ORIGINAL ARTICLE

Reduced γ-Aminobutyric Acid$_A$–Benzodiazepine Binding Sites in Insular Cortex of Individuals With Panic Disorder

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Context: Benzodiazepine drugs are highly effective anxiolytic medications, but the role of the benzodiazepine–γ-aminobutyric acid$_A$–chloride ion channel macromolecular complex in the pathophysiologic mechanism of anxiety is not well understood. Previous human imaging studies have indicated involvement of specific regions of the brain in anxiety disorders, especially the frontal-prefrontal, temporal, and cingulate cortical and the limbic areas.

Objective: To identify potential abnormalities of brain benzodiazepine receptor binding number and distribution in anxiety disorders.

Setting and Participants: At the University of Michigan positron emission tomography facility, 11 individuals with *DSM-IV*–defined anxiety syndrome panic disorder were compared with 21 unaffected healthy control subjects.

Design and Main Outcome Measure: In a between-group comparison, we used positron emission tomography and the benzodiazepine receptor ligand flumazenil labeled with carbon 11 to assess the regional brain pattern of receptor binding.

Results: We observed decreased binding specifically in the insular cortex bilaterally. No binding abnormality was observed in any other brain region, and there was no evidence of abnormal cerebral blood flow anywhere in the brain. Individuals with panic disorder and comorbid depression, indicative of a more severe disorder, had the lowest binding. No significant correlations were observed for binding with age, sex, or duration of disorder.

Conclusions: A previous smaller study with the same ligand reported a probable binding abnormality in the right insula. Because γ-aminobutyric acid is a major inhibitory neurotransmitter in the brain and because benzodiazepines facilitate this effect of γ-aminobutyric acid, decreased benzodiazepine binding is consistent with localized brain activation (ie, loss of inhibition). Because the insula is strongly involved in visceral-somatic afferent and efferent function, activation of the insula is consistent with the occurrence of the physical symptoms prominently associated with panic disorder.

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ANXIETY DISORDERS ARE among the most common psychiatric syndromes. There is substantial evidence of the involvement of the benzodiazepine–γ-aminobutyric acid$_A$ (GABA$_A$)–chloride ion channel macromolecular complex in human anxiety states.[1] It is well established that benzodiazepine medication can reduce both normal and pathologic anxiety symptoms.[2,3] An inverse agonist of the benzodiazepine receptor, a β-carboline, is anxiogenic.[4] Saccadic eye movement responses to benzodiazepine administration are abnormal in individuals with panic disorder.[5] Some[6] but not all[7] studies have suggested that subjects with panic disorder (hereinafter referred to as panic subjects) have abnormal subjective and/or physiologic reactions to administration of the benzodiazepine antagonist flumazenil. The possibility of the existence of an endogenous ligand for the benzodiazepine receptor, which might be abnormal in pathological anxiety conditions, has been considered.[8] Clinical observation and some research[5] suggest that the sedative effects of benzodiazepines are affected by the intensity of anxiety being experienced, although other studies have not always supported that observation.[9] Animal models of anxiety support benzodiazepine receptor involvement as well.[10,11]

Thus, there is strong evidence implicating benzodiazepines in the effective treatment of anxiety states but uncertainty regarding the nature of the involve-

ment of the benzodiazepine receptor in the pathophysiologic mechanism of anxiety. To advance understanding of the nature of this involvement, we used positron emission tomography (PET) to compare the central nervous system distribution volume (DV) (proportional to receptor binding) and blood-brain barrier transport coefficient (proportional to cerebral blood flow) of the positron-labeled benzodiazepine receptor ligand flumazenil labeled with carbon 11 ([^{11}C]flumazenil) in people with the anxiety syndrome panic disorder with those in unaffected control subjects. Previous studies of panic disorder (eg, Reiman et al,[12] Fontaine et al,[13] and De Cristofaro et al[14]) most strongly implicate frontal-prefrontal and temporal cortical areas and the cingulate gyrus in the pathophysiologic features of panic disorder. Many of the structures of the limbic system are contained in these regions. We hypothesized that any abnormalities observed would be found in these regions.

