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Purinergic Receptors of the Central Nervous System: Biology, PET Ligands, and Their Applications

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Abstract

Purinergic receptors play important roles in central nervous system (CNS). These receptors are involved in cellular neuroinflammatory responses that regulate functions of neurons, microglial and astrocytes. Based on their endogenous ligands, purinergic receptors are classified into P1 or adenosine, P2X and P2Y receptors. During brain injury or under pathological conditions, rapid diffusion of extracellular adenosine triphosphate (ATP) or uridine triphosphate (UTP) from the damaged cells, promote microglial activation that result in the changes in expression of several of these receptors in the brain.

Imaging of the purinergic receptors with selective Positron Emission Tomography (PET) radioligands has advanced our understanding of the functional roles of some of these receptors in healthy and diseased brains. In this review, we have accumulated a list of currently available PET radioligands of the purinergic receptors that are used to elucidate the receptor functions and participations in CNS disorders. We have also reviewed receptors lacking radiotracer, laying the foundation for future discoveries of novel PET radioligands to reveal these receptors roles in CNS disorders.

Keywords

purinergic receptors, central nervous system, PET ligands, biology, neuroinflammation

Introduction

The cell surface purinergic receptors (purinoceptors) are plasma membrane proteins found in nearly all mammalian tissues including the central nervous system (CNS).[1] The history of the purinergic receptors goes back to early 20th century when, for the first time, an observation was made that purines effected cardiovascular physiology.[2] Almost half a century later, these receptors were classified based on their endogenous ligands into P1 and P2 categories.[3]

P1 or adenosine receptors (ARs) are a family of G protein–coupled receptors (GPCRs) with 4 subtypes: A_1, A_{2A}, A_{2B}, and A_3. P2 receptors are subgrouped into the ligand-gated ion channel receptors P2X with 7 receptor subtypes: $P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, $P2X_5$, $P2X_6$, $P2X_7$, and P2Y, which are G protein-coupled metabotropic receptors with 8 subtypes: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ (Figure 1).[4] Burnstock has recently published an excellent review article on purinergic receptors, their distributions, and functions revealing the importance of these receptors in physiological system.[5] Purinergic receptors play major roles in CNS disorders including Alzheimer disease (AD), Parkinson disease (PD), Huntington disease (HD), frontotemporal dementia (FD), amyotrophic lateral sclerosis (ALS), multiple scleroses (MS), traumatic brain injury (TBI), stroke, cerebral ischemia, epilepsy, psychiatric diseases, sleep disorder, and neuropathic pain.[1,3,6,7]

In the CNS, adenosine 5′-triphosphate (ATP), an energy source for neurons and glial cells, also acts as an extracellular purinergic signaling molecule that controls communication between brain cells.[8] The steady state concentration of cytosolic ATP is high, ranging between 5 and 10 mM, and very low (nM) in the extracellular space.[9] Under pathological conditions and CNS insults such as trauma, ischemic stroke, epileptogenic seizures, cellular stress, neuroinflammation, and neurodegenerative disorders, high concentration

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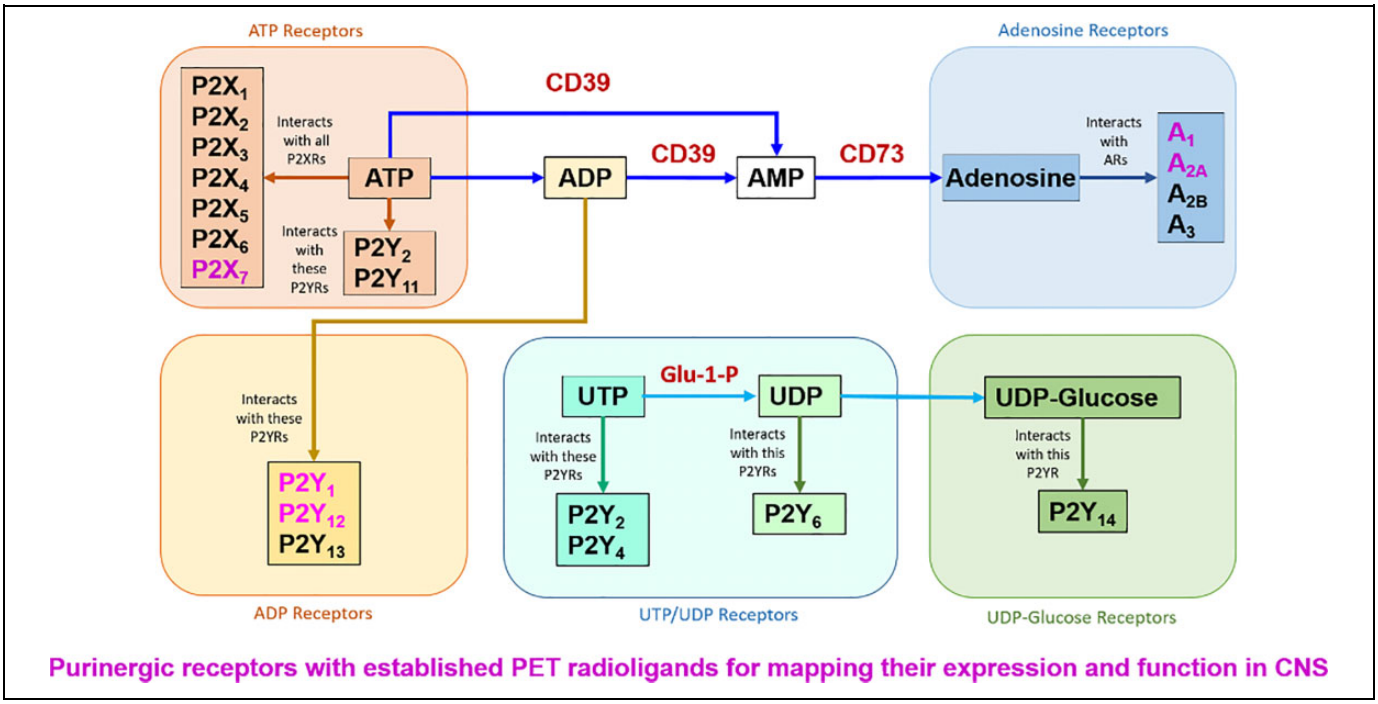

Figure 1. Purinergic receptor subfamilies and their endogenous ligands.

of ATP is released to the extracellular region as a danger signal creating a cascade of events that eventually damages the neurons.[10,11]

High level of extracellular ATP from the damaged cells enforces microglia to undergo chemotaxis to the site of injury in order to remove cell debris from these sites.[12] Microglial activation[13] results in upregulation of $P2X_4$ and $P2X_7$[14,15] and downregulation of $P2Y_{12}$ receptor expression.[16] This balance between the expression of $P2X_4$, $P2X_7$, and $P2Y_{12}$ receptors dictate the destiny of microglia.[17] A relative expression levels of $P2X_4$ and $P2X_7$ receptors are positive indicator of microglial activation, while $P2Y_{12}$ receptor is a negative predictor.[18] Additionally, upon release of the large amount of ATP (hundreds of μmol), $P2Y_1$ and $P2X_7$ receptors facilitate movement of ramified microglia to the damage site, while $P2Y_6$ receptor, a normally expressed receptor on the activated microglia, intervenes the process of phagocytosis.[3,19] Furthermore, extracellular ATP can be converted to adenosine via ectonucleotidases CD39 and CD73 that are present in microglia[20] and in turn activates ARs as well.[21,22]

While novel ligands of some purinergic receptors are currently used as pharmacological tools to define and modify actions of these receptor subtypes in the CNS,[23] there is still a growing need to clearly understand these receptors' roles in the brain, specifically as it relates to neuroinflammation and neurodegeneration. Positron emission tomography (PET) imaging has advanced our understanding of the functions of purinergic receptors in healthy and pathological brains.[24,25] Herein, we have reviewed the significance of the purinergic receptors in the CNS and accumulated a comprehensive list of the existing PET radioligands that have been used as tools for understanding the functions of these receptors.

Adenosine, Receptors and Functions in the CNS

Adenosine has been widely recognized as an inhibitory modulator of the CNS.[26] It acts as a homeostatic modulator at synapses[26,27] and participates in neurotransmitter release,[28] neuronal excitability, synaptic plasticity,[29] and local inflammatory processes.[30,31] Adenosine is complicated in neurobiology of learning and memory[29,32,33] by overstimulating the *N*-methyl-D-aspartic acid (NMDA) receptors[34,35] that influence long-term potentiation (LTP) and long-term depression (LTD).[29] Additionally, adenosine participates in modulation of neurotransmissions exerted by dopamine (DA) and acetylcholine (Ach).[36-48] Consumption of drugs of abuse and psychostimulants, either acutely or chronically, has shown to modify adenosine level in the brain.[49] As such, a more clear understanding of the involvement of adenosine signaling pathway during addiction might help to explore potential treatments for substance use dependence.[50] Several reports have indicated the involvement of adenosine in neuropathological conditions including stroke,[51,52] epilepsy,[53] PD,[54-57] and other neurodegeneration disorders.[31]

Extracellular adenosine binds to its 4 receptor subtypes A_1, A_{2A}, A_{2B}, and A_3 to exert its effect in the CNS.[30] A_1R and $A_{2A}R$ have high affinities of 70 and 150 nM, respectively, while $A_{2B}R$ and A_3R have a distinctly lower affinities of 5100 and 6500 nM, respectively, for adenosine.[58] All ARs are

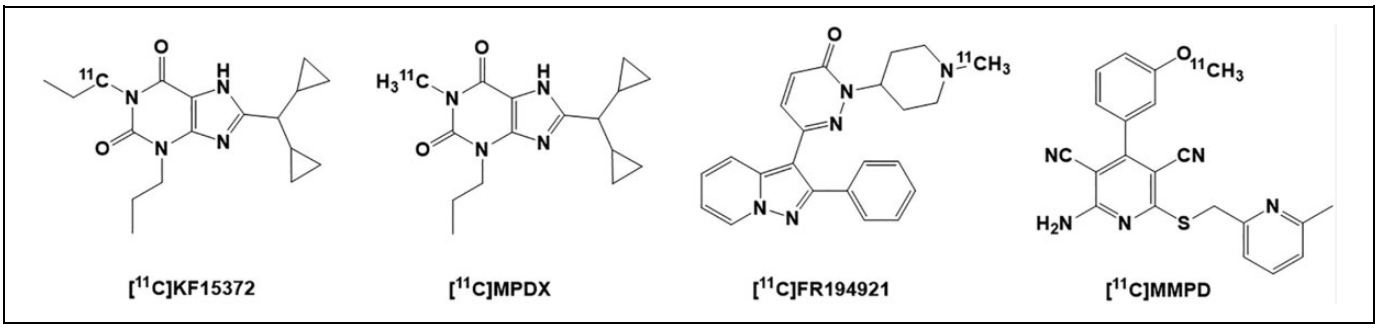

Figure 2. Structures of the adenosine A_1 receptor [^{11}C] PET radioligands: [^{11}C]KF15372, [^{11}C]MPDX, [^{11}C]FR194921, and [^{11}C]MMPD. PET indicates positron emission tomography.

present on neurons,[52] astrocytes,[59] oligodendrocytes,[60] and microglia.[61]

In the brain, A_1 and A_{2A} are the major ARs.[58] A_1 receptor, the most abundant subtype, is widely distributed in the cortex, hippocampus, and cerebellum, while A_{2A} receptor is mainly localized in the striatum and olfactory bulb.[30] Presynaptically, A_1 and A_{2A} interact with adenosine to modulate the release of neurotransmitters.[62] Postsynaptically, adenosine decreases cellular excitability through activation of A_1Rs or inhibition of A_{2A}Rs.[63] Thus, A_1Rs impose an inhibitory brake on excitatory transmission, while A_{2A} receptors engage in promoting excitatory effect.[64] In general, A_1R is considered a neuroprotective and A_{2A} receptor is designated as a neurodegenerative receptor.[65] Consequently, adenosine mainly effects brain functions through interaction with these 2 receptors, A_1 and A_{2A},[64] and a fine balance between inhibitory action of A_1 and excitatory function of A_{2A} receptors influences the neuromodulatory effect of adenosine.

