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Molecular cloning and characterization of the human A_3 adenosine receptor

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ABSTRACT **The human A_3 adenosine receptor was cloned from a striatal cDNA library using a probe derived from the homologous rat sequence. The cDNA encodes a protein of 318 amino acids and exhibits 72% and 85% overall identity with the rat and sheep A_3 adenosine receptor sequences, respectively. Specific and saturable binding of the adenosine receptor agonist N^6-(4-amino-3-[^{125}I]iodobenzyl)adenosine [^{125}I]ABA was measured on the human A_3 receptor stably expressed in Chinese hamster ovary cells with a K_d = 10 nM. The potency order for adenosine receptor agonists was *N*-ethylcarboxamidoadenosine (NECA) ≥ (*R*)-N^6-phenyl-2-propyladenosine [(*R*)-PIA] > N^6-cyclopentyladenosine (CPA) > (*S*)-N^6-phenyl-2-propyladenosine [(*S*)-PIA]. The human receptor was blocked by xanthine antagonists, most potently by 3-(3-iodo-4-aminobenzyl)-8-(4-oxyacetate)phenyl-1-propylxanthine (I-ABOPX) with a potency order of I-ABOPX > 1,3-dipropyl-8-(4-acrylate)phenylxanthine ≥ xanthine amino congener ≫ 1,3-dipropyl-8-cyclopentylxanthine. Adenosine, NECA, (*R*)- and (*S*)-PIA, and CPA inhibited forskolin-stimulated cAMP accumulation by 30–40% in stably transfected cells; I-ABA is a partial agonist. When measured in the presence of antagonists, the dose–response curves of NECA-induced inhibition of forskolin-stimulated cAMP accumulation were right-shifted. Antagonist potencies determined by Schild analyses correlated well with those established by competition for radioligand binding. The A_3 adenosine receptor transcript is widespread and, in contrast to the A_1, A_{2a}, and A_{2b} transcripts, the most abundant expression is found in the lung and liver. The tissue distribution of A_3 mRNA is more similar to the widespread profile found in sheep than to the restricted profile found in the rat. This raises the possibility that numerous physiological effects of adenosine may be mediated by A_3 adenosine receptors.**

The actions of adenosine are mediated through guanine nucleotide-binding protein (G-protein)-coupled receptors that were initially classified into A_1 and A_2 subtypes based on pharmacological criteria and coupling to adenylyl cyclase (1). Further pharmacological classification of adenosine receptors prompted subdivision of the A_2 class into A_{2a} and A_{2b} subtypes on the basis of high and low affinity, respectively, for adenosine and the agonists *N*-ethylcarboxamidoadenosine (NECA) and 2-[4-(2-carboxyethyl)phenyl]ethylamino-5′-*N*-ethylcarboxamidoadenosine (CGS21680) (2, 3). The existence of A_1, A_{2a}, and A_{2b} subtypes has been confirmed by cloning and functional characterization of expressed bovine, canine, rat, and human receptors (4). A fourth subtype, A_3, had remained pharmacologically undetected until its recent identification by molecular cloning. The rat A_3 sequence, tgpcr1, was first cloned from rat testis by Meyerhof *et al.* (5). Subsequently, the functional expression of a rat striatal cDNA encoding the identical receptor confirmed the sequence as an adenosine receptor subtype on the basis of the binding of the radioligand N^6-2-(4-amino-3-[^{125}I]iodophenyl)-ethyladenosine [^{125}I]APNEA and the agonist-induced inhibition of cAMP accumulation with a potency order of NECA = (*R*)-N^6-phenyl-2-propyladenosine [(*R*)-PIA] > CGS21680 (6). The rat receptor exhibited a unique pharmacology relative to the other adenosine receptor subtypes and was reported not to bind the xanthine antagonists 1,3-dipropyl-8-cyclophenylxanthine (DPCPX) and xanthine amine congener (XAC). The rat A_3 adenosine receptor transcript is primarily expressed in the testis (5). Recently, the sheep homolog of the A_3 receptor was cloned from hypophysial pars tuberalis (7). The sheep receptor is 72% identical to the rat receptor, binds the radioligand N^6-(4-amino-3-[^{125}I]iodobenzyl)adenosine ([^{125}I]ABA), and is also coupled to inhibition of cAMP. The agonist affinity order of the sheep receptor is I-ABA > NECA ≥ (*R*)-PIA ≫ N^6-cyclopentyladenosine (CPA). The pharmacology of xanthine antagonists was extensively studied and the sheep receptor was found to exhibit high affinity for 8-phenylxanthines with *para*-acidic substitutions. In contrast to the rat transcript, the expression of the sheep A_3 adenosine receptor transcript is widespread and is most abundant in the lung and spleen.

