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GABAergic Inhibition of Endogenous Dopamine Release Measured *in vivo* with ^{11}C-Raclopride and Positron Emission Tomography

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Extensive neuroanatomical, neurophysiological, and behavioral evidence demonstrates that GABAergic neurons inhibit endogenous dopamine release in the mammalian corpus striatum. Positron emission tomography (PET) studies in adult female baboons, using the dopamine D_2-specific radiotracer ^{11}C-raclopride, were undertaken to assess the utility of this imaging technique for measuring these dynamic interactions *in vivo*. ^{11}C-raclopride binding was imaged prior to and following the administration of either γ-vinyl-GABA (GVG), a specific suicide inhibitor of the GABA-catabolizing enzyme GABA transaminase, or lorazepam, a clinically prescribed benzodiazepine agonist. Striatal ^{11}C-raclopride binding increased following both GVG and lorazepam administration. This increase exceeded the test/retest variability of ^{11}C-raclopride binding observed in the same animals. These findings confirm that changes in endogenous dopamine concentrations resulting from drug-induced potentiation of GABAergic transmission can be measured with PET and ^{11}C-raclopride. Finally, this new strategy for noninvasively evaluating the functional integrity of neurophysiologically linked transmitter systems with PET supports its use as an approach for assessing the multiple mechanisms of drug action and their consequences in the human brain.

Advances in radiochemistry and positron emission tomography (PET) have made it feasible to study neurotransmitter systems *in vivo*. Previously, the study of neurotransmitter activity in the human brain was limited to indirect measures (metabolites in plasma or cerebrospinal fluid) of individual neurotransmitter systems. Therefore, many neuropsychiatric diseases have been attributed to single neurotransmitter deficits. The quantitative capabilities intrinsic to the technology make PET a suitable experimental tool for measuring not only the regional neuroanatomical distribution of specific receptors and their subtypes, but also for measuring the dynamic properties of these receptors and their intrinsic abilities for modulating the release and inhibition of other functionally linked neurotransmitters. This approach of assessing the *responsiveness* of an endogenous neurotransmitter to drug intervention by measuring an increase or decrease in the specific binding of a radiotracer with which it competes has been used with several neurotransmitter receptor ligands (Dewey et al., 1990b, 1991; Logan et al., 1991). In the present study, ^{11}C-raclopride was chosen as an appropriate ligand because *in vitro* studies have shown that its binding is especially sensitive to alterations in endogenous dopamine concentrations due to its K_d for the dopamine D_2 receptor (Seeman et al., 1989; Inoue et al., 1991).

The use of PET to study neurotransmitter interactions represents an opportunity to extend basic neurophysiological findings in animals to cerebral physiology in the human brain. Furthermore, PET investigations of neurotransmitter interactions may result in the development of alternative therapeutic strategies.

Our initial studies established the feasibility of the PET technique for investigating neurotransmitter interactions *in vivo* in both the human and primate brain (Dewey et al., 1988, 1990b). These studies focused on interactions between dopamine and ACh in the extrapyramidal motor system. In the present report, we have extended these findings to include an examination of the responsiveness of the extrapyramidal dopamine system to pharmacological manipulation of GABA. By taking advantage of two mechanisms by which GABAergic transmission can be manipulated, we have examined the inhibition of endogenous dopamine release following either an increase in endogenous GABA or the potentiation of existing levels. GABA's modulatory role in endogenous dopamine release in this motor pathway has been well documented. Studies using neurochemical, neurophysiological, and behavioral methods have demonstrated that GABAergic, dopaminergic, and cholinergic neurons interact to form a multisynaptic feedback mechanism by which the CNS maintains control of endogenous striatal dopamine levels (Fig. 1). Neuroanatomical studies have shown that striatal GABAergic neurons project, via the striatonigral and pallidonigral pathways, onto dopaminergic neurons within the pars compacta region of the substantia nigra, which they tonically inhibit (Bunney and Aghajannian, 1976; Kubota et al., 1987). The inhibition of pallidal GABAergic input to dopamine-containing neurons in the ventral tegmental area results in an increase in dopamine activity in the nucleus accumbens and olfactory tubercle, resulting in the production of a "psychosis-like" syn-

