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[^{3}H]MRS 1754, a selective antagonist radioligand for A_{2B} adenosine receptors

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Abstract

MRS 1754 [*N*-(4-cyanophenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)-phenoxy]acetamide] is a selective antagonist ligand of A_{2B} adenosine receptors. This is the least well-defined adenosine receptor subtype, and A_{2B} antagonists have potential as antiasthmatic drugs. For use as a radioligand, MRS 1754, a *p*-cyanoanilide xanthine derivative, was tritiated on the propyl groups in a two-step reaction using a *p*-carboxamido precursor, which was dehydrated to the cyano species using trifluoroacetic anhydride. [^{3}H]MRS 1754 (150 Ci/mmol) bound to recombinant human A_{2B} adenosine receptors in membranes of stably transfected HEK-293 cells. Specific binding was saturable, competitive, and followed a one-site model, with a K_D value of 1.13 ± 0.12 nM and a B_{max} value of 10.9 ± 0.6 pmol/mg protein. Specific binding utilizing 0.7 nM [^{3}H]MRS 1754 was > 70% of total binding. The affinity calculated from association and dissociation binding constants was 1.22 nM (N = 4). Binding to membranes expressing rat and human A_1 and A_3 adenosine receptors was not significant, and binding in membranes of HEK-293 cells expressing human A_{2A} receptors was of low affinity (K_D > 50 nM). The effects of cations and chelators were explored. Specific binding was constant over a pH range of 4.5 to 6.5, with reduced binding at higher pH. The pharmacological profile in competition experiments with [^{3}H]MRS 1754 was consistent with the structure–activity relationship for agonists and antagonists at A_{2B} receptors. The K_i values of XAC (xanthine amine congener) and CPX (8-cyclopentyl-1,3-dipropylxanthine) were 16 and 55 nM, respectively. NECA (5′-*N*-ethylcarboxamidoadenosine) competed for [^{3}H]MRS 1754 binding with a K_i of 570 nM, similar to its potency in functional assays. Thus, [^{3}H]MRS 1754 is suitable as a selective, high-affinity radioligand for A_{2B} receptors.

Keywords: G protein-coupled receptors; Tritium purines; Xanthines; Adenosine analogues

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Abbreviations: BOP-Cl, bis(2-oxo-3-oxazolidinyl)phosphinic chloride; CGS 15943, 9-chloro-2-(2-furanyl) [1, 2, 4]triazolo[1,5-*c*]quinazolin-5-amine; CGS 21680, 2- [4-[(2-carboxyethyl]phenyl]ethyl-amino]-5′-*N*-ethylcarbamoyladenosine; CHO cells, Chinese hamster ovary cells; CPA, N^6-cyclopentyladenosine; CPX, 8-cyclopentyl-1,3-dipropylxanthine; DAX, 1,3-diallyl-8-cyclohexylxanthine; DMF, dimethylformamide; HEK cells, human embryonic kidney cells; IABOPX, 3-(4-amino-3-iodobenzyl)-8-(phenyl-4-oxyacetate)-1-propylxanthine; K_D, dissociation constant; K_i, equilibrium inhibition constant; MRS 1754, *N*-(4-cyanophenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)-phenoxy]acetamide; NECA, 5′-*N*-ethylcarboxamidoadenosine; *R*-PIA, *R*-N^6-phenylisopropyladenosine; SAR, structure–activity relationship; SCH 58261, 5-amino-7-(2-phenylethyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine; SPA, N^6-*p*-sulfophenyladenosine; TEA, triethylamine; XAC, 8- [4-[[[[(2-aminoethyl]amino]carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthine; XCC, 8- [4-[(carboxymethyl]oxy]phenyl]-1,3-dipropylxanthine; and ZM 241385, 4-(2-[7-amino-2-{furyl}{1,2,4}triazolo{2,3-*a*}{1,3,5}triazin-5-ylamino]ethyl)phenol.

1. Introduction

Four extracellular G protein-coupled receptors for adenosine have been identified: A_1, A_{2A}, A_{2B}, and A_3 [1]. A_{2B} receptors, which are coupled to stimulation of adenylyl cyclase [2, 3] and also lead to a rise in intracellular calcium [4], are involved in the control of vascular tone, cell growth and gene expression, mast cell degranulation, and intestinal water secretion. Activation of A_{2B} receptors in human retinal endothelial cells may lead to neovascularization by a mechanism involving increased angiogenic growth factor expression [5]. Selective xanthine antagonists of the A_{2B} receptor have been reported recently [6, 7]. Such antagonists are potentially useful in the treatment of asthma [8, 9] and intestinal disorders [10].

