EFFECT OF METHYLERGOBASINE MALEATE ON
SERUM GONADOTROPHIN AND PROLACTIN IN HUMANS

By

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ABSTRACT

Intramuscular injection of 0.2 mg methylergobasine maleate (Methergin®, Sandoz) in women on day 3 post-partum, in regularly menstruating women and in adult men, is followed within 30 to 75 min by a 50% decrease in serum prolactin concentration: the levels remain low until 180 min and increase between 180 and 240 min. The amplitude of the decrease is the same when prolactin is measured in terms of the same serum prolactin standard by a homologous ovine assay and by a homologous human assay. However, in the case of regularly menstruating women and of men serum prolactin concentration is some three times higher when estimated by the ovine assay than when estimated by the human assay. This difference between assay results obtained by the two radioimmunoassay methods could be due to heterogeneity of serum prolactin. However, non-specific effects of serum are not excluded. In regularly menstruating women and in men, intramuscular injection of 0.2 mg methylergobasine maleate is followed within 45 to 75 min by a 50% decrease in immunoreactive serum LH concentration without concomitant change in immunoreactive FSH. The depression of LH secretion lasts for 1 to 2 h. The circulating levels of HCG in post-partum women are not modified after intramuscular injection of Methergin. In humans as in animals and in in vitro studies, inhibition of prolactin and LH release induced by ergot drugs are likely due to both an indirect effect via the hypothalamus and to a direct effect on the pituitary cells.
Finally, these data suggest that, because of its interference with prolactin secretion, intensive treatment with ergot drugs during the post-partum period may impair lactation.

Several ergot drugs have been shown to inhibit prolactin secretion in animals (Meites & Clemens 1972). It has also been reported that 2-Br-α-ergocryptine, an ergot drug free of uterotonic or vascular effects, inhibits prolactin secretion in humans (del Pozo & Flückiger 1973).

The purpose of the present study was to investigate whether methylergobasine maleate, an ergot drug, widely used in the post-partum for its uterotonic effect would suppress prolactin secretion in women after delivery, in regularly menstruating women and in adult men.

It has also been shown that, in rats, ergot drugs interfered with gonadotrophin secretion (Wutke et al. 1971). Therefore, serum HCG, LH and FSH were measured in addition to prolactin at frequent intervals before and after injection of methylergobasine maleate.

**MATERIAL AND METHODS**

Four women aged 28–32 on day 3 post-partum, four regularly menstruating women aged 28–45 between day 7 and day 23 of the menstrual cycle and six normal men aged 18–24 were investigated. A catheter was inserted in an antecubital vein and the first blood sample was collected between 9.00 and 9.30 a.m.; 30 min later 0.2 mg of methylergobasine maleate (Methergin®, Sandoz S.A., Basel) was injected intramuscularly. Blood samples were collected at frequent intervals during four hours as indicated in Figs. 1 and 2. Blood was allowed to clot at room temperature and kept overnight at 4°C. Then serum was separated by centrifugation and stored at −20°C.

Serum HCG, LH, FSH and prolactin were measured by double antibody radioimmunoassay methods with delayed addition of the tracer. Highly purified HCG (E 201-2, Istituto Serono, Rome, Italy), human FSH (H-FSH 1575-C, National Institute of Arthritis, Metabolism and Digestive Diseases, USA), ovine prolactin (LER-860-2) and human prolactin (HPR VLS-1, National Institute of Arthritis, Metabolism and Digestive Diseases, USA) preparations were labelled with iodine-125 (The Radiochemical Centre, Amersham, Great Britain) according to Greenwood et al. (1963) as modified by Robyn et al. (1971). Anti HCG serum (No. 327; Petrusz et al. 1971b), anti-ovine FSH serum (L’Hermite & Midgley 1971), anti-ovine prolactin serum (No. 770: L’Hermite et al. 1972a) and anti-human prolactin serum (VLS-1, National Institute of Arthritis, Metabolism and Digestive Diseases, USA) were used at final dilutions of 1:1000000, 1:4000, 1:180000 and 1:400000, respectively. All these antisera were raised in rabbits. The second antibody was a sheep anti-rabbit immunoglobulin serum (No. 1608-3) at a final dilution of 1:250.

The radioimmunoassays were conducted as previously described for HCG, LH, FSH, and prolactin (Robyn et al. 1971; L’Hermite & Midgley 1972a; Nokin et al. 1972; Robyn et al. 1973; Sinha et al. 1973; L’Hermite & Midgley 1971).

The assay calculations were performed according to the recommendations of Rodbard et al. (1968); linearization of the standard inhibition curves was achieved by logit
transformation of the ratio between the amount of labelled hormone bound to the antibody in presence of unlabelled hormone and the amount of labelled hormone bound in absence of it. All assay parameters including slopes (b), Y intercepts (a), index of precision (g; Gaddum 1933), validity tests for regression (t-test) and for linearity (F test) and estimates of hormone concentrations with standard deviations on triplicates were calculated by programmes (Robyn et al., to be published) run on the Control Data Corporation (CDC 6500) University Computer.

