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Gender-related differences in functional assessment of serotonergic system in healthy young subjects

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Introduction. Prolactin stimulation test with serotonergic stimulants has been widely used in the study of diverse psychiatric disorders. However, the characterization of this response in normal subjects is still incomplete.

Objective. To compare the response to serotonin stimulation using dexfenfluramine, a specific serotonergic agent, in young healthy men and women, controlling the menstrual cycle.

Methods. A total of 10 women and 9 men, who were given 30 mg of dexfenfluramine orally, were studied and their levels of prolactin were measured on an hourly basis for a five-hour period. Baseline, maximum and delta values of prolactin were compared for both groups.

Results. According to the age groups studied (mean age for men: 19.9 ± 2.5 years old; mean age for women: 20 ± 1.5 years old), the prolactin maximum level and the response to prolactin (ΔPRL) were significantly higher in women (p-values: 0.02 and 0.04, respectively).

Conclusion. Young healthy women show a greater response to stimulation with dexfenfluramine than young healthy men. Clinical and biological implications of this observation are discussed in the context of the currently available research papers.

Key words:
Serotonin. Stimulation. Challenge. Prolactin. Estrogen. Gender.

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Diferencias de género en la exploración funcional del sistema serotoninérgico en jóvenes sanos

Introducción. Las pruebas de estimulación de prolactina con agonistas serotoninérgicos han sido ampliamente utilizadas en el estudio de diversas patologías psiquiátricas; sin embargo, la caracterización de su respuesta en sujetos normales es aún incompleta.

Objetivo. Comparar la respuesta a la estimulación serotoninérgica utilizando dexfenfluramina, un agente serotoninérgico específico, en hombres y mujeres jóvenes sanos, controlando el ciclo menstrual en estas últimas.

Métodos. Se estudió a 10 mujeres y 9 hombres, a quienes se les administró 30 mg de dexfenfluramina por vía oral, midiendo los niveles de prolactina cada hora por un período de 5 h. El nivel basal, el nivel máximo y la variación de prolactina fueron comparados en ambos grupos.

Resultados. En los grupos etarios estudiados (edad promedio para los hombres: 19,9 ± 2,5 años; edad promedio para las mujeres: 20 ± 1,5 años), el nivel máximo de prolactina y la respuesta a prolactina (ΔPRL) fueron significativamente mayores en mujeres (valor p: 0,02 y 0,04, respectivamente).

Conclusión. Las mujeres jóvenes sanas muestran una mayor respuesta a la estimulación con dexfenfluramina que los hombres jóvenes sanos. Las implicancias clínicas y biológicas de esta observación se discuten en el contexto de la literatura.

Palabras clave:
Serotonina. Estimulación. Prueba. Prolactina. Estrógeno. Género.

INTRODUCTION

The serotonergic function has been associated to different body functions such as sleep, eating, sexuality and control of impulses. Serotonergic dysfunctions have been identified in psychiatry in several disorders, such as mood, obsessive-compulsive, eating and personality ones[1-3]. Drugs that increase serotonergic transmission, such as selective serotonin reuptake inhibitors, are used in the treatment of these diseases.

Different strategies have been used to examine the serotonergic function in clinical populations. For example, serotonin levels and its metabolites in some body fluids (blood, CSF, urine) were measured[4,5] One of the limitations

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of these studies is that they represent a static measurement of serotonergic function. In order to achieve a dynamic examination, stimulation tests with substances such as metachlorophenylpiperazine (m-CPP), flesinoxan and fenfluramine have been developed[6]. One of those used most frequently is fenfluramine, a racemic mixture of d and l-fenfluramine. However, the studies have observed that d-fenfluramine is more specific as a serotonergic agonist[7,8].

Even though the serotonergic stimulation tests have been widely used in the study of different psychiatric diseases, the characterization of its response in normal subjects is still incomplete. Few investigations have been made on this subject and the conclusions in regards to a difference in the response of prolactin in relationship to gender are contradictory. Some authors have observed a greater response of prolactin in women compared to men[9], while others find that the difference is not significant[10]. The different methods used have made it difficult to reach a conclusion on this problem.

