Imaging Serotonergic Neurotransmission in Depression: Hippocampal Pathophysiology May Mirror Global Brain Alterations

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The recent development of [carbonyl-11C]WAY-100635 for serotonin (5-HT)1A and [18F]setoperone and [18F]altanserin for 5-HT2A positron emission tomography receptor imaging has allowed studies of 5-HT neurotransmission in depressive disorders. The hippocampus is likely to be an important brain structure in the pathophysiology of depression because it may mediate both cognitive deficits and hypercortisolemia found in this disorder. Decreased 5-HT1A binding was reported in the medial temporal cortex, which receives dense 5-HT innervation, and also throughout neocortical regions. Because the 5-HT1A antagonist pindolol may hasten antidepressant effects of selective serotonin reuptake inhibitor medications, its receptor occupancy has been measured in both presynaptic and postsynaptic sites. The results are controversial but suggest that pindolol has preferential occupancy of somatodendritic autoreceptors in the raphe. The results of 5-HT2A receptors are mixed, with one showing a significant decrease in the right orbitoinsular cortex and three not detecting a significant change. The disparate findings in patients with depression almost certainly reflect the heterogeneity of the disorder, and we highlight the utility of the hippocampus as a useful target region not only to compare depressed subjects with healthy subjects but also to correlate findings with cognitive function and activity of the limbic–hypothalamic–pituitary axis system.

Introduction

Abnormalities in several neurotransmission systems, including dopamine, serotonin (5-hydroxytryptamine; 5-HT), norepinephrine, acetylcholine, and substance P, may be relevant to the pathophysiology of depression. The 5-HT system has been the most extensively studied, in part because of the therapeutic efficacy of selective serotonin reuptake inhibitor (SSRI) medications. The hypothesis that depression is caused by low 5-HT transmission was derived from the findings of largely peripheral measures, such as low plasma L-tryptophan, reduced 5-HT content and serotonin transporters in blood platelets, and low CSF levels of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA; reviewed in Maes and Meltzer 1995). These results have been replicated with sufficient consistency to justify direct measurements in brain tissue. With the development of tracers for 5-HT receptors and transporters, several postmortem studies have been performed in brain tissue from suicide victims, a portion of which had depressive disorders. As reviewed previously (Staley et al 1998), the results of these studies vary significantly. One potential source of this variability was that both suicidal attempt and depression may be relevant to the alterations in 5-HT system, and, in some studies, retrospective case histories of depression were missing or inaccurate.

Brain imaging studies of the 5-HT system in well-characterized living patients will be useful both to assess abnormalities and to delineate the potential roles of 5-HT neurotransmission in specific pathophysiologic features of the disorder (e.g., patients with and without suicidal features or those with and without cognitive deficits). Among more than a dozen 5-HT receptor subtypes, tracers suitable for PET imaging have been developed for 5-HT1A ([carbonyl-11C]WAY-100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexane carboxamide trihydrochloride)) and 5-HT2A ([18F]setoperone and [18F]altanserin) receptors. This article will review the ongoing PET studies of 5-HT1A and 5-HT2A receptors as well as preliminary single photon emission computed tomography (SPECT) investigations of serotonin transporter (SERT). Because the hippocampus is likely to play an important role in the pathophysiology of depression (including cognitive dysfunction and hypercortisolemia), these studies will be reviewed with the orientation that this
brain structure will be a useful target to examine abnormalities and covariance of symptomatology in this disorder.

**Stress, Corticosteroids, and the Hippocampal 5-HT<sub>1A</sub> Receptor**

Serotonin 1A receptors are densely distributed in the hippocampus. Increased activity of the hypothalamic–pituitary–adrenal (HPA) axis has been consistently reported in severe depression, and recent animal studies suggest that interactions among stress, corticosteroids, and hippocampal 5-HT<sub>1A</sub> receptor play a critical role in depression.

