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Dose-dependent Enhancement of In Vivo *$GABA_A$–Benzodiazepine Receptor Binding by Isoflurane*

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Background: **Abundant *in vitro* and animal model data suggest the postsynaptic γ-aminobutyric acid receptor type A ($GABA_A$-R) is an important target for volatile general anesthetics, but the relevance of these models is untested in humans. Because benzodiazepines have also been shown to act *via* a specific $GABA_A$-R site, they provide sensitive probes for the $GABA_A$-R. Availability of the ^{11}C-labeled benzodiazepine ligand, flumazenil, allowed us to quantitatively test in humans whether the volatile anesthetic isoflurane affects $GABA_A$-Rs *in vivo* in a dose-dependent manner.**

Methods: **^{11}C-flumazenil positron emission tomography scans were obtained in 12 healthy subjects while awake (control condition) and anesthetized with either 1.0 or 1.5 minimum alveolar concentration isoflurane (n = 7 and 5, respectively; isoflurane conditions). Regions of interest included areas of high, intermediate, and low $GABA_A$–benzodiazepine site density. For each subject and experimental condition, the binding of ^{11}C-flumazenil, expressed as distribution volume (which linearly correlates to maximal binding site density and apparent ligand affinity), was obtained by curve fitting using a two-compartment model.**

Results: **The ratio of distribution volume increased significantly in each examined region during the isoflurane conditions compared with control conditions ($P < 0.01$, one-tailed *t* test). Furthermore, the increases in ratio of distribution volume during the 1.5–minimum alveolar concentration isoflurane condition were significantly greater than those measured during 1.0 minimum alveolar concentration isoflurane inhalation ($P < 0.002$, one-tailed *t* test).**

Conclusions: **Isoflurane exposure appeared to enhance receptor-specific ^{11}C-flumazenil binding in a dose-dependent manner. The results suggest the possibility that a conformational change of the $GABA_A$-R is involved in the mechanism of action of isoflurane in the living human brain.**

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GENERAL anesthesia is a behavioral state characterized by loss of consciousness and immobility in response to pain stimulation. Abundant *in vitro* and animal model data support that the postsynaptic γ-aminobutyric acid receptor type A ($GABA_A$-R) is an important target for volatile general anesthetics (reviewed by Tanelian *et al.*[1] and Franks and Lieb[2]). Volatile anesthetics enhance GABA binding at clinically relevant concentrations[3] by increasing ligand affinity, which appears to be a mechanism for the augmentation of GABA-evoked chloride currents by volatile agents, including isoflurane.[4,5] Isoflurane also enhances GABA-gated Cl^- currents *in vitro* in a concentration-dependent manner at therapeutically relevant concentrations,[6] and potentiation of GABA-mediated Cl^- currents correlates highly with the anesthetizing potency of isoflurane.[7] Although such studies provide strong support for the $GABA_A$-R hypothesis of anesthetic action, direct supporting evidence from living organisms, especially from humans, is lacking.

Recent advances in functional imaging techniques, such as positron emission tomography (PET), have made it possible to investigate the receptor targets of drug action on the molecular level by measuring the maximal binding site density (B_{max}), apparent equilibrium dissociation constant (K_d), and distribution volume (DV) of a radiolabeled receptor ligand, the latter parameter incorporating the ratio B_{max} and K_d. PET can directly assess benzodiazepine ligand interactions with brain $GABA_A$-R.[8] When labeled with the positron emitting isotope, ^{11}C, the binding characteristic (DV) of the benzodiazepine ligand, flumazenil, can be directly observed. This information, together with careful consideration of potential confounders, such as changes in regional cerebral blood flow (rCBF), radioligand metabolism, and nonreceptor ligand binding in the brain, allow us to quantitatively test in humans whether a volatile general anesthetic affects $GABA_A$-Rs *in vivo*.

Benzodiazepine agonists are potent sedative hypnotics–anxiolytics that exert their actions by enhancing GABA-mediated neurotransmission *via* a specific benzodiazepine-binding site integral to the $GABA_A$-R macromolecule. The binding of benzodiazepines to the $GABA_A$-R is enhanced by GABA binding to its low-affinity sites,[9,10] where GABA exerts its physiological effect on Cl^- flux.[11] Likewise, isoflurane potentiates benzodiazepine binding in a concentration- and chloride-dependent fashion by increasing the

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affinity of the binding site for its ligand. These observations demonstrate the tight coupling between $GABA_A$-R conformation and benzodiazepine binding, indicating that benzodiazepines are appropriate and sensitive probes to reflect the effects of anesthesia on the $GABA_A$-R.

The $GABA_A$-R hypothesis of anesthetic action would predict that the volatile anesthetic, isoflurane, administered to volunteers at two anesthetizing concentrations, enhances ^{11}C-flumazenil binding to human cortical $GABA_A$-Rs in a dose-dependent manner. To test this, we assessed the effect of 1.0 and 1.5 minimum alveolar concentration (MAC) isoflurane on ^{11}C-flumazenil binding to brain $GABA_A$-R in anesthetized human volunteers compared with nonanesthetized controls using PET.

Materials and Methods

Subjects

After obtaining approval from the Institutional Review Board of the University of Pittsburgh (No. 950389), informed consent was obtained in 12 healthy volunteers who were free from neurologic, psychiatric, and substance abuse disorders. Each subject was randomized to one of two separate groups: 1.0 MAC isoflurane (n = 7) and 1.5 MAC isoflurane (n = 5). In each group, subjects were studied during two experimental conditions: awake (control condition) and anesthetized with either 1.0 MAC isoflurane (mean age, 23 ± 4 yr; four women) or 1.5 MAC isoflurane (mean age, 28 ± 7 yr; three women) (isoflurane conditions).

