Synthesis and Evaluation of No-Carrier-Added 8-Cyclopentyl-3-(3-[^{18}F]fluoropropyl)-1-propylxanthine ([^{18}F]CPFPX): A Potent and Selective A_1-Adenosine Receptor Antagonist for in Vivo Imaging

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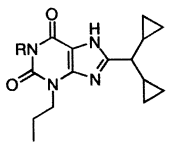
This report describes the precursor synthesis and the no-carrier-added (nca) radiosynthesis of the new A_1 adenosine receptor (A_1AR) antagonist [^{18}F]8-cyclopentyl-3-(3-fluoropropyl)-1-propylxanthine (CPFPX), **3***, with fluorine-18 (half-life = 109.6 min). Nucleophilic radiofluorination of the precursor tosylate 8-cyclopentyl-3-(3-tosyloxypropyl)-7-pivaloyloxymethyl-1-propylxanthine, **2,** with nca [^{18}F]KF under aminopolyether-mediated conditions (Kryptofix 2.2.2/K_2CO_3) followed by deprotection was straightforward and, after formulation, gave the radioligand ready for injection with a radiochemical yield of 45 ± 7%, a radiochemical purity of >98% and a specific radioactivity of >270 GBq/μmol (>7.2 Ci/μmol). Preparation time averaged 55 min. The synthesis proved reliable for high batch yields (~7.5 GBq) in routine production (n = 120 runs). The radiotracer was pharmacologically evaluated in vitro and in vivo and its pharmacokinetics in rodents determined in detail. After iv injection a high accumulation of radioactivity occurred in several regions of mouse brain including thalamus, striatum, cortex, and cerebellum. Antagonism by the specific A_1AR antagonists 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and N^6-cyclopentyl-9-methyladenine (N-0840), but not with the A_2AR antagonist 3,7-dimethyl-1-propargylxanthine (DMPX), indicated specific and reversible binding of the radioligand to A_1AR in cortical and subcortical regions of interest. In mouse blood at least two polar metabolites formed rapidly (50% at 5 min after tracer application). However, chromatographic analyses of brain homogenate extracts taken 60 min pi showed that >98% of radioactivity was unchanged radioligand. Chromatographic isolation and reinjection of peripherally formed radioactive metabolites revealed no accumulation of radioactivity in mouse brain, probably due to the polarity of the metabolites. These preliminary results suggest that nca [^{18}F]CPFPX is a useful radioligand for the noninvasive imaging of the brain A_1AR.

Introduction

The four kinds of adenosine receptors (ARs) are the A_1AR, A_{2A}AR, A_{2B}AR, and A_3AR.[1] The most intensively studied subtypes are the high-affinity A_1AR and A_{2A}-AR, which adenosine activates in nano- to sub-micromolar concentrations. The clinical importance of the A_1AR makes it an attractive target for in vivo radionuclide imaging. This receptor is distributed heterogeneously throughout the human brain with particularly high densities in the hippocampus, striatum, and cerebral cortex.[2] Interruptions of the blood supply of the brain cause a profound loss of receptor density,[3] a change that could be used for the evaluation of patients with stroke or other circulatory disturbances. A_1ARs in the heart inhibit generation of the heartbeat[4] and slow or even block impulse conduction from the atria to the ventricles.[5] The A_1AR initiates the protective effect of preconditioning in response to transient ischemia in brain and heart.[6,7] Accordingly, positron-emission-tomography (PET), using high affinity, highly selective radioligands, could be a useful investigative and perhaps even diagnostic tool.



R = $CH_3CH_2{}^{11}CH_2$ [^{11}C]KF15372
$CH_3{}^{11}CH_2$ 1-[^{11}C-ethyl]KF15372
$^{11}CH_3$ 1-[^{11}C-methyl]KF15372

Figure 1. Structures of PET radioligands for the A_1AR.

The literature describes twelve adenosine receptor antagonists labeled with carbon-11. A_1AR antagonists (Figure 1) include [^{11}C]KF15372 ([1-propyl-^{11}C]8-dicyclopropylmethyl-1,3-dipropylxanthine)[8] and its ethyl and methyl derivatives [^{11}C]EPDX and [^{11}C]MPDX.[9] A_{2A}AR antagonists (Figure 2) include the 8-styrylxanthines [^{11}C]CSC,[10] [^{11}C]KF17837, [^{11}C]KF18446, [^{11}C]-KF19631,[11] [^{11}C]IS-DMPX [^{11}C]BS-DMPX,[12] [^{11}C]-KF21213,[13] and [^{11}C]KW-6002[14] and the nonxanthine compound [^{11}C]SCH442416.[15]

So far [^{11}C]KF15372 is the only A_1AR ligand evaluated as a candidate for the imaging of the A_1AR by PET.[8] The combination of a time-consuming multistep

