Effects of Age and Gender on CNS Serotonergic Responsivity in Normal Adults

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The effects of age and gender on central nervous system (CNS) serotonergic responsivity were assessed with a neuroendocrine challenge test in 30 normal adults. Subjects ≥30 years of age, compared with younger subjects, exhibited decreased prolactin secretion in response to a 60-mg oral dose of dl-fenfluramine hydrochloride, an indirect serotonin agonist. Furthermore, women had greater prolactin responses than men. As prolactin secretory capacity appears to be stable through midlife, the age-associated decrease in fenfluramine-induced prolactin release suggests a decline in CNS serotonergic responsivity. In contrast, the finding of greater prolactin release in women than in men probably reflects the effects of nonserotonergic modulatory influences at the level of the lactotroph. Age and gender effects must be considered in studies of the CNS serotonergic system.

Introduction

Alterations in the function of the neurotransmitter serotonin have been reported in a number of psychiatric disorders, most notably major depression (van Praag 1986), obsessive-compulsive disorder (Insel et al. 1985), autistic disorder (Anderson 1987, McBride et al. 1989), and in the susceptibility to aggressive, impulsive (Coccaro et al. 1989), or suicidal behavior (Mann et al. 1986, van Praag 1986). In recent years, neuroendocrine challenge tests have been widely used to evaluate the physiological responsivity of central serotonergic neuron systems and postsynaptic serotonin receptors in persons with psychiatric disorders. However, comparatively little attention has been devoted to the effects of age and gender on serotonin-mediated neuroendocrine responses in normal subjects.

Associations between age or gender and the hormonal responses of normal subjects to serotonin agonists have been briefly noted in the literature. We and others have described an age-related decline in prolactin secretion following challenge with the indirect serotonin agonists fenfluramine (McBride et al. 1986; Asnis et al. 1988) and tryptophan (Heninger et al. 1984). Women have been reported to have greater prolactin responses to the direct serotonin receptor agonist *m*-chlorophenylpiperazine (*m*-CPP) than men (Mueller et al. 1985; Charney et al. 1988).

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In the present study, the prolactin response to fenfluramine was used to assess the effects of age and gender on central nervous system (CNS) serotonergic responsivity. The study differs in a number of respects from previous studies that have reported a relationship between age or gender and serotonin-mediated neuroendocrine responses in healthy individuals. A larger number of subjects were evaluated, and subjects spanned a wider range of ages (21–74 years). Furthermore, plasma drug levels were measured in order to determine if potential age- and gender-associated differences in the magnitude of the hormonal response represent pharmacodynamic versus pharmacokinetic effects of drug administration.

Administered as a single dose, fenfluramine enhances serotonergic transmission by stimulating release of serotonin from the presynaptic neuron and by blocking serotonin reuptake (Borroni et al. 1983). Fenfluramine was chosen as the challenge agent in this study because (1) the neuroendocrine response reflects the functional status of both pre- and postsynaptic serotonergic neurons, and thus provides a measure of net serotonergic responsivity; (2) prolactin secretion in response to moderate doses of fenfluramine appears to be mediated by the drug's specific effects on serotonergic neurons (Quattrone et al. 1978; Fuller et al. 1982; Quattrone et al. 1983); and (3) sensitive assays for fenfluramine and the pharmacologically active metabolite norfenfluramine are available.

Methods

Subjects

Thirty adults (18 men, 12 women) without personal or family histories of psychiatric or neurological disorder participated in the study. All were of normal weight and in excellent physical health. The mean age of the group was 35.0 ± 15.0 (SD) years; the mean ages of the male and female subjects were 36.2 ± 15.2 and 33.1 ± 15.7 years, respectively. All subjects were drug-free at testing and denied recent drug ingestion or histories of substance abuse. None of the female subjects had taken oral contraceptives in the preceding year. Most subjects were either hospital employees or medical students. Written informed consent was obtained as required by the New York Hospital-Cornell Medical Center Committee on Human Rights in Research.

Fenfluramine Challenge Test

The fenfluramine challenge test was performed in the Laboratory of Psychopharmacology utilizing a placebo-controlled design. Each subject received a 60-mg oral dose of dl-fenfluramine hydrochloride (Pondimin) and placebo on different days. Food and fluids other than water were not permitted after 11 PM on nights preceding testing. Studies began at 8 AM with insertion of an indwelling catheter into an antecubital vein. An intravenous infusion of 5% dextrose and 0.45% sodium chloride (200 cc/hr) was administered to prevent dehydration and hormonal changes due to the hypoglycemic effect of fenfluramine. Blood samples for measurement of plasma prolactin levels were drawn (via the catheter) both 15 min prior to and immediately preceding the ingestion of fenfluramine or placebo at 9 AM (hour 0), and then hourly for 5 successive hr. Plasma was also obtained at hr 0, 3, and 5 for assay of fenfluramine and norfenfluramine levels. A total of 100 cc of whole blood was required for all studies. Subjects rested quietly throughout the test.

Although a number of subjects described impaired concentration, drowsiness, or mild dysphoria, anxiety, or elation following fenfluramine ingestion, none manifested more dramatic changes in mental status such as agitation or psychotic symptoms. Prolactin responses to fenfluramine challenge were not correlated with subjects' subjective reports of their emotional state or level of arousal, rated on a 10-item Lickert scale. Somatic complaints included nausea, headache, fatigue, and lightheadedness, all minor in severity.