Positron Emission Tomography Scanning

Positron emission tomography scans were performed with a Siemens 951R/31 scanner (Siemens, CTI PET Systems, Inc., Knoxville, TN) collecting 31 parallel slices over an axial field of view of 10.8 cm with an inherent detector resolution of approximately 6 mm full-width half-maximum in both axial and transaxial directions. Each subject was positioned in the scanner with the lowest image plane approximately parallel to and 1 cm above the canthomeatal line. A transmission scan, collected during exposure of a ^{68}Ge/^{68}Ga ring source, was used to correct for radiation attenuation by the head. For the ^{11}C-flumazenil study, a single bolus injection of high-specific-activity ^{11}C-flumazenil (> 11,100 GBq, 481–592 MBq per study) was used during awake (control) and anesthetized (isoflurane) conditions. Each dynamic ^{11}C-flumazenil PET scan was composed of 20 frames of varying lengths, acquired during approximately 90 min (0–23 min: 14 frames; 23–93 min: 6 frames). To determine the time course of total ^{11}C-flumazenil radioactivity in plasma for DV estimation, approximately 20 arterial blood samples were obtained during the initial 2 min of the study, and 20 more samples were collected over the remainder of the 90-min time frame of the study. A ^{15}O-water rCBF study was performed before each ^{11}C-flumazenil study and consisted of an intravenous injection of 1,850 MBq ^{15}O-water followed by a 3-min dynamic PET scan. To construct an arterial time–activity curve for quantitative rCBF measurements, arterial blood was continuously sampled and assayed (Siemens, CTI PET Systems, Inc.). A 20-min interval was imposed after each blood flow scan for virtually complete ^{15}O decay to occur. A delay of 130 min was allowed between ^{11}C-flumazenil injections for decay and anesthesia procedures.

Two intravenous catheters and a radial artery catheter were inserted before the scanning session. After the awake scans, subjects were anesthetized with 1.0 or 1.5 MAC isoflurane. The anesthetic was administered from an isoflurane vaporizer through a conventional semiclosed anesthesia delivery system with continuous monitoring of inspired and expired concentrations by infrared analysis. Electrocardiogram, heart rate, arterial blood pressure, respiratory rate, arterial oxygen saturation, axillary temperature, inspired oxygen, and expired carbon dioxide concentration were monitored and strictly controlled during administrations. After induction of anesthesia with isoflurane only, the trachea was intubated, and controlled ventilation was initiated to maintain an expired carbon dioxide concentration of 35 ± 5 mmHg. A phenylephrine infusion (80 μg/ml in saline) was used to maintain mean arterial blood pressure at the preinduction, awake level.

Subjects' PET studies were centered[12] and coregistered.[13] Both rCBF and ^{11}C-flumazenil PET images were registered to individual magnetic resonance images to facilitate accurate anatomic localization of regions of interest using the algorithm and software described by Woods *et al.*[14] Magnetic resonance imaging scans were obtained using a 1.5 Tesla G.E. Signa system (Milwaukee, WI). Region-of-interest templates were created on the magnetic resonance imaging plane best intersecting the region, using reference points defined in the stereotactic atlas of Talairach and Tournoux.[15] Regions of interest included areas of high (frontal cortex: gyrus frontalis inferior; temporal cortex: gyrus temporalis inferior; occipital cortex: gyrus occipitalis lateralis), intermediate (cerebellar cortex, thalamus), and low (pons) $GABA_A$–benzodiazepine site density.[16,17]

Derivation of the Binding Parameter

The derivation of DV, as a reflection of both nonreceptor- and receptor-specific ligand binding, requires pharmacokinetic models that describe the movement of tracer among individual brain compartments (see Appendix). For each subject and experimental condition, DV and the kinetic parameter K_1, which combines flow and transport across the blood–brain barrier, were obtained by fitting the observed PET data to the modeled ^{11}C-flumazenil concentration curve and solving the dif-

Table 1. Physiologic Variables

	Pulse (beats/min)		MAP (mmHg)		Spo_2 (%)	
Group	Control	Isoflurane	Control	Isoflurane	Control	Isoflurane
1.0 MAC	76.57 ± 4.61	72.57 ± 4.16	76.86 ± 9.81	78.86 ± 5.76	99.43 ± 0.53	99.57 ± 0.53
1.5 MAC	78.57 ± 3.26	74.14 ± 5.87	77.29 ± 7.41	80.29 ± 4.82	99.29 ± 0.76	99.43 ± 0.79

Data are mean ± SD.

MAP = mean arterial pressure; Spo_2 = oxygen saturation measured by pulse oximetry; MAC = minimum alveolar concentration.

ferential equations of the two-compartment model[18,19] using nonlinear least squares curve fitting[20] and Marquardt's method of minimization.[21] ^{11}C-flumazenil metabolism was monitored in the plasma by quantifying the time course of unmetabolized parent compound radioactivity in arterial blood samples obtained at 5, 10, 15, 45, and 75 min after injection using high-performance liquid chromatography.[22] rCBF was calculated from the ^{15}O-water brain tissue PET data and plasma tracer activity curve using similar methods.[23] Because DV incorporates both receptor-specific (B_{max}/K_D,) and nonspecific binding, it is preferable to monitor the ratio of DV (DV_{RATIO}), a parameter that reflects exclusively receptor-specific binding. To do so, individual regional DV values are normalized to the DV value for nonspecific binding ($DV_{NONSPECIFIC}$). Then:

$$DV_{RATIO} = DV_{BRAIN\ REGION}/DV_{NONSPECIFIC}. \quad (1)$$

Because there is no $GABA_A$-R–free area in the human brain,[16,17] such a DV value was obtained as follows. Regional ^{11}C-flumazenil DV values for each subject, measured in control and isoflurane conditions (both 1.0 and 1.5 MAC), were correlated with the human $GABA_A$–benzodiazepine site density data of Braestrup *et al.*,[16] using least squares regression ($P < 0.001$). The Y intercepts of the obtained lines represent the DV values of a theoretical $GABA_A$–benzodiazepine site–free area ($DV_{NONSPECIFIC}$) in each subject.

