Benzodiazepine receptors mediate regional blood flow changes in the living human brain

($H_2^{15}O$ positron-emission tomography)

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ABSTRACT We studied the effects of a high-affinity γ-aminobutyric acid (GABA)-benzodiazepine-receptor agonist (lorazepam) and an antagonist (flumazenil) in humans, using $H_2^{15}O$ positron-emission tomography. Administration of lorazepam to healthy volunteers caused time- and dose-dependent reductions in regional cerebral blood flow and self-reported alterations in behavioral/mood parameters. Flumazenil administration reversed these changes. These observations indicated that benzodiazepine-induced effects on regional cerebral blood flow and mood/behavior are mediated at some level through GABA-benzodiazepine receptors, although the specific mechanism remains unclear. The approach described here provides a method for quantifying GABA-benzodiazepine-receptor-mediated neurotransmission in the living human brain and may be useful for studying the role of these receptors in a variety of neuropsychiatric disorders.

Benzodiazepines (diazepam and related compounds) have been widely used as antianxiety, sedative–hypnotic, anticonvulsant, and muscle-relaxant agents for over three decades. Their neural effects are mediated through specific receptors that modulate the effects of the major inhibitory neurotransmitter in brain, γ-aminobutyric acid (GABA) (1–3). These benzodiazepine receptors are part of a heterooligomeric receptor complex composed of membrane-spanning polypeptide subunits that form intrinsic chloride ion channels (4) and contain distinct binding sites for GABA as well as a number of sedative–hypnotic drugs. When benzodiazepines interact with their receptors, GABA-mediated chloride ion conductance is potentiated, and the net result is enhancement of GABA-mediated inhibitory synaptic events (5). While the binding of benzodiazepines to their receptors is clearly the initial step in the chain of events that culminates in the pharmacological actions of these drugs, little is known about the brain regions or neural circuits and neurotransmitters that may be involved in producing these effects.

Changes in the activity of neurons in a given brain region, whether excitatory or inhibitory, result in altered regional cerebral blood flow (rCBF) and substrate utilization (6). We reasoned that, given the widespread distribution of GABAergic neurons and their corresponding receptors, it would be possible to observe changes in rCBF by increasing or decreasing GABA-mediated inhibitory events. The structural association between benzodiazepine receptors and the GABA receptor itself, coupled with the relative safety of benzodiazepines, suggested an alternative way of studying GABAergic neurotransmission. In fact, previous work indicates that ad-

ministration of benzodiazepines is associated with decreases in rCBF (7, 8) and glucose utilization (9, 10), similar to the reported effects of GABA or GABA-receptor agonists in laboratory animals (11, 12).

Benzodiazepine receptors have been implicated in a number of neuropsychiatric disorders, including anxiety and panic disorder (11) and epilepsy (12). Their pathophysiologic role, however, remains unclear, due, at least in part, to a dearth of suitable methods for studying these receptors in the living human brain. To date, *in vivo* studies of benzodiazepine, as well as other receptor systems in humans, have consisted mainly of attempts to measure distribution patterns and receptor numbers using radiolabeled antagonists or agonists and positron-emission tomography (PET) (13). The role of such receptor "mapping" studies in the elucidation of causal mechanisms in neuropsychiatric disorders would be considerably enhanced if coupled with strategies that yield information about the functional "activity" of the receptors *in vivo*.

In this report, we describe one such approach. As in traditional pharmacological experiments, our strategy was to identify a quantifiable receptor-mediated (or receptor-associated) physiologic response by measuring the changes induced by selective receptor agonists or antagonists. We hypothesized that the administration of a benzodiazepine-receptor agonist would cause a dose-dependent decrease in rCBF which, if receptor-mediated, would be blocked or reversed by a selective receptor antagonist. To evaluate this hypothesis, we measured rCBF in normal human volunteers using $H_2^{15}O$ PET, before and after the administration of a benzodiazepine-receptor agonist and a selective benzodiazepine-receptor antagonist. Several scans could be obtained on each subject during a single study session because of the short half-life ($t_{1/2}$, 123 sec) of $H_2^{15}O$, and therefore we were able to perform time and dose–response studies in each subject using baseline measurements to quantify drug/receptor-induced alterations in rCBF.

