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Short Communication

Surrogate Markers for Cerebral Blood Flow Correlate With [^{18}F]-Fallypride Binding Potential at Dopamine $D_{2/3}$ Receptors in Human Striatum

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ABSTRACT Positron emission tomography (PET) with the high affinity dopamine $D_{2/3}$ receptor ligand [^{18}F]-fallypride affords estimates of the binding potential (BP_{ND}) in extra-striatal regions of low receptor abundance, but the sufficient recording time for accurate measurements in striatum has been called into question. We have earlier argued that transient equilibrium measurements are obtained in striatum with [^{18}F]-fallypride PET recordings of 3 h duration, which may be the practical limit for clinical investigations without interrupted scanning. However, the high extraction fraction of [^{18}F]-fallypride predicts flow-dependence of tracer delivery to brain, which may be a source of variance of the apparent BP_{ND} in regions of high binding. To test this prediction, we conducted a retrospective analysis of [^{18}F]-fallypride PET data from a group of 50 healthy volunteers (age 18–58 years [mean ± SD: 32.6 ± 10.6), who had participated in clinical studies without arterial input measurements. We used the initial 120-s integral (AUC) of the venous confluence (VC) as a surrogate marker for cerebral blood flow (CBF) and tested for correlations between regional estimates of BP_{ND} calculated by the simplified reference tissue model (SRTM) and the individual VC-AUC. The magnitude of BP_{ND} in a high binding region (putamen), but not in a low binding region (thalamus) correlated positively with VC-AUC, suggesting that approximately 9% of the variance in the [^{18}F]-fallypride BP_{ND} in putamen can be attributed to individual differences in this surrogate marker for CBF, a contribution equal in magnitude to the effects of age on BP_{ND} in putamen of the present healthy control group. **Synapse 67:199–203, 2013.**

INTRODUCTION

Positron emission tomography (PET) investigations with high affinity dopamine $D_{2/3}$-ligands enable the quantification of receptor availability in extrastriatal regions. The affinity of [^{18}F]fallypride to dopamine D_2 and D_3 receptors is comparable to that of [^{11}C]-FLB 457, and some 10-fold higher than the affinities of [^{11}C]raclopride or [^{11}C]-(+)-PHNO. Whereas the latter ligand shows preferential binding to D_3-receptors ostensibly in an agonist binding state, the antagonist [^{18}F]fallypride binds with comparable affinity to D_2 and D_3 receptors, irrespective to their coupling to intracellular G-proteins (e.g., Cumming, 2011, Gallezot et al., 2012; Graff-Guerrero et al. 2009; Mukherjee et al., 1999). Whereas [^{11}C]-FLB 457 is suited for

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quantification of binding potentials (BP_{ND}) only in regions of low receptor abundance, the longer physical half-life of fluorine-18 enables BP_{ND} measurements also in high binding regions with [^{18}F]-fallypride. This affords the possibility of measuring the $D_{2/3}$-receptor availability in striatal and extrastriatal regions with a single PET recording, albeit lasting at least 3 h (Kegeles et al., 2010; Zald et al., 2010). Indeed, the time to equilibrium binding of [^{18}F]-fallypride binding in striatum is such that its kinetics might be considered to be poised between the conditions of reversible and irreversible binding models, as is the case for the butyrophenone ligand [^{11}C]-NMSP (Mach et al., 1995; Rosa-Neto et al., 2004). Insofar as the binding of any tracer with sufficiently high association rate constant (k_3) relative to the dissociation rate constant (k_4) is potentially vulnerable to bias arising from tracer delivery effects, it must be considered that cerebral blood flow (CBF) may be a factor influencing the estimation of [^{18}F]-fallypride BP_{ND} in regions with high receptor concentrations, whereas this influence should be less important in low-binding regions. Although vulnerability to CBF effects of BP_{ND} for the lower affinity congener [^{11}C]-raclopride has been established in cat striatum (Hassoun et al., 2003), there have been no studies specifically testing the possible relationship between individual differences of CBF and the apparent [^{18}F]-fallypride BP_{ND} in human.

We have earlier used the influx of the serotonin $5HT_{1A}$ [^{18}F]-MPPF ligand during the first minute of rat PET recordings as a surrogate index of the magnitude of K_1, the unidirectional tracer clearance across the blood–brain barrier (la Fougère et al., 2010), which is itself a function of CBF, reduced by the extraction fraction of the tracer. Based upon that approach, one might elect to employ the initial influx of [^{18}F]-fallypride to brain as a surrogate of CBF. However, preliminary analysis of [^{18}F]-fallypride kinetics suggested that its rate of association to dopamine receptors is so rapid that specific binding may contribute to the striatal signal within the first minutes of the PET recording. Given that a relevant fraction of the early [^{18}F]-fallypride uptake in receptor-rich regions might be due to $D_{2/3}$-receptor-binding, which ultimately determines the BP_{ND} measured in prolonged recordings, any observed intercorrelation between these parameters might be attributed to early specific binding rather than to CBF effects. Therefore, we elected in the present investigation to utilize as our main image-derived surrogate of the unmeasured global or regional CBF the initial area under the curve (AUC) measured for [^{18}F]-fallypride in the venous confluence (VC), a vascular structure also known as the torcular herophili. Although we focus on BP_{ND} in the putamen and thalamus as representative high- and low-binding regions, we also examined this relationship in the caudate nucleus (NC) and the inferior temporal lobe (GTi).

