Differential effects of adrenalectomy on the prolactin-induced suppression of LH and FSH secretion after castration in male rats

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Hyperprolactinaemia inhibits gonadotrophin secretion in males and females of many species. The aim of this study was to determine the role of the adrenal gland in mediating the inhibitory effects of prolactin by contrasting the effects of acute hyperprolactinaemia on LH and FSH secretion in adrenal-intact and adrenalectomized rats with and without physiological corticosterone replacement. Adult male rats were administered purified ovine prolactin every 12 h (2.4 mg per injection s.c.) beginning at the time of castration. Blood samples were collected every 3 h for 36 h, then every 12 h until 10 days after castration. Ovine prolactin significantly reduced LH secretion in all groups from approximately 15 to 48 h after castration. In contrast, plasma FSH concentrations were reduced by ovine prolactin from 21 to 48 h only in the adrenal-intact rats and not in the adrenalectomized or adrenalectomized plus corticosterone groups. In all groups, ovine prolactin inhibited endogenous prolactin secretion in rats by short-loop autocrine feedback as soon as 3 h after the first ovine prolactin injection and throughout the 10 days of the study. Adrenalectomy *per se*, with or without corticosterone replacement, also had a differential effect on LH and FSH secretion after castration, causing only a transient delay in the rise in LH after castration, but inducing a significant and long-lasting inhibition of FSH secretion. The results demonstrate that ovine prolactin-induced suppression of LH secretion after castration occurs with or without the adrenal glands. Suppression of FSH secretion after castration by ovine prolactin, however, may involve an adrenal component. The rise in FSH after castration, but not of LH, requires the involvement of stimulatory factors from the adrenal gland. The adrenal gland, therefore, appears to have a differential role in the control of LH and FSH secretion following castration and also in mediating the inhibitory effect of hyperprolactinaemia on gonadotrophin secretion. In contrast to the transient suppression of LH and FSH by acutely administered ovine prolactin, endogenous prolactin secretion in rats is continuously suppressed by exogenously induced hyperprolactinaemia.

Introduction

Hyperprolactinaemia has been shown to impair reproductive function in males and females of many species, largely by inhibition of pituitary secretion of LH and FSH (Evans *et al.*, 1982). It is likely that this anti-gonadotrophic action of prolactin is mediated by a direct effect on the hypothalamic mechanisms controlling the secretion of LHRH, as most (Weber *et al.*, 1983; Koike *et al.*, 1984, 1991; Sarkar and Yen, 1985; Voogt *et al.*, 1987) but not all (Brar *et al.*, 1985) studies examining LHRH concentrations in the pituitary portal blood have demonstrated significant suppression of LHRH release by hyperprolactinaemia. High prolactin concentrations are reported to decrease the amount of LH released by electrical stimulation of the hypothalamus (Smith and Bartke, 1987) and to reduce both the frequency and amplitude of LH pulses (Cohen-Becker *et al.*,

1986; Fox *et al.*, 1987; Larsen and Odell, 1987), findings which suggest a central site of action. In addition, there is evidence that prolactin may have some action at the level of the pituitary (Cheung, 1983; Garcia *et al.*, 1985; Fox *et al.*, 1987) or the gonad (Larsen *et al.*, 1990) to further inhibit gonadotrophin secretion. It has also been suggested that at least part of the inhibitory effects of prolactin on gonadotrophin secretion might be mediated by the adrenal gland, which contains a high concentration of prolactin receptors (Posner *et al.*, 1974; Frantz *et al.*, 1974; Davies *et al.*, 1980; Dube *et al.*, 1980). Hyperprolactinaemia is associated with a significant increase in adrenal weight (Vasquez and Kitay, 1978; Lamberts *et al.*, 1981; McMurtry and Wexler, 1981; Drago and Continella, 1985; Weber *et al.*, 1987) and plasma concentrations of corticosterone (Witorsch and Kitay, 1972; Svare *et al.*, 1979; Eldridge and Lymangrover, 1984; Drago and Continella, 1985; Drago *et al.*, 1985; Weber *et al.*, 1987), dehydroepiandrosterone (DHEA) (Higuchi *et al.*, 1984), DHEA-sulfate (Higuchi *et al.*, 1984), progesterone (Eldridge and Lymangrover, 1984; Piva *et al.*, 1973), aldosterone

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(Eldridge and Lymangrover, 1984) and androstendione (Eldridge and Lymangrover, 1984) are all reported to be increased by high prolactin. Although some studies, including our own (Park and Selmanson, 1991), have demonstrated the anti-gonadotrophic effect of prolactin in the absence of the adrenal gland (McNeilly *et al.*, 1980; Hodson *et al.*, 1980; Voogt *et al.*, 1987; Weber *et al.*, 1987; Kooy *et al.*, 1989), others have reported that adrenalectomy reduces (Greeley and Kizer, 1979) or abolishes (Weber *et al.*, 1982, 1983) the inhibitory effects of prolactin on gonadotrophin secretion.

