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Review Article

Assessment of adenosine A_{2A} receptors with PET as a new diagnostic tool for neurological disorders

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Introduction

Adenosine is an endogenous modulator of several physiological functions in the central nervous system (CNS) as well as in peripheral organs. Its effects are mediated by 2 major subtypes of receptors: adenosine A_1 receptors, which exhibit a higher affinity for adenosine and inhibit adenylyl cyclase, and adenosine A_2 receptors, which exhibit a lower affinity for adenosine and stimulate adenylyl cyclase. Recent advances in molecular biology and pharmacology have demonstrated the presence of at least 4 subtypes: A_1, A_{2A}, A_{2B} and A_3 receptors (1-4).

From the first demonstration of dopamine D_2 receptors in the human brain (5), many neuroreceptors in humans and other animals have been visualized *in vivo* by positron emission tomography (PET) and single photon emission computed tomography (SPECT) with the corresponding radioligands (6-9). In turn, the use and value of PET as a tool in CNS drug discovery and development is growing (10). PET assessment of the adenosine receptor system in the CNS may provide us with a new tool for diagnosing neurological disorders as well as increase our understanding of neurotransmission in general. Therefore, since 1995, several PET ligands have been proposed for mapping the adenosine A_1 (11-17) and A_{2A} receptors (18-27) and adenosine uptake sites (28) in the CNS. Several radioligands are found to be promising for application to human PET studies. This article reviews the current development of PET ligands for adenosine A_{2A} receptors.

Design of adenosine A_{2A} receptor ligands

The first adenosine receptor antagonist described in the literature as being A_{2A}-selective was 3,7-dimethyl-1-propylxanthine (DMPX); however, it has low affinity and low A_{2A}-selectivity *versus* A_1 receptors (29). Shimada *et al.* have discovered that introduction of the styryl group in the 8 position of xanthines was critical in achieving compounds endowed with selective A_{2A} receptor antagonistic properties (30, 31). The representative compound, (*E*)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine (KF17837), has been widely used for pharmacological and neurochemical studies as a selective antagonist for adenosine A_{2A} receptors. Another compound, (*E*)1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methylxanthine (KW-6002), is currently being developed for the treatment of Parkinson's disease (4, 32). *N*-Alkyl and *O*-methoxy groups in the styryl group in the 8 position of these xanthines can be easily labeled with a positron emitter, ^{11}C ($t_{1/2}$ = 20 min), via [^{11}C]alkyl iodide. Six PET radioligands including [^{11}C]KF17837 were prepared by *N*- or *O*-methylation with [^{11}C]methyl iodide.

Müller *et al.* introduced brominated and chlorinated styryl groups in the 8 position of DMPX to produce A_{2A} selectivity (33, 34). (*E*)-8-(3-Bromostyryl)-3,7-dimethyl-1-propargylxanthine (BS-DMPX) and (*E*)-8-(3-chlorostyryl)-3,7-dimethyl-1-propargylxanthine (CS-DMPX) had slightly lower affinity for the adenosine A_{2A} receptors than KF17837. BS-DMPX can potentially be labeled with another positron emitter ^{75}Br ($t_{1/2}$ = 1.7 h) or ^{76}Br ($t_{1/2}$ = 16.1 h) in addition to ^{11}C. These findings led to the idea that the iodinated derivative (*E*)-3,7-dimethyl-8-(3-iodostyryl)-1-propargylxanthine (IS-DMPX) may also

Table I: The affinity and calculated lipophilicity of adenosine A_{2A} receptor antagonists labeled with a positron emitter and related antagonists.

