Measurement of plasma LH concentrations in aged male rodents by a radioimmunoassay and a radioreceptor assay

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Summary. Circulating plasma concentrations of LH from young mature (3-4 months old), middle-aged (15-18 months old) and aged (31-32 months old) male C57BL/6 mice, Syrian hamsters (3-4, 19-20 and 24-25 months old), Fischer 344 rats (3-4, 18-19 and 28-29 months old), Chinese hamsters (3-4, 19-20 and 29-30 months old) and Mongolian gerbils (3-4 and 19-22 months old) were analysed using a radioimmunoassay (RIA) and a radioreceptor assay (RRA). Male rats exhibited the greatest changes with advancing age: the oldest rats had an almost undetectable quantity of plasma LH, as measured by both assays. In contrast, the oldest male Syrian hamsters had significantly higher levels of LH than did younger animals. A significant decrease occurred in the amounts of LH detectable by RRA in middle-aged Chinese hamsters which was not evident with the RIA. There were no statistically significant differences in LH levels of C57BL/6 mice and gerbils with increasing age. The mean RRA:RIA ratios indicated that age-related differences in LH concentrations resulted from physiological changes in the secretion or the metabolic clearance of LH and not from changes in the biological potency of LH.

Introduction

Parkening, Collins & Smith (1980) have shown that concentrations of plasma luteinizing hormone (LH), as measured by RIA, in aged female mice (cyclic and constant dioestrous 16-20 months old) are significantly higher than those of young mature (2-4 months old) females during oestrus. Plasma LH levels in aged (13-17 months old) anoestrous Syrian hamsters were also statistically higher than those found in younger (3-5 months old) dioestrous females (Parkening, Collins, Lau & Saksena, 1982b). In contrast, levels of plasma LH from aged (14-18 months old) repetitively pseudopregnant Wistar rats were significantly lower than those of younger (3-5 months old) dioestrous females (Parkening *et al.*, 1982a). When plasma from the three species of animals was analysed in a radioreceptor assay (RRA) the younger females had concentrations of LH similar to those measured by RIA for that species (Parkening *et al.*, 1982a). This was also true for aged Wistar rats but for aged oestrous and constant dioestrous mice and aged anoestrous hamsters, the RRA levels of LH were significantly lower than those found by RIA (Parkening *et al.*, 1982a). This suggested that age-related changes in the pituitary of these species may result in qualitative changes in the LH molecule. Such a molecule, detectable by RIA, may have little biological potency. Because of the differences amongst the aged females of the various species examined it was of interest to determine whether similar differences existed in the LH concentrations of aged male rodents.

Materials and Methods

Young mature (3–4 months old), middle-aged (15–18 months old) and aged (31–32 months old) C57BL/6 mice acquired from Timco Breeding Laboratories (Houston, Texas), Syrian hamsters (3–4, 19–20 and 24–25 months old) purchased from Sasco Inc. (Omaha, Nebraska), Fischer 344 rats (3–4, 18–19, 28–29 months old) acquired from the National Institute on Aging Colony maintained by Charles River Breeding Laboratories, Wilmington, Massachusetts, and Chinese hamsters (3–4, 19–20 and 29–30 months old) and Mongolian gerbils (3–4 and 19–22 months old) born and raised in our colony at The University of Texas Medical Branch (stock lines from Chick Line Company, Vineland, New Jersey and Tumberbrook Farm, West Brookfield, Massachusetts) were used in these studies. The animals were housed 2–3 per cage in separate animal rooms provided with uniform temperature (21–23°C) and lighting (14 h light : 10 h darkness) and provided with food and water *ad libitum*. The diet of both species of hamster and the gerbils was supplemented once a week with lettuce and carrots. All of the animals except the rats had been used for breeding purposes during some stage of their life.

All blood samples were collected between 10:00 and 12:00 h and the plasma was stored frozen (–80°C) for 1 week before analysis. The mice and Chinese hamsters were bled unanaesthetized by cardiac puncture; the Syrian hamsters were bled under light ether anaesthesia from the abdominal vena cava, and the rats and gerbils were bled by decapitation. Sufficient plasma was acquired from each animal to permit simultaneous assay for LH concentrations by RIA and RRA.