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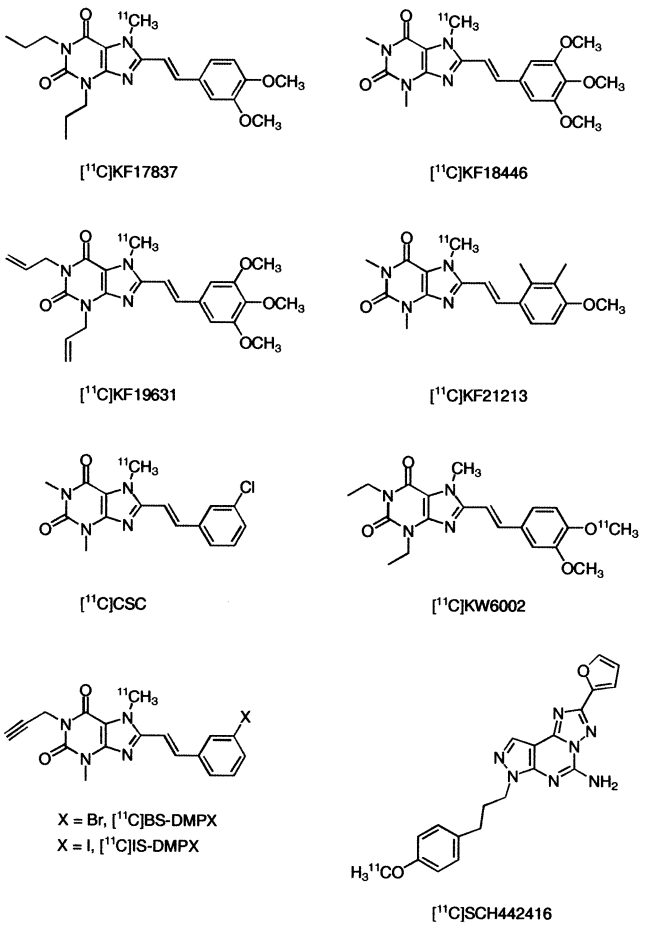


Figure 2. Structures of PET radioligands for the A_{2A}AR.

radiosynthesis and the short physical half-life of carbon-11 ($t_{1/2}$ = 20.4 min) resulted in a low (ca. 5%) radiochemical yield (RCY) of [^{11}C]KF15372 and a specific activity that ranged between 10 and 56 GBq/μmol (0.3−1.5 Ci/μmol). Studies in mice showed that brain uptake after iv injection was 1.9% of the injected dose (ID)/g after 5 min and fell thereafter. The content of radioligand was highest in hippocampus, cerebral and cerebellar cortex, and striatum. The administration of cold ligand reduced the content of radioligand to about 45% of control. Although the preliminary data look promising, the rather low RCY, low specific activity, and need to restrict imaging to near the site of production potentially limit the use of [^{11}C]KF15372. Its two derivatives ([^{11}C]EPDX and [^{11}C]MPDX) did not prove useful in preclinical animal studies.[9]

For PET studies, fluorine-18 ($t_{1/2}$ = 109.6 min) offers significant advantages in comparison to the short 20 min half-life of carbon-11. The 110 min half-life of fluorine-18 allows sufficient time for isotope incorporation into the tracer molecule and its purification to a final product suitable for human administration and permits the preparation of multidose batches for distribution by a cyclotron-based radiopharmacy ("satellite concept"). Fluorine-18 is also the lowest energy positron emitter (0.635 MeV, 2.4 mm positron range), a physical attribute that translates into PET images having the highest spatial resolution of all positron-emitting radionuclides. To circumvent the limitations of carbon-11 we undertook the development of an A_1AR ligand labeled with fluorine-18 that could be a useful ligand for

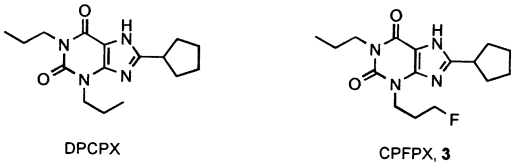
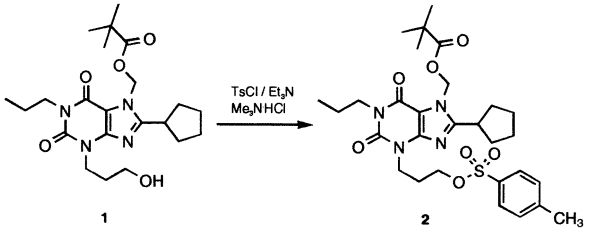


Figure 3. Structures of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and 8-cyclopentyl-3-(3-fluoropropyl)-1-propylxanthine (CPFPX, **3**).

Scheme 1. Synthesis of Precursor Tosylate **2** for ^{18}F-Labeling



quantitative in vivo mapping of human brain A_1AR receptors by PET.

This report describes the synthesis of the precursor 8-cyclopentyl-3-(3-tosyloxypropyl)-7-pivaloyloxymethyl-1-propylxanthine, **2**, and the nca radiofluorinated form of the new A_1AR antagonist 8-cyclopentyl-3-(3-fluoropropyl)-1-propylxanthine (CPFPX, **3**), previously developed in a systematic SAR study.[16] That ligand is a fluorine isostere of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), which, owing to its high selectivity and affinity for the A_1AR, is the prototypical A_1AR antagonist (Figure 3).[17−19] The present studies determined the pharmacological properties and pharmacokinetics of nca [^{18}F]CPFPX, **3***, its in vivo binding characteristics to A_1-AR, the effects of antagonists or carrier ligand on the distribution within the brain and the metabolic stability of the radioligand. An earlier report[16] described the synthesis and some of the in vitro pharmacological characteristics of unlabeled CPFPX**, 3**.

Results and Discussion

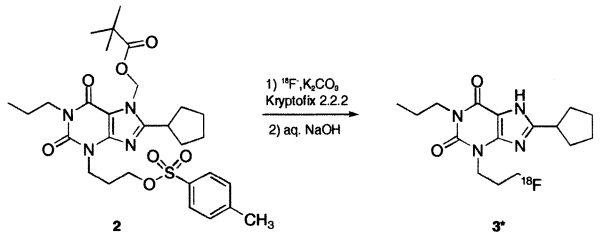
Radiochemical Synthesis. The synthesis of the precursor for radiolabeling, 8-cyclopentyl-3-(3-tosyloxyopropyl)-7-pivaloyloxymethyl-1-propylxanthine **2**, started from 8-cyclopentyl-3-(3-hydroxypropyl)-7-pivaloyloxymethyl-1-propylxanthine**, 1**.[16] Tosylation of the hydroxyl group of **1** using TsCl/Et_3N and catalytic Me_3N·HCl as a combined base[20] gave the N-7-protected precursor **2** in a yield of 91% (Scheme 1).

