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RADIOIMMUNOASSAY OF SERUM LUTEINIZING HORMONE RELEASING HORMONE (LH-RH) AFTER INTRA-NASAL ADMINISTRATION AND EVALUATION OF THE PITUITARY GONADOTROPHIC RESPONSE

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SUMMARY

Serum levels of LH-RH, LH and FSH were measured by radioimmunoassay at frequent intervals in four young men following the intranasal administration of 2 mg synthetic LH-RH. The initial rise in serum LH-RH was seen at 2·5 min and the peak was reached at 15 min. There was a rapid response in serum LH, the peak occurring between 30 and 45 min, while FSH levels did not change significantly. It was calculated that 1·25% of the dose was absorbed. The intranasal administration of LH-RH appears to have considerable therapeutic potential.

INTRODUCTION

Although it has been customary to administer hypothalamic releasing hormones such as LH-RH by injection (nearly always intravenous) the possibility of achieving serum levels sufficient to cause pituitary stimulation without the necessity for injection would have potential therapeutic usefulness. The aims of the present study were to measure the serum levels of LH-RH after intranasal administration, so as to estimate the amount and pattern of absorption, and to evaluate its biological effectiveness, by assessing the response of pituitary luteinizing hormone (LH) and follicle stimulating hormone (FSH).

MATERIALS AND METHODS

Subjects tested

Four healthy men, aged 24–25 years, volunteered for this study. Blood samples were drawn from an indwelling catheter placed in an antecubital vein at −15, 0, 2·5, 5, 7·5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240, and 270 min, and subsequently every half hour for 5 hr following the administration of LH-RH. The blood samples were immediately

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placed at 4°C, the serum was separated at the completion of the experiment and was stored at −20°C until the time of assay.

Preparation and administration of LH-RH

Synthetic LH-RH was provided by Dr K. Bruch of Farbwerke Hoechst AG in ampoules containing 2 mg of dry powder. The contents of one ampoule were dissolved in 1 ml physiological saline, and were administered drop-wise, ten drops into each nostril at 0·5 sec intervals. 10 μl of the solution was kept for confirmation of the dose administered.

Radioimmunoassay methods

Serum LH-RH was measured by a newly developed radioimmunoassay (Burger & Franchimont, 1974) using synthetic LH-RH for radioiodination and reference standard, and an antiserum made against a LH-RH–bovine serum albumin conjugate. Separation of bound and free hormone was achieved by adsorption of the free hormone onto uncoated charcoal. The assay was specific for the LH-RH molecule, there being no cross-reaction with LH, FSH, vasopressin, oxytocin, angiotensin, insulin, glucagon, or thyrotropin releasing hormone (TRH). The N-terminal tripeptide and C-terminal heptapeptide of LH-RH showed 1·0 and 3·5% cross reactivity respectively while the free acid derivative showed 0·4% reactivity. Within assay precision varied between 7 and 12% and all samples from one subject were measured within a single assay, at two dilutions, parallelism of the dilutions with the standard curve being observed. Assay sensitivity was 10–20 pg/ml serum. Serum LH and FSH were measured as described previously (Franchimont, 1966, 1968) using the MRC standards (68/39 and 68/40) for reference.

RESULTS

Basal levels of LH-RH

Basal levels were undetectable in four of the eight basal samples and varied between 10 and 50 pg/ml in the remainder.

Effect of LH-RH administration on its serum levels

As shown in Fig. 1, an elevation in serum LH-RH was detectable in the first sample, 2·5 min after the nasal drops had been instilled, the concentration at this time varying from 25 to 175 pg/ml. The mean peak value of 322 pg/ml was attained at 15 min, following which the levels fell progressively to return to the basal concentrations at 120 min.

The amount of the administered dose which was absorbed was calculated as follows: the disappearance curve of an intravenous dose of 100 μg LH-RH (Burger & Franchimont, 1974) was plotted on cartesian co-ordinates, and the area beneath the curve was determined. Using the same co-ordinates, the area beneath the curve as shown in Fig. 1 was determined and compared with the former: it was calculated that 25 μg LH-RH was absorbed, representing 1·25% of the dose.

Effects on gonadotrophin release

The first increase in serum LH (Fig. 2) was seen 5 min after LH-RH was instilled, and the peak (6·1 mIU/ml) occurred between the 30th and 45th minute. The levels subsequently declined slowly but were still above the baseline concentration at 4·5 hr.

There was no significant change in FSH, the basal value of 2·4 mIU/ml being statistically no different from the 'peak' of 3·6 mIU/ml at 120 min.