MATERIALS AND METHODS

Subjects. The Clinical Research Subpanel of the National Institute of Mental Health and the Radiation Safety Committee of the National Institutes of Health approved the study protocol. Normal male volunteers [$n = 12$; mean age (SD), 31.7 (10.3) yr; handedness, right $n = 10$, left $n = 2$; mean weight (SD), 81.2 (10.6) kg] participated. Each subject gave informed consent. All were screened for a history of alcohol or drug

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Abbreviations: POMS, profile of mood states; rCBF, regional cerebral blood flow; PET, positron-emission tomography; ROI, regions of interest; WBBF, whole brain blood flow; GABA, γ-aminobutyric acid.
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abuse, psychiatric disorders, and significant medical problems and underwent a physical examination, routine blood work, urinalysis, and electrocardiogram. They were asked to avoid alcohol, medications of any kind, caffeine, and nicotine for 48 hr before the study and not to ingest anything except water for at least 6 hr before the study.

Benzodiazepine Drugs. Lorazepam (Ativan), a highly potent benzodiazepine-receptor agonist (a 3-hydroxy-1,4-benzodiazepine derivative), and flumazenil (Ro 15-1788, an imidazobenzodiazepinone compound), a selective benzodiazepine-receptor antagonist, were used. Flumazenil has no benzodiazepine-like actions, except in high doses, although it blocks or reverses all the known pharmacological effects of benzodiazepine agonists (14). Both drugs were administered i.v. (through an infusion pump; rate, 1 mg/min) to minimize the pharmacokinetic variability often encountered with other routes of drug administration.

Experimental Design. Each subject underwent a "sham" scan (in which the entire procedure for blood-flow measurement was done with normal saline instead of $H_2^{15}O$) and two resting $H_2^{15}O$ PET scans, followed by either time-course or dose–response studies. These serial PET scans were done at ≈15-min intervals so that residual radioactivity from the preceding scan was minimal. Baseline sensory conditions (eyes patched, dimmed room lighting, reduced noise) were imposed 15 min before the study started and continued until the end. Subjects were given no information about the sham nature of the first scan or the sequence of the other studies. Heart rate, blood pressure, and respiration were continuously monitored. Time–activity curves for $H_2^{15}O$ were obtained from arterial blood samples as described (15). Arterial pO_2, pCO_2, pH, standard bicarbonate, and plasma levels of lorazepam (16) were measured at the end of each scan. Plasma flumazenil levels were not determined.

PET Scans. These were done on a Scanditronix PC 1024-7B scanner (seven-slice; reconstructed in-plane resolution, 6.5 mm; axial resolution, 10–12 mm; slice thickness, 13.75 mm). The PET scan procedure and methods of data analysis have been described (15). In outline, whole brain blood flow (WBBF, ml·100 g^{-1}·min^{-1}) and rCBF (ml·100 g^{-1}·min^{-1}) values were obtained from regions of interest (ROI) placed on brain slices. Differences were demonstrated by comparing postdrug states to baseline values using the *t* test (two-tail). The Bonferroni correction for dependent means (17) was used to examine the relationships of the responses within ROIs. Inter-ROI relationships were not examined because such statistical inferences are of limited value in this type of experimental paradigm where a large number of measurements were made on a small number of subjects.

Time-Course Studies. After the sham and resting scans, each subject received an i.v. injection of lorazepam (*n* = 3; 60 μg/kg; maximum dose, 4 mg) or flumazenil (*n* = 4; 120 μg/kg). In each subject, five serial $H_2^{15}O$ PET scans were done at ≈15-min intervals, starting 2–5 min after the end of the drug infusion. In this way, the time course of the effect of a single i.v. dose of lorazepam or flumazenil was studied over a 60-min period.

Dose–Response Studies. After sham and resting scans, each subject (*n* = 5) was treated with four incremental doses of lorazepam (2.5, 5, 10, and 20 μg/kg). A $H_2^{15}O$ PET scan was done ≈5 min after each lorazepam dose; this time point was chosen for reasons of logistical practicality. A longer interval between lorazepam administration and cerebral blood flow measurement was not feasible because it would have prolonged the study considerably and decreased the probability of subject compliance. We note however that the responses measured using this paradigm are the effects of cumulative incremental doses of lorazepam, rather than a true dose–response study that would require the effects of the different drug doses to be studied in different subjects. Such a study

design would not have served our intent, which was to make within-subject assessments of the dose–response effects of lorazepam. About 20 min after administration of the highest dose of lorazepam (20 μg/kg), the subjects received flumazenil (80 μg/kg), and two $H_2^{15}O$ PET scans were done ≈2 and 17 min later.

Profile of Mood States (POMS). Detailed evaluation of the behavioral and cognitive effects in conjunction with the PET studies was not feasible because our experimental protocol required all subjects to be immobilized in the PET scanner with arterial and venous catheters and patched eyes for the study duration. Furthermore, there was a time constraint because the interval between the end of a scan and the start of the next scan was ≤15 min. Therefore, we administered a version of the POMS (18) immediately after the sham scan and after each subsequent scan. Customarily, a subject reads a list of 72 adjectives commonly used to describe momentary mood states from a questionnaire and provides a written response. In this study, the administration of the POMS was modified to suit the dictates of the protocol—i.e., the adjectives were read aloud to subjects, and they indicated how they felt at that moment, on a five-point scale, ranging from "not at all" (0 point) to "extremely" (4 points).