Data Analysis

DV_{RATIO} and $DV_{NONSPECIFIC}$ values were compared between control and isoflurane conditions with a paired *t* test at $P < 0.05$. DV_{RATIO} changes (DV_{RATIO}), during the 1.0- and 1.5-MAC isoflurane conditions were compared with a paired *t* test at $P < 0.05$. Individual ΔDV_{RATIO} and K_1 changes (ΔK_1); ΔDV_{RATIO} and rCBF changes (ΔrCBF); and ΔK_1, and ΔrCBF values during the 1.0- and 1.5-MAC isoflurane conditions were correlated using least squares regression ($P < 0.05$) in the sampled brain regions.

Results

In every subject of both experimental groups, phenylephrine was used to maintain mean arterial blood pressure at the preinduction, awake level. The total amount of phenylephrine used for each subject varied between 1.4 and 4.1 mg. Physiologic variables in subjects did not change significantly between the control and either one of the isoflurane conditions (table 1). Figure 1 illustrates that there is a higher concentration of bound ^{11}C-flumazenil in $GABA_A$–benzodiazepine site–rich areas of brain (darker shade of red) during the 1.0- and 1.5-MAC isoflurane conditions *versus* control conditions (first, second, third, and fourth rows, respectively). Furthermore, during the 1.5-MAC isoflurane condition, bound ^{11}C-flumazenil exceeded that observed during 1.0-MAC isoflurane inhalation (third and fourth *vs.* first and second rows). Figure 1 also demonstrates that the difference in bound ^{11}C-flumazenil concentration during 1.0 and 1.5 MAC isoflurane *versus* control conditions, as well as during 1.5 *versus* 1.0 MAC isoflurane is more pronounced in areas of higher $GABA_A$–benzodiazepine site density (occipital cortex [first and third rows] *vs.* thalamus [second and fourth rows] *vs.* pons [second and fourth rows]). Figure 2 illustrates the close correlation between measured and modeled ^{11}C-flumazenil (kilobecquerel per milliliter) concentration-*versus*-time curves in three selected brain regions. This is strong evidence for the accuracy of the two-compartment model in describing ^{11}C-flumazenil kinetics during our specific experimental conditions. Figure 2 also illustrates the differential increase of tissue radiotracer concentration during the 1.0-MAC isoflurane condition compared with control in the occipital cortex, cerebellum, and pons, which have high, moderate, and low $GABA_A$–benzodiazepine site density, respectively.[16] Table 2 shows the averaged regional ^{11}C-flumazenil DV_{RATIO}, calculated from DV, blood-to-brain transfer constant (K_1; see Methods), and rCBF values from right and left regions for all brain areas (individual data are shown in table as a Web Enhancement). Regional ^{11}C-flumazenil DV values for each subject, measured in control and 1.0- and 1.5-MAC isoflurane conditions, were correlated with the human $GABA_A$–benzodiazepine site density data of Braestrup *et al.*[16] using least squares regression ($P < 0.001$). The Y intercepts of the lines obtained represent the DV values of a theoretical $GABA_A$-R–free area and, consequently, nonspecific ligand binding ($DV_{NONSPECIFIC}$) in each subject. Sample regression lines are shown in figure 3 for subject 1 for both the control and 1.0-MAC isoflu-