Radiofluorination of tosylate **2** with [^{18}F]fluoride used aminopolyether-supported (Kryptofix 2.2.2/K_2CO_3)[21,22] nucleophilic aliphatic substitution with nca [^{18}F]KF, followed by removal of the 7-pivaloyloxymethyl (POM) protecting group with NaOH (Scheme 2). In over 100 runs synthesis time averaged 55 min.

After sterile formulation the target compound**,** 8-cyclopentyl-3-(3-[^{18}F]fluoropropyl)-1-propylxanthine, **3***, was obtained ready for injection with a RCY of 45 ± 7% with respect to initial [^{18}F]fluoride ion activity, a radiochemical and chemical purity of >98% and a specific activity of >270 GBq/μmol (7.2 Ci/μmol).

[^{18}F]CPFPX showed excellent in vitro stability at room temperature in aqueous solution. Up to 8 h after

Scheme 2. Nucleophilic, Cryptate-Mediated Radiosynthesis of nca [^{18}F]CPFPX (**3***)



preparation, neither HPLC nor TLC analysis of the tracer gave any indication of disintegration products.

In Vitro Studies. Radioligand Binding. Previous in vitro binding studies on bovine cortex membranes,[16] using the tritiated antagonist [^{3}H]DPCPX (K_D = 0.15 nM) as the reference ligand and *R*-PIA [N^6-(1*R*-methyl-2-phenylethyl)adenosine] for the determination of unspecific binding, indicated high affinity and selectivity of **3** for the A_1AR (K_i = 0.18 nM, A_{2A}/A_1 > 500).

The present binding studies and saturation experiments using [^{3}H]CPFPX and either rat or pig cortex membranes or human cloned A_1ARs revealed a high affinity of **3** for the A_1AR K_D-values were 0.63 nM, 1.37 nM, and 1.26 nM for the rat, pig, and human rA_1AR, pA_1AR, and hA_1AR, respectively. Binding assays using rat striatal membranes with [^{3}H]CGS21680 (K_D = 11.6 nM) as the radioligand determined the binding profile to the rA_{2A}AR. The K_i of **3** was 812 nM, indicating a low affinity for the A_{2A}AR and an A_1/A_{2A} selectivity >1200-fold At the cloned hA_{2A}AR the K_D of [^{3}H]CPFPX was 940 nM, an A_1/A_{2A} selectivity of >700. Figure 4 shows representative binding curves for the human A_1-AR and A_{2A}AR. The data from the rat and human receptors confirm the estimates of affinity and selectivity from bovine cortex membranes.[16] Although the A_3-AR has substantial amino acid homology and an agonist pharmacological profile resembling that of the A_1AR, there is no evidence that it exists in the human brain.[23] For that reason and because this project aims at the development of an imaging agent for *human* brains, we did not measure the hA_1AR/hA_3AR selectivity ratio.

In Vivo Studies. NMRI mice served for preliminary in vivo evaluation of pharmacokinetics. Table 1 shows the organ distribution of **3*** between 2 and 60 min pi.

Clearance of radioactivity from the blood proceeded slowly by a process having biexponential kinetics, the rapid initial phase having a rate of 0.58 min^{-1} and the slower phase a rate of 0.005 min^{-1}. The slow clearance probably reflects binding to plasma proteins rather than uptake by blood cells. Ultrafiltration studies showed that in human plasma 86 ± 2% of **3*** bound to proteins larger than 30 kDa. All organs took up tracer rapidly; accumulation was highest in the liver, lungs, and kidneys, maintaining a plateau between 10 and 40 min, then falling in all tissues except the intestine, where ^{18}F-activity accumulated to a high extent at later time points (about 9% ID/g at 60 min pi). Such a pattern is consistent with excretion through the liver, a major route for the alkylxanthines.[24] Uptake of radioactivity in femur was very low, 2.3% at 60 min pi, evidence that the label is metabolically stable.

Figure 5 depicts the kinetics of the accumulation of **3*** in mouse brain. Uptake of **3*** by brain (Figure 5, curve labeled *baseline*) was rapid, peaking at 2.4% of ID at 2 min and remaining essentially constant for 40 min before declining to 1.5% ID at 60 min. This relatively high uptake correlates with the lipophilicity of **3***; the octanol−water distribution parameter log *P* was 2.1, indicating that **3*** is sufficiently lipophilic to cross the blood−brain barrier.[25] Similarly, the administration of unlabeled A_1AR antagonists N^6-cyclopentyl-9-methyladenine (N-0840) 20 min before or DPCPX 10 min after the administration of **3*** decreased brain uptake by 70−80% (Figure 5, curves labeled *blocking* and *displacement*, respectively). Reducing the specific activity of **3*** by 900-fold also decreased brain uptake to the level of unspecific binding (data not shown). However, neither the preadministration nor coadministration of the selective A_{2A}AR antagonist 3,7-dimethyl-1-propargylxanthine (DMPX)[26] affected brain uptake of **3***. These findings indicate that **3*** binds specifically and reversibly to the A_1AR in cortical and subcortical regions of interest.

