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Effect of the Anxiolytic Drug Buspirone on Prolactin and Corticosterone Secretion in Stressed and Unstressed Rats

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URBAN, J. H., L. D. VAN DE KAR, S. A. LORENS AND C. L. BETHEA. *Effect of the anxiolytic drug buspirone on prolactin and corticosterone secretion in stressed and unstressed rats.* PHARMACOL BIOCHEM BEHAV **25**(2) 457–462, 1986.—Buspirone is an atypical anxiolytic drug that exerts its action at a receptor site other than the GABA-benzodiazepine-chloride ionophore complex. The present study examined the effect of buspirone on plasma prolactin and corticosterone levels in both control and stressed rats. In unstressed rats, buspirone produced dose-dependent increases in plasma prolactin and corticosterone levels. The minimal doses of buspirone which led to significant elevations in plasma prolactin and corticosterone levels were 1.0 and 2.0 mg/kg (IP), respectively. The effect of buspirone on both hormones was maximal 30 minutes after injection. The plasma levels of prolactin and corticosterone were significantly elevated in rats that were stressed using a conditioned fear paradigm. Buspirone produced a dose-dependent attenuation of the stress-induced increase in prolactin secretion. The stress-induced increase in corticosterone secretion was inhibited by the 0.5 mg/kg (IP) dose but not by the 2.0 mg/kg (IP) dose of buspirone, which increased corticosterone secretion both in stressed and unstressed rats. These data suggest that the effect of buspirone on plasma prolactin and corticosterone levels may be mediated by two different mechanisms of action.

Non-benzodiazepine anxiolytics Buspirone Prolactin Corticosterone Stress Serotonin 5-HT_{1A}

PROLACTIN and corticosterone secretion are regulated differentially by central monoaminergic systems. Tuberoinfundibular dopaminergic neurons inhibit prolactin secretion [2] whereas norepinephrine [3] and serotonin [4, 46, 50] neurons facilitate the release of prolactin from the pituitary. ACTH and corticosterone secretion is stimulated by serotonergic [7,45] and dopaminergic [8] neurons. Noradrenergic pathways inhibit the release of corticosteroids [44].

Prolactin and corticosterone are stress-sensitive hormones. The effect of stress on these hormones may be mediated by biogenic amines [6,33] or GABAergic receptors [18]. The benzodiazepines are a class of anxiolytic drugs that enhance GABA transmission through their interaction with the GABA-benzodiazepine-chloride ionophore complex [26,28]. Administration of benzodiazepines has been shown to attenuate the stress-induced increases in prolactin and corticosterone secretion [13, 21, 25, 48].

The non-benzodiazepine anxiolytic drug, buspirone, does not exert its action at the GABA-benzodiazepine receptor complex [31] and does not possess the anticonvulsant and muscle relaxant properties characteristic of the benzodiazepines [12, 40, 41]. The mechanism of the anxiolytic action of buspirone is not well understood. However, buspirone is known to affect dopaminergic [20, 32, 52], serotonergic [11, 16, 51] and noradrenergic [34,42] neurotransmission. The present studies were designed to test whether buspirone could alter the plasma levels of prolactin and corticosterone both in stressed and unstressed rats.

METHOD

Male Sprague-Dawley rats (275–300 g) were purchased from Sasco-King Animal Laboratories (Oregon, WI). The rats were housed in conventional cages (2 per cage) in a temperature (22–25°C), humidity (50–55%) and illumination (12:12 light/dark cycle; lights on at 07:00 hr) controlled room. Water and rat chow (Wayne Lab Blox, Allied Mills, Inc., Chicago, IL) were available ad lib. All experiments were conducted between 12:00 and 15:00 hours because plasma corticosterone levels are low and constant during this period