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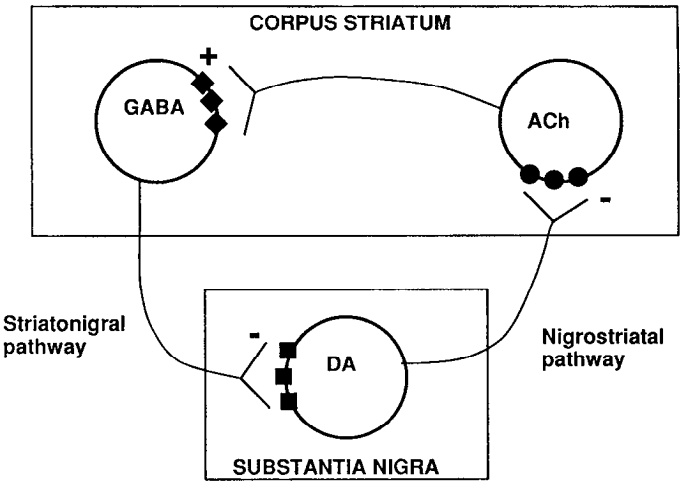

Figure 1. Schematic representation of the multisynaptic nigrostriatal loop. GABAergic neurons in the corpus striatum project onto dopaminergic neurons in the substantia nigra, which they inhibit (−). These dopaminergic neurons inhibit (−) some cholinergic interneurons in the corpus striatum. *DA,* dopamine. *Solid circles* on the *ACh* neuron represent dopamine D_2 receptors; *solid boxes* on *GABA* neuron represent muscarinic receptor (M_1 and M_2); *solid boxes* on *DA* neuron represent GABA receptor.

drome in cats (Stevens et al., 1974). These studies reveal the functional significance of GABAergic and dopaminergic interactions in the mesencephalic dopamine system. Furthermore, they support the need for a noninvasive method that can evaluate the functional integrity of these dynamic systems in patients suffering from CNS diseases originating from their disruptions.

Materials and Methods

In order to examine GABA's modulatory role in endogenous dopamine release, we have examined the striatal binding of the D_2 ligand ^{11}C-raclopride following the separate administration of two different drugs that have previously been shown to enhance GABAergic transmission.

Baboon PET studies. Adult female baboons (*Papio anubis,* 14.5–16.5 kg) were prepared for PET scanning as detailed previously (Dewey et al., 1990a). Animals were initially immobilized with ketamine hydrochloride (10 mg/kg) and subsequently maintained on gas anesthesia using isoflurane, nitrous oxide, and oxygen for the duration of the PET study. The specific dopamine D_2 ligand ^{11}C-raclopride was utilized in these studies and was synthesized as described previously (specific activity, 500–900 mCi/μmol; 26–42 mg; Farde et al., 1986). In all studies, dynamic PET scanning commenced simultaneously with ^{11}C-raclopride injection and was performed for 60 min in a Computer Technology Imaging positron tomograph [model 931-08/12; 15 slices, 6.5 mm slice thickness, full width at half-maximum (FWHM)] with an in-plane resolution of 6.0 × 6.0 mm (FWHM) using the following scanning protocol: 10 scans for 1 min each followed by 10 scans of 5 min each.

After radiotracer administration, arterial blood was sampled continuously for the first 2 min using an automated device (Ole Dich, Hvidovre, Denmark) and then manually at 5.0, 10.0, 30.0, and 60.0 min postinjection. Selected plasma samples (1.0, 5.0, 10.0, and 30.0 min postinjection) were analyzed for the presence of unchanged radiotracer.

Assay of ^{11}C-raclopride in plasma. Briefly, 0.5 cc of acetonitrile was added to each plasma sample. Samples were sonicated and centrifuged. Unchanged raclopride concentrations were determined by high-pressure liquid chromatography (HPLC) of the supernatant previously spiked with unlabeled raclopride. The HPLC system consisted of a Waters Nova-Pak C_{18} column (3.9 × 300 mm) and a mobile phase of 40:60: 0.5 CH_3CN/0.01 M ammonium formate/glacial acetic acid at a flow rate of 0.09 ml/min. Detection of the raclopride peak was by ultraviolet absorption at 254 nm. Raclopride eluted at approximately 9 min. A 50–100 ml standard of each supernatant was removed prior to HPLC injection and used to determine column recovery.

Pharmacologic intervention. Two pharmacologic strategies were selected to potentiate GABAergic transmission. In the first series of studies, γ-vinyl-GABA (GVG, or Vigabatrin) was administered, as a specific suicide inhibitor of the GABA-catabolizing enzyme GABA transaminase (GABA-T; EC 2.6.1.19) (Schechter et al., 1977). GABA transaminase is the primary catabolic enzyme of GABA, and its inhibition has been shown to elevate GABA concentrations significantly in the CNS. GVG is an anticonvulsant drug whose irreversible inhibition of GABA transaminase has been shown to elevate central GABA levels specifically, maximally between 3 and 4 hr following parenteral administration (Palfreyman et al., 1981). GVG's effects on endogenous GABA levels have been confirmed in several studies in which the protection against experimental or epileptic seizures is achieved concomitantly with an elevation in GABA levels in the CNS or cerebral spinal fluid as observed in animals (Schechter et al., 1977; Piredda et al., 1987; Bernasconi et al., 1988; Valin et al., 1991) and human epileptic patients (Schechter et al., 1984).

