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8-Cyclopentyl-1,3-dipropylxanthine (DPCPX) – a selective high affinity antagonist radioligand for A_1 adenosine receptors

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Summary. The properties of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) as an antagonist ligand for A_1 adenosine receptors were examined and compared with other radioligands for this receptor. DPCPX competitively antagonized both the inhibition of adenylate cyclase activity via A_1 adenosine receptors and the stimulation via A_2 adenosine receptors. The K_i-values of this antagonism were 0.45 nM at the A_1 receptor of rat fat cells, and 330 nM at the A_2 receptor of human platelets, giving a more than 700-fold A_1-selectivity. A similar A_1-selectivity was determined in radioligand binding studies. Even at high concentrations, DPCPX did not significantly inhibit the soluble cAMP-phosphodiesterase activity of human platelets. [^{3}H]DPCPX (105 Ci/mmol) bound in a saturable manner with high affinity to A_1 receptors in membranes of bovine brain and heart, and rat brain and fat cells (K_D-values 50–190 pM). Its nonspecific binding was about 1% of total at K_D, except in bovine myocardial membranes (about 10%). Binding studies with bovine myocardial membranes allowed the analysis of both the high and low agonist affinity states of this receptor in a tissue with low receptor density. The binding properties of [^{3}H]DPCPX appear superior to those of other agonist and antagonist radioligands for the A_1 receptor.

Key words: Adenosine receptors – Adenylate cyclase – Phosphodiesterase – Xanthines – Radioligands

Introduction

Adenosine modulates a great variety of biological functions both in the nervous system and in peripheral tissues. Its most prominent actions are the inhibition of neurotransmitter release and of neuronal firing (see Dunwiddie 1985 for a review), the inhibition of platelet aggregation and a pronounced vasodilatation (see Berne et al. 1983 for a review). Most of these effects appear to be mediated via specific cell surface receptors. On the basis of both pharmacological and biochemical studies these receptors have been subdivided into two subtypes, termed A_1 and A_2 adenosine receptor (Van Calker et al. 1978; Londos et al. 1980). The A_1 receptor mediates an inhibition, and the A_2 receptor a stimulation of adenylate cyclase activity. In addition, there is evidence for an A_1 receptor coupled directly to potassium channels (Böhm et al. 1986). For the A_2 receptor, Bruns et al. (1986) have proposed a subdivision into A_{2a} (corpus striatum) and A_{2b} (peripheral tissues) subtypes.

The A_1 receptor has been extensively studied with radioligand binding techniques. However, whereas several agonist radioligands recognize A_1 receptors with high affinity and specificity, such as [^{3}H]PIA (Schwabe and Trost 1980), [^{3}H]CHA (Bruns et al. 1980), or the radioiodinated ^{125}I-HPIA (Schwabe et al. 1982) and ^{125}I-N^6-p-aminobenzyladenosine (Linden et al. 1985), the only antagonist radioligand available so far, [^{3}H]DPX, has a low affinity and poor selectivity (Bruns et al. 1980).

It has been shown that a 1,3-dipropyl-substitution of xanthines leads to a higher affinity at adenosine receptors (Bruns et al. 1983). Recently, Jacobson et al. (1986) have synthesized a 1,3-dipropyl-8-phenylxanthine derivative, [^{3}H]XAC, which binds with relatively high affinity to A_1 receptors. A second approach to increase both the affinity and the selectivity of xanthines for the A_1 receptor is by means of a 8-cyclopentyl substitution. Thus, 8-cyclopentyltheophylline has a 1,000-fold higher affinity and a 30-fold higher selectivity for A_1 receptors than theophylline itself (Bruns et al. 1986). It is possible that the effects of the two approaches are additive; if so, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) would be an antagonist with enhanced affinity and selectivity for A_1 adenosine receptors. Such an antagonist would improve the pharmacological analysis of adenosine receptor-mediated effects, and – as a radioligand – allow the study of A_1 receptors without interference from the guanine nucleotide binding protein N_i (Lohse et al. 1984a).

We have used DPCPX to block A_1 receptors selectively in autoradiographic experiments with rat brain (Lee and Reddington 1986). More recently, Bruns et al. (1987) have shown high affinity binding of tritiated DPCPX to rat brain membranes.

