Development of PET/SPECT Ligands for the Serotonin Transporter

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INTRODUCTION

There is clear evidence in rodents and nonhuman primates that the widely abused "designer" drug, 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"), causes loss of central serotonergic (5-hydroxytryptamine [5-HT]) neurons (Commins et al. 1986; Ricaurte et al. 1988*a*). Doses of MDMA that approximate those typically taken by humans have been shown to be toxic to serotonergic nerve fibers in the monkey brain (Ricaurte et al. 1988*b*; Slikker et al. 1988). Of similar or greater concern are neurochemical studies showing that the anorectic agent fenfluramine causes serotonergic damage in rodents as well as in primates (Harvey et al. 1975; Schuster et al. 1986). Currently, fenfluramine is a clinically prescribed drug in both the United States and Europe. It is used to treat obesity with doses that closely approach those that produce neurotoxic effects in animals (0.2 to 2.0 mg/kg). At this time, it is not known whether regeneration of 5-HT neurons occurs in nonhuman primates and, if so, what the timeframe for recovery is.

Long-term neurotoxic damage caused by MDMA and fenfluramine appears to be restricted to the presynaptic element of the serotonergic nerve fiber. Recent investigations on the effect of MDMA on postsynaptic 5-HT_2 receptors in the rat brain have shown that, although there is an initial down-regulation of these postsynaptic receptors, it is of short duration. Approximately 1 week after administration of MDMA (eight subcutaneous injections [20 mg/kg], twice daily for 4 days), the number of 5-HT_2-ligand binding sites returns to control levels (Scheffel et al. 1992*a*). In contrast, 5-HT uptake sites, located

on presynaptic nerve endings in the rat brain, recover very slowly after MDMA treatment: their number remains decreased for up to 12 months (Battaglia et al. 1988*a*).

Several methods have been employed to test for serotonin neurotoxicity in humans, including measurement of the serotonin metabolite 5-hydroxyindoleacetic acid in cerebrospinal fluid (Moir et al. 1970) and endocrine challenge using intravenously administered L-tryptophane (Heninger et al. 1984). All these tests are indirect and cannot accurately reflect the state of the central serotonergic neuron. Therefore, a presynaptic marker is needed to indicate serotonergic neurotoxicity in the living human brain.

This chapter describes work performed in the authors' laboratories in the development and in vivo testing of radiolabeled ligands for the serotonin uptake site suitable for single photon emission computed tomography (SPECT) or positron emission tomography (PET) imaging. Several compounds with known in vitro affinity for the 5-HT uptake site were labeled with [^{123}I] for SPECT studies or with either [^{11}C] or [^{18}F] for investigations using PET. The radiotracers were characterized in rodents for their in vivo kinetics, distribution, and specificity of binding to the 5-HT uptake site.

PAROXETINE, N-[^{11}C]METHYL PAROXETINE, AND [^{18}F]FLUOROALKYLATED PAROXETINE ANALOGS

Paroxetine, first described in the late 1970s (Petersen et al. 1977; Buus Lassen 1978), is an antidepressant drug with high affinity and selectivity for the 5-HT uptake site in platelets and presynaptic neurons in the central nervous system (CNS) (Habert et al. 1985; Marcusson et al. 1988). In its tritiated form, paroxetine has been used to characterize the binding to the 5-HT uptake site (Mellerup et al. 1983; Mellerup and Plenge 1986) and to determine the density and distribution of 5-HT uptake sites in the rat and human brain (Battaglia et al. 1988*b*; Cortes et al. 1988).

Studies by Scheffel and Hartig (1989) indicated that [^{3}H]paroxetine is a potent and selective agent for in vivo labeling of cerebral serotonin uptake sites. Furthermore, Scheffel and Ricaurte (1990) demonstrated

that paroxetine can serve as an in vivo indicator of MDMA neurotoxicity. They concluded that labeling paroxetine with [^{11}C] ($t_{1/2}$ = 20 min) or [^{18}F] ($t_{1/2}$ = 110 min) would yield a useful PET tracer for imaging and quantitating 5-HT uptake sites in the CNS of living mammals and humans. However, introduction of a positron emitter label into the authentic paroxetine molecule has not been accomplished yet.

