Pergamon

Pharmacology Biochemistry and Behavior, Vol. 48, No. 2, pp. 429–436, 1994

0091-3057/94 $6.00 + .00

0091-3057(93)E0056-A

ICV Injection of the Serotonin 5-HT_{1B} Agonist CP-93,129 Increases the Secretion of ACTH, Prolactin, and Renin and Increases Blood Pressure by Nonserotonergic Mechanisms

LOUIS D. VAN DE KAR,[1] MARIA C. ALVAREZ SANZ, JOSEPH M. YRACHETA, KAYOKO KUNIMOTO, QIAN LI, ANDREW D. LEVY AND PETER A. RITTENHOUSE

Department of Pharmacology, Stritch School of Medicine, Loyola University Chicago, 2160 S. First Avenue, Maywood, IL 60153

VAN DE KAR, L. D., M. C. ALVAREZ SANZ, J. YRACHETA, K. KUNIMOTO, Q. LI, A. D. LEVY AND P. A. RITTENHOUSE. *ICV Injection of the serotonin 5-HT_{1B} agonist CP-93,129 increases the secretion of ACTH, prolactin, and renin and increases blood pressure by nonserotonergic mechanisms.* PHARMACOL BIOCHEM BEHAV **48**(2) 429–436, 1994.—This study tested whether a new serotonin (5-HT_{1B}) agonist, 3-(1,2,5,6-tetrahydro-4-pyridyl)-5-propoxypyrrolo[3,2-*b*]pyridine (CP-93,129), could be used to study the potential role of 5-HT_{1B} receptors in the secretion of adrenocorticotropic hormone (ACTH), prolactin, and renin. CP-93,129 has a high affinity for 5-HT_{1B} receptors but low affinity for other 5-HT receptor subtypes. In addition, CP-93,129 does not readily cross the blood–brain barrier. The secretion of ACTH, prolactin, and renin is known to be increased after activation of 5-HT receptors. ICV injections of CP-93,129 (100 μg/kg) increased the plasma concentrations of ACTH, prolactin, and renin. CP-93,129 also increased blood pressure and reduced heart rate. To determine whether these effects of CP-93,129 are centrally mediated, we compared them with IP injection of the same dose of CP-93,129. IP-injected CP-93,129 did not alter blood pressure or heart rate and did not elevate plasma prolactin and renin concentrations. To determine whether 5-HT_{1B} receptors mediate the central effects of CP-93,129, rats were pretreated with the 5-HT antagonists *l*-propranolol or metergoline prior to ICV injections of doses of CP-93,129 (0–100 μg/kg). The 5-$HT_{1A/1B/2A/2C}$ antagonist metergoline (0.5 mg/kg, IP) failed to inhibit the CP-93,129-induced elevation of ACTH, prolactin, or renin concentrations. In contrast, the 5-$HT_{1A/1B/\beta}$ antagonist *l*-propranolol (20 μg/kg, ICV) inhibited the renin but not the ACTH or prolactin responses to ICV CP-93,129. However, the same dose of *l*-propranolol injected intraarterially (IA) also inhibited the effect of ICV-injected CP-93,129 on renin concentrations and potentiated the CP-93,129-induced reduction of heart rate, suggesting that this inhibition is mediated by peripheral β rather than central 5-HT_{1B} receptors. In conclusion, although CP-93,129 increases the secretion of ACTH, prolactin, and renin by CNS mechanisms, the data suggest that these effects are mediated by activation of nonserotonin receptors.

ACTH Prolactin Renin Blood pressure Serotonin Receptor

THE present studies were designed to investigate the role of central 5-HT_{1B} receptors in the secretion of adrenocorticotropic hormone (ACTH), prolactin, and renin. Serotonergic stimulation of several hormones is mediated by distinct 5-HT receptor subtypes. Activation of 5-HT_{1A} receptors increases ACTH, oxytocin, and possibly prolactin secretion, while activation of 5-$HT_{2A/2C}$ receptors increases ACTH, prolactin, oxytocin, vasopressin, and renin secretion (19,26,31,36). The possible involvement of 5-HT_{1B} receptors in the regulation of hormone secretion is presently unclear, although an involvement in stimulating prolactin secretion has been suggested (38,39). The present studies were undertaken to test whether the putative 5-HT_{1B} agonist CP-93,129 increases the secretion of several hormones by activating 5-HT_{1B} receptors. CP-93,129 is a drug structurally related to the indole 5-HT agonist RU 24969. Both drugs have a very high affinity for 5-HT_{1B} receptors, but CP-93,129 has a very low affinity for 5-HT_{1A} and 5-HT_{2C} receptors, while RU 24969 has a high affinity for 5-HT_{1A} and a moderate affinity for 5-HT_{2C} receptors (21,22, 28). We previously have demonstrated that RU 24969 increases the secretion of ACTH, prolactin, and renin by activating either 5-HT_{1A}, 5-$HT_{2A/2C}$, or 5-HT_{1B} receptors (38). Since the reported affinity of CP-93,129 for 5-HT_{1A}, 5-HT_{2C}, and 5-HT_{2A} receptors is much lower than the affinity of RU

[1] To whom requests for reprints should be addressed.

24969, we believed that it could function as a tool to establish the role of 5-HT_{1B} receptors in neuroendocrine function. CP-93,129 has an additional advantage: It does not penetrate the blood–brain barrier readily (28). Thus, we compared the effect of central (ICV) versus peripheral administration of CP-93,129 on plasma hormones. Since renin secretion is strongly influenced by changes in blood pressure (11,14) and the renin angiotensin system is an important regulator of blood volume and blood pressure (17,24,42), we also measured blood pressure and heart rate after injections of CP-93,129.

METHODS

Animals

Male Sprague–Dawley rats (225–275 g) were purchased from Harlan (Indianapolis). The rats were housed in a lighting- (12-h light–dark, lights on at 0700), humidity-, and temperature-controlled room. Food and water were available ad lib. All procedures were conducted in accordance with the NIH *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 of the rats was collected into centrifuge tubes containing 0.5 ml of a 0.3-M ethylenediaminetetraacetic acid (EDTA; pH 7.4) solution. The blood was centrifuged at 1000 × *g* for 25 min at 4°C and stored at −40°C until hormone determinations were completed.