METHODS

SUBJECTS

We studied 32 subjects, including 11 with active panic disorder, of whom 7 also had agoraphobia, and 21 healthy, unaffected control subjects. All participants underwent screening and diagnosis according to *DSM-IV* criteria, based on findings of the Structured Clinical Interview for *DSM-IV* administered by 1 of us (G.C.H.). Five of the panic subjects also had comorbid depression, including 1 with current major depressive disorder, 1 with current dysthymia, and 3 with major depressive disorder in remission at the time of study. Controls had no history of any psychiatric disorder and neither did any of their first-degree relatives. All participants denied lifetime diagnoses of alcohol or other drug abuse and were free of any substances known to affect PET results at the time of study. All participants except 1 with panic disorder were benzodiazepine naïve; that individual had last received a benzodiazepine medication more than 1 month before the study. All participants were free of any other medical disorders known to affect PET findings at benzodiazepine binding sites, and results of screening neurological examinations were normal. The sex distributions were 9 women and 2 men in the panic group, and 8 women and 13 men in the control group. Mean±SD ages were 31±8 years (range, 22-45 years) for the panic group and 31±8 years (range, 18-44 years) for the control group. For the panic group, the mean±SD interval since the initial diagnosis was 74±64 months (range, 6-184 months). The mean±SD duration of current panic episodes was 15±13 months (range, 1-36 months). The study was approved by the University of Michigan institutional review board and the Committee for the Use of Radioactive Isotopes in Humans. After a full explanation of the study, all participants gave written informed consent.

PET DATA ACQUISITION

The PET data were acquired in the 3-dimensional mode (septa retracted) on a commercially available scanner (ECAT EXACT-47; Siemens-CTI, Knoxville, Tennessee), which images a 15.8-cm axial field of view and permits reconstruction of 47 contiguous 3.375-mm-thick tissue sections. Participants were comfortably positioned supine in the gantry of the scanner with eyes and ears unoccluded. They underwent radial artery and contralateral antecubital venous catheterizations approximately 30 minutes before scanning.

All PET sessions were performed with the infusion equilibrium technique previously developed in our laboratory.[15] Benzodiazepine receptor determination began after intravenous administration of a bolus containing 45% of the total administered [^{11}C]flumazenil dosage, followed by continuous infusion of the remaining tracer at a constant rate for 60 minutes. The total dose administered to each subject was approximately 0.025 Ci (approximately 9.25×10^8Bq) of [^{11}C]flumazenil containing less than 27 µg of mass. Arterial plasma sampling and dynamic emission scanning of the brain were conducted for 60 minutes, with the scan duration sequence of 4×0.5, 3×1, 2×2.5, 4×5, and 3×10 minutes. Fiduciary radioactive markers were affixed to each subject's scalp before tracer injection, and the original dynamic PET data were realigned to a predefined common orientation. An automated routine located the positions of the markers in individual images during the sequence and then reoriented each image to correct for subject motion (drift).

Blood samples were obtained at 10-second intervals throughout the first 2 minutes and again at 3, 5, 7.5, 10, 20, 30, 40, 50, and 60 minutes. Samples at 1 and 2 minutes and thereafter were processed chromatographically to determine authentic, unmetabolized flumazenil levels after the addition of tritiated flumazenil as an internal standard for recovery.[16] The image and plasma data were analyzed with a 2-compartment tracer kinetic model, resulting in the voxel-by-voxel calculation of the blood-to-tissue transport rate (K_1, calculated as milliliters of blood per minute per milliliter of brain tissue, proportional to the product of cerebral blood flow and capillary surface area).[16] The tissue DV (calculated as milliliters of plasma per milliliter of brain tissue, proportional to benzodiazepine binding site density) was determined from the final 30 minutes of data, when a steady-state level of tracer in arterial plasma and the brain was achieved, as described previously.[15]

STATISTICAL DATA ANALYSIS

Population-averaged binding maps were produced with voxel-by-voxel stereotactic procedures for binding-site summation analyses. Each parametric image set was scaled spatially and transposed into a common stereotactically defined coordinate space by a sequence of operations. First, rotation and tilt in the images were evaluated and the interhemispheric plane was identified. The location of the line joining the anterior and posterior commissures (AC-PC line) was estimated, followed by linear translation, rotation, and image scaling to the coordinate space defined by the human brain atlas of Talairach and Tournoux.[17] Finally, gray matter boundaries in the images were adjusted to those of the stereotactic reference by nonlinear (plastic) image deformation, using a 3-dimensional extension of the thin-plate spline transformation.[18] The individual-subject DV maps were then normalized to the pons DV, whereas the K_1 maps were normalized to the global mean cerebral and cerebellar gray matter K_1 (weighted by volume). The pons was used for DV normalization because previous research indicates that this region demonstrates predominantly nonspecific binding.[19]

Regions of interest (ROIs), positioned as specified by the atlas,[17] were then sampled from the PET data. Specifically, based on previous findings[12-14] in panic disorder, we analyzed the frontal cortex (Brodmann areas [BAs] 8-11 and 44-47), the cingulate cortex (BAs 23, 24, 26, and 29-31), the mesial temporal cortex (BAs 27, 28, 34-36, and 38), and the lateral temporal cortex (BAs 20-22, 34, and 42). Other regions assessed by the ROI method, hypothesized to be unaffected, included the parietal cortex (BAs 5, 7, 39, and 40), the occipital cortex (BAs 17-19), the primary sensorimotor cortex (BAs 1-4 and 6), and the caudate nucleus, putamen, thalamus, cerebellar hemisphere, and cerebellar vermis.

