SPECT Imaging of the Benzodiazepine Receptor: Feasibility of In Vivo Potency Measurements From Stepwise Displacement Curves

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Iodine-123-labeled Ro 16-0154 is a high affinity, reversibly binding radiotracer for the benzodiazepine (BZ) receptor. Brain uptake of this radioligand was relatively stable and showed high ratios of specific-to-nonspecific uptake, with greater than 90% displaced by intravenous administration of BZ receptor agents. Repeated injections of increasing doses of each of five BZ drugs (Ro 16-0154, Ro 15-1788, clonazepam, alprazolam, and diazepam) yielded stepwise displacement curves, which were analyzed to measure the in vivo potencies of these agents. The relatively long half-life of ^{123}I and the stable biologic uptake of the radiotracer allowed such potency estimations in just one experiment following a single injection of radioligand. The in vivo potencies of these five agents were highly correlated with their affinities for the BZ receptor determined with in vitro homogenate binding. A single crystal probe provided potency measurements virtually identical to simultaneously performed SPECT imaging studies. In conclusion, stepwise displacement by agents administered following the injection of the radioligand ^{123}I-Ro 16-0154 provided a reliable means of measuring the in vivo potencies of BZ receptor agents. This in vivo determination may predict the clinical potency of BZ drugs than in vitro homogenate estimations, because the in vivo measure provides the summed effects of receptor affinity, plasma protein binding, penetration of the blood-brain barrier, and metabolism of the displacing agent.

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Ro 16-0154 (ethyl 7-iodo-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4] benzodiazepine-3-carboxylate) is an iodine-containing antagonist of the BZ receptor (*1*). The binding of ^{125}I-Ro 16-0154 to brain tissue homogenates prepared from human and monkey brain has been shown to be reversible, saturable, of high affinity (Kd = 0.5 n*M* at 37°C), and to have a high ratio of specific to nonspecific binding (~40:1) (*2*). Preliminary studies with ^{123}I-Ro 16-0154 in human (*1*) and non-human (*3*) primates suggest that this radioligand is a useful SPECT probe of the BZ receptor in the central nervous system. The maximal uptake into monkey brain is high (approximately 7% at 120 min postinjection) and relatively stable for an additional 240 min. Approximately 90% of brain uptake can be displaced by the closely related BZ antagonist Ro 15-1788 (0.1–0.2 mg/kg i.v.), even up to 360 min postinjection of the radioligand. The fact that the radioligand can actually be displaced (rather than requiring pretreatment blockade of uptake) and that it has a long period of apparent steady-state suggested the feasibility of measuring potency by in vivo displacement studies following repeated injections of increasing doses of displacer. The purpose of this study was to evaluate this stepwise displacement method to measure the potency of agents at the central BZ receptor.

SPECT is generally thought to be less accurate than PET to provide absolute quantitation of the concentration of radiotracer (*4*). For this reason, SPECT may be less suited than PET for measurement of receptor number, which is expressed in absolute units like pmole/g of tissue. On the other hand, receptor affinity can be determined from relative measurements of radioactive concentrations (*5*). "Affinity" is measured in an *absolute* unit of concentration (e.g., p*M*) but is dependent upon only a *relative* change of the radioactive signal (e.g., to half of its original value). In a manner exactly analogous to in vitro receptor binding studies, we have examined the ability of SPECT to provide an in vivo measurement of drug affinity, which is typically referred to as "potency" and is expressed as the ED_{50} or dose of drug required to displace 50% of radiotracer binding.

MATERIALS AND METHODS

Iodine-123-Ro 16-0154 (Fig. 1) was prepared by oxidative radioiodination of the tributyltin precursor (ethyl 7-tributyl-tin-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4] benzodiazepine-3-carboxylate), following a no-carrier-added method of io-

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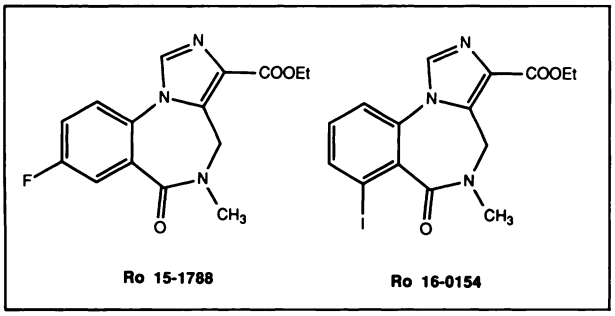

FIGURE 1. Chemical structures of Ro 16-0154 and Ro 15-1788.

dination described elsewhere (*6*). Using the same batch of precursor and identical methods, McBride et al. (*6*) measured the specific activity of product to be greater than 180,000 Ci/mmol. Tracer was separated from the precursor on an isocratic HPLC system with C-18 reverse-phase column and a mobile phase of 55% methanol/water. The final product was formulated in 6 ml of sterile saline containing 3% ethanol.

A total of 28 SPECT scanning experiments were performed with twelve baboons (10-kg female *Papio anubis*) and four rhesus monkeys (10-kg male *Macaca mulatta*). Animals were intravenously anesthetized with pentobarbital and aligned with a laser light for imaging in planes parallel to the cantho-meatal (CM) line. The head was immobilized with a "bean bag" which is molded around the head and hardens on evacuation (Olympic Medical, Seattle, WA). Iodine-123-Ro 16-0154 (9.8 ± 1.0 mCi, expressed as mean ± s.e.m.) was injected intravenously, and brain uptake was monitored in serial 2-min acquisitions with the 810X Brain Imager (Strichman Medical Equipment, Medfield, MA). Images were attenuation corrected assuming uniform attenuation equal to that of water in an ellipse drawn around the brain.