Thus, the role of the adrenal gland in mediating the prolactin-induced suppression of gonadotrophin secretion after castration remains uncertain. In an attempt to clarify this problem, we contrasted inhibitory effects of acute hyperprolactinaemia on the rise of both LH and FSH after castration in adrenal-intact male rats and in adrenalectomized male rats, with or without replacement of physiological concentrations of corticosterone. In addition, because the inhibitory effect of moderate hyperprolactinaemia on the gonadotrophin rise after castration is thought to be transient (Beck and Wuttke, 1977; Beck *et al.*, 1977; Carter *et al.*, 1983; Smith and Bartke, 1987; Park and Selmanson, 1991) as opposed to the irreversible suppression of gonadotrophins caused by tumour concentrations of prolactin (Hodson *et al.*, 1980; Login *et al.*, 1982; Voogt *et al.*, 1987; Kooy *et al.*, 1989), we further examined the duration of the inhibitory effect of prolactin on LH and FSH secretion in this acutely hyperprolactinaemic model. Finally, we compared the short-loop autofeedback inhibition of endogenous prolactin secretion by acute hyperprolactinaemia with the antigonadotrophic effects, to examine the possibility that a common mechanism may regulate both inhibitory effects.

Materials and Methods

Male, Sprague-Dawley rats, weighing 250–300 g, were purchased from Zivic-Miller Laboratories (Allison Park, PA), and maintained under a 14 h light:10 h dark schedule with free access to food and water. Between 09:00 and 11:00 h on experimental day 0, rats were castrated under ketamine (Parke Davis, Morris Plains, NJ)/acepromazine (PromAce, Aveco Co. Inc., Ford Dodge, IA) anaesthesia (113.2 mg ketamine kg⁻¹; 0.68 mg acepromazine kg⁻¹), and implanted with an indwelling right atrial cannula as described by Selmanson and Wise (1981). Some animals were also bilaterally adrenalectomized during the same operation. Half of the adrenalectomized animals received replacement of corticosterone by means of a s.c. implanted 50% corticosterone pellet, which provides physiological mean corticosterone concentrations of approximately 60 ng ml⁻¹, as described by Park and Selmanson (1991). All adrenalectomized animals were maintained on 0.9% saline. Hyperprolactinaemia was induced by s.c. injection of 2.4 mg purified ovine prolactin (NIDDK-oPRL-19; biopotency 31 iu mg⁻¹) in a 50% polyvinylpyrrolidone (PVP):50% NaHCO₃ buffer (0.01 mol l⁻¹, pH 8.6) every 12 h beginning at the time of castration, while control rats received an injection of the PVP diluent only. Castrated rats were assigned to six different experimental groups: (i) adrenal intact, PVP-treated (*n* = 11), (ii) adrenal intact, ovine prolactin treated (*n* = 10), (iii) adrenalectomized, PVP-treated (*n* = 12), (iv) adrenalectomized, ovine prolactin-treated

(*n* = 9), (v) adrenalectomized plus corticosterone, PVP-treated (*n* = 11), and (vi), adrenalectomized plus corticosterone, ovine prolactin-treated (*n* = 13).

Blood samples were collected from all groups by means of the previously implanted atrial cannulae at the time of surgery (time 0) and every 3 h for 36 h, and an additional sample was collected 48 h after castration. Samples taken during the dark period of the light:dark cycle were collected under red light illumination. In the adrenal intact and adrenalectomized plus corticosterone groups sample collection was continued after 48 h; a sample was taken every 12 h until 10 days after castration, to examine the effects of hyperprolactinaemia on LH and FSH secretion over a longer period. Only those animals from which all samples could be taken throughout the first 8 days of the experiment were used for statistical analysis of samples between 60 and 192 h after castration. This reduced the number of animals to six PVP- and nine ovine prolactin-treated rats in the adrenal intact group, and eight PVP- and eight ovine prolactin-treated rats in the adrenalectomized plus corticosterone group for this part of the experiment. On the sixth day after castration, additional samples were collected from the ovine prolactin-treated rats at intervals of 3 h, to compare the pattern of plasma ovine prolactin concentrations following the twelfth ovine prolactin injection with that observed from 24 to 36 h after castration. Animal protocols were approved by the University of Maryland, School of Medicine IACUC.