Adenosine receptors undergo different activities during neurodegeneration progression.[66] While both A_1 and A_{2A} receptors have shown upregulation in the frontal cortex,[67] the A_1R expression was reduced in hippocampus, specifically in dentate gyrus, and in CA1, but not in CA3 region.[68] Additionally, studies of brain of patients with AD have revealed reduction of striatal A_1Rs in this population.[31] Several studies have shown that both A_1R and A_{2A}R may be involved in metabolism of amyloid β (Aβ) protein and A_1 agonists and A_{2A} antagonists might serve as an effective therapy for treating patients with AD.[69,70] Moreover, there are some evidences that support the cross talk between A_1Rs and A_{2A}Rs in other age-related disorders.[71-73]

Both A_1 and A_{2A} receptors are also expressed in endothelial cells of the primary human brain, suggesting that modulation of these receptors can alter blood–brain barrier (BBB) and result in abnormal brain permeability that could interfere with drug delivery into the CNS.[74]

Provided the roles of A_1 and A_{2A} receptors in brain pathologies, the availability of scientific tools such as specific PET radioligands for evaluation of these receptors functions under normal and pathophysiological conditions would be desirable and could help elucidate novel therapeutic strategies.[75]

Adenosine A_1R and Functions in the CNS

A_1 is the most abundant AR subtype in the brain with broad distribution in neurons of the cortex, hippocampus, and cerebellum.[31,58] Several studies have shown that activation of adenosine A_1R promoted neuroprotection, induced sedation, reduced anxiety, inhibited seizures,[76] and reduced A_1R exacerbated neuronal damage.[58] Significant reduction in A_1R expression was detected in layers of the dentate gyrus in the brain of AD subjects,[68,77] providing evidence that A_1R agonists might be an effective therapy for treatment of AD even at late stages of the disease.[78] Additionally, A_1R agonists or adenosine reuptake inhibitors have shown to decrease the extent of brain damage in most brain injuries.[78,79] There are evidences of increased microglial proliferation; enhanced matrix metalloproteinase 12 (MMP-12) expression, inducible nitric oxide synthase, and proinflammatory interleukin-1β (IL-1β); and exacerbated demyelination in MS and neuronal injury in A_1R knockdown animal models.[80,81] The positive effect of A_1Rs activation in the CNS suggests that this receptor could be one of the most promising targets for the development of novel drugs with neuroprotective effect for the treatment of neurological and psychiatric disorders.[69]

Several A_1R agonist have been reported to date; most of them have only minimal brain penetration. A nonselective agonist MRS5474 has shown antidepressant and anticonvulsant activities.[82] While not optimum to fully map all the functions of the A_1R in the brain, several ^{11}C and ^{18}F PET radioligands of the receptor have been evaluated for imaging of the A_1R in the brain as described herein.

[^{11}C] PET radioligands of adenosine A_1R. As shown in Figure 2, several ^{11}C PET radioligands of the A_1R have been developed and tested thus far. The first PET radiotracer was developed by ^{11}C radiolabeling of the xanthene-derived A_1R antagonist KF15372, [^{11}C]KF15372, and showed a specific and reversible brain uptake but an unacceptable high fraction of nonspecific binding that limited its use in preclinical evaluation of the A_1R.[83,84] [^{11}C]MPDX, another analog of A_1R antagonist KF15372, was developed to measure regional A_1R densities in the brain of rodent and in patients with diffuse axonal injury, a model for TBI.[85] It detected an increase in A_1R expression in

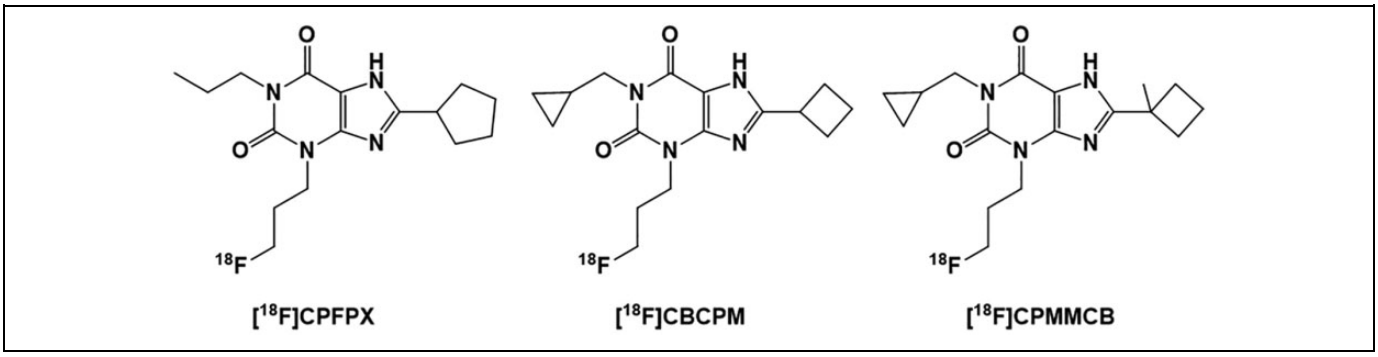

Figure 3. Structures of the adenosine A_1 receptor [^{18}F] PET radioligands: [^{18}F]CPFPX, [^{18}F]CBCPM, and [^{18}F]CPMMCB. PET indicates positron emission tomography.

areas surrounding the injuries in the brain, emphasizing on neuroprotective and neuromodulatory effects of A_1R in TBI.[85] Moreover, [^{11}C]MPDX was also used to investigate the cerebral density of A_1Rs in early stages of PD and showed a higher binding potential in the temporal lobe of the patients with PD compared to the healthy controls.[86] Similarly, [^{11}C]MPDX was used for mapping of the A_1Rs in the brain of aged human compared to the young subjects and showed a significantly lower BP_{ND} in the frontal, temporal, occipital, parietal cortices, and thalamus of aged subjects.[87] [^{11}C]MPDX is currently the most widely used PET agent for imaging the A_1Rs in human brain.[83,85] Interestingly, [^{11}C]MPDX was employed to identify the selective antagonists (DPCPX and caffeine) and agonist (*N*6-cyclopentyladenosine [CPA]) binding sites on the A_1Rs and suggested that different ligands (agonists and/or antagonists) bind to A_1Rs allosterically.[88]

The first nonxanthine ^{11}C PET ligand of A_1 R was [^{11}C]FR194921, an analog of a potent A_1R antagonist FR194921 (K_i = 2.9 nM).[89,90] The PET imaging with [^{11}C]FR194921 showed selective accumulation of A_1Rs in the hippocampus, cerebral cortex, striatum, thalamus, and cerebellum of the rat brain.[89] However, the specific binding of [^{11}C]FR194921 was not as high as expected.[89] Recently, a highly potent partial A_1R agonist 2-amino-4-(3-methoxyphenyl)-6-(((6-methylpyridine-2-yl)methyl)thio)pyridine-3,5-dicarbonitrile was labeled with ^{11}C to produce [^{11}C]MMPD and showed brain uptake that was consistent with A_1R.[91] [^{11}C]MMPD is currently under further evaluation for participation of A_1R in sleep mechanisms.[91]

[^{18}F] PET radioligands of adenosine A_1R. Few ^{18}F PET radioligands have been developed and evaluated for imaging of the A_1R as shown in Figure 3. Of these, [^{18}F]CPFPX has shown high affinity and selectivity for A_1R[6,92]; however, due to the high in vivo metabolism, this radiotracer exhibited a short biological half-life of only about 10 minutes.[84] Despite this fact, [^{18}F]CPFPX has been used for imaging of A_1Rs in the human brain[93] and is currently a standard PET radioligand for evaluation of the A_1R density in CNS disorders such as sleep–wake research.[84,94-96]

In order to improve metabolic stability inherent in [^{18}F]CPFPX, two additional fluorinated PET analogs [^{18}F]CBCPM and [^{18}F]CPMMCB were developed and tested.[84] In vitro autoradiographic studies of rat brain slices with [^{18}F]CBCPM and [^{18}F]CPMMCB revealed accumulation of both compounds in regions known to have a high A_1R expression. However, in vitro metabolite studies using human liver microsomes identified comparable metabolic instabilities for these radioligands, similar to that of the parent ligand [^{18}F]CPFPX.[84]

Importantly, both [^{11}C]MPDX or [^{18}F]CPFPX are inverse agonist of the A_1R. [^{11}C]MPDX did not compete with either endogenous or exogenous agonist in receptor binding but did show an increased binding potential without enhanced tracer delivery to the brain.[88] Despite stated limitations, these tracers[85] have presented promising imaging tools for mapping of A_1R in the brain[86,87,96]. A list of all aforementioned A_1R PET radioligands is presented in Table 1.

Adenosine A_{2A}R and Functions in the CNS

Highly expressed in the basal ganglia, A_{2A}Rs specially reside on GABAergic neurons of the striatum.[58] These receptors are also expressed at low level in hippocampus, cortex, and other brain regions, and the extrastriatal increase in A_{2A}Rs has been detected in pathological challenge models and animal models of neuroinflammation.[97] Several studies have revealed an increased level of A_{2A}R expression in hippocampal neurons of patients with AD and in animal models of cognition.[98,99] The same studies reported that inhibition or genetic deletion of A_{2A} receptors enhanced memory function in the brain.[100] A_{2A} receptors are also expressed in areas of the brain that is rich in DA,[101] providing a possibility of being considered as a target for developing drugs that prevent addiction.[102]

Inhibition of A_{2A}R has resulted in a complete shift of LTD to LTP, supporting a major role of A_{2A}Rs in cognitive deficits.[103] Inhibition of A_{2A}R has also been promising in reduction of excitotoxicity in neurons[104,105] and in movement diminished motor symptoms in PD.[54,55,106-111] Additionally, in vitro studies of A_{2A}R antagonists have shown to prevent Aβ-induced neurotoxicity and synaptotoxicity,[99,100] while A_{2A} receptor

Table 1. Adenosine A_1 Receptor PET Ligands for CNS Studies.