In this report, we describe the cloning and pharmacological characterization of a human A_3 adenosine receptor subtype.¶ The human A_1, A_{2a}, and A_{2b} adenosine receptor cDNAs have been cloned (8–11); however, the tissue distribution of receptor transcripts has not been previously presented. The distribution of A_1, A_{2a}, A_{2b}, and A_3 receptor transcripts has now been determined in human tissues.

MATERIALS AND METHODS

Compounds. NECA, (*R*)-PIA, (*S*)-PIA, DPCPX, and XAC were from Research Biochemicals. 1,3-Dipropyl-8-(4-acrylate)phenylxanthine (BW-A1433) was a gift from Susan Daluge of the Burroughs Wellcome Company. [^{125}I]ABA (12), I-ABA (12), and 3-(3-iodo-4-aminobenzyl)-8-(4-oxyacetate)phenyl-1-propylxanthine [I-ABOPX (BW-A522)] (13) were synthesized as described.

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Abbreviations: [^{125}I]ABA, N^6-(4-amino-3-[^{125}I]iodobenzyl)adenosine; [^{125}I]APNEA, N^6-2-(4-amino-3-[^{125}I]iodophenyl)ethyladenosine; NECA, *N*-ethylcarboxamidoadenosine; CGS21680, 2-[4-(2-carboxyethyl)phenyl]ethylamino-5′-*N*-ethylcarboxamidoadenosine; (*R,S*)-PIA, (*R,S*)-N^6-phenyl-2-propyladenosine; CPA, N^6-cyclopentyladenosine; I-ABOPX, 3-(3-iodo-4-aminobenzyl)-8-(4-oxyacetate)phenyl-1-propylxanthine; BW-A1433, 1,3-dipropyl-8-(4-acrylate)phenylxanthine; XAC, xanthine amine congener; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; p[NH]ppG, 5′-guanylylimidodiphosphate; G protein, guanine nucleotide-binding protein; CHO, Chinese hamster ovary.

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¶The HS-21a sequence reported in this paper has been deposited in the GenBank data base (accession no. L22607).

Probe Synthesis and cDNA Library Screening. Rat brain cDNA was used as template in a PCR amplification reaction, according to the GeneAmp protocol (Perkin–Elmer/Cetus), containing 50 pmol each of two primers (5′-CCCAAGCTTATGAAAGCCAACAATACC) and (5′-TGCTCTAGACTCTGGTATCTTCACATT) based on the published rat A_3 adenosine receptor sequence (6). Forty cycles of 40 sec at 94°C, 1 min at 55°C, and 3 min at 72°C were performed. The resulting 788-bp fragment was subcloned into pBluescript II KS+ (Stratagene) and verified by the dideoxynucleotide chain-termination method (14) using Sequenase (United States Biochemical). For screening, the DNA fragment was labeled with [α-^{32}P]dCTP (15). A human striatal cDNA library (Stratagene) constructed in λZAP II was transferred to Hybond-N (Amersham) and hybridized in a solution containing the rat A_3 probe, 30% formamide, 5× SSPE (0.9 M NaCl/50 mM sodium phosphate, pH 7.7/5 mM EDTA), 5× Denhardt's solution, 0.5% SDS, and 50 μg of sonicated salmon testis DNA per ml at 42°C. Filters were washed in 2× SSC (1× SSC = 0.15 M NaCl/15 mM sodium citrate, pH 7) at 55°C. Positively hybridizing phage were plaque purified and inserts were subcloned by *in vivo* excision into pBluescript II SK− (Stratagene). Double-stranded plasmid was sequenced using Sequenase.