Blood samples were immediately centrifuged at 800 *g* for 2 min and the plasma stored at -20°C. The red blood cells were resuspended in 0.9% saline and stored at 4°C until they were reinjected into the same animal following collection of a later sample. Plasma concentrations of LH, FSH, rat prolactin and ovine prolactin were measured in all samples by double-antibody radioimmunoassay, using reagents provided by the NIDDK National Hormone and Pituitary Program. Details of the assay methods for LH are given by Park and Selmanson (1991), for rat prolactin by Selmanson and Selmanson (1983) and for ovine prolactin by Selmanson and Gregerson (1984). Reference preparations rLH-RP-3, rPRL-RP-3 and oPRL-I-1 were used for these three assays, respectively, providing a sensitivity defined as 15% displacement (i.e. 85% binding) of 0.024 ng per tube for LH, 0.04 ng per tube for rat prolactin and 0.025 ng per tube for ovine prolactin. All samples were measured in a single assay for each hormone, with an intra-assay coefficient of variation in the linear portion of the standard curve (15–85% binding) of 5.6% for LH, 5.4% for rat prolactin and 2.2% for ovine prolactin. For FSH the method outlined by the NIDDK was followed. Plasma samples (25–50 µl aliquots) were added to the rabbit anti-rat FSH primary antibody (anti-rFSH-S-11, 1:125 000 final tube dilution) in a volume of 350 µl. The tubes were incubated for 2 days at 4°C, and then 50 µl of ¹²⁵I-FSH (about 10 000 c.p.m. per tube) was added. After a further incubation for 2 days at 4°C, 100 µl goat anti-rabbit immunoglobulin antiserum (1:75 dilution) in 0.05 mol EDTA l⁻¹ was added to precipitate the bound FSH. The tubes were incubated for 3 days at 4°C, and bound FSH was then separated from free by centrifugation. These conditions gave 22–25% total binding, and nonspecific binding was less than 1% of the total radioactivity measured. The reference preparation was rFSH-RP-2, which is 45 times more potent than rFSH-RP-1. Calculating the least detectable dose as 15% displacement, the

Fig. 1. Profile of plasma ovine prolactin (OPRL) concentrations following s.c. injection of 2.4 mg ovine prolactin every 12 h ($n = 32$). $\bigcirc$ = Undetectable.

sensitivity of the assay was 0.08 ng per tube. All samples were measured in a single assay, and the intra-assay coefficient of variation was 4.6%.

Data were compared by two-factor analyses of variance with repeated measures on one factor (time), followed by the Newman-Keuls multiple range test for *post hoc* comparisons between means. Data are expressed as means $\pm$ SEM, except where the standard error bar lies within the symbol. All statements of statistical significance refer to a probability level of less than 5% (i.e. $P < 0.05$).

Results

Ovine prolactin-induced hyperprolactinaemia

Ovine prolactin was readily detectable in the plasma by 3 h after the first subcutaneous injection and concentrations continued to rise throughout the first 12 h (Fig. 1). After the second injection 12 h after castration, plasma ovine prolactin concentrations rose further to a new peak 3–6 h later (15–18 h after castration), then fell to a plateau about 600–700 ng ml⁻¹ immediately before the next injection. Prolactin was maintained at least at this value throughout the experiment by continued twice daily injections (Fig. 1). Rats treated with 4 mg ovine prolactin day⁻¹ have been reported to exhibit decreased ovine prolactin clearance rates after 9 days of exposure to ovine prolactin (Diamond *et al.*, 1980). More frequent collection of blood samples (every 3 h) following the injections at 24 h and again at 144 h after castration demonstrated that injection of ovine prolactin resulted in a peak of ovine prolactin in the plasma of approximately 1500 ng ml⁻¹ over the high baseline concentrations of 600–700 ng ml⁻¹. The pattern of increase and then clearance of ovine prolactin following subcutaneous injection did not change over at least the first six days of the experiment

Fig. 2. Effect of ovine prolactin on the increase in LH secretion after castration. Note the differences in scale on the ordinate axes. (●) Vehicle-treated control rats; (○) ovine prolactin-treated rats. (a) In adrenal-intact rats, LH concentrations were significantly suppressed by ovine prolactin at 18, 21, 24, 30, 33 and 48 h ($P < 0.05$); $n = 11$ controls; 10 ovine prolactin-treated rats. (b) In adrenalectomized rats, LH concentrations were significantly suppressed by ovine prolactin at 6, 15, 18, 21, 27 and 30 h ($P < 0.05$); $n = 12$ controls; nine ovine prolactin-treated rats. (c) In adrenalectomized plus corticosterone rats, LH concentrations were significantly suppressed by ovine prolactin at 6, 12, 18, 21, 24, 27, 33, 36 and 48 h ($P < 0.05$); $n = 11$ controls; 13 ovine prolactin-treated rats.