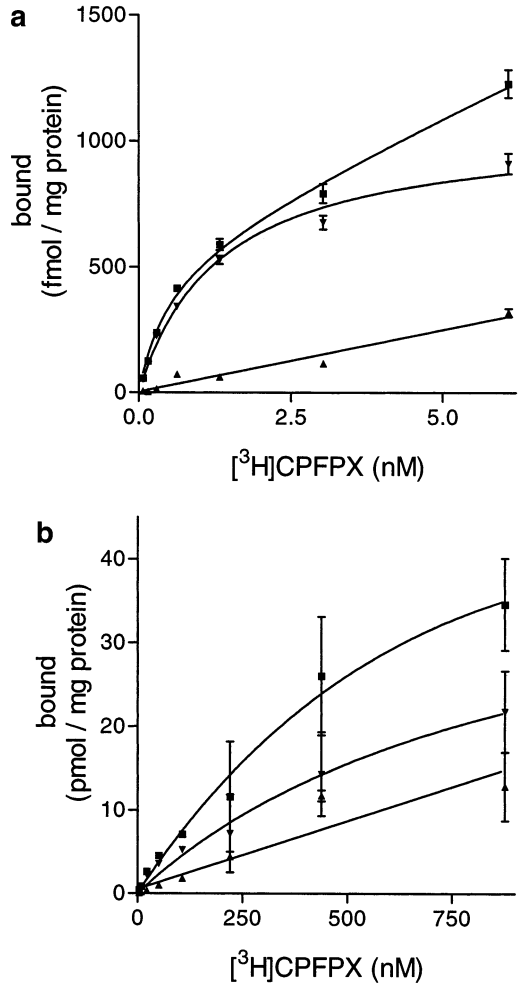


Figure 4. Equilibrium binding of **3*** to the cloned hA_1AR (Figure 4a) and hA_{2A}AR (Figure 4b) expressed in CHO cells. These results are representative of three separate experiments. Note the difference in the ordinate axis scales.

Figure 6 shows the heterogeneous regional distribution of **3*** in mouse brain 10 min pi. The radioactivity preferentially accumulated in regions of known high of A_1AR density,[27,28] namely, hippocampus, frontal cortex, and striatum, to an extent of 4.5 ± 0.4%, 4.2 ± 0.3%, and 3.9 ± 0.3% of the ID/g, respectively. Such a

Table 1. Biodistribution of nca **3*** in NMRI Mice[a]

	tissue fluorine-18 activity, % ID/g					
time pi	2 min	5 min	10 min	20 min	40 min	60 min
blood	11.2 ± 0.91	6.53 ± 1.02	5.21 ± 1.11	4.13 ± 1.07	3.92 ± 0.88	1.55 ± 0.32
brain	2.45 ± 0.14	2.37 ± 0.09	2.44 ± 0.11	2.39 ± 0.13	2.52 ± 0.07	1.55 ± 0.12
intestine	2.07 ± 0.14	2.21 ± 0.18	3.08 ± 0.31	3.51 ± 0.24	3.74 ± 0.21	9.13 ± 0.56
pancreas	3.58 ± 0.20	3.35 ± 0.31	4.29 ± 0.66	4.58 ± 0.66	3.22 ± 0.57	1.03 ± 0.18
liver	9.34 ± 1.01	7.44 ± 1.01	8.64 ± 0.59	9.93 ± 1.17	9.61 ± 0.89	4.36 ± 0.77
kidneys	4.68 ± 0.23	3.81 ± 0.67	6.10 ± 1.21	6.05 ± 0.82	5.95 ± 0.71	2.63 ± 0.41
heart	4.64 ± 0.16	3.71 ± 0.41	3.83 ± 0.27	4.11 ± 0.31	4.23 ± 0.34	1.63 ± 0.22
lungs	5.35 ± 0.68	4.22 ± 0.84	5.79 ± 0.78	5.75 ± 0.48	5.70 ± 0.61	2.43 ± 0.18
spleen	2.25 ± 0.08	1.56 ± 0.09	1.77 ± 0.16	1.78 ± 0.13	1.79 ± 0.20	0.84 ± 0.09
bones	0.37 ± 0.03	1.40 ± 0.12	1.71 ± 0.08	2.02 ± 0.13	2.18 ± 0.17	2.27 ± 0.12

[a] Each datum represents mean ± SEM of four mice at each time point. Dose of **3*** was 10−15 MBq or 0.15−0.2 nmol per mouse.

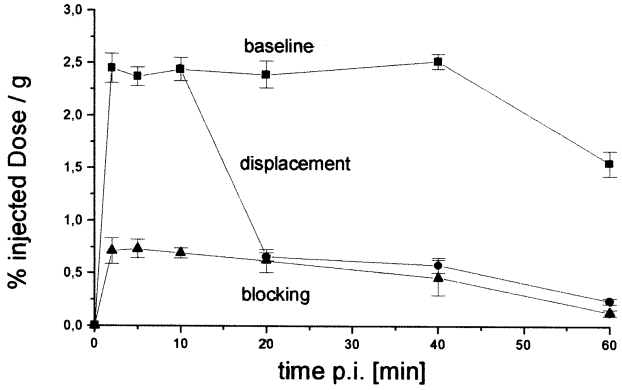


Figure 5. Kinetics of mouse brain uptake of compound [^{18}F]-CPFPX (**3***) in vivo (10−15 MBq/mouse, 0.15−0.2 nmol **3**/mouse, $n = 4$ animals for each time point, the upper curve (*baseline*) represents the kinetics of radioactivity uptake in untreated animals. **T**he other curves were obtained after a *displacement* experiment using DPCPX (1 mg/kg 10 min pi) or a *blocking* experiment using N-084 **(**1 mg/kg**,** 20 min before tracer injection**)**.

