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Increased responsiveness of the hypothalamic-pituitary axis to steroid feedback effects in ovariectomized rats treated neonatally with monosodium L-glutamate

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Summary. Chronic ovariectomized rats treated neonatally with MSG showed reduced circulating concentrations of LH coupled with elevated hypothalamic LHRH stores. Despite the apparent loss of LHRH secretion, the small pituitary glands showed an increased density of LHRH receptors and normal responsiveness to the releasing hormone. The positive feedback effects of progesterone on LH release in oestrogen-primed animals was greatly exaggerated reflecting the build-up of hypothalamic LHRH stores without loss of pituitary responsiveness to LHRH.
Key words. Monosodium L-glutamate; luteinizing hormone; luteinizing hormone-releasing hormone; ovariectomized rats; steroid feedback.

Rats treated neonatally with the neurotoxin, monosodium L-glutamate (MSG), have permanent lesions of the arcuate nucleus which cause a disruption of the neuroendocrine regulation of gonadal function and hypogonadism, despite normal serum levels of LH[1,2]. Since there appears to be no impairment in the release mechanism for LH-releasing hormone (LHRH)[3] or in the pituitary responsiveness to LHRH[4] it is likely that MSG treatment disrupts the neuronal circuits mediating steroid feedback control of LHRH secretion. Indeed, adult rats treated neonatally with MSG show an attenuated rise in LH release after ovariectomy[5,6] and a reduction of the positive feedback effects of chronic oestrogen administration to ovariectomized rats[7].

In these experiments we have further investigated the effects of MSG-induced arcuate lesions on steroid feedback mechanisms in ovariectomized rats. The negative and positive feedback action of exogenously administered steroids has been assessed and the results have been correlated with measurements of hypothalamic LHRH content, pituitary LH responses to LHRH and pituitary LHRH receptors.

Material and methods. Female Porton Wistar pups were injected i.p. with 4g MSG/kg in 0.9% saline on days 2, 4, 6, 8 and 10 after birth; control rats received an equivalent volume of isosmotic NaCl (10% w/v). After weaning at approximately 3 weeks of age, the sexes were separated and they were housed under controlled conditions of light (lights on 06.00–18.00 h) and temperature (22 °C); food and water was available ad libitum. When they were 3 months old they were ovariectomized and three weeks later used for experimentation.

One group of animals was stunned and decapitated, trunk blood was collected for hormone measurements, and the pituitaries and hypothalami dissected out. The hypothalami measured approximately 5 × 4 × 2 mm with the rostral and caudal limits being defined by the optic chiasma and mamillary bodies respectively. Immediately after dissection, the tissue was placed in 1 ml ice cold saline containing 1mM bacitracin (Sigma), disrupted by sonication and the separated extract stored at −20 °C until assayed for LHRH.

The pituitaries were hemisected and one half was used to investigate the responses of perfused glands to LHRH and the other half for the measurement of pituitary LHRH receptors. Hemipituitaries were placed on 200-µl volume perspex chambers and perfused with Krebs ringer bicarbonate (KRB) containing 2 g glucose/l and 2.5 g bovine serum albumen/l. The perfusate was constantly gassed with 95% O_2/5% CO_2 and delivered to the tissue at a rate of 0.2 ml/min. After an initial 2 h stabilization period, 10-min fractions were collected for a further 2-h period during which time two 5-min pulses of 10 ng LHRH (Cambridge Research Biochemicals Ltd., Harston CB2 5NX)/ml KRB was delivered to the gland with an interval of 1h betwwen each pulse. Perfusate samples were stored at −20 °C.

Pituitary LHRH receptors of individual hemi-pituitaries were measured by binding of the LHRH-analogue [D-Ser(t-Bu)[6]] des-Gly[10]-N-ethylamide (buserelin, Hoechst (UK) Ltd., Middlesex House, Hounslow) using a method described previously[1]. Protein estimation of individual homogenates were determined by the method of Lowry et al.[9].

