

# Elevated Brain Serotonin Turnover in Patients With Depression

## Effect of Genotype and Therapy

David A. Barton, MBBS, FRANZCP; Murray D. Esler, MBBS, PhD; Tye Dawood, PhD; Elisabeth A. Lambert, PhD; Deepak Haikerwal, MBBS; Celia Brenchley, BSc; Florentia Socratous, BSc; Jacqueline Hastings, PhD; Ling Guo, MD; Glen Wiesner, PhD; David M. Kaye, MBBS, PhD; Richard Bayles, BSc(Hon); Markus P. Schlaich, MD; Gavin W. Lambert, PhD

**Context:** The biological basis for the development of major depressive disorder (MDD) remains incompletely understood.

**Objective:** To quantify brain serotonin (5-hydroxytryptamine [5-HT]) turnover in patients with MDD.

**Design:** Patients with depression were studied both untreated and during administration of a selective serotonin reuptake inhibitor (SSRI) in an unblinded study of sequential design. Healthy volunteers were examined on only 1 occasion. Direct internal jugular venous blood sampling was used to directly quantify brain serotonin turnover. The effect of serotonin transporter (5-HTT) genotype on brain serotonin turnover was evaluated and the influence of SSRI therapy on serotonin turnover was investigated.

**Setting:** Participants were recruited from the general community following media advertisement. Experimental procedures were performed in the research catheterization laboratory of a major training hospital and medical research institute.

**Participants:** Studies were performed in 21 patients fulfilling the *DSM-IV* and *International Statistical Classification of Diseases, 10th Revision* diagnostic criteria for MDD and in 40 healthy volunteers.

**Interventions:** Treatment for patients consisted of SSRI administration for approximately 12 weeks.

**Main Outcome Measures:** Brain serotonin turnover before and after SSRI therapy.

**Results:** Brain serotonin turnover was significantly elevated in unmedicated patients with MDD compared with healthy subjects (mean [SD] internal jugular venoarterial 5-hydroxyindoleacetic acid plasma concentration difference, 4.4 [4.3] vs 1.6 [2.4] nmol/L, respectively;  $P = .003$ ). Analysis of the influence of the 5-HTT genotype in MDD indicated that carriage of the *s* allele compared with the *l* allele was associated with greater than a 2-fold increase in brain serotonin turnover (mean [SD] internal jugular venoarterial 5-hydroxyindoleacetic acid plasma concentration difference, 6.5 [4.7] vs 2.7 [2.9] nmol/L, respectively;  $P = .04$ ). Following SSRI therapy, brain serotonin turnover was substantially reduced (mean [SD] internal jugular venoarterial 5-hydroxyindoleacetic acid plasma concentration difference, 6.0 [4.0] nmol/L prior to treatment vs 2.0 [3.3] nmol/L following therapy;  $P = .008$ ).

**Conclusions:** Brain serotonin turnover is elevated in unmedicated patients with MDD and is influenced by the 5-HTT genotype. The marked reduction in serotonin turnover following SSRI treatment and the accompanying improvement in symptoms suggest that high brain serotonin turnover may be a biological substrate of MDD.

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### Author Affiliations:

Department of Psychological Medicine, Alfred Hospital, Monash University (Dr Barton), and Human Neurotransmitters Laboratory (Drs Barton, Esler, Dawood, E. A. Lambert, Haikerwal, Hastings, Guo, Wiesner, Schlaich, and G. W. Lambert, Mss Brenchley and Socratous, and Mr Bayles) and Wynn Department of Metabolic Cardiology (Dr Kaye), Baker Heart Research Institute, Melbourne, Victoria, Australia.

**T**HE ETIOLOGY OF MAJOR DEPRESSIVE DISORDER (MDD) has been linked to brain monoaminergic neuronal dysfunction.<sup>1,2</sup> Of particular interest is the role of brain serotonin (5-hydroxytryptamine [5-HT]) in MDD. The diversity of roles played by serotonin coupled with the lack of a demonstrated relationship between different clinical presentations and biochemical abnormalities has hampered the development of sensitive markers of the disease. Indeed, brain serotonin-releasing neu-

rons subserve diverse although incompletely understood functions related to emotions and behavior, feeding and adiposity,<sup>3</sup> and light stimulation.<sup>4</sup> We have previously demonstrated influences of obesity and feeding<sup>5</sup> and season and sunlight<sup>6</sup> on brain serotonin turnover in humans. The principal means of intraneuronal metabolism of serotonin is via oxidative deamination by monoamine oxidase resulting in formation of 5-hydroxyindoleacetic acid (5-HIAA) (**Figure 1**). Deamination followed by reduction, conjugation with sulfate or glucuronide, and

