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A simple probe measures the pharmacokinetics of [^{125}I]RTI-55 in mouse brain in vivo

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Abstract

A simple external radiation detector system was used to assess brain dopamine and serotonin transporters in mice in vivo using [^{125}I]RTI-55. The results were compared to ex vivo dissection data. Methyl 3β-(4-iodophenyl) tropane-2β-carboxic acid methyl ester (RTI-55 or β-CIT), a high-affinity cocaine antagonist, binds to presynaptic dopamine and serotonin transporters in the brain. Radiotracer accumulation increased for the first 150 min after intravenous injection and then remained constant. When unlabeled RTI-55 was injected, either before or 60 min after radiotracer administration, a significant decrease in tracer accumulation was observed. [^{125}I]RTI-55 binding was also displaced by blocking doses of 1-[2-[*bis*(4-fluorophenyl)methoxy]ethyl]-4-[3-phenylpropyl]piperazine dihydrochloride (GBR 12909) and paroxetine. The results were similar to the ex vivo dissection data. The results demonstrate the feasibility of using the probe detector system to study the presynaptic transporter system in vivo in the mouse brain. The technique is applicable to other cerebral neurotransmitter systems. © 1997 Elsevier Science B.V.

Keywords: Single photon detection system; Dopamine transporter; 5-HT (5-hydroxytryptamine, serotonin) transporter; GBR 12909; Paroxetine; RTI-55

1. Introduction

A simple probe radiation detector system has been proposed as a useful tool in the design, development and monitoring of drug–receptor interactions (Bice et al., 1986; Lee et al., 1988; Villemagne et al., 1994; Kim et al., 1997). The high sensitivity system, coupled with a highly specific radiotracer, is particularly useful when it is necessary to measure receptor occupancy with high temporal resolution. This detection system has been successfully used to evaluate drug–receptor interaction in mice (Sasaki et al., 1993; Liu et al., 1997). Similar measurements can also be obtained with high-resolution imaging devices such as positron emission tomography (PET), but often this is not necessary, either because the distribution of the tracer is diffuse or because its location is known (Jeffries et al., 1995). If non-specific binding is low the signal to noise ratio will be high.

The tracer used in the studies described in this paper is [^{125}I]RTI-55 (β-CIT). RTI-55 has been characterized with in vitro assays as a high affinity analog of cocaine, which binds to dopamine and serotonin transporters (Boja et al., 1992a,b; Carroll et al., 1995). RTI-55 is approximately 100 times more potent than cocaine in inhibiting specifically bound [^{3}H]WIN 35428 (Boja et al., 1990; Boja et al., 1991) and 20–50 times more potent than cocaine as a psychomotor stimulant (Cline et al., 1992). In vivo studies in rodents (Scheffel et al., 1992) and nonhuman primates (Shaya et al., 1992; Laruelle et al., 1994) showed that the tracer readily penetrates the blood–brain barrier and labels dopamine transporter sites (highly concentrated in the striatum) and, to a lesser degree, serotonin transporter sites (located primarily in midbrain structures such as hypothalamus and thalamus) (Carroll et al., 1995). Accumulation of [^{125}I]RTI-55 in serotonin transporter rich areas paralleled the density of [^{3}H]serotonin uptake sites in these regions and could be inhibited by pre-injection of the serotonin blocker paroxetine but not by the dopamine uptake site inhibitor GBR 12909 (Scheffel et al., 1992).

Studies have shown that only drugs known to act on dopamine and serotonin uptake sites are able to compete with [^{125}I]RTI-55. GBR 12909, a dopamine transporter inhibitor, was competitive, but haloperidol, a dopamine D_2 receptor antagonist, was not. Paroxetine, a serotonin trans-

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porter inhibitor, was competitive but maprotiline, which binds to the norepinephrine transporter, was not (Carroll et al., 1995). Binding of [^{125}I]RTI-55 in the hypothalamus/midbrain region was specific for serotonin transporters, as evidenced by inhibition with serotonin transporter inhibitors such as paroxetine (Scheffel et al., 1992; Carroll et al., 1995).

