Objective: This study was undertaken to measure serotonergic modulation of dopamine in vivo by using positron emission tomography (PET), a radiotracer for the striatal dopamine D_2 receptor ([^{11}C]raclopride), and a pharmacologic challenge of the serotonin system (d,l-fenfluramine).

Method: Two PET studies using [^{11}C]raclopride were performed in 11 normal male subjects before administration of the serotonin-releasing agent and reuptake inhibitor fenfluramine (60 mg p.o.) and 3 hours afterward. A graphical analysis method was used with the [^{11}C]raclopride data to derive the distribution volume of D_2 receptors. Plasma levels of fenfluramine, norfenfluramine, homovanillic acid (HVA), cortisol, and prolactin were determined.

Results: Levels of fenfluramine and prolactin were elevated 2 hours after fenfluramine administration and remained significantly elevated during the second scan, while levels of HVA and cortisol were not altered significantly during the time of scanning. A significant decrease in the specific binding (striatum) and the nonspecific binding subtracted from the specific binding (striatum minus cerebellum) of [^{11}C]raclopride was observed. The rate of metabolism of [^{11}C]raclopride and the nonspecific binding (cerebellum) were not significantly altered by the fenfluramine intervention.

Conclusions: The observed decrease in [^{11}C]raclopride binding is consistent with an increase in dopamine concentrations and with the ability of serotonin to stimulate dopamine activity. The ability to measure serotonergic modulation of dopamine in vivo may have implications for the study of etiologic and therapeutic mechanisms in schizophrenia, major depressive disorder, obsessive-compulsive disorder, and substance abuse.
attributable to differences in the binding affinities of the two radiotracers for the D₂ receptor, and therefore the sensitivity of ligand binding to alterations in endogenous dopamine concentrations would differ as well (5). This observation led to a series of PET and SPECT studies which demonstrated that radiotracer binding could be altered by pharmacologically manipulating dopamine concentrations (6–9). The research strategy developed from these initial studies represents the most direct, noninvasive method available to measure neurotransmitter concentrations in the living brain. In the case of schizophrenia, the potential importance of studying dopamine concentrations rather than receptor density is supported by the evidence that 1) schizophrenic patients do not consistently show alterations in dopamine receptor numbers in comparison with normal control subjects (1–3); 2) the degree of striatal D₂ receptor occupancy by antipsychotic medications is not indicative of therapeutic response to antipsychotic treatment (10, 11); and 3) the time course of occupancy of the striatal D₂ receptor is much more rapid (several hours) than the time course of clinical response (several weeks) (12, 13). Therefore, in vivo ligand-binding measurements of the striatal D₂ receptor in isolation suggest that symptoms and response to treatment may be related to other factors, such as the integrity of extrastriatal dopamine systems or the ability of other functionally linked neurotransmitter systems to modulate dopamine function or to be modulated by dopamine. These lines of evidence supported the development of a method using PET to study dynamic aspects of dopaminergic function and to evaluate other neurotransmitters that modulate dopamine activity.

Using ligands with different affinities for the D₂ receptor and pharmacologic agents that directly altered dopamine concentrations by different mechanisms of action, initial studies demonstrated the sensitivity of PET for detecting alterations in radiotracer binding secondary to pharmacologic alteration of dopamine concentrations (6–8). Subsequent studies in the adult baboon and in human subjects demonstrated that PET could be used to measure the modulation of dopamine by the primary neurotransmitters known to modulate its activity (e.g., γ-aminobutyric acid, acetylcholine, serotonin) (14–16). These results were remarkably consistent with the basic neuroanatomic and neurophysiologic data concerning the modulation of dopamine in the rat and nonhuman primate brain (17–19).

The application of this PET approach to neuropsychiatric disease states will permit us to evaluate the hypothesis that neuropsychiatric symptoms are a function of the inability of one neurotransmitter system to modulate the activity of another functionally linked system. Specifically, the intent of the present study was to develop a method to measure serotonin modulation of dopamine concentrations in normal subjects. The experimental paradigm involved performing a baseline scan with the selective D₂ radiotracer [¹¹C]raclopride, administering the serotonin reuptake inhibitor and releasing agent fenfluramine, and then repeating the [¹¹C]raclopride study 3 hours later, when plasma fenfluramine concentrations were at the highest levels and an increase in prolactin release was observed. In regard to the selection of a pharmacologic challenge agent, fenfluramine was chosen from among the other serotonin agonists because of its relatively good selectivity for the serotonin system (in comparison with other catecholamines), its relatively good absorption after a single dose, and its safety and tolerability (20). The interaction between serotonin and dopamine systems has been hypothesized to underlie symptoms and response to treatment in several neuropsychiatric disorders, including schizophrenia, obsessive-compulsive disorder, affective disorders, and substance abuse. The results of the present study have been presented in preliminary form (21).

