GnRH-TEST AFTER HIGH-DOSE MPA IN WOMEN WITH POST-MENOPAUSAL LH-PREFERENTIAL RELEASE

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SUMMARY

The association between post-climacteric LH-preferential release after GnRH-Test and the occurrence of benign or malignant estrogen dependent diseases makes the Authors evaluate the variations induced in such type of hypophyseal response by MPA, administered in the same doses as in the hormonal therapy of cancer.

MPA lowered both the basal gonadotropin secretion and the amplitude of the response to the neurohormone, suggesting the hypothesis of a possible direct inhibitory action of the hormone on the hypophysis.

The persistence of LH-preferential release after the ten day treatment with MPA 200 mg daily might be explained by the lack of modifications induced by the hormone on the levels of cytoplasmic E2-receptors, on which seems based LH-preferential release.

In previous reports ([1, 2]) we analysed the clinical and diagnostic significance of LH-preferential release by the hypophysis of post-menopausal women undergoing GnRH-Test; in the present study we try to evaluate the variations induced in the pituitarian response to GnRH by the administration of high dose (H-D) MPA for a relatively short period.

MATERIAL AND METHODS

We tested by GnRH (Relisorm - Serono 100 mcg i.v. rapidly), before and after H-D MPA treatment, 15 post-menopausal patients admitted to the Obstetric and Gynecological Dept. of Padua University: of them, 5 were affected with endometrial cancer and 10 with different malignancies always arising in the genital tract. All they were tested during the period of clinical staging, before undergoing primary surgery. Histology of the uterus confirmed the presence of endometrial cancer in the 5 above mentioned patients, while no endometrial pathology could be found in the others.

The study required 12 days: in days 1st and 12th the patients were GnRH-tested; from the 2nd to the 11th day they assumed MPA 100 mg twice a day per os. The test was performed between 08.00 and 10.00: plasma samples were drawn, from an indwelling catheter placed in the cubital vein, before the injection and at the 15th, 30th, 45th, 60th, 90th minute from it.

The samples were immediately centrifuged and sera were stored at −20 °C until RIA.

The hormonal values of each patient were graphically represented to examine the variations induced by H-D MPA in the hormonal basal levels and in the type of response to GnRH.

RESULTS

Before Progestin treatment, LH was preferentially released in 10 patients (among them the 5 with endometrial cancer), while the 5 remaining patients manifested a FSH-preferential release: the type of response was neither correlated with body weight nor with actual Estradiol plasma levels, as previously reported.

Figs. 1 and 2 exemplify the variations induced by H-D MPA on the hypophy-

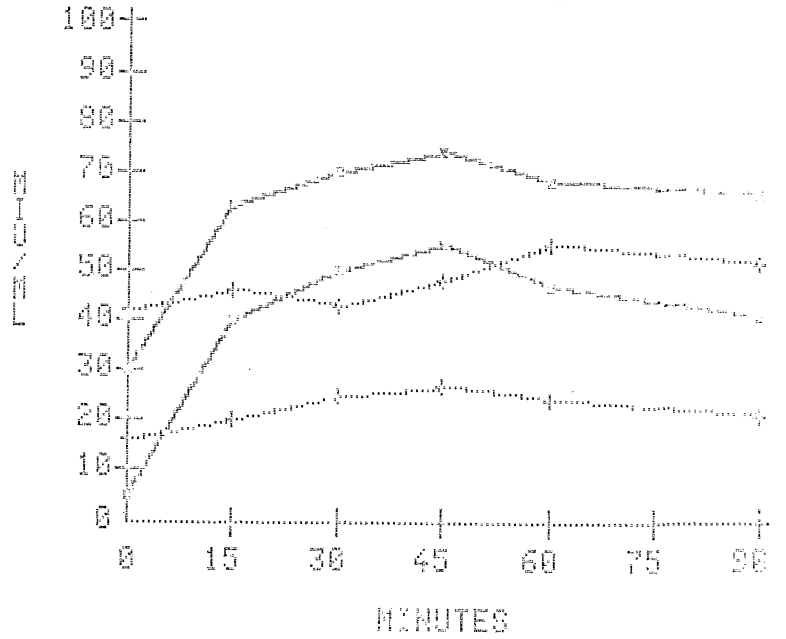
seal basal secretion and on the gland response to GnRH in the patients who respectively exhibited, before the treatment, a preferential release of LH and FSH.