(Fig. 1), and the high baseline ovine prolactin was maintained for the entire 10 days of the experiment (data not shown).

Effect of ovine prolactin on LH secretion after castration

After castration, plasma LH concentrations rose rapidly in all groups (Fig. 2). This effect was most marked in the adrenal intact, PVP-treated group, in which LH increased from basal values of 0.3 ng ml⁻¹ to a peak of approximately 3 ng ml⁻¹ by 24 h (Fig. 2a). Ovine prolactin treatment delayed and reduced the rate of this castration-induced LH rise ($P < 0.05$). Mean plasma LH concentrations were significantly less than PVP-treated controls at most timepoints from 18 to 60 h after castration (Fig. 2a; Table 1). By 72 h after castration, the suppression of LH by ovine prolactin was no longer evident

Table 1. Long-term effects of ovine prolactin treatment on LH secretion after castration in male rats

Time after castration (h)	Adrenal intact		Adrenalectomy plus corticosterone	
	Vehicle ^a	Ovine prolactin	Vehicle ^a	Ovine prolactin
60	2.9 ± 0.1	2.4 ± 0.1*	2.5 ± 0.3	2.3 ± 0.2
72	2.1 ± 0.2	2.3 ± 0.2	2.3 ± 0.3	2.0 ± 0.2
84	2.0 ± 0.2	2.3 ± 0.3	2.0 ± 0.2	2.5 ± 0.1
96	2.3 ± 0.1	2.2 ± 0.3	2.3 ± 0.3	1.8 ± 0.0
108	2.2 ± 0.2	2.2 ± 0.1	2.3 ± 0.2	1.9 ± 0.1
120	2.0 ± 0.2	2.1 ± 0.3	1.6 ± 0.2	1.7 ± 0.2
132	3.2 ± 0.5	2.4 ± 0.3	1.9 ± 0.3	1.8 ± 0.2
144	2.0 ± 0.2	2.0 ± 0.3	1.8 ± 0.2	1.5 ± 0.2
156	2.8 ± 0.5	2.3 ± 0.4	2.4 ± 0.7	1.5 ± 0.3
168	2.7 ± 0.6	1.9 ± 0.6	2.0 ± 0.5	1.5 ± 0.3
180	3.4 ± 1.1	1.0 ± 0.1*	3.1 ± 0.7	1.3 ± 0.7
192	3.1 ± 0.9	1.5 ± 0.4*	2.5 ± 0.4	1.4 ± 0.9

*Significantly different from PVP-treated control rats ($P < 0.05$).

^a50% polyvinylpyrrolidone: 50% NaHCO₃ buffer (0.01 mol l⁻¹, pH 8.6).

(Table 1). Analysis of variance of the repeated hormone measurements from 60 to 192 h after castration, however, revealed that the LH concentrations in the ovine prolactin-treated group were still significantly lower than the PVP-treated controls throughout this period ($P < 0.05$), even though only a few of the differences between individual pairs of means attained statistical significance (Table 1). Adrenalectomy, with or without corticosterone replacement, did not prevent the prolactin-induced inhibition of LH secretion in the first 48 h after castration (Fig. 2b, c). Ovine prolactin treatment significantly suppressed LH secretion compared with the PVP-treated controls at most timepoints from 15 to 30 h after castration in the adrenalectomized rats and from 12 to 48 h after castration in adrenalectomized plus corticosterone treated rats. As in adrenal intact rats, the prolactin-induced suppression of LH secretion after castration had diminished by 60–72 h after castration. LH concentrations from 60 to 192 h after castration in ovine prolactin-treated adrenalectomized plus corticosterone rats tended to be lower than the PVP-treated controls throughout the 8 day experimental period (Table 1), but this difference did not attain statistical significance ($F = 4.46$; $df = 1/11$; $P = 0.0518$).