Table. Normalized Flumazenil Transport and Binding Parameters in the Indicated Regions of Interest[a]

	Binding Parameters, Mean ± SD			
	Subjects With Panic Disorder (n = 11)		Healthy Control Subjects (n = 21)	
Region of Interest	K_1	DV	K_1	DV
Anterior cingulate cortex	1.10 ± 0.04	5.49 ± 0.69	1.11 ± 0.02	5.45 ± 0.57
Frontal cortex	0.95 ± 0.04	4.69 ± 0.57	0.92 ± 0.03	4.82 ± 0.45
Sensorimotor cortex	0.96 ± 0.03	4.52 ± 0.54	0.98 ± 0.05	4.54 ± 0.54
Parietal cortex	1.00 ± 0.03	4.95 ± 0.63	1.01 ± 0.03	4.91 ± 0.61
Lateral temporal cortex	0.97 ± 0.03	5.02 ± 0.67	0.97 ± 0.03	5.03 ± 0.52
Mesial temporal cortex	0.80 ± 0.04	4.15 ± 0.59	0.78 ± 0.03	4.30 ± 0.40
Occipital cortex	1.10 ± 0.07	5.42 ± 0.70	1.15 ± 0.05	5.30 ± 0.64
Caudate nucleus	1.01 ± 0.08	2.82 ± 0.37	1.03 ± 0.08	2.96 ± 0.33
Putamen	1.13 ± 0.09	3.15 ± 0.35	1.12 ± 0.06	3.20 ± 0.33
Thalamus	1.14 ± 0.08	2.84 ± 0.39	1.10 ± 0.08	2.87 ± 0.33
Cerebellar hemisphere	1.10 ± 0.05	4.05 ± 0.63	1.10 ± 0.10	3.96 ± 0.61
Cerebellar vermis	1.03 ± 0.05	3.76 ± 0.56	1.01 ± 0.10	3.62 ± 0.52

Abbreviations: DV, distribution volume (calculated as milliliters of plasma per milliliter of brain tissue, proportional to ligand binding); K_1, blood-to-tissue transport rate constant (calculated as milliliters of blood per minute per milliliter of brain tissue, proportional to cerebral blood flow).

[a] Brodmann areas used to define cerebral cortical regions are indicated in the "Statistical Data Analysis" subsection of the "Methods" section. DV data are normalized within-subject to that of the pons, and K_1 data are normalized within-subject to the whole-brain gray matter average. No region differs between the panic disorder and control groups.

Normalized transport and binding maps were additionally analyzed on a voxel-by-voxel basis. The voxelwise analysis was constrained to include only the 21 068 voxels in the following a priori hypothesized ROIs (as defined in the previous paragraph): frontal cortex, cingulate cortex, and mesial temporal cortex (including the hippocampus and amygdala). Parametric DV and K_1 maps were assessed for significant between-group differences using the Statistical Parametric Mapping 99 program (SPM99; Wellcome Department of Imaging Neuroscience, University College London, London, England) with a 2-sample *t* test.[20] Familywise error-corrected thresholds, which control the chance of 1 or more false-positive results, were found using a nonparametric permutation method, the Statistical Nonparametric Mapping Toolbox (SnPM; Biostatistics Department, School of Public Health, University of Michigan, Ann Arbor).[21] The nonparametric method has been found to be more powerful than the standard, random-field–theory parametric method.[22] Cluster-size inference was performed by defining contiguous voxels above a $P=.001$ intensity threshold.

Post hoc tests were used to assess any relationship between any brain region found to show abnormal binding in panic subjects, with sex, age, duration of panic disorder, duration of the present episode, and the presence vs absence of current or past comorbid depression. Unless otherwise indicated, data are expressed as mean±SD.

RESULTS

No panic subject experienced panic attacks during PET. Analyses of the [^{11}C]flumazenil K_1 parameter showed no significant group differences in mean gray matter transport (panic disorder group vs control group, 0.34±0.03 vs 0.32±0.07 mL of plasma per minute per milliliter of brain tissue). In ROI- (**Table**) and voxel-based analyses (not shown), there were no significant group differences in normalized K_1.

