An improved in-vitro model for the study of gonadotrophin secretion from immature rat pituitaries

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Studies on the regulation of release of the gonadotrophic hormones from the anterior pituitary have in general utilized incubations of hemipituitaries derived from adult male rats (Wakabayashi, Date & Tamaoki, 1973). The small magnitude of stimulation (2–3-fold) of hormonal release by gonadotrophin-releasing hormone (GnRH) has led investigators to search for a more sensitive model using pituitaries from pro-oestrous rats (Ferland *et al.*, 1975) and anterior pituitary monolayer cultures (Tang & Spies, 1975) or dispersed cells (Nakano, Fawcett & McCann, 1976). The present report describes a simple and sensitive model for the study of gonadotrophic hormone release.

Pituitaries were taken from 12-day- and 2-month-old male and female Wistar-derived rats. Each pituitary was placed in 1 ml Krebs–Ringer bicarbonate buffer (KRB) pH 7.4, containing glucose (1 mg/ml), and initially incubated for 2 h at 37°C under an atmosphere of 95% O₂:5% CO₂. The medium was then changed to 1 ml KRB or 1 ml KRB containing GnRH (Hoechst) and incubation was continued for the time indicated. At the end of the incubation period, aliquots of the medium were analysed for LH and FSH content by radioimmunoassay (Daane & Parlow, 1971) using the kits kindly supplied by the NIAMDD, Rat Pituitary Hormone Program, NIH, Bethesda, U.S.A. Results are expressed in terms of the RP-1 reference preparations. The sensitivity of the assays was 10 ng/ml and 50 ng/ml for LH and FSH, respectively. The inter-assay coefficient of variation was 5%.

Table 1. Effect (as % of control) of increasing doses of GnRH on FSH and LH release from pituitaries of immature and adult rats during a 4-h incubation

Pituitary donor	FSH			LH		
	GnRH (10 ⁻⁹ M)					
	0.25	1.0	5.0	0.25	1.0	5.0
Immature females	120 ± 18	325 ± 44**	760 ± 40**	220 ± 20**	550 ± 80**	980 ± 40**
Immature males	180 ± 20**	300 ± 32**	600 ± 60**	180 ± 20**	585 ± 62**	940 ± 80**
Pro-oestrous females	131 ± 25	175 ± 28*	310 ± 23**	210 ± 15**	340 ± 40**	785 ± 60**
Adult males	90 ± 15	130 ± 17	160 ± 30*	180 ± 10**	310 ± 35**	346 ± 51**

Values are mean ± S.E.M. for 12 determinations. Means were compared with control values by Student's *t* test. * *P* < 0.02; ** *P* < 0.001.

Incubation of pituitaries with increasing concentrations of GnRH for 4 h resulted in a dose-related increase in the release of LH and FSH. The stimulation of LH and FSH release was more pronounced when pituitary donors were immature rather than adult rats (Table 1). The kinetics of gonadotrophin release were studied by incubating pituitaries with 5 nM (mature rats) or 1 nM (immature rats) GnRH for various periods (Text-fig. 1). A significant stimulation of LH release by GnRH occurred from adult pituitaries after 90 min of incubation, whereas enhanced gonadotrophin release from immature pituitaries was evident even after 20 min. The maximal relative increase of LH (8-fold) and FSH (3-fold) release from pituitaries of adult rats was observed in pro-oestrous females after 4 h of incubation with GnRH, whereas a more marked stimulation of gonadotrophin secretion (LH, 33-fold; FSH, 5.5-fold) was observed in pituitaries of immature females after 90 min of incubation. The absolute increment of LH in the immature rat was similar to that seen in the adult, even

Text-fig. 1. Effect of GnRH (open columns) on LH and FSH release from pituitary glands of immature and adult female and male rats. Concentration of GnRH: 1×10^{-9} M for the immature rats and 5×10^{-9} M for the mature rats. Stippled columns represent control incubations. Vertical bars indicate S.E.M. for 18 determinations; means were compared with control values by Student's *t* test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

though a lower dose of GnRH (1 nm compared to 5 nm) was used, whereas the absolute increment of FSH was greater in the immature animal.

Pituitaries derived from immature rats provide an improved model for the study of gonadotrophin release because they respond to low doses of GnRH after a short period of incubation with an augmented stimulation of LH and FSH release.

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