RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Neuroleptic Binding Sites: Specific Labeling in Mice with [^{18}]Haloperidol, A Potential Tracer for Positron Emission Tomography

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Haloperidol labeled with fluorine-18 ($T_{1/2}$ = 110 min, positron emission 97%), prepared yielding .04 Ci/millimole by the Balz-Schiemann reaction, was evaluated in a murine model as a potential radiotracer for noninvasive determination, by positron-emission tomography, of regional concentrations of brain dopamine receptors in patients. As the haloperidol dose in mice was increased from 0.01 to 1000 μg/kg, the relative concentration of [^{18}F]haloperidol (μCi per g specimen/μCi per g of body mass), at one hour after injection decreased from 30 to 1.0 in the striatum and from 8.0 to 1.0 in the cerebellum. The striatal radioactivity, plotted as relative concentration against log of dose, decreased sigmoidally, presumably reflecting competition between labeled and unlabeled haloperidol for a single class of accessible binding sites. Because the cerebellum is relatively deficient in dopamine receptors, the observed decrease in cerebellar radioactivity may reflect a saturable component of haloperidol transport into brain. The high brain concentrations and the unexpectedly high striatum-to-cerebellum concentration ratios (>4 at haloperidol doses ≤1 μg/kg) suggest that [^{18}F]haloperidol warrants further investigation as a potential radiotracer for dopamine receptors.

J Nucl Med 24: 408–416, 1983

Aberrant dopaminergic neuronal transmission has been implicated in the pathogenesis of various neurologic disorders, including Parkinsonism (*1–3*), Huntington's chorea (*1,4,5*), and tardive dyskinesia (*6–10*), as well as of certain neuroses (*11*) and psychoses (*12–15*). Characterization of dopamine receptors has been accomplished largely using radiometric binding assays of synaptic membrane preparations derived from animal or postmortem brain specimens (*16–19*). The highly invasive and nonphysiologic nature of these procedures severely limits their applicability to man and makes interpretation of results somewhat problematic. Consequently there is great interest in the potential utilization of positron-emission tomography (PET) (*20,21*), in conjunction with a suitable radiotracer for dopamine receptors, for the noninvasive determination of regional concentrations of brain dopamine receptors in vivo.

Specific dopamine receptors are present in brain in nanomolar concentrations (*22*), and therefore will be saturated at comparably low brain concentrations of a dopaminergic ligand. In contrast, nonspecific binding sites are practically nonsaturable (*23–26*). A potential dopamine-receptor-binding radiotracer must have a sufficiently high specific activity to yield an adequate photon flux for statistically significant radiation counting at ligand doses that do not saturate the specific binding sites. Because theoretical maximum specific activity is inversely proportional to half-life, dopaminergic ligands labeled with short-lived radionuclides—most notably carbon-11 ($T_{1/2}$ = 20.4 min, positron emission 100%) and fluorine-18 ($T_{1/2}$ = 110 min, positron emission 97%)—have great potential as dopamine receptor-binding radiotracers. For compounds singly labeled with

Received Sept. 24, 1982; revision accepted Dec. 17, 1982.

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carbon-11 or fluorine-18, the theoretical maximum specific activities are 9.2×10^6 and 1.7×10^6 Ci/mmole, respectively.