There was no significant group difference in the parametric [^{11}C]flumazenil DV of the pons (panic disorder group vs control group, 1.10±0.24 vs 1.13±0.19 mL of plasma per milliliter of brain tissue). Furthermore, there was no significant difference in global (weighted average) gray matter parametric [^{11}C]flumazenil DV (panic disorder group vs control group, 5.2±0.9 vs 5.4±0.9 mL of plasma per milliliter of brain tissue). Analyses of [^{11}C]flumazenil binding normalized within the subject to the pons showed no significant differences in the ROI analyses (Table).

The voxelwise analysis identified significant clusters in the left and right insular cortical regions (cluster sizes, k=67 and k=53 [$P=.02$ and $P=.03$, respectively, corrected for multiple comparisons]). **Figure 1** illustrates the regions of effect, and **Figure 2** shows a horizontal profile through these insular regions. Both of these clusters were significant also by their maximum intensities ($t=4.19$ [$P=.05$] for coordinates x=–38, y=–11, and z=16; $t=4.21$ [$P=.047$] for coordinates x=32, y=–2, and z=16), again after correction for multiple comparisons. No differences in global normalized DV were present (panic disorder group vs control group, 3.68±0.14 vs 3.73±0.09 mL of plasma per milliliter of brain tissue; $t=1.03$ [$P=.31$]).

Right and left insular regions found to be significant were used for the post hoc tests. Correlations between binding and age, total panic disorder duration, and duration of the current episode were not statistically significant. Inclusion of the 1 panic subject who had prior benzodiazepine exposure did not skew the results. The mean of binding (DV) in all panic subjects was 3.19 mL of plasma per milliliter of brain tissue (range, 2.42-4.31 mL of plasma per milliliter of brain tissue); the value for the benzodiazepine-exposed subject was 3.29 mL of plasma per milliliter of brain tissue.

The analyses of variance for the right and the left insulae as dependent variables, with diagnosis (all panic