The radioiodination of LH was conducted according to the procedures of Lee & Ryan (1973) by reacting 10 µg rat LH-I-5 with 1 mCi Na¹²⁵I (Amersham, Arlington Heights, Illinois) in the presence of 25 µg chloramine-T in 25 µl buffer (0.05 M-PBS) for 30 sec at 4°C. The reaction was terminated with 25 µg sodium metabisulphite in 25 µl buffer. The labelled hormone was purified by chromatography on a Sephadex G-75 column and the labelled LH fraction with the highest immunoreactivity was used for the RIA and RRA. Both assays were begun on the same day.

For RIA studies, single samples of plasma, except for duplicate samples from the Syrian hamsters and rats, were analysed by using a protocol similar to that of Niswender, Reichert, Midgley & Nalbandov (1969). Details of the assay procedures have been published elsewhere (Parkening *et al.*, 1980, 1982a; Parkening, Calcote & Collins, 1981). Maximum binding of the iodinated hormone by the antiserum was 28.7%. Samples were analysed in one assay for the rat and gerbil. For the mouse and both species of hamsters the inter-assay coefficient of variance was 6.7%. The intra-assay coefficient of variance was less than 10%.

The RRA was conducted according to the procedures of Lee & Ryan (1972) and was identical to the methods published previously (Parkening *et al.*, 1980), except that 1% Celite was added to the buffer used to wash the membrane pellets. Single samples were assayed for all species except for duplicate samples of the Syrian hamster and rat. The maximum binding of the iodinated hormone by the homogenate in the absence of the standard or sample was 24.5%. As in the RIAs rat and gerbil samples were analysed in one assay and the inter-assay coefficient of variance for the other species was 15.1%. The intra-assay coefficient of variance for all species was less than 10%.

All data were statistically evaluated by one-way analysis of variance or the Student's *t* test.

Results

As shown in Text-fig. 1, the mean ($\pm$ s.e.m.) slopes of the dose-response curves from 4 separate determinations for plasma LH of each species (mouse, 2.56 ± 0.16 ; Syrian hamster, 2.20 ± 0.16 ; rat, 2.26 ± 0.08 ; Chinese hamster, 2.23 ± 0.15 ; gerbil, 2.29 ± 0.19) and the standard (2.42 ± 0.09) were not statistically significantly different when tested by one-way analysis of variance (*F* ratio = 1.43).

No differences existed in plasma levels of LH measured by RIA or RRA when comparing older mice or gerbils with levels obtained from their younger counterparts (Table 1). However, there were

Text-fig. 1. A comparison of the parallelism between the dose-response curves for NIAMDD-rat LH-I-5 and plasma from 5 species of male laboratory rodents. The RRA utilized 5 mg equivalents of rat ovarian receptors and ^{125}I -labelled rat LH. The slopes of the curves shown are similar to the mean slopes calculated from 4 different curves for each species or standard (see text).

Table 1. Plasma concentrations of LH as measured by radioimmunoassay (RIA) and an ovarian radio-receptor assay (RRA) in young mature and aged male rodents

Species and age (months)	No. of animals	Mean $\pm$ s.e.m. LH conc. (ng/ml)		Mean $\pm$ s.e.m. RRA:RIA
		RIA	RRA	
C57BL/6 mice				
3-4	10	12.7 $\pm$ 0.7	10.9 $\pm$ 0.7	0.82 $\pm$ 0.05
15-18	16	15.1 $\pm$ 1.3	10.5 $\pm$ 0.7	0.74 $\pm$ 0.05
31-32	5	9.2 $\pm$ 1.7	6.7 $\pm$ 1.9	0.70 $\pm$ 0.08
Syrian hamsters				
3-4	8	10.2 $\pm$ 1.3* (5.24)	8.5 $\pm$ 1.0** (8.65)	0.88 $\pm$ 0.06
19-20	9	13.0 $\pm$ 2.3	8.7 $\pm$ 0.8	0.74 $\pm$ 0.07
24-25	7	19.0 $\pm$ 1.8	17.6 $\pm$ 3.0	0.90 $\pm$ 0.08
Fischer 344 rats				
3-4	20	9.1 $\pm$ 0.9*** (29.63)	8.3 $\pm$ 0.9*** (25.27)	0.94 $\pm$ 0.06*** (13.87)
18-19	19	6.1 $\pm$ 0.8	5.7 $\pm$ 0.6	1.01 $\pm$ 0.06
28-29	16	1.1 $\pm$ 0.1	1.6 $\pm$ 0.2	1.66 $\pm$ 0.18
Chinese hamsters				
3-4	11	6.7 $\pm$ 0.7	5.4 $\pm$ 0.4* (4.91)	0.87 $\pm$ 0.07
19-20	14	5.0 $\pm$ 0.4	3.6 $\pm$ 0.2	0.75 $\pm$ 0.05
29-30	7	6.5 $\pm$ 1.4	6.3 $\pm$ 1.4	0.96 $\pm$ 0.12
Mongolian gerbils				
3-4	20	17.6 $\pm$ 1.3	15.8 $\pm$ 1.1	0.91 $\pm$ 0.05
19-22	22	18.3 $\pm$ 1.1	15.9 $\pm$ 1.1	0.87 $\pm$ 0.03