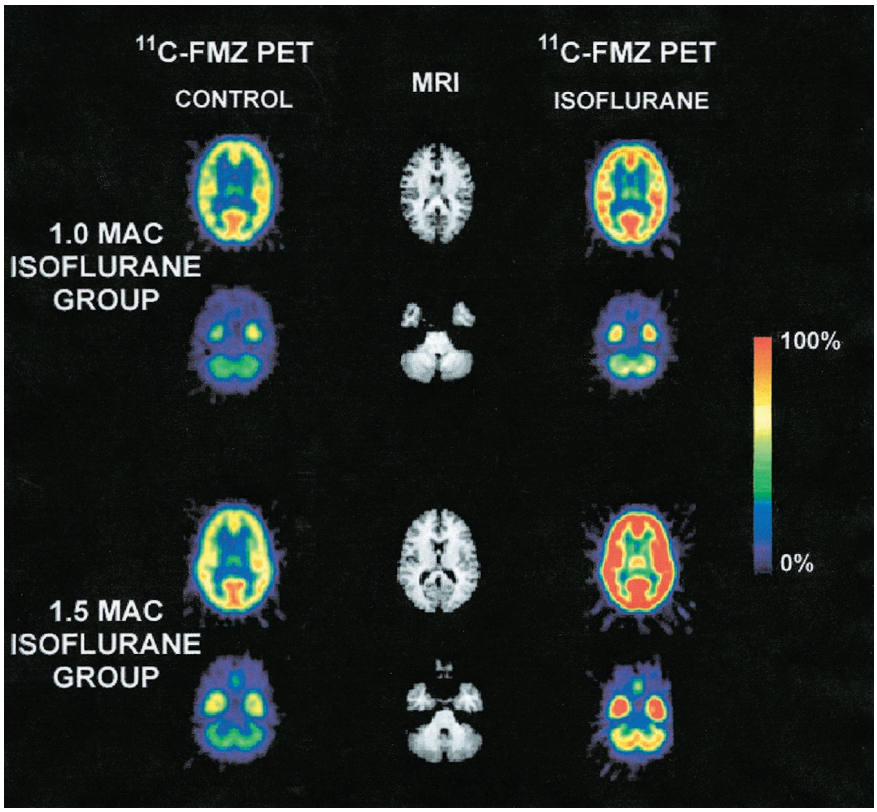

Fig. 1. Summed positron emisson tomography (PET; summed during 20–80 min after ^{11}C-flumazenil [^{11}C-FMZ] injection) of ^{11}C-flumazenil distribution in two representative volunteers during awake conditions (control) and when anesthetized with 1.0 minimum alveolar concentration (MAC) isoflurane (*top row*; subject 1 of 1.0-MAC isoflurane group) and 1.5 MAC isoflurane (*bottom row*; subject 1 of 1.5-MAC isoflurane group). The plane of both PET and the corresponding magnetic resonance scans is 4 cm (*top row*) and 2 cm (*bottom row*) superior to the canthomeatal line and shows the frontal, temporal, and occipital cortical areas, as well as the thalamus. The color scale is shown with red corresponding to 99.9 kBq/ml brain tissue. MRI = magnetic resonance image.

rane conditions, demon-strating the significant correlation between regional DV values and $GABA_A$-R density ($P < 0.001$). The individual $DV_{NONSPECIFIC}$ values were then averaged across subjects for each experimental condition (control, 1.0 MAC isoflurane, and 1.5 MAC isoflurane), and the corresponding averages were used to calculate regional DV_{RATIO} values in each subject. DV_{RATIO} increased significantly in each examined brain region during both the 1.0- and 1.5-MAC isoflurane conditions compared with control conditions ($P < 0.01$, one-tailed *t* test). Furthermore, the increases in DV_{RATIO} noted during the 1.5-MAC isoflurane condition were significantly greater than those obtained during the 1.0-MAC isoflurane condition ($P < 0.002$, one-tailed *t* test), indicating a dose-related effect of isoflurane on $GABA_A$-R ligand binding (table 2). Importantly, the comparison of $DV_{NONSPECIFIC}$ values between control and 1.0-MAC isoflurane conditions, as well as control and 1.5-MAC isoflurane conditions (table 3), revealed no significant differences in nonspecific binding in the presence of either concentration of isoflurane (paired two-tailed *t* test; $P = 0.45$). There was no significant difference found between the plasma concentration of unmetabolized ^{11}C-flumazenil of the control and isoflurane conditions in the two experimental groups at the 5-, 10-, 15-, 45-, and 75-min time points ($P = 0.31$; paired, two-tailed *t* test). Similarly, using least squares regression, there was no significant correlation found between individual ΔDV_{RATIO} and ΔK_1, and ΔDV_{RATIO} and ΔrCBF in any of the sampled brain regions ($P = 0.28$ and $P = 0.39$, respectively).

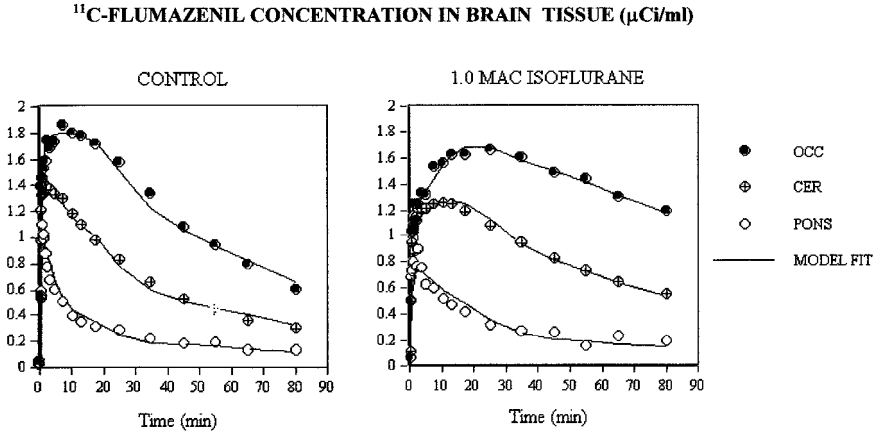

Fig. 2. Model fits for ^{11}C-flumazenil positron emission tomography data from the occipital cortex (OCC), cerebellum (CER), and pons, areas of high, intermediate, and low $GABA_A$–benzodiazepine site density, respectively, in the same subject as in the top row of figure 1. Each data point indicates a single measurement. Similar curve fits were obtained for each region of interest in every subject. MAC = minimum alveolar concentration.

