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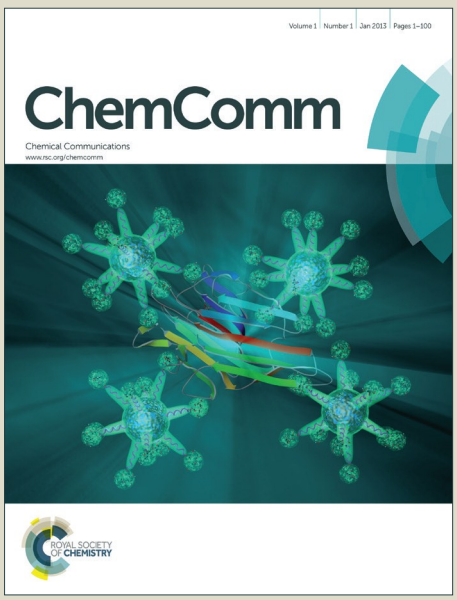

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FEATURE ARTICLE

PET tracers for imaging brain α7 nicotinic receptors: an update

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S. Chalon,[a,*] J. Vercouillie,[a] D. Guilloteau, [a] F. Suzenet,[b] S. Routier[b,*]

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Abstract. Positron emission tomography (PET) molecular imaging of brain targets is a powerful tool to diagnose, follow up, and develop treatments and personalized medicine in a number of acute and chronic brain disorders. The availability of β+ emitter tracers labelled with [^{11}C] or [^{18}F] having optimal characteristics of affinity and selectivity for alpha-7 nicotinic receptors (α7R) has received considerable attention, due to the major implication of these receptors in brain functions. The aim of this review is to identify the interest and need for the *in vivo* exploration of α7R by PET molecular imaging, which tools are currently available for this and how to progress.

1. Introduction.

Positron emission tomography (PET) molecular imaging of brain targets such as receptors, transporters, or abnormal proteins is a powerful tool to diagnose, follow up, and develop treatments and personalized medicine in a number of acute and chronic brain disorders. This method requires the availability of tracers labelled with β+ emitters such as [^{11}C] or [^{18}F] that have optimal characteristics of affinity and selectivity for the target, passage through the blood-brain-barrier (BBB), kinetics and *in vivo* metabolism, allowing specific *in vivo* quantification of the target. The alpha-7 nicotinic receptor (α7R) is a target of great interest due to its major implications in a number of brain disorders, and in recent years many efforts have been deployed to obtain an efficient PET tracer for it.

The aim of this review is to identify the interest and need for the *in vivo* exploration of α7R by PET molecular imaging, the tools that are currently available for this and how to progress.

1.1. The α7 nicotinic receptor, a multiple actor of brain function.

The brain cholinergic system is mainly constituted of long neurons with cell bodies in the basal forebrain and projections in the cortex, hippocampus and amygdala. It regulates a number of processes which are crucial for a large variety of physiologic and cognitive functions. These effects are mainly mediated through neuronal nicotinic receptors belonging to the family of ligand-gated ion channels. These receptors are structurally composed of 5 subunits belonging to the α subfamily (9 subunits, α2-α10 except α8 in mammalian) and/or β subfamily (3 subunits, β2-β4), this composition determining their pharmacological and functional properties. In the human brain, the heteropentameric α4β2 and homopentameric α7 subtypes are predominant.[1] α7R has several pharmacological characteristics such as a high permeability to calcium, low sensitivity to acetylcholine and high affinity for α-bungarotoxin[2].

Nicotinic receptors are present in several areas of the central nervous system (CNS). On neurons, they are localized preferentially pre-synaptically, where they regulate the release of several neurotransmitters, but they can also be post-synaptic.[3] Besides their neuronal localization, α7R are also present and functional on glial cells.[4]