Images were reconstructed in planes parallel to the CM line. Because the highest concentration of radioactivity was consistently localized in the occipital area, the plane through this region (approximately 15 mm rostral to the CM line) was selected for detailed kinetic analyses determined from repeated 2 min acquisitions. Regions of interest (ROIs) were outlined which roughly corresponded to right and left occipital, parietal, striatal, and frontal regions (Fig. 2). This ROI template was then applied to all slices from that study. Data were analyzed as the average regional radioactivity, expressed as "counts/ 2 min/ pixel." Radioactivity measurements were decay-corrected to time of injection.

In three experiments, a single crystal NaI probe was used which has a collimator with a broad field of view. This device (Thyro-Count, Kemble Instruments, Inc., Hamden, CT) is normally used for thyroid uptake measurements. While the animal was in the SPECT scanner, the thyroid probe was directed at the head at a distance of approximately 20 cm. Activity was recorded at one min intervals.

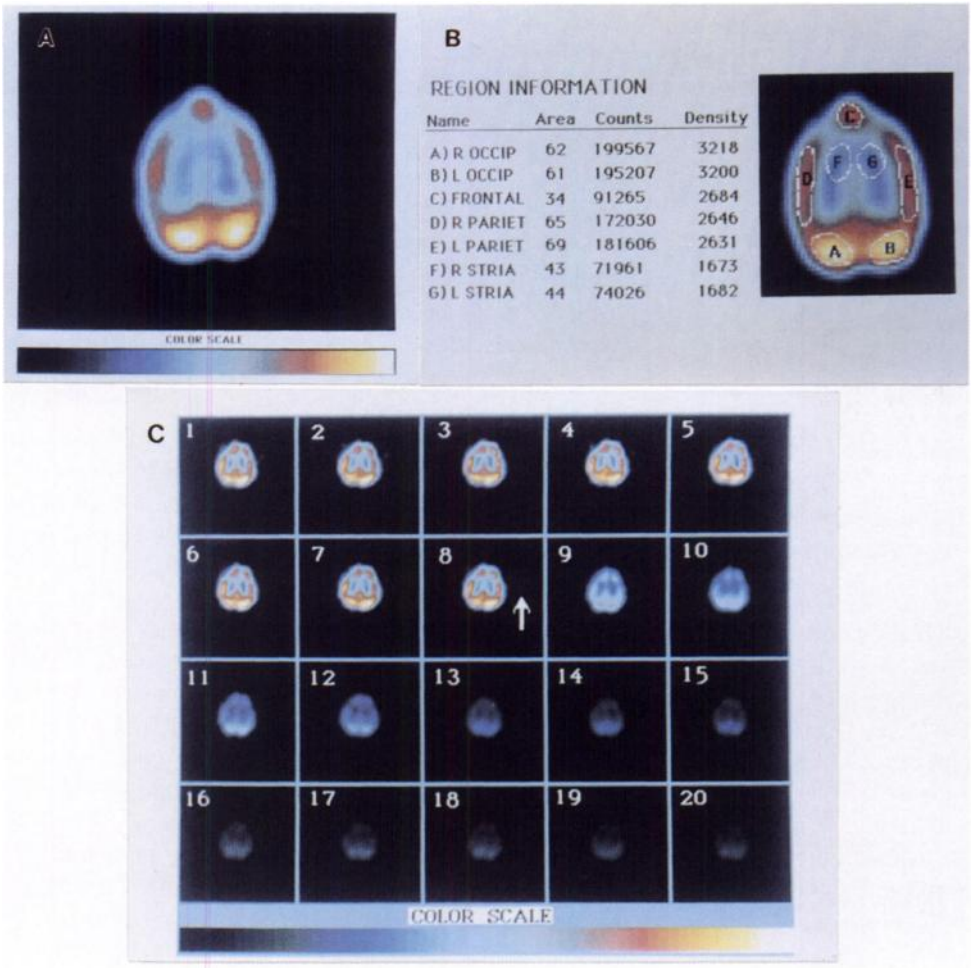

FIGURE 2. (A) Transaxial slice through the baboon brain at the level of the occipital cortex and striatum approximately 70 min postinjection 16 mCi ^{123}I-Ro 16-0154. This image was reconstructed from data acquired by the Strichman 810X over 2 min from a plane parallel and approximately 15 mm superior to the cantho-meatal line. (B) Typical ROIs drawn on the computer screen are used for determination of concentration of radioactivity. The area of the region is expressed in pixels, and the density in counts/2 min/pixel, with each pixel corresponding to approximately 1.6 × 1.6 mm. (C) Repeat pictures from serial 2-min acquisitions through the same area of baboon brain, with slice 1 starting approximately 60 min postinjection of 15 mCi ^{123}I-Ro 16-0154. Between slices 8 and 9, the administration of nonradioactive Ro 15-1788 (0.1 mg/kg i.v.) caused a rapid decrease of almost 90% of radioactivity from brain. This washout of radioactivity is consistent with displacement of radioligand bound to the benzodiazepine receptor.