Haloperidol and spiroperidol—potent neuroleptics of the butyrophenone series, widely used in the management of schizophrenia—have been shown to bind specifically, reversibly, and with high affinity to synaptic membrane preparations in vitro (*22*). Demonstration in vivo of specific spiroperidol binding sites has been accomplished using specifically tritiated spiroperidols of high specific activity, namely 1-phenyl-[3-^{3}H]spiroperidol (sp. ac. 8–15 Ci/mmole) (*27–30*) and 1-phenyl-[4-^{3}H]spiroperidol (30 Ci/mmole) (*31,32*), and also *p*-[^{77}Br]bromospiroperidol (5–10 Ci/mmole) (*33,34*). Radiochemical stability of the tritiated ligands in vivo has been demonstrated by thin-layer chromatography (TLC) (*27,29,31*). In the case of 1-phenyl-[^{3}H]spiroperidol, the striatum-to-cerebellum radioactivity concentration ratio at 1 hr after injection decreased from 8.8 to 1.0 as the spiroperidol dose was increased from 44 to 2000 μg/kg (*27,29*). Because dopamine receptors are abundant in the striatum and relatively deficient in the cerebellum (*22*), this decrease in the striatum-to-cerebellum ratio presumably reflects saturation of striatal dopamine receptors and the ensuing dose-dependent decrease in the proportion of specifically bound spiroperidol in brain. In contrast, demonstration in vivo of specific haloperidol binding sites using tritiated haloperidol has not yet been accomplished. The specific activities of randomly labeled tritiated haloperidol (0.042 Ci/mmole) and of 4′-fluorobutyrophenone-[2′-^{3}H]-haloperidol (0.1 Ci/mmole), though adequate for pharmacokinetic studies (*35–40*), are apparently too low for the discrimination of specifically localized from nonspecifically localized haloperidol in brain (*37,40*). Though of adequately high specific activity, the unexpected absence of selective striatal localization of 4-(4-chlorophenyl)-[3-^{3}H]-haloperidol (8–18 Ci/mmole) at a haloperidol dose of only 5 μg/kg (*30*) suggests, perhaps, that it may undergo spontaneous de-tritiation in vivo.

We have prepared [^{18}F]haloperidol by the Balz-Schiemann reaction and have evaluated it in a murine model as a radioligand for the specific labeling in vivo of neuroleptic binding sites. Because of the technical constraints imposed by a short-lived, cyclotron-produced radionuclide such as fluorine-18, we have concurrently evaluated [G-^{3}H]haloperidol as a convenient model radioligand for the determination of the dose-dependent regional concentrations of haloperidol in brain. [G-^{3}H]Haloperidol is commercially available and has been used in receptor binding assays in vitro (*41,42*). [^{18}F]-Haloperidol prepared by the Balz-Schiemann reaction includes carrier because fluorine-18 is introduced by isotopic exchange with stable fluorine in the corresponding tetrafluoroborate diazonium salt (*43–44*). (Schmall B, Tilbury RS, Nisselbaum, JS, unpublished data). Although the resulting specific activity 400 mCi/mmole appears to be somewhat low for receptor studies in vivo, such studies are feasible in the "partial-body counting facility" a high-sensitivity, low-background radiation counting facility designed and installed in our center's biophysics laboratory (*45*).

MATERIALS AND METHODS

Chemicals. All commercially obtained chemicals and solvents were of reagent grade and were used as supplied. Haloperidol in crystalline form was a gift.* [G-^{3}H]-Haloperidol (14 Ci/mmole) was obtained commercially. The radiochemical purity (97%) was verified by TLC on silica gel† in chloroform/methanol/ammonium hydroxide (98:2:1).

Animals. All experiments used male and female B6 mice, maintained on a 12-hr light-dark cycle, with free access to food and water and housed 8–10 to a cage. The mice were approximately six months old and weighed 20–25 g each.

Preparation and analysis of "de-fluorinated haloperidol". This compound, 4-[4-(4-chlorophenyl)-4-hydroxypiperidino] butyrophenone, appears to be a ligand binding to dopamine receptors (*46*), and was therefore prepared as an analytical standard for high-pressure liquid chromatography. It was synthesized by the N-alkylation of 4-(4-chlorophenyl)-4-hydroxypiperidine with γ-chlorobutyrophenone, and was purified by recrystallization from 95% ethanol (mp:125–128°C; Ref. *46*: 128–130°C). Thin-layer chromatography of the product yielded a single spot ($R_f = 0.75$). The identity of the product was verified by chemical ionization mass spectrometry.