In a second group of animals, the response to exogenous steroid treatment was investigated. On day 21 at 12.00 h, a blood sample was obtained by cardiac puncture under ether anesthesia just

Effects of neonatal MSG treatment on body and pituitary weight, gonadotrophic hormone secretion, hypothalamic LHRH content and pituitary LHRH receptors in adult ovariectomized rats. Values give mean ± SEM and group numbers are given in parenthesis. †, $p < 0.005$ and * $p < 0.05$ compared with control; Student's t-test

	MSG Treated	Control
Ovarian weight at ovariectomy (mg)	43.2 ± 3.9† (10)	69.3 ± 6.2
Pituitary weight (mg)	16.8 ± 1.18† (10)	33.8 ± 1.42 (9)
Body: tail lenght ratio	0.73 ± 0.01† (10)	0.94 ± 0.02 (10)
Serum LH (µg/l)	116 ± 29† (8)	404 ± 0.02 (7)
Serum prolactin (µg/l)	53 ± 29 (8)	21 ± 3 (7)
Hypothalamic LHRH (pg)	1212 ± 87[6] (6)	362 ± 78 (6)
Pituitary LHRH receptors:		
a) fmoles/hemi-pituitary	43.5 ± 4.3 (10)	54.0 ± 6.6 (9)
b) fmoles/mg protein	65.7 ± 7* (10)	41.0 ± 6.8 (9)

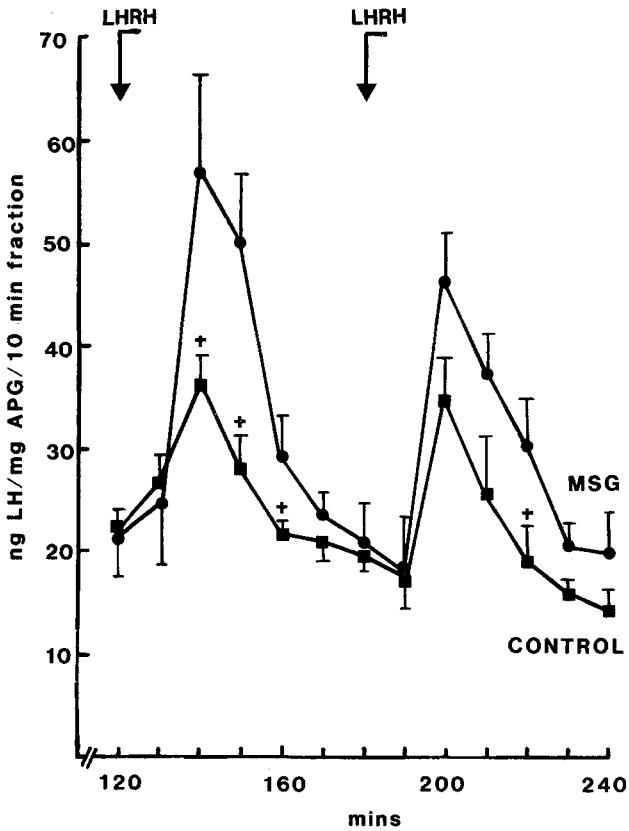

Figure 1. The LH responses of perfused anterior hemipituitary glands (APG) to two 5-min pulses of 10 ng/ml LH-releasing hormone (LHRH). Closed circles give the mean values obtained from ovariectomized adult rats treated neonatally with MSG (n = 8), open circles the controls (n = 7). Bars represent the SEM. $^{+}p < 0.05$, Student's t-test.

prior to a s.c. injection of 80 µg/kg 17 β-oestradiol benzoate (Sigma) dissolved in corn oil. Seventy-two hours later a second blood sample was taken before 10 mg/kg progesterone (Sigma) in corn oil was injected s.c.; two further blood samples (0.5 ml) were taken 5 and 6 h after the progesterone treatment.

LH and prolactin concentrations were measured by a double antibody radioimmunoassay using the reagents and protocol supplied by the National Hormone and Pituitary Program, Baltimore, MD, USA. The hormone concentrations are expressed in terms of NHPP rat LH–RP1 and rat prolactin – RP3, and the sensitivities of the assays were 10 µg/l and 1 µg/l respectively. The inter- and intracoefficients of variation of the assays were 10.1 and 9.0% for LH and 10.4 and 9.0% for prolactin. The concentration of LHRH in the hypothalamus was measured by a single antibody radioimmunoassay[10] using synthetic LHRH (Cambridge Research Biochemicals Ltd.) as standard. The antiserum to LHRH was a gift of Dr H. M. Fraser (M. R. C. Unit of Reproductive Biology, Edinburgh) and the sensitivity of the assay was > 30 ng/l; inter- and intra-assay variations were 10.7 and 7.0% respectively. Statistical significance was determined by an unpaired Student's t-test.