Receptor	PET ligand	Affinity (nM)	Status	References
A_1R	[^{11}C]KF15372	3.0 (K_i)	Exhibited high fraction of nonspecific binding that limited its use in preclinical evaluation of the A_1 receptor.	83
A_1R	[^{11}C]MPDX	4.2 (K_i, r)	Used to study A_1R function in patients with AD. Studied in patients with TBI and in patients with early stages of PD. Currently, the most widely used PET agent for imaging the A_1R in human brain.	85-87
A_1R	[^{11C}]FR194921	4.96 (K_i, r) 2.91 (K_i, h)	Showed acceptable BBB permeability, but relatively low specific binding in the rodent brain that limited its further use.	89,90
A_1R	[^{11}C]MMPD	0.49 (K_i, r) 0.8 (K_i, h)	Exhibited an A_1R partial agonist activity. Showed BBB permeability. Currently under evaluation in sleep mechanisms.	91
A_1R	[^{18}F]CPFPX	3.49 (K_i, r) 0.18 (K_i, h) 4.4 (K_d, r) 1.26 (K_d, h)	Used to study sleep deprivation in humans. Fast metabolic degradation. An inverse agonist of the A_1 receptor.	84,93-96
A_1R	[^{18}F]CBCPM	8.86 (K_i)	Exhibited low nonspecific binding. Metabolic degradation rate similar to [18]CPFPX.	84
A_1R	[^{18}F]CPMMCB	3.73 (K_i)	Exhibited low nonspecific binding. Metabolic degradation rate similar to [18]CPFPX.	84

Abbreviations: AD, Alzheimer disease; A_1R, adenosine A_1 receptor; BBB, brain–blood barrier; CNS, central nervous system; h, human; m, mice; PD, Parkinson disease; PET, positron emission tomography; r, rat.

agonists increased Aβ production.[100] However, study of APP/PS1 mice treated with A_{2A} receptor antagonist istradefylline, an anti-Parkinson drug, showed an increase in $A\beta_{42}$ accumulation in cortical, but not in the hippocampal neurons.[112] The underlying relationship between amyloid deposition, AD progression, and adenosine remains unclear and require more clarification.[97,113,114] Nevertheless, there is an indication that activation A_{2A} receptor can result in microglia activation and antagonists of A_{2A} receptor can reverse this process.[115] Some studies have suggested that A_{2A} receptor inhibition might also contribute to control of astrogliosis as well,[116] and selective elimination of A_{2A} receptors from astrocytes has resulted in memory improvement in animal models of AD.[113] Therefore, in addition to microglia, astrocytes might also be a responsible culprit, associating A_{2A} receptor with neuroinflammatory and neurodegenerative diseases.

Interestingly, excitotoxicity prevention by the A_{2A} receptor antagonist appears to be time dependent, and while A_{2A} receptor antagonist SCH58261 completely blocked the induced glutamate release in rat striatum,[117] its effect was reversed 2 weeks after the treatment.[105] Remarkably, this spontaneous glutamate release in response to SCH58261 treatment was different in young rats compared to the aged ones.[104] Additionally, recent study suggested that, although A_{2A} receptor antagonists initially protected against transient ischemic injury, this protective effect disappeared 7 days after ischemia and despite continued treatment with the antagonist.[118]

Application of pharmacological tools of the A_{2A} receptors have shown a significant benefit in treating several CNS disorders,[119] and thus, PET imaging of the A_{2A} receptors has been useful to study in vivo expression of A_{2A} receptors in normal and under pathophysiological brains.

[^{11}C] PET radio ligands of adenosine $A_{2A}R$. Several ^{11}C radioligands of the A_2R have been developed for PET imaging of this receptor as shown in Figure 4. These most studied ligands are 2 xanthine-derived compounds, [^{11}C]TMSX ([^{11}C]KF18446) and [^{11}C]KF21213, and 2 nonxanthene compounds, [^{11}C]SCH442416 and [^{11}C]Preladenant.[25] Within the xanthene-based PETs, [^{11}C]TMSX has been successfully evaluated in vivo in rodent (mice and rat) and in nonhuman primate (monkeys) and has detected A_{2A}Rs in the brain with good striatum/cerebellum uptake ratio in the above animal species.[25,120-122] Another xanthine PET ligand [^{11}C]KF21213 also displayed good striatal/cerebellum uptake ratio in rodent (10.5 at 60 minutes) but showed a lower signal to noise ratio in nonhuman primate brain.[121,123] Among the latter 2 xanthene-derived PET radioligands, [^{11}C]TMSX has been the most suitable radiotracer for mapping the A_{2A}Rs and exhibited the highest binding potential in the striatum.[120] Currently, [^{11}C]TMSX is the most broadly used PET imaging radioligand for visualization of A_{2A} receptor in the brain and therefore is considered the gold standard for brain imaging of the A_{2A} receptors.[121,122] A major consideration when using this tracer is the fact that dosing and blood sampling need to be performed under dimmed light due to [^{11}C]TMSX photoisomerization.

To overcome the photoisomerization issue inherent with xanthene radiotracer [^{11}C]TMSX, a potent, selective, and reversible nonxanthene $A_{2A}R$ antagonist SCH442416 was radiolabeled to produce [^{11}C]SCH442416 and exhibited a good striatum/cerebellum uptake ratio with slow rate of metabolism in rat.[124] In rhesus monkeys, [^{11}C]SCH442416 was rapidly accumulated in the brain, with twice as much radioactivity concentration in the striatum than in the cerebellum, but it showed a high nonspecific binding activity in monkey brain.[124] [^{11}C]SCH442416 has been used to study receptor occupancy and involvement of striatal A_{2A}Rs in the brain of PD patients with dyskinesia.[125,126] Both A_{2A}Rs antagonists [^{11}C]TMSX and [^{11}C]SCH442416 have already been used in multiple studies in human.[122,124] Preladenant, a PD drug, was also

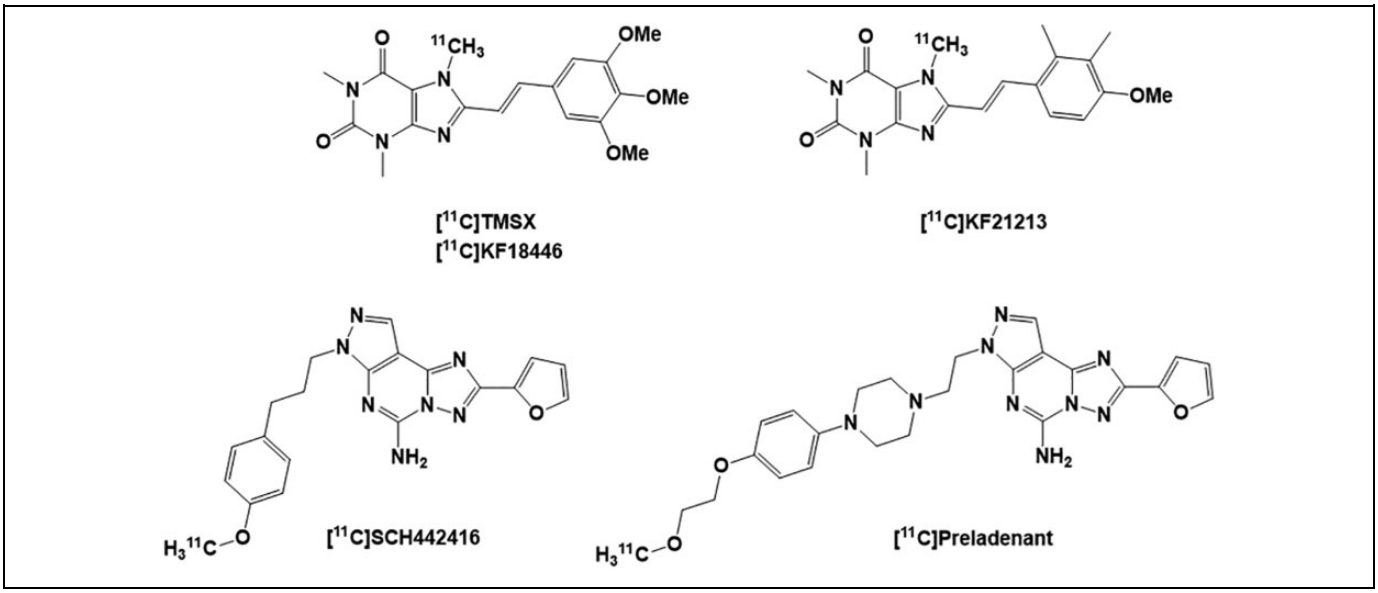

Figure 4. Structures of the adenosine A_{2A} receptor [^{11}C] PET radioligands: [^{11}C]TMSX, [^{11}C]KF21213, [^{11}C]SCH442416, and [^{11}C]Preladenant. PET indicates positron emission tomography.

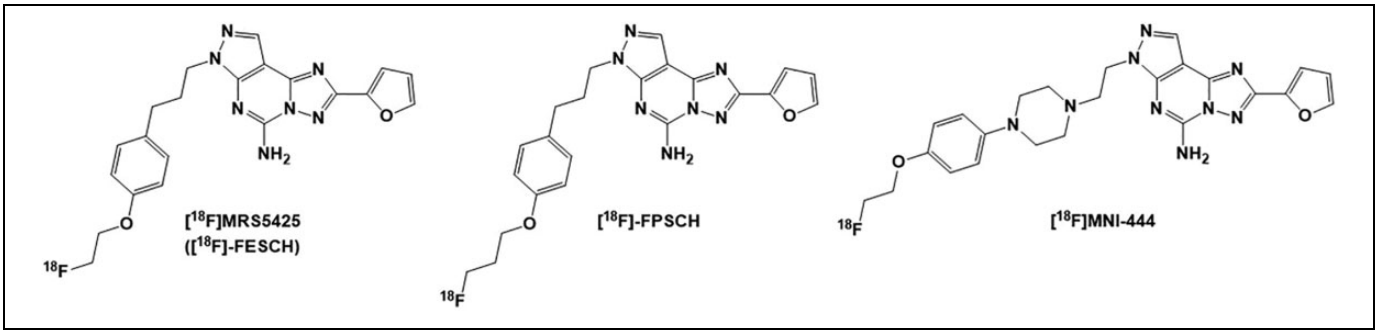

Figure 5. Structures of the adenosine A_{2A} receptor [^{18}F] PET radioligands: [^{18}F]MRS5425 ([^{18}F]-FESCH), [^{18}F]-FPSCH, and [^{18}F]MNI-444. PET indicates positron emission tomography.