As to gonadotropin basal levels, we could observe a slight, though significant ($p<0.05$), diminution of FSH plasma levels, while LH dropped in a highly significant way ($p<0.001$).

As to the type of response, the LH preferential release persisted in all the patients where it was present before MPA: we observed an important diminution in the values of both gonadotropins, but the trend of the curves was the same as in the pre-treatment test (fig. 1).

In one patient we excluded from our study (she was affected with endometrial cancer stage IV and received 1000 mg of MPA orally for 40 days) we observed the just described variation after 10 days of treatment, while at the 40th day the gonadotropin curves were overlapping, flattened down to hardly detectable levels.

In the patients with pre-treatment FSH preferential release, H-D MPA induced a gonadotropin drop as well, with persistence of the previous type of response (fig. 2).



Dark lines = LH; light lines = FSH; Upper lines: before MPA; lower lines: after MPA.

Fig. 1. — FSH and LH release after GnRH-Test in patients presenting a post-climacteric LH preferential release, before and after MPA-treatment.

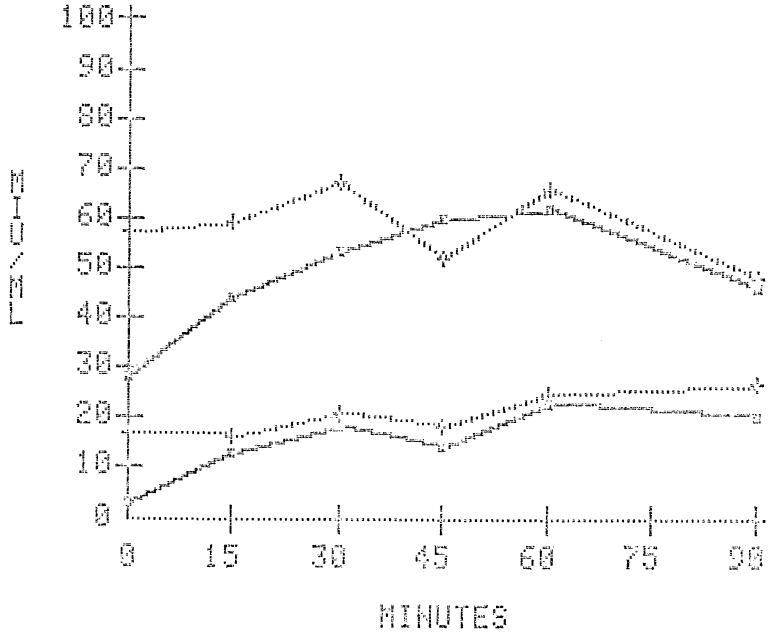
DISCUSSION

A drop in LH basal levels after H-D MPA was reported by Mayer ([3]), Sadoff ([4]), Montanari ([5]), Bonte ([6]); a concomitant reduction in FSH levels was observed by Franchimont ([7]) and Toppozada ([8]) after administration of MPA 150 mg i.m., and by Iannotta ([9]) after an 8 day oral administration of a daily dose of MPA 500 mg.

However, when we examine the response to GnRH-Test after a treatment with H-D MPA, we find in literature an

evident disagreement: on one hand ([7, 8]), the finding of an unchanged response to the neurohormone in patients with MPA-lowered gonadotropin basal levels led the Authors to suppose the Progestin acts at the hypothalamic level, as also believed by Yen ([10]), Goodman ([11]), Lu ([12]), Pohl ([13]) and Moss ([14]); on the other hand, Monto an higher concentration of E2-induced hypophyseal GnRH receptors, function of the intensity and length of previous estrogen impregnation.

It is evident from our data that MPA, administered according to our schedule, fails in normalizing the factors underlying the LH preferential release.



Dark lines = LH; light lines = FSH; Upper lines: before MPA; lower lines: after MPA.

Fig. 2. — FSH and LH release after GnRH-Test in patients presenting a post-climacteric FSH preferential release, before and after MPA-treatment.

tanari ([5]) and Iannotta ([9]) obtained results similar to ours.

The association between post-climacteric LH preferential release after GnRH-Test and the occurrence of benign and malignant estrogen-dependent diseases ([1, 2, 15]), made us consider with the greatest attention the variations induced by MPA, the main therapeutic aid in such diseases, on the responses of patients presenting a pre-treatment LH preferential release.

