for LHRH mRNA grain counting.

RESULTS

The PMSG treatment was effective in all the animals used for the *in situ* hybridization studies, as assessed by substantial uterine enlargement in each rat, and a significant increase in plasma LH concentrations. The mean ± SEM plasma LH concentrations in the PMSG-treated animals was 25.4 ± 7.9 and 13.9 ± 4.4 ng NIH–LH–S18/ml ($n = 10$ per group) in saline- and prazosin-treated rats, respectively. In animals not treated with PMSG the plasma LH concentrations were 1.7 ± 0.1 ng NIH–LH–S18/ml ($n = 5$) (data from ref. 9). The large scatter in the present LH values is probably due to (i) the fact that for humane reasons trunk blood was taken under sodium pentobarbitone anaesthesia which is a powerful blocker of LH release [26], and (ii) the fact that single LH values were obtained at different times during the expected time of the LH surge.

There was a significant decrease in numbers of cells expressing LHRH mRNA in prazosin-treated animals when compared with matched saline-treated animals (Table 1; Fig. 1). However, there was no significant between-group difference in the grain counts of LHRH mRNA expressing cells (Table 1; Fig. 1). The number of POMC mRNA expressing cells was higher in prazosin- compared with saline-treated rats in each of 5 out of 6 matched pairs (Table 1; Fig. 1). However, in one pair (#5), the cell counts were decreased in the prazosin-treated rat, even though the grain counts/cell were higher in the prazosin- compared with the

Table 1. The cell counts and silver grain counts per cell in PMSG-treated animals treated with either prazosin or saline

	Total cell counts			Mean grain counts		
LHRH	Saline	Prazosin	% Change (Saline = 100%)	Saline	Prazosin	% Change (Saline = 100%)
Pair 1	251	214	−14.7	87.49	89.97	2.8
Pair 2	432	353	−18.3	83.85	71.78	−14.4
Pair 3	442	337	−23.8	115.13	95.99	−16.7
Pair 4	218	171	−21.6	95.16	95.35	0.20
Pair 5	227	208	−8.4	83.46	70.69	−15.4
Mean ± SEM	314 ± 51	257 ± 37	−17 ± 3	93 ± 6	85 ± 6	−9 ± 4
	$P = 0.02$ (paired t-test)	$P = 0.05$ (Wilcoxon signed-rank)		NS (paired t-test)	NS (Wilcoxon signed-rank)	
POMC						
Pair 1	1652	2008	21.5	78.79	107.35	36.2
Pair 2	1354	1560	15.2	78.34	104.77	33.7
Pair 3	1494	1904	27.4	83.58	101.40	21.3
Pair 4	1898	2202	16.0	78.23	85.20	8.9
Pair 5	2025	1656	−28.3	84.37	95.04	12.6
Pair 6	1540	1576	2.3	76.58	80.32	4.9
Mean ± SEM	1661 ± 104	1818 ± 107	9 ± 8	80 ± 1	96 ± 5	20 ± 5
Mean ± SEM (pair 5 excluded)	1588 ± 91	1850 ± 125	17 ± 4			
All 6 pairs	NS (paired t-test)	$P = 0.025$ (Wilcoxon signed-rank)		$P = 0.014$ (paired t-test)	$P = 0.025$ (Wilcoxon signed-rank)	
Pair 5 excl.	$P = 0.016$ (paired t-test)	$P = 0.05$ (Wilcoxon signed-rank)				

NS = not significant.

The data were collected from 5 pairs of experimentally-matched animals processed by *in situ* hybridization histochemistry for LHRH mRNA and 6 pairs of experimentally-matched animals processed by *in situ* hybridization histochemistry for POMC mRNA.