Subjects and technical staff were not informed whether fenfluramine or placebo was administered on a particular day. Placebo tablets identical to fenfluramine tablets were supplied by A.H. Robins Company. In cases where fenfluramine was administered on the first test day, placebo studies were not performed for at least 1 week. The maximum interval between studies with active drug and placebo was 1 month.

Plasma prolactin levels were determined by an immunoradiometric assay using kits purchased from Hybritech Incorporated. The minimum detectable prolactin concentration is 0.3 ng/ml, and the interassay coefficient of variation is 4%.

Plasma levels of fenfluramine and norfenfluramine were measured by a gas-liquid chromatographic method (Krebs et al. 1984). The minimum detectable concentrations of fenfluramine and norfenfluramine are 2.0 ng/ml and 5.0 ng/ml, respectively. Coefficients of variation are 3.4% for fenfluramine and 6.8% for norfenfluramine.

Data Analysis

The net change in plasma prolactin levels (ΔPRL) between hr 0 and 5 served as the outcome measure of the fenfluramine challenge test. Because resting prolactin levels varied significantly with time during placebo studies, fenfluramine-induced changes in hourly plasma prolactin levels were determined by subtracting an individual's plasma prolactin levels during the placebo trial from time-matched levels following administration of active drug. Baseline prolactin levels (average of levels at 8:45 and 9:00 AM) were not significantly different on the 2 study days ($F = 0.11$, df = 1,29, ns).

A repeated measures analysis of variance (ANOVA) was used to assess the effects of age and gender on fenfluramine-induced prolactin release. The analysis was performed both without and with an index of subjects' plasma drug levels as a covariate. Additional repeated measures ANOVA were performed to evaluate the effects of age and gender on (1) resting prolactin levels during the placebo trial, and (2) plasma drug levels.

The average of summed levels of fenfluramine and norfenfluramine at hr 3 and hr 5 was used as the index of subjects' plasma drug levels. As we have discussed elsewhere (McBride et al. 1989), this index is a close approximation of both an individual's peak drug level and the drug level between hr 3 and 5, the period of maximal prolactin secretion during the fenfluramine challenge test.

In order to derive a measure of each subject's fenfluramine-induced prolactin response for use in correlational analyses, net changes in plasma prolactin levels were quantified by calculating the area under the curve ($AUC_{\Delta PRL}$) using the trapezoid rule. The correlation between values for $AUC_{\Delta PRL}$ and the peak change in prolactin levels was 0.95 ($p < 0.001$).

Neither values for $AUC_{\Delta PRL}$ nor the index of plasma drug levels were significantly correlated with body weight ($r = -0.29$, df = 28, ns and $r = -0.25$, df = 28, ns, respectively).

The nonparametric rank method of Spearman was used to generate correlation coef-

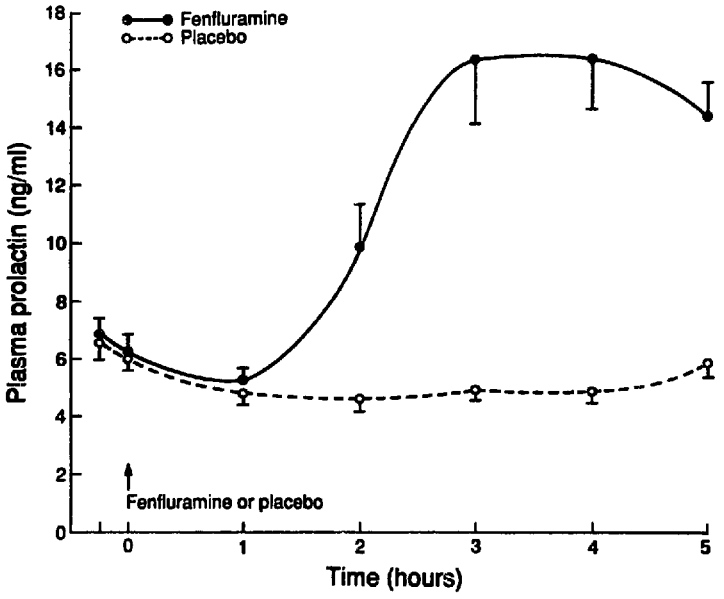

Figure 1. Plasma prolactin levels (mean ± SEM) in 30 normal adults following a 60-mg oral dose of fenfluramine (solid lines) and placebo (broken lines).

ficients for the relationships between age and fenfluramine-induced prolactin release because values were not normally distributed.

Standard deviations are reported as indices of group variability unless otherwise noted.

Results

Fenfluramine-Induced Prolactin Release

Figure 1 illustrates mean plasma prolactin levels following administration of a 60-mg oral dose of dl-fenfluramine hydrochloride or placebo to 30 healthy adults. Fenfluramine resulted in a significant increase in plasma prolactin levels (treatment × time interaction: $F = 24.38$, df = 5,145, $p < 0.001$) which peaked 3–4 hr after drug ingestion.

Effect of Age

Figure 2 depicts the relationship between age and the amount of prolactin released in excess of placebo levels during the fenfluramine challenge test, expressed as the area under the curve ($AUC_{\Delta PRL}$). Overall, net prolactin release declined with age (Spearman's rank correlation coefficient: $r_s = -0.59$, n = 30, $p < 0.001$). Correlation coefficients were similar in men ($r_s = -0.63$, n = 18, $p < 0.01$) and women ($r_s = -0.55$, n = 12, $p = 0.07$), although the correlation in women fell just short of statistical significance.

