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The ability of prolactin to change the sensitivity of the pituitary of the lactating rat to luteinising hormone releasing hormone *in vitro*

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Abstract. The ability of prolactin to influence the responsiveness of the lactating rat pituitary to luteinising hormone releasing hormone has been examined *in vitro*. The pituitary responsiveness *in vivo* to luteinising hormone releasing hormone decreased as a function of increase in the lactational stimulus. Prolactin inhibited the spontaneous *in vitro* release of luteinising hormone and follicle stimulating hormone to a small extent, from the pituitary of lactating rats with the suckling stimulus. However, it significantly inhibited the release of these two hormones from luteinising hormone releasing hormone-stimulated pituitaries. The responsiveness of pituitaries of rats deprived of their litter 24 h earlier, to luteinising hormone releasing hormone was also inhibited by prolactin, although minimal. It was concluded that prolactin could be influencing the functioning of the pituitary of the lactating rat by (a) partially suppressing the spontaneous release of gonadotropin and (b) inhibiting the responsiveness of the pituitary to luteinising hormone releasing hormone.

Keywords. Pituitary responsiveness; luteinising hormone releasing hormone; prolactin; lactational stimulus.

1. Introduction

Our earlier studies carried out *in vivo* in lactating rodents (Muralidhar *et al* 1977) and subhuman primates (Maneckjee *et al* 1976) showed that prolactin has a direct inhibitory effect on pituitary luteinising hormone (LH) secretion. This effect, however, was shown to be dependent on the presence of minimal suckling stimulus. In the lactating rat the intensity of suckling can be regulated by varying the number of pups and it is known that endogenous prolactin levels increase in proportion to the intensity of suckling stimulus (Ford and Melampy 1973). It has been reported by Minaguchi and Meites (1967) that post-partum lactating rats have less luteinising hormone releasing hormone (LHRH) activity in the hypothalamus and less LH content in their pituitary compared to non-lactating rats, suggesting that suckling inhibits LHRH secretion with consequent reduction in pituitary LH secretion. In addition, LHRH has been shown to release lesser amount of LH both *in vivo* and *in vitro* in post-partum lactating rats, as compared to normal cycling female rats (Lu *et al* 1976). We have, however, recently observed that when the lactating rat or monkey is under minimal suckling stimulus, injection of LHRH brings about a significant increase in blood levels of LH and that this effect could be blocked by simultaneous

injection of exogenous prolactin (Muralidhar *et al* 1977 and Maneckjee *et al* 1976). This effect cannot be seen in lactating rats under intense suckling stimulus (e.g. those suckling 8 pups) as the response to exogenous LHRH itself was not significant (Muralidhar *et al* 1977). Based on this, we suggested that the inhibition of LH secretion during intense suckling may be due to alteration in the sensitivity of the pituitary to LHRH, brought about by high levels of prolactin present in circulation at this time.

It is possible that the *in vivo* response to LHRH and prolactin injections may be influenced by the presence of endogenous LHRH (even though the effect during lactation may be minimal); alternatively, under *in vivo* situation the pituitary may be influenced by other factors making it difficult to delineate the role of prolactin. It was therefore considered important to determine the ability of the pituitaries of lactating rats, subjected to varying suckling intensities, to release LH and follicle stimulating hormone (FSH) in response to synthetic LHRH and prolactin *in vitro*.

2. Materials and methods

Pregnant female albino rats of our Institute colony were housed in individual cages a few days prior to the expected date of parturition. The day after post-partum estrus was considered as day 1 of lactation. At birth, the litter size was adjusted to 8 or 2 pups per rat. In another group of rats, the litters were withdrawn from the mothers 24 h prior to removal of the pituitaries. On day 10 of lactation, groups of rats suckling varying numbers of pups were decapitated at about 1000 a.m., the pituitaries removed immediately, hemisected and weighed. Each hemipituitary was incubated in 2 ml of medium-199 containing $NaHCO_3$ and 0·2% bovine serum albumin at a pH of 7·4. Incubations were carried out at 37°C in a Dubnoff metabolic shaker (50 cycles/min). Each flask prior to incubation was flushed with O_2 for several min and stoppered tightly. After 30 min pre incubation, the medium was removed and replaced with 2 ml of fresh medium-199 in the presence or absence of 20 ng of synthetic LHRH (Ay-24, 301-4, Ayerst Research Laboratory, Montreal, Canada), flushing with O_2 was repeated and incubation continued for an additional 2 h. Hemipituitaries from the same animals were used as control and experimental. Each group had 6 animals.

To study the effect of prolactin on pituitary responsiveness to LHRH added *in vitro* 1 h prior to removal of the pituitaries, lactating rats suckling 2 pups each and those which were deprived of their litters on day 9 of lactation, were given an injection of 500 μg ovine-prolactin (NIH-P-S5) in 0·1 ml of 0·9% NaCl on day 10 of lactation. The pituitaries were then incubated as in the previous experiment in the presence and absence of LHRH.