Surgery

Implantation of ICV cannulae and intrafemoral catheters was performed under pentobarbital anesthesia (50 mg/kg, IP). Animals were pretreated with 50 mg/kg (IP) ampicillin to prevent infection and 0.2 mg/kg atropine methyl bromide to reduce excessive secretions. The ICV cannulae (Plastics One, Roanoke, VA) were implanted stereotaxically (0.5 mm caudal, 4.5 mm ventral, and 1.4 mm lateral from bregma), then anchored onto four jewelers screws with dental cement and secured with stylets. Animals were allowed two weeks recovery from surgery before the experiments.

Catheters (PE 50 tubing) were inserted into the descending aorta through the femoral artery and led SC to exit between the scapulae. This procedure was carried out in rats anesthetized with pentobarbital (50 mg/kg, IP). The rats received an infusion of saline (2 ml/kg, IV), and the catheters were filled with a 50% sucrose solution containing 1000 units/ml heparin. Arterial blood pressure recordings were made 24 h following catheter implantation.

Drugs

CP-93,129 (donated by Pfizer Inc., Groton, CT) was dissolved in saline and injected ICV in a volume of 50 μl/kg or IP in a volume of 1 ml/kg. Control rats received saline in the same volume. Metergoline (donated by Pharmitalia, Turin, Italy) was dissolved in 0.1% ascorbate and injected IP in a volume of 2 ml/kg. Control rats received this solution as the vehicle injection. *l*-Propranolol was purchased from RBI (Natick, MA) and dissolved in saline.

Experimental Protocols

1. Time course of ICV injections. In the first experiment we determined the time course of effects of ICV-injected CP-93,129. Rats were implanted with chronic ICV cannulae as described above. Two weeks later the rats were implanted with a catheter in their femoral artery as described above and were tested 24 h after the catheterization. Blood pressure and heart rate were monitored beginning 45 min before the start of the ICV injection of either saline or CP-93,129 (100 μg/kg) and for an additional 60 min postinjection. Blood samples (0.6 ml) were withdrawn and replaced with an equal volume of saline at 0, 5, 15, and 30 min after the injection. The blood was collected in tubes containing 0.06 ml EDTA. The rats were killed by decapitation at 60 min postinjection and their trunk blood was collected.

2. Time course of IP injections. In a second experiment the time course of IP injection of CP-93,129 was determined. The procedure was as described above for ICV injection except that no ICV cannulae were implanted and CP-93,129 was injected IP at the same dose (100 μg/kg).

3. Pretreatment with metergoline. Rats were implanted with chronic ICV cannulae as described above. Two weeks later the rats first received an injection of metergoline (0.5 mg/kg, IP) or vehicle 75 min before the rats received an ICV injection of CP-93,129 (0, 10, 50, or 100 μg/kg, ICV). The rats were decapitated 15 min after the injection of CP-93,129. The 0.5-mg/kg dose of metergoline is based on previous studies in which the hormone responses to various 5-HT agonists were inhibited (4,5,12,13,23).

4. Pretreatment with ICV l-Propranolol. Rats were implanted with chronic ICV cannulae as described above. *l*-Propranolol (RBI, Natick, MA) was injected (20 μg/kg, ICV) in a volume of 10 μl/kg 15 min before the injection of CP-93,129 (10, 50, or 100 μg/kg, ICV). The rats were decapitated 15 min after the injection of CP-93,129. Control rats received saline injection. The dose of *l*-propranolol used in the present study is 50 times lower than IV or IP doses used by other investigators to inhibit the renin responses to several stimuli (1,2,29).

5. Pretreatment with intraarterial (IA) l-Propranolol. Rats were implanted with chronic ICV cannulae as described above. Two weeks later the rats were implanted with a catheter in their femoral artery as described above and were tested 24 h after the catheterization. Blood pressure and heart rate were monitored beginning 45 min before the start of the injection of either saline or *l*-propranolol and for an additional 30 min after the injection of *l*-propranolol. *l*-Propranolol (RBI, Natick, MA) was injected at a dose of 20 μg/kg IA. CP-93,129 (100 μg/kg, ICV) was injected 15 min after the injection of *l*-propranolol and the rats were killed by decapitation 15 min after the latter injection for the collection of trunk blood.

Biochemical Determinations

Plasma ACTH radioimmunoassay. Direct ACTH radioimmunoassay of plasma samples (5–50 μl) was performed as previously described (8). Briefly, the ACTH antiserum was obtained from IgG Corp. (Nashville, TN). ACTH standards (1-39) were obtained from Calbiochem (San Diego) and [^{125}I]ACTH from Incstar (Stillwater, MN). The sequence recognition of the antiserum is 5-18. In addition, this antiserum does not significantly recognize lipotropin, α-MSH (melanocyte stimulating hormone), β-MSH, β-endorphin, β-ACTH 11-24, or ACTH 1-16-amide. The minimum detectable amount is 0.25 pg/tube and the intra- and interassay variabilities are 4.2% and 14.6%, respectively.

Plasma prolactin radioimmunoassay. Prolactin radioimmunoassay was performed with reagents provided by the National Institute of Arthritis, Diabetes, Digestive and Kidney

Disorders (NIADDK). Antirat prolactin serum (rPRL-S-9) was used at a dilution of 1 : 7500 as described previously (37). Briefly, NIADDK rat prolactin (preparation rPRL-I-5) was used for iodinated tracer, and NIADDK rat prolactin (preparation rPRL-RP-3) was used as the reference preparation. The sensitivity of the assay is 30 pg/tube and intra- and interassay variabilities are 6.8% and 13.6%, respectively. All the samples from one experiment were determined together in one assay.

Plasma renin concentration. Plasma renin concentration was measured by radioimmunoassay for generated angiotensin I (ANG I). A saturating concentration of renin substrate was added to the plasma to allow generation of ANG I at maximal velocity. Renin substrate is obtained from plasma of rats that were nephrectomized and received a dexamethasone injection (0.2 mg/rat) 24 h before sacrifice. The details of this assay were described by us elsewhere (30). The radioimmunoassay of ANG I was performed with antiserum at a dilution of 1 : 16 000 and a total binding of 30% as previously described (30). The sensitivity limit of the RIA is 10 pg ANG I and the intraassay variability is 4.4%. The interassay variability is 12.6%.