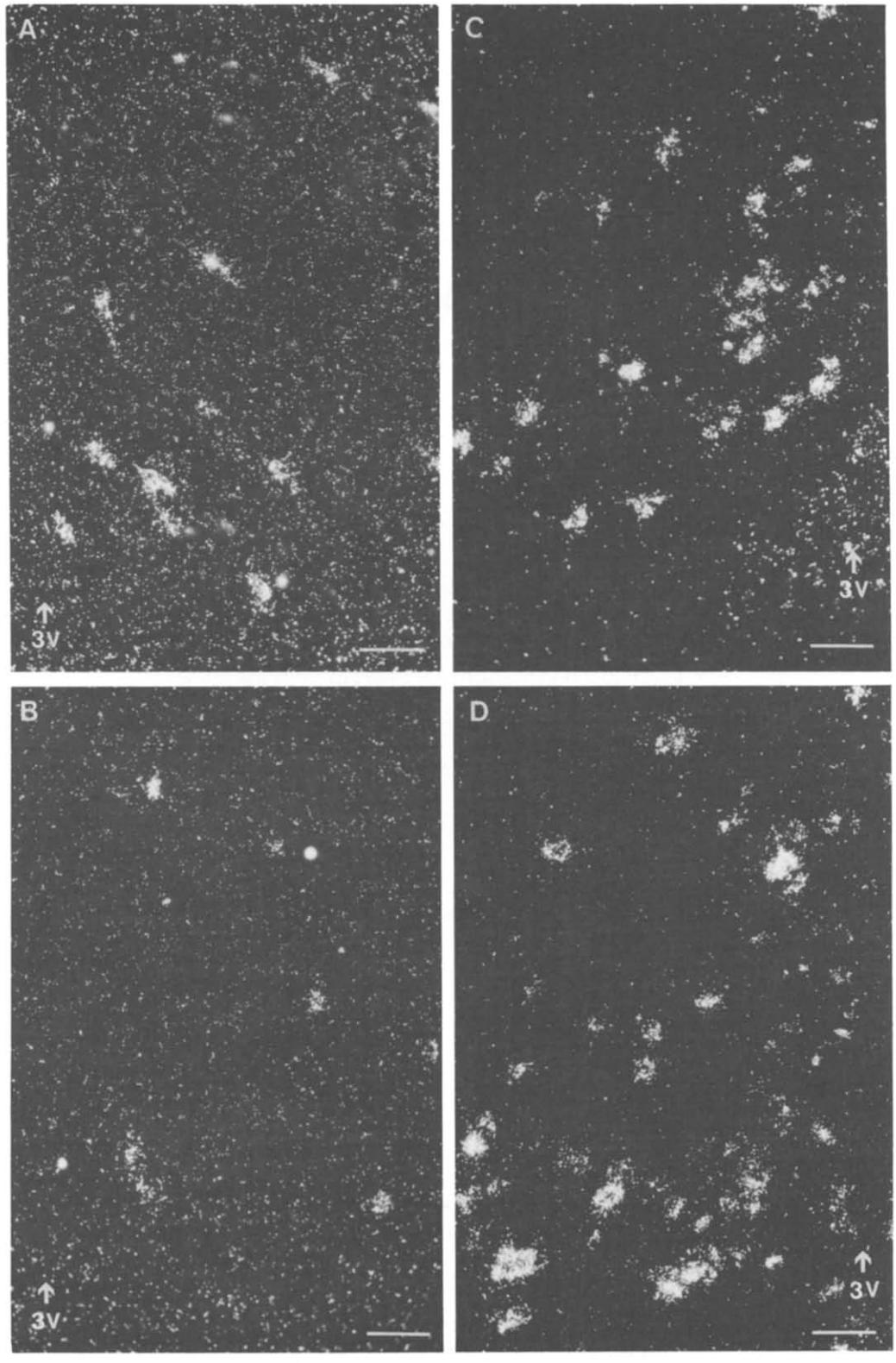

Fig. 1. *In situ* hybridization histochemistry showing autoradiographs of LHRH mRNA expressing neuronal cell bodies in the preoptic area of a matched pair of rats treated with either saline (A) or prazosin (B) and POMC mRNA expressing cell bodies in the rostral arcuate nucleus from a matched pair of rats treated with either saline (C) or prazosin (D). The sections are coronal and the third cerebral ventricle (3V) is shown for orientation. The calibration bar = 50 μm.

