Increased activation of the central benzodiazepine receptor (BZR) appears to play an important role in hepatic encephalopathy (HE). However, there is controversy regarding whether the density or affinity of BZRs is altered. A previous positron emission tomography (PET) study using the BZR antagonist \[^{11}C\]flumazenil (FMZ) found two- to threefold greater cerebral cortical tracer uptake in recurrent HE, but did not account for impaired FMZ metabolism due to liver disease or assess the relative contributions of tracer delivery versus BZR binding. We hypothesized that correcting for these factors would affect estimations of BZR binding in HE.

Nine patients with recurrent HE and 13 age-comparable controls were studied with \[^{11}C\]FMZ PET. After intravenous administration of \[^{11}C\]FMZ PET, arterial blood samples were collected, and PET images were acquired over 60 minutes. FMZ transport and binding maps were calculated for each subject by using a physiological tracer kinetic model. In agreement with the previous report, we found that FMZ reached a much higher level and was retained longer in the HE cerebral cortex despite similar total blood radioactivity levels in the two groups. However, the patients showed impaired hepatic metabolism of FMZ. After physiological modeling incorporating these data, significant increases in BZR binding were found in the thalamus (13%), cerebellum (20%), and pons (23%). There were minor, statistically insignificant increases in cerebral cortical (10%), putamen (12%), and whole brain (12%) BZR binding in patients with recurrent HE. These findings are in general agreement with results of autopsy studies, confirming a lack of major increases in cortical or basal ganglia BZR binding in HE. They emphasize that physiological tracer modeling should be used and altered peripheral radioligand metabolism considered in future PET studies of HE.

Hepatic encephalopathy (HE) is a disabling consequence of advanced liver disease, but the pathophysiology of HE remains uncertain. Several lines of evidence support a role for increased activity of the central benzodiazepine receptor (BZR) in HE. The BZR is a component of the \(\gamma\)-aminobutyric acid (GABA\(\_\alpha\)) chloride channel. Activation of the BZR results in an increased sensitivity of the GABA\(\_\alpha\) receptor to agonists and increased activity of the GABA\(\_\alpha\) chloride channel.

Increases in the density of GABA\(\_\alpha\) and BZRs have been shown in animals with hepatic failure induced by galactosamine. This model of HE also showed changes in the affinity of the GABA\(\_\alpha\) receptor with increasing severity of HE. Finally, the central nervous system concentrations of endogenous BZR agonists are elevated in rat and rabbit models of HE in fulminant hepatic failure. An increase in BZR tone has been implicated in HE in humans also. Endogenous benzodiazepine-like ligand ("endozapine") concentrations are increased in plasma, cerebrospinal fluid, and brain tissue of patients with chronic HE. The source of these circulating endogenous factors has not been determined, although colonic flora have been proposed as a possible source. Additionally, \[^{11}C\]flumazenil (FMZ) has been shown in clinical studies to reverse both acute and chronic HE.

Although activation of central BZRs appears to contribute to HE, the status of the receptor itself is unclear. There has been controversy about whether part of the increase in BZR activity in HE is due to a change in the density or agonist affinity of the BZR. Samson et al. reported the results of a positron emission tomography (PET) study using the BZR antagonist \[^{11}C\]FMZ in 4 patients with recurrent HE in which they found a two- to threefold increase in cerebral radioactivity compared with normal control subjects. They attributed the findings to an increase in density of central BZRs. However, Butterworth et al. in a carefully conducted postmortem study, found no increase in the affinity or density of GABA or BZRs in the brains of patients with alcoholic cirrhosis who died with grade IV HE.

The report from Samson et al. states that blood \(^{11}C\) potassium were "roughly" similar between HE and control subjects, but did not separate \[^{11}C\]FMZ from its radiolabeled metabolites in plasma. It has since been shown that metabolism of FMZ is impaired in patients with advanced liver disease. Intact FMZ readily crosses the blood-brain barrier. However, its major metabolite, the polar acid RO15-3890, has been shown to not cross the blood-brain barrier in human studies. If peripheral FMZ metabolism were impaired, it should result in an increase in intact plasma FMZ pool available for transport into the brain. Failure to correct PET data for arterial FMZ concentration in patients with altered hepatic func-
tion would then result in overestimation of BZR density. We considered that this was probably the reason for the increase in brain FMZ activity reported previously. In the present studies, we have extended these earlier FMZ PET observations with the application of a quantitative tracer kinetic method for estimation of FMZ binding to GABA_A receptors that accounts for effects on peripheral radiotracer metabolism by direct measurement and chromatography of arterial plasma.21-23

