Blunted Fenfluramine-Evoked Prolactin Secretion in Hypertensive Rats

Sean D. Stocker, Matthew F. Muldoon, Alan F. Sved

Abstract—Plasma prolactin (PRL) levels after acute administration of fenfluramine (FEN) have been used as a probe of brain serotonin activity. FEN-evoked increases in PRL levels inversely correlate with arterial blood pressure (ABP) in humans (Muldoon et al. Hypertension. 1998;32:972-975), thereby suggesting that brain serotonin activity may be reduced in hypertension. The present study sought to determine whether the relation between FEN-evoked PRL levels and ABP was present in two rat models of hypertension. Experiments were performed in awake male rats that were instrumented with femoral arterial and venous catheters 2 days before experiments. FEN (3.0 mg/kg IV) significantly increased plasma PRL levels in both spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY); however, FEN-evoked PRL levels were significantly lower in SHR compared with WKY, though baseline levels were similar between strains. Similar results were obtained in rats with chronic hypertension produced by figure-8 renal wrap plus contralateral nephrectomy. In contrast, the increase in PRL levels evoked by the serotonin receptor agonist m-CPP or the dopamine receptor antagonist eticlopride did not differ between SHR and WKY, indicating that PRL secretion is not generally blunted in chronic hypertensive rats. Furthermore, FEN-evoked PRL levels were not attenuated in rats made acutely hypertensive by an infusion of the α-adrenergic agonist phenylephrine. Thus, the present findings are consistent with the human data and suggest that chronic hypertension is associated with a presynaptic alteration in brain serotonin function. (Hypertension. 2003;42[part 2]:662-666.)

Key Words: brain ■ blood pressure ■ central nervous system ■ hypertension, chronic ■ hypertension, experimental

Brain serotonin participates in the regulation of sympathetic outflow and arterial blood pressure (ABP).1,2 Pharmacological stimulation of serotonin receptors produces complex changes in cardiovascular parameters, including increases and decreases in ABP.3-6 Moreover, activation of neuronal populations that contain serotonin has been reported to produce increases or decreases in ABP and sympathetic nerve activity.1,7 On the other hand, changes in ABP have been reported to alter serotonin neurotransmission in several brain nuclei.8-15 Studies in hypertensive rats have indicated that chronic hypertension may be associated with changes in serotonin function.11,13-16 However, the role of brain serotonin systems in the pathophysiology of chronic hypertension is poorly understood.

The increases in plasma prolactin (PRL) and cortisol levels after acute administration of fenfluramine (FEN) have been used as indices of brain serotonin function.16,17 As FEN increases serotonin release and decreases its reuptake by neurons, FEN increases serotonin-mediated neurotransmission, thereby increasing the secretion of PRL and cortisol. Differences in PRL and cortisol responses to FEN are thought to reflect differences in ongoing serotonin activity. In a study of 270 healthy human subjects, FEN-evoked increases in plasma PRL levels were inversely correlated to baseline ABP.18 The strength of this correlation was as high as other known risk factors for hypertension, such as obesity. Moreover, those subjects with the smallest FEN-evoked PRL response had a 2.6-times greater possibility to have an elevated blood pressure (≥135/85).18 These data suggest that brain serotonin activity may be reduced in humans with hypertension.

In the present study, we sought to determine whether the relation between FEN-evoked plasma PRL levels and baseline ABP was present in two rat models of chronic hypertension—the spontaneously hypertensive rat (SHR) and the Grollman renal wrap rat. If plasma PRL levels after FEN were blunted in hypertensive rats, this would suggest that central serotonin activity is reduced in hypertensive rats and provide an animal model to investigate the mechanisms underlying these differences as well as the role of central serotonin in hypertension. In both forms of experimental hypertension examined, plasma PRL levels in response to FEN were attenuated significantly in comparison to normotensive control rats. In marked contrast, FEN-evoked PRL levels were not attenuated in rats made acutely hypertensive by an infusion of the α-adrenergic agonist phenylephrine (PE). Additional experiments provide evidence that PRL secretion is not generally blunted in hypertensive rats, thereby suggesting that disturbed lactotroph function does not account for the attenuated PRL response to FEN.
Methods