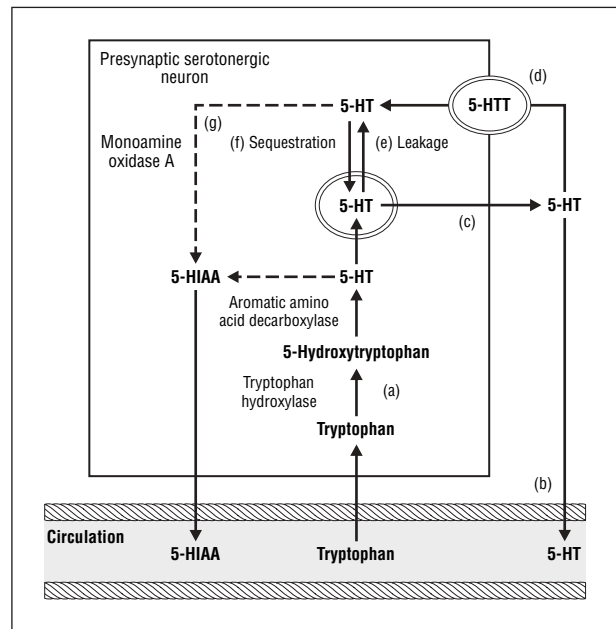
N-acetylation represent minor metabolic pathways of serotonin.<sup>10,11</sup> Brain serotonin turnover, where *turnover* refers to the rate of synthesis of the transmitter, is difficult to assess in humans. The brain is the source of less than 10% of 5-HIAA found in plasma and urine,<sup>12</sup> rendering peripheral venous plasma measurements and urine collections unsuitable for studying brain serotonin turnover.

Given our group's longstanding expertise in direct intravenous catheterization techniques, we used a method originally described by Maas et al<sup>13-15</sup> with percutaneously placed venous sampling catheters positioned high in an internal jugular vein in order to quantify the turnover of central nervous system monoamine neurotransmitters. Earlier investigations from our group documenting similar cerebral norepinephrine and metabolite internal jugular venous overflows in healthy subjects and in patients with autonomic failure in whom there was existing biochemical evidence of almost complete postganglionic sympathetic denervation<sup>16</sup> provided the justification that our internal jugular venous metabolites measured emanate from central neurons and not from cerebrovascular sympathetic nerves.<sup>17</sup> In previous articles, we documented the importance of subcortical norepinephrine in the regulation of sympathetic activity in patients with heart failure<sup>18</sup> and hypertension<sup>19</sup> and reported reduced brain norepinephrine and dopamine turnover in patients with treatment-refractory depression.<sup>20</sup> In this study, we examined brain serotonin turnover in healthy subjects and in unmedicated patients with MDD. To facilitate interpretation of brain serotonin turnover measurements in the patients with MDD, serotonin transporter (5-HTT) genotyping was also performed. The 5-HTT gene (GenBank accession No. SLC6A4) is subject to a 44-base pair deletion-insertion promoter region polymorphism that may influence gene activity.<sup>21</sup> We hypothesized that the presence of the short form of the gene might increase serotonin turnover rates through impairment of neuronal serotonin reuptake. In addition, in 11 patients with MDD, jugular venous sampling was performed twice, first while patients were untreated and then during treatment with a selective serotonin reuptake inhibitor (SSRI). This was done to ascertain whether treatment response under SSRI dosing was associated with normalization of any abnormality in brain serotonin turnover that might be present.

## METHODS

### PARTICIPANTS

Data were obtained from 21 patients (9 men and 12 women; mean [SD] age, 45 [12] years) fulfilling the *DSM-IV* and *International Statistical Classification of Diseases, 10th Revision* diagnostic criteria for MDD and from 40 healthy volunteers (31 men and 9 women; mean [SD] age, 41 [19] years). Patients with MDD and healthy volunteers were recruited through media advertisements. Patients were either newly diagnosed or currently untreated after a relapse and had not been receiving antidepressants or benzodiazepines for at least 4 weeks prior to the study (5 weeks if they had been receiving fluoxetine hydrochloride). Eight of the patients had never been treated with



**Figure 1.** Diagrammatic representation of the relative contributions to intraneuronal serotonin (5-hydroxytryptamine [5-HT]) turnover. At steady state, the rate of serotonin synthesis (a) by definition must equal the rate of serotonin turnover, where turnover is equal to the sum of the rates of spillover of serotonin to plasma (b) and intraneuronal metabolism of serotonin (g). In cerebrospinal fluid, 5-hydroxyindoleacetic acid (5-HIAA) concentrations are approximately 200 times that of serotonin,<sup>7</sup> thereby rendering 5-HIAA levels an approximation of serotonin turnover. The availability of serotonin for intraneuronal metabolism is dependent on the balance between serotonin release (c) and the entry of serotonin into the axoplasm via the processes of intraneuronal reuptake (d) and leakage (e) and removal (f) from the axoplasm. Vesicular serotonin transport occurs via the action of the vesicular monoamine transporter. Alterations in vascular permeability, which is influenced by the sympathetic nervous system,<sup>8,9</sup> may influence the dynamics of capillary exchange and appearance of 5-HIAA in plasma. 5-HTT indicates serotonin transporter.