saline-treated rat in this pair. Means are, therefore, given with and without pair #5 included; non-parametric statistical analysis shows that in either case the cell counts were significantly greater in prazosin- compared with saline-treated rats, and paired *t*-test shows a high level of significance when pair #5 is exluded. In order to avoid a type 2 statistical error we infer, therefore, that prazosin treatment significantly increased the number of POMC mRNA cells in the rostral arcuate nucleus. The silver grain counts in POMC mRNA expressing cells were also significantly increased in the prazosin- compared with the saline-treated animals (Table 1; Fig. 1). This point is also illustrated by Fig. 2 which shows that the silver grain counts of POMC mRNA expressing cells of a prazosin-treated animal showed a shift to the right compared with those in a matched saline-treated control animal. In contrast, there was no difference in the distribution of silver grain counts of LHRH mRNA expressing cells in a prazosin-treated compared with a matched, saline-treated animal (Fig. 2). Similar data were obtained for the other pairs of animals.

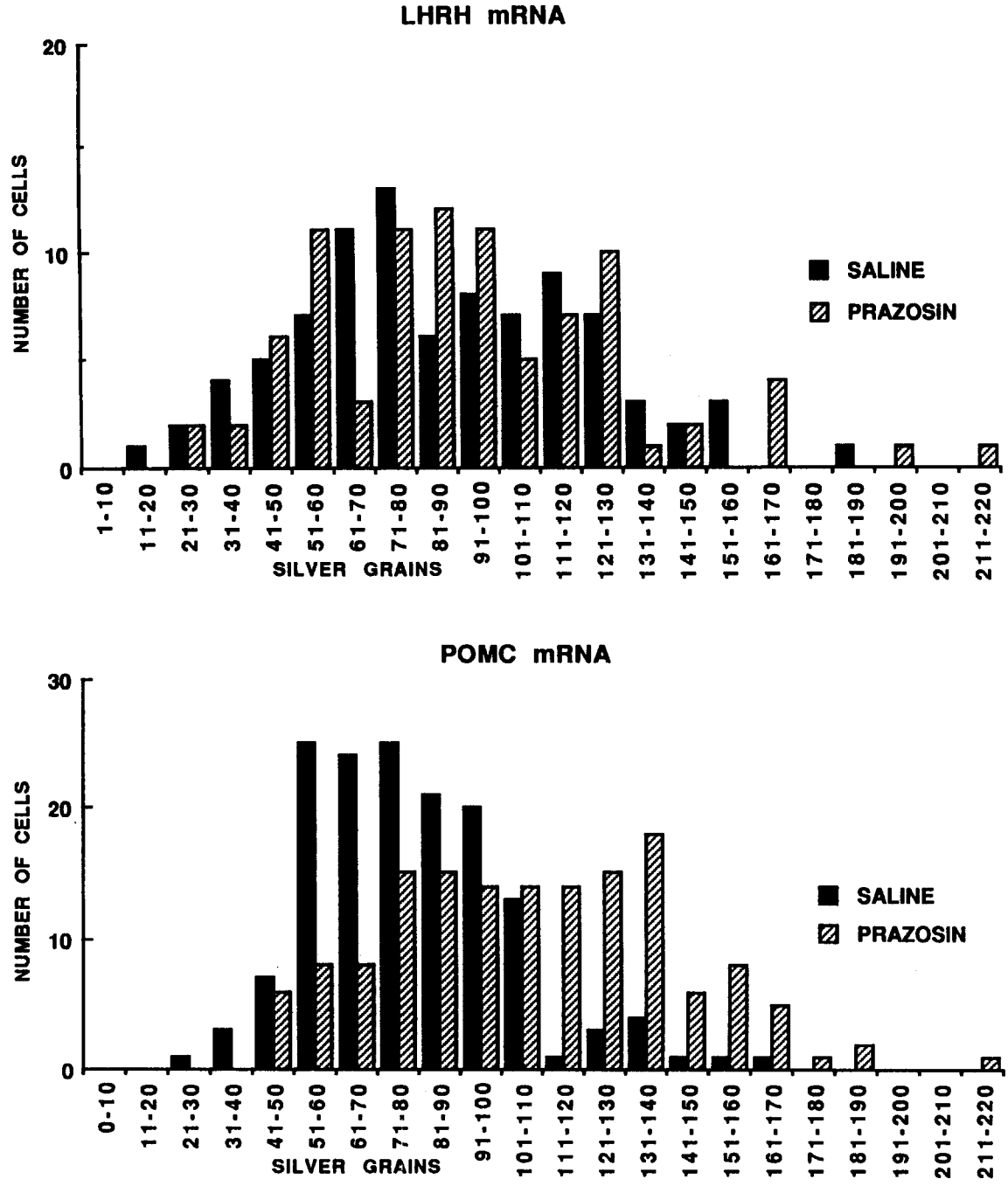

Fig. 2. Frequency distribution histogram of silver grain counts of LHRH mRNA expressing cells from a pair of experimentally matched animals and for comparison a distribution histogram of silver grain counts of POMC mRNA expressing cells from a matched pair of animals.

DISCUSSION

These results show for the first time that α_1 blockade with prazosin increases the number of POMC mRNA expressing cells and the concentrations of POMC mRNA per cell in the rostral arcuate nucleus. The results also confirm our preliminary observations [20] that α_1 adrenergic blockade with prazosin significantly reduces the number of hypothalamic cells which express LHRH mRNA in immature rats treated with PMSG. Our findings on LHRH mRNA are similar to those in ovariectomized adult rats treated with estrogen [18] with the exception that in the latter (i) the percentage reduction in the number of cells was greater than in the present study and (ii) Weesner *et al.* [18, 27] also found that α_1 blockade significantly decreased the concentration of LHRH mRNA as assessed by grain counts/cell. The reason for this difference may be that in the study by Weesner *et al* [18, 27], in which prazosin was administered by hypothalamic implant, hypothalamic neurons may have been exposed to concentrations of prazosin which were higher than those in the present study, in which prazosin was administered i.p. However, although our LH data must be interpreted cautiously for reasons outlined in the Results (they are based on measurements in single samples taken from animals anaesthetized with sodium pentobarbitone) they do show that prazosin attenuated the PMSG-induced LH surge. Lower dosages of prazosin than those used here, also administered systemically, blocked the pulsatile LHRH release in the rhesus monkey [28] and surge, but not basal, LH release in conscious adult female rats [19].