PATIENTS AND METHODS

Subjects. Nine patients with recurrent HE and 13 age-comparable controls were studied. The patients were all undergoing evaluation for liver transplantation and took lactulose daily for control of symptoms of chronic HE. Clinical details of these 9 patients are presented in Table 1. The severity of liver disease was estimated using the Child-Pugh score.24 Three patients had liver disease from ethanol, two had cryptogenic cirrhosis, and one each had autoimmune hepatitis, hepatitis C, primary sclerosing cholangitis, and type III glycogen-storage disease. Cirrhosis is an unusual sequela of type III glycogen-storage disease, and the clinical details of this patient have been reported previously.25 Five patients had transjugular intrahepatic portosystemic shunts (TIPS), and one had a distal splenorenal shunt for control of variceal hemorrhaging. Patency of the TIPS was confirmed by Doppler ultrasonography within 2 weeks of the study. No patient had received sedatives for at least 5 weeks before the study. All 9 had had recurrent episodes of acute or chronic HE, although none had greater than grade I HE on the day of the study.

There were 7 men and 6 women in the control group, all of whom were free of significant general medical, neurological, and psychiatric illness and had no history of first-degree relatives with familial neurological or psychiatric disease. The median age was 59 years (mean SD, 57 ± 7 years; range, 45-65 years) and was not significantly different from that in the patient group.

All subjects were screened to exclude history of head trauma with loss of consciousness, recent drug or alcohol abuse or dependence, smoking, excessive consumption of caffeine, and, in the case of control subjects, use of centrally acting medications within 12 months of participation. The studies were approved by the University of Michigan Institutional Review Boards governing studies in- ventations of the terminal FMZ concentrations were determined as previously described.21

The blood and PET data were analyzed according to a two-compartment, two-parameter tracer kinetic model, resulting in pixel-by-pixel parametric images of tracer transport (K_1) and binding (represented by the total tracer tissue distribution volume [DV]). Prior studies have identified the physiological sensitivity and specificity of these parameters and indicate a lack of confounding effects of cerebral blood flow on estimates.22,23,26

After calculation of the K_1 and DV images, we spatially deformed individual brains to a common reference anatomic format. The deformation procedure makes use of the K_1 image for anatomic feature definition and adjusts both the scale and the shape of each brain with sequential application of linear and nonlinear thin-plate spline deformations as described previously.23 Average images of K_1 and of DV were then constructed for the HE and control groups. These were sampled according to prospectively designated regions of interest (ROIs), and were additionally explored on a voxel-by-voxel basis for unanticipated local differences.28

Statistics. Blood FMZ and metabolism indices and brain ROI data were assessed with pairwise Student’s t tests. Exploratory pixel-by-pixel analyses employed correction for multiple parallel comparisons, assuming 9-mm^3 resolution in the reoriented images, corresponding to approximately 980 independent gray-matter resolution elements in the PET images.29 A statistical threshold of P < .05 was used to identify significant group differences in all analyses.

RESULTS

The integrated arterial 11C activity from unmetabolized, intact [11C]FMZ was similar for the two groups over the hour of the PET imaging study (Table 2). However, the arterial 11C activity due to polar, radiolabeled, FMZ metabolites was significantly greater in the control group. When arterial tracer time courses were examined in detail, substantial elevations of the terminal FMZ concentrations were observed in HE, despite similar total arterial radioactivity levels in the two groups (Fig. 1). At 60 minutes postinjection, the percentage of arterial 11C activity due to authentic FMZ was almost 70% higher in the patients than in the control group.

