Tissue distribution of adenosine receptor mRNAs in the rat

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1   A degree of ambiguity and uncertainty exists concerning the distribution of mRNAs encoding the four cloned adenosine receptors. In order to consolidate and extend current understanding in this area, the expression of the adenosine receptors has been examined in the rat by use of *in situ* hybridisation and the reverse transcription-polymerase chain reaction (RT-PCR).

2   In accordance with earlier studies, *in situ* hybridisation revealed that the adenosine A_1 receptor was widely expressed in the brain, whereas A_{2A} receptor mRNA was restricted to the striatum, nucleus accumbens and olfactory tubercle. In addition, A_1 receptor mRNA was detected in large striatal cholinergic interneurones, 26% of these neurones were also found to express the A_{2A} receptor gene. Central levels of mRNAs encoding adenosine A_{2B} and A_3 receptors were, however, below the detection limits of *in situ* hybridisation.

3   The more sensitive technique of RT-PCR was then employed to investigate the distribution of adenosine receptor mRNAs in the central nervous system (CNS) and a wide range of peripheral tissues. As a result, many novel sites of adenosine receptor gene expression were identified. A_1 receptor expression has now been found in the heart, aorta, liver, kidney, eye and bladder. These observations are largely consistent with previous functional data. A_{2A} receptor mRNA was detected in all brain regions tested, demonstrating that expression of this receptor is not restricted to the basal ganglia. In the periphery A_{2A} receptor mRNA was also found to be more widely distributed than generally recognised. The ubiquitous distribution of the A_{2B} receptor is shown for the first time, A_{2B} mRNA was detected at various levels in all rat tissues studied. Expression of the gene encoding the adenosine A_3 receptor was also found to be widespread in the rat, message detected throughout the CNS and in many peripheral tissues. This pattern of expression is similar to that observed in man and sheep, which had previously been perceived to possess distinct patterns of A_3 receptor gene expression in comparison to the rat.

4   In summary, this work has comprehensively studied the expression of all the cloned adenosine receptors in the rat, and in so doing, resolves some of the uncertainty over where these receptors might act to control physiological processes mediated by adenosine.

Keywords: Adenosine; adenosine receptor; RT-PCR; tissue distribution

Introduction

Currently four different adenosine receptors are recognised, designated A_1, A_{2A}, A_{2B} and A_3. This classification is based on both their protein sequences and affinity for a variety of ligands (Fredholm *et al.*, 1994). These receptors play an integral role in modulating the metabolic activity and function of many tissue and cell types. Despite the pivotal role that adenosine receptors play in a wide range of cellular functions, the anatomical distribution of the cloned receptors' expression remains poorly defined.

Adenosine A_1 receptor distribution is perhaps the most extensively characterised. In the CNS, the presence of A_1 receptor protein has been demonstrated by ligand binding (Alexander & Reddington, 1989; Weber *et al.*, 1990) and immunohistochemistry (Rivkees *et al.*, 1995), while the mRNA has been detected by *in situ* hybridisation (Reppert *et al.*, 1991). However, less is known of the distribution of the other receptors.

In the rat, A_{2A} receptors are believed to be concentrated in the striatum, nucleus accumbens, olfactory tubercle and lateral segment of the globus pallidus (Jarvis & Williams, 1989; Parkinson & Fredholm, 1990). In the striatum, the A_{2A} receptor is thought to be exclusively expressed in the GABAergic striatopallidal neurones (Schiffman *et al.*, 1991), although there is now evidence of a functionally active receptor in striatal cholinergic neurones (Kurokawa *et al.*, 1994). It has become apparent that the A_{2A} receptor may also be present in other brain areas including the hippocampus (Sebastiao & Ribeiro, 1992), the nucleus tractus solitarius (Barraco *et al.*, 1991) and the cerebral cortex (Kirk & Richardson, 1995). It would therefore appear as though the adenosine A_{2A} receptor may be more widely expressed in the CNS than was originally thought.