One of the main reasons that explain the differences between these studies is the wide range of ages of the subjects. This point is especially relevant because the serotonergic system capacity of response decreases as age increases[9,10].

Recent studies have used narrower age groups, focusing on the changes related with age in the serotonergic function described by McBride et al.[9] and Muldoon et al.[10] These studies indicate that gender has no impact on the response of elderly subjects to d-fenfluramine.[11]. On the other hand, a study conducted in 14.4 ± 1.5 year old adolescents showed significant differences according to gender in their response to mCPP.[12]

In addition, the different conclusions of the previous publications[9,10] on influence of gender in prolactin response may be due to the large variations in estrogen levels in the variations of prolactin response in accordance to the menstrual cycle phase[13].

This study aims to compare the response of prolactin to the serotonergic system stimulation using dexfenfluramine, a specific serotonergic agent, in healthy and young men and women (average age: 20 years; range: 17-25 years), controlling the menstrual cycle in the women. Our hypothesis which is based on the literature is that women have a greater prolactin response than men in this age group.

This study differs from other previous ones because it is the first one to focus on this specific age group (17-25 years), in order to elucidate if there is a difference according to gender to serotonergic stimulation. In addition, it also differs because it uses the most specific serotonergic agonist, that is, dexfenfluramine[7,8], and because it controls the menstrual cycle in accordance with the findings of O'Keane et al.[13]

METHODOLOGY

Subjects

A total of 29 young healthy subjects (10 women and 9 men) with no background of medical or psychiatric disease were studied. All had given their consent and participated voluntarily and had been informed on the study. The study was approved by the Ethics Committee of the Hospital Clínico of the University of Chile and respected the principles stated in the Declaration of Helsinki. The Goldberg questionnaire was used to rule out the presence of psychiatric disease[14,15]. All the women were studied within their first 10 days of the menstrual cycle to control the variations related with the cycle. This period corresponds to the time when the estrogen levels and response to d-fenfluramine are the least[13]. None of them were pregnant or were taking contraceptive pills. In addition, none of the subjects studied were taking any type or drug or medicine.

Procedures

The subjects followed a low monoamine diet for 72 hours and were fasting at the time of the test. The test began at 8.30 a.m. with a 30-minute period of rest after which intravenous catheter was placed in the forearm. They remained at rest in bed for 45 minutes when the first two blood samples were drawn in periods separated by 10 minutes, corresponding to the baseline level. At this time, corresponding to time 0, 30 mg of oral dexfenfluramine were administered. After, blood samples were drawn every hour, up to a total of five. During the entire period, the subjects remained at rest in bed and they were only allowed to eat a light meal at 4 hours of the administration of fenfluramine. The blood samples were centrifuged for 10 minutes at 3,500 rpm. The plasma was stored at 20 ºC. Prolactin levels were measured using the Abbot Meia Axsym. Low sensitivity limit for the prolactin test is 0.6 ng/ml and inter-trail variation coefficient was 4.5%.

Statistical analysis

The average values and standard deviations were calculated for each significant variable such as age, baseline prolactin, maximum prolactin value (the maximum obtained in any of the five samples) and the delta-prolactin (the difference between the maximum value of prolactin and the baseline value of prolactin for each subject, corresponding to the «prolactin response»).

Considering the small size of the group and that the prolactin values for each time did not follow a normal distribution, the data were analyzed using non-parametric tests. The Kruskal-Wallis test was used to determine if there was a significant variation in the time for each group (men and women). Wilcoxon t function was used to search for signifi-

cant differences between the groups of men and women for each hour of measurement, especially in the baseline, maximum and delta-prolactin values.

RESULTS

Both the group of women as that of men showed significant increases in the prolactin values during the five hour follow-up in accordance with the results of the Kruskal-Wallis test applied to the seriated measurements (p-value: 0.001 for women and 0.001 for men).

The baseline value of prolactin was not significantly different between men and women (p-value: 0.59). However, as shown in table 1, in agreement with Wilcoxon t function, the maximum value of average prolactin was significantly greater (p-value: 0.02) in women (39.39 ng/ml) than in men (21.39 ng/ml). The delta-prolactin value was also significantly greater (p-value: 0.04) in women (24.35 ng/ml) than in men (10.02 ng/dl). The standard deviation was greater in women than in men for all the prolactin values.