Expression Construct and Transfection. The 1.7-kb HS-21a cDNA was subcloned as a *Sal* I–*Bam*HI fragment into the expression vector pCMV5 (16) creating the vector pCMV5-A3. Chinese hamster ovary (CHO) cells stably expressing the human HS-21a cDNA were prepared by cotransfection of 15 μg of pCMV5-A3 and 1 μg of pWLneo (Stratagene) using the calcium phosphate method. Neomycin-resistant colonies were selected in 1 mg of G418 per ml (GIBCO) and screened for expression of HS-21a by [^{125}I]ABA binding.

Binding Studies. Membranes were prepared from stable CHO cell lines as described (17). Pellets were resuspended in 5 mM Hepes, pH 7.4/5 mM $MgCl_2$/0.1 mM benzamidine at a protein concentration of 1–2 mg/ml and were incubated with adenosine deaminase (Boehringer Mannheim), 2 units/ml at 37°C for 20 min. Saturation binding of [^{125}I]ABA was carried out on 50 μg of membranes for 120 min at 25°C in a total volume of 100 μl. The assay was terminated by rapid filtration and three washes with ice-cold binding buffer. The specific activity of [^{125}I]ABA, initially 2200 Ci/mmol (1 Ci = 37 GBq), was reduced to 100 Ci/mmol with nonradioactive I-ABA for saturation analysis. Nonspecific binding was measured in the presence of 1 μM I-ABA or 400 μM NECA. The K_d and B_{max} values were calculated by the EBDA program (18). Competition binding of agonists and antagonists was determined with [^{125}I]ABA (0.17–2.0 nM, 2000 Ci/mmol). Data were analyzed and competition curves were constructed from the mean of triplicate determinations by GRAPH PAD INPLOT, Version 3.0 (Graph Pad Software, San Diego). K_i values were calculated using the Cheng–Prusoff derivation (19).

cAMP Studies. Determinations were made on stably transfected CHO cells in suspension as described (7). Supernatants (500 μl) were acetylated and acetyl-cAMP was measured by automated radioimmunoassay (20). Antagonist dissociation constants were estimated from pA_2 values as described by Schild (21).

Northern Blot Analysis. Human poly(A)$^+$ RNA (Clontech) was fractionated on a 1% agarose/formaldehyde gel (22), transferred to Hybond-N membranes, and hybridized in 5× SSPE, 5× Denhardt's solution, 0.5% SDS, and 50 μg of sonicated salmon testis DNA per ml with 30% formamide (for A_1, A_{2a}, and A_{2b}) or 50% formamide (for HS-21a) at 42°C. DNA probes corresponding to nucleotides 512–1614, 936–2168, and 321–1540 of accession nos. X68485 (A_1), X68486 (A_{2a}), and X68487 (A_{2b}), respectively, and a 1.7-kb *Sal* I–*Bam*HI fragment of HS-21a were labeled with [α-^{32}P]dCTP by the random priming method. Filters were washed under high stringency conditions in 0.1× SSC at 65°C.

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          1                    I                    50
human     MPNNSTALS. .LANVTYITM EIFIGLCAIV GNVLVICVVK LNPSLQTTTF
sheep     MPVNSTAVS. .WTSVTYITV EILIGLCAIV GNVLVIWVVK LNPSLQTTTF
rat       MKANNTTTSA LWLQITYVTM EAAIGLCAVV GNMLVIWVVK LNRTLRTTTF
Consensus M--N-T--S- -----TY-T- E--IGLCA-V GN-LVI-VVK LN--L-TTTF

         51        II                  III       100
human     YFIVSLALAD IAVGVLVMPL AIVVSLGITI HFYSCLFMTC LLLIFTHASI
sheep     YFIVSLALAD IAVGVLVMPL AIVISLGVTI HFYSCLFMTC LMLIFTHASI
rat       YFIVSLALAD IAVGVLVIPL AIAVSLEVQM HFYACLFMSC VLLVFTHASI
Consensus YFIVSLALAD IAVGVLV-PL AI--SL---- HFY-CLFM-C --L-FTHASI

