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Probing the cholinergic system to understand neurodegenerative diseases

“...the discovery of new radioligands is a real challenge for chemists and radiochemists.”

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The cholinergic system plays an important role in number of cognitive functions, including memory, selective attention and emotional processing [1]. Acetylcholine (ACh) is a ubiquitous neurotransmitter with widespread innervation in the cortex, subcortical structures and the cerebellum. The three main projection systems of the CNS are the nucleus basalis of Meynert, the pedunculopontine nucleus and cholinergic neurons in the striatum. ACh is produced from choline and acetate, as starting substrates, by action of the choline acetyltransferase and produced ACh is stored into synaptic vesicles by vesicular ACh transporter (VACHT). The two important classes of the postsynaptic cholinergic receptors are the nicotinic receptors (nAChRs) and the muscarinic receptors (mAChRs). The nAChRs are ligand-gated ion channels permeable to cations such as Na, K and Ca. Neuronal nAChRs are homopentameric and heteropentameric combinations of α and β subunits. Among the nAChRs subtypes in the CNS, $\alpha 4\beta 2$ and $\alpha 7$ nAChRs are predominant in the brain and play a role in the neurodegenerative processes [2]. Muscarinic receptors belong to the family of G-protein-coupled receptors (GPCRs) and there are five subtypes of receptors, named M1–M5. M1, M3 and M5 receptors are coupled with Gq proteins, whereas M2 and M4 receptors are coupled with G_{i/o} proteins. M1 receptors are abundantly expressed in brain including the cerebral cortex, hippo-

campus and striatum, and they are consequently involved in the learning and memory processes [3].

Altered levels of ACh or modified receptors expression and function in the nervous system have been described in several neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease – especially with dementia (PDD) [4,5]. AD is the most common age-related form of dementia and is characterized by progressive memory loss, decline in language skills, behavioral disturbances and other cognitive impairments. In 2015, WHO estimated 47.5 million people affected by AD worldwide and the prevalence of dementia is expected to reach 135.5 million in 2050. AD has been described as a complex and multifactorial disease and histologically characterized by extra neuronal amyloid- β protein deposits and intraneuronal hyperphosphorylated T-protein aggregation. Other cellular hallmarks include oxidative stress, dyshomeostasis of biometals, a loss of synapses and cell death, especially in the cholinergic neurons network [6]. The cholinergic hypothesis submits that the loss of cognitive and memory associated with AD are based on the observation of a deficiency of ACh in the CNS [7]. Therefore, the most widely used concept for AD drug development during these last 20 years, is especially based on increasing the brain ACh levels by inhibiting acetylcholinesterase (AChE), the enzyme that hydrolyses ACh in choline and acetate. Although their efficacies are moder-

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ate, AChE inhibitors (tacrine, galantamine, rivastigmine and donepezil) are the sole approved drugs as palliative treatments in order to restore the cognitive functions of AD patients. Besides, M1 agonists and positive allosteric modulator (PAM), as well as, $\alpha 7$ agonists and PAMs are also developed as therapeutic approaches to increase cholinergic function in neurodegenerative processes.

In order to understand the role of each receptor and enzyme involved in the cholinergic system and in the neurodegenerative processes, molecular imaging techniques such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT) using specific radioligands have been applied for *in vivo* imaging, while applications of the fluorescence imaging have been developed for *in vitro* and *in cellulo* studies. The latter, cheaper and safer than PET/SPECT imaging system, is a good alternative to localize the receptors as well as to lead kinetic and thermodynamic studies on live or fixed tissues.

“...fluorescence resonance energy transfer assays have been reported to probe human M1 receptors using fluorescent positive allosteric modulator.”