METHODS

The study group consisted of 50 drug-free, healthy volunteers, 30 of whom had participated in previously published [^{18}F]-fallypride PET studies (Gründer et al., 2006; Vernaleken et al., 2008, 2010, 2011). The same scanning protocol was used in all cases, and all subjects had given informed consent for participation in approved research protocols. Subjects were right handed of mean age 32.6 ± 10.6 years (range: 18–58 years; 40 ♂ + 10 ♀). In brief, following a 10-min transmission scan, 3 h emission recordings were initiated at the start of an intravenous infusion of [^{18}F]-fallypride (150–250 MBq), administered as a slow bolus lasting 30 s. Dynamic emission recordings were obtained using the Siemens ECAT EXACT PET scanner, operating in 3D mode; the sequence always consisted of three 20 s frames, followed by frames of progressively increasing duration, to a total of 39 frames in 180 min. After attenuation correction, reconstruction (filtered back-projection, 4 mm Hanning filter), and spatial normalization, all as described in the citations above, 180 min time-radioactivity curves were extracted using standard volume of interest (VOI) templates for the cerebellum, the medial thalamus, and the putamen, and converted to standardized uptake value (SUV) units. A 6 mm diameter spherical VOI was placed over the VC near the posterior cerebellum. The VC-VOI was initially placed on the normalized template and then manually adjusted to capture the early venous phase (frames 1–6 = 0–2 min) for each subject. The VOI diameter of 6 mm was chosen based on the mean size of the human VC and its main tributaries (e.g., sinus rectus: 7.1 mm; Liu et al., 2010). The AUCs during the first 120 s (AUC_{120}) were calculated for putamen, thalamus, and VC, and converted to SUV units. The magnitude of [^{18}F]-fallypride BP_{ND} was calculated in thalamus and putamen by the simplified reference tissue method (SRTM) (Lammertsma and Hume, 1996) implemented in PMOD (PMOD Technologies Ltd., Zurich) using the entire 180 min recordings, with the cerebellum serving as a reference region. The thalamus and putamen VOIs were obtained from a VOI template used in several previous investigations (Vernaleken et al., 2008, 2010, 2011). Correlation coefficients were calculated between the individual magnitudes of BP_{ND}, age, and the initial VC-AUCs using a simple linear model, and a multi-linear model with age as a nuisance variable. Furthermore, the VC-AUC was correlated with the graphically estimated time-to-equilibrium (see Vernaleken et al., 2011). All statistical analyses were performed using SPSS (v18.0; IBM Corp., Armonk, NY).

