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Enhancement of ^{18}F-Fluorodeoxyglucose Metabolism in Rat Brain Frontal Cortex Using a β3 Adrenoceptor Agonist

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Abstract

We report the use of β3-adrenergic receptor mediated activation of rat brain frontal cortex using mirabegron (a selective β3-adrenoceptor agonist), measured by ^{18}F-FDG PET/CT. Another β3-agonis t, CL 316,243, did not have this effect due to impermeability through the blood brain barrier (BBB), while atomoxetine, a norepinephrine transporter blocker, did increase ^{18}F-FDG uptake in the frontal cortex. Mirabegron exhibited a dose-dependent increase in frontal cortex ^{18}F-FDG uptake. These findings suggest a possible use of selective β3-adrenoceptor agonists in reversing regional glucose hypometabolism in the brain.

Keywords

β3-Adrenoceptor; ^{18}F-FDG; frontal cortex; Alzheimer's disease

Introduction

β3 adrenergic receptors, regulators of fatty acid metabolism (Bartelt et al., 2011), are mainly in brown adipose tissue (BAT), but are also found in white adipose tissue, myocardium, skeletal muscle, liver, and brain (Coman et al., 2009; Ursino et al., 2009). Expression of β3 adrenoceptors mRNA in the brain is lower than in BAT (Summer et al., 1995). β3 adrenoceptor agonists have been shown to enhance ^{18}F-FDG uptake in BAT in vivo (Mirbolooki et al., 2011). β3 adrenoceptor-induced glucose metabolism is of potential interest for obesity and diabetes (Mirbolooki et al., 2013).

Our previous work with CL316,243, a β3 adrenoceptor selective agonist, showed high levels of ^{18}F-FDG activation in vivo in BAT but not in the brain due to its BBB impermeability (Mirbolooki et al., 2011). Intracranial injections of CL316,243 along with injections of $A\beta_{1–42}$ have been reported to study β-amyloid induced amnesia in chicks. Results of this

Conflict of Interest
The authors declare no conflict of interest in the work presented here.

Statement of Authors Contributions
The rat experiments were carried out by Dr. Reza Mirbolooki, Kimberly Schade analyzed the PET data, prepare figures and contributed to writing the manuscript, Dr. Cristian Constantinescu analyzed the PET data for drug effects, Dr. Min-Liang Pan evaluated the dosing of rats with drugs and ^{18}F-FDG and Dr. Jogeshwar Mukherjee was involved in planning the experiments and writing the manuscript. All authors read and approved the final manuscript.

study demonstrated rescued memory loss suggesting a role that β3 adrenoceptor agonists may play in Alzheimer's disease (AD) (Gibbs et al., 2008). Mirabegron, 2-(2-Amino-1,3-thiazol-4-yl)-*N*-[4-(2-{[(2*R*)-2-hydroxy-2-phenylethyl]amino}ethyl)phenyl]acetamide, is a β3 adrenoceptor selective agonist (Takasu et al., 2007) that shows some permeability across the BBB ().

In order to assess brain effects of a β3 adrenoceptor selective agonist, we quantitatively analyzed ^{18}F-FDG uptake in rats treated with mirabegron and compared its effects to those treated with CL316,243 and a presynaptic NET inhibitor, atomoxetine, in the brain and interscapular brown adipose tissue (IBAT) using PET/CT. The following procedures were implemented: 1. Compare each drug's effect on the brain; 2. Evaluate dose effects of mirabegron on the frontal cortex, and compare these results to those in IBAT.

Materials and Methods

An Inveon Multimodality (MM) CT scanner (Siemens Medical Solutions Inc) was used for CT acquisitions in combined PET/CT experiments as previously described (Mirbolooki et al., 2013). The Inveon PET and CT scanners were placed in the "docked mode" for combined PET/CT experiments. All in vivo images were analysed by Inveon Research Workplace (IRW) software (Siemens Medical Solutions Inc) and PMOD Software (PMOD Technologies). Radioactivity was counted using a Capintec CRC-R dose calibrator. All animal studies were approved by the Institutional Animal Health Care and Use Committee of University of California-Irvine.

