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Comment on “In Vivo $^{18}\text{F}]$GE-179 Brain Signal Does Not Show NMDA-Specific Modulation with Drug Challenges in Rodents and Nonhuman Primates”

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Abstract

Schoenberger and colleagues (2018; ACS Chem. Neurosci. 9, 298-305) recently reported attempts to demonstrate specific binding of the positron emission tomography (PET) radiotracer, \[^{18}\text{F}]\text{GE-179}, to NMDA receptors in both rats and Rhesus macaques. GE-179 did not work as expected in animal models; however, we disagree with the authors’ conclusion that “the \[^{18}\text{F}]\text{GE-179} signal seems to be largely nonspecific”.

It is extremely challenging to demonstrate specific binding for the use-dependent NMDA receptor intrachannel ligands such as \[^{18}\text{F}]\text{GE-179} in animals via traditional blocking, due to its low availability of target sites \(B'_{\text{max}}\). Schoenberger and colleagues anaesthetised rats and rhesus monkeys using isoflurane, which has an inhibitory effect on NMDA receptor function and thus would be expected to further reduce the \(B'_{\text{max}}\).

The extent of glutamate release achieved in the provocation experiments is uncertain, as is whether a significant increase in NMDA receptor channel opening can be expected under anaesthesia.

Prior data suggest that the uptake of di-substituted arylguanidine-based ligands such as GE-179 can be reduced by phencyclidine binding site antagonists, if injection is performed in the absence of ketamine and isoflurane anaesthesia, e.g. with GE-179’s antecedent, CNS 5161 (Biegon et al., 2007), and with GMOM (van der Doef et al., 2016). However, the extent of non-specific uptake remains uncertain.

Keywords

\[^{11}\text{C}]\text{CNS 5161}, \[^{18}\text{F}]\text{GE-179}, \text{isoflurane}, \text{ketamine}, \text{NMDA, PET}.
Graphic for Table of Contents

\[ \text{[\textsuperscript{18}F]GE-179} \]

\[ \text{[\textsuperscript{18}F]PK-209} \]

\[ \text{[\textsuperscript{[11]}C]ONS 5161} \]

\[ \text{[\textsuperscript{[123]}I]CONS 1261} \]

\[ \text{[\textsuperscript{[11]}C]GMOM} \]
Introduction

Alterations in N-methyl D-aspartate (NMDA) receptor activation are implicated in the pathophysiology of several neuropsychiatric disorders, including epilepsy, schizophrenia and traumatic brain injury. Imaging NMDA receptor activation in vivo has proven challenging. \[^{18}\text{F}\]GE-179\(^2\) is a candidate positron emission tomography (PET) radiotracer for this purpose that has shown expected changes in both rat and human studies\(^3\)–\(^5\). Schoenberger and colleagues recently reported attempts to demonstrate specific binding of \[^{18}\text{F}\]GE-179 to NMDA receptors in both rats and Rhesus macaques\(^6\) in well-founded experiments simultaneously combining PET and magnetic resonance imaging (MRI). Although the experiments conducted provide solid evidence that GE-179 does not work as expected in animal models, we disagree with the authors’ conclusion that “the \[^{18}\text{F}\]GE-179 signal seems to be largely nonspecific”, for the reasons outlined below.

The challenge of evaluating use-dependent PCP-site radiotracers

Unlike most other neuroreceptor radiotracers, \[^{18}\text{F}\]GE-179 uptake is expected to reflect not only receptor distribution but also receptor “state”, i.e. it should exhibit “use-dependency”. The proportion of NMDA receptors that are in the open state at any one point of time in healthy rodents, macaques and humans is unknown and the estimates of the probability of channel opening vary considerably (e.g. 0.002\(^6\) – 0.3\(^7\)). We believe that it is extremely challenging to demonstrate specific binding for \[^{18}\text{F}\]GE-179 in animals via traditional blocking as in \(^6\), due to its low and inconstant availability of target sites (\(B^\text{max}_\text{max}\)).

Effects of anaesthesia on PCP-site availability

GE-179 \(\left(\text{N-[2-chloro-5-(2-fluoroethylsulfanyl)phenyl]-N'}\text{-methyl-N'}\text{-}(3-methylsulfanylphenyl)guanidine}\right)^2\) is one of several putative di-substituted aryguanidine-based ligands with selectivity for the intrachannel phencyclidine (PCP) binding site of the NMDA receptor. Other molecules in this class that have been radiolabelled for imaging purposes include CNS 1261\(^7\), CNS 5161\(^8\), GMOM\(^9\) and
PK-20910 (Figure 1). The PCP binding site only becomes available when the receptor is in the “open” state, i.e. on simultaneous binding of both the agonist glutamate and a co-agonist such as glycine, accompanied by cell depolarisation.

