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[^{3}H]ZM241385—an antagonist radioligand for adenosine A_{2A} receptors in rat brain

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Abstract

Binding of the novel adenosine A_{2A} receptor-selective antagonist radioligand [2-^{3}H]-4-(2-[7-amino-2-{2-furyl}{1,2,4}triazolo{2,3-*a*}{1,3,5,}triazin-5-yl amino]ethyl)phenol ([^{3}H]ZM241385) was examined using particulate preparations and frozen sections of rat brain. In membranes from the rat striatum, binding was saturable, reversible and temperature-dependent. Analysis of saturation isotherms indicated that [^{3}H]ZM241385 bound with high affinity (K_d of 0.84 nM), high density (1680 fmol mg protein^{-1}) and with a high proportion of specific binding (93% at 1 nM radioligand). Examination of competition profiles indicated that [^{3}H]ZM241385 bound to sites with an A_{2A} adenosine receptor-like rank order. The presence of guanosine 5′-(3-thio)-triphosphate failed to alter either [^{3}H]ZM241385 binding or agonist competition for [^{3}H]ZM241385 binding. Autoradiographic analysis of [^{3}H]ZM241385 binding to frozen sections of rat brain indicated specific binding to the rat striatum of similar affinity (K_d of 0.43 nM) and susceptibility to adenosine receptor ligands. At 2 nM [^{3}H]ZM241385, specific binding comprised 95 ± 1% total binding. In the hippocampus and frontal cortex, binding of [^{3}H]ZM241385 failed to saturate and was of lower density. Taken together, these results indicate that [^{3}H]ZM241385 should prove to be a useful radioligand in the characterisation of adenosine A_{2A} receptors.

Keywords: Adenosine A_{2A} receptor; Striatum, rat; Radioligand binding; Autoradiography

1. Introduction

Adenosine A_{2A} receptors appear to be most dense in the brain, particularly in the striatum, nucleus accumbens and olfactory tubercle (Fredholm, 1977; Ongini and Fredholm, 1996; Svenningsson et al., 1997). In these regions, they have been suggested to play a role in the control of movement and the behavioural effects of caffeine. Medicinal chemistry has generated ligands with appreciable A_{2A}-selectivity (Alexander and Peters, 1999). Thus, agonists based on adenosine (e.g., 2-(4-[2-carboxyethyl]-phenylamino)adenosine-5′-*N*-ethyluronamide [CGS21680]) and non-xanthine heterocyclic antagonists (e.g., 5-amino-2-(2-furyl)-7-phenylethyl-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5*c*]-pyrimidine (SCH58261) and 4-(2-[7-amino-2-{2-furyl}{1,2,4}triazolo{2,3-*a*}{1,3,5,}triazin-5-yl amino]ethyl)phenol (ZM241385)), departing from the archetypal adenosine receptor antagonist structure of the methylxanthines, have gained wide acceptance as means of defining A_{2A} receptors (Ongini and Fredholm, 1996). Visualisation of adenosine A_{2A} receptors by radioligand binding has proved problematic, however, due to binding to other sites, limited availability of radioligands or the necessity for radioiodination (Ongini and Fredholm, 1996). Autoradiographic analysis of adenosine A_{2A} receptors in the central nervous system has typically been conducted using agonist radioligands such as 5′-*N*-ethylcarboxamidoadenosine ([^{3}H]NECA) (Alexander and Reddington, 1989) or [^{3}H]CGS21680 (Jarvis et al., 1989a). However, these radioligands also appear to bind to sites distinct from A_{2A} receptors (e.g., Cunha et al., 1996). Recently, the A_{2A}-selective antagonist SCH58261 has been radiolabelled and, though not yet generally available, has been shown to be useful in assays of binding to membranes and sections (Fredholm et al., 1998; Zocchi et al., 1996).