In the next series of studies, we administered lorazepam (Ativan), a potent and clinically prescribed benzodiazepine agonist. Lorazepam does not change endogenous GABA levels but instead produces a receptor-mediated potentiation of preexisting GABA concentrations as benzodiazepine receptors are structurally and functionally linked to GABA receptors (Marangos et al., 1982). The benzodiazepine recognition site is one component of the GABA-receptor ionophore complex. Benzodiazepines increase the affinity of GABA for the receptor complex, which results in enhanced chloride channel permeability (Olsen, 1981). In rats, for example, benzodiazepine drugs decrease nigrostriatal dopamine release and turnover (Rastogi et al., 1980; Wood et al., 1982) and potentiate the cataleptic effects of neuroleptics (Keller et al., 1976).

Design of studies. In the first series of experiments ($n = 6$) we examined the reproducibility of ^{11}C-raclopride binding using a test/retest experimental protocol in which each animal served as its own control. This provided a baseline measurement upon which the significance of changes in subsequent experimental studies could be assessed (Fowler and Wolf, 1989). In the second series of studies, performed using the *same* animals under the *same* experimental conditions (i.e., anesthetic agent, depth of anesthesia as determined by respiratory and cardiac rate as well as blood pressure, body temperature, position in estrous cycle as determined by constant charting of menses, time of day, etc.), GVG was intravenously administered (300 mg/kg, 40 cc over 3 min) immediately following completion of the first dynamic scan. After a period of 3.5 hr, a second ^{11}C-raclopride scan was performed. At this time after GVG administration, GABA levels in baboon cerebrospinal fluid have been shown to be elevated by 300% (Valin et al., 1991). In the third series of studies, again performed under identical experimental conditions, lorazepam was intravenously administered (0.75 mg in study 171B, 1.0 mg in study 172B, and 1.25 mg in study 177B) 30 min prior to a second ^{11}C-raclopride scan. At 30 min following intravenous administration, lorazepam has been shown to achieve brain : plasma equilibrium, maximal receptor occupancy (95%), and maximal clinical effect (Miller et al., 1987; Greenblatt et al., 1989; Greenblatt and Sethy, 1990). Animals remained in the PET gantry between scans, and vital signs were monitored and recorded throughout the length of both scanning periods. In every case, animals recovered for at least 3 weeks prior to their second set of PET scans.

Region of interest selection. Regions of interest (ROIs) for the corpus striatum were drawn directly on the PET image to encompass the entire structure on every slice upon which it appeared and were appropriate in size for the resolution of the tomograph. This multiplanar method of ROI selection reduces differences that may arise due to movement of the animal within the gantry during the scanning interval (Dewey et al., 1990b; Bendriem et al., 1991). ROIs were then copied directly from the first scan to the appropriate slices of the second. By examining placement of the ROIs on the second scan, changes could be made, if necessary, in ROI position only. In addition, an infrared motion-monitoring device (Tri-Tronics, Smarteye model SD) was utilized in order to detect any movement of the animal's head (>1.0 mm) at any time during the experiment. The cerebellar ROIs were drawn at the level of the vermis and included both cortical gray and white matter. This region served as a measure of nonspecific binding, as the density of D_2 receptors in this region is negligible.

Data analysis. Receptor availability as a function of changes in endogenous dopamine concentration was analyzed using a graphical technique specifically designed for reversible systems that directly gives a linear function of the free receptor concentration known as the distribution volume (Logan et al., 1989). In this analysis, a plot of $\int_0^t \mathrm{ROI}(t)$

Table 1. Kinetic parameters for ^{11}C-raclopride in baboon brain (test/retest)

		Distribution volume					
Baboon	Study	ST	% Change[a]	CB	% Change	ST/CB	% Change
Clovis	146A	1.45		0.53		2.74	
	146B	1.47	1	0.52	−2	2.83	3
	153A	1.51		0.49		3.05	
	153B	1.66	10	0.47	−4	3.45	13
	164A	1.43		0.48		3.00	
	164B	1.51	6	0.47	−2	3.19	6
Oral	147A	3.67		1.23		3.00	
	147B	3.60	−2	1.15	−6	3.13	4
	160A	3.45		1.11		3.12	
	160B	3.80	10	1.15	4	3.31	6
Karm	149A	3.32		1.05		3.16	
	149B	3.49	5	1.10	5	3.17	0

[a] % Change = $-[(A - B)/A] \times 100$.