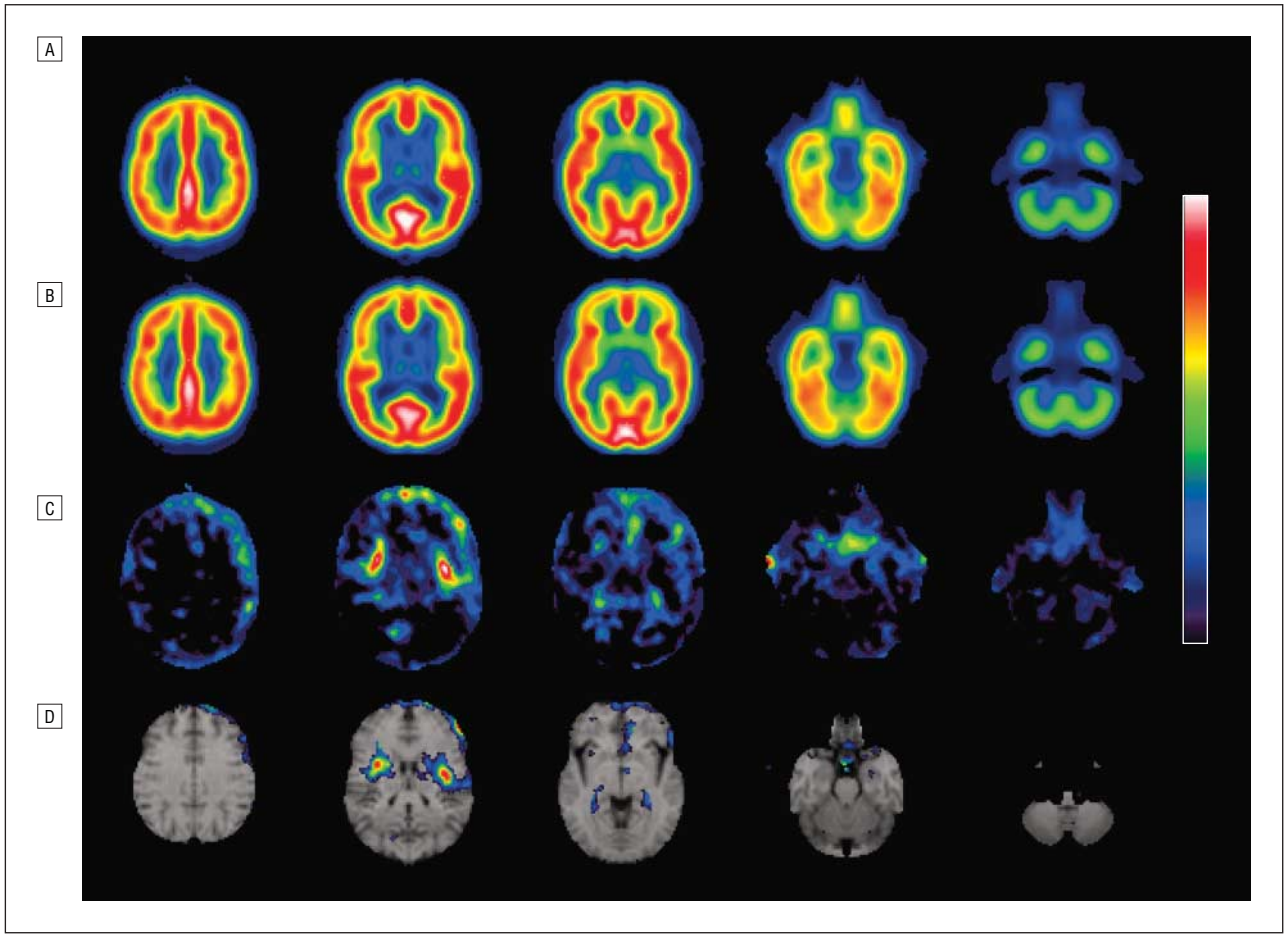

Figure 1. This figure presents binding images of flumazenil labeled with carbon 11 ([^{11}C]flumazenil) distribution volume (DV) (calculated as milliliters of plasma per milliliter of brain tissue, proportional to ligand binding) for control subjects and for subjects with panic disorder (panic subjects), as well as subtraction images derived from averaged images of the 2 groups and *t* statistical images for the subtraction images. A, Regional distribution of the average [^{11}C]flumazenil DV in 5 horizontal slices from 21 unaffected healthy control subjects. B, Average DV data from 11 panic subjects. C, Subtraction of the DV data of the panic subjects from those of controls, showing prominent localized decreased binding bilaterally in the insular cortex of panic subjects. D, Distribution of the *t* statistic depicting the panic disorder vs control differences, superimposed on a reference structural T1-weighted magnetic resonance image to facilitate localization. The parametric images are displayed in pseudocolor according to the scale at right, with highest values in white and red and lowest values in blue and violet. Flumazenil DV (rows A and B) ranges from 0 to 7 mL of plasma per milliliter of brain tissue. The difference images (row C) are depicted at a range of 0 to 1 mL of plasma per milliliter of brain tissue. The *t* statistical images (row D) are shown with an approximate level of .05 uncorrected threshold, windowed from 1.7 to 4.5.

subjects vs all controls) and sex as independent variables, showed robust effects for diagnosis (right insula, $F_{1,28}=7.69$ [$P=.01$]; left insula, $F_{1,28}=7.96$ [$P=.009$]). In these analyses of variance, sex did not approach statistical significance. In addition, when only female subjects were included, despite the decrease in sample size, when panic subjects were compared with controls, $t_{15}=2.76$ ($P=.01$) for the right insula and $t_{15}=2.86$ ($P=.01$) for the left.

Figure 3 shows individual binding scores for the right and left insulae for controls, panic subjects without depression, and panic subjects with present or past depression. For both insulae, the depressed panic subjects as a group appear to have lower binding than did nondepressed panic subjects. The analyses of variance for these 3 groups were performed for the right and left insulae. For the right insula, $F_{2,29}=8.66$ ($P=.001$); and for the left, $F_{2,29}=12.51$ ($P<.001$). Post hoc Fisher protected least significant difference tests demonstrated that, for the right insula, controls differed significantly from panic subjects without depression ($P=.02$) and from panic subjects with depression ($P<.001$). For the left insula, post hoc tests were significant for controls vs panic subjects with depression ($P<.001$), with a trend for controls vs panic subjects without depression ($P=.07$).

COMMENT

This study used PET and the positron-labeled antagonist of the benzodiazepine binding site, [^{11}C]flumazenil, to compare the number and distribution of brain benzodiazepine binding sites in panic subjects with those in controls. In all subjects, binding in all other specific regions analyzed was normalized to the pons, a brain region that demonstrates least specific binding of identifiable gray matter structures.[19] No significant difference in whole brain [^{11}C]flumazenil binding or in major structural regions was observed, but a focal decrease in the dorsal insular cortex was demonstrated on voxel-based analyses, supporting the hypothesis that an abnormality would be found in the frontotemporal cortex. Corti-