For HCG, the results were expressed in IU/ml with reference to the second international standard. For LH and FSH the results were expressed in mIU/ml with reference to a human pituitary gonadotrophin preparation (HHG-B; Petrusz et al. 1971a) standardized against the second international reference preparation of human menopausal gonadotrophin. For prolactin, the results were expressed in milli-Units with reference

![Graph](image)

**Fig. 1.**
Mean serum prolactin in women at day 3 post-partum as measured by a homologous ovine radioimmunoassay (●—●) and by a homologous human radioimmunoassay (○—○) before and after im injection of 200 µg methylergobasine maleate (Methergin®, Sandoz) at 0 min. One Unit (U) is the amount of immunoreactive prolactin contained in 1.0 ml of a pool of sera rich in prolactin collected from post-partum women.

Vertical bars represent standard error of the means.
Mean serum prolactin in regularly menstruating women as measured by a homologous ovine radioimmunoassay (●—●) and by a homologous human radioimmunoassay (○—○) before and after im injection of 200 µg methylergobasine maleate (Methergin®, Sandoz) at 0 min. Vertical bars represent standard error of the means.

to a pool of sera rich in prolactin collected in the early post-partum: one milli-Unit (mU) is the amount of immunoreactive prolactin contained in one µl of this pool. In the homologous ovine assay 1.0 mU is equivalent to 2.3 milli-ampoule (with 95% fiducial limits at 2.9 and 1.8) of the research human serum prolactin standard (MRC 71/167) from the Medical Research Council (London, Great Britain). In the homologous human assay, 1.0 mU of the laboratory standard is equivalent to 3.26 µU
Mean serum prolactin in men as measured by a homologous ovine radioimmunoassay (●—●) and by a homologous human radioimmunoassay (○—○) before and after injection of 200 μg methylergobasine maleate (Methergin®, Sandoz) at 0 min. Vertical bars represent standard error of the means.

(with 95% fiducial limits at 4.57 and 2.40 of the research human pituitary prolactin standard (MRC 71/222) from the Medical Research Council (London, Great Britain) and 1.0 mU of the laboratory standard is equivalent to 2.71 milli-ampoule (with 95% fiducial limits at 2.05 and 3.35) of the research human serum standard (MRC 71/167).

All samples from the same subject were tested in triplicates in the same assay. The experimental data were submitted to a variance analysis with calculation of the between subjects and the within subjects variations using factorial arrangements of treatments (Snedecor 1956). The distribution of the individual values within each treatment group has been tested by a Rankit test (Martin 1973). The distribution of the individual values was log-normal for HCG, LH, FSH and prolactin serum concentrations. All statistical analyses were conducted on log transforms. The homogeneity of variance among the treatment groups was based on Bartlett’s tests (Snedecor 1956).
Mean serum HCG and FSH in women at day 3 post-partum before and after im injection of 200 µg methylergobasine maleate (Methergin®, Sandoz) at 0 min. Vertical bars represent standard error of the means.

RESULTS

1. Early post-partum

The $\chi^2$ values for the Bartlett’s tests were 0.6 for HCG, 6.7 for prolactin as measured by the ovine homologous assay, 3.5 for prolactin as measured by the human homologous assay and 0.5 for FSH. None of these values was significant at a level of probability of 0.05 with the 12 degrees of freedom involved: thus the variances of the treatment groups could be assumed to be homogeneous. Serum prolactin as measured by the ovine homologous assay was some 1.5 times lower than when measured by the human homologous assay.
Mean serum LH and FSH in regularly menstruating women before and after injection of 200 μg methylergobasine maleate (Methergin®, Sandoz) at 0 min. Vertical bars represent standard error of the means.

(Fig. 1). There was a highly significant correlation ($r = 0.57; P < 0.001$) between the results obtained by the two radioimmunoassay methods for human prolactin.

As indicated in Fig. 1, a significant decrease in serum prolactin occurred between 45 and 60 min after injection of the ergot drug. During 2 to 3 h, the values remained at some 50% of the control levels obtained before or at the
time of injection. Then the mean serum prolactin concentration started rising again: in both homologous assays, it was significantly higher after 240 min than the control levels at 120 min.

As indicated in Fig. 4, there were no significant changes in HCG serum concentration after injection of methylergobasine maleate when using the control levels at 0 min as reference. However, the levels at 60 and 75 min were significantly higher than those at –30 and 240 min (Fig. 4).