Non-selective radioligands have been used to character-

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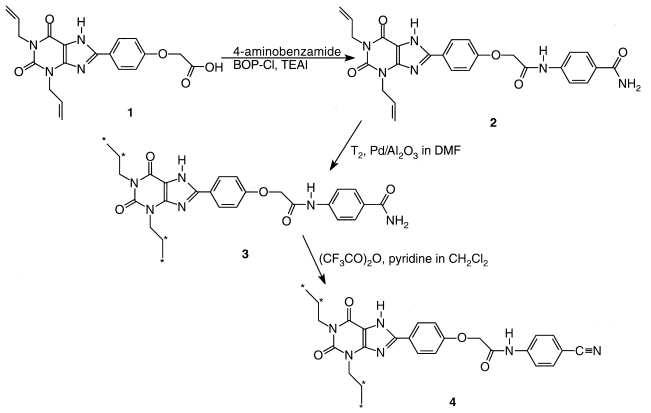

Fig. 1. Synthesis of [^{3}H]MRS 1754, **4,** in a multi-step reaction sequence. *p*-Carboxamidoaniline was condensed with XCC, **1,** to give the amide, **2.** The 1,3-diallyl groups were tritiated to give **3.** Dehydration gave the final product, **4,** which was purified using TLC.

ize recombinant human A_{2B} receptors overexpressed in HEK-293 cells. These include: ^{125}I-IABOPX (^{125}I-3-(4-amino-3-iodobenzyl)-8-(phenyl-4-oxyacetate)-1-propylxanthine) [11], [^{3}H]CPX [9], and [^{3}H]ZM 241385 ([^{3}H]4-(2-[7-amino-2-{furyl}{1,2,4}triazolo{2,3-*a*}{1,3,5}triazin-5-ylamino]ethyl)phenol) [12]. Based on these binding assays, we have identified several new compounds with improved potency and selectivity for human A_{2B} receptors [13, 14], culminating with aniline derivatives of the 8-phenylxanthine carboxylic congener, XCC (**1,** Fig. 1) [6]. A *p*-cyanoanilide derivative in this series, MRS 1754 (*N*-(4-cyanophenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)-phenoxy]acetamide, **4,** Fig. 1), was 400-, 245-, and 123-fold more selective for human A_{2B} receptors than human $A_1/A_{2A}/A_3$ receptors, although less selective than rat A_1/A_{2A} receptors. This antagonist at a 100 nM concentration was shown to completely inhibit calcium mobilization stimulated by 1 μM NECA in HEK-293 cells expressing human A_{2B} receptors [6]. In the present study, this selective antagonist for the A_{2B} adenosine receptor, MRS 1754, has been prepared in tritiated form and shown to be a selective, high-affinity radioligand useful for characterizing recombinant human A_{2B} receptors.

2. Materials and methods

2.1. Synthesis

2.1.1. Preparation of ^{3}H-labelled MRS 1754

2.1.1.a N-(4-(aminocarbonyl)phenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl)-phenoxy]acetamide (2). A solution of **1** [15] (2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-diallyl-1*H*-purin-8-yl)-phenoxy]acetic acid, 38 mg, 0.1 mmol), 4-aminobenzamide (27 mg, 0.2 mmol), BOP-Cl (30 mg, 0.12 mmol), and TEA (20 μL, 0.206 mmol) in 2 mL of anhydrous DMF:CH_2Cl_2 (1:1 mixture) was stirred at room temperature for 24 hr. The mixture was evaporated to dryness under reduced pressure, and the residue was recrystallized from a solution of $CHCl_3$:MeOH (10:1) and washed with a solution of TEA in MeOH to afford 5 mg of **2.** ^{1}H NMR (DMSO-d_6) 4.52 and 4.66 (2d, 4H, J = 3.9 Hz, 2X –NCH_2–), 4.83 (s, 2H, –OCH_2–), 5.04–5.17 (m, 4H, 2X =CH_2), 5.83–6.03 (m, 2H, 2X –CH=), 7.14 (d, 2H, J = 8.8 Hz, Ar), 7.26 (bs, 1H, –NH_2), 7.71 (d, 2H, J = 8.8 Hz, Ar), 7.85 (m, 3H, Ar and –NH_2), 8.09 (d, 2H, J = 8.8 Hz, Ar), 10.35 (s, 1H, –NH–). HRMS (EI, M^+) for $C_{26}H_{24}N_6O_5$: Calc. 500.1808. Found 500.0825.

