J. Steroid Biochem. Molec. Biol. Vol. 37, No. 3, pp. 421–430, 1990
Printed in Great Britain.

0960-0760/90 $3.00 + 0.00

Steroids and Aging

NEUROENDOCRINE REGULATION OF PULSATILE LUTEINIZING HORMONE SECRETION IN ELDERLY MEN

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Summary—Leydig cell function is driven by LH, secreted in a pulsatile manner by the anterior pituitary in response to episodic discharge of hypothalamic LHRH into the pituitary portal circulation, under control of a yet to be defined neural mechanism, the "hypothalamic LHRH pulse generator". The normal aging process in elderly men is accompanied by a decline in Leydig cell function. Whereas primary testicular factors undoubtedly play an important role in the decrease of circulating (free) testosterone levels with age, recent studies demonstrated that aging also affects the central compartment of the neuroendocrine cascade. Hypothalamic alterations comprise changes in the regulation of the frequency of the LHRH pulse generator with an inappropriately low frequency relative to the prevailing androgen impregnation and opioid tone, and with an increased sensitivity to retardation of the LHRH pulse generator by androgens. As observed by some authors in basal conditions and by others after endocrine manipulations, LH pulse amplitude seems also to be reduced in elderly men as compared to young subjects. This is most probably the consequence of a reduction in the amount of LHRH released by the hypothalamus. Indeed, challenge of the gonadotropes with low, close to physiological doses of LHRH in young and elderly men reveals no alterations in pituitary responsiveness when looking at either the response for immunoreactive LH or bioactive LH. Deconvolution analysis on data obtained after low-dose LHRH suggests a markedly prolonged plasma half-life of LH in elderly men, a finding which may explain the paradoxical increase of mean LH levels in face of the reduced or unchanged frequency and amplitude of LH pulses.

INTRODUCTION

The normal aging process in men is accompanied by a progressive decline in endocrine gonadal function [1–3]. It is firmly established that primary testicular factors play an important, and probably predominant, role in the decline of Leydig cell function in elderly men. However, it has been pointed out in more recent studies that aging also affects higher compartments of the hypothalamo–pituitary–Leydig cell axis. Our intention is to give an outline of the neuroendocrine regulation of Leydig cell function in elderly men, as can be distilled from the information presently available.

Proceedings of the XIV Meeting of the International Study Group for Steroid Hormones, Rome, Italy, 30 November–2 December 1989.

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NEUROENDOCRINE REGULATION OF LEYDIG CELL FUNCTION

Pituitary control of Leydig cell function

Leydig cell function is driven by pituitary luteinizing hormone (LH). In men, as is the case in women and in all mammals studied to date, LH is secreted by the gonadotropes in a pulsatile manner. The occurrence of discrete pulses of increased LH concentration in the systemic circulation can be readily demonstrated by immuno- or bioassay of LH on serially obtained blood samples [4, 5].

In healthy young men, the mean LH interpulse interval is about 120 min. However, there is considerable variation in LH pulse frequency both within and between individuals, with intervals as short as 30 min and as long as 480 min being recorded [6, 7]. Large within and between subject variations are also observed for the amplitude of the LH pulses [6].

Episodic exposure of the Leydig cells to pulses of LH results, in turn, in intermittent increases in testosterone secretion. Although the occurrence of pulses of testosterone can be demonstrated by repeated sampling from either the spermatic veins or the peripheral circulation, the pattern of testosterone pulsatility is much less clear than is the case for LH. Nevertheless, a temporal relationship between LH pulses and increments in testosterone plasma levels has been reported, with estimates of time-lag between LH and testosterone secretion ranging from 10 to 40 min [6, 7].

Hypothalamic control of LH secretion

The pulsatile mode of LH secretion is caused by the episodic discharge of a bolus of hypothalamic luteinizing hormone-releasing hormone (LHRH) into the pituitary portal circulation. The neuronal mechanism which governs the rhythmic activation of the LHRH cells to release the neuropeptide from their nerve-terminals in the median eminence is unknown but has, for convenience, been called the "LHRH pulse generator". In the rhesus monkey, an animal model close to the human, it has been shown that the "LHRH pulse generator" is localized in the mediobasal hypothalamus and that its proper functioning at an adequate frequency is essential for maintenance of normal reproductive function in both sexes [8, 9].