To test the linearity of recorded SPECT measurements vs activity, a simple phantom was constructed with a plastic cylinder (200 ml total volume, approximately 9.0 cm diameter and 7.0 cm length) which had smaller cylindrical inserts (5 ml each, approximately 1.0 cm diameter and 3.4 cm length) to represent three brain areas in each hemisphere. The total radioactivity in the phantom (1 mCi) and concentrations in each vial and the background area were selected to approximate those previously measured from postmortem analysis following ^{123}I-Ro 16-0154 scans in monkeys (*7*). Ratios of radioactivity were: occipital (30), frontal (15), parietal (25), and background (1). ^{99m}Tc was used as the radionuclide for these measurements. Its photon emission (140 keV) is similar in energy to that of ^{123}I (159 keV). The phantom was repeatedly scanned for 2 min over a 24-hr period of time. Thus, the physical decay of the radionuclide was used to generate known varying concentrations at several time points over a 24-hr period.

Drugs were obtained from the following sources: Ro 15-1788 and Ro 16-0154 (Hoffmann-La Roche, Basel, Switzerland); diazepam and clonazepam (Sigma, St. Louis, MO); lorazepam (Upjohn, Kalamazoo, MI). Agents were dissolved in ethanol, then diluted 10–20-fold in normal saline, and administered intravenously over 30-60 sec.

RESULTS

Brain Uptake

Following injection of ^{123}I-Ro 16-0154, the occipital area showed the highest uptake of radioligand (Fig. 2). The SPECT slice through this area (approximately 15 mm above the cantho-meatal line) was chosen for detailed pharmacological studies. A template of ROIs was placed over repeated slices obtained with a 2-min data acquisition. Artifacts caused by head movement were minimized by immobilizing the monkey's head in a vacuum pack "bean bag" and by having the animal anesthetized.

The regional distribution of radioactivity corresponded to the known distribution of BZ receptors in primate brain (*8*). The distribution of radioactivity was determined at the time of maximal brain uptake using typical regions shown in Figure 2B. Relative concentrations of radioactivity in various brain regions were: occipital (100%); temporoparietal (66% ± 4%), frontal (66% ± 4%); striatal (46% ± 2%); and cerebellar (65% ± 4%), expressed as mean ± s.e.m. for the first eight animals studied.

Temperature Dependence

The uptake of radioactivity into brain following the infection of ^{123}I-Ro 16-0154 reached an apparent steady-state in about 120 min and remained relatively stable for another 240 min. During the course of these studies with ^{123}I-Ro 16-0154, we serendipitously discovered a temperature dependence of clearance of brain radioactivity, because barbiturate anesthesia tends to lower body temperature. We found that animals with hypothermic core temperatures of 32–34°C (measured with deep rectal probe) had a slower clearance of radioligand than animals at normothermic temperatures of 36–37°C maintained with a heated circulating water blanket. Subsequent to this discovery (about midway in the studies), experiments were performed at controlled and monitored hypothermic core temperatures (32–34°C). These animal studies with monitored hypothermic temperatures were combined with previous unmonitored experiments in which core temperatures were retrospectively thought to have been hypothermic because of the effects of pentobarbital anesthesia.

The temperature dependence of the brain clearance of ^{123}I-Ro 16-0154 was measured as the %decrease of radioactivity in the 60 min period after peak values were reached. At hypothermic temperatures, the brain washout was 3.3% ± 1.1% per hour (n = 5) compared to 14% ± 0.8% per hour for normothermic animals (n = 4). Lower temperatures were used for the studies described here because they provided a more stable baseline against which to assess the effects of stepwise displacements. For studies performed at normothermic temperatures, the baseline values were declining and could be estimated from linearly projected values expected at those times if no displacing agent had been administered. With a hypothermic washout of only 3.3% per hour, such projected estimates had minimal effects on ED_{50} measurements and were not used for the values reported here.

Stepwise Displacement Studies

Approximately 90% of brain radioactivity was displaced by the intravenous administration of the structurally-related BZ antagonist Ro 15-1788 (0.2 mg/kg) (Fig. 2C and 3C). Thus, even up to 6 hr postinjection of radioligand, the vast majority of brain radioactivity appeared to be associated with the BZ receptor and, therefore, displaceable by BZ agents. This long period of relatively stable brain uptake of ^{123}I-Ro 16-0154 was used as a baseline against which to compare the effects of increasing doses of five BZ receptor drugs (Figs. 3–4). These stepwise curves were analyzed using the residual activity after the final dose of displacer as a measure of non-displaceable radioligand (typically 5%–10% of peak levels). The non-displaceable level was subtracted from each point to provide a measure of specifically bound radioligand. The percent decrease of specific uptake following each dose of displacer was measured at the plateau level in the 5–10-min period prior to the next displacing dose. The ED_{50} dose of displacer required to inhibit 50% of specific uptake was determined from a straight line fit of the log-probit plot of percent displacement versus log of cumulative dose.

Stepwise displacement studies were performed two to three times for each of five BZ agents: two antagonists (Ro 16-0154 and Ro 15-1788) and three agonists (diazepam, alprazolam, and clonazepam). The reproducibility of ED_{50} measurements was studied in different animals and varied by less than three fold (Table 1). The relative order of in vivo potencies of the five agents were: Ro 16-0154 > Ro 15-1788 >> clonazepam ≈ alprazolam > diazepam.

