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European Journal of Pharmacology 274 (1995) 141–149

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Neuroendocrine profile of the potential anxiolytic drug S-20499

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Received 22 November 1994; accepted 25 November 1994

Abstract

The neuroendocrine profile of the serotonin 5-HT_{1A} receptor agonist and potential anxiolytic drug (+)-4[*N*-(5-methoxy-chroman-3-yl)*N*-propylamino]butyl-8-azaspiro-(4, 5)-decane-7,9-dione (S-20499) was examined in conscious male rats. S-20499 (0.01–20 mg/kg i.p.) dose-dependently elevated plasma adrenocorticotropin (ACTH) and corticosterone concentrations, with maximal effects observed at 15–30 and 30–60 min respectively. S-20499 also reduced plasma prolactin concentration, and did not alter plasma renin activity. S-20499 (1 mg/kg i.p.) also reduced blood pressure and heart rate within 10 min, suggesting reduced sympathetic output. Pretreatment with the 5-HT_{1A} receptor antagonists (–)-pindolol (0.3 mg/kg i.p.) or spiperone (0.01 or 3 mg/kg s.c.) significantly attenuated the stimulatory effects of S-20499 on plasma ACTH and/or corticosterone concentrations. The data suggest that S-20499 stimulates the hypothalamic-pituitary adrenal axis by activating 5-HT_{1A} receptors, although activation of dopamine D_2 receptors may contribute to these responses. Like other 5-HT_{1A} receptor agonists, S-20499 does not increase renin secretion. Additionally, it reduces prolactin secretion, presumably by acting as a weak dopamine D_2 receptor agonist in the pituitary.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); ACTH (adrenocorticotropin); Corticosterone; Prolactin; Renin; Blood pressure; Heart rate

1. Introduction

In recent years, several 5-HT_{1A} receptor agonists have been studied for use for the treatment of anxiety disorders. It has been suggested that these 5-HT_{1A} anxiolytic drugs may have some advantages over the benzodiazepine anxiolytics, possessing less severe side effects and lower abuse potential (Eison, 1989). These novel anxiolytics (e.g. buspirone, ipsapirone and gepirone) share some common characteristics in that they are 5-HT_{1A} receptor agonists (Levy and Van de Kar, 1992; Eison, 1990). However, each has a unique pharmacological profile, which could produce differential physiological effects, and possibly differences in their efficacies or side effects. S-20499 ((+)-4[*N*-(5-methoxy-chroman-3-yl)*N*-propylamino]butyl-8-azaspiro-(4, 5)-decane-7, 9-dione) is a drug that is under investigation for its potential anxiolytic actions (Porsolt et al., 1992; Barrett and Vanover, 1993). S-20499 is a potent 5-HT_{1A} receptor agonist (K_i = 0.19 nM) (Kidd et al., 1993) and a weak dopamine D_2 receptor agonist (K_i = 70 nM) (M. Lesourd, personal communication).

Neuroendocrine pharmacology is an approach that can serve several functions in the study of psychotherapeutic drugs. First, because the secretions of many hormones are differentially influenced by several neurotransmitters in the brain (Levy and Van de Kar, 1992), the influence of drugs on several neurotransmitter pathways and receptors can be assessed simultaneously by examining the secretions of multiple hormones. Thus, the acute neuroendocrine profile can be utilized to examine the interactions of a potential therapeutic agent with several neurotransmitter systems. Additionally, neuroendocrine pharmacology could potentially be used to assess the therapeutic actions of drugs on some disorders, particularly anxiety. Because several hormone (e.g. adrenocorticotropin (ACTH), prolactin and renin) are secreted in response to stress (Van de Kar et al., 1991), this approach could potentially be used in humans and in animal models to ascertain the anxiolytic actions of these drugs (File, 1990; Rittenhouse et al., 1992; Johnson et al., 1992).

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SSDI 0014-2999(94)00719-5

Serotonergic and dopaminergic neurons have some common but also some different effects on the secretion of several hormones. Activation of 5-HT, and dopamine D_2 receptors increases ACTH and corticosterone secretion (Fuller and Snoddy, 1984; Levy et al., 1994; Borowsky and Kuhn, 1992). However, activation of 5-HT receptors increases, while activation of dopamine D_2 receptors decreases the secretion of prolactin (Ben-Jonathan, 1985; Levy et al., 1994). 5-HT receptor activation increases renin secretion while dopamine neurons have no known influence on the secretion of renin (Van de Kar, 1991).