GBR 12909 is a high affinity, long-acting dopamine uptake inhibitor with an unusually high selectivity for the dopamine transporter (Melia and Spealman, 1991; Scheffel et al., 1991; Izenwasser et al., 1994; Rothman et al., 1995). It inhibits [^{3}H]dopamine uptake with high affinity and selectivity (Van der Zee et al., 1980; Heikkila and Manzino, 1984; Andersen, 1989) and dissociates very slowly from the dopamine uptake site (Rothman et al., 1991). Administration of large doses of GBR 12909 produce only modest elevations in intrasynaptic dopamine in the striatum in in vivo microdialysis studies (Westerink et al., 1987; Rothman et al., 1991). Cocaine and mazindol have lower potency and are shorter-acting dopamine uptake blockers compared to GBR 12909 and RTI-55 (Pogun et al., 1991; Elmer et al., 1996). GBR 12909 is more selective for dopamine transporter sites relative to the serotonin transporter sites while cocaine and RTI-55 are more potent in pharmacodynamic effects at the serotonin transporter sites than at the dopamine transporter sites (Elmer et al., 1996).

The antidepressants fluoxetine, sertraline and paroxetine are specific inhibitors of serotonin uptake. Their inhibitory effect on serotonin uptake has been observed both in vitro and in vivo (Scheffel et al., 1994).

The physiological and pharmacological characteristics of neurotransmitter systems are usually examined in vitro (Boja et al., 1992a). However, in vivo approaches are essential to determine the pharmacokinetic characteristics of neuroreceptors. The present study reports the evaluation of the pharmacological characteristics of [^{125}I]RTI-55 in vivo in mice and their correlation with ex vivo data.

2. Materials and methods

2.1. Animals

Male CD-1 mice (Charles River, Wilmington, DE, USA) weighing 25–30 g were used. They were kept in a climate controlled room on a light–dark cycle with unlimited access to food and water. Experiments were carried out during daylight hours. The mice were anesthetized by intraperitoneal (i.p.) injection of a carbamic acid ethyl ester (urethane) saline solution of 1.8 g/kg (0.35 ml). Anesthesia was maintained with additional urethane (0.18 g/kg) when necessary.

2.2. Drugs and radioactive materials

[^{125}I]RTI-55 (specific activity 2200 Ci/mmol) was used in these experiments. It was prepared by the method first reported by Carroll et al. (1991). Radiochemical purity and specific activity were determined by high-performance liquid chromatography (HPLC) with electrochemical detection. Blocking drugs were obtained as follows: Unlabeled RTI-55 (3β-(4-iodophenyl)-tropane-2β-carboxylic acid methyl ester) was a gift from F.I. Carroll of the Research Triangle Institute; GBR 12909 from Research Biochemicals International (Natick, MA, USA) and paroxetine from Beecham Pharmaceuticals (Epsom, UK).

2.3. Methods

To characterize the kinetics and distribution of the in vivo binding sites of [^{125}I]RTI-55, mice were injected intravenously into a tail vein with 15 μCi (0.15 ml) of [^{125}I]RTI-55.

For the displacement studies, either unlabeled RTI-55 (5 mg/kg mouse body weight) ($n = 3$), GBR 12909 (5 mg/kg) ($n = 3$), paroxetine (5 mg/kg) ($n = 3$) or a combination of GBR 12909 and paroxetine (5 mg/kg each) ($n = 3$) was administered i.v. 60 min after injection of the radiotracer. In control studies, saline (0.15 ml) was injected 60 min after the radiotracer ($n = 5$). For the blocking study, RTI-55 (5 mg/kg) was injected 5 min before the injection of the radiotracer ($n = 3$).

2.3.1. In vivo studies

The anesthetized mouse was positioned in a restraining device. A commercially available computer-based radiation detection system, designed for thyroid uptake clinical studies (Capintec, Ramsey, NJ), with a NaI crystal ($2 \times 2 \times 2$ inch) and fitted with two removable, single-hole (5 and 67 mm in diameter, respectively) lead collimators, each one 28 mm thick, was used. The collimators were positioned to touch the skin of the left temporal region and aimed at the striatum (Fig. 1). A stereotaxic atlas of the mouse brain was used to determine this position (A/P, +0.5; M/L, 1.0; D/V, −2.0; relative to bregma and dura) (Montemurro and Dukelow, 1972; Nakahara et al., 1993). Brain radioactivity was measured every minute for 210 min.