As shown in Figure 2, the age-related decline in fenfluramine-induced prolactin release was not linear, but rather largely complete by the end of the third decade of life. Values for $AUC_{\Delta PRL}$ were not correlated with age in either subjects <30 years ($r_s = 0.15$, n = 15, ns) or ≥30 years ($r_s = -0.07$, n = 15, ns). On the basis of these findings, subjects were divided into groups age <30 years and ≥30 years for all analyses employing repeated measures ANOVA.

A comparison of fenfluramine-induced changes in plasma prolactin levels in subjects <30 years versus ≥30 years by ANOVA confirmed that net prolactin release was sig-

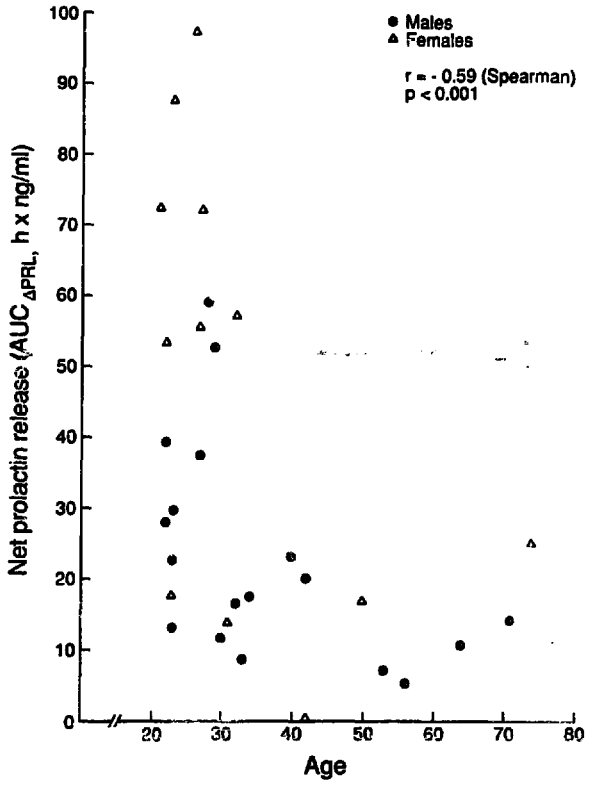

Figure 2. The relationship between age and net fenfluramine-induced prolactin release, quantified oy the area under the curve ($AUC_{\Delta PRL}$), in 30 normal adults.

nificantly decreased in the older age group (treatment × age interaction: F = 22.04, df = 1,26, $p < 0.001$). Figure 3 illustrates the time course of stimulated prolactin secretion in subjects <30 years versus ≥30 years stratified by gender as well as age. Net prolactin responses were approximately three times greater in younger compared with older subjects of the same gender (men: $AUC_{\Delta PRL}$ = 35.2 ± 15.3 versus 13.4 ± 6.3 hr × ng/ml, respectively, t = 3.78, df = 16, $p < 0.005$; women: $AUC_{\Delta PRC}$ = 65.6 ± 27.3 versus 21.1 ± 24.4 hr × ng/ml, respectively, t = 2.91, df = 10, $p < 0.02$).

Not only the magnitude, but the temporal pattern of fenfluramine-induced prolactin

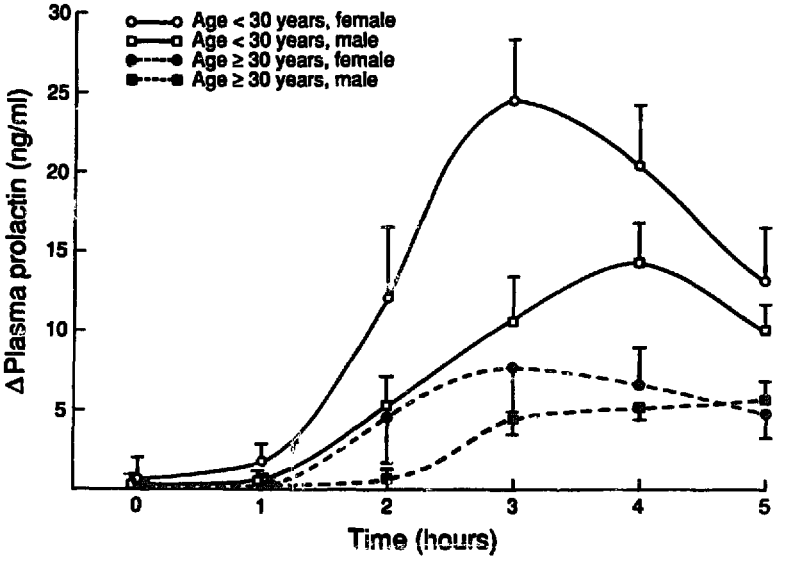

Figure 3. The time course of net fenfluramine-induced prolactin release (mean ± SEM) in 30 normal adults stratified by age and gender. The number of subjects in each group was as follows: women <30 years: 7; men <30 years: 8; women ≥30 years: 5; men ≥30 years: 10.