The identification of α7R throughout the brain originates from *in situ* hybridization for mRNA, and autoradiography, western blotting and immunohistochemistry for protein expression although there is not always concordance between the localization of mRNA and α7R protein.[5] The most widely used *in vitro* α7R tracers are the potent antagonists [^{125}I]α-Bungarotoxin ([^{125}I]α-Bgt) and [^{3}H]methyllycaconitine ([^{3}H]MLA). These tools have made it possible to detect a fairly similar α7R distribution in the human and rodent brain. In the human brain, the density of [^{125}I]α-Bgt binding sites is high in the cortex (temporal), hippocampus, amygdala, forebrain (nucleus basalis of Meynert), thalamus and hypothalamus, medium in the cerebellum, and very low in the caudate-putamen.[6] In the rat brain, binding studies with [^{125}I]α-Bgt and [^{3}H]MLA show a high density of α7R in the cortex, hypothalamus, hippocampus, inferior colliculus and several brain stem nuclei, while the thalamus, striatum and cerebellum have a very low signal.[7]

The physiological functions of brain α7R are varied. A key feature of α7R is its high permeability to calcium[8], inducing the activation of multiple calcium-dependent kinases resulting in short- and long-term changes in synaptic plasticity.[9] One consequence of this is the role of α7R in the regulation of neuron survival, and thus in neurodegenerative diseases associated to apoptosis. Another major role of α7R is the modulation of transmitter release in various brain regions. Due to its localization at the presynaptic level, it is for example involved in the release of glutamate[10] and GABA.[11]

Although α7R appear to have important physiological functions, transgenic mice constitutively deficient in these receptors lacked [^{125}I]α-Bgt binding sites, as expected, but had surprisingly normal growth, general appearance and neuroanatomy.[12] Similarly, the deficiency in α7R specifically in astrocytes did not result in any abnormality in physical and behavioural parameters.[13] However, the mice showed impaired attention[14] and appeared to present dysfunctions in the dopamine-related effects of stimulation by nicotine.[15]

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Besides their effects on neurotransmission, α7R are involved in the modulation of neuroinflammatory processes, and agonists of these receptors are more efficient than acetylcholine at inhibiting the inflammatory signalling and production of pro-inflammatory cytokines from immune cells.[16] One hallmark of neuroinflammation is the activation of microglia. *In vitro* studies on primary cultured microglial cells have shown that they are able to synthetize acetylcholine.[17] They also express α7R and can modulate the TNF-α release evoked by LPS through these receptors, this effect being counteracted by α-Bgt.[18] This link between α7R and neuroinflammation has a particular significance in Alzheimer's disease (AD) as activated microglia are often co-localized with amyloid plaques, and seem to exacerbate tau pathology.[2] All this suggests that acetylcholine may regulate neuroinflammation through microglial α7R, resulting in a shift from proinflammatory to a neuroprotective effect, even if the precise mechanisms are not totally understood.[19]

1.2. α7R and brain disorders.

Presynaptic α7R are essential for cognitive processing. A physiological decline in these receptors associated to age has been described in several brain areas,[20] and they are involved in a number of brain disorders such as drug addiction, schizophrenia and neurodegenerative diseases such as Alzheimer's and Parkinson's disease.

1.2.1. Schizophrenia

A large body of epidemiological, genetic and pathophysiological evidence links schizophrenia to the α7R, as reviewed by Young and Geyer.[21] The density of these receptors was found to be reduced in several specific brain regions,[22] and this decrease was related to the degree of cognitive dysfunction.[23] However, other factors may impact this density such as the treatment (antipsychotic), smoking or age. Besides, preclinical studies examined the capability of agonists and positive allosteric modulators (PAM) of α7R to improve behavioral abnormalities associated with schizophrenia.[21,24] The beneficial effects of several of these compounds were evidenced in relevant animal models[24,25], confirming that the stimulation of α7R could enhance cognitive processes. In humans, the proof-of-concept of this role was first demonstrated in a cohort of non-smoking schizophrenic patients with the partial agonist DMXB-A[26]. From that time, several clinical trials involving different compounds were conducted with either reserved or promising still ongoing trials.[25]

In this context, *in vivo* PET exploration of α7R in different sub-populations of patients (e.g. smoking or non-smoking, first episode untreated or chronically treated) would be highly valuable to assess the links between these receptors and symptoms (positive/negative), cognitive performance and outcome, and adapt the treatment accordingly.