The antagonist ZM241385 has proved effective as a means of identifying adenosine A_{2A} receptor-mediated responses both in vivo and in vitro (Keddie et al., 1996; Poucher et al., 1995). A tritiated version has recently become available, and has been shown to label over-expressed adenosine A_{2B} receptors in human embryonic

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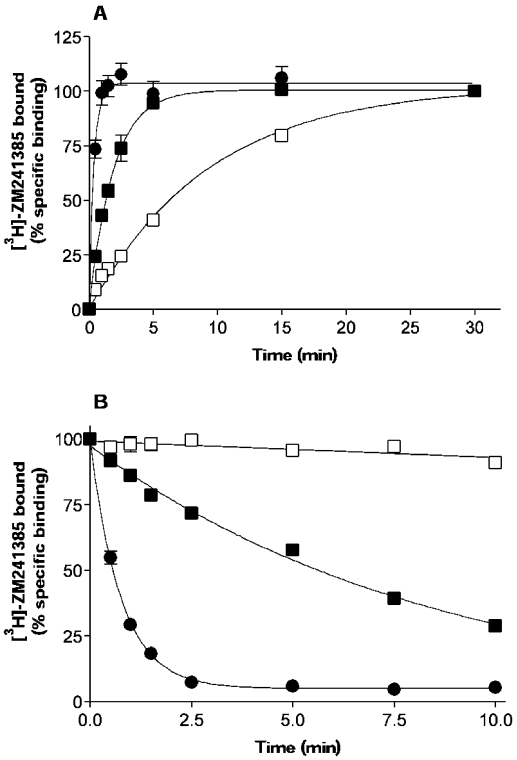

Fig. 1. Binding of [^{3}H]ZM241385 to rat brain membranes. Kinetic analysis of association (A) and dissociation (B) was conducted using membranes from rat striatum in the presence of 0.9–1.4 nM [^{3}H]ZM241385 at 4°C (□), 22°C (■) and 37°C (●). Dissociation was initiated by the addition of 5 mM theophylline. Data are means and S.E.M. from single replicates using six separate membrane preparations. Where error bars are not visible, they are smaller than the symbol.

kidney (HEK) 293 cells (Ji and Jacobson, 1999). Here, we report binding of [^{3}H]ZM241385 to adenosine A_{2A} receptors in particulate preparations and frozen sections from rat brain. Some of these findings have been presented in preliminary form to the British Pharmacological Society (Alexander, 1998; Millns and Alexander, 1999).

2. Methods

2.1. Binding of [^{3}H]ZM241385 to particulate preparations

Binding of [^{3}H]ZM241385 was typically conducted over 30 min at room temperature, in TE buffer (50 mM Tris, 1 mM EDTA, pH 7.4) containing adenosine deaminase (1 U ml^{-1}) and Triton X-100 (0.01%), harvesting bound ligand by rapid filtration over GF/B filters (Alexander et al., 1994). Following dissection of particular regions from rat brain (Wistar, 200–350 g, either sex), membranes were prepared by repeated homogenisation and centrifugation as before (Alexander et al., 1994). Saturation analysis was conducted over the nominal radioligand concentration range of 0.25–16 nM, while competition curves were conducted at 1 nM nominal [^{3}H]ZM241385 concentration, using 5 mM theophylline to define non-specific binding. Association kinetic analyses were conducted in the presence of 0.9–1.4 nM [^{3}H]ZM241385 at 4°C, room temperature (ca. 22°C) and 37°C. After 60 min incubation with radioligand at the indicated temperature, dissociation analysis was performed using the addition of 5 mM theophylline to initiate dissociation. Data reported are means ± S.E.M. of at least three separate experiments.

2.2. Autoradiography of [^{3}H]ZM241385 binding

Parasagittal (20 μm) sections of rat (Wistar, 150–300 g, either sex) frozen brain were cut using a cryostat and mounted on gelatin-subbed slides. After drying, sections were exposed to [^{3}H]ZM241385 over 90 min at room temperature, in buffer (50 mM Tris, 1 mM EDTA, pH 7.4) containing adenosine deaminase (1 U ml^{-1}). After rapid washing and drying, sections were apposed to photographic film (Hyperfilm, LKB) for 8–15 weeks. [^{3}H]-Labelled standards exposed alongside the tissue allowed conversion of grain density to fmol mm^{-2}. Saturation analysis was conducted over the nominal radioligand concentration range of 0.25–8 nM, while competition curves were conducted at 2 nM, defining non-specific binding by 1 mM theophylline. Data reported are means ± S.E.M. from three separate experiments using 3–8 tissue sections per animal (striatum, frontal cortex and hippocampus) or 4–10 sections in total (nucleus accumbens and olfactory tubercle). Statistical significance of competition for [^{3}H]ZM241385 binding was assessed using repeated measures ANOVA, with Dunnett's multiple comparison test.