Inspection of cerebral tracer time activity curves revealed characteristic shape differences between HE and control subjects, with relatively greater peak tracer uptake and more

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**Table 1. Clinical Details of 9 Patients With Recurrent HE Studied With [11C]FMZ PET**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Sex</th>
<th>Etiology</th>
<th>PT (s)</th>
<th>Albumin (g/dL)</th>
<th>Bilirubin (μmol)</th>
<th>Ascites</th>
<th>Encephalopathy</th>
<th>Child-Pugh Score</th>
<th>Shunt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33/M</td>
<td>Glycogen-storage disease type 3</td>
<td>14</td>
<td>3.6</td>
<td>17</td>
<td>Mild</td>
<td>Mild</td>
<td>5</td>
<td>TIPS</td>
</tr>
<tr>
<td>2</td>
<td>38/M</td>
<td>Ethanol</td>
<td>13.6</td>
<td>3.3</td>
<td>17</td>
<td>Mild</td>
<td>Mild</td>
<td>6</td>
<td>TIPS</td>
</tr>
<tr>
<td>3</td>
<td>56/F</td>
<td>Primary sclerosing cholangitis</td>
<td>13.7</td>
<td>3.6</td>
<td>31</td>
<td>Mild</td>
<td>Moderate</td>
<td>6</td>
<td>TIPS</td>
</tr>
<tr>
<td>4</td>
<td>62/F</td>
<td>Hepatitis C</td>
<td>14.1</td>
<td>3.4</td>
<td>20</td>
<td>Moderate</td>
<td>Moderate</td>
<td>8</td>
<td>TIPS</td>
</tr>
<tr>
<td>5</td>
<td>60/F</td>
<td>Cryptogenic</td>
<td>15.8</td>
<td>3.0</td>
<td>49</td>
<td>Moderate</td>
<td>Moderate</td>
<td>9</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>49/M</td>
<td>Cryptogenic</td>
<td>15.2</td>
<td>3.5</td>
<td>22</td>
<td>Marked</td>
<td>Moderate</td>
<td>9</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>53/F</td>
<td>Autoimmune chronic hepatitis</td>
<td>12.4</td>
<td>2.8</td>
<td>54</td>
<td>Mild</td>
<td>Severe</td>
<td>11</td>
<td>Distal splenorenal</td>
</tr>
<tr>
<td>8</td>
<td>53/M</td>
<td>Ethanol</td>
<td>14.6</td>
<td>3.0</td>
<td>51</td>
<td>Marked</td>
<td>Moderate</td>
<td>11</td>
<td>TIPS</td>
</tr>
<tr>
<td>9</td>
<td>52/M</td>
<td>Ethanol</td>
<td>20.5</td>
<td>2.1</td>
<td>75</td>
<td>Marked</td>
<td>Moderate</td>
<td>14</td>
<td>No</td>
</tr>
</tbody>
</table>

Abbreviation: PT, prothrombin time.
protracted retention in HE (Fig. 1C), similar to those previously reported. However, after compartmental physiological model applications to the activity curves, there were no significant differences either in whole brain or in regional FMZ $K_1$ in HE versus the normal control groups (Table 3). There was a trend toward increased whole brain average $[^{11}C]$FMZ DV in patients with recurrent HE that was not statistically significant ($P = .065$; Table 3, Fig. 2). Additionally, there was no correlation between the severity of liver disease (Fig. 3) or the presence of a portosystemic shunt and whole brain $[^{11}C]$FMZ DV. When specific ROIs were examined, patients with HE showed modest (13%-23%) but statistically significant increases in $[^{11}C]$FMZ DV in the thalamus, cerebellum, and pons (Fig. 4, Table 3). Although there were trends toward increased FMZ DV (10%-12%) in the putamen and cerebral cortex of the patient group, these were not statistically significant. The changes in the putamen were representative of those occurring elsewhere in the basal ganglia. The voxel-by-voxel analysis of FMZ DV confirmed that the largest between-group differences were in the thalamic and cerebellar regions but did not suggest additional areas of change that had been overlooked in the a priori ROI analyses.

### DISCUSSION

The metabolism of drugs, including tracers such as $[^{11}C]$FMZ, may be impaired in patients with recurrent HE. We found increased arterial FMZ levels in the later phases of PET imaging sessions and reduced levels of polar FMZ metabolites in the plasma of patients with HE. These changes indicate either impaired metabolism of tracer doses of $[^{11}C]$FMZ or altered peripheral volumes of distribution for FMZ and its metabolites. Decreased peripheral FMZ metabolism has recently been reported in patients with advanced liver disease and is the most likely explanation for these changes. Whereas our patient group showed a trend toward greater integrated $[^{11}C]$FMZ plasma activity over the 60 minutes of the study, the reduction in plasma metabolite activity was more obvious. The most significant effect of altered FMZ metabolism was a greater percentage of circulating $^{11}$C activity attributable to $[^{11}C]$FMZ in patients than in controls during the last half of the PET sessions.