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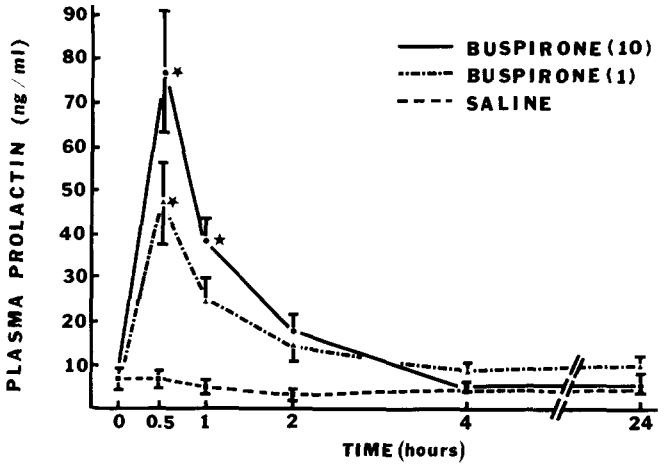

FIG. 1. The time course of the effect of saline (2.0 ml/kg, IP) and buspirone (1.0 or 10.0 mg/kg, IP) on plasma prolactin levels. Each data point represents mean±S.E.M. of 8 rats. *Significant difference from saline control groups, $p<0.01$ (ANOVA and Duncan's new multiple range test).

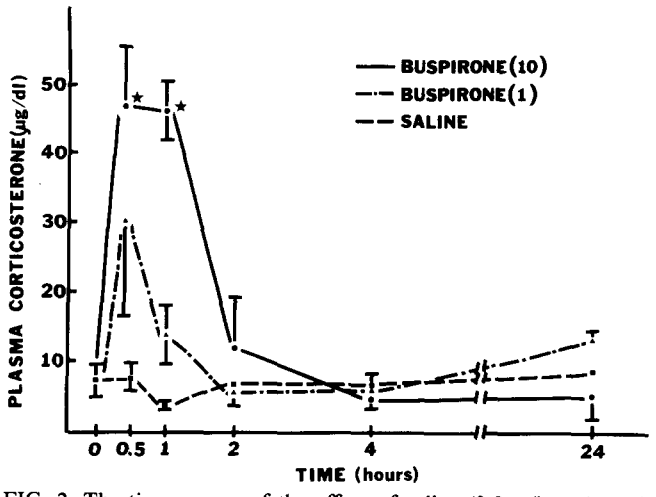

FIG. 2. The time course of the effect of saline (2.0 m/kg, IP) and buspirone (1.0 or 10.0 mg/kg, IP) on plasma corticosterone levels. Each data point represents mean±S.E.M. of 8 rats. *Significant difference from saline control groups, $p<0.01$ (ANOVA and Duncan's new multiple range test).

with minimal diurnal change [27,39]. We do not know of any evidence for a circadian rhythm of prolactin secretion in male rats.

In the first experiment, buspirone (1.0 and 10.0 mg/kg) or saline (2.0 ml/kg) were administered intraperitoneally (IP), and the rats were sacrificed at different times after injection. In the second experiment, rats received injections of saline (2.0 ml/kg, IP) or buspirone (0.1–50.0 mg/kg, IP) and were sacrificed 30 minutes post-injection. In both experiments, the rats were replaced in their home cage immediately following injection and were sacrificed by decapitation in an area adjacent to the animal quarters.

The conditioned emotional or fear response (CER) was elaborated in a rectangular chamber (49 cm long × 23 cm wide × 28 cm high) with a grid floor composed of stainless steel rods (7.6 mm in diameter) spaced 1.3 cm apart. The front wall of the chamber was constructed from clear Plexiglas. The remaining walls and ceiling of the chamber were made of white Plexiglas. Illumination was provided by a fluorescent lamp (20 W) mounted outside the rear wall. The chamber was located in a sound attenuated room 7.5 meters from the animal quarters. Scrambled constant current shock was delivered through the grid floor by a Grayson-Stadler shock generator. The rats were carried individually to the stress room in a plastic cage identical to their home cage. Three minutes following placement in the chamber, the experimental animals received an inescapable foot shock (1.0 mA DC for 10 sec). Immediately thereafter the rats were returned to their home cage. Control rats were treated identically except that shock was not administered at any time. This procedure was repeated, once a day, for 3 consecutive days. By the third day it was quite apparent that the stressed rats had learned that placement in the chamber would be followed by a shock. In contrast to control animals, the stressed rats defecated, urinated and alternated between freezing and jumping behaviors [47–49]. On the fourth day, the rats received an intraperitoneal injection of saline (2.0 ml/kg) or buspirone (0.5 or 2.0 mg/kg) 30–45 min prior to being placed in the stress chamber for 3 minutes. Instead of receiving shock, however, they were removed and immediately sacrificed by decapitation in a room located 3.0 meters from the stress room.

