Abnormal gonadotrophin release from pituitaries of muscular dystrophic mice and hamsters

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Summary. LHRH-stimulated LH and FSH secretion was studied in hemipituitaries, *in vitro*, obtained from several dystrophic mouse mutants (male: 129/ReJ-dy; 129B6F₁/J-dy; C57BL/6J-dy and C57BL/6J-dy^{2J}; female: 129B6F₁/J-dy) and a dystrophic hamster mutant (male and female CHF-147). Without exception, pituitary tissue from dystrophic animals released significantly more FSH than did tissue obtained from controls. LH secretion was more variable; in the male mice release was inhibited, whereas in the male dystrophic hamsters secretion was elevated above normal. The female mouse mutant pituitary released more LH whereas in the female hamster LH secretion was normal.

The reduction in body weight of the mutants studied could have contributed to the observations of impaired anterior pituitary function.

Introduction

The extensive literature devoted to the investigation of muscular dystrophy emphasizes the preoccupation of most research workers' studies on nerve and muscle. However, disorders of the endocrine system in general, and of the reproductive system in particular, appear to be a recognized feature of human myotonic dystrophy (see reviews by Harper, 1979; Morley & Melmed, 1979) and occurs in approximately 80% of men with this disease. Serum levels of gonadotrophins and prolactin varying from normal to highly abnormal are known to occur in these patients (Morley & Melmed, 1979; Canal *et al.*, 1982; Mahler & Parizel, 1982; Nappi *et al.*, 1982; Barreca *et al.*, 1983). The hypogonadism is accompanied by damaged or fibrosed seminiferous tubules. Leydig cells, however, are often normal in appearance (Morley & Melmed, 1979). In women the same degree of hypogonadism is apparently not present although amenorrhoea, oligomenorrhoea and early menopause have been described (Morley & Melmed, 1979).

Investigations into the reproductive systems of mutant dystrophic mice and hamsters are rare although it is known that these animals are difficult to breed (Heiniger & Dorey, 1980). A further brief report suggests that mice of the 129/ReJ-dy strain demonstrate a delay in sexual maturation (Harman, Tassoni, Curtis & Hollinshead, 1963).

In this paper we have examined the responsiveness of the anterior pituitary of mice and hamsters to stimulation with LHRH *in vitro*.

Materials and Methods

Experimental animals

The following male mouse mutants were obtained in groups of 6 from the Jackson Laboratory, Bar Harbor, ME, U.S.A.: 129/ReJ-dy, 129B6F₁/J-dy, C57BL/6J-dy and C57BL/6J-dy^{2J}. An

additional group of 6 female 129B6F₁/J-dy mutants was also examined. All mice were 5–7 weeks old and were shipped with an equal number of age-matched controls. Male and female dystrophic hamsters (CHF-147) were obtained from Canadian Hybrid Farms, Halls Harbour, Centreville, Nova Scotia, Canada. Control hamsters were of the golden Syrian strain of the same age (5 months).

The animals were allowed to acclimatize for at least 7 days under controlled lighting conditions (lights on 07:00–19:00 h). Food and water were freely available. We did not take into account the stage of the oestrous cycle.

Culture technique

Animals were killed by decapitation at 09:00–10:00 h. The brain was quickly removed and processed as described elsewhere (Wilkinson & Khan, 1982). Because of the cost of these animals we routinely examined pituitary tissue from animals that were also used for binding studies. Under binocular magnification the anterior pituitary was dissected free of the neural lobe and was then bisected *in situ*. Each half was placed on a separate stainless-steel grid in an organ culture dish containing 0.5 ml Medium 199 (Gibco, NY, U.S.A.) supplemented with Hepes buffer (0.01 M; Sigma, St Louis, MO, U.S.A.; sodium pyruvate (0.001 M) and 50 Units penicillin + 50 µg streptomycin/ml).

Cultures were incubated at 37°C in a water-saturated atmosphere of 5% CO₂/95% air for 120 min to allow stabilization of gonadotrophin release. The medium was then discarded and replaced, with or without LHRH (4 ng/ml; 3.4×10^{-9} M; Sigma), i.e. one half of each pituitary was stimulated with LHRH while the remaining half was used to determine basal (unstimulated) secretion. Each group normally consisted of 6 halves. Incubation was stopped at 120 min and 100 µl culture medium were diluted 2-fold with assay buffer (0.01 M-sodium phosphate in 0.15 M-sodium chloride containing 0.1% gelatin and 0.01% thimerosal; pH 7.6) before freezing at –80°C. LH and FSH levels were determined by radioimmunoassay with kits from NIAMDD (LH and FSH RP-1 standards) (Wilkinson, Moger & Selin, 1980). The sensitivities were 1.9 ng/tube for LH and 7.8 ng/tube for FSH. Samples from each experiment were always assayed in a single assay to avoid interassay variability. Intra-assay variation was 5% (LH) and 7.6% (FSH). These assays have been previously validated for use in the hamster (Berndtson & Desjardins, 1974) and mouse (Beamer, Murr & Geschwind, 1972). Statistical analysis was by Student's *t* test.

Results

The results in Tables 1 and 2 indicate that the dystrophic animals were always significantly smaller than the controls. This observation is in general agreement with the report by Cosmos, Butler, Mazliah & Allard (1980) on growth curves for the mouse mutant 129/ReJ-dy. However, pituitary gland weights were not consistently reduced except in the male and female CHF-147 hamsters. In female mice of the 129B6F₁/J-dy strain and in the female hamsters uterine weights were abnormal. However, the reduction in weight of the mouse uterus was not significant when corrected for body weight. In contrast, the dystrophic hamster uteri were significantly enlarged. We have not observed any differences in ovarian and testicular weights between dystrophic and normal animals (results not shown).