Preparation and analysis of [^{18}F]haloperidol. This was prepared from the corresponding tetrafluoroborate diazonium salt (chemical yield 20–30%, radiochemical yield 0.5–1.0%) by the Balz-Schiemann reaction using aqueous [^{18}F]fluoride, as developed by Kook et al. (*43*) and adapted by Schmall and Bigler (*44*). [^{18}F]Fluoride was produced in our compact medical cyclotron via the nuclear reaction $^{16}O(^3He,p)^{18}F$ by the irradiation of water with 22-MeV helium-3 ions (*47,48*). Chemical and radiochemical purity was greater that 95%, as determined by reverse isotope dilution analysis, thin-layer chromatography, and high-pressure liquid chromatography. Reverse isotope dilution analysis was accomplished by chromatographing an admixture of [^{18}F]-haloperidol and a large excess of unlabeled haloperidol on neutral alumina (Brockman activity 1, 80–200 mesh) and comparing its specific activity before and after chromatography. Analysis of the product by thin-layer chromatography on silica gel in chloroform/methanol/ammonium hydroxide (98:2:1) and in chloroform/ethyl acetate/formic acid (5:4:1) yielded single

radioactive spots (R_f = 0.75 and 0.36, respectively) identical to those of haloperidol. High-pressure liquid chromatography of the product was carried out using an analytical reverse-phase (PAX 5-1025 ODS-3) column and a solvent system of 0.1 m*M* ammonium acetate/methanol (65:35). Mass and radioactivity were measured simultaneously using a refractometer and a NaI(Tl) well counter with logarithmically scaled radiation meter, respectively, interfaced to a dual-trace strip-chart recorder. The retention time for defluorinated haloperidol was 11 min (k′ = 1.2) and for haloperidol 12 min (k′ = 1.4). High-pressure liquid chromatography yielded a single mass peak and a single radioactive peak, co-eluting with precisely the same retention time as haloperidol. There was no detectable defluorinated haloperidol in the [^{18}F]haloperidol preparation. The specific activity of [^{18}F]haloperidol, determined using ultra-violet spectrophotometry and a calibrated NaI(Tl) well counter, was 0.2–0.4 Ci/mmole at the time of injection.

Injection and dissection procedure. Mice were injected under light ether anesthesia with either [G-^{3}H]- or [^{18}F]haloperidol in 0.1 ml acidified, ethanolic isotonic saline via the retro-orbital venous plexus. The doses were 1.0, 10, 25, 100, and 1000 μg/kg for [G-^{3}H]haloperidol and 0.010, 0.10, 1.0, 10, 25, 100, and 1000 μg/kg for [^{18}F]haloperidol, adjusted before injection as necessary by the admixture of an appropriate amount of unlabeled haloperidol with the radioagent. There were four mice per dosage group. Each mouse was killed at 1 hr by cervical dislocation and immediately decapitated. The entire brain was removed and placed on an ice-cold metal surface. The striatum and the cerebellum were excised according to the method of Glowinski and Iversen (*49*). Each tissue specimen was placed on a tared piece of weighing paper, the paper and specimen weighed, and the wet mass of the specimen determined by difference.

Counting of tritium in tissue specimens. Each tissue specimen was placed in a 20-ml glass counting vial. Enough tissue solubilizer (typically 0.5 ml) was added to each vial to immerse the tissue completely. The specimens were incubated for 48 hr at 50°C. After cooling, 15 ml of scintillation cocktail[‡] were added to each vial and the radioactivity counted in an automatic beta counter using a preset "tritium" energy window. The counting efficiency for tritium was 58%. Knowing the mass and the activity per unit mass of the radioactive solution injected into each mouse, the injected dose of activity was determined. After correcting for background, the amount of radioactivity in each tissue specimen was expressed as relative concentration (μCi found per g specimen/μCi injected per g body mass (*50*).

