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EDITORIAL COMMENTARY

Testing for radioligand sensitivity to endogenous neurotransmitter release

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Over the last decade, important advances in our understanding of the regulation of the dopaminergic transmitter system in the human brain have been achieved. This knowledge has for a large part been obtained through the use of PET and SPECT radioligands susceptible to pharmacologically or physiologically evoked alterations in endogenous dopamine levels [1]. The results of these studies have revealed, for example, enhanced amphetamine-evoked dopamine release in the brain of patients with schizophrenia [2, 3], leading to a reassessment of the role of presynaptic factors in the pathophysiology of schizophrenia [4].

The mechanism behind the observed reduction in in vivo radiotracer binding in conjunction with a stimulus that increases the endogenous release of neurotransmitter is still disputed. Suggested explanations for this sensitivity, so far most consistently observed with certain dopamine $D_{2/3}$ receptor antagonist ligands, include neurotransmitter competition at the receptor-ligand level, internalization of the receptor and/or radioligand-selective binding to different affinity states of the receptor. In addition to simple competition from dopamine, agonist-induced internalization of membrane-bound dopamine receptors has also been used to explain changes in benzamide binding after amphetamine-evoked dopamine release [1]. Indeed, there can occur uncoupling of the temporal changes of extracellular dopamine concentrations measured by microdialysis and the declines in benzamide radioligand binding measured by PET; reduced binding can be of longer duration than the interval of increased dopamine release [5]. With a single PET measurement, a decline in D_2 receptor binding could be caused by a change in both receptor density and/or radioligand affinity. In vivo measurements have confirmed that following a dopamine releasing pharmacological challenge, a change in radioligand affinity does occur [6]. Ex vivo, the absolute concentration of benzamide binding sites declines after amphetamine challenge in rats [7], consistent with receptor internalization in addition to simple competition.

A major drawback with the currently available $D_{2/3}$ receptor radioligands suitable for detection of changes in endogenously released dopamine is that only striatal release is detectable. For many purposes, it would be valuable to have access to radioligands susceptible to extrastriatal dopamine release, as this would allow studies of cortical dopamine release. This requires that one moves from the relatively low-affinity radioligands to radioligands with higher affinity, such as ^{11}C-FLB457.

In this issue of the *European Journal of Nuclear Medicine and Molecular Imaging*, Aalto et al. [8] report a study of exemplary design showing that extrastriatal binding of the $D_{2/3}$ receptor radioligand ^{11}C-FLB457 is not susceptible to increases in dopamine evoked by oral treatment with d-amphetamine. This state-of-the-art study, with a randomized, double-blind, placebo-controlled design, was properly powered to ensure that differences in cerebral ^{11}C-FLB457 binding that were larger than 11% should have been detected. Further, since reference-tissue modelling of high-affinity D_2 receptor PET radioligands has been questioned in several papers [9, 10], the authors also appropriately conducted arterial sampling and confirmed the absence of any significant decline in ^{11}C-FLB457 binding.

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Their finding is seemingly in contrast to the previous observation of Montgomery et al. [11], who in ten subjects undergoing a methylphenidate challenge observed an average decline of between 3% and 7% in the distribution volume of ^{11}C-FLB457. Their observation was only statistically significant when quantification was done with arterial input measurements, and not with the less invasive reference tissue model.

So what can we conclude regarding the ability to measure endogenous dopamine release with ^{11}C-FLB457 PET?

^{11}C-FLB457 PET may eventually be able to quantitatively pick up declines in extrastriatal ^{11}C-FLB457 binding induced by a challenge that acutely releases dopamine. There are, however, a number of conditions that must be fulfilled to achieve solid and statistically significant findings. Firstly, a high specific radioactivity of ^{11}C-FLB457 is a necessity. A significant association between distribution volumes and the amount of "cold" injected FLB457 has been demonstrated [11], and even injected amounts between 0.5 and 1 μg are associated with underestimation of the binding. Radiosynthesis of ^{11}C-FLB457 at a specific radioactivity that enables injection of less than 0.1 μg is complicated, and if unsuccessful, the amount of "cold" FLB457 injected will need to be accounted for. Secondly, the lack of an appropriate brain region without $D_{2/3}$ receptors [9, 10] requires arterial input sampling, even though this is complicated and associated with increased noise levels. Thirdly, given the presumably large increases in cerebral neurotransmitter levels invoked by the pharmacological treatment, with more physiological (smaller) alterations in cerebral neurotransmitter levels, e.g. a change in binding below 5%, inclusion of a very large group of subjects would be required.

It is unclear if localized cerebral changes in ^{11}C-FLB457 binding are more readily detected. Aalto et al. have previously detected a reduced ^{11}C-FLB457 binding confined to the ventrolateral frontal cortex bilaterally and in the left hippocampus and amygdala in response to a working memory task [12].

The full range of factors influencing receptor binding in vivo remains to be revealed. So far, it has been difficult to consistently identify a similar susceptibility to alterations in endogenous neurotransmitter levels for other receptors than the $D_{2/3}$ receptor. No susceptibility has been clearly demonstrated in clinical studies for dopamine D_1 [13, 14] and 5-HT_{2A} receptors [15, 16]. It is predicted that agonist rather than the much more commonly used antagonist radioligands should in principal reveal the physiologically relevant fraction of receptors in living brain, and should possess superior sensitivity to physiological fluctuations in endogenous neurotransmitter levels. Indeed, the vulnerability of the binding of the dopamine agonist *N*-[^{3}H]propylnorapomorphine ([^{3}H]NPA) in mouse striatum to amphetamine challenge has been shown to exceed that of [^{11}C]raclopride [17]. A relatively greater vulnerability of [^{11}C]NPA has also been demonstrated in a recent PET study in nonhuman primates [18]. Thus, agonist ligands based upon the NPA structure have properties superior to those of benzamide antagonists for the detection of perturbed dopamine release, and should consequently be more sensitive probes for the study of the pathophysiology of diverse neuropsychiatric conditions. A European effort is underway to coordinate the development and validation of radiotracers for detection of endogenous neurotransmitter release. Scientists interested in joining the consortium are encouraged to contact Gerry Dawson at gdawson@p1vital.com.

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