Statistical Analysis

The radioimmunoassay data were extrapolated from standard curves by a computer program (RIA-AID, Robert Maciel Associates, Arlington, MA). All the data are represented as group means and the standard errors of the means (SEMs). The hormone data were analyzed by a two-way analysis of variance (ANOVA) or, when appropriate, by a two-way ANOVA with repeated measures. All the blood pressure and heart rate data were analyzed by a two-way ANOVA with repeated measures. Group means were compared by Newman-Keuls multiple range test (33). Because of a lack of homogeneity of variance in the ACTH data, analysis for this hormone was performed after conversion to the $\log_{10}$ base. For all the statistical analyses we used a computer program (NWA STATPAK, Portland, OR).

RESULTS

1. Time Course of ICV-Injected CP-93,129

Figure 1 shows the effect of CP-93,129 (100 μg/kg, ICV) on plasma ACTH, prolactin, and renin concentrations over 60 min. Plasma ACTH concentration, $F(4, 76) = 22.41$, $p < 0.001$, was elevated within 5 min and returned to control levels by 60 min postinjection. Plasma prolactin concentration, $F(4, 72) = 11.91$, $p < 0.001$, was elevated more slowly, reaching a peak at 15 min, but it returned to control levels within 30 min postinjection. In contrast, plasma renin concentration was elevated by 15 min postinjection, $F(4, 69) = 9.85$, $p < 0.001$, and remained elevated for the 60-min duration postinjection. Figure 2 demonstrates the blood pressure and heart rate responses to ICV injection of CP-93,129. Blood pressure, $F(5, 80) = 3.52$, $p < 0.01$, rose within 5 min but was only significantly elevated at 15 min and stayed elevated for the duration of the determinations, 60 min postinjection. Heart rate was reduced at 15 min postinjection but returned to control levels at 60 min postinjection.

2. Time Course of IP-Injected CP-93,129

Figure 3 shows that plasma prolactin and renin concentrations were not significantly altered by IP injection of CP-93,129 (100 μg/kg). In contrast, plasma ACTH concentration was slowly elevated, reaching a significant elevation at 60 min postinjection. This is a different time course compared with ICV injection of CP-93,129. However, there was a similar though nonsignificant increase in plasma ACTH observed in the rats that received saline injections, suggesting that the increase could also be partly due to stress. Figure 4 shows that IP injection of CP-93,129 did not produce significant changes in blood pressure and heart rate.

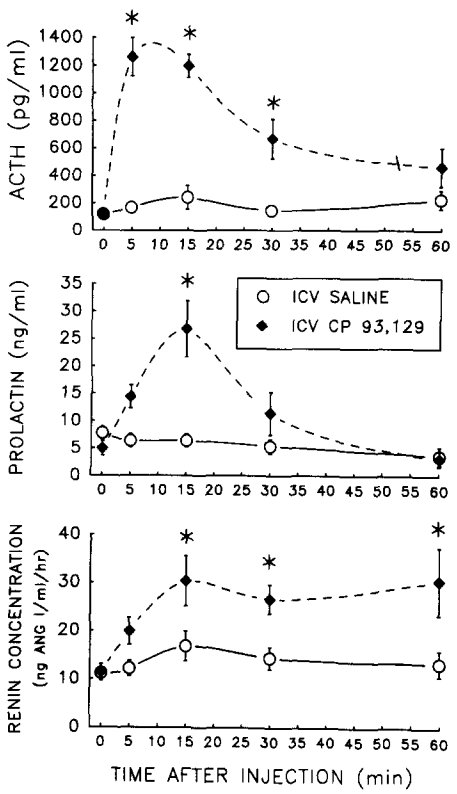

FIG. 1. ICV injection of CP-93,129 (100 μg/kg) increases plasma adrenocorticotropic hormone (ACTH), prolactin, and renin concentrations. The data represent mean ± SEM of 10 rats per group. *Significant difference from the saline group, $p < 0.05$ (two-way ANOVA with repeated measures and Newman-Keuls test).

3. Pretreatment With Metergoline

Figure 5 demonstrates that ICV injection of CP-93,129 (0, 10, 50, 50, or 100 μg/kg, ICV) produced a dose-dependent increase in plasma ACTH, prolactin, and renin concentrations, $F(3, 53) = 14.67$, $p < 0.001$; $F(3, 51) = 48.9$, $p < 0.001$; $F(3, 54) = 6.67$, $p < 0.001$, respectively. Pretreatment with metergoline (0.5 mg/kg, IP) did not reduce any of the hormone responses to CP-93,129. In the case of plasma renin, metergoline pretreatment enhanced the response to CP-93,129 at the highest dose (Fig. 5).

4. Pretreatment With ICV l-Propranolol

As can be seen in Fig. 6, the dose-response effects of CP-93,129 (0, 10, 50, or 100 μg/kg, ICV) on plasma ACTH and prolactin concentrations were not significantly inhibited by pretreatment with *l*-propranolol (20 μg/kg, ICV). In contrast, *l*-propranolol significantly inhibited the renin response to CP-93,129: interaction, $F(3, 46) = 3.270$, $p < 0.03$. Subsequent

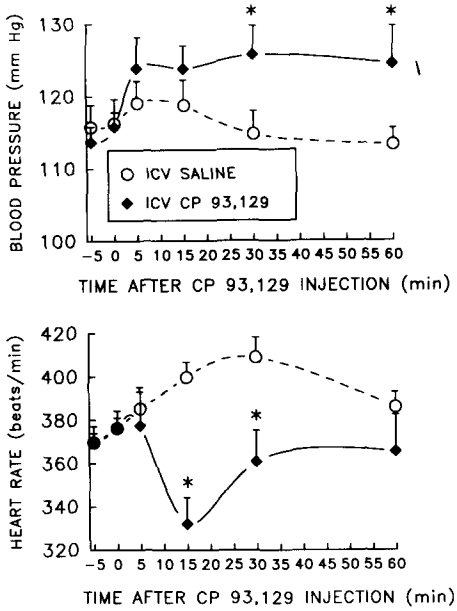

FIG. 2. ICV injection of CP-93,129 (100 μg/kg) increases blood pressure and decreases heart rate. The data represent mean ± SEM of 10 rats per group. *Significant difference from the saline group, $p < 0.05$ (two-way ANOVA with repeated measures and Newman-Keuls test).