Data obtained from the probe system were averaged every 15 min and normalized for mouse weight and injected dose. Data were displayed as the percentage of the normalized activity at 60 min after injection. Data were analyzed by repeated measure analysis of variance (ANOVA). Comparisons were made between saline and drug injected animals. Differences were considered significant if P-values were less than 0.05.

2.3.2. Ex vivo dissection studies

For validation of probe measurements, mice were killed by cervical dislocation at 210 min after the tracer injection. The brain was dissected into cerebellum, olfactory tubercle, hypothalamus, striatum, hippocampus, thalamus, superior colliculi, frontal cortex, parietal cortex and the rest of the brain. Radioactivity was measured with an automated

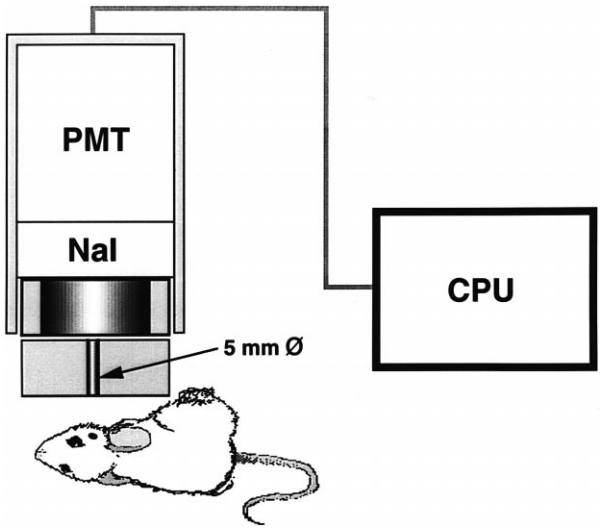

Fig. 1. The probe system was set in perpendicular position to the experimental animal (mouse). The 28 mm thick collimator facing the animal consisted of two opposing lead blocks. The collimator hole was 5 mm in diameter. A second collimator on the face of the NaI crystal was 28 mm thick and had a collimator hole with 67 mm in diameter. Abbreviations: CPU, DOS-based personal computer; NaI, sodium iodide crystal; PMT, photomultiplier.

gamma-counter. A ratio was obtained by comparing the radioactivity concentration of each specific region to that of the cerebellum. These values provide an estimate of specific to nonspecific binding since dopamine and serotonin uptake sites are almost absent in the cerebellum.

Data from the dissection studies were analyzed by applying ANOVA with post-hoc Dunnet's test to the region-to-cerebellum (R/CB) values. Comparisons were made between saline and drug injected animals. Differences were considered significant if the *P*-value was less than 0.05.

3. Results

3.1. Displacement and blockade studies with unlabeled RTI-55

Fig. 2 shows the [^{125}I]RTI time–activity curves over a 210 min period in mice injected with saline (controls), pre-injected with 5 mg/kg of unlabeled RTI-55 (blocking studies) or injected with the same dose of unlabeled RTI-55 at 60 min after the tracer injection (displacement studies).

Control studies showed continuous accumulation of the [^{125}I]tracer in the field of view of the probe over the period of observation; a plateau was reached at ~150 min post injection (593 cpm/kg per μCi). At 210 min, the normalized radioactivity was 597 cpm/kg per μCi.

When unlabeled RTI-55 was administered 60 min after the injection of the radiotracer, radioactivity fell rapidly from 544 to 416 cpm/kg per μCi at 60 min after unlabeled RTI-55 administration (i.e., 120 min after administration of the radiotracer) reaching 347 cpm/kg per μCi at the end of the study. Total radioactivity displacement amounted to 42% of controls. Blocking the monoaminergic transporters five min before injection of [^{125}I]RTI-55 resulted in 358 cpm/kg per μCi (40% inhibition) at the end of the study (Fig. 2). Displacement and pre-blockade studies at 210 min were significantly different from controls ($P < 0.001$).