Several analogs of paroxetine that could be labeled more easily with [^{11}C] or [^{18}F] than authentic paroxetine were prepared (Villemagne et al. 1989; Suehiro et al. 1991). However, in vitro binding assays demonstrated a considerable loss of affinity of these analogs: N-(methyl) paroxetine displaced [^{3}H]paroxetine binding to 5-HT uptake sites 25 times less potently than authentic paroxetine; N-(3-fluoroethyl) and N-(3-fluoropropyl) paroxetine had 424 and 1,220 times lower potencies, respectively (table 1).

TABLE 1. *Potencies of paroxetine and analogs in inhibiting [^{3}H]paroxetine binding to 5-HT uptake sites in rat cortical homogenates. K_i values are means of four assays performed in triplicate.*

Inhibitor	K_i(M)
Paroxetine*	5.9×10^{-11}
N-(methyl) paroxetine†	1.5×10^{-9}
N-(2-fluoroethyl) paroxetine*	2.5×10^{-8}
N-(3-fluoropropyl) paroxetine*	7.2×10^{-8}

SOURCE: *Suehiro et al. 1991; †Plenge et al. 1987

Similar results were obtained in in vivo studies using N-([^{11}C]methyl) paroxetine and N-(3-[^{18}F]fluoropropyl) paroxetine (figure 1). After intravenous (IV) injection of these high specific activity tracers into mice, the radioactivity concentrations in different areas of the brain were measured by dissecting the brain regions and weighing and counting the tissue samples. Ratios of the radioactivities in the hypothalamus, an area known to contain high densities of 5-HT uptake

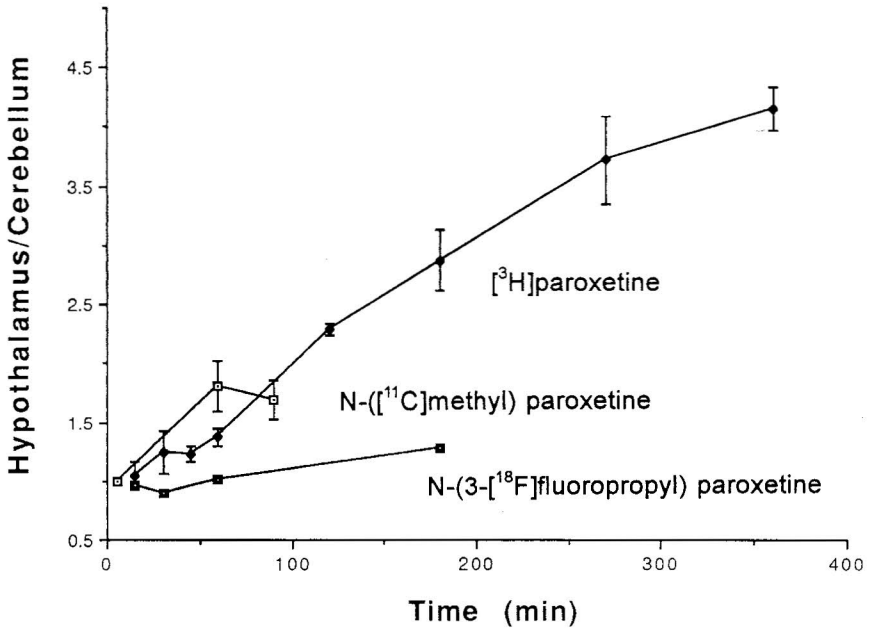

FIGURE 1. *Comparison of the in vivo binding of [^{3}H]paroxetine with that of its analogs, N-([^{11}C]methyl) paroxetine and N-(3-[^{18}F]fluoropropyl) paroxetine. Time course of hypothalamus to cerebellar radioactivity ratios in the mouse brain.*

KEY: ♦ = [^{3}H]paroxetine; □ =N-([^{11}C] methyl) paroxetine;
■ = N-(3-[^{18}F]fluoropropyl) paroxetine

sites, and in the cerebellum, a region relatively devoid of binding sites, were calculated to estimate total/nonspecific binding. With N-([^{11}C]methyl) paroxetine, the highest hypothalamus-to-cerebellar ratio was 1.8:1 at 60 min after administration. For N-(3-[^{18}F]fluoropropyl) paroxetine, the hypothalamus-to-cerebellar ratio climbed to only 1.3:1 at 3 hours after injection. Ratios for [^{3}H]paroxetine (Scheffel and Hartig 1989) were plotted for comparison.

