Differences in the metoclopramide-induced prolactin release related to age at first full-term pregnancy or nulliparity

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To investigate if an association exists in parous women between metoclopramide-induced prolactin (PRL) release and chronological age at first full-term pregnancy and to compare their response to a group of non-parous women, we studied 139 healthy, non-lactating women aged 15.8–48.2 years, on days 18–22 of their menstrual cycle (except two post-menopausal women). There were 61 parous women divided according to chronological age at first full-term pregnancy: 22 were aged ≤20.0 years, 25 were aged 20.1–29.9 years and 14 were aged >30 years. There were also 50 nulliparous women and 28 women who had experienced only first trimester abortions. Three basal blood samples were obtained before oral metoclopramide (10 mg) was administered; subsequently samples were taken at 60, 90 and 120 min. Duplicate serum PRL determinations were performed by radioimmunoanalysis and the area under the curve (AUC) calculated. In parous women, the PRL release related to age at first full-term pregnancy increases. Materials and methods

Introduction

Among the several long-term effects of a first full-term pregnancy on the subsequent hormonal environment of a woman (Musey et al., 1987a), prolactin (PRL) has received special attention. Earlier studies have described that both basal and stimulated (Musey et al., 1987b; Espinosa de los Monteros et al., 1991) serum PRL concentrations are lower in parous than in nulliparous women. Furthermore, the metoclopramide-stimulated PRL release in the latter group was augmented linearly as chronological age increased (Parra et al., 1993).

In addition, age at first full-term pregnancy as a major reproductive factor in human breast cancer has received wide acceptance for many years (MacMahon et al., 1973; Miller and Bulbrook, 1986). Women who have had their first full-term pregnancy before 22 years of age have a much lower risk for breast cancer than women with a first full-term pregnancy after the age of 30 years and than nulliparous women (MacMahon et al., 1973; Kwa et al., 1981; Henderson et al., 1982). Also, women who had exclusively repeated first trimester abortions have an elevated risk for breast cancer (Pike et al., 1981). However, others have recently proposed age at last full-term pregnancy as a high parity (Lund, 1991; Kalache et al., 1993) as protective factors for human breast cancer. Considering the significant morbidity associated with breast cancer, it seems justified to investigate associated risk factors, among which a high serum PRL concentration has been repeatedly suggested (Kwa et al., 1981; Yu et al., 1981; Henderson et al., 1982).

Based on the above information, we reasoned that if a first full-term pregnancy has long-term lowering effects on serum PRL concentrations, then the lower stimulated PRL response in parous women may show a gradual increase as chronological age at first full-term pregnancy increases. Thus, the present cross-sectional study was undertaken to investigate the existence of possible differences in the metoclopramide-induced PRL release among parous women with different chronological ages at the time of their first full-term pregnancy and to compare them with a group of nulliparous women and with another group of women who had experienced only first trimester abortions.

Materials and methods

Subjects

A total of 139 clinically healthy volunteer women, aged 15.8–48.2 years, with a history of regular menses at least 1 year before the study (except two post-menopausal women aged 42.5 and 48.2 years), cycle length 28–30 days, of 4–6 days duration, and without a family history of breast cancer (mother or sister) were studied. None had been regularly ingesting any medication known to increase serum PRL concentrations, including oral contraceptives, during the 6 months prior to the study. In addition, no obstetric event or abortion or lactation had occurred in the previous 6 months. At the time of the study, 31 women were using an intrauterine device (Copper T model T Cu-380 A; Finishing Enterprises Inc., North Tonawanda, NY, USA) known not to modify PRL response to metoclopramide (Parra et al., 1991). 41 women were using barrier contraceptive methods, 27 women were not using any birth control method and 40 women had never had sexual intercourse. Women with an active sexual life were asked not to have sexual intercourse for 1 week before the study. All subjects were included after giving written informed consent, and the protocol was approved by the Institutional Review Board of the Instituto Nacional de Perinatología (México City, México).
Before entering the study, all women had normal basal serum total triiodothyronine, free thyroxine, thyroid-stimulating hormone, cortisol, 17α-hydroxyprogesterone and free testosterone concentrations.