In the present study we report the use of DPCPX to block and label A_1 adenosine receptors selectively in several tissues, and compare it with DPX and XAC, the other antagonist radioligands for this receptor.

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Abbreviations: CHA, N^6-cyclohexyladenosine; DPX, 1,3-diethyl-8-phenylxanthine; HPIA, N^6-p-hydroxyphenylisopropyladenosine; NECA, 5'-N-ethylcarboxamidoadenosine; PIA, N^6-phenylisopropyladenosine; XAC ("xanthine amine congener"), 8-{4-[([{(2-aminoethyl)amino}carbonyl]methyl)oxy]phenyl-1,3-dipropyl-xanthine. 8-Cyclopentyl-1,3-dipropylxanthine is abbreviated DPCPX (from 1,3-dipropyl-8-cyclopentylxanthine), as used by Lee and Reddington (1986)

Materials and methods

Materials. [^{3}H]PIA and [^{3}H]DPX were purchased from New England Nuclear (Dreieich, FRG). Carrier-free Na^{125}I and [α-^{32}P]ATP were from Amersham (Braunschweig, FRG). [α-^{32}P]ATP was purified by ion-exchange chromatography on Dowex 1 × 8 (Serva, Heidelberg, FRG) as described by Walseth and Johnson (1979). 4(6)-Amino-1,3-diallyluracil was from Aldrich (Milwaukee, WI, USA). XAC was a kind gift of Dr. Entzeroth, Thomae, Biberach, FRG. Forskolin was donated by Dr. Metzger, Hoechst, Frankfurt, FRG. Sephadex G25 superfine and QAE-Sephadex A25 were obtained from Pharmacia, Freiburg, FRG. All other compounds were from standard sources or as described previously (Lohse et al. 1984a, b).

Synthesis of [^{3}H]DPCPX and DPCPX. 1,3-Diallyl-8-cyclopentyl-xanthine was prepared as a precursor for [^{3}H]DPCPX. A magnetically stirred solution of 4-amino-1,3-diallyluracil (5 g, 22 mmol) in 30 ml acetic acid:water 4:1 (v/v) was cooled in an ice bath to 5°C. The dropwise addition, over 10 min, of an ice-cold solution of $NaNO_2$ (1.6 g, 23 mmol) in 10 ml water generated crystalline 4-amino-1,3-diallyl-5-nitrosouracil. The thick suspension was stirred an additional 15 min, filtered, and the product thoroughly washed with ice water. A suspension of the nitrosouracil in 200 ml boiling water was treated portionwise with solid $Na_2S_2O_4$ until the purple colour disappeared. The clear solution was filtered, cooled and extracted with ethyl acetate (5 × 25 ml); the combined extracts were dried ($MgSO_4$) and evaporated in vacuo to yield the diaminouracil as a pale yellow oil, 3.5 g (71%). A solution of the diaminouracil and cyclopentanecarboxylic acid (5.7 g, 50 mmol) in 200 ml dry toluene was heated at reflux in a flask fitted with a Dean-Stark trap until HPLC showed the disappearance of the starting material (8 h). After evaporation in vacuo and one coevaporation with 50 ml toluene to remove traces of cyclopentanecarboxylic acid, the residue was boiled in 50 ml 1 N NaOH for 2 h and cooled. Acidification with 2 N HCl precipitated the product which was filtered, dissolved in NaOH and reprecipitated with HCl. Crystallization from ethanol/water after decolorizing with charcoal gave 3.3 g (50%) of product, mp 175–177°C. Analysis calculated for $C_{16}H_{20}N_4O_2$, FW 300.36: C 63.98, H 6.71, N 18.65; found: C 64.16, H 6.76, N 18.62. UV λ_{max} (ϵ): 275 nm (12,200). ^{1}H NMR (DMSO-d_6, δ ppm from tetramethylsilane): 1.7, m, 8H, cyclopentyl $-CH_2-$; 3.1, m, 1H, cyclopentyl $-CH<$; 4.4, t, 4H, $-\underline{CH_2}\,CH=CH_2$; 5.1, m, 4H, $-CH_2CH=\underline{CH_2}$; and 5.9, m, 2H, $-CH_2\underline{CH}=CH_2$. Catalytic reduction of the product to give [^{3}H]DPCPX (specific activity 105 Ci/mmol) was done by Amersham International, Cardiff, UK. The compound will be available from Amersham.