5-HT receptors are currently divided into seven major classes (5-HT_{1-7}) and several subclasses (5-HT_{1A-F}, 5-HT_{2A-2C}) (Humphrey et al., 1993). 5-HT_{1A} receptor activation increases the secretion of ACTH, corticosterone, oxytocin and prolactin (Gilbert et al., 1988; Koenig et al., 1988; Kellar et al., 1992; Bagdy and Kalogeras, 1993; Levy et al., 1994). In contrast, renin secretion is not increased by activation of 5-HT_{1A} receptors (Van de Kar, 1991). Additionally, 5-HT_{2A} and/or 5-HT_{2C} receptor activation increases the secretion of several hormones including ACTH, corticosterone, prolactin, renin, oxytocin and vasopressin (Levy et al., 1994). 5-HT_3 receptors also appear to have a minor role in the secretion of prolactin (Jorgensen et al., 1992; Levy et al., 1993) but not ACTH or renin (Levy et al., 1993). Thus, the concomitant measurement of plasma ACTH, prolactin and renin can provide insight into the selectivity of putative 5-HT_{1A} receptor agonists.

The present study examined the acute neuroendocrine profile of S-20499. To determine whether 5-HT_{1A} receptors mediate the hormone responses to S-20499, the ability of two 5-HT_{1A} receptor antagonists to inhibit S-20499 effects was examined. These antagonists were (−)-pindolol and spiperone, which exhibit different pharmacological profiles regarding their affinities for other receptors. (−)-Pindolol is a 5-HT_{1A}/5-HT_{1B}/β-adrenoceptor antagonist and spiperone is a 5-HT_{1A}/5-HT_{2A}/dopamine D_2 receptor antagonist (Zifa and Fillion, 1992; Leysen et al., 1993; Roth et al., 1992). Thus, comparing their effect would reveal which common effects could be due to 5-HT_{1A} receptor involvement and which might be due to the side effects of these antagonists.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (225–275 g) were purchased from Harlan (Indianapolis, IN, USA). The rats were housed in a lighting-(12 h light-12 h dark; lights on at 7.00 h), humidity- and temperature-controlled room. Food and water were available ad libitum. Animals were housed two per cage, except following surgery (for intra-arterial catheter implantation) when they were single housed. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals as approved by the Loyola University Institutional Animal Care and Use Committee. In all the experiments in which the rats were decapitated, trunk blood was collected into chilled centrifuge tubes containing 0.5 ml of a 0.3 M EDTA (pH 7.4) solution. The blood was centrifuged at $1000 \times g$ for 25 min at 4°C and stored at −70°C until radioimmunoassays were performed.

2.2. Surgery

Catheters (polyethylene tubing (PE-50)) were inserted under pentobarbital anesthesia into the descending aorta through the femoral artery and led subcutaneously to exit between the scapulae. Catheters were filled with a 50% sucrose solution containing 1000 units/ml heparin. Arterial blood pressure recordings were made 24 h following catheter implantation. The solution containing 50% sucrose was removed and replaced with heparinized saline (1000 units/ml heparin). The tubing was then connected to a Statham blood pressure transducer and blood pressure was recorded on a Grass polygraph.

2.3. Drugs

All drugs (except for spiperone) were dissolved in isotonic saline and were administered intraperitoneally (i.p.). Spiperone (Sigma, St. Louis, MO, USA) was initially dissolved in 95% ethanol, then diluted with saline to a 10% ethanol-saline solution. Spiperone (0, 0.01 or 3 mg/kg) was injected subcutaneously (s.c.). All injections were in a volume of 1 ml/kg, and control groups received the appropriate vehicle. S-20499 (0.1–20 mg/kg) was donated by Institut de Recherches Internationales Servier (Courbevoie, France). (−)-Pindolol was purchased from RBI (Natick, MA, USA) and was injected in a dose of 0.3 mg/kg i.p.

2.4. Experimental protocols

2.4.1. Time course of S-20499

Male rats ($n = 8$ per group) were injected with S-20499 (5 mg/kg i.p.) or saline. Rats were killed by decapitation 15, 30, 60, 120 or 240 min later, and trunk blood was collected for radioimmunoassays of plasma hormone concentrations.

2.4.2. Dose-response effects of S-20499

S-20499 (0.1–20 mg/kg i.p.) or saline was administered, and blood samples were collected as described above, 30 min post-injection.

2.4.3. Cardiovascular effects of S-20499

S-20499 (1 mg/kg i.p.) or saline (1 mg/kg i.p.) was administered in rats implanted with femoral artery catheters for measurements of blood pressure and heart rate. Blood pressure and heart rate were monitored, beginning 45 min before and terminating 30 min after injections.

2.4.4. Effects of the 5-HT_{1A}/5-HT_{1B}/β-adrenoceptor antagonist (−)-pindolol on the hormone responses to S-20499

(−)-Pindolol (0.3 mg/kg i.p.) was administered 30 min prior to S-20499 (2–10 mg/kg i.p.) or saline. Blood samples were collected 30 min after S-20499 or saline injections. The dose of (−)-pindolol was based on a prior study (Koenig et al., 1987), in which hormone responses to the 5-HT_{1A} receptor agonist 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin) was antagonized by (−)-pindolol.