radiolabeled with ^{11}C to produce [^{11}C]Preladenant.[126-128] Studies of this PET tracer in the brain of monkey showed an uptake that is consistent with the distribution of A_{2A}Rs with highest uptake in the putamen and the caudate, respectively.[128] The lowest uptake of [^{11}C]Preladenant was observed in the cerebellum. Estimated binding potential values of [^{11}C]Preladenant with different scan durations were similar (4.3-5.3 in A_{2A}R-rich regions).[128] Preinjection with nonradiolabeled Preladenant reduced the tracer uptake in regions rich in A_{2A}R and pretreatment with caffeine reduced tracer uptake in the striatum in a dose-dependent manner. [^{11}C]Preladenant PET is a suitable tool to study A_{2A}R occupancy in the brain.[128] The regional distribution of [^{11}C]Preladenant PET is consistent with known A_{2A}R densities in the brain.[126]

[^{18}F] PET radio ligands of adenosine A_{2A}R. Few ^{18}F PET radioligand derivatives of potent and selective A_{2A}R antagonist SCH442416 were developed for imaging of the A_{2A}Rs. These PET ligands include [^{18}F]MRS5425 ([^{18}F]-FESCH),[129,130] [^{18}F]-FPSCH,[130] and [^{18}F]MNI-444[131] as shown in Figure 5. The A_{2A}R-mediated uptake of [^{18}F]MRS5425 was higher in the striatum of the 6-OHDA lesion-induced rats compared to that of the normal rats, making [^{18}F]MRS5425 a suitable PET radiotracer for imaging of PD patients.[129]

A fluoropropyl analog [^{18}F]-FPSCH was also developed and studied for mapping of the A_{2A} receptors expression in rat brain.[130] Both [^{18}F]-FESCH and [^{18}F]-FPSCH showed similar striatum/cerebellum ratios post injection as well as reversible binding in the brains of rat.[130] However, dynamic PET imaging for 60 minutes, under baseline and blocking conditions, demonstrated [^{18}F]MRS5425 ([^{18}F]-FESCH) to be the most suitable ^{18}F PET radioligand for quantifying A_{2A} receptor expression in rat brain.[130]

Another highly potent nonxanthene ^{18}F PET radioligand analog of SCH442416, [^{18}F]MNI-444 (K_i = 2.8 nM, human recombinant A_{2A}Rs) was developed to noninvasively monitor

Table 2. Adenosine A_{2A} Receptor PET Ligands for CNS Studies.

Receptor	PET ligand	Affinity (nM)	Status	References
$A_{2A}R$	[^{11}C]TMSX ([^{11}C]KF18446)	5.9 (K_i, r)	Used widely and considered a gold standard PET ligand for mapping A_2R. Has been studied in human subjects and in patients with PD, HD, and MS.	25,121,122
$A_{2A}R$	[^{11}C]KF21213	3.0 (K_i, r)	Possessed high in vitro selectivity (A_{2A}/A_1 >3300). Good striatal/cerebellum uptake ratio in rodent, but lower signal to noise ratio in nonhuman primate brain.	25,123
$A_{2A}R$	[^{11}C]SCH442416	0.048 (K_i, h) 0.5 (K_i, r)	Studied in patients with PD who suffer from the levodopa-induced dyskinesia. The first suitable nonxanthine $A_{2A}R$ PET ligand.	25,124
$A_{2A}R$	[^{11}C]Preladenant	1.1 (K_i, h) 2.5 (K_i, r)	Studied in rat, rhesus monkeys, and human with PD. First human study was published in 2017.	25,125,126,128
$A_{2A}R$	[^{18}F]MRS5425 ([^{18}F]-FESCH)	12.4 (K_i)	Used for quantifying A_{2A} receptor expression in the rat brain and showed higher concentration in the striatum of the 6-OHDA lesion induced in rats, possibly a suitable PET radiotracer for imaging of PD.	129,130
$A_{2A}R$	[^{18}F]-FPSCH	53.6 (K_i)	Propyl analog of [^{18}F]FESCH and very similar in property, but less suitable PET.	130
$A_{2A}R$	[^{18}F]MNI-444	2.8 (K_i, h)	Exhibited superior property for studying and mapping the $A_{2A}R$ in the brain. Used as PET and SPECT radiopharmaceutical to study human brain. Showed high uptake, rapid kinetics, and high target/nontarget ratios in the brain, consistent with A_{2A} receptor distribution.	25,131-133

Abbreviations: $A_{2A}R$, adenosine 2A receptor; CNS, central nervous system; h, human; HD, Huntington disease; m, mice; MS, multiple sclerosis; 6-OHDA, 6-hydroxydopamine; PD, Parkinson disease; PET, positron emission tomography; r, rat.

A_{2A} receptor densities and functions in the brain of patients with PD.[131] [^{18}F]MNI-444 radioligand has shown high uptake, rapid kinetics, and high target/nontarget ratios in the brain, consistent with A_{2A} receptor distribution.[131,132] Thus far, [^{18}F]MNI-444 has turned out to be a superior imaging tracer among all the ^{18}F PET radioligands for studying and mapping the $A_{2A}R$ in the brain.[133] A list of all A_{2A} receptor PET radioligands is presented in Table 2.

P2X Receptors and Functions in the CNS

P2X receptors (P2XRs) are a family of 7 fast-acting subreceptors $P2X_1$ to $P2X_7$. These nonselective cation-gated channels receptors exhibit high Ca^{2+} permeability upon activation by extracellular ATP.[134,135]. P2X receptors are widely distributed on non-neuronal and neuronal cells and participate in numerous physiological as well as pathophysiological processes.[15] Several studies have suggested the change in P2XRs expression under neuroinflammatory, nerve transmission, and pain sensation conditions.[136] Activation of some P2XRs has been associated with various pathological disorders of CNS including neuroinflammation and neurodegeneration.[36]

With the exception of $P2X_7R$ that is only activated by high concentration of ATP (hundreds of μM), other P2X receptor subtypes are usually activated at high nM to low μM ATP concentration.[10,11]. In the CNS, P2XRs participate in modulation of neurotransmission, neuron-glia communication, inflammation, and apoptosis.[136-139] Adenosine 5′-triphosphate released under physiological conditions modulate synaptic plasticity by acting on P2X receptors via Ca^{2+}-dependent interaction with the NMDA receptors that facilitate LTP in the hippocampus.[140] In general, overexpression of the $P2X_3$, $P2X_4$, and $P2X_7$ receptors have been detected in CNS disorders and their antagonists could potentially be useful therapies for the treatment of CNS diseases including neurodegeneration and brain injuries.[6,136,141] Among subtypes of the P2XRs, $P2X_7R$ has been the focus of many studies as a therapeutic target for treating brain disorders.[142] Herein, we focus on 3, $P2X_3$, $P2X_4$ and $P2X_7$, receptors and review their existing PET radioligands.

$P2X_3$ Receptor and Functions in the CNS

$P2X_3$ receptors, either as a homomeric $P2X_3$ or a combination of $P2X_2$-$P2X_3$ receptors, are primarily expressed on nociceptive sensory neurons[143] and mediate the ATP nociceptive signaling.[144] In the spinal cord, released ATP from injured cells facilitates glutamate release from primary afferent neurons by its action at the presynaptic $P2X_3$ receptors.[144,145] $P2X_3$ knockout animals have shown to exhibit a reduction of activity of afferent nerves and nociceptive signaling,[146] and $P2X_3$ receptor expression downregulation by antagonist A-317491 has resulted in reduced mechanical hyperalgesia and neuropathic pain,[147,148] supporting the effect of ATP on peripheral nerve afferents.[146]

Thus far, few antagonists of $P2X_3$ and $P2X_{2/3}$ have been identified. One of them, A-317491, has shown to reduce mechanical allodynia and thermal hyperalgesia following chronic nerve constriction.[149,150] AF-353 is another $P2X_3$ receptor antagonist that has shown similar potency for human and rat recombinant $P2X_3$ homotrimers ($IC_{50} = 8.7$ and $IC_{50} = 8.9$ nM, respectively).[151] A prodrug version of AF-353, (RO-51), has been developed to treat urological dysfunction and chronic pain.[152] A recently marketed $P2X_3R$ antagonist, gefapixant (AF-219, MK-7264), is used for reduction of exaggerated, persistent, and frequent urge to cough as a result of hypersensitized sensory neurons, triggered by injury or infection.[153-155] Recently, a series of 5-hydroxy pyridine derivatives were synthesized and evaluated for their activities at $hP2X_3$ receptors.[156] One of the compounds in this series, prodrug

28, has shown antiallodynic activity in spinal nerve ligation and chemotherapy-induced peripheral neuropathy in rats.[156] This and other data on the $P2X_3R$ antagonists indicate that targeting the $P2X_3$ receptors could be a promising treatment for neuropathic pain. Thus far, there is no identified PET radioligand for evaluation of the $P2X_3$ receptors, to the best of our knowledge.

$P2X_4$ Receptor and Functions in the CNS:

$P2X_4$ receptor, the first identified P2X receptor, is widely expressed in peripheral nervous system and CNS.[157] $P2X_4$ receptors are one of the most abundantly expressed functional purinergic receptors found on glial cells and most neurons[17,158] and are upregulated on activated microglia after brain and spinal cord injuries.[159] Similar to $P2X_7R$, $P2X_4R$ facilitate ion efflux through cell membrane and induces activation of inflammasomes.[137] Supporting evidences indicate that P2X4 receptors physically couple with $GABA_A$ receptors as well as with the $P2X_2$ receptors and this cross talk may play a role in regulating synaptic signaling and plasticity of neurons.[160] Alcohol abuse is known to enhance neuroinflammation through $P2X_4Rs$ activation[161] and there are suggestions of implication of $P2X_4Rs$ in tolerance to morphine and hyperalgesia induction by morphine.[161,162] $P2X_4$ receptors are upregulated in TBI[6], in acute experimental encephalomyelitis (EAE) rodent model of multiple sclerosis[17] and following hypoxia and ischemia events.[163] In neurons, $P2X_4R$ has shown to stimulate activation of the inflammasome caspase-1 resulting in cytokines IL-18 and IL-β release, and in $P2X_4R$ knockout mice, impaired inflammasome signaling was reported to couple to the reduction of IL-β level.[164] Inhibition of $P2X_4$ receptors by antagonists prior to cerebral ischemia has resulted in an attenuation of the neuroinflammation response and health of neuronal tissue.[165] Additionally, P2X4 receptor upregulation has been reported in several rodent models including mechanical allodynia,[14] superoxide dismutase 1-mutation models of ALS,[166] EAE model of multiple sclerosis,[17] post spinal cord injury,[164] formalin-induced inflammatory pain,[167] TBI,[168] and ischemia.[165] These data support the central role that P2X4 receptors play in coordinating the microglial response to cellular injuries and/or diseases.