Although lorazepam has pronounced antianxiety and sedative effects, we anticipated difficulties in quantifying such responses in subjects who are immobilized with patched eyes and unaware of the type of drug being administered. We also expected considerable variability in the subjects' responses because the test setting and procedure were different from the usual situations in which the POMS is administered. Therefore, difference (difference = baseline minus postdrug) scores for each scale in each subject (at each time point or after each dose) were examined. These data were expressed as the percentage difference (difference/baseline × 100) from baseline values. In addition, the sum of the difference scores was also determined for each subject. These summed differences were expressed as percentage difference from baseline scores and used to quantify change in mood and behavior. Such an approach has been useful in revealing trends that might otherwise be obscured by artifactual variability attributable to test procedure and setting (C.-E.J., unpublished data).

RESULTS

Heart rate, blood pressure, and respiration remained within the normal range in all subjects at all times. Arterial pO_2, pCO_2, pH, and standard bicarbonate measured at the end of each scan showed no significant changes.

Effects on WBBF and rCBF. *Time course of lorazepam.* After i.v. administration of a single dose of lorazepam, plasma levels rose rapidly to 108 ng/ml at 5 min after i.v. administration and then gradually decreased (Fig. 1 *Inset*). WBBF fell below 80% of baseline values in 20 min ($P < 0.05$, *t* test, two-tail) and remained at this level over the next 40 min (Fig. 1). rCBF was significantly reduced in 80% of the regions sampled at 5 min and in all regions at 20 min (Table 1). These observations concur with findings in experimental animals and humans (19), which have shown that peak plasma levels occurred within 5 min after i.v. administration, while behavioral changes were slower in onset, with peak effects at 15–30 min.

Time course of flumazenil. In contrast, after the administration of a single dose of flumazenil, WBBF did not differ significantly from baseline values (Fig. 1). Approximately 15 min after i.v. flumazenil administration, rCBF increased in the cingulate and frontal areas, as well as in a few other cortical regions, and in the cerebellum and corpus striatum, although, interestingly, not in the thalamus, where lorazepam-induced decreases were most apparent (Table 1). This result concurs with studies in humans, using [11C]flumazenil PET (20), which
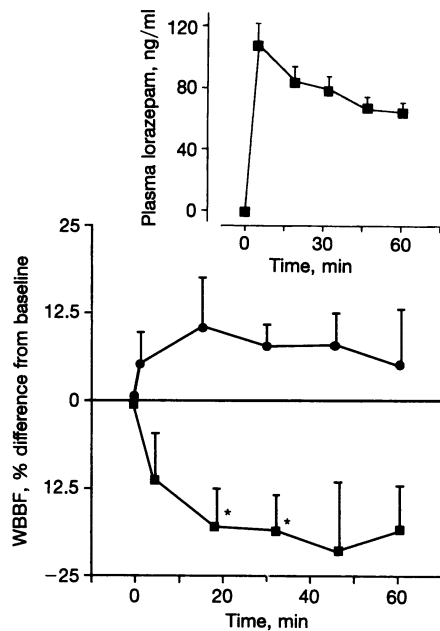
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FIG. 1. Time course: Effect of benzodiazepine administration on WBBF. Time (min) after i.v. benzodiazepine administration (lorazepam, 60 μg/kg; flumazenil, 120 μg/kg) is plotted on the abscissa; percentage difference from baseline values is plotted on the ordinate. WBBF values below and above baseline values are shown as negative (−) and positive (+) numbers, respectively. *, Significant difference from baseline ($P < 0.05$; t test, two-tail). Error bars represent the SEM. (*Inset*) Plasma concentration of lorazepam when WBBF was measured; all levels differed significantly from baseline. ■, Lorazepam (n = 3); ●, flumazenil (n = 4).

have shown that peak cerebral cortical levels of the radiotracer are reached in 5–10 min.