LH pulsatility as correlate of hypothalamic episodic LHRH release

Characteristic, abrupt increases in multi-unit electrical activity, associated with the initiation of each pulse of LH as monitored by serial venous blood sampling, have been recorded from the mediobasal hypothalamus of rhesus monkeys [10]. An unambiguous unitary relationship between these characteristic volleys of hypothalamic multi-unit electrical activity and plasma LH pulses has been observed under various experimental conditions, including pharmacological manipulations of LH pulse frequency [11]. In other experiments, a similar one-to-one relationship has been observed between LHRH pulses measured in portal blood and simultaneously monitored LH pulses in the systemic circulation [12, 13]. Furthermore, pulsatile intravenous administration of exogenous LHRH to rhesus monkeys with lesioned hypothalamus and abolished endogenous LHRH secretion or, to humans with absent LHRH secretion due to a congenital defect, reliably elicits pulses of LH, provided the frequency of LHRH administration does not markedly depart from the range of LH pulse frequencies observed in physiological situations [9, 14, 15]. From these observations, it can be inferred that the frequency of LH pulses, which can be monitored in the peripheral circulation, usually is a reliable reflection of the frequency of the hypothalamic "LHRH pulse generator".

Reports that (endogenous or exogenous) LHRH pulses may occasionally not be followed by a detectable LH pulse [12, 15], should not invalidate the essence of the latter conclusion. A matter for more concern is that the validity of any conclusion regarding the frequency of the "LHRH pulse generator" based on estimates of LH pulse frequencies is critically dependent on the soundness of the applied methodology for monitoring of LH pulsatility. The importance, for valid LH pulse frequency estimates, of factors such as LH assay characteristics, sampling frequency and pulse-identification criteria is now well recognized [16]. However, continuous refinement of the applied techniques may paradoxically introduce other, conceptually more troublesome problems. Indeed, high blood sampling frequencies and the use of highly performing LH dosage techniques may lead to the detection of small, statistically fully validated LH pulses which, although real, no longer correspond to episodes of activation of the "LHRH pulse generator", but rather to LHRH independent variations in the rate of LH secretion, shifts in body fluids, or sampling artefacts. Clearly, the limitation inherent to the indirect monitoring of "LHRH pulse generator" activity through monitoring of LH pulse frequency, the only available approach for studies in the human, will not be overcome by pushing farther the sophistication of LH pulses detection methods.

While the interpretation of LH pulse frequency seems relatively straight forward, interpretation of LH pulse amplitude is far more complicated. The ampitude of LH pulses is probably determined by a complex interplay of multiple factors which include the intrinsic responsiveness of the gonadotropes, the size of the released LHRH bolus, the frequency of LHRH stimulation, the magnitude of—and the time elapsed since—the preceding LHRH bolus [14, 17]. Moreover, the interpretation of

LH pulse amplitude is further complicated by preferential release of biologically active forms of LH during stimulation of the gonadotropes by either endogenous or exogenous pulses of LHRH, as indicated by an intrapulse increase of the ratio of bioactive-over immunoreactive-LH serum concentrations [5, 18]. Clearly, monitoring of changes in LH pulse amplitude do not allow, *per se*, any conclusion regarding either hypothalamic LHRH release or pituitary responsiveness.

Circadian rhythm of LH and testosterone secretion

The occurrence of a diurnal variation in LH secretion has been an inconstant finding among the studies which addressed this question. This may be due to differences in the applied methodology or to differences in subject selection criteria, since inspection of data from individual subjects reveals a nycthemeral rhythm in some subjects and absence of circadian variations in others [6, 19]. A diurnal variation in testosterone levels across study populations of young men has been a more constant finding [3, 19, 20], but again, this circadian rhythm is not present in all young individuals [6, 19].

Feedback regulation of LH secretion by sex steroids

In rhesus monkeys, bilateral orchidectomy results, within 24 h, in an acceleration of the LH pulse frequency from 1 pulse every 2–4 h to the typical circhoral rhythm of the long-term castrate. Similarly, LH pulse frequency is higher in men with primary testicular hypogonadism than in eugonadal men. Both in the castrated monkeys and in the hypogonadal men, testosterone replacement progressively decelerate LH pulsatility and restores a pattern of pulsatile LH characteristic for the eugonadal status [9, 21]. In eugonadal men, administration of androgens results in a further deceleration of LH pulsatility to frequencies lower than the physiological rhythm [22, 23], whereas administration of a pure anti-androgen accelerates LH pulsatility [24]. Foregoing observations clearly indicate that testosterone exerts a negative feedback action on the frequency of the hypothalamic "LHRH pulse generator". Plant [9] provided elegant indirect evidence indicating that this modulation of the frequency of episodic LHRH secretion by testosterone is, in fact, the major mechanism underlying the negative feedback control of LH secretion by the testes.