Blocking studies of putative PCP-site NMDA-selective radiotracers are confounded by the use of general anaesthesia. In reference 6, the rats were anaesthetised via isoflurane inhalation, and the macaques were anaesthetised with ketamine and xylazene, with maintenance via isoflurane inhalation. The use of anaesthesia facilitates the acquisition of high-quality images. However, what is the effect of isoflurane, and the other anaesthetics used on $B_{\text{max}}'$?

Isoflurane and similar volatile anaesthetics have complex mechanisms of action which include a well-described inhibitory effect on NMDA receptor function$^{11-25}$, and which is possibly mediated in part via competitive antagonism at the glutamate$^{26}$ or glycine binding sites$^{24, 26-28}$. Such inhibition would be expected to reduce the already-low $B_{\text{max}}'$ of PCP-site radiotracers such as $[^{18}\text{F}]\text{GE-179}$. Demonstration of signal blockade in such circumstances would be extremely difficult.

Effects of methamphetamine on PCP site availability

In an attempt to increase $B_{\text{max}}'$ via provocation of NMDA receptor channel opening, Schoenberger and colleagues$^6$ injected methamphetamine two minutes prior to injection of $[^{18}\text{F}]\text{GE-179}$ in rat studies and at 48 minutes p.i. in macaques studies, i.e. presumably administered after the induction of anaesthesia. Although single- dose methamphetamine may induce glutamate release$^{29-30}$, there is some evidence that suggests the effect on glutamate release is negligible$^{31-32}$. As the authors acknowledged, amphetamine and methamphetamine can actually directly inhibit the NMDA receptor$^{33-35}$. It is not clear whether significant glutamate release was actually achieved, and whether and when a significant increase in NMDA receptor channel opening can be expected under the competing influences of anaesthesia and perhaps methamphetamine.
Complementary studies of putative PCP-site radiotracers

We interpret the experiments reported in reference 6 in the context of the relevant studies for similar ligands (Figure 1). In short, partial blockade of radiotracer uptake has been achieved, and modest enhancement of the signal with challenge has been reported, as summarised below.

Awake rats

GE-179 is a derivative of CNS 5161 (N-[2-chloro-S-(2-methylsulfanyl)phenyl]-N’-methyl-N’-(3-methylsulfanylphenyl) guanidine); which has a low inhibition constant (K_i) of 1.9 ± 0.6 nM versus MK-801. In non-sedated rats, pre-treatment with cold MK-801 (3mg/kg intraperitoneal) reduced the
cortex-to-cerebellum uptake ratio from 1.45 to 1.20 (i.e. ~17%), approximately, at 90 minutes post-
injection of $[^3]$H]CNS 5161$^{37}$. Whilst complete activation of the NMDA channel is unlikely with
pharmacological manipulation at doses that do not elicit seizures, pre-treatment with NMDA
40mg/kg five minutes prior to injection of radiotracer increased the uptake ratio from 1.45 to 1.60
(i.e. ~10%), approximately, with larger increases (~31%) seen in the hippocampus. Approximately
17% blockade of signal has been seen in rats that were not sedated at the time of injection with

Pre-treatment of a baboon with (cold) MK-801 (after induction of anaesthesia with ketamine
and maintenance with isoflurane) did not significantly reduce $[^{11}]$C]GMOM binding$^9$, and similar to
the results presented in reference $^6$, a slight increase was actually observed. Crucially, however, pre-
treatment of awake rats with MK-801 (1 mg/kg intravenous) five minutes prior to injection produced
a uniform decrease in binding of up to 28%$^9$. The discrepant findings between awake rats and
anaesthetised baboons are consistent with an anaesthesia-induced reduction of $B'_{max}$, in vivo. Pre-
treatment of the awake rats with the co-agonist D-serine produced increases in binding of up to
24%, whereas the NR2B-selective antagonist Ro25-6981 produced decreases of up to 38%. Blockade
of the binding of a $[^{18}]$F]PK-209, a $[^{11}]$C]GMOM derivative, has also been seen with MK-801 pre-
treatment in awake rats$^{10}$, and in Rhesus macaques that were anaesthetised using agents other
than ketamine and isoflurane$^{38}$. Awake humans

A uniform decrease in $[^{11}]$C]GMOM influx constant of approximately 66% was observed in six healthy,
non-sedated human participants following early and prolonged administration of the low-affinity
PCP-site antagonist, S-ketamine$^{39}$. The decrease in radioactivity concentration (kBq/ml), as opposed
to influx constant, was not quantified but appeared to be modest (see “Data Analysis section below)
– opposed by a slight (7%) increase in perfusion/extraction and accumulation in the non-specific
compartment ($V_{NS}$; 10%). A reduction in volume-of-distribution ($V_i$) of approximately 20% has also
been observed with $[^{123}]$I]CNS-126$^{40,41}$. 8
Data Analysis