**METHOD**

Potential subjects underwent medical evaluation (physical and laboratory testing, toxicology screening, physical examination), psychiatric evaluation, and magnetic resonance imaging scans with a Siemens 1.5-T magnetom Vision scanner. They were excluded from the study on the basis of past or current substantial medical, psychiatric, or neurologic disease; substance abuse; a family history (first- or second-degree relatives) of psychiatric illness or substance abuse; or use of prescription or over-the-counter medications with CNS effects (e.g., antihistamines, cold medications) within the past month. Eleven right-handed male subjects were included; their mean age was 26.45 years (SD = 4.11, range = 22–35). After a complete description of the study to the subjects, written informed consent was obtained. The subjects fasted after midnight and were given a standard breakfast 3 hours before scanning.

For each subject, two [¹¹C]raclopride studies were performed on the same day. After a baseline [¹¹C]raclopride scan, d,l-fenfluramine (60 mg p.o.) was administered, and a second [¹¹C]raclopride scan was performed 3 hours after the fenfluramine was given. The timing of the second scan was chosen to coincide with the maximal plasma level of fenfluramine, the maximal effect of fenfluramine on prolactin levels, and effects on glucose metabolic rates, as previously reported in normal subjects (20, 22, 23).

The [¹¹C]raclopride studies were performed on a Computer Technology Inc. 931-08/12 tomograph as described previously (7). [¹¹C]Raclopride was synthesized by the reaction of 11C-labeled methyl iodide with norraclopride (24). Prior to scanning, catheters were placed in an antecubital vein for radiotracer injection and in a radial artery for blood sampling. [¹¹C]Raclopride was injected intravenously (4.01–13.67 mCi, specific activity = 0.5–1.5 Ci/μmol) and scanning began immediately. Subjects were scanned in a quiet, dimly lit room, with eyes open and ears unoccluded. Each [¹¹C]raclopride scan was performed for 60 minutes, and the 4-hour interval between radiotracer injections was sufficient to allow for decay of the radiotracer. The scanning protocol involved 10 1-minute scans followed by 10 5-minute scans. Continuous blood samples were obtained by an automated device (Olu Dich, Denmark) for the first 2 minutes after injection, at the peak of radioactivity in blood; for the remainder of the study, manual sampling was used. Selected plasma samples (at 1, 5, 30, and 60 minutes) were analyzed for the presence of unchanged [¹¹C]raclopride, as described previously (25).

Plasma samples were obtained for analyses of levels of fenfluramine, its metabolite norfenfluramine, the dopamine metabolite homovanillic acid (HVA), prolactin, and cortisol. The analytical methods and their coefficients of variation have been described previously (22). Plasma samples were obtained prior to and at the end of the first [¹¹C]raclopride injection; 1, 2, and 3 hours after fenfluramine administration (before the second [¹¹C]raclopride injection); and 4 hours after fenfluramine (at the end of the second [¹¹C]raclopride scan). The
TABLE 1. Plasma Fenfluramine and Norfenfluramine Levels of 11 Normal Subjects in a PET Study With [11C]Raclopride

<table>
<thead>
<tr>
<th>Time Since Fenfluramine Administration</th>
<th>Fenfluramine (ng/ml)</th>
<th>Norfenfluramine (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>1 hour</td>
<td>24.7</td>
<td>28.4</td>
</tr>
<tr>
<td>2 hours</td>
<td>68.7a</td>
<td>20.9</td>
</tr>
<tr>
<td>3 hours (before scan)</td>
<td>70.1a</td>
<td>12.4</td>
</tr>
<tr>
<td>4 hours (after scan)</td>
<td>62.6b</td>
<td>13.5</td>
</tr>
</tbody>
</table>

aSignificantly different from baseline (p<0.001).
bSignificantly different from baseline (p<0.05).