Values that are statistically significantly different within each group, as determined by one-way analysis of variance (F-ratios are given in parentheses): * P < 0.05, ** P < 0.002, *** P < 0.001.

significant differences in the LH concentrations in the 3 age groups of the Syrian hamsters, rats and Chinese hamsters (Table 1). The oldest Syrian hamsters (24–25 months old) had higher concentrations of LH, as measured by both assays, than did the younger animals. In contrast, the rats showed a progressive decline in LH concentrations with increasing age in both assays. A drop in the quantity of LH measurable by RRA in 19–20-month-old Chinese hamsters also resulted in statistically significant differences within this group.

Comparing RIA and RRA values within individual age groups of each species, statistically significant differences (Student's *t* test) existed in 15–18-month-old mice ($P < 0.005$), 28–29-month-old rats ($P < 0.05$) and 19–20-month-old Chinese hamsters ($P < 0.005$).

A comparison of the mean RRA : RIA ratios of each species indicated differences only within ageing rats (Table 1).

Discussion

The present study indicates that there is apparently very little change in the biological potency of the plasma LH measured by RRA in the aged male rodents examined. This is in contrast to the results for females of some of the same species; in females the RRA : RIA ratio of LH decreases significantly with age, suggesting that there is a decrease in the biological potency of LH (Parkening *et al.*, 1982a). The lower LH values found by RIA in the old Fischer 344 male rats were confirmed by RRA. The increase in the mean RRA : RIA ratio of the 28–29-month-old male rats probably results from the small quantity of detectable LH, since the limits of sensitivity of the RIA assay (1 ng LH/tube) were approached. In rats, therefore, at least those of the Fischer 344 strain, the decreased LH levels result from lower plasma levels in the aged animal and not from changes in the potency of LH secreted by the anterior pituitary gland.

The lower levels of LH detectable in ageing male Fischer 344 rats was not unexpected in view of a similar report by Bethea & Walker (1979). RIA studies with other strains of rats indicate that LH levels decrease with advancing age in intact rats, castrated male rats receiving exogenous hormones, and in LH-RH-treated rats (Shaar, Euker, Riegle & Meites, 1975; Riegle & Meites, 1976; Miller & Riegle, 1978b; Gray, 1978; Saksena & Lau, 1979). Testosterone has also been shown to decline in rats older than 14 months of age whether measured *in vivo* or *in vitro* (Ghanadian, Lewis & Chisholm, 1975; Chan, Leathem & Esashi, 1977; Gray, 1978; Miller & Riegle, 1978a). Miller & Riegle (1978a) reported that the testicular response to hCG, as measured by serum testosterone concentrations, was reduced in aged male rats, 20–30 months of age, although if 5 i.u. hCG/100 g body-weight were given s.c. for 7 days, the levels of testosterone were similar in young mature and aged rats, an indication that the testes of aged animals still had the ability to secrete testosterone. It has been suggested that the primary cause of reduced testosterone levels in the aged male rat results from inadequate levels of gonadotrophins. Sufficient LH-RH appears to be present in hypothalamic extracts from aged male rats (Riegle, Meites, Miller & Wood, 1977), but the concentrations of hypothalamic dopamine and catecholamines, which are thought to regulate the release of LH-RH, are reduced (Miller, Shaar & Riegle, 1976; Simpkins, Mueller, Huang & Meites, 1977). Conn, Cooper, McNamara, Rogers & Shoenhardt (1980) suggested that the LH molecule in the aged male rat (NIH hybrids of the Dublin strain) may have a higher molecular weight (determined by gel filtration) than that found in younger males. It was postulated that this higher molecular weight form of LH originates because of increased glycosylation which alters the structure of the LH molecule in the pituitary and circulation. However, even if the LH molecule is altered in male Fischer 344 rats it is still capable of binding to ovarian receptor sites, as shown by the RRA in the current study.