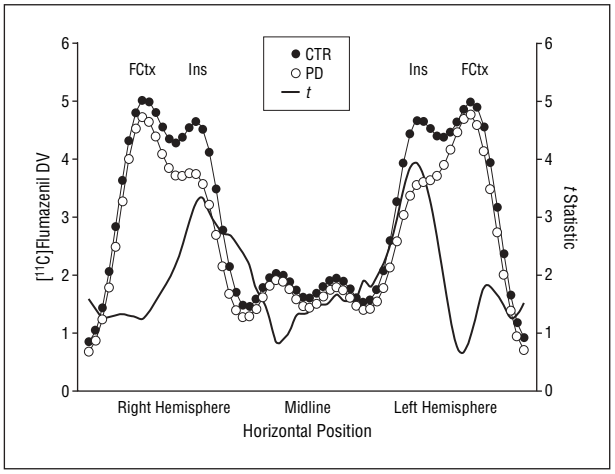

Figure 2. Horizontal profiles of the distribution volume (DV, calculated as milliliters of plasma per milliliter of brain tissue, proportional to ligand binding) of flumazenil labeled with carbon 11 ([11C]flumazenil) through the region of greatest difference in the left insula for panic disorder (PD) vs control (CTR) subjects, and the *t* statistic for the group difference. Data depict the group mean flumazenil DV from 21 control subjects and 11 subjects with panic disorder. FCtx indicates frontal cortex; Ins, insula.

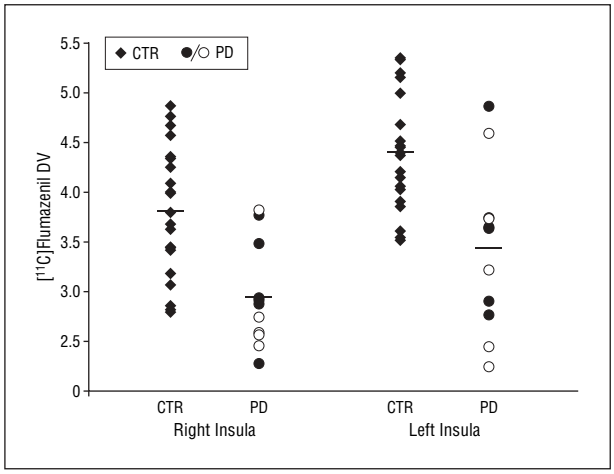

Figure 3. Individual subject distribution volumes (DVs, calculated as milliliters of plasma per milliliter of brain tissue, proportional to ligand binding) of flumazenil labeled with carbon 11 ([11C]flumazenil) for the right and left insulae for 21 control (CTR) subjects and 11 subjects with panic disorder (PD). Horizontal lines are at the mean for each group. Solid circles represent 6 subjects with PD who have no history of depression; open circles, 5 subjects with PD and a history or present diagnosis of major depressive disorder or dysthymia.

cal regions, including the insular cortex, which is contiguous with frontal, parietal, and temporal cortices, have high densities of [11C]flumazenil benzodiazepine binding sites.[16,19] No corresponding difference in the [11C]flumazenil transport coefficient (K_1) was seen, indicating that the apparent binding decrease is not merely attributable to a cerebral structural change (ie, atrophy). Age did not affect results. In the present and one previous study,[23] there were unequal percentages of male and female participants in the panic vs the control groups, but in neither study did this account for the differences observed. Finally, normalization to whole brain or to another specific brain region might have yielded different results, but use of a region with minimal specific binding is usually considered optimal, and the previous study[23] did not normalize to this region but observed an abnormality in the insula nonetheless.

Our results suggested that the difference between controls and panic subjects might have been due, at least in part, to comorbid depression present in some of the panic subjects. However, a previous single-photon emission computed tomography (SPECT) study of subjects with major depression[24] and a postmortem autoradiographic study of depressed suicide victims[25] did not find any binding abnormality. Previous studies[26] have reported that individuals with comorbid anxiety and depression have more severe anxiety disorders than those without comorbidity. It seems likely that the present findings represent a severity marker, with comorbid individuals having a more severe overall panic syndrome associated with a larger decrease in binding, and not an effect of depression per se.

Several previous studies have used SPECT and the SPECT benzodiazepine binding ligands iomazenil labeled with iodine 123 or NNC 13-8241 to assess the status of brain benzodiazepine binding sites in subjects with anxiety disorders.[27-34] Ligand binding measures were decreased in 5 of these 8 studies. However, there are inconsistencies in the apparent regions affected. In 1 previous study, increased iomazenil binding was reported. Finally, there was a lack of healthy controls in 3 of the studies. Potential explanations for these variable results include variable techniques for extraction of brain regional measures and use of imaging procedures that may not be entirely specific for ligand binding vs effects of ligand transport.