In addition to providing a measure of in vivo potency, the stepwise displacement curves provided pharmacokinetic information on the time required by the displacing

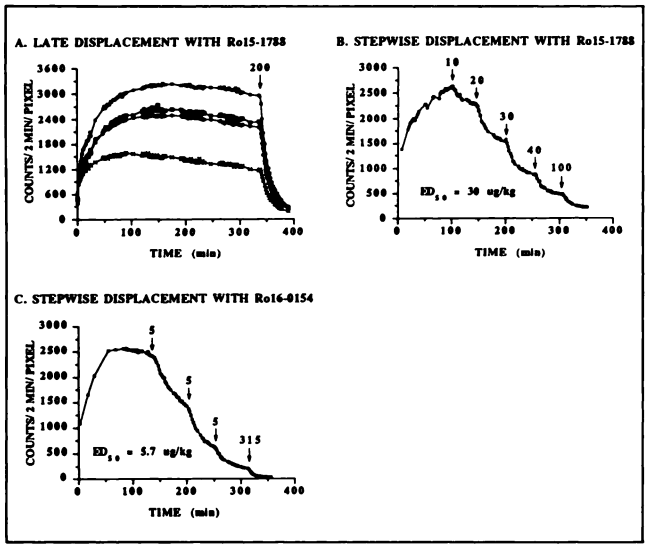

FIGURE 3. Time course of brain radioactive densities in monkeys following intravenous injection of ^{123}I-Ro 16-0154 at time 0. (A) The animal was injected with 15 mCi of ^{123}I-Ro 16-0154, and serial 1–2-min scans were obtained from the transaxial slice approximately 15 mm rostral to the cantho-meatal line. Listed from highest to lowest, regional activities were: occipital (□); frontal (□); parietal (◆); and striatal (×). Ro 15-1788 (200 μg/kg i.v.) caused a rapid decrease of almost 90% of brain radioactivity. (B) Following injection of 14.9 mCi ^{123}I-Ro 16-0154, nonradioactive Ro 15-1788 was injected intravenously with the individual doses indicated in units of μg/kg. "Specific" uptake was defined as the difference between "Total" uptake (graphed above) and "Nondisplaceable" uptake remaining after the last dose of Ro 15-1788. The ED_{50} of the displacing agent (i.e., Ro 15-1788) was defined as the cumulative dose required to displace 50% of the "Specific" uptake and was determined from a log-probit plot to be 30 μg/kg. The ED_{50}'s determined from three other regions (parietal, frontal, and striatal) were virtually identical to that shown above for the occipital area. (C) Following injection of 12.4 mCi ^{123}I-Ro 16-0154, nonradioactive Ro 16-0154 was injected intravenously with the individual doses indicated in units of μg/kg. The ED_{50} was calculated for this occipital area as described above and found to be 7 μg/kg. Comparison of panels B and C shows that in vivo displacement of the benzodiazepine radioligand caused by Ro 15-1788 is more rapid but less potent than that with Ro 16-0154.

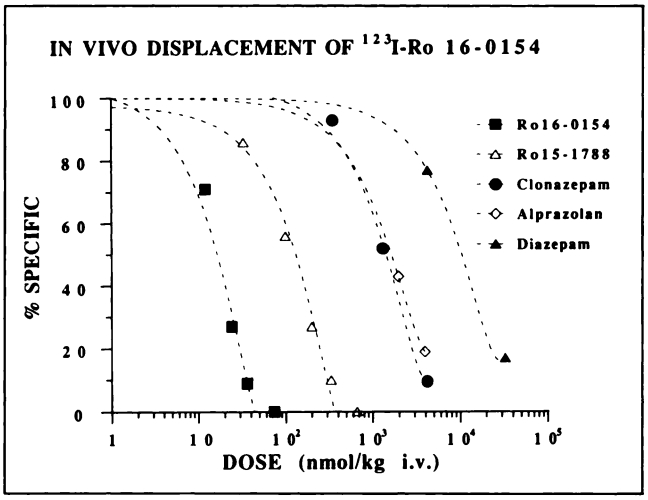

FIGURE 4. The in vivo displacement of ^{123}I-Ro 16-0154 by five benzodiazepine receptor agents is plotted as the %specific uptake in the occipital area vs cumulative dose of displacer. Each curve was determined from one scanning session in which stepwise increasing doses of the displacer were administered (as in Fig. 2B and C). The relative in vivo potencies of these agents were: Ro 16-0154 > Ro 15-788 > clonazepam ≈ alprazolam > diazepam.

agent to reach the brain's BZ receptor. For example, the displacement curves of Ro 16-0154 were more shallow than those of Ro 15-1788 (Fig. 3), indicating that Ro 16-0154 was relatively slow in gaining access to the BZ receptor. To gain a measure of this pharmacokinetic parameter, the individual displacement curves were fit to exponential curves and the half-life was calculated (Table 1). The speed of drug access to the BZ receptor was *not* correlated with in vivo potency. For example, Ro 15-1788 caused a more rapid displacement but was four fold less potent than Ro 16-0154.

Correlation of In Vivo Drug Potency with In Vitro Receptor Affinity

The affinity of these five agents for the BZ receptor has previously been measured in vitro with homogenate binding studies using ^{125}I-Ro 16-0154 (*2*). The inhibition constant (K_i, which is inversely related to affinity) was determined using primate brain and incubation temperature of 37°C. The in vivo measure of potency (ED_{50}) was highly correlated with the in vitro measure of receptor affinity (K_i), when displayed as either a linear-linear (r = 0.98) or the more stringent log-log plot (r = 0.94; Fig. 5).