$dt/\mathrm{ROI}(T)$ vs. $\int_0^T \mathrm{Cp}(t)\,dt/\mathrm{ROI}(T)$ [where $\mathrm{ROI}(T)$ and $\mathrm{Cp}(T)$ represent tissue and plasma radiaoctivity, respectively, at time T due to ^{11}C-raclopride] becomes linear after some time with a slope (K_R) that is equal to the steady state distribution volume. For the case in which tissue binding is due to both specific binding to free receptors and nonspecific binding, the steady state distribution volume becomes

$$K_R = (K_1/k_2)(1 + \mathrm{NS} + (B_{\max} - \mathrm{RL})/K_d), \quad (1)$$

where K_1 and k_2 are plasma to tissue and tissue to plasma transport constants, respectively. Since the transport constants appear as the ratio, the dependence upon blood flow cancels (Patlak and Fenstermacher, 1975; Lassen and Gjedde, 1983; for the relationship between K_1 and blood flow). NS is the ratio of binding constants for nonspecific binding, $B_{\max}$ is the total D_2 receptor concentration, RL represents the endogenous neurotransmitter concentration that occupies receptors and hence reduces the number of free receptors, and K_d is the raclopride receptor equilibrium dissociation constant.

For the cerebellum, the distribution volume is given by

$$K_R = (K_1/k_2)(1 + \mathrm{NS}), \quad (2)$$

assuming no specific binding to receptors. In Equations 1 and 2, we have explicitly separated the transport and nonspecific binding terms, although if nonspecific binding is rapidly reversible, it is included in k_2. It is frequently assumed that the ratio K_1/k_2 is the same for both the corpus striatum and cerebellum (Wong et al., 1986a), so that the striatum to cerebellum (CB) ratio gives

$$\frac{K_R(\mathrm{ST})}{K_R(\mathrm{CB})} = \frac{B_{\max} - \mathrm{RL}}{K_d(1 + \mathrm{NS})} + 1, \quad (3)$$

which is independent of the transport constants. In Equation 3 we have assumed that the nonspecific binding is the same for both the striatum and cerebellum. It has been suggested that this might not be the case for ^{11}C-raclopride (Farde et al., 1988). Farde et al. (1988) found the distribution ratio of ^{11}C-FLB472 (the pharmacologically inactive enantiomer of raclopride) for striatum to cerebellum to be 1.35 (rather than 1 as in Eq. 1), although they suggest that this difference may be due to inaccuracies of the measurement process. In any case, Equation 3 is a linear function of the available receptor concentration. Our results are presented in terms of K_R for the striatum and cerebellum as well as their ratios (Tables 1, 2). A blood volume contribution of 4.5% was subtracted from the ROI radioactivity prior to analysis.

The statistical analysis was designed to address the hypothesis that the difference in receptor availability was greater for the perturbation conditions relative to the test/retest variability of the ligand. Therefore, the receptor availability data ([(scan A − scan B)/scan A] × 100) for the striatal, cerebellar, and striatal/cerebellar distribution volumes were subject to univariate analysis of variance (ANOVA). Separate ANOVAs were performed for the GVG and lorazepam conditions, relative to the test/retest condition.

Results

Immediately following administration of ^{11}C-raclopride, radioactivity accumulated bilaterally in the corpus striatum alone. In all control studies, radioactivity reached a peak value within 5 min postinjection and began to clear from the corpus striatum, reaching a value of approximately 25% of the peak at 60 min. Cerebellar radioactivity reached a peak value within 5 min and began to clear rapidly, reaching values of less than 10% of the peak by 60 min. In the test/retest studies, we observed a variability in our K_R measurement for ^{11}C-raclopride binding of 10% or less in the corpus striatum and cerebellum (Table 1). This magnitude of variation in repeated measures without intervention is consistent with test/retest measurements of other radiotracers in the baboon brain, including ^{18}F-*N*-methylspiroperidol (^{18}F-NMSP), ^{11}C-benztropine (Dewey et al., 1990a), and ^{11}C-cocaine (Fowler et al., 1989). When GVG was administered 3.5 hr prior to the second scan, ^{11}C-raclopride binding increased in the corpus striatum by an average of 28% (Table 2), while there was no effect observed in the cerebellum (Table 2, Fig. 2). This increase in striatal receptor availability and in the ratio of striatal/cerebellar receptor availability, as measured by the distribution volume and the ratios of the distribution volumes, respectively, was statistically significant compared with the test/retest variability ($F[1,8] = 37.61$, $p < 0.001$, and $F[1,8] = 19.92$, $p < 0.01$, respectively). Cerebellar receptor availability was not significantly altered ($F[1,8] = 0.90$, $p > 0.1$). Lorazepam administration at a dose of 0.75 mg produced an increase in striatal radiotracer binding of 15%, 1.0 mg increased radiotracer binding by 49%, and 1.25 mg increased radiotracer binding by 49% as well (Table 2). Unlike GVG, lorazepam administration resulted in changes in the cerebellum that exceeded our stability measurements in this region. Therefore, in order to examine changes in striatal radiotracer binding in light of changes occurring in the cerebellum, we have also reported these data as the ratio of K_R in the striatum to K_R in the cerebellum in order to eliminate any effect due to drug-induced changes in the ratio K_1/k_2. Consistent with the GVG results, the increase in striatal and striatal/cerebellar receptor availability in the lorazepam studies was statistically significant ($F[1,8] = 16.43$, $p < 0.01$; $F[1,8] = 8.15$, $p < 0.02$); however, the effect in cerebellum was also significant