The 139 women were divided into groups: a parous group (n = 61) subdivided into three groups according to the women’s chronological age at the time of their first full-term pregnancy; one nulliparous group (n = 50); and one group of women who had experienced only first trimester abortions (n = 28). In addition, as in a previous report (Parra et al., 1993), each group was subdivided according to the woman’s chronological age at the time of testing: ≦25.0 and >25.0 years. This seemingly arbitrary division may have biological relevance because there is evidence that the growth of the lean tissue mass in humans ceases at ~20–25 years of age (Reba et al., 1968; Forbes, 1972) and that, after the age of 25 years, the lean body mass decreases at a slow but steady rate (Forbes, 1972). Therefore, the final group classification was as follows: group 1, 22 women who had their first full-term pregnancy (≧36.0 weeks of gestation) before 20.0 years of age, with a chronological age at the time of testing of ≦25.0 (n = 12) or >25.0 years (n = 10); group 2, 25 women who had their first full-term pregnancy between 20.1 and 29.9 years of age, with a chronological age at the time of testing of ≦25.0 years (n = 3) or >25.0 years (n = 22); group 3, 14 women who had their first full-term pregnancy after 30.0 years of age, with a chronological age at the time of testing of >25.0 years (n = 14); group 4, 50 nulliparous women (Parra et al., 1993), with a chronological age at the time of testing of ≦25.0 (n = 20) or >25.0 years (n = 30); and group 5, 28 women who had only experienced one to five first trimester abortions but never a full-term pregnancy, with a chronological age at the time of testing of ≦25.0 (n = 1) or >25.0 years (n = 27).

The term ‘parous’ will be used to encompass all women in groups 1–3, while ‘non-parous’ will refer to women in groups 4 and 5. The term ‘nulliparous’ will be used exclusively for those women in group 4.

**Experimental protocol**

All women, except the two post-menopausal women (included in group 4), were studied between days 18 and 22 after day 1 of their last menstrual cycle. After a 10–12 h overnight fast, an indwelling peripheral catheter was placed in the antecubital region between 08.00 and 08.30 h and kept permeable with a slow i.v. drip of a 0.85% saline solution. After a 30 min rest, three basal blood samples were obtained at 15 min intervals (~30, ~15 and 0 min), and thereafter at 60, 90 and 120 min after the administration of a single 10 mg oral dose of metoclopramide. The 30 min sample was avoided because the PRL response was most variable at this sampling time, presumably because of individual variations in drug absorption (Espinosa de los Monteros et al., 1991). Elimination of this sampling value did not preclude a valid interpretation of the total response (Parra et al., 1991, 1993).

Duplicate serum determinations of PRL were carried out in all samples, and serum oestradiol and progesterone measurements were made only in the pool of the three basal samples using commercially available radioimmunoassay kits (PRL and progesterone, Kodak Clinical Diagnostics Ltd, Amersham, UK; oestradiol, Diagnostic Products Corporation, Los Angeles, CA, USA). The intra- and interassay coefficients of variation were PRL ≦4.8 and ≦6.5%, oestradiol ≦6.5 and ≦8.2%, and progesterone ≦5.4 and ≦9.0% respectively.

In each woman the following clinical parameters were registered: chronological age at the time of testing, body mass index (BMI), obstetric history (number of pregnancies, vaginal deliveries, Caesarean sections or abortions), duration of lactation for the first and all subsequent (cumulative) children, chronological age at first full-term pregnancy, the time elapsed between first full-term pregnancy and testing and the time elapsed between the last obstetric event registered and testing. Serum PRL concentrations were recorded at each sampling time. In addition, the 2 h area under the PRL curve (AUC-PRL) was calculated using a trapezoidal method (Zenobi et al., 1992). AUC-PRL values were used for all calculations as a more valid measurement for a provocative test, because we have previously obtained (Parra et al., 1991, 1993) similar conclusions regardless of the way the results were expressed.

The values expressed in the text, tables and figures represent means ± SEM.

**Statistical analysis**

A one-way analysis of variance and Student’s paired and unpaired t-tests were used for within- and between-groups comparisons. The level of significance for all statistical tests was 0.05. Pearson’s correlation analysis was used to study the inter-relationships between the different clinical parameters, AUC-PRL values and serum oestradiol concentrations.

**Results**

**Clinical data (Table I)**

Groups 1 and 4 were the youngest, followed by groups 2 and 5; group 3 was the oldest (P < 0.001). However, all groups had a similar BMI. Group 2 had the greatest mean pregnancy rate when compared with groups 1 and 3 (P < 0.001), and group 5, by definition, had the highest number of abortions (P < 0.001).