Two previous PET studies assessed benzodiazepine binding in panic disorder with [11C]flumazenil. One study[35] found no difference between 10 subjects with anxiety disorders, including 5 panic subjects, and controls in any of the several cortical regions analyzed or in the cerebellum. The insular cortex was not specifically reported. The other study[23] described a significant global decrease in whole brain benzodiazepine binding, with the largest decreases on the right side in the orbitofrontal cortex and the insula. These latter focal changes, however, did not achieve statistical significance.

The findings of the present study extend and partially confirm these observations with PET and [11C]flumazenil. In agreement with 1 of the previous PET studies,[23] we found decreases in insular cortex benzodiazepine binding, which in our study were statistically significant, localized, and bilateral. However, we found no evidence of a significant global binding reduction or other significant focal changes in cerebral cortical benzodiazepine binding. The present results agree with the findings of the other PET study[35] that there is no frontal cortical or whole cortex decrease in [11C]flumazenil binding. Thus, the one consistent finding among the regions assessed by [11C]flumazenil PET in these 3 studies is a decrease in binding in the insular cortex. Although the temporal lobe region was reported to be abnormal in several of the SPECT studies, none commented specifically on the insular region. The present study has more statistical power than either of the other 2 previous PET [11C]flumazenil studies, involving larger panic (11 vs 7 and 5) and control (21 vs 8 and 10) groups. The differ-

ence in whole brain results between the present study and the previous one that reported whole brain differences[23] might reflect a difference in methods. That study used a bolus injection of [^{11}C]flumazenil, whereas the present study used a steady-state method involving an initial bolus plus a continuous infusion during imaging.

Because of differences in the specific anxiety disorders studied, control groups involved, image methods and ligands used, and brain regions reported, the results obtained are difficult to compare or to interpret. It will be necessary to have more comparable studies before additional questions can be addressed, such as whether there are differences in brain regions involved in different anxiety disorders or in anxiety disorders comorbid with other syndromes, or whether abnormalities in particular brain regions can be associated with other aspects of the disorder such as symptoms or syndrome severity or treatment response.

Several previous studies have reported changes in cerebral blood flow associated with anxiety, including panic disorder,[36] although methods and findings among studies differ. Structural abnormalities have also been reported in the brains of individuals with panic disorder, including the temporal lobe.[13,37] The present study did not replicate such findings. A normal K_1 parameter in this study indicates that, in the panic subjects studied herein, there was no evidence of any change in cerebral blood flow and, thus, no evidence of atrophic or other structural changes. In other words, macroscopic structural changes do not appear to account for the decrease in benzodiazepine receptor binding that was observed. Furthermore, normal cerebral blood flow, coupled with the absence of any panic attacks during the study, suggests that subjects were at most mildly anxious during scanning.

The finding of decreased [^{11}C]flumazenil binding indicates that there is a decreased number of unoccupied benzodiazepine binding sites in the insular cortex in panic disorder at the time of imaging (ie, not during actual attacks). As already noted, this is unlikely to be due to tissue loss. Because none of the controls had prior benzodiazepine exposure and only 1 panic subject had such exposure (several weeks before the study and with typical results) and because subjects from neither group had substantial alcohol exposure, decreased numbers of binding sites due to pharmacologically induced receptor downregulation is unlikely to account for the results. Possible explanations include (1) increased benzodiazepine receptor occupancy by some other substance (eg, an endogenous ligand), (2) a decreased number of neurons or synapses bearing benzodiazepine–$GABA_A$–chloride ion receptor binding sites (despite the lack of detectable macroscopic atrophy), and/or (3) a decreased number or affinity of binding sites per neuron or synapse. The results of this study cannot determine which of these possibilities is correct. Finally, it cannot be determined whether the development of decreased benzodiazepine binding is associated with the underlying pathophysiologic mechanism of panic disorder (a possible cause of panic) or is the result of panic attacks per se (an effect). Future studies of individuals at risk for panic disorder or who have only very recently developed panic disorder might aid in resolution of this question, as might postmortem studies.

A different anxiety disorder, posttraumatic stress disorder, did not show this abnormality of [^{11}C]flumazenil binding.[38] Using SPECT and [^{123}I]iomazenil binding, one previous posttraumatic stress disorder study[39] reported decreased binding in the prefrontal cortex but another study[40] did not find any abnormality. In addition, a binding study of a serotonin 1A receptor ligand[41] reported decreased binding in the cingulate and raphe, but specifically not in the insula. The results of these studies indicate that, among the ligands and disorders assessed, this insular abnormality is specific to [^{11}C]flumazenil benzodiazepine ligand binding and to panic disorder.