In ex vivo studies, the values of R/CB ranged between 1.80 to 6.35 (Fig. 3). There was a high accumulation of [^{125}I]RTI-55 in olfactory tubercle (R/CB = 5.68) and striatum (R/CB = 6.35) in the control study, regions known to

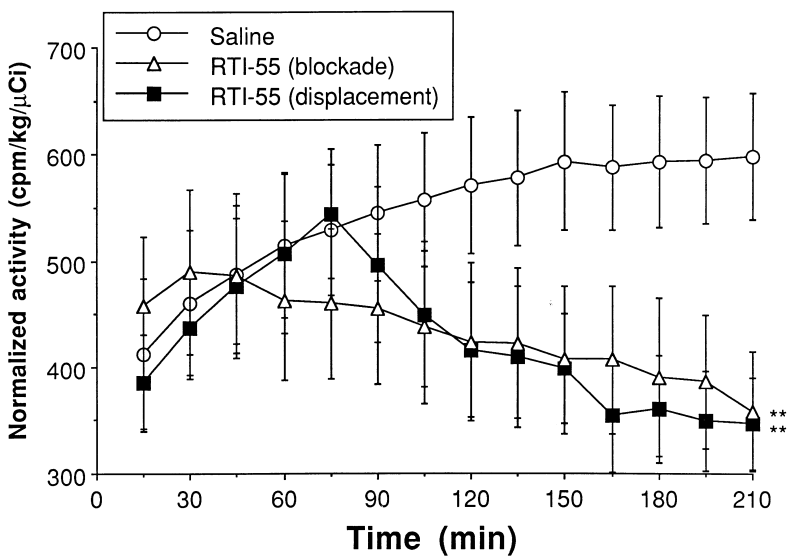

Fig. 2. Probe time-radioactivity curves obtained after i.v. administration of [^{125}I]RTI-55 to controls (○; $n = 5$). Blocking studies were carried out by i.v. administration of unlabeled RTI-55 (5 mg/kg) 5 min prior to [^{125}I]RTI-55 injection (Δ; $n = 3$) and displacement studies by i.v. administration of unlabeled RTI-55 (5 mg/kg) 60 min after [^{125}I]RTI-55 injection (■; $n = 3$). Values are mean ± S.E.M. of cpm/kg per μCi injected ($^{**}P < 0.01$).

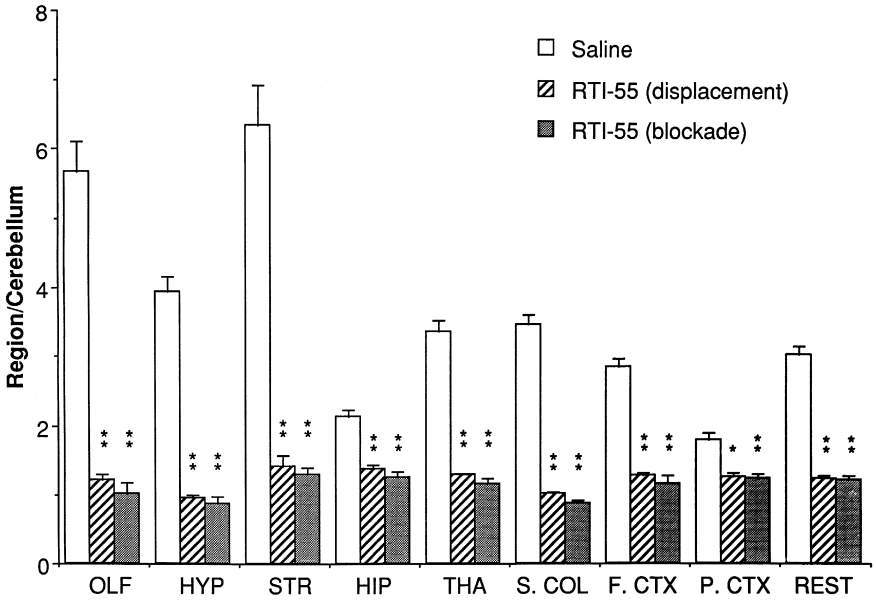

Fig. 3. Region-to-cerebellar ratios of [^{125}I]RTI-55 obtained from mice used in the probe studies and sacrificed at 210 min after injection. Unlabeled RTI-55 (5 mg/kg) was administered at 60 min after injection of the tracer for the displacement study and at 5 min before tracer injection for the blockade study. Values are mean ± S.E.M. of tissue-to-cerebellar ratios. Abbreviations: OLF, olfactory tubercle; HYP, hypothalamus; STR, striatum; HIP, hippocampus; THA, thalamus; S.COL, superior colliculi; F.CTX, frontal cortex; P.CTX, parietal cortex ($^{*}P < 0.05$, $^{**}P < 0.01$).