It is, of course, possible that the effects of α_1 adrenergic blockade on LHRH mRNA and POMC mRNA expressing cells are functionally unrelated. However, these findings provide further support for the view that endogenous opioids play a key role in controlling the biosynthesis and release of LHRH [3, 6, 29] and that several different pathways, possibly multisynaptic, may be involved in E_2 stimulation of LHRH biosynthesis and release [3, 29]. That is, E_2 stimulation of LHRH mRNA biosynthesis may involve (i) α_1 adrenergic-dependent stimulation of LHRH neurons and/or (ii) disinhibition of LHRH neurons consequent on α_1 adrenergic-dependent inhibition of POMC neurons which, in the absence of high E_2 levels, normally inhibit LHRH biosynthesis and release. In addition to the α_1 adrenoreceptor mechanisms outlined above, we have recently shown that a 5-HT_2 receptor mechanism also plays a crucial role in stimulating LH, and presumably LHRH release [19]. The results of these pharmacological and *in situ* hybridization studies are supported by immunocytochemical data. Thus, for example, (i) there is a dense concentration of α_1 adrenergic receptors in the medial hypothalamus [30] and (ii) double immunostaining for LHRH and either dopamine-β-hydroxylase (DBH) (for adrenergic neurons) or 5-HT showed that there is a close proximity between LHRH cell bodies and DBH and 5-HT-containing nerve terminals and between LHRH and 5-HT nerve fibres [14]. Ultrastructural and lesion studies suggest that LHRH neurons are innervated by POMC and dopaminergic, but not noradrenergic, neurons [4, 15]. Noradrenergic neurons could exert their effects on LHRH neurons by GABA in addition to POMC neurons. Glutamic acid decarboxylase (GAD) containing neurons (presumably GABA neurons) receive projections from putative noradrenergic fibres and could by way of their terminals on LHRH neurons mediate the action of noradrenergic neurons [15]. Data obtained from push–pull cannula [31] or intracerebral infusion [32] techniques suggest that E_2-induced inhibition of GABA release may result in disinhibition of LHRH neurons. Whether this is due to a direct action of E_2 on GABA neurons or E_2-induced noradrenergic inhibition of GABA neurons [15], or both, remains to be determined.