Table 2. Kinetic parameters for ^{11}C-raclopride in baboon brain (control/intervention)

		Distribution volume					
Baboon	Study	ST	% Change[a]	CB	% Change	ST/CB	% Change
Clovis	166A	1.22		0.43		2.84	
	166B[b]	1.64	34*	0.42	−2	3.90	37**
Oral	170A	2.95		0.97		3.01	
	170B[b]	3.71	26*	1.03	6	3.62	20**
Karm	165A	2.94		1.09		2.70	
	165B[b]	3.60	22*	1.11	2	3.24	20**
	172A	2.76		1.00		2.76	
	172B[c]	4.13	49**	1.15	15***	3.59	30**
Elly	171A	4.10		1.30		3.15	
	171B[d]	4.70	15**	1.39	7***	3.39	8**
Spunky	177A	1.78		0.56		3.20	
	177B[e]	2.64	49**	0.68	22***	3.90	22**

[a] % Change = $-[(A - B)/A] \times 100$.
[b] Pretreated with GVG (300 mg/kg).
[c] Pretreated with lorazepam (1.0 mg).
[d] Pretreated with lorazepam (0.75 mg).
[e] Pretreated with lorazepam (1.25 mg).
* Statistically different from test/retest studies ($p < 0.001$).
** Statistically different from test/retest studies ($p < 0.01$).
*** Statistically different from test/retest studies ($p < 0.02$).

($F[1,8] = 16.63$, $p < 0.01$). It is important to reiterate that the effect in striatum remains statistically significant, when expressing the data as the ratio of striatum to cerebellum. No movements of the baboons' heads during or between scans were detected. There were no effects of GVG or lorazepam on respiratory or heart rate or on blood pressure, and the recovery from anesthesia was unremarkable. GVG and lorazepam produced no abnormalities in motor coordination, consistent with that seen in patients suffering from chronic dopamine depletion. The rate of systemic metabolism of the labeled raclopride and the subsequent values of the metabolite-corrected plasma input functions were unaltered by either drug administration.

A lower bound on the estimate of the amount of dopamine bound to receptors in the control studies can be calculated with the following assumpations: (1) negligible displacement of dopamine by raclopride (this should be a reasonable assumption due to the short half-time of raclopride in tissue following a bolus injection and to the similarity of K_d values for both dopamine and raclopride), (2) the cerebellum containing only nonspecific binding, and (3) negligible binding of dopamine to receptors in the second experiment after either treatment. The equilibrium equation for dopamine in the synapse can be written as

$$(B_{max} - RL) = K'_d RL/L, \quad (4)$$

where RL is the concentration of receptors occupied by dopamine, L is the concentration of free dopamine in the synapse and K'_d is the equilibrium dissociation constant for dopamine with the D_2 receptor. This can be related to K_R for the experiments with and without either pretreatment assuming NS is the same for both such that

$$\frac{(B_{max} - RL)}{B_{max}} = \frac{K'_d}{B_{max}}\frac{RL}{L} = \frac{K^A_R(ST)/K^A_R(CB) - 1}{K^B_R(ST)/K^B_R(CB) - 1}, \quad (5)$$

where K_d and (1 + NS) cancel from the right side of Equation 5. Forms of this equation have appeared previously (Farde et al., 1988). From the first and last terms in Equation 5, RL/B_{max} = 0.37 and 0.24 for experiments 166 and 165, respectively, indicating that at least 25–40% of the receptors were occupied by dopamine. The amount of free dopamine was estimated from L/K'_d, which is 0.59 and 0.32 for experiments 166 and 165, respectively. The total dopamine in the synapse (L + RL) is on the order of 6–10 nM assuming dopamine's K_d = 5 nM (Seeman et al., 1990) and B_{max} = 20 nM. These same calculations, carried out assuming that the distribution ratio of nonspecific binding for the striatum/cerebellum is 1.35 as suggested by Farde et al. (1988), would give estimates of synaptic dopamine concentrations of 8–12 nM. The estimates, however, are based upon several assumptions and should be viewed accordingly. These values of synaptic dopamine concentrations represent a lower estimate since it is doubtful, based upon the lack of any behavioral abnormalities following GVG or lorazepam pretreatment, that all the dopamine has been removed from the synapse following GVG or lorazepam pretreatment.