Among the 61 parous women (groups 1–3) 31 were uniparous, 29 had experienced two or three pregnancies, while only one woman had had four pregnancies. Of the parous women, 36 lactated for <6 months after the first child, nine lactated for between 7 and 15 months, and 16 women did not lactate at all. In all, 30 women had a cumulative lactation of <6 months, 17 lactated for between 7 and 18 months, and 14 women never lactated. By definition, chronological age at first full-term pregnancy was clearly different between parous groups 1, 2 and 3 (P < 0.0001). In the parous group the time elapsed between the first full-term pregnancy or the last obstetric event and testing was inversely proportional to chronological age at first full-term pregnancy, as expected.

**Serum oestradiol**

Basal serum oestradiol concentrations are shown in Table I. Group 1 had the highest concentration (P ≦ 0.04), with similar concentrations among groups 2–4. However, groups 3 and 4 had lower concentrations than group 5 (P < 0.05 and <0.01 respectively). In group 1, the oestradiol concentration in women aged ≦25.0 years at the time of testing (502 ± 69 pmol/l) was slightly lower than that in women aged >25.0 years (708 ± 102 pmol/l, P = 0.05). In group 4, women aged ≦25.0 years at the time of testing had lower oestradiol concentrations (209 ± 33 pmol/l) than those aged >25.0 years (367 ± 40 pmol/l, P = 0.02). As can be seen, women in group 1 aged both ≦25.0 and >25.0 years had respectively a higher serum oestradiol concentration than the corresponding women in group 4 (P ≦ 0.002).

215
A. Parra et al.

Table I. Clinical data and basal serum oestradiol concentrations in the 139 women studied

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Serum testosterone (nmol/l) at different sampling times (min)</th>
<th>Area under curve (AUC) (ng/ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>−30</td>
<td>−15</td>
</tr>
<tr>
<td>1</td>
<td>22</td>
<td>7.3 ± 0.6^a</td>
<td>7.0 ± 0.6^a</td>
</tr>
<tr>
<td>&lt;25.0 years</td>
<td>12</td>
<td>6.5 ± 0.4</td>
<td>6.3 ± 0.6</td>
</tr>
<tr>
<td>&gt;25.0 years</td>
<td>10</td>
<td>8.2 ± 1.1</td>
<td>7.7 ± 0.9</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>7.1 ± 0.6^a</td>
<td>6.0 ± 0.2^a</td>
</tr>
<tr>
<td>&lt;25.0 years</td>
<td>3</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>&gt;25.0 years</td>
<td>22</td>
<td>7.3 ± 0.7</td>
<td>6.0 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>7.0 ± 1.0^a</td>
<td>7.5 ± 1.2</td>
</tr>
<tr>
<td>&lt;25.0 years</td>
<td>0</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>&gt;25.0 years</td>
<td>14</td>
<td>7.0 ± 1.0</td>
<td>7.5 ± 1.2</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>10.4 ± 0.6</td>
<td>9.3 ± 0.6</td>
</tr>
<tr>
<td>&lt;25.0 years</td>
<td>20</td>
<td>8.7 ± 0.8^a</td>
<td>8.2 ± 0.7^a</td>
</tr>
<tr>
<td>&gt;25.0 years</td>
<td>30</td>
<td>11.6 ± 0.8^d</td>
<td>10.0 ± 0.8^a</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>9.6 ± 1.0</td>
<td>8.9 ± 0.8</td>
</tr>
<tr>
<td>&lt;25.0 years</td>
<td>1</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>&gt;25.0 years</td>
<td>27</td>
<td>9.8 ± 1.0^e</td>
<td>9.0 ± 0.8^e</td>
</tr>
</tbody>
</table>

See Table I for the definition of groups.

^p < 0.05 versus groups 4 and 5.

^p < 0.01 versus groups 2–5.

^p < 0.05 versus groups 3–5.

^p < 0.01 versus group 5.

^p < 0.05 versus group 4.

^p < 0.03 versus group 4 aged >25 years.

^p < 0.04 versus groups 2 and 3 aged >25 years.

Progestrone

Except for the two post-menopausal women in group 4, the other 137 women had a serum progesterone concentration ≧12.7 nmol/l, indicative of ovulation, without significant differences between groups (data not shown).