**Limbic–Hypothalamic–Pituitary–Adrenal Axis Function in Depression**

Because mood disorders are frequently associated with high levels of the stress hormone cortisol, abnormalities in the system regulating its secretion may be pathogenic in the disorder. The secretion of cortisol is regulated by the limbic–hypothalamic–pituitary–adrenal (LHPA) axis. The neurons in the medial parvocellular division of the paraventricular nucleus of the hypothalamus (mpPVN) synthesize corticotropin-releasing hormone (CRH), which stimulates the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary corticotropes; ACTH activates synthesis and release of glucocorticoids such as corticosterone and cortisol from the adrenal cortex. The hippocampus provides negative feedback to this HPA axis. The removal of the hippocampus reduces but does not eliminate the efficacy of cortisol inhibition (Sapolsky et al 1990). The hippocampus is distinguished from other feedback sites, including the hypothalamus and pituitary, by the high expression of both mineralocorticoid (MR) and glucocorticoid (GR) receptors (Jacobson and Sapolsky 1991), enabling it to modulate the HPA axis over a wide range of corticosteroid levels. The increased response of the LHPA axis to stress after hippocampal damage or antagonism of hippocampal GR (Feldman and Conforti 1980; Sapolsky et al 1984) suggests these receptors also contribute significantly to HPA axis regulation.

Decades of research have led to the conclusion that a subgroup of depressed patients exhibit hypercortisolemia due to LHPA dysregulation. Evidence supporting this assertion includes, but is not limited to, dexamethasone nonsuppression (Carroll et al 1981; Rush et al 1996), abnormal 24-hour secretion of plasma cortisol (Murphy 1991; Sachar et al 1973), and elevated secretion of 24-hour urinary free cortisol (Anton 1987; Rothschild et al 1993). Hypercortisolemia may be associated with more severe, recurrent illness with specific neuropsychologic impairments (Force 1987; Nemeroff and Evans 1984; Rothschild et al 1993).

The mechanism underlying the hypercortisolemia of depression is multifactorial. Possible causes include 1) increased secretion of CRH from mpPVN, 2) enhanced pituitary secretion of ACTH, 3) enhanced adrenal secretion of cortisol, 4) decreased sensitivity to negative feedback at the level of the hypothalamus, pituitary, or hippocampus (Krishnan et al 1991; McAllister-Williams et al 1998; Nemeroff 1996; Nemeroff et al 1992).

**5-HT, Corticosteroids, and Hippocampal Neurogenesis**

Studies in laboratory animals have shown that glucocorticoids, which are released during stress, are associated with damage to neurons of the hippocampus, (McEwen et al 1992; Sapolsky 1996; Sapolsky et al 1990). Monkeys who died spontaneously following exposure to severe stress were found on autopsy to have multiple gastric ulcers, consistent with exposure to chronic stress, and hyperplastic adrenal cortices, consistent with sustained cortisol release. These monkeys also had damage to the CA3 subfield of the hippocampus (Uno et al 1989). Studies in a variety of animal species indicate that stress and increased glucocorticoid exposure results in decreased hippocampal dendritic branching (Watanabe et al 1992; Woolley et al 1990), alterations in hippocampal synaptic terminal structure (Magarinos et al 1997), and a loss of hippocampal neurons (Uno et al 1990).

Contrary to prior beliefs, neurogenesis (i.e., cell division with the production of new neurons) is known to occur in the adult as well as the developing brain. Gould and colleagues demonstrated neurogenesis initially in rodent hippocampus (Cameron et al 1993). Neurogenesis was subsequently demonstrated in nonhuman primate (Gould et al 1998) and even human hippocampus (Erickson et al 1998). Stress has been shown to reduce neurogenesis in the CA3 hippocampal region of adult rodents and primates (Gould et al 1998). Furthermore, reduction of corticosteroids by adrenalectomy restored neurogenesis in the hippocampus of aged rats (Cameron and McKay 1999). The implication of these findings for both depression and stress disorders is that the associated elevation of cortisol may not only damage existing neurons, but also decrease the production of new neurons. In addition, a vicious cycle could be initiated in which cortisol-induced damage of the hippocampus further reduces the ability of this structure to inhibit additional release of cortisol.