Figure 1 shows the time course of the prolactin response to the d-fenfluramine stimulation test. In spite of the differences in the already mentioned values, the time pattern observed was similar for men and women. A peak was reached at the third and fourth hour, after which the levels tended to become stabilized. The chart illustrates a greater response in women that becomes clear in the fourth hour of the measurement.

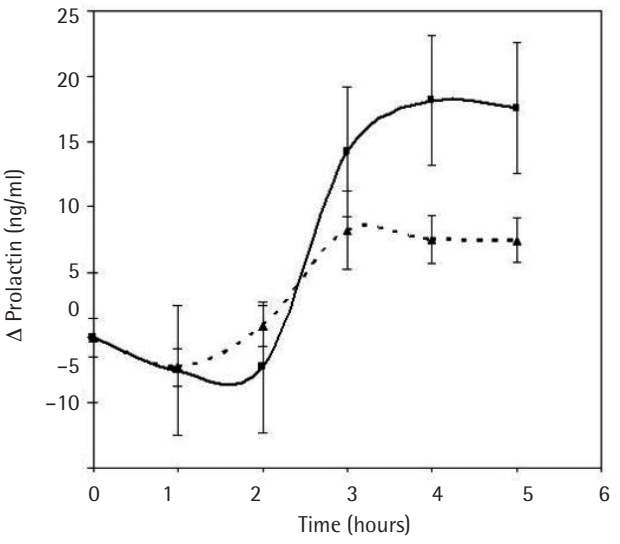

Figure 1 *Variation of the response of prolactin in relationship to time. The solid line curve that joins the boxes corresponds to Δ-prolactin (ΔPRL) of the women. The broken line that joins the triangles corresponds to the ΔPRL of the men. The standard deviations of each curve are demonstrated. Women have significantly greater response of prolactin that is made clear in the fourth and fifth hour of the follow-up.*

DISCUSSION

A difference was found in the response of prolactin to stimulation with d-fenfluramine between healthy young men and women and the response was significantly greater in women. This difference would be present at least until the age of 20 years and, in accordance to other authors, seems to decrease in older subjects[9-11] probably due to the effects of aging in the serotonin transporter binding sites[16-18]. The results obtained in this study are consistent with the evidence of a difference related with gender in the serotonergic system. This has been demonstrated in genetic, biochemical and neuroendocrine studies, both in animal as well as human models[2,3,19-21].

Table 1 **Average values of baseline prolactin, maximum prolactin, and delta-prolactin expressed in ng/ml and age corresponding to groups of men and women, with the p-values obtained**

	Men		Women		p-value
	X	SD	X	SD	
Age	19.9	2.53	20	1.56	0.55
Baseline prolactin	11.37	2.96	15.04	11.18	0.59
Maximum prolactin	21.39	4.79	39.39	16.46	0.02
Δ Prolactin	10.02	4.96	24.35	17.38	0.04

Significant p-values (< 0.05) are found for the maximum prolactin and d-prolactin. X: average. SD: standard deviation.

There are genetic factors that should be taken into account. A recent study that measured serotonin blood levels is consistent with an inheritable sexual dimorphism, that is a result of the combination of loci dependent on gender and other independent factors[19]. On the other hand, the polymorphism of the serotonin transporter promoter region (5HTTLPR) interacts especially with gender, in agreement with the Cadoret et al. study[20]. This author found that men with this short variant were more prone to have greater symptoms in relationship to different behavior disorders. On the contrary, it was associated with lower levels of symptoms in women who had the short allele.

The influence of estrogens in serotonergic function is already clear in intra-uterine life. Studies on embryogenic cell lines have demonstrated expression of alpha and beta estrogen receptors in prenatal serotonergic cells and that estrogens reduce growth of the dendrites of these cells. This

effect may play a role in the formation of a sexually dimorphic pattern in serotonergic innervation[21].