        101                          IV          150
human     MSLLAIAVDR YLRVKLTVRY KRVTTHRRIW LALGLCWLVS FLVGLTPMFG
sheep     MSLLAIAVDR YLRVKLTVRY RRVTTQRRIW LALGLCWLVS FLVGLTPMFG
rat       MSLLAIAVDR YLRVKLTVRY RTVTTQRRIW LFLGLCWLVS FLVGLTPMFG
Consensus MSLLAIAVDR YLRVKLTVRY --VTT-RRIW L-LGLCWLVS FLVGLTPMFG

        151                          V           200
human     WNMKLTSEYH RNVTFLSCQF VSVMRMDYMV YFSFLTWIFI PLVVMCAIYL
sheep     WNMKLSSA.D ENLTFLPCRF RSVMRMDYMV YFSFFLWILV PLVVMCAIYF
rat       WNRKVTLELS QNSSTLSCHF RFVVGLDYMV FFSFITWILI PLVVMCIIYL
Consensus WN-K------ -N---L-C-F --V---DYMV -FSF--WI-- PLVVMC-IY-

        201                          VI          250
human     DIFYIIRNKL SLNLSNSKET GAFYGREFKT AKSLFLVLFL FALSWLPLSI
sheep     DIFYIIRNRL SQSFSGSRET GAFYGREFKT AKSLLLVLFL FALCWLPLSI
rat       DIFYIIRNKL SQNLTGFRET RAFYGREFKT AKSLFLVLFL FALCWLPLSI
Consensus DIFYIIRN-L S-------ET -AFYGREFKT AKSL-LVLFL FAL-WLPLSI

        251                VII                   300
human     INCIIYFNGE VPQLVLYMGI LLSHANSMMN PIVYAYKIKK FKETYLLILK
sheep     INCILYFDGQ VPQTVLYLGI LLSHANSMMN PIVYAYKIKK FKETYLLILK
rat       INFVSYFNVK IPEIAMCLGI LLSHANSMMN PIVYACKNKK VQRNHFVILR
Consensus IN---YF--- -P------GI LLSHANSMMN PIVYA-K-KK -------IL-

        301                  321
human     ACVVCHPSDS LDTSIEKNSE *
sheep     ACVMCQPSKS MDPSTEQTSE *
rat       ACRLCQTSDS LDSNLEQTT. *
Consensus AC--C--S-S -D---E---- -
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FIG. 1. Deduced amino acid sequence of HS-21a and alignment with sheep and rat A_3 adenosine receptor sequences. Putative transmembrane domains are indicated by solid lines along with numbered designations (I–VII). Gaps in the sequences are represented by dots.

RESULTS

Cloning. The screening of a human striatal cDNA library with a cDNA probe derived from the rat A_3 sequence resulted in the identification of several positively hybridizing clones (17 positives/1 × 10^6 recombinants screened). One of the clones, designated HS-21a, contained a 1.7-kb insert and upon sequencing was found to have a complete open reading frame corresponding to 318 amino acids (Fig. 1) and 265 bp and 517 bp of 5′ and 3′ untranslated sequence, respectively. An in-frame stop codon (TAG) was found upstream of the proposed initiating methionine at position −72. A hydrophilicity plot of the translated sequence predicted seven transmembrane domains, which is a common feature of the G-protein-coupled family of receptors (23). Three putative N-linked glycosylation sites are found in the amino terminus at Asn-3, Asn-4, and Asn-12 and a fourth site is found in the second extracellular loop at Asn-160. The HS-21a translated sequence has 72% and 85% overall identity with the rat and sheep A_3 receptors, respectively (Fig. 1). Among the three species, the most similar regions of sequence are the transmembrane domains. In addition, all three species have a short amino-terminal sequence containing potential N-linked glycosylation sites and sequence that is more similar between human and sheep than between human and rat. The rat carboxyl-terminal sequence is considerably different in sequence from the human and sheep. All three species contain a cysteine residue in the carboxyl-terminal region (Cys-303 of HS-21a) that is conserved among all species of A_1, A_{2b}, and A_3 receptors but is not found in the A_{2a} receptor (7). This residue may serve as a palmitoylation site involved in the formation of a fourth intracellular loop (24). Comparison of the translated HS-21a sequence with the cloned human adenosine receptor subtype sequences yielded identity scores of 49%, 43%, and 40% for the A_1, A_{2a}, and A_{2b} subtypes, respectively. All four human adenosine receptor sequences contain a potential glycosylation site in the second extracellular loop; however, only the HS-21a sequence contains sites near the amino terminus.