Table 1. Plasma Levels of Fenfluramine and Norfenfluramine (Mean ± SD) in Groups Stratified by Age and Gender

Fenfluramine (ng/ml)

	Hour 3		Hour 5	
	Age < 30 years	Age ≥ 30 years	Age < 30 years	Age ≥ 30 years
Men	46.7 ± 11.3 (n = 8)	48.0 ± 12.5 (n = 10)	47.6 ± 9.8 (n = 8)	44.0 ± 15.6 (n = 10)
Women	54.8 ± 13.4 (n = 7)	58.9 ± 7.2 (n = 5)	52.6 ± 11.2 (n = 7)	53.1 ± 11.6 (n = 5)

Norfenfluramine (ng/ml)

	Hour 3		Hour 5	
	Age < 30 years	Age ≥ 30 years	Age < 30 years	Age ≥ 30 years
Men	12.0 ± 3.7 (n = 8)	10.5 ± 3.1 (n = 10)	14.2 ± 3.5 (n = 8)	11.6 ± 4.6 (n = 10)
Women	12.2 ± 5.3 (n = 7)	13.1 ± 3.7 (n = 5)	12.8 ± 3.2 (n = 7)	15.9 ± 6.3 (n = 5)

Repeated measures ANOVA, effects of age and gender on overall plasma drug levels (fenfluramine plus norfenfluramine): effect of age: $F = 0.01$, df = 1,26, ns; effect of gender: $F = 3.76$, df = 1,26, $p = 0.07$; age × gender interaction: $F = 0.56$, df = 1, 26, ns.

release differed in subjects <30 years versus those ≥30 years (treatment × age × time interaction: $F = 6.73$, df = 5,130, $p < 0.001$). The initial increase in prolactin levels occurred up to 1 hr earlier in younger than in older individuals, and prolactin levels began to return toward baseline somewhat sooner in younger subjects (see Figure 3).

Table 1 lists mean plasma levels of fenfluramine and its active metabolite, norfenfluramine, at hr 3 and 5 following drug ingestion in subjects <30 years versus ≥30 years subgrouped by gender. Age was not associated with the total amount of drug (fenfluramine plus norfenfluramine) present in plasma (drug level × age interaction: $F = 0.01$, df = 1,26, ns); the rate of change in drug concentration across sampling points (drug level × age × time interaction: $F = 0.68$, df = 1,26, ns); or the ratio of norfenfluramine to fenfluramine (ratio × age interaction: $F = 0.02$, df = 1,26, ns). Thus, group differences in the magnitude and timing of fenfluramine-induced changes in plasma prolactin levels appear to reflect pharmacodynamic rather than pharmacokinetic effects of drug administration, although the fact that plasma drug levels are not available at all time points makes it impossible to eliminate the possibility that differences in fenfluramine absorption and metabolism contributed to the observed differences in the temporal pattern of responses.

Effect of Gender

Repeated measures ANOVA revealed significantly greater fenfluramine-induced prolactin release in women than in men (treatment × gender interaction: $F = 6.50$, df = 1,26, $p < 0.02$; see Figure 3). Net prolactin responses were one and a half to two times greater in women than in men belonging to the same age group, although the difference was statistically significant only in the younger group (subjects <30 years: $AUC_{\Delta PRC} = 65.6 \pm 27.3$ versus 35.2 ± 15.3 hr × ng/ml, respectively, $t = 2.71$, df = 13, $p < 0.05$;

subjects ≥30 years: $AUC_{\Delta PRC}$ = 21.1 ± 24.4 versus 13.4 ± 6.3 hr × ng/ml, respectively, t = 0.69, df = 13, ns).

A trend toward higher plasma drug levels was found in women compared with men (drug level × gender interaction: F = 3.76, df = 1,26, p = 0.07; see Table 1). When the average of summed levels of fenfluramine and norfenfluramine at the two sampling points was used as a covariate, the effect of gender on the magnitude of fenfluramine-induced prolactin release remained statistically significant (treatment × gender interaction: F = 4.47 df = 1,25, $p < 0.05$). Thus, the finding of more robust prolactin responses in women reflects a pharmacodynamic effect of drug administration, although an additional pharmacokinetic effect may also contribute.

The timing of fenfluramine-induced prolactin secretion varied as a function of gender as well as age (treatment × gender × time interaction: F = 3.20, df = 5,130, $p <$ 0.01). Prolactin levels increased more rapidly in women than in men, and declined earlier (see Figure 3). A pharmacokinetic contribution to this result cannot be ruled out, but men and women did not differ significantly in either the rate of change in drug concentration across sampling points (drug level × gender × time interaction: F = 0.29, df = 1,26, ns) or the ratio of norfenfluramine to fenfluramine (ratio × gender interaction: F = 3.17, df = 1,26, ns).

Interactions Between Age and Gender

Age and gender did not have a significant interactive effect on the magnitude or timing of fenfluramine-induced prolactin secretion (treatment × age × gender interaction: F = 2.53, df = 1,26, ns; treatment × age × gender × time interaction: F = 0.94, df = 5,130, ns). Thus, men and women appear to exhibit similar age-associated changes in prolactin responses to fenfluramine challenge.

Effects of Age, Gender, and Time on Resting Prolactin Levels

Repeated measures ANOVA indicated a significant effect of gender and a trend toward a significant effect of age on resting prolactin levels during the placebo trial (prolactin × gender interaction: F = 15.04, df = 1,26, $p < 0.001$; prolactin × age interaction: F = 3.74, df = 1,26, p = 0.07). Women exhibited higher resting prolactin levels than men, and younger subjects a trend toward higher levels than older subjects. Effects of age and gender were not interactive (prolactin × age × gender interaction: F = 0.29, df = 1,26, ns). Resting prolactin levels differed with time in the total pool of subjects (prolactin × time interaction: F = 8.24, df = 5,130, $p < 0.001$; see Figure 1). Younger subjects had a greater morning drop in resting prolactin levels than older subjects (prolactin × age × time interaction: F = 2.55, df = 5,130, $p < 0.05$). Significant interactions were not found between gender and time, or age, gender, and time (prolactin × gender × time interaction: F = 0.46, df = 5,130, ns; prolactin × age × gender × time interaction: F = 1.75, df = 5,130, ns).

