Effect of Porcine Follicular Fluid on LH and FSH Secretion in Rats

REIKO DEMURA, TOMOHARU SUZUKI,* SAEKO NAKAMURA,*
HIROMI KOMATSU and HIROSHI DEMURA

*Department of Internal Medicine and *Radioassay Center,
Tokyo Women's Medical College, Tokyo 162*

DEMURA, R., SUZUKI, T., NAKAMURA, S., KOMATSU, H. and DEMURA, H. *Effect of Porcine Follicular Fluid on LH and FSH Secretion in Rats.* Tohoku J. exp. Med., 1988, **156** (2), 157-163 — To investigate a mode and a site of action of inhibin on FSH and LH secretion, in vivo and in vitro effects of porcine follicular fluid (PFF) on FSH and LH secretion were studied in male rats. PFF administered i.v. suppressed basal FSH levels for 1-8 hr, but did not alter LH levels. On the contrary, PFF given either 1 or 6 hr in advance, suppressed LH-RH stimulated LH responses as well as LH-RH induced FSH responses. In cultured pituitary cells PFF exerted a partial suppressive effect on LH release. PFF suppressed LH release only under the maximal stimulation with 10^{-10} M LH-RH and 0.1 mM 3-isobutyl-1-methylxanthine (IBMX) to a lesser degree compared with uniform and greater suppression on FSH release regardless of the experimental conditions. Above results showed a selective suppressive effect of PFF on FSH release and synthesis with a less inhibitory effect on LH release mainly at the pituitary level. ——— inhibin; porcine follicular fluid; FSH; LH; LH-RH

It has been widely demonstrated that follicular or testicular preparations with definite inhibin activities suppressed FSH secretion in rats (Marder et al. 1977; Schwartz and Channing 1977) as well as in other species (Channing et al. 1981; Rettori et al. 1982). However, effects of inhibin on LH release are still controversial. To investigate the effect of inhibin on LH and FSH secretion, in vivo and in vitro effects of porcine follicular fluid (PFF) on LH and FSH secretion were studied in male rats under various conditions.

MATERIALS AND METHODS

Mature Wistar male rats weighing approximately 250 g were given 200 μ l of porcine follicular fluid (PFF) treated with dextran coated charcoal as reported previously (Demura et al. 1987a, b) through an indwelling atrial catheter under urethan anesthesia. The same amount of porcine serum albumin (PSA) dissolved in the same amount of saline were given as control. Blood samples were drawn through the catheter before and 1, 2, 4, 6 and 8 hr after administration of PFF and PSA for measurements of plasma LH and FSH. To investigate the effect of PFF on LH-RH stimulated LH and FSH release, LH-RH test was preformed by injecting 200 ng LH-RH to 5 normal rats and PFF pretreated rats. PFF was given 1 or 6 hr in advance to i.v. administration of 200 ng of LH-RH to 4 and 7 rats,

Received July 6, 1988; revision accepted for publication September 6, 1988.

respectively. Blood samples were obtained 10, 20, 30 and 40 min after LH-RH for measurements of plasma LH and FSH. For in vitro studies, the effect of various amount of PFF on the secretion of LH and FSH from dispersed pituitary cells under basal as well as 10^{-10} M LH-RH stimulation with and without 0.1 mM 3-isobutyl-1-methylxanthine (IBMX). Preparation of rat anterior pituitary cells for monolayer culture was reported previously (Demura et al. 1987a, b).

LH and FSH in plasma and culture media were measured by radioimmunoassay (RIA) using RIA kits kindly supplied by NIADDK, Baltimore, MA, USA. The minimal detectable levels for LH and FSH were 0.1 ng/ml and 1.0 ng/ml, respectively. Inter- and intra-assay variances were less than 15% and 10%, respectively for both LH and FSH. Statistical analyses were performed by Student's *t*-test.

RESULTS

Basal plasma levels of LH and FSH after a single i.v. injection of 200 μ l of PFF and PSA are shown in Fig. 1. Plasma FSH became significantly low at 6 and 8 hr after PFF, but LH levels stayed unchanged. FSH (Fig. 2) and LH (Fig. 3) responses to LH-RH were significantly suppressed by 1 and 6 hr prior administrations of PFF. The maximal responses of LH and FSH appeared at 30 min after LH-RH injection in every LH-RH tests except for one performed 6 hr after PFF, in which LH peak was seen at 20 min. Suppressions of maximal response by PFF administered 1 and 6 hr in advance were 66 and 72% for FSH and 58 and 80% for LH, respectively. There was no significant difference between the maximal response of both LH and FSH in LH-RH tests performed 1 and 6 hr after

Fig. 1. Effects of i.v. administration of PFF on basal plasma levels of LH (Δ) and FSH ($\blacktriangle$) as compared with control administration of PSA on LH ($\circ$) and FSH ($\bullet$). Each point represents mean $\pm$ S.E. ($n=5$, each group). * $p < 0.05$ against corresponding values in the PSA group.

Fig. 2. Effects of PFF, given 1 hr (▲) and 6 hr (■) in advance, on plasma FSH response to 200 ng of LH-RH as compared with a control test (○). Each point represents mean $\pm$ S.E. ($n=4$ for ▲, 7 for ■ and 5 for ○). * $p<0.05$; ** $p<0.01$ against corresponding values in the control test.