Counting of fluorine-18 in tissue specimens. For tissue specimens from mice injected with haloperidol doses of 1 μg/kg or less, each specimen was placed in a small polyethylene tube and the radioactivity counted by annihilation coincidence detection. This utilized the partial-body-counting facility, a radiation counter using two heavily shielded NaI(Tl) crystals 23-cm in diameter by 13 cm thick. When operated in coincidence (coincidence resolving time: 100 nsec) and with a crystal separation of 4 cm, the background counting rate is 0.7–0.8 cpm and the overall counting efficiency for 0.511-MeV annihilation photons is ~20%. For tissue specimens from mice with haloperidol doses of 10 μg/kg or greater, each specimen was placed in a 20-ml glass counting vial and the radioactivity counted in an automatic gamma counter with an energy window spanning 450–570 keV. After correcting for background and for decay (to the time of injection), the amount of radioactivity in each specimen was expressed as relative concentration.

Analysis of the chemical form of radioactivity in vivo. The striata and the cerebella of several mice injected with [G-^{3}H]haloperidol or with [^{18}F]haloperidol were combined in chloroform and homogenized manually with a glass homogenizer. Each of the resulting suspensions was filtered and an aliquot of each of the respective filtrates admixed with haloperidol. Each of the resulting solutions was analyzed by TLC.

RESULTS

Striatal and cerebellar H-3 concentrations as a function of haloperidol dose. Figure 1 shows the relative concentrations of tritium in the striatum and cerebellum of mice, as a function of dose, 1 hr after the administration of [G-^{3}H] haloperidol. As the log dose of haloperidol is increased from 1.0 to 1000 μg/kg, the relative concentrations of tritium in the striatum and in the cerebellum remain constant at a value of approximately 2.0.

Striatal and cerebellar F-18 concentrations as a function of haloperidol dose. Figure 2 shows the relative concentrations of F-18 in the striatum and cerebellum of mice as a function of dose 1 hr after the administration of [^{18}F]haloperidol. As the log dose of haloperidol is increased from 0.01 to 1000 μg/kg, the relative concentrations of fluorine-18 in the striatum and in the cerebellum decrease in a sigmoidal fashion. The dose-dependent fall-off in the striatum is much more rapid than that in the cerebellum. The striatum-to-cerebellum fluorine-18 concentration ratio, shown in Fig. 3 as a function of log dose of haloperidol, likewise decreases in sigmoidal fashion.

Chemical form of radioactivity in vivo. Thin-layer chromatography of tritium in mouse brain yielded a chromatogram on which the radioactivity was diffusely distributed. The absence of a notable radioactive spot co-migrating with haloperidol suggests that [G-^{3}H]-haloperidol has undergone substantial de-tritiation in

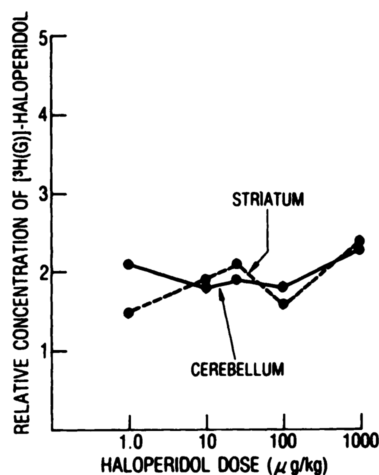

FIG. 1. Relative concentrations of [G-^{3}H]haloperidol in striatum and cerebellum of mice at 1 hr after injection, as a function of administered dose of haloperidol. Error bars represent standard error of the mean.

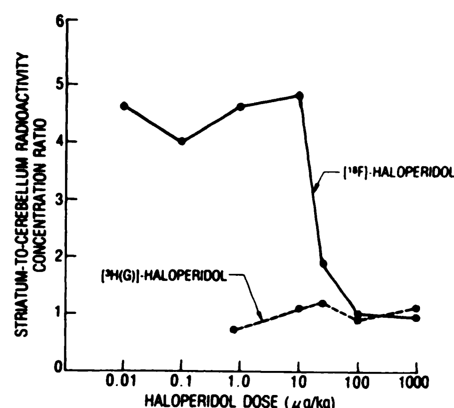

FIG. 3. Striatum-to-cerebellum radioactivity concentration ratios in mice at 1 hr after injection of [G-^{3}H]- and of [^{18}F]-haloperidol as a function of administered dose of haloperidol. Error bars represent standard error of the mean.

vivo. In contrast, TLC of F-18 in mouse brain yielded a chromatogram having a single radioactive spot, comigrating precisely with haloperidol (R_f = 0.75) and accounting for at least 80% of the radioactivity on the chromatogram. Because only a single radioactive spot was observed, [^{18}F]haloperidol probably accounts for the remaining radioactivity as well.