Both postsynaptic 5-HT<sub>1A</sub> receptor in the hippocampus and LHPA axis function have been proposed to play an important role in the pathophysiology of mood disorders. Strong evidence suggests that the interactions between
The Postsynaptic 5-HT1A Receptor in Depression: Postmortem Studies

Based on the animal studies and the hypothesis described above, 5-HT1A receptor density in hippocampus is expected to be decreased in depression, particularly in patients with high cortisol levels. The results of postmortem studies have not been consistent, however. Two groups did not find a change in 5-HT1A density in hippocampus in suicide victims with major depression using [3H]8-hydroxy-2-[di-n-propyl-amino]tetralin (8-OH-DPAT) autoradiography (Lowther et al 1997; Stockmeier et al 1997). One group reported a significantly decreased hippocampal 5-HT1A mRNA, decreased hippocampal MR mRNA, and a decreased hippocampal MR/GR ratio in suicide victims with a history of major depression, changes similar to those found in animals subjected to chronic unpredictable stress (Lopez et al 1998).

A few groups also studied the density of 5-HT1A receptors in frontal cortex of suicide victims using [3H]8-OH-DPAT. Two did not find a change in receptor density (Lowther et al 1997; Stockmeier et al 1997), whereas two found an increased receptor density (Matsubara et al 1991; Arango et al 1995), one of which found an increase only in nonviolent suicide (Matsubara et al 1991). Only one of these studies confirmed an ongoing episode of depression at the time of suicide, however (Stockmeier et al 1997). The increased 5-HT1A receptor densities reported by the two groups may not necessarily contradict the hypothesis regarding hippocampal pathology in depression. Regulation of 5-HT1A receptors in brain may be regionally selective. For example, hippocampal 5-HT1A receptors, because their colocalization with MR and GR (Joels et al 1991), may be more sensitive to circulating corticosteroids, whereas receptors in the prefrontal cortex may be less responsive to corticosteroids and more responsive to local 5-HT concentrations.

Results of postmortem studies of suicide victims should not be equated with the pathophysiology of depression. Some suicide victims have primary psychiatric diagnoses other than depression (e.g., schizophrenia and personality disorder). Furthermore, abnormalities in the 5-HT system may have a unique pathophysiologic role in suicidality,
which is distinct from, but interactive with, that of depression. For example, lower 5-HIAA levels were reported in more medically damaging suicide attempts (Mann et al. 1996). Because of the difficulty of retrospectively determining diagnoses and symptomatology in suicide victims, PET studies of living patients more easily provide complete characterizations, which may be assessed relative to in vivo neurochemical measurements.

The Postsynaptic 5-HT\textsubscript{1A} Receptor in Depression: PET Studies

Positron emission tomography studies focusing on 5-HT\textsubscript{1A} receptor have been hampered by the lack of an appropriate tracer. A major advance was the development of the highly selective antagonist WAY-100635 (Fletcher et al. 1994). This tracer was subsequently labeled with [\textsuperscript{11}C] at either the O-methyl or carbonyl positions. Carbonyl labeling is preferable, although more difficult, because it does not produce radiolabeled lipophilic metabolites which enter the brain and increase nondisplaceable activity (Osman et al. 1998). On the other hand, the O-methyl labeled tracer does produce a radiolabeled lipophilic metabolite that enters the brain and binds to 5-HT\textsubscript{1A} and \(\alpha_1\)-adrenoceptors (Osman et al 1996).

One potential problem of [\textit{carbonyl-}{\textsuperscript{11}}C]WAY-100635 is the generation of an active metabolite, [\textit{carbonyl-}{\textsuperscript{11}}C]desmethyl-WAY-100635, which also binds to 5-HT\textsubscript{1A} receptors (Osman et al. 1998; Pike et al. 1998). However, the production of the desmethy metabolite appears to be negligibly small in human (Osman et al 1998; Pike et al. 1998). In fact, the desmethyl compound itself (i.e., [\textit{carbonyl-}{\textsuperscript{11}}C]desmethyl-WAY-100635) can be used as a 5-HT\textsubscript{1A} receptor probe (Pike et al. 1998). Although [\textit{carbonyl-}{\textsuperscript{11}}C]WAY-100635 seems well suited as a PET tracer, improved ligands are under development, including [\textit{fluoro-cyclohexyl-}{\textsuperscript{18}}F]FCWAY (fluoro-cyclohexyl WAY analog; Lang et al. 1999), which showed high specific-to-nondisplaceable ratio and suitable pharmacokinetic characteristics for PET studies in nonhuman primates (Carson et al. 2000) and has technical advantages of the longer lived \(\textit{\textsuperscript{18}}F\) nuclide (\(T_{1/2}\) 110 min) compared with \(\textit{\textsuperscript{11}}C\) (\(T_{1/2}\) 20 min). Because of the short half-life of [\textit{carbonyl-}{\textsuperscript{11}}C]WAY-100635, the reliability of the late time point data (even 60 min after injection) may be poor, particularly the pharmacokinetics of the radiotracer’s concentration in plasma. The use of the longer physical half-life of \(\textit{\textsuperscript{18}}F\) should ameliorate these difficulties.