Animals
Adult male rats were housed individually in a temperature-controlled room (22°C to 23°C) with a 12-hour/12-hour light-dark cycle (lights on at 8 AM). Tap water and Purina Laboratory Chow were available ad libitum except where noted. SHR and Wistar-Kyoto rats (WKY) were obtained from Charles River Laboratories and weighed 290 to 360 g (13 to 15 weeks of age) at the time of experiments. Additional experiments were performed in adult male Sprague-Dawley rats (Harlan Laboratories) weighing 285 to 400 g.

General Procedures
At least 48 hours before experiments, catheters were implanted into the left femoral artery (microrenthane tubing, 0.012-inch ID and 0.025-inch OD, Braintree Scientific) and vein (silastic, 0.020-inch ID and 0.037-inch OD) while rats were anesthetized with halothane (2% to 3% in 100% O₂). All catheters were tunneled subcutaneously to exit between the scapulae, filled with heparinized saline, and flushed daily (arterial, 1000 U/mL; venous, 40 U/mL). Rats were injected with an antibiotic (Dual-cillin; 30 000 U/mL IM) and fitted with an infusion harness (Harvard Apparatus) that allowed the catheters to pass outside the cage while protected by a steel spring.

At least 1 hour before experiments began, rats were weighed and returned to the home cage without food. Water was removed immediately before baseline recording of ABP and heart rate (HR). ABP was recorded by connecting the arterial line to a Statham pressure transducer (Grass Instruments) and a polygraph chart recorder (Grass Instruments, Model 7). The pulsatile ABP signal was filtered electronically to obtain mean ABP (MAP); HR was obtained through a tachograph (Grass Instruments, Model 7P44) triggered by the pulsatile ABP.

Genetic Hypertension
After a 20-minute recording of baseline MAP and HR, SHR and WKY rats received a bolus injection of D-FEN (3.0 mg/kg IV; Sigma). This dose of FEN was selected from previous studies demonstrating that FEN administered to rats produced a significant increase in synaptic serotonin levels19 and significant and sustained increases in plasma PRL levels.20,21 Blood samples (0.3 mL) were collected from the previous sample resuspended in isotonic saline, whereas subsequent samples were replaced with red blood cells from the previous sample resuspended in isotonic saline warmed at 37°C.

An additional group of SHR and WKY rats were treated with the serotonin receptor agonist 1-(m-chlorophenyl)piperazine (m-CPP) or the dopamine receptor antagonist eticlopride (ETC) to determine whether chronic hypertension affects the ability of lactotrophs to secrete PRL. After a 20-minute recording of baseline MAP and HR, SHR and WKY rats received a bolus injection of either m-CPP (2 mg/kg IV; Sigma) or ETC (0.1 mg/kg IV; Sigma). Blood samples (0.3 mL) were collected from the arterial line as described above at 5 minutes before and 5, 10, and 30 minutes after D-FEN. Samples from a single experiment were analyzed in the same assay.

Renal Wrap Hypertension
Plasma PRL levels in response to FEN also were determined in a second model of hypertension, the Grollman model of renal hypertension.22 Adult male Sprague-Dawley rats were anesthetized with halothane (2% to 3% in 100% O₂), and a figure-8 Grollman renal wrap with suture and contralateral nephrectomy were performed. Control rats received a unilateral nephrectomy only. All rats were treated with antibiotic (Dual-cillin; 30 000 U/mL IM) and returned to home cages. Approximately 10 to 12 days later, rats were instrumented with arterial and venous catheters as described above, and experiments began 2 days later. Renal wrap (RW) rats with a baseline MAP >135 mm Hg were considered hypertensive RW rats.