Therefore, $P2X_4$ receptor antagonists might have potentials for the treatment of neuropathic pain,[23] epilepsy, stroke, multiple sclerosis, and neurodegenerative diseases such as PD and AD.[159,169] Paroxetine, a selective serotonin reuptake inhibitor, has shown to behave as an allosteric antagonist of $P2X_4Rs$ at high concentrations ($IC_{50} = 2.45\ \mu M$, rat, and $IC_{50} = 1.87\ \mu M$, human).[170] Thus far, attempts to identify potent and selective antagonist of $P2X_4Rs$ have resulted in the discovery of allosteric ligands with low potency and poor aqueous solubility. Among these antagonists is the benzodiazepine derivative 5-BDBD ($IC_{50} = 0.5\ \mu M$)[171] and its analogs[172] that possessed allosteric antagonism, but low potency at $P2X_4R$.[172] The urea derivative BX-430 was another allosteric $P2X_4$ receptor antagonist with low potency ($IC_{50} = 0.54\ \mu M$).[173] An additional allosteric $P2X_4Rs$ antagonist is the high lipophilic and poor soluble carbamate PSB-12054 with good selectivity and reasonable potency at human $P2X_4Rs$ but much less potency at rat and mice $P2X_4Rs$.[174] An analog of PSB-12054, PSB-12062 with better solubility, was developed later and showed equal potency at human, rat, and mouse and good selectivity for $P2X_4R$ versus $P2X_1$, $P2X_3$, and $P2X_7$ receptors.[175] Recently, a new diazepine antagonist NP-1815-PX with reasonable potency and selectivity at $P2X_4Rs$ ($IC_{50} = 0.26\ \mu M$, $hP2X_4R$, concentration dependent)[176] has shown an antiallodynic effect and suppression of mechanical allodynia in mice with traumatic nerve damage without affecting acute nociceptive pain and motor function, suggesting that microglial $P2X_4Rs$ could potentially act as an important target for treating chronic pain.[176] Nippon Chemiphar has reported the discovery of yet another potent antagonist of the $P2X_4Rs$, NC-2600 for the treatment of neuropathic pain. Phase I evaluation of NC-2600 has been completed and phase II evaluation is underway. NC-2600 is believed to be the first-in-class candidate to control pain by targeting glial cells. NC-2600 is currently under safety/tolerability studies. To our best knowledge, lack of highly potent $P2X_4Rs$ ligands has limited efforts to develop PET ligand for this receptor.

$P2X_7$ Receptor and Functions in the CNS

$P2X_7R$ is regarded as an important silent receptor as its expression is only upregulated when ATP concentration increases to a high level,[177] suggesting the high relevance of $P2X_7Rs$ in pathological conditions.[178] $P2X_7Rs$ are expressed on presynaptic neurons, astrocytes, and oligodendrocytes, but its highest concentrations is expressed on microglia where it releases proinflammatory cytokine IL-1β, a key mediator of chronic inflammation and chronic pain.[6,178] Several studies of the $P2X_7$ receptors have shown involvement of this receptor in animal models of neuroinflammatory diseases including AD,[137,179,180] PD,[181] HD,[182] FD,[1] ALS,[183] MS,[184] TBI,[15] cerebral ischemia,[6] epilepsy,[185] depression,[186,187] anxiety, and bipolar disorders.[178] Astrocytic $P2X_7Rs$ expression has also shown to be involved in the neurotoxic phenotype model of ALS.[188]

Stimulation of $P2X_7Rs$ by high level of ATP (hundreds of μM) produces a large transmembrane pores, permeable to large molecular sizes of up to 900 Da, promoting further increase in extracellular ATP release that can lead to activation of caspases and result in cell death.[189] $P2X_7$ receptor expression in the CNS could be increased with systemic administration of bacterial lipopolysaccharide (LPS), providing a realistic mechanism similar to systemic infection in the brain.[6] Genetic deficiency and pharmacological inhibition of $P2X_7$ receptors have shown to attenuate hyperactivity induced by amphetamine in the model of manic bipolar disorder.[190] Mood stabilizer drugs such as lithium and valproate reversed ATP-induced cell death in the hippocampus, an action that is probably mediated by $P2X_7$ receptors.[191,192]

Discovery of a number of potent and selective $P2X_7Rs$ antagonists has been instrumental in studying the receptor in

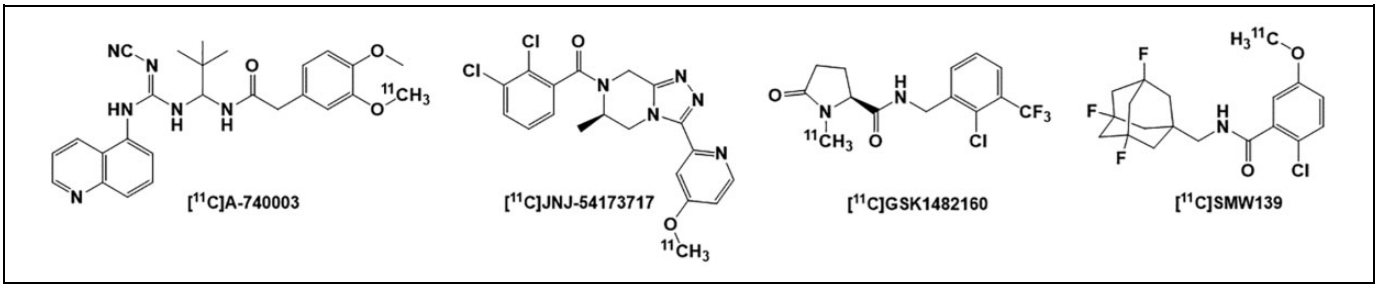

Figure 6. Structures of the $P2X_7$ receptor [^{11}C] PET radioligands: [^{11}C]A-740003, [^{11}C]JNJ-54173717, [^{11}C]GSK1482160, and [^{11}C]SMW139. PET indicates positron emission tomography.

human and rodent. Some of these ligands including AZD9056[193] and CE-224535[194] were developed for the treatment of inflammation but failed to exhibit benefits in patients.[194] Other existing and understudy ligands of the $P2X_7$ receptors include A438079, A740003, A804598, A839977, AZ1060612, AZ11645373, GSK1482160, and GW791343.[9,195]

Some $P2X_7$ receptor antagonists were specifically developed to study disorders of the CNS. These are the brain penetrant benzamides GSK1482160,[196] JNJ-42253432,[197] JNJ-47965567,[198] triazoles JNJ-54232334 and JNJ-54140515,[199] JNJ-54166060,[200] JNJ-54173717,[201] JNJ-54175446,[202] and JNJ-55308942.[203] These molecules have demonstrated $P2X_7$ receptor antagonist activities in rodent and human. Three of these molecules, GSK1482160, JNJ-54175446, and JNJ-55308942, have already moved into clinical trials for evaluation of the disorders of CNS.[187]

Association of $P2X_7R$ activation with pro-inflammatory phenotype of microglia in CNS diseases makes $P2X_7R$ an interesting and valuable biomarker of inflammation.[9] Development of useful PET radioligands for imaging the $P2X_7Rs$ in CNS can potentially enable studies of the pharmacology and functional role of this receptor in neuroinflammation and evaluate the effect of therapeutic agents in treating neuroinflammatory and neurodegenerative diseases. Fortunately, an ample number of potent and selective $P2X_7R$ ligands has presented opportunities to develop a few ^{11}C and ^{18}F PET radioligands of the receptor as described herein.[24,204]

[^{11}C] PET radioligands of $P2X_7R$. Several antagonists of the $P2X_7R$ have been radiolabeled with ^{11}C for evaluation of the receptor expression and function as shown in Figure 6. The selective $P2X_7R$ antagonist A-740003 ($IC_{50} = 18$ nM, rP2X7R and $IC_{50} = 40$ nM, hP2X7R) was radiolabeled with ^{11}C to produce [^{11}C]A-740003,[205] but showed low biodistribution and poor brain permeability.[205] The first brain penetrable ^{11}C PET radioligand for quantification of $P2X_7R$ expression in the brain was [^{11}C]JNJ-54173717. This tracer showed high potency in humanized rat $P2X_7R$ ($IC_{50} = 4.2$ nM, hP2X7R)[206] and excellent uptake in the $hP2X_7R$ overexpressing striatum area that was reduced by pretreatment with nonradioactive antagonists JNJ-54173717 and JNJ-42253432, suggesting selective $P2X_7R$ binding of this radiotracer in the brain.[24] Additionally, [^{11}C]JNJ-54173717 displayed high brain uptake in rhesus monkey, an indication of BBB penetrability to study receptor expression levels in neurodegenerative disorders in humans.[206] Another potent $P2X_7$ receptor antagonist, benzamide GSK1482160 was also radiolabeled with ^{11}C to produce PET radioligand [^{11}C]GSK1482160 (Ki = 2.63 nM, $IC_{50} = 3$ nM, $hP2X_7$ and $K_d = 1.15 \pm 0.12$ nM, hP2X7R).[207-209] Evaluation of [^{11}C]GSK1482160 in mouse model of LPS-induced neuroinflammation showed increased uptake of 3.6-fold compared with saline-treated mice in all studied organs (2.9- to 5.7-fold).[208] In the EAE rat model of MS, [^{11}C]GSK1482160 uptake was high in rat lumbar spinal cord and the highest uptake was measured at the EAE peak stage.[209] Micro-PET studies of [^{11}C]GSK1482160 in rhesus monkey has shown high tracer retention and a homogeneous brain distribution.[209] All of these studies strongly correlated the [^{11}C]GSK1482160 uptake with the $P2X_7$ R overexpression on activated microglia and its participation in neuroinflammation.[209] Another ^{11}C PET radioligand of $P2X_7$ R antagonist was developed by radiolabeling of the SMW139 ($K_i = 32$ nM, $hP2X_7R$)[210] and was evaluated in a humanized rat model to study the expression of $P2X_7R$ in striatum.[210] Even though [^{11}C]SMW139 did not detect overexpression of the $P2X_7$ R in postmortem brain of patients with AD,[210,211] this PET radioligand has entered clinical evaluation in patients with MS and is currently the first in human to study neuroinflammation in patients with MS.[210,212]

[^{18}F] PET radioligands of $P2X_7R$. Thus far, there are reports of 3 known ^{18}F radioligands for evaluation of the $P2X_7R$ expression as shown in Figure 7. An analog of a potent $P2X_7R$ antagonist A-804598 was radiolabeled with ^{18}F to yield [^{18}F]EFB that showed high affinity for human and rat $P2X_7R$.[213] However, this PET tracer suffered from a low brain uptake in both healthy and LPS-treated rats that limited its application for brain imaging of the receptor.[213] Another PET radioligand [^{18}F]JNJ-64413739 was developed by ^{18}F radiolabeling of a potent and selective $P2X_7$ R antagonist JNJ-64413739 (Ki = 2.7 nM, rat cortex, Ki = 15.9 nM, hP2X7R). [^{18}F]JNJ-64413739 has shown to be an effective PET ligand for mapping of $P2X_7R$ in human brain.[214] The PET imaging studies with [^{18}F]JNJ-