Discussion

These data represent the first *in vivo* demonstration of GABAergic inhibition of endogenous striatal dopamine release with PET. Enhanced GABAergic inhibition induced by GVG or benzodiazepine administration results in a decrease in striatal dopamine concentrations (Palfreyman et al., 1978). This decrease is consistent with our measured increase in ^{11}C-raclopride binding, as there is less endogenous dopamine to compete effectively with labeled raclopride for the D_2 receptor.

The use of a suicide inhibitor of GABA-T provides a unique approach for investigating the modulatory role of GABA on endogenous dopamine release, as this strategy does not involve the direct perturbation of any specific neurotransmitter receptor

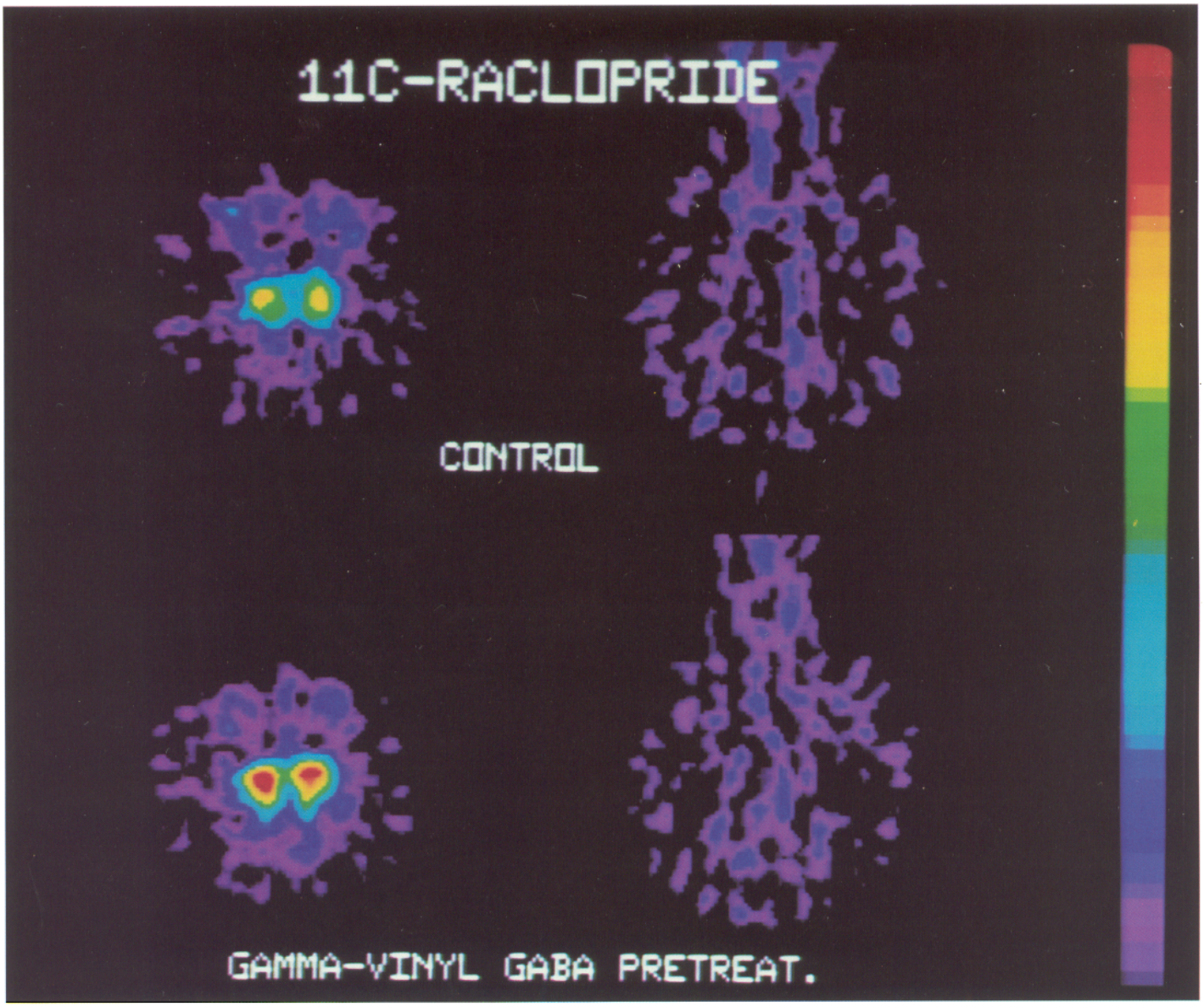

Figure 2. Transaxial PET images of ^{11}C-raclopride binding in the baboon brain 60 min following radiotracer administration. Images on the *left* are at the level of the corpus striatum, while images on the *right* are at the level of the cerebellum. The *top two images* are taken from a control study (165A), and the *bottom two images* were taken 4.5 hr following GVG administration (165B).