After a 20-minute recording of baseline MAP and HR, hypertensive RW (n=9) and control rats (n=8) received a bolus injection of FEN (3.0 mg/kg IV; Sigma). Blood samples (0.3 mL) were collected as described above 5 minutes before and 10, 30, and 60 minutes after FEN. At least 3 days later, a subset of these rats received ETC (0.1 mg/kg IV), and blood samples (0.3 mL) were collected as described above.

Acute Hypertension
Additional experiments were performed in rats made acutely hypertensive. After a 20-minute baseline recording of MAP and HR, male Sprague-Dawley rats were infused continuously with the α-adrenergic agonist PE (10 μg/kg per minute IV; Sigma) or isotonic saline (SLN; 0.5 mL/h IV) for 45 minutes. FEN was given 15 minutes after the initiation of the PE or SLN infusion. Blood samples (0.3 mL) were collected at baseline, 10 minutes after the initiation of the PE or SLN infusion, and 10 and 30 minutes after FEN. Every rat received the infusion of PE and SLN separated by 3 days; half of the rats received the infusion of PE first.

Determination of Plasma PRL Levels
Plasma PRL levels were determined by radioimmunoassay, with the use of materials generously provided by the National Hormone and Pituitary Program (Torrance, Calif). Briefly, 25-μL plasma aliquots were incubated for 24 hours at room temperature (22°C to 23°C) with a rabbit antibody to rat PRL (rPRL-S-9; final dilution, 1:420 000) and ~10 000 counts/min of 125I-labeled PRL (New England Nuclear-DuPont). Subsequently, the antibody was precipitated with the use of a secondary antibody procedure. The tubes were centrifuged (3000g, 25 minutes), the supernatant was aspirated, and the remaining pellets were counted in a gamma scintillation counter (4470 Wizard, Wallac Inc). PRL values were calculated from standard curves generated with known values of rat PRL (rPRL-RP-3, National Hormone and Pituitary Program). Duplicate plasma PRL samples were analyzed, and the averaged values were expressed as nanograms per milliliter. All samples from a single experiment were analyzed in the same assay.

Statistical Analysis
All data are expressed as mean±SEM. Plasma PRL levels were log-transformed and analyzed by a 2-way ANOVA with repeated measures (Systat, SPSS). When significant F values were obtained for the group factor, independent t tests with a layered Bonferroni correction were performed. Within-group effects were compared by a repeated-measures ANOVA followed by paired t tests with layered Bonferroni correction to compare each time with baseline values. MAP and HR were analyzed similarly. A probability value of <0.05 was considered statistically significant in all comparisons.

Results

Genetic Hypertension
Initial experiments were performed to determine whether D-FEN–evoked increases in PRL levels would be attenuated in SHR versus WKY rats. As expected, MAP values of SHR rats were significantly elevated compared with those values of WKY rats (Figure 1B). Administration of D-FEN caused significant increases in plasma PRL levels in both SHR and WKY rats (Figure 1A); however, plasma PRL levels of SHR rats were significantly less than those of WKY rats at every time after D-FEN (Figure 1A). There were no differences in baseline PRL levels between SHR and WKY rats. D-FEN produced a transient increase in MAP that was of similar magnitude and duration in both SHR and WKY rats (Figure 1B). No differences in HR were observed between SHR and...
WKY rats (baseline: 375±6 versus 361±8 bpm, respectively), and D-FEN did not alter HR in either group.

Because plasma PRL levels in response to D-FEN were significantly attenuated in SHR compared with WKY rats, additional experiments were performed to determine whether this relationship was present in a second model of hypertension: the Grollman renal wrap model.22

As expected, hypertensive RW rats had a significantly elevated baseline MAP compared with control rats (Figure 4B). Administration of FEN significantly increased plasma PRL levels in both hypertensive RW and control rats (Figure 4A); however, plasma PRL levels were significantly attenuated in hypertensive RW rats compared with control rats at every time after FEN (Figure 4A). It is noteworthy that 3 additional RW rats with an average baseline MAP <135 mm Hg (127±3 mm Hg) had an increase in PRL secretion after FEN that was similar to normotensive control rats (10 minutes: 57±9 pg/mL; 30 minutes: 61±8 pg/mL; 60 minutes: 30±4 pg/mL). Baseline plasma PRL levels did not differ between groups.