Rats were purchased from Harlan Laboratories Inc. (male adult, approx. 300 g) and housed under controlled temperatures of 22°C ± 1°C, in a 12-h light–dark cycle, on at 6 AM, with water ad libitum. All rats were fasted for 17 hours before ^{18}F-FDG administration. Control rats were given normal saline while the other groups of rats (n=2) were administered 0.5 mg/kg of atomextine, mirabegron, or CL316,243, iv via the tail vein, 30 mins prior to ^{18}F-FDG. Under 2% isoflurane anesthesia, the rats were administered ^{18}F-FDG (22 ± 4 MBq). Following the injections, rats were awake for 60 minutes and subsequently placed in the supine position in a rat holder and anesthetized with 2% isoflurane for upper-body PET imaging (brain and IBAT). The rat holder was placed on the PET/CT bed and all animals had a CT scan after the PET scan for attenuation correction and anatomical delineation of PET images. Separate 30 min scans (60 mins post-^{18}F-FDG injections, 19 ± 4 MBq) were used for dose-dependence (0.03, 0.3, 3.0 mg/kg, iv via the tail vein, 30 mins prior to ^{18}F-FDG, n=2) of mirabegron.

All images were calibrated in units of Bq/cm^3 by scanning a Ge-68 cylinder (6 cm diameter) with known activity and reconstructing the acquired image with parameters identical to those of ^{18}F-FDG images. The magnitude of ^{18}F-FDG activation was expressed as standard uptake values (SUV) which was defined as the average ^{18}F-FDG activity in each volume of interest, VOI (in kBq/cm^3) divided by the injected dose (in MBq) times the body weight of each animal (in Kg). The SUV values were thus expressed in units of [kBq/cm^3/(MBq/Kg)].

Results

Brain uptake of ^{18}F-FDG occurred under all the four conditions, i.e., control and the three drugs. Compared to controls, the behavior of the three drugs on the brain uptake were very different. As we have previously observed, CL316,243 had a significant reduced brain uptake with the SUV ratio versus control in the range of 0.4 to 0.5 (Figure-1). Atomoxetine on the other hand had a lowering effect on the whole brain, except on the frontal cortex, where the SUV ratio compared to control was over 1.2. Mirabegron showed almost a similar whole brain uptake of ^{18}F-FDG. Frontal cortex showed the highest increase, with SUV ratio versus control exceeding 1.4 (Figure-1). This divergent effect of the three drugs is evident in the group effect seen in Figure-1. There were no left-right differences for the three drugs.

Figure-2a shows that there is an increase in the amount of ^{18} F-FDG uptake, specifically in the frontal cortex, with increasing doses of mirabegron. The frontal cortex SUV following doses of 0.03 mg/Kg, 0.3 mg/Kg, and 3.0 mg/Kg mirabegron were 4.97, 5.96, and 6.36 respectively. Frontal cortex showed a 20% increase in ^{18}F-FDG uptake compared to controls. Along with the dose-dependent effect seen in the frontal cortex, there is also an increase in ^{18} F-FDG uptake in IBAT with increasing doses of mirabegron (Figure-2b). For a dose of 0.03 mg/kg, the SUV was 1.03, while doses of 0.3 mg/kg and 3.0 mg/kg had SUVs of 2.03 and 3.12, respectively.

Discussion

Presence of β3 adrenoceptor mRNA levels in rat brain regions have been reported (Summers et al., 1995). Comparing β3 adrenoceptor mRNA in BAT at 100%, the frontal cortex contains 3.1% while the cerebellum only contains 0.18%. While the mechanism of β3 adrenoceptor activation is via the uncoupling protein (UCP1) in adipocytes, β3 activation in the brain may influence other pathways including serotonin synthesis (Ursino et al., 2009). Therefore, a β3 agonist may be expected to activate brain regions, similar to our observations in IBAT (Mirbolooki et al., 2011). The effect of CL316,243 is remarkable in activating ^{18}F-FDG uptake in BAT but shows a reduced SUV in the brain compared to control. Since CL316,243 is unable to permeate the BBB, brain effects cannot be expected. However, the reduction in brain SUV may be due to lower plasma ^{18}F-FDG because of activation and consumption of ^{18}F-FDG by BAT. On the other hand, atomoxetine permeates into the brain, affecting norepinephrine levels; therefore, it shows a more selective increase in ^{18}F-FDG in the frontal cortex compared to other regions of the brain that are similar to controls. The atomoxetine-induced norepinephrine effect also exhibits increased ^{18}F-FDG uptake in BAT due to activation of β3 adrenoceptors by norepinephrine (Mirbolooki et al., 2013).