Effect of ovine prolactin on FSH secretion after castration

In adrenal intact rats, plasma FSH increased from basal concentrations between 5 and 10 ng ml⁻¹ to a peak of approximately 25 ng ml⁻¹ by 24 h after castration (Fig. 3a). This high FSH concentration was then maintained throughout the 8 days of the experiment (Table 2). Ovine prolactin treatment delayed this castration-induced FSH rise ($P < 0.05$), and mean plasma FSH concentrations were significantly lower than PVP-treated controls at most timepoints from 6 to 48 h after castration. By 60 h after castration, the prolactin-induced suppression of FSH was no longer evident, and from 60 to 192 h after castration

plasma concentrations of FSH were not significantly different in the PVP- and ovine prolactin-treated groups (Table 2). In contrast to the LH results presented above, removal of the adrenal gland completely abolished the inhibitory effects of prolactin on the FSH rise after castration (Fig. 3b), an effect not prevented by replacement of physiological concentrations of corticosterone (Fig. 3c). There was no significant difference between plasma FSH concentrations in the PVP-treated or ovine prolactin-treated groups of adrenalectomized and adrenalectomized plus corticosterone treated rats in the first 48 h after castration. Furthermore, there was little evidence of an inhibitory effect of ovine prolactin on FSH in adrenalectomized plus corticosterone treated rats after sustained hyperprolactinaemia with no significant effect of ovine prolactin on plasma FSH from 60 to 192 h after castration (Table 2).

Effect of adrenalectomy on LH and FSH secretion after castration

Plasma LH and FSH concentrations in the three PVP-treated control groups (Fig. 4) are replotted from Figs 2 and 3 to demonstrate the effect of removal of the adrenal gland with or without corticosterone replacement on the increase in concentrations of these gonadotrophins after castration. Adrenalectomy significantly delayed the rise of both gonadotrophins ($P < 0.05$), an effect that was particularly marked in the case of FSH (Fig. 4b). Replacement of corticosterone did not reverse the effects of adrenalectomy, as there were no significant differences between the adrenalectomized and adrenalectomized plus corticosterone treated groups for either LH or FSH. In the case of plasma LH, adrenalectomy caused only a transient delay in the rise after castration. LH concentrations in both groups of adrenalectomized rats were significantly lower than in the adrenal-intact rats at most time points from 9 to 33 h after castration, but by 36 h after castration LH concentrations were not different from those of the adrenal-intact rats (Fig. 4a). In contrast, the effect of

Fig. 3. Effect of ovine prolactin on the increase in FSH secretion after castration. Note the differences in scale on the ordinate axes. (●) Vehicle-treated control rats; (○) ovine prolactin-treated rats. (a) In adrenal-intact rats, FSH concentrations were significantly suppressed by ovine prolactin at 6, 9, 21, 24, 27, 33 and 48 h ($P < 0.05$); $n = 11$ controls; 10 ovine prolactin-treated rats. (b) and (c) show results from adrenalectomized and adrenalectomized plus corticosterone treated rats, respectively. There were no significant differences between ovine prolactin-treated rats and the PVP-treated controls in these two groups. For (b), $n = 12$ controls; nine ovine prolactin-treated rats. For (c), $n = 11$ controls; 13 ovine prolactin-treated rats.

adrenalectomy on FSH had not reversed by 48 h after castration. Plasma concentrations of FSH in both groups of adrenalectomized rats were significantly lower than those of adrenal-intact animals by 3 h after castration, and then at all time points up to 48 h. In adrenalectomized plus corticosterone treated rats, FSH concentrations continued to be significantly lower than in the adrenal-intact controls throughout the 8 days of this study (Table 2).

Effect of ovine prolactin treatment on endogenous rat prolactin secretion

Plasma rat prolactin concentrations in adrenalectomized and adrenalectomized plus corticosterone treated rats were not significantly different from adrenal intact controls, so all groups

were combined to illustrate the effect of ovine prolactin treatment on endogenous prolactin secretion. Rat prolactin concentrations in PVP-treated rats were very low during surgery and in the immediate post-surgery blood sample (3 h), but then rose rapidly to reach a peak at approximately 14 ng ml^{-1} between 18 and 24 h after castration (Fig. 5). Rat prolactin concentrations then declined to a steady basal concentration of $6\text{--}8 \text{ ng ml}^{-1}$ throughout the remainder of the experiment. The ovine prolactin treatment significantly reduced prolactin secretion as early as 3 h after the first ovine prolactin injection ($P < 0.05$), and maintained full suppression of endogenous prolactin secretion throughout the 10 days of the experiment (Fig. 5). There was no evidence of a reduction of this effect with time.

