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Evaluation of radioiodinated (2*S*,α*S*)-2-(α-(2-iodophenoxy)benzyl) morpholine as a radioligand for imaging of norepinephrine transporter in the heart

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Abstract

Introduction: The norepinephrine transporter (NET) is located presynaptically on noradrenergic nerve terminals and plays a critical role in the regulation of the synaptic norepinephrine (NE) concentration via the reuptake of NE. Changes in NET have been recently reported in several cardiac failures. Therefore, a NET-specific radioligand is useful for in vivo assessment of changes in NET density in various cardiac disorders. Recently, we developed a radioiodinated reboxetine analogue, (2*S*,α*S*)-2-(α-(2-iodophenoxy)benzyl)morpholine ((*S*,*S*)-IPBM), for NET imaging. In the current study, we assessed the applicability of radioiodinated (*S*,*S*)-IPBM to NET imaging in the heart.
Methods: The NET affinity and selectivity were measured from the ability to displace specific [^{3}H]nisoxetine and (*S*,*S*)-[^{125}I]IPBM binding to rat heart membrane, respectively. To evaluate the distribution of (*S*,*S*)-[^{125}I]IPBM in vivo, biodistribution experiment was performed in rats. With the use of several monoamine transporter binding agents, pharmacological blocking experiments were performed in rats.
Results: In vitro binding assays showed that the affinity of (*S*,*S*)-IPBM to NET was similar to those of the well-known NET-specific binding agents, nisoxetine and desipramine. Furthermore, (*S*,*S*)-[^{125}I]IPBM binding was inhibited by nisoxetine and desipramine, but not by dopamine or serotonin transporter binding agents. These data indicated that (*S*,*S*)-IPBM had high affinity and selectivity for NET in vitro. Biodistribution studies in rats showed rapid and high uptake of (*S*,*S*)-[^{125}I]IPBM by the heart and rapid clearance from the blood. The heart-to-blood ratio was 31.9 at 180 min after the injection. The administration of nisoxetine and desipramine decreased (*S*,*S*)-[^{125}I]IPBM accumulation in the heart, but injection of fluoxetine and GBR12909 had little influence.
Conclusions: Radioiodinated (*S*,*S*)-IPBM is a potential radioligand for NET imaging in the heart.

Keywords: Norepinephrine transporter; Sympathetic nervous function; SPECT; Radioiodination; (*S*,*S*)-IPBM; Heart

1. Introduction

The norepinephrine transporter (NET) is located presynaptically on noradrenergic nerve terminals and plays a critical role in the regulation of synaptic concentrations of norepinephrine (NE) in the noradrenergic nervous system by the reuptake of NE [1]. In the heart, NET exists in sympathetic nerve terminals. Changes in NET have been reported in several cardiac failures [2–7]. Furthermore, this NET has been recently noted as a therapeutic target [8–10]. Two pieces of information are necessary for estimating this NET function precisely. One is the transport activity of NET, and the other is the density of NET in sympathetic nerve terminals. To evaluate the first information, [^{123}I]meta-iodobenzylguanidine (MIBG) and [^{11}C]hydroxyephedrine, NET substrates, have been widely used for the imaging of cardiac

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sympathetic nervous function in various cardiovascular diseases [11–13]. These radiolabeled NET substrates are taken up by sympathetic nerve terminals, stored into vesicles and secreted to sympathetic clefts, and therefore images of these NET substrates reflect the overall function of nervous terminals. The information on NET density could be also important for predicting the effect of NET targeting therapy and/or understanding the condition of cardiac diseases.

A radioligand bound to NET could be potentially useful for studying the role of the NE reuptake system in these diseased states and for predicting the therapeutic effect of NET targeting drugs. Therefore, the development of a radioligand to bind NET has been of great interest.

We recently synthesized radioiodinated (*R*)-*N*-methyl-3-(2-iodophenoxy)-3-phenylpropamine ((*R*)-MIPP) as a NET binding agent. However, this radioligand had a slight affinity to serotonin transporter (SERT) in vivo in the brain and heart [14,15]. More recently, we developed radioiodinated (2*S*,α*S*)-2-(α-(2-iodophenoxy)benzyl)morpholine ((*S*,*S*)-IPBM), which was more selective for NET compared with (*R*)-MIPP in the brain [15,16].

In this study, the applicability of radioiodinated (*S*,*S*)-IPBM to NET imaging in the heart was evaluated by investigating the affinity and selectivity for NET in the heart and biodistribution including pharmacological blocking experiments and comparison with MIBG.

