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In vivo evaluation of [^{123}I]-4-iodo-*N*-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)butyl)-benzamide: a potential sigma receptor ligand for SPECT studies

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Abstract

In this study, in vivo evaluation in mice and rabbits of [^{123}I]-4-iodo-*N*-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)butyl)-benzamide ([^{123}I]-BPB), a potential radioligand for visualisation of the sigma receptor by single photon emission computed tomography (SPECT), is reported. The compound possesses appropriate lipophilicity (log P=2.2) and binds sigma-1 and sigma-2 receptors (pKi=6.51 and 6.79, respectively). In mice, this new radioiodinated tracer exhibited high brain uptake (4.99% ID/g tissue at 10 min postinjection) and saturable binding (3.06% ID/g tissue at 10 min postinjection) as determined by pretreatment with unlabeled [^{123}I]-BPB. A metabolite study demonstrated no (less than 5%) labeled metabolites in the brain. In rabbits, regional brain distribution was investigated and the tracer displayed high, homogeneous central nervous system uptake. Selectivity was assessed by competition experiments with known sigma ligands. Metabolite analysis showed no (less than 8%) labeled metabolites in the rabbit brain. In conclusion, our findings indicate that [^{123}I]-BPB is not a suitable tracer for visualisation of D_3 receptors while its potential for sigma receptor imaging is severely hampered by its affinity for dopamine receptors.

Keywords: Sigma receptors; Dopamine D_3 receptors SPECT; ^{123}I

1. Introduction

Sigma receptors were first postulated by Martin et al. [1] in 1976 based on the actions of SKF 10,047 (*N*-allylnormetazocine) and related benzomorphans. The name "σ" originated from the first letter "S" in SKF 10,047, which was thought to be the prototypic ligand for this protein. Later experiments however proved SKF 10,047 to be a nonselective compound, contributing to the confusion surrounding the early understanding of the pharmacology of sigma receptors. After initially being classed as opioid receptors, sigma sites were eventually understood to be unique entities unlike any other known mammalian protein [2]. Much of the initial interest in sigma receptors was driven by their potential role in the actions of antipsychotic drugs. This was due mainly to the high affinity of many typical neuroleptics for these sites [3]. In 1995, it was also discovered that sigma receptors are expressed in a wide variety of tumor cell lines, leading to the hypothesis that labeled sigma receptor ligands could serve as possible diagnostic agents for certain types of cancer [4]. Biochemical and pharmacological studies indicate the existence of multiple sigma receptor subtypes. Currently, two subtypes of sigma receptors are known, namely, sigma-1 (σ_1) and sigma-2 (σ_2). Drug selectivity patterns and pharmacological responses distinguish the subtypes. The (+)-isomer of benzomorphans and morphinans interacts with both subtypes of receptors with similar affinities. However, the relative interactions of the (−)-isomers of these compounds can be discriminating. At the σ_1 subtype, the (+)-isomer has a better affinity compared with the (−)-isomer, whereas at

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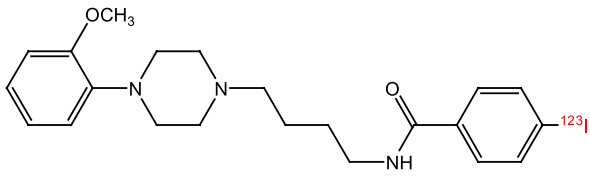

Fig. 1. [^{123}I]-BPB.

the σ_2 subtype, the (−)-isomer has a better affinity compared with the (+)-isomer [5]. Our initial goal was to synthesize an iodine 123-labeled SPECT tracer for in vivo visualisation of the dopamine D_3 receptor. The selection of [^{123}I]-4-iodo-*N*-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)butyl)-benzamide ([^{123}I]-BPB) (Fig. 1) was based on a paper published by Murray et al. [6] in 1995. In their article, it was noted that 4-bromo-*N*-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)butyl)-benzamide has a high affinity for the dopamine D_3 receptor (pKi=9.3) and demonstrated a 100-fold selectivity for D_3 over D_2 (pKi=7.4) and D_4 (pKi=7.3) receptors and that, in addition, this ligand was 1000-fold selective for D_3 versus D_1 receptors. The brominated derivative also showed some affinity for $5HT_{1A}$ (pKi=7.9) and α_1 (pKi=7.9) receptors. From these data, it was assumed that the iodinated derivative would be selective for the dopamine D_3 receptor, especially since D_3 ligands with larger groups in the 4 position of the carboxybenzyl also showed D_3 selectivity (e.g., aminobenzyl: pKi D_3=9.7; pKi D_2=7.7) [6].

