Inverse Relationship Between Fenfluramine-Induced Prolactin Release and Blood Pressure in Humans

Matthew F. Muldoon, Alan F. Sved, Janine D. Flory, James M. Perel, Karen A. Matthews, Stephen B. Manuck

Abstract—Although substantial evidence from experimental animals suggests that augmentation and reduction in serotonergic neurotransmission both affect arterial blood pressure (BP), it is unknown whether “tonic” central serotonergic activity is related to resting BP variability in humans. We tested this hypothesis in a community sample by evaluating the relationship between resting BP and a neuropharmacologic index of brain serotonergic activity (the fenfluramine challenge test). Subjects were 270 generally healthy men and women aged 25 to 60 years who were not receiving prescribed antihypertensive or psychotropic medications. The sample included 216 non-Hispanic whites and 47 blacks. Resting systolic BP ranged from 85 to 161 mm Hg and diastolic from 58 to 98 mm Hg. Each subject received 0.55 to 0.65 mg/kg D,L-fenfluramine hydrochloride, and the plasma prolactin concentration was measured over 3.5 hours. Analyses revealed a linear, inverse relationship between the maximum fenfluramine-induced prolactin rise and systolic and diastolic BP in whites: \( r = -0.36 \) and \( r = -0.29 \), respectively \( (P<0.001 \) for both). These relationships were not observed in the black participants. In whites, the prolactin response to fenfluramine remained a significant predictor of systolic and diastolic BPs in multivariate models including age, gender, body mass index, physical activity, smoking, and alcohol consumption \( (P\leq0.001) \). When compared with subjects in the highest quartile of prolactin response, individuals whose prolactin responses to fenfluramine comprised the lowest quartile were 2.6 times more likely to have a resting systolic/diastolic BP of \( >135/85 \) mm Hg. These data reveal that in white but not black adults, fenfluramine-induced prolactin release correlates inversely with BP and may indicate a role of central serotonergic activity in the pathogenesis of hypertension. \( \textit{Hypertension}. \ 1998;32:972-975. \)

Key Words: blood pressure ■ central nervous system ■ serotonin ■ fenfluramine ■ prolactin

As part of a clinical investigation of the neurobehavioral correlates of cardiovascular disease risk, we sought to examine the relationship between brain serotonergic function and resting blood pressure (BP). Effects of central serotonergic activity on BP are suggested by a series of studies in experimental animals. Pharmacological stimulation of some brain serotonin (5-hydroxytryptamine [5-HT]) receptors produces robust, if complex, changes in cardiovascular parameters, including both hypertensive and hypotensive responses. Conversely, experimental manipulation of BP has also been found to influence serotonergic neural transmission in certain brain loci. However, it has never been demonstrated whether central serotonergic activity, under resting or basal conditions, is related to individual differences in BP.

In the present study, central serotonergic activity was assessed with a neuroendocrine challenge with D,L-fenfluramine hydrochloride. Fenfluramine enhances serotonin neurotransmission by both inducing presynaptic release of serotonin stores and inhibiting synaptic reuptake. Activation of serotonergic receptors in the hypothalamus in turn promotes the pituitary release of prolactin into the circulation. Therefore, the rise in plasma prolactin concentration after fenfluramine administration reflects "net" serotonergic responsiveness, as influenced by variability in presynaptic events (ie, synthesis, storage, release, and reuptake) and activation of hypothalamic 5-HT receptors. In this article we report an inverse correlation between interindividual variability in prolactin response to fenfluramine and resting BP that is independent of traditional risk factors for hypertension.

Methods

Subjects

The 270 adult participants described in this report were recruited from the Pittsburgh area through media advertisements and local distribution of brochures and posters. Exclusion criteria for participation included age \( \geq 60 \) years; diastolic BP \( >99 \) mm Hg; presence of congestive heart failure, angina, stroke, cancer, or hepatic or renal insufficiency; and use of psychotropic medications. Also excluded from the present analyses were persons receiving antihypertensive medications. The protocol was approved by the University of Pittsburgh Institutional Review Board, and subjects gave informed consent.
BP measurements were obtained at 2 screening appointments, separated by 1 to 3 weeks. Subjects arrived between 8 and 10 AM following an overnight fast. After the subject had rested in the seated position for 20 minutes, a registered nurse obtained a single BP measurement in the right arm using a mercury sphygmomanometer and a regular, large, or extra-large adult cuff, according to the subject’s arm circumference. The average of the readings from the 2 screening visits was used as the subject’s resting BP. Self-reported measures of alcohol consumption, smoking, and physical activity were also obtained.