Discussion

This is the first study to systematically investigate the role of the adrenal gland in mediating the antigonadotrophic effects of acutely induced hyperprolactinaemia. The results reveal that removal of the adrenal gland has a differential effect on secretion of the gonadotrophins LH and FSH following castration, and also on the prolactin-induced suppression of each gonadotrophin. This finding suggests that adrenal-derived factors may be involved in stimulating the rise in FSH secretion, but not in LH secretion, after castration and that the inhibition of castration-induced FSH secretion by acute hyperprolactinaemia may be mediated by an inhibition of these adrenal factors.

The suppression of LH secretion after castration by acute and chronic hyperprolactinaemia has been demonstrated in numerous studies (Grandison *et al.*, 1977; Login *et al.*, 1982; Smith and Bartke, 1987; Park and Selmánoff, 1991). Our results confirm this inhibitory effect and, in addition, clearly demonstrate that the adrenal gland is not involved in mediating this prolactin-induced suppression. The absence of an adrenal involvement in mediating the acute inhibitory effect of prolactin is consistent with several other studies that have demonstrated inhibition of LH secretion by chronic hyperprolactinaemia in adrenalectomized rats, using different experimental models involving pathophysiological concentrations of prolactin from pituitary tumours (Hodson *et al.*, 1980; Voogt *et al.*, 1987; Kooy *et al.*, 1989) or more physiological concentrations from pituitary transplants under the kidney capsule (McNeilly *et al.*, 1980). However, some evidence for adrenal involvement in prolactin-induced suppression of LH secretion has been reported. Greeley and Kizer (1979) reported that acute hyperprolactinaemia inhibits LH release in response to exogenous LHRH, but only in the presence of the adrenals. Our results suggest that if there is such an adrenal-mediated effect of prolactin on the pituitary, it plays only a minor role in the regulation of LH secretion after castration. Weber *et al.* (1982, 1983) reported that a prolactin- and ACTH-secreting pituitary tumour inhibited LHRH secretion into the portal blood (Weber *et al.*, 1983) and reduced pituitary LH and FSH secretion (Weber *et al.*, 1982, 1983), but that these responses were prevented by adrenalectomy. It is possible that the small amounts of ACTH secreted from their tumour, which may contribute together with prolactin to a sixfold increase in adrenal size (Weber *et al.*, 1982) and a 2.7-fold increase in corticosterone secretion (Carlson *et al.*, 1985), may account for the observed adrenal effect. However, it is difficult to reconcile the absence of

Table 2. Long-term effects of ovine prolactin treatment on FSH secretion after castration in male rats

Time after castration (h)	Adrenal intact		Adrenalectomy plus corticosterone	
	Vehicle ^a	Ovine prolactin	Vehicle ^a	Ovine prolactin
60	21.3 ± 2.4	18.8 ± 2.0	15.1 ± 1.3	15.7 ± 1.1
72	19.3 ± 2.9	18.4 ± 2.8	14.1 ± 0.9	14.4 ± 1.3
84	19.4 ± 4.0	16.3 ± 1.1	15.0 ± 1.2	14.9 ± 1.3
96	24.3 ± 4.1	17.5 ± 1.3	14.0 ± 0.9	12.0 ± 1.5
108	20.2 ± 2.6	18.8 ± 1.6	12.8 ± 1.2	13.6 ± 0.9
120	23.8 ± 4.4	17.9 ± 0.6	13.8 ± 1.8	12.0 ± 1.7
132	20.1 ± 3.3	16.1 ± 1.7	11.6 ± 1.5	12.6 ± 2.0
144	16.9 ± 1.8	17.9 ± 2.9	9.5 ± 1.8	10.7 ± 1.8
156	17.5 ± 2.5	16.1 ± 2.4	11.3 ± 1.7	10.2 ± 0.8
168	20.0 ± 1.6	18.0 ± 2.5	13.4 ± 1.0	9.6 ± 0.8
180	22.6 ± 5.7	14.2 ± 1.8	14.4 ± 1.8	10.4 ± 2.7
192	28.0 ± 4.9	17.4 ± 1.8	15.5 ± 3.3	8.1 ± 1.5

^a50% polyvinylpyrrolidone: 50% NaHCO₃ buffer (0.01 mol l⁻¹, pH 8.6).