DISCUSSION

In the current study, evidence for the specific labeling in vivo of neuroleptic binding sites in mouse brain with [^{18}F]haloperidol has been presented. Because dopamine receptors are abundant in the striatum and relatively rare in the cerebellum (*22*), the dose-dependent decrease in the relative concentration in the striatum and in the striatum-to-cerebellum concentration ratio is consistent with receptor-mediated localization of [^{18}F]haloperidol in the striatum (Figs. 2 and 3). At haloperidol doses of ≤1 μg/kg—that is, at doses below those necessary to saturate specific binding sites—a relatively large fraction of haloperidol in brain is specifically bound and is therefore localized in the striatum. As the haloperidol dose is increased (1–100 μg/kg), approaching and eventually exceeding that necessary to saturate the specific binding sites, an increasing proportion of haloperidol is bound nonspecifically. At high haloperidol doses (≥100 μg/kg)—that is, at doses that greatly oversaturate the specific binding sites—nonspecific binding predominates, with a notable absence of selective striatal localization of F-18. This is because nonspecific binding is generally considered to be as likely to occur in the cerebellum as it is in the striatum, since neuroleptics readily localize in biological membranes because of their high lipid solubility (*23,26*). Thin-layer chromatography of F-18 in mouse brain suggests that most of the radioactivity represented intact [^{18}F]haloperidol. The apparent absence of any substantial extra-hepatic metabolism of haloperidol (*35,39,51,52*) and the rapid blood clearance (*53*) and excretion (*35,39,51,52*) of haloperidol metabolites, are consistent with this supposition. Because the carbon-fluorine bond is the single strongest bond formed by carbon (*54*), spontaneous de-fluorination of haloperidol in vivo is highly unlikely.

The results obtained with [G-^{3}H]haloperidol are markedly different from those obtained with [^{18}F]haloperidol. At all haloperidol doses, the relative concentration of tritium in the striatum and in the cerebellum maintains a constant low value of approximately 2.0. Even at low haloperidol doses, where demonstrable binding to specific receptors is expected to occur, selective striatal localization of tritium is not observed. Thin-layer chromatography of tritium in mouse brain suggests that practically none of the radioactivity represented haloperidol. Moreover, the relative concen-

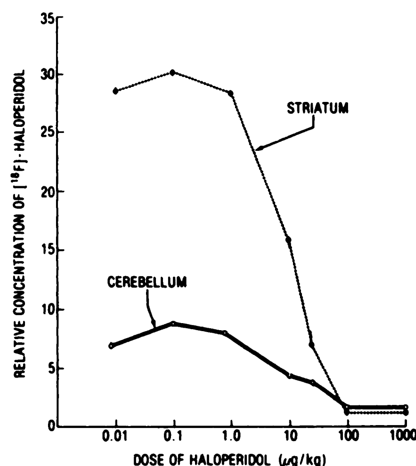

FIG. 2. Relative concentrations of [^{18}F]haloperidol in striatum and cerebellum of mice at 1 hr after injection, as a function of administered dose of haloperidol. Error bars represent standard error of the mean.

tration of tritium in mouse brain 1 hr after the administration of ^3H_2O (1.1) (unpublished results) compares with that 1 hr after the administration of [G-^{3}H]haloperidol (1.9). It appears, therefore, that the radioactivity in vivo following the administration of [G-^{3}H]haloperidol was largely "free" tritium, that is, tritiated water. Thus, [G-^{3}H]haloperidol is not useful for the specific labeling in vivo of neuroleptic binding sites because of the lability of the tritium atoms. A critical, but oftentimes ignored, consideration in such an experiment is clearly the stability of the tracer to spontaneous radiochemical degradation (e.g., detritiation) under the actual experimental conditions.