Table 2. Regional Model Parameters Relating to ^{11}C-flumazenil Binding

Brain Region	DV_{RATIO} Control	DV_{RATIO} Isoflurane	ΔDV_{RATIO} (%)	K_1 ($ml \cdot g^{-1} \cdot min^{-1}$) Control	K_1 ($ml \cdot g^{-1} \cdot min^{-1}$) Isoflurane	ΔK_1 (%)	rCBF ($ml \cdot 100^{-1}g$ brain tissue $\cdot min^{-1}$) Control	rCBF ($ml \cdot 100^{-1}g$ brain tissue $\cdot min^{-1}$) Isoflurane	ΔrCBF (%)
1.0 MAC isoflurane group (n = 7)									
Occipital	12.20 ± 4.47	15.17 ± 4.61	27.67 ± 18.84	0.4077 ± 0.0671	0.2738 ± 0.0438	−31.60 ± 13.32	62.65 ± 18.66	52.85 ± 22.98	−3.58 ± 67.52
Temporal	9.89 ± 2.97	12.47 ± 3.43	28.73 ± 15.88	0.3096 ± 0.0410	0.2343 ± 0.0267	−23.32 ± 12.73	48.93 ± 7.82	50.55 ± 17.00	7.06 ± 43.44
Frontal	9.89 ± 3.77	12.47 ± 4.96	25.25 ± 14.88	0.3614 ± 0.0489	0.2683 ± 0.0377	−25.19 ± 11.14	57.43 ± 7.80	54.86 ± 23.48	−1.80 ± 46.89
Thalamus	5.94 ± 2.20	7.22 ± 2.64	24.12 ± 16.29	0.3987 ± 0.0829	0.2551 ± 0.0343	−34.48 ± 11.34	62.61 ± 17.10	53.16 ± 19.89	−7.73 ± 48.02
Cerebellum	6.92 ± 1.99	8.21 ± 2.18	22.31 ± 15.30	0.3931 ± 0.0801	0.2594 ± 0.0430	−32.80 ± 10.88	54.88 ± 7.32	52.51 ± 18.49	−0.26 ± 46.07
Pons	2.31 ± 0.97	2.65 ± 1.12	16.22 ± 5.52	0.2726 ± 0.0998	0.2148 ± 0.0577	−16.27 ± 23.23	46.18 ± 3.42	55.65 ± 14.92	22.49 ± 39.29
1.5 MAC isoflurane group (n = 5)									
Occipital	12.73 ± 3.69	25.80 ± 2.81	115.15 ± 60.07	0.3808 ± 0.0426	0.3477 ± 0.0893	−7.88 ± 26.78	67.60 ± 7.41	46.66 ± 15.96	−32.01 ± 18.61
Temporal	10.66 ± 1.64	19.78 ± 1.88	87.25 ± 19.08	0.2926 ± 0.0582	0.2272 ± 0.0670	−23.85 ± 7.99	48.05 ± 8.75	31.89 ± 13.19	−35.14 ± 18.04
Frontal	10.04 ± 1.45	19.78 ± 1.36	99.76 ± 26.82	0.3213 ± 0.0466	0.2433 ± 0.1039	−25.69 ± 22.13	53.38 ± 8.66	33.98 ± 15.36	−37.33 ± 22.82
Thalamus	5.84 ± 0.85	11.12 ± 0.71	93.17 ± 27.48	0.3722 ± 0.0588	0.2736 ± 0.0787	−26.67 ± 22.78	60.34 ± 10.85	44.96 ± 13.79	−26.19 ± 16.32
Cerebellum	8.24 ± 1.00	14.84 ± 1.07	81.45 ± 17.08	0.3449 ± 0.0658	0.3417 ± 0.0832	−0.97 ± 15.81	59.29 ± 11.20	54.93 ± 17.86	−8.98 ± 15.07
Pons	2.70 ± 0.24	4.13 ± 0.59	81.61 ± 13.34	0.2130 ± 0.0727	0.2422 ± 0.1011	19.85 ± 28.90	47.71 ± 5.49	50.21 ± 14.90	4.55 ± 26.96

Data are mean ± SD. For all brain areas, ^{11}C-flumazenil distribution volume ratio (DV_{RATIO}), blood-to-brain transfer constant (K_1), and regional cerebral blood flow (rCBF) values from right and left regions were averaged.

MAC = minimum alveolar concentration; occipital = gyrus occipitalis lateralis; temporal = gyrus temporalis inferior; frontal = gyrus frontalis inferior; cerebellum = cerebellar cortex.

To examine the dependence of DV estimates on changes in rCBF, brain activity curves were also simulated using a mathematical model that allows for direct input of rCBF[24] and the measured ^{11}C-flumazenil plasma activity curves. The simulated brain curves, using rCBF values from 25 to 400% of normal gray matter rCBF (20–320 $ml \cdot 100\ g^{-1} \cdot min^{-1}$) and keeping all other parameters constant, were then analyzed with the aforementioned two-compartment model. Estimated DV varied by only ± 2%, indicating the stability of DV_{RATIO} even in the presence of substantial rCBF fluctuations.

Discussion

These data identify a dose-dependent effect of the general anesthetic isoflurane on ^{11}C-flumazenil DV_{RATIO} in humans *in vivo*. Specifically, it is shown that receptor-specific ^{11}C-flumazenil binding increases significantly in brain areas of high, medium, and low $GABA_A$–benzodiazepine site density in the presence of 1.0 and 1.5 MAC isoflurane compared with control conditions. Furthermore, the increases in radioligand binding observed in the presence of 1.5 MAC isoflurane were significantly greater than those measured during anesthesia with 1.0 MAC isoflurane, suggesting a dose-related effect of isoflurane on $GABA_A$-R ligand binding (table 2).