Non-aromatizable androgens appear to decelerate LH pulse frequency as effectively as testosterone, which suggests that the negative feedback effects of testosterone on the pulse generator are exerted through an androgen receptor-dependent mechanism and do not require prior aromatization of testosterone to estrogens [9, 22, 23]. Consistent with the view that aromatization of testosterone does not play a major role in the negative feedback effects of this steroid on the "LHRH pulse generator", are the observations that in the female, LH pulse frequency in the follicular phase of the cycle is not slower than in castrated females, despite exposure to increasing estradiol concentrations. Moreover, the effects of androgen administration on LH pulsatility in eugonadal men were not reproduced when estradiol was infused [22, 23]. More difficult to reconcile with foregoing conclusions are the observations of enhanced LH pulse frequencies in normal men treated with compounds, such as clomiphene citrate and tamoxifen HCl, which possess anti-estrogen properties [25, 26]. Although the latter findings may suggest that some estrogen activity is necessary for full expression of the feedback action of androgens on the "LHRH pulse generator", their interpretation is difficult since the mechanisms underlying the effects of these anti-estrogenic compounds are unknown and may involve actions not mediated by estrogen receptors.

As far as the relative importance of the feedback effects of androgens and estrogens at the pituitary level is concerned, quite opposite conclusions seem to hold true when compared to the effects of these steroids at the level of the "LHRH pulse generator". Neither the amplitude of spontaneous LH pulses, nor the LH response to exogenous LHRH seem to be reduced by androgen administration in young men [22, 23, 27]. LH pulse amplitude is also unaffected by administration of an anti-androgen [24]. In rhesus monkeys with abolished endogenous LHRH secretion by hypothalamic lesioning, orchidectomy had no marked effects on the amplitude of the LH pulses in response to administration of exogenous LHRH at a fixed pulsatile regimen [9]. Estrogen administration to young men, on the contrary, results in a reduction of the amplitude of endogenous LH pulses and of the LH response to exogenous LHRH [22, 23, 27]. This, taken together with the lack of effect of such a treatment on the frequency of LH pulses,

suggests a negative feedback effect of estrogens at the level of the pituitary. In the female primates it has been well documented that the main feedback effects of estrogens are exerted at the level of the pituitary [14].

Role of opioidergic systems in androgen feedback regulation of LH

Several lines of evidence support the concept that the negative feedback action of androgens at the level of the "LHRH pulse generator" is mediated, at least in part, by increased opioidergic activity [9]. Endogenous opioid blockade accelerates LH pulse frequency in eugonadal men [22, 28–30]. The effect of opioid receptor blockade on the frequency of the "LHRH pulse generator" is no longer demonstrable in hypoandrogenic men [31] nor in men pretreated with an anti-androgen [24]. Furthermore, the response to opioidergic blockade can be reinstated by androgen substitution therapy [31] and the effect of 5α-dihydrotestosterone (DHT) to slow down the frequency of pulsatile LH in eugonadal men can be overridden by simultaneous opioid receptor blockade [22]. Besides modulatory effects on the frequency of the hypothalamic "LHRH pulse generator", endogenous opiates may also have additional effects at other levels of the reproductive axis [32].

DECLINE OF LEYDIG CELL FUNCTION IN ELDERLY MEN

It is no longer a subject of controversy that Leydig cell function declines in elderly men. There is an age-dependent decrease in the plasma levels of testosterone, apparent free testosterone and bioavailable (not bound to SHBG) testosterone [1–3, 26]. As a consequence of a reduction in the nycthemeral variations in plasma testosterone levels, the relative deficiency in Leydig cell function is more apparent when assessing morning testosterone levels or mean 24 h testosterone levels [3, 19, 20]. Primary testicular factors undoubtedly play an important role in this decline of testosterone levels with age, a view supported by observations in elderly men of decreased numbers of Leydig cells, of impaired testicular perfusion and of altered testicular steroid biosynthesis. The testosterone response to hCG administration is clearly lower in elderly men than in young men, as is also the testosterone response to increased endogenous LH secretion during treatment with clomiphene citrate. Consistent with a primary testicular deficiency in aging men is the moderate, but significant, rise in gonadotropins which accompanies the decline in testosterone levels [1–3, 26, 27, 33].