2. Materials and methods

2.1. Materials

[^{125}I]MIBG was supplied by Daiichi Radioisotope Laboratories (Tokyo, Japan). Sodium [^{125}I]iodide (643.8 GBq/mg) and [^{3}H]nisoxetine (2.96 TBq/mmol) were purchased from New England Nuclear (Boston, MA, USA). All chemicals used in this study were of reagent grade.

2.2. Radiosynthesis

(*S*,*S*)-[^{125}I]IPBM was obtained by a halogen exchange reaction with sodium [^{125}I]iodine according to the methods of Kanegawa et al. [15]. Briefly, (*S*,*S*)-BPBM was added to a mixture of sodium [^{125}I]-iodine, ammonium sulfate and copper (II) sulfate pentahydrate in a vial. The reaction mixture was heated for 45 min at 130°C. After cooling, the reaction mixture was extracted with methanol and filtered with a 0.22-μm filter. The filtered extract was applied to a reverse-phase high-performance liquid chromatography (HPLC) column (Cosmosil 5C$_{18}$-AR-300 Packed Column, 250×10 mm id, Nacalai Tesque, Kyoto, Japan) and eluted with 20 mM phosphate buffer (pH 2.5)/acetonitrile=72:28 at a flow rate of 2.0 ml/min [R_t=42 min for (*S*,*S*)-BPBM, 58 min for (*S*,*S*)-IPBM]. An adequate amount of ethanol was added to the separated (*S*,*S*)-[^{125}I]-IPBM fraction.

The radiochemical purity of the labeled compound was determined by analytical HPLC. Analytical HPLC was performed on a 150×4.6 mm id, Cosmosil AR-300 column (Nacalai Tesque, Kyoto, Japan) eluted with 20 mM phosphate buffer (pH 2.5)/acetonitrile=72:28 at a flow rate of 1.0 ml/min (R_t=15.0 min).

2.3. In vitro binding assay

The preparation of synaptosomal membranes from rat hearts was carried out according to a previously reported method with some modifications [17]. Briefly, the heart ventricles were homogenized in 20 volumes of ice-cold 250 mM sucrose buffer (5 mM Tris/HCl; 1 mM $MgCl_2$; 250 mM sucrose) with a 30-s burst, using a Polytron PT10-35 set at speed 6. The homogenate was centrifuged at 750×*g* for 10 min. The pellet was discarded and the supernatant recentrifuged at 20,000×*g* for 20 min. The resulting pellet was resuspended in 10 volumes of ice-cold 50 mM Tris-HCl buffer (50 mM Tris/HCl, 5 mM KCl, 120 mM NaCl, pH 7.4) and recentrifuged to give a pellet that was resuspended in 5 vol of the Tris-HCl buffer. The suspension was stored at −80°C until use. The protein concentration was measured by the Lowry method [18].

[^{3}H]Nisoxetine and (*S*,*S*)-[^{125}I]IPBM competition assays were performed according to the methods of Raisman et al. [17] with some modification. The assays were carried out by incubating 400 μl of the heart preparation (0.25 mg/ml) with [^{3}H]nisoxetine (2.5 nM) and various concentrations of competitors in 150 μl of 50 mM Tris-HCl buffer (50 mM Tris/HCl, 5 mM KCl, 120 mM NaCl, pH 7.4). Incubation was performed for 30 min at 25°C. At the end of the incubation, the mixture was poured into 5 ml of ice-cold Tris-HCl buffer, rapidly filtered through Whatman GF/B fiber filters and washed with 3×5 ml of buffer. For [^{3}H]nisoxetine, the radioactivity bound to the filter was measured with a liquid scintillation counter (2500-TR, Packard). For (*S*,*S*)-[^{125}I]IPBM, the radioactivity bound to the filters was measured with a NaI well scintillation counter (Cobra II Auto-Gamma, Packard). All incubations were performed in triplicate. Nonspecific binding was determined in the presence of 1 mM nisoxetine. IC_{50} values were determined from displacement curves of the percent inhibition of [^{3}H]nisoxetine and (*S*,*S*)-[^{125}I]IPBM binding vs. the inhibitor concentration using the GraphPad Prism software (GraphPad Software, San Diego, CA, USA).

(*S*,*S*)-[^{125}I]IPBM saturation assays were performed according to our previous method for brain with some modification [16]. Four hundred microliters of membrane (0.25 mg/ml) was mixed with 50 μl of (*S*,*S*)-[^{125}I]IPBM (0.01–3.0 nM). Incubation was performed for 60 min at 25°C. At the end of the incubation, the mixture was poured into 5 ml of ice-cold Tris-HCl buffer, rapidly filtered through Whatman GF/B fiber filters and washed with 3×5 ml of buffer, and the radioactivity was measured with a NaI well scintillation counter. Nonspecific binding was determined in the presence of 1 mM nisoxetine. K_D value was determined with GraphPad Prism.