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Synthesis and radiopharmacological evaluation of 2′-(4-fluorophenyl)-21-[^{18}F]fluoro-20-oxo-11β,17α-dihydroxy-pregn-4-eno[3,2-*c*]pyrazole as potential glucocorticoid receptor ligand for positron emission tomography (PET)

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Abstract—The radiosynthesis and the radiopharmacological evaluation of pyrazolo steroid 2′-(4-fluorophenyl)-21-[^{18}F]fluoro-20-oxo-11β,17α-dihydroxy-pregn-4-eno[3,2-*c*]pyrazole **[^{18}F]-2** is described. The radiolabeling was accomplished in 3–4% decay-corrected radiochemical yield within 80 min at an specific radioactivity of 0.8–1.2 Ci/μmol. Biodistribution studies in male Wistar rats showed an initial brain uptake of 0.25 ± 0.03% ID/g after 5 min, which remained constant over 60 min. The radiopharmacological evaluation of compound **[^{18}F]-2** was completed with autoradiography using rat brain sections and micro-PET imaging.

1. Introduction

Endogenous corticosteroids are implicated in the regulation of a variety of essential physiological functions, such as energy metabolism, mineral balance and the defense reaction of the body against stressful stimuli.[1,2] There is accumulating evidence that corticosteroids, especially glucocorticoids, play an important role in several neuropsychiatric disorders, such as severe depression and anxiety.[3] Glucocorticoids are produced in the adrenal gland, and their production is controlled via the hypothalamo-pituitary-adrenocortical (HPA) axis, a classical closed-loop endocrine system. It has been suggested that a disturbed HPA axis regulation may be a primary causal factor in depression. Glucocorticoids mainly exert their effects by binding to the classical intracellular glucocorticoid receptor (GR). Due to the lipophilic nature of glucocorticoids they not only act on peripheral organs but also readily enter the brain where they bind to GRs. The presence of GRs in the human brain was demonstrated by autoradiographic studies, and highest concentrations of GRs are found in regions involved in the feedback regulation of the hormonal stress response, such as the paraventricular hypothalamus, hippocampus, and pituitary.[4,5]

The use of GR ligands, which are appropriately labeled with short-lived positron-emitting radioisotopes, such as ^{11}C ($t_{1/2}$ = 20.4 min) and ^{18}F ($t_{1/2}$ = 109.6 min), would allow the non-invasive in vivo imaging of brain GRs by means of positron emission tomography (PET). In this context, PET studies of brain GRs would provide important information on the neurobiological basis of GR-mediated abnormalities of HPA axis function.

To date several attempts have been made to synthesize glucocorticoids labeled with the short-lived positron emitter fluorine-18 ($t_{1/2}$ = 109.6 min) to image brain GRs.[6–12] Some of the compounds exhibited excellent in vitro GR binding. However, none of the investigated compounds were suitable for imaging brain GRs due to its rapid in vivo defluorination and their insufficient ability to cross the blood–brain barrier, which have prevented an accumulation of these compounds in the brain.

Recently we have synthesized a series of novel 4-fluorophenyl pyrazolo steroids showing different substitution

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patterns at the D-ring of the steroid skeleton.[12] The binding affinities of the compounds for the GR were determined in a competitive radiometric receptor-binding assay. Some compounds show good binding affinities of up to 56% in comparison to the high affinity GR ligand dexamethasone.

In this paper we report the radiosynthesis and radiopharmacological evaluation of 2′-(4-fluorophenyl)-21-[^{18}F]fluoro-20-oxo-11β,17α-di-hydroxy-pregn-4-eno-[3,2-*c*]pyrazole **[^{18}F]-2** as potential PET ligand for imaging brain glucocorticoid receptors.

2. Radiochemistry

The radiosynthesis of 2′-(4-fluorophenyl)-21-[^{18}F]fluoro-20-oxo-11β,17α-di-hydroxy-pregn-4-eno-[3,2-*c*]pyrazole **[^{18}F]-2** is depicted in Figure 1.

The radiofluorination was accomplished by [^{18}F]fluoride ion displacement of iodide labeling precursor **1**. The synthesis of iodide labeling precursor **1** was reported recently.[12] The radiosynthesis of ^{18}F-labeled GR ligand **[^{18}F]-2** was carried out in a nucleophilic fluorination module (Nuclear Interface, Münster). Starting activities of [^{18}F]fluoride in the range of 150–200 mCi were used. After drying the kryptofix®/K_2CO_3/[^{18}F]fluoride solution the iodide precursor **1** (4–5 mg in 1 mL of acetonitrile) was added. The use of iodide precursor **1** was shown to be superior to a corresponding mesylate labeling precursor with respect to higher radiochemical yields.[12] The reaction mixture was heated at 80 °C for 20 min. After cooling the reaction mixture was transferred from the reaction vessel onto a semi-preparative C-18 column (Axxiom, 9.4 mm × 250 mm, 60:40 acetonitrile/water, 5 mL/min). The fraction eluting at 7-8 min was collected, diluted with water (10 mL), and passed through a C-18 Sep-Pak Light cartridge. The cartridge was washed with water (3 mL) and the product was eluted with ethanol (0.5 mL). Addition of 0.9% saline (4.5 mL) gave 10% EtOH solution in saline suitable for the animal experiments.