Fig. 6.
Mean serum LH and FSH in men before and after im injection of 200 µg methyl-
ergobasine maleate (Methergin®, Sandoz) at 0 min. Vertical bars represent standard error of the means.
No significant changes in FSH serum levels were observed during the entire period of investigation (Fig. 4).

2. In regularly menstruating women

The $\chi^2$ values for the Bartlett's tests were 6.3 for LH, 12.7 for prolactin as measured by the ovine homologous assay, 1.6 for prolactin as measured by the human homologous assay and 0.6 for FSH. None of these values was significant at a level of probability of 0.05 with the 12 degrees of freedom involved: thus the variances of the treatment groups could be assumed to be homogeneous. Serum prolactin measured by the ovine homologous assay was some three times higher than when measured by the human homologous assay (Fig. 2). There is a highly significant correlation ($r = 0.458; P < 0.001$) between the results obtained by the two radioimmunoassay methods for human serum prolactin. There was a decrease in serum prolactin starting to be significant 75 min after intramuscular injection of 0.2 mg methylergobasine maleate (Fig. 2). After 240 min, the serum levels of prolactin were rising again and they were no longer significantly different from the control values found before or at the time of injection of the ergot drug (Fig. 2).

A significant fall in serum LH occurred within 75 min after injection of 0.2 mg methylergobasine maleate (Fig. 5). After 240 min, LH values were not significantly different from the control levels. The LH values were lower at 0 min than at -30 min but the difference was not significant ($F = 0.29; P > 0.05$). No significant, nor systematic changes in FSH serum concentrations were encountered during the period of investigation (Fig. 5).

3. In adult men

The $\chi^2$ values for the Bartlett's tests were 9.2 for LH, 9.4 for prolactin as measured by the ovine homologous assay, 1.4 for prolactin as measured by the human homologous assay and 12.5 for FSH. None of these values was significant at a level of probability of 0.05 with the 12 degrees of freedom involved: thus the variances of the treatment groups could be assumed to be homogeneous. As in regularly menstruating women serum prolactin levels measured by the ovine homologous assay were some three times higher than serum prolactin levels measured by the human homologous assay (Fig. 3). However, there was a highly significant correlation ($r = 0.432; P < 0.001$) between the results obtained by the two radioimmunoassay methods for human serum prolactin. As indicated in Fig. 3, with both systems, serum prolactin decreased significantly 45 min after injection of 0.2 mg methylergobasine maleate and until 120 min remained 2 times lower than before injection. At 180 min, serum prolactin concentration was not significantly different from the control values found before or at the time of injection, at least when determined with the ovine homologous assay.
As in regularly menstruating women, a significant fall in serum LH was also observed between 60 and 105 min after intramuscular injection of the ergot drug (Fig. 6): the values were 3 times lower than the control levels. After 105 min, serum LH increases again and at 120, 180 and 240 the values are not significantly different from the control levels. No significant nor systematic changes in FSH serum concentration were encountered during the period of investigation (Fig. 6).

**DISCUSSION**

The amino acid sequence of human prolactin has some 80 % homology with that of ovine prolactin (Lewis et al. 1972; Niall et al. 1973). L’Hermite et al. (1972a) measured human prolactin in serum by a radioimmunoassay initially developed by Davis et al. (1971) for ovine prolactin, using a pool of human sera rich in prolactin as standard. Serum levels of human prolactin as evaluated by this method showed a circadian periodicity (Nokin et al. 1972) different from that of TSH (Vanhaelet et al. 1973) and from that of cortisol or ACTH (Leclercq et al. 1973); they rise after TRH injection (L’Hermite et al. 1972b) during treatment with psychotrophic drugs (L’Hermite et al. 1972a) and they decrease after administration of 2-Br-a-ergocryptine and L-Dopa (Rozenweig et al. 1973; Badawi et al. 1974).

These data were similar to those reported by other groups using human homologous or heterologous assay methods (see Human Prolactin 1973).

In the present study, a significant fall in serum prolactin occurs within 30 to 75 min after injection of 0.2 mg methylerygobasine maleate in women on day 3 post-partum, in regularly menstruating women and in men: the levels remain low until 180 min and increase again between 180 and 240 min after injection. The amplitude of the decrease is very similar as evaluated by both the ovine homologous and the human homologous radioimmunoassays; an excellent correlation exists between the assay results obtained by both methods. However, the absolute values of serum prolactin concentration expressed in terms of the same laboratory standard, i.e. a pool of sera collected from lactating women in the early post-partum are very different from one method to another. The values are some 3.0 times higher with the ovine homologous assay in blood samples collected from men and regularly menstruating women but they are some 1.5 times lower with the ovine homologous assay in blood samples collected from women in the post-partum period. Such discrepancies could be due to the heterogeneity of prolactin in the laboratory standard or to non-specific effects of serum. Turkington (1973) reported that human prolactin is heterogeneous in serum at least in patients with high concentrations of prolactin secreted by pituitary tumour cells. Such a heterogeneity has also
been observed in pituitary and in amniotic fluid prolactin preparations (Friesen 1973). In these conditions, the homologous ovine assay may not detect exactly the same population of prolactin molecules as the homologous human assay. Differences in the population of prolactin molecules as found during the early post-partum when the levels are very high and in men or in menstruating women exhibiting much lower levels, may account for the discrepancies observed between the absolute values in serum prolactin concentrations obtained by the two radioimmunoassays. It is of interest to consider that the difference between assay results is minimal, i.e. 1.5 times lower with the homologous ovine assay than with the human one, when serum samples from post-partum women are tested against the laboratory standard which is a pool of sera collected after delivery.

