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# *In vivo* evaluation of a new $^{18}$F-labeled PET ligand, [$^{18}$F]FEBU, for the imaging of $I_2$-imidazoline receptors

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ABSTRACT

*Introduction:* The functions of $I_2$-imidazoline receptors ($I_2$Rs) are unknown, but evidence exists for their involvement in various neuropsychiatric disorders. Although a few positron emission tomography (PET) $I_2$R ligands have been developed, of which [$^{11}$C]FTIMD and [$^{11}$C]BU99008 were evaluated as PET $I_2$R imaging ligands in monkeys, no human PET imaging study using an $I_2$R-selective PET ligand has been conducted yet. Thus, we synthesized an $^{18}$F-labeled $I_2$R-selective ligand (BU99018 or FEBU, *Ki* for $I_2$Rs = 2.6 nM), and evaluated its application using rodents in PET imaging *in vivo* toward the development of a clinically-useful $I_2$R PET imaging ligand.
*Methods:* [$^{18}$F]FEBU was synthesized by the reaction of its precursor and [$^{18}$F]fluoroethyl bromide. A biodistribution and brain PET study were conducted in mice and rats respectively.
*Results:* [$^{18}$F]FEBU was successfully synthesized yielding a radioactivity suitable for injection (10.1 ± 5.3% at the end of the irradiation ($n$ = 10) based on $^{18}F^-$). The specific activity at end of synthesis (EOS) was 40–147 TBq/mmol ($n$ = 10). The radiochemical purity was >99% at EOS and remained >99% for 90 min after EOS. In mice brain uptake was relatively high. In the blocking study with the co-injection of the high-affinity $I_2$R ligand BU224 (1 mg/kg b.w.) brain uptake was significantly decreased 30 min post-injection. In the PET studies the radioactivity was highly accumulated in the $I_2$R-rich hypothalamus. Pretreatment with BU224 (1 mg/kg b.w.) significantly decreased the radioactivity in the hypothalamus to 23% of that of the control from 60 to 90 min post-injection.
*Conclusion:* [$^{18}$F]FEBU was sufficiently stable as a PET ligand and had a relatively high specific binding affinity for $I_2$Rs in rats and mice.



## 1. Introduction

Imidazoline receptors (IRs), also known as imidazoline binding sites, are proposed to represent certain actions of the antihypertensive drug clonidine and its analogs, and are distinct from adrenergic receptors [1]. At present, IRs are categorized into at least three subtypes ($I_1$, $I_2$, and $I_3$) based on their available physiologic functions and pharmacologic roles [1–3]. The $I_1$-imidazoline receptors ($I_1$Rs) are encoded by a non-G-protein-coupled protein called imidazoline receptor antisera-selected protein [4], and possess hypotensive activity [5]. Clonidine and structurally related imidazoline compounds have preferential affinity for $I_1$Rs. The $I_2$Rs are located mostly on the outer membrane of mitochondria [6], although $I_2$R proteins have not been encoded. The $I_2$R ligands described to date belong to four chemical families: imidazolines, guanidines, 2-aminoimidazolines, and carbolines [7]. The $I_3$R is emerging and has a robust pharmacology and function, with strong evidence to suggest that it modulates $K_{ATP}$ channels [8].

IRs have a broad tissue distribution in various species including humans, and are present in the central nervous systems (CNS) and in peripheral organs such as the kidneys, lungs, and heart [9]. Functions associated with $I_2$Rs are not known, but evidence exists for their involvement in various CNS disorders, such as depression [10,11], Alzheimer's disease [12], Huntington's disease [13], Parkinson's disease [14], aging [15], and glial cell tumors [16]. It is possible that the changes in $I_2$R density are directly or indirectly related with a particular disease. In addition, selective $I_2$R ligands promote food intake [17] and may therefore alter eating behavior.