2.3. Chemicals

[^{3}H]ZM241385 and SCH58261 were kind gifts from Tocris-Cookson (Bristol, UK) and Dr. Silvio Dionisotti,

Table 1
Kinetic parameters for binding of [^{3}H]ZM241385 to rat striatal particulate preparations

Temperature (°C)	Association parameters			Dissociation parameters			Calculated K_d (nM)
	Maximal binding (% specific binding)	$t_{1/2}$ (min)	K_{obs} (min^{-1})	Maximal displacement (% specific binding)	$t_{1/2}$ (min)	K_{obs} (min^{-1})	
4	103 ± 1	6.50 ± 0.37	0.108 ± 0.006	nc	nc	nc	nc
22	101 ± 3	1.36 ± 0.13	0.529 ± 0.046	100 ± 3	6.05 ± 0.27	0.115 ± 0.005	0.27 ± 0.04
37	104 ± 4	0.27 ± 0.01	2.602 ± 0.134	95 ± 1	0.52 ± 0.02	1.339 ± 0.048	1.57 ± 0.19

Data are means ± S.E.M. of individual estimations of [^{3}H]ZM241385 binding to rat striatal membranes ($n = 6$). nc: Values could not be determined.

Schering-Plough (Milan, Italy), respectively. Adenosine receptor ligands were obtained from RBI Sigma (Poole, UK). All other reagents were obtained either from Sigma or Fischer Scientific (Loughborough, UK).

3. Results

3.1. Binding of [^{3}H]ZM241385 to particulate preparations

At room temperature (ca. 22°C), binding of [^{3}H]ZM241385 to striatal membrane preparations was rapid and reversible (Fig. 1; Table 1). Using parameters derived by the computer program Prism, a dissociation constant of 0.27 ± 0.04 nM could be calculated. At 4°C, both association and dissociation rates were reduced, such that there was less than 10% displacement 10 min after addition of 5 mM theophylline. Consequently, although an apparent half-life of association could be determined, dissociation parameters could not be calculated (Fig. 1; Table 1). At 37°C, both association and dissociation rates were increased, such that binding reached steady state within 60 s and could be completely displaced within 5 min. At this temperature, a K_d of 1.57 ± 0.19 nM could be calculated. Analysis using Prism indicated that a single component model was fit better than a two component model for [^{3}H]ZM241385 binding to and dissociation from striatal membranes at 22°C and 37°C.

Saturation isotherms using neostriatal membranes gave estimates of the K_d value for [^{3}H]ZM241385 of 0.84 ± 0.05 nM with a B_{max} value of 1680 ± 66 fmol mg protein^{-1} (Fig. 2A). At 1 nM radioligand, non-specific binding was 7 ± 1% total binding. Using cerebellar membranes, [^{3}H]ZM241385 binding was displaceable (at 1 nM, a binding density of 19 ± 8 fmol mg protein^{-1} could be calculated with non-specific binding representing 67 ± 12% total binding) but not saturable (Fig. 2B). Agonists competed for [^{3}H]ZM241385 binding to neostriatal membranes (Fig. 2C) with monophasic profiles and a rank order of affinity (pK_i value; Hill slope): NECA (6.46 ± 0.07, -1.03 ± 0.06) > CGS21680 (5.37 ± 0.07; -1.01 ± 0.02) > CPA (N^6-cyclopentyladenosine; 74 ± 3% control binding at 10^{-4} M). Binding of [^{3}H]ZM241385 was unaffected in the presence of 10 μM guanosine-5′-(3-thio)-triphosphate (GTP-γ-S; 100 ± 6%), as was the affinity of NECA (6.42 ± 0.10; -1.10 ± 0.17). Antagonist competition (Fig. 2D) showed the rank order: SCH58261 (8.70 ± 0.19; -1.07 ± 0.10) > XAC (xanthine amine congener; 7.47 ± 0.03; -0.99 ± 0.11) > DPCPX (8-cyclopentyl-1,3-dipropylxanthine; 6.56 ± 0.05; -1.23 ± 0.24) > theophylline (4.68 ± 0.03; -1.07 ± 0.09).