2.1.1b [^{3}H]N-(4-(Aminocarbonyl)phenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl)-phenoxy-]acetamide (3). 1.7 mg (3.4 μM) of **2** was dissolved in 2 mL DMF, and 1 mL ethanol was added. The compound was reduced in an atmosphere of tritium gas utilizing 10 mg of 5% Pd/Al_2O_3 for 4 hr at room temperature. After removal of labile tritium by evaporation several times with DMF-EtOH, an assay indicated ~400 mCi of crude product. TLC on LKC5F plates using $CHCl_3$:MeOH (50:5, v/v) followed by scanning showed a radiochemical purity of ~80%. The crude product was purified by preparative TLC using $CHCl_3$:MeOH (50:3, v/v) as the developing solvent. The purified product was dissolved in ethanol and submitted for mass spectral analysis for specific activity determination (150 Ci/mmol). The purified material (125 mCi) was stored in 10 mL ethanol at −20° and had a radiochemical purity of > 98%. The product was compared with the corresponding unlabeled form of **3,** for which spectroscopic data were reported [6].

2.1.1c [^{3}H]N-(4-Cyanophenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl)-phenoxy]acetamide (4, [^{3}H]MRS 1754). 10 mCi of purified **3** was dissolved in 200 μL of methylene chloride, and 20 μL of pyridine was added. The reaction mixture was cooled to −76°, and 6 μL of trifluoroacetic anhydride was added. The reaction was warmed to room temperature, and an aliquot was removed for TLC analysis [$CHCl_3$:MeOH (50:2, v/v)]. To the crude reaction mixture (~60% product) was added 200 μL methanol and 200 μL TEA, followed by rotary evaporation to dryness. The product was purified by TLC using $CHCl_3$:MeOH (50:1, v/v) as solvent. The product was eluted from the plate using ethanol and stored at ~1 mCi/mL in ethanol at −20°. TLC [$CHCl_3$:MeOH (50:1, v/v)] indicated a radiochemical purity of > 97% (yield: 5.2 mCi). The product was compared with a corresponding unlabeled form of **4,** for which spectroscopic data have been reported [6].

2.2. Pharmacological methods

A 20 nM stock solution of [^{3}H]MRS 1754 was prepared in an equivolume mixture of DMSO and assay medium,

which consisted of 50 mM Tris buffer containing 5 mM Mg^{2+} and 1 mM EDTA, at pH 6.5. Membranes from HEK-293 cells stably expressing the human A_{2B} receptor, prepared as reported [6] or obtained from a commercial source (Batch 1365, Receptor Biology, Inc., Beltsville, MD), were studied. Glass incubation tubes contained a total volume of 100 μL, consisting of a suspension in Tris buffer (as above) containing membranes (30 μg protein, stored at −80°) and [^{3}H]MRS 1754 (final concentration 0.7 nM), and a solution of the competing compound, where applicable. Nonspecific binding was determined in the presence of 100 μM NECA (RBI-Sigma). SCH 58261 (5-amino-7-(2-phenylethyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine) was the gift of Dr. Ennio Ongini, Schering-Plough, S.p.a. All non-radioactive compounds were initially dissolved in DMSO, and diluted with buffer to the final concentration, with the amount of DMSO in the final assay tubes being consistently 4.5%.

Incubations were terminated by rapid filtration over Whatman GF/B filters, which had been presoaked in 0.5% polyethyleneimine, using a Brandell cell harvester. The tubes were rinsed three times with 2 mL of ice-cold Tris buffer (pH 6.5).

For saturation studies, the concentration of [^{3}H]MRS 1754 ranged from 0.1 to 20 nM. For competition experiments, at least six different concentrations of competitor, spanning three orders of magnitude adjusted appropriately for the ic_{50} of each compound, were used. The ic_{50} values, calculated with the nonlinear regression method implemented in the Prism program (GraphPAD), were converted to apparent K_i values using the Cheng–Prusoff equation [16].

3. Results

The A_{2B} receptor-selective xanthine antagonist MRS 1754 [6] was synthesized in tritiated form (Fig. 1) for use as a radioligand in a two-step tritiation sequence. The precursor 1,3-diallyl amide, **2,** was prepared from the corresponding carboxylic acid, **1,** reported previously [15]. An intermediate tritiated carboxamide derivative, **3,** was dehydrated using trifluoroacetic anhydride, and the final product, **4,** was purified using TLC. [^{3}H]MRS 1754 was found to bind specifically to the human A_{2B} receptor expressed in HEK-293 cells, using 100 μM NECA to define nonspecific binding. Since MRS 1754 is a relatively hydrophobic molecule and has low aqueous solubility, a stock solution of the radioligand in 50% aqueous DMSO was prepared, which allowed the concentration of the dissolved xanthine to remain constant.