1.2.2. Alzheimer's disease

In AD, cholinergic neurons are particularly vulnerable as they preferentially accumulate the β-amyloid peptide (Aβ) and neurofibrillary tangles.[27] Modifications in the number of α7R during AD have not been clearly assessed. Increased levels have been described in early stages,[28] which could be due to a compensatory mechanism and/or consequence of the desensitization due to the known association of α7R with Aβ. Indeed, a role of α7R in AD could also be related to the potential and complex interactions between the receptors and both fibrillary and oligomeric Aβ.[29] It has been recently proposed that in early AD, the activation of α7R through interaction with soluble Aβ oligomers may have a neuroprotective and neurotrophic effect whereas in later stages the accumulation of Aβ induces a functional blockade of α7R.[30]

At advanced stages of AD, no change or various reductions of α7R have been observed, depending on the methods used and the brain regions considered.[31] In view of their multiple roles in AD, α7R appear as highly promising targets for diagnosis and therapy due to 1) their change in density during the course of the disease, 2) the evidence of links between them and specific signaling cascades, neurodegeneration and neuroinflammation, 3) the potential neuroprotective effects provided by positive allosteric modulators and/or agonists of these receptors.[32]

Although PET imaging in AD has exploded in recent years with the development of [^{11}C]- and [^{18}F]-labeled tracers of β-amyloid plaques[33] and more recently of phosphorylated Tau,[34] complementary PET exploration of other molecular targets, in particular α7R, has still a prominent place due to their early and multiple implication in the course of the disease.

2. Development of a PET tracer of α7 nicotinic receptors: which approaches and results?

The first PET tracer used for imaging nicotinic receptors in AD was [^{11}C]nicotine, which showed significantly reduced brain accumulation in diseased subjects, but had drawbacks such as high level of non-specific binding, rapid metabolism and rapid washout from the brain.[35] From that time, a number of new [^{11}C]- and [^{18}F]-labeled compounds have been developed, given that [^{18}F]-labeled tracers are more suitable for extensive clinical application due to the half-life of [^{18}F] (109.6 *vs* 20.4 min for [^{11}C]). The development of PET tracers for α7R imaging already been reviewed.[36] As most of these tracers have not yet been adapted for clinical application, this challenge is still topical and new data have recently emerged.

In the last few years, new α7R PET tracers were developed such as [^{11}C]CHIBA-1001 and several other 1,4-diazabicyclo[3.2.2.]nonane derivatives, i.e. [^{11}C]NS-14492, [^{18}F]NS-10743 and [^{18}F]NS-14490 (Fig.1).[37] Although they passed through the BBB, the *in vivo* specific binding of these radioligands was globally modest, with a ratio of less than two between the brain regions with the highest density in α7R such as the cortex and hippocampus, and the cerebellum defined as the non-specific binding region.

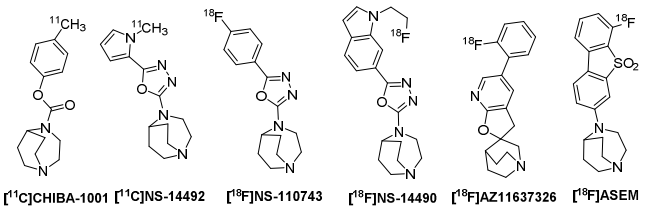

Fig.1 Structure of recently developed α7R PET tracers.

Another chemical family was investigated, leading to the spirofuropyridine derivative called AZ11637326, a high affinity ligand of α7R (Kd = 0.2 nM) which, labeled with [^{3}H] and evaluated in rat brain, showed very promising properties with a good brain penetration and a ratio between the regions of interest and the cerebellum around 5.[38] However, the fluorinated analog [^{18}F]AZ11637326 showed disappointing

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results both in rodents and the monkey regarding *in vivo* specific binding.[39]

The best α7R PET tracers described today are two fluorinated derivatives of dibenzo[*b,d*]thiophendioxide called ASEM and its para-isomer.[40] Both had high selectivity vs. other nicotinic subtypes and 5-HT_3 receptors. [^{18}F]ASEM had the highest affinity (Ki of 0.4 vs. 1.3 nM) and showed a high uptake in the mouse brain (3-4% injected dose/g tissue in the regions of interest at 90 min post i.v. injection), a brain distribution in agreement with the α7R localization (a ratio of the cortex and hippocampus to the cerebellum around 5), and an *in vivo* specific binding blocked by α7R ligands. [^{18}F]ASEM also appeared as a highly reliable α7R tracer in the baboon, with BP_{ND} values of 3.9 to 6.6 in the target regions such as the thalamus, cortex and hippocampus as well as in preliminary studies performed in humans.[41]

Conclusions and future directions.