The inferences drawn from the *in situ* hybridization studies assume that changes in peptide mRNA levels are always reflected by changes, in the same direction, in peptide release. This is certainly the case for LHRH [1] and also seems likely for POMC/β-endorphin. As assessed by the measurement of β-endorphin release into hypophysial portal vessel blood, which presumably reflects β-endorphin release from nerve terminals in the median eminence, the data of Sarkar and Yen [33] suggest that, in the rat, LHRH and β-endorphin release are inversely related, consistent with the notion that decreased β-endorphin release permits the surge release of LHRH. But, this finding was not confirmed by other studies in the rat [34] or rhesus monkey [35, 36], although our studies in the rat did show a decline in β-endorphin release during the afternoon of proestrus [34]. The latter is correlated with a significant decrease in the tissue concentrations of β-endorphin in the MPOA and arcuate nucleus [37] and suggests that "overflow" of peptide (β-endorphin) into hypophysial portal vessel blood does reflect the pattern of β-endorphin release at synapses on LHRH cell bodies or at axo–axonal contacts in the median eminence.

The present studies do not, of course, exclude the involvement of other neurotransmitter systems in the E_2 stimulation of LHRH release. Galanin, for example, has been implicated because it is co-localized with LHRH [38] and its concentration in the median eminence and anterior pituitary gland is significantly increased by PMSG-induced E_2 secretion [39]. However, prazosin, while reducing the E_2-stimulated increase in LHRH mRNA, had no effect on galanin mRNA concentrations [27].

In summary our data show importantly that, in the prepubertal female rat, an α_1 adrenoreceptor mechanism is involved in mediating the E_2 inhibition of POMC mRNA synthesis as well as in the E_2 stimulation of

LHRH mRNA synthesis. Taken together with data in the adult rat, the reduced concentrations of POMC mRNA may result in reduced β-endorphin release at synapses on LHRH cell bodies or nerve terminals resulting in the disinhibition of LHRH neurons. This is one of several mechanisms by which E_2, in its positive feedback mode, could stimulate LHRH mRNA synthesis and LHRH release.