As an example of neuroendocrine studies that support a gender-related difference in the serotonergic system, this d-fenfluramine induced sensation of fullness is different in male and female rats. The minimum effective dose with anorexigenic effect is lower in females and the magnitude and duration of this effect in the females is greater than in males.[3] In humans, Soloff et al.[2] observed that men with borderline personality disorder had decreased prolactin response, but this was not true in women. Impulsivity and aggressivity were inversely related with the delta prolactin and with the maximum prolactin value only in men.

These findings coincide with those found by McBride et al.[9] who found that fenfluramine induced prolactin release was greater in women than in men. However, it is important to stress that in the mentioned study, the youngest patient was 21 years old, older than the average age of the subject group that participated in our study. In relationship to the prolactin release time pattern, we did not find the pattern described by these authors (women reaching the peak earlier, with a faster decrease than in the man). In our study, the prolactin levels tended to become stabilized towards the end of the test, which is consistent with the patterns described by other authors[6].

Our results do not agree with those obtained by Muldoon et al.[10] who did not find that fenfluramine induced prolactin release was greater in women than in men. However, the conclusions may be interpreted as complementary: the age group analyzed by Muldoon et al. was between 25 and 60 years and our study analyzed the group immediately younger than that, between 17 and 25 years of age. In this sense it is important to stress that this is the first study that directly investigates the situation in young patients of approximately 20 years of age (with standard deviations of 2.53 for men and 1.56 for women).

One of the contributions of this study is that, on the contrary to others, d-fenfluramine was used instead of fenfluramine. This made it possible to have a more specific stimulation of the serotonergic system. Fenfluramine induced prolactin release may also be mediated by the catecholaminergic system, given that it is associated to an increase in the homovanillic acid (HVA) level[22]. This may lead to confusion in the interpretation of the possible differences in serotonergic function between men and women[7,8].

Another contribution of this study is that the effects of the menstrual cycle were controlled, since estrogen concentration is variable during the cycle and influences the response of prolactin to stimulants such as d-fenfluramine. Other works have demonstrated that the response to d-fenfluramine is the greatest in the middle of the cycle, minimum during the early and intermediate follicular phase in the pre-menstrual period, with a strict relationship to the levels of circulating estrogen[13]. One of the limitations of this study may be that it did not measure estrogen levels. However, given that O'Keane et al.[13] demonstrated that the response to d-fenfluramine was the lowest during the early follicular face (that occurs in the first days of the cycle), performing the d-fenfluramine test during these days makes it possible to control the phrase-dependent variations in the response of prolactin.

Although McBride et al.[9] found a greater response pattern in women, that study did not control the menstrual cycle. Thus, their results could have been affected by the time period of the cycle in the women studied. The fact that our study only considered women during the days corresponding to the lowest response of prolactin, and that in spite of this, significant differences were found (greater response in women than in men), strongly suggests that these differences cannot be explained by cyclic increases of estrogens dependent on the menstrual cycle in the group studied. That is, presumably, healthy 20 year-old young women have a greater average pattern of response of prolactin then healthy 20 year-old men, independently of time period of the woman's menstrual cycle.

One of the limitations of this study is that the weight of the subjects was not controlled as a variable to adjust the d-fenfluramine dose administered to each individual. Some studies indicate weight as an inversely related factor to the response of prolactin to fenfluramine[10]. Studies that have analyzed the pharmacokinetics of d-fenfluramine and its metabolites have concluded that there is a significant difference in the volume of distribution between obese and non-obese subjects, but not in other variables such as bioavailability, clearance or half-life[23]. In relationship to this, the longer follow-up (5 hours post-administration of the drug vs. 3.5 hours in other studies)[10] could lessen this weakness. Muldoon et al. determined that the blood concentration of the drug is an independent variable of body weight[10]. Thus, not measuring the levels of d-fenfluramine and its metabolites in blood may represent an additional limitation.

Finally, we could conclude that our results support the fact that there is a gender-related difference in stimulation of prolactin with dexfenfluramine in the 17 to 25 year old age group and that the literature strongly suggests that this corresponds to differences due to gender in the serotonergic system that should be present at least until this age. Future research should complete these findings by controlling weight and measuring the blood levels of dexfenfluramine. However, this study will be useful in d-fenfluramine stimulation tests to investigate young patients with different psychiatric disorders such as borderline personality disorder, contributing to an adequate interpretation of the results.

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