Correlation Between the Average Resting Plasma Prolactin Level and Fenfluramine-Induced Release ($AUC_{\Delta PRL}$)

In the total pool of subjects, values for $AUC_{\Delta PRL}$ were positively correlated with the average resting plasma prolactin level on the placebo day (r = 0.41, df = 28, $p <$ 0.05). However, this correlation partly reflects the fact that women had both higher

resting and higher stimulated prolactin levels than men. When the genders were considered separately, values for $AUC_{\Delta PRL}$ were not significantly correlated with resting prolactin levels in either women ($r = 0.11$, df = 10, ns) or men ($r = 0.46$, df = 16, ns).

Discussion

The present study demonstrated a significant age-related decline in fenfluramine-induced prolactin release which appeared to be largely complete by approximately age 30 years. Furthermore, women exhibited greater prolactin responses to fenfluramine challenge than men. These differences represent pharmacodynamic effects of fenfluramine, although a pharmacokinetic effect may also contribute to enhanced prolactin responses in women compared with men. Age and gender did not have a significant interactive effect on the magnitude of fenfluramine-induced prolactin release.

The findings of the present study not only underscore the need to carefully control for age and gender in clinical studies employing neuroendocrine probes, but suggest potential age- and gender-associated differences in CNS serotonergic responsivity. However, fenfluramine-induced prolactin responses of differing magnitude may reflect variability in the secretory capacity of the pituitary lactotroph as well as specific effects of the drug on central serotonergic transmission. Thus, information concerning age- and gender-related changes in the function of both serotonergic neurons and the lactotroph must be considered before concluding that a difference in serotonergic responsivity is the most likely explanation for a difference in fenfluramine-induced prolactin release.

Data from humans and other mammalian species indicate that some markers of central serotonergic neuron function do vary with age and gender. Normal aging appears to be associated with a decline in the function of postsynaptic serotonergic neurons. Several studies have demonstrated a linear decrease in the number of postsynaptic serotonin$_2$ (5-HT_2) receptors in human frontal cortex or hippocampus between the third and ninth decades of life (Bennett et al. 1979; Allen et al. 1983; Marcusson et al. 1984b; Wong et al. 1984; Mann et al. 1985). The relationship between age and the density of postsynaptic serotonin$_1$ (5-HT_1) receptors in human frontal cortex is less clear; though two studies have described an age-related decline in receptor number (Allen et al. 1983; Marcusson et al. 1984a), two others found no association between receptor density and age (Bennett et al. 1979; Mann et al. 1985). Effects of age on 5-HT_1 receptor subtypes have yet to be assessed. Impaired postsynaptic neuron responsivity in senescence has been suggested by reports that exposure of dentate granule and hippocampal neurons to serotonin evoked less membrane hyperpolarization in preparations obtained from old versus young rats (Segal 1982; Baskys et al. 1987).

Indices for the following markers of presynaptic serotonergic neuron function appear to be relatively stable across the lifespan in humans: concentrations of serotonin and its metabolite 5-hydroxyindoleacetic acid throughout the brain, with the apparent exception of the medulla oblongata, where an age-related increase in serotonin has been described (Carlsson et al. 1980; Bucht et al. 1981; Wester et al. 1984); serotonin uptake (Allen et al. 1983); and monoamine oxidase A (MAO-A) activity (Carlsson et al. 1980; Mann and Stanley 1984). An age-related increase in cortical MAO-B activity has been reported (Fowler et al. 1980; Mann and Stanley 1984), but the functional significance of this finding remains unclear. Most serotonergic neurons in rat brain appear to contain

MAO-B (Levitt et al. 1982), but serotonin appears to be selectively metabolized by MAO-A rather than MAO-B except when present in high concentrations (Fowler and Tipton 1984). [^{3}H]-Imipramine binding in human frontal cortex has been variably reported as increased (Severson et al. 1985) or unchanged (Stanley et al. 1982; Allen et al. 1983) with advancing age, although the age ranges were limited in the studies that did not observe an age effect.

Little information is available concerning the effect of gender on measures of central serotonergic neuron function in humans. In one study, concentrations of serotonin and 5-hydroxyindoleacetic acid did not differ significantly between men and women in 15 of 16 brain regions (Bucht et al. 1981). Higher levels of 5-hydroxyindoleacetic acid, though, have been reported in the cerebrospinal fluid of women (Asberg et al. 1973; von Knorring et al. 1986). Studies in rodents have pointed to increased serotonin turnover in women versus men. Higher concentrations of serotonin, 5-hydroxyindoleacetic acid, and tryptophan have been found in the brains of female rats (Carlsson et al. 1985). Serotonergic neurons in female rat brain appear to have greater storage capacity for serotonin, greater activity of the enzymes tryptophan hydroxylase and 5-hydroxytryptophan decarboxylase, and a higher rate of serotonin synthesis (Carlsson et al. 1985). Evidence suggests that ovarian steroids may increase brain serotonin concentration and turnover (Munaro 1978), whereas androgens may inhibit serotonin-mediated responses (Fischette et al. 1984).