Several $^{11}$C-labeled PET ligands have been developed for $I_2$Rs [18–23]. Recently, we developed [$^{11}$C]FTIMD (Fig. 1) for imaging of $I_2$Rs [20]. [$^{11}$C]FTIMD showed specific binding to $I_2$Rs in rat and monkey brains, but its binding specificity appears to be relatively low [20,24]. More recently, Kealey et al. developed [$^{11}$C]BU99008 (Fig. 1) as a more potent PET ligand for $I_2$R imaging [23]. [$^{11}$C]BU99008 displayed a relatively high brain penetration and specific binding in the porcine and rhesus brain [23,25]. However, no human PET imaging study using a

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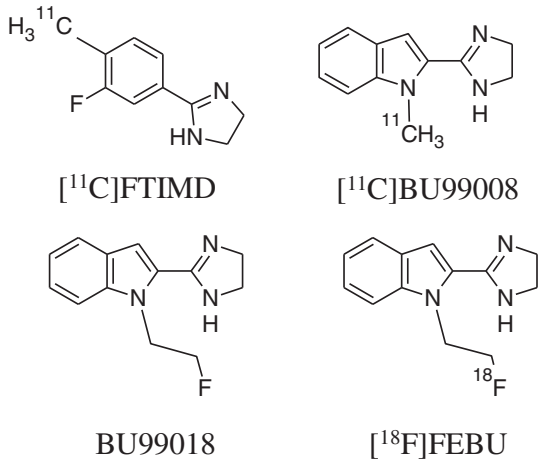


**Fig. 1.** Chemical structure of four imidazoline $I_2$ receptor PET ligands.

selective $I_2$R PET ligand has been conducted yet. Therefore, the objective of this study was to develop clinically useful PET ligand for imaging of $I_2$Rs. Recently, Tyacke et al. synthesized a high affinity $I_2$R ligand, a fluoroethyl analog of BU99008 (2-[4,5-Dihydro-1*H*-imidazol-2-yl]-1-[2-fluoroethyl]-1*H*-indole, BU99018 or FEBU, *Ki* for $I_2$Rs = 2.6 nmol/L) (Fig. 1) [26]. $^{18}$F-labeled BU99018, however, was not further developed as a PET ligand because of its poor stability in solution, particularly in methanol [26].

Here, we synthesized [$^{18}$F]FEBU (Fig. 1) for the first time to assess its radiochemical stability as a PET ligand, and evaluated its potential as an $I_2$R-specific PET imaging agent.

## 2. Materials and methods

### 2.1. General

All reagents and organic solvents were purchased commercially and used without further purification. BU224 hydrochloride, 2-BFI hydrochloride, and moxonidine hydrochloride were purchased from Tocris Bioscience (Bristol, UK). Efaroxan hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Proton nuclear magnetic resonance ($^1$H–NMR) and carbon-13 nuclear magnetic resonance ($^{13}$C–NMR) spectra were recorded on a JNM-AL-300 spectrometer (Jeol, Tokyo, Japan). Fast-atom bombardment mass spectra (FABMS) were obtained on a NMS-SX 102-SX spectrometer (Jeol, Tokyo, Japan). The FEBU and BU precursor were synthesized in-house according to a procedure reported previously [26] and were identified by NMR and FABMS, which were consistent with the data described previously [26].

Preparative high-performance liquid chromatography (HPLC) and analytical HPLC were performed using a Jasco HPLC system (Jasco, Tokyo, Japan). Effluent radioactivity was monitored using a NaI(Tl) scintillation detector system. If not otherwise stated, radioactivity was determined using an IGC-3R Curiemeter (Aloka, Tokyo, Japan).

Male inbred strain of laboratory mice (ddY, aged 7–9 weeks) and male Sprague–Dawley (SD) rats (aged 7–9 weeks) were purchased from Japan SLC Inc. (Shizuoka, Japan). Animals were maintained and handled in accordance with recommendations of the U.S. National Institutes of Health and the guidelines of the National Institute of Radiological Sciences (Chiba, Japan). Animal studies were approved by the Animal Ethics Committee of the National Institute of Radiological Sciences.