antidepressants prior to this trial. The remaining patients had previously received antidepressant medications; 10 of them had stopped receiving medication more than 12 months prior to the investigation (2 of whom had previously been receiving fluoxetine), 1 of them had stopped 9 months prior, and 2 of them had stopped 6 weeks prior. Following initial telephone screening, patients were interviewed by a psychiatrist (D.A.B.) using a structured clinical interview (Mini International Neuropsychiatric Interview [MINI]<sup>22</sup>). The 17-item Hamilton Depression Scale (HAM-D),<sup>23</sup> Spielberger's State and Trait Anxiety Inventories,<sup>24,25</sup> and the Beck Depression Inventory (BDI)<sup>26</sup> were used to assess severity and monitor response to treatment. Patients were eligible for inclusion if they fulfilled criteria for MDD on the MINI, had HAM-D and BDI scores of 18 or higher, and were assessed as having MDD as the primary illness at psychiatric interview. Patient selection attempted to minimize psychiatric comorbidity. Possible comorbidity was evaluated with the Anxiety Disorders Interview Schedule for *DSM-IV*,<sup>27</sup> which allows for discrimination between anxiety disorders as well as for the determination of primary and secondary diagnoses based on the participant's responses and severity scores on measures of symptoms. Ratings were considered to be clinically significant with a score of 4 or higher on the 8-point Likert-type scale, where 2 is mild, 4 is moderate, 6 is severe, and 8 is very severe. Patients with comorbid panic or anxiety disorders were included in the study if the primary diagnosis was depression and any panic or anxiety was secondary to their depression. All of the participants had a clinical examination to exclude any previously undiagnosed medical

conditions. Patients were excluded if they had coexisting heart disease, diabetes, medicated hypertension, alcohol or drug abuse or dependence, or infectious disease; had a comorbid psychotic disorder, eating disorder, mental retardation, personality disorder, or epilepsy; or had a current high suicide risk. Suicide risk was assessed using the MINI, the BDI (question 9), and evaluation of current risk by a psychiatrist (D.A.B.) during the initial clinical interview. Patients having previously failed to respond to SSRI treatment at the maximum tolerated dose for at least 4 weeks were excluded from the study. Four of the patients with MDD were current smokers and none in the control group were smokers.

Initial research studies were performed within 10 days of a confirmed diagnosis of MDD. Following the initial investigation, 11 patients commenced treatment with an SSRI (citalopram hydrobromide,  $n=7$ ; sertraline hydrochloride,  $n=2$ ; fluoxetine,  $n=2$ ). The choice of SSRI was based on clinical grounds and was made by the psychiatrist in consultation with the participant. The dosage was determined according to clinical response. No structured psychotherapy was provided to the patient either within or external to the study. Repeat research studies with blood sampling from the same internal jugular vein were performed after approximately 12 weeks of therapy (mean [SD] duration of therapy, 98 [7] days). Patients were examined weekly for the purposes of the study or more frequently if required on clinical grounds. Significant clinical improvement was defined as a decrease of more than 50% in HAM-D scores and remission was defined as a HAM-D score lower than 8.

By appropriate selection and matching of a healthy reference population, with matching of healthy subjects and patients with MDD in terms of body mass index (calculated as the weight in kilograms divided by the height in meters squared) and sunlight hours on the study day, we were able to avoid a confounding effect of serotonergic neuronal systems activated by sunlight<sup>6</sup> and in obesity.<sup>5</sup> The mean (SD) body mass index was 25 (1) in patients with MDD and 24 (1) in healthy subjects. With the assistance of the Melbourne Bureau of Meteorology,<sup>6</sup> healthy volunteers were chosen from a large pool of subjects to achieve matching for sunlight hours on the day of the catheter sampling study (mean [SD] sunlight hours, 6.6 [0.8] hours in patients with MDD and 6.0 [0.6] hours in the reference population). Healthy subjects were studied on only 1 occasion.

### CENTRAL VENOUS CATHETERIZATION PROCEDURE

All of the catheter studies were performed with subjects in the supine position according to previously described methods.<sup>17,20</sup> Caffeinated beverages, alcohol, and tobacco smoking were prohibited for the 12 hours preceding the catheter study. Under local anesthesia, the radial artery was cannulated for arterial pressure monitoring and blood sampling. A venous introducer sheath was placed in the antecubital fossa. Subsequently, a 6F coronary sinus angiographic catheter (Cordis Europa, Roden, the Netherlands) was introduced via the antecubital venous sheath and placed under fluoroscopic control in the right ( $n=19$  patients with MDD,  $n=28$  control subjects) or left ( $n=2$  patients with MDD,  $n=12$  control subjects) internal jugular vein. The antecubital access on the same side as the sampled jugular vein was used in each case. The face and scalp have a rich blood supply and the venous drainage into the internal jugular vein is typically via the supratrochlear and supraorbital veins, which combine to form the facial vein.<sup>28</sup> Given that the facial vein enters the internal jugular vein near the greater cornu of the hyoid bone, the internal jugular venous catheter

was advanced beyond the mandibular angle upstream to points of entry of veins draining the face and neck to minimize any contamination of the cerebral venous effluent.<sup>28</sup> The catheter was used for sampling internal jugular vein blood. Thirty milliliters of blood was simultaneously obtained from the arterial and internal jugular catheters.

### MEASUREMENT OF BRAIN SEROTONIN TURNOVER

The metabolism of serotonin is achieved almost exclusively via the actions of monoamine oxidase and aldehyde dehydrogenase, resulting in the formation of 5-HIAA. Venoarterial plasma 5-HIAA concentration gradients were used as indicators of brain serotonin turnover.<sup>12</sup> Plasma 5-HIAA concentrations were determined by high-performance liquid chromatography coupled with coulometric detection as previously described.<sup>12</sup> The interassay and intra-assay coefficients of variation for 5-HIAA were  $\pm 6\%$  and  $\pm 2\%$ , respectively. The assay was linear within the physiological range, with a sensitivity (signal to noise ratio of 3) of 50 pg.