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REFERENCES

1. Rosie R., Thomson E. and Fink G.: Oestrogen positive feedback stimulates the synthesis of LHRH mRNA in neurons of the rostral diencephalon of the rat. *J. Endocr.* **124** (1990) 285–289.
2. Shivers B. D., Harlan R. E., Morrell J. E. and Pfaff D. W.: Absence of oestradiol concentration in cell nuclei of LHRH-immunoreactive neurones. *Nature* **304** (1983) 345–347.
3. Fink G.: The G W Harris Lecture. Steroid control of brain and pituitary function. *Q. J. Exp. Physiol.* **73** (1988) 257–293.
4. Leranth C., MacLusky N. J., Shanabrough M. and Naftolin F.: Immunohistochemical evidence for synaptic connections between pro-opiomelanocortin-immunoreactive axons and LHRH neurons in the preoptic area of the rat. *Brain Res.* **449** (1988) 167–176.
5. Chen W.-P., Witkin J. W. and Silverman A.-J.: Beta-endorphin and gonadotropin-releasing hormone synaptic input to gonadotropin-releasing hormone neurosecretory cells in the male rat. *J. Comp. Neurol.* **286** (1989) 85–95.
6. Rosie R., Thomson E., Blum M., Roberts J. L. and Fink G.: Oestrogen positive feedback reduces arcuate proopiomelanocortin messenger ribonucleic acid. *J. Neuroendocr.* **4** (1992) 625–630.
7. Roberts J. L., Wilcox J. N. and Blum M.: The regulation of proopiomelanocortin gene expression by estrogen in the rat hypothalamus. In *Neuroendocrine Molecular Biology* (Edited by G. Fink, A. J. Harmar and K. W. McKerns). Plenum Press, New York (1986) pp. 261–270.
8. Sawyer C., Markee J. and Everett J.: Further experiments on blocking pituitary activity in the rabbit and rat. *J. Exp. Zool.* **113** (1950) 659–682.
9. Sarkar D. K. and Fink G.: Gonadotropin-releasing hormone surge: possible modulation through postsynaptic α-adrenoreceptors and two pharmacologically distinct dopamine receptors. *Endocrinology* **108** (1981) 862–867.
10. Weiner I., Findell P. R. and Kordon C.: Role of classic and peptide neuromediators in the neuroendocrine regulation of LH and prolactin. In *The Physiology of Reproduction* (Edited by E. Knobil, J. Neill *et al.*). Raven Press, New York (1988) pp. 1235–1281.
11. Sar M. and Stumpf W. E.: Central noradrenergic neurons concentrate estradiol-H-3. *Nature* **289** (1981) 500–502.
12. Hoffman G. E.: Relationship of catecholamines and LHRH—light microscopic study. *Brain Res. Bull.* **9** (1982) 417–430.
13. Hoffman G. E.: Organization of LHRH cells—differential apposition of neurotensin, substance P and catecholamine axons. *Peptides* **6** (1985) 439–461.
14. Jennes L., Beckman W. C., Stumpf W. E. and Grzanna R.: Anatomical relationships of serotoninergic and noradrenalinergic projections with the GnRH system in septum and hypothalamus. *Exp. Brain Res.* **46** (1982) 331–338.
15. Leranth C., MacLusky N. J., Shanabrough M. and Naftolin F.: Catecholaminergic innervation of luteinizing hormone releasing hormone and glutamic acid decarboxylase immunopositive neurons in the rat medial preoptic area—an electron microscopic double immunostaining and degeneration study. *Neuroendocrinology* **48** (1988) 591–602.
16. Sarkar D. K. and Fink G.: Mechanism of the first spontaneous gonadotrophin surge and that induced by pregnant mare serum and effects of neonatal androgen in rats. *J. Endocr.* **83** (1979) 339–354.
17. Coen C. W. and Coombs M. C.: Effects of manipulating catecholamines on the incidence of the preovulatory surge of luteinizing hormone and ovulation in the rat: evidence for a necessary involvement of hypothalamic adrenaline in the normal or "midnight" surge. *Neuroscience* **10** (1983) 187–206.
18. Weesner G. D., Krey L. C. and Pfaff D. W.: α_1 Adrenergic regulation of estrogen-induced increases in luteinizing hormone releasing hormone messenger RNA levels and release. *Molec. Brain Res.* **17** (1993) 77–82.
19. Dow R. C., Williams B. C., Bennie J., Carroll S. and Fink G.: A central 5-HT_2 receptor mechanism plays a key role in the proestrous surge of luteinizing hormone in the rat. *Psychoneuroendocrinology* **19** (1994) in press.
20. Rosie R., Thomson E. and Fink G.: An alpha one noradrenergic mechanism mediates oestrogen stimulation of luteinizing hormone releasing hormone mRNA synthesis. *J. Endocr. Invest.* **14** (1991) 175.
21. Horn A. M. and Fink G.: Effects of 5-hydroxytryptamine uptake blockers on the release of LH and prolactin in several different experimental steroid models in the rat. *J. Endocr.* **104** (1985) 397–406.
22. Pellegrino L. J., Pellegrino A. S. and Cushman A. J.: *A Stereotaxic Atlas of the Rat Brain.* Plenum Press, New York (1979).
23. Seeburg P. H. and Adelman J. P.: Characterization of cDNA for precursor of human luteinizing hormone releasing hormone. *Nature* **311** (1984) 666–668.
24. Maxam A. M. and Gilbert W.: A new method for sequencing DNA. *Proc. Natn. Acad. Sci. U.S.A.* **74** (1977) 560–564.
25. Rosie R., Wilson H. and Fink G.: Testosterone induces an all-or-none, exponential increase in arginine vasopressin mRNA in the bed nucleus of stria terminalis of the hypogonadal mouse. *Molec. Cell. Neurosci.* **4** (1993) 121–126.
26. Brown-Grant K. and Greig F.: A comparison of changes in the peripheral plasma concentrations of luteinizing hormone and follicle stimulating hormone in the rat. *J. Endocr.* **65** (1975) 389–397.
27. Weesner G. D., Bergen H. T. and Pfaff D. W.: Differential regulation of luteinizing hormone-releasing hormone and galanin messenger ribonucleic acid levels by alpha$_1$-adrenergic agents in the ovariectomized rat. *J. Neuroendocr.* **4** (1992) 331–336.
28. Gearing M. and Terasawa E: The alpha$_1$-adrenergic neuronal system is involved in the pulsatile release of luteinizing hormone-releasing hormone in the ovariectomized female rhesus monkey. *Neuroendocrinology* **53** (1991) 373–381.
29. Kalra S and Kalra P.: Opioid–adrenergic–steroid connection in regulation of luteinizing hormone secretion in the rat. *Prog. Neuroendocr.* **38** (1984) 418–426.
30. Young W. S. I. and Kuhar M. J.: Noradrenergic α_1 and α_2 receptors: light microscopic autoradiographic localization. *Proc. Natn. Acad. Sci. U.S.A.* **77** (1980) 1696–1700.
31. Jarry H., Perschl A. and Wuttke W.: Further evidence that preoptic anterior hypothalamic GABAergic neurons are part of the GnRH pulse and surge generator. *Acta Endocr.* **118** (1988) 573–579.
32. Herbison A E. and Dyer R. G.: Effect on luteinizing hormone secretion of GABA receptor modulation in the medial preoptic area at the time of proestrous luteinizing hormone surge. *Neuroendocrinology* **53** (1991) 317–320.
33. Sarkar D. K. and Yen S. S. C.: Changes in β-endorphin-like immunoreactivity in pituitary portal blood during the estrous cycle and after ovariectomy in rats. *Endocrinology* **116** (1985) 2075–2079.
34. Seward W. J., Lim A., Alder B., Copolov D., Dow R. C. and Fink G.: Hypothalamic release of atrial natriuretic factor and β-endorphin into rat hypophysial portal plasma: relationship to oestrous cycle and effects of hypophysectomy. *J. Endocr.* **131** (1991) 113–125.