3.2. Autoradiography of [^{3}H]ZM241385 binding

Saturable specific binding was observed in the caudate putamen, nucleus accumbens and olfactory tubercle (Fig. 3). Analysis of saturation isotherms in the caudate putamen

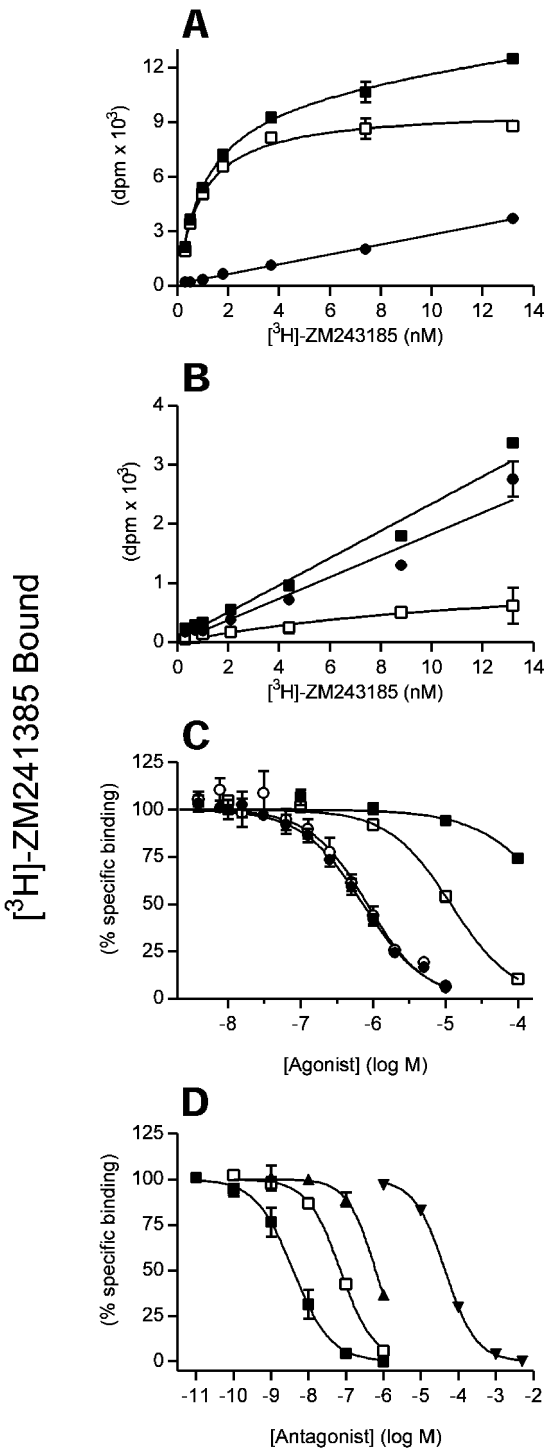

Fig. 2. Binding of [^{3}H]ZM241385 to rat brain membranes. Saturation analysis was conducted using membranes from rat striatum (A) or cerebellum (B). Total (■), non-specific (●) and specific (□) binding are indicated. Competition for [^{3}H]ZM241385 binding was conducted using either agonists (C) [NECA in the absence (●) and presence (○) of 10 μM GTP-γ-S, CGS21680 (□) and CPA (■)] or antagonists (D) [SCH58261 (■), XAC (□), DPCPX (▲) and theophylline (▼)]. Data are means and S.E.M. from individual experiments representative of three (A and B) or the means and S.E.M. from three to six separate membrane preparations (C and D). Where error bars are not visible, they are smaller than the symbol.