An evaluation of the effects of age and gender on lactotroph function requires basic knowledge of the processes that modulate prolactin secretion. Prolactin secretion is enhanced or attenuated by a number of neurotransmitters, neuropeptides, and hormones, but is primarily under tonic inhibitory control (Kato et al. 1985; Ben-Jonathan 1985). Dopamine, which interacts directly with dopamine$_2$ receptors on the lactotroph, appears to be the most physiologically important prolactin-inhibiting factor (Ben-Jonathan 1985). In contrast to dopamine, serotonin does not appear to exert a direct effect on the lactotroph (MacLeod and Login 1977; Kato et al. 1985; Ben-Jonathan 1985). Indirect evidence suggests that serotonin promotes prolactin release by stimulating secretion of a hypothalamic prolactin-releasing factor into the portal circulation (Meltzer et al. 1982; Kato et al. 1985). Estrogens have been shown to enhance prolactin synthesis, storage, and release, and to increase the number of lactotrophs (Ben-Jonathan 1985).

The prolactin response to thyrotropin-releasing hormone (TRH), a neuropeptide that directly stimulates prolactin secretion via a Ca^{2+}/protein kinase-C pathway (Martinez de la Escalera and Weiner 1988), has been widely used to evaluate lactotroph secretory capacity in healthy individuals as well as patient populations. The test is sensitive to differences in dopaminergic activity (Burrow et al. 1977; Carlson 1986), most likely because dopamine inhibits the lactotroph Ca^{2+}/protein kinase pathway (Martinez de la Escalera and Weiner 1988). A large-scale study of normal subjects has revealed that the prolactin response to TRH is stable in both men and women until at least the seventh decade (Jacobs et al. 1973), a finding that suggests that the capacity of the lactotroph to respond to provocative challenge remains intact through midlife. Women exhibit greater prolactin secretory capacity than men throughout the life-span. Maximal TRH-induced prolactin secretion in women is approximately twice that in men in the elderly (Jacques et al. 1987) as well as younger adults (Jacobs et al. 1973).

Not only studies of the prolactin response to TRH, but of the human nigrostriatal system suggest that central dopaminergic function is stable through midlife. Changes in measures of dopaminergic neuron function after the sixth decade include decreased levels of dopamine and the synthetic enzyme tyrosine hydroxylase in the basal ganglia, a loss

of dopaminergic neurons in the substantia nigra, and a decrease in striatal dopamine$_2$ receptor number (Morgan et al. 1987; Carlsson 1985). Of note, an age-related decline in tuberoinfundibular dopaminergic activity would be expected to result in enhanced rather than inhibited lactotroph secretory capacity. Little is known about gender differences in dopaminergic function, although animal studies suggest that chronic exposure to estrogens may damage dopaminergic neurons (Meites et al. 1987).

Interpreted in the light of known effects of age on serotonergic neurons and the lactotroph, our finding of reduced fenfluramine-induced prolactin release in adults ≥30 years versus <30 years of age is consistent with the hypothesis that CNS serotonergic responsivity declines during young adulthood. It is unlikely that the observed age-associated decrease in prolactin release is due to a decline in lactotroph secretory capacity, as the prolactin response to TRH is stable at least until age 60 years. The attenuated prolactin responses of older subjects, though, might conceivably be explained by an age-related reduction in the density of one or more postsynaptic serotonin receptor subtypes in the hypothalamus. This possibility is supported by the fact that 5-HT$_2$ receptor number begins to decline in other brain regions during the third decade. The fact that the age-associated decrease in fenfluramine-induced prolactin release appears to be complete at a substantially earlier age than age-associated decreases in the densities of cortical serotonin receptors might be explained by regional differences in the effect of age on receptor number; a superimposed reduction in the function of a receptor-linked signal transducing system(s); or the fact that existing studies of the effect of age on 5-HT$_1$ and 5-HT$_2$ receptor binding indices may not have detected early age-associated changes in the densities of serotonin receptor subtypes. Although most markers of presynaptic serotonergic neuron function do not appear to vary with age, a presynaptic contribution to the age-dependent decline in prolactin responses must still be considered. At this juncture, the behavioral and cognitive consequences of a decline in CNS serotonergic responsivity during early adulthood are unclear.

Although significantly greater fenfluramine-induced prolactin secretion was found in women than in men, the present study does not provide support for enhanced CNS serotonergic responsivity in women. Because women have roughly twofold greater prolactin responses to both fenfluramine and TRH, it is probable that the difference in fenfluramine-induced prolactin release between the sexes reflects gender-related differences in modulatory inputs at the level of the lactotroph. It is difficult to ascribe the greater prolactin secretory capacity of women solely to acute effects of estrogens. Though we did not document a statistically significant difference in fenfluramine-induced prolactin secretion in women versus men ≥30 years of age (this may reflect the relatively small sample size coupled with a large variance in female subjects), the gender difference in TRH-induced prolactin secretion is clearly maintained well after menopause, when estrogen levels are low. The possibility that women may have lesser tuberoinfundibular dopaminergic activity than men must be considered, particularly in light of evidence that estrogens may exert a toxic effect on dopaminergic neurons.

In summary, the findings of this study suggest an age-related decline in CNS serotonergic responsivity which is largely complete by the end of the third decade of life. A gender-associated difference in serotonergic responsivity was not supported by the model used. Future studies of the effect of age on serotonin-mediated neuroendocrine responses should include adolescents (and, if possible, prepubertal children) in order to determine whether changes in responsivity are first observed in adulthood, or begin during an earlier phase of development. Neuroendocrine challenge studies with direct serotonin receptor

agonists may point to age- or gender-dependent differences in the function of postsynaptic serotonin receptor subtypes in the hypothalamus. Additional human postmortem brain studies are needed to evaluate the effects of age and gender on the serotonergic system at the molecular and cellular levels, and to identify regional differences. Once pharmacologically specific radiotracers become available, in vivo studies utilizing positron emission tomography should prove useful in evaluating age- or gender-associated differences in serotonin receptor density throughout the brain.

