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[^{3}H]8-Ethyl-4-methyl-2-phenyl-(8*R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]-purin-5-one ([^{3}H]PSB-11), a Novel High-Affinity Antagonist Radioligand for Human A_3 Adenosine Receptors

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Abstract—This study describes the preparation and binding properties of [^{3}H]PSB-11, a novel, potent, and selective antagonist radioligand for human A_3 adenosine receptors (ARs). [^{3}H]PSB-11 binding to membranes of Chinese hamster ovary (CHO) cells expressing the human A_3 AR was saturable and reversible. Saturation experiments showed that [^{3}H]PSB-11 labeled a single class of binding sites with high affinity ($K_D = 4.9$ nM) and limited capacity ($B_{max} = 3500$ fmol/mg of protein). PSB-11 is highly selective versus the other adenosine receptor subtypes. The new radioligand shows an extraordinarily low degree of non-specific binding rendering it a very useful tool for studying the (patho)physiological roles of A_3 ARs.

The adenosine receptor (AR) family comprises four distinct subtypes, A_1, A_{2A}, A_{2B}, and A_3.[1] The ARs are G protein-coupled and may either inhibit (A_1, A_3) or stimulate (A_{2A}, A_{2B}) adenylate cyclase activity. Coupling to other second messenger systems has been described, including phospholipase C stimulation (A_1, A_{2B}, A_3).[1] The A_3 AR is unique for it shows large species differences between rat and human A_3 AR with respect to amino acid sequence, ligand affinity, and tissue distribution/level of expression.[2] The A_3 AR has become a new target for drug development,[3] since it may play a role in pathological conditions such as inflammatory diseases, including allergies, asthma, and rhinitis, ischemias and glaucoma.[2–4]

Only a few radioligands suitable for the characterization of A_3 ARs have become available;[5] all of them are affected with drawbacks. The current standard radioligand for A_3 ARs is [^{125}I]AB-MECA, an *agonist* structurally derived from adenosine.[6] In addition, the non-selective agonist [^{3}H]NECA, and the A_1-selective agonist [^{3}H]*R*-PIA have been used in binding studies with recombinant human A_3 ARs.[7] The first antagonist radioligand for human A_3 ARs has recently been developed by Baraldi and co-workers.[8,9] The [^{3}H]-labeled pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine derivative [^{3}H]MRE 3008F20 is very potent at human A_3 ARs ($K_D = 0.80$ nM) and selective versus the other AR subtypes. However, the compound is highly lipophilic and exhibits a high degree of non-specific binding (ca. 25% at the K_D value).[9] The radioligand has not become generally available so far. However, an antagonist radioligand is urgently needed to study the (patho)physiological role of A_3 ARs.

Recently, 2-phenyl-substituted imidazo[2,1-*i*]purin-5-ones have been found to possess high affinity for human A_3 ARs.[10,11] The compounds were shown to be antagonists at the ARs. 8-Ethyl-4-methyl-2-phenyl-(8*R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]-purin-5-one (PSB-11) exhibited a K_i value of 2.3 nM at human A_3 ARs and was several hundred-fold selective versus A_1, A_{2A} and A_{2B} ARs.[10]

PSB-11 was subsequently characterized as an antagonist with inverse agonistic activity at human A_3 ARs in [^{35}S]GTPγS binding studies.[11] The compound PSB-11 has now been prepared in tritiated form and characterized as a novel A_3 antagonist radioligand.

[^{3}H]PSB-11 was prepared by catalytic hydrogenation of the trichlorophenyl precursor PSB-10 using tritium gas (Fig. 1). The radiolabeling was performed by Nycomed Amersham, Buckinghamshire, UK through Amersham Pharmacia Biotech Europe GmbH, Freiburg, Germany.

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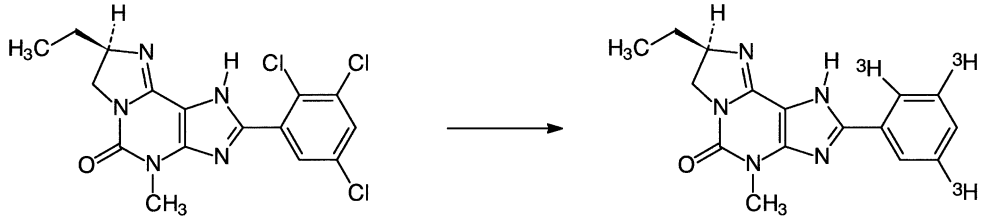

Figure 1. Preparation of [^{3}H]PSB-11 from the trichlorophenyl precursor PSB-10 by catalytic hydrogenation.