[G-^{3}H]haloperidol is prepared commercially by catalytic exchange in the presence of tritiated water. Though nominally "generally labeled," the principal site of labeling is presumably the methylene group in the α position to the carbonyl function. The carbonyl function tends to increase the acidity of the α-hydrogen atoms (*55*). In order to determine whether its lability in vivo was due simply to the acidity of the α-hydrogen atoms, [G-^{3}H]haloperidol was incubated at 37°C for 1 hr at various hydrogen-ion concentrations (pH 3, 7, and 10). The radiochemical purity, as determined by TLC, was in all instances comparable to the specified value (97%) for the commercial preparation (maintained at 4°C). Consistent with its utility in receptor-binding assays in vitro, conducted under physiologic conditions (37°C, pH 7.4), the de-tritiation of [G-^{3}H]haloperidol in vivo is not simply a temperature- or pH-dependent process.

Based principally on the differential binding affinities of dopaminergic ligands, the existence of distinct classes, or subsets, of dopamine receptors has been hypothesized (*22,56–58*). For example, the putative "dopamine-2 (D2)" receptor, which exhibits a thousand times the affinity for antagonists as for agonists, has been implicated in the pathogenesis of schizophrenia (*13–15,22*), as well as in the antipsychotic effect of neuroleptics (*15,22,26*). The dose-dependent sigmoidal decrease in the relative concentration of [^{18}F]haloperidol in the striatum and in the striatum-to-cerebellum concentration ratio appears to be conceptually consistent with a single class of accessible binding sites in vivo. Multiple classes of accessible binding sites in vivo would be expected to yield a dose-response curve (i.e., tissue concentration against log dose) consisting of a number of distinct sigmoidal components. Earlier studies, though suggestive of a single class of binding sites in vivo, have not unambiguously identified the shape of the dose-response curve because of the limited range (>1 μg/kg) of neuroleptic doses studied (*27,29,31,32*). The current series, which includes haloperidol doses as low as 0.01 μg/kg, yielded an unambigously sigmoidal dose-response curve, thus suggesting the existence of a single class of accessible binding sites in vivo.

In contrast to the striatum, dopamine receptors are relatively deficient in the cerebellum (*22*). Hollt et al. (*27*), Hollt and Schubert (*29*), and Kuhar et al. (*31*), all found a marked dose-dependent decrease in the striatal concentration of tritiated spiroperidol, but did not observe any decrease in the cerebellar concentration. It is unlikely, therefore, that the dose-dependent decrease in the relative concentration of [^{18}F]haloperidol in the cerebellum (Fig. 2) is receptor-mediated. Tewson et al. (*59*) found that pretreatment of a monkey with a pharmacologic dose of haloperidol reduced the fraction of no-carrier-added [^{18}F]haloperidol extracted by the brain during a single capillary transit following injection into the internal carotid artery. Because cerebral blood flow was not altered, the observed decrease in extraction fraction (i.e., from 0.7 to 0.4) was explained by postulating that [^{18}F]haloperidol competes with excess unlabeled haloperidol for a carrier-mediated transport system at the blood-brain barrier. This postulated saturable component of haloperidol transport into brain could also explain the dose-dependent decrease in the relative concentration of [^{18}F]haloperidol in the cerebellum. Consistent with this hypothesis is the finding that the cerebellar concentration of haloperidol in dogs decreased by nearly 50% as the haloperidol dose was increased from 40 to 630 μg/kg (*37*).