Northern blot analysis of rat tissue mRNA demonstrated the greatest expression of the A_{2B} receptor in caecum, large intestine, and urinary bladder, with lesser amounts in brain, spinal cord and lung (Stehle *et al.*, 1992). Central expression was investigated in the same study by *in situ* hybridisation; however, a strong signal was only detected in the hypophyseal pars tuberalis. Yet the ability of the A_{2B} receptor to stimulate adenylyl cyclase in brain slices has led to the suggestion that the A_{2B} receptor is in fact present throughout the brain (Daly *et al.*, 1983).

The distribution of the A_3 receptor in the three species from which it has been cloned would appear to vary. In the rat, A_3 receptor mRNA was only detected in the testis (Meyerhof *et al.*, 1991; Zhou *et al.*, 1992) by *in situ* hybridisation, although by reverse transcription-polymerase chain reaction (RT-PCR), A_3 expression was also found in the heart, the lung and a limited number of areas in the CNS (Zhou *et al.*, 1992). In contrast, significant expression of the human and sheep A_3 receptor mRNAs have been observed in many peripheral tissues, with lower levels in the central nervous system and testis (Linden *et al.*, 1993; Salvatore *et al.*, 1993). It is therefore generally accepted that this receptor shows a species-specific tissue distribution (Linden *et al.*, 1993).

Current data on the distribution of the adenosine receptors can therefore be seen to be both incomplete and confused. In order to consolidate this situation and extend current understanding of the distribution of these important receptors, adenosine receptor gene expression has been examined in the rat by *in situ* hybridisation and the more sensitive technique of RT-PCR, thereby identifying tissues and cells in which these important receptors may be functionally active.

Methods

Collection of tissues

Male and female Sprague-Dawley rats (8–12 weeks) (Tucks, Rayleigh, Essex), maintained in a 12 h light-dark cycle with free access to laboratory rodent chow were killed by cervical dislocation. Tissue was rapidly dissected out, snap frozen in isopentane cooled by solid CO_2 and stored at $-70°C$ for subsequent sectioning and total RNA extraction.

In situ *hybridisation*

Cryostat sections (10 μm) were thaw-mounted onto poly-L-lysine-coated slides, fixed in phosphate buffered 4% paraformaldehyde and stored in 95% ethanol at 4°C. The basic method used for *in situ* hybridisation has been described previously (Sirinathsinghji & Dunnett, 1993). Briefly, sense and antisense oligodeoxyribonucleotide probes complementary to the rat adenosine receptors were synthesised on a Biosearch 8700 DNA synthesiser and purified on 8% polyacrylamide/8 M urea preparative sequencing gels. The sequence of the antisense probes (Table 1) were unique to the individual rat adenosine receptors. Probes were labelled with α-[^{35}S]-dATP (> 1000 Ci mmol^{-1}, Amersham International plc, Amersham, Bucks.) by terminal deoxynucleotidyl transferase (Pharmacia, Milton Keynes, Bucks.) at 32°C for 1 h. The labelled probe was applied to sections in hybridisation buffer containing 4 × standard sodium citrate (SSC), 50% deionised formamide. The slides were incubated overnight at 42°C before being washed in 1 × SSC containing 0.1% sodium thiosulphate for 1 h at 55°C and dehydrated through alcohol. Slides were exposed to X-ray film (Hyperfilm-βmax, Amersham) for 7 days, dipped in Ilford K5 emulsion (Amersham), and stored desiccated at 4°C for 2 weeks. After development, sections were

stained with haematoxylin and eosin and mounted with a coverslip.

Northern blots

Total RNA was isolated from tissue samples by acid guanidinium thiocyanate-phenol-chloroform extraction (Chirgwin *et al.*, 1979). Poly(A$^+$) RNA was selected by oligo(dT) cellulose chromatography, denatured in glyoxal, and separated by electrophoresis on a 1.2% formaldehyde agarose gel prior to transfer onto Hybond N membrane (Amersham). RNA was fixed to the membrane by u.v. irradiation and molecular mass markers were made visible by ethidium bromide staining. The antisense probes used for *in situ* hybridisation were 3'-end labelled with [^{32}P]-dATP (6000 Ci mmol^{-1}; NEN) and terminal deoxynucleotidyl transferase (Pharmacia). After prehybridisation at 42°C for 4 h, the blots were hybridised overnight at 42°C with the ^{32}P-labelled probe in hybridisation buffer under conditions similar to those described for *in situ* hybridisation, except that dextran sulphate was omitted from the hybridisation buffer. After washing in 1 × SSC/0.1% sodium dodecyl sulphate at room temperature for 30 min and then at 55°C for 30 min, the blot was wrapped in Saran wrap and exposed to Kodak XAR X-ray film at $-70°C$ in X-ray cassettes containing intensifying screens.