Results and discussion. Rats treated neonatally with MSG showed the expected reduction in ovarian and pituitary weights, small atrophied optic nerves and a stunting of body growth associated with increased subcutaneous fat (table). The low circulating concentrations of LH in MSG treated animals was correlated with an elevated hypothalamic content of LHRH (table), the mean value being more than three times higher than controls. This implies that reduced gonadotrophin secretion is not caused by a lack of LHRH but some impairment in the control of its release. Previous studies also report that neonatal MSG treatment lowers the mean circulating levels of LH[5] and attenuates the amplitude[6] or frequency[11] of pulsatile LH secretion in adult ovariectomized rats.

Despite the reduction in pituitary gland size, the in vitro pituitary LH responses to pulses of LHRH and the total number of pituitary LHRH receptors (table) were not significantly different between MSG-treated and control animals. In fact both the amount of LH released per mg of tissue (fig. 1) and the density of pituitary receptors per mg protein (table) is actually increased in MSG-treated rats after ovariectomy. An enhanced LHRH-induced LH release has been reported after neonatal MSG treatment from both intact[1,4] and ovariectomized rats[12] as well as an increase in the pituitary concentration of LH[4,13]. Thus, despite the reduced gonadotroph size[13] cells retain normal concentrations of LHRH receptors and immunoassayable LH.

The positive feedback effects of progesterone in oestrogen primed MSG-treated rats was greatly exaggerated in the majority of animals (Group 1) although in 3 of the rats (Group 2) there was virtually no response at all (fig. 2). While there is no apparent explanation for these divergent results, they may be related to the precise extent of the MSG-induced lesions of the arcuate nucleus.

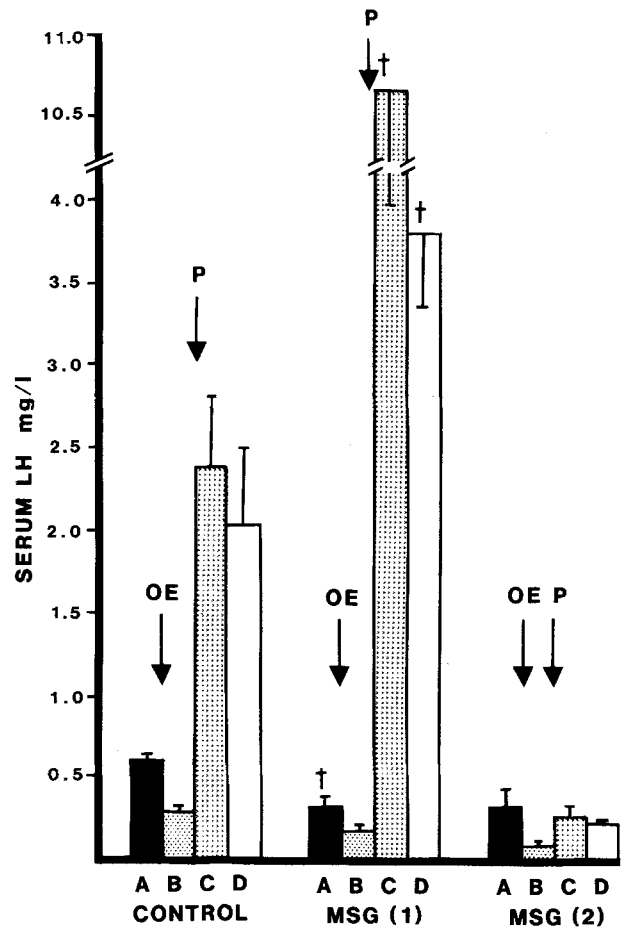

Figure 2. Feedback effects of exogenous steroids on LH secretion in ovariectomized rats treated neonatally with MSG. The first blood sample (A) was taken 3 weeks after ovariectomy just prior to 80 µg/kg oestradiol benzoate (OE). The second blood sample (B) was taken 72 h later and immediately before 10 mg/kg progesterone (P). The last two blood samples (C and D) were obtained 5 and 6 h after the progesterone treatment. Values show means and bars represent SEM's. $^{+}p < 0.05$ MSG (1) (N = 6) vs control (n = 8) Student's t-test. MSG (2) were excluded from the analysis because of the small group size (n = 3).