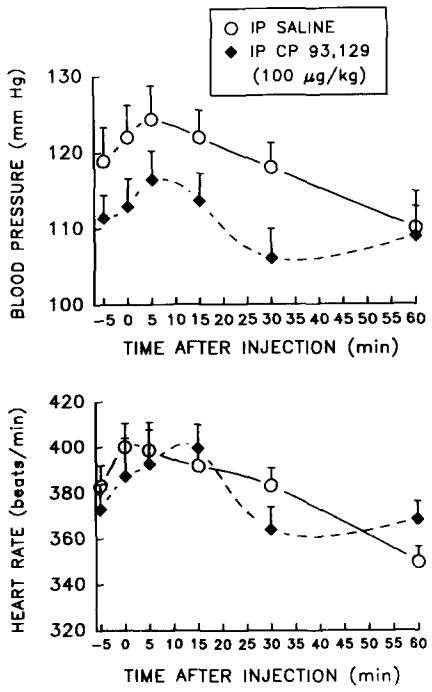

FIG. 4. IP injection of CP-93,129 (100 μg/kg) does not alter blood pressure or heart rate. The data represent mean ± SEM of five rats per group.

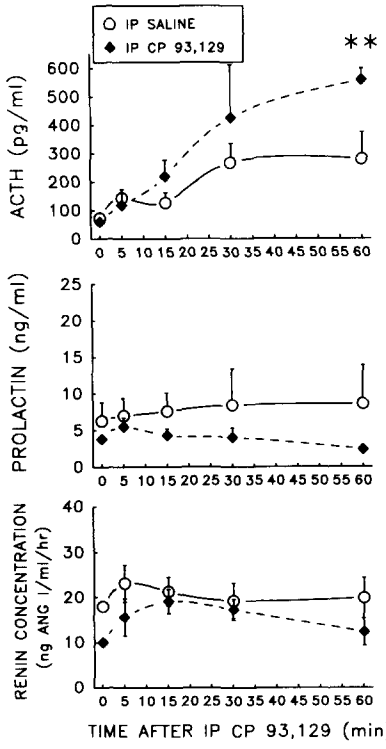

FIG. 3. IP injection of CP-93,129 (100 μg/kg) increases plasma adrenocorticotropic hormone (ACTH) but not prolactin or renin concentrations. The data represent mean ± SEM of five rats per group. *Significant difference from the saline group, $p < 0.05$ (two-way ANOVA with repeated measures and Newman-Keuls test).

Newman-Keuls tests revealed that this inhibition was observed at the 50- and 100-μg/kg doses of CP-93,129 ($p < 0.05$).

5. *Pretreatment With IA l-Propranolol*

This experiment was conducted to determine whether the ability of ICV *l*-propranolol to inhibit the effect of CP-93,129 was mediated by central or peripheral receptors. Figure 7 shows the hormone responses to a single dose of CP-93,129 (100 μg/kg, ICV) in rats pretreated with an IA injection of *l*-propranolol (20 μg/kg). Both the renin, $F(1, 31) = 6.83$, $p < 0.014$, and ACTH, $F(1, 28) = 4.096$, $p < 0.0526$, responses to CP-93,129 were significantly inhibited by *l*-propranolol. In contrast, the prolactin response to CP-93,129 was unaffected by pretreatment with *l*-propranolol. The blood pressure and heart rate responses to *l*-propranolol and CP-93,129 are presented in Fig. 8. CP-93,129 increased blood pressure and decreased heart rate. Pretreatment with an IA injection of *l*-propranolol did not alter the blood pressure-elevating effects of CP-93,129. However, the suppression of heart rate induced by CP-93,129 was potentiated in rats pretreated with *l*-propranolol. *l*-Propranolol alone produced a transient (5 min) and statistically nonsignificant reduction in heart rate.

DISCUSSION

The results presented in this study suggest that CP-93,129 can stimulate the secretion of several hormones and increase blood pressure by acting at a CNS site. However, the data do not support a role for 5-HT_{1B} receptors as mediators of these effects of CP-93,129. We compared two 5-HT antagonists, metergoline and *l*-propranolol, that have a high affinity for 5-HT_{1B} receptors (43) and found that they did not consistently inhibit the effects of CP-93,129 on any of the hormones

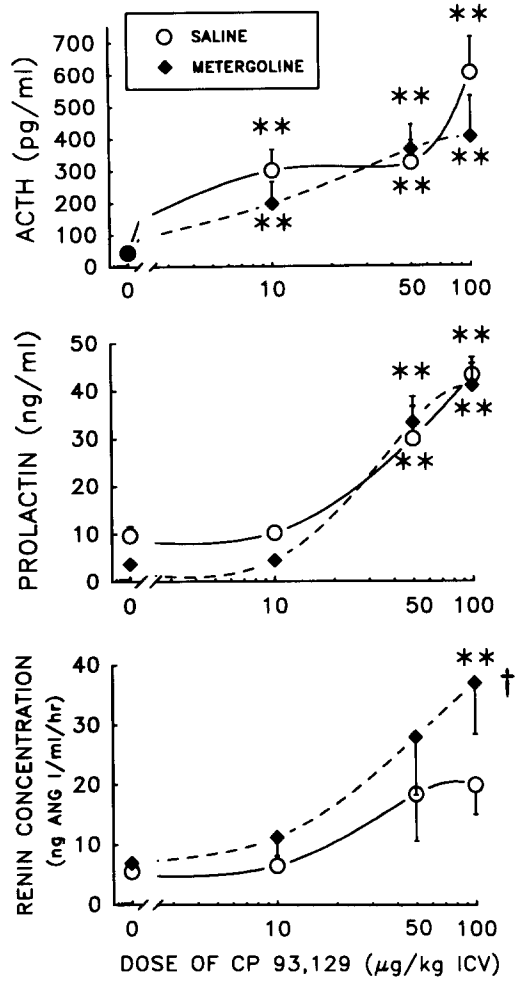

FIG. 5. Pretreatment with metergoline (0.5 mg/kg, IP) does not inhibit the effect of ICV injection of CP-93,129 (0, 10, 50, or 100 μg/kg) on plasma adrenocorticotropic hormone (ACTH), prolactin, or renin concentrations. The data represent mean ± SEM of seven to eight rats per group. *Significant difference from the saline group, $p < 0.05$ (two-way ANOVA and Newman-Keuls test).

measured. Although *l*-propranolol reduced the renin response to CP-93,129, metergoline did not inhibit any of the hormone responses to CP-93,129. Since metergoline is a potent 5-$HT_{2A/2C}$ antagonist, we also conclude that these receptors do not mediate the endocrine effects of CP-93,129.

