Prolactin-Releasing Effect of Buspirone in Developing and Adult Male and Female Rats

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Abstract. **The prolactin-releasing effects of buspirone, an azaspirodecanedione anxiolytic drug unrelated to the benzodiazepines in structure and pharmacologic properties, was examined in developing and adult male and female rats. The possibility that effects of this drug on hormone release could be modulated by neonatal brain sexual differentiation was also evaluated.**

A single injection of buspirone, 2 or 10 mg/kg body wt, increased serum prolactin (PRL) levels in both sexes; the increase was significant from Day 12 onward. The PRL-releasing effect increased with age. No significant sexual differences were observed in younger rats, but in peripubertal and adult animals, the hyperprolactinemic response was higher in the female. Neonatal androgenization of females or orchidectomy of males failed to modify the PRL-releasing action of buspirone.

Serum titers of luteinizing hormone and follicle-stimulating hormone were not modified by buspirone at any age.

The present results show for the first time the ontogeny of the PRL-releasing effect of buspirone in male and female rats, and provide evidence that the response is higher in the female and that the effect does not depend on brain sexual differentiation.

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Evidence from physiologic, biochemical, pharmacologic, and immunohistologic studies indicates that the release of anterior pituitary hormones is controlled by brain neurotransmitter systems (1). Neurotropic drugs acting on those brain controlling systems are able to modify prolactin (PRL) and gonadotropin secretion. These include antipsychotic drugs, such as haloperidol (2–4), as well as anxiolytic agents, such as benzodiazepines (5–8).

Buspirone, 8-[4-[4-(2-pyrimidinyl)-1-piperazinyl]-butyl]-8-azaspiro-(4,5)-decane-7,9-dione hydrochloride, is a new anxiolytic agent structurally unrelated to benzodiazepines. Both are used with the same clinical goal of alleviating anxiety, although these two drugs each appear to interact with different central neurotransmitter systems (9–12). Buspirone can affect anterior pituitary secretion; for example, it has been shown that it releases PRL in the adult male rat (13, 14). In men, buspirone given orally elevated plasma levels of PRL and growth hormone (15). Effects on growth hormone, thyrotropin, and corticotropin release have been reported in different species (15, 16). All previous studies have been performed in adulthood and in only one sex. However, there is evidence for developmental and sexual differences in brain neurotransmitter control of PRL and gonadotropin secretion in rats. (17–20). Therefore, it was of interest to determine the ontogeny of the PRL-releasing effect of buspirone and whether there were any sex differences, as well as the influence of brain sexual differentiation on this response.

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Materials and Methods

Sprague-Dawley rats were housed in an air-conditioned room with lights on at 0700 hr and off at 1900 hr. They were given free access to laboratory chow and tap water. Experiments were always performed between 1000 and 1200 hr to prevent variations due to the circadian pattern of pituitary hormone secretion. Two sets of experiments were done.

Female and male rats were used at the ages of 4, 12, 20, 28, 38, and 60 (adults) days. Pups younger that 22 days of age were allowed to remain with their mothers, undisturbed until the experiment. The remaining pups were weaned at 22 days of age and housed in groups until adulthood. Adult females presenting different vaginal smears were randomized among control and experimental groups.

To examine the effects of brain sexual differentiation, a group of females were injected subcutaneously on the day of birth with 100 μg of testosterone propionate dissolved in corn oil (androgenized rats) and used at 60 days of age. They presented closed vaginas at this time. A group of males was gonadectomized under cold anesthesia on the day of birth (neonatally gonadectomized rats) and used when they were 60 days old. Female littermates injected with oil and sham-orchidectomized males were used as controls.

Buspirone (CIBA-Geigy, Buenos Aires, Argentina) was injected intraperitoneally at doses ranging from 0.40 to 10.00 mg/kg. Saline-injected rats were used as controls. After injection, animals were left undisturbed for 15 min and then quickly decapitated to avoid stress. Trunk blood was collected and sera were frozen and kept at −20°C for radioimmunoassay determinations. For 4-day-old rats, each sample consisted of sera pooled from two or three animals.