The striatal and the cerebellar concentrations of haloperidol, as indicated in Table 1, are much greater than those of other neuroleptics, including spiroperidol (*27–32*), *p*-bromospiroperido (*33,34*), and pimozide (*28,30,60*). The striatal and the cerebellar concentrations are not inconsistent with the whole-brain concentrations determined by tissue distribution studies in rats using either 4′-fluorobutyrophenone-[2′-^{3}H]haloperidol (*37–39*) or [^{18}F]haloperido (*53*), and by external scintigraphy in dogs and in monkeys using [^{18}F]haloperidol (*45,53*, unpublished data). For a given level of statistical accuracy, the greater brain concentrations of haloperidol will permit reduction of the dose of radioactivity, or the duration of data acquisition, for the determination by PET of regional receptor concentrations. Alternatively, for a given dose of radioactivity, the greater brain concentrations of haloperidol will serve to increase the statistical accuracy of regional receptor concentration measurements. As indicated in Table 2, the brain-to-blood concentration ratios are likewise much greater for haloperidol (*39,53,61*) than for other neuroleptics, including pimozide (*61*), trifluperidol (*61*), bromperidol (*53,62*), and spiroperidol (*63*). This will serve to increase further the statistical accuracy of regional receptor concentration measurements by reducing the correction for the vascular component of radioactivity within brain tissue.

If one assumes that striatal and cerebellar radioactivities represent total and free ligand, respectively, the maximum total-to-free (i.e., striatum-to-cerebellum) haloperidol concentration ratio in vivo (4.6, Table 1) is

TABLE 1. STRIATAL AND CEREBELLAR CONCENTRATIONS OF VARIOUS RADIOLABELED NEUROLEPTICS UNDER CONDITIONS OF MAXIMAL SPECIFIC BINDING

Radioligand	Dose (μg/kg)	Species	Time after injection (hr)	Relative concentration			Reference
				Striatum	Cerebellum	Stratium-to-cerebellum ratio	
[^{18}F]haloperidol	1	mouse	1 (iv)	28	7.9	4.6	Current study
1-phenyl-[3-^{3}H]-spiroperidol	5	mouse	0.5 (iv)	0.32	0.070	4.5	27
1-phenyl-[3-^{3}H]-spiroperidol	5	rat	2 (iv)	0.50	0.058	8.6	28, 30
1-phenyl-[3-^{3}H]-spiroperidol	4.4	mouse	1 (iv)	0.14	0.015	8.8	29
1-phenyl-[4-^{3}H]-spiroperidol	10	rat	1 (iv)	0.63	0.10	6.3	31
1-phenyl-[4-^{3}H]-spiroperidol	2	rat	2 (iv)	0.71	0.14	5.1	32
[^{77}Br]bromospiroperidol	7.5	rat	2 (iv)	0.93	0.34	2.7	33
[^{77}Br]bromospiroperidol	2.5*	cat	5 (iv)	—	—	4.7†	34
				—	—	3.8‡	
1-(4,4)-bis-(p-fluorophenyl)butyl-[3,4-^{3}H]-pimozide	20	rat	2 (iv)	0.38	0.13	2.9	28, 30
1-(4,4)-bis-(p-fluorophenyl)butyl-[3,4-^{3}H]pimozide	10	dog	6 (sc)	1.1	0.38	2.9	37
1-(4,4)-bis-(p-fluorophenyl)butyl-[3,4-^{3}H]-pimozide	2	mouse	2 (iv)	0.18	0.094	1.9	60

* 200 μCi [^{77}Br]bromospiroperidol (specific activity: 7.5 Ci/mmole) administered to a cat (5 kg assumed body mass).
† Determined by seven-pinhole single-photon emission tomography.
‡ Determined by tissue distribution study.

TABLE 2. BRAIN-TO-BLOOD CONCENTRATION RATIOS OF VARIOUS RADIOLABELED NEUROLEPTICS

Radioligand	Dose (μg/kg)	Species	Time after injection (hr)	Brain to-blood concentration ratio	Reference
4′-fluorobutyrophenone-[2′-^{3}H]-haloperidol	40	rat	1 (sc)	26	39
4′-fluorobutyrophenone-[2′-^{3}H]-haloperidol	5000	rat*	0.5 (iv)	51†	61
[^{18}F]haloperidol	10	rat	1 (iv)	12	53
1-(4,4)-bis-(p-fluorophenyl)butyl-[3,4-^{3}H]-pimozide	5000	rat*	0.5 (iv)	2.1†	61
4′-fluorobutyrophenone-[2′-^{3}H]-trifluperidol	5000	rat*	0.5 (iv)	21†	61
[^{82}Br]bromperidol	200	rat	1 (iv)	2.2	53, 62
[^{11}C]spiroperidol	0.05‡	mouse	0.5 (iv)	0.67	63