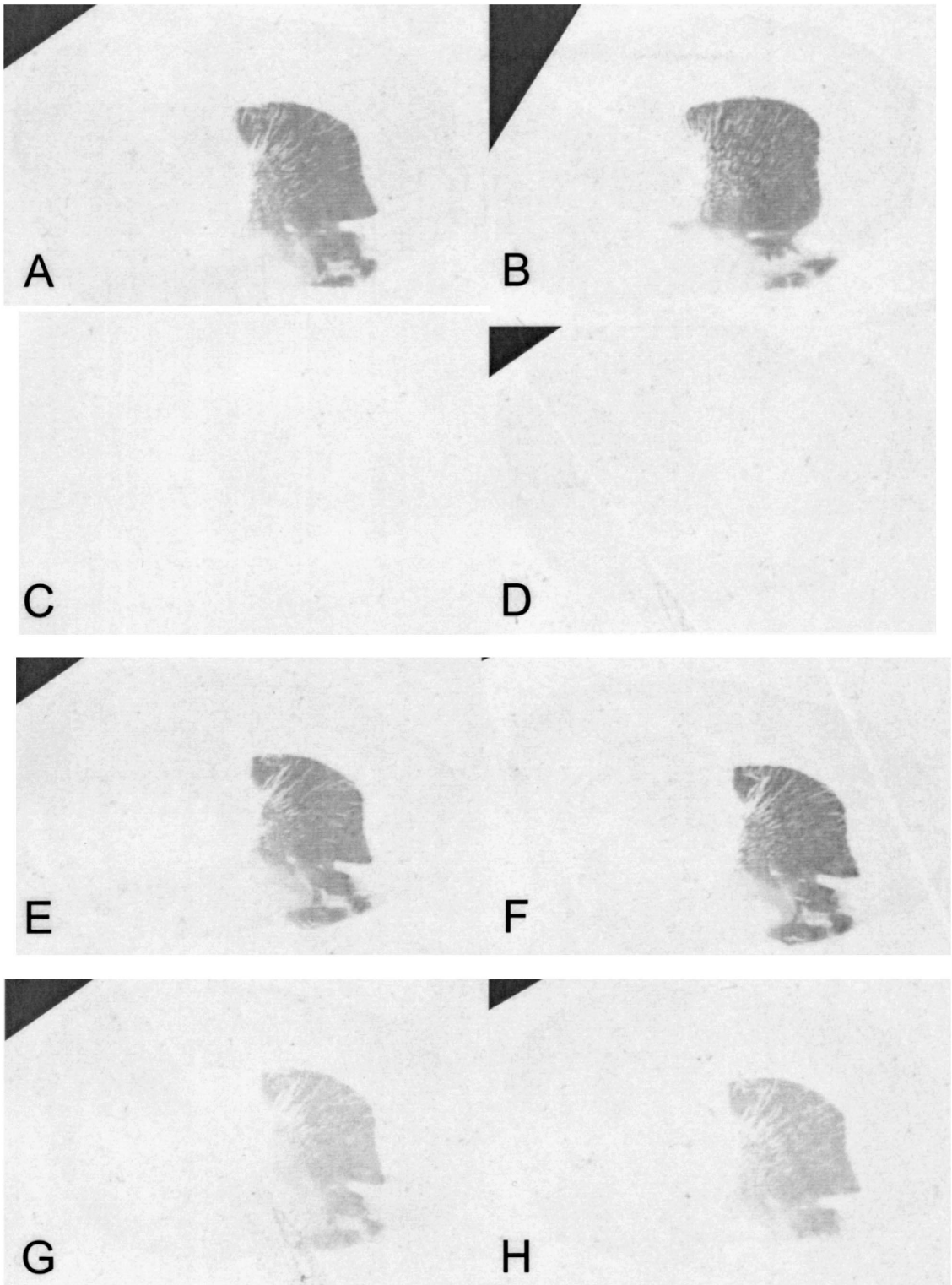

Fig. 3. Binding of [^{3}H]ZM241385 to rat brain parasagittal frozen sections. Competition for [^{3}H]ZM241385 binding was conducted in the absence of competing ligand (A), or in the presence of 100 nM DPCPX (B), 1 mM theophylline (C), 100 nM SCH58261 (D), 100 nM XAC (E), 10 μM CPA (F), 10 μM NECA (G) or 10 μM CGS21680 (H).

showed the radioligand to have a K_d value of 0.43 ± 0.06 nM, with a B_{max} value of 293 ± 22 fmol mm^{-2} ($n = 3$). Although fewer sections were obtained in which the nucleus accumbens and olfactory tubercle could be examined, analysis of this preliminary evidence showed high affinity binding (K_d values of 0.15 and 0.16 nM, respectively) with relatively high density (B_{max} values of 212 and 222 fmol mm^{-2}, respectively). In all three brain

regions, the proportion of specific binding of 2 nM [^{3}H]ZM241385 was high (95% total binding). The rank order of agonist and antagonist competition for [^{3}H]ZM241385 binding in sections of the caudate putamen was comparable to that observed in striatal membranes: (i.e., NECA ≥ CGS21680 > CCPA and SCH58261 ≫ XAC = DPCPX) (Figs. 3 and 4A). A similar pattern was observed for agonist and antagonist competition in the nucleus accumbens and olfactory tubercle (analysis not shown).

In contrast, the specific binding of [^{3}H]ZM241385 to extrastriatal areas was weak and failed to saturate. In the frontal cortex and hippocampus, for example, specific binding of 2 nM [^{3}H]ZM241385 was 2–3 fmol mm^{-2} (39% and 38% total binding, respectively). Analysis of binding in the hippocampus and frontal cortex indicated that the A_{2A}-selective ligands CGS21680 and SCH58261 competed well for [^{3}H]ZM241385 binding, while the A_1-selective ligands CCPA and DPCPX were poor competitors (Fig. 4A). The non-selective agonist NECA was also effective at competing for [^{3}H]ZM241385 binding in the frontal cortex and hippocampus. However, XAC significantly enhanced binding of [^{3}H]ZM241385 in the frontal cortex and hippocampus (Fig. 4A).