complex. Given that GVG's mechanism of action is enzymatically and not receptor mediated, our results indicate that the increase in ^{11}C-raclopride binding represents GABA's direct effect on endogenous dopamine release and not an effect on the D_2 receptor itself. Unlike GVG, however, lorazepam's mechanism of action does involve the direct perturbation of the GABA receptor complex (Kanto and Klotz, 1982). Nevertheless, a direct competition between lorazepam and labeled raclopride for the D_2 receptor would produce a decrease in radiotracer binding and not the measured *increase* observed here. Increasing the dose of lorazepam from 0.75 to 1.0 or 1.25 mg produced a larger increase in striatal ^{11}C-raclopride binding. While this is consistent with a dose–response function, these two studies were performed using two different animals. These data may represent preexisting differences in endogenous neurotransmitter concentrations, given our previous findings of differential effects of similar doses of unlabeled benztropine on ^{11}C-benztropine binding across human subjects (Dewey et al., 1990a). As paired studies (control vs. drug pretreated) were always performed on the same day, small fluctuations in specific activity could not account for the differences we observed, as studies performed on the same day consistently had specific activities that were very similar. The possibility must be considered that the observed increase in ^{11}C-raclopride binding following drug administration is partially attributable to increases in regional cerebral blood flow. However, several lines of evidence argue against such an interpretation. While Edvinsson et al. (1980) have shown in rats *in vivo* that GABA and GABAergic agonists increase blood flow in the cerebral cortex, they observed no changes in cerebellum or subcortical structures including the thalamus and corpus striatum. Lai et al. (1988) were unable to observe an effect of GABA on cerebral arteries isolated from the primate brain. Mathew and Wilson (1991) have reported decreases in cerebral blood flow following benzodiazepine administration to humans.

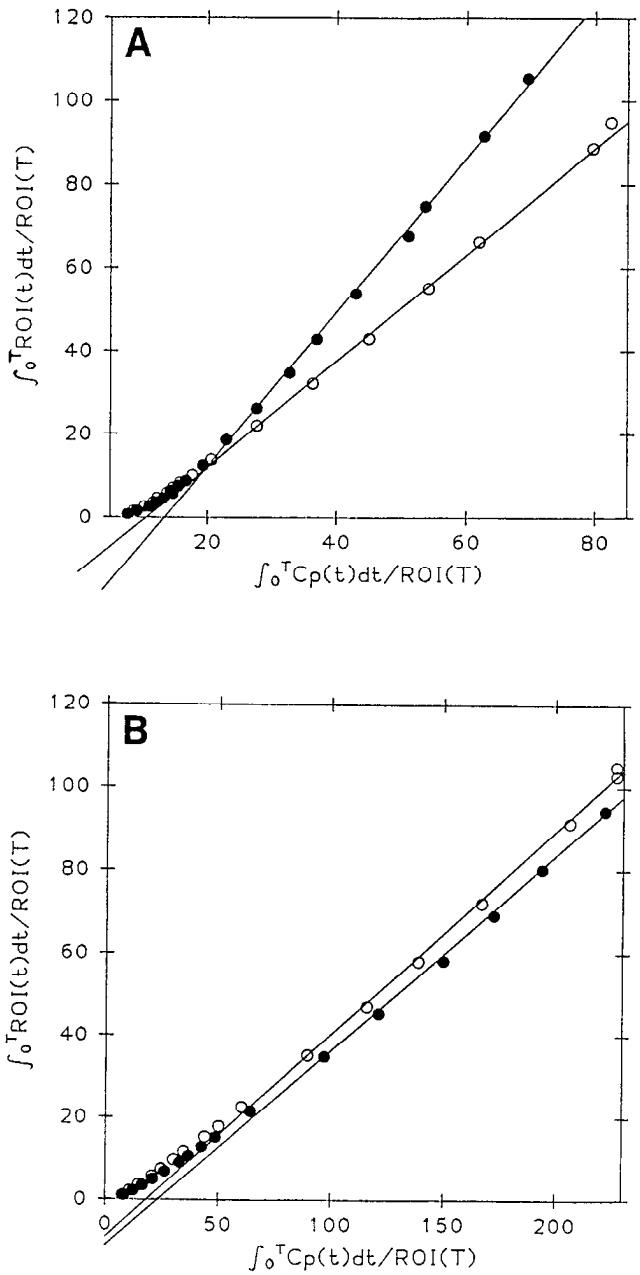

Figure 3. *A*, Graph of the distribution volume for the corpus striatum from BE166. *Open circles* represent the control study (BE166A), and *solid circles* represent the effects of GVG on ^{11}C-raclopride binding (BE166B). *B*, Graph of the distribution volume for the cerebellum from BE166. *Open circles* represent the control study (BE166A), and *solid circles* represent the effects of GVG on ^{11}C-raclopride binding (BE166B).