A PET radiotracer useful for quantitative imaging of a brain target (receptor, transporter, normal or abnormal protein), must fulfil a number of properties such as a high affinity and selectivity for the target, ability to pass through the BBB, lack of active radiometabolites, brain kinetics adapted to the radioactive half-life, and high specific radioactivity.[42]

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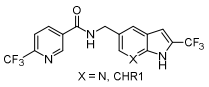
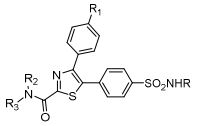
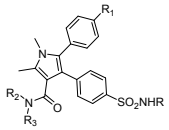
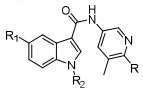
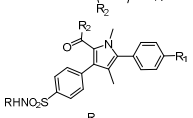
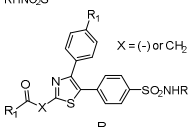
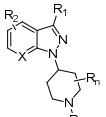
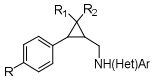
1	US20150038517	Proximagen	
2	WO2014203150	Lupin	
3	WO2014203150	Lupin	
4	WO2014172759	Bionomics	
5	WO2014195848	Lupin	
6	WO2014072957	Lupin	
7	US20140121243	Dainippon Sumitomo Pharma	
8	WO2014019023	Merck Sharp & Dohme	
9	US20140057921	Novartis	

Table 1 Structure of newly patented α7R ligands (Patentscope used).

The crossing of the BBB is of course an indispensable requirement for a useful PET brain tracer, and the passive brain entry of a compound is mainly influenced by its lipophilicity but also by its non-specific binding.[42,43] In the case of α7R, the selectivity of potential ligands is more complex because of its high structural homology with 5-HT3 receptors[36,44], and its potential heteromeric form due to the assembly of α7 with β2 or α7dup as recently described ([25]for review). The relatively low density of α7Rs in target brain regions in human,[22] non-human primate and rodent brain[7], and their potential intra-cellular localization represent additional difficulties for a tracer to allow in vivo quantification of the functional receptor. In addition to these requirements linked to the physico-chemical and pharmacological properties of the "vector" part of the tracer, it is also necessary to have available precursors which will allow the introduction of fluor-18 in one-step, because of the short half-life of this β+ emitter.

The comparison of some characteristics of [^{18}F]AZ11637326[39] and [^{18}F]ASEM[40] shows that under the same assay conditions, the latter exhibits superior binding affinity to α7R[40], with a Ki of 0.4 vs. 3.3 nM. In addition, the specific radioactivity was more than 100 times higher for [^{18}F]ASEM39[40] than for [^{18}F]AZ11637326.[39] This property is probably highly relevant to reduce *in vivo* competition between the labelled and stable form of the ligand which is of particular value in the case of low density targets.

It appears that despite the huge potential interest to quantify brain α7R by PET, the numerous attempts to obtain a useful tracer are globally disappointing, with to date a unique promising candidate. However, since 2014 several patents described new α7R ligands (Table 1). While some of them follow the well-known pharmacophoric model (i.e., a strong basic centre followed by the attachment of a hydrogen bound donor-acceptor, highly electron rich, and terminated by an aryl structure), a novel generation of ligands has nevertheless appeared.

It is now time to plan clinical studies with the most promising radioligand [^{18}F]ASEM in order to evaluate the status of α7R in different pathological situations such as prodromal stages of AD (MCI) and PD, or schizophrenia, and to explore these receptors under different treatments, in particular aimed at the modulation of neuroinflammation.

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[a] UMR Inserm U930, Université François-Rabelais de Tours, F-37000 Tours, France. sylvie.chalon@univ-tours.fr

[b] Univ. Orleans, CNRS, UMR7311, Institut de Chimie Organique et Analytique, rue de Chartres, BP 6759, F-46067 Orléans Cedex 2, France. sylvain.routier@univ-orleans.fr

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