Reverse transcription PCR (RT-PCR)

PCR primers were synthesised from the adenosine receptor cDNA sequences (Table 1). Total RNA (10 μg) was reverse transcribed in the presence of an anchored oligo dT-primer $T_{17}(A,G,C)$, by use of M-MLV reverse transcriptase (Gibco BRL, Paisley, Renfrewshire) to manufacturer's recommendations. Single stranded cDNA products were denatured and subjected to PCR amplification (30 cycles). Each PCR cycle consisted of denaturing at 94°C for 45 s, annealing at 64°C for 45 s, and extension at 72°C for 60 s. The reaction mixture contained; 0.5 mM dNTPs, 1.2 u AmpliTaq DNA polymerase (Applied Biosystems, Warrington, Cheshire), 0.5 u Perfect match (Stratagene, Cambridge, Cambs.), 1 × PCR buffer (3.5 mM Mg^{2+}, pH 8.8). Amplification of β-actin and sucrase-isomaltase mRNA served as controls. The PCR products were separated on 2.5% agarose gels. To aid comparison of the distribution of the receptor mRNAs, all RT-PCR reactions were performed on the same RNA preparation.

Table 1 Adenosine receptor-specific antisense probes, PCR primers and PCR product sizes

Receptor	Accession number	Probe/ Primer	Nucleotides	Sequence	Product size	Restriction Digest	Fragment sizes
A$_1$	M64299	Probe	1050-1006	5'-GGC GAT CTT CAG CTC CTT CCC GTA GTA CTT CTG GGG GTC ACC GGA-3'			
		Forward	630-649	5' CTC CAT TCT GGC TCT GCT CG 3'	207	HaeIII	101, 37, 69
		Reverse	836-818	5' ACA CTG CCG TTG GCT CTC C 3'			
A$_{2A}$	L08102	Probe	632-588	5'-CGC CAT CCG AAT TCC ACT CCG GTA CAA TGG CTT GGT GAC AGG TGT-3'			
		Forward	407-423	5' CCA TGC TGG GCT GGA ACA 3'	150	HaeIII	87, 63
		Reverse	556-536	5' GAA GGG GCA GTA ACA CGA ACG 3'			
A$_{2B}$	M91466	Probe	619-575	5'-CAC TTC ACA GGG CAG CAG CTC TTA TTC GTG ATG CCA TCC CCA GGT-3'			
		Forward	139-156	5' TGG CGC TGG AGC TGG TTA 3'	160	XhoI	71, 89
		Reverse	298-281	5' GCA AAG GGG ATG GCG AAG 3'			
A$_3$	M94152	Probe	366-321	5'-GTA GGT GAT TTG CAA CCA CAA GGC ACT CGT CGT GGT ATT GTT GGC-3'			
		Forward	238-256	5' AGA AGC TAG GTC CAC TGG C 3'	665	AluI	52, 6, 135, 222, 132, 14,
		Reverse	902-880	5' GCA CAT GAC AAC CAG GGG GAT GA 3'			100

The fragment sizes given are those predicted from the receptor sequence upon complete digestion with the indicated restriction enzyme.