	Affinity, K_i (nM)*		Selectivity	Ref.	Lipophilicity+
	A_1	A_{2A}	A_1/A_{2A}		cLog P
KF17837	62	1.0	62	(31)	4.03
KW-6002	150	2.2	68	(32)	2.97
KF21213	> 10000	3.0	> 3300	(25)	3.12
KF19631	860	3.5	250	(22)	2.70
KF18446	1600	5.9	270	(22)	1.55
CSC	28000	54	520	(32)	2.97
DMPX	12000	8600	1.4	(33)	–0.34
BS-DMPX	2300	7.7	300	(24)	2.82
IS-DMPX	> 10000	8.9	> 1100	(24)	3.08
SCH 58261	121	2.3	53	(36)	3.54
SCH 442416#	1815	0.50	3630	(26)	3.84
ZM 241385	510	0.91	560	(23)	2.82

*The affinity of all compounds for the adenosine A_1 and A_{2A} receptors was determined using the rat forebrain membrane and [^{3}H]-*N*^{6}-cyclohexyladenosine as a radioligand and the rat striatal membrane and [^{3}H]CGS 21680, respectively. #The radioligands used for measuring affinities for A_1 and A_{2A} receptors were [^{3}H]DPCPX and [^{3}H]SCH 58261, respectively. +cLogP was calculated by Daylight PC models ver.4.72.

have a high affinity for the A_{2A} receptors (24). [^{11}C]- and [^{123}I]-labeled IS-DMPX may be applied to studies with PET and SPECT, respectively.

A number of nonxanthine heterocycles have also been synthesized as A_{2A} receptor antagonists starting from the nonselective adenosine antagonist CGS 15943, a triazoloquinazoline. Representative ligands with a high and selective affinity are SCH 58261, 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]-pyrimidine (35, 36) and ZM 241385, 4-(2-[7-amino-2-{2-furyl}{1,2,4}triazolo{2,3-*a*}{1,3,5}triazin-5-yl-amino]ethyl)-phenol (37). These ligands do not have an appropriate synthon to label with positron emitters; however, Todde *et al.* used SCH 442416 with its 4-methoxyphenylpropyl group and prepared [^{11}C]SCH 442416 by *O*-[^{11}C]methylation (26). The iodinated ZM 241385 ([^{125}I]ZM 241385) was reported as a selective radioligand (38), and a [^{123}I]-labeled analog may be applicable to SPECT.

Table I summarizes the *in vitro* affinity and selectivity for the adenosine receptors and lipophilicity of the ligands described above. Among the compounds labeled with a positron emitter, the highest affinity for the adenosine A_{2A} receptors was found with SCH 442416, followed by KF17837, KW-6002 and KF21213. SCH 442416, KF21213 and IS-DMPX showed superior A_{2A} selectivity. KF19631, KF18446, CSC and BS-DMPX showed moderate selectivity, but their affinities for the A_1 receptors were too low to bind *in vivo*. All compounds except for DMPX and KF18446 have a high lipophilicity evaluated by cLog P values.

Radiosynthesis

The positron emitting adenosine A_{2A} receptor ligands described in the literature (18-27) are summarized in Figure 1. All ligands were prepared by *N*^{7}-methylation of the xanthine or 4-*O*-methylation of the styrlyl group of respective demethyl compounds with [^{11}C]methyl iodide, followed by HPLC separation. The radiochemical yields were high for clinical use except for the syntheses of [^{11}C]BS-DMPX and [^{11}C]IS-DMPX (24). The specific radioactivity was also high enough for receptor-ligand binding studies *in vivo*. All procedures were carried out under dim light to prevent isomerization of the compounds (39).

Localization of adenosine A_{2A} receptors in the brain

Adenosine A_{2A} receptors are present at a high density in the striatum, nucleus accumbens and olfactory tubercle where dopamine D_1 and D_2 receptors are also localized in very high densities (40, 41). In contrast, adenosine A_1 receptors are widely distributed throughout the entire brain. The density of the binding sites of a standard A_{2A} receptor ligand [^{3}H]CGS 21680 was over 10 times higher in the rat stratum as compared to other tissues (40). The binding sites of [^{3}H]CGS 21680 in the hippocampus and cortex (42-44) may be distinctly different from the classical adenosine A_{2A} receptors present in the striatum, and may also be different from other defined receptor subtypes. The reported B_{max} and K_d values (respectively) are 353 pmol/mg protein and 58 nM in the hippocampus, 264 pmol/mg protein and 58 nM in the cortex and 419 pmol/mg protein and 17 nM in the striatum (44). The cortical binding sites (atypical A_{2A} receptors) of [^{3}H]CGS 21680 were clearly discriminated with another selective adenosine A_{2A} receptor antagonist SCH58261 (45).