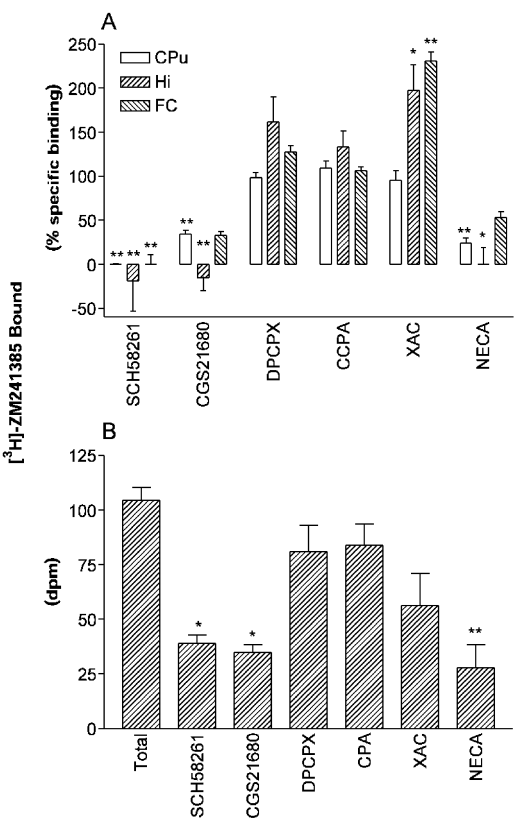

Fig. 4. Binding of [^{3}H]ZM241385 to rat brain (A) frozen sections and (B) hippocampal membranes. Competition for specific [^{3}H]ZM241385 binding in striatum (CPu), hippocampus (Hi) and frontal cortex (FC) by antagonists (SCH58261, XAC and DPCPX present at 100 nM) and agonists (CGS21680, NECA and CPA present at 10 μM). Data are means ± S.E.M. for (A) 11–15 sections from three donor animals or (B) four donor animals. $^*P < 0.05$, $^{**}P < 0.01$ vs. control binding, analysis of variance (ANOVA) plus Dunnett's comparison.

Binding of [^{3}H]ZM241385 to hippocampal membranes was conducted to examine whether the enhancement of [^{3}H]ZM241385 binding by XAC could be reproduced. Competition for binding in membranes generally showed a similar pattern to that observed in sections, in that NECA, CGS21680 and SCH58261 competed well, while CPA and DPCPX were poor competitors (Fig. 4B). XAC displayed an intermediate level of competition consistent with the degree of competition for [^{3}H]ZM241385 binding observed in striatal membranes (Fig. 2D).

4. Discussion

The present study describes binding of the novel antagonist radioligand [^{3}H]ZM241385 to particulate and frozen section preparations of the rat brain. Binding is of high affinity, specific, saturable, temperature-dependent and reversible with characteristics expected of an adenosine A_{2A} receptor.

In comparison with other antagonist radioligands used to label A_{2A} receptors, [^{3}H]ZM241385 shows similar or higher affinity in the rat striatum (e.g., [^{3}H]SCH58261 0.7 nM, Zocchi et al., 1996; [^{125}I]ZM241385 1.4–1.6 nM, Palmer et al., 1995; [^{3}H]KF17837S 7 nM, Nonaka et al., 1994) and a similar or greater proportion of specific binding ([^{3}H]SCH58261 92%, Zocchi et al., 1996; [^{125}I]ZM241385 70–85%, Palmer et al., 1995; [^{3}H]KF17837 60–70%, Nonaka et al., 1994). The binding density of [^{3}H]ZM241385 (1680 fmol mg^{-1}) is similar to that obtained with [^{3}H]KF17837S (1300 fmol mg protein^{-1} (Nonaka et al., 1994)), but markedly higher than that obtained using agonist radioligands (e.g., 375 fmol mg^{-1} (Jarvis et al., 1989b)) or [^{3}H]SCH58261 (971 fmol mg protein^{-1} (Zocchi et al., 1996)). The apparent homogeneity of [^{3}H]ZM241385 binding in the rat striatum (as evidenced by single component kinetic analysis, the near-unity Hill slopes and the near-identical level of maximal displacement for the competing ligands) suggests that binding sites other than the A_{2A} receptor are not being visualised using this ligand, a feature of the currently available agonist radioligands (Ongini and Fredholm, 1996; Ralevic and Burnstock, 1998).