Thus, in a typical experiment 200 mCi of [^{18}F]fluoride could be converted into 7 mCi of ^{18}F-labeled corticosteroid **[^{18}F]-2** (3–4% decay-corrected radiochemical yield) after HPLC purification at an specific radioactivity of 0.8–1.2 Ci/μmol at the end of synthesis. The complete radiosynthesis including HPLC separation was accomplished within 80 min after EOB, and the radiochemical purity exceeded 98%.

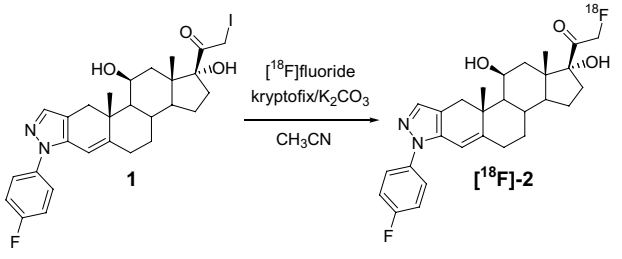

Figure 1. Radiosynthesis of compound **[^{18}F]-2**.

3. Animal experiments[13]

3.1. Biodistribution

The male Wistar rats were housed under standard conditions with free access to standard food and tap water. The animals (body weight 184 ± 17 g) were intravenously injected with 50 μCi **[^{18}F]-2** in 0.5 mL saline with 2% ethanol. Animals were sacrified at 5 min and 60 min post injection. Blood, pituitary, adrenals, and the major organs were collected, wet-weighed, and counted in a gamma counter (Packard). The percent injected dose per gram (%ID/g) was determined for each sample. For each animal, radioactivity of the tissue samples was calibrated against a known aliquot of injectate. Values are quoted as mean ± standard deviation (SD) for a group of four animals. The specific localization of the radiotracer on glucocorticoid binding sites was investigated by injecting 10 mg/kg of hydrocortisone dissolved in 0.5 mL 10% ethanol/saline (blocked) or 0.5 mL 10% of ethanol/saline (control) intraperitoneal (ip) 1 h before the application of the radiotracer. Biodistribution data of compound **[^{18}F]-2** are shown in Table 1.

Blood clearance was very fast and the radioactivity concentration in the blood reached its final level (0.07 ± 0.01% ID/g) after 5 min. There was rapid adrenal uptake, which remained constant over 1 h. The systemic clearance was predominantly governed by hepatobiliary elimination. Compared to other large organs relatively high uptake was found in the pancreas, brown fat, and harderian glands. The radioactivity concentration in the pituitary (0.61 ± 0.23% ID/g at 5 min; 0.52 ± 0.04% ID/g at 60 min) and thymus (0.31 ± 0.04% ID/g at 5 min; 0.37 ± 0.03% ID/g at

Table 1. Radioactivity expressed as percent injected dose per gram tissue in different organs after single intravenous injection of 50 μCi **[^{18}F]-2** in 0.5 mL saline with 2% ethanol (n = 4 for each time point)