Prolactin (Table I)

The mean fasting values were similar among groups 1, 2 and 3, and also between groups 4 and 5. In general, basal concentrations were significantly lower in the former than in the latter groups.

In response to the oral metoclopramide, group 1 had significantly lower concentrations than group 2 at 60 min, than group 3 at 60 and 90 min and than groups 4 and 5 throughout the test. There were no significant differences either between groups 2 and 3 or between groups 4 and 5; nevertheless, group 2 had lower concentrations than groups 4 and 5 at 120 min, while group 3 had lower concentrations only when compared with group 5 at 60 min. Considering chronological age at the time of testing, in group 1 the PRL response was similar among women aged ≤ and >25 years, but in group 4 women aged ≥25.0 years had a lower response than those aged >25.0.
Figure 1. Relationships between the serum prolactin area under the curve and (A) chronological age at the time of testing in parous (●; groups 1–3) and non-parous women (○; groups 4 and 5), and (B) chronological age at the time of testing in parous women from group 1 aged ≤25.0 (●) and >25.0 (○) years.

Correlation analysis
Chronic age at the time of testing had a positive correlation with AUC-PRL in parous and non-parous women, but the slope was 43% greater in the latter group (Figure 1A). Among parous women, only in those of group 1 were differences observed in this correlation: a positive correlation existed for women aged ≤25.0 years ($y = 0.1689 + 0.3366x$, $r = 0.607$, $P = 0.018$), while a negative correlation existed for women aged >25.0 years ($y = 17.3898 - 0.3026x$, $r = -0.628$, $P = 0.019$) (Figure 1B). Using an intersecting lines technique (Mellits, 1968), the estimated point of intersection was calculated as between 26 and 27 years of age. In addition, in parous women there was a significant positive linear correlation between chronological age at the time of the first full-term pregnancy and AUC-PRL (Figure 2A).

In parous women aged ≤25.0 years who were allocated to groups 1 and 2, the AUC-PRL had a positive correlation with the time elapsed between the first full-term pregnancy and testing, but in women aged >25.0 years the same variables had a negative correlation and the estimated point of intersection was calculated as between 9 and 10 years (Figure 2B). Basal serum oestradiol concentrations showed a weak positive correlation with chronological age at the time of the first full-term pregnancy only in women aged ≤25.0 years ($r = 0.451$, $P = 0.048$), but the same correlation was negative in those women aged >25.0 years ($r = -0.409$, $P = 0.003$) (data not shown). On the other hand, in non-parous women (regardless of their chronological age), serum oestradiol had a positive linear correlation with AUC-PRL ($r = 0.449$, $P < 0.0001$).

Discussion
Contrary to a previous report (Musey et al., 1987c), our study demonstrated that, as a general phenomenon, the 2 h AUC-

years throughout the test. Women aged ≤25.0 years in groups 1 and 4 had similar responses; however, women aged >25.0 years in group 1 had the lowest response, with intermediate concentrations in groups 2 and 3, and the highest response in groups 4 and 5.

The mean AUC-PRL for women in group 1 was the lowest ($P < 0.01$), with similar intermediate values for groups 2–4, and the highest value in group 5 when compared with groups 2 and 3 ($P < 0.01$), but the mean AUC-PRL for group 5 was not different from that in group 4. Again, in women aged ≤25.0 years, no difference was observed between groups 1 and 4. However, for women aged >25.0 years, group 1 had the lowest value ($P = 0.03$), groups 2 and 3 were similar, but clearly lower than groups 4 ($P = 0.029$) and 5 ($P = 0.035$); the two latter groups were similar.