To optimize specific binding, the pH dependence (Fig. 2), temperature dependence, and optimal amount of protein (Fig. 3) were determined. In the range of pH 4.5 to 6.5, the level of specific binding of 0.7 nM [^{3}H]MRS 1754 was constant, whereas raising the pH (6.5 to 8.0) decreased the

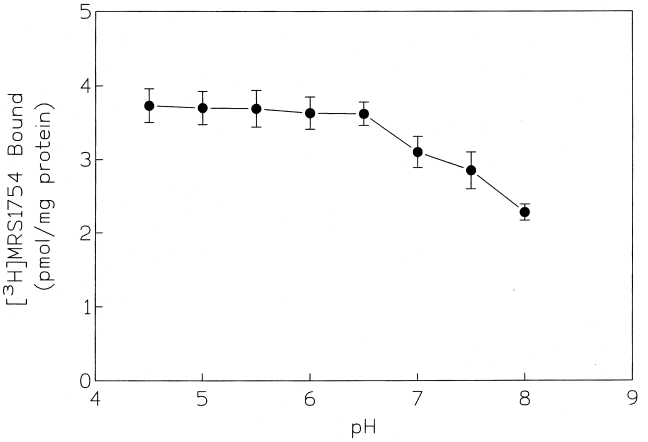

Fig. 2. pH dependence of specific binding of [^{3}H]MRS 1754 to human A_{2B} receptors. [^{3}H]MRS 1754 (0.7 nM) was incubated with membranes for 60 min at 25°. The curve represents the mean ± SEM of four determinations.

level by approximately 10–15% for each 0.5 pH unit. The specific binding increased linearly with increasing amounts of protein (2–30 μg) present in each tube (Fig. 3). At 25°, the level of specific binding of 0.7 nM [^{3}H]MRS 1754 was nearly identical to that at 37°; thus, subsequent binding was carried out at 25°. Using optimal ligand binding conditions, the association and dissociation binding kinetics were determined (Fig. 4), using 100 μM NECA to induce dissociation. Binding reached equilibrium at 40 min and remained constant for the following 80 min. The standard time of incubation selected for subsequent experiments was 60 min. The kinetics of the association appeared monophasic with a $T_{1/2}$ value of 7.65 ± 0.28 min. At equilibrium, the nonspecific binding did not exceed 30% of the total [^{3}H]MRS 1754 bound. The association and dissociation rate constants were 0.022 ± 0.003 min^{-1} nM^{-1} and 0.027 ± 0.001 min^{-1}, respectively, resulting in a kinetic K_D (k_{-1}/k_1) value of 1.22 nM (N = 4), in good agreement with the equilibrium determination.

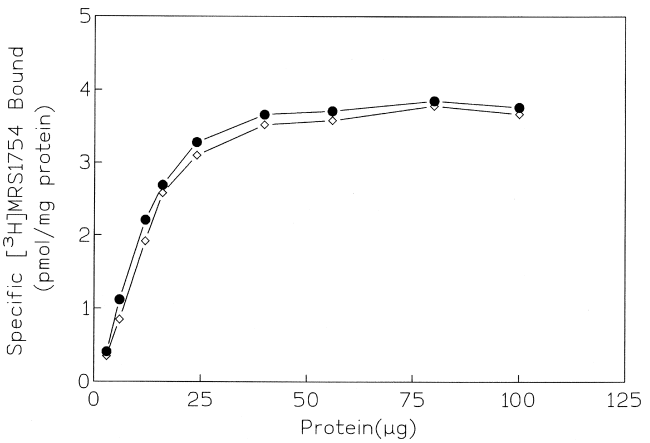

Fig. 3. Dependence of the specific binding of [^{3}H]MRS 1754 to the human A_{2B} receptor on the amount of protein present in each assay tube at 25° (●) or 37° (◇). [^{3}H]MRS 1754 (0.7 nM) was incubated with membranes for 60 min (N = 2).