EVIDENCE FOR ALTERED REGULATION OF LH SECRETION IN ELDERLY MEN

There have been reports of a somewhat delayed and protracted pattern of LH secretion in response to exogenous LHRH [2, 33] and of a decreased bio/immuno LH ratio in the peripheral circulation in basal conditions as well as after LHRH stimulation in elderly men [34, 35]. Furthermore, there is a marked blunting of the circadian rhythmicity in blood testosterone levels in the elderly [3, 19, 20]. Such findings have been put forward as arguments for the occurrence of changes in the hypothalamo–pituitary compartment of the gonadal axis in elderly men. The fact that, in face of persistently decreased (free) testosterone levels, LH secretion fails to increase further such as to restore androgen levels typical of young men, seems a more fundamental argument in support of this point of view and suggests a resetting of the sensitivity of the system to the negative feedback actions of testosterone. Indeed, the maximal secretory capacity of the gonadotropes in elderly men seems rather intact [2, 23, 27, 33] and the testicular response to hCG, albeit reduced, shows the existence of a residual secretory capacity of the Leydig cells [2]. As will be discussed in the next section, detailed study of the pulsatile patterns of LH secretion confirmed that the aging proces affects not only the testicular, but also the central compartment of the hypothalamo–pituitary gonadal axis.

NEUROENDOCRINE REGULATION OF PULSATILE LH SECRETION IN ELDERLY MEN

LH pulsatility in elderly men

Several groups have performed comparative studies of the patterns of episodic LH release in young and in elderly men. The results of these studies were not uniform. Several studies failed to demonstrate changes in the frequency of pulses of immunoreactive LH [18, 23, 26] or bioactive LH [35], while other studies showed a reduced pulse frequency for bioactive [36] or immunoactive LH in elderly men [27, 37]. Whereas we found a significant decrease in the frequency of immunoreactive LH pulses in elderly men in an earlier study [27], when using

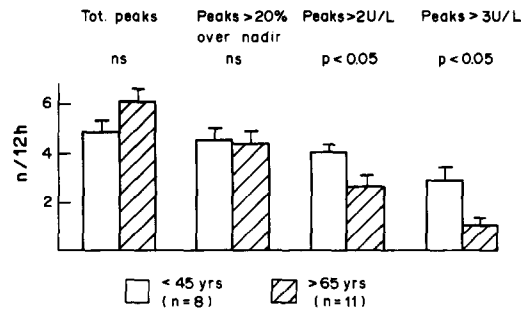

Fig. 1. LH pulse frequency in young and elderly monks as assessed by (oligoclonal) RIA on serum samples obtained at 10 min intervals. Mean ± SE for all pulses (increase >3 times dose-adjusted intra-assay t.v.), for pulses with >20% increase above nadir, pulses with amplitude >2 U/l and pulses with ampitude >3 U/l. Adapted from the data in Ref. [30].

a radioimmunoassay with polyclonal antibody for assay of LH on samples obtained at 20 min intervals [27], we found no significant difference in LH pulse frequency in a later, study with use of a more specific oligoclonal immunoradiometric assay for LH and a higher sampling frequency at 10 min intervals [30]. The contradiction between the results of these two studies may be only apparent and probably resides in the methodological differences. Indeed, it is likely that in the first study, mainly pulses with larger amplitude were detected as a consequence of the lower sampling frequency and the less performing LH dosage technique. The second study, while showing an equal total number of LH pulses in young and elderly men, showed a clear reduction in the number of pulses with larger amplitude (> 2 U/l) (Fig. 1).