Attempts to derivatize paroxetine for SPECT have been made. Mathis and coworkers (1991) recently reported on several iodinated paroxetine

analogs, of which (±)-2'-[^{125}I]iodo paroxetine appeared to be the most promising. However, subsequent in vivo experiments showed that this tracer penetrated the blood-brain barrier rather poorly, and nonspecific binding was too high for the tracer to be useful for SPECT studies.

[^{11}C]CITALOPRAM, [^{11}C]FLUOXETINE, AND CIS-[^{11}C]DDPI

Citalopram, fluoxetine, and cis-N,N,-dimethyl-3-(2',4'-dichlorophenyl)-indanamine (cis-DDPI) are known to be potent and selective serotonin uptake site blockers (Hyttel 1982; Fuller and Wong 1977; Bøgesø et al. 1985). These compounds were radiolabeled with [^{11}C] to assess their potential use as PET ligands.

[^{11}C]Citalopram, [^{11}C]fluoxetine, and cis-[^{11}C]DDPI were synthesized from their respective precursors and [^{11}C]iodomethane (Dannals et al. 1990; Kilbourn 1988; Suehiro et al. 1992*a*). The biological activity of these compounds was studied in mice (Scheffel et al. 1990; Suehiro et al. 1992*a*). [^{11}C]Citalopram, [^{11}C]fluoxetine, and cis-[^{11}C]DDPI penetrated the blood-brain barrier well with 1.3, 3.1, and 3.6 percent of the injected dose (%D), respectively, in the whole mouse brain at 15 min after IV injection.

As shown in figure 2, [^{11}C]fluoxetine did not exhibit any specific binding to 5-HT uptake sites during the time of observation (15 to 90 min after injection). At no time after administration did [^{11}C]fluoxetine distribution agree with that of 5-HT uptake site densities, nor did preinjection of the high-affinity uptake site blocker paroxetine (1 mg/kg) cause any inhibition of the accumulation of the tracer. The reasons for the failure of [^{11}C]fluoxetine to bind to 5-HT uptake sites in vivo are unknown. However, it is possible that metabolism of the radioligand occurred before specific binding in the CNS could take place. Conversely, cis-[^{11}C]DDPI showed specific accumulation in areas rich in 5-HT uptake sites (e.g., the hypo-thalamus, olfactory tubercles, and prefrontal cortex). This binding could be blocked 60 to 80 percent by preinjection of 1 mg paroxetine/ kg bodyweight. However, because nonspecific binding was high and the clearance of this nonspecifically bound fraction was slow, the ratio between total to nonspecific binding reached only 1.4:1 at 60 to 90 min after injection (figure 2).

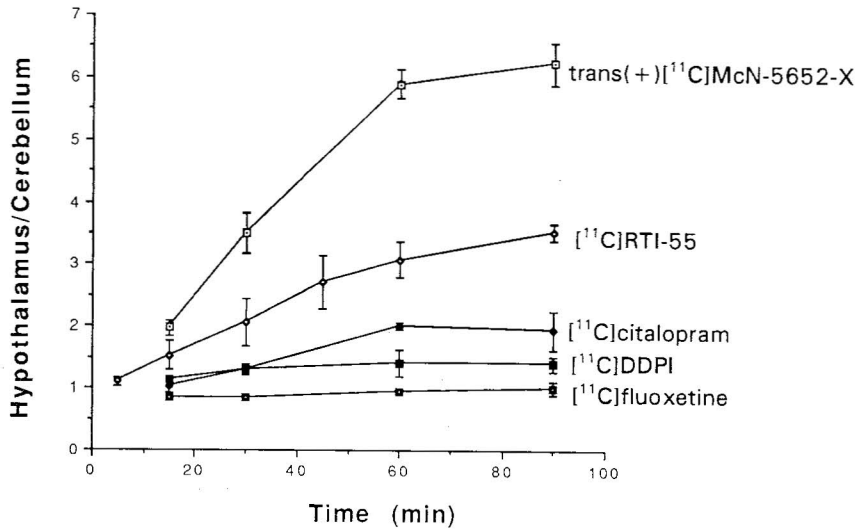

FIGURE 2. *Hypothalamus-to-cerebellar ratios of [^{11}C] concentrations at different times after IV injection of 300 to 500 μCi of either [^{11}C]fluoxetine, cis-[^{11}C]DDPI, [^{11}C]citalopram, [^{11}C]RTI-55, or trans(+)[^{11}C]McN-5652-X into mice weighing 25 to 35 g. The specific activity at time of injection of each of the tracers was approximately 3,000 mCi/μmol. Data are means ±1 standard deviation, and n = 3 to 4 mice for each time point.*