This type of response in the patients we tested had been previously ascribed ([1, 15])

The synthesis of hypophyseal GnRH receptors seems to increase when Estradiol is present ([15]); on the contrary, there is still uncertainty about the effect of GnRH on the synthesis of its own receptors: some Authors ([10, 16, 17, 18]) believe that the neurohormone, which is present in higher levels in post-menopausal plasma ([7]), would increase their number and consequently its own stimulating capacity. According to others ([19, 20]), on the contrary, GnRH, in high close doses, would diminish the number of its own receptors:

even in this case, however, GnRH stimulating capacity would be increased due to a self-priming effect of the neurohormone on its own receptors.

There would be, in this way, a dissociation between hypophyseal concentration of GnRH receptors and the gland capacity to respond to GnRH, with the suggestion that an important role in gonadotropin secretion may be played by still unknown factors ([20]).

Experimental data ([7]) point out an increased LH-release after GnRH administration to post-menopausal women pretreated with Ethynil-Estradiol; Geller ([15]), as well, concludes that GnRH in menopause provokes a LH preferential release when Estradiol activity is present, while FSH release prevails in an estrogen-free medium.

A timely combination of E2 and GnRH activities seems then indispensable for the increase of the hypophyseal response to the neurohormone ([21, 22]); quite recently ([23]), the existence of a very short feed-back system has been proposed, through which GnRH would enhance the specific intrapituitarian estrogen binding and so increase LH release in spite of an eventual drop in GnRH-receptors; however, no conclusion seems possible about the increase or diminution of hypophyseal cytoplasmic GnRH-receptors, the levels of which seem to vary too rapidly.

A greater contribution to our comprehension of the mechanism responsible for post-menopausal LH preferential release, and of the reason why MPA fails in normalizing the response, might come from the analysis of the role played by hypophyseal cytoplasmic receptors to Estradiol. Their levels seem scarcely affected by progesterone ([24]) and might be then a much more reliable marker of the degree of past estrogen impregnation, on which seems dependent the occurrence of estrogen related diseases. *In vitro* experiments on hypophyseal cells show that chronic E2 administrations induce an increase of gonadotropin reserve and enhance hypophyseal sensitivity to GnRH ([10, 22]) with an augmented release of LH by the neurohormone, though gonadotropin basal secretion is lowered ([11]).

A low dose progesterone administration in the pre-ovulatory period ([10, 25]) or to estradiol primed subjects elicits a LH-surge, and increases the release of LH by GnRH in E2 pre-treated women ([26]) without any variation in circulating GnRH levels ([11]).

However, when E2 pre-treatment is effected by the administration of very large doses ([7, 27]), the capacity of releasing LH by GnRH-injection is lost. Similarly, the chronic administration of progesterone *in vitro* inhibits estradiol stimulation on gonadotropin producing cells ([11]), and diminishes the amount of stored gonadotropins in them.

The reduction of gonadotropin basal levels and the quantitative depression of their dismission by GnRH observed in our patients after MPA treatment, lead us to hypothesize a direct inhibitory action of the hormone on the hypophysis, in contrast with the opinion of other Authors ([7, 8, 10, 11, 12, 13, 14]). MPA might inhibit the estradiol induced hypophyseal synthesis and storage of gonadotropins, and consequently reduce the amount of them ready to be released ([22]). Moreover, it might also inhibit the E2-induced pituitarian sensitization to GnRH.

The above mentioned experiments ([7, 10, 11]) seem to support our hypothesis, even if Convey ([22]) denies any relation between the stored and the ready to be released LH pools; however, these pools may appear to be linked: in fact, increased LH concentrations were found in the hypophysis of animals given GnRH-antagonists in the pre-ovulatory phase ([28]), and a continuous infusion of GnRH in post-menopause led to a down-regulation of hypophyseal gonadotropin secretion ([29]).

After the attempt to explain gonadotropin depression, we must face the most

difficult questions: which is the exact basis of post-menopausal LH-preferential release, and why does it persist after HD-MPA treatment?

The rapid variations of hypophyseal cytoplasmic GnRH-receptors after close repeated GnRH administrations ([20]), and the lack of any steady correlation ([14, 22]) between the levels of hypophyseal GnRH-receptors and gonadotropin release by the neurohormone injection make hardly sustainable a direct influence of their concentration on the type and degree of response.

However, Geller's ([15]) observations remain very important: in fact, he found that post-menopausal LH-preferential release was associated to the presence of increased concentrations of E2-receptors in the cytoplasma of target-cells.

The synthesis of these receptors seems genetically determined and their distribution seems uniform in all the target tissues of the same subject ([30]); moreover, they seem to share a common mechanism of action ([24]).