The blood of the decapitated rats was collected into centrifuge tubes containing 0.5 ml of a 0.3 M EDTA (ethylene diamine tetra-acetate; pH 7.4) solution. The plasma was stored at −40°C until hormone determinations were performed.

Corticosterone was measured by a radioimmunoassay using procedures and corticosterone antisera from Radioassay Systems Laboratories (Carson, CA) on unextracted plasma in which binding proteins had been denatured by boiling [1].

Prolactin was determined by radioimmunoassay using reagents and antiserum that were provided by the NIADDK. Anti-rat prolactin serum S-8 was used at a dilution of 1:5000 and rat prolactin I-5 was radioiodinated. Rat prolactin RP-2 served as the reference preparation [46, 47, 50].

Buspirone was donated by Bristol Myers Company (Evansville, IN), and was dissolved in 0.9% NaCl solution.

Statistical analysis of the data was performed by an analysis of variance (ANOVA) and a Duncan's new multiple range test [38]. When variances were proportional to the squares of the treatment means, a logarithmic transformation of the data was performed, and the data were then analyzed by ANOVA and Duncan's new multiple range test [38].

RESULTS

As shown in Fig. 1, both the 1.0 and 10.0 mg/kg (IP) doses of buspirone produced a significant increase in plasma prolactin levels 30 minutes post-injection, $F(9,50)=22.6$ for the 10.0 mg/kg dose, and $F(9,67)=8.8$ for 1.0 mg/kg. The 10.0 but not the 1.0 mg/kg dose of buspirone also increased corticosterone levels 30 minutes after administration (Fig. 2). This effect was statistically significant for the 10.0 mg/kg dose, $F(9,47)=14.7$, but not for the 1.0 mg/kg dose, $F(9,55)=1.4$, of buspirone. Both prolactin and corticosterone levels were still significantly elevated one hour after administration of the higher (10.0 mg/kg IP) dose of buspirone. Significant hormonal effects of buspirone were not observed 2–24 hours post-injection.

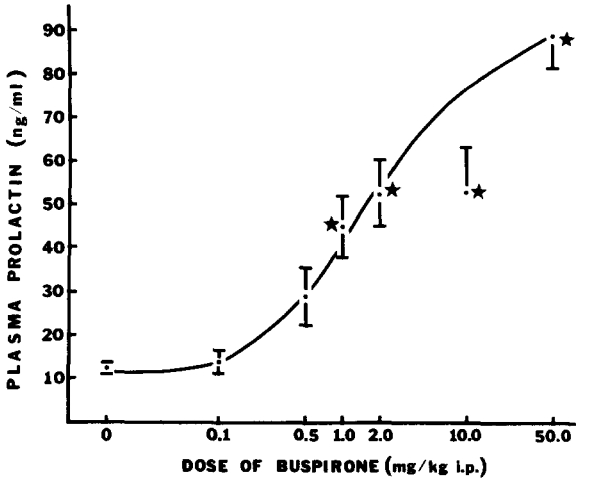

FIG. 3. Dose response effects of buspirone (0.1–50.0 mg/kg, IP) on plasma prolactin levels 30 minutes after administration. Each data point represents mean±S.E.M. of 8 rats. *Significant difference from saline control groups, $p<0.01$ (ANOVA and Duncan's new multiple range test).

