Rat metabolism

Awake rats were injected intravenously with 225–250 μCi of [^{18}F]FP-TZTP or [^{18}F]FP-TZTP plus 50 nmoles of FP-TZTP and one animal sacrificed at each of the time points: 5, 10, 15, 30, 45, or 60 min. At 5 and 10 min, only blood was collected. At the other time points, blood and brain were removed, weighed, and counted to determine the total activity. Serum was separated and extracted with an equal volume of acetonitrile. Brain samples were placed in an equal volume of acetonitrile and homogenized with 15–30 sec bursts. Following centrifugation, the radioactive content of the supernatant and pellets were determined. The supernatants were chromatographed on TLC plates, developed with the system described for determining metabolites in primates, and placed on a phosphorimaging plate overnight. The plates were scanned for the [^{18}F]FP-TZTP and its metabolites the next day using a Fuji Bioimaging Analysis System 1500. Extraction efficiency was evaluated from a single rat injected with 600 μCi and sacrificed at 15 min. Serum and brain samples were extracted with an equal volume of acetonitrile as described above. The brain and blood pellets were then extracted a second time with an equal volume of acetonitrile. The pellets remaining after the second extraction were extracted with the same volume of 0.1 M EDTA. Another sample of brain and serum was extracted in four times the volume of acetonitrile. The pellets and supernatants were assessed for radioactivity from each extraction. The supernatants from each extraction were chromatographed on TLC and the radiochromatograms assayed with the Fuji Bio-imaging Analysis System 1500 for radiochemical components.

RESULTS

Radiochemistry

The radiosyntheses of [^{18}F]FP-TZTP and [^{18}F]FETZTP follow the same scheme (Fig. 1) as described in our previous communication (Kiesewetter et al., 1995a). Yields for the two compounds are equivalent.

In vivo biodistribution in rats

[^{18}F]FE-TZTP displayed very high uptake in the brain of awake rats (Fig. 2). The uptake was similar to the previously reported results of [^{18}F]FP-TZTP in awake rats at 60 min. However, the observed inhibition of uptake upon coinjection of 50 nmol unlabeled P-TZTP was approximately half that observed for [^{18}F]FPTZTP. For example, in the caudate, thalamus, and pons, inhibition of [^{18}F]FE-TZTP uptake was 23%, 27%, 34%, respectively; while inhibition of [^{18}F]FP-TZTP uptake in the same regions was 68%, 70%, and 67%, respectively (Kiesewetter et al., 1995a). The uptake of [^{18}F]FE-TZTP in the heart was unexpectedly quite low and very little blocking was observed. Because of these observations with [^{18}F]FE-TZTP, we decided to further evaluate [^{18}F] FP-TZTP as our primary M_2 radioligand. In order to enhance our understanding of the in vivo muscarinic binding properties of [^{18}F]FP-TZTP, we conducted coinjection studies with the muscarinic ligand L-687, 306 (3-(3- cyclopropyl-1,2,4-oxadiazol-5-yl)-1-azabicyclo[2.2.1]heptane) (Freedman et al., 1993). In rats, we observed that coinjection of L-687,306 at a dose of 500 nmol per rat inhibited the uptake of [^{18}F]FP-TZTP at 60 min (Fig. 3).

In order to evaluate M_2 selective binding in the heart (where theM_2 subtype predominated), we extended our past work on the time course of uptake to examine shorter times. The biodistribution of [^{18}F]FP-TZTP at 15 min with and without coinjection of P-TZTP (Fig. 4A) was similar to the results of our previous 60-min study (Kiesewetter et al., 1995a). Uptake in the heart does not exceed the blood level and specific binding in the heart is small, at 15 min as demonstrated by the minimal inhibition in the coinjection study. However, at 5 min we did observe inhibition of uptake in the heart (55%) upon coinjection of unlabeled P-TZTP (Fig. 4B).

Autoradiography in rats

We conducted high resolution in vivo autoradiography in the rat with a mass dose of 0.7 nmol. The uptake per gram into the brain in our 15-min distribution study (Fig. 4a) in the brain averaged less than 1%. This gave a maximum brain concentration of 5 nM. Since the concentration of the M_2 subtype in the cerebellum of rats is 16 nM, significant receptor occupancy is present. Our high resolution autoradiograms showed anatomical distribution with excellent gray/white matter definition. The slice shown in Figure 5A is similar in anatomical location to published photomicrograph slices of antibody localization of the muscarinic subtypes (Levey, 1993). Qualitatively, the uptake of [^{18}F]FP-TZTP in the slice matched the pattern of localization of the M_2 antibody better than the other subtypes. Autoradiograms obtained after coinjection of radiolabeled and nonradiolabeled ligand showed a much reduced uptake and no contrast between gray and white matter (Fig. 5B).