The conclusion that CP-93,129 increases the secretion of several hormones by a non-5-HT_{1B} receptor mechanism seems surprising, considering the high affinity of CP-93,129 for 5-HT_{1B} receptors and its very low affinity for 5-HT_{1A}, 5-HT_{2C}, 5-HT_{1D}, and 5-HT_{2A} receptors (21,22). Although CP-93,129 has been characterized as a selective 5-HT_{1B} agonist, with respect to other 5-HT receptor subtypes (21,22), its ability to interact with nonserotonergic neurons and receptors has not been reported. While the current data suggest that the hormone and cardiovascular actions of CP-93,129 are predominantly mediated in the brain, it is unclear which neurotransmitter receptors are involved. There is a structural similarity between CP-93,129 and RU 24949, a drug that binds with high affinity to 5-HT_{1A} and 5-HT_{1B} and with modest affinity for 5-HT_{2C} but not 5-HT_{2A} receptors (43). RU 24969 increases the secretion of several hormones by activating 5-HT_{1A}, 5-$HT_{2A/2A}$, and/or 5-HT_{1B} receptors (32,38,39,41).

There were differences in the time course effect of ICV-injected CP-93,129 for ACTH and prolactin as compared with plasma renin and blood pressure. While the ACTH and prolactin responses were transient, the renin and blood pressure responses were prolonged. Both ACTH and renin have relatively short plasma $t_{1/2}$s of 5-15 min (3,20,27). Thus, differences in plasma half-life could not explain these differences in the responses to ICV-injected CP-93,129. The difference in time course between ACTH and prolactin on the one hand and renin and blood pressure on the other suggests that distinct mechanisms mediate the ACTH and prolactin responses to CP-93,129 when compared with the mechanisms that mediate the effect of CP-93,129 on renin and blood pressure.

It is likely that the ability of *l*-propranolol to inhibit the renin response to ICV-injected CP-93,129 represents inhibition of β-adrenoceptors rather than inhibition of 5-HT_{1B} receptors in the brain. It is well known that inhibition of peripheral β-adrenoceptors reduces renin secretion (7,18,29,40).

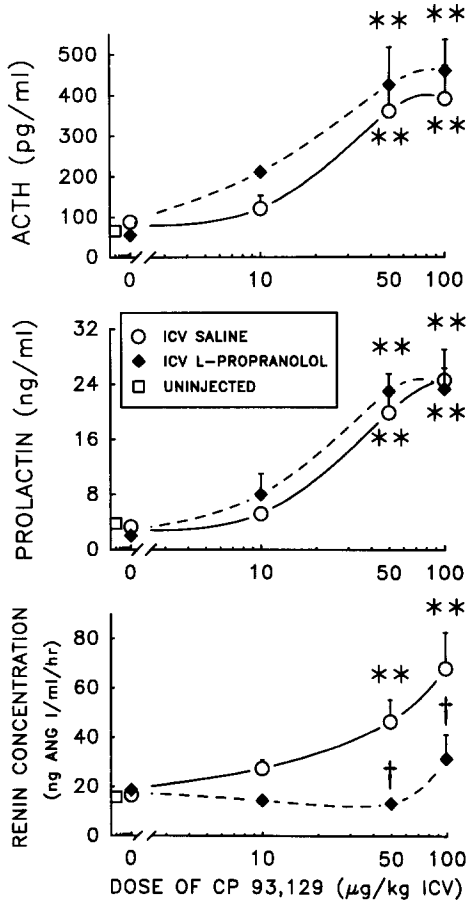

FIG. 6. Pretreatment with ICV *l*-propranolol (20 μg/kg) does not inhibit the effect of ICV injection of CP-93,129 (0, 10, 50, or 100 μg/kg) on plasma adrenocorticotropic hormone (ACTH) or prolactin concentrations but inhibits the renin response. The data represent mean ± SEM of seven to eight rats per group. *Significant difference from the saline group, $p < 0.05$ (two-way ANOVA and Newman-Keuls test). †Significant difference from the control group injected with the same dose of CP-93,129, $p < 0.05$ (two-way ANOVA and Newman-Keuls test).

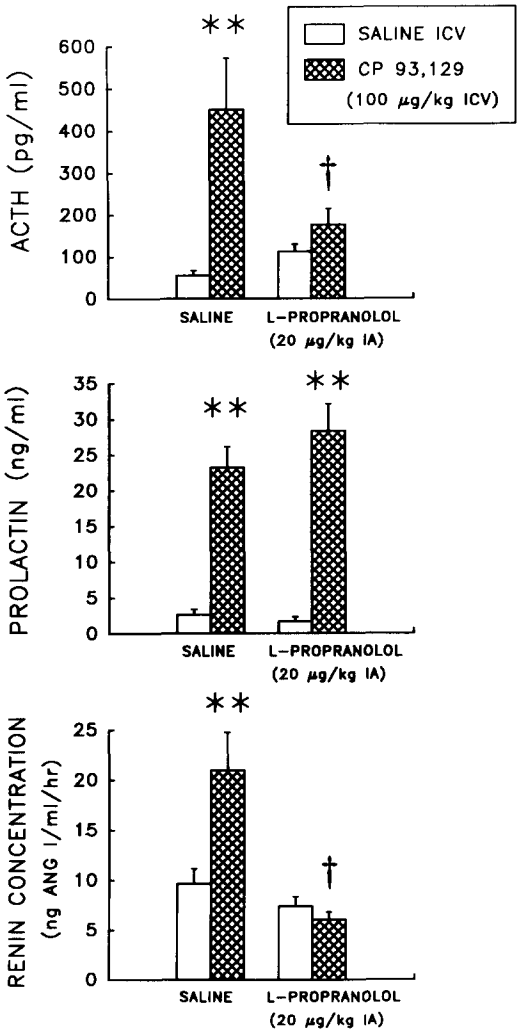

FIG. 7. Pretreatment with intraarterial *l*-propranolol (20 μg/kg) does not inhibit the effect of ICV injection of CP-93,129 (100 μg/kg) on plasma prolactin concentrations but inhibits the ACTH and renin responses. The data represent mean ± SEM of seven to eight rats per group. *Significant difference from the saline group, $p < 0.05$ (two-way ANOVA and Newman- Keuls test). †Significant difference from the control group injected with the same dose of CP-93,129, $p < 0.05$ (two-way ANOVA and Newman-Keuls test).