Single-Dose Displacement

The analysis of the stepwise displacement curves uses the cumulative dose. The accuracy of the cumulative dose analysis depends upon the assumption that an insignificant amount of previous doses of displacer has been removed from the brain. To test the accuracy of this method for the displacing agent Ro 15-1788, we compared the displace-

TABLE 1
Displacement of ^{123}I-Ro 16 0154 in Non-Human Primate Brain*

	ED_{50} (nmole/kg)		Exponential fit†	
Displacer	Mean	(Individual values)	$T_{½}$	r Value
Ro16-0154	15.4	(13.9, 17.0)	15.1 ± 2.2	0.992 ± 0.002
Ro 15-1788	52.8	(33.0, 59.3, 98.9)	11.2 ± 0.5	0.994 ± 0.002
Clonazepam	1, 330	(950, 1,710)	11.3 ± 0.2	0.995 ± 0.001
Alprazolam	2,800	(1,720, 3,890)	12.5 ± 2.0	0.989 ± 0.002
Diazepam	10,700	(10,200, 11,200)	13.0 ± 1.2	0.994 ± 0.001

* Stepwise increasing doses of the displacer were administered intravenously, and the ED_{50} was calculated from a log-probit plot using the cumulative dose. The displacement curves from individual doses were fit to exponential curves, and half-lives and r values were calculated.

† Expressed as mean ± s.e.m.

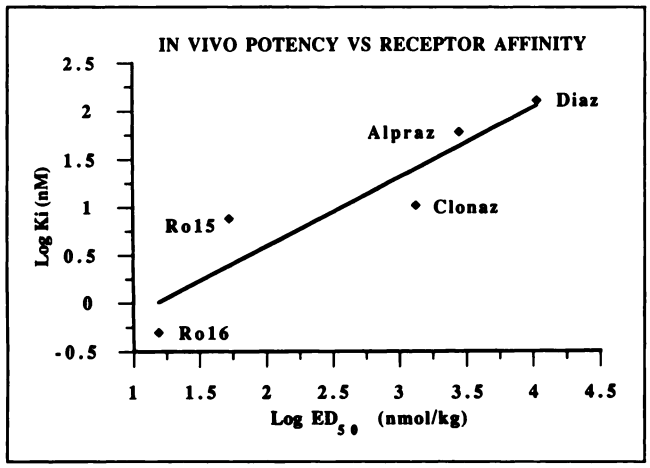

FIGURE 5. Correlation of the in vivo ED_{50} doses and in vitro K_i concentrations of five BZ agents. The ED_{50} of each agent was determined from stepwise displacement of ^{123}I-Ro 16-0154 in monkey brain. The K_i values were determined from homogenate binding studies of ^{125}I-Ro 16-0154 binding to membranes prepared from human and monkey cerebral cortex, which gave virtually identical results (2). The K_i value represents the mean of three separate determinations; the ED_{50} value, the mean of two to three separate experiments. A linear fit determined with least-squares analysis method had a correlation coefficient r = 0.92. The displacing agents were: Ro16 = Ro 16-0154; Ro15 = Ro 15-1788; Clon = clonazepam; Alpraz = alprazolam; and Diaz = diazepam.

ment caused by a single injection of 20 μg/kg intravenously to the result determined by stepwise displacement above, which was the average of three separate experimental measurements. Since stepwise curves typically used two initial doses of 10 μg/kg Ro 15-1788 separated by 40–60 min, the experimental question was: Does a single dose of 20 μg/kg Ro 15-1788 equal that of two doses of 10 μg/kg administered at different times?

The administration of 20 μg/kg Ro 15-1788 caused displacement of 41% (mean value of two separate measurements of 39 and 42%). This value was similar to the average displacement value of stepwise curves in three experiments (62%). If previous doses of Ro 15-1788 had been cleared from brain, the stepwise displacement should, in fact, have been less than that from a single dose. This study suggests that, within the accuracy of the measurements and for time intervals of 40–60 min, cumulative dose analysis is acceptable for stepwise displacement curves for Ro 15-1788.

Single-Crystal Probe

Since the in vivo displacement studies showed that the radioligand ^{123}I-Ro 16-0154 had a very favorable ratio of specific-to-nonspecific binding (with approximately 90% of radioactive signal displaceable), we used a less sophisticated, single-crystal thyroid probe to concurrently measure in vivo potency. The shape of the displacement curve measured with this thyroid probe determined in three separate experiments) was similar to that determined with regional brain SPECT data, as well as to the total counts in the entire SPECT slice (Fig. 6). Representative data from one such simultaneous experiment showed virtually identical displacement curves and ED_{50} values.