A high density of the adenosine A_{2A} receptors in the striatum was also confirmed in postmortem human brains

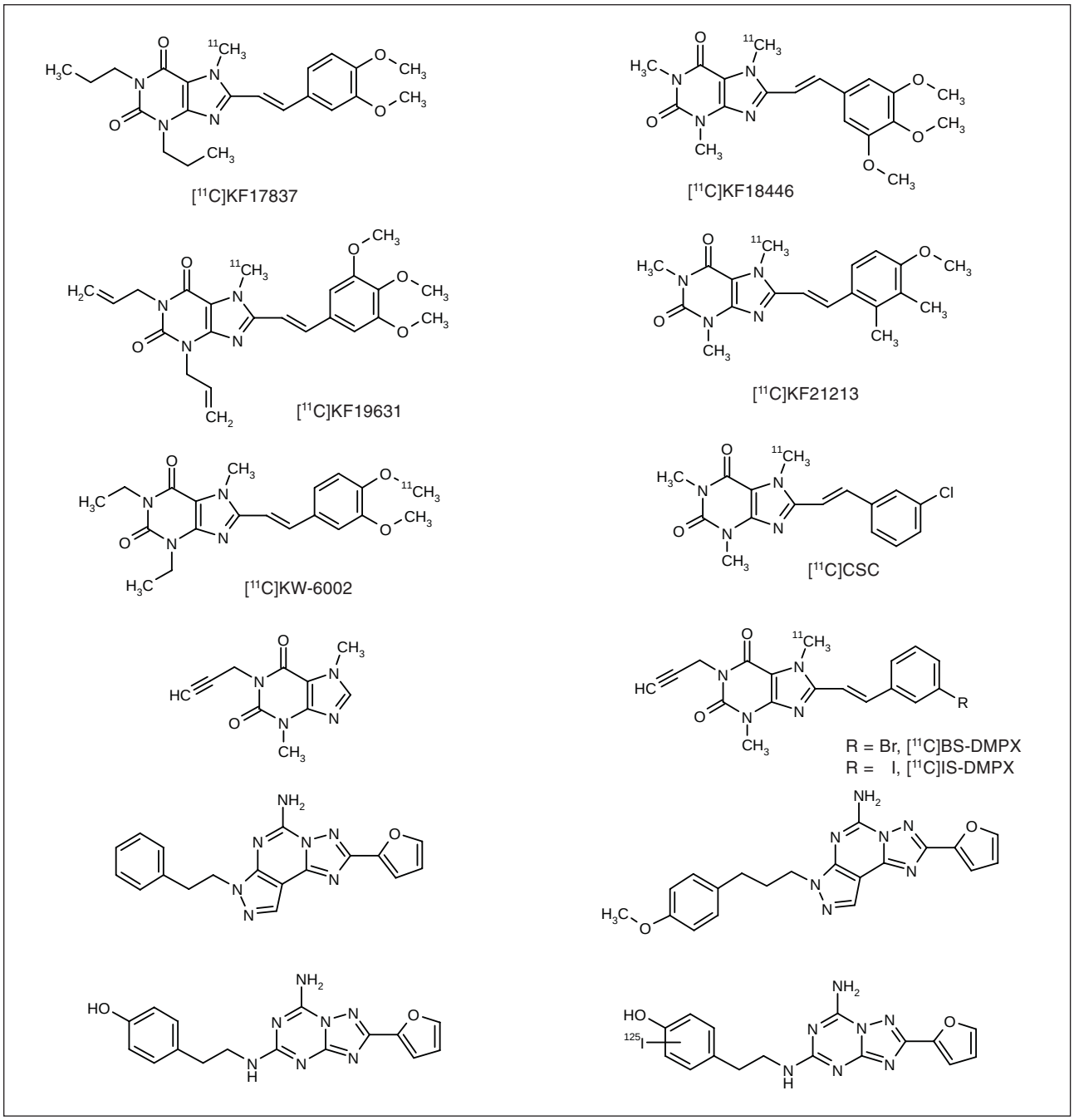

Fig. 1. Chemical structures of positron emitting adenosine A_{2A} receptor ligands proposed for PET studies and 4 other adenosine A_{2A} antagonists.

using a membrane binding assay (46) and autoradiography (47). The binding of [^{3}H]CGS 21680 or [^{3}H]SCH 58261 to extrastriatal regions such as the thalamic nuclei and throughout the cerebral cortex (although to a much lesser extent than the striatal binding seen in the rat brain) demonstrates that the adenosine A_{2A} receptors seem to be more widely distributed in the human brain than previously recognized.