Dose response. As expected, mean plasma levels of lorazepam correlated significantly with the dose administered (correlation coefficient, 0.98; four doses; n = 5; $P < 0.001$) and remained elevated for >30 min after administration of the highest dose. Flumazenil administration did not affect plasma lorazepam level (Fig. 2C). WBBF decreased to ≈14% of the baseline value after the highest dose of lorazepam (Fig. 2A). The WBBF reduction correlated significantly with the dose of lorazepam (correlation coefficient, 0.99; n = 5; $P < 0.001$). After flumazenil administration, WBBF increased to the baseline level, and the plasma lorazepam level remained elevated. WBBF measurements at 2 and 17 min after flumazenil administration were significantly higher ($P < 0.05$, t test, two-tail) than WBBF measured after the highest dose (20 μg/kg) of lorazepam.

rCBF was reduced in a dose-dependent manner in the thalamus, cerebellum, corpus striatum, and some cortical areas, including the cingulate and frontal regions (Table 2). Flumazenil administration resulted in a return to near baseline (or slightly above) values in the regions sampled (Table 2) with significant increase only in the cingulate region. Comparisons (t test, two-tail) between the highest lorazepam dose (20 μg/kg) and the postflumazenil states showed significant increase in the cingulate and frontal areas, and in the thalamus, cerebellum ($P < 0.001$), and corpus striatum ($P < 0.01$) at 2 and 17 min after flumazenil administration.

Effects on POMS. Review of the POMS scores for individual subjects showed considerable variation in response. Consequently, no significant differences were seen when group scores (time course of lorazepam, time course of flumazenil; dose response) were examined by repeated measures ANOVA for each scale. The test setting and mode of administration of the POMS undoubtedly contributed to response variability to

Table 1. Time course: lorazepam and flumazenil

	rCBF, % difference from baseline									
	Time (min) after lorazepam administration					Time (min) after flumazenil administration				
ROI	5.0	19.2	32.8	46.9	60.9	1.7	16.1	31.2	46.5	61.3
Cingulate	−14.1†	−25.2‡	−23.4‡	−14.1‡	−25.2‡	16.1‡	19.5‡	17.0‡	12.7‡	7.0
	(4.0)	(2.8)	(2.8)	(5.0)	(2.9)	(3.5)	(4.5)	(2.7)	(2.4)	(5.3)
Frontal, left	−15.0*	−22.1‡	−24.2‡	−15.0‡	−22.1‡	4.9	13.0*	10.0‡	11.4‡	5.3
	(3.9)	(2.4)	(2.1)	(3.9)	(2.7)	(2.8)	(4.4)	(2.4)	(2.5)	(4.1)
Frontal, right	−16.9‡	−24.3‡	−26.8‡	−16.9‡	−24.3‡	4.2	11.6†	12.2‡	8.6‡	5.8
	(2.9)	(2.4)	(1.9)	((3.7)	(2.4)	(2.8)	(4.4)	(2.4)	(2.5)	(4.1)
Temporal, left	−10.8*	−19.9‡	−24.4‡	−10.8‡	−19.9‡	4.4	8.7†	4.1*	5.8	1.6
	(14.9)	(10.9)	(8.2)	(14.9)	(11.9)	(2.2)	(3.3)	(2.2)	(2.3)	(3.7)
Temporal, right	−11.1*	−19.7‡	−21.4‡	−11.1‡	−19.7‡	5.5	10.4†	5.1*	7.2†	2.5
	(4.0)	(3.3)	(2.6)	(4.2)	(3.2)	(2.6)	(3.2)	(1.7)	(2.3)	(3.4)
Parietal, left	−9.7	−16.5†	−18.7†	−9.7*	−16.5*	2.2	4.8	3.5	8.9	3.7
	(5.4)	(3.9)	(2.9)	(6.1)	(4.7)	(2.8)	(5.2)	(3.2)	(4.6)	(6.5)
Parietal, right	−10.6	−20.5*	−29.9‡	−10.6	−20.5*	5.6	4.7	7.5†	9.9†	5.2
	(5.1)	(3.6)	(1.7)	(5.9)	(4.1)	(2.9)	(3.1)	(2.0)	(2.8)	(5.8)
Occipital, left	−10.0*	−22.6‡	−16.2†	−10.0†	−22.6†	2.6	4.5	3.4	8.4*	4.0
	(3.5)	(4.1)	(3.3)	(5.6)	(4.3)	(3.2)	(3.4)	(2.3)	(3.3)	(3.9)
Occipital, right	−9.7*	−18.3‡	−15.5†	−9.7‡	−18.3†	1.4	8.8*	3.2	7.3*	2.0
	(3.1)	(3.2)	(3.4)	(6.1)	(3.9)	(2.9)	(3.2)	(2.8)	(3.1)	(3.2)
Thalamus	−16.8*	−24.1†	−29.8†	−16.8†	−24.1†	8.0	10.3	5.8	9.1	11.7
	(5.6)	(4.5)	(4.6)	(5.3)	(3.7)	(3.6)	(5.5)	(4.8)	(4.7)	(7.4)
Cerebellum	−17.8‡	−20.2‡	−22.5‡	−24.9‡	−19.3‡	5.2	16.2†	11.6‡	10.2‡	6.8
	(2.1)	(2.7)	(2.4)	(3.8)	(3.2)	(2.9)	(5.1)	(2.4)	(2.1)	(3.4)
Corpus striatum	−10.0†	−19.0‡	−19.9‡	−22.8‡	−17.5‡	6.8	14.0†	11.4‡	11.2†	6.4*
	(3.2)	(3.1)	(3.7)	(3.2)	(3.5)	(2.1)	(3.5)	(2.8)	(2.8)	(5.3)