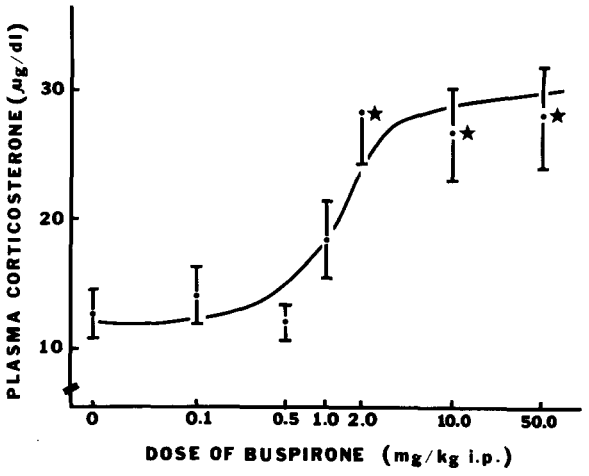

FIG. 4. Dose response effects of buspirone (0.1–50.0 mg/kg, IP) on plasma corticosterone levels 30 minutes post-administration. Each data point represents mean±S.E.M. of 8 rats. *Significant difference from saline control groups, $p<0.01$ (ANOVA and Duncan's new multiple range test).

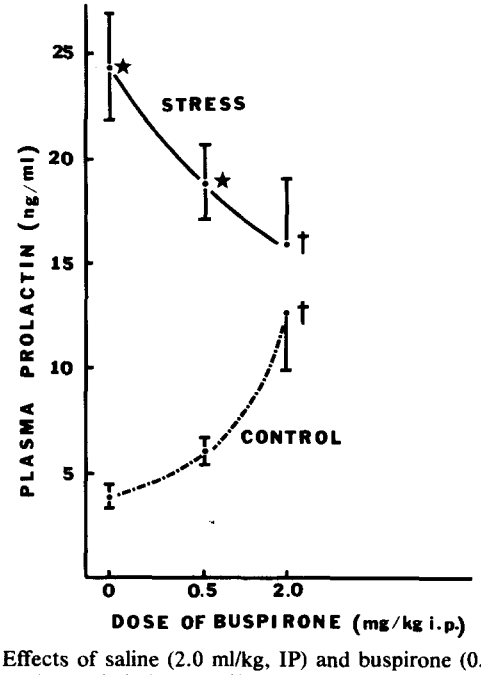

FIG. 5. Effects of saline (2.0 ml/kg, IP) and buspirone (0.5 or 2.0 mg/kg, IP, 33–48 min before sacrifice) on prolactin levels in stressed and non-stressed rats. Each data point represents mean±S.E.M. N=6 for the buspirone (0.5, 2.0 mg/kg IP) doses and n=8 for the saline groups. *Significant differences from the corresponding control groups, $p<0.01$ (ANOVA and Duncan's new multiple range test). †Significant difference from the corresponding saline group, $p<0.05$ (ANOVA and Duncan's new multiple range test).

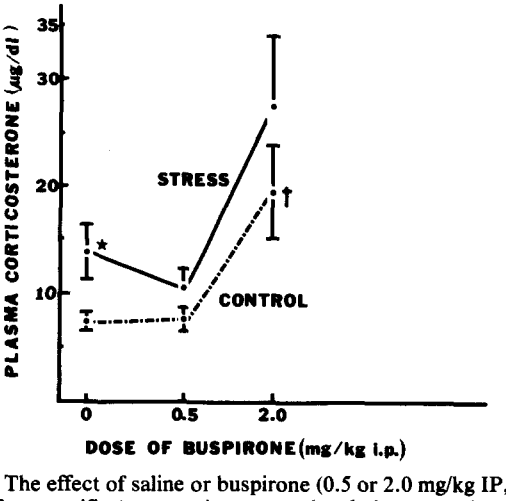

FIG. 6. The effect of saline or buspirone (0.5 or 2.0 mg/kg IP, 33–48 min before sacrifice) on corticosterone levels in stressed and non-stressed rats. Each data point represents mean±S.E.M. n=6 for the buspirone (0.5, 2.0 mg/kg IP) doses and n=8 for the saline groups. *Significant difference from the corresponding control group, $p<0.01$ (ANOVA and Duncan's new multiple range test). †Significant difference from the corresponding saline groups, $p<0.01$ (ANOVA and Duncan's new multiple range test).