Quantitation of PET receptor imaging often requires concurrent analysis of the parent tracer in arterial plasma. Errors in these plasma measurements confound the receptor outcome values and may be particularly problematic for short-lived isotopes such as \(\textit{\textsuperscript{11}}C\). Lammertsma has developed a reference tissue model for the analysis of neuroreceptor levels that does not require plasma measurements (Lammertsma and Hume 1996). This method uses a brain region devoid of receptors (e.g., cerebellum) as a substitute for arterial plasma levels of the radiotracer. An extension of this method by Gunn et al. (1998) provides an image in which individual pixel values correlate with receptor densities. Images of groups (e.g., depressed patients vs. healthy subjects) can then be analyzed by a pixel-based method, such as statistical parametric mapping (SPM), to survey all regions and pixels of the brain. Both the tissue reference model and its extension to pixelwise measurements were developed to analyze 5-HT\textsubscript{1A} receptor images and are certainly significant advances in PET image analysis. Nevertheless, controversy still exists as to whether these methods can be accurately applied to [\textit{carbonyl-}{\textsuperscript{11}}C]WAY-100635 images because the cerebellum may not reflect nondisplaceable uptake or may show significant differences between subjects or between groups. The report from a recent meeting on PET imaging of the 5-HT\textsubscript{1A} receptor provides a discussion of these technical issues (http://www.ki.se/org/way/).

The first 5-HT\textsubscript{1A} receptor imaging studies in depressed patients were performed at Hammersmith Hospital, where the tracer [\textit{carbonyl-}{\textsuperscript{11}}C]WAY-100635 was developed (Sargent et al. 1999). To date, two complete studies have been published using a reference tissue analytic method: one from Hammersmith Hospital (Sargent et al. 2000) and one from University of Pittsburgh (Drevets et al. 1999). Sargent et al (2000) found moderate reductions (about 10\%) of 5-HT\textsubscript{1A} receptor levels throughout brain including medial temporal cortex (hippocampus and amygdala) in unmedicated depressed patients. Drevets et al (1999) found a greater 27\% decrease of binding potential in medial temporal cortex and a 42\% decrease in the dorsal raphe of seven unmedicated depressed patients (Table 1). The difference in these decreases were not correlated with plasma cortisol nor with 24-hour urinary free cortisol levels. There was a difference in the magnitude of decreases between these two studies, and there was also a difference in subject population: Drevets et al included patients with a history of bipolar disorder, whereas Sargent et al did not. Sargent et al (2000) also studied the effects of SSRI treatment. The reduced levels of 5-HT\textsubscript{1A} receptors were not altered in 10 of the subjects, who were scanned again during SSRI treatment. Half of these 10 patients were considered SSRI responders, but the resulting sample size was probably inadequate to assess potential normalization of receptor levels with remission of depression.