have high concentration of dopamine transporters. Unlabeled RTI-55 blocked and displaced the specific binding of [^{125}I]RTI-55 from all regions of the brain, with R/CB values ranging from 0.87 to 1.42. The greatest displacement of [^{125}I]RTI-55 was observed in olfactory tubercle (R/CB = 1.21) hypothalamus (R/CB = 0.96) and striatum (R/CB = 1.42). Statistically significant decreases were observed in all brain regions when compared to the control study (Fig. 3).

3.2. Displacement study with monoamine re-uptake antagonists

Fig. 4 shows the time–radioactivity curves obtained in mice injected at 60 min after administration of [^{125}I]RTI-55

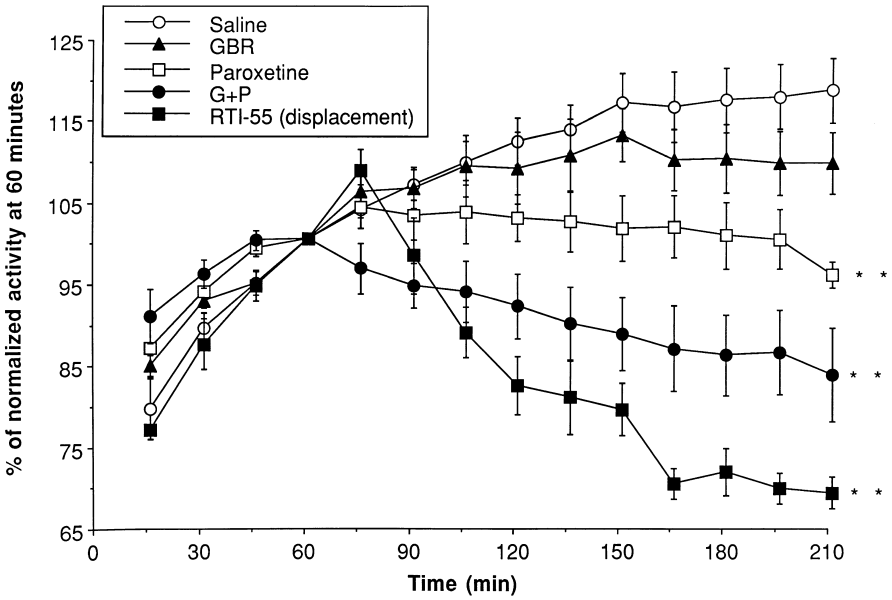

Fig. 4. Probe time-radioactivity curves expressed as % of the normalized counts at 60 min post radiotracer injection of [^{125}I]RTI-55. Curves are displacement studies carried out by i.v. administration of different drugs 60 min after [^{125}I]RTI-55 injection: Saline (○: $n = 5$), GBR 12909 (5 mg/kg; ▲: $n = 3$), paroxetine (5 mg/kg; □: $n = 3$), both GBR 12909 plus paroxetine (G + P) (5 mg/kg; ●: $n = 3$), unlabeled RTI-55 (5 mg/kg; ■: $n = 3$). Values are means ± S.E.M. ($^{**}P < 0.01$).