Taken together, the results of the foregoing studies indicate that if any deceleration of the "LHRH pulse generator" occurs in elderly men, this phenomenon is, anyhow, limited and inconstant. Although one may be tempted to label those findings as "negative results", they do in fact support the concept of an altered regulation of LH secretion at the level of the pulse generator in elderly men. Indeed, in none of the studies on LH pulsatility in elderly men is there any indication of an increased LH pulse frequency, the expected outcome in face of the decreased testosterone levels, if the neuroendocrine regulatory mechanisms operating in young men, were fully operational in the elderly. Consequently, the relatively inappropriate rise of LH levels in elderly men can be explained, at least in part, by the failure of the "LHRH pulse generator" to speed-up to a higher frequency than that typical of eugonadal young subjects.

Another characteristic of LH pulsatility that we should consider, is the LH pulse amplitude. When using an oligoclonal immunoradiometric assay for LH dosage and short sampling intervals of 10 min [30], we found that the mean LH pulse amplitude, the maximal pulse amplitude and the frequency of pulses with large amplitude are significantly higher in young men as compared to the elderly.

As this phenomenon can be expected to be amplified when looking at bioactive LH levels [18], these observations could have direct implications as far as stimulation of Leydig cell function is concerned.

Amplitude of immunoreactive LH pulses in studies by Tenover *et al.* [19, 26] and of bioactive LH pulse in the study by Urban *et al.* [35] were not different between young and elderly men when studied in basal conditions, although both authors observed smaller pulse amplitudes in elderly men than in young men after treatment with anti-estrogens. In an earlier section we pointed out that interpretation of changes in LH pulse amplitude is difficult as they may result from altered hypothalamic inputs, changed pituitary responsiveness or a combination of both.

Hypothalamic and pituitary sensitivity to sex steroid feedback action

The absence of acceleration of the "LHRH pulse generator" in response to the decreased testosterone levels may suggest an increased sensitivity of the "pulse generator" to steroid hormone feedback action, a view supported by the observations of Winters *et al.* [23] of an increased suppressive effect of androgen infusion in elderly men. When testing this hypothesis, we found that similar increases in DHT levels in young and elderly men, achieved by percutaneous administration of this steroid (125 mg/day, for 10 days), resulted in a more marked suppression of LH, testosterone, and estradiol plasma levels in the elderly subjects, while the LH response to exogenous LHRH after treatment was not significantly affected in the younger subjects and was enhanced in elderly men [27] (Fig. 2). Estradiol treatment (1.5 mg/day, percutaneously for 10 days) had a more limited suppressive effect on basal LH and testosterone levels than DHT administration and was not different in young and elderly men, whereas the LH response to exogenous LHRH was unaffected in younger subjects and moderately suppressed in elderly men [27]. These

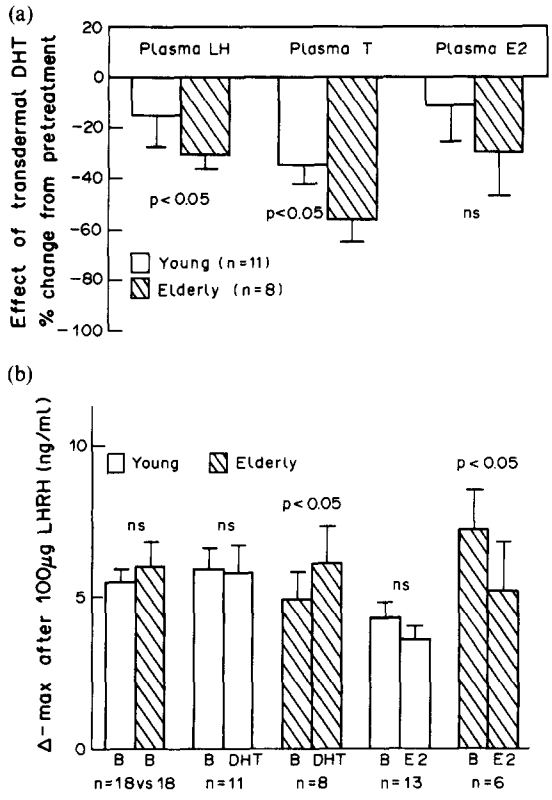

Fig. 2. (a) Effect of transdermal DHT (125 mg/day for 10 days) on basal levels of LH, testosterone (T) and estradiol (E2) in young (<32 yr) and elderly (>65 yr) monks. Mean ± SE. (b) Maximal LH response (Δ-MAX) to 100 μg LHRH i.v. in young and elderly monks in basal conditions (2 bars on the left), before (B) and after (DHT) 10 days transdermal DHT (125 mg/day), before (B) and after (E2) 10 days transdermal E2 (1.5 mg/day). Mean ± SE, Adapted from the data in Ref. [27].

results are in good agreement with the findings by Winters *et al.* [23] of a more marked deceleration of LH pulse frequency and an increased response to exogenous LHRH in elderly men after a 4-day infusion of DHT (7 mg/day). Besides an effect on LH pulse frequency, testosterone or DHT infusion also resulted in the elderly subjects, but not in young men, in a reduction of LH pulse amplitude.