Many efforts have been made to develop radioligands for imaging human brain nAChRs [8]. Radioligands including [^{11}C]-nicotine, 2-[^{18}F]-FA and [^{18}F]-AZAN have been used in clinical studies for *in vivo* imaging of $\alpha 4\beta 2$ nAChRs within the brain of AD and PDD patients [9]. [^{11}C]-nicotine presents a nonspecific binding of $\alpha 4\beta 2$ nAChRs, whereas 2-[^{18}F]-FA or [^{18}F]-AZAN, developed by Hordi *et al.*, are more suitable radioligands for imaging the brain distribution of $\alpha 4\beta 2$ nAChRs in human. Although numerous $\alpha 7$ nAChRs agonists have been developed to treat AD, schizophrenia and depression [10], the development of efficient $\alpha 7$ nAChRs radioligands is more challenging and, nowadays, none of the developed PET or SPECT radiotracers for imaging human brain $\alpha 7$ nAChRs shows an efficient binding selectivity for a clinical development. Regarding the fluorescent probes, there are only two fluorescent dyes for imaging nAChRs. Fluorescent epibatidine agonists were ideally suited for nAChRs *in vitro*, *in cellulo* or even native tissues [11], and, more recently, 3-amino-quinuclidine derivatives have been reported as partial agonists of $\alpha 7$ nAChRs. Besides, fluorescent ACh derivatives have also been examined and showed a subnanomolar affinity toward Torpedo nAChRs [12].

Numerous PET/SPECT radioligands for imaging mAChRs, including [^{11}C]-NMPB or [^{123}I]-QNB, have been reported for AD and PDD patients, nevertheless all these radiotracers showed a low selectivity toward

mAChRs [13]. Regarding fluorescent imaging of these GPCRs, fluorescence resonance energy transfer assays have been reported to probe human M1 receptors using fluorescent PAM [14].

AChE is known for decades as a reliable marker for brain cholinergic nerve termini, and the most commonly used radiotracers to image the distribution of AChE in brain are two radiolabeled lipophilic ACh analogs, [^{11}C]-PMP and [^{11}C]-MP4A, that diffuse readily into brain [15]. Radiolabeling of AChE inhibitors have also been undertaken for mapping cerebral AChE activity. While distribution of [^{11}C]-methyl-tacrine and [^{11}C]-donepezil did not correlate with brain AChE distribution due to a nonspecific binding, (-)-[^{11}C]-galantamine showed an accumulation in the AChE-abundant regions of brain. Nevertheless, none of these radiolabeled AChE ligands are used for imaging human brain AChE in clinical studies [16]. Besides, we have prepared three [^{18}F]-huprines known as potent and selective AChE inhibitors. The radiotracers showed binding in the rat striatum, rich in AChE but low binding in the surrounding cortex, poor in AChE [17]. Regarding the fluorescent imaging of AChE, we have recently developed two near infrared fluorescent probes HupNIR1 and HupNIR2 based on the huprine scaffold and cyanine 5.0 dye. These probes are the first ones described in the literature showing a great selectivity and sensitivity for active AChE even in the brain region displaying a low AChE concentration as striatum [18].

The vesicular cholinergic transporters (VACHT) are localized in the membrane of the presynaptic vesicle where ACh is accumulated, and play an important role in the regulation of cholinergic activity [19]. Many PET/SPECT radiotracers targeting VACHT have been developed, nevertheless [^{123}I]-IBVM, an analog of vesamicol, is the unique radiotracer used for human *in vivo* imaging [20]. Regarding the fluorescent VACHT imaging, experiments have been investigated using green fluorescent protein-tagged VACHT in living cholinergic cells [21].

Many PET or SPECT radioligands have been developed to understand the role of receptors or enzyme involved in the dysfunction of cholinergic system observed in neurodegenerative diseases such as AD and PDD. Numerous existing radiotracers show a non-specificity or a low affinity toward the target, and are unusable for *in vivo* imaging. Therefore, the discovery of new radioligands is a real challenge for chemists and radiochemists. Besides, the development of fluorescent selective probes for receptors involved in the dysfunction of cholinergic system is also required by biologists and neurobiologists.

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