### 5-HTT GENOTYPING

The human 5-HTT, encoded by a single gene on chromosome 17q11.2, is expressed in both the brain and peripheral blood cells. A 5-HTT-linked gene promoter region (5-HTTLPR) insertion-deletion polymorphism with long (*l*) and short (*s*) forms has been demonstrated to influence expression and function of the 5-HTT.<sup>21,29,30</sup>

Genomic DNA was isolated from whole blood using the Flexigene DNA Kit (Qiagen, Hilden, Germany). Genotyping for the 5-HTTLPR was performed after amplification by polymerase chain reaction using sense and antisense primers as previously described.<sup>21,30</sup> The polymerase chain reaction products were resolved and the bands visualized by UV illumination. The identity of each band was confirmed by automated DNA sequencing.

### PLASMA CATECHOLAMINE DETERMINATIONS

Given that activation of the sympathetic nervous system may be responsible for stress-related increases in brain tryptophan concentration,<sup>31</sup> arterial norepinephrine and epinephrine concentrations were used as a marker of sympathetic nervous and sympathoadrenal activation. Plasma concentrations of norepinephrine and epinephrine were determined by high-performance liquid chromatography with electrochemical detection.<sup>32</sup>

### ETHICS CONSIDERATIONS

Given that this study involved the percutaneous placement of a central venous catheter and an arterial cannula in participants for whom this was not clinically indicated, comment on research ethics is in order. The central issues in clinical research ethics are the following: (1) the quality of the research, (2) the potential for harm to an individual from the experiment, and (3) the degree of safeguarding of the participants' autonomy. In this study, we investigated aspects of brain serotonergic function in patients with MDD that are potentially of clinical importance using well-established research methods. There is no less invasive method for validly doing this than we applied. In our extensive research experience with central venous catheters<sup>6,20,33-35</sup> involving in excess of 2000 studies performed over more than 20 years, we have found the procedure

to be invariably associated with negligible risk. Cardiologists who are experts in the technique always perform the procedures. The process of written consent, to which an honest, open, and explicit participant information sheet was central, conformed to the standards expected to preserve the autonomy of the participants. The research protocol conformed to the relevant guidelines of the National Health and Medical Research Council of Australia and was approved by the Alfred Hospital ethics review committee. Written informed consent was obtained from each subject prior to the study. Patients with MDD were not paid. Healthy subjects were reimbursed US \$100.

## STATISTICAL ANALYSES

All of the results unless otherwise specified are expressed as mean (standard deviation). Tests of significance were carried out using analysis of variance (1-way analysis of variance for comparison between patients with MDD and control subjects and repeated-measures analysis of variance for the effect of therapy) or distribution-free nonparametric tests. The possible relationship between variables was evaluated using least squares linear regression analysis. All of the statistical tests were 2-tailed and statistical significance was set at a probability level of .05.

## RESULTS

Patients with MDD were moderately to severely depressed with a mean (SD) HAM-D score of 25 (4) and a mean (SD) BDI score of 29 (8). They also had high levels of both state (mean [SD] score, 57 [12]) and trait (mean [SD] score, 62 [7]) anxiety. Approximately half of the patients had a positive family history of depression. On average, patients had experienced 2.3 previous episodes of depression, with 64% having had 2 or more previous episodes. In 3 patients this was their first episode. Eighteen percent of the population had a current episode duration shorter than 3 months, whereas 59% had a current episode duration longer than 12 months. In all of the patients, the primary disorder was MDD. Seven of the patients with MDD had comorbid (secondary) panic disorder and 10 had social phobia. No patients had generalized anxiety disorder or obsessive-compulsive disorder. Clinician-rated suicide risk (assessed during the clinical interview from the MINI) indicated that 14 of the patients were considered to be at low risk, 6 were at medium risk, and 1 was at high risk. The 1 patient at high risk was rated high on the MINI due to a previous suicide attempt but was felt not to be currently at significant risk by the psychiatrist at the initial interview. Analysis of the hemodynamic data and arterial plasma norepinephrine and epinephrine concentrations revealed no difference between patients with MDD and healthy subjects (**Table**).

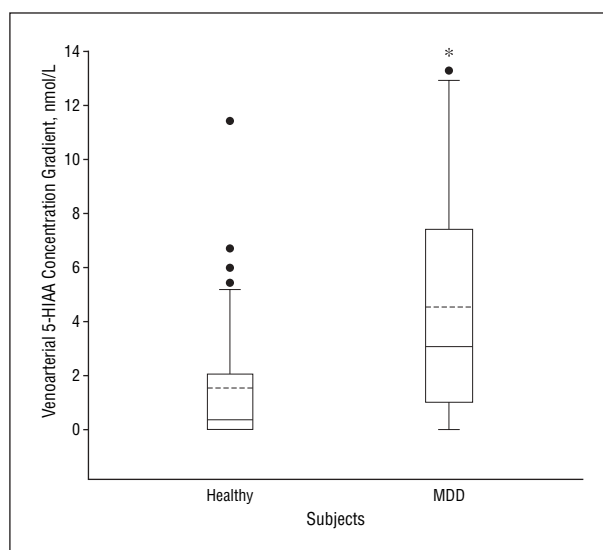
The arterial 5-HIAA plasma concentration did not differ between groups (mean [SD], 46 [21] nmol/L in patients with MDD vs 42 [19] nmol/L in control subjects;  $P = .48$ ). Indicative of elevated brain serotonin turnover, the internal jugular venoarterial 5-HIAA plasma concentration gradient was significantly elevated in unmedicated patients with MDD as compared with control subjects (**Figure 2**) (mean [SD], 4.4 [4.3] vs 1.6 [2.4] nmol/L, respectively;  $P = .003$ ). In patients with MDD,