The synthesis of unlabelled DPCPX from 4-amino-1,3-dipropyl-uracil (Papesch and Schroeder 1951) followed the same route as the preparation of the diallyl congener. Product obtained in 43% yield as silky crystals and a mp of 191–193°C. Analysis calculated for $C_{16}H_{24}N_4O_2$, FW 304.39: C 63.13, H 7.95, N 18.41; found C 62.94, H 8.03, N 18.44. UV λ_{max} (ϵ): 274 nm (12,100). ^{1}H NMR (DMSO-d_6, δ ppm from tetramethylsilane): 0.85, t, $-CH_2CH_2\underline{CH_3}$; 1.85, m, 12H, cyclopentyl $-CH_2-$ and $-CH_2\underline{CH_2}CH_3$; and 3.90, m, 5H, cyclopentyl $-CH<$ and $-\underline{CH_2}\,CH_2CH_3$.

[^{3}H]DPCPX, DPCPX and XAC were diluted in 50 mM Tris-HCl, pH 7.4, containing either 0.1% 3-((3-cholamidopropyl)dimethylammonio)-1-propanesulfonate (radioligand binding experiments) or 0.1% bovine serum albumin (adenylate cyclase experiments) to reduce adsorption to surfaces. These additions alone did not affect the respective assays.

Preparation of ^{125}I-HPIA. N^6-p-Hydroxyphenylisopropyl-adenosine (HPIA) was iodinated according to Hunter and Greenwood as described earlier (Schwabe et al. 1982), and purified by gel filtration on Sephadex G25 superfine.

Preparation of membranes. Rat brain membranes from whole forebrains of male Wistar rats, from rat corpus striatum dissected according to Glowinski and Iversen (1966), and membranes from bovine cerebral cortex were prepared as described previously (Lohse et al. 1984b).

Human platelet membranes were prepared from fresh platelet-rich plasma according to Hoffman et al. (1982).

Rat fat cells were isolated from the epididymal fat pads of male Wistar rats as described by Honnor et al. (1985), and fat cell membranes were prepared by the method of McKeel and Jarett (1970); however, the density gradient centrifugation was omitted ("crude membrane fraction", P3).

Bovine myocardial membranes ("crude membrane fraction") were prepared as described previously (Lohse et al. 1985).

All membranes were resuspended in 50 mM Tris-HCl, pH 7.4, frozen in liquid nitrogen, and stored at −80°C. Their protein content was determined with a modified Lowry method (Peterson 1977).

Radioligand binding. The binding of [^{3}H]DPCPX to membranes of various tissues was carried out in an assay volume of 250 µl Tris-HCl, pH 7.4, containing 0.2 nM [^{3}H]DPCPX unless stated otherwise. The protein content ranged from 10 µg (bovine and rat brain and rat fat cells) to 100 µg (bovine myocardium). The incubation lasted for 2 h at 25°C and was terminated by filtration through Whatman GF/B glass-fibre filters, and the filter radioactivity was determined by liquid scintillation counting.

Binding of [^{3}H]PIA, [^{3}H]DPX and ^{125}I-HPIA was measured under the same conditions as described earlier (Lohse et al. 1984a, 1985). The radioligand concentrations in saturation experiments varied from 0.01 nM to 100 nM for [^{3}H]PIA, 5 nM to 200 nM for [^{3}H]DPX, and 0.05 nM to 2 nM for ^{125}I-HPIA.

Nonspecific binding was defined by the addition of 10 µM R-PIA or 1 mM theophylline; for all four radioligands, the nonspecific binding determined with the two competitors was the same.

Binding of [^{3}H]NECA was done as described by Bruns et al. (1986), using 50 nM N^6-cyclopentyladenosine to block A_1 receptors and 100 µM N^6-cyclopentyladenosine to define nonspecific binding. Under these conditions, binding of [^{3}H]NECA to A_1 receptors was not detectable, and nonspecific binding was about 30% of total binding in the presence of 50 nM N^6-cyclopentyladenosine.