Although previous experiments have not shown exaggerated steroid feedback effects after MSG-treatment[7,14] the present results agree with the corroborative data on hypothalamic LHRH stores and LHRH receptors. It is suggested that while the destruction of the tuberoinfundibular dopaminergic system[2], or other calecholaminergic or peptidergic neurones[15], after MSG treatment reduces basal release of LHRH, the hypothalamus can be triggered to release substantial amounts of LHRH provided it is challenged with appropriate steroid feedback signals. The resultant LH surge is consequently amplified by the accumulated stores of LHRH which acts on a pituitary gland that has retained normal levels of LHRH receptors and responsiveness to LHRH, despite the reduction in gonadotroph size.

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17β-Estradiol-sensitivity of cultured myometrial cells

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Summary. The estrogen sensitivity of cells cultured from the rat myometrium was studied by growing the cells in the absence or presence of 1 nM 17β-estradiol. Following a time lag of ~ 10 days, exposure to estrogen resulted in increased incorporation of radiothymidine by the cells. Estrogen treatment also decreased isoproterenol-dependent and GTP-dependent adenylate cyclase activity, but had no effect on basal activity. These cultured cells have been shown previously to have some properties of uterine smooth muscle. The effects estrogen has in vitro, therefore, may reflect important properties in vivo that account for the mechanism by which the sex steroid decreases the sensitivity of the myometrium to isoproterenol.
Key words. β-Adrenergic catecholamines; estrogen; cAMP synthesis; myometrium.

The sex steroid hormones have well characterized effects on the patterns of uterine motility and their regulation by adrenergic catecholamines[1]. Estrogen (E_2) in general, has been associated with both an increase in uterine contractions and a decrease in sensitivity to the relaxing effects of β-adrenergic catecholamines[2,3]. The characterization of the mechanism that explains the effect of E_2 on β-adrenergic catecholamine sensitivity has remained ellusive.

Although E_2 decreases myometrial sensitivity, it increases β-adrenergic receptors in the rat[4]. One reason for these disparities may be that E_2 effects more than one cell type in vivo[3,5]. This complexity might be circumvented in tissue culture using myometrial cells with more homogeneous properties, and smooth muscle cells from the myometrium have been cultured in several laboratories. Although the cultured cells may lose their contractile properties, they retain other important properties of uterine smooth muscle cells. These include sensitivity to oxytocin and relaxin and receptors for acetylcholine and β-adrenergic catecholamines[6-9].

Myometrial cells growing in tissue culture also retain E_2 receptors and nuclear estrogen-binding sites[10]. Sex steroid sensitivity in vitro might include effects on β-adrenergic receptor-mediated cAMP production which could reflect the way E_2 effects uterine motility in vivo.

Materials and methods. Cell culture. Smooth muscle cells were obtained from the uteri of 21-day-old Sprague-Dawley rats and grown in cell culture as described by Fortier et al.[7]. Cells were grown to the stage of confluent monolayers in growth medium with normal serum, then changed to the same medium with charcoal extracted medium. Cells passaged in culture 4–6 times were used in the experiments described here.

Radiothymidine incorporation. 17β-Estradiol (E_2) was added on successive days at a final concentration of 1 nM to cultures which had been maintained in medium containing charcoal extracted serum. The cell culture medium was changed every 4 days and during these routine changes fresh E_2 was added to the cultures which had been growing in it previously. At the culmination of the experiment, which lasted up to 35 days, the medium was discarded and all of the cultures received fresh medium which contained 10 μCi per ml [^{3}H]thymidine (NEN, Boston). Incorporation of the radioisotope was allowed to continue for 3 h after which the radioactive medium was discarded and the cultures washed 3 times with phosphate buffered saline.

The contents of each culture dish was suspended with the aid of a plastic scraper in 1 ml of 0.2 N NaOH and the suspensions heated at 90 °C for 15 min. The solubilized contents were chilled and an equal volume of ice cold 20% TCA was added. The acid insoluble fraction was allowed to precipitate overnight at 4 °C. The precipitates were collected by vacuum filtration onto glass fiber filters, washed with 5% ice cold TCA, dried, dissolved in 90% NCS (Amersham) and counted in toluene based scintillation fluid.

Adenylate cyclase activity. In parallel experiments, cells were incubated in the absence or presence of estradiaol for 8 days then