The serum PRL response to oral metoclopramide, either at different sampling times or expressed as AUC, was similar in parous women who did ($n = 45$) or did not ($n = 16$) lactate either to their first child ($9.2 ± 0.3$ and $10.2 ± 0.6$ ng/ml/min respectively) or to any subsequent child ($n = 47$; $9.2 ± 0.3$ ng/ml/min, and $n = 14$, $10.4 ± 0.7$ ng/ml/min respectively). Among women who lactated to their first child, those aged ≤25.0 years ($n = 12$) had lower AUC-PRL values than those aged >25.0 years ($n = 33$) ($7.6 ± 0.4$ versus $9.8 ± 0.4$ ng/ml/min respectively, $P = 0.007$), and the same was true for cumulative lactation ($P = 0.005$). Considering only parous women aged >25.0 years, those who lactated to their first child ($n = 33$) had slightly lower AUC-PRL values than those who did not ($n = 13$) ($9.8 ± 0.4$ versus $10.8 ± 0.6$ ng/ml/min respectively, $P = 0.07$), and the same difference was observed for cumulative lactation ($P = 0.061$). A similar comparison could not be made among women aged ≤25.0 years because of the small number of young non-lactating women ($n = 3$).
Figure 2. Relationships between the serum prolactin area under the curve and (A) chronological age at the time of their first full-term pregnancy in 61 parous women (groups 1–3), and (B) the time elapsed between the first full-term pregnancy and testing in parous women from groups 1 and 2, aged ≤ 25.0 (●) and >25.0 (○) years.

The PRL response throughout the metoclopramide test showed clear differences between group 1 and the other groups at an increasing number of sampling times as one moved from group 2 to group 5 (Table II). Furthermore, when the AUC-PRL value for each group was analysed, group 1 had the lowest and group 5 had the highest values, without significant differences between groups 2–4. Although women aged ≤25.0 years in groups 1 and 4 (no other group comparisons were valid because of the small number of women) had a similar AUC-PRL value, when women aged >25.0 years of age were considered alone, a clear difference emerged between the lowest value in group 1, the similarly intermediate values in groups 2 and 3, and the similarly highest values in groups 4 and 5. These results would indicate that the long-term PRL lowering effect of a first full-term pregnancy became more obvious after the women had reached a chronological age >25.0 years, or after a certain ‘lag period’ had occurred. For this reason, it was interesting to note that in parous women the correlation between the time from the first full-term pregnancy and testing and the AUC-PRL value was positive in women aged ≤25.0 years, while it was negative in those aged >25.0 years, with an estimated point of intersection of between 9 and 10 years (Figure 2B). If the mean chronological age at first full-term pregnancy in group 1 was 17.6 years, this would indicate that in such women the PRL lowering effect of the early pregnancy will theoretically start to show at between 26 and 27 years of age, an age identical to that observed in parous women of group 1 when chronological age at the time of the study and AUC-PRL value were correlated (Figure 1B). As an overall interpretation, it seems that after the age of 25 years, for a similar chronological age, parous women would have a greater dopaminergic tone than non-parous women, and thus a lower PRL response to oral metoclopramide.

Finally, considering only parous women, the highest basal serum oestradiol concentration was observed in the early parous women, who were also the youngest at the time of the study (group 1), while the lowest concentration was found in women who had experienced their first full-term pregnancy after the age of 20 years and were also aged >25.0 years at the time of the study (groups 2 and 3). These results suggest a change in the level of influence of oestradiol upon PRL release via the tuberoinfundibular dopaminergic neurons, depending on the biological ‘momentum’ of a parous woman. In young women (aged ≤25.0 years) who had experienced a first full-term pregnancy before 20.0 years of age (as in group 1), high and/or sustained serum oestradiol concentrations do not decrease but may in fact favour the PRL-inhibiting effect of
dopamine, as demonstrated to occur in monkeys with pituitary stalk transection (Bethea, 1985) and in normal and agonadal women (Judd et al., 1979), and thus the lower PRL response to metoclopramide. However, in women aged >25.0 years and who had experienced a first full-term pregnancy after the age of 20.0 years, the older the woman, the lower the mean basal serum oestriadiol concentration, the lower the dopaminergic tone and the higher the PRL response to metoclopramide (as in groups 2 and 3).

The PRL response was similar regardless of whether or not the women had lactated. Perhaps this was because of the small number of patients studied in terms of past history of lactation and of the fact that the study was not specifically designed for that purpose.

In conclusion, our study has demonstrated the existence of gradual and progressive differences in the metoclopramide-induced PRL response in parous women according to their chronological age at the time of their first full-term pregnancy, and such a response was lower than in nulliparous women or women with only first trimester abortions. The long-term influence of an early parity, and of a prolonged nulliparity — respectively beneficial and detrimental — upon the PRL response seems to require more time to emerge clearly: either a chronological age >25.0 years or at least 9–10 years after the first full-term pregnancy, especially in early parous women.

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