Kaler & Neaves (1981) suggested that circulating plasma LH and testosterone values in 3- and 24-month-old male Sprague–Dawley rats remained relatively constant when considering that the

body weight had increased almost 50% in the older rats. They concluded that the lower testosterone levels in 24-month-old rats resulted from a dilution of the hormone within an expanded volume of plasma and therefore considered that the amounts of LH had actually increased in the older rats. Plasma dilution of circulating LH was not a factor in the present study because the 28–29-month-old Fischer 344 rats weighed less (mean $\pm$ s.e.m. 375.9 ± 42.3 g) than the 18–19-month-old rats (422.5 ± 96.1 g). The mean $\pm$ s.e.m. testicular weight on a 100 g body weight basis in 28–29-month-old rats was twice (2.2 g) that of 3–4-month-old (1.1 g) and 18–19-month-old (1.0 g) rats. Morphologically, however, most testes of the oldest rats contained numerous opaque seminiferous tubules and accumulations of a clear fluid beneath the tunica albuginea. The fluid was an important component of the increased weight of the testis: 3 of the 16 28–29-month-old rats had one extremely atrophied testis. The low LH concentrations in the oldest rats probably led to low testosterone values because there was a marked reduction in seminal vesicle weight (57.2 ± 5.0 mg compared with 367.3 ± 13.2 and 303.1 ± 15.0 mg in 3–4- and 18–19-month-old rats, respectively).

The patterns of LH secretion by C57BL/6 mice in the present study agree with the findings of Finch *et al.* (1977) who reported no major differences in the concentrations of circulating LH in 12- and 28-month-old male C57BL/6 mice. Similar levels of serum LH have also been found in young mature and 24-month-old CB F₁ mice, provided the older males were robust and sexually active (Bronson & Desjardins, 1977). In contrast to the aged rat, testosterone concentrations in 6-, 12-, 20- and 28-month-old C57BL/6 mice (Eleftheriou & Lucas, 1974), 8–11- and 29–30-month-old C57BL/6 mice (Nelson, Latham & Finch, 1975) and in young mature and 24-month-old CB F₁ mice (Bronson & Desjardins, 1977) were similar, provided the males were visibly healthy and free of pathological lesions.

The higher concentrations of LH in 24–25-month-old Syrian hamsters were unexpected, since there were no statistically significant differences in the values in 3–4- and 19–20-month-old animals. The testes from the oldest Syrian hamsters were normal in appearance and weight. It is not known whether the differences between age groups are a reflection of the small number of animals or whether the Leydig cells are steroidogenically deficient.

The results obtained in this study with younger males of each species agree with those reported for young male rats by Solano, Dufau & Catt (1979), who used an in-vitro bioassay consisting of cultured rat interstitial cells in which testosterone release was stimulated by rat LH. Solano *et al.* (1979) found that the LH levels determined by bioassay and RIA were identical, provided a highly purified LH preparation was used as a standard for the assays; if the NIAMDD LH-RP-1 standard was used significantly higher levels of LH were obtained in the bioassay than in the RIA. Similar results have been reported for rhesus monkeys when LH levels were measured by an RIA and RRA (employing porcine granulosa cells in culture) with ordinary NIAMDD standards and a purified LH standard (Sakai & Channing, 1979). We also experienced similar effects when using the NIAMDD LH-RP-1 standard in the RRA chosen for our studies, which is why the more purified NIAMDD LH-I-5 was selected for the standard and for radioiodination. Solano *et al.* (1979) suggested that the discrepancies in their study resulted from immunoreactive material in the LH-RP-1 standard that was biologically inactive, thereby causing an artificially low estimate of the immunoreactive LH present in samples assayed against this standard.

From the present study it appears that LH secretion in the male Chinese hamster, gerbil and C57BL/6 mouse is unaffected by ageing; whereas LH secretion increases with age in the aged Syrian hamster and decreases with age in senescent rats. Additional studies with a larger number of animals are needed to check this finding for male Syrian hamsters, but the age-related changes occurring in the hypothalamic–pituitary–testicular complex of the rat are apparently different from those of other laboratory rodents.

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