Characterization of HS-21a Expressed in CHO Cells. The radioligand, [^{125}I]APNEA, was previously used to characterize rat A_3 adenosine receptors (6). In preliminary experiments, high nonspecific [^{125}I]APNEA binding to CHO cell membranes, which interfered with the measurement of specific binding to expressed receptors, was observed. Specific and saturable binding of the adenosine receptor agonist [^{125}I]ABA was measured on membranes prepared from the stably transfected cells (Fig. 2*A*). The specific binding of [^{125}I]ABA could be prevented by either 1 μM nonradioactive I-ABA or 400 μM NECA. No specific binding of [^{125}I]ABA

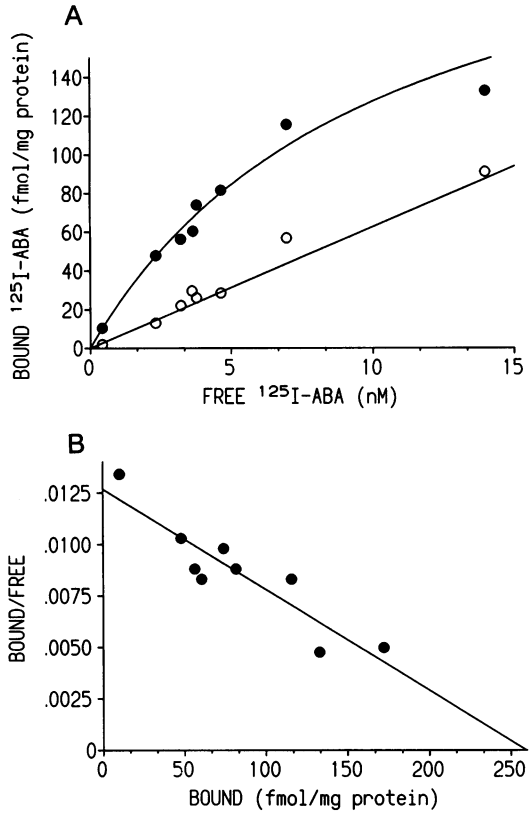

FIG. 2. Equilibrium binding and Scatchard analysis of [^{125}I]ABA to membranes prepared from HS-21a stably transfected CHO cells. (*A*) Specific (●) and nonspecific (○) binding. Nonspecific binding was measured in the presence of 1 μM I-ABA. (*B*) Scatchard transformation of the specific binding.