Fenfluramine Challenge
Participants reported to the laboratory in the morning after a 12-hour fast, and a 20-gauge heparin-locked venous catheter was inserted. After a 30-minute adaptation period, a heparinized blood sample was obtained for determination of baseline prolactin concentration. Subjects then received 30 to 60 mg of d,l-fenfluramine hydrochloride orally to achieve a dose in the range of 0.55 to 0.65 mg/kg body wt. Subsequent blood samples for plasma prolactin were drawn 1, 2, 2.5, 3, and 3.5 hours later. Additional samples were taken at 2.5 and 3.5 hours for measurement of plasma fenfluramine and norfenfluramine concentrations. This protocol is shorter than the standard 5-hour fenfluramine test used in psychiatric research because of practical constraints on participation and scheduling in our sample of generally healthy volunteers. In a sample of 42 individuals, we found that peak prolactin concentrations over 3.5 and 5 hours had a correlation of 0.91, indicating high concordance between assessments based on the abbreviated and standard sampling intervals (S.B.M., J.D.F., M.F.M., unpublished data, 1997). All blood samples were centrifuged immediately, separated, and stored at −70°C until analysis. Methods for determination of plasma prolactin, fenfluramine, and norfenfluramine (the principal active metabolite) have been described elsewhere.

Analysis
Peak prolactin change was calculated as the arithmetic difference between the highest prolactin value obtained after drug administration and the prolactin concentration at baseline. The resulting distribution of prolactin change scores was normalized by logarithmic transformation and adjusted for covariation with initial values to yield a baseline-free index of prolactin response to fenfluramine. Although prolactin levels were not collected after ingestion of placebo in this study, prior research has shown that this calculation of peak prolactin response to fenfluramine is correlated very highly with both placebo-adjusted prolactin responses and the calculated area under the curve (r>0.90). Associations between prolactin response to fenfluramine and BP were examined using standard parametric correlational and multivariate linear regression analyses. Pearson correlation coefficients were compared using Fisher’s z transformation. BP and hypertensive status according to quartiles of peak prolactin response were compared by 1-way ANOVA (with contrasts among group means performed using Tukey’s honestly significant difference procedure) and χ² analysis.

Results
The 270 subjects included 145 men and 125 women (216 non-Hispanic whites, 47 blacks, 4 Asians, and 3 Hispanics). Sample characteristics included the following: mean age, 45 years (range, 24 to 60 years); mean education, 15.5 years; mean body mass index (BMI), 27 kg/m² (range, 18 to 41 kg/m²); 62% married; 79% employed full or part-time; 16% smokers; and mean 3.4 alcoholic drinks consumed per week. Among the women, none were pregnant or lactating; 40 (32%) were postmenopausal, and 12 were taking prescribed hormone replacement therapy. Systolic BP ranged from 85 to 161 mm Hg, and diastolic BP varied from 58 to 98 mm Hg. A total of 31 subjects met the criteria for stage I or II hypertension. The mean±SE baseline plasma prolactin concentration was 3.6±0.3 ng/mL, and the mean maximum level after fenfluramine was 7.1±0.2 ng/mL. The mean plasma concentrations of fenfluramine plus norfenfluramine did not correlate significantly with resting BP, indicating that fenfluramine bioavailability did not vary systematically with BP.

Correlations between BP and age, BMI, and maximum prolactin response to fenfluramine are provided in the Table. As is commonly observed, systolic and diastolic BP increased with increasing age and with increasing BMI. Weak relationships were observed between BP and self-reported alcohol consumption (r=0.10 to r=0.11) and between BP and basal prolactin concentration (r=−0.13 to r=−0.17). More notable and unique to this investigation, systolic and diastolic BPs both correlated negatively with maximum prolactin response to fenfluramine (P<0.001). Because determinants of BP variability may vary according to ethnicity, blacks and non-Hispanic whites, the major races represented in this sample, were also analyzed independently. The inverse relationship between BP and peak prolactin response was highly significant in non-Hispanic whites but not in blacks. This difference in correlations between blacks and whites was statistically significant (P<0.01 for both systolic and diastolic BPs).

Multivariate linear regression analyses were then conducted on the data from the non-Hispanic whites to determine whether the relationship between BP and prolactin response to fenfluramine was independent of recognized predictors of BP. Age, gender, BMI, physical activity, smoking, and alcohol consumption were first entered into regression models for systolic and diastolic BPs. Maximum prolactin response was then entered into the model for systolic BP (β=−0.266, P<0.001) and into the model for diastolic BP (β=−0.199, P=0.001). Because women in this study varied with respect to menopausal status, secondary analyses were conducted in women only. When menopausal status was entered into the multivariate regression models, maximum prolactin response was a significant predictor of both systolic and diastolic BPs.