Fig. 4. Effect of adrenalectomy, with or without corticosterone replacement, on LH and FSH secretion after castration. The results represent the vehicle-treated controls from the six different experimental groups, and have been replotted from Figs 2 and 3 for ease of comparison. (○) Adrenal intact rats (*n* = 11); (▲) adrenalectomized plus corticosterone treated rats (*n* = 11); (□) adrenalectomized rats (*n* = 12). (a) LH concentrations in adrenalectomized rats were significantly suppressed compared with adrenal intact controls at 3, 9, 12, 15, 18, 21, 33 and 48 h (*P* < 0.05). LH concentrations were significantly suppressed in adrenalectomized plus corticosterone rats at 12, 15, 18, 24, 30 and 33 h (*P* < 0.05). (b) FSH concentrations were significantly suppressed in both adrenalectomized and adrenalectomized plus corticosterone treated rats from 3 to 48 h after castration (*P* < 0.05).

Fig. 5. Effect of ovine prolactin on endogenous rat prolactin (rPRL) secretion in castrated rats. Rat prolactin concentrations were significantly suppressed from 3 to 192 h after castration (*P* < 0.05). (●) Vehicle-treated control rats (*n* = 34); (○) ovine prolactin-treated rats (*n* = 32).

an inhibitory effect of hyperprolactinaemia in their adrenalectomized rats with our present results, and with subsequent results from their laboratory (Voogt *et al.*, 1987; Kooy *et al.*, 1989) and others (Hodson *et al.*, 1980; Lamberts *et al.*, 1981) using different pituitary tumours to induce chronic hyperprolactinaemia, which clearly demonstrate prolactin inhibition of LH secretion in adrenalectomized rats.

The inhibitory effect of prolactin on FSH secretion after castration has not been studied as comprehensively as that on LH. Prolactin has been known to influence FSH secretion since the 1930s (Bates *et al.*, 1937). Most recent studies have measured a decrease in basal FSH concentrations in response to chronic hyperprolactinaemia (McNeilly *et al.*, 1978, 1983; Fox *et al.*, 1987), although one study reported a prolactin-induced increase

in FSH (Tresguerres and Esquifino, 1981) and acute hyperprolactinaemia did not effect basal FSH secretion (Selmanoff, 1985). However, in contrast to the results of the study reported here, several investigators have reported that high prolactin does not inhibit the castration-induced rise in FSH secretion (McNeilly *et al.*, 1980; Carter *et al.*, 1983; Smith and Bartke, 1987), or that the inhibition of FSH secretion by prolactin requires the presence of the testes (McNeilly *et al.*, 1983). Indeed, Smith and Bartke, 1987 suggested that the absence of an effect of prolactin on the rise in FSH secretion after castration was evidence for differential control of LH and FSH by the hypothalamus. In these previous studies, however, blood samples were usually collected several days after castration. It is likely, therefore, that these studies missed the period immediately after castration when prolactin exerted a clear inhibition of FSH secretion in the study reported here. When a blood sample was collected 24 h after castration in one of these studies, FSH concentrations were significantly suppressed in hyperprolactinaemic rats compared with controls, and no significant difference was seen 4 days after castration (McNeilly *et al.*, 1983). Thus, acutely increased prolactin concentrations inhibit the FSH rise after castration in a manner similar to the inhibition of LH. The onset and duration of the prolactin-induced suppression are similar for both gonadotrophins, suggesting a common mechanism, such as inhibition of LHRH secretion.

In contrast to the LH results described above, however, removal of the adrenal completely abolished the inhibitory effect of ovine prolactin on castration-induced FSH secretion. It is possible, therefore, that prolactin is acting via an adrenal-mediated pathway that specifically inhibits FSH but not LH secretion. Such a mechanism may not involve corticosterone, as replacement of corticosterone did not restore the prolactin inhibition. We cannot rule out the possibility that the failure of the corticosterone treatment to reverse the effects of adrenalectomy was due to the constant release of the hormone from a pellet, as opposed to the normal physiological pattern of secretion, with circadian variations in corticosterone concentration. Alternatively, it is possible that the inability of prolactin to suppress FSH secretion in adrenalectomized rats is due to an inhibitory effect of the adrenalectomy itself. Adrenalectomy has been shown to delay the rise in both LH and FSH after castration by approximately 12–24 h (Schwartz and Justo, 1977), which was thought to be caused by a neurally mediated stress response resulting in suppression of LHRH release (Ringstrom and Schwartz, 1984). This delayed rise of LH and FSH after castration in adrenalectomized rats was repeated in our study, and our results further demonstrate that the effect is particularly marked and long lasting in the case of FSH. The continued suppression of FSH secretion after castration in adrenalectomized rats throughout the 8 days of the present experiment is unlikely to be caused by stress alone. A substance from the adrenal might therefore be involved in stimulating FSH secretion in the castrated rat. Although Ringstrom and Schwartz (1984) found that cortisol reversed the inhibitory effect of adrenalectomy on FSH (but not LH) secretion, our failure to restore FSH to adrenal-intact concentrations using replacement of physiological concentrations of corticosterone suggests that the absence of glucocorticoid is not the major cause of the inhibition induced by adrenalectomy. Another possibility is that adrenal progesterone, which has been reported to stimulate FSH release