Kelly et al. (1983) demonstrated that while GABA agonists decreased cortical cerebral blood flow *in vivo*, vasculature supplying subcortical structures including the thalamus and corpus striatum were less sensitive to GABA agonists. These effects are contrary to the observed increase in ^{11}C-raclopride binding reported in this study. Finally, the validity of our graphical analysis method was confirmed by comparing results from the compartmental analysis of studies BE166A and B. Using a three-compartmental (four-parameter) model (Logan et al., 1991), the distribution volumes were found to be 1.26 and 1.69 for the A and B studies, respectively. These values are in good agreement with those calculated from the graphical analysis method (Table 2). The graphical analysis for BE166A and B is illustrated in Figure 3. Values for the transport constant K_1 (striatum) were found to be 0.16 and 0.14 (ml/gm/min) for the A and B studies, respectively, indicating that the uptake should not be particularly sensitive to changes in cerebral blood flow [assuming blood flow values of 0.5 (ml/gm/min) or greater]. Our interpretation of these data is further strengthened by the finding that two drugs that effectively enhance GABAergic transmission by different neurochemical mechanisms produce a significant increase in striatal ^{11}C-raclopride binding.

Taken with our previous PET studies in humans and baboons, our data are consistent with neurophysiological evidence that dopaminergic neurons project either directly or indirectly onto cholinergic neurons in the corpus striatum, bilaterally, which they inhibit. Decreases in ^{18}F-NMSP binding in the striatum following benztropine administration (Dewey et al., 1990b) suggest that cholinergic neurons inhibit striatal dopamine release, presumably through their excitatory input to GABA-containing neurons (Pletscher, 1976). These PET studies uniquely demonstrate the importance of examining not only pathologic changes in separate components of the extrapyramidal motor system (i.e., ACh, dopamine, GABA, etc.) but the multisynaptic consequences of their disruptions as well. Alterations in this multisynaptic feedback loop, for example, have been implicated in the pathogenesis of several CNS diseases (McGeer and McGeer, 1984). These studies also emphasize the importance of assessing receptor availability, as it reflects endogenous neurotransmitter activity *in vivo*. Furthermore, studies designed to quantitate B_{max} values in various disease states need to be viewed with caution, as fluctuations in endogenous neurotransmitter concentrations, due directly to aberrant alterations in the functional integrity of other interactive neurotransmitter systems, may ultimately affect B_{max} measurements.

PET studies using different radiolabeled traces specific for the dopamine D_2 receptor (raclopride and NMSP) in schizophrenic patients have yielded controversial results (Farde et al., 1986; Wong et al., 1986b). While methodological differences, including different radiotracers, may in part explain these inconsistencies, preexisting differences in endogenous dopamine concentrations may provide an alternative explanation. Our PET studies of dopamine D_2 receptor availability as a function of GABAergic inhibition may provide insight into the inconsistencies found between these studies. The possibility exists, for example, that differences in synaptic dopamine concentrations resulting from an altered responsiveness of this system to other functionally linked neurotransmitters may produce these differences. A likely mechanism for this is the insufficient inhibition of endogenous dopamine release by GABAergic neurons in the striatum. In fact, a GABA hypothesis of schizophrenia has been proposed that suggests that an inadequacy of GABAergic inhibition of nigral dopaminergic neurons results in an increase in endogenous striatal dopamine levels (Roberts, 1972; Van Kammen et al., 1982). Benzodiazepine administration, therefore, has been extensively studied as a treatment strategy for neuroleptic-resistant schizophrenic patients (Johnson, 1985; Greenstein et al., 1986).

The studies described in this report demonstrate that the pharmacological manipulation of GABAergic transmission represents an effective route for decreasing endogenous dopamine release. As a result, these data provide direct *in vivo* neurophysiological evidence that furthers our understanding of the

findings that benzodiazepine drugs are potentially useful adjuncts to the effective neuroleptic management of schizaphrenic illness (Wolkowitz and Pickar, 1991), as their efficacy may be related to their ability to inhibit dopamine release through GABAergic interactions. Finally, this new strategy of elevating endogenous GABA levels and using PET to measure dopaminergic response may be useful in evaluating not only the functional integrity of both GABAergic and dopaminergic neurons in patients but the multiple mechanisms of drug actions and their consequences in the human brain as well.

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