Evaluation of PET radioligands in animal models

Following the injection of radioligands into experimental animals, the radioactivity in brain tissues includes free, receptor-bound and nonspecifically bound ligands and labeled metabolites. In general, the time-activity curves in the tissues are primarily dependent on the affinities of the radioligands. The receptor-specific binding of the

Table II: Striatal uptake, in vivo *selectivity and* in vivo *specific binding of positron emitting (PET) radioligands for adenosine A_{2A} receptors.*

	Uptake* Striatum	*In vivo* selectivity Uptake ratio		*In vivo* specific binding+ Striatum	Cortex	Cerebellum
	(SUV)	Striatum/Cortex	Striatum/Cerebellum	(%)	(%)	(%)
[^{11}C]KF17837	0.82 (m, 15)	2.1 (m, 60) 1.5 (r, 15)#	2.0 (m, 60) 1.2 (r, 15)#	43 (m, 15)	32 (m, 15)	44 (m, 15)
[^{11}C]KF19631	0.33 (m, 15)	1.6 (m, 60) 1.5 (r, 15)*	1.2 (m, 60) 1.2 (r, 15)*	31 (m, 15)45	(m, 15)	46 (m, 15)
[^{11}C]KF18446	1.54 (m, 15) 1.68 (r, 15)*	2.7 (m, 15) 3.2 (r, 15)#	2.8 (m, 15) 2.7 (r, 15)#	72 (m, 15)	37 (m, 15)	38 (m, 15)
[^{11}C]CSC	ND	1.3 (r, 15)#	ND	ND	ND	ND
[^{11}C]KF21213	1.40 (m, 15)	8.6 (m, 60) 4.0 (r, 15)#	10.5 (m, 60) 3.7 (r, 15)#	69(m, 15)	12^{ns} (m, 15)	7^{ns} (m, 15)
[^{11}C]KW-6002	2.9 (r, 15)	1.9 (r, 75)	1.1 (r, 75)	88 (r, 75)	76 (r, 75)	87 (r, 75)
[^{11}C]BS-DPMX	0.90 (m, 15)	1.7 (m, 60)	1.2 (m, 60)	51 (m, 15)	43 (m, 15)	49 (m, 15)
[^{11}C]IS-DPMX	0.70 (m, 15)	1.6 (m, 60)	1.2 (m, 60)	17^{ns} (m, 15)	29 (m, 15)	26 (m, 15)
[^{11}C]SCH 442416	1.15 (r, 15)	4.6 (r, 15)	4.6 (r, 15)	ND	ND	ND

*Uptake was normalized as the standardized uptake value (SUV, [tissue activity/total injected activity] x [g weight/g tissue weight]), assuming the body weight of rats and mice was 300 g and 35 g, respectively, when the other parameter, the percentage of tissue activity of the injected dose per gram of tissue, was used. In parentheses "m" and "r" represent the uptake in rat and mouse brain, respectively, killed at the time indicated. Tissue uptake was measured using the tissue dissection method or #by *ex vivo* autoradiography. +The reduced percentages of the uptake by the blockade with selective appropriate adenosine A_{2A} ligand. ND, not determined; ns, not significant as compared to the control and blocked animals.

radioligand *in vivo* is usually evaluated as the difference between the uptake by the target tissue and that by a reference tissue that is devoid of the receptors, or the difference between the tissue uptake in the baseline and that in the blockade with an excess amount of nonradioactive selective ligand, which is administered as a pretreatment, coinjection or posttreatment (Table II).