(Campbell *et al.*, 1977), may act to promote FSH secretion after castration. Regardless of the precise mechanism involved, plasma FSH concentrations were significantly reduced in both adrenalectomized groups in the present experiment, demonstrating that the adrenal gland plays a differential role in the control of LH and FSH secretion after castration. The fact that prolactin could not further suppress FSH secretion in these adrenalectomized animals may indicate that in the absence of adrenal-mediated stimulation of FSH secretion following castration, the hypothalamic mechanism(s) controlling FSH release was insensitive to this inhibitory stimulus. The magnitude of the inhibition of FSH after castration by either ovine prolactin or adrenalectomy was similar to that of adrenal-intact, PVP-treated controls.

In all groups of animals, prolactin was very low during surgery and for the first 3 h after castration compared with the basal prolactin concentrations of approximately 7 ng ml^{-1} in castrate male rats (Park and Selmanoff, 1991). This was surprising, as it was expected that prolactin concentrations would be high owing to the surgical stress (Gala, 1990). The low concentrations of prolactin found during surgery suggests that the anaesthesia regimen used in the present study suppressed prolactin secretion. Ketamine has been reported to cause a slight, nonsignificant decrease in basal prolactin secretion (Lawson and Gala, 1974), but its effects in combination with acepromazine are not known. After the first 3 h of the experiment, prolactin concentrations in PVP-treated control animals rose to a peak of about 14 ng ml^{-1} between 12 and 24 h after castration. It is likely that this rise in prolactin concentrations was caused by a chronic post-surgical stress response (Gala, 1990). After 24 h prolactin concentrations fell to relatively normal basal values of $6\text{--}8 \text{ ng ml}^{-1}$. Injection of exogenous ovine prolactin significantly inhibited both the post-surgery rise in prolactin concentrations, and reduced basal prolactin secretion in all groups of rats. This effect was first evident within 3 h of the first ovine prolactin injection, and was maintained continuously throughout the 10 days of this experiment. This rapid and long-lasting inhibitory effect of exogenous ovine prolactin on endogenous rat prolactin secretion is consistent with the known effect of prolactin to inhibit basal (Selmanoff and Gregerson, 1984; Selmanoff, 1985; Park and Selmanoff, 1991) and stress-induced prolactin secretion (Selmanoff, 1985; Grattan and Averill, 1991).

The fact that this ovine prolactin-induced inhibition of prolactin secretion was maintained indefinitely contrasts with the apparent reduction of the prolactin-induced suppression of LH and FSH secretion after castration despite continued hyperprolactinaemia. Either the two effects may therefore be mediated by different mechanisms, or the sensitivity of a single inhibitory feedback mechanism may differ markedly for prolactin compared with the gonadotrophins. The fact that much higher concentrations of prolactin produced by pituitary tumours can produce complete and irreversible inhibition of gonadotrophin secretion (Hodson *et al.*, 1980; Login *et al.*, 1982; Voogt *et al.*, 1987; Kooy *et al.*, 1989) is consistent with the second of these possible explanations. There was some suggestion that the ovine prolactin was still having an inhibitory effect on LH throughout the period of this experiment, as plasma LH concentrations tended to be slightly lower in the ovine prolactin-treated groups than in the PVP-treated controls. It is possible that more intensive blood sampling to examine the

pulsatile pattern of hormone-secretion might reveal prolactin-induced changes, even after mean concentrations have become statistically indistinguishable.

This study has demonstrated that the prolactin-induced suppression of LH secretion does not depend on the presence of the adrenal gland. Prolactin-induced suppression of FSH secretion, however, may involve an adrenal component. The mechanism(s) controlling this prolactin-induced suppression of gonadotrophin secretion is much less sensitive than is the auto-regulatory feedback control of prolactin on its own secretion. The castration-induced rise in FSH, but not in LH, depends on the presence of the adrenal glands, demonstrating that the adrenal gland plays a differential role in regulating LH and FSH secretion following castration, and also in mediating the inhibitory effects of hyperprolactinaemia on gonadotrophin secretion.

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