**Table. Hemodynamic Variables<sup>a</sup>**

Hemodynamic Variable	Patients With MDD	Healthy Subjects	P Value
Blood pressure, mean (SD), mm Hg			
Systolic	134 (2)	135 (3)	.82
Diastolic	72 (2)	70 (2)	.42
Heart rate, mean (SD), beats/min	65 (2)	65 (2)	.96
Arterial norepinephrine concentration, mean (SD), pg/mL	321 (51)	220 (17)	.22
Arterial epinephrine concentration, mean (SD), pg/mL	62 (5)	59 (9)	.40

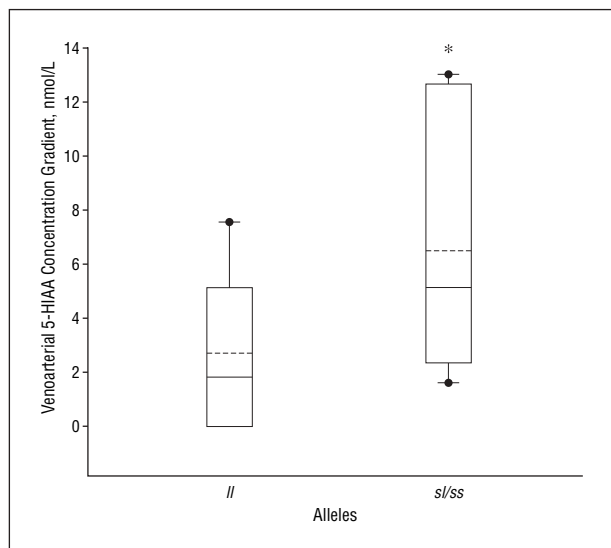
SI conversion factors: For norepinephrine, convert picograms per milliliter to picomoles per liter, multiply by 5.911. For epinephrine, convert picograms per milliliter to picomoles per liter, multiply by 5.459.

<sup>a</sup>Hemodynamic variables and arterial catecholamine plasma concentrations during the catheter procedure.



**Figure 2.** Box plot indicating the internal jugular venoarterial 5-hydroxyindoleacetic acid (5-HIAA) concentration gradient in healthy subjects and in unmedicated patients with major depressive disorder (MDD). The box represents the median, 25th, and 75th percentiles and the error bars indicate the 10th and 90th percentiles. Outliers are shown by solid dots. The mean is represented by the dotted line. Differences between groups were analyzed using the Mann-Whitney rank sum test. \* $P < .01$ .

there was a trend only for the internal jugular venous 5-HIAA concentration gradient to be related to the severity of depression as indicated by the HAM-D rating ( $r = 0.35$ ;  $P = .12$ ). No effect of sex occurred on the internal jugular 5-HIAA concentration gradient in patients with MDD (mean [SD], 4.2 [4.9] nmol/L for women vs 4.6 [3.9] nmol/L for men;  $P = .82$ ). The internal jugular venous 5-HIAA concentration gradient was not quantitatively linked to the assessed risk of suicide ( $P = .54$ ). The mean (SD) internal jugular 5-HIAA concentration gradient was 5.6 (3.8) nmol/L in patients with comorbid panic disorder, whereas it was 3.5 (4.2) nmol/L in patients with comorbid social phobia. This difference was not significant ( $P = .32$ ). In all of the subjects combined, there was no relationship between the internal jugular 5-HIAA concentration gradient and arterial norepinephrine ( $P = .18$ )



**Figure 3.** Box plot indicating the internal jugular venoarterial 5-hydroxyindoleacetic acid (5-HIAA) concentration gradient in unmedicated patients with major depressive disorder taking into account the serotonin transporter genotype. The box represents the median, 25th, and 75th percentiles and the error bars indicate the 10th and 90th percentiles. Outliers are shown by solid dots. The mean is represented by the dotted line. Differences between groups were analyzed using analysis of variance. \* $P < .05$ .