Radioimmunoassay. PRL and follicle-stimulating hormone were measured using kits provided by National Institute of Diabetes, Digestive, and Kidney Diseases. Luteinizing hormone was determined with the radioimmunoassay developed by Niswender *et al.* (21). Results were expressed in terms of PRL RP 3 standard. Inter- and intraassay coefficients of variation for PRL were 11.4% and 8.1%, respectively.

Statistical Analysis

Results were expressed as mean ± SE of seven to 15 samples. Data were analyzed by two-way analysis of variance for the effects of dose and sex, followed by a Dunnet's test for individual comparisons between means compared with respective controls when F of interaction was significant, or between groups of means if interaction was found to be not significant. The level of significance chosen was $P < 0.05$.

Results

The effects of acute injection of buspirone, at different doses, on serum PRL levels in prepubertal and adult male and female rats are shown in Figure 1.

Basal levels of PRL were similar to those described earlier (2, 3, 5, 6). Buspirone did not alter serum PRL significantly in 4-day-old rats. At the doses of 2 and 10 mg/kg, it increased serum PRL in male and female animals significantly from Day 12 onward. The releasing effect increased with age; a lower dose of 0.4 mg/kg released PRL only in 38-day-old and adult animals. No significant sex differences in PRL release were observed in younger rats. However, in peripubertal and adult animals, the PRL-releasing effect was higher in the female. For example, in postpubertal animals, buspirone at 2.0 mg/kg body wt increased serum PRL to 182.12 ± 26.31 ng/ml ($n = 18$) in females and to 52.70 ± 5.86 ng/ml ($n = 15$) in males ($P < 0.01$). A clear dose-response relationship was seen in adult females (in ng/ml; saline, 11.24 ± 1.64; 0.4 mg/kg of buspirone, 79.96 ± 15.46; 2.0 mg/kg of buspirone, 182.12 ± 26.31; 10.0 mg/kg of buspirone, 323.77 ± 21.93 (7–18 rats/group).

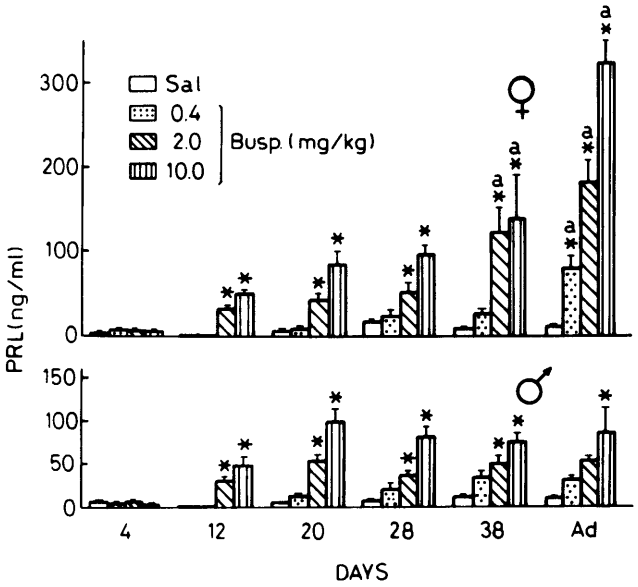

Figure 1. Effect of an acute injection of buspirone (Busp), at different doses, on prolactin secretion in prepubertal and adult (Ad) male and female rats. The height of the bar indicates the mean and the vertical line 1 SE (7–18 rats/group). *$P < 0.05$ between buspirone- and saline (Sal)-injected rats. [a]$P < 0.05$ between males and females.

Serum levels of luteinizing hormone and follicle-stimulating hormone in prepubertal male and female rats, at different ages, were not significantly modified by buspirone treatment (data not shown).

Neonatal androgenization of females (Fig. 2) did not reduce the PRL-releasing effect of buspirone, and, on the contrary, an increment was observed. Neonatal orchidectomy did not modify the releasing effect of buspirone in adult males.