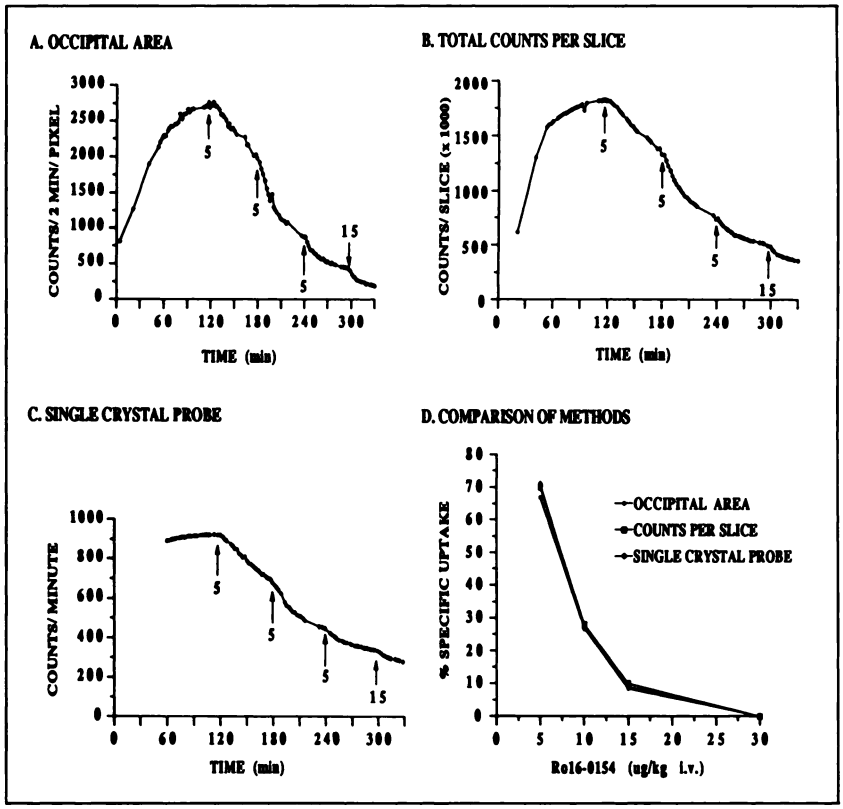

FIGURE 6. Displacement of ^{123}I-Ro 16-0154 simultaneously measured with SPECT and single crystal devices. (A) Following the injection of 14.5 mCi ^{123}I-Ro 16-0154 at time 0, stepwise doses of nonradioactive Ro 16-0154 (μg/kg i.v.) were administered at times indicated with arrows. Curve displays regional radioactivity concentrations in occipital area in exactly the same manner as Figures 1 and 2. (B) The total counts per slice through the occipital area are displayed. (C) A single crystal probe (typically used to monitor thyroid radioactivity) was aimed at the animal's head at a distance of approximately 20 cm, and one min serial counts recorded. By adjusting the direction of the monitor, it was clear that the relatively high baseline counts after the final injection of Ro 16-0154 (15 μg/kg) derived in large part from the torso and was caused by the broad field of view of this probe. (D) Displacement measured by these three methods is plotted as %specific uptake versus the cumulative dose of Ro 16-0154. "Specific" uptake was defined as the excess radioactivity above that present after the final dose of displacing agent. The three curves show virtually complete overlap and estimate the ED_{50} as 7 μg/kg.

Linearity of SPECT Measurements

As discussed above, these in vivo potency measurements do not require absolute quantitation of the radioactive signal but do assume linearity of the measurement. To test the linearity of SPECT measurements at varying concentrations of radioactivity, a simple phantom was created to approximate the distribution and concentrations of radioactivity found in the monkey displacement studies. The phantom was filled with ^{99m}Tc and imaged at six time points (five acquisitions of 2 min each) during a 24-hr period. The physical decay of the radionuclide resulted in radioactivity levels which varied over 90% and which were similar to the decrease of radioactivity found in the in vivo displacement experiments.

The linearity of SPECT measurements was then assessed in two ways: (1) ROI data were fit to an exponential curve and the calculated half-life was compared to the known value for ^{99m}Tc and (2) decay-corrected ROI data were fit to a straight line and the slope calculated.

Data from the "occipital" and "frontal" regions of the phantom were representative of all areas and are graphically presented in Figure 7. Region of interest data were fit to an exponential curve, with a half-life of 346 min for the "frontal" area ($r^2 = 0.998$) and half-life of 354 min for the "occipital" area ($r^2 = 0.997$), which closely approximated the exponential decay of ^{99m}Tc ($T_{1/2}$ = 362 min). When data were decay-corrected and fit to a straight line, the slopes were: frontal, −0.042 and occipital, −0.026 (expressed as cpm/pixel/min). These results closely approximated absolute linearity which would have slope = 0.