%ID/g tissue time p.i.	Control		Blocked	
	5 min	60 min	5 min	60 min
Blood	0.07 ± 0.01	0.06 ± 0.00	0.06 ± 0.01	0.08 ± 0.01
Pituitary	0.61 ± 0.23	0.61 ± 0.36	0.52 ± 0.04	0.82 ± 0.14
Brown fat	0.81 ± 0.10	0.91 ± 0.21	0.80 ± 0.10	0.91 ± 0.15
Brain	0.24 ± 0.02	0.24 ± 0.04	0.26 ± 0.04	0.25 ± 0.04
Pancreas	0.94 ± 0.09	0.85 ± 0.10	0.85 ± 0.09	0.85 ± 0.12
Spleen	0.43 ± 0.05	0.41 ± 0.06	0.40 ± 0.04	0.44 ± 0.06
Adrenals	1.73 ± 0.37	1.36 ± 0.34	1.23 ± 0.25	1.96 ± 0.10
Kidney	0.77 ± 0.11	0.66 ± 0.10	0.66 ± 0.12	0.87 ± 0.13
Fat	0.45 ± 0.12	0.63 ± 0.12	0.82 ± 0.10	0.31 ± 0.07
Muscle	0.29 ± 0.06	0.32 ± 0.05	0.31 ± 0.04	0.32 ± 0.10
Heart	0.62 ± 0.06	0.61 ± 0.09	0.63 ± 0.10	0.66 ± 0.09
Lung	0.64 ± 0.08	0.60 ± 0.11	0.60 ± 0.08	0.66 ± 0.04
Thymus	0.31 ± 0.04	0.38 ± 0.07	0.37 ± 0.03	0.40 ± 0.12
Hard. glands	0.67 ± 0.22	0.92 ± 0.29	1.09 ± 0.17	0.55 ± 0.05
Liver	2.02 ± 0.48	1.44 ± 0.38	1.30 ± 0.35	2.15 ± 0.31
Femur	0.26 ± 0.04	0.33 ± 0.07	0.34 ± 0.05	0.22 ± 0.02
Testis	0.09 ± 0.03	0.18 ± 0.07	0.18 ± 0.03	0.07 ± 0.01

60 min) as the potential target organs was not increased in comparison to the other main tissues. In all organs, excluding the liver, changes of the activity concentration within the first hour after injection were relatively low. The compound was also accumulated in the brain (0.25 ± 0.1% ID/g) at all time points.

The described biodistribution reflects predominantly non-specific distribution of compound **[^{18}F]-2** according to its high lipophilicity (log $P_{o/w}$ 3.9).[12] The low accumulation of radioactivity in the bone 0.26 ± 0.04% ID/g after 5 min and 0.33 ± 0.07% ID/g after 60 min, respectively, is indicative of a low in vivo defluorination of **[^{18}F]-2** in comparison to similar compounds reported in the literature.[6,9,10]

The blocking experiments were performed to establish glucocorticoid binding site mediated uptake. However, the administration of hydrocortisone as blocking agent revealed no specific receptor-mediated uptake in the target tissues. Moreover, hydrocortisone administration seems to cause a displacement of the radiotracer **[^{18}F]-2** from low affinity binding sites in the fat and bone marrow resulting in an increase of radioactivity found in the elimination organs such as liver and kidneys.

3.2. Ex vivo autoradiography[14]

Figure 2 shows an autoradiogram and the corresponding histological section of the in vivo distribution of **[^{18}F]-2** in the median horizontal planes of the rat brain at 60 min after injection.

The highest concentration was found in the pituitary gland, cerebellar nuclei, plexus choroideus, and inferior colliculi. This is in agreement with studied performed by Ahima and Harlan using ^{3}H-labeled corticosteroids when they found high densities of GRs in all regions of the cerebellar cortex.[15] However, high levels of GRs expressed in the hippocampus as also reported by Ahima and Harlan and other groups could not be found in our experiments. This finding is unexpected, since

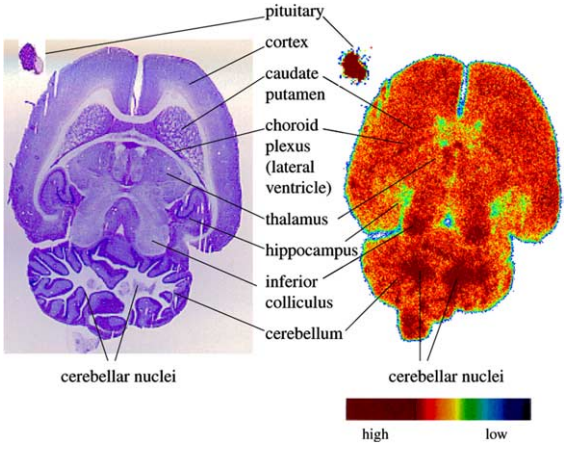

Figure 2. Digital autoradiograph (right) of a rat brain section at 60 min after single iv application of the **[^{18}F]-2** and the corresponding histological section (left).

Table 2. Activity distribution of **[^{18}F]-2** in one control and one blocked (hydrocortisone) rat brain after single iv injection of 0.9 mCi **[^{18}F]-2** at 60 min after injection determined by ex vivo autoradiography

Region	Control	Blocked
Pituitary	1.34 ± 0.12	1.36 ± 0.13
Cerebellar nuclei	0.96 ± 0.06	0.94 ± 0.05
Inferior colliculi	0.92 ± 0.04	0.89 ± 0.01
Plexus choroideus	0.90 ± 0.05	0.83 ± 0.04
Cortex	0.69 ± 0.04	0.70 ± 0.08
Hippocampus	0.59 ± 0.02	0.59 ± 0.06

Data are given as mean of regions of interest in four levels with 10 sections each in %ID/g tissue ± SD.

the hippocampus is known as region of high GRs expression.[4,5] In this context, McEwen et al.[16] have pointed out that the uptake and the retention of several ^{3}H-labeled corticosteroids in tracer doses, which were used in their study, also differed to that of ^{3}H-labeled corticosterone, which showed high accumulation in the hippocampus.