Previously reported increases in cerebral cortical $[^{11}C]$FMZ uptake observed with PET in patients with HE have been interpreted as evidence of increased BZR availability. Our data reproduce the prior observation of comparable total blood activity levels and distinctly different cerebral tracer activities in HE. However, when the arterial plasma concentrations of unmetabolized tracer are used in physiological estimations of $[^{11}C]$FMZ transport and BZR binding, the two- to threefold increases in binding suggested by the cerebral activity levels alone are now shown to reflect predominantly the effects of elevated and protracted blood tracer availability in HE. When the arterial plasma concentration of unmetabolized $[^{11}C]$FMZ was used in the physiological calculations of $[^{11}C]$FMZ transport and binding, there were trends toward increased global (whole brain), cerebral cortical, and basal ganglial binding in HE that were not statistically significant and are considerably lower in magnitude than previously reported. Failure to correct cerebral tracer data for altered blood levels in this instance results in a substantial overestimation of FMZ binding, emphasizing the need to chromatographically determine arterial ligand concentrations in future PET studies of HE.

We found a trend toward increased global (whole brain)
### Table 3. [11C]Flumazenil Transport (K₁) and Binding (DV) for Whole Brain and Selected Regions of Interest in Control and Patient Groups

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>FMZ Transport (K₁)</th>
<th>FMZ Binding (DV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls (±SD)</td>
<td>Patients (±SD)</td>
</tr>
<tr>
<td>Cerebral Cortex</td>
<td>0.349 (0.077)</td>
<td>0.392 (0.062)</td>
</tr>
<tr>
<td>Putamen</td>
<td>0.368 (0.082)</td>
<td>0.420 (0.068)</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.448 (0.106)</td>
<td>0.509 (0.072)</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.371 (0.081)</td>
<td>0.377 (0.055)</td>
</tr>
<tr>
<td>Pons</td>
<td>0.319 (0.076)</td>
<td>0.344 (0.062)</td>
</tr>
<tr>
<td>Global Gray matter</td>
<td>0.330 (0.066)</td>
<td>0.347 (0.047)</td>
</tr>
</tbody>
</table>

NOTE. Parametric estimates of FMZ transport (K₁) and binding (DV) from a 2-compartment kinetic model were sampled from stereotactically-placed ROI as described previously. Values represent means and SD for K₁ (mL plasma/mL brain/min) or DV (mL plasma/mL brain). Volume-weighted average of cerebral cortical activity in the scanned field-of-view.

* Volume-weighted average of cerebral gray matter in the scanned field-of-view as determined on the basis of K₁ values.

† P < 0.05, ‡ P < 0.01, †† P = 0.065 vs. control.

FMZ binding in HE that was not statistically significant and was not correlated with the severity of liver disease in individual patients as gauged by their Child-Pugh scores. It should be noted, however, that 6 of the 9 patients had porto-systemic shunts, and the lack of correlation may be due to confounding favorable effects of the shunts on ascites and hepatic biochemical function. Additionally, the multifactorial nature of exacerbations of chronic HE is well recognized, and alterations in central BZR density or affinity might reflect recent biochemical fluctuations to a greater extent than the long-term effects of hepatic insufficiency. Nevertheless, our data do not support a major role of elevated whole brain BZR density in the clinical phenomenology of HE responsiveness to BZR antagonists. We found insignificant, minor trends toward increased FMZ binding in the cerebral cortex and basal ganglia of patients with HE. Our binding data are thus in general agreement with the findings of the autopsy study of Butterworth et al., which reported a lack of change in either the density or the affinity of BZRs in the cerebral cortex or caudate nucleus of patients who died of HE. Animal studies have also failed to detect a significant increase in the density of BZRs or GABA_A receptors in HE.