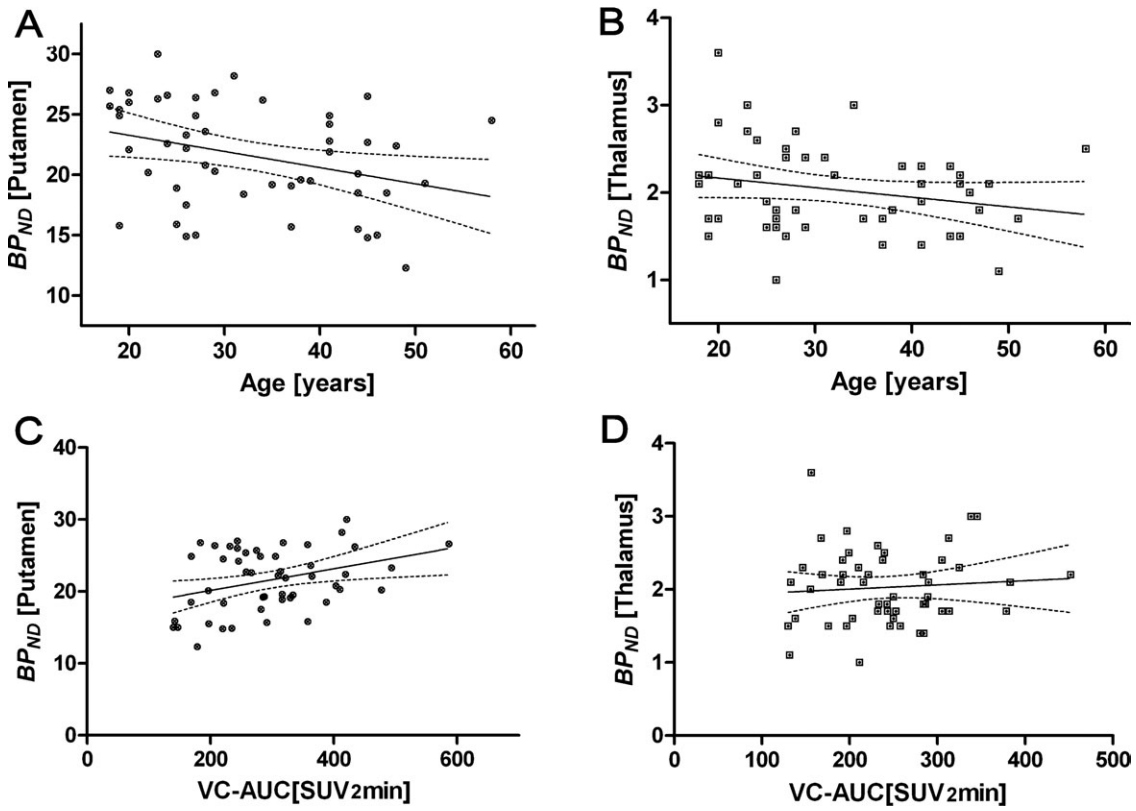

Fig. 1. Correlations between individual [^{18}F]-fallypride BP_{ND} with age in the putamen (**A**; $r = -0.33$; $P = 0.020$) and thalamus (**B**; $r = -0.23$; $P = 0.115$) in a series of 50 healthy subjects. The dependence of BP_{ND} in putamen on the initial (2 min) VC-AUC (**C**; $r = 0.305$; $P = 0.031$) accounted for 9% of the variance of BP_{ND} in putamen ($r^2 = 0.093$), but there was no significant relationship between BP_{ND} in thalamus and the VC-AUC (**D**; $r = -0.086$; $P = 0.522$).

RESULTS

The [^{18}F]-fallypride BP_{ND} ranged from 12.3 to 30.0 (mean ± SD: 21.6 ± 4.3) in putamen and from 1.0 to 3.6 (mean ± SD: 2.0 ± 0.5) in the thalamus. The mean VC-AUC_{120} was 282 ± 70 (SD) SUV units, and there was no significant correlation between age and VC-AUC_{120} ($r = -0.142$; $P = 0.325$). The VC-AUC_{120} correlated highly with corresponding initial AUCs for putamen (mean AUC_{120} 297 ± 98; $r = 0.711$, $P < 0.0001$), thalamus (mean AUC_{120} 242 ± 71; $r = 0.837$, $P < 0.0001$), and cerebellum time activity curve (TAC) ($r = 0.783$, $P < 0.0001$). There was no significant correlation between the VC-AUC and the time-to-equilibrium. Pearson's correlation analyses revealed a significant inverse relationship between putamen BP_{ND} and age (Fig. 1A: $r = -0.33$; $P = 0.020$), and a weaker non-significant relationship for thalamus BP_{ND} (Fig. 1B: $r = -0.226$; $P = 0.115$). The mean age-corrected putamen BP_{ND} was 25 ± 5.

The magnitude of individual VC-AUC_{120} correlated with BP_{ND} in putamen ($r = 0.305$; $P = 0.031$) as well as NC ($r = 0.322$; $P = 0.023$), but not in thalamus ($r = 0.086$; $P = 0.522$) or Gti ($r = -0.026$; $P = 0.863$) (Figs. 1C and 1D). A multiple linear regression using VC-AUC as an independent parameter and age as a nuisance variable (using SSTYPE I: VC-AUC → age → VC-AUC_{120} x age) gave the following relationships for the putamen BP_{ND} (VC-AUC_{120}: Wald-χ^2 = 5.31, df = 1, $P = 0.026$; age: Wald-χ^2 = 4.72, df = 1, $P = 0.035$; VC-AUC_{120} × age: Wald-χ^2 = 0.78, df = 1, $P = 0.381$). For the thalamus, no significant relationships were observed using the GLM (VC-AUC_{120}: Wald-χ^2 = 0.43, df = 1, $P = 0.516$; age: Wald-χ^2 = 2.30, df = 1, $P = 1.05$; VC-AUC_{120} × age: Wald-χ^2 = 0.90, df = 1, $P = 0.310$).