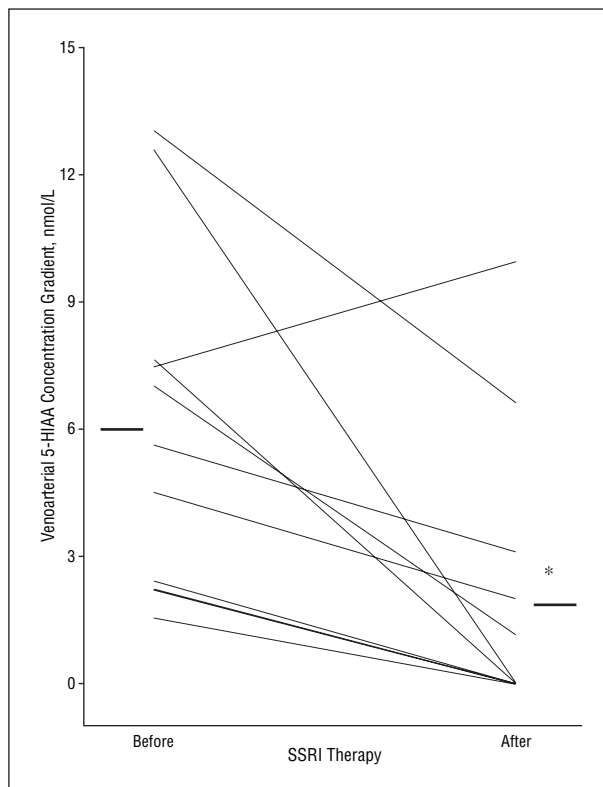
or epinephrine ( $P = .91$ ) level, systolic ( $P = .77$ ) or diastolic ( $P = .25$ ) blood pressure, or heart rate ( $P = .88$ ).

Analysis of the influence of the 5-HTT genotype in MDD indicated that carriage of the *s* allele compared with the *l* allele was associated with greater than a 2-fold increase in brain serotonin turnover (**Figure 3**) (mean [SD] internal jugular venoarterial 5-HIAA plasma concentration difference, 6.5 [4.7] vs 2.7 [2.9] nmol/L, respectively;  $P = .04$ ). Nine patients had the *ll* genotype, 7 had the *sl* genotype, and 4 had the *ss* genotype. In 1 patient we were unable to amplify and resolve a fragment of appropriate size. Three of the 4 current smokers carried the *s* allele; hence, there was a trend for the current smokers to have a greater serotonin turnover than the non-smokers (mean [SD] internal jugular venoarterial 5-HIAA plasma concentration difference, 7.5 [3.6] vs 3.7 [4.2] nmol/L, respectively;  $P = .08$ ).

In the 11 patients in whom we obtained matching post-SSRI treatment internal jugular vein blood samples, SSRI treatment resulted in a marked improvement in clinician- and patient-rated symptoms (during treatment: mean [SD] HAM-D score, 7 [5],  $P < .001$ ; mean [SD] BDI score, 9 [5],  $P < .001$ ; mean [SD] Trait and State Anxiety Inventory scores, 42 [12] and 40 [11], respectively, both  $P < .001$ ). Brain serotonin turnover was substantially reduced in all but 1 patient following SSRI therapy (**Figure 4**) (mean [SD] internal jugular venoarterial 5-HIAA plasma concentration difference, 6.0 [4.0] nmol/L prior to treatment vs 2.0 [3.3] nmol/L following therapy;  $P = .008$ ).

#### COMMENT

In this study, we used direct internal jugular venous blood sampling to examine brain serotonin turnover in pa-



**Figure 4.** Internal jugular venoarterial 5-hydroxyindoleacetic acid (5-HIAA) concentration gradient in patients with major depressive disorder before and after selective serotonin reuptake inhibitor (SSRI) therapy. The means are represented by the horizontal lines. Differences between groups were analyzed using repeated-measures analysis of variance. \* $P < .01$ .

tients with MDD. Perhaps surprisingly, brain serotonin turnover was substantially elevated in unmedicated patients with MDD, with serotonin turnover being particularly prominent in those patients who carried the short allele of the serotonin transporter. Given that arterial norepinephrine and epinephrine levels, blood pressure, and heart rate were not elevated during testing in the patients with MDD as well as the lack of an association between brain serotonin turnover and these parameters, it would seem that the difference between groups is not due to elevated situational stress in the patients with MDD. Following effective therapy with an SSRI, brain serotonin turnover was reduced to the level observed in healthy subjects.

Recent data linking fenfluramine-stimulated serotonin release and diminished dysfunctional attitudes as well as showing an association between dysfunctional attitudes and an up-regulation of cortical serotonin 2A binding potential in patients with MDD<sup>36,37</sup> support the conventional view that MDD is caused by a relative reduction in brain monoaminergic neuronal activity. While our combined observations of elevated internal jugular venous 5-HIAA concentration in unmedicated patients and reduced serotonin turnover following SSRI therapy appear to run counter to this thesis, an elevated rate of serotonin metabolism could represent a process by which the availability of serotonin to be released neuronally is lowered. Interestingly, Gjerris et al<sup>7</sup> in their initial cerebrospinal fluid (CSF) study actually documented elevated

serotonin levels in lumbar CSF in patients with depression. More recently, Sullivan and colleagues<sup>38</sup> documented elevated CSF 5-HIAA levels in patients with MDD with comorbid panic disorder. While the study by Sullivan and colleagues was confined to examinations in female patients, we found no difference in internal jugular 5-HIAA concentrations between sexes. Consistent with elevated serotonin turnover in MDD, in an experimental model of depression, chronic stress was associated with an up-regulation of tryptophan hydroxylase expression in the dorsal raphe nucleus.<sup>39</sup> Cerebrospinal fluid 5-HIAA levels are typically normal in nonsuicidal depressed patients but low in patients with a predisposition to violent suicide.<sup>40,41</sup> Normal CSF 5-HIAA concentration in patients with MDD<sup>42-44</sup> and in suicide attempters has also been described.<sup>45</sup> Rosa-Neto and colleagues<sup>46</sup> recently estimated serotonin synthesis using  $\alpha$ -[<sup>11</sup>C]methyl-L-tryptophan, a synthetic analog of tryptophan taken up by serotonergic neurons, and concluded that reduced serotonin synthesis occurs in some parts of the brain in patients with MDD. These conflicting results may reflect diagnostic heterogeneity of the patients examined or the imprecision of using lumbar CSF as an index of brain neurotransmitter turnover. Lumbar CSF concentrations reflect only a small portion of total 5-HIAA production by the central nervous system.<sup>47</sup> Moreover, there exists a substantial rostrocaudal 5-HIAA concentration gradient in CSF,<sup>48</sup> with serotonin in lumbar CSF being derived from both the brain and the terminals of descending serotonergic neuronal projections to the spinal cord.<sup>49,50</sup>