It might then seem reasonable to hypothesize that increased levels of cytoplasmic E2-receptors are present also in the hypophysis of the women presenting a LH-preferential release after GnRH-Test in menopause. Their concentration might depend on the intensity of previous estrogen impregnation and, particularly, on the degree of endocrine umbalancement occurring in the peri-menopausal period ([2]). Their persistence beyond their inducing stimulus ([31]) and the total lack of Progesterone modulation would permit an efficacious action to even low levels of Estradiol, and consequently assure the maintenance of the estrogen-priming underlying LH-preferential release ([2]).

This chronic unopposed estrogen stimulation would increase the LH concentration in the reserve-pool and the hypophyseal sensitivity to GnRH, with a consequent enhancement of LH-release and, possibly ([7]), a diminution of FSH-release.

The same conditions in the target-tissues would lead to dysplastic and neoplastic growth.

Provided this is the basis of LH-preferential release, how can we explain HD-MPA failure in normalizing hypophyseal response to GnRH?

Of course, we can only try to formulate hypotheses, basing on the most recent data in literature ([24, 30, 31, 32, 33, 34, 35, 36, 37]) which seem to give further insight into the exact interactions between hormones and receptors: Markaverich ([24]), for instance, studying E2-induced uterine growth in ovarectomized rats, found that it was significantly inhibited by Progesterone, without any variation in cytoplasmic E2-receptor concentration. The treatment, however, significantly ($p < 0.05$) reduced the synthesis of type II E2-receptors, induced in the nucleus by the complex E2/cytoplasmic E2-receptor, which seem indispensable ([24, 32]) for estradiol to be effective. Moreover, there was a sort of parallelism between the degree of inhibition of the synthesis of type II receptors and the diminution of uterine growth response.

King ([33, 34]), on the other hand, studying the endometrium of post-menopausal women pre-treated with estradiol and then given progestins, observed a significant drop in the levels of nuclear E2-receptors without any change in the levels of cytoplasmic E2-receptors; these results were confirmed also by researches in animals ([35]). However, progestins induced a rise in the levels of 17-beta-E2-dehydrogenase activity which seems to parallel nuclear receptor drop ([36, 37]).

Due to the uniformity of receptor distribution ([30]) and mode of action ([24]), which is plausible in the different hormone target tissues of the same individual, we can hypothesize that progesterone effect may be identical also at the hypophyseal level.

The significant reduction, even if not abolition, induced by progesterone on the synthesis of nuclear E2-receptors or on the

translocation of cytoplasmic receptors into the nucleus might lead to a diminution of gonadotropin synthesis and storage; in the same way, progestins may interfere with E2 sensitization of the hypophysis to GnRH; at last, the increased 17-beta-E2-dehydrogenase activity, reducing the amount of active estradiol and so inhibiting its binding to the receptor, concurs in the blockade of estrogen-dependent metabolic steps; all these factors would lead to a depression of the hypophyseal response to GnRH, even in presence of an estrogen-primed substratum on which estrogens are still potentially dangerous. In fact, progesterone administration seems uneffective in modifying the levels of cytoplasmic E2-receptors and progestin withdrawal might restart estrogen hyperactivity.

On the other hand, higher cytoplasmic E2-receptor levels in the hypophysis of post-menopausal women seem the likely basis of post climacteric LH-preferential release after GnRH-Test ([2]): their persistence even after progesterone-treatment might explain why the type of response is unchanged.

However, many steps in these processes are far from being cleared for instance, the doses and the length of the treatment may be important: in the case we treated by MPA 1.0 g/day for 40 days, the response at 10 days was quite similar to our patients', while at the end of the treatment every response was abolished. We can consequently suppose that different doses and administration schedules might have led to different results, and this may be important when we program prophylactic treatments at doses which are much lower than those employed in this study.

Anyway, we would like to set forth a suggestion: if post-climacteric LH-preferential release after GnRH-Test is associated with the presence of estrogen-dependent disease ([1, 2, 15]) and may disclose the presence of the substratum at risk for their occurrence even in otherwise healthy women, the type of response to GnRH-Test during or after a progestin treatment might help in understanding both the degree of estrogen hyperactivity suppression and the moment in which the substratum at risk becomes normal.

Whether these observations may be useful or not in the follow-up of MPA-treated hormone-dependent malignancies or in the progestin prophylaxis of menopauses at risk, might be the object of further investigations.

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