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Imaging of I_2-imidazoline receptors by small-animal PET using 2-(3-fluoro-[4-^{11}C]tolyl)-4,5-dihydro-1*H*-imidazole ([^{11}C]FTIMD)

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Abstract

Introduction: Imidazoline receptors (IRs) have been established as distinct receptors, and have been categorized into at least two subtypes (I_1R and I_2R). I_2Rs are associated with depression, Alzheimer's disease, Huntington's disease and Parkinson's disease. A few positron emission tomography (PET) probes for I_2Rs have been synthesized, but a selective PET probe has not been evaluated for the imaging of I_2Rs by PET. We labeled a selective I_2R ligand 2-(3-fluoro-4-tolyl)-4,5-dihydro-1*H*-imidazole (FTIMD) with ^{11}C and performed the first imaging of I_2Rs by PET using 2-(3-fluoro-[4-^{11}C]tolyl)-4,5-dihydro-1*H*-imidazole ([^{11}C]FTIMD).
Methods: [^{11}C]FTIMD was prepared by a palladium-promoted cross-coupling reaction of the tributylstannyl precursor and [^{11}C]methyl iodide in the presence of tris(dibenzylideneacetone)dipalladium(0) and tri(*o*-tol)phosphine. Biodistribution was investigated in rats by tissue dissection. [^{11}C]FTIMD metabolites were measured in brain tissues and plasma. Dynamic PET scans were acquired in rats, and the kinetic parameters estimated.
Results: [^{11}C]FTIMD was successfully synthesized with a suitable radioactivity for the injection. Co-injection with 0.1 mg/kg of cold FTIMD and BU224 induced a significant reduction in the brain-to-blood ratio 15 and 30 min after the injection. In metabolite analysis, unchanged [^{11}C]FTIMD in the brain was high (98%) 30 min after the injection. In PET studies, high radioactivity levels were observed in regions with a high density of I_2R. The radioactivity levels and V_T values in the brain regions were prominently reduced by 1.0 mg/kg of BU224 pretreatment as compared with control.
Conclusion: [^{11}C]FTIMD showed specific binding to I_2Rs in rat brains with a high density of I_2R.

Keywords: Imidazoline receptors; I_2; FTIMD; ^{11}C; PET

1. Introduction

Imidazoline receptors (IRs), also known as imidazoline binding sites, were proposed to represent certain actions of the antihypertensive drug clonidine and its analogs, which produce pharmacological effects in the central nervous system (CNS) by interaction not only with α_2-adrenoceptors (α_2-AR) but also with an imidazoline binding site [1]. Such IRs were deemed to be pharmacologically distinct from α_2-AR because they were not activated by catecholamines [1]. IRs have been categorized into at least two subtypes (I_1R and I_2R) based on the available physiologic functions and pharmacologic tools [2], and a third class, I_3 binding sites, has been proposed [3]. I_1Rs are encoded by a non-G-protein-coupled protein called imidazoline receptor antisera-selected protein [4], and are involved in hypotensive activity [5].

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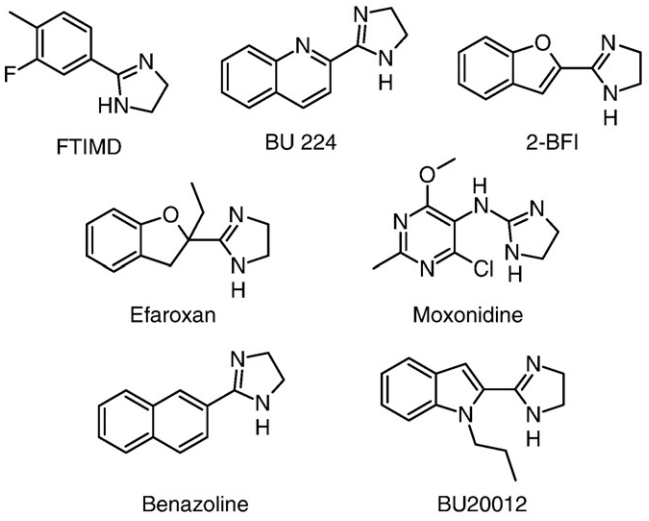

Fig. 1. Chemical structure of imidazoline receptor ligands.