In contrast to cortical and basal ganglia BZR binding, we detected statistically significant increases in [11C]FMZ binding in the thalamus, pons, and cerebellum. These localized increases in [11C]FMZ binding could be due to a variety of factors. Only unbound (free) plasma FMZ is available to cross the blood-brain barrier. From this pool, only unmetabolized FMZ has been shown to cross the intact blood-brain barrier. FMZ has a relatively low binding of plasma protein binding; approximately 40% is plasma protein bound in normal volunteers. Although patients with advanced liver disease have been shown to have reduced plasma protein binding of [11C]FMZ, we believe that the potential increase of free plasma [11C]FMZ in the patient group has not had a major impact on our data. If increased free FMZ were important in distinguishing our groups, we would expect global rather than regional effects on DV. Furthermore, effects on both blood-brain transport (K₁) as well as FMZ binding (DV) should be observed in this instance. Our data indicate substantial regional variations in DV increases, from only 10% in the cerebral cortex to more than 20% in the pons and cerebellum and reveal only minor effects on K₁ (2%-14%) that appear maximal in the basal ganglia and thalamus, and least in the cerebellum where DV is most significantly increased. This means that significant permeability changes in the blood-brain barrier are unlikely.
FIG. 4. Group summation analyses of BZR binding in control and HE subjects. \([11C]FMZ\) DV maps from individual subjects were anatomically standardized and summed, resulting in group mean binding maps from patients (top row) and control subjects (middle row). Voxel-by-voxel comparison results in a \(t\)-statistical map of between-group differences (bottom row). Transaxial images, oriented parallel to the anterior commissure–posterior commissure line, are depicted from supraventricular (left column) to posterior fossa (right column) levels. Parametric FMZ DV (mL plasma/mL brain) and \(t\) values are represented in pseudocolor, according to the scale. Largest changes in FMZ DV are apparent in the cerebellum (right image column), whereas highest \(t\) values are observed in the ventral thalamus/hypothalamus (middle column) and cerebellum. Although the cerebellar, thalamic, and pontine DV changes are statistically significant in prospective ROI analyses, none of the voxel-by-voxel changes exceed the adjusted \(t\) threshold corresponding to the \(P<.05\) level of significance (calculated \(t\) threshold, 6.97).

The major pathway for metabolism of FMZ in normal human subjects is via ester hydrolysis, resulting in a carboxylic acid metabolite designated Ro 15-3890.\(^{32,33}\) Ro 15-3890 is more polar than FMZ and is therefore unable to cross the blood-brain barrier. Persson et al.\(^{20}\) directly verified this prediction in studies of the cerebral uptake of \([11C]Ro 15-3890\) in healthy volunteers, demonstrating no detectable entry into the brain after its systemic administration. Furthermore, we have confirmed in similar studies that the major radioactive metabolites of \([11C]FMZ\) do not enter the brain.\(^{21}\) Formation of a hydroxyl ester at the same position, or N-demethylation (potentially resulting in \(^{11}CO_2\)), are both theoretical metabolic pathways for FMZ, but evidence of either of these products has not been found in baboon or human \([11C]FMZ\) PET studies.\(^{32,33}\) Increased permeability of the blood-brain barrier to polar \([11C]FMZ\) metabolites or the production of abnormal, less polar labeled metabolites could lead to increased brain activity, mimicking increased BZR binding in HE. However, this effect, as with altered plasma protein binding, is predicted to result in global changes rather than the regional increases in FMZ DV we observed.

Our data indicate increased \textit{in vivo} binding of \([11C]FMZ\) in discrete subcortical brain regions, particularly the cerebellar cortex and thalamus, in HE. Under conditions of trace binding site occupancy, as conducted in our studies, the DV binding measure is proportional to the ratio of binding site density (Bmax) to affinity (Kd).\(^{21,23}\) Thus, our findings could indicate either increased binding site numbers or affinity in the thalamus and cerebellum. FMZ, a BZR antagonist, is not associated with shifts in binding affinity induced by other ligands of the GABA\(_A\) complex and is not generally recognized to show affinity differences in pathological neurodegenerative states. We thus believe that the most likely explanation for increased FMZ DV in our studies is increased BZR density. Previous PET measures of cerebral blood flow and glucose metabolism in HE have suggested subcortical increases, particularly in the cerebellum, thalamus, and caudate.\(^{34}\) Although we find no evidence of significant FMZ DV change in the caudate nucleus, the overlap of our binding data with the metabolic changes in these regions suggests that they may be preferentially involved in the cerebral response to hepatic failure.

In conclusion, we have shown that there is no significant increase in whole brain or in cerebral cortical \([11C]FMZ\) binding in patients with recurrent HE, although there are regional increases in the thalamus and cerebellar cortex. These regional changes are most likely a result of increases in BZR density, but parallel or independent increases in BZR affinity cannot be entirely excluded. Further study is now indicated to determine the cellular localization and neurochemical mechanisms responsible for the regional increases in central BZR binding in recurrent HE.

REFERENCES