Discussion

The present results show for the first time the ontogeny of the PRL-releasing effect of buspirone in male and female rats, as well as the lack of effect of brain sexual organization on this response. In adult male rats, the PRL-releasing effect of buspirone has been described and this action has been attributed to the blockade of the hypothalamic/pituitary dopami-

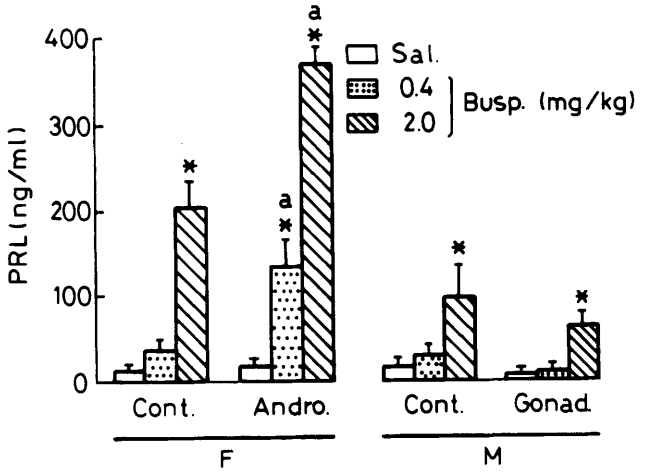

Figure 2. Effect of neonatal androgenization (Andro) of females (F) and of neonatal gonadectomy (Gonad) of the males (M) on the prolactin-releasing effect of buspirone (7–10 rats/group). $^{*}P < 0.05$ between buspirone- and saline (Sal)-injected rats. $^{a}P < 0.05$ between androgenized and control females.

nergic system (13, 15) and to a serotoninergic action (14).

If buspirone acted through an antidopaminergic mechanism at the pituitary level, or through a serotoninergic brain mechanism, similar patterns of maturation, and sex differences, could be expected. Dopaminergic inhibition of PRL release increases from birth to puberty. This has been shown using two different dopaminergic blocking agents, pimozide (22) and haloperidol (2). As early as the first week of life, haloperidol is able to release PRL in females and in males. The PRL-releasing effect increases with age both in females and males, although higher levels are always obtained in females from 20 days of age onward. This sexual difference does not appear to be caused by a process of hypothalamic differentiation occurring during the fetal or early neonatal period of life, but it is caused by a modulating action of estrogen during the course of sexual development (17). Neither androgenization of female rats nor castration of males at birth is effective in reversing the female or male patterns of PRL response to pimozide (22). Such a pattern is only reversed when estrogen is administered to male rats or when the main source of estrogen in the female is eliminated by long-term ovariectomy. On the other hand, serotonin released PRL in 12-day-old rats, after the twentieth postnatal day the PRL-releasing effect of this indoleamine was more evident in the male, and a male-differentiated brain was more sensitive to the PRL-releasing effect of serotonin than a female-differentiated brain, irrespective of the hormonal environment (19, 20).

Therefore, there seems to be a close similarity between the present results and the ontogeny and sex differences of the hyperprolactinemic response to antidopaminergic drugs. The similarity in the maturation and the sex differences favor the hypothesis of a dopaminergic involvement in the mechanisms of action of buspirone. The PRL-releasing effects of antidopaminergic drugs are modified by estradiol. We can speculate that this is also the case for buspirone. The sexual differences are first found when estradiol secretion increases as the female approaches puberty, and in the androgenized female, high estrogen secretion has been described which can account for the high PRL secretion observed in this experimental group (23, 24). This hypothesis warrants further study.

Serum luteinizing hormone and follicle-stimulating hormone levels did not change in response to buspirone, using the present doses and in the experimental conditions studied. This could explain the lack of information in the literature on the effect of buspirone on gonadotropins, although effects of this drug on the release of other pituitary hormones have been explored.

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