Estradiol infusion (45 μg/day for 4 days) had only limited effects with only a non-significant reduction in LH pulse amplitude and LH response to exogenous LHRH in both age groups, and no alteration of LH pulse frequency [23].

Taken together, these data indicate a higher sensitivity for retardation of the hypothalamic "LHRH pulse generator" by androgens in elderly men. The somewhat enhanced response of LH to exogenous LHRH during androgen treatment in elderly men is most probably secondary to the increased interpulse intervals with, as a consequence, availability of a larger releasable pool of LH.

The reduced amplitude of spontaneous LH pulses in elderly men during androgen treatment, observed by Winters *et al.* [23], contrasts, with the increased response to exogenous LHRH. Although somewhat surprising, this finding seems in accordance with our observation of a reduced number of pulses with large amplitude in untreated elderly men [30]. To explain the apparently opposite responses to endogenous and exogenous LHRH stimulation, one has to assume that the aging process affects the hypothalamus at an additional site, besides the pulse generator, with reduction of the size of the quantum of LHRH released episodically into the pituitary portal circulation. Alternatively, aging might induce more subtle changes at the pituitary level which were not revealed when using large doses of LHRH to challenge the gonadotropes. Consistent with the latter possibility are observations of a delayed LH response to LHRH [33], and of a somewhat increased pituitary sensitivity to estradiol feedback in elderly men [27].

Prevailing opioid tone in elderly men

Since the negative feedback action of androgens on the "LHRH pulse generator" is mediated, at least in part, by increased opioid tone, we studied the influence of an anti-opiod, naltrexone, on the pattern of pulsatile LH secretion in young and elderly men [30]. Indeed, one could hypothesize that the higher sensitivity of the "LHRH pulse generator" to the negative feedback action of androgens in elderly men is the result of either an increased opioid tone relative to the prevailing testosterone levels or of an increased sensitivity of the pulse generator for opioidergic retardation of its frequency. In both cases one would expect that endogenous opioid blockade will result in a similar increase in LH pulse frequency in young and elderly men, unlike what is observed in other situations with primary testicular hypogonadism. Whereas oral administration of naltrexone (2 × 40 mg the day before, and 1 × 40 mg on the morning of blood sampling) induced, as expected, a significant increase in LH pulse frequency and sum of all LH pulse amplitudes in young men, this treatment failed to alter these parameters in the elderly men, with only a marginal increase in basal LH levels being observed in these subjects (Fig. 3). LH pulse amplitude in elderly

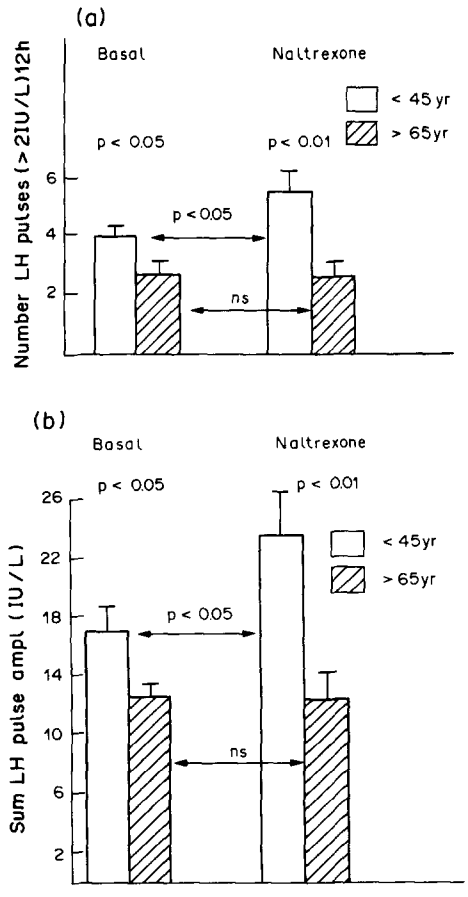

Fig. 3. (a) LH pulse frequency for pulses with amplitude > 2 IU/l in young and elderly monks in basal conditions and during treatment with naltrexone (2 × 40 mg p.o. the day before, and 1 × 40 mg on the morning of blood sampling). Mean ± SE. (b) Sum of the amplitudes of all LH pulses in young and elderly monks in basal conditions and during treatment with naltrexone. Mean ± SE. Adapted from the data in Ref. [30].

men was also unaffected by the naltrexone treatment.