Clonidine and structurally-related imidazoline compounds have preferential affinity for I_1Rs. I_2Rs are located mostly on the outer membrane of mitochondria [6], although I_2R proteins have not been encoded. Idazoxan-like imidazoline compounds have a preferential affinity for I_2Rs.

IRs are widely distributed throughout the tissues of various species including humans, and are present in the central and peripheral nervous systems and in various organs such as the kidney, lung, and heart [7]. In autoradiogram of rat brains using high-affinity I_2Rs ligands [^{3}H]2-BFI and [^{3}H] BU224, high density of I_2Rs were observed in the arcuate nuclei, interpeduncular nuclei, pineal gland, and the ependymal cell layer lining the ventricles [8,9]. Functions associated with I_2Rs 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 likely that the changes in the density are directly or indirectly related with a particular disease. These studies have led to the proposal that I_2R ligand may provide a useful probe for investigating these conditions using PET imaging studies. In addition, I_2Rs have been considered to be associated with monoamine oxidase (MAO) enzyme protein [17,18], although I_2R ligands inhibit MAO activity at micromolar concentrations [19–21]. Also, I_2Rs have been considered to be distinct from the active site of MAO [22,23]. Selective I_2R ligands with inhibitory activity against MAO may be valuable for the treatment of depression, Alzheimer's diseases, Parkinson disease, and Huntington's disease.

Several I_2R ligands have been synthesized [24,25]. Among them, 2-BFI (Fig. 1) and BU224 (Fig. 1) have high affinity for I_2R (Table 1) [24,26–28]. However, these ligands are practically inaccessible with a typical ^{11}C-labeling technique. Roeda et al. synthesized [^{11}C]benazoline (Fig. 1) as a selective I_2R ligand for PET (Table 1) by the condensation of a ^{11}C-labeled carboxylic acid with ethylenediamine [29,30], but in vivo experiments and preclinical PET studies are not yet reported. Hudson et al. synthesized [^{11}C]BU20012 (Fig. 1) and its derivatives as selective I_2R ligands for PET (Table 1) [31], but they have not reported an in vivo evaluation by PET using these ligands. Consequently, it appears that a suitable I_2R ligand for PET has not been reported.

Anastassiadou et al. synthesized 2-aryl-imidazoline compounds as selective IR ligands [32]. Of these, 2-(3-fluoro-4-tolyl)-4,5-dihydro-1*H*-imidazole (FTIMD) has a high and selective affinity for I_2R (K_i for I_2R, 8.0 nM; I_1R/I_2R>3388; K_i for α_1-AR and α_2-AR, >10 μM) [32]. Here, we labeled the FTIMD, a selective high-affinity I_2R imaging agent, with ^{11}C by the palladium-promoted cross-coupling reaction. The aim of this work was to characterize the binding kinetics of new I_2R imaging agent.

2. Materials and methods

2.1. Materials

All reagents and organic solvents were purchased commercially and used without further purification.

Table 1
In vitro imidazoline receptors (I_1R and I_2R) and α-adrenoceptor affinities of IR ligands

Ligand	Binding affinity *K*i (nM)					
	I_1R	I_2R	α_1-AR	α_{2A}-AR	α_{2B}-AR	α_{2C}-AR
FTIMD	>10,000[a]	3.0[a]	>10,000[a]	>10,000 (α_2)[a]		
BU 224	42[b]	3.7[c]	n.a.	4000[b]	3600[b]	500[b]
2-BFI	67[b]	3.4[c]	n.a.	8500[b]	5200[b]	1480[b]
Moxonidine	4.2[b]	>10,000[c]	n.a.	13[b]	9.5[b]	16[b]
Efaroxan	52[b]	>10,000[c]	n.a.	9.8[b]	9.9[b]	180[b]
Benazoline	n.a.	0.85[d]	2300[d]	>10,000 (α_2)[d]		
BU20012	n.a.	3.1[e]	n.a.	210 (α_2)[e]		

n.a., data not available.
[a] Ref. [32].
[b] Ref. [26].
[c] Ref. [24].
[d] Ref. [30].
[e] Ref. [31].