Mirabegron is a selective β3 adrenoceptor agonist in clinical use for overactive bladder and thus its effects were expected to be in the periphery. Compared to CL316,243, mirabegron has better agonist potency for human β3 adrenoceptors (Ki of CL316,243 = 3 nM (Mirbolooki et al., 2011) and mirabegron = 40 nM (AusPAR, 2014); Emax of CL316,243 = 69% (IC_{50}=7.36 nM) and mirabegron = 72% (IC_{50}=1.71 μM) for rat bladder contractions induced by electrical field stimulation (Caltabiano et al., 2013)). Our initial interest was to

evaluate the effects of mirabegron BAT activation. Brain effects were not anticipated since BBB permeability of mirabegron appears to be very low. However, contrary to this expectation, mirabegron showed significant brain effects as well as effects on IBAT. Effects on both the organs are consistent with the β3 adrenoceptor mediated activation and a dose-dependency of ^{18}F-FDG uptake was observed (Figure-2). The highest effect of mirabegron in the brain was in the frontal cortex, while the effect on the whole brain ^{18}F-FDG uptake was lower. These findings suggest that mirabegron and/or its metabolites may permeate into the brain, albeit at small levels, enough to cause activation of different brain regions.

Activation of β3 adrenoceptor to increase regional brain metabolism may be of therapeutic value in various brain disorders, including neurodegeneration. Amibegron is another selective β3 adrenoceptor agonist that crosses BBB and has anti-depressant like properties such as its ability to increase serotonin synthesis (Stemmelin et al., 2010). Brain PET studies with amibegron are planned in order to ascertain the effect of β3 adrenoceptor agonists on ^{18}F-FDG metabolism.

Conclusion

In conclusion, mirabegron showed an increase in uptake of ^{18}F-FDG in the frontal cortex in a dose dependent manner. It is likely that this increase in ^{18}F-FDG may be due to several mechanisms including increased serotonin-mediated activity as well as activation of astrocytes mediated by adrenergic agonists (Hertz et al., 2013). Our results suggests that β3-adrenoceptor agonist may have a specific role in enhancing frontal cortex glucose metabolism, consistent with the improved cognition using a β3 adrenoceptor agonist (Gibbs et al., 2008). This enhancement of glucose metabolism by β3 adrenoceptor agonists may have potential value in CNS disorders, such as AD which shows brain regions deprived of ^{18}F-FDG uptake.

Acknowledgments

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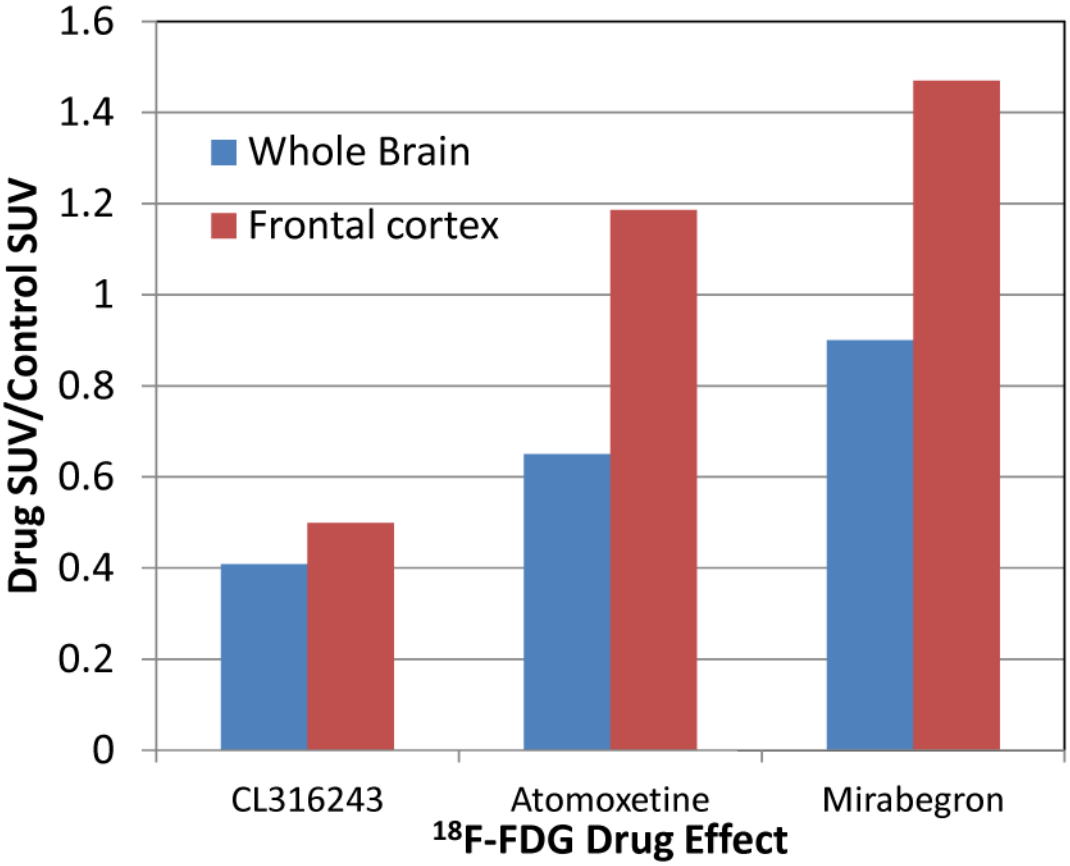

Figure 1.
Ratio of ^{18}F-FDG SUV of drug treatment versus control in whole brain (Global) and frontal cortex (FC) regions for rats treated with CL316,243 (0.5 mg/kg), atomoxetine (0.5 mg/kg), and mirabegron (0.5 mg/kg).