Adenylate cyclase assays. The activity of adenylate cyclase of rat fat cell and human platelet membranes was determined as described earlier (Klotz et al. 1985). The adenylate cyclase

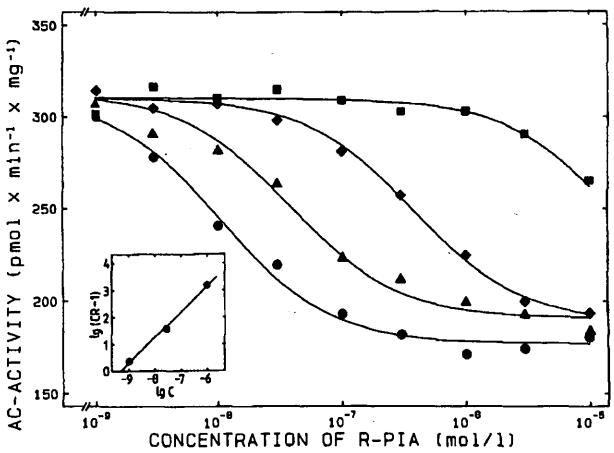

Fig. 1. Antagonism of R-PIA-induced inhibition of rat fat cell adenylate cyclase by DPCPX. The adenylate cyclase activity of rat fat cell membranes was measured in the presence of the indicated concentrations of R-PIA, and the following concentrations of DPCPX: 0 nM (●), 1 nM (▲), 30 nM (◆), and 1,000 nM (■). The inset shows the Schild plot of the data; *CR*, ratio of the IC_{50}-values (calculated from simultaneous fitting of all curves to the Hill equation) in the presence and absence of DPCPX (concentration ratio); *C*, concentration of DPCPX (log M)

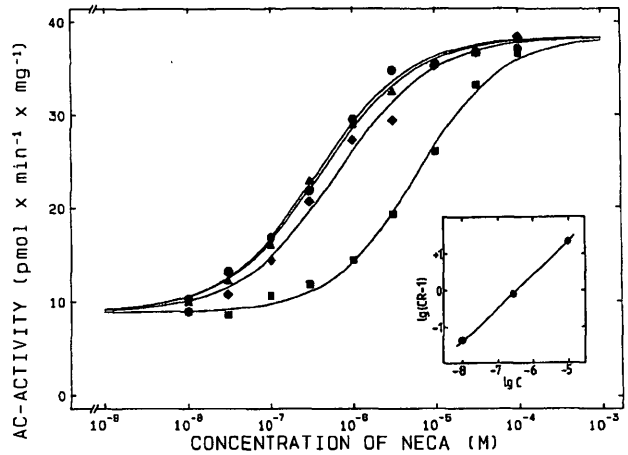

Fig. 2. Antagonism of NECA-induced stimulation of human platelet adenylate cyclase by DPCPX. The adenylate cyclase activity of human platelet membranes was measured in the presence of the indicated concentrations of NECA, and the following concentrations of DPCPX: 0 nM (●), 10 nM (▲), 300 nM (◆), and 10,000 nM (■). The inset shows the Schild plot of the data; *CR*, ratio of the IC_{50}-values (calculated from stimultaneous fitting of all curves to the Hill equation) in the presence and absence of DPCPX (concentration ratio); *C*, concentration of DPCPX (log M)

assays with rat fat cell membranes were done in the presence of 10 μM forskolin.

Phosphodiesterase assay. Phosphodiesterase activity was measured in the supernatant of human platelet lysates after centrifugation at 100,000 × *g* for 60 min. The assay was carried out was described by Bauer and Schwabe (1980) using 1 μM [^{3}H]cAMP as the substrate and QAE-Sephadex A25 to separate the reaction products.

Data analysis. Radioligand binding data were analyzed with the non-linear curve-fitting program SCTFIT (De Lean et al. 1982). Kinetic data were fitted to mono- or multiexponential equations as previously described (Lohse et al. 1984a). Concentration-response curves were fitted by non-linear regression to the Hill equation as previously described (Lohse et al. 1986).

Results

Effects on adenylate cyclase

To evaluate the effects of the different xanthines at A_1 and A_2 receptors we investigated their capacity to block the adenosine receptor-mediated modulation of adenylate cyclase in subtype-selective cells. Rat fat cells were used as a model for the A_1 receptor, and human platelets for the A_2 receptor.