In view of the observed acceleration of LH pulse frequency in elderly men during treatment with clomiphene citrate [26], it is unlikely that the absence of response to opiod blockade would be due to an intrinsic inability of the "LHRH pulse generator" to fire at a higher frequency. The foregoing results rather indicate that the opioid tone is decreased in elderly men, as is the case in other hypoandrogenic states, and thus allow to reject the working hypothesis of an increased opioid tone relative to the prevailing testosterone levels in elderly men.

Reassessment of pituitary responsiveness to LHRH in elderly men

In view of the decreased mean LH pulse amplitude (or lower frequency of LH pulses with large amplitude) in elderly men observed by us under basal conditions and by Tenover *et al.* [26] during androgen administration, it seemed important to settle more definitely the question whether there is any impairment of pituitary responsiveness to LHRH in elderly men. Therefore, we reassessed in 10 young (< 45 yr) and 10 elderly (> 65 yr) healthy men, the responsiveness of the gonadotropes by consecutive intravenous administration, at 120 min intervals, of small increasing doses of 0.625, 1.25, 2.5 and 5 μg LHRH. The mean responses for immunoreactive LH (oligoclonal IRMA) and bioactive LH (mouse Leydig cell bioassay Ref. [38]) in young and elderly men are depicted in Fig. 4. The responses of immunoreactive LH to LHRH stimulation were similar in young and elderly subjects. Moreover, the response of bioactive LH was higher in elderly men than in young subjects after all doses (not significant for the lower dose). It is interesting to note that administration of these low doses of LHRH resulted in an appropriate [18] increase in bio/immuno LH ratio in both young and in

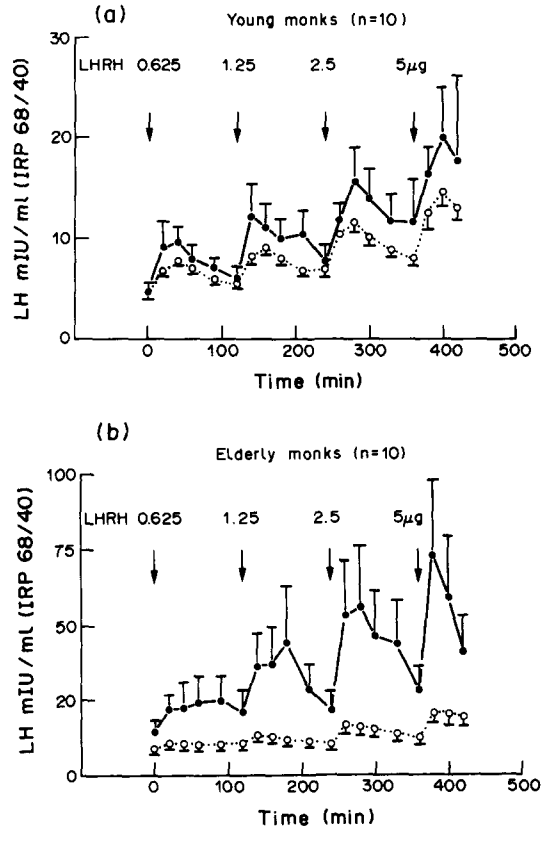

Fig. 4. LH levels measured by (oligoclonal) RIA (open circles) and by mouse Leydig cell bioassay (filled circles) after consecutive i.v. administration of 0.625, 1.25, 2.5 and 5 μg LHRH to (a) young monks (< 45 yr) and (b) elderly monks (> 65 yr). Mean ± SE.