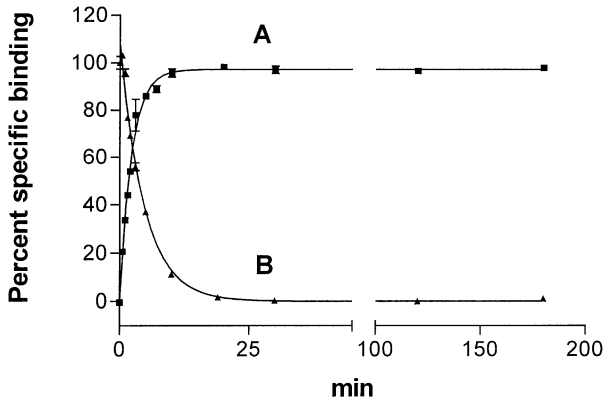

Figure 2. Kinetics of [^{3}H]PSB-11 binding (0.5 nM) to membranes of Chinese hamster ovary cells expressing the human A_3 adenosine receptor at 25 °C: (A) association curve; (B) dissociation curve; dissociation was achieved by the addition of 100 μM *R*-PIA after 2 h of preincubation.

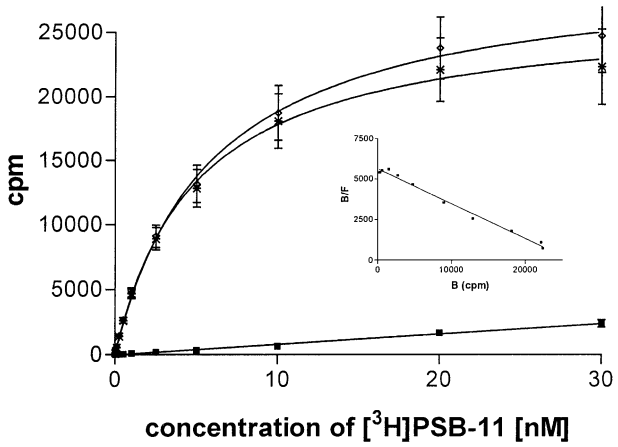

Figure 3. Saturation curve for [^{3}H]PSB-11 binding to membranes of Chinese hamster ovary cells expressing the human A_3 adenosine receptor and corresponding Scatchard plot. The following binding parameters were calculated: $K_D = 4.9 \pm 0.2$ nM, $B_{max} = 3500$ fmol/mg protein.

The specific activity was 53 Ci/mmol (1.96 TBq/mmol). Radiochemical purity was found to be 99.6% as determined by HPLC (column: Hypersil ODS 5 μm, 250 × 4.6 mm, solvents: (A) methanol/water/triethylamine (10:90:1), (B) methanol/triethylamine (100:1), gradient: 30 to 100% B over 15 min, flow rate: 1 mL/min, UV detection at 254 nm, elution at 5 min. The structure was confirmed by FAB-MS in comparison with non-labeled compound (PSB-10).

Kinetic studies using CHO cell membranes expressing the human A_3AR[7] were performed using 0.5 nM [^{3}H]PSB-11 in a total volume of 500 μL containing 70 μg of protein. Both association and dissociation appeared monophasic (Fig. 2). Equilibrium was reached after less than 10 min. The binding was rapidly reversed after the addition of 100 μM of *R*-PIA (Fig. 2).

Saturation experiments using 10 different concentrations ranging from 0.05 to 30 nM showed that [^{3}H]PSB-11 bound to a single class of binding sites with limited capacity exhibiting a K_D value of 4.9 ± 0.2 nM and an apparent B_{max} value of 3.5 pmol/mg protein (Fig. 3).

Competition experiments with selected agonists and antagonists using 0.5 nM of [^{3}H]PSB-11 showed a rank order of potencies typical for the human A_3 AR:[1,2,4,9] (R)-N^6-phenylisopropyladenosine (*R*-PIA) $\geq$ 5′-*N*-ethylcarboxamidoadenosine (NECA) > 2-chloroadenosine (CADO) > N^6-cyclopentyladenosine (CPA) > 2-[4-(carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine (CGS-21680). AR antagonists showed the following order of potency: PSB-11 >> 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) > caffeine, 3,7-dimethyl-1-propargylxanthine (DMPX).

At a concentration of 0.5 nM, total binding corresponded to ca. 2700 cpm, and non-specific binding amounted to only 1–2% of total binding. Non-specific binding was only slightly higher at the K_D value of [^{3}H]PSB-11 (2.5 ± 0.1% at 5 nM). This extraordinarily low degree of non-specific binding is probably due to the imidazoline ring containing a basic nitrogen atom which can be protonated at physiological pH values, conferring high polarity and increased water-solubility to the molecule.

In conclusion, we have developed a novel antagonist radioligand, [^{3}H]PSB-11, for human A_3 ARs which exhibits high receptor affinity and selectivity and an extraordinarily low degree of non-specific binding. These properties will render it a useful pharmacological tool, for example, for investigating native tissues expressing low densities of A_3 ARs.

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References and Notes

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