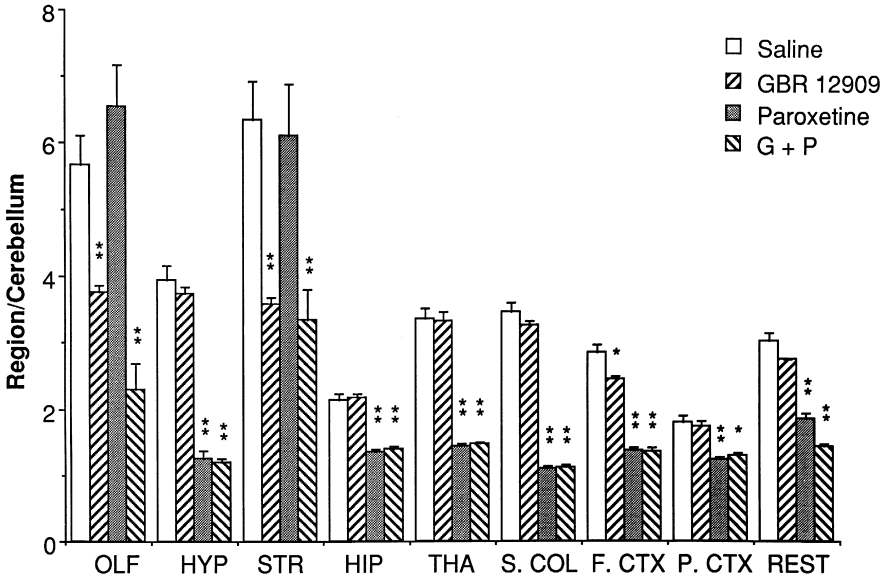

Fig. 5. Region-to-cerebellar ratio of [^{125}I]RTI-55 accumulation at 210 min after injection. GBR 12909 (5 mg/kg), paroxetine (5 mg/kg) and both drugs together were administered at 60 min after injection of the tracer for the displacement study. Values are means ± S.E.M. Abbreviations: OLF, olfactory tubercle; HYP, hypothalamus; STR, striatum; HIP, hippocampus; THA, thalamus; S.COL, superior colliculi; F.CTX, frontal cortex; P.CTX,: parietal cortex; G + P, GBR 12909 and paroxetine ($^{*}P < 0.05$, $^{**}P < 0.01$).

with saline ($n = 5$), GBR 12909 ($n = 3$), paroxetine ($n = 3$), the combination of GBR 12909 and paroxetine ($n = 3$) or unlabeled RTI-55 ($n = 3$). The data are expressed as the percentage of activity measured in each case at 60 min after tracer injection.

A blocking dose of GBR 12909 (5 mg/kg) decreased radioactivity at 210 min by 7% and paroxetine (5 mg/kg) by 19%. A combination of GBR 12909 and paroxetine reduced [^{125}I]RTI-55 binding at 210 min by 29%. Unlabeled RTI-55 (5 mg/kg) displaced [^{125}I]RTI-55 binding at 210 min by 42% (Fig. 4). Statistical significance was observed in the studies with paroxetine ($P < 0.01$) and the combination of GBR 12909 and paroxetine ($P < 0.01$) in repeated measure ANOVA.

Post mortem dissection revealed the greatest displacement with GBR 12909 in the regions with high concentration of dopamine transporters; olfactory tubercle (34%, $P < 0.01$) and striatum (44%, $P < 0.01$), and less in other regions such as the frontal cortex (14%, $P < 0.05$) (Fig. 5). These results are in agreement with those by Cline et al. (1992). Paroxetine displaced [^{125}I]RTI-55 in the regions rich in serotonin transporters; hypothalamus, hippocampus, thalamus, superior colliculus, frontal cortex and parietal cortex by 31 to 68% ($P < 0.01$). These results are consistent with previously published data (Scheffel et al., 1992a). Although administration of the combination of GBR 12909 and paroxetine displaced [^{125}I]RTI-55 in all regions of the brain (parietal cortex: $P < 0.05$, other regions: $P < 0.01$), the displacement in the primarily dopaminergic areas of olfactory tubercle and striatum was less (Fig. 5) than when unlabeled RTI-55 was used for the displacement (Fig. 3).

4. Discussion

A simple probe system was used to determine the in vivo binding of an [^{125}I]labeled cocaine analog, RTI-55, to dopamine and serotonin transporters in the mouse brain. [^{125}I]RTI-55, one of the most potent inhibitors of dopamine and serotonin uptake reported to date, was tested in CD-1 mouse brain to characterize the time-course and to determine the localization and pharmacological specificity of [^{125}I]RTI-55 binding in vivo in mouse brain.