In the dose-response experiment, saline and different doses of buspirone were administered to the rats 30 minutes before sacrifice. Both prolactin (Fig. 3) and corticosterone (Fig. 4) demonstrated dose-dependent increases in response to buspirone administration, $F(6,49)=16.6$ for prolactin, and $F(6,49)=10.1$ for corticosterone. Prolactin levels were significantly increased after administration of the 1.0 mg/kg dose of buspirone, whereas the minimal dose needed to increase corticosterone levels was 2.0 mg/kg, possibly because of the high variance in the 1.0 mg/kg group.

Both prolactin and corticosterone levels were elevated in the rats that were stressed by subjecting them to the conditioned emotional response paradigm, $F(5,33)=14.0$ for prolactin, and $F(5,33)=5.1$ for corticosterone. Buspirone produced a dose-dependent attenuation of the stress-induced increase in prolactin secretion (Fig. 5).

The stress-induced increase in corticosterone secretion was inhibited by the 0.5 mg/kg dose of buspirone. The higher

(2.0 mg/kg) dose of buspirone produced a significant elevation in corticosterone levels. However, there was no significant difference between the stressed rats that received buspirone (2.0 mg/kg) and those that received saline injections. There also was no difference in corticosterone levels between stressed and unstressed rats which received the 2.0 mg/kg dose of buspirone (Fig. 6).

DISCUSSION

The results of the first two experiments demonstrate that buspirone produces dose-dependent increases in plasma prolactin and corticosterone levels in unstressed rats. It is not clear whether these effects are mediated by a common or by different mechanisms of action since their dose-response profiles are so similar.

Studies by Meltzer *et al.* [22] suggest that buspirone increases prolactin levels by blocking post-synaptic dopamine (D_2) receptors at the pituitary level. Wood *et al.* [52] suggest that buspirone has a neuroleptic mechanism of action which accounts for its antagonism of dopamine-induced behaviors and the increase in prolactin levels.

Buspirone possesses different mechanisms of action which may account for the increase in corticosterone levels. Dopamine is known to stimulate ACTH and corticosterone secretion via a central mechanism of action [8, 9, 17]. For example, administration of the dopamine agonist LY14185 has been reported to stimulate central D_2 dopamine receptors selectively [43] and cause dose-dependent increases in corticosterone secretion [9]. These same doses also produce a suppression of serum prolactin levels [9]. Therefore, it is not likely that buspirone increases corticosterone levels via a dopaminergic mechanism because dopamine neurotransmission has opposite effects on prolactin and corticosterone secretion and both hormones are not likely to be elevated simultaneously via this mechanism of action.

The firing of neurons in the locus coeruleus is increased after iontophoretic or systemic administration of buspirone [34,42]. Thus, buspirone might intensify the inhibitory effect of norepinephrine on corticosterone secretion [44]. Our findings do not support this view.

Enhanced serotonergic transmission also produces increases in prolactin levels which are mediated by the dorsal raphe nucleus [4, 46, 50]. However, Meltzer *et al.* [22] suggested that it is unlikely that buspirone stimulates prolactin secretion through a serotonergic mechanism since the increase in prolactin could not be blocked by pretreatment with the non-selective serotonin antagonists cyproheptadine and cinanserin. Cinanserin and cyproheptadine have a very low affinity for 5-HT_{1A} receptors [37]. Furthermore, dorsal raphe firing rate is decreased by administration of buspirone [51], possibly via activation of 5-HT_{1A} receptors [29]. If buspirone reduced serotonergic neurotransmission, it would be expected that plasma prolactin levels would either be decreased or unchanged. Our results are inconsistent with this hypothesis. On the other hand, buspirone may increase prolactin secretion via activation of postsynaptic 5-HT_{1A} receptors. Serotonin acts as a stimulus for the release of corticosterone [7,45]. Administration of the serotonin type I_A (5-HT_{1A}) receptor agonist 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin) or the 5-HT_{1B} agonist mCPP (1-(3-chlorophenyl) piperazine) produces dose-dependent increases in corticosteroid levels ([14,19]; Lorens and Van de Kar, submitted for publication). Buspirone recently has been shown to bind to 5-HT_{1A} receptors [15, 24, 29] with heavier binding occurring in the hypothalamus [24]. These observations suggest that buspirone increases corticosterone and prolactin levels by acting on 5-HT_{1A} receptors in the hypothalamus.