Figure 1 shows the effect of different concentrations of DPCPX on the inhibition of adenylate cyclase activity in rat fat cell membranes. Increasing concentrations of DPCPX cause a marked shift of the concentration-response curve of R-PIA to the right without markedly altering the shape of the curve. This indicates a competitive antagonism. The Schild plot gives a K_i-value for the competition of 0.45 nM.

Table 1. Antagonism of adenosine receptor-mediated modulation of adenylate cyclase activity by xanthines. The antagonism at adenosine receptors was determined from Schild plots as in Figs. 1 and 2. Values are geometric means from 3 experiments; 95% confidence intervals were within 0.2 log units. Data for DPX and theophylline are from Schwabe et al. (1985)

Xanthine	A_1 receptor K_i (nM)	A_2 receptor K_i (nM)	A_1-selectivity $K_i(A_2)/K_i(A_1)$
DPCPX	0.45	330	735
XAC	4.4	24	5.5
DPX	170	210	1.2
Theophylline	9,500	13,000	1.4

The effects of DPCPX on the stimulation of adenylate cyclase activity in human platelet membranes was investigated in a similar manner (Fig. 2). DPCPX competitively antagonized the stimulation by NECA, but although 10 times higher concentrations of DPCPX were used than in Fig. 1 the shift was clearly less marked. The K_i-value calculated from the Schild plot is 330 nM.

The K_i-values of XAC, DPX and theophylline were determined in analogous experiments and are given in Table 1. It can be seen that DPCPX has both the highest affinity and selectivity for A_1 receptors. In fact, the other xanthines are barely selective at all.

Effects on radioligand binding

Radioligand binding studies were done to evaluate the effects of DPCPX and the other xanthines on the proposed A_{2a} subtype of the A_2 receptor, as measured by the binding of [^{3}H]NECA to striatal membranes after blockade of A_1 receptors (Bruns et al. 1986). The K_i-values obtained from competition experiments were compared with those at the

Table 2. Competition for [^{3}H]PIA binding to rat brain membranes and [^{3}H]NECA binding to rat striatal membranes. K_i-values are geometric means calculated from 3 competition curves with 8 concentrations of the competitor. 95% confidence limits were within 0.15 log units

Xanthine	[^{3}H]PIA K_i (nM)	[^{3}H]NECA K_i (nM)	A_1-selectivity $\frac{K_i\,[^3H]NECA}{K_i\,[^3H]PIA}$
DPCPX	0.3	340	1,130
XAC	3.5	24	6.9
DPX	70	550	7.9
Theophylline	11,000	32,000	2.9

Table 3. Inhibition of human platelet cAMP-phosphodiesterase activity by xanthines. The inhibition of phosphodiesterase activity was measured at a substrate concentration of 1 μM cAMP and 7 to 10 different concentrations of the xanthines. For IC_{50}-values above 100 μM (limits of solubility for these xanthines), the percent inhibition at 100 μM is given. Values are means from 3 experiments

Xanthine	IC_{50} (μM)	Inhibition at 100 μM (%)
DPCPX	(> 100)	9.8
XAC	(> 100)	18.5
DPX	(> 100)	31.4
Theophylline	90	

A_1 receptor from competition for [^{3}H]PIA binding to rat brain membranes (Table 2). Again DPCPX proved to be a very A_1-selective compound (1,130-fold), whereas the other three xanthines had only a slight A_1-selectivity.

Effect on cAMP-phosphodiesterase

Apart from blocking adenosine receptors, methylxanthines are also well-known inhibitors of cyclic nucleotide phosphodiesterases. Therefore, we investigated the effects of DPCPX, XAC and DPX on soluble human platelet cAMP-phosphodiesterase activity under conditions measuring largely low K_m cAMP-phosphodiesterase (type III; Weishaar et al. 1985). All three compounds produced only a minor inhibition of phosphodiesterase activity in concentrations up to 100 μM; the least effect was observed for DPCPX (Table 3). These concentrations are several orders of magnitude higher than those effective in blocking adenosine receptors. In contrast, theophylline inhibited the phosphodiesterase activity at concentrations only 10 times higher than those required to block adenosine receptors.