Although the decreased binding was expected from the hypothesis described above, several confounding factors in the PET findings must be considered. Postmortem studies used the agonist [\textit{\textsuperscript{3}}H]8-OH-DPAT, which predom-
inantly binds to the high-affinity state of the receptor, whereas the PET studies used an antagonist $[^{11}\text{C}]\text{WAY-100635}$, which binds to both high- and low-affinity states. The affinity state is regulated by coupling and uncoupling to a G-protein. The extent of G-protein coupling in vivo is unknown under normal conditions, and the level of one G-protein subunit was reported to be decreased in prefrontal cortex of depressed suicide victims, with the hippocampus not examined (Pacheco et al 1996). Therefore, changes in G-protein coupling to the 5-HT$_{1A}$ receptor may be a source of the discrepancy between some postmortem studies and these PET studies. Furthermore, in vivo extracellular levels of 5-HT may modify radioligand binding by either blocking access of the tracer to the receptor or modifying its cellular trafficking, which is known to exist for most G-protein coupled receptors (Bloch et al 1999). One study showed a lower level of total tissue 5-HT levels in the right hippocampus of suicide victims with depression (Cheetham et al 1989), whereas another did not (Owen et al 1986). The influence of extracellular 5-HT levels on the PET measurement of $[^{11}\text{C}]\text{WAY-100635}$ binding are currently under investigation and not fully understood (Parsey et al 1999); however, if depression is associated with low 5-HT neurotransmission (and low synaptic 5-HT concentrations), then the decreased uptake of $[^{11}\text{C}]\text{WAY-100635}$ in depressed patients could not be explained by 5-HT blockade of the receptor and, thereby reduced binding access of the radiotracer. The effects of agonists and antagonists on cellular trafficking are complex and may well modify the ability of the tracer to bind to an internalized and potentially modified receptor. Nevertheless, with these caveats in mind, the first two 5-HT$_{1A}$ receptor imaging studies in depression both reported the predicted decrease in receptor binding, but in a global and not regionally selective manner.

### The Presynaptic 5-HT$_{1A}$ Receptor

The presynaptic 5-HT$_{1A}$ receptors in the raphe nuclei regulate the release of 5-HT through a negative feedback mechanism. Blier and de Montigny (1994) have hypothesized that abnormally increased function of the somatodendritic 5-HT$_{1A}$ receptor has an etiologic role in depression and that a common mechanism of action of several antidepressant treatments is the downregulation of presynaptic 5-HT$_{1A}$ receptor function. That is, the inhibition of 5-HT reuptake by SSRIs does not lead to increased 5-HT levels in terminal synapses until an adaptive desensitization of inhibitory 5-HT$_{1A}$ autoreceptors has occurred. In a series of studies, De Montigny, Blier, and colleagues have demonstrated that the antidepressant mechanism of action of several classes of antidepressant medications are related to downregulation of dorsal raphe 5-HT$_{1A}$ receptors, resulting in a net increase in forebrain 5-HT transmission (Blier and de Montigny 1998).

Consistent with this hypothesis, the concomitant use of a 5-HT$_{1A}$ receptor antagonist with antidepressants may hasten medication response. Recent therapeutic studies have evaluated whether coadministration of pindolol (an antagonist at both $\beta$-adrenoreceptors and 5-HT$_{1A}$ receptors) and an SSRI will hasten antidepressant drug response. Some studies showed accelerated therapeutic response by concurrent use of pindolol (Bordet et al 1998; Perez et al 1997; Zanardi et al 1997), but others did not (Berman et al 1997; Perez et al 1999). This discrepancy may indicate that concurrent use of pindolol is effective in only a subgroup of patients, which has not yet been clearly identified.

#### Positron Emission Tomography Studies of Presynaptic 5-HT$_{1A}$ Receptors

As mentioned above, two studies with $[^{11}\text{C}]\text{WAY-100635}$ have reported decreased presynaptic 5-HT$_{1A}$ receptor levels in the raphe region of depressed patients. Sargent et al (2000) reported a 14% decrease; Drevets et al (1999) reported a 42% decrease (Table 1). Both of these in vivo results are contrary to the 5% to 30% increase (the increase was dependent on the rostral-to-caudal level) of postmortem 5-HT$_{1A}$ receptor binding in dorsal raphe reported by Stockmeier et al (1998). As described in the section on postsynaptic receptor imaging, difference of tracers (agonist vs. antagonist) and the
influence of extracellular 5-HT must be considered as sources of this discrepancy. In fact, tissue 5-HT levels in midbrain are more than twice as that in terminal regions (Dewar et al 1992). Furthermore, in vivo imaging methods such as PET have intrinsic limitations in their abilities to measure structures as small as the raphe nuclei. Decreased 5-HT1A receptor binding measured with PET may actually reflect a decrease in the number of 5-HT neurons and shrinkage of raphe without change in the density of the receptors per remaining neuron. In the opposite direction, a postmortem study reported a 32% significant increase in neuronal density without change in the volume of dorsal raphe in suicide victims primarily with major depression (Underwood et al 1999). If substantiated, this increased neuronal density may help explain the increased number of 5-HT1A receptors in the postmortem study of Stockmeier et al (1998).