In the rat experiments, Schoenberger et al inferred the absence of an effect from the failure to observe ‘a meaningful change in the whole-brain TAC’. Whilst in some experiments the displacement is so clear simple assessment of the whole-brain time-activity curve will suffice, a more rigorous quantitative analysis of the data is usually required to establish the presence of an effect. Drug competition can cause changes of the bioavailability of the PET tracer due to changes of the peripheral metabolism, alterations of the delivery, binding to peripheral sites, etc. Therefore model-based quantification of regional tracer binding in brain tissue is usually preferred over simpler methods when the expected effect sizes are in the order of a few per cent. The possibility cannot be excluded that the authors missed small but measurable effects in their in vivo rat experiments because they did not calculate quantitative measures of regional [18F]GE-179 binding.

Discussion

Taken together, these data suggest that the uptake/binding of di-substituted arylguanidine-based NMDA-selective radiotracers can be reduced by PCP site antagonists, if injection is performed in the absence of ketamine and isoflurane anaesthesia. We expect that this should be the case for [18F]GE-179, particularly since it has already been reported for its antecedent [11C]CNS-516137. Moreover, increased uptake/binding in non-sedated specimens has been demonstrated via direct provocation of channel opening with the agonist NMDA37 and alternatively with the co-agonist D-serine9. Increased uptake/binding has also been demonstrated in conditions in which increased “endogenous” NMDA receptor channel opening is expected, such as deep brain stimulation44, dyskinesias45, epilepsy5-44, and cerebral ischaemia46. There is good evidence, therefore, that these radiotracers specifically bind to the PCP site in vivo, and we suggest that the divergent findings in are explicable by the use of isoflurane and/or ketamine anaesthesia. A within-subject paired study design, in which [18F]GE-179 is administered prior to anaesthesia for one scan and subsequent to anaesthesia in the other, would allow this hypothesis to be tested. If confirmed and if it proves
valuable to perform pharmacological (or other) challenge before the induction of anaesthesia, this would advantage F-18 labelled agents such as $^{[18F]}$GE-179 over those limited by the short radioactive half-life of C-11 (e.g. $^{[11C]}$GMOM).

The extent of blockade or alternatively the extent of enhancement that has been achieved thus far, in terms of change in radioactivity concentration, has been modest. The results presented in reference 39 suggest that alterations in perfusion/extraction can confound the detection of blockade (and presumably enhancement). The modest alterations in signal might also have resulted in part from incomplete receptor blockade/enhancement; for example, Van der Doef and colleagues estimated that their ketamine dosing regimen (total 0.3 mg/kg over 135 minutes) resulted in an average occupancy of the PCP binding site of only approximately 19%39. Hence, it is not immediately apparent that non-specific binding should be particularly marked for GE-179, given its lipophilicity (LogD$_{7.4}$ = 2.5 ± 0.1). However, the low volume of distribution observed for the second compartment ($V_s$) of kinetic models is consistent with low specific binding in healthy specimens.

In conclusion, we believe that the experiments described in reference 6, which contrast with those of several related studies9-10, 37, 39-41, 47-48, do not adequately resolve the question of specific versus nonspecific binding for $^{[18F]}$GE-179 and similar radiotracers. Evaluation of NMDA receptor-selective radiotracers is a challenging endeavour that will require continued experimental innovation. The data to date suggests that diarylguanidine-based PCP-site tracers are sensitive to channel opening in awake specimens, whereas the extent of non-specific uptake remains uncertain. $^{[18F]}$GE-179 and $^{[11C]}$GMOM might still find use in clinical populations in which marked alterations in channel opening probability are expected.

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Conflicts of Interest

CJM, DARDB, WT, DJB, JSD, MJK, and AH have conducted a study that used [18F]GE-179 and was supported in part by GE Healthcare Ltd. EH, FL and JS are employees of GE Healthcare Ltd. EA and WT were employees of GE Healthcare Ltd. CJM, JSD, and MJK have received fees from GE Healthcare Ltd, but have never been employees of the organisation. JSD has also received fees from UCB Pharma, Eisai, and GSK. The remaining authors do not declare any conflicts of interest.

References


22. Nishikawa, K., and Maciver, M. B. (2000) Excitatory synaptic transmission mediated by NMDA receptors is more sensitive to isoflurane than are non-NMDA receptor-mediated responses. *Anesthesiology* 92, 228-36.