Binding of [^{3}H]DPCPX

[^{3}H]DPCPX bound rapidly to membranes from various tissues. Both association and dissociation appeared monophasic. Using rat brain membranes at 25°C, we observed an association constant $k_{+1} = 0.09\ nM^{-1}\ min^{-1}$, leading to approximate equilibrium after 30 min at a radioligand concentration of 0.2 nM. The dissociation at 25°C occurred with a time constant $k_{-1} = 0.045\ min^{-1}$, corresponding to a half-time of 15 min. This gives a kinetic K_D of 0.5 nM. At 0°C, both association and dissociation were about 10 times

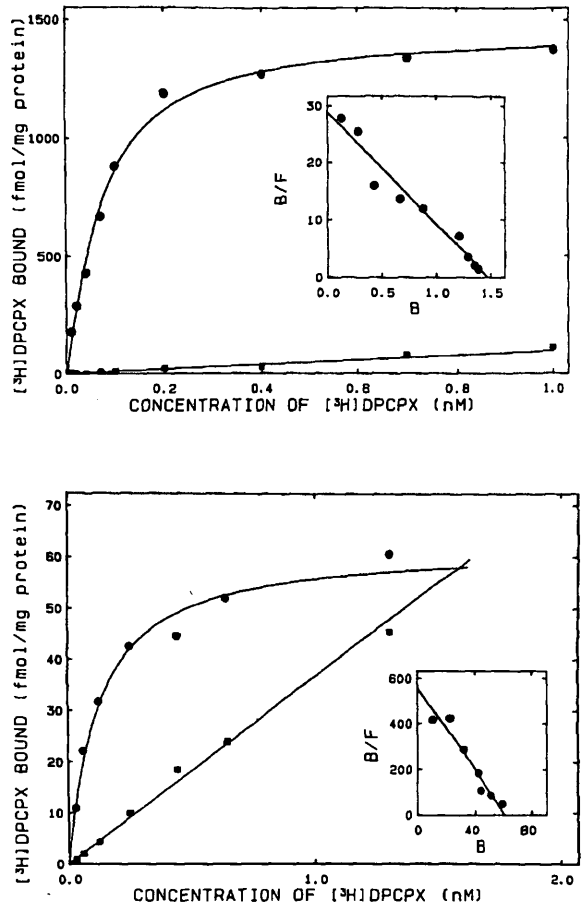

Fig. 3. Saturation of [^{3}H]DPCPX binding to bovine cerebral cortex (*top*) an myocardial (*bottom*) membranes. Given are specific (●) and nonspecific (■; in the presence of 1 mM theophylline) binding. The insets show the Scatchard plots of the data; *B*, radioligand specifically bound (pmol/mg protein, top; fmol/mg protein, bottom); *F*, free radioligand (nM)

slower: $k_{+1} = 0.008\ nM^{-1}\ min^{-1}$, $k_{-1} = 0.004\ min^{-1}$, $K_D = 0.5$ nM.

Saturation experiments with [^{3}H]DPCPX were done with membranes from different tissues containing A_1 receptors (Fig. 3, Table 4). The binding was of high affinity with K_D-values ranging from 50 to 190 pM, bovine tissues having a somewhat higher affinity. Nonspecific binding was negligible in experiments with brain and fat cell membranes. The low nonspecific binding is most notable in bovine myocardial membranes, as the low content of A_1 receptors of this tissue has so far allowed reliable studies only with radioiodinated ligands (Linden et al. 1985; Lohse et al. 1985).

In general, the B_{max}-values were in fairly good agreement with those for the antagonist [^{3}H]DPX and the sum of high and low affinity B_{max}-values for the agonist [^{3}H]PIA. In bovine heart, A_1 receptors were not reliably detected with the latter two ligands. With ^{125}I-HPIA only high affinity agonist binding was observed in this tissue, and the B_{max}-value was only 60% of the value obtained with [^{3}H]DPCPX. The higher B_{max}-values of [^{3}H]DPCPX binding together with the linear Scatchard plot suggest by analogy to other tissues (Lohse et al. 1984a) that [^{3}H]DPCPX binds also to the

Table 4. Binding parameters of different tissues for [^{3}H]DPCPX, [^{3}H]DPX and [^{3}H]PIA. The binding parameters were obtained from 3 separate saturation experiments containing at least 7 (antagonists) or 13 (agonists) different concentrations of radioligand. Values for [^{3}H]PIA are the sums of high and low agonist affinity states. Data are given as means ± SEMs or with 95% confidence limits