Numbers represent means, and SEMs are in parentheses. Lorazepam dose was 60 μg/kg (n = 3); flumazenil dose was 120 μg/kg (n = 4). rCBF percentage difference from baseline is calculated as follows: (ROI baseline − ROI scan)/(ROI baseline) × 100. *, $P < 0.05$ (t test, two-tail); †, $P < 0.01$ (t test, two-tail) and $P < 0.05$ with Bonferroni correction for dependent means, five comparisons; ‡, $P < 0.002$ (t test, two-tail) and $P < 0.01$ with Bonferroni correction, five comparisons. Plasma levels of lorazepam corresponding to these time points are shown in Fig. 1 *Inset*.
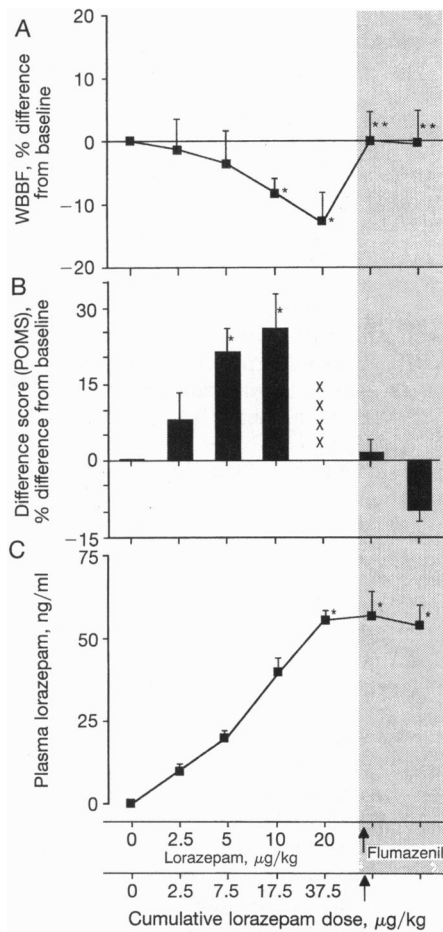
FIG. 2. In *A–C*, the abscissa represents i.v. administered incremental doses of lorazepam (2.5, 5, 10, and 20 μg/kg; cumulative doses are shown below each point) and flumazenil (one dose, 80 μg/kg). The gray area denotes points after flumazenil administration. Error bars represent SEMs. (*A*) Dose response: Effect of lorazepam and flumazenil on WBBF. WBBF values (depicted on ordinate) below and above baseline values are shown as negative (−) and positive (+) numbers, respectively. *, Significant decrease from baseline ($n = 5$; $P < 0.05$; *t* test, two-tail). **, WBBF at 2 and 17 min after flumazenil administration was significantly higher than after the preceding lorazepam (20 μg/kg) dose ($P < 0.05$; *t* test, two-tail). (*B*) Dose response: Effect of lorazepam and flumazenil on mood and behavior (POMS). The four vertical Xs denote that the subjects were drowsy and unable to do the POMS. For each dose in each subject, the difference from baseline for each POMS scale was summed to obtain a global difference score, expressed as percentage difference from baseline for that subject at that dose. Each column represents the mean percentage difference score for that dose. Differences below and above baseline values are shown as negative (−) and positive (+) numbers, respectively. *, Significant difference from baseline ($n = 5$; $P < 0.05$; *t* test, two-tail). (*C*) Dose response: Plasma concentration of lorazepam. The ordinate represents the corresponding plasma lorazepam concentration. All levels were significantly higher than baseline (zero). *, No significant difference between points.

the POMS because of stress related to the test situation. We emphasize however that benzodiazepine-induced changes in mood and behavior were consistently seen in all subjects, even though it was not possible to resolve the data into significant differences in individual POMS scales. Summed-difference scores (expressed as percentage difference from baseline) and the time taken to complete the POMS were used to quantify these observed effects.