DISCUSSION

The [^{18}F]-fallypride BP_{ND} findings in the present analysis are comparable to results of other authors using this ligand (e.g., Slifstein et al., 2010). As expected, we detected in this group of 50 drug-free subjects a robust age dependence in the magnitude of [^{18}F]-fallypride BP_{ND} in putamen, which declined by approximately 13% per decade, although there was no significant age dependence in the thalamus. Our earlier failure to detect age-related changes in the striatal [^{18}F]-fallypride BP_{ND} in a different group of

14 healthy volunteers (Rominger et al., 2012) doubtless reflects the lesser statistical power of that study. Relative to the group mean value, the VC-AUC_{120}, our surrogate parameter for global CBF, showed a 3.3% loss per decade, which failed to reach statistical significance. Others have reported significant age-dependent global CBF declines of comparable magnitude in a group of healthy women, but not in men (Bertsch et al., 2009). Most likely, the lack of significant age-dependence of our CBF surrogate is due to the considerable dispersion in the VC-AUC_{120} measurements, as well as the low number of female participants.

The VC-AUC_{120} is an image-derived index of the availability of [^{18}F]-fallypride in circulation. Since the measurement is made on the venous side of brain circulation, the radioactivity concentration might be reduced by initial extraction of the tracer to brain. Nonetheless, we see a very high agreement and correlation between VC-AUC_{120} and the corresponding initial AUCs measured in brain tissue, which is doubtless a reflection of the very rapid equilibration of free [^{18}F]-fallypride across the blood–brain barrier as reported in living non-human primate (Vandehey et al., 2010). Due to the rather high plasma/tissue extraction rate of [^{18}F]-fallypride (K_1: 50 mL hg^{-1} min^{-1}), the AUC_{120} measured in the VC is related of the unmeasured arterial input during that same interval. In spite of the several sources of variance, the relative standard deviation (CoV) of our VC-AUC_{120} measurement (25%) were only slightly higher than that reported for the [^{18}F]-fallypride K_1 in brain of non-human primates (20%; cited above), or comparable to the relative standard deviation of global CBF in an [^{123}I]IMP-SPECT study of healthy humans (Inoue et al., 2006). Our choice of the VC-VOI as a CBF surrogate measure was intended to avoid bias arising from specific ligand binding, which might occur in the initial minutes of the PET recording. In as much as the correlations between VC-AUC and the tissue AUC_{120} values were highly significant, we expect that these effects were likely small, such that early tissue TACs are also a function of CBF.

The main finding of the present analysis is a significant correlation between the age-corrected [^{18}F]-fallypride BP_{ND} in representative high binding regions (putamen and NC) with the corresponding image-derived surrogate of mean global CBF, i.e. VC-AUC_{120}. Within this group of subjects, who were free of any drug treatment altering central nervous system function, approximately 9% of the variance in BP_{ND} in high-binding regions could be explained by VC-AUC_{120}, a contribution similar in magnitude to the well-know effect of age on dopamine $D_{2/3}$ receptor availability. Using the multiple linear regression analysis with sequential sum of square calculations, age still explains some variance of BP_{ND} after correction for VC-AUC_{120}. Together with the lack of significant age-effects on our VC-AUC_{120} results, these data do not suggest that the age-related decline of BP_{ND} is mainly driven by a global decline of CBF in the aging brain.

In a previous combined [^{11}C]-raclopride/[^{15}O]H_2O-PET study of cats, very large increases of global CBF evoked by the anaesthetic halothane in comparison to the awake state resulted in bias in the apparent BP_{ND} in striatum for the lower affinity dopamine $D_{2/3}$ receptor ligand [^{11}C]-raclopride (Hassoun et al., 2003). Here we find preliminary evidence that individual differences in global brain perfusion of healthy awake humans may bias the estimation of [^{18}F]-fallypride BP_{ND} in striatum. Despite the provisional nature of our CBF surrogate, our observation has face validity, given that the apparent dependence of BP_{ND} estimates on tracer delivery was present in striatum, but not in thalamus or the GTi, where 3 h is indisputably sufficient time for obtaining a transient equilibrium of the specific binding. While the absence of a significant correlation in the low-binding regions does not prove independence from CBF, since covariance of BP_{ND} is worse in extrastriatal regions (CoV 0.20 in putamen versus 0.25 in thalamus), a parsimonious explanation for the lack of association between BP_{ND} in low binding regions and our CBF surrogate is that equilibrium binding is more perfectly obtained. We have early shown that transient equilibrium was obtained within approximately 2 h in putamen, such that truncation of [^{18}F]-fallypride scans to 2 h caused only a 6% decrease of the mean striatal BP_{ND} estimate (Vernaleken et al., 2011). Accordingly, truncation to 2 h in the present investigation caused only a small decline in the correlation between VC-AUC_{120} and putamen-BP_{ND} ($r_{180\ min}$ = 0.305; r_{120min} = 0.281). Present observations with our surrogate, image-derived indicator of global CBF may merit further investigation in a study of prospective design, perhaps employing hybrid PET/MRI technology for simultaneous PET recordings and spin-labeling CBF measurements, or by serial PET studies of CBF and [^{18}F]-fallypride binding.

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