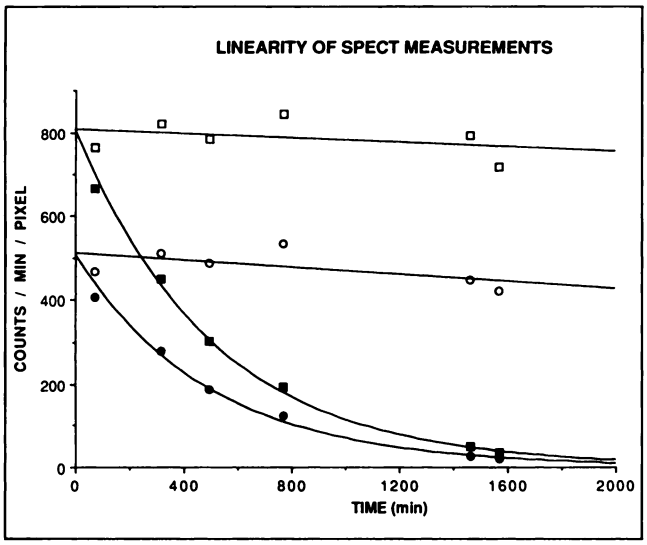

FIGURE 7. Activity (cpm/pixels) in the "occipital" and "frontal" areas measured over a 24-hr period from a ^{99m}Tc filled phantom, created to approximate the distribution of ^{123}I-Ro 16-0154 in monkey brain. At each of six time points, the phantom was imaged five times with a 2-min acquisition time. Filled symbols represent mean activity measured in the occipital (■) and frontal (●) areas; open symbols, decay-corrected activity in occipital (□) and frontal (○) areas. The measured activity was fitted to an exponential curve which had $T_{1/2}$ of 346 min and 354 min for frontal and occipital regions, respectively. Corrected activity was fitted to a straight line which had a slight negative slope of −0.042 and −0.026 cpm/pixel per min for frontal and occipital areas, respectively. Error bars were not included in graph, because they were smaller than the size of the symbol denoting the mean. The average ratio of s.d.-to-mean for the six time points was 1.6% for occipital and 2.3% for frontal.

DISCUSSION

The major finding of these studies is that SPECT imaging using the BZ receptor radioligand ^{123}I-Ro 16-0154 in conjunction with stepwise displacements can provide a measure of the in vivo potency (i.e., ED_{50}) of displacing drugs. Estimation of ED_{50} from three to five stepwise increasing doses of displacer was performed in one experiment following a single injection of radioligand. ED_{50} values for five BZ receptor drugs were highly correlated with their receptor affinities determined with in vitro homogenate binding using ^{125}I-Ro 16-0154. The accuracy of the SPECT scanning results was dependent upon a linear measurement of radioactive densities, which was demonstrated for this particular camera. Finally, the accuracy of the SPECT measurements was confirmed with concurrent measurements using a single crystal detector.

Receptor Reserve

These in vivo displacement studies confirm previous reports of a major receptor reserve for the BZ binding site (*9*). This conclusion derives from the expected receptor occupancy required for effects induced by both agonists and antagonists. For example, maximal sedative and anticonvulsant effects of the agonist diazepam occur at doses of 300–1,000 nmole/kg intravenously (*10*). The in vivo displacement studies with ^{123}I-Ro 16-0154 suggest that these doses would correspond to only 10%–30% receptor occupancy (Fig. 4). The remaining 70%–90% of the binding sites are referred to as "spare receptors" which comprise the "receptor reserve." The rationale for this terminology is that the entire reserve could theoretically be inactivated without diminishing the efficacy (or peak response) of agonist drugs. In the case of significant receptor reserve, in vivo potency estimations (like ED_{50} measurements described here) will suggest that agonists are unusually weak relative to clinically or behaviorally effective doses.

The situation is reversed for antagonists. For example, the ED_{50} of the antagonist Ro 15-1788 required to block agonist-induced anti-convulsant effects is 2.8 mg/kg intravenously (*11*), which would correspond to approximately 90% receptor occupancy (Fig. 4). The antagonist must block nearly the entire receptor reserve before effectively diminishing the action of the agonists. Thus, in vivo potency estimations (like ED_{50} measurements) may suggest that antagonists are unusually strong relative to clinically or behaviorally effective doses.

A major reserve for the BZ receptor has significant implications for brain imaging studies, whether performed with PET or SPECT. For example, saturation studies (for

determination of Bmax and Kd) require significant receptor occupancy (certainly greater than 50%) by the radioligand. If the tracer is an agonist, then supramaximal doses of sedating agents need to be administered, and such studies may be impossible in human subjects. Comparable saturation studies with an antagonist radioligand would be expected to have minimal pharmacological side effects, because the highest dose of the antagonist would barely occupy the full receptor reserve.

For the studies reported here, the radioligand itself occupies only a small percentage of receptors. The radionuclide (^{123}I-NaI) is provided no-carrier-added, and the use of a tributyltin precursor (in contrast to iodine isotope exchange) produces product with very high specific activity (>180,000 Ci/mmol). A dose of 10 mCi ^{123}I-Ro 16-0154 is estimated to occupy less than 0.2% of the BZ receptors in monkey brain (*3*). Thus, these studies used radiotracer doses which would be expected to have no pharmacologic effects.

Effect of Barbiturate Anesthesia

The BZ receptor is a drug binding site on the alpha subunit of the multimeric $GABA_A$, receptor, which itself forms a chloride ionophore and may be the major inhibitory neurotransmitter receptor in mammalian brain (for review see reference *12*). BZ agonists act allosterically to enhance GABA-induced inhibition mediated by increased chloride conductance. Barbiturates are thought to act via a separate binding site but also function to enhance GABA-induced inhibition. The BZ binding site is distinct from that for GABA and barbiturates, and BZ drugs are *not* displaced by either of these agents (*12*). In fact, binding studies have shown no effect of GABA or barbiturates to displace ^{123}I-Ro 16-0154 binding to tissue homogenates (*2*). Thus, endogenous GABA or the barbiturate used for anesthesia (i.e., pentobarbital) would not directly affect these brain imaging studies. However, both GABA and barbiturates have been shown to act allosterically to modulate the affinity of agonists, but *not* that of antagonists (*13,14*). Since Ro 16-0154 is a potent antagonist with only weak inverse agonist properties (Hunkeler W, personal communication), neither endogenous GABA nor the barbiturate anesthetic would be expected to modulate the affinity of ^{123}I-Ro 16-0154. Nevertheless, both GABA and pentobarbital may be acting allosterically to enhance by a factor of 2–3 the affinity of the agonists (diazepam, alprazolam, and clonazepam) used in these studies (*14*). The potential effect of pentobarbital has been examined in two baboons, each of which was scanned twice (first with isoflurane anesthesia and 1–2 wk later with pentobarbital) for measurement of the in vivo potency of diazepam to displace ^{123}I-Ro 16-0154. The ED_{50} values were: animal 1 (1.7 and 1.4) and animal 2 (1.5 and 1.6 mg/kg i.v.) for isoflurane and pentobarbital, respectively. These results suggest that pentobarbital at doses used in these studies (3–4.5 mg/kg i.v. every 30 min) had no significant effect on the potency of agonists.