[^{11}C]Citalopram was the most promising of the three candidate ligands. Hypothalamus-to-cerebellar ratios rose to 2.0:1 at 60 min (figure 2). Regional [^{11}C]citalopram distribution at this time was in agreement with 5-HT uptake site densities, and preadministration of a blocking dose of paroxetine did significantly inhibit tracer binding. Although these results were encouraging, the ratios between specific and nonspecific binding were not high enough to guarantee successful imaging of 5-HT uptake sites by PET.

TRANS(±)[^{11}C]McN-5652-Z AND (+)[^{11}C]McN-5652-X

Trans(±)McN-5652-Z was described by Shank and colleagues (1968) as a highly potent serotonin uptake inhibitor. In addition, several stereoisomers of this compound were isolated and tested for their binding to presynaptic monoaminergic neurotransmitter uptake sites (Shank et al. 1968). Trans(+)McN-5652-X was shown to exhibit the highest affinity toward the 5-HT uptake site (K_i for inhibition of [^{3}H]5-HT = 0.4 nM), followed by trans(±)McN-5652-X (K_i = 0.6 nM) and cis McN-5655 (K_i = 16.6 nM); the trans(-)McN-5652-W isomer was approximately 150 times less potent (K_i = 58.4 nM) than the corresponding (+) isomer. All these compounds showed moderate in vitro affinity for the norepinephrine (NE) uptake site (i.e., five times lower than for the 5-HT uptake site) and little or no affinity for the dopamine (DA) uptake site.

Trans(±)McN-5652-Z, trans(+)McN-5652-X, trans(-)McN-5652-W, and cis McN-5655-Z were labeled recently with [^{11}C] (Suehiro et al. 1992*b*, in press). The synthesis of these tracers was performed by S-methylation of the respective precursors with [^{11}C]iodomethane. The specific activity determined at the end of synthesis ranged from 3,300 to 4,250 mCi/μmol.

As shown in figure 2, [^{11}C]McN-5652-X, the trans(+) isomer, far exceeded all previously tested radioligands in specific binding to the 5-HT transporter in the mouse brain. Ratios between hypothalamus and cerebellar radioactivity concentrations reached 5.9:1 and 6.2:1, respectively, at 60 and 90 min after IV injection of the tracer. In contrast, [^{11}C]McN-5652-W, the trans(-) isomer, displayed little specific binding. For this compound, the ratio of the radioactivity concentration in the hypothalamus to the nonspecifically bound radioactivity concentration in the cerebellum was 1.3:1 at 60 min and 1.9:1 at 90 min after administration.

[^{11}C]McN-5652-Z, the racemic (±) mixture of the optical isomers (+) and (-) [^{11}C]McN-5652, was prepared to study the selectivity of in vivo binding to the 5-HT uptake site in the mouse model. Blocking doses of drugs known to bind to specific monoamine neurotransmitter sites were injected intravenously 5 min before tracer injection. Tissue-to-cerebellar radioactivity ratios in different brain regions were deter-