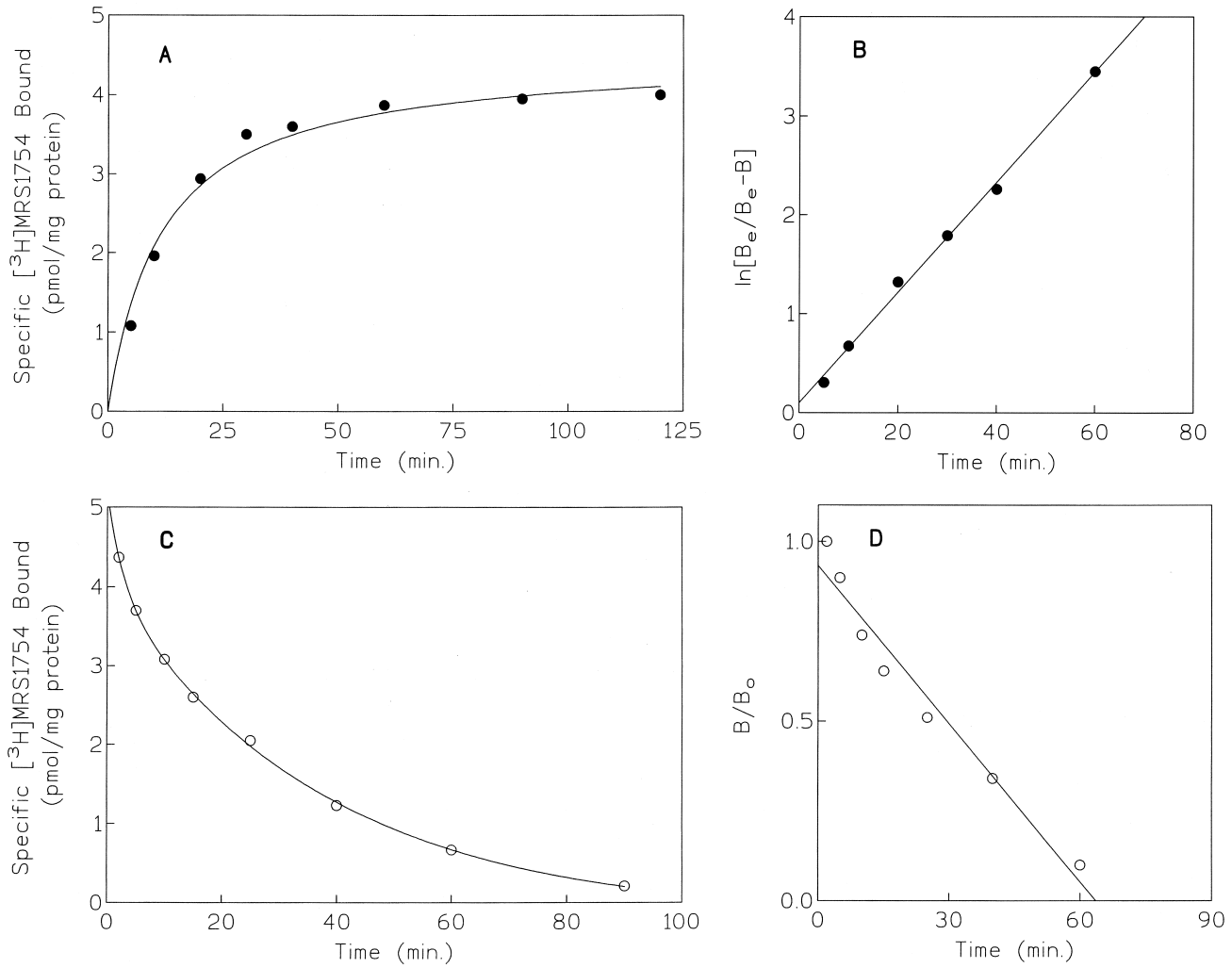

Fig. 4. Association (A, B) and dissociation (C, D) kinetics of [^{3}H]MRS 1754 binding to HEK-293 cell membranes expressing the human A_{2B} receptor. [^{3}H]MRS 1754 (0.7 nM) was incubated with membranes (30 μg protein) at 25°. Dissociation was initiated by the addition of 100 μM NECA. The results shown are representative of three separate determinations.

The effects of cations and chelators on specific [^{3}H]MRS 1754 binding to human A_{2B} receptors expressed in HEK-293 cell membranes were studied (Table 1). The presence of the divalent cations Zn^{2+} ($\geq$1 mM) and Mn^{2+} (1 mM) significantly decreased the amount of specific binding of 0.7 nM [^{3}H]MRS 1754, whereas Na^+ had no effect. The effect of Zn^{2+} to inhibit binding appeared to be concentration-dependent, with an IC_{50} of $\sim$1 mM. The cations Ca^{2+} or Mg^{2+} (10 mM) caused a 20% reduction in the amount of specific binding of 0.7 nM [^{3}H]MRS 1754, and the addition of chelating agents in the presence of Ca^{2+} (EGTA or EDTA) or Mg^{2+} (EDTA) restored the full degree of binding.