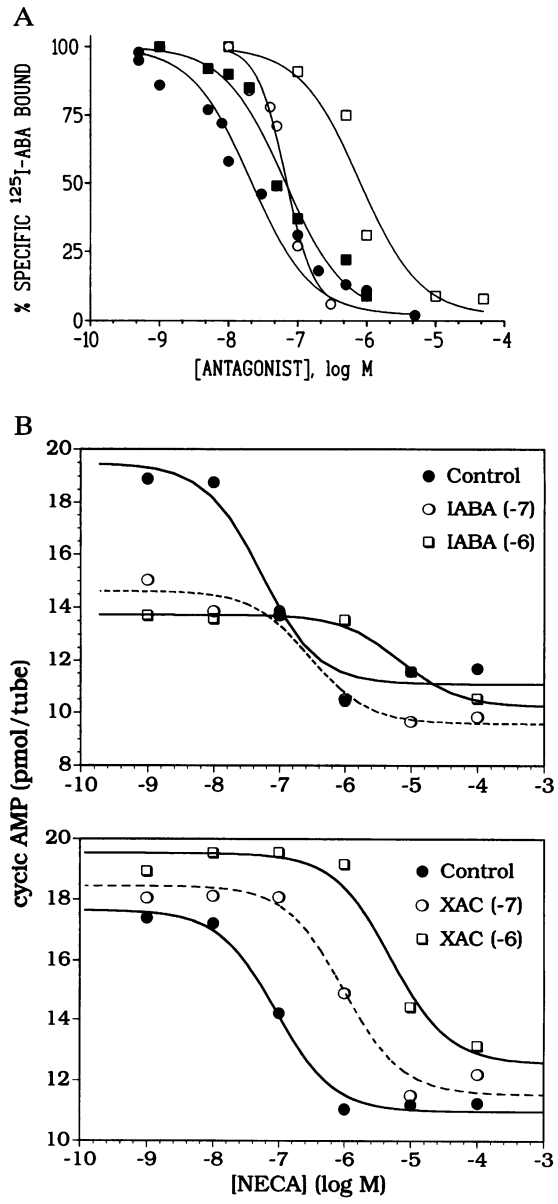

FIG. 3. (*A*) Competition by antagonists for [^{125}I]ABA binding to membranes prepared from HS-21a stably transfected CHO cells. ●, I-ABOPX; ■, BW-A1433; ○, XAC; □, DPCPX. (*B*) Competition by I-ABA and XAC of NECA-inhibited cAMP accumulation in CHO cells. ED_{50} values were used to construct Schild plots. Each figure is representative of two or three experiments.

Table 1. Comparison of agonist and antagonist affinities determined in binding and functional cAMP assays

Compound	Binding, pK_d or pK_i	cAMP, pK_a or pK_i
Agonist		
I-ABA	8.0	7.46
NECA	7.59	6.89
(*R*)-PIA	7.47	6.14
CPA	7.05	6.8
(*S*)-PIA	6.49	6.04
Antagonist		
I-ABOPX	7.74	8.13
XAC	7.15	7.66
BW-A1433	7.26	7.0
DPCPX	6.12	5.36

was measured on membranes prepared from nontransfected CHO cells. The specific binding of [^{125}I]ABA measured in the presence of 100 μM 5'-guanylylimidodiphosphate (p[NH]ppG) was reduced by 44% relative to the specific binding measured in the absence of the uncoupling reagent (data not shown). [^{125}I]ABA binds to membranes prepared from the HS-21a stable CHO cells with a dissociation constant of 10 nM (B_{max} = 258 fmol/mg of protein), with a Hill coefficient of 0.99, indicating binding to a single class of high-affinity sites (Fig. 2*B*).

The competition of adenosine receptor agonists and antagonists for binding to HS-21a receptors was determined. The K_i values for agonists were 26 nM for NECA, 34 nM for (*R*)-PIA, 89 nM for CPA, and 320 nM for (*S*)-PIA, resulting in a potency order profile of NECA > (*R*)-PIA > CPA > (*S*)-PIA. In contrast to the insensitivity of adenosine receptor antagonists reported for the rat A_3 adenosine receptor subtype (6), a number of xanthine antagonists exhibited competition with [^{125}I]ABA for binding to the HS-21a receptor (Fig. 3*A*). The K_i values for antagonists were 18 nM for I-ABOPX, 55 nM for BW-A1433, 70 nM for XAC, and 750 nM for DPCPX, resulting in a potency order profile of I-ABOPX > BW-A1433 > XAC > DPCPX.

cAMP Studies. The ability of the HS-21a receptor stably expressed in CHO cells to couple to the cAMP regulatory system was measured using adenosine as an agonist. Adenosine (10 μM) produced a 30% inhibition of the forskolin-stimulated increase in cAMP. In the absence of forskolin, adenosine had no effect on the cAMP levels. In nontransfected CHO cells, adenosine had no effect on cAMP levels when measured with or without forskolin treatment.