Non-specific effects of serum may also be involved in the differences found between the results obtained by the two radioimmunoassays for prolactin.

We could not so far get any “prolactin free” serum in order to investigate whether non-specific effects would be greater in one assay system than in another. Usually significant and sometimes even high serum levels or prolactin are still found after hypophysectomy (Friesen et al. 1973; L’Hermite & Robyn 1974). After chronic administration of high doses of 2-Br-a-ergocryptine, no complete suppression of prolactin secretion is obtained: some 30% of the pre-treatment levels are still detectable (Rozencweig et al. 1973). Non-specific effects of serum would not explain the difference found by the two radioimmunoassays between men and non-pregnant women on the one hand and post-partum women on the other.

A circadian periodicity in serum prolactin concentration has been described (Nokin et al. 1972; Sassin et al. 1972). Such periodicity seems to disappear during the last trimester of pregnancy but is present again on day 3 of the post-partum (Robyn et al. 1973): however, the periodic decrease found post-partum between 9.0 a.m. and 1.0 p.m., i.e. the period of the investigation, was only of some 15% whereas that observed after injection of methylergobasine maleate was of some 50%.

It can be concluded that, in this case, the decrease in serum concentration was likely due to an inhibition of prolactin secretion by the ergot drug. It has been reported that inhibition of prolactin secretion achieved in the post-partum by 2-Br-a-ergocryptine was effective in suppressing puerperal lactation. Although prolactin inhibition induced by methylergobasine maleate is of less amplitude and of shorter time than that induced by 2-Br-a-ergocryptine (Rozencweig et al. 1973), further studies are required to elucidate a possible effect on lactation of an intensive treatment with ergot drugs during post-partum.

In women on day 3 post-partum there is still between 770 and 940 mU of HCG per ml of serum. A significant increase in HCG serum levels occurs
during the period of observation. It seems, however, that it is not related to
the ergot drug since it apparently starts before injection. This 13% rise in
serum HCG takes place at the same time of the day as the circadian rise
observed during the last trimester of pregnancy (Pujol-Amat et al. 1973). It
remains to be established whether a circadian periodicity exists in serum
HCG concentration even after delivery.

In regularly menstruating women and in men, methylergobasine maleate is
also less effective than 2-Br-a-ergocryptine as inhibitor of prolactin secretion.
Due to its uterotonic and vascular effects, the doses cannot be increased as
much as for 2-Br-a-ergocryptine (Rozencweig et al. 1973), which is free of
such side effects.

In regularly menstruating women and in men, methylergobasine maleate in¬
duces within 60 to 75 min after injection a significant decrease in serum LH
lasting 180 to 240 min without any concomitant change in serum FSH levels.
Such inhibition of LH secretion induced by ergot drugs has also been reported
in rats (Wuttke et al. 1971) where ergocryptine can block ovulation (Kraicer
& Strauss 1970). In contradistinction to the effects of methylergobasine maleate
in humans, Gorski (1966) and Kordon (1967) concluded that in rats ergo¬
cornine did not inhibit the tonic secretion of pituitary gonadotrophins, but only
the cyclic one. However, Wuttke et al. (1971) have shown that, in rats, ergo¬
cornine increases the prolactin inhibiting factor (PIF) and decreases the LH
releasing hormone (LH-RH) in the hypothalamus. These data together with
those reported in this study suggest that, not only in animals but also in
humans, prolactin and LH secretions may be interrelated at a hypothalamic or
at a supra-hypothalamic level. However, ergocornine and also 2-Br-a-ergo¬
cryptine inhibits prolactin secretion from rat and human foetal pituitaries in
culture (Pastee¡s et al. 1971) and from cultured rat pituitary tumour cells
(Tashjian & Hoyt 1971). Nagasawa et al. (1973) reported that 2-Br-a-ergo¬
cryptine counteracts prolactin secretion promoted by dibutyryl-adenosine 3',5'
monophosphate in rats. Thus in addition to their effects mediated by the hypo¬
thalamus, it appears that ergot drugs also exert a direct inhibition on the
pituitary, at least on the lactotrophs.

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