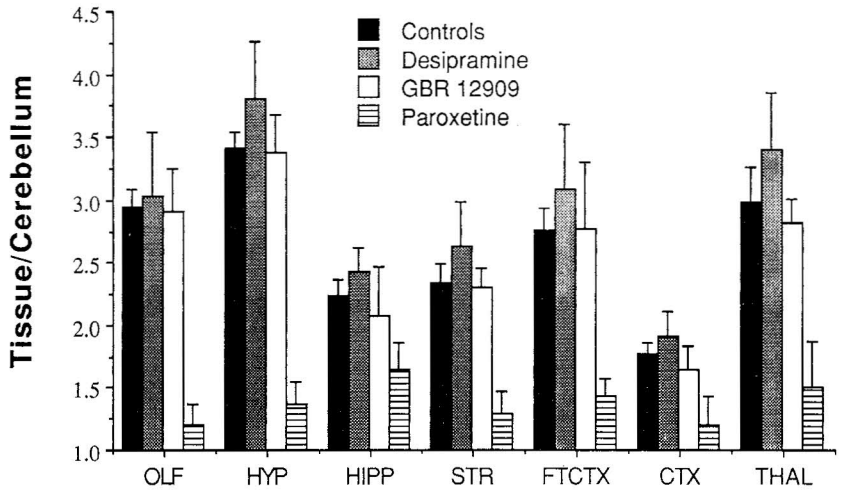

FIGURE 3. *Effect of blocking doses (5 mg/kg) of paroxetine (specific for the 5-HT uptake site), GBR 12909 (DA uptake site), and desipramine (NE uptake site) on [^{11}C]McN-5652-Z binding. The drugs were injected intravenously 5 minutes before administration of the tracer. Tissue-to-cerebellar ratios were determined 60 minutes after tracer injection. Data are means ±1 standard deviation (n = 4).*

KEY: OLF = olfactory tubercles; HYP = hypothalamus; HIPP = hippocampus; STR = striatum; FTCTX = prefrontal cortex; CTX = cortex; THAL = thalamus

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mined 60 min after tracer injection and compared with those of saline-injected controls. The results of these studies are shown in figure 3.

Paroxetine (5 mg/kg) significantly decreased the specific binding of [^{11}C]McN-5652-Z in all areas examined. In contrast, GBR 12909, a DA uptake site-specific drug, and desipramine, which blocks the NE

uptake sites, had no effect. The results indicate that [^{11}C]McN-5652-Z is a highly selective in vivo label for the serotonin transporter.

The ability of [^{11}C]McN-5652-Z to detect serotonergic damage in the CNS was shown in rats lesioned with the known 5-HT neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) (200 µg 5,7-DHT in 10 µL 0.01 percent ascorbic acid, injected intracerebroventricularly after NE uptake sites were blocked by pretreatment of the animals with desipramine [25 mg/kg, IV]) 13 days before the tracer study. [^{11}C]McN-5652-Z was injected intravenously 60 min before sacrifice of the animals. As shown in figure 4, specific [^{11}C]McN- 5652-Z binding (expressed as %D/g tissue minus %D/g in cerebellum) was reduced by 50 to 90 percent in different brain regions of the 5,7-DHT lesioned rats. Moreover, the regional decreases in [^{11}C]McN-5652-Z binding after 5,7-DHT treatment corresponded with those observed using [^{3}H]paroxetine as the in vivo indicator. These results indicate that [^{11}C]McN-5652-Z can be used to quantify the extent of serotonergic damage.

[^{125}I]-RTI-55 AND [^{11}C]-RTI-55

Recently, several cocaine analogs have been synthesized, radiolabeled, and tested for their in vitro and in vivo affinities for monoaminergic neurotransmitter uptake sites. (For a recent overview of this work, see Boja et al. 1992*a*). Of these compounds, [$^{123/125}$I] 2ß-carbomethoxy-3ß-(4-iodophenyl)tropane ([$^{123/125}$I]RTI-55) (Carroll et al. 1991; Neumeyer et al. 1991) was found to bind to both the DA and 5-HT transporters in homogenate binding assays (Boja et al. 1992*b*) as well as after IV injection in the brain of rodents and baboons (Cline et al. 1992; Innis et al. 1991; Scheffel et al. 1992*b*). The affinity of RTI-55 was approximately 100 times greater than that of (-) cocaine for both the DA and the 5-HT transporters (Boja et al. 1992*b*).

Studies in rats demonstrated that the in vivo distribution of [^{123}I]RTI-55 in CNS areas rich in serotonin uptake sites correlated well with regional densities of [^{3}H]serotonin uptake sites (figure 5). Maximal target-to-nontarget ratios (up to 7.4) in 5-HT uptake site-rich areas in the rat brain were reached 5 hours after IV injection (figure 6). However, as mentioned before, [^{123}I]RTI-55 labels not only 5-HT but