Applying positron emission tomographic scanning methods in parallel with the jugular venous technique would be instructive. Positron emission tomographic studies, ideally using a variety of serotonergic ligands, should provide regional measures of receptor occupancy that would be complementary to the measures of serotonin turnover based on jugular venous sampling. Indicative of the complexity of serotonergic regulation in the brain, positron emission tomographic examinations have revealed influences of sex<sup>51</sup> and age<sup>52</sup> on binding potential of various radiolabeled ligands. While serotonin 2 binding potential is not increased in untreated depressed subjects who have not made recent suicide attempts,<sup>53</sup> recent articles<sup>36,37</sup> have documented elevated cortical serotonin 2 receptor binding potential in patients with dysfunctional attitudes.

To appropriately interpret our results, one must appreciate the mechanisms involved in neurotransmitter turnover. The terms *release*, *spillover*, and *turnover* are often used interchangeably but do refer to distinct processes (Figure 1). To maintain neuronal stores of neurotransmitter under steady-state conditions, the rate of serotonin synthesis must equal the rate of serotonin turnover, where turnover specifically denotes the depletion of previously synthesized stores of serotonin. Hence, in the brain, cerebral serotonin turnover may be estimated from the sum of differences in rates at which serotonin and its metabolites enter and leave the cerebral circulation. In CSF, 5-HIAA concentrations are approximately 200 times that of serotonin,<sup>7</sup> thereby rendering 5-HIAA levels an approximation of serotonin turnover. Given that 5-HIAA is effectively a terminal metabolite, the prime de-

terminants of outward flux at steady state are the brain concentration and production rate. Infusion of radiolabeled 5-HIAA would have allowed a more formal application of the Fick principle with measurement of the outward flux of the metabolite from the brain based on isotope dilution. 5-Hydroxyindoleacetic acid is produced by intraneuronal deamination (via monoamine oxidase A) of serotonin after either leakage of transmitter into the axoplasm from storage vesicles or reuptake of transmitter after exocytotic release; the contribution of the latter process is reflected by the decrease in 5-HIAA production after neuronal uptake blockade. Using carbon 11-labeled harmine and positron emission tomography, Meyer et al<sup>54</sup> demonstrated a substantial elevation in monoamine oxidase A density in patients with MDD. Given the dependence of serotonin turnover on intraneuronal deamination, this observation is consistent with our data showing a substantial elevation in internal jugular venous 5-HIAA overflow in patients with MDD. It is important to point out that elevated monoamine oxidase A activity and a concomitant increase in serotonin turnover in the brain do not necessarily translate to a reduction in brain serotonin release. Increased metabolism without a concomitant elevation in the synthesis rate would lead to a rapid depletion of neurotransmitter stores.

Elevated internal jugular 5-HIAA overflow in patients with MDD could occur as a result of augmented neuronal firing or secondary to either increased vesicular leakage or reduced capacity of vesicular uptake and subsequent intraneuronal metabolism. This latter explanation would be consistent with the proposal of reduced serotonergic activity being of primary importance in the etiology of MDD. Decreased vesicular monoamine transporter 2 protein expression in the brain in an animal model of depression has been described.<sup>55</sup> Evidence implicating a role for vesicular monoamine transporter 2 in clinical MDD is scant.

Given that serotonin may be accumulated into, metabolized, and released (as 5-HIAA) from sympathetic nerves in cerebral blood vessels<sup>56</sup> and by uptake and metabolism by endothelial cells of the skeletal muscle vascular bed,<sup>57,58</sup> it is important to consider possible extracerebral sources of 5-HIAA in plasma. Previous studies have demonstrated that up to 50% of intra-arterially infused serotonin is removed in a single passage through the hind limb of anesthetized dogs.<sup>57</sup> Shepro and Hechtman<sup>57</sup> proposed that it reflected uptake and metabolism by the endothelial cells of the skeletal muscle vascular bed. Moreover, tritium-labeled serotonin may be incorporated into nerve endings and subsequently released, spontaneously or by electrical stimulation,<sup>59</sup> with the radioactivity being predominantly associated with 5-HIAA.<sup>56</sup> This indicates that serotonin taken up into sympathetic nerves is subjected to intraneuronal oxidative deamination by monoamine oxidase A. While possible, we think it is unlikely that either the cerebrovascular sympathetic nerves or the vascular endothelium contributed markedly to the determined internal jugular 5-HIAA concentration gradients or accounted for the difference between values in healthy subjects and patients with MDD. Using regional blood sampling from a variety of vascular beds coupled with radiotracer-derived measure-

ments of the rate of spillover of norepinephrine to plasma, we have previously demonstrated a significant relationship between regional sympathetic activity and 5-HIAA overflow only from the hepatosplanchnic bed.<sup>12</sup> There was no relationship between sympathetic activity and 5-HIAA overflow from the skeletal muscle vascular bed. Importantly, with regard to a possible endothelial source of 5-HIAA, while studies using animal preparations<sup>57,59</sup> have documented marked uptake of serotonin, 2 studies<sup>60,61</sup> using human vascular preparations have demonstrated only a minor capacity of the human vascular endothelium to take up serotonin.