It is possible that either a new population of $GABA_A$-Rs are unmasked by isoflurane or that ligand affinity is increased in existing $GABA_A$-Rs. The latter notion is consistent with findings from numerous *in vitro* experiments. The involvement of GABA in anesthesia is suggested by *in vivo* studies showing that GABA agonists that penetrate the blood–brain barrier can obtund rats.[25] *In vitro* animal studies have shown that volatile anesthetics enhance GABA binding at clinically relevant concentrations. Isoflurane, enflurane, and halothane enhance GABA-gated Cl^- currents *in vitro* in a concentration-dependent manner at therapeutically relevant concentrations,[6] and the potentiation of GABA-mediated Cl^- currents highly correlates with their anesthetizing potency.[7] The volatile anesthetic–activated Cl^- current can be blocked by bicuculline and picrotoxin,

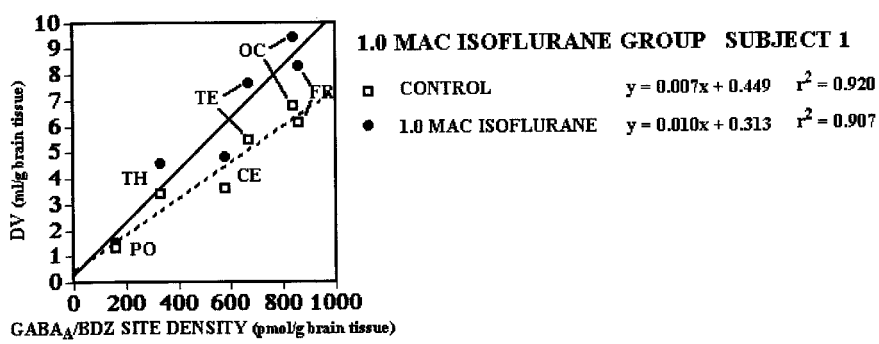

Fig. 3. Least squares regression lines of the regional ^{11}C-flumazenil distribution volume ratios of subject 1 in the 1.0–minimum alveolar concentration (MAC) isoflurane group measured during control and isoflurane conditions *versus* human $GABA_A$–benzodiazepine ($GABA_A$/BZD) density data published by Braestrup *et al.*[16] Each data point represents one measurement. CE = cerebellar cortex; FR = gyrus frontalis inferior; OC = gyrus occipitalis lateralis; PO = pons; TE = gyrus temporalis inferior; TH = thalamus.

Table 3. Regional Model Parameters Relating to Nonreceptor ^{11}C-flumazenil Binding

Subject	$DV_{NONSPECIFIC}$ Control	$DV_{NONSPECIFIC}$ Isoflurane
1.0 MAC isoflurane group		
1	0.449	0.313
2	0.564	0.871
3	0.726	0.580
4	0.966	0.831
5	0.537	0.413
6	0.251	0.233
7	0.414	0.476
Mean ± SD	0.558 ± 0.2317	0.531 ± 0.2454
1.5 MAC isoflurane group		
1	0.932	0.910
2	0.690	0.615
3	0.372	0.290
4	0.546	0.569
5	0.328	0.393
Mean ± SD	0.573 ± 0.2468	0.555 ± 0.2378

$DV_{NONSPECIFIC}$ = DV value for nonspecific binding; MAC = minimum alveolar concentration.

indicating that the current is mediated by $GABA_A$-Rs.[26] As demonstrated by receptor binding studies, an increase in the affinity of the binding site for GABA appears to be a mechanism for the augmentation of GABA-evoked Cl^- currents by volatile agents.[4,5] At clinically relevant concentrations, halothane stimulates Cl^- flux[27] and, at the same concentrations, increases $GABA_A$-R affinity for muscimol.[28] Likewise, isoflurane, halothane, and enflurane potentiate benzodiazepine binding in a concentration- and chloride-dependent fashion by increasing the affinity of the binding site for its ligand. Our group recently showed that inbred mice strains sensitive to benzodiazepine agonists (known to act at $GABA_A$-R) are cross-sensitive to several volatile general anesthetics,[29,30] and that while ubiquitous deletion of the β_3 $GABA_A$-R gene affects anesthetic potency in mice,[31] deletion of α_6 does not.[32]

Because benzodiazepines have been shown to exert their sedative effect *via* binding to a specific site of the $GABA_A$ receptor, they provide sensitive probes for the macromolecule. Clinical studies support the pharmacologic relevance of this interaction in humans; plasma concentrations associated with sedation generally correspond to those required to occupy the benzodiazepine site *in vitro*.[33] Thus, a benzodiazepine ligand is an appropriate and relevant probe for central $GABA_A$-Rs. Benzodiazepines increase Cl^- uptake in a concentration-dependent manner in the presence of GABA.[34] Benzodiazepine agonists also enhance GABA-mediated transmission and the response to exogenously applied GABA.[35,36] The binding of benzodiazepines to the $GABA_A$-R is enhanced by GABA binding to its low-affinity sites,[9,10] where GABA exerts its physiological effect on Cl^- flux.[11,37] These observations suggest a tight coupling between $GABA_A$-R function and benzodiazepine binding, indicating that the latter provides an appropriate and relevant probe for the functional state of the $GABA_A$-R. Although only the binding of benzodiazepines agonists is affected by GABA *in vitro*, the binding of the benzodiazepines antagonist flumazenil is similarly enhanced *in vivo*.[38] The lack of agreement between *in vitro* and *in vivo* approaches may be partly caused by the presence of residual endogenous GABA in *in vitro* membrane preparations that might mask the effect of added GABA on flumazenil binding.[39] Furthermore, flumazenil uptake *in vivo* has been shown to be different than that *in vitro*,[40] which could affect ligand binding measurements.