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References

Allen SJ, Benton JS, Goodhardt MJ, et al (1983): Biochemical evidence of selective nerve cell changes in the normal ageing human and rat brain. *J Neurochem* 41:256–265.

Anderson GM (1987): Monoamines in autism: An update of neurochemical research on a pervasive developmental disorder. *Med Biol* 65:67–74.

Asberg M, Bertilsson L, Tuck D, Cronholm B, Sjoquist F (1973): Indoleamine metabolites in the cerebrospinal fluid of depressed patients before and during treatment with nortriptyline. *Clin Pharmacol Ther* 14:277–286.

Asnis G, Eisenberg J, van Praag HM, Lemus CZ, Harkavy Friedman JM, Miller AH (1988): The neuroendocrine response to fenfluramine in depressives and normal controls. *Biol Psychiatry* 24:117–120.

Baskys A, Niesen CE, Carlen PL (1987): Altered modulatory actions of serotonin on dentate granule cells of aged rats. *Brain Res* 419:112–118.

Ben-Jonathan N (1985): Dopamine: A prolactin-inhibiting hormone. *Endocrinol Rev* 6:564–589.

Bennett JP, Enna SJ, Bylund DB, Gillin JC, Wyatt RJ, Snyder SH (1979): Neurotransmitter receptors in frontal cortex of schizophrenics. *Arch Gen Psychiatry* 36:927–934.

Borroni E, Ceci A, Garattini S, Mennini T (1983): Differences between D-fenfluramine and D-norfenfluramine in serotonin presynaptic mechanisms. *J Neurochem* 40:891–893.

Bucht G, Adolfsson R, Gottfries CG, Roos B-E, Winblad B (1981): Distribution of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in human brain in relation to age, drug influence, agonal status and circadian variation. *J Neural Transm* 51:185–203.

Burrow GN, May PB, Spaulding SW, Donabedian RK (1977): TRH and dopamine interactions affecting pituitary hormone secretion. *J Clin Endocrinol Metab* 45:65–72.

Carlson HE (1986): Carbidopa plus L-dopa pretreatment inhibits the prolactin (PRL) response to thyrotropin-releasing hormone and thus cannot distinguish central from pituitary sites of prolactin stimulation. *J Clin Endocrinol Metab* 63:249–251.

Carlsson A (1985): Neurotransmitter changes in the aging brain. *Dan Med Bull* 32(Suppl):40–43.

Carlsson A, Adolfsson R, Aquilonius S-M, et al (1980): Biogenic amines in human brain in normal aging, senile dementia, and chronic alcoholism. In Goldstein M et al. (eds), *Ergot Compounds and Brain Function: Neuroendocrine and Neuropsychiatric Aspects*. New York: Raven Press, pp 295–304.

Carlsson M, Svensson K, Eriksson E, Carlsson A (1985): Rat brain serotonin: Biochemical and functional evidence for a sex difference. *J Neural Transm* 63:297–313.

Charney DS, Goodman WK, Price LH, Woods SW, Rasmussen SA, Heninger GR (1988): Serotonin function in obsessive-compulsive disorder: A comparison of the effects of tryptophan and *m*-chlorophenylpiperazine in patients and healthy subjects. *Arch Gen Psychiatry* 45:177–185.

Coccaro EF, Siever LJ, Klar HM, et al (1989): Serotonergic studies in patients with affective and personality disorders: Correlates with suicidal and impulsive aggressive behavior. *Arch Gen Psychiatry* 46:587–599.

Fischette CT, Biegon A, McEwen BS (1984): Sex steroid modulation of the serotonin behavioral syndrome. *Life Sci* 35:1997–2006.

Fowler CJ, Tipton KF (1984): On the substrate specificities of two forms of monoamine oxidase. *J Pharm Pharmacol* 36:111–115.

Fowler CJ, Wiberg A, Oreland L, Marcusson J, Winblad B (1980): The effect of age on the activity and molecular properties of human brain monoamine oxidase. *J Neural Transm* 49:1–20.

Fuller RW, Snoddy HD, Clemens JA, Molloy BB (1982): Effect of norfenfluramine and two structural analogues on brain 5-hydroxyindoles and serum prolactin in rats. *J Pharm Pharmacol* 34:449–450.

Heninger GR, Charney DS, Sternberg DE (1984): Serotonergic function in depression: Prolactin response to intravenous tryptophan in depressed patients and healthy subjects. *Arch Gen Psychiatry* 41:398–402.

Insel TR, Mueller EA, Alterman I, Linnoila M, Murphy DL (1985): Obsessive-compulsive disorder and serotonin: Is there a connection? *Biol Psychiatry* 20:1174–1188.

Jacobs LS, Snyder PJ, Utiger RD, Daughaday WH (1973): Prolactin response to thyrotropin-releasing hormone in normal subjects. *J Clin Endocrinol Metab* 36:1069–1073.

Jacques C, Schlienger JL, Kissel C, Kuntzmann C, Sapin R (1987): TRH-induced TSH and prolactin responses in the elderly. *Age Ageing* 16:181–188.