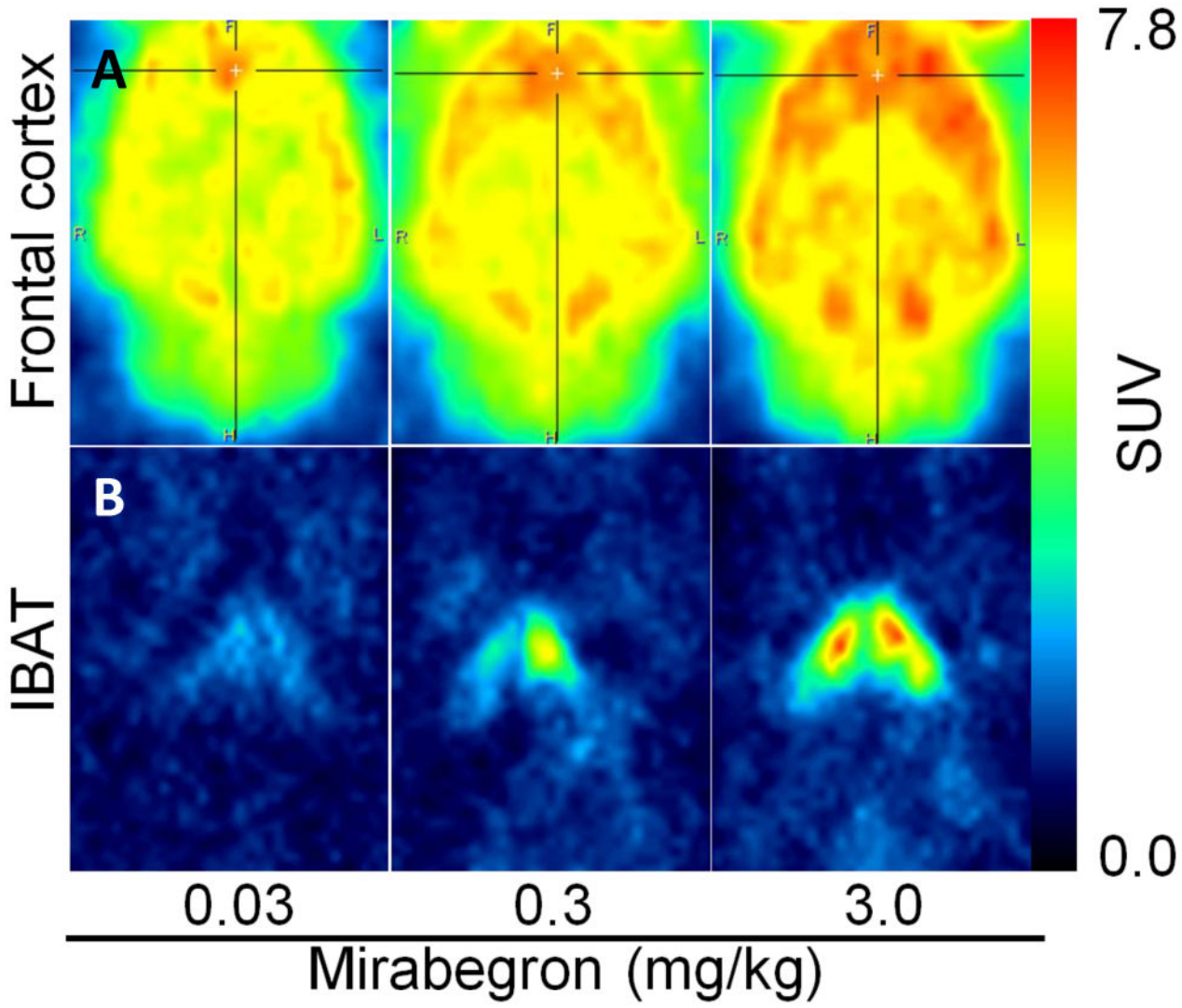

Figure 2.

Dose-dependent effect of mirabegron on ^{18}F-FDG uptake in the brain regions including frontal cortex (A: upper row) and interscapular brown adipose tissue (B: lower row). Images are normalized to show measures of SUV of ^{18}F-FDG in the different conditions.