Restriction digest

Following RT-PCR, 15 μg of amplified adenosine receptor cDNA was digested to completion at 37°C for 1 h with the specific endonucleases shown in Table 1 (Biolabs, New England, U.S.A.). The digestion products were then separated on 2.5% agarose gels, and the fragment sizes predicted from the receptor sequences are shown in Table 1.
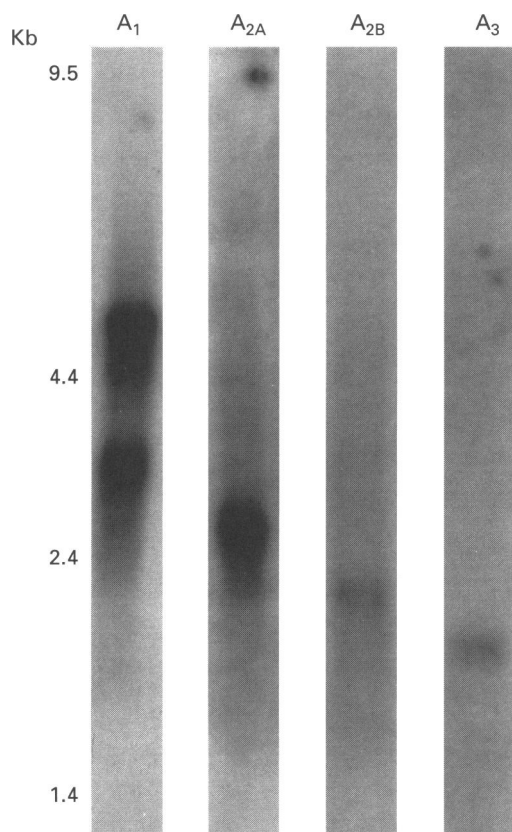

Figure 1 Autoradiograph of Northern blots by use of adenosine receptor specific anti-sense probes. Each ^{32}P-labelled probe was hybridised to 10 μg of poly(A$^+$) RNA extracted from whole rat brain. An RNA ladder was used to assess the sizes of mRNAs species detected.

Results

In situ hybridisation histochemistry was used initially to investigate the expression of adenosine receptor mRNAs in rat tissues. The specificity of the antisense probes was checked by Northern blot analysis. Each antisense probe recognised RNA transcripts of the appropriate size: 3.5 kb and 5.5 kb for the A_1 receptor (Reppert *et al.*, 1991), 2.6 kb for the A_{2A} receptor (Fink *et al.*, 1992), 2.2 kb for the A_{2B} receptor (Stehle *et al.*, 1992) and 1.8 kb for the A_3 receptor (Meyerhof *et al.*, 1991; Figure 1). In addition, when complementary sense probes were used under identical conditions to those used for antisense probes no specific hybridisation was observed (Figure 2).

In situ hybridisation with the A_1 receptor probe revealed a similar distribution pattern to that previously observed (Mahan *et al.*, 1991; Reppert *et al.*, 1991), with the highest level of A_1 mRNA detected in the cortex, hippocampus and cerebellum (Figure 2). Examination of emulsion dipped slides revealed that, with the exception of the caudate putamen, the cellular localisation of A_1 receptor mRNA was also in accordance with previous findings (Reppert *et al.*, 1991). In the caudate putamen the A_1 receptor gene was found to be expressed exclusively in the large neurones (approximately 30 μm in diameter) (Figure 3). *In situ* hybridisation of the A_{2A} receptor probe confirmed the strong expression of the A_{2A} receptor gene in the striatum, olfactory tubercle and nucleus accumbens (Jarvis & Williams, 1989; Parkinson & Fredholm, 1990), with apparently no expression in other areas of the brain (Figure 2). Emulsion dipped slides showed that the mRNA was concentrated in the medium sized neurones (approximately 10 μm in diameter) of the caudate putamen (Figure 3). Examination of the large striatal neurones revealed specific hybridisation in 13/50 neurones examined. The concentration of silver grains over these cells appeared less than that over the medium sized cells, but considerably greater than background (Figure 3). The A_{2B} receptor probe revealed no specific hybridisation pattern in the rat brain, but there did appear to be weak uniform binding of probe at levels slightly above background (Figure 2). By use of *in situ* hybridisation, significant expression of the A_3 receptor mRNA was only detected in the testis, where the probe hybridised specifically to the spermatocytes and the spermatids (data not shown). No specific hybridisation was detectable in the CNS (Figure 2), or any other peripheral tissues (data not shown).