Kato Y, Matsushita N, Ohta H, Tojo H, Shimatsu A, Imura H (1985): Regulation of prolactin secretion. In Imura H (ed), *The Pituitary Gland*. New York: Raven Press, pp 261–278.

Krebs HA, Cheng LK, Wright GJ (1984): Determination of fenfluramine and norfenfluramine in plasma using a nitrogen-sensitive detector. *J Chromatogr* 310:412–417.

Levitt P, Pintar JE, Breakefield XO (1982): Immunocytochemical demonstration of monoamine oxidase B in brain astrocytes and serotonergic neurons. *Proc Natl Acad Sci USA* 79:6385–6389.

MacLeod RM, Login IS (1977): Regulation of prolactin secretion through dopamine, serotonin and the cerebrospinal fluid. *Adv Biochem Psychopharmocol* 16:147–157.

Mann JJ, Petito C, Stanley M, McBride PA, Chin J, Philogene A (1985): Amine receptor binding and monoamine oxidase activity in postmortem human brain tissue: Effect of age, gender, and postmortem delay. In Burrows GD, Norman TR, Dennerstein L (eds), *Clinical and Pharmacologic Studies in Psychiatric Disorders*. London: John Libbey & Co, pp 37–39.

Mann JJ, Stanley M (1984): Postmortem monoamine oxidase enzyme kinetics in the frontal cortex of suicide victims and controls. *Acta Psychiatr Scand* 69:135–139.

Mann JJ, Stanley M, McBride PA, McEwen BS (1986): Increased serotonin and B-adrenergic receptor binding in the frontal cortices of suicide victims. *Arch Gen Psychiatry* 43:954–959.

Marcusson JO, Oreland L, Winblad B (1984a): Effect of age on human brain serotonin (S-1) binding sites. *J Neurochem* 43:1699–1705.

Marcusson JO, Morgan DG, Winblad B, Finch GE (1984b): Serotonin-2 binding sites in human frontal cortex and hippocampus: Selective loss of S-2A sites with age. *Brain Res* 311:51–56.

Martinez de la Escalera G, Weiner RI (1983): Mechanism(s) by which the transient removal of dopamine regulation potentiates the prolactin-releasing action of thyrotropin-releasing hormone. *Neuroendocrinology* 47:186–193.

McBride PA, Anderson GM, Hertzig ME, et al (1989): Serotonergic responsivity in male young adults with autistic disorder: Results of a pilot study. *Arch Gen Psychiatry* 46:213–221.

McBride PA, Kream J, Anderson G, Mann JJ (1986): Effect of age upon measures of central and

peripheral serotonergic function in human subjects (Abstract). Society for Neuroscience Annual Meeting, p 1313.

Meites J, Goya R, Takahashi S (1987): Why the neuroendocrine system is important in aging processes. *Exp Gerentol* 22:1–15.

Meltzer HY, Wiita B, Tricou BJ, Simonovic M, Fang V, Manov G (1982): Effect of serotonin precursors and serotonin agonists on plasma hormone levels. In Ho BT, Schoolar JC, Usdin E (eds), *Serotonin in Biological Psychiatry*. New York: Raven Press, pp 117–139.

Morgan DG, May PC, Finch CE (1987): Dopamine and serotonin systems in human and rodent brain: Effects of age and neurodegenerative disease. *J Am Geriatr Soc* 35:334–345.

Mueller EA, Murphy DL, Sunderland T (1985): Neuroendocrine effects of *M*-chlorophenylpiperazine, a serotonin agonist, in humans. *J Clin Endocrinol Metab* 61:1179–1184.

Munaro NI (1978): The effect of ovarian steroids on hypothalamic 5-hydroxytryptamine neuronal activity. *Neuroendocrinology* 26:270–276.

Quattrone A, Di Renzo GF, Schettini G, Tedeschi G, Scopacasa F (1978): Increased plasma prolactin levels induced in rats by *d*-fenfluramine: Relation to central serotonergic stimulation. *Eur J Pharmacol* 49:163–167.

Quattrone A, Tedeschi G, Aguglia U, Scopacasa F, Di Renzo GF, Annunziato L (1983): Prolactin secretion in man, a useful tool to evaluate the activity of drugs on central 5-hydroxytryptaminergic neurons: Studies with fenfluramine. *Br J Pharmacol* 16:471–475.

Segal M (1982): Changes in neurotransmitter actions in the aged rat hippocampus. *Neurobiol Aging* 3:121–124.

Severson JA, Marcusson JO, Osterburg HH, Finch CE, Winblad B (1985): Elevated density of [^{3}H]imipramine binding in aged human brain. *J Neurochem* 45:1382–1389.

Stanley M, Virgilio J, Gershon S (1982): Tritiated imipramine binding sites are decreased in the frontal cortex of suicides. *Science* 216:1337–1339.

van Praag HM (1986): Indoleamines in depression and suicide. *Prog Brain Res* 65:59–71.

von Knorring L, Oreland L, Haggendal J, Magnusson T, Almay B, Johansson F (1986): Relationship between platelet MAO activity and concentrations of 5-HIAA and HVA in cerebrospinal fluid in chronic pain patients. *J Neural Transm* 66:37–46.

Wester P, Hardy JA, Marcusson J, et al (1984): Serotonin concentrations in normal aging human brains: Relation to serotonin receptors. *Neurobiol Aging* 5:199–203.

Wong DF, Wagner HN, Dannals RF, et al (1984): Effects of age on dopamine and serotonin receptors measured by positron tomography in the living human brain. *Science* 226:1393–1396.