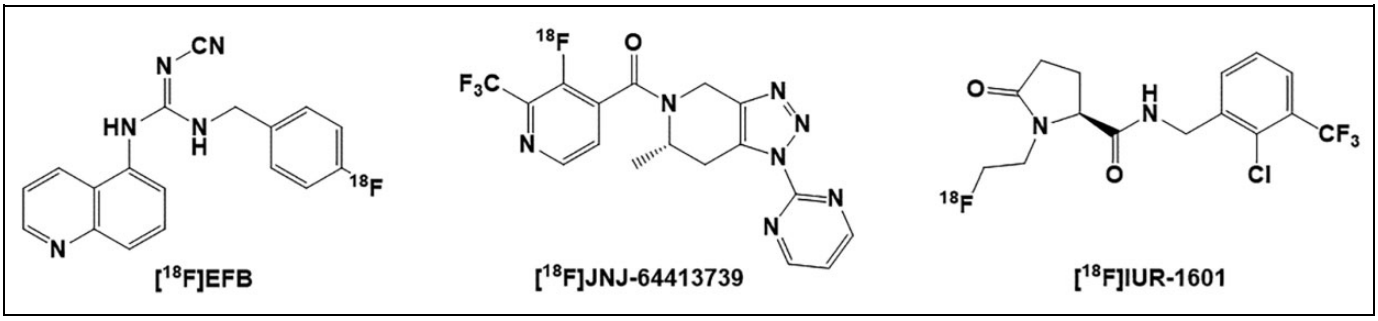

Figure 7. Structures of the $P2X_7$ receptor [^{18}F] PET radioligands: [^{18}F]EFB, [^{18}F]JNJ-64413739, and [^{18}F]IUR-1601. PET indicates positron emission tomography.

Table 3. $P2X_7$ Receptor PET Ligands for CNS Studies.

Receptor	PET ligand	Affinity (nM)	Status	References
$P2X_7R$	[11C]A-740003	18 (IC_{50}, r) 40 (IC_{50}, h)	Showed low brain uptake and a moderate metabolic rate.	205
$P2X_7R$	[^{11}C]JNJ-54173717	1.6 ± 0.1 (K_i, r) 4.2 (IC_{50}, h) 7.6 (IC_{50}, r)	Entered rat brain and showed excellent uptake in the human P2X7R overexpressing striatum. Showed high initial brain uptake in nonhuman primates.	201,206
$P2X_7R$	[^{11}C]GSK1482160	2.63 ± 0.6 (K_i) 1.15 ± 0.12 (K_d) 3 (IC_{50}, h)	Possessed high P2X7 selectivity and good blood–brain barrier permeability. Studied in mouse model of LPS-induced neuroinflammation and EAE rat model of MS. Showed a high tracer retention and a homogeneous brain distribution in rhesus monkey. In preclinical evaluation.	196,207-209
$P2X_7R$	[^{11}C]SMW139	32 ± 5 (K_i, h) 24.5 ± 5.5 (IC_{50}, h) 158 ± 44 (IC_{50}, m)	Entered clinical trial to study neuroinflammation in patients with MS.	210-212
$P2X_7R$	[^{18}F]EFB	2.88 (K_i, h) 36.1 (K_i, r) 547 (K_i, m)	Exhibited low brain uptake due to limited compatibility of the cyanoguanidine moiety with BBB entry in rats. Limited solubility.	213
$P2X_7R$	[^{18}F]JNJ-64413739	15.9 ± 2.0 (K_i, h) 2.7 ± 1.1 (K_i, r) 1.0 ± 0.2 (IC_{50}, h) 2.0 ± 0.6 (IC_{50}, r)	Showed dose-dependent competitive binding with JNJ-54175446 in monkey PET studies. A potential imaging biomarker of central neuroinflammation. Entered clinical trial in 2017.	214-217
$P2X_7R$	[^{18}F]IUR-1601	4.31 (K_i) 7.86 (IC_{50})	Evaluated in *in vitro* assays and is currently under evaluation in 5XFAD animal model of AD.	218

Abbreviations: AD, Alzheimer disease; BBB, brain–blood barrier; CNS, central nervous system; h, human; LPS, lipopolysaccharides; m, mice; MS, multiple sclerosis; PET, positron emission tomography; $P2X_7R$, $P2X_7$ receptor; r, rat.

64413739 in nonhuman primate showed engagement of the tracer with the $P2X_7R$. In vitro blocking experiments of [^{18}F]JNJ-64413739 with 2 known $P2X_7R$ antagonists demonstrated inhibition of the tracer binding to rat brain tissue sections in a dose-dependent manner.[214-216] While [^{18}F]JNJ-64413739 may be a useful tool for imaging of neuroinflammation, lack of a reference region in image analysis (ie, similar to TSPO) might hinder its use as an optimum PET radiotracer for detection of neuroinflammation.[214,217] Most recently, our team has synthesized a novel ^{18}F radioligand [^{18}F]IUR-1601, the fluoroethyl analog of GSK1482160.[218] [^{18}F]IUR-1601 has been successfully evaluated in vitro and is currently under evaluation in 5XFAD animal model of AD. A list of all $P2X_7$ receptor PET radioligands is presented in Table 3.

P2Y Receptors and Functions in the CNS

The metabotropic P2Y receptors are a family of GPCRs with 8 subtypes: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$, with ubiquitous expression and effect in body.[18,219] In the CNS, P2Y receptors are localized on neurons, microglia, astrocytes, and oligodendrocytes where they have important physiological roles in glial-cell communication, neurotransmission, and neurogenesis.[220,221] The hippocampus expresses $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{12}$ receptors in addition to all the P2X receptor subtypes.[67]

In contrast to the ion channel P2X receptors, P2YRs are activated by several endogenous ligands including the adenine nucleotides: ADP (acting on $P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$) and ATP (acting on $P2Y_2$ and $P2Y_{11}$), and the uridine nucleotides

UTP (acting on $P2Y_2$ and $P2Y_4$), UDP (acting on $P2Y_6$), and the UDP-glucose (acting on $P2Y_{14}$).[222]

Several studies have revealed that during brain injury and under pathological conditions, neurons,[223] astrocytes,[189] and microglia[224] release high concentration of ATP that acts as a neuromodulator of the P2Y receptors.[134,225] P2Y receptor activation then induces fast synaptic transmission through postsynaptic P2X receptors in the brain.[135] Therefore, P2Y receptors affect the release of number of neurotransmitters[225] through actions on calcium influx.[226]

The P2Y receptors, individually or in combination, participate in many biological conditions. $P2Y_1R$ has a complex role in modulation of DA release, even though there is no evidence of its existence in the dopaminergic terminals of the prefrontal cortex.[227,228] $P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$ receptors specifically block the release of noradrenaline in the spinal cord,[229] the hippocampus,[230] and in the cortex,[228] while these same receptors inhibit the release of serotonin in the cortex.[231] $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_{12}$, and $P2Y_{13}$ receptors have also shown to inhibit the release of glutamate in the spinal cord,[226] the hippocampus synapses, and the cerebral cortex.[221] $P2Y_{12}$ receptor is known as a protective receptor that stimulates microglial migration toward neuronal damage.[16] Functional studies have demonstrated the involvement of P2Y receptors in seizure pathology, as well.[232]

Some of the P2Y receptors have prominent roles in neurodegenerative diseases. For example, during neuronal injuries, $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors regulate the phagocytic activity of microglia upon leaked UTP and UDP from injured hippocampal cells.[233] Microglia execute the uptake of cellular debris specifically through $P2Y_6$ receptor.[233] $P2Y_1$, $P2Y_4$, and $P2Y_{12}$ are prominent P2YRs in the brain and represent favorable targets for treating neuroinflammatory diseases and neurodegenerative disorders including AD.[46,226]

Activation of some P2YRs has shown to inhibit the excitatory transmission mediated by postsynaptic NMDA receptors and increase the inhibitory action of the $GABA_A$ receptors prompting LTP.[226,234] In the CA1 region of hippocampus, released ATP from astrocytes has shown to result in LTD of synapses from neighboring neurons via activation of the presynaptic P2Y receptors, indicating participation of ATP from activated astrocytes in this form of plasticity.[140] In a specific region of the brain, the medial habenular nucleus that is involved in depression, stress, and nicotine withdrawal[234,235] an application of UTP or UDP resulted in LTP of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated currents, apparently through activation of presynaptic $P2Y_4R$.[226]

There has been suggestions that activation of $P2Y_2$ and $P2Y_4$ receptors may be useful in treating neurodegenerative diseases.[18,221] Studies of rat primary cerebellar neurons has provided evidence that $P2Y_{13}$ receptor activation protected neurons against oxidative stress-induced death.[236] $P2Y_1$ receptor has specifically emerged as a new target for treating cognitive dysfunction in CNS.[226,237,238]

Overall, investigations of the P2Y family receptors have been challenging due to the lack of potent, selective, and high-specific-radioactivity PET radioligands for these receptors. Herein, we present the subfamily of P2Y receptors and their ligands that are known to have important functions in the CNS.

