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Synthesis and bioevaluation of [^{18}F]4-fluoro-*m*-hydroxyphenethylguanidine ([^{18}F]4F-MHPG): A novel radiotracer for quantitative PET studies of cardiac sympathetic innervation

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ABSTRACT

A new cardiac sympathetic nerve imaging agent, [^{18}F]4-fluoro-*m*-hydroxyphenethylguanidine ([^{18}F]4F-MHPG), was synthesized and evaluated. The radiosynthetic intermediate [^{18}F]4-fluoro-*m*-tyramine ([^{18}F]4F-MTA) was prepared and then sequentially reacted with cyanogen bromide and NH_4Br/NH_4OH to afford [^{18}F]4F-MHPG. Initial bioevaluations of [^{18}F]4F-MHPG (biodistribution studies in rats and kinetic studies in the isolated rat heart) were similar to results previously reported for the carbon-11 labeled analog [^{11}C]4F-MHPG. The neuronal uptake rate of [^{18}F]4F-MHPG into the isolated rat heart was 0.68 ml/min/g wet and its retention time in sympathetic neurons was very long ($T_{1/2}$ >13 h). A PET imaging study in a nonhuman primate with [^{18}F]4F-MHPG provided high quality images of the heart, with heart-to-blood ratios at 80–90 min after injection of 5-to-1. These initial kinetic and imaging studies of [^{18}F]4F-MHPG suggest that this radiotracer may allow for more accurate quantification of regional cardiac sympathetic nerve density than is currently possible with existing neuronal imaging agents.

Radioiodinated *m*-iodobenzylguanidine (MIBG, Fig. 1) was one of the first radiopharmaceuticals developed for scintigraphic imaging of presynaptic sympathetic nerve fibers in the human heart.[1] Clinical studies with MIBG have demonstrated significant changes in the regional distribution of cardiac sympathetic nerves in many diseases, including congestive heart failure, diabetic autonomic neuropathy, myocardial infarction, cardiac arrhythmias, sudden cardiac death and Parkinson's disease.[2] Several radiotracers for noninvasive imaging of cardiac sympathetic innervation with positron emission tomography (PET) have also been developed, including [^{11}C]-(−)-*m*-hydroxyephedrine ([^{11}C]HED, Fig. 1),[3] [^{11}C]-(−)-epinephrine ([^{11}C]EPI, Fig. 1),[4] [^{11}C]-(−)-phenylephrine,[5] 6-[^{18}F]fluorometaraminol ([^{18}F]6-FMR Fig. 1),[6] 6-[^{18}F]fluorodopamine,[7] *m*-[^{76}Br]bromobenzylguanidine[8] and *p*-[^{18}F]fluorobenzylguanidine ([^{18}F]PFBG).[9]

All of these tracers are structural analogs of the neurotransmitter norepinephrine. They are initially transported into cardiac sympathetic neurons as substrates of the norepinephrine transporter (NET). Once inside the neurons, to varying degrees these compounds are taken up into norepinephrine storage vesicles by the second isoform of the vesicular monoamine transporter (VMAT2).[10] While the rapid neuronal uptake of these agents results in high quality images of the heart, this also causes problems in extracting accurate quantitative measures of regional sympathetic nerve density from the myocardial kinetics of these agents. NET transport of these tracers is a fast process and because of this their neuronal uptake rates are rate-limited by delivery from plasma to the extracellular spaces near the neurons. Extraction of these tracers from plasma is governed primarily by blood flow, so radiotracers with these kinetic properties are often called 'flow-limited tracers'. Since NET transport is not the rate-limiting step in the uptake of the tracer, it is not possible to extract an estimate of NET transport rate of the tracer from its myocardial kinetics, as measured from the acquired PET scan data. This is unfortunate, since a regional estimate of the NET transport rate of the tracer would likely serve as a sensitive measure of the regional density of presynaptic nerve terminals. The inability to apply tracer kinetic analysis approaches forces us to rely instead on semi-quantitative measures of tracer retention, such as the heart-to-medistinum ratio (HMR) for MIBG and the retention index (RI) for [^{11}C]HED.[10] While these have been found to be useful, another consequence of the rapid neuronal uptake rate of these compounds is that measures of tracer retention tend to be insensitive to regional nerve losses until those losses become fairly severe. Thus current tracers likely do not detect early denervation—which may be important in providing therapies designed to halt further cardiac denervation.

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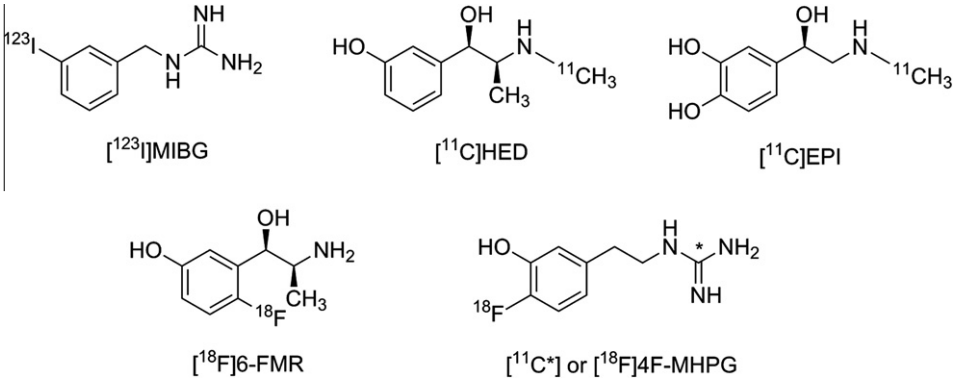