I-ABA produced only about half as much inhibition of forskolin-stimulated cAMP accumulation in CHO cells as did NECA (Fig. 3*B Upper*) and other agonists (PIA and CPA). Furthermore, in the presence of I-ABA, the dose–response curve of NECA to lower cAMP was right-shifted. These data indicate that I-ABA is a partial agonist in this system. Dose–response curves of NECA-induced inhibition of forskolin-stimulated cAMP accumulation were also right-shifted in the presence of competing xanthine antagonists (Fig. 3*B Lower*). The pK_i values determined by competitive binding for various agonists and antagonists are compared in Table 1 with the pK_a values determined by Schild analysis in the functional cAMP assay. The potency order profiles were nearly identical for the binding and functional assays; however, the K_a of agonists to lower cAMP were consistently higher (i.e., lower potency) than K_i values determined from competitive binding assays. Although the conditions of these assays differ, these data suggest that recombinant A_3 receptors are not well coupled to inhibition of cAMP accumulation in CHO cells.

Distribution of Tissue Expression. Poly(A)$^+$ RNA from a number of human tissues was evaluated by Northern blot analyses to establish the distribution of tissue expression for the HS-21a transcript (Fig. 4*A*). A 2-kb transcript was most abundantly expressed in lung and liver, with moderate amounts observed in brain and aorta. Low levels of expression were also observed in testis and heart. No expression was detected in spleen or kidney. The profile of lung = liver ≫ brain = aorta > testis > heart determined for HS-21a is considerably different from the tissue distribution of the other human adenosine receptor subtypes (Fig. 4*B*). A human A_1 transcript (2.9 kb) is expressed in brain, heart, kidney, and lung, with the most abundant expression observed in the brain. A second hybridizing band of 4.3 kb is also observed in lower amounts in the brain. The A_{2a} adenosine receptor transcript (2.8 kb) is equally expressed in brain, heart, and kidney, with slightly higher levels of expression detected in the lung. [Two hybridizing bands were observed when the full-length A_{2a} coding sequence was used as a probe and may be the result of cross-hybridization with the A_1 transcript (upper band)]. The human brain mRNA utilized in the Northern analysis was prepared from the brainstem, pons, cerebellum, telencephalon, diencephalon, and mesencephalon regions of the brain and does not represent enriched transcripts from those regions of the brain in which the most abundant expression of A_1 in the cortex (25) and A_{2a} in caudate, putamen, and nucleus accumbens (26) has been indicated by radioligand binding or *in situ* hybridization studies. For the human A_{2b} subtype, two hybridizing transcripts of 1.7 kb and 2.1 kb were observed in brain, heart, and lung and no expression was observed in the kidney. From the comparison of the distribution of human adenosine receptor transcripts, it can be concluded that the subtype transcripts are widely distributed but differ from each other in the abundance found in particular tissues.

DISCUSSION

We have cloned a cDNA from human striatum designated HS-21a that encodes a human A_3 adenosine receptor. The cDNA is homologous with rat (5, 6) and sheep clones (7) and encodes a receptor that couples to inhibition of cAMP accumulation when stably expressed in CHO cells. [^{125}I]ABA, previously used as a radioligand for A_1 adenosine receptors (12), was found to be suitable for detecting recom-

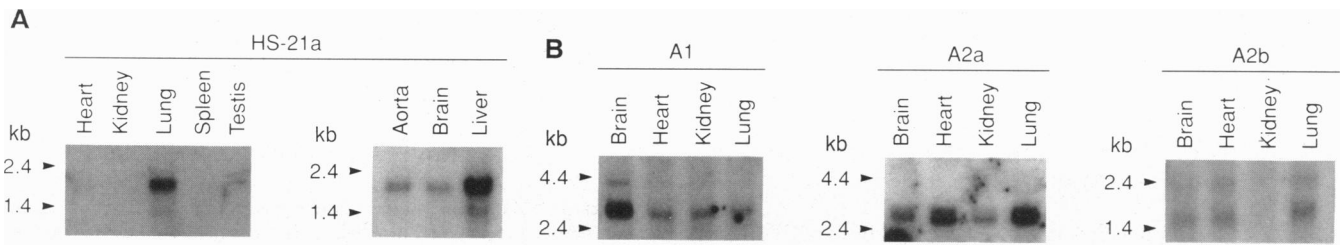

FIG. 4. Northern blot analysis of the four human adenosine receptor subtypes. (*A*) Five micrograms of poly(A)$^+$ RNA from various human tissues probed with HS-21a. The two blots shown were transferred, hybridized, and exposed separately. (*B*) Seven and one-half micrograms of poly(A)$^+$ RNA from various human tissues probed with either A_1, A_{2a}, or A_{2b}. Each blot was transferred and exposed separately.