Although agonist affinities estimated using [^{3}H]-ZM241385 appear to be lower than those estimated in reports describing other [^{3}H]-labelled antagonists, the same rank order is maintained throughout, and antagonist affinities are similar to those obtained using either agonist or antagonist radioligands (Bruns et al., 1987; Nonaka et al., 1994; Zocchi et al., 1996). The reasons for such a discrepancy are obscure.

Using an autoradiographic approach, the rank orders of agonist and antagonist competition, together with the high affinity of [^{3}H]ZM241385 are generally consistent with the radioligand binding to adenosine A_{2A} receptors in the rat

striatum, nucleus accumbens and olfactory tubercle and in other brain regions of lower density. Using agonist radioligands such as [^{3}H]CGS21680, binding in the hippocampus and cerebral cortex is apparent, although at lower levels than in the striatum (Cunha et al., 1994; Johansson and Fredholm, 1995; Kirk and Richardson, 1995; Cunha et al., 1996). The nature of these binding sites has been questioned, although it is apparent from molecular techniques that mRNA for adenosine A_{2A} receptors is present in these brain regions (Dixon et al., 1996). The present study shows that [^{3}H]ZM241385 binding in sections of hippocampus and frontal cortex exhibits characteristics compatible with binding sites in the striatal regions and therefore are likely to reflect genuine A_{2A} receptors, albeit at approximately 1% of the level observed in the striatum. The variability associated with binding in the former regions presumably reflects the low expression levels of binding expressed.

Although XAC was a potent inhibitor of [^{3}H]ZM241385 binding to striatal membranes with a pK_i value of approximately 7.5, binding of [^{3}H]ZM241385 in the presence of 100 nM XAC was unchanged in striatal and enhanced in hippocampal and frontal cortex sections (Figs. 3 and 4A). In hippocampal membranes, where displaceable binding is greatly reduced compared to the striatum, however, XAC competed to a degree consistent with that observed in striatal membranes. This suggests a complex interaction between XAC and [^{3}H]ZM241385 binding solely in the autoradiographic assay. It will be of interest to determine whether this interaction is reproduced by other analogues of XAC.

The relative ease of handling and increased duration of usage of [^{3}H] radioligands compared to radio-iodinated ligands, combined with the chemically indistinguishable nature of [^{3}H]ZM241385 compared to the well characterised "cold ligand" ZM241385 (Ralevic and Burnstock, 1998), means that [^{3}H]ZM241385 should prove to be a useful ligand in the characterisation of adenosine A_{2A} receptors either in membrane binding or autoradiographic techniques.

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References

Alexander, S.P.H., 1998. Binding of the A_{2A} adenosine receptor antagonist radioligand [^{3}H]-ZM241385 to rat brain homogenates. Br. J. Pharmacol. 125, 27 pp.

Alexander, S.P.H., Peters, J., 1999. TiPS receptor and ion channel nomenclature supplement 1999. Trends Pharmacol. Sci. 10, 1–98.

Alexander, S.P., Reddington, M., 1989. The cellular localization of adenosine receptors in rat neostriatum. Neuroscience 28, 645–651.

Alexander, S.P.H., Curtis, A.R., Hill, S.J., Kendall, D.A., 1994. A_1 adenosine receptor inhibition of cyclic AMP formation and radioligand binding in the guinea-pig cerebral cortex. Br. J. Pharmacol. 113, 1501–1507.

Bruns, R.F., Fergus, J.H., Badger, E.W., Bristol, J.A., Santay, L.A., Hays, S.J., 1987. PD 115,199: an antagonist ligand for adenosine A_2 receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 335, 64–69.

Cunha, R.A., Johansson, B., Van der Ploeg, I., Sebastiao, A.M., Ribeiro, J.A., Fredholm, B.B., 1994. Evidence for functionally important adenosine A_{2a} receptors in the rat hippocampus. Brain Res. 649, 208–216.

Cunha, R.A., Johansson, B., Constantino, M.D., Sebastiao, A.M., Fredholm, B.B., 1996. Evidence for high affinity binding sites for the adenosine A_{2a} receptor agonist [^{3}H]-CGS 21680 in the rat hippocampus and cerebral cortex that are different from striatal A_{2a} receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 353, 261–271.