* Ovariectomized rats.
† Cerebral cortex-to-plasma ratio.
‡ Based on estimate of specific activity of no-carrier-added [^{11}C]spiroperidol.

greater than that in vitro (1.2–1.4) (*41,64*). One of the assumptions implicit in this comparison—the utilization of the ligand concentration in the cerebellum as an estimate of the free ligand concentration in the striatum—may perhaps require rigorous validation at some point. In comparison with haloperidol, the maximum striatum-to-cerebellum spiroperidol concentration ratio in vivo (4.5–8.8, Table 1) is not inconsistent with the maximum total-to-free spiroperidol concentration ratio in vitro (5–15) (*41,64*). These data suggest, perhaps, that at least for haloperidol the receptor-binding affinity in vivo may be somewhat greater than that determined in vitro.

In contrast to the striatum-to-cerebellum concentration ratio, the unusually high brain concentrations (Table 1) and brain-to-blood concentration ratios (Table 2) are probably not related to receptor binding. Because tissue localization of a radiotracer is such a complex phenomenon, it should not be expected that target tissue concentrations of a putative receptor-binding radiotracer will always be correlated with receptor-binding affinity. For example, domperidone—a highly potent and specific dopamine antagonist in vitro (*65*)—is completely excluded from brain tissue by the blood-brain barrier because it is highly basic ($pK_a = 11$) and therefore highly charged at physiologic pH (66, P. Seeman, personal communication). Bioavailability to brain (determined by brain extraction efficiency and thus net lipid solubility in vivo), localization in and redistribution from other tissues [particularly the lungs (*67,68*)], and metabolism and excretion, are most likely responsible for the observed differences in brain concentrations among neuroleptics. In particular, the postulated carrier-mediated transport system for haloperidol may be largely responsible for its unusually high brain concentrations.

[^{18}F]Haloperidol has already proven useful in noninvasive pharmacokinetic studies. For example, the distributions of pharmacologic haloperidol doses in a monkey (*44,53*) and in a dog (unpublished data) have been determined by two-dimensional longitudinal imaging and by PET. Because near-maximal receptor binding is attained by several hours after injection (*27–34,60*), the 110-min half-life of F-18 makes it well suited for radiotracers binding to dopamine receptors. The striatal concentrations and the brain-to-blood concentration ratios are much greater for haloperidol than for any other neuroleptic studied thus far. Most importantly, perhaps, the maximum striatum-to-cerebellum concentration ratio in vivo appears to be greater than that expected on the basis of receptor-binding assays in vitro. No-carrier-added [^{18}F]haloperidol has been prepared, though in low yield, by the triazene reaction (*59,69*) and by deaminative fluorination (unpublished data). The current study suggests that [^{18}F]haloperidol warrants further investigation as a potential dopamine receptor-binding radiotracer. For haloperidol, as well as for other dopaminergic ligands, the mechanisms of tissue localization and the nature of specific and nonspecific binding sites require further elucidation.

FOOTNOTES

* Generously provided by McNeil Laboratories, Fort Washington, PA.

† 1381 Silica gel Eastman Chromagram Sheet, Eastman Kodak Company, Rochester, NY.

‡ Insta-gel liquid scintillation cocktail, Packard Instrument Company, Inc., Downers Grove, IL.

ACKNOWLEDGMENTS

We express our appreciation to Messrs. Richard Lee and J. Robert Dahl for the production of fluorine-18.

This investigation was supported in part by the U.S. Department of Energy Contract no. EE-77-S-02-4258 and by the National Cancer Institute Grant No. CA-09022.

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