Comparison with PET Imaging

In vivo potency of drugs for the BZ receptor have been previously performed with PET using ^{11}C-Ro 15-1788 (*15–17*). For such potency measurements, SPECT offers several advantages over PET. First, the brain uptake of ^{123}I-Ro 16-0154 is more stable for a longer period of time than the PET radioligand ^{11}C-Ro 15-1788. As mentioned above, the clearance of ^{123}I-Ro 16-0154 from brain at normothermic temperatures is approximately 14% per hour after peak values have been reached. Data are not reported in a similar fashion for comparable PET studies, but estimation from the reported graphical data suggests a washout of almost 60% per hour (*15*). The stable period of brain uptake of the SPECT radioligand may derive, in part, from the 10-fold higher affinity of Ro 16-0154 over Ro 15-1788 (*2*). The use of ^{123}I-Ro 16-0154 allows a longer and more stable baseline against which to measure the effects of displacing agents.

Second, the longer half-life of ^{123}I (13 hr) compared to ^{11}C (20 min) allows longer individual experiments and, thus, multiple injections of displacing agents. For example, our studies with ^{123}I-Ro 16-0154 frequently lasted 6 hr, during which time a comparable ^{11}C-labeled compound would have decayed by 18 half-lives.

Thus, because of the long biologic and physical properties of ^{123}I-Ro 16-0154, measurement of ED_{50} determined from three to five displacing doses could be measured in one experiment in a single animal. Comparable PET studies with ^{11}C-Ro 15-1788 required separate experiments for each dose of the displacing agent (*15*).

Requirements of the Imaging Device

As discussed above, in vivo potency measurements do not require absolute quantitation of the radioactive signal. Rather, our ED_{50} estimations merely had the implicit assumption that the measurements were linear. The phantom study showed that fixed locations within the imaging field of the Strichman 810X device provided a linear correlation between true and observed activity in the concentration ranges expected for these non-human primate experiments.

Pharmacokinetics

The in vivo displacement studies provided a measure of the rate with which BZ drugs reach their target sites in the central nervous system. The kinetics of displacement was measured as the half-life of the exponential fit of the displacement curve. In general, the ability of in vivo displacement studies to provide such pharmacokinetic measurements depends in part on the relative rates of brain uptake of the displacing drug and the off-rate of the radioligand from the receptor. If the off-rate of the radioligand is many times larger than the rate of drug entry into brain, then brain uptake would not be rate-limiting and would not be significantly reflected in the slope of the displacement curve.

Homogenate binding studies with ^{125}I-Ro 16-0154 have shown that dissociation of the radioligand is exponential

with a half-life of approximately 2 min at 37°C (*2*). Assuming a similar in vivo rate, the prolonged brain uptake of ^{123}I-Ro 16-0154 must correlate with repeated binding and re-binding of the radioligand. Such a rapid receptor off-rate would provide excellent temporal resolution for the measurement of the brain uptake of displacing drugs.

Comparison of In Vivo and In Vitro Results

The in vivo potencies (measured as ED_{50} values in displacing ^{123}I-Ro 16-0154) were highly correlated with in vitro receptor affinities (measured as the K_i values for homogenate displacement of ^{125}I-Ro 16-0154). Although this correlation was strong ($r = 0.94$), it was not perfect. These discrepancies can be clearly seen in the comparison of clonazepam and alprazolam, whose in vivo potencies differ two-fold, but whose in vitro receptor affinities differ six-fold (Fig. 5). The cause for these discrepancies may well be different bioavailabilities of the compounds. That is, the in vivo displacement represents the combined effects of receptor affinity *plus* metabolism, lipophilicity, plasma-protein binding, first-pass effects, and penetration of the blood-brain barrier. In vitro measurements have artificially simple experimental conditions which focus only on receptor affinity. In vivo potency measurements would be expected to have a stronger correlation to behavioral or clinical potencies of the receptor agents than in vitro receptor affinity. For example, clonazepam shows approximately two-fold greater clinical potency than alprazolam (*18*), which is more consistent with the ED_{50} measurements rather than the in vitro K_i values which differ by six-fold (Fig. 5).

CONCLUSION

Iodine-123-Ro 16-0154 is a useful SPECT probe for the BZ receptor in primate brain. Uptake of this radioligand was relatively stable for several hours after peak levels are achieved. Increasing doses of intravenously administered BZ receptor agents produced stepwise displacement curves which can be analyzed to provide a measure of the in vivo potency of the displacer and the rate with which these drugs reach their target sites in brain. The relatively stable brain uptake of the tracer allowed such estimations in a single experiment with one injection of radioligand. Furthermore, nonspecific uptake was low enough (less than 10%) that potency measurements could be performed with a single-crystal probe.

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