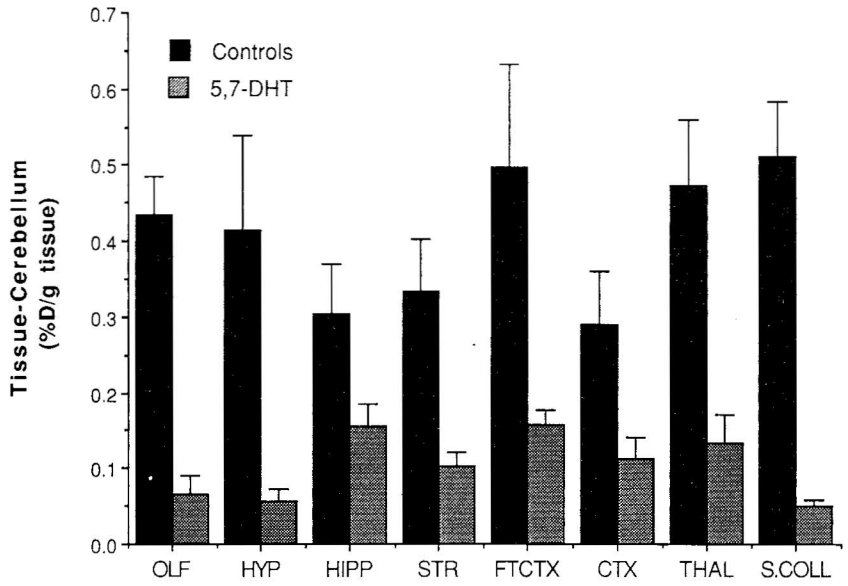

FIGURE 4. *Effect of 5,7-DHT lesioning on the specific binding (%D/g tissue-%D/g cerebellum) of [^{11}C]McN-5652-Z at 60 minutes after tracer injection. Rat brains were lesioned by intracerebroventricular injection of 5,7-DHT 13 days before the tracer study. Data are means ±1 standard deviation; n = 3.*

KEY: OLF = olfactory tubercles; HYP = hypothalamus; HIPP = hippocampus; STR = striatum; FTCTX = prefrontal cortex; CTX = cortex; THAL = thalamus; S.COLL = superior colliculi

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DA transporters as well. At the time when [^{123}I]RTI-55 specific binding in 5-HT uptake site-rich areas was highest (the hypothalamus-to-cerebellar ratio was 7.4:1), the striatal-to-cerebellar ratio was 22.7:1.

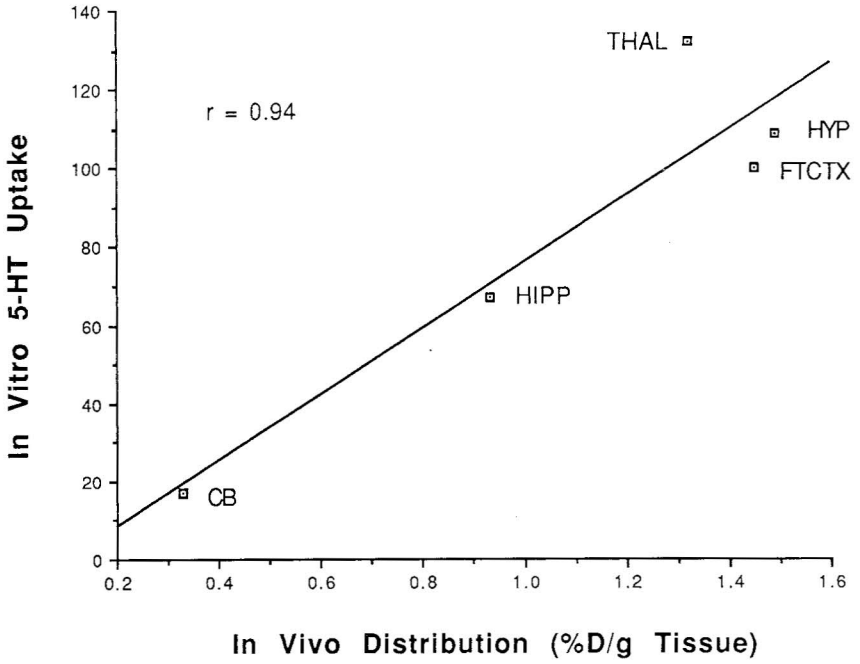

FIGURE 5. *Comparison between [^{123}I]RTI-55 concentrations (%D/g tissue) in various regions of the rat brain at 2 hours after IV injection and the proportional distribution of [^{3}H]serotonin uptake in synaptosomal preparations of these regions as published by Leysen (1985), where [^{3}H]serotonin uptake in the frontal cortex is set at 100 percent.*

KEY: CB = cerebellum, HIPP = hippocampus, THAL = thalamus, FTCTX = prefrontal cortex, HYP = hypothalamus