Time course of lorazepam. After lorazepam administration, subjects were less alert and more confused than their baseline state. The time taken to complete the POMS increased after lorazepam administration [mean (SEM) min; sham and resting

Table 2. Lorazepam: Dose response and reversal with flumazenil

	rCBF, % difference from baseline					
	Lorazepam dose, μg/kg					
ROI	2.5	5.0	10.0	20.0	Postflumazenil	
Cingulate	−6.6*	1.9	−12.1‡	−16.1‡	7.1*	3.0
	(2.5)	(4.0)	(2.6)	(3.1)	(3.2)	(3.8)
Frontal, left	−2.9*	7.3	−9.9‡	−13.9‡	0.1	−1.3
	(1.3)	(4.0)	(2.4)	(3.1)	(2.9)	(2.9)
Frontal, right	−4.0	5.6	−10.5‡	−11.8†	1.9	−0.8
	(2.1)	(4.0)	(2.6)	(3.5)	(2.9)	(3.3)
Temporal, left	0.1	14.0*	−2.7	−6.7*	−2.5	1.9
	(2.5)	(6.2)	(2.8)	(3.1)	(2.2)	(2.9)
Temporal, right	−2.2	8.7	−6.4*	−9.3†	−3.5	0
	(2.3)	(5.2)	(2.9)	(2.4)	(1.8)	(2.5)
Parietal, left	−5.1	4.9	−8.9	−9.9	−3.3	−0.7
	(2.4)	(6.6)	(5.1)	(6.1)	(3.8)	(4.3)
Parietal, right	−9.1*	1.1	−7.7	−9.3	−2.7	−1.7
	(3.3)	(5.1)	(4.9)	(5.6)	(3.3)	(4.7)
Occipital, left	−3.2	18.8*	−3.6	−8.1*	−4.1	−3.5
	(2.5)	(7.4)	(3.7)	(3.6)	(2.9)	(4.9)
Occipital, right	−4.2	20.5*	−3.6	−10.3†	−5.4	−3.8
	(2.8)	(7.2)	(3.6)	(3.1)	(3.2)	(5.2)
Thalamus	−8.5	−7.4	−18.7‡	−23.1‡	0.9	2.7
	(4.0)	(4.1)	(2.6)	(3.4)	(5.6)	(5.3)
Cerebellum	−5.6*	1.7	−14.9‡	−14.8‡	2.5	3.4
	(2.0)	(5.0)	(1.3)	(1.8)	(3.9)	(3.1)
Corpus striatum	−4.6	2.9	−10.5‡	−11.6†	3.1	0.7
	(2.3)	(4.0)	(2.0)	(3.6)	(2.6)	(3.6)

Numbers represent means, and SEMs are in parentheses. rCBF percentage difference from baseline is calculated as follows: (ROI drug − ROI baseline)/(ROI baseline) × 100. *, $P < 0.05$ (*t* test, two-tail); †, $P < 0.00833 = P < 0.05$; and ‡, $P < 0.00166 = P < 0.01$ with Bonferroni correction for dependent means (six comparisons). Plasma levels of lorazepam measured after administration of each dose are shown in Fig. 2*C*.

scans, 4.6 (0.3); scans 3–7, 5.6 (0.01), 7.1 (2.1), 6.9 (2.2), 7.1 (2.1), 5.6 (1.6)], indicating that responses were delayed after lorazepam administration. Summed-difference scores [mean (SEM)] were significantly increased at 5 min [87.6 (15.3)], 19 min [63.4 (9.7)], 33 min [77.8 (12.4)], 47 min [71.4 (15.7)], and 61 min [70.7 (11.8)] min after lorazepam administration ($P < 0.01$ at all time points; *t* test, two-tail).

Time course of flumazenil. The administration of a single flumazenil dose produced no consistent mood or behavioral change, and no significant changes were demonstrated in summed-difference scores or the time taken to complete the POMS.

Dose response. All subjects showed obvious dose-dependent changes in mood and behavior (decreased alertness, delayed responses, increased confusion and fatigue) after lorazepam administration, which were rapidly reversed by flumazenil administration. The summed-difference score increased incrementally after each of the first three doses of lorazepam (2.5, 5, 10 μg/kg) (Fig. 2*B*). After the fourth dose (20 μg/kg), three subjects were too drowsy to take the POMS, and the remaining two were unable to complete the POMS.

Flumazenil was then i.v. infused at the rate of 1 mg/min. All subjects rapidly became alert (within 2 min) and remained thus until the end of the study ≈20 min later. The summed-difference score returned to near-baseline values and became even lower at the end of the last scan (Fig. 2*B*).