In vivo *affinity*

After injection of [^{11}C]KF17837, [^{11}C]KF21213 or [^{11}C]SCH 442416 into mice or rats, the radioactivity accumulates in the striatum being highly enriched with adenosine A_{2A} receptors for 15-30 min and then decreases. These time-activity curves represent a reversible ligand-receptor binding in the time frame of PET measurement with [^{11}C]-labeled tracers. Although [^{11}C]KW-6002 and [^{11}C]KF19631 have lower affinities than these 3 radioligands, they accumulated for 60-75 min. [^{11}C]KF18446 and other radioligands with weaker affinities decreased following the initial uptake. [^{11}C]CSC, with a lower affinity (K_i = 54 nM), was rapidly washed out from the brain, suggesting little receptor binding *in vivo*.

In vivo *selectivity*

Among 9 radioligands investigated, the highest *in vivo* selectivity evaluated with the uptake ratios of striatum to cortex and striatum to cerebellum was found with [^{11}C]KF21213, followed by [^{11}C]SCH 442416, [^{11}C]KF 18446 and [^{11}C]KF17837. The finding that the adenosine A_{2A} receptors are more scarce in the cerebellum as compared to the cerebral cortex is mostly reflected in the distribution profile of [^{11}C]KF21213 (striatum-to-cortex ratio < striatum-to-cerebellum). The small striatum-to-cortex and striatum-to-cerebellum ratios for other radioligands are inconsistent with the density of the adenosine A_{2A} receptors measured *in vitro* (44).

In vivo *specific binding*

In the case of [^{11}C]KF21213 and [^{11}C]KF18446, the specific binding was much greater in the striatum than the cortex and cerebellum. In the other radioligands, a similar or even higher specific binding was evaluated in the cortex and cerebellum compared with that in the striatum by the blockade studies; however, the reduction does not reflect specific binding of the ligands to the adenosine A_{2A} receptors because of the few receptors in these tissues as described above. As for [^{11}C]KF21213 and [^{11}C]KF18446, no *in vivo* affinity for adenosine A_1 receptors was found.

Metabolism

A slow peripheral degradation of 2 xanthine compounds, [^{11}C]KF18446 and [^{11}C]KW-6002, was confirmed with metabolite analysis in plasma, but most radioactivity was detected as unchanged in the striatum. The findings demonstrate that the PET signal in the striatum reflects free, receptor-bound and nonspecifically bound radioligand when they are applied to PET. The labeled metabolites of [^{11}C]SCH 442416 were also found in plasma, but not investigated in the striatum.

Candidate PET ligands for human studies

So far, [^{11}C]KF18446, [^{11}C]KF21213 and [^{11}C]SCH 442416 are the radioligands of choice for PET studies, although the specific binding of [^{11}C]SCH 442416 has not yet been evaluated in a blockade study (26). These 3 radioligands showed suitable pharmacokinetic properties as [^{11}C]-labeled tracers ($t_{1/2}$ = 20 min), *i.e.*, the receptor-ligand binding in the brain reaches an equilibrium state during the time scale for PET measurement. Among the 3, only [^{11}C]KF18446 has been successfully applied to PET imaging of the adenosine A_{2A} receptors in the monkey striatum (22). The lipophilicity of [^{11}C]KF18446 (cLog P = 1.55) made it much better at penetrating the blood-brain barrier in the monkey brain than [^{11}C]KF17837 (cLog P = 4.03) (20, 22). The higher lipophilicity of [^{11}C]KF21213 (cLog P = 3.12) and [^{11}C]SCH 442416 (cLog P = 3.84) may result in less penetration into the brain.

[^{11}C]KF21213 and [^{11}C]SCH 442416 showed superior *in vivo* properties as compared to [^{11}C]KF18446; however, [^{11}C]KF18446 with a weaker affinity is still of interest as a diagnostic tool. In the radioligands with a lower affinity, the receptor-radioligand binding may be influenced by endogenous neurotransmitters (48). For example, the binding of [^{11}C]raclopride to striatal dopamine D_2 receptors was significantly altered by drugs that modify synaptic dopamine levels (49) and by physiological stimulation (50). Therefore, it would be interesting to investigate whether the kinetics of the receptor binding of [^{11}C]KF18446 are affected by changes in the endogenous adenosine concentration in brain disorders such as ischemia.