Despite the fact that [^{123}I]RTI-55 is not selective for the serotonin uptake site, it is anticipated that the tracer will be useful for quantitating 5-HT uptake sites in vivo by SPECT. In rats, neurotoxic doses of fenfluramine caused decreases of 66 percent (in the hypothalamus) to 83 percent (in the superior colliculi) of specific [^{123}I]RTI-55 binding in all areas except in the striatum and the olfactory tubercles (regions rich in DA transporters) (Scheffel et al. 1992*b*). In baboons, Innis and colleagues (1991) and Laruelle and coworkers (1992) observed specific

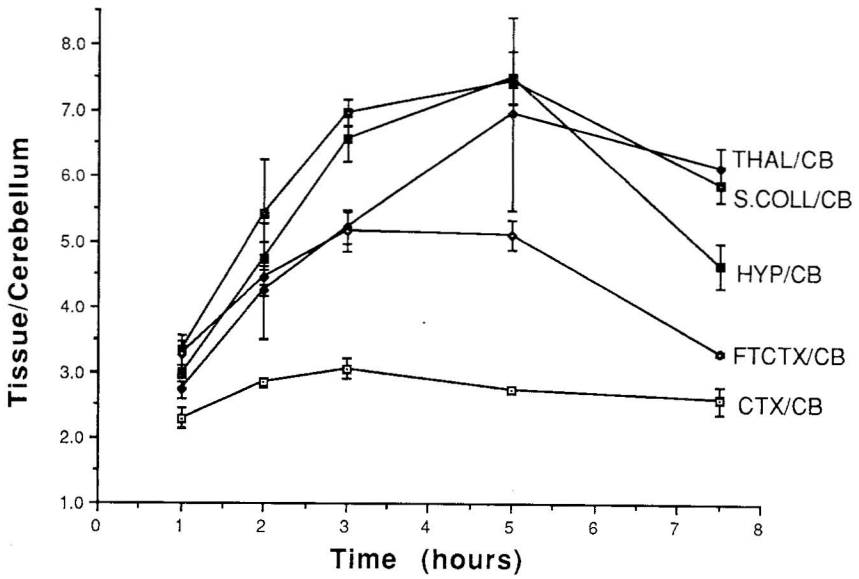

FIGURE 6. *Time course of tissue-to-cerebellar radioactivity ratios in selected areas of the rat brain after IV injection of [^{123}I]RTI-55. Data are means ± SEM; n = 2 to 4 rats/time point.*

KEY: THAL = thalamus; CB = cerebellum; S.COLL = superior colliculi; HYP = hypothalamus; FTCTX = prefrontal cortex; CTX = cortex

SOURCE: Scheffel et al. 1992*b*. Copyright 1992 by Wiley-Liss.

binding of [^{123}I]RTI-55 in the midbrain that was associated primarily with 5-HT transporters. A more selective SPECT agent for the serotonin transporter has recently been described by Mathis and colleagues (1992). Studies with [^{125}I]-iodo-6-nitroquipazine showed excellent target-to-nontarget ratios in 5-HT transporter-rich regions of the rat brain; a hypothalamus-to-cerebellar ratio of 6.3:1 at 6 hours after injection was reported.