For illustrative purposes, non-Hispanic white subjects were divided into quartiles of peak prolactin response to fenfluramine. As shown in the Figure, mean systolic and diastolic BPs adjusted for age, gender, and BMI varied with quartile of prolactin response (P<0.05), with individuals in the lowest quartile having higher BPs than those in the highest quartile of prolactin response (P<0.05). Finally, the prevalence of high-normal BP or clinically defined hypertension (BP ≥135/85 mm Hg) also differed by quartile [χ²(3)=8.5, P<0.05]; subjects’ likelihood of having elevated BP was 2.6 times higher in the lowest quartile compared to the highest quartile of peak prolactin response.
greater in the lowest, relative to the highest, prolactin response quartile (bottom panel of Figure).

Discussion

We found that smaller prolactin responses to fenfluramine were associated with elevated resting BPs in data collected for a relatively large community sample not receiving drugs known to affect prolactin release or BP. This relationship was strongest in non-Hispanic whites. Notably, prolactin response to fenfluramine explained as much of the sample variability in resting BP as did several well-recognized determinants of BP (specifically, age and BMI). The association between prolactin response and BP was linear and persisted after adjustment for other predictors of BP (ie, age, gender, BMI, physical activity, and alcohol consumption). Compared with individuals in the highest quartile of prolactin response, subjects in the lowest quartile were 2 to 3 times more likely to have resting BP in the high-normal or hypertensive range. Finally, this relationship was at least weaker in black than in white participants, possibly reflecting differences in the etiology of essential hypertension among racial groups. However, this latter finding was based on a sample of blacks less than one fourth the size of that of whites.

Interpretation of these results depends on the validity of the D,L-fenfluramine challenge as an index of brain serotonergic activity. Numerous studies in animals and humans have shown that plasma prolactin levels increase after administration of serotonin precursors, releasing agents, and direct agonists. When administered acutely, fenfluramine serves as a serotonin agonist by stimulating serotonin release from storage granules in presynaptic neurons, by blocking serotonin reuptake, and possibly by activating postsynaptic receptors. In rodents, prolactin responses to fenfluramine are prevented by acute lesions of the raphe nuclei, and in humans and experimental animals, prolactin responses are dose-related and blocked by pretreatment with serotonin antagonists. Thus, the fenfluramine challenge does not distinguish between presynaptic and postsynaptic processes but instead permits assessment of the overall function of neuronal circuits utilizing serotonin. In addition, prolactin responses to the serotonin-selective D-fenfluramine isomer have been found to correlate highly with responses to D,L-fenfluramine. The topographical pattern of neuronal activation on positron-emission tomography induced by racemic fenfluramine is also similar to that observed after administration of D-fenfluramine. Finally, several reports indicate that prolactin response to fenfluramine has acceptable reproducibility and correlates significantly with cerebrospinal fluid 5-hydroxy indole–acetic acid.

Although fenfluramine-evoked prolactin release is mediated by serotonergic systems, it is possible that the response is also influenced by other factors involved in the control of prolactin secretion. For example, blunted fenfluramine-evoked prolactin release might reflect an attenuation of stimulus-secretion coupling in lactotrophs or enhanced dopaminergic inhibition. However, the functional status of lactotrophs and their tonic inhibition by dopamine, as assessed by prolactin secretion after infusion of thyrotropin-releasing hormone, are unrelated to fenfluramine-induced changes in circulating prolactin levels. Some previous research has suggested that hypertension is associated with elevated basal prolactin concentration and exaggerated prolactin response to the dopaminergic antagonist metaclopramide. However, prolactin responses in individuals with a modest BP elevation in this study were blunted and therefore are inconsistent with a general increase in basal and stimulus-evoked prolactin release. Moreover, the prolactin responses in this study were adjusted for any covariation with baseline prolactin levels. Our findings therefore point to a unique and most likely serotonergic mechanism for prolactin responses to fenfluramine.

To the extent that fenfluramine-induced prolactin release serves as an index of hypothalamic-pituitary serotonergic activity, the present findings extend prior research demonstrating that brain serotonin plays a role in BP regulation in laboratory animals. Serotonergic neurons modulate autonomic nervous activity and vasopressin and renin release, and augmentation of serotonin release in obese individuals with fenfluramine may decrease autonomic sympathetic ac-
tivity.20 However, as described in the classic article by Page,21 the actions of serotonin injected into the cerebrospinal fluid of rats increases BP by activating 5-HT₁ receptors, whereas stimulation of 5-HT₂ receptors, many of which are autoreceptors located presynaptically, decreases BP, particularly in hypertensive animals. Furthermore, in experimental animals elevations or reductions in BP cause alterations in serotonin neurotransmission, likely reflecting compensatory adjustments to raised or lowered BP.22 In conclusion, the inverse association seen here between fenfluramine-induced prolactin release and BP may reflect either a causal relationship involving 5-HT, a permissive effect of diminished serotonergic activity, or, alternatively, blunted prolactin responses may serve as a marker for a separate unidentified disturbance more directly responsible for BP dysregulation.

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References
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