Similar to SHR and WKY rats, FEN produced a significant increase in MAP at 2 minutes in both hypertensive RW and control rats (Figure 4B); however, MAP was not different from baseline values for the remainder of the test in both groups.

Renal Wrap Hypertension

Because PRL levels after D-FEN were blunted significantly in SHR compared with WKY rats, additional experiments were performed to determine whether this relationship was present in a second model of hypertension: the Grollman renal wrap model.22

As expected, hypertensive RW rats had a significantly elevated baseline MAP compared with control rats (Figure 4B). Administration of FEN significantly increased plasma PRL levels in both hypertensive RW and control rats (Figure 4A); however, plasma PRL levels were significantly attenuated in hypertensive RW rats compared with control rats at every time after FEN (Figure 4A). It is noteworthy that 3 additional RW rats with an average baseline MAP <135 mm Hg (127±3 mm Hg) had an increase in PRL secretion after FEN that was similar to normotensive control rats (10 minutes: 57±9 pg/mL; 30 minutes: 61±8 pg/mL; 60 minutes: 30±4 pg/mL). Baseline plasma PRL levels did not differ between groups.

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Figure 2. Mean±SEM values of plasma PRL levels (A) and MAP (B) in SHR and WKY rats treated with the serotonergic receptor agonist m-CPP (2 mg/kg IV). Administration of m-CPP significantly increased MAP in both SHR and WKY rats (Figure 2B). The peak increase in MAP was larger in SHR versus WKY rats (46±2 mm Hg versus 32±2 mm Hg, respectively; P<0.001); however, this difference in MAP was not significant when the values were expressed as a percent change of baseline MAP values (25±2% versus 21±2%, respectively). A 2-way ANOVA revealed a significant interaction (group×time, P<0.01) as MAP of SHR remained elevated above baseline levels for the remainder of the test, whereas MAP of WKY rats was elevated only at 2 and 5 minutes after m-CPP (Figure 2B). Furthermore, m-CPP transiently increased HR in SHR rats from baseline levels at 2 and 5 minutes (364±13 to 408±14 and 403±14 bpm; P<0.05), but HR did not significantly change in WKY rats (baseline: 356±10 bpm).

In addition to m-CPP, ETC was administered to further evaluate whether differences in PRL levels in response to D-FEN were due to changes in PRL-secreting lactotrophs, as ETC acts directly on the pituitary to block dopamine-mediated inhibition PRL secretion. ETC markedly increased PRL levels in both SHR and WKY rats (Figure 3), and no differences in plasma PRL levels were observed between SHR and WKY rats. ETC did not affect MAP (Figure 3B) or HR (data not shown) in either group (P>0.2 from overall ANOVAs).

Renal Wrap Hypertension

Because PRL levels after D-FEN were blunted significantly in SHR compared with WKY rats, additional experiments were performed to determine whether this relationship was present in a second model of hypertension: the Grollman renal wrap model.22

As expected, hypertensive RW rats had a significantly elevated baseline MAP compared with control rats (Figure 4B). Administration of FEN significantly increased plasma PRL levels in both hypertensive RW and control rats (Figure 4A); however, plasma PRL levels were significantly attenuated in hypertensive RW rats compared with control rats at every time after FEN (Figure 4A). It is noteworthy that 3 additional RW rats with an average baseline MAP <135 mm Hg (127±3 mm Hg) had an increase in PRL secretion after FEN that was similar to normotensive control rats (10 minutes: 57±9 pg/mL; 30 minutes: 61±8 pg/mL; 60 minutes: 30±4 pg/mL). Baseline plasma PRL levels did not differ between groups.