Dixon, A.K., Gubitz, A.K., Sirinathsinghji, D.J.S., Richardson, P.J., Freeman, T.C., 1996. Tissue distribution of adenosine receptor mRNAs in the rat. Br. J. Pharmacol. 118, 1461–1468.

Fredholm, B.B., 1977. Activation of adenylate cyclase from rat striatum and tuberculum olfactorium by adenosine. Med. Biol. 55, 262–267.

Fredholm, B.B., Lindstrom, K., Dionisotti, S., Ongini, E., 1998. [^{3}H]SCH 58261, a selective adenosine A_{2A} receptor antagonist, is a useful ligand in autoradiographic studies. J. Neurochem. 70, 1210–1216.

Jarvis, M.F., Jackson, R.H., Williams, M., 1989a. Autoradiographic characterization of high-affinity adenosine A2 receptors in the rat brain. Brain Res. 484, 111–118.

Jarvis, M.F., Schulz, R., Hutchison, A.J., Do, U.H., Sills, M.A., Williams, M., 1989b. [^{3}H]CGS 21680, a selective A_2 adenosine receptor agonist directly labels A_2 receptors in rat brain. J. Pharmacol. Exp. Ther. 251, 888–893.

Ji, X.-D., Jacobson, K.A., 1999. Use of the triazolotriazine [^{3}H]ZM 241385 as a radioligand at recombinant human A_{2B} adenosine receptors. Drug Des. Discovery 16, 217–226.

Johansson, B., Fredholm, B.B., 1995. Further characterization of the binding of the adenosine receptor agonist [^{3}H] CGS 21680 to rat brain using autoradiography. Neuropharmacology 34, 393–403.

Keddie, J.R., Poucher, S.M., Shaw, G.R., Brooks, R., Collis, M.G., 1996. In vivo characterisation of ZM 241385, a selective adenosine A_{2A} receptor antagonist. Eur. J. Pharmacol. 301, 107–113.

Kirk, I.P., Richardson, P.J., 1995. Further characterization of [^{3}H]-CGS 21680 binding sites in the rat striatum and cortex. Br. J. Pharmacol. 114, 537–543.

Millns, P.J., Alexander, S.P.H., 1999. Autoradiographic analysis of the binding of the antagonist radioligand [^{3}H]-ZM241385 to A_{2A} adenosine receptors in rat striatum. Br. J. Pharmacol. 128, 152 pp.

Nonaka, H., Mori, A., Ichimura, M., Shindou, T., Yanagawa, K., Shimada, J., Kase, H., 1994. Binding of [^{3}H]KF17837S, a selective adenosine A_2 receptor antagonist, to rat brain membranes. Mol. Pharmacol. 46, 817–822.

Ongini, E., Fredholm, B.B., 1996. Pharmacology of adenosine A_{2A} receptors. Trends Pharmacol. Sci. 17, 364–372.

Palmer, T.M., Poucher, S.M., Jacobson, K.A., Stiles, G.L., 1995. ^{125}I-4-(2-[7-Amino-2-{2-furyl}{1,2,4}triazolo{2,3-*a*}{1,3,5}triazin-5-ylmino]ethyl)phenol, a high affinity antagonist radioligand selective for the A_{2a} adenosine receptor. Mol. Pharmacol. 48, 970–974.

Poucher, S.M., Keddie, J.R., Singh, P., Stoggall, S.M., Caulkett, P.W.R., Jones, G., Collis, M.G., 1995. The in vitro pharmacology of ZM 241385, a potent, non-xanthine, A_{2a} selective adenosine receptor antagonist. Br. J. Pharmacol. 115, 1096–1102.

Ralevic, V., Burnstock, G., 1998. Receptors for purines and pyrimidines. Pharmacol. Rev. 50, 413–492.

Svenningsson, P., Hall, H., Sedvall, G., Fredholm, B.B., 1997. Distribution of adenosine receptors in the postmortem human brain: an extended autoradiographic study. Synapse 27, 322–335.

Zocchi, C., Ongini, E., Ferrara, S., Baraldi, P.G., Dionisotti, S., 1996. Binding of the radioligand [^{3}H]-SCH 58261, a new non-xanthine A_{2a} adenosine receptor antagonist, to rat striatal membranes. Br. J. Pharmacol. 117, 1381–1386.