Tissue	[^{3}H]DPCPX		[^{3}H]DPX	[^{H}]PIA
	K_D (pM)	B_{max} (fmol/mg protein)		
Rat fat cells	190 (160–230)	580±50	540± 80	560±100
Rat brain	180 (160–200)	1080±30	1170± 50	1040± 30
Bovine brain	51 (46–56)	1460±50	1450±110	1120± 80
Bovine heart	110 (95–130)	62± 5	nd	(38±4[a])

[a] Value obtained with 0.05–2 nM ^{125}I-HPIA, allowing detection of a state of high affinity only. nd, not detectable

Table 5. Competition for [^{3}H]DPCPX and ^{125}I-HPIA binding to bovine myocardial membranes. Values are geometric means of 3 separate concentration-response curves with at least 8 (antagonists) or 12 (agonists) concentrations of competitor; 95% confidence intervals were within 0.15 log units

Competitor	[^{3}H]DPCPX		^{125}I-HPIA
Antagonists	K_i (nM)		K_i (nM)
DPCPX	0.07		0.06
XAC	0.08		0.2
DPX	1.7		3.4
Theophylline	3,800		12,300
Agonists[a]	K_H (nM)	K_L (nM)	K_H (nM)
R-PIA	0.19	3.1	0.53
S-PIA	3.0	83	4.6
NECA	5.9	125	14.3

[a] For agonists: K_H, K_i-value of the high agonist affinity state; K_L, K_i-value of the low agonist affinity state; the percentage of receptors in the high agonist affinity state was 65% to 70%. ^{125}I-HPIA labels only the high agonist affinity state, therefore only K_H-values can be calculated for agonists

low affinity agonist binding state without differentiating between the two states.

This was confirmed in competition experiments for [^{3}H]DPCPX binding to bovine myocardial membranes (Table 5): Antagonists competed for [^{3}H]DPCPX binding with slope factors of 0.95–1.05, suggesting only one state of homogeneous affinity. Among the antagonists, DPCPX was slightly more potent than XAC. Agonists competed for [^{3}H]DPCPX binding with shallow curves (n_H 0.55–0.75), which could be fitted significantly better to a two-state model ($p < 0.01$, F-test). The K_L-values were 15 to 30 times higher than the K_H-values. In general, there was good agreement of the K_i-values of competition for [^{3}H]DPCPX and ^{125}I-HPIA binding (K_H-values in the case of agonists), suggesting that [^{3}H]DPCPX does indeed bind to A_1 receptors.

GTP in concentrations up to 100 μM did not affect [^{3}H]DPCPX binding in any of the tissues studied. In the presence of 100 μM GTP, agonists competed for the binding of [^{3}H]DPCPX to bovine myocardial membranes with K_i-values similar to the K_L-values determined in the absence of GTP (R-PIA 2.2 nM, CHA 5.4 nM, S-PIA 52 nM, NECA 140 nM).

The high affinity of the 8-phenyl-substituted xanthines, XAC and DPX, for the bovine heart A_1 receptor was not observed with rat brain membranes. DPCPX, XAC and DPX competed for the binding of [^{3}H]DPCPX to rat brain membranes with K_i-values of 0.2, 3.5, and 60 nM, respectively, which agree relatively well with the values obtained from competition for [^{3}H]PIA binding (see Table 2).

Discussion

DPCPX appears to be the only A_1-selective compound among the antagonists proposed as radioligands for the A_1 adenosine receptor. On the basis of adenylate cyclase studies it has a more than 700-fold selectivity for the A_1 subtype, compared to 5.5 for XAC and 1.2 for DPX. In a previous study (Schwabe et al. 1985), the high-affinity antagonist 1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine (PACPX – Bruns et al. 1983) was identified as the most A_1-selective xanthine; however, both the affinity and the selectivity of DPCPX are 10 times higher. In addition, the high non-specific binding of tritiated PACPX to membranes and filters preclude its use as a radioligand for A_1 receptors (Bruns et al. 1983).

The K_i-value of DPCPX at human platelet A_2 receptors is similar to its K_i-value in competing for the binding of [^{3}H]NECA to striatal membranes. This suggests that it does not differentiate between the putative A_{2a} (striatum) and A_{2b} (peripheral tissues) receptors. In similar radioligand binding studies, Lee and Reddington (1986) observed an A_1-selectivity by a factor of 500, and Bruns et al. (1987) by a factor of 740, which is in good agreement with our data from Table 2.