Postmortem human brain studies and the prospect for PET studies

A limited number of postmortem human studies on adenosine A_{2A} receptors have been published. In patients with Huntington's disease in which selective degeneration of the striatopallidal neurons is one of the pathological features, the density of adenosine A_{2A} receptors was found to be significantly reduced in the striatum (51, 52). The loss of adenosine A_{2A} receptor binding in the caudate nucleus, putamen and external globus pallidus was more predominant than that of dopamine D_2 receptor binding (52).

In patients with Parkinson's disease characterized by selective degeneration of nigrostriatal dopamine neurons, adenosine A_{2A} density was not significantly affected (51). On the other hand, a significant decrease in the level of adenosine A_{2A} receptor mRNA was found in the anterior and posterior caudate nucleus and anterior dorsal putamen of parkinsonian patients who were receiving treatment with dopaminergic drugs and died. In contrast, a significant increase was observed in the substantia nigra pars reticulata when compared to age-matched controls (53). However, chronic administration of haloperidol, a typical neuroleptic drug, increased the density of adenosine A_{2A} receptors in the rat striatum (54).

Thus far a large number of PET studies have been performed to characterize the degeneration of nigrostriatal and striatopallidal neurons in patients with neurological disorders (6-9). The markers used for the nigrostriatal neurons were dopamine synthesis and the density of the dopamine transporters, which were evaluated with radiolabeled precursors of dopamine such as [^{18}F]6-fluorodopa and [^{18}F/^{11}C]-labeled dopamine transporter ligands such as [^{18}F/^{11}C]β-CFT, respectively. The den-sity of dopamine D_2 receptors was usually measured using selective D_2 ligands such as [^{11}C]raclopride for evaluating striatopallidal neurons. Adenosine A_{2A} receptors can be used as a marker for the diagnosis of neurological disorders because they are coexpressed with the dopamine D_2 receptors on GABAergic-enkephalin neurons (55, 56). However, a number of experiments on signal transduction, gene expression, neurotransmitter release and behavioral responses showed that adenosine A_{2A} receptors effects opposed dopamine D_2 receptor mediated-effects (4), although A_{2A}-D_2 receptor interaction was also suggested (57, 58). The information derived from PET studies targeting the adenosine A_{2A} receptors would be different from the PET data obtained previously. In a preliminary PET study using a rat model in which excitotoxin quinolinic acid was unilaterally injected into the striatum, the binding of [^{11}C]KF18446 to adenosine A_{2A} receptors decreased slightly more than the binding of [^{11}C]raclopride to dopamine D_2 receptors (59).

PET and SPECT are used to differentially diagnose Parkinson's disease from parkinsonian syndromes such as multiple system atrophy, progressive supranuclear palsy, corticobasal degeneration or diffuse Lewy body disease to a certain extent, even though these syndromes have some particular neurochemical and metabolic profiles. However, clinical variability in the symptoms, therapeutic responses and prognosis for Parkinson's disease cannot be explained only by the evaluation of metabolic profiles and the dopaminergic system. Furthermore, parkinsonian syndromes with the exception of idiopathic Parkinson's disease showed degeneration of postsynaptic as well as presynaptic dopaminergic functions (7, 9), but the use of PET and SPECT to distinguish between the parkinsonian syndromes has barely been established. Therefore, the diagnosis of these neurological disorders by PET targeting of adenosine A_{2A} receptors is of great interest. It is also expected that PET will provide important information on the pathological features of parkinsonian syndromes.

Also of interest is that the receptor-specific binding of the adenosine A_{2A} ligand [^{11}C]KF-18446 but not that of the dopamine D_2 receptor ligand [^{11}C]raclopride was found in the rat globus pallidus by *ex vivo* autoradiography (60). So far, no PET study has been performed which focuses on the globus pallidus probably due to its small structure and low metabolic activity. Using PET with radioligands for adenosine A_{2A} receptors may enable imaging of the pallidal terminals projecting from the striatum.

Conclusions

[^{11}C]KF18446, [^{11}C]KF21213 and [^{11}C]SCH 442416 have the potential to be used as radioligands for PET mapping of adenosine A_{2A} receptors in the human brain. PET studies would contribute to the understanding of the adenosine receptor system in the CNS *in vivo*, and provide a new diagnostic tool for neurological disorders.

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