$P2Y_1$ Receptor and Functions in the CNS

$P2Y_1$ receptor is one of the most abundant P2Y receptor subtype in brain tissues with large expression on neurons of the cerebellum,[237] cerebral cortex, and ischemia-sensitive regions of the hippocampus that is predominantly implicated in AD.[239] $P2Y_1R$ is also expressed on oligodendrocytes and astrocytes in the brain and optic nerves.[240,241] Human $P2Y_1R$ is activated by ADP ($EC_{50} = 10$ nM),[220,221] and ADP activation of the receptor induces platelet activation making this receptor as an important antithrombotic drug target.[242] Like $P2X_7$ receptor, $P2Y_1$ receptor also mediates activation of microglia after brain injuries and insult.[243]

There are reports of $P2Y_1$ receptor upregulation in CNS under pathological conditions such as mechanical injury,[244] ischemia,[245] and neurodegeneration.[246] Additionally, hyperactivity of astrocytic $P2Y_1$ receptors have been detected in animal models of AD[246,247] and increased expression of the receptor has been observed in hippocampus and cortex of postmortem brain sections in patients with AD.[248] $P2Y_1R$ is also upregulated after stroke and TBI and inhibition of the receptor has been shown to reduce cognition deficit resulted from these conditions.[249] Indeed, antagonists of the $P2Y_1$ receptor have shown to reduce neuronal injury and improve spatial memory in rat model of TBI.[250,251]

Inhibition of astrocytic $P2Y_1R$ has resulted in cytokine and chemokine transcriptional suppression and brain protection.[247,250] Blocking of hippocampal $P2Y_1$ receptors has shown to enhance synaptic signaling and might be responsible for promotion of antioxidant mechanism that consequently results in pro-survival pathways.[249,252] $P2Y_1R$ antagonists have also shown to mediate and upregulate the oxidoreductase enzymes by increasing tolerance to hydrogen peroxide.[253] A recent study has shown that $P2Y_1$ agonist MRS2365 initiated nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) release after stroke and enhanced neuroinflammatory responses, while $P2Y_1$ receptor antagonist MRS2179 attenuated inflammation and reduced the infarct size.[250,251] Furthermore, $P2Y_1$ antagonist has shown to help patients with schizophrenia to experience reduction in unnecessary information and noise entering their brain.[254]

Ironically, there is an evidence that $P2Y_1Rs$ may also promote axonal elongation to offset the neurotoxic effects of neurofibrillary tangles and have a neuroprotective effect in patients with AD.[255] Nevertheless, there are still more supporting data that $P2Y_1R$ antagonist could potentially be appropriate candidates for the treatment of neurodegenerative diseases.[247-249]

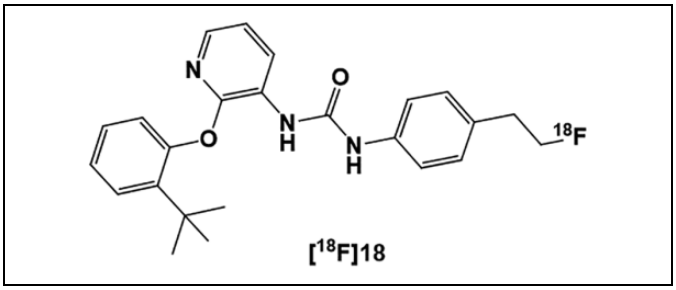

Figure 8. [^{18}F]Radiolabeled P2Y$_1$R PET ligand [^{18}F]18. PET indicates positron emission tomography.

PET radioligand of P2Y$_1$R. Overall, investigation of the P2Y family receptors has been challenging due to the absence of potent, selective, and high-specific-radioactivity PET radioligands. Recently, a highly potent (IC_{50} = 10 nM) P2Y$_1$R antagonist (compound 18) was identified and radiolabeled with [^{18}F] ([^{18}F]18) as shown in Figure 8. Although [^{18}F]18 exhibited fast in vivo metabolism, its high potency and unique allosteric binding mode has provided an opportunity to investigate it as a potential PET tracer for mapping the P2Y$_1$ receptor.[256] Additionally, highly potent, selective, and high specific radioligand [^{32}P]MRS2500 has been used successfully to measure human P2Y$_1$ receptor expression in Sf9 insect cell membrane.[257]

P2Y$_2$ Receptor and Functions in the CNS

One of the most studied receptors in this family is the P2Y$_2$R, with a wide distribution in all cells in human body and particularly in immune cells.[258] In the brain, P2Y$_2$ receptor is expressed on neurons, microglia, and astrocytes.[12,259] Under normal brain conditions, there is a low expression of P2Y$_2$R on neurons, but it can be upregulated to exert neuroprotective effects against the release of pro-inflammatory cytokine IL-1β as a result of P2X$_7$R expression on activated microglia.[260] In the AD mouse model TgCRND8, genetic deletion of P2Y$_2$ receptor has shown to enhance early AD pathology, while activation of the receptor enhanced phagocytosis and degradation of the Aβ peptide.[261] Furthermore, activation of P2Y$_2$R has proven to result in degradation of amyloid precursor protein by α-secretase, yielding to soluble APPα peptide that prevented production and accumulation of the neurotoxic A$\beta_{1\text{-}42}$.[262,263] In studies that compared brain neocortex and parietal cortex of postmortem patients with AD to those of the normal aged controls, the low level of P2Y$_2$R expression was associated with neuropathology and synapse loss in patients with AD,[263] presenting additional support for neuroprotective function of P2Y$_2$R in AD pathology.[264] Additionally, activation of the P2Y$_2$R has shown to promote neurite outgrowth.[265] These studies suggest that loss of neuroprotective functions of P2Y$_2$R might contribute to disease pathogenesis in AD, and therefore, targeting the P2Y$_2$Rs with agonist might be a promising strategy to boost neuroprotection in neurodegenerative diseases.

P2Y$_2$R is equally activated by ATP and UTP (EC_{50} = 0.3-3 μM),[221] suggesting its close proximity to conditions such as inflammation and apoptosis.[258] All known agonists of P2Y$_2$R are derivatives of ATP and UDP (UDPpS, MRS2698, INS37217, INS48823, α,β-methylene-UDP, 5-bromo-UTP, PSB-1114). One such agonist INS365 (diquafosol, EC_{50} = 100 nM) has been approved as an ophthalmic solution for the treatment of dry eye syndrome.[266] PSB1114 is another known P2Y$_2$R agonist that possesses 60-fold selectivity over the P2Y$_4$R or the P2Y$_6$R.[267] Two of the P2Y$_2$R agonists INS37217 and MRS2698 are currently in clinical trials for treating cystic fibrosis.[258] Thus far, there are no reports of any PET radioligand for imaging of this receptors.

P2Y$_4$ Receptor and Functions in the CNS

The P2Y$_4$R is present in all cells of the brain, including neurons,[268] astrocytes,[269] and microglia.[270] However, the functional role of the receptor is still ambiguous. It is believed that P2Y$_4$ R might complement the P2Y$_2$R since both receptors are present in glial end feet in vicinity of the blood vessel walls.[271] Human P2Y$_4$R is stimulated by UTP (EC_{50} = 73 nM), but not by ATP.[272] However, both nucleotides activate the rat and mouse P2Y$_4$ receptors. In microglia, P2Y$_4$ receptors are involved in ATP triggered pinocytosis that results in the uptake of soluble A$\beta_{1\text{-}42}$, and either P2Y$_4$ knockdown or ATP deficiency has shown to decrease this process.[273] Hence, in addition to the P2Y$_{12}$ receptor-mediated "find me" signal[16] and the P2Y$_6$ receptor-mediated "eat me" signal,[233] P2Y$_4$ receptors facilitate "drink me" signal that enables uptake of soluble Aβ by microglia.[273] Therefore, activation of P2Y$_4$ receptor in AD may have a neuroprotective effect possibly through uptake of A$\beta_{1\text{-}42}$.[273,274]

Thus far, there has been no report of a selective P2Y$_4$R agonists or antagonists. Nonselective P2Y agonists UTPγS,[275] 5-bromo-UTP,[276] INS365, INS37217, and INS45973 also exhibit agonist activity for the P2Y$_4$R.[277,278] Recently, an anthraquinone derivative was synthesized and showed selective and noncompetitive antagonist activity at the hP2Y4Rs (IC_{50} = 233 nM).[279] To the best of our knowledge, there has been no report of any PET radioligand for mapping of the P2Y$_4$Rs thus far.

P2Y$_6$ Receptor and Functions in the CNS

The P2Y$_6$ receptor is distributed on both immune and nonimmune cells and plays an important role in mammalian innate immunity.[280] It is preferentially activated by UDP (EC_{50} = 15 nM).[221] Under conditions that cause neuronal damage or in response to LPS, UDP leakage from damaged cells facilitates uptake and removal of cellular debris by activation of the microglial P2Y$_6$ receptors,[6,281] especially in PD.[221] Indeed, P2X$_6$R is regarded as a potential clinical biomarker of PD and other neuroinflammatory diseases.[282]

Additionally, UDP has shown to promote feeding through activation of P2Y$_6$ receptors in AgRP neurons. These neurons are known to be involved in systemic insulin resistance which is an onset of obesity-associated hyperphagia.[283] Moreover,

hypothalamic UDP concentrations have shown to be increased in obesity disorder.[283]

Inhibition of $P2Y_6R$ has proven to be a potential therapeutic strategy for treatment of neuroinflammation, PD,[282] feeding disorders, and systemic insulin resistance in obesity condition.[283] Potent and selective nonnucleotide $P2Y_6R$ antagonist MRS2578 (IC50 = 37 nM, $hP2Y_6$ R and IC50 = 98 nM, $rP2Y_6R$) has shown to inhibit UDP-induced phagocytosis and prevent LPS-induced neuronal loss in mixed neuronal/glial cultures.[284] MRS2578 specifically lacks any antagonist activity at $P2Y_{1,2,4,11}$ receptors.[285,286] Recently, a novel selective $hP2Y_6R$ antagonist TIM-38 was reported with low potency ($IC_{50} = 4.3$ μM).[287] TIM-38 could be a useful pharmacological tool and a starting point for the development of therapeutic agents against $P2Y_6$ receptor-implicated disease. Activation of $P2Y_6R$ by either its endogenous ligand UDP or selective agonist MRS-2693 has shown to promote production of proinflammatory cytokines IL-6 and IL-8 and contribute to phagocytosis of neurons.[288,289] To the best of our knowledge, there has been no report of any PET radioligand for mapping of the $P2Y_6Rs$.

$P2Y_{12}$ Receptor and Functions in the CNS

$P2Y_{12}$ receptor is activated by endogenous agonist ADP (EC_{50} = 60 nM).[221] It acts as a regulator of blood clotting; therefore, it is targeted for the treatment of thromboembolisms.[290] In normal brain, $P2Y_{12}R$ expression level is high on M2 type microglia[291] but downregulates under pathological conditions or after LPS treatment.[291,292] Indeed, expression of $P2Y_{12}$ in microglia was undetectable 24 hours after injury.[16] During microglial transition from highly ramified to an amoeboid state, low level of $P2Y_{12}$ receptors is an indication of the receptor role in early responses of microglia to the brain injury.[16] Immunohistochemical studies of postmortem brains from patients with AD and MS have shown reduction of $P2Y_{12}$ receptor expression on microglia near the injury sites.[291] Therefore, $P2Y_{12}$ receptor could potentially act as a valuable biomarker for detecting the activity of human microglia during CNS pathologies in neurodegenerative diseases.[291] $P2Y_{12}$ is also expressed on astrocytes of the rat cortex and hippocampal pyramidal neurons and on oligodendrocytes where is involved in myelination.[271,293]

Within the P2Y receptor family, both $P2Y_{12}$ and $P2Y_6$ receptors[233] control microglia activation and migration to the injury site; however, $P2Y_{12}R$ expression is decreased, while $P2Y_6R$ expression is increased.[294,295] $P2Y_{12}$ receptor also participates in a crosstalk with A_3R to perform the process extension of microglia,[296] suggesting the nucleotides action on $P2Y_{12}$ as a primary target to induce microglial chemotaxis early on in response to CNS injury. Therefore, $P2Y_{12}R$ can potentially be targeted for the treatment of neurodegenerative diseases.[16]

A wide variety of antithrombotic $P2Y_{12}R$ antagonists such as ticlopidine (Ticlid), clopidogrel (Plavix), ticagrelor (Brilinta), prasugrel (Effient), ticagrelor (AR-C 69931),[297] and

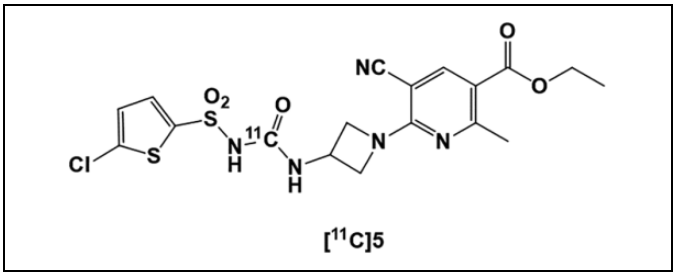

Figure 9. [^{11}C]Radiolabeled $P2Y_{12}R$ PET ligand [^{11}C]5. PET indicates positron emission tomography.