binantly expressed human A_3 adenosine receptors. The pharmacological profile of the human receptor and distribution of transcript expression is more similar to the sheep than the rat homolog.

The rat A_3 adenosine receptor differs from the human and sheep receptors in that it was reported not to bind the xanthine antagonists, XAC and DPCPX (6). The sheep (7) and now the human A_3 adenosine receptors have been found to bind both antagonists and also have high affinity for 8-phenylxanthines with *para*-acidic substitutions. I-ABOPX was found to have the highest affinity as an A_3 adenosine receptor antagonist with K_i values of 2 and 18 nM on sheep and human receptors, respectively. A limited number of xanthine analogs were evaluated in the pharmacological characterization of the rat A_3 receptor and it is possible that the rat A_3 receptor may bind acidic xanthines as is the case with the human and sheep A_3 homologs. A few significant differences in ligand binding exist between the human and sheep receptors. I-ABA appears to be a full and partial agonist, respectively, for lowering cAMP in CHO cells transfected with sheep and human receptors. The human receptor has a generally higher affinity for all of the agonists and a preference for CPA over (*S*)-PIA. The antagonist affinity order profiles are similar between human and sheep receptors; however, the human homolog exhibits a higher affinity for XAC.

The potency order profiles of agonist and antagonist binding to the A_3 receptor differ substantially from the profiles established for the other cloned human adenosine receptor subtypes (4). All subtypes of human adenosine receptors are blocked by xanthine antagonists such as BW-A1433, XAC, and DPCPX but can be distinguished by their differences in affinities and potency order profiles for these ligands. The human A_3 sequence is more similar to the A_1 than A_{2a} and A_{2b} sequences. Pharmacologically, the A_3 subtype contrasts with the A_1 subtype by its preference for phenyl over cyclopentyl substitutions at the N6 and C8 positions of agonists and antagonists, respectively, but retains a structure–activity relationship similar to that proposed for A_1 agonists and antagonists in which parallel changes in potency are observed when phenyl substitutions are incorporated at these positions (27, 28). A similar relationship has been suggested to exist for the sheep A_3 receptor (7).

The tissue distribution of the human A_3 adenosine receptor transcript was found to be more similar to the sheep than the rat homolog. A comparison of the distribution of human adenosine receptor transcripts suggests that the A_1, A_{2a}, A_{2b}, and A_3 subtypes are all expressed in a number of tissues, but the pattern of transcript distribution is variable. It is likely that transcript expression will be found in other human tissues not yet examined. For example, abundant A_{2b} transcript expression has been found in the large intestine, cecum, and urinary bladder of the rat, in addition to moderate expression in the brain and lung (29).

The physiological role of the A_3 adenosine receptor has not yet been defined. Recently, the A_3 subtype was suggested to mediate *in vivo* hypotension in the angiotensin II-supported circulation of the pithed rat (30). On the basis of the *in situ* localization of A_3 mRNA within the central luminal regions of rat testis seminiferous tubules (5), a role in reproduction and spermatogenesis has been proposed. The abundant mRNA observed in the human and sheep lung suggests that the A_3 subtype may also mediate a physiological action in the pulmonary system. Adenosine has been shown to mediate vasodilation and vasoconstriction in the pulmonary vasculature (31, 32) and induces bronchoconstriction in asthmatics but not in normal patients (33). The establishment of the pharmacological profile for the A_3 receptor in the human and the sheep and the availability of subtype-selective ligands will facilitate the identification of the physiological functions mediated by the A_3 adenosine receptor subtype.

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