35. Wardlaw S. L., Wehrenberg W. B., Ferin M., Antunes J. L. and Frantz A. G.: Effect of sex steroids on β-endorphin in hypophyseal portal blood. *J. Clin. Endocr. Metab.* 55 (1982) 877–881.
36. Wehrenberg W. B., Wardlaw S. L., Frantz A. G. and Ferin M.: β-Endorphin in hypophyseal portal blood: variations throughout the menstrual cycle. *Endocrinology* **111** (1982) 879–881.
37. Kerdelhué B., Parnet P., Lenoir V., Schirar A., Gaudoux F., Levasseur M.-C., Palkovits M., Blacker C. and Scholler R.: Interactions between 17β-estradiol and the hypothalamo–pituitary β-endorphin system in the regulation of the cyclic LH secretion. *J. Steroid Biochem. Molec. Biol.* 30 (1988) 161–168.
38. Merchenthaler I., Lopez F. J. and Negro-Vilar A.: Colocalization of galanin and luteinizing hormone-releasing hormone in a subset of preoptic hypothalamic neurons: anatomical and functional correlates. *Proc. Natn. Acad. Sci. U.S.A.* 87 (1990) 6326–6330.
39. Gabriel S. M., Koenig J. I. and Kaplan L. M.: Galanin-like immunoreactivity is influenced by estrogen in peripubertal and adult rats. *Neuroendocrinology* **51** (1990) 168–173.