Under optimized equilibrium conditions (60-min incubation at 25° with 30–35 μg protein/tube, in Mg^{2+}-containing medium), binding of [^{3}H]MRS 1754 to membranes of HEK-293 cells expressing the human A_{2B} receptor was saturable and was best described by a one-site model (GraphPad, Prism). The percent of specific binding using a 0.7 nM concentration of the radioligand was $>$ 70% of total binding. A representative saturation isotherm and a Scatchard transformation of the same data are shown in panels A and B of Fig. 5. The B_{max} value was 10.9 $\pm$ 0.6 pmol/mg protein (N = 4). The K_D value obtained from the saturation experiments was 1.13 $\pm$ 0.12 nM, which was in good agreement with the K_D value of 1.22 $\pm$ 0.22 nM determined in kinetic studies.

Levels of binding of [^{3}H]MRS 1754 that was displaceable by 100 μM NECA in membranes expressing other adenosine receptor subtypes, e.g. rat and human A_1 and A_3 adenosine receptors (Table 2), were not significant. The only significant binding was to membranes expressing A_{2B} receptors, and this binding displayed characteristics of the A_{2B} subtype. Radioligand binding in membranes of HEK-293 cells expressing human A_{2A} receptors was, in all experiments, either undetectable or not greater than 8% of the total binding and, when observed, was not displaceable using the A_{2A} selective antagonist SCH 58261.

The pharmacological profile for known adenosine receptor ligands in competition for [^{3}H]MRS 1754 binding was

Table 1
Effect of cations and chelators on [^{3}H]MRS 1754 binding

Reagent	Concn (mM)	Bound (% of control)
EDTA	1	97 ± 3
Mg^{2+}	1	90 ± 7
	5	79 ± 5
	10	79 ± 3
Mg^{2+}(+ EDTA)	1	116 ± 8
Ca^{2+}	1	93 ± 5
	10	78 ± 7
Ca^{2+}(+ EDTA)	1	105 ± 4
Ca^{2+}(+ EGTA)	1	110 ± 1
Zn^{2+}	1	52 ± 2
	5	36 ± 9
	10	23 (N = 1)
Mn^{2+}	1	75 ± 7
Na^+	10	101 ± 0.5
	100	101 ± 1

Data are means ±SD from three separate determinations measured in duplicate. Control value was 100% (coresponding to 3600 cpm). Nonspecific binding was determined in the presence of 100 μM NECA. [^{3}H]MRS 1754 was present at a final concentration of 0.7 nM. The concentration of EDTA or EGTA was 1 mM.

Table 2
Binding of a single concentration of [^{3}H]MRS 1754 in membranes of cells expressing four adenosine receptor subtypes

Cell-Subtype	Bound (pmol/mg protein)	Reference[a]
CHO-hA_1	0,22 ± 0.09	[17]
HEK-hA_{2A}	[b]	[9]
HEK-hA_{2B}	6.89 ± 0.63	[9,12,14]
CHO-hA_3	0.02 ± 0.02	[26]
Rat brain-rA_1	0.17 ± 0.04	[6,15]
CHO-rA_3	0.06 ± 0.07	[15]

Data are means ± SD from 3–6 separate determinations measured in duplicate. Nonspecific binding was determined in the presence of 100 μM NECA. [^{3}H]MRS 1754 was present at a concentration of 0.7 nM, unless indicated.

[a] References describe the source of each preparation and the pharmacological characteristics of each receptor. These citations also demonstrate that substantial binding to the indicated receptors was observed using radioligands having high affinity for each receptor. Thus, the expression levels of A_1, A_{2A}, and A_3 receptors were much higher than indicated by the level of binding of [^{3}H]MRS 1754.

[b] Binding was either undetectable or ≤ 8% of total binding, using 5–10 μg protein/tube (N = 10). This did not represent specific binding to A_{2A} receptors, since binding was not displaceable by the A_{2A} selective antagonist SCH 58261. The concentration of [^{3}H]MRS 1754 was 1.0 nM.

consistent with the SAR for antagonists (Fig. 6A) and agonists (Fig. 6B) noted previously at A_{2B} receptors [6, 11, 12]. The most potent displacer of [^{3}H]MRS 1754 binding (Table 3) was MRS 1754, itself, with a K_i value of 1.45 nM. This value was consistent with the observed K_D value and with a K_i value of 1.97 nM [6], determined by competition for binding of ^{125}I-IABOPX or [^{3}H]ZM 241385. The potent xanthine derivatives XAC and CPX, and the triazoloquinazoline CGS 15943 [18], had K_i values of 16, 55, and 34 nM, respectively. The triazolotriazine ZM 241385 [19] had a K_i value of 145 nM, somewhat less potent than previously determined [7, 12]. Another potent xanthine derivative, XCC [14], which is a precursor of MRS 1754, had a K_i value of 54 nM in binding to human A_{2B} receptors, similar to the value reported previously [12]. Enprofylline (3-propylxanthine) and theophylline were roughly equipotent in displacing binding of [^{3}H]MRS 1754. Alloxazine, which has been reported to be moderately selective (10-fold) for A_{2B} versus A_1 and A_{2A} receptors [20], had a K_i value of 2.04 μM. DAX, a xanthine derivative of interest as a treatment for cystic fibrosis [21], displaced binding with a K_i value of 408 nM. The Hill coefficients (n_H) in the competition experiments were in the range of 0.8 to 1.0 for antagonists and agonists.