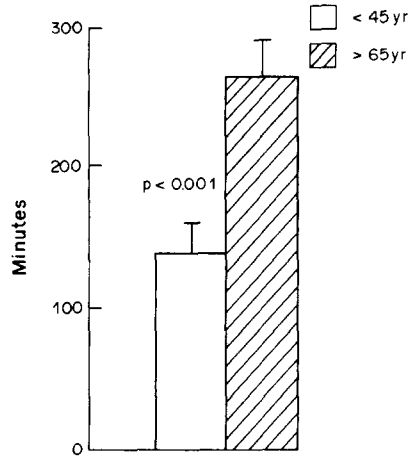

Fig. 5. Plasma half-life (elimination phase) of immunoreactive LH in young and elderly monks as estimated by deconvolution analysis applied to the data presented in Fig. 4. Mean ± SE.

elderly subjects. In contrast with previous reports of altered spontaneous and LHRH-stimulated release of biologically active LH in elderly men [34, 35], we found a significantly higher bio/immuno LH ratio both, in basal conditions and after LHRH stimulation in the elderly men as compared to the young subjects. This is in line with what would be expected in a situation of relative hypoandrogenism. These results indicate that the responsiveness of the gonadotropes to LHRH is well preserved in elderly men. Foregoing findings give credence to the hypothesis of an additional hypothalamic defect, besides the altered regulation of the frequency of the "LHRH pulse generator", as the explanation for the observation of less frequent pulses of large amplitude in elderly men. Possibly, the size of the LHRH bolus released in response to activation of the "pulse generator" is reduced. A reduced *in vitro* LHRH release from the hypothalamus of aged rats has been reported [39]. Possible underlying mechanisms could include altered LHRH synthesis or processing, reduced hypothalamic LHRH cell mass, loss of synchronous firing of LHRH neurons, or altered regulation of LHRH release at the nerve endings.

Plasma half-life of LH in elderly men

Paradoxical findings which emerge from the detailed study of LH secretion in elderly men are the consistently elevated mean LH levels on the one hand with decreased, or at best unchanged, LH pulse frequency and decreased LH pulse amplitude on the other hand. Mathematical modelling and deconvolution analysis applied to the data obtained after repeated LHRH challenge in young and elderly men, presented in the previous section, revealed that the plasma half-life of immunoreactive LH is markedly prolonged in elderly men when compared to young subjects (Fig. 5). This observation offers an explanation for the apparent contradiction between increased mean LH levels and decreased or unchanged LH pulsatility but do not exclude the possibility of increased, non-pulsatile basal LH secretion in elderly men.

CONCLUSIONS

Primary testicular factors play an important role in the decline of Leydig cell function in elderly men but there is overwhelming evidence indicating that the aging process also affects the central compartment of the gonadal axis. The alterations in neuroendocrine regulation of Leydig cell function in elderly men seem to be primarily localized at the hypothalamic level. These hypothalamic alterations comprise changes in the regulation of the frequency of the "LHRH pulse generator", with an inappropriately low frequency relative to the prevailing decreased androgen impregnation and opioid tone and with an increased sensitivity to the negative feedback effects of androgens. Further hypothalamic alterations may include a reduction in the size of the bolus of LHRH released episodically in response to activation of the "LHRH pulse generator". Finally, hypothalamic alterations may also underly the marked blunting in testosterone circadian rhythm and the, less well documented, loss of circadian rhythm in LH secretion.

The mechanisms underlying the altered neuroendocrine regulation of Leydig cell function at the hypothalamic level remain to be elucidated. The hypothesis of an increased opioid tone in elderly men can be rejected. Other possible mechanisms that should be explored in further studies include opioid-independent androgen effects, intrinsically reduced functional capacity of the neuronal circuitry involved in episodic LHRH release and altered regulation by other neurotransmitters previously shown to play a role in the regulation of LHRH secretion. Aging is known to affect several hypothalamic neurotransmitter systems [40]. Central α-adrenergic systems, believed to play a role in the regulation of the LHRH pulse generator in several mammal

species [11], is a less likely candidate in this regard, since pharmacological studies failed to show a significant role of these systems in the regulation of LH pulse frequency in men [41, 42]. More work is also needed to evaluate the implications of the markedly prolonged half-life of LH in the systemic circulation of elderly men.

Acknowledgements—We gratefully thank Dr Guy Thomas, Faculté de Médecine Lariboisiere Saint Louis, Paris, for mathematical analysis of the data on repeated administration of low-dose LHRH.

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