As for the non-specific receptor-mediated uptake in peripheral target tissues, pre-injection of hydrocortisone nor dexamethasone (data not shown) resulted in no blocking of the **[^{18}F]-2** uptake in brain regions studied (Table 2). This result can be explained by the fact that we have used non-adrenalectomized rats in our experiments. Thus, the endogeneous adrenal steroid capacity of the animals was high during the experiments.

3.3. MicroPET imaging studies

PET imaging was performed using a micro-PET® P4 primate model scanner (CTI Concorde Microsystems Inc. Knoxville, TN). The raw data were sorted into 3D sinograms followed by Fourier re-binning and 2D iterative image reconstruction (OSEM2D). No correction for recovery and partial volume effects was applied. General anesthesia of the animal was induced and maintained by inhalation of halothane (3%) and N_2O (65%) in O_2. The animal were positioned with the brain in the center of the 22 cm transaxial and 8 cm axial field of view (FOV) of the scanner. The rats were injected with 300 μCi of **[^{18}F]-2** via the tail vein and scarified at 2 h p.i. by intravenous application of KCl. The rat was scanned for 15 min, and the raw data were framed into 30 frames with attenuation correction. 2-D projection of saggital, transversal and coronal micro-PET images are displayed in Figure 3.

The brain is clearly visible in the micro-PET images, which is consistent with the brain biodistribution data and ex vivo autoradiography data of the brain. Prominent uptake was also observed in the harderian glands.

In summary, ^{18}F-labeled corticosteroid **[^{18}F]-2** as novel ligand for studying brain GRs could be synthesized in sufficient radiochemical yield. The radiopharmacological investigation of **[^{18}F]-2** revealed promising brain uptake and low in vivo radiodefluorination compared to other PET radioligands for brain GRs known in the literature. However, the uptake of the compound into

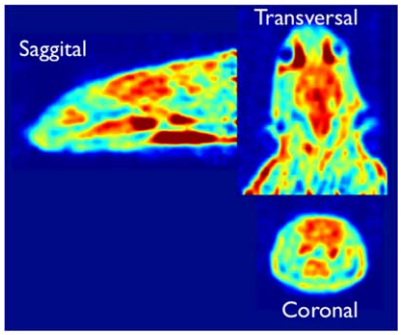

Figure 3. Coronal, saggital, and transversal rat mircoPET images 30 min after injection of 300 μCi of **[^{18}F]-2** (15 min acquisition).

target tissues seems to be not receptor mediated. For further experiments, adrenalectomized animals will be used to reduce endogeneous steroid capacity during the study.

Acknowledgements

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13. All animal experiments were carried out in compliance with the German law relating to conduct of animal experimentation.
14. Male Wistar rats (body weight 100 ± 6 g) were intravenously injected with approximately 1 mCi **[^{18}F]-2** in a volume of 0.5 mL saline with 10% ethanol. To investigate the effect of hydrocortisone on the radioactivity localization in the rat brain further animals received an injection of 10 mg/kg of hydrocortisone ip 1 h prior to the radiotracer injection. The animals were sacrificed by CO_2 inhalation at 60 min after the radiotracer injection. The brains were removed and quickly frozen by immersion in isopentane/dry ice solution at −50 °C. The frozen brains were weighed and the activity concentration was determined, using a γ-well counter (Isomed 2000, Germany). Then the stage-mounted brains were cut on a cryostat microtome (CM 1850, Leica Instruments, Germany) into 40 μm sagittal sections, which were thaw-mounted onto microscope slides (Super Frost, Menzel, Germany), dried under a continuous cold air stream and exposed to imaging plates (BAS-SR Imaging Plate, FUJI, Japan) together with a tissue activity standard overnight. The imaging plates were scanned in the bio-imaging analyzer BAS 5000 (FUJI, Japan). The data were recorded as photostimulated luminescence values (PSL), which are proportional to the radioactivity of the measured sample. The data analysis was carried out with the software program AIDA (Raytest, Germany). After exposure, the brain sections were stained with cresyl violet to match anatomical with functional information. Regions of interest (ROI) were drawn over pituitary, choroid plexus, hypothalamus, and several nuclei of the thalamus, colliculi, caudate putamen, hippocampus, frontal cortex, and cerebellum.
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