TABLE 2. Plasma Homovanillic Acid (HVA), Prolactin, and Cortisol Levels of 11 Normal Subjects in a PET Study With [11C]Raclopride

<table>
<thead>
<tr>
<th>Time</th>
<th>HVA (ng/ml)</th>
<th>Prolactin (ng/ml)</th>
<th>Cortisol (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before scan 1 (baseline: 65 minutes before fenfluramine)</td>
<td>6.8 1.6</td>
<td>4.5 0.9</td>
<td>8.6 5.0</td>
</tr>
<tr>
<td>After scan 1 (5 minutes before fenfluramine)</td>
<td>7.1 1.7</td>
<td>5.1a 1.1</td>
<td>10.3a 3.8</td>
</tr>
<tr>
<td>1 hour after fenfluramine</td>
<td>7.0 2.0</td>
<td>4.9 1.7</td>
<td>6.5a 1.8</td>
</tr>
<tr>
<td>2 hours after fenfluramine</td>
<td>7.6 2.0</td>
<td>6.5 2.4</td>
<td>11.0 3.3</td>
</tr>
<tr>
<td>Before scan 2 (3 hours after fenfluramine)</td>
<td>8.2 2.8</td>
<td>7.4a 3.4</td>
<td>9.9 2.9</td>
</tr>
<tr>
<td>After scan 2 (4 hours after fenfluramine)</td>
<td>8.1 2.9</td>
<td>9.3b 2.0</td>
<td>9.9 3.5</td>
</tr>
</tbody>
</table>

aSignificantly different from baseline (p<0.05).
bSignificantly different from baseline (p<0.001).

correction, i.e., by dividing the probability level p<0.05 by the number of post hoc ANOVAs (N=23), which resulted in the use of p=0.002 as the corrected level of significance for the post hoc tests.

RESULTS

The results of the plasma analyses for levels of fenfluramine and norfenfluramine are shown in table 1, and the results of the plasma analyses for levels of HVA, prolactin, and cortisol are shown in table 2. Significant overall effects of time were obtained for the plasma levels of fenfluramine (F=25.26, df=3, 40, p<0.001), norfenfluramine (F=103.9, df=3, 40, p<0.001), HVA (F=3.76, df=5, 40, p<0.05), prolactin (F=6.70, df=5, 40, p<0.001), and cortisol (F=6.90, df=5, 40, p<0.001).

Post hoc ANOVAs revealed a significant increase in fenfluramine levels from 1 to 4 hours after administration of the drug (table 1) (from 1 to 2 hours: F=39.98, df=1, 10, p<0.001; from 1 to 3 hours: F=25.11, df=1, 10, p<0.001; from 1 to 4 hours: F=12.95, df=1, 10, p<0.05) and a slight decline in levels from 3 to 4 hours (beginning and end of the second scan; F=6.58, df=1, 10, p<0.05). Post hoc ANOVAs revealed a similar pattern of change for norfenfluramine (table 1): an increase from 1 to 4 hours after administration (from 1 to 2 hours: F=152.2, df=1, 10, p<0.001; from 1 hour to 3 hours: F=162.1, df=1, 10, p<0.001; from 1 hour to 4 hours: F=87.2, df=1, 10, p<0.001) and no significant change during the time of the second scan (F=1.16, df=1, 10, p=0.30). It is important to note that the norfenfluramine levels represented a small fraction (less than one-third) of the fenfluramine levels.

Cortisol levels were elevated from baseline levels at the end of the first scan (F=8.34, df=1, 10, p<0.05), were decreased 1 hour after fenfluramine administration (F=10.98, df=1, 10, p<0.05), and were significantly elevated 2 hours compared to 1 hour after fenfluramine administration (F=28.0, df=1, 10, p<0.001) (table 2). These increases in cortisol occurred after the morning cortisol peak and may represent a response to fenfluramine challenge. Prolactin levels were elevated after the first scan (F=6.02, df=1, 10, p<0.05) and from 3 to 4 hours after fenfluramine administration, including the interval of the second scan (from baseline to 3 hours: F=7.62, df=1, 10, p<0.05; from baseline to 4 hours: F=83.4, df=1, 10, p<0.001). None of the subjects reported any behavioral change after fenfluramine administration, including dizziness, fatigue, nausea, appetite suppression, change in mood or anxiety level, or difficulties in concentration or cognitive function.