Another set of hemipituitaries from lactating rats suckling 2 pups each was incubated in medium-199 containing both prolactin (50 μg) and LHRH under identical conditions described above. A control group of hemipituitaries from rats suckling 2 pups or those which were deprived of their litters on day 9 were incubated in medium-199.

LH and FSH released into the incubation medium were assayed by the double-antibody radioimmunoassay procedure (Muralidhar and Moudgal 1976) using the NIAMDD rat LH and FSH kits. The sensitivity of the LH assay was 5 ng, while

that of the FSH assay was 10 ng. The results are expressed as ng of LH or FSH (NIH-RP-standard-1) released per mg pituitary in 2 h.

All results were analysed by Student's '*t*' test. The actual amounts of LH and FSH released into medium (±S.E.M.) by the control and experimental groups were used for these computations. In some cases, the results were found significant only at 90% confidence limits due to large variations in *in vitro* response. These have been indicated at appropriate places.

3. Results

3.1. *Ability of LHRH to release LH and FSH from hemipituitaries in vitro as a function of lactational stimulus in vivo*

Addition of LHRH *in vitro* to pituitaries appeared to bring about a differential response, in terms of release of LH and FSH, depending upon the suckling stimulus (table 1). While the response of the pituitaries from mothers suckling 2 pups to LHRH did not differ very much from those of the 0 pup group, the pituitaries from rats suckling 8 pups each showed clearly a reduced response in terms of amounts of both FSH and LH released.

3.2. *Effect of in vivo prolactin administration in lactating rats on pituitary responsiveness to LHRH in vitro*

In lactating rats, suckling 2 pups each, *in vivo* administration of 500 μg ovine prolactin, 1 prior to removal of pituitaries brought about a significant reduction in the amount of LH and FSH released from the hemipituitaries in response to LHRH *in vitro*. In the absence of prolactin the net amount of LH and FSH released was 264 and 164 ng/mg pituitary/2 h. However, on administration of prolactin *in vivo*, the amount of LH and FSH released was significantly reduced. Even the basal amount which is normally released in the absence of LHRH was inhibited to a marked extent (table 2).

Table 1. Influence of suckling stimulus on the ability of the pituitary to release LH and FSH in response to *in vitro* LHRH stimulation.

No. of pups	LH released (ng/mg pituitary/2h)			FSH released (ng/mg pituitary/2h)		
	−LHRH	+LHRH	Net amount of LH released	−LHRH	+LHRH	Net amount of FSH released
8	442±171	1180±220*	738	446±24	566±88*	120
2	497±179	1759±590	1262	1080±500	1686±600	606
0	392±80	1800±480	1408	690±390	1440±440	750

No. of hemipituitaries in each group = 6. LHRH = 20 ng/2 ml of medium
LH and FSH standards used were the NIAMDD RP—I preparation. Values given are mean ± SEM.

*Significantly different from the 0 pup control only at $p = 0{\cdot}05-0{\cdot}1$.

Prolactin also appeared to inhibit the response of pituitaries obtained from mothers deprived of their litters 24 h previously (0 pup group) to LHRH, but the extent of inhibition was significantly less compared to that seen in the 2 pup group (table 2).

3.3. *Effect of addition of prolactin in vitro on the responsiveness of the pituitaries from lactating rats to LHRH in vitro*

A significant reduction in LH and FSH released by hemipituitaries of rats suckling 2 pups each was observed when 50 μg ovine prolactin was added to the incubation medium along with LHRH (table 3). This reduction was similar to that observed when prolactin was administered *in vivo*. When hemipituitaries from rats which had their litters withdrawn 24 h prior to removal of the pituitary were incubated in the presence of prolactin and LHRH *in vitro* (table 4) there was less reduction in

Table 2. Effect of *in vivo* prolactin administration on pituitary release of LH and FSH in response to addition of LHRH *in vitro*.

	2 pup group			0 pup group		
	−LHRH	+LHRH	Net amount released	−LHRH	+LHRH	Net amount released
			LH released ng/mg pituitary/2h			
−PRL	588±162*	852±214*	264	620±236*	972±200*	352
+PRL	368±141	176±40	−	386±56	658±36	272
			FSH released ng/mg/pituitary/2h			
−PRL	468±148	632±192†	164	568±52*	788±200†	220
+PRL	600±220	368±164	−	320±92	388±96	68

Ovine prolactin (PRL 500 μg) was injected 1 h prior to removal of pituitaries. No. of hemipituitaries per group = 6.
LH & FSH standards used were the iodination grade hormones supplied by the NIAMDD, Bethesda, MD., USA
*Significantly different from the +PRL group at $p<0.05$.
†Significantly different from the +PRL group only at $p = 0.05$ to 0·10.
All values are expressed as mean ± SEM
−Signifies inhibition of gonodotropin release.