Since IA injection of the same dose of *l*-propranolol produced the same effect and potentiated the reduction of heart rate (a β_1-adrenoceptor-mediated phenomenon), the data suggest that peripheral β-adrenoceptors were inhibited by this dose of *l*-propranolol regardless of the route of administration. Another possibility is that receptors in circumventricular organs could mediate the effects of CP-93,129 on renin and ACTH secretion. Finally, metergoline, which is not a β-antagonist, did not inhibit the effect of CP-93,129 on plasma renin concentration, confirming that this is not a 5-HT_{1B} receptor- but more likely a β-adrenoceptor-mediated effect.

The difference between the hormonal and cardiovascular responses to ICV as compared with the IP injection of CP-93,129 suggests that these responses are mediated by brain mechanisms. This suggestion is also in agreement with the reported poor ability of CP-93,129 to cross the blood-brain barrier (28). However, it is conceivable that the low IP dose used by us could be insufficient to stimulate the secretion of hormones because of rapid metabolism. The slow increase in plasma ACTH after IP injection of CP-93,129 could be due to the stress of the procedure itself because saline injections also produced a small rise in plasma ACTH and no significant difference was observed between CP-93,129 and the saline-injected rats at the same time points.

An interesting phenomenon that deserves discussion is the ability of IA-injected *l*-propranolol to inhibit the ACTH response to ICV CP-93,129, while the same dose of *l*-propranolol injected ICV does not influence this response to ICV-injected CP-93,129. One possible interpretation of these results is based on the observation that peripherally injected CP-93,129 increased ACTH secretion. Thus, CP-93,129 might increase ACTH secretion by activating peripheral (or circumventricular) but not central 5-HT_{1B} receptors. Since peripherally injected *l*-propranolol could inhibit the activation of these 5-HT_{1B} receptors, this interpretation would suggest that ICV-

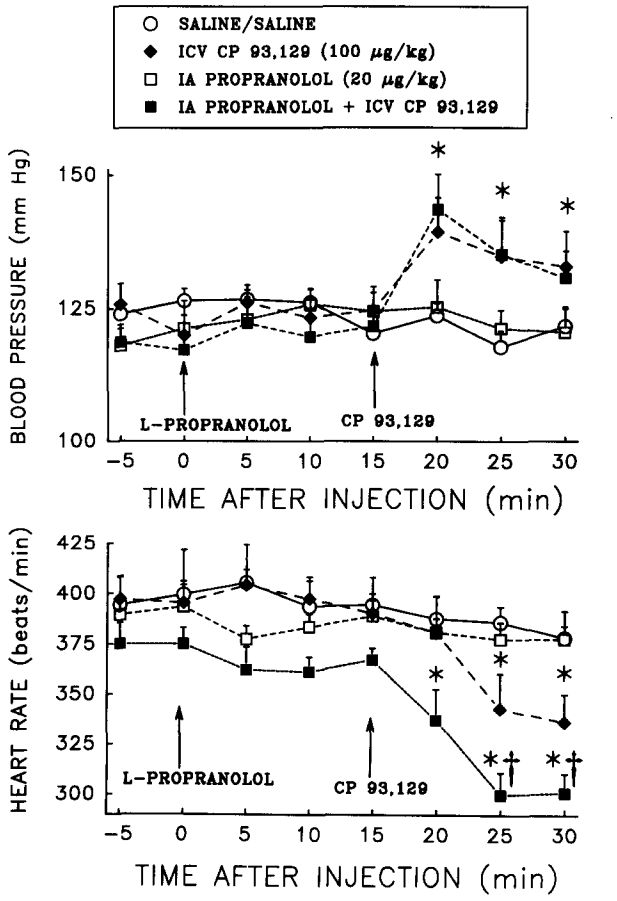

FIG. 8. Pretreatment with *l*-propranolol (20 μg/kg intraarterially) does not inhibit the effect of ICV injection of CP-93,129 (100 μg/kg) on blood pressure but potentiates the suppression of heart rate induced by CP-93,129. The data represent mean ± SEM of 8-10 rats per group. *Significant difference from the saline group, $p < 0.05$ (two-way ANOVA with repeated measures and Newman-Keuls test). †Significant difference from the control group injected with the same dose of CP-93,129, $p < 0.05$ (two-way ANOVA with repeated measures and Newman-Keuls test).

injected CP-93,129 can leak towards the periphery. There is no information confirming or contradicting leakage of CP-93,129 from the cerebrospinal fluid towards the peripheral circulation. An alternative interpretation of the data is that activation of β-adrenoceptors is responsible for the effects of CP-93,129 on ACTH secretion. Activation of β-receptors is known to increase the secretion of ACTH, possibly by peripheral mechanisms such as increased concentration of epinephrine in the blood (6,9,10,25,34,35). Thus, it is possible that the IA injection of *l*-propranolol inhibited peripheral β-adrenoceptors more effectively than the central injection of the same dose of *l*-propranolol.

It is interesting to compare ACTH with the renin response to CP-93,129. In the case of renin, both peripheral and central injection of the same dose of *l*-propranolol inhibited the effect of CP-93,129. However, the peripheral route seems more efficacious because the renin response to CP-93,129 was completely blocked, while ICV injection of *l*-propranolol inhibited but did not completely block the effect of CP-93,129. Consequently, it is not clear whether the ability of IA injection of *l*-propranolol to inhibit the ACTH response to CP-93,129 reflects antagonism at peripheral β-adrenoceptors. It is unlikely that this antagonism, by *l*-propranolol, represents effects at 5-HT_{1A} receptors because CP-93,129 has very low affinity for this receptor and metergoline, a drug with high affinity for 5-HT_{1A} and 5-HT_{1B} receptors, did not inhibit the effect of CP-93,129 on plasma ACTH.