2.4.5. Effects of the 5-HT_{1A}/5-HT_{2A}/dopamine D_2 receptor antagonist spiperone on the hormone responses to S-20499

Spiperone (0.01 or 3 mg/kg s.c.) or its vehicle (10% ethanol in saline) were administered 30 min prior to S-20499 (2–10 mg/kg i.p.) or saline. Blood samples were collected 30 min after S-20499 or saline injections. The choice of spiperone doses was based on several studies (Hoyer and Schoeffter, 1991; Koenig et al., 1988; Millan et al., 1993; Stubbs et al., 1991; Przegalinski et al., 1989; Blier et al., 1993), suggesting that the low dose would preferentially antagonize dopamine D_2 receptors, while the higher dose would also block 5-HT_{1A} receptors.

2.5. Biochemical determinations

2.5.1. Plasma ACTH radioimmunoassay

This was performed on unextracted plasma samples using an ACTH antiserum from IgG Corporation (Nashville, TN, USA). The sequence recognition of the antiserum is the 5–18 segment of ACTH. This antiserum does not significantly recognize α-melanocyte stimulating hormone, β-melanocyte stimulating hormone, β-endorphin, β-lipotropin, ACTH-(11–24) or ACTH-(1–16)-amide. ACTH-(1–39) standards were obtained from Calbiochem (San Diego, CA, USA). In this double antibody radioimmunoassay, samples or standards were incubated with the ACTH antibody (final dilution 1:30000, 22% total binding) at 4°C for 24 h in a 0.01 M phosphosaline buffer (PBS, pH 7.6) containing 1% bovine serum albumin (Sigma, St. Louis, MO, USA), 0.025 M EDTA, 0.5% normal rabbit serum (Calbiochem, San Diego, CA, USA) and 250 KIU/ml aprotinin (Sigma Chemical Co., St. Louis, MO, USA). [^{125}I]ACTH (2000 cpm, Incstar, Stillwater, MN, USA) was added and incubated for 24 h at 4°C in a total volume of 0.3 ml. The second antibody (goat anti-rabbit-γ-globulin, Calbiochem, San Diego, CA, USA) was added at a final dilution of 1:50 and incubated overnight at 4°C. Cold PBS (1.5 ml) was added to the tubes and they were then centrifuged at 15000 × *g* at 4°C for 40 min. The radioactivity of the pellet was counted by a Micromedic 4/200 plus gamma counter. The sensitivity of the assay is 0.25 pg/tube and the intra- and inter-assay variations are 4.2% and 14.6% respectively.

2.5.2. Plasma corticosterone radioimmunoassay

This was performed on un-extracted plasma samples in which corticosterone-binding proteins have been denatured by boiling, as previously described (Van de Kar et al., 1985a). The assay is based on procedures and antiserum (final dilution 1:11200, 46% total binding) from ICN Biochemicals (Irvine, CA, USA). The radioactive [^{3}H]corticosterone tracer was obtained from Du Pont NEN Research Products (Boston, MA, USA). The sensitivity is 0.02 ng/tube and the intra- and inter-assay variabilities are 4.5% and 11.9% respectively.

2.5.3. Plasma prolactin radioimmunoassay

This was performed with reagents provided by the National Institute of Diabetes, Digestive and Kidney disorders (NIDDK). Briefly, rat prolactin (NIDDK preparation rPRL-I-6) was iodinated by the lactoperoxidase method (Marchalonis, 1969), using Na^{125}I from Du Pont NEN (Boston, MA, USA). The reaction was stopped by addition of phosphate buffer containing 1% bovine serum albumin. [^{125}I]Prolactin was purified by Sephadex G-50 chromatography (18 × 1.5 cm column) followed by Sephadex G-100 chromatography (56 × 0.9 cm column, before use for the assay). Rat prolactin (NIDDK preparation rPRL-RP-3) was used as standard. The double antibody radioimmunoassay was performed in a 0.01 M phosphosaline buffer (pH 7.6) at 4°C. Anti-rat prolactin serum (rPRL-S-9) was used at a dilution of 1:22500 and 35% total binding. Briefly, plasma samples (2 × 10 and 2 × 50 μl) were incubated with the antiserum for 24 h at 4°C. [^{125}I]Prolactin (freshly purified by Sephadex G-100 chromatography) was added (20000 cpm/tube) and incubated for 72 h at 4°C. The [^{125}I]prolactin-bound antibody was precipitated with the second antibody (final dilution 1:110, goat anti-rabbit γ-globulin, Calbiochem) combined with 5% polyethylene glycol and incubated for 20 min at 4°C, then centrifuged for 15 min at 15000 × *g*. The radioactivity of the pellet was detected using a Micromedic 4/200 plus gamma counter. The sensitivity of the assay is 0.02 ng/tube and the intra- and inter-assay variabilities are 4.8% and 13.6% respectively (Li et al., 1993).