Fig. 3. Effects of PFF, given 1 hr (▲) and 6 hr (■) in advance on plasma LH response to 200 ng of LH-RH as compared with a control test (○). Each point represents mean $\pm$ S.E. ($n=4$ for ▲, 7 for ■ and 5 for ○). * $p<0.05$; ** $p<0.01$ against corresponding values in the control test.

Fig. 4. Effect of PFF on FSH (upper panel) and LH (lower panel) releases from cultured pituitary cells under basal conditions ($\circ$) and in response to stimulation with 10^{-10} M LH-RH in the absence ($\bullet$) and presence ($\blacktriangle$) of 0.1 mM IBMX. Releases are expressed in % of those in control medium without PFF. Serially diluted PFF was added to the cells. Each point represents mean $\pm$ S.E. of 5 samples $\times$ 4 wells.

Fig. 5. Effect of 1/9 diluted PFF on FSH release from cultured anterior pituitary cells under various conditions (shadow column) and control medium (white column). Each column represents mean $\pm$ S.E. of 5 samples $\times$ 4 wells. Numbers on the shadow column show % of FSH secretion with PFF vs. control. * $p < 0.01$ against corresponding control.

Fig. 6. Effect of 1/9 diluted PFF on LH release from cultured anterior pituitary cells under various conditions (shadow column) and control medium (white column). Each column represents mean $\pm$ s.e. of 5 samples $\times$ 4 wells. Numbers on the shadow column show % of LH secretion with PFF vs. control.

* $p < 0.01$ against corresponding controls.

PFF administration.

In vitro studies with cultured pituitary cells, PFF suppressed basal as well as LH-RH stimulated FSH secretion in a dose dependent manner with and without 0.1 mM IBMX (Fig. 4). On the contrary, LH secretion was suppressed by PFF at higher concentrations than 1/81 only when stimulated by 10^{-10} M LH-RH and/or 0.1 mM IBMX (Fig. 4). Fig. 5 summarizes % inhibition of FSH secretion by 1/9 diluted PFF under basal condition and in response to 10^{-10} M LH-RH with and without 0.1 mM IBMX. By addition of 0.1 mM IBMX, 10^{-10} M LH-RH or both of them, basal FSH secretion increased gradually with a significant difference, but PFF inhibited FSH secretion equally to less than one half of the basal levels under any conditions. Fig. 6 summarized effects of 1/9 diluted PFF on LH secretion under the same conditions as those in FSH experiments. Basal LH secretion tended to increase by an addition of either IBMX or LH-RH and increased significantly by an addition of both of them. PFF inhibited LH secretion with a significant difference only under the maximal stimulation with 0.1 mM IBMX and 10^{-10} M LH-RH to 75.3% of the basal level.

DISCUSSION

The present study firstly demonstrated that PFF inhibited FSH secretion mainly at the pituitary level as reported by many investigators (Franchimont et al. 1975; Hopkinson et al. 1977; Labrie et al. 1978; Lorenzen et al. 1978; Depaolo et al. 1979; Lumpkin et al. 1981). At the same time, the long-lasting effect on basal FSH and its time course being maximal at several hours after PFF administration indicated that PFF suppressed not only FSH release but also its

synthesis. de Jong et al. (1979) showed that Sertoli cell factors inhibited the incorporation of ^3H -uridine into immunoprecipitable FSH fraction in cultured pituitary cells and suggested an inhibition of FSH synthesis by inhibin.

Secondly, it was clearly demonstrated that PFF possessed a partial suppressive effect on LH secretion especially when LH secretion was under stimulation. The effect of inhibin on LH secretion has been controversial. Though a number of investigators (Baker et al. 1976; de Jong et al. 1979; Depaolo et al. 1979; Lagace et al. 1979; Shander et al. 1980; Shiraishi 1986) reported a less selective effect of inhibin on LH release. Koiter et al. (1983) reported that PFF did not alter LH release even after LH-RH, or Huang and Miller (1984) reported that PFF rather intensified an LH-RH mediated LH release. Rush et al. (1981) reported that PFF suppressed basal LH secretion during proestrous in female rats. These inconsistent results may be partly due to differences in a potency or a purity of inhibin activity in the preparations used for the experiments. A slight discrepancy in the suppressible effect of PFF on LH secretion in *in vivo* and *in vitro* in the present study could be due to a difference in a potency of inhibin activity or secretagogues used for both studies. Besides, an extrapituitary effect of PFF could not be ruled out as a modulatory factor for *in vivo* LH-RH action, as Demoulin et al. (1979), Lumpkin et al. (1981) and Moodbidri et al. (1981) suggested. Further studies must be needed to clarify a site and a mode of action of inhibin using a pure material.

In summary, a partial suppressive effect of PFF on LH release at the pituitary level was demonstrated together with a selective effect on FSH release and probably on its synthesis. Extra-pituitary site of action of PFF could not be ruled out.

Acknowledgments

We thank the Pituitary Hormone Distribution Program, NIADDK, USA for kindly supplying RIA kits for rat LH and FSH. This work was partly supported by a grant-in aid for Specific Diseases from the Ministry of Health and Welfare, Japan.

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