[^{18}F]FP-TZTP in monkeys

We conducted brain distribution studies on rhesus monkeys using PET. Fig. 6 shows the time–activity data

following bolus injection of [18F]FP-TZTP in a control study. Rapid uptake of the tracer was seen in all brain regions, followed by clearance from the brain. Clearance was more rapid and approached a lower level in the cerebellum than in cortical regions and thalamus.

Figure 7 shows data from a second study where 80 nmol/kg of unlabeled FP-TZTP was administered i.v. 45 min postinjection.Adistinct change in slope was seen in the net efflux. To estimate the magnitude of displacement, the data before 45 min was fit by a compartment model (Carson et al., 1996) and extrapolated forward to 90 min. Comparison of the measured radioactivity from 70 to 90 min postinjection to the extrapolated control data showed displacements of 20%, 36%, and 41% in cerebellum, cortex, and thalamus, respectively. Unlabeled P-TZTP was also effective as a displacer (data not shown).

FP-TZTP metabolism

We examined the metabolites in the plasma of the monkey in order to determine the percent parent over the time course of the study. Plasma was extracted with acetonitrile and analyzed by TLC and/or HPLC. The parent compound degrades quickly in vivo, representing 62 6 8%, 23 6 3%, and 11 6 3% (n 5 6) of extracted plasma activity at 10, 30, and 60 min, respectively. A comparison of TLC and HPLC for primate plasma was performed in a limited set of studies. We observed an excellent correlation (r 5 0.99) in the parent percentage measurements between TLC (T) and HPLC (H) with a relationship of

$$H = 23.9 + 1.04\,T$$

For samples between 15 and 50 min postinjection, H underestimated T by a mean of 5%.We did note a trend that TLC values measured with the Bioscan Imaging scanner tended to underestimate HPLC values, while values measured with the Fuji hosphorimager tended to overestimate them.

During our experiment in monkeys, three metabolite peaks on TLC increased in concentration while the parent decreased (Fig. 8). One of these components (Met #1) is nearly as lipophilic as the parent compound on TLC, raising the question of its uptake into brain. To evaluate the level of this metabolite in brain, we studied metabolism in the serum and brain of rats. We examined the metabolite profile in rats out to 60 min postinjection in both serum and brain.Aplot of the time course of the fraction of parent compound is shown in Figure 9. In the serum, the parent compound rapidly disappears and metabolites rapidly increase. However, parent compound in the brain represents greater than 95% of the extracted radioactivity through 30 min and greater than 90% at 45 and 60 min. When parent fraction was analyzed in a study involving coinjection of radiolabeled compound with a 50 nmol blocking dose of nonradioactive ligand, the parent compound represented greater than 90% of the extracted radioactivity from the brain at 15 min, but was reduced to only 62% at 45 min. Parent compound concentrations in the serum were not different.

In evaluation of the metabolite proportions, one must know the extraction efficiency of the procedure. The extraction efficiency observed in the above metabolism studies for rat serum ranged from 82% at 5 min to 53% at 60 min. We examined the 15-min time point using multiple extractions to determine the nature of the nonextracted radioactivity. From serum, the first extraction collected 52% of the total counts and the second volume extracted an additional 5% of the original total activity. Thus, the sum of two equal volume extractions was 60% of the serum radioactivity. TLC analysis shows that the percent of parent [18F]FP-TZTP in each of the two acetonitrile extractions is the same. A single four-volume extraction of serum with acetonitrile resulted in an even lower extraction efficiency of 27%; however, the total percent parent compared with the two extraction study is the same.

Extraction efficiency from the rat brain tissue was constant, ranging from 67–75% over the same time course. In the brain at 15 min, two serial extractions with one volume of acetonitrile resulted in 84% efficiency. Both supernatants showed the same 97% parent Extraction of brain tissue with four volumes of acetonitrile results in a 92% efficiency, which is shown by TLC to also be 97% parent.