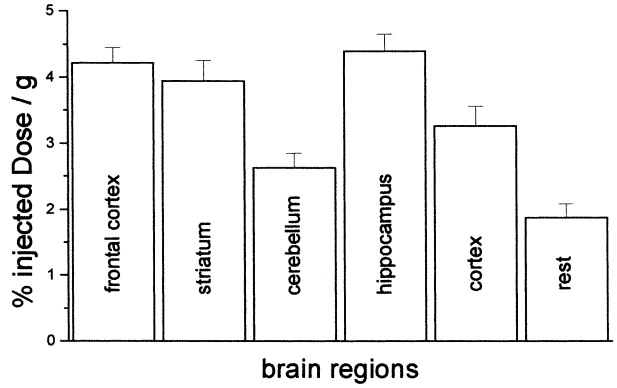


Figure 6. Distribution of radioactivity in various regions of mouse brain 10 min after injection of **3*** (10−15 MBq or 0.15−0.2 nmol per mouse, $n = 6$ animals for each point**)**.

distribution agrees closely with autoradiographic data on the distribution of A_1ARs in experimental animals.[27]

Metabolism of [^{18}F]8-Cyclopentyl-3-(3-fluoropropyl)-1-propylxanthine, 3*. To clearly identify the radioactive species in the brain and to get information about the metabolic stability of **3*** in vivo, homogenates of brains harvested 60 min after tracer administration were analyzed by radio-TLC. Intact **3*** accounted for >98% of the radioactivity. By contrast, over that interval mouse blood metabolized the radioligand at a significant rate, giving at least two polar metabolites (Figure 7). The chromatographic system separated **3*** from its metabolites cleanly. Typical R_f values for the

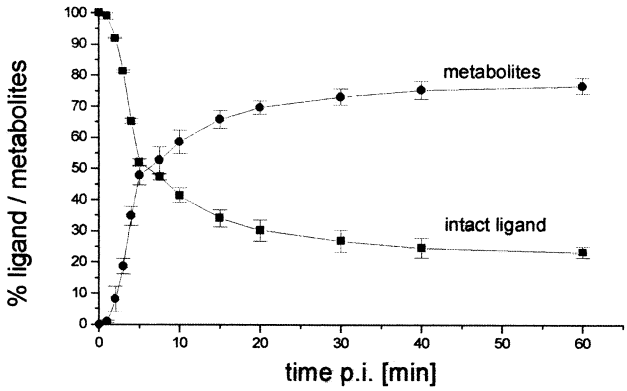


Figure 7. Kinetics of metabolite formation in mouse blood after the injection of **3*** (10−15 MBq or 0.15−0.2 nmol per mouse, $n = 4$ animals for each point).

metabolites were <0.3 and that of **3*** > 0.7. The biological half-life of **3*** was in the range of 5−7 min; thus, by 60 min pi about 75% of the radioligand in the periphery had undergone metabolism.

Isolation by TLC of the labeled metabolites from mouse blood obtained 30 min pi, reinjection into a second mouse and subsequent analysis of the recipient's brain tissue revealed no accumulation of radioactivity. Thus, the metabolites formed do not penetrate the blood−brain barrier, probably because of their polarity. Despite these encouraging results in mice, PET studies of the CNS in humans must still address the question of possible interference with the signal of radiotracer **3*** by radioactive metabolites formed in peripheral tissues.

Conclusion

In summary, we report a method for the high yield radiosynthesis of nca [^{18}F]8-cyclopentyl-3-(3-fluoropropyl)-1-propylxanthine, **3***, an A_1AR antagonist with affinity in the subnanomolar range that exhibits a >1200-fold selectivity for the rA_1AR over the rA_{2A}AR and a >700-fold selectivity for the hA_1AR over the hA_{2A}-AR. The radiochemical yield was 45 ± 7% and the radiochemical purity exceeded 98%. In the mouse, brain uptake was high; specific binding to the A_1AR accounted for 70−80% of the radioactivity. Radioligand in the brain was stable, but ligand in the blood underwent degradation to at least two polar metabolites, which, however, did not cross the blood−brain barrier.

Experimental Section

Chemistry. Materials and Methods. Melting points were measured on an Electrothermal apparatus and are uncor-

rected. Elemental analyses were performed by the Zentralabteilung für chemische Analysen at the Forschungszentrum Juelich, and are within ± 0.4% of the calculated composition. Mass spectra (MS), ESI, were obtained on a Finnigan Automass III mass spectrometer (Thermo Quest, Egelsbach). Thin-layer chromatography (TLC) employed precoated silica sheets (4 × 8 cm, Polygram, Macherey-Nagel, Düren) developed with ethyl acetate/hexane 50/50 (v/v). ^{1}H and ^{13}C NMR spectra were obtained at 200.13 and 50.32 MHz, respectively, by means of a Bruker DPX-200 spectrometer (Avance 200) in ≈5% solution at 25 °C. Chemical shifts are given in δ ppm using the residual proton signals of the appropriate deuterated solvents as a reference ($\delta_{H(CDCl3)} = 7.30$, $\delta_{C(CDCl3)} = 77.48$). The multiplicity symbols s, d, t, and m refer to singlet, dublet, triplet, and multiplet, respectively.