Among agonists (Fig. 6B), as in functional assays [20, 22], NECA was more potent than N^6-substituted analogues

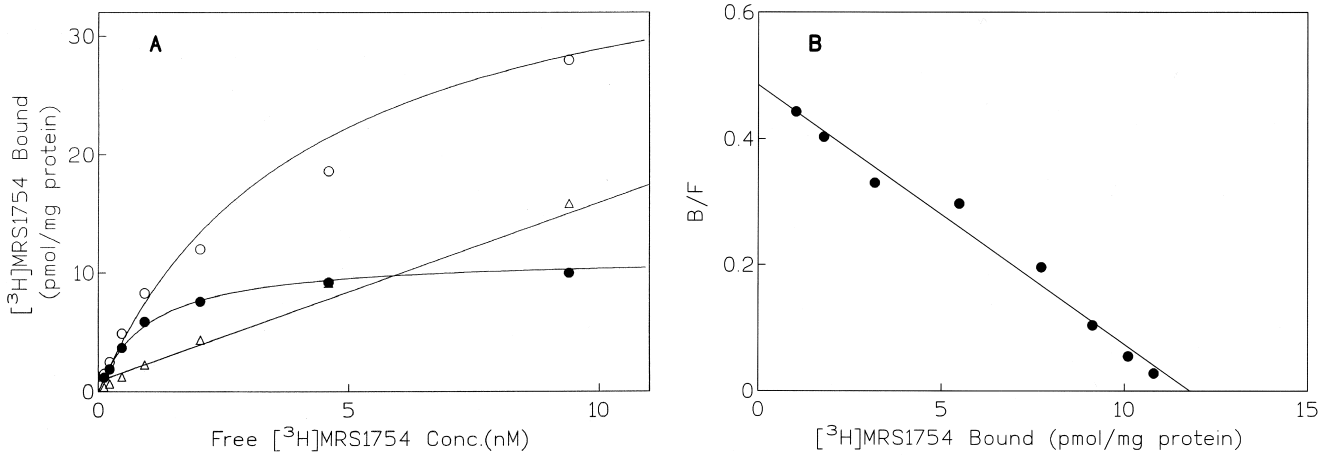

Fig. 5. (A) Saturation isotherm of [^{3}H]MRS 1754 binding to human A_{2B} receptors expressed in HEK-293 cell membranes, and (B) Scatchard analysis of the same data. [^{3}H]MRS 1754 (0.1 to 20 nM) was incubated with 30 μg of membranes for 60 min at 25°. Total binding (○), specific binding (●), and nonspecific binding (Δ), determined using 100 μM NECA, are shown in (A). Four experiments were carried out; data from one representative experiment are shown.