None of the antagonist A_1 receptor radioligands had major effects on the cAMP-phosphodiesterase activity of human platelet lysates. This agrees with the observation that 8-phenyltheophylline is a weak inhibitor of phosphodiesterases (Smellie et al. 1979). The difference between the concentrations needed to block the A_1 receptor and those that inhibit phosphodiesterase activity was largest for DPCPX (> 100,000) and least for DPX (> 600), which is still high compared with the difference of less than 10 for theophylline.

Tritiated DPCPX has been proposed as a radioligand by Bruns et al. (1987). [^{3}H]DPCPX of high specific activity (over 100 Ci/mmol) can be obtained by catalytic reduction of the 1,3-diallyl-analogue. [^{3}H]DPCPX appears to label the A_1 adenosine receptor selectively in a number of tissues, as evidenced by the competition for [^{3}H]DPCPX binding and the similar binding capacities compared to other A_1 receptor radioligands. The high affinity, rapid kinetics and low nonspecific binding allow the detection of A_1 receptors in tissues with low receptor densities such as the heart. This enables the investigation of the different agonist affinity states of the receptor, which hitherto has not been accessible

Table 6. Comparison of antagonist radioligands for A_1 adenosine receptors. The data were obtained from saturation experiments with rat brain membranes

Ligand	Specific activity (Ci/mmol)	Affinity K_D (nM)	Nonspecific binding (% of total at K_D)
[^{3}H]DPCPX	105	0.18	1.3
[^{3}H]XAC[a]	103	1.2	20
[^{3}H]DPX	13	68	40

[a] Binding parameters for [^{3}H]XAC are from Jacobson et al. (1986)

to binding studies with radioiodinated agonists (Linden et al. 1985; Lohse et al. 1985). It shows that the high and low agonist affinity states in bovine heart have characteristics similar to those in bovine brain: the difference between K_H- and K_L-values is 15 to 30-fold, compared to a factor of 90 to 150 in rat brain (Lohse et al. 1984a). A difference of only 20-fold has also been observed for bovine brain (Lohse et al. 1984a). Secondly, the bovine heart A_1 receptor has a high affinity for N^6-substituted adenosine analogues (S-PIA > NECA). In addition, it has a similar high affinity for 8-phenyl-substituted xanthines that has been observed for the bovine brain A_1 receptor (Schwabe et al. 1985). This difference between bovine and rat A_1 receptors is much less pronounced for the 8-cyclopentyl-substituted DPCPX.

Table 6 summarizes the binding properties of the antagonist radioligands for the A_1 receptor. The specific activity that can be obtained for [^{3}H]DPCPX and [^{3}H]XAC is the same, since both compounds are prepared by catalytic reduction of their 1,3-diallyl-analogues, and is substantially higher than that of DPX. However, the affinity of [^{3}H]DPCPX is much higher than that of [^{3}H]XAC and [^{3}H]DPX; consequently, there is considerably less nonspecific binding at K_D. In fact, when half of the receptors are saturated with [^{3}H]DPCPX, the nonspecifically bound radioactivity is only slightly above the counter background. This high signal to noise ratio makes [^{3}H]DPCPX also superior to the radioiodinated agonists for studies of tissues with low receptor densities, unless a limited tissue supply necessitates the still higher specific activity of radioiodinated ligands. Thus the nonspecific binding of ^{125}I-HPIA to bovine myocardial membranes at K_D is 65% (Lohse et al. 1985), and that of ^{125}I-N^6-p-aminobenzyladenosine to rat heart membranes at K_D about 50% of the total binding (Linden et al. 1985). In contrast, the nonspecific binding of [^{3}H]DPCPX to bovine myocardial membranes at K_D is only 12%. Finally, [^{3}H]DPCPX is the only antagonist radioligand that is selective for A_1 receptors (Table 1). Although the agonist radioligand [^{3}H]N^6-cyclopentyladenosine (Williams et al. 1986) has a similar A_1 selectivity, its specific activity is less than half that of [^{3}H]DPCPX, its affinity considerably lower, and its nonspecific binding at K_D is several times higher.

These properties indicate that DPCPX and [^{3}H]DPCPX will be useful tools in the study of A_1 adenosine receptors.

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