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Evaluation of the binding characteristics of [^{18}F]fluoroproxyfan in the rat brain for in vivo visualization of histamine H_3 receptor

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Abstract

Histamine H_3 receptors play an important role in biological functions. The aim of this research was to examine whether histamine H_3 receptors can be visualized in vivo and in vitro with [^{18}F]3-(1*H*-imidazol-4-yl)propyl 4-fluorobenzyl ether (fluoroproxyfan). [^{18}F]Fluoroproxyfan was synthesized with high specific activity using [^{18}F]benzyl bromide. The binding of [^{18}F]fluoroproxyfan to rat brain homogenates was higher in the striatum and thalamus and was lowest in the cerebellum. The in vitro autoradiographic study successfully demonstrated the specific binding of [^{18}F]fluoroproxyfan to the H_3 receptor in the rat brain. In accordance with the in vitro bindings, the in vivo distribution of [^{18}F]fluoroproxyfan was heterogeneous in the rat brain. In the blocking experiments, the heterogeneous distribution disappeared in the presence of large amounts of fluoroproxyfan. These data suggest that [^{18}F]fluoroproxyfan can be potentially useful to image histamine H_3 receptor noninvasively in the human brain by positron emission tomography.

Keywords: Histamine H_3 receptor; Binding; Autoradiography; Positron emission tomography (PET); Fluoroproxyfan; Biodistribution

1. Introduction

Histamine is a well-known neurotransmitter that plays various important roles through four distinct G-protein-coupled receptors named simply as H_1, H_2, H_3 and H_4 [1]. The histamine H_3 receptor has been firstly determined to be a presynaptic autoreceptor regulating the synthesis and release of histamine in the central nervous system (CNS) through negative feedback [2–6]. Moreover, H_3 receptors were found to act as a heteroreceptor regulating a modulatory effect on the release of other neurotransmitters in the CNS including dopamine, GABA, serotonin and acetylcholine [7,8]. Consequently, the H_3 receptor might be a therapeutic target in CNS disorders such as Parkinson's and Alzheimer's diseases, as well as memory, learning and sleep disorders [9–12]. From this point of view, many different leads in the development of H_3 receptor agonists or antagonists are reported to regulate synthesis and release of histamine and to inhibit the release of various neurotransmitters [13,14].

To measure the distribution and density of histamine receptors, various imaging probes have been used to visualize histamine receptors in the human brain by positron emission tomography (PET) [15–19]. We previously reported histamine H_1 receptors in humans visualized by PET and [^{11}C]doxepin, a radioligand for H_1 receptors [20]. Using this technique, we revealed the age-related decline of histamine receptor binding [21], its correlation to the cognitive deficits of Alzheimer's patients [22], the decrease of H_1 receptor binding in the frontal and anterior cingulate cortex areas in patients with depression [23] and the interaction between histamine receptor occupancy and cognitive impairment induced by sedative antihistamines [24,25]. Specifically, PET is a powerful tool to measure the drug receptor occupancy in the human brain since such occupancy is closely related to the efficacy of therapy [26,27]. In this way, histamine H_1 receptors have been examined for biological functions using PET [28]. Equally, it

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has been attempted to obtain details about H_3 receptors using PET. Several radiolabeled ligands toward H_3 receptors have already been synthesized and evaluated as imaging probes for PET, but unfortunately, these probes were not suitable as PET ligands for H_3 receptors because of a low uptake or high nonspecific binding in the rodent brain [29–31].

3-(1*H*-Imidazol-4-yl)propyl 4-iodobenzyl ether (iodoproxyfan) is a promising antagonist for H_3 receptors because of its high selectivity and high affinity for these receptors [32]. 3-(1*H*-Imidazol-4-yl)propyl 4-fluorobenzyl ether (fluoroproxyfan) is an analogue of iodoproxyfan, and it has been reported that fluoroproxyfan has comparable selectivity and affinity toward H_3 receptors as well as iodoproxyfan [33]. Although we already demonstrated that [^{18}F]fluoroproxyfan can be successfully synthesized using [^{18}F]benzyl bromide [34], its binding characteristics and use as an imaging probe have not been evaluated yet.

In the present study, we describe the biological evaluation, in vitro and in vivo, of [^{18}F]fluoroproxyfan.

2. Materials and methods

2.1. General methods

Fluoroproxyfan and desbenzyl precursor were synthesized according to our previous article [34]. Chemicals and solvents were obtained commercially and used without further purification.