All solvents and reagents in the highest state of purity were obtained commercially from Sigma-Aldrich, Deisenhofen, Germany. *N*,*N*-Dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were distilled under argon and stored in a light-proof container over 4 Å molecular sieves. Dichloromethane (DCM) and 1,2-dichloroethane (DCE) were dried over 4 Å molecular sieves. Other solvents and reagents were used as supplied by the vendor. 8-Cyclopentyl-3-(3-hydroxypropyl)-7-pivaloyloxymethyl-1-propylxanthine, **1**, and CPFPX, **3**, were prepared as described.[16] Recrystallization from CH_3CN purified **3**. Sigma-Aldrich, supplied 2-chloroadenosine, DPCPX, N-0840, and DMPX. Fluka, Buchs, Switzerland, supplied 2-hydroxypropyl-β-cyclodextrin. Stock solutions of DPCPX, N-0840, and DMPX were prepared by dissolving these ligands in 45% (w/v) aqueous 2-hydroxypropyl-β-cyclodextrin. Solubilities in this medium were 0.7, 50, and 220 μM, respectively.

HPLC on a LiChrosphere RP C-18 column eluted with with CH_3CN/water, 70/30 (v/v) at a flow rate of 1 mL/min and monitoring UV absorbance of the efluent at 254 nm established the purity of precursor 2. In this system the capacity factor (k'-value)[28] of **2** was 9.93. Semipreparative radio-HPLC used a C-18 column (Nucleosil 100−10, 250 × 8 mm) eluted with acetonitrile/water, 65/35 (v/v) at a flow rate of 20 mL/min. In this system the k'-values of the precursor tosylate **2** and the ligand **3** were 1.65 and 4.50, respectively. For continuous measurement of radioactivity, the outlet of the UV detector was connected to a NaI(Tl) well-type scintillation detector, and the recorded data were processed by a software system (Nuclear Interface, Datentechnik für Strahlungsmessgeräte GmbH, Münster).

Quality control of the product employed a RP 18 Select B column (250 × 4 mm) eluted with acetonitrile/aqueous KH_2PO_4 (3.3 g/L), 70/30 (v/v) at a flow rate of 1 mL/min. Under these conditions the k'-value for **3*** was 2.03. A second system consisted of a Supersphere Si-60 column eluted with ethanol/hexane, 5/95 (v/v) at a flow rate of 1 mL/min. Under these conditions the k'-value for **3*** was 2.51. Calculations of the specific activity of **3*** used HPLC assays of the radioactivity and UV absorption under the product peak of aliquots of the labeled product referred to a standard curve.

Preconditioning of cartridges for solid-phase extraction (Sep-Pak Environmental C_{18}, 500 mg, Millipore Waters, Eschborn) with ethanol and water followed the manufacturer's directions.

Radio-TLC for metabolic studies used SIL G-25 UV_{254} glass-plates 20 × 10 cm and 10 × 10 cm (Macherey-Nagel, Düren) and ethyl acetate/hexane 50/50 (v/v) as an eluent. The plates were analyzed using an InstantImager (Canberra-Packard, Dreieich).

8-Cyclopentyl-3-(3-tosyloxypropyl)-7-pivaloyloxymethyl-1-propylxanthine, 2. Under argon a solution of *p*-toluenesulfonic acid chloride (1.14 g, 6 mmol) in dry 1,2-DCE (6 mL) was added dropwise over 5 min via a syringe to a stirred mixture of 8-cyclopentyl-3-(3-hydroxypropyl)-7-pivaloyloxymethyl-1-propylxanthine, **1** (2.31 g, 5 mmol), Et_3N (1.67 mL, 12 mmol), and Me_3N·HCl (71.7 mg, 0,75 mmol) in dry 1,2-DCE (6 mL) cooled to 0−5 °C. The mixture was stirred at that temperature for 2 h with monitoring by TLC (R_f^{educt} 0.56, $R_f^{product}$ 0.88, $R_f^{tosyl\ chloride}$ 0.92). Water (12 mL) was added, the organic phase separated and the aqueous phase extracted with DCM (2 × 20 mL). The combined organic extracts were washed with water (2 × 50 mL), 1 M citric acid (100 mL), and brine (100 mL), dried over Na_2SO_4, and evaporated to an oil (3.4 g) that was taken up in MeOH (10 mL). Cooling to 0 °C for 2 h precipitated the product as a colorless solid. Recrystallization from MeOH and air-drying gave colorless needles of tosylate **2** (2.68 g, 91%), mp 107−108 °C (MeOH), which HPLC showed was >99.5% pure.

^{1}H NMR ($CDCl_3$) δ: 0.96 (t, 3H, $CH_2CH_2CH_3$); 1.23 (s, 9H, $C(CH_3)_3$); 1.52−2.28 (m, 12H, $CH_2CH_2CH_3$, $CH_2CH_2CH_2OTos$ and cyclopentyl CH_2); 2.47 (s, 3H, CH_3Tos); 3.26 (m, 1H, cyclopentyl *H*-1); 3.94 (t, 2H, $CH_2CH_2CH_3$); 4.12 (m, 4H, $CH_2CH_2CH_2OTos$ and $CH_2CH_2CH_2OTos$); 6.26 (s, 2H, CH_2OPOM); 7.36 (d, 2H Tos, C-3); 7.74 (d, 2H Tos, C-2). ^{13}C NMR ($CDCl_3$) δ: 11.73 (N^1CH_3), 22.1 ($TosCH_3$), 26.19 (CH_3CH_2), 27.34 ($(CH_3)_3$), 33.06 (CH_2CH_2OTos), 36.72 (*C*H), 39.23 ($C(CH_3)_3$), 40.47 (N^1CH_2), 43.23 (N^3CH_2), 106.77 (*C*-5), 128.29, 130.25, 133.33, 145.21 (C_6H_4), 148.11 (*C*-8), 151.46 (*C*-4), 154.76 (*C*-2), 159.95 (*C*-6), 177.68 (*C*=O). MS (*m*/*e*): 589.2 ($M^+ + 1$, 100%). Anal. Calcd for $C_{29}H_{40}N_4O_7S$ (588.72): C, 59.16; H, 6.85; N, 9.52. Found: C, 59.15; H, 6.87; N, 9.50.