2.5.4. Plasma renin activity

Plasma renin activity was measured by radioimmunoassay for generated angiotensin I, as previously described (Richardson Morton et al., 1989). The following were added to the plasma samples (150 μl) in order to generate angiotensin I: 0.5 M phosphate buffer pH 6.0 (0.1 ml), and 5 μl each of phenylmethylsulfonyl fluoride and 8-hydroxyquinoline (to a final concentration of 2.5 and 3.4 mM, respectively) and 50 μl of water. The mixture was incubated at 37°C for 1 h. The reaction was stopped by addition of 0.14 ml of cold water and boiling for 3 min. The antiserum against angiotensin I was used at a dilution of 1:16000 with total binding of 30%. The sensitivity limit of the radioimmunoassay is 15 pg angiotensin I per tube. Intra-assay variability was 4.4%, with inter-assay variability 12.6% (Richardson Morton et al., 1989).

2.6. Statistical analysis

The hormone data were extrapolated from standard curves by a computer program (RIA-AID, Robert Maciel Associates, Arlington, MA, USA). The data are represented as group means and the standard errors of the means (S.E.M.). The data were analyzed by either one-way (dose-response experiments) or two-way analyses of variance (ANOVA). Group means were compared by Newman-Keuls' test, or by Dunnett's test (for the dose-response experiment).

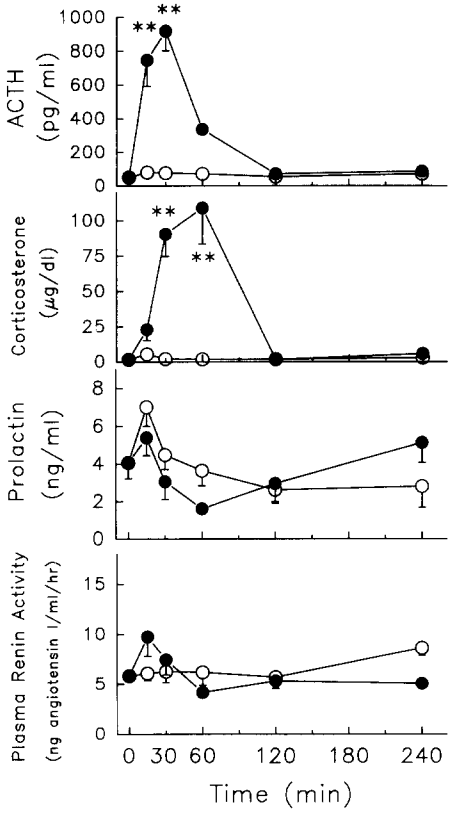

Fig. 1. Time course effect of S-20499 (5 mg/kg i.p.) or saline on plasma concentrations of ACTH, corticosterone and prolactin, and plasma renin activity. The data represent mean ± S.E.M. of 8 rats per group. Plasma samples were collected from separate groups of rats at 0 min (uninjected), 15 min, 30 min, 60 min, 2 h and 4 h post-injection. (○) Saline; (●) S-20499. ** Significant vs. saline ($P < 0.01$, Newman-Keuls' test).

3. Results

The time course of endocrine responses to S-20499 (5 mg/kg i.p.) was examined. S-20499 increased plasma ACTH concentrations ($F(4,68) = 11.96$, $P < 0.001$), with maximal effects observed 15–30 min after injection (Fig. 1). S-20499 also increased plasma corticosterone concentrations ($F(4,67) = 12.71$, $P < 0.001$), with maximal effects observed 30–60 min post-injection (Fig. 1). S-20499 produced a small reduction of plasma prolactin concentration (main effect of S-20499: $F(1,69) = 4.83$, $P < 0.05$), which did not attain statistical significance based on Newman-Keuls' tests (Fig. 1). S-20499 did not consistently modify plasma renin activity (Fig. 1).

The dose-response effects of S-20499 were evaluated 30 min post-injection (Fig. 2). S-20499 dose-dependently increased plasma concentrations of ACTH ($F(6,45) = 9.32$, $P < 0.001$) and corticosterone ($F(6,41) = 5.54$, $P < 0.001$). S-20499 significantly reduced plasma prolactin concentration ($F(6,48) = 2.94$, $P < 0.05$). Plasma renin activity was not significantly modified by S-20499 ($F(6,49) = 0.98$).