Subjects' response times became prolonged after administration of incremental doses of lorazepam. This result was reflected in the time required to complete the POMS [sham and resting scans; mean (SEM) min, 3.7 (0.3)], which increased after lorazepam administration [2.5 μg/kg, 4.1 (0.4) min; 5 μg/kg, 4.3 (0.4) min; 10 μg/kg [5.0 (0.8) min]. After fluma-

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zenil, the time taken to complete the POMS [3.8 (0.4), 3.4 (0.5) min] was comparable to baseline values. There were significant correlations between summed-difference scores and reduction in WBBF (correlation coefficient, 0.94; $n = 5$, $P < 0.01$) and the time taken to complete the POMS (2.5, 5, and 10 μg/kg; correlation coefficient, 0.94; $n = 5$; $P < 0.01$).

DISCUSSION

We have shown that administration of the benzodiazepine-receptor agonist lorazepam resulted in dose-dependent decreases in rCBF as well as alterations in mood and behavior. These parameters returned to near-baseline values after administration of the benzodiazepine-receptor antagonist flumazenil. The finding that flumazenil reversed the dose-dependent effects of lorazepam on rCBF and behavior indicated that the lorazepam-induced changes are mediated, at some level, through the GABA-benzodiazepine receptor. The specific mechanisms that cause lorazepam-induced changes in rCBF are unclear. These changes may be directly mediated through benzodiazepine receptors or, alternatively, they may be secondary to benzodiazepine effects on other neurotransmitter systems.

While the concept of studying the effects of pharmacological agents on blood flow in conjunction with measures of physiological changes is not entirely new (21), our study is distinct for the following reason. The strategy used here—i.e., the demonstration of benzodiazepine-receptor agonist-induced dose-dependent changes that can be reversed by administration of a benzodiazepine-receptor antagonist—has not, to our knowledge, been previously used.

In this report of regional benzodiazepine-receptor dependent effects in the intact human brain, the largest lorazepam-induced reductions in rCBF were seen in the thalamus. The density of benzodiazepine receptors is highest in the cerebral cortex, intermediate in the cerebellum and limbic structures, and lowest in the thalamus and brain stem (22, 23). This regional distribution suggests that the effects on rCBF occur "downstream" from the sites of maximal receptor density—i.e., benzodiazepines bind to their receptors and produce changes in the activity of neurons in regions removed from, but connected to, areas of high receptor density.

In our study, the administration of flumazenil produced an increase in rCBF in some cortical areas, including the cingulate region, an integral part of the limbic system, which is generally implicated in the regulation of a variety of emotional and viscerosomatic functions, including fear and anxiety (24). While the significance of this finding is not clear, it lends itself to speculation that administration of this benzodiazepine-receptor antagonist may result in a change in some endogenous benzodiazepine-like ligand (25).

Studies in humans, using the xenon-inhalation technique (26–28), have demonstrated benzodiazepine-induced changes in rCBF, although this method lacks the spatial resolution of PET. Several investigators have used [^{18}F]fluorodeoxyglucose PET to demonstrate decreases in cerebral glucose metabolism after administration of various benzodiazepines (29–32). The study designs were not comparable to our experimental paradigm, and regional changes were either not apparent or not statistically significant (31, 32).

In conclusion, our study attempts to replicate traditional pharmacological and biochemical approaches in living human brain and demonstrates the feasibility of using functional imaging techniques for *in vivo* pharmacological studies in humans. Similar paradigms using appropriate agonists and antagonists may be developed to study other neurotransmitter systems in humans. The approach presented here can be generalized to define a variety of relevant issues in humans, such as the mode of action of major tranquilizers, mechanisms of action of drugs of addiction, as well as pathophysiology of neuropsychiatric disorders. This method can also be combined with studies of the distribution, rate of synthesis, turnover, and concentration of neurotransmitters and their receptors, in strategies designed to study the influence of neurotransmission on cellular activity and, ultimately, human behavior.