Positron emission tomography receptor imaging would seem particularly well suited to measure 5-HT1A occupancy by pindolol and the hypothesis that pindolol has higher affinity/occupancy of presynaptic (i.e., raphe) than postsynaptic (i.e., hippocampal) sites. 5-HT1A receptor occupancy by pindolol in human subjects has been measured by three groups. Following oral administration, receptor occupancy was dose related. Occupancies in the presynaptic site were as follows: 17 to 40% for 7.5 to 10 mg and 39 to 64% for 20 to 30 mg; and in postsynaptic regions 12 to 24% for 7.5 to 10 mg and 42 to 46% for 20 to 30 mg (Table 2). Using [carbonyl-11C]WAY-100635, two of the three studies reported that pindolol had higher occupancy of presynaptic than postsynaptic sites (Andree et al 1999; Martinez et al 2000; Rabiner et al 2000). Rabiner et al showed a higher occupancy of pindolol at presynaptic receptors at a dose of 10 mg (37% vs. 13%) but not at the doses of 5 and 20 mg given orally 2 hours before the PET study (Table 2; Rabiner et al 2000) indicating that the appropriate dose range of pindolol is small. Using a larger sample size, Martinez et al showed higher occupancy at the presynaptic site by both 7.5 and 30 mg given after 1 week of treatment with 7.5 mg q.d. The difference in the occupancy in pre- and postsynaptic sites was greater after 7.5 mg. The results by these two groups may indicate that differential occupancies are achieved by a relatively low dose.

Table 2. Receptor Occupancy by Pindolol Measured in Positron Emission Tomography

<table>
<thead>
<tr>
<th>Author</th>
<th>Dose of pindolol (mg)</th>
<th>n</th>
<th>Presynaptic (%)</th>
<th>Postsynaptic (%)</th>
<th>Postsynaptic regions</th>
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<td>Andree et al 1999</td>
<td>10</td>
<td>3</td>
<td>17</td>
<td>24</td>
<td>Frontal and temporal cortices</td>
</tr>
<tr>
<td>Martinez et al 2000</td>
<td>7.5 (4 hours after a dose)</td>
<td>8</td>
<td>40</td>
<td>18</td>
<td>Cortices</td>
</tr>
<tr>
<td></td>
<td>7.5 (10 hours after a dose)</td>
<td>8</td>
<td>38</td>
<td>12</td>
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<td></td>
<td>30</td>
<td>8</td>
<td>64</td>
<td>42</td>
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</tr>
<tr>
<td>Rabiner et al 2000</td>
<td>5</td>
<td>3</td>
<td>10</td>
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<td>Cortices</td>
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The 5-HT2A Receptor

5-HT2A Receptor Density

As reviewed previously (Staley et al 1998), more than 10 studies have reported the densities of 5-HT2A receptors in prefrontal cortex of suicide victims. Many studies used the antagonist [3H]ketanserin, which has only moderate (2- to 140-fold, most findings are close to the lower end) selectivity for the 5-HT2A vs. 5-HT2C receptor. About half of the studies did not detect a significant change; the other half showed significantly increased binding; and only one detected a significant decrease. As in other 5-HT markers, however, the potentially differential effects of suicide and depression were difficult to discriminate in these postmortem studies.