The binding of [^{125}I]RTI-55 increased up to 150 min and then plateaued. This finding is consistent with the data obtained by Cline et al. (1992), who reported that [^{125}I]RTI-55 peaked in striatum and olfactory tubercle at ~120 min after injection. The saturability of the binding was demonstrated by both blockade and displacement of [^{125}I]RTI-55 by unlabeled RTI-55.

Selectivity for the dopamine transporter and serotonin transporter was demonstrated by pharmacological studies that indicated [^{125}I]RTI-55 was displaced by GBR 12909, a highly selective dopamine uptake inhibitor and paroxetine, a potent serotonin transporter inhibitor. In the probe study, displacement of [^{125}I]RTI-55 was significantly greater with paroxetine (5 mg/kg) than with GBR 12909 (5 mg/kg). This might be due to the fact that the striatum is a deep

structure that contains a high density of dopamine transporters, while serotonin transporters are primarily located in the brain cortex, therefore closer to the detector's surface. Although the concentration of radioactivity is higher in the striatum, the value obtained by the probe might be underestimated because of attenuation of gamma rays by brain structures overlying the region from which the radioactivity emanates. This might also explain the difference observed between the in vivo probe results and the ex vivo data.

GBR 12909 significantly inhibited [^{125}I]RTI-55 binding in striatum and olfactory tubercles using ex vivo measurements in agreement with a previous report (Cline et al., 1992). GBR 12909 had no significant effect on [^{125}I]RTI-55 binding in hypothalamus, a region with high density of serotonin uptake sites. On the other hand, paroxetine, a potent ligand for serotonin uptake sites (Scheffel and Hartig, 1989), caused significant decreases in [^{125}I]RTI-55 binding in hypothalamus, but not in striatum or olfactory tubercle. The combination of GBR 12909 and paroxetine displaced [^{125}I]RTI-55 less than unlabeled RTI-55. This was also observed in the probe studies, where the combination of GBR 12909 and paroxetine displaced [^{125}I]RTI-55 by 29% which was less than the observed displacement with unlabeled RTI-55 (42%). Though the displacement due to paroxetine in serotonin transporter-rich areas is similar to the one observed by unlabeled RTI-55 (68 and 75% in the hypothalamus for paroxetine and unlabeled RTI-55, respectively), the difference observed when used in combination with GBR 12909 might be mainly attributed to the inability of GBR 12909 to fully displace [^{125}I]RTI-55 in dopamine transporter-rich areas (44 and 78% in the striatum for GBR 12909 and RTI-55, respectively). Similar findings were obtained when GBR 12909 was used to displace [^{3}H]cocaine from the dopamine transporter binding site (Boja et al., 1992a). The probe results demonstrate that these drugs displaced the binding of RTI-55, although the degree of displacement measured by the probe method was much smaller than the one determined ex vivo. Differences between the probe and ex vivo results are probably due to the fact that the probe samples the radioactivity from all the brain regions in its field of view, while the ex vivo data is a region-specific sampling of the data.

Measurements of brain receptor occupancy and the effect of drug interactions can be assessed in vivo in mice using ^{125}I-labeled radioligands and a simple radiation detection system. The probe system has a high temporal resolution and is exceptionally sensitive, which allows detection of ^{125}I soft gamma (35.4 keV) and X-ray (27.2–31.7 keV) emissions. Furthermore, the ability to employ the probe with ^{125}I is particularly valuable since the radioisotope is readily available at low cost and has a long half-life ($t_{1/2} = 60$ days). On the other hand, the main disadvantage of the probe system resides in its inability to examine the regional differences between the structures under its field of view. However, this problem can be circumvented by using selective radioligands with known specific regional distributions in the mouse brain.

The probe system makes it possible to characterize drug pharmacokinetics and the time-course of pharmacological effects in the same experimental animal, which minimizes the number of animals required for a study. Multiple determinations at various pharmacokinetically interesting time points must be obtained for a given radioligand to be characterized in vivo by traditional methods. About 250 mice would have been necessary to obtain the data shown in Figs. 2 and 4 by dissection, while only 20 animals were used for the probe study. The probe system should prove useful in testing new drugs with minimal use of animals and drug.

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