Changes in blood pressure and heart rate were examined following S-20499 (1 mg/kg i.p.). S-20499 produced small reductions in blood pressure ($F(8,112) = 1.76$, $P = 0.093$) and heart rate ($F(8,112) = 2.69$, $P < 0.01$) which were observed from 10–15 min post-injection ($P < 0.05$, Newman-Keuls' test; Fig. 3). Note that this dose of S-20499 produced minimal changes in hormone secretions (see Fig. 2).

To determine whether 5-HT_{1A} receptors mediate the endocrine responses of S-20499, the ability of two 5-HT_{1A} receptor antagonists to inhibit the responses to S-20499 (2–10 mg/kg i.p.) was evaluated. The 5-HT_{1A}/5-HT_{1B}/β-adrenoceptor antagonist (–)-pindolol (0.3 mg/kg i.p.) inhibited the S-20499-induced elevation of plasma ACTH ($F(3,48) = 4.91$, $P < 0.01$). (–)-Pindolol produced a similar trend on the corticosterone response to S-20499, although the two-way interaction failed to achieve statistical significance ($F(3,47) = 2.604$, $P = 0.063$; Fig. 4). (–)-Pindolol reduced prolactin concentration, and reduced the ability of S-20499 to suppress plasma prolactin concentration ($F(3,51) = 3.83$, $P < 0.05$). (–)-Pindolol increased plasma renin activity ($F(1,51) = 42.96$, $P < 0.001$). This effect was not clearly altered by S-20499.

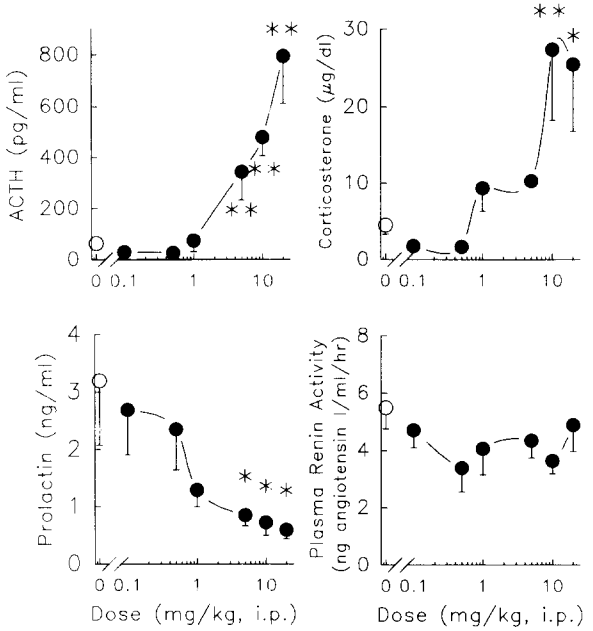

Fig. 2. Dose-response effect of S-20499 (0, 0.1, 0.5, 1, 5, 10 or 20 mg/kg i.p.) on plasma ACTH, corticosterone and prolactin concentrations, and plasma renin activity. The data represent mean ± S.E.M. of 8 rats per group. Plasma samples were collected 30 min post-injection. (○) Saline; (●) S-20499. Significant vs. saline (* $P < 0.05$ or * * P 0.01, respectively, Dunnett's test).

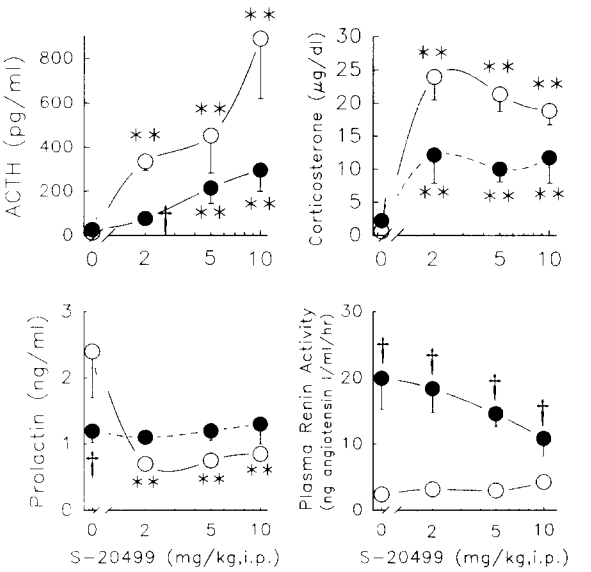

Fig. 4. The influence of (−)-pindolol (0.3 mg/kg s.c.) on the ACTH, corticosterone, prolactin and renin responses to S-20499 (0, 2, 5 or 10 mg/kg i.p.). The data represent mean ± S.E.M. of 8 rats per group. (−)-Pindolol or vehicle was administered 30 min prior to S-20499 (or saline). Plasma samples were collected 30 min after S-20499 injections. S-20499 increased plasma ACTH ($F(3,48) = 25.96$, $P < 0.001$) and corticosterone ($F(3,47) = 15.54$, $P < 0.01$) concentrations. S-20499 decreased plasma prolactin concentration ($F(3,51) = 3.78$, $P < 0.05$). (○) Saline; (●) (−)-pindolol. * * Significant vs. saline ($P < 0.01$, Newman-Keuls' test). † Significant effect of (−)-pindolol ($P < 0.05$, Newman-Keuls' test).