Without exception, pituitary tissue from dystrophic mice and hamsters released significantly more FSH than did control tissue after stimulation with LHRH (Tables 1 & 2). LH secretion was variable; e.g. tissue from 129B6F₁/J-dy female mice released more LH whereas in the males, LH release was reduced or unaffected compared to controls. In contrast, in the hamster, pituitaries from males released more LH whereas those from females exhibited normal secretion.

In a separate experiment, the pituitary content of LH and FSH was determined. In pituitaries of 129/ReJ-dy males (N = 8), LH values (796 ± 207 µg/mg tissue) were significantly lower ($P < 0.005$) than in control glands (1272 ± 158 µg/mg tissue). FSH content was identical in pituitaries from dystrophic and control mice (177 ± 19 and 179 ± 16 µg/mg tissue respectively).

Table 1. Body weights, hemipituitary weights and LHRH-stimulated LH and FSH release in male dystrophic mice and hamsters

Mouse/hamster strain	Body wt (g)	Hemipituitary wt (mg)	FSH†	LH†
Mouse 129/ReJ-dy	10.6 ± 0.2***	0.26 ± 0.02***	27 200 ± 12 000*	6400 ± 1200
Control	24.0 ± 0.5	0.48 ± 0.05	10 600 ± 1400	9000 ± 1200
Mouse 129B6F ₁ /J-dy	15.1 ± 1.6*	0.54 ± 0.12	7700 ± 450**	2300 ± 800**
Control	21.6 ± 2.5	0.44 ± 0.02	5200 ± 600	4000 ± 750
Mouse C57BL/6J-dy	16.8 ± 0.7**	0.42 ± 0.04	12 500 ± 1100***	2800 ± 650**
Control	24.8 ± 0.9	0.54 ± 0.04	3000 ± 450	4900 ± 500
Mouse C57BL/6J-dy ^{2j}	21.7 ± 0.3***	0.70 ± 0.12	13 400 ± 1500***	2750 ± 70*
Control	26.5 ± 0.4	0.77 ± 0.02	6250 ± 850	3400 ± 400
Hamster CHF-147	109 ± 5*	1.8 ± 0.1*	1500 ± 217*	2051 ± 262*
Control	121 ± 3	2.6 ± 0.3	1063 ± 58	1242 ± 250

Values are mean ± s.e.m. for 6 observations.

† Values of LH and FSH are ng/ml. mg⁻¹ and represent stimulated secretion, i.e. total hormone released minus basal (unstimulated) values at 2 h after addition of LHRH.

* $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$ compared to control values.

Table 2. Body weights, hemipituitary weights, uterine weights and LHRH-stimulated LH and FSH release in female dystrophic mice and hamsters

Mouse/hamster strain	Body wt (g)	Hemipituitary wt (mg)	Uterine wt (mg)	FSH†	LH†
Mouse 129B6F ₁ /J-dy	17.2 ± 0.2***	0.80 ± 0.04**	48.8 ± 6.9***	6000 ± 1100*	3600 ± 400*
Control	26.1 ± 0.1	1.10 ± 0.10	83.0 ± 4.5	2700 ± 150	1900 ± 400
Hamster CHF-147	98 ± 1**	2.5 ± 0.1**	395 ± 31**	732 ± 78***	1154 ± 125
Control	122 ± 2	3.3 ± 0.2	279 ± 18	442 ± 56	917 ± 61

Values are mean ± s.e.m. for 6 observations.

† Values are ng/ml. mg⁻¹ for stimulated secretion (see Table 1).

* $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$ compared to control values.

Discussion

In their review of gonadal dysfunction in systemic disorders, Morley & Melmed (1979) note that studies in patients with myotonic dystrophy show no consistent abnormalities of urinary gonadotrophin levels. However, the use of radioimmunoassay has revealed that, at least in men, basal serum LH and FSH as well as LHRH-stimulated levels are significantly elevated above normal (Harper, Penny, Foley, Migeon & Blizzard, 1972; Febres *et al.*, 1975; Sagel, Distiller, Morley & Isaacs, 1975; Takeda & Ueda, 1977). Our results for dystrophic mice and hamsters reveal some similarities. For example, LHRH-stimulated FSH secretion from hemipituitaries was consistently and significantly higher in all mutants examined. On the other hand, we observed no large differences in basal (unstimulated) FSH release (results not shown). This latter observation reflects an influence of our culture technique, i.e. the medium from the initial preincubation (120 min) is routinely discarded, a step which obscures any differences in basal secretion.

Our results show that FSH release is selectively increased, because in all the mutants except the female 129B6F₁/J-dy, LH output is either reduced or unchanged.

In view of the significantly lower body weights seen in all the mutants examined, studies on the effects of body mass on pituitary response to LHRH are obviously desirable. In men, loss of body weight through starvation results in a blunted pituitary response to LHRH (Klibenski, Beitins, Badger, Little & McArthur, 1981). Similar results are seen in the rat (Howland, 1980). In women,

severe weight loss is associated with reduced basal and LHRH-stimulated release of gonadotrophins (see for example Warren, 1977), although more recent studies suggest that sub-maximal doses of LHRH can give peak responses of FSH which are significantly greater than normal (Vigersky, Loriaux, Andersen, Mecklenburg & Vaitukaitis, 1976; Travaglini *et al.*, 1976). These studies indicate that the lower body weight of the dystrophic animals could contribute to the abnormal pituitary response to LHRH.

In conclusion, our results suggest that there are some similarities between the effects of muscular dystrophy in animals and myotonic dystrophy in man, at least insofar as anterior pituitary function is concerned.

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