Studies using microdialysis suggested that CP-93,129 acts at 5-HT_{1B} autoreceptors to reduce 5-HT release in the brain (15). Their conclusion was based on the ability of methiothepin to block the effects of CP-93,129. However, methiothepin has an affinity for 5-HT_{1B} receptors similar to that of metergoline or propranolol (16). Thus, our data do not support the conclusion that 5-HT_{1B} (auto)receptors mediate the hormone responses to CP-93,129.

In conclusion, the results of this study suggest that CP-93,129 can increase the secretion of ACTH, prolactin, and renin and increase blood pressure by activating brain mechanisms. The exact nature of the receptors activated by CP-93,129 remain unclear, but it seems unlikely that they are 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2C}, or 5-HT_{2A} receptors. For these reasons, CP-93,129 does not seem to be a good drug for studying the role of 5-HT_{1B} receptors in neuroendocrine function.

ACKNOWLEDGEMENTS

The authors thank Pfizer Inc. (Groton, CT) for the donation of CP-93,129 and Pharmitalia (Turin, Italy) for the donation of metergoline. Supported by USPHS grants DA04865 and MH45812 and the American Heart Association of Metropolitan Chicago.

REFERENCES

1. Alper, R. H.; Ganong, W. F. Pharmacological evidence that the sympathetic nervous system mediates the increase in secretion of renin produced by p-chloroamphetamine. Neuropharmacology 23:1237–1240; 1984.
2. Alper, R. H.; Lee, C.; Brody, M. J. Hemodynamic pharmacology of p-chloroamphetamine, a serotonin agonist, in conscious rats. J. Pharmacol. Exp. Ther. 243:446–454; 1987.
3. Assaykeen, T. A.; Otsuka, K.; Ganong, W. F. Rate of disappearance of exogenous dog renin from the plasma of nephrectomized dogs. Proc. Soc. Exp. Biol. Med. 127:306–310; 1968.
4. Aulakh, C. S.; Wozniak, K. M.; Haas, M.; Hill, J. L.; Zohar, J.; Murphy, D. L. Food intake, neuroendocrine and temperature effects of 8-OHDPAT in the rat. Eur. J. Pharmacol. 146:253–259; 1988.
5. Bagdy, G.; Sved, A. F.; Murphy, D. L.; Szemeredi, K. Pharmacological characterization of serotonin receptor subtypes involved in vasopressin and plasma renin activity responses to serotonin agonists. Eur. J. Pharmacol. 210:285–289; 1992.
6. Berkenbosch, F.; Vermes, I.; Buijs, R. M.; Tilders, F. J. H. Vasopressin is not involved in the catecholamine-induced release of ACTH, alpha-MSH and beta-endorphin from the rat pituitary gland. Neuroendocrinology 37:117–121; 1983.
7. Blair, M. L.; Hisa, H.; Sladek, C. D.; Radke, K. J.; Gengo, F. M. Dual adrenergic control of renin during nonhypotensive hemorrhage in conscious dogs. Am. J. Physiol. Endocrinol. Metab. 260:E910–E919; 1991.
8. Carnes, M.; Brownfield, M. S.; Kalin, N.; Lent, S.; Barksdale, C. M. Episodic secretion of ACTH in rats. Peptides 7:219–224; 1986.
9. Chaouloff, F.; Gunn, S. H.; Young, J. B. Central 5-hydroxytryptamine$_2$ receptors are involved in the adrenal catecholamine-releasing and hyperglycemic effects of the 5-hydroxytryptamine indirect agonist *d*-fenfluramine in the conscious rat. J. Pharmacol. Exp. Ther. 260:1008–1016; 1992.
10. Chaouloff, F.; Laude, D.; Baudrie, V. Ganglionic transmission is a prerequisite for the adrenaline-releasing and hyperglycemic effects of 8-OH-DPAT. Eur. J. Pharmacol. 185:11–18; 1990.
11. Fray, J. C. S.; Lush, D. J.; Valentine, A. N. D. Cellular mechanisms of renin secretion. Fed. Proc. 42:3150–3154; 1983.
12. Gartside, S. E.; Cowen, P. J. Mediation of ACTH and prolactin responses to 5-HTP by 5-HT2 receptors. Eur. J. Pharmacol. 179: 103–109; 1990.
13. Gartside, S. E.; Ellis, P. M.; Sharp, T.; Cowen, P. J. Selective 5-HT_{1A} and 5-HT_2 receptor-mediated adrenocorticotropin release in the rat: Effect of repeated antidepressant treatments. Eur. J. Pharmacol. 221:27–33; 1992.
14. Hackenthal, E.; Paul, M.; Ganten, D.; Taugner, R. Morphology, physiology, and molecular biology of renin secretion. Physiol. Rev. 70:1067–1116; 1990.
15. Hjorth, S.; Tao, R. The putative 5-HT_{1B} receptor agonist CP-93,129 suppresses rat hippocampal 5-HT release in vivo: Comparison with RU 24969. Eur. J. Pharmacol. 209:249–252; 1991.
16. Hoyer, D.; Schoeffter, P. 5-HT receptors: Subtypes and second messengers. J. Recept. Res. 11:197–214; 1991.
17. James, G. D.; Pickering, T. G.; Laragh, J. H. Ambulatory blood pressure variation is related to plasma renin activity in borderline hypertensive men. Am. J. Hypertens. 4:525–528; 1991.
18. Johnson, M. D.; Shier, D. N.; Barger, A. C. Circulating catecholamines and control of plasma renin activity in conscious dogs. Am. J. Physiol. 236:H463–H470; 1979.
19. Kellar, K. J.; Hulihan-Giblin, B. A.; Mulroney, S. E.; Lumpkin, M. D.; Flores, C. M. Stimulation of serotonin$_{1A}$ receptors increases release of prolactin in the rat. Neuropharmacology 31: 643–647; 1992.
20. Kim, S.; Iwao, H.; Nakamura, N.; Ikemoto, F.; Yamamoto, K. Fate of circulating renin in conscious rats. Am. J. Physiol. 252: E137–E146; 1987.
21. Koe, B. K.; Lebel, L. A.; Fox, C. B.; Macor, J. E. Binding and uptake studies with [^{3}H]CP-93,129, a radiolabeled selective 5-HT_{1B} receptor ligand. Drug Dev. Res. 25:67–74; 1992.
22. Koe, B. K.; Lebel, L. A.; Fox, C. B.; Macor, J. E. Characterization of [^{3}H]CP-96,501 as a selective radioligand for the serotonin 5-HT_{1B} receptor: Binding studies in rat brain membranes. J. Neurochem. 58:1268–1276; 1992.
23. Koenig, J. I.; Gudelsky, G. A.; Meltzer, H. Y. Stimulation of corticosterone and beta-endorphin secretion in the rat by selective 5-HT receptor subtype activation. Eur. J. Pharmacol. 137:1–8; 1987.
24. Laragh, J. H.; Sealey, J. E. Abnormal sodium metabolism and plasma renin activity (renal renin secretion) and the vasoconstric-