Preliminary SPECT and PET 5-HT2A receptor imaging studies were performed in patients with depression using [2-123I]ketanserin and [11C]-N-methyl spiperone. These tracers suffer from major limitations of high nonspecific uptake or no selectivity relative to dopamine D2 receptors. Recently, several PET studies of depression have been performed using a new generation of tracers, [18F]setoperone and [18F]altanserin. The former has 100 times greater affinity for 5-HT2A than 5-HT2C receptors but only 10- to 50-fold higher affinity for 5-HT2 compared with D2 receptors. Even this 10- to 50-fold selectivity may be inadequate in the striatum, where the density of D2 receptors is so much higher than that of 5-HT2 receptors. Therefore, [18F]setoperone is useful to study cortex where the density of D2 receptors is low. On the other hand, [18F]altanserin has more than 100-fold selectivity relative to dopamine D2 receptors but only 20-fold selectivity relative to 5-HT2C receptors (Tan et al 1999). Even this modest selectivity relative to 5-HT2C receptors may be adequate for quantitative studies, when combined with the relatively low density of these sites in neocortex. One
limitation of $[^{18}\text{F}]$altanserin is that it produces radioactive lipophilic metabolites, which probably cross the blood–brain barrier. Intersubject variability in peripheral metabolism may introduce different levels of lipophilic metabolites in brain and may affect measures of specific-to-nondisplaceable ratio, in which cerebellum is used as a receptor-poor region. These metabolites have been partially characterized (Baldwin et al 1998). Furthermore, a deuterated analog of $[^{18}\text{F}]$altanserin has been synthesized to decrease production of these metabolites (Tan et al 1997, 1999), and a method using bolus plus constant infusion has been instituted to correct for the presence of any residual radiolabeled metabolites that have entered the brain (van Dyck et al, in press).

Using $[^{18}\text{F}]$altanserin, a significant reduction in tracer uptake was detected in right postero-lateral orbitofrontal and anterior insular cortices in medication-free depressed patients (Biver et al 1997); however, three studies using $[^{18}\text{F}]$altanserin (Meltzer et al 1999) and $[^{18}\text{F}]$setoperone (Attar-Levy et al 1999; Meyer et al 1999b) did not detect a significant change in medication-free depressed patients (Table 1). Unfortunately, some of these studies suffer from major methodologic limitations. By applying large volumes of interest (VOIs), Meyer et al (1999b) and Attar-Levy et al (1999) may have overlooked small regions with significant changes. In fact, postmortem studies indicated that changes in receptor density often are modest in magnitude and anatomically restricted to only one or two Brodmann areas (Arango et al 1997). Therefore, pixel-based analysis such as SPM may be necessary to detect these changes. Some readers may doubt the results by Biver et al because they initially analyzed relative changes in receptor density by applying global normalization and by using only brain data without correction for plasma tracer levels; however, they did post hoc VOI analyses for the region detected by SPM and corrected the data using the uptake in cerebellum. Therefore, the significant decrease they detected may be true. Nevertheless, lipophilic metabolites of $[^{18}\text{F}]$altanserin may enter the brain and contribute activity in nondisplaceable compartment. Therefore, the measurement using cerebellum as a reference region is not justified if there is a systematic difference in the metabolism of $[^{18}\text{F}]$altanserin between depressed patients and healthy subjects.

In summary, the results of 5-HT$_{2A}$ receptor imaging in depression report either decreased levels or no change, in apparent contradiction to postmortem studies that have often found increased 5-HT$_{2A}$ receptor levels. Limitations in technical aspects of the 5-HT$_{2A}$ receptor PET studies justify caution in the generalizability of these findings and support the utility of studies with larger sample sizes to examine potential correlates of depressive symptomatology and subtypes with the imaging results.

**The Effect of Antidepressants on 5-HT$_{2A}$ Receptors**

The 4- to 6-week time lag between initiating antidepressant treatment and clinical response suggest that alterations in the 5-HT system occurring during this period are relevant to the therapeutic mechanism of the medications. Animal studies have shown that administration of tricyclic antidepressants and monoamine oxidase inhibitors cause a downregulation in the number of 5-HT$_{2}$ receptors (Cowen 1990; Eison and Mullins 1996), whereas the results with SSRIs are mixed (Beasley et al 1992; Cadogan et al 1993; Eison and Mullins 1996). Two PET $[^{18}\text{F}]$setoperone studies were conducted to investigate changes in 5-HT$_{2A}$ receptors before and after 3 to 4 weeks of desipramine (Yatham et al 1999) or clomipramine (Attar-Levy et al 1999) treatment in depressed patients. Both of these studies showed significant decreases in tracer binding in almost all cortical regions (Table 3), which is consistent with prior animal experiments. The PET studies have significant methodologic limitations, however. The analysis by Yatham et al (1999) was based on the assumption of no substantial change in global binding, although the results showed such global alterations with desipramine treatment. In addition, because the analysis used global normalization in SPM, the results did not indicate a decrease in absolute quantity of binding potential but a decrease relative to the binding potential in the whole brain. Potential changes in the clearance of tracer were not taken into account in the measurement by Attar-Levy et al (1999). Because the affinity of 5-HT at 5-HT$_{2A}$ receptors is low compared with that at 5-HT$_{1A}$ receptors, potential changes of extracellular 5-HT levels induced by antidepressants probably did not affect the measurements. In fact, a single oral administration of 20 mg paroxetine did not change the binding of $[^{18}\text{F}]$setoperone (Meyer et al 1999a).