We report here the in vivo evaluation of [^{123}I]-BPB in mice and rabbits. In mice, a biodistribution study was carried out to assess passage across the blood–brain barrier, saturable binding in the brain and potential interference from metabolites. In rabbits, regional brain distribution and competition studies with different sigma ligands, as well as a metabolite analysis, were performed.

2. Materials and methods

2.1. Chemicals and radiochemicals

All chemical reagents were purchased from Sigma-Aldrich (St. Louis, Mo, USA) and used without further purification. Solvents were purchased from Lab-Scan Analytical Sciences (Dublin, Ireland). [^{123}I] Sodium iodide (in 0.05 M NaOH) was purchased from Bristol-Myers Squibb Pharma (Brussels, Belgium). S-(−)-sulpiride, BD-1008 and ifenprodil were purchased from Tocris Cookson Ltd. (Bristol, UK). 1-(3-fluoropropyl)-4-((4-cyanophenoxy)-methyl)piperidine [7] (FPS) was kindly provided by Dr. R. N. Waterhouse (Columbia University).

2.2. Chemistry

Synthesis and radiosynthesis of [^{123}I]-BPB were performed as previously described [8]. Radiochemical yield was 83±3% (n=10), radiochemical purity was >95% while specific activity was >2.96 Ci/μmol.

2.3. Animal studies

All animal experiments were conducted according to the regulations of Belgian laws and the local ethical committee.

2.4. Biodistribution and blocking studies in mice

Approximately 37 kBq (1 μCi) of [^{123}I]-BPB, dissolved in 200 μL of ethanol/water (5:95), was injected in the tail vein of male white mice [20–25 g, 4–5 weeks old, Naval Medical Research Institute (NMRI)]. At 20 and 40 s, 1, 1.5, 2, 3, 5, 10, 20 and 40 min and 1, 2, 3, 6, 9, 15, 24 and 48 h postinjection, animals (n=3) were sacrificed by decapitation after halothane anesthesia administration. Blood and fatty tissue were taken, organs were excised and excretion was collected. All tissues were weighed and counted for radioactivity with a Packard Cobra gamma-ray spectrometer equipped with five 1×1-in. NaI(Tl) crystals. The concentration of radioactivity was expressed as a percentage of the injected dose per gram of tissue plus or minus the S.D. (% ID/g tissue±S.D.) and decay corrected. The excretion results (urine plus feces) were expressed as a percentage of the injected dose plus or minus the S.D. and decay corrected. To determine the specific tissue uptake of the labeled compound, displacement studies were performed. Thirty-nine animals (13 time points, 3 mice/time point) received a tail vein injection of 1 mg of cold product per kilogram of body weight, dissolved in 150 μL ethanol/water (5:95); 10 min thereafter, 1 μCi of the labeled compound dissolved in 50 μL of ethanol/water (5:95) was injected. The animals were sacrificed at the same time points as for the biodistribution up to 3 h postinjection. All tissues were treated as previously described. The concentration of radioactivity in tissues and excretion were expressed as percentage of the injected dose per gram of tissue and percentage of the injected dose per gram, respectively.

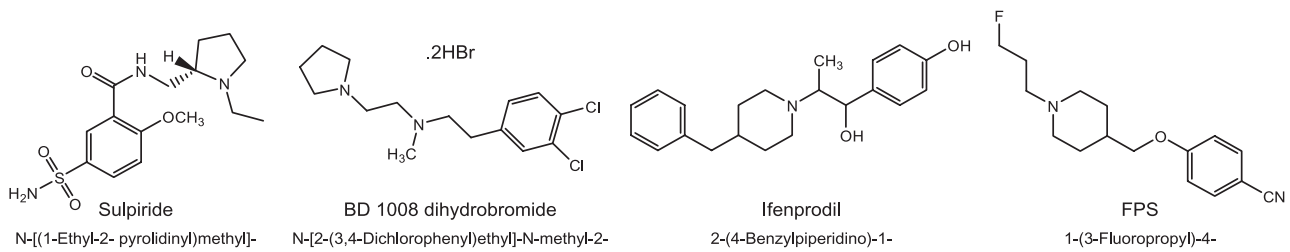

Fig. 2. Structures of S-(−)-sulpiride, BD-1008, ifenprodil and FPS.