Imaging the binding of the ^{11}C-labeled benzodiazepines ligand flumazenil with PET allows the direct noninvasive and quantitative assessment of general anesthetic effects on $GABA_A$ receptors in the intact human brain. ^{11}C-flumazenil has become the ligand of choice for *in vivo* studies for the following reasons: (1) as an antagonist, it is devoid of significant sedative effects[41]; (2) its binding is specific for the central benzodiazepines site, with virtually no binding to mitochondrial benzodiazepine sites[42]; (3) it is associated with minimal nonspecific (nonreceptor) binding[43,44]; (4) despite rapid peripheral metabolism, radioactive metabolites do not penetrate the blood–brain barrier to confound signals[44]; and (5) it can be synthesized with sufficiently high specific activity, and both radio- and chemical purity, to make *in vivo* studies feasible.[8] Given these advantages, ^{11}C-flumazenil has enabled demonstration of altered benzodiazepine site distribution in both Huntington disease[45] and epilepsy.[46]

Although with significant variance, our data show decreases in rCBF in the presence of 1.0 and 1.5 MAC isoflurane in most subjects and brain regions (table 2). This is unexpected in light of human data in the literature showing mostly no change in rCBF either when the mean arterial blood pressure was pharmacologically maintained at preisoflurane levels,[47] as in the current experiment, or it was not supported.[48,49] Beyond the differences in measurement techniques, this discrepancy could partly be caused by the fact that, in these experiments, baseline measurements were made during isoflurane-free anesthesia as opposed to our awake baseline condition, in addition to using nitrous oxide and other agents as part of the anesthetic regimen, in contrast to our single-anesthetic approach. The use of phenylephrine for blood pressure support in these subjects of intact cerebrovascular physiology is an unlikely explanation for the observed changes in rCBF.[50]

The dose-dependent effect of isoflurane on ^{11}C-flumazenil binding described could, in principle, be caused by the direct effect of isoflurane on rCBF, leading to altered radioligand delivery to the examined brain areas. The lack of correlation between rCBF and ^{11}C-flumazenil binding, or ΔK_1 and ^{11}C-flumazenil binding, however, rules out this possibility. In addition, computer-simu-

lated ^{11}C-flumazenil DV_{RATIO} brain curves using rCBF values from 25 to 400% of normal gray matter rCBF (20–320 ml · 100 g^{-1} · min^{-1}), showed only trivial variation in DV_{RATIO}. This is in agreement with previous human studies showing that, in the occipital cortex, ^{11}C-flumazenil DV_{RATIO} does not change significantly during visual stimulation of an intensity that is known to increase rCBF by 30%.[51]

^{11}C-flumazenil protein binding differences caused by isoflurane (the main source of nonspecific binding) could potentially confound results by perturbing radiotracer availability. Although direct information is not available, there is *in vitro* evidence that isoflurane decreases diazepam binding to human serum albumin.[52] Furthermore, because nonspecific binding is linearly related to the amount of radioligand delivered to the imaged brain compartments, any change in the latter, for example, because of altered plasma protein binding or rCBF, would affect the former linearly. It follows that nonspecific binding serves as a cumulative index of radioligand delivery. As shown in table 3, however, no significant differences were observed in nonreceptor ligand binding between the two experimental conditions in either experimental group, indicating that the observed changes in binding were not confounded by altered ligand delivery.

These data provide support for the effect of isoflurane on $GABA_A$-Rs *in vivo* in humans, consistent with numerous *in vitro* experiments. Possible mechanisms of these observations include the direct effect of isoflurane on $GABA_A$-Rs, resulting in increased affinity for benzodiazepine ligands, or an indirect effect leading to an increase in GABA concentrations and consequent enhancement of benzodiazepine binding. Although the relevance of endogenous benzodiazepine remains questionable in healthy subjects, despite the demonstration of their presence in patients with hepatic encephalopathy,[53] the possibility that isoflurane decreases the binding of these substances, leading to increased availability of flumazenil binding sites, cannot be ruled out. Isoflurane has recently been shown, using ^{18}F-deoxyglucose PET, to decrease regional cerebral metabolism in humans.[54] Combination of receptor imaging with ligands such as ^{11}C-flumazenil, with metabolic imaging *via* ^{18}F-deoxyglucose, has the potential to yield new insights into how drug effects are translated into altered brain function during the most clinically relevant circumstance—the intact, living human brain.

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Appendix

Kinetic Principles for Quantitative Receptor Imaging

Assessment of cerebral neuroreceptors *in vivo* has recently become feasible by combining the use of radiolabeled ligands and dynamic PET scanning. The derivation of the parameters DV, as a reflection of receptor binding, B_{max}, and apparent K_d, requires pharmacokinetic models that describe the movement of tracer among individual brain compartments. The distribution of ^{11}C-flumazenil in humans has previously been quantified using a conventional two-compartment model,[18,19] where C_P represents unmetabolized radiotracer in intracapillary (plasma) spaces (kilobecquerel per milliliter), C_1 represents free and nonspecifically bound radiotracer in the extravascular brain compartment (kilobecquerel per milliliter); C_2 represents radiotracer specifically bound to its receptor (kilobecquerel per milliliter); C_T represents a single brain tissue compartment incorporating free and nonspecifically and specifically bound drug fractions (fig. 4).