**The 5-HT Transporter**

Because the 5-HT transporter in platelet has been considered a model of its counterpart in brain, many studies have...
been conducted to compare [3H]imipramine and [3H]paroxetine binding between depressed patients and healthy subjects. Although the results were mixed, the majority (65–75%) of studies have confirmed reductions in platelet SERT levels in patients with major depression. A meta-analysis has shown this effect to be robustly significant across studies, including those examining high-affinity binding sites (Ellis and Salmond 1994). Consistent with findings of decreased platelet SERT binding, and as reviewed previously (Staley et al 1998), two postmortem studies have reported a significantly decreased 5-HT transporter levels in hypothalamus.

Using [123I]β-CIT and SPECT, the binding potential in hypothalamus and midbrain was measured in patients. Medication-free depressed patients showed a significant decrease of 18% (Malison et al 1998). A significant decrease was also detected in midbrain in alcoholism, and the decrease was significantly correlated with depressive symptoms (Heinz et al 1998). Therefore, decreased SERT levels in the midbrain may be related to depressive symptomatology.

A significant limitation of [123I]β-CIT is its lack of selectivity for serotonin versus dopamine transporters (i.e., SERT vs. DAT). Although studies in nonhuman primates suggest that the majority of activity detected in the “midbrain” region is associated with SERT (Laruelle et al 1993), a more selective tracer should be used in patients with depression. In addition, [123I]β-CIT does not show measurable specific binding in cortical regions. Therefore, efforts have been focused on the development of improved PET and SPECT tracers selective for SERT and capable of measuring the relatively low cortical levels above the nonspecific uptake of the tracer. The following tracers are recently developed: [11C] and [18F]McN5652 (Suehiro et al 1993, 1996), [11C]-labeled cocaine analogs with high selectivity (Helfenbein et al 1999), [123I] and [76Br]nitroguapazine (Jagust et al 1996; Lundkvist et al 1999), [123I]IDAM (Kung et al 1999), and [123I]ODAM (Acton et al 1999). Most of these tracers suffer from some limitations such as slow kinetics, insufficient specific-to-nondisplaceable ratio, or need for additional validation studies. Among them, [123I]IDAM is perhaps the most promising at the moment. Nevertheless, none of these tracers have been shown to provide measurable specific binding in cortical regions.

Conclusions

The development of suitable PET tracers for 5-HT1A and 5-HT2A receptors has recently allowed studies to further elucidate probable abnormalities of serotonergic neurotransmission in depression. These studies have consistently shown decreased 5-HT1A receptor binding in pre- and postsynaptic brain areas in depressed patients, but much more mixed results in cortical 5-HT2A receptor levels. The latter finding may well be related to the heterogeneity of depressive disorders and could reflect selected symptoms present in subsets of patients (e.g., suicidality). Preliminary studies have also reported decreased SERT binding in the brain stem of depressed patients, but the tracer ([123I]β-CIT) also binds to dopamine transporters and therefore is not selective for SERT. Although preliminary, these studies are clearly promising and justify further efforts. For example, study of larger sample sizes would help determine whether these brain markers are associated with subtypes of the disorder or with specific clinical symptoms. New tracers would also be useful, including probes selective for SERT or entirely new tracers (e.g., glucocorticoid, CRH or substance P receptors) of likely relevance to depressive pathophysiology. Finally, because of the documented abnormalities in the LHPA axis in depression (including hypercortisolemia and cognitive deficits), the hippocampus is likely to be a valuable target area for several of these studies. PET studies of molecular alterations in the hippocampus can be connected meaningfully to pathophysiologic features of the disorder. The changes in this region might mirror global changes and can be used either as a model for or an important contrast to changes in other brain regions.

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