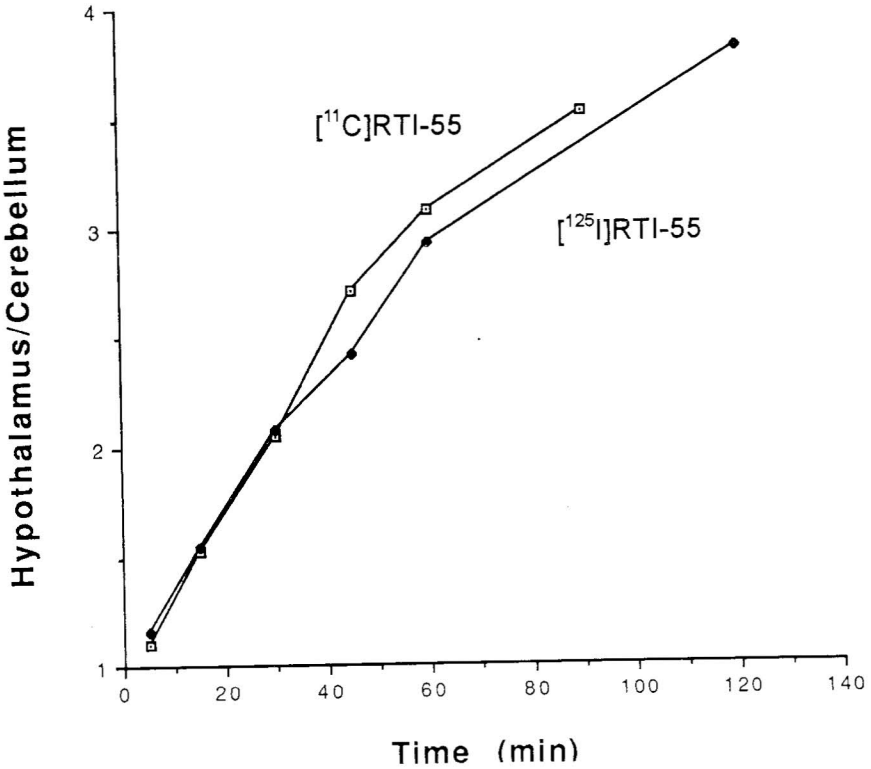

FIGURE 7. *Time course for hypothalamus-to-cerebellar ratios in the mouse brain after injection of either [^{125}I]RTI-55 or [^{11}C]RTI-55*

SOURCE: [^{125}I]RTI-55 data are from Cline and colleagues (1992).

RTI-55 recently was labeled with [^{11}C] (Dannals, unpublished results), and studies in mice were performed to compare the specificity and selectivity of [^{11}C]RTI-55 binding to 5-HT uptake sites with that of [^{123}I]RTI-55. Similar results were obtained with both tracers (figure 7). [^{11}C]RTI-55 is being evaluated in nonhuman primates as a PET tracer for 5-HT and DA uptake sites.

SUMMARY

There is a great need for PET and SPECT ligands with high affinity and selectivity for the serotonin uptake site. These imaging agents would be useful in screening human populations at risk (e.g., individuals exposed to neurotoxic amphetamines such as MDMA and fenfluramine). Moreover, these radioligands would allow the study of serotonergic function in the normal living human brain, and they also would be useful in the examination of altered serotonergic neurotransmission in diseases such as depression and obsessive-compulsive and other neuropsychiatric disorders.

Over the past several years, a number of radioligands have been studied in several laboratories for their in vivo binding to 5-HT uptake sites. Although [^{3}H]paroxetine showed promising binding characteristics, conversion of authentic paroxetine into a PET or SPECT tracer turned out to be difficult and has not been achieved yet. Analogs of paroxetine displayed considerable loss of binding affinity and were, therefore, not useful for imaging purposes. For [^{11}C]fluoxetine, [^{11}C]citalopram, and cis-[^{11}C]DDPI, target-to-nontarget (hypothalamus-to-cerebellar) ratios remained less than 2.0:1 over a 90-min period after injection. The most promising PET agents identified today are [^{11}C]RTI-55 and [^{11}C]McN-5652-X. [^{11}C]RTI-55 labels both 5-HT and DA uptake sites. [^{11}C]McN-5652-X is highly selective for 5-HT uptake sites, and its distribution is consistent with the neuroanatomical distribution of the 5-HT uptake site. Because [^{11}C]McN-5652-Z is a racemic mixture of two stereoisomers, of which the (+) isomer (McN-5652-X) binds to the 5-HT uptake site in vivo and the (-) isomer (McN-5652-W) does not, the possibility exists that regional-specific binding can be determined by subtracting nonspecific binding of the (-) isomer from total radioactivity counts obtained with the (+) isomer. [^{11}C]McN-5652-X is the best PET radioligand for the 5-HT uptake site described thus far. This tracer warrants further testing in nonhuman primates. Efforts are underway to obtain an investigational new drug application for use of the tracer in humans.

Promising candidates as SPECT imaging agents for the 5-HT uptake site are [^{123}I]RTI-55 and [^{123}I]-iodo-6-nitroquipazine. Both agents are under intense investigation in different laboratories in the United States.

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ACKNOWLEDGMENTS

This work was supported by U.S. Public Health Service grants DA-05477, NS-15080, and CA-32845. Marigo Stathis and Christine Steinert provided work in biodistribution studies in rodents and assistance with baboon studies. Robert Smoot assisted with the operation of the cyclotron. Dr. Hayden T. Ravert helped to prepare the [^{11}C] and [^{18}F] tracers.

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