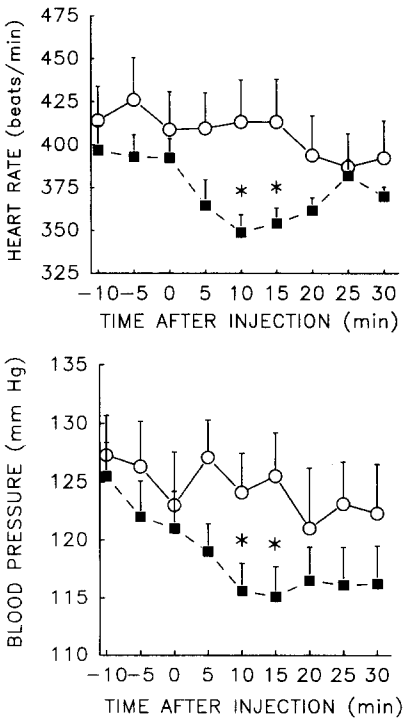

Fig. 3. Effect of S-20499 (1 mg/kg i.p.) or saline on blood pressure and heart rate. The data represent mean ± S.E.M. of 8 rats per group. (○) Saline; (■) S-20499. * Significant vs. saline ($P < 0.05$, Newman-Keuls' test).

The 5-HT_{1A}/5-HT_{2A}/dopamine D_2 receptor antagonist spiperone inhibited the effect of S-20499 on plasma ACTH (main effect of spiperone: $F(2,75) = 3.30$, $P < 0.05$) and corticosterone (two-way interaction: $F(6,75) = 3.13$, $P < 0.01$) (Fig. 5). Observe that the low dose of spiperone (0.01 mg/kg) was sufficient to inhibit the ACTH and corticosterone responses to S-20499 ($P < 0.05$, Newman-Keuls' test). Additionally, both doses of spiperone produced similar elevations of plasma prolactin concentrations ($F(2,81) = 262.8$, $P < 0.001$). While administration of S-20499 alone reduced prolactin concentrations (from 2.19 ng/ml to 0.21 ng/ml (at the 2 mg/kg dose)), it was unable to reduce prolactin levels in spiperone pretreated groups. S-20499 produced a small increase in plasma renin activity ($F(3,81) = 3.63$, $P < 0.05$) in this experiment. The increases are most clearly observed in groups receiving spiperone (Fig. 6).

4. Discussion

The results of the present study suggest that S-20499 increases the secretion of ACTH and corticosterone primarily by activating 5-HT_{1A} receptors. Additionally,

in agreement with previous studies, activation of 5-HT_{1A} receptors did not alter renin secretion. Furthermore, S-20499 reduced prolactin secretion, which likely reflects dopamine D_2 receptor agonist actions.

The ability of S-20499 to increase plasma ACTH and corticosterone concentrations is consistent with previous reports indicating that activation of 5-HT_{1A} receptors stimulates the hypothalamic-pituitary-adrenal axis (Lorens and Van de Kar, 1987; Koenig et al., 1988; Gilbert et al., 1988; Chaouloff, 1993). The ACTH and/or corticosterone responses to S-20499 were inhibited by (−)-pindolol and spiperone. Both spiperone (K_i = 23–74 nM) and (−)-pindolol (K_i = 19 nM) are 5-HT_{1A} receptor antagonists (Zifa and Fillion, 1992). However, spiperone also is a 5-HT_{2A} and a dopamine D_2 receptor antagonist while (−)-pindolol is a 5-HT_{1B} receptor and β-adrenoceptor antagonist (but not a 5-HT_{2A} or D_2 receptor antagonist) (Hoyer and Schoeffter, 1991; Leysen et al., 1993; Roth et al., 1992; Albus et al., 1985). Thus, comparing the effect of these two antagonists on the hormone responses to S-20499 can provide insight into the involvement of 5-HT_{1A} receptors mediating the endocrine effects of S-20499. The data suggest that these responses are mediated by 5-HT_{1A} receptors. However, the ACTH and corticosterone responses to S-20499 were antagonized by the low dose of spiperone (0.01 mg/kg). This dose was chosen because it was expected to block D_2 receptors, but have minimal antagonist actions at 5-HT_{1A} receptors (Hoyer and Schoeffter, 1991; Koenig et al., 1988; Millan et al., 1993; Stubbs et al., 1991; Przegalinski et al., 1989; Blier et al., 1993). The ability of the low dose of spiperone to antagonize the ACTH and corticosterone responses to S-20499 suggests that dopamine D_2 receptor activation contributes to these actions of S-20499. A stimulatory role of dopamine D_2 receptors on the hypothalamic-pituitary-adrenal axis has been established previously (Fuller and Snoddy, 1984; Borowsky and Kuhn, 1992).