Radiosynthesis of nca [^{18}F]-8-Cyclopentyl-3-(3-fluoropropyl)-1-propylxanthine, 3*. Radiosyntheses were performed in a 5 cm lead shielded compartment using a computer-assisted, fully automated radiofluorination device.[21] Under argon the precursor tosylate **2** (4 mg, 6.8 μmol) was dissolved in dry DMSO (500 μL) and placed in the reactor of the automated device. The reactor contained dry kryptate consisting of Kryptofix 2.2.2 (9 mg, 24 μmol), K_2CO_3 (1.6 mg, 12 μmol), and dry [^{18}F]KF (typically 33 GBq, 900 mCi). The reaction mixture was heated to 85 °C for 10 min and cooled to room temperature. Aqueous 2 N NaOH (200 μL, 400 μmol) was added, and the hydrolysis of the POM group proceeded for 3 min at room temperature. The reaction mixture was diluted with sterile water (10 mL) containing glacial acetic acid (400 μL, 7 mmol), and the reaction mixture was passed over a Sep-Pak cartridge. The crude product was eluted with acetonitrile (3 mL), the acetonitrile solution injected onto a semipreparative HPLC column, and the fraction containing the product collected. Further purification and formulation of the radioligand proceeded by diluting the HPLC fraction (typically 20−25 mL) with sterile water (120 mL) and passing the solution over a second Sep-Pak cartridge. Washing the cartridge with water (10 mL) removed residual organic solvents**.** The product was eluted with ethanol (0.8 mL) followed by isotonic saline (7.5 mL) through a sterile filter into a sterile vial. Routinely **3*** was ready for injection in about 55 min in batches of 7.5 ± 0.5 GBq (200 ± 20 mCi, $n = 120$).

In Vitro Studies. Binding Assays Using Cloned Human A_1AR. Binding experiments used membranes from CHO-K1 cells stably expressing the human A_1 adenosine receptor (obtained from Euroscreen, Brussels, Belgium). Aliquots of thawed membranes were resuspended in 20 mM HEPES buffer, pH 7.4, to a final protein concentration of 461 μg/mL. Saturation experiments were carried out in triplicate in a final volume of 250 μL in 1.5 mL Eppendorf micro test tubes containing**,** in 10 μL, from 0.12 to 8 nM [^{3}H]CPFPX (produced in-house, specific activity 2.2 GBq/μmol, 59 mCi/μmol), 20 mM HEPES buffer, pH 7.4 (220 μL), containing 10 mM $MgCl_2$, 100 mM NaCl and diluted membranes (20 μL). Unspecific binding was determined in the presence of 10 μM DPCPX. After incubation for 90 min at 20 °C, centrifugation at 4 °C for 5 min and 48000*g* separated bound from free radioactivity. Saturation curves were analyzed by a computer-assisted curve-fitting program (Prism version 3.0, GraphPad Software, Inc.).

Binding Assays Using Cloned Human A_{2A}AR. Binding experiments used membranes from HEK-293 cells stably expressing the human A_{2A} adenosine receptor (Biotrend, Köln, Germany). Aliquots of thawed membranes were resuspended in 50 mM Tris buffer to a final protein concentration of 0.6 mg/mL. Saturation experiments were in triplicate in a final volume of 200 μL in 1.5 mL Eppendorf micro test tubes containing, in 10 μL, from 3.3 to 876 nM [^{3}H]CPFPX (produced in-house, specific activity 2.2 GBq/μmol, 59 mCi/μmol), 50 mM

Tris buffer pH 7.4 (170 μL), containing 10 mM $MgCl_2$, adenosine deaminase (2 U/mL), and diluted membranes (20 μL). Unspecific binding was measured in the presence of 50 μM chloroadenosine. After incubation for 90 min at 20 °C centrifugation at 8 °C for 5 min and 48000*g* separated bound from free radioactivity. Saturation curves were analyzed by a computer-assisted curve-fitting program (Prism version 3.0, GraphPad Software, Inc.).

Binding Studies Using Rat and Pig Brain Membranes. *Corpora striata* (for A_{2A}AR assays) and frontal *cortices* (for A_1-AR assays) were dissected from rat and pig brain and the tissue was homogenized for 1 min in 20 volumes of ice-cold 50 mM Tris−HCl buffer, pH 7.4 containing 10 mM $MgCl_2$, soybean trypsin inhibitor (20 μg/mL), bacitracin (200 μg/mL), and benzamidine HCl (160 μg/mL) by means of an Ultra Turrax at 20000 rpm. The homogenate was centrifuged at 48000*g* for 10 min at 4 °C (Beckmann Optima L, SW41Ti rotor). The pellet was suspended in 20 volumes of Tris·HCl, pH 7.4, containing adenosine deaminase (2 U/mL) and Trypsin Inhibitor (20 μg/mL), then was incubated for 30 min at 37 °C. After centrifugation at 48000*g* for 10 min at 4 °C, the resulting pellet was diluted in 20 volumes of 50 mM Tris-HCl, pH 7.4, containing 10 mM $MgCl_2$. Aliquots of the homogenate (1 mL) were stored at − 80 °C. The assays were performed in triplicate by incubating aliquots of the membrane fractions (65−120 μg protein/assay) in Tris·HCl, pH 7.4, containing adenosine deaminase (2 U/mL), [^{3}H]CPFPX, and cortical homogenates for the A_1AR or striatal homogenates for A_{2A}AR, in a total assay volume of 200 μL. Incubation was carried out at 20 °C for 90 min. In saturation experiments, the nonspecific binding was defined as the binding in the presence of either 10 μM CPDPX or 50 μM 2-chloroadenosine for studies of the A_1AR and A_{2A}AR, respectively. In A_{2A}AR competition experiments [^{3}H]CGS21680 was used in a concentration of 1.5 nM. Centrifugation at 48000*g* for 6 min at 8 °C separated bound from free ligand. Supernatants were discarded, the pellets washed with 1 mL ice cold buffer and dissolved by incubating in Solvable (500 μL, Canberra-Packard) for 120 min at 50 °C. Aliquots of 450 μL were placed in scintillation vials with scintillation cocktail (10 mL, Ultima Gold XR, Canberra-Packard). Radioactivity was measured in a liquid scintillation analyzer. Protein estimation was performed with a commercial assay (Bio-Rad DC Protein Assay) after solubilization in 15% NH_4OH containing 2% SDS (v,w); human serum albumin served as a standard. A computer-assisted curve-fitting program (GraphPad Prism, version 3.0) calculated IC_{50}, K_i, and K_D.