The development of molecular, biochemical, and imaging techniques has yielded significant insights into the functioning of the serotonin transporter. Our finding of elevated internal jugular 5-HIAA overflow in depressed patients carrying the *s* allele of the 5-HTT gene is in agreement with the recent findings by Kishida and colleagues,<sup>62</sup> who observed elevated 5-HIAA levels in the CSF of patients with MDD carrying the *s* allele. While it has been observed that 5-HTT expression and serotonin uptake are impaired with the presence of the short-form haplotype,<sup>21,30</sup> studies in postmortem brain tissue demonstrated no relationship between 5-HTT binding potential and genotype in suicide victims.<sup>63</sup> Moreover, using positron emission tomography, Shioe et al<sup>64</sup> failed to reveal an association between 5-HTT binding and genotype in healthy individuals. This discrepancy in findings may be owing to the fact that functional single nucleotide variants exist in the *s* and *l* alleles.<sup>65</sup> Indeed, a single-nucleotide variant (A to G) of the *l* allele has been shown to influence transporter function, with the long G (L<sub>G</sub>) variant being functionally similar to the *s* allele.<sup>66</sup> Indeed, Praschak-Rieder and colleagues<sup>67</sup> recently demonstrated that the long *A/A* variant was associated with elevated serotonin transporter binding density in the putamen compared with subjects who carried the L<sub>G</sub> allele. While we did not take into account the possibility of variation in the *l* allele, given that we observed elevated internal jugular 5-HIAA levels in patients who carried the *s* allele, further consideration of whether the *ll* patients carried the L<sub>G</sub> or L<sub>A</sub> allele may have strengthened our association. Our observation that current smokers, most of whom carried the *s* allele, tended to have higher serotonin turnover than nonsmokers is difficult to interpret given that tobacco smoking is associated with a diminution in brain monoamine oxidase A<sup>68</sup> activity. On balance, the 5-HTT promoter polymorphism seems not to be associated with smoking behavior.<sup>69</sup>

The mechanisms underpinning the therapeutic effect of SSRIs remain incompletely understood. The knowledge that neuronal uptake blockade following antidepressant administration occurs rapidly but therapeutic effects may take from 2 to 6 weeks to occur<sup>70</sup> has been difficult to reconcile both in terms of the etiology of the disease and the therapeutic mechanism of SSRIs. In agreement with our results documenting a reduction in internal jugular venous overflow of 5-HIAA following SSRI administration, improvement in symptoms coupled with a reduction in CSF 5-HIAA levels in patients with MDD following therapy with citalopram,<sup>71,72</sup> venlafaxine hydrochloride,<sup>73</sup> fluvoxamine, and fluoxetine<sup>43,74</sup> have pre-

viously been described. Citalopram administration in chronically stressed rats normalizes the elevated expression of tryptophan hydroxylase.<sup>39</sup> The commonly held view that desensitization of inhibitory presynaptic serotonin 1A autoreceptors is implicit in the therapeutic effect of SSRIs is not consistent with these data documenting a reduction in indices of brain serotonergic activity following long-term SSRI treatment. Given that serotonin 1A autoreceptors on the cell body do retain the capacity to inhibit serotonin release even after long-term SSRI administration (for review, see the article by Hjorth et al<sup>75</sup>), the reduction in internal jugular venous 5-HIAA levels that we have documented following treatment with SSRIs may reflect a long-term activation of serotonin 1A autoreceptors leading to a reduction in neuronal firing and serotonin release. Perhaps contrary to this view, Parsey and colleagues,<sup>76</sup> using positron emission tomography, demonstrated a reduced serotonin 1A binding potential in patients with MDD who were previously exposed to antidepressant medications compared with drug-naïve patients. Given the recent demonstration that serotonergic neurons may be heterogeneous with respect to their susceptibility to serotonin 1A-induced inhibition,<sup>77</sup> the integration of these results may be more complicated than previously thought.

In this study, using direct internal jugular venous sampling, we have demonstrated that brain serotonin turnover is elevated in unmedicated patients with MDD. Interestingly and consistent with the hypothesis that the activity of the 5-HTT is influenced by genotype, carriage of the 5-HTT *s* allele was associated with an approximately 2-fold increase in serotonin turnover. Whether elevated brain serotonin turnover occurs as a result of increased neuronal activity or enhanced vesicular leakage and subsequent intraneuronal metabolism remains unknown.

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**Correspondence:** Gavin W. Lambert, PhD, Human Neurotransmitters Laboratory, Baker Heart Research Institute, PO Box 6492, St Kilda Road Central, Melbourne, Victoria 8008, Australia (gavin.lambert@baker.edu.au).

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