S-20499 reduced plasma prolactin concentration. The ability of S-20499 to reduce prolactin concentrations was inhibited by both the low and high doses of spiperone. However, because spiperone alone produces robust increases in prolactin concentrations, it is difficult to conclude that this is a D_2 mediated response of S-20499. It is interesting to note that (−)-pindolol alone reduced prolactin concentrations, and S-20499 was unable to further reduce this effect. Because these low levels are near the detection limits of the assay, it becomes difficult to conclude that the prolactin response to S-20499 was blocked by (−)-

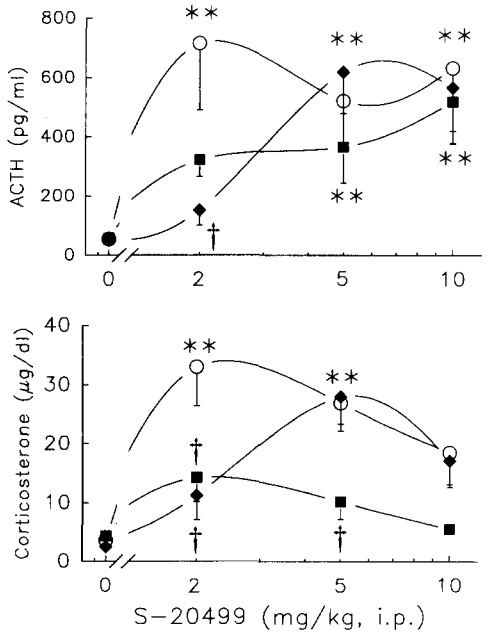

Fig. 5. The ability of spiperone (0.01 or 3 mg/kg s.c.) to inhibit the ACTH and corticosterone responses to S-20499 (0, 2, 5 or 10 mg/kg i.p.). The data represent mean ± S.E.M. of 8 rats per group. Spiperone (or vehicle) was administered 30 min prior to S-20499 (or saline). Plasma samples were collected 30 min after S-20499 injections. S-20499 increased plasma ACTH ($F(3,80) = 19.5$, $P < 0.001$) and corticosterone ($F(3,75) = 11.9$, $P < 0.001$) concentrations. (○) Vehicle; (◆) 0.01 mg/kg spiperone; (■) 3 mg/kg spiperone. **Significant vs. saline ($P < 0.05$, Newman-Keuls' test). † Significant effect of spiperone ($P < 0.05$, Newman-Keuls' test).

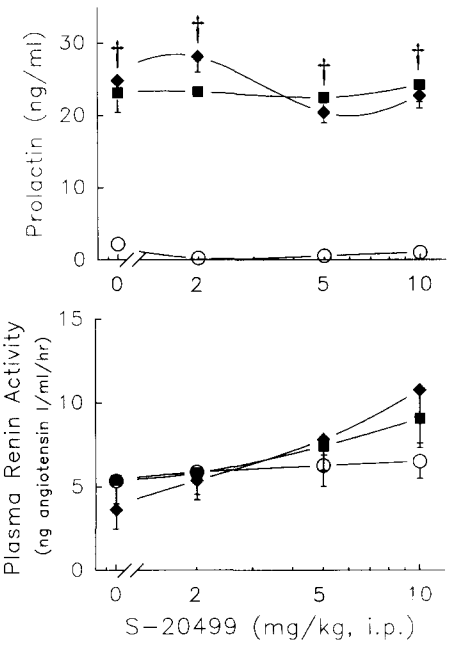

Fig. 6. The effect of spiperone (0.01 or 3 mg/kg s.c.) and S-20499 (0, 2, 5 or 10 mg/kg i.p.) on plasma prolactin concentration and plasma renin activity. The data represent mean ± S.E.M. of 8 rats per group. Spiperone (or vehicle) was administered 30 min prior to S-20499 (or saline). Plasma samples were collected 30 min after S-20499 injections. (○) Vehicle; (◆) 0.01 mg/kg spiperone; (■) 3 mg/kg spiperone. Significant vs. saline (*$P < 0.05$ or **P0.01, respectively, Newman-Keuls' test). † Significant vs. vehicle pretreated rats that received the same dose of S-20499 ($P < 0.05$, Newman-Keuls' test).

pindolol. It is likely that S-20499 reduced prolactin secretion due to agonist actions at D_2 receptors. Lesourd (personal communication), has suggested that S-20499 exhibits weak dopamine D_2 receptor agonist activity.