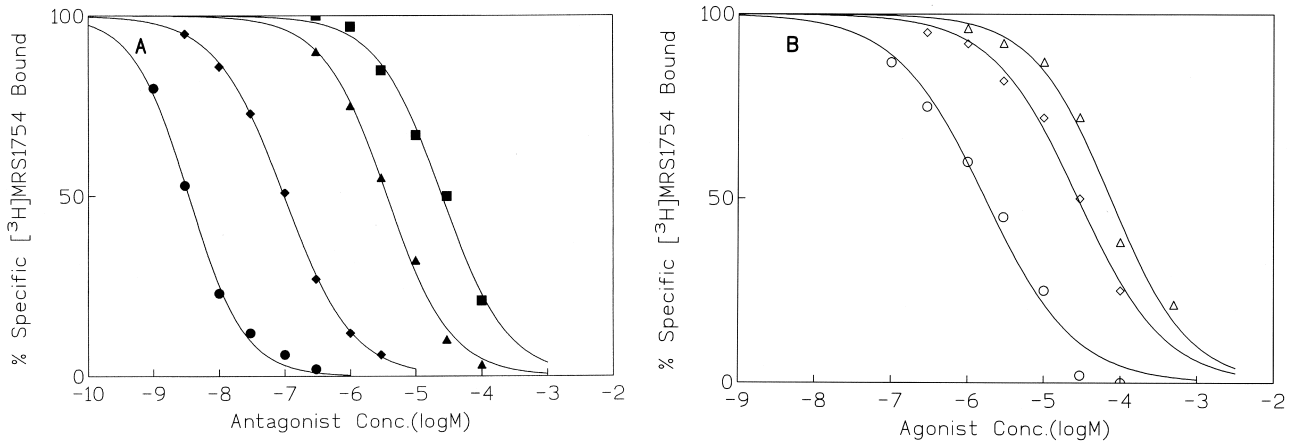

Fig. 6. Effects of (A) the antagonists MRS 1754 (●), CPX (◆), alloxazine (▲), and enprofylline (■) and (B) the agonists NECA (○), *R*-PIA (◇), and SPA (Δ) on radioligand binding to human A_{2B} receptors expressed in HEK-293 cell membranes. Membranes (30 μg) were incubated for 60 min at 25° with 0.7 nM [^{3}H]MRS 1754. Nonspecific binding was determined with 100 μM NECA. The data are representative of four experiments.

in binding competition. The A_{2A}-selective agonist CGS 21680 (2-[4-[(2-carboxyethyl)phenyl]ethyl-amino]-5′-*N*-ethylcarbamoyladenosine) did not displace [^{3}H]MRS 1754 binding significantly, even at a concentration of 100 μM, which is consistent with functional studies showing this agonist to be inactive at A_{2B} receptors and selective for the A_{2A}-receptor subtype [3].

4. Discussion

Following synthesis by an efficient multi-step method, [^{3}H]MRS 1754 was shown to bind with high affinity to a single class of binding sites in membranes of HEK-293 cells expressing the human A_{2B} receptor. The pharmacological characteristics of this binding site resemble the functional characteristics of A_{2B} receptors [7, 11, 19, 20, 22]. [^{3}H]MRS 1754 is selective for the A_{2B} receptor, with very low affinity for A_1 and A_3 receptors of both humans and rats. In cells expressing human A_{2A} receptors, the low levels of binding of [^{3}H]MRS 1754 were demonstrated not to represent binding to this receptor subtype. Thus, due to its high affinity and selectivity, [^{3}H]MRS 1754 has advantages over [^{3}H]CPX, [^{3}H]ZM 241385, and ^{125}I-IABOPX as a radioligand for A_{2B} receptors.

Theophylline is widely used as an antiasthmatic drug, although its mechanism of action is uncertain. The related xanthine enprofylline (3-propylxanthine) [9, 13], which is also therapeutically efficacious in the treatment of asthma, was earlier thought to act through a non-adenosine receptor-mediated mechanism due to its low affinity at A_1 and A_{2A} receptors. However, the discovery that enprofylline has greater than anticipated affinity and slight selectivity at the A_{2B} subtype [9] supports the hypothesis that A_{2B} receptor antagonism may contribute to the antiasthmatic activity of xanthines [8, 23, 24]. This hypothesis was strengthened by functional effects of A_{2B} receptor activation observed in mast cells of dogs, mice, and humans [8, 25]. Thus, potent and/or selective A_{2B} receptor antagonists may provide new therapeutic agents.

In conclusion, [^{3}H]MRS 1754 binding to recombinant human A_{2B} receptors in membranes is a practical method for characterizing these receptors and their ligands in recombinant systems. This radioligand is yet to be characterized in cells and tissues endogenously expressing A_{2B} receptors and in the presence of other subtypes of adenosine receptors. Also, the affinity of MRS 1754 at A_{2B} receptors in other species is yet to be determined. The development of binding assays for this subtype of adenosine receptors that are useful with cell membranes will aid in the elucidation of

Table 3
K_i values for displacement of [^{3}H]MRS 1754 binding to human A_{2B} receptors expressed in HEK-293 cell membranes

Compound	K_i(nM)
Antagonists	
MRS 1754	1.45 ± 0.21
XAC	16.0 ± 0.7
CGS 15943	34.2 ± 1.0
XCC	53.6 ± 3.8
CPX	54.6 ± 12.1
ZM 241385	145 ± 15
DAX	408 ± 54
Alloxazine	2,040 ± 570
Enprofylline	19,800 ± 6,120
Theophylline	15,200 ± 4,100
Agonists	
NECA	570 ± 170
R-PIA	13,900 ± 3,400
SPA	24,700 ± 7,500
CPA	20,600 ± 7,500
CGS 21680	14 ± 2% displacement at 100 μM

Specific binding was approximately 75% of total binding. Values are means ±SEM of 3–7 separate experiments.

the SAR of A_{2B} receptor agonists and antagonists, which are currently being synthesized [6,16,22,26].

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