Lipophilicity. The octanol−water partition coefficient (log *P*) assessed the lipophilicity of **3*** as follows: to ca. 2−3 MBq of **3*** purified by HPLC was added 50 mM Tris·HCl, pH = 7.4 (1 mL), saturated with *n*-octanol. Buffer-saturated *n*-octanol (1 mL) was added, and the mixture was vortexed for 10 min at room temperature. The layers were separated by centrifugation (2 min at 6800*g*), and radioactivity in 500 μL aliquots of the aqueous and organic phases was measured in an automatic gamma counter. The procedure was repeated twice by the addition of fresh buffer (500 μL) to the octanol phases and fresh octanol (500 μL) to the aqueous phases until constant partition values were obtained. The partition coefficient was calculated as $\log P = \log(cpm_{octanol}/cpm_{buffer})$.

Protein Binding. The binding of **3*** to plasma proteins was determined by adding **3*** (2−3 MBq) to human plasma (5 mL). After incubation at 37 °C for intervals of 0, 5, 15, or 30 min, triplicate 300 μL aliquots were centrifuged at 14000*g* for 14 min at room temperature through Microcon YM 30 filters (MW cutoff 30 kDa, Millipore Corp., Bedford, MA). Radioactivity in 100 μL aliquots of the filtrates was measured in a gamma counter and compared to radioactivity in 100 μL aliquots of incubated plasma. The filter membranes retained <0.1% of the radiotracer.

In Vivo Studies. Animal Studies. General. All studies (permission No. 23.203.2 KFA 4/92) were performed on female NMRI (Naval Medical Research Institute) mice (~30 g), which had access to food and water ad libitum. Injections were through the tail vein in unanesthetized animals. The injected doses were 0.15−0.2 nmol **3*** having a specific radioactivity >270 GBq/μmol. For blocking and displacement experiments, unlabeled DPCPX, N-0840, or DMPX, 1 mg/kg body weight, were administered either 20 min before or 10 min after tracer injection. For biodistribution studies, the animals were sacrificed by cervical dislocation at 2, 5, 10, 20, or 60 min after injection ($n = 4$ per time). Organs of interest were excised and weighed, and radioactivity was counted for 1 min in a gamma counter.

For the measurements of the cerebral distribution of radiotracer **3*** at the above-mentioned times ($n = 6$ animals per time), the animals were killed, and the brains were rapidly removed and dissected over ice to obtain the regions of interest. These regions were weighed and counted for 1 min in the gamma counter. The tissue uptake of ^{18}F-activity was expressed as the percentage of injected dose per gram tissue (% ID/g).

Metabolite Isolation and Analysis. For the determination of in vivo metabolic stability of **3*** blood samples from mice (30 μL each, taken via the tail vein 60 min after tracer injection) were diluted with methanol/DCM (80/20 v/v, 30 μL), vortexed for 20 s at room temperature and centrifuged for 1 min at 14000*g*, and 10−15 μL samples of the clear supernatant were spotted onto TLC plates. Brains were processed at 0−5 °C by extracting the entire brain for 1 min in a blender with methanol/DCM (1 mL/g tissue). After centrifugation at 15000*g* for 5 min at 4 °C, aliquots of the supernatants (typically 15−20 μL each) were spotted onto TLC plates, which were developed and analyzed as described above. Measurements of radioactivity in both the pellets and supernatants showed that recovery of radioactivity was over 97%.

Penetration of the Blood−Brain Barrier by Metabolites of 3*. Isolation of the Metabolites of 3*. Radiotracer **3*** (60−75 MBq) was injected iv into three mice. After 40 min, blood samples (100 μL each) were drawn from the tail vain, pooled, and extracted with DCM/methanol (300 μL) as described above. After centrifugation, the clear supernatant was separated, concentrated to a volume of ≈50 μL at 40−45 °C under a stream of argon, and spotted onto a TLC plate. After development, the fractions having a R_f value <0.3 were cut out of the plate and extracted from silica gel by vortexing twice with methanol (150 μL). The combined methanolic extracts obtained by centrifugation were evaporated to dryness, reconstituted in physiological saline containing 5% ethanol (200 μL), and filtered through a sterile filter into a sterile vial. The solution containing the metabolites was injected iv into two mice, incubation was allowed to proceed for 30 min, and then the brains were removed and analyzed by radio-TLC as described above.

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