Preparative HPLC purification was performed on a YMC ODS column (YMC ODS A-324, YMC Co., Ltd., Kyoto, Japan; 10 mm i.d.×30 cm long) with a solvent system of 0.1 M ammonium formate–acetonitrile (65/35) at a flow rate of 5.0 ml/min. QC HPLC analysis was performed on a LiChrosorb column (LiChrosorb RP-18, Merck; 4 mm i.d.×25 cm long) with a solvent system of 0.05 M ammonium acetate–acetonitrile (60/40) at a flow rate of 2.0 ml/min.

Male Wistar rats (Japan SLC, Shizuoka, Japan; 5 weeks) were used in these studies. They were fed food and water ad libitum. The animal study was carried out according to the protocol approved by the Animal Care Committees of Cyclotron and Radioisotope Center, Tohoku University.

2.2. Synthesis of [^{18}F]fluoroproxyfan

Radiosynthesis was carried out as described in our previous article [34]. [^{18}F]Fluoroproxyfan was produced from 4-[^{18}F]fluorobenzyl bromide and purified by preparative HPLC (Fig. 1). Usually, the desired product was eluted at 6–8 min. Synthesized cold amounts of fluoroproxyfan were calculated by measuring peak areas from a standard curve using an analytical HPLC.

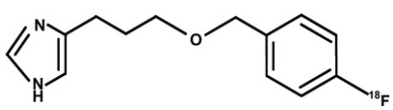

Fig. 1. Chemical structure of [^{18}F]fluoroproxyfan.

The radioactivity of [^{18}F]fluoroproxyfan was approximately 37–259 MBq (1–7 mCi) after 60 min irradiation, and radiochemical yield was calculated to be 8–11% based on 4-[^{18}F]fluorobenzyl bromide after decay correction. The specific activity of [^{18}F]fluoroproxyfan was 412 GBq/μmol (11.1 Ci/μmol) at the end of synthesis (90 min after the bombardment). The radiochemical purity was more than 99%.

2.3. In vitro binding study

The rats were anesthetized by diethyl ether and sacrificed. Whole brains were removed and immediately dissected into six brain tissues (cerebral cortex, striatum, hippocampus, cerebellum, midbrain and brain stem). Each tissue was weighed and homogenized in 10 volumes with 50 mM Na/K phosphate buffer (pH 6.8). The suspensions were centrifuged at 50,000×*g* for 20 min, and then, the supernatant was decanted. This was repeated twice and the pellets were stored at −80°C until use.

At the time of the experiments, the pellets were washed again with the phosphate buffer and resuspended in a final concentration of approximately 10 mg of original tissue in 400 μl buffer. These suspensions were incubated with 50 μl of [^{18}F]fluoroproxyfan (74 kBq; 2 μCi) and 50 μl buffer (for total binding) or 100 μM fluoroproxyfan (for nonspecific binding). The incubation was performed for 20 min at 37°C, and the incubation mixtures were terminated by rapid filtration on Whatman GF/B glass filters, which were washed twice with the ice-cold buffer. The radioactivity was counted with a gamma counter in the range of 400–600 keV.

2.4. In vitro autoradiography

Wistar rats were sacrificed, and the brains were quickly removed and frozen in powdered dry ice. Serial coronal and sagittal sections, 20 μm in thickness, were cut from the frozen brains in a −20°C cryostat and dried by cold air on glass coverslips for 2–3 min and then stored at −80°C until use. Brain sections were incubated for 30 min at room temperature in the 50 mM Na/K phosphate buffer (pH 6.8) containing [^{18}F]fluoroproxyfan (1.2 MBq; ca. 0.5 pM). Following incubation, sections were washed twice in the same buffer and then dipped into water for 2–3 s. Nonspecific binding was calculated in the presence of fluoroproxyfan (50 μM). Brain sections obtained were exposed to universal-type imaging plates for 10 min (Fuji Photo Film Co., Ltd., Tokyo). Autoradiographic images were obtained using an imaging plate system (Fujix Bio-Imaging Analyzer BAS5000, Fuji Photo Film Co., Ltd.).

2.5. In vivo distribution study

The rats were injected intravenously with 1.30–1.85 MBq (35–50 μCi; ca. 3.5–5 pmol) of [^{18}F]fluoroproxyfan in 0.2 ml of physiological saline via tail vein. The rats were killed at the five time points (5, 15, 30, 60 and 120 min). Samples of blood, organs of interest and eight brain tissues (cerebral cortex, striatum, hippocampus, thalamus, hypothalamus,