Table 3 shows the percentage of unchanged [11C]raclopride in plasma 1, 10, 30, and 60 minutes after injection. While the main effect of time was significant...
(F=52.9, df=3, 21, p<0.001), the main effect of study condition (scan 1 or scan 2) was not significant (F=5.64, df=1, 10, p<0.05), nor was the interaction between time and condition (F=2.70, df=3, 21, p=0.10). The relevant comparisons between the two conditions at the same time point revealed differences at 30 and 60 minutes that did not exceed the probability value corrected for the number of post hoc tests, p<0.002 (F=6.18, df=3, 21, p=0.03, and F=5.54, df=3, 21, p=0.04, respectively). This indicates that the rate of metabolism of [11C]raclopride was slightly slower (3%–4%) after fenfluramine administration.

The distribution volume for the striatum minus that for the cerebellum of the individual subjects is shown in table 4 and figure 1. It is important to note that all of the subjects demonstrated a decrease in striatal distribution volume and striatal-minus-cerebellar distribution volume. After fenfluramine treatment the distribution volume for the striatum (baseline mean=1.45, SD=0.20; postchallenge mean=1.26, SD=0.1) was significantly decreased (F=36.53, df=1, 10, p<0.001), as was the distribution volume of the striatum minus that of the cerebellum (baseline mean=1.04, SD=0.19; postchallenge mean=0.85, SD=0.11; F=41.51, df=1, 10, p<0.001). The measures of K1 (the parameter representing ligand delivery that is sensitive to changes in blood flow) in the striatum were not significantly altered (baseline mean=0.08, SD=0.01; postchallenge mean=0.08, SD=0.01; F=2.74, df=1, 10, p<0.10), nor were those in the cerebellum (baseline mean=0.09, SD=0.05; postchallenge mean=0.07, SD=0.02; F=0.69, df=1, 10, p>0.40), and the distribution volume for the cerebellum (baseline mean=0.41, SD=0.50; postchallenge mean=0.41, SD=0.50) was not significantly altered (F=0.50, df=1, 10, p>0.50).

Finally, correlations between the receptor availability measures and the plasma measures were performed. No significant correlations were obtained between the baseline or posttreatment [11C]raclopride values or magnitude of change in [11C]raclopride binding and any of the plasma measurements, including drug and metabolite, HVA, prolactin, and cortisol levels and drug dosage (corrected for body weight).

**DISCUSSION**

The results of this study demonstrated that in normal human subjects, a fenfluramine-induced increase in serotonin concentrations resulted in a decrease in striatal [11C]raclopride binding, consistent with an increase in dopamine concentrations. The magnitude of decrease in [11C]raclopride binding was greater than the test-retest variability for [11C]raclopride determined on the same day (in the baboon) or on different days (in the human being) (14, 25). At the time of the increase in dopamine, an increase in plasma fenfluramine and norfenfluramine levels and prolactin concentrations was observed. It has been hypothesized that the ability of fenfluramine to release prolactin is indicative of an interaction between serotonin and dopamine (31). However, serotonin may also stimulate prolactin release directly (32). The magnitude of change in prolactin release was not correlated with the change in [11C]raclopride binding. This may be due to a differential time course of the effects of fenfluramine on dopamine compared with prolactin release. Both the lack of an effect of fenfluramine on plasma HVA and the earlier effect of fenfluramine on cortisol levels relative to prolactin levels are consistent with the findings of previous studies (33, 34).

There is considerable neuroanatomic evidence for serotonin-dopamine interactions in both the nigrostriatal and mesocorticolimbic dopamine systems (19, 35–39). Serotonergic innervation of the nigrostriatal pathway is derived from the dorsal raphe nuclei (36, 38), and there is anatomic evidence for serotonergic modulation of dopamine in the ventral tegmental area (37, 39). In addition, neurons of the dorsal raphe project to the nucleus accumbens and medial prefrontal cortex, and both areas receive dopaminergic input from the ventral tegmental area (39). If a suitable radiotracer for measuring dopamine receptor availability in the target areas of the mesolimbic dopamine projections (prefrontal cortex, cingulate gyrus) is developed, it may be possible...
to measure serotonergic modulation of dopamine in these areas directly.

Several issues should be considered in the interpretation of the present results. To address the potential problems related to oral administration of d,l-fenfluramine, plasma levels of fenfluramine and its metabolite norfenfluramine were measured to determine whether the plasma levels of fenfluramine and norfenfluramine may have a direct effect on dopamine concentrations or on D2 binding, which would confound data interpretation (40). The affinities of fenfluramine for binding to the D2 receptor and dopamine transporter are relatively high (Ki > 10,000 nM and Ki > 1,100 nM, respectively) (41, 42). Thus, it is not likely that blockade of the D2 receptor or dopamine transporter contributed substantially to the results obtained.