MRS-2395[298] have been developed for the treatment of platelet aggregation.[269,299] Inhibition of the $P2Y_{12}R$ through knockdown of expression or by pharmacological inhibitor has resulted in less neuronal injury.[294] The direct effects of the $P2Y_{12}$ antagonists on cardiovascular system may indirectly heal neural injury and CNS diseases.[16] Therefore, inhibition of both $P2Y_6$ and $P2Y_{12}$ receptors with their antagonists may prevent phagocytosis of salvageable cells and could be a promising path in treating neuroinflammation-induced neurodegeneration.[251]

PET Radioligand of $P2Y_{12}R$. Since $P2Y_{12}$ receptors are the only identified target exclusively expressed on M2-type microglia, PET imaging of this receptor could help detect the precise role of microglial phenotype in each stage of neuroinflammation and identify stages of the neurodegeneration diseases. Thus, an antagonist of $P2Y_{12}R$ (sulfonylureas compound 5, with IC_{50} = 6 nM)[300] was radiolabeled with ^{11}C to produce [^{11}C]5, as shown in Figure 9 and used as a PET tracer for evaluation of the $P2Y_{12}$ receptor[301] function in MS disease progression.[24,302] Unfortunately, [^{11}C]5 was shown to be an unstable tracer that metabolized rapidly in plasma and in an ex vivo biodistribution study in rats, and only very low brain uptake of this radioligand was detected in this study.[302] Therefore, its use for PET imaging of the $P2Y_{12}$ receptor is not favored.

$P2Y_{13}$ Receptor and Functions in the CNS

$P2Y_{13}$ receptor is one of the most recently identified nucleotide receptor on neurons.[303] Like $P2Y_1$ and $P2Y_{12}$, $P2Y_{13}$ receptor belongs to a group of P2Y receptors responding to endogenous nucleotides ADP.[304] $P2Y_{13}Rs$ are specifically present in cerebellar astrocytes, microglia, and granule neurons where they, and not the $P2Y_1$ receptors, participate in the ADP-evoked calcium responses with $P2Y_{13}$ expression higher in microglia than in the astrocytes.[305] In granule neurons, $P2Y_{13}$ receptors have been coupled to PI3K/Akt pathway that prevents neuronal death.[304] Additionally, $P2Y_{13}$-mediated ERK1/2 signaling has shown to trigger activation of CREB, suggesting an antiapoptotic act of the $P2Y_{13}$ receptor against glutamate neurotransmitter toxicity.[304] $P2Y_{13}$ receptors are implicated in the release of acetylcholine from synapses and play key roles in neuronal cell differentiation and axonal elongation.[305,306]

Remarkably, activation of microglial $P2Y_{12}$ and $P2Y_{13}$ receptors following inflammation induces the release of paracrine mediators via upregulation of the $P2Y_1$ and $P2Y_{12}$ receptors on proliferated astroglia, and upon reduction of inflammation and microglia phenotype change, both $P2Y_{12}$ and $P2Y_{13}$ have shown to be downregulated on astrocytes.[305]

While ADP is the known endogenous agonist of $P2Y_{13}$ ($EC_{50} = 60$ nM),[221] 2-MeSADP, a nonselective $P2Y_{12/13}$ agonist, is even more potent at this receptor.[271] However, inosine 5′-diphosphate sodium salt (IDP) is the preferential selective $P2Y_{13}$ agonist with 5-fold more potency for $hP2Y_{13}$ over the $P2Y_{12}$ receptor.[306] Furthermore, IDP with $EC_{50} = 9.2$ nM is more potent at murine $P2Y_{13}$ than at human $P2Y_{13}$ ($EC_{50} = 552$ nM).[306] Inosine 5′-diphosphate sodium salt is currently considered as a potent $P2Y_{13}$ receptor agonist.[306]

Among the $P2Y_{13}$ receptor antagonists, there are some nonselective $P2Y_{12/13}$ antagonist including a highly potent $P2Y_{12}$ antagonist AR-C69931 ($IC_{50} = 0.4$ nM) and 2-MeSAMP.[221] However, nonnucleoside MRS-2211 is a selective antagonist of $P2Y_{13}$ and displays high selectivity over $P2Y_1$ and $P2Y_{12}$ receptors.[307]

$P2Y_{14}$ Receptor and Functions in the CNS

The $P2Y_{14}$ receptor is preferentially expressed in hematopoietic stem cells of both humans and mice.[308] While physiological functions of this receptor remain to be established, expression of the $P2Y_{14}$ receptor has been detected in immune cells, suggesting its connotation with inflammation.[309] Most of the data on $P2Y_{14}$ is associated with its peripheral effects, but there are indications of its expression in human astrocytes[310] and rat cortical and cerebellar astrocytes.[311] Increased $P2Y_{14}$ receptor expression in LPS-mediated microglial activation also suggests its role in CNS inflammatory responses.[312] In mice, $P2Y_{14}$ deficiency has not shown to carry a noticeable CNS effect under homeostatic conditions, but showed reduced tolerance to glucose and insulin secretion deficiency.[313] A variety of factors including aging, radiation therapy, consecutive exposure to chemotherapy, and repeated bone marrow transplantation have shown to increase senescence in animals lacking $P2Y_{14}$ receptor.[314]

Therapeutic effect of the $P2Y_{14}R$ activation on CNS diseases are not fully elucidated yet. The $P2Y_{14}R$ is activated by UDP-glucose ($EC_{50} = 80$ nM).[221] This endogenous ligand is not prone to hydrolysis and acts as an extracellular proinflammatory mediators.[315] UDP also acts as a $P2Y_{14}$ R agonist, overlapping with the $P2Y_6R$. Several analogs of UDP including MRS2802 and MRS2905 have exhibited high potency and selectivity at the $P2Y_{14}$ over the $P2Y_6$ and other P2Y receptors.[316] Releases of nucleotide-sugars in astrocytes play an important role in maintaining the normal status of the cell via $P2Y_{14}$ receptors.[317]

Potential $P2Y_{14}R$ antagonists are dihydropyridopyrimidine base compound with analogs acting as noncompetitive antagonists of the receptor.[318] Another set of $P2Y_{14}$ R antagonists are naphthoic acid and derivatives that inhibited [^{3}H]UDP binding to the $P2Y_{14}R$, suggesting orthosteric antagonism for $P2Y_{14}$ receptors.[319] A selective and highly potent competitive antagonist PPTN that was converted to a prodrug has shown to increase bioavailability allowing further studies of this receptor.[320] PPTN has shown to inhibit chemotaxis of human neutrophils in cell line expressing $P2Y_{14}$ receptor.[320] An analog of Alexa Fluor 488 (AF488), MRS4174 has also exhibited selectivity and a remarkably high binding activity of 80 pM at the $P2Y_{14}R$.[320] There has been no report of any PET radioligand for mapping of the $P2Y_{14}$Rs.

Concluding Remarks

Existing evidences indicate that chronic inflammation mediated by modulation of neurons and activation of microglia and astrocytes plays significant roles in CNS disorders and specifically in neurodegenerative diseases. Decades of research toward the discovery and development of treatments for these diseases, especially the neurodegeneration, while successful to some extent, still faces hurdles. The probability that some failed therapies have engaged wrong targets might be a possible explanation. Preclinical findings suggest that elucidation of target engagement of drugs in CNS disorders via PET imaging of the known brain biomarkers can assist to track disease progression, guide drug development, and monitor therapies for the treatment of these disorders. This task requires having access to the number of receptor-selective molecular probes. Especially in early stage of neurodegenerative diseases, in addition to evaluation of cerebrospinal fluid and plasma samples of an individual, PET imaging of pro-inflammatory biomarker of the same individual may help identify the causes of inflammation and potentially assist developing an efficient translational application of relevant therapeutic interventions. Purinergic receptors present promising potential for PET imaging of the neurological disorder biomarkers. These receptors have experienced an exciting journey since the discovery of their first member in early 20th century. Currently, a number of ^{11}C and ^{18}F PET radioligands of the adenosine, particularly the A_1 and A_{2A} receptors, and the fast synaptic P2X receptor subtypes, in particular, the $P2X_7$ receptor have helped to elucidate the expression and functions of these purinergic receptors in CNS disorders. Despite emerging facts regarding participation of the P2Y signaling in the brain, their functions are not fully recognized. This is largely due to lack of availability of selective nonnucleotide and brain penetrable ligands to be radiolabeled as PET radiotracer for evaluation of their expression and functions in the brain. However, a list of P2Y receptor ligands have been mentioned in this review to enlighten and guide interested scientists in discovering novel PET ligand for noninvasive approach to evaluate the P2Y receptor contribution in the brain disorders and especially the neurodegeneration diseases.

Authors' Note

H. Zarrinmayeh has over 20 years of research experience as a medicinal chemist in pharmaceutical industry where she designed and

discovered lead drug candidates for the treatment of various disorders including cancer and especially the diseases and disorders of the CNS. Upon joining Indiana University Radiology and Imaging Sciences Department, Dr. Zarrinmayeh resumed her research in the area of the design and development of novel $P2X_7$ receptor PET radioligand for evaluation of neuroinflammation and assessment of neurodegeneration. Her contribution has yielded to the discovery of a novel ^{18}F PET radioligands for evaluation of the $P2X_7$, a biomarker of neuroinflammation in CNS disorders. Dr. Territo has more than 20 years of experience in physiology, pharmacology, medical imaging, and biomarker development in support of phenotyping and therapeutic response in both pharmaceutical industry (10 years) and academia (+10 years), where his experiences led to the development of translational imaging biomarkers in the area of neuroscience, oncology, and cardiovascular diseases. At IUSM, Dr. Territo's research has incorporated both Tracer Development and Validation and Pre-Clinical Imaging techniques. The Tracer Development and Validation Lab was established to support development of novel imaging tracers by integration of molecular methods, physiology, pharmacology, imaging, and analysis modeling. Dr. Territo oversees the in vitro, in vivo, and ex vivo imaging studies of ^{11}C and ^{18}F PET radioligands and is involved in study analysis and statistical modeling of the data from these studies.

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