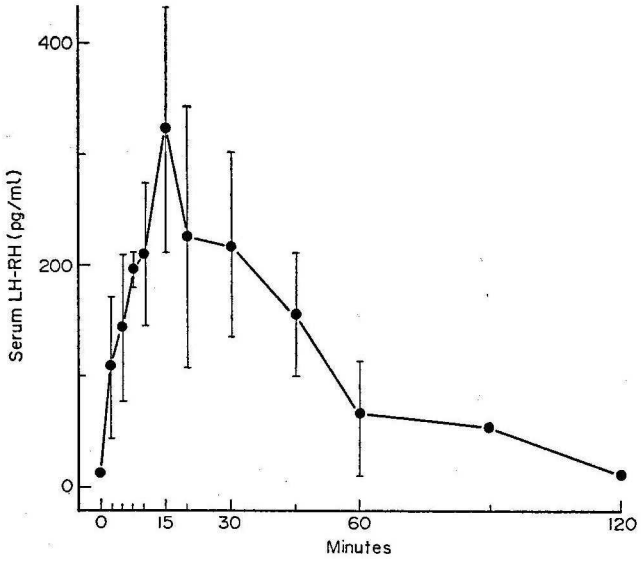

FIG. 1. Mean serum LH-RH levels (±1 SD) following intranasal LH-RH administration to four normal men.

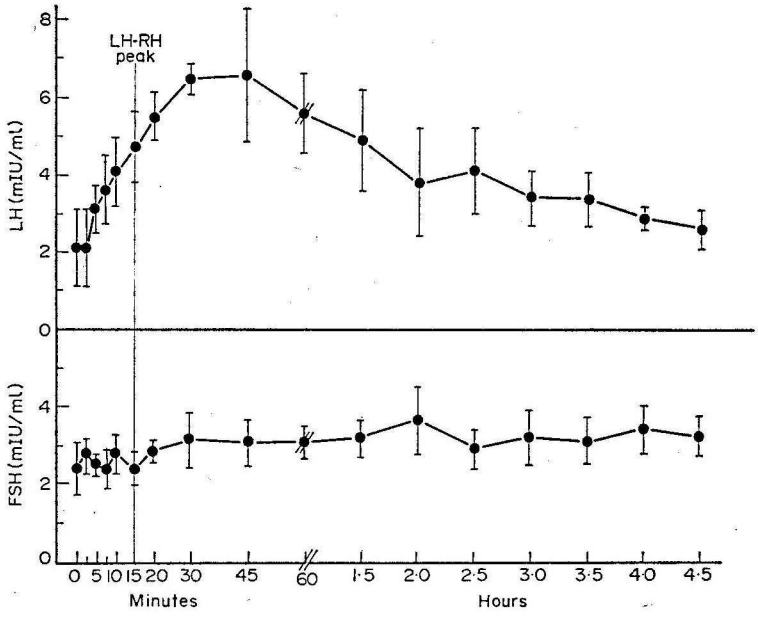

FIG. 2. Mean serum LH and FSH levels (±1 SD) following intranasal LH-RH administration to four normal men.

DISCUSSION

As was recently shown by Solbach & Wiegelmann (1973) intranasal administration of LH-RH can result in significant pituitary stimulation. These authors administered 2·0 mg LH-RH dissolved in 0·5 ml of distilled water by the intranasal route to five healthy males, aged 20–25 years. The peak of LH was found 30 min later. The values were in the same range as the peaks observed after the intravenous injection of 50 μg LH-RH.

In the present study, the effectiveness of intranasal LH-RH administration has been confirmed, and furthermore, the actual serum LH-RH levels achieved have been measured. It is clear that absorption is immediate and progressive with a subsequent gradual decline, in contrast to the sharp rise and fall which follows intravenous administration.

The relatively small amount absorbed (1·25%) was no doubt partly due to the fact that the dose was given in a total of twenty drops: administration in a more concentrated form might ensure a higher degree of local absorption, and less chance of losses via the gastro-intestinal tract.

It was noteworthy that the interval of 15–30 min between the giving of LH-RH and the subsequent LH peak was of the same order as that seen following intravenous dosage. The peak LH values achieved were approximately 50% of those seen after a single intravenous dose of 25 μg LH-RH, but the values remained elevated for a longer period, reflecting the pattern of LH-RH absorption. The characteristics of the responses described here suggest that intranasal administration of this releasing hormone could well be of considerable therapeutic interest.

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