For diffusion limited tracers, the kinetic parameter K_1 (milliliter per minute per milliliter) combines flow and transport across the blood–brain barrier and is thus defined as:

$$K_1 = rCBF \cdot (1 - e^{-PS\, f_1/rCBF}) \quad (2)$$

where PS is the brain permeability–surface area product for plasma (milliliter per minute per milliliter), and f_1 is the free fraction of tracer in the blood.[55,56] The k_2 (per minute) parameter in the schematic describes the transport of the free and nonspecifically and specifically bound tracer across the blood–brain barrier back into the intravascular compartment. The kinetic parameters k_3 and k_4 describe the binding to and dissociation from receptors, where k_4 is assumed to be equivalent to the first-order dissociation rate constant, k_{off}. The parameter, k_3, is a pseudo–first-order association rate constant, and:

$$k_3 = k_{on} \cdot f_2 \cdot [B_{max} - C_2(t)] \quad (3)$$

where k_{on} is the bimolecular association rate constant (nanomolar per minute), f_2 is the fraction of the radiotracer free from nonspecific binding,[24] and B_{max} is the density of receptors available for binding (nanomolar). When only tracer doses are used with negligible receptor occupancy, k_3 simplifies to:

$$k_{on} \cdot f_2 \cdot B_{max} \quad (4)$$

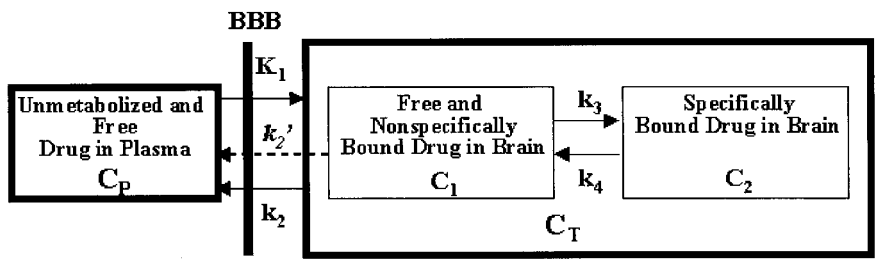

Fig. 4. Two-compartment model of ^{11}C-flumazenil distribution. BBB = blood–brain barrier.

Derivation of Distribution Volume and Ratio of Distribution Volume Parameters

Distribution volume is defined at equilibrium as the ratio of brain and blood radiotracer concentrations, *e.g.*, for a two-compartment model and the aforementioned definitions of C_P, C_T, C_1, and C_2:

$$DV = \frac{C_1 + C_2}{C_T} = \frac{C_T}{C_P} \quad (5)$$

Its advantage is that DV can be mathematically derived using differential model equations and the individual kinetic parameter estimated, without the need for actual physical equilibrium during the PET study.[18] DV (milliliter/milliliter) is calculated from the model parameters:

$$DV = \frac{K_1}{k_2} = \frac{K_1}{k_2'(1+k_3/k_4)} \quad (6)$$

where k_2' (per minute; see schematic) describes the transport of only the free and nonspecifically bound tracer fractions across the blood–brain barrier back into the intravascular compartment. The model parameters are derived from the differential equations of a two-compartment model as follows. Because the PET-measured brain tissue concentration of the radiotracer (C_T) is also described by the differential equations of the two-compartment pharmacokinetic model, after fitting the measured and modeled concentration curves, the equations can be solved for K_1 and k_2. The curve-fitting procedure compares total compartment activity[57] with the observed PET data and minimizes this difference, thereby producing optimal estimates of K_1, k_2, k_3, and k_4 using nonlinear least squares curve fitting[21] and Marquardt's method of minimization.[21]

Because DV incorporates both receptor-specific ligand binding, as the ratio of B_{max} and K_d, as well as nonspecific binding, it must be normalized to the DV value of a brain area that is devoid of receptors to obtain a binding parameter that exclusively reflects receptor-specific binding. Because there is no area in the human brain that would be completely devoid of $GABA_A$-R–benzodiazepine sites, this reference DV value is computed by correlating regional DV values of the various experimental conditions with human data of $GABA_A$-R–benzodiazepine site density from the literature,[16,17] and obtaining the intercepts of the regression lines with the DV axis in each subject for each experimental condition. The obtained value represents the DV of a theoretical brain area that is devoid of $GABA_A$-R–benzodiazepine sites by which regional DV values are normalized. The obtained DV_{RATIO} values reflect exclusively the receptor-specific component of ^{11}C-flumazenil binding characteristics, incorporating both B_{max} and K_d.

Derivation of Absolute Regional Cerebral Blood Flow

The arterial ^{15}O-water time–activity raw PET scan data for rCBF computation provide images of regional cerebral radioactivity concentration summed over the duration of the scan (T_1 to T_2), providing the integral (C) of an instantaneous count rate [$C_i(T)$ in counts per second times unit weight]. Both the PET scan data and arterial blood concentrations [$C_a(t)$] were corrected for radioactive decay. rCBF was then calculated by solving an equation described by Herscovitch *et al.*[58] using custom software:

$$C_i(T) = rCBF \cdot \int_0^T C_a(t) \cdot e^{[-(rCBF/\lambda)(T-t)]} dt \quad (7)$$

$$C = \int_{T_1}^{T_2} C_i(T) dt \quad (8)$$

where λ is the equilibrium brain:blood partition coefficient for water, which, on average, is 0.9 for gray and white matter.[58] Dispersion and timing delays inherent in using a continuous fluid monitor were corrected.[23] In each region of interest, calculated individual percentage ^{11}C-flumazenil DV_{RATIO} differences between control and isoflurane conditions were correlated with the corresponding percentage rCBF alterations using least squares regression. All computational tasks were executed on a SparcStation 2 workstation (Palo Alto, CA).

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