### 2.2. Radiosynthesis

[$^{18}$F]FEBU was synthesized by fluoroethylation of the BU precursor with [$^{18}$F]fluoroethyl bromide in the presence of tetrabutylammonium hydroxide (TBAOH) (Fig. 2) using an automated synthesis system [27]. [$^{18}$F]Fluorine anion was produced from the cyclotron (CYPRIS HM-18; Sumitomo Heavy Industries, Tokyo, Japan) by the $^{18}$O(p, n)$^{18}$F reaction on over 98 atom % $H_2^{18}O$ (ROTEM Industries, Arava, Israel) using 18 MeV protons (14.2 MeV on target) and was separated from $H_2^{18}O$ using the Sep-Pak Accell Plus QMA Plus Light cartridge (Waters, Milford, MA, USA). [$^{18}$F]Fluorine anion was eluted from the cartridge with a mixture of aqueous potassium carbonate (2.8 mg/0.2 mL) and a solution of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8,8,8]hexacosane (Kryptofix 222, 7.5 mg) in acetonitrile (0.2 mL), and transferred into a reaction vessel in the hot cell. The aqueous [$^{18}$F]fluorine anion solution was dried at 120 °C for 15 min to remove water and acetonitrile. Subsequently, trifluoromethanesulfonic acid 2-bromoethyl ester [28] (8 μL) in *o*-dichlorobenzene (0.4 mL) was added to the radioactive mixture. 2-[$^{18}$F]Fluoroethyl bromide in this vessel was distilled under a nitrogen flow (90–100 mL/min) at 130 °C for 5 min and bubbled into another vessel containing the BU precursor (1.0 mg) and 1 mol/L TBAOH in methanol (6 μL) in anhydrous *N*,*N*-dimethylformamide (DMF, 0.3 mL) at room temperature, and the reaction mixture was heated and maintained at 60 °C for 10 min. Preparative HPLC purification was performed on an InertSustain Phenylhexyl column (5 μm, 10 mm internal diameter [i.d.] × 250 mm length; GL Sciences, Tokyo, Japan) using a mobile phase of acetonitrile/50 mmol/L ammonium acetate solution (20:80, vol./vol.) at a flow rate of 4 mL/min. The retention times of the BU precursor and [$^{18}$F]FEBU were approximately 13 min and 18 min, respectively. HPLC fractions of [$^{18}$F]FEBU were collected into a flask to which Tween 80 (75 μL) in ethanol (0.3 mL) and 25% ascorbic acid (0.1 mL) had been added before radiosynthesis. The fractions were subsequently evaporated to dryness and the residue was dissolved in physiological saline.

The products were analyzed by HPLC with radioactivity and ultraviolet detection at 300 nm using a Capcell Pak C18 UG 80 column (4.6 mm i.d. × 250 mm length; Shiseido, Tokyo, Japan). Elution was performed using a mixture of acetonitrile, water, and triethylamine (50:50:0.01, vol./vol./vol.) at a flow rate of 1.5 mL/min. The retention times of [$^{18}$F]FEBU and BU-precursor were 5.3 and 9.4 min, respectively.

### 2.3. Chemical and radiochemical stability

The chemical stability of FEBU and the radiochemical stability of [$^{18}$F]FEBU were analyzed by HPLC as is represented by the chemical and radiochemical purity, respectively, in the HPLC chromatograms. The chemical stability of FEBU was measured almost immediately and 90 min after its dissolution in 50% aqueous acetonitrile (approximately 10 mmol/L). The radiochemical stability of the [$^{18}$F]FEBU injection was measured at the end of radiosynthesis (EOS) and 90 min thereafter. The chemical and radiochemical stability were analyzed by HPLC as described above.

### 2.4. Biodistribution in mice

Mice (age: 7–8 weeks; weight: 33–38 g; 4 mice per time point) received an intravenous injection of [$^{18}$F]FEBU (5.6 MBq/0.093 nmol). Mice were sacrificed by cervical dislocation 5, 15, 30, 60, or 90 min after the injection.

The effects of the co-injection of imidazoline and adrenergic receptor ligands on tissue distribution were also investigated. Mice (age: 7–8 weeks; weight: 34–38 g; 4 mice per group) received an intravenous co-injection of [$^{18}$F]FEBU (2.8 MBq/0.048 nmol) and one of the following four ligands (1.0 mg/kg b.w.): BU224 ($I_2$R ligand), 2-BFI ($I_2$R ligand), moxonidine ($I_1$R/$\alpha_2$-adrenoceptor (AR) ligand), and efaroxan ($I_1$R/$I_3$R/$\alpha_2$-AR ligand). Mice were sacrificed by cervical dislocation 30 min after the injection.

Blood samples were collected by heart puncture. Organs or tissues were dissected and weighed. The radioactivity in the samples was counted in an automatic gamma counter (Wizard 3" 1480, PerkinElmer,