The results of our third experiment suggest that buspirone attenuates the stress-induced increase in prolactin and corticosterone secretion. The duration of the CER paradigm (3 minutes) is too short to observe a maximal effect on prolactin and corticosterone secretion. We recently have completed a series of experiments to determine the time course of the effect of different stressors on plasma prolactin and corticosterone levels. We found that plasma prolactin and corticosterone levels reach a maximum at 12 and 22 minutes, respectively, after the initiation of stress. Thus the increases that were observed in the CER paradigm were on the rising phase of the hormonal responses, and resulted in a 70% and 500% increase in plasma corticosterone and prolactin levels, respectively. Nevertheless, buspirone attenuated the stress-induced rise in both hormones.

In the control rats, buspirone caused a dose-dependent increase in plasma prolactin levels, although this effect was not as pronounced as in the previous two experiments. The difference between this experiment and the previous two experiments is that the rats were sacrificed 33–48 minutes after the administration of buspirone, and thus after the maximal effect had been reached and was subsiding (see Fig. 1).

The effect of buspirone on stress-induced corticosterone and prolactin secretion may be mediated through a different mechanism than the effect of buspirone on these hormones in unstressed rats. Administration of buspirone has been shown to be clinically effective in the treatment of anxiety [10, 12, 40] and equally as effective as diazepam in the treatment of these disorders [12, 40, 41]. Buspirone is not equally effective in all animal models of anxiety [35], but it attenuates the startle response in rats that were subjected to a fear paradigm [5] and is active in shock-suppressed drinking in rats and key pecking in pigeons [35]. Since buspirone increases prolactin and corticosterone levels in unstressed rats, it might be expected that buspirone and stress would have an additive effect on prolactin and corticosterone levels. However, such an additive effect was not observed. In fact, the dose of buspirone (2.0 mg/kg) that caused a significant increase in prolactin secretion in unstressed rats, reduced stress-induced prolactin secretion by 40%. Stress-induced corticosterone secretion was inhibited by the lower (0.5 mg/kg) dose of buspirone, but the higher dose of buspirone (2.0 mg/kg) produced an increase in corticosterone levels. This increase in corticosterone levels could mask any anxiolytic action of buspirone that might otherwise be seen in the stressed rats. From these results, it seems likely that buspirone can selectively decrease the stress response of prolactin and corticosterone, and stimulate the resting levels of these hormones by separate mechanisms of action.

Various biogenic amines have been postulated to mediate the anxiolytic effect of buspirone. The locus coeruleus is believed to be involved in the mediation of the stress response [30]. Consistent with this view, administration of benzodiazepines has been shown to decrease the firing rate of locus coeruleus neurons [30,34]. In contrast, buspirone increases the activity of locus coeruleus neuron [34,42]. Lesions of the dorsal raphe nucleus do not block the stress-induced increase in prolactin secretion [47]. Therefore, it is not likely that buspirone alters the stress-induced increase in prolactin secretion via serotonergic neurons located in the

dorsal raphe nucleus. Mioduszewski and Critchlow [23] have reported that a telencephalic pathway projecting to the mediobasal hypothalamus via the medial forebrain bundle mediates the stress-induced increase in prolactin levels. Studies by Siegel *et al.* [36] suggest that the medial forebrain bundle also plays a role in mediating the corticosterone response to stress. It is possible that buspirone acts on this pathway to attenuate the response of these hormones to stress. Administration of benzodiazepines lowers the stress-induced secretion of prolactin and corticosterone [21, 25, 48] but not of renin [48]. Buspirone, on the other hand, prevented the increase in renin secretion in rats that were subjected to stress by a conditioned emotional response paradigm [49], and attenuates stress-induced prolactin and corticosterone secretion. These observations lend further support to the hypothesis that buspirone acts at a different site than the benzodiazepine anxiolytics.

In summary, the results of the present study suggest that buspirone produces dose-dependent increases in prolactin and corticosterone secretion in non-stressed rats, possibly by different mechanisms of action; and that the stress-induced increases in these hormone levels are attenuated by low doses of buspirone.

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