Previous studies have implicated the insular cortex in panic disorder.[42,43] There is substantial plausibility that the insula would be involved in panic disorder and possibly in anxiety more generally. The insula, often considered part of the limbic system,[43] is highly involved in visceral-autonomic sensory (and motor) function, and anxious individuals commonly demonstrate symptoms referable to the gastrointestinal tract and, especially, the cardiovascular and respiratory systems and physiologic changes associated with these systems.[44,45] Because GABA is a major inhibitory neurotransmitter in the central nervous system,[3] a decrease in benzodiazepine binding associated with a decrease in the inhibitory functioning of the benzodiazepine–$GABA_A$–chloride ion channel macromolecular complex would be consistent with the prominence of such symptoms and pathophysiologic changes in anxiety states.

A number of studies have investigated the potential molecular mechanisms of involvement of GABA and benzodiazepines in animal models of anxiety. It has been reported[46] that mice with a knockin mutation of the $GABA_A$ receptor $\alpha 2$ subunit were insensitive to the anxiolytic effects of a benzodiazepine, whereas mutations of the $\alpha 3$ subunit were not. Modification of the $\gamma 2$ subunit can also affect anxietylike behavior.[47] Furthermore, several strains of mice with knockouts of the $serotonin_{1A}$ receptor produced changes in the $\alpha 1$ and $\alpha 2$ subunits of the $GABA_A$ receptor that, perhaps mediated by these $GABA_A$ changes, resulted in increased anxiety.[48] A knockout of the mouse $\alpha 2$ adrenergic receptor can also apparently affect anxietylike behavior.[49] Genetic research concerning this adrenergic receptor in anxiety is less well developed. There is evidence of abnormal function of the $\alpha 2$ adrenoreceptor in anxiety disorders.[50] Neurosteroid substances, including brain corticotropin-releasing hormone systems and adrenocortical feedback onto the brain,[51] as well as estrogen-related effects,[52] are potentially important because of their effects on GABA function and animal anxiety models.[53] These observations suggest the potential importance of GABA-related molecular mechanisms to the occurrence of anxiety, including anxiety disorders. All of these studies must be interpreted carefully, however, because it is not clear how relevant animal models of anxietylike changes are to human anxiety disorders.[10,54]

Despite the concern about animal models, the association between GABA and serotoninergic function does suggest the potential relevancy of changes in serotoninergic function to human anxiety disorders and the findings of the present study. Mice with serotonin knockouts have changes in bodily autonomic function,[55] raising

the possibility that serotoninergic changes are related, at least indirectly, to visceral-autonomic dysfunction and insular activity in anxiety disorders. A PET study in humans demonstrated abnormalities of serotoninergic receptors, but not of the insula, however.[41] Individuals with panic with agoraphobia had an association with a polymorphism of the serotonin 1A receptor.[56] Finally, serotonin 1A PET receptor binding was associated with anxiety symptoms in depressed individuals,[57] and a serotonin 1A polymorphism was associated with anxious and depressive personality traits.[58]

The amygdala is strongly involved in fear mechanisms,[59] thus potentially implicating it as well as the insula in anxiety and anxiety disorders,[60] including panic disorder.[61] Benzodiazepine receptors are present in the amygdala.[62,63] Benzodiazepines and $GABA_A$-active agents administered directly to the amygdala affect function in animal models of anxiety states,[60,61] including flumazenil.[64] Furthermore, there are functional and anatomical connections between the amygdala and the insula.[43,65] All of these findings suggest that benzodiazepine receptor binding might be expected to be abnormal in panic disorder. However, we did not find any such abnormality. These results do not imply that amygdaloid function is necessarily normal in panic disorder, only that no benzodiazepine receptor-binding abnormality was observed.

In conclusion, a number of imaging studies[12-14] of people with panic disorder, including the present investigation, have reported changes in the insula or in brain regions such as the temporal pole that could encompass and represent abnormalities specifically in the insular cortex, in most cases demonstrated under resting conditions (ie, not occurring specifically during panic attacks). Putative involvement of the insula has substantial face validity because of its known involvement in visceral-autonomic physiologic mechanisms and its linkages with limbic structures, including the amygdala. Decrease in benzodiazepine binding is consistent with hyperactivity of insular function. Further research is needed to verify this finding and to characterize the nature of this benzodiazepine–$GABA_A$–chloride ion receptor complex abnormality and its role in general in anxiety and anxiety disorders and specifically in panic disorder.

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