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Figure 1. Mean±SEM values of plasma PRL levels (A) and MAP (B) in SHR and WKY rats given D-FEN (3.0 mg/kg IV). Administration of D-FEN significantly increased plasma PRL levels from baseline values in both SHR and WKY rats (P<0.01); however, plasma PRL levels were significantly attenuated in SHR compared with WKY rats at every time after d-FEN. *Significant difference from baseline values within a treatment group (P<0.05).
Furthermore, FEN caused significant bradycardia in both RW and control rats at 5 to 20 minutes (data not shown; \( P \), 0.05). Baseline HR values did not differ between RW and control rats (406 ± 6 versus 412 ± 12 bpm, respectively).

Because the blunted PRL response to FEN in RW rats may be due to a change in the PRL-secreting lactotrophs, a subset of hypertensive RW (n = 7) and control rats (n = 6) were given ETC. Plasma PRL levels increased markedly in hypertensive RW and control rats at 10 and 30 minutes after ETC (\( P \), 0.01); however, plasma PRL levels did not differ between hypertensive RW and control rats at baseline (2.3 ± 0.5 versus 3.3 ± 1.5 ng/mL, respectively), 10 minutes (138 ± 11 versus 120 ± 12 ng/mL, respectively), and 30 minutes (159 ± 8 versus 124 ± 22 ng/mL, respectively). ETC had no effect on MAP and HR in either group.

**Acute Hypertension**

A final set of experiments was performed to determine whether FEN-evoked increases in PRL levels would be blunted in rats made acutely hypertensive by an infusion of the \( \alpha \)-adrenergic agonist PE. As expected, the infusion of PE significantly raised MAP above baseline values and MAP of rats infused with SLN (Figure 5B). However, FEN-evoked increases in PRL levels were not blunted in rats infused with PE at 10 or 30 minutes after FEN (Figure 5A).

It is noteworthy that FEN significantly decreased MAP in rats made acutely hypertensive by an infusion of PE (Figure 5B). In contrast, FEN caused a transient but significant increase in MAP in rats infused with SLN at 2 minutes (Figure 5B), though this response was significantly smaller than that observed in similar Sprague-Dawley rats with unilateral nephrectomy (Figure 4). As expected, the infusion of PE produced a significant bradycardia, whereas the infusion of SLN did not alter HR (Figure 5C). However, administration of FEN significantly decreased HR in rats infused with SLN at 2, 5, and 10 minutes (Figure 5C). FEN did not affect HR in rats infused with PE.

**Discussion**

In a study of healthy adults, increases in plasma PRL levels after acute administration of FEN were inversely correlated with baseline MAP, thereby suggesting that hypertension might be associated with a lower FEN-evoked PRL secretion and therefore a decrease in brain serotonin activity.\(^{18}\) The present study sought to determine whether this relationship exists in rat models of hypertension. These findings clearly demonstrate that FEN-evoked PRL levels were blunted markedly in two rat models of chronic hypertension but not altered in rats made acutely hypertensive by an infusion of PE. Furthermore, additional experiments indicate that the blunted FEN-evoked PRL response in chronic hypertensive rats is not a direct reflection of impaired pituitary PRL secretion. Therefore, the present findings are consistent with observations in humans and suggest that brain serotonin activity is reduced in rats with chronic hypertension.

The present experiments assessed brain serotonin activity by a neuroendocrine challenge to FEN. Several studies in animals and humans have demonstrated that plasma PRL levels increase unilaterally after acute administration of FEN.
after acute administration of serotonin precursors, serotonin releasing agents, and direct serotonin receptor agonists. Acute administration of FEN enhances synaptic serotonin levels by blocking reuptake and stimulating release from presynaptic terminals and additionally may activate postsynaptic receptors. The increase in synaptic levels of serotonin in the hypothalamus stimulates the secretion of PRL. The dose of FEN used in the present study is within the reported range of doses that produce dose-dependent increases in synaptic serotonin levels and plasma PRL levels. The increase in plasma PRL levels after FEN is blocked either by pretreatment with serotonin receptor antagonists or reuptake inhibitors or lesions of the raphe nuclei. Indeed, treatments that interfere with central serotonin neurons (eg, chronic fenfluramine, parachlorophenylalanine) reduce or eliminate FEN-evoked PRL secretion in a manner that is consistent with this neuroendocrine challenge test, accurately reflecting brain serotonin-mediated neural transmission. Therefore, the PRL response is dependent on serotonin mechanisms and provides an index of overall serotonin activity or net responsivity, but it does not distinguish between presynaptic versus postsynaptic mechanisms.