It is important to note that these results obtained in human subjects are in the opposite direction to the results obtained previously in PET studies in the anesthetized baboon and microdialysis studies in freely moving rats (16). The discrepancy between baboon and human results is most likely due to a difference between the experimental conditions. The different route of administration and the onset and time course of effects produced by the different pharmacologic agents used across studies may explain the discrepancy. In the baboon PET studies, administration of the serotonin 5-HT2A antagonist altanserin resulted in a decrease in [11C]raclopride binding, while administration of the selective serotonin reuptake inhibitor citalopram resulted in an increase in [11C]raclopride binding. The microdialysis results in freely moving rats reported in those studies were consistent with the baboon PET findings. However, the majority of other in vivo microdialysis studies (in anesthetized and freely moving rats) have demonstrated that serotonin agonists (including fenfluramine) stimulate dopamine release (43–46), consistent with the results of the present study. Consistent with the present results, a recent study that measured the effect of fenfluramine on [11C]raclopride binding in the anesthetized baboon demonstrated a 33% reduction in specific binding (Mathis et al., unpublished data). Furthermore, a recently published study (47) demonstrated that acute administration of citalopram decreased [11C]raclopride binding in human subjects, consistent with the fenfluramine results; however, the effect was much smaller (8% decrease) than the fenfluramine effect (consistent with the greater release of serotonin produced by fenfluramine compared with citalopram shown by in vivo microdialysis), and neuroendocrine levels were not measured.

We cannot rule out the possibility that physiologic processes such as receptor internalization, which may influence the apparent maximum number of binding sites, may occur during the time frame of the experiment and influence the results (48, 49).

There is a concern that the decrease in [11C]raclopride binding may be attributed to a regionally specific decrease in cerebral blood flow (CBF) in the striatum compared with the cerebellum that is produced by fenfluramine administration, which would selectively alter the delivery of the [11C]raclopride to the striatum. This issue has been addressed in several ways. First, changes in CBF would not confound the interpretation of the individual distribution volumes, since the distribution volume as derived is independent of flow. The flow terms appear in the numerator and denominator and thus cancel out. The validity of the assumptions of the distribution volume analysis have been addressed experimentally in the analysis of two reversible tracers ([11C]flumazenil and [11C]raclopride) for two different neurotransmitter systems, and the assumption that flow contributes to the distribution volume is not supported (28, 50). Nonetheless, the kinetic parameter most affected by changes in CBF is K1. As presented in the Results section, the K1 values for [11C] raclopride binding in the striatum and cerebellum were not significantly altered by fenfluramine administration, and the cerebellar distribution volume was not significantly altered. Finally, recent studies have measured the effects of fenfluramine on regional CBF with the use of PET and [15O]H2O, and the results indicate that the striatum and cerebellum are affected proportionately (Smith et al., manuscript in preparation). The regionally selective effects of fenfluramine on CBF have been observed in the frontal cortex and cingulate gyrus (51, 52), areas not imaged in the present study. Thus, the observed decrease in [11C]raclopride binding is probably not attributable to change in regional ligand delivery due to a
regionally selective effect of fenfluramine on CBF in the striatum or cerebellum. The study of serotonergic modulation of dopamine function has implications for etiologic and treatment mechanisms in several neuropsychiatric disease states, including schizophrenia, affective disorders, obsessive-compulsive disorder, and substance abuse (e.g., cocaine dependence). It has been hypothesized that an imbalance between serotonin and dopamine systems occurs in these disease states, in part on the basis of the greater therapeutic efficacy of treatments that alter both systems rather than each system individually (53–58). The importance of studying interactions between neurotransmitter systems has been evaluated by previous studies that have compared multiple neurotransmitter metabolites and levels in CSF and plasma (59, 60).

The hypothesis which is the basis for the application of this experimental paradigm with PET to neuropsychiatric disease is that the inability of a neurotransmitter system to influence another functionally linked system, which may be etiologically relevant, may underlie symptoms. Restoration of this modulatory capacity may represent a more efficacious approach to treatment. In the study of neuropsychiatric disease states, measuring the response to pharmacologic challenge may be more revealing than studying the static properties of these systems (numbers of receptors). The use of the experimental strategy developed for normal subjects to evaluate neurotransmitter abnormalities in neuropsychiatric disorders will enable us to test an alternative hypothesis, namely, that serotonergic modulation of dopamine is altered, rather than to study the dopamine system in isolation.

REFERENCES