The role of 5-HT_{1A} receptors in prolactin secretion has been evaluated with less than conclusive results. Several 5-HT_{1A} receptor agonists have been shown to elevate plasma prolactin concentrations (Kellar et al., 1992; Li et al., 1993). However, it has not been conclusively demonstrated that these effects are mediated by 5-HT_{1A} receptors (Aulakh et al., 1988). One characteristic of the effects of most 5-HT_{1A} receptor agonists on prolactin secretion is that this is a brief response (Kellar et al., 1992). Thus, it is likely that 5-HT_{1A} receptors stimulate prolactin secretion, but further studies are necessary to definitively establish this conclusion. In the present study, S-20499 did not produce a significant elevation in prolactin concentration at any time point. The lack of a stimulatory effect of S-20499 on prolactin secretion may reflect inhibitory effects of D_2 receptor activation by S-20499, which could mask any possible 5-HT_{1A} stimulatory actions on this hormone. Thus, it is likely that the prolactin response to S-20499 is mediated by activation of dopamine D_2 receptors, although this conclusion must be kept tentative.

Renin secretion was not altered by S-20499. We have previously reported that several 5-HT_{1A} receptor agonists, such as buspirone, ipsapirone and 8-OH-DPAT, do not increase renin secretion (Lorens and Van de Kar, 1987; Van de Kar et al., 1985b). Thus, the present observations are consistent with the hypothesis that activation of 5-HT_{1A} receptors does not increase renin secretion. Although S-20499 did not alter renin secretion, there was a tendency for it to reduce some of the effects of (−)-pindolol. (−)-Pindolol alone produced robust elevations of plasma renin activity. This effect was unexpected, because other β-adrenoceptor antagonists (e.g. propranolol) generally reduce plasma renin activity (Keeton and Campbell, 1980). While it is unlikely that this effect is mediated by antagonism of 5-HT_{1A} receptors, the magnitude of the renin response to (−)-pindolol was reduced (albeit nonsignificantly) by the high dose of S-20499. Subsequent evaluation of these effects may further clarify the pharmacological profiles of (−)-pindolol and S-20499. Spiperone exposed a small stimulatory effect of S-20499 on renin secretion. However, this was observed only at the low dose of spiperone. Additionally, the effects were quite variable at the high dose of S-20499. Therefore, it remains doubtful whether this effect has any physiological significance.

The lack of effect of S-20499 on renin secretion is interesting in light of our finding that this drug reduces heart rate and blood pressure. Sympathetic activation increases, while reduction in sympathetic outflow reduces renin secretion (Hackenthal et al., 1990). The present cardiovascular data suggest that S-20499 reduces sympathetic outflow. However, this is not followed by changes in renin secretion. One possibility is that the reduced blood pressure compensates for the changes in sympathetic traffic to the kidneys. There is an inverse relationship between blood pressure and renin secretion (Fahri et al., 1982). Thus, the reduction in blood pressure could produce a moderate increase in renin secretion to compensate for a moderate reduction as a consequence of reduced sympathetic output. Cardiovascular effects of 5-HT_{1A} receptor agonists have been reported previously. Several 5-HT_{1A} receptor agonists reduce blood pressure and heart rate (Stubbs et al., 1991; Mir et al., 1988; Bjork et al., 1991). This action appears to be mediated by postsynaptic 5-HT_{1A} receptors located in the ventral medulla (Helke et al., 1993). S-20499 (as well as several other 5-HT_{1A} receptor agonists, such as 8-OH-DPAT) reduces both blood pressure and heart rate. Similar to S-20499, 8-OH-DPAT does not modify renin secretion (Lorens and Van de Kar, 1987; Bagdy et al., 1992).

In conclusion, the data from the present study indicate that the potential anxiolytic drug S-20499 increases secretion of ACTH and corticosterone by activation of 5-HT_{1A} receptors, although its agonist actions at D_2 receptors may contribute to this response. Additionally, S-20499 reduced prolactin secretion, which likely reflects weak agonist actions at dopamine D_2 receptors. Further studies are needed to address this hypothesis. Finally, S-20499 did not alter renin secretion, which probably reflects both a noninvolvement of 5-HT_{1A} receptors in renin secretion, and the opposing actions of reduced blood pressure and reduced sympathetic activity on renin secretion.

Acknowledgements

The authors thank Kayoko Kunimoto and Joseph Yracheta for their excellent technical assistance. Supported by Institut de Recherches Internationales Servier, and the Loyola University Chicago Neuroscience and Aging Institute.

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