1. Braestrup, C. & Squires, R. F. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3805–3809.
2. Möhler, H. & Okada, T. (1977) *Science* **198**, 849–851.
3. Haefely, W. & Polc, P. (1983) in *Anxiolytics: Neurochemical, Behavioral and Clinical Perspectives*, eds. Malick, J. B., Enna, S. J. & Yamamura, H. I. (Raven, New York), pp. 113–145.
4. Schofield, P. R., Darlison, M. G., Fujita, N., Burt, D. R., Stephenson, F. A., Rodriguez, H., Rhee, L. M., Ramachandran, J., Reale, V., Glencourse, T. A., Seeburg, P. H. & Barnard, E. A. (1987) *Nature (London)* **328**, 221–227.
5. Olsen, R. W. (1982) *Annu. Rev. Pharmacol. Toxicol.* **22**, 245–277.
6. Sokoloff, L. (1981) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40**, 2311–2316.
7. Rockoff, M. A., Naughton, K. V. H., Shapiro, H. M., Ingvar, M., Ray, K. F., Gagnon, R. L. & Marshall, L. F. (1980) *Anesthesiology* **53**, 215–218.
8. Kelly, P. A. T., Ford, I. & McCulloch, J. (1986) *Neuroscience* **19**, 257–265.
9. Kelly, P. A. T. & McCulloch, J. (1982) *J. Neurochem.* **39**, 613–624.
10. Palacios, J. M., Kuhar, M. J., Rapoport, S. I. & London, E. D. (1981) *Eur. J. Pharmacol.* **71**, 333–336.
11. Nutt, D. J., Glue, P., Lawson, C. & Wilson, S. (1990) *Arch. Gen. Psychiatry* **47**, 917–925.
12. Savic, I., Roland, P., Sedvall, G., Persson, A., Pauli, S. & Widen, L. (1988) *Lancet* **ii**, 863–868.
13. Blomquist, G., Pauli, S., Farde, L., Eriksson, L., Persson, A. & Halldin, C. (1990) *Eur. J. Nucl. Med.* **16**, 257–265.
14. Bonetti, E. P., Pieri, L., Cumin, R., Schaffner, R., Pieri, M., Gamzu, E. R., Muller, R. K. M. & Haefely, W. (1982) *Psychopharmacology* **78**, 8–18.
15. Matthew, E., Andreason, P., Carson, R. E., Herscovitch, P., Pettigrew, K., Cohen, R., King, C., Johanson, C.-E. & Paul, S. M. (1993) *J. Cereb. Blood Flow Metab.* **13**, 748–754.
16. Greenblatt, D. J., Franke, K. & Shader, R. I. (1978) *J. Chromatogr.* **146**, 311–330.
17. Miller, R. G., Jr. (1966) *Simultaneous Statistical Influence* (McGraw-Hill, New York), pp. 67–70.
18. McNair, D., Lorr, M. & Droppleman, L. (1971) *Profile of Mood States: Manual* (Educational and Industrial Testing Service, San Diego).
19. Miller, L. G., Greenblatt, D. J., Paul, S. M. & Shader, R. I. (1987) *J. Pharmacol. Exp. Ther.* **240**, 1516–1522.
20. Pappata, S., Samson, Y., Chavoix, C., Prenant, C., Maziere, M. & Baron, J. C. (1988) *J. Cereb. Blood Flow Metab.* **8**, 304–313.
21. Grasby, P. M., Friston, K. J., Bench, C. J., Frith, C. D., Paulesu, E., Cowen, P. J., Liddle, P. F., Frackowiak, R. S. J. & Dolan, R. (1992) *Eur. J. Neurosci.* **4**, 1203–1212.
22. Braestrup, R. F., Albrechtsen, R. & Squires, R. E. (1977) *Nature (London)* **269**, 702–704.
23. Young, W. S. & Kuhar, M. J. (1979) *Nature (London)* **280**, 393–395.
24. Papez, J. W. (1958) in *Reticular Formation of the Brain*, ed. Jasper, H. H. (Little, Brown, Boston), p. 591.
25. Rothstein, J. D., Garland, W., Puia, G., Guidotti, A., Weber, R. J. & Costa, E. (1992) *J. Neurochem.* **58**, 2102–2115.
26. Mathew, R. J., Wilson, W. H. & Daniel, D. G. (1985) *Biol. Psychiatry* **20**, 1109–1116.
27. Forster, A., Juge, O., Louis, M. & Nahory, A. (1987) *Anesth. Analg.* **66**, 309–313.
28. Mathew, R. J. & Wilson, W. H. (1991) *Psychiatry Res. Neuroimaging* **40**, 125–134.
29. Volkow, N. D., Wang, G.-J., Hitzemann, R., Fowler, J. S., Wolf, A. P., Pappas, N., Biegon, A. & Dewey, S. L. (1993) *Am. J. Psychiatry* **150**, 417–422.
30. Buchsbaum, M. S., Wu, J., Haier, R., Hazlett, E., Ball, R., Katz, M., Sokolski, K., Lagunas-Solar, M. & Langer, D. (1987) *Life Sci.* **40**, 2393–2400.
31. Foster, N. L., VanDerSpek, A. F. L., Aldrich, M. S., Berent, S., Hichwa, R. H., Sackellares, J. C., Gilman, S. & Agranoff, B. W. (1987) *J. Cereb. Blood Flow Metab.* **7**, 415–420.
32. de Wit, H., Metz, J., Wagner, N. & Cooper, M. (1991) *Neuropsychopharmacology* **5**, 33–41.