Although the neuroendocrine challenge with FEN appears to be dependent on serotonin mechanisms, the attenuation of FEN-evoked PRL levels in hypertensive rats may be due to a number of factors mediating PRL release. For example, the blunted PRL response to FEN may reflect an alteration in PRL-secreting lactotrophs in chronic hypertensive rats. However, no differences were observed in plasma PRL levels in response to ETC and m-CPP between chronically hypertensive and normotensive rats in the present study. These findings suggest that chronic hypertension does not impair the ability of lactotrophs to secrete PRL in the range of plasma levels studied here and therefore cannot explain the blunted response to FEN. Consistent with this notion, Sowers and colleagues reported that the change in PRL levels was similar between SHR and WKY rats in response to thyrotrophin-releasing hormone or haloperidol. Furthermore, the increase in PRL levels after the serotonin receptor agonist m-CPP was similar between SHR and WKY rats, thereby suggesting that the differences in FEN-evoked PRL levels in chronic hypertensive rats is not due to changes in the serotonin postsynaptic receptor or other receptors downstream from the serotonin presynaptic neuron. Collectively, these observations suggest that the apparent differences in FEN-evoked PRL levels between hypertensive and normotensive rats cannot be explained by an alteration in PRL-secreting lactotrophs and further suggests that chronic hypertension may be associated with presynaptic changes in serotonin neurons.

In both rat models of chronic hypertension, plasma PRL levels in response to FEN were attenuated significantly in hypertensive compared with normotensive rats. In marked contrast, this relationship was not observed in rats made acutely hypertensive by an infusion of PE and receiving FEN. Although acute changes in ABP have been reported to alter serotonin neurotransmission, the present findings suggest that a reduction in brain serotonin activity or responsivity only occurs in chronic hypertension. It is noteworthy that changes in serotonin metabolism have been reported in young SHR in the hypothalamus and areas of the hindbrain involved in cardiovascular regulation, but these differences were not detectable in the adult. In addition, several investigators have reported changes in cardiovascular parameters evoked by stimulation of serotonin receptors in the central nervous system. Therefore, it is interesting to speculate that the decrease in serotonin function contributes to the pathogenesis of chronic hypertension in these two models. Hypertension in both SHR and RW rats is associated with an elevation in sympathetic outflow and ABP in these models. However, the present results do not provide a direct link between increased sympathetic outflow and brain serotonin function in chronic hypertension. It is possible that the decrease in serotonin function results after the appearance of the hypertension or is dependent on other factors unrelated to the elevated sympathetic outflow in either model.

In summary, the present findings demonstrate that plasma PRL levels in response to FEN are attenuated significantly in chronic hypertensive rats in comparison with normotensive rats. These findings suggest that chronic hypertension may be associated with presynaptic changes in serotonin neurons and that these changes may contribute to the alteration in PRL release. Further studies are needed to elucidate the mechanisms underlying these changes and their role in the pathogenesis of chronic hypertension.
two rat models of chronic hypertension. These observations are similar to the reported inverse correlation between FEN-evoked PRL secretion and baseline MAP in humans.\textsuperscript{18} Collectively, these observations suggest that the activity of brain serotonin pathways is reduced in chronic hypertension, although it is not clear whether this results from an increased clearance or reuptake, increased inactivation, or decreased synthesis or release of serotonin. Whether changes in the activity of these pathways contribute to the pathogenesis of hypertension or follow the onset of hypertension in these two rat models remains unclear.

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References


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