Table 3. Effect of prolactin added *in vitro* on pituitary LH and FSH release in response to LHRH stimulation *in vitro*.

No. of pups	LH released ng/mg pituitary/2h			FSH released ng/mg pituitary/2h		
	−PRL	+PRL	%Inhibition	−PRL	+PRL	%Inhibition
2	852±214	172±40*	79·8	632±196	224±64*	64.5
0	972±200	676±224	30·5	788±200	542±86	31.2

Ovine PRL (50 μg) added *in vitro* along with 100 ng LHRH/ml medium.
No. of hemipituitaries in each group = 6.
LH and FSH standards used were the same as in tables 2 and 3.
*Significantly different from the control at $p<0.001$.
All values are expressed as mean ± SEM.

Table 4. Effect of prolactin addition *in vitro* on spontaneous gonadotropin release from rat pituitaries.

No. of pups	LH released ng/mg pituitary/2h			FSH released ng/mg pituitary/2h		
	−PRL	+PRL	% Inhibition	−PRL	+PRL	%Inhibition
2	500±179	325±54*	35	480±150	443±205	8
0	488±71	319±132†	39	345±195	310±42	10

Ovine PRL (50 μg) added per ml medium.
No. of hemipituitaries in each group = 3.
LH and FSH standards used were the same as in tables 2 and 3.
*Significantly different from the controls at $p = 0{\cdot}05$.
†Significantly different from the control at $p = 0{\cdot}05$ to 0·10.
All values expressed are mean ± SEM.

release of LH and FSH (30% compared to 80% reduction in the 2 pup groups. The results in table 4 show that prolactin is capable of suppressing the basal levels (in the absence of LHRH) of LH and FSH released from the pituitary. While this suppression is not significant in the case of FSH (only by 10%), in the case of LH the release is reduced by 39%.

4. Discussions

The present *in vitro* study was undertaken with a view to obtain an insight into the mechanism by which prolactin influences secretion of gonadotropins in the lactating rat. The results clearly show that the *in vitro* responsiveness of the pituitary to LHRH is a function of the lactational state of the rat. Assuming that the number of pups suckling have a direct correlation to the degree of suckling stimulation, it is seen that with intense suckling stimulus (e.g. 8 pup group) the responsiveness to LHRH is lowered (table 1).

It has earlier been shown by us that injection of prolactin to intact lactating rats could influence the pituitary responsiveness to exogenous LHRH administration. This effect, however, could only be shown in the presence of minimal suckling stimulus (Muralidhar *et al* 1977). Prolactin inhibits the basal levels of secretion of both LH and FSH; LH, being inhibited to a greater extent than FSH (table 4). The present *in vitro* studies suggest that the pituitaries of the lactating rat are already 'primed' since they react to prolactin addition *in vitro* in a manner similar to that observed by injecting prolactin *in vivo*. The ' suckling stimulus ' is not yet understood clearly in biochemical terms. Further, prolactin added *in vitro* to pituitaries removed from rats suckling 2 pups inhibits the normal responsiveness of these to LHRH (table 3).

The *in vivo* studies showed that prolactin was less effective in inhibiting pituitary responsiveness to LHRH in rats deprived of their pups. The *in vitro* studies with such pituitaries clearly show that prolactin is still able to exercise its inhibitory effect, although at a much lower level (50% of that normally seen in the 2 pup group). It is interesting to find that prolactin added *in vitro* is able to inhibit the small extent of spontaneous release of LH and FSH that occurs when the pituitaries are incubated without any LHRH (tables 2 and 4). There appears to be no difference between the

pituitaries of rats suckling 0 and 2 pups as far as the ability of prolactin to inhibit the spontaneous release of gonodotropins (table 4). The inhibition observed, following incubation of pituitaries of rats deprived of their litter, with LHRH and prolactin, can mostly be accounted for by this direct effect of prolactin on gonadotropin release (in both cases it is approximately 30% — compare the '0 pup' group in tables 3 and 4). However, the inhibition in gonadotropin release observed in the 2 pup group appears to be due to prolactin affecting pituitary responsiveness to LHRH (compare tables 3 and 4).

The mechanism by which prolactin affects gonodotropin release is not clear at this moment. The present experiments do not distinguish between inhibition of synthesis and/or release of gonodotropins by prolactin. However, it is possible to conclude from the present studies that prolactin has a direct effect on pituitary gonadotropin release. This effect is accentuated during lactation and addition of LHRH does not totally over ride prolactin inhibition.

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