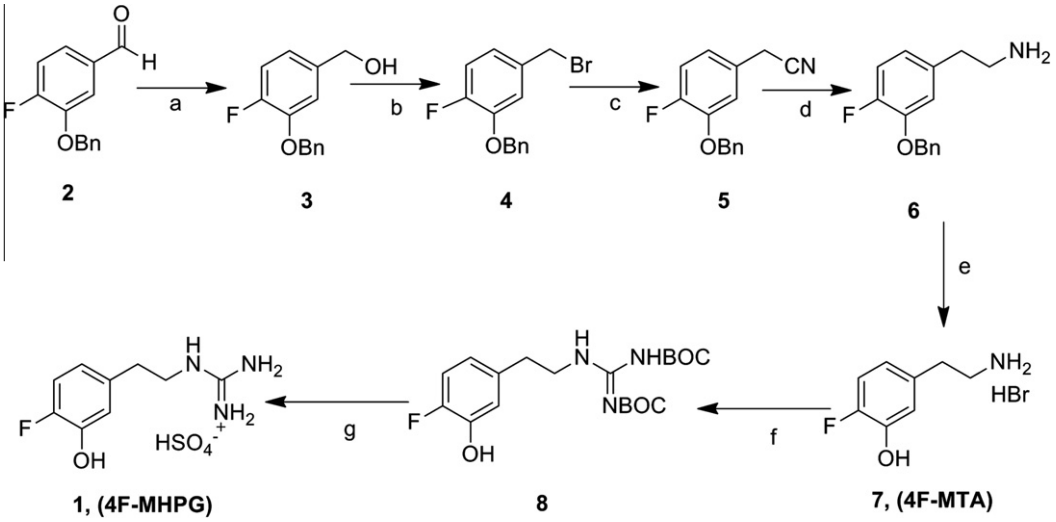
Figure 1. Structures of cardiac sympathetic nerve imaging agents.

The only way to overcome these obstacles to accurate and sensitive detection of cardiac denervation is to develop a new tracer that possesses optimal kinetics for tracer kinetic analyses. Such tracers would provide more accurate and sensitive measures of regional nerve density, allowing detection of denervation earlier in the course of diseases that cause nerve damage, such as diabetic autonomic neuropathy and heart failure. We hypothesized that a new radiolabeled NET substrate should possess two kinetic properties to be better suited for tracer kinetic analyses: (1) a slower neuronal uptake rate; and (2) a very long neuronal retention time, through efficient vesicular storage. We believed that compounds with these kinetic properties could be found among the various guanidine derivatives that show potent pharmacological effects on cardiac sympathetic neurons. In particular, we chose to study phenethylguanidines because some of these compounds are known to be potent depletors of cardiac norepinephrine stores in vivo, due to their avid uptake and retention inside norepinephrine storage vesicles.[11]

Evaluations of a series of carbon-11 labeled phenethylguanidines have previously been reported.[12] Among the compounds tested, [^{11}C]-4-fluoro-*m*-hydroxy-phenethylguanidine ([^{11}C]4F-MHPG, Fig. 1) was found to possess the desired kinetic properties of a slower NET transport rate and a very long neuronal retention time. Also, microPET studies in rhesus macaque monkeys demonstrated that this agent had favorable in vivo imaging properties and kinetics. These encouraging results led us to attempt the synthesis of fluorine-18 labeled 4-fluoro-*m*-hydroxy-phenethylguanidine ([^{18}F]4F-MHPG). While the short half-life of carbon-11 (20.1 min) limits its wide spread application due to the requirement of an on-site cyclotron, the longer half-life of fluorine-18 (109.8 min) allows for imaging studies at imaging centers without a cyclotron through distribution by commercial vendors. The longer half-life of fluorine-18 also allows for imaging times up to several hours after injection into a patient and permits multiple patients doses to be dispensed from a single synthesis batch.

We describe here the preparation of a new fluorine-18 radiotracer ([^{18}F]4F-MHPG) for imaging cardiac sympathetic innervation with PET and the results of initial bioevaluation studies in rats and a non-human primate. Biological data for [^{18}F]4F-MHPG are compared to previous results obtained with [^{11}C]4F-MHPG.

Reference standards for HPLC and in vitro studies, 4F-MHPG **1** and 4-fluoro-*m*-tyramine (4F-MTA, **7**), were prepared from the starting material 3-benzyloxy-4-fluorobenzaldehyde **2** (Scheme 1). The benzaldehyde **2** was reduced with $NaBH_4$ to afford the benzyl alcohol **3**. The alcohol **3** was converted to the benzyl bromide **4** by reaction with PBr_3 and then followed by treatment with sodium cyanide in DMSO to generate the benzyl cyanide **5**. Reduction of cyano group with borane and deprotection of the benzyl group with 48% HBr was carried out to provide 4F-MTA **7**. Condensation of 4F-MTA with 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea[13] and then followed by deprotection under mild acidic condition afforded 4F-MHPG **1.** Another reference standard,



Scheme 1. Reagents and conditions: (a) $NaBH_4$, THF, 0 °C to rt, N_2, 4 h, 72%; (b) PBr_3, CH_2Cl_2, rt, overnight, 37%; (c) NaCN, DMSO, rt, 3 h, 90%; (d) 1.0 M BH_3/THF complex, THF, reflux, 2 h, 69%; (e) HBr (48%), 130 °C, N_2, 6 h, 97%; (f) 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea, $HgCl_2$, Et_3N, DMF, 0 °C to rt, 24 h, 87%, (g) 1.0 N HCl, CH_2Cl_2/MeOH, rt, 24 h, 87%.