tion volume hypothesis: Implications for pathogenesis and treatment of hypertension and its vascular consequences (heart attack, stroke). Clin. Chem. 37:1820–1827; 1991.
25. Laude, D.; Baudrie, V.; Martin, G. R.; Chaouloff, F. Effects of the 5-HT_1 receptor agonists DP-5-CT, CGS 12066B, and RU 24969 on plasma adrenaline and glucose levels in the rat. Naunyn Schmiedebergs Arch. Pharmacol. 342:378–381; 1990.
26. Li, Q.; Rittenhouse, P. A.; Levy, A. D.; Alvarez Sanz, M. C.; Van de Kar, L. D. Neuroendocrine responses to the serotonin$_2$ agonist DOI are differentially modified by three 5-HT_{1A} agonists. Neuropharmacology 31:983–989; 1992.
27. Lopez, F. J.; Negro-Vilar, A. Estimation of endogenous adrenocorticotropin half-life using pulsatility patterns: A physiological approach to the evaluation of secretory episodes. Endocrinology 123:740–746; 1988.
28. Macor, J. E.; Burkhart, C. A.; Heym, J. H.; Ives, J. L.; Lebel, L. A.; Newman, M. E.; Nielsen, J. A.; Ryan, K.; Schulz, D. W.; Torgersen, L. K.; Koe, B. K. 3-(1,2,5,6-Tetrahydropyrid-4-yl)-pyrrolo[3,2-*b*]pyrid-5-one: A potent and selective serotonin (5-HT_{1B}) agonist and rotationally restricted phenolic analogue of 5-methoxy-3-(1,2,5,6-tetrahydropyrid-4-yl)indole. J. Med. Chem. 33:2087–2093; 1990.
29. Porter, J. P. Electrical stimulation of paraventricular nucleus increases plasma renin activity. Am. J. Physiol. 254:R325–R330; 1988.
30. Richardson Morton, K. D.; Van de Kar, L. D.; Brownfield, M. S.; Bethea, C. L. Neuronal cell bodies in the hypothalamic paraventricular nucleus mediate stress-induced renin and corticosterone secretion. Neuroendocrinology 50:73–80; 1989.
31. Rittenhouse, P. A.; Bakkum, E. A.; Van de Kar, L. D. Evidence that the serotonin agonist, DOI, increases renin secretion and blood pressure through both central and peripheral 5-HT_2 receptors. J. Pharmacol. Exp. Ther. 259:58–65; 1991.
32. Saydoff, J. A.; Rittenhouse, P. A.; Van de Kar, L. D.; Brownfield, M. S. Enhanced serotonergic transmission stimulates oxytocin secretion in conscious male rats. J. Pharmacol. Exp. Ther. 257:95–99; 1991.
33. Steel, R. G. D.; Torrie, J. H. Principles and procedures of statistics with special reference to the biological sciences. New York: McGraw-Hill; 1960.
34. Tilders, F. J. H.; Berkenbosch, F. CRF and catecholamines; their place in the central and peripheral regulation of the stress response. Acta Endocrinol. 276:63–75; 1986.
35. Tilders, F. J. H.; Berkenbosch, F.; Vermes, I.; Linton, E. A.; Smelik, P. G. Role of epinephrine and vasopressin in the control of the pituitary-adrenal response to stress. Fed. Proc. 44:155–160; 1985.
36. Van de Kar, L. D. Neuroendocrine pharmacology of serotonergic (5-HT) neurons. Annu. Rev. Pharmacol. Toxicol. 31:289–320; 1991.
37. Van de Kar, L. D.; Bethea, C. L. Pharmacological evidence that serotonergic stimulation of prolactin secretion is mediated via the dorsal raphe nucleus. Neuroendocrinology 35:225–230; 1982.
38. Van de Kar, L. D.; Carnes, M.; Maslowski, R. J.; Bonadonna, A. M.; Rittenhouse, P. A.; Kunimoto, K.; Piechowski, R. A.; Bethea, C. L. Neuroendocrine evidence for denervation supersensitivity of serotonin receptors: Effects of the 5-HT agonist RU 24969 on corticotropin, corticosterone, prolactin and renin secretion. J. Pharmacol. Exp. Ther. 251:428–434; 1989.
39. Van de Kar, L. D.; Lorens, S. A.; Urban, J. H.; Bethea, C. L. Effect of selective serotonin (5-HT) agonists and 5-HT_2 antagonist on prolactin secretion. Neuropharmacology 28:299–305; 1989.
40. Van de Kar, L. D.; Richardson-Morton, K. D. Serotonergic regulation of renin release is not mediated by the autonomic nervous system, but involves beta adrenoceptors. Neuropharmacology 25: 487–492; 1986.
41. Van de Kar, L. D.; Urban, J. H.; Brownfield, M. S. Serotonergic regulation of renin and vasopressin secretion. In: Paoletti, R.; Vanhoutte, P. M.; Brunello, N.; Maggi, F. M., eds. Serotonin. From cell biology to pharmacology and therapeutics. Dordrecht, The Netherlands: Kluwer Academic Publishers; 1990: 123–129.
42. Van den Meiracker, A. H.; Admiraal, P. J. J.; Man in 't Veld, A. J.; Derkx, F. H. M.; Ritsema van Eck, H. J.; Mulder, P.; Van Brummelen, P.; Schalekamp, M. A. D. H. Prolonged blood pressure reduction by orally active renin inhibitor RO 42-5892 in essential hypertension. Br. Med. J. 301:205–210; 1990.
43. Zifa, E.; Fillion, G. 5-Hydroxytryptamine receptors. Pharmacol. Rev. 44:401–458; 1992.