The PCR primers used are listed in Table 1. The specificity of each PCR amplification was confirmed by restriction digest (Figure 4). When assessing the presence or absence of receptor mRNA transcripts in tissue samples the amplification of β-
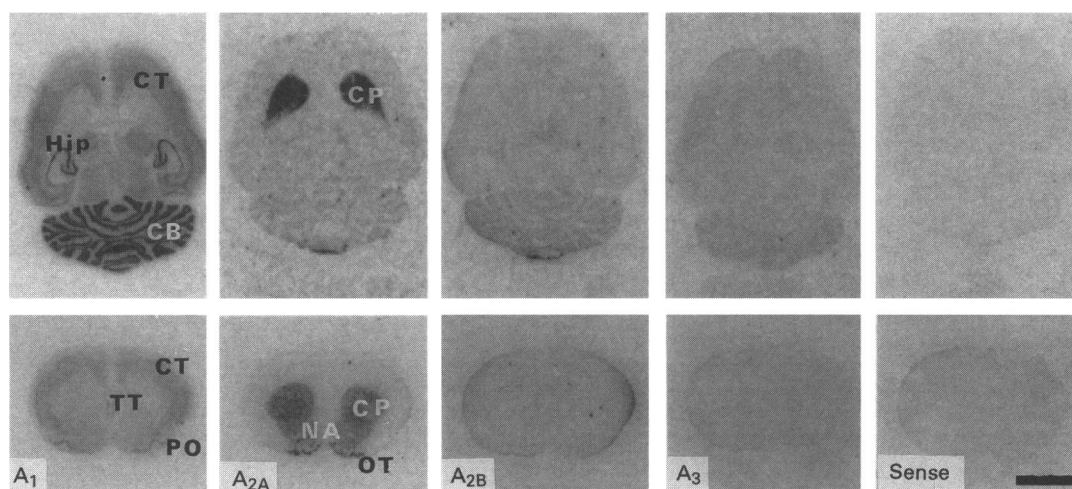

Figure 2 *In situ* hybridisation of adenosine receptor specific oligonucleotide probes. X-ray autoradiographs of horizontal (top) and vertical (bottom) sections of rat brain after hybridisation of ^{35}S-labelled adenosine receptor anti-sense probes and a representative sense probe. Brain areas showing specific hybridisation are labelled: CT, cortex; Hip, hippocampus; CB, cerebellum; CP, caudate putamen; TT, taenia tecta; PO, primary olfactory cortex; NA, nucleus accumbens; OT, olfactory tubercle. Scale bar = 0.5 cm.

actin and sucrase-isomaltase mRNAs were used as controls (Dixon *et al.*, 1995); β-actin cDNA was used to confirm the integrity of RNA preparations, thus guarding against false negatives; amplification of the intestine-specific enzyme sucrase-isomaltase (Chandrasena *et al.*, 1992) was used in order to control for false positives due to over sensitive PCR conditions, or amplification of genomic DNA contamination.

RT-PCR revealed expression of the adenosine A_1 receptor gene in all the brain areas tested (Figure 5). In the periphery the A_1 receptor transcript was also observed in the heart, aorta, spleen, kidney, liver, testis, eye and bladder. In each case the amount of amplified product was lower than that observed in the CNS. Very low levels of amplification product were detected in samples from the lung, uterus and stomach, while none were observed in samples derived from the skin, skeletal muscle, jejunum and proximal colon.

RT-PCR revealed a much wider distribution of the adenosine A_{2A} receptor transcript than previously described, with an amplification product being observed in all areas of the CNS (Figure 5). High levels of amplification product were observed in the striatum, nucleus accumbens, and thalamus with lower levels in every other brain region tested. In the periphery A_{2A} receptor mRNA transcript was also detected in RNA samples derived from many different tissues. The highest degree of amplification product was seen in the eye and skeletal muscle, with lower amplification in the heart, lung, bladder, and uterus. Even lower levels were detected in the aorta, spleen, stomach, testis, skin, kidney and liver. No amplification product was observed in samples from the jejunum and proximal colon.

RT-PCR revealed the adenosine A_{2B} receptor gene to be uniformly expressed throughout the CNS. In the periphery the highest amount of amplification product was seen in samples from the proximal colon followed by the eye, lung, uterus and bladder, lower levels in the aorta, stomach, testis, and skeletal muscle. Lowest levels of product were detected in the jejunum, kidney, heart and skin with trace levels detectable in the spleen and liver (Figure 5).

RT-PCR revealed the presence of A_3 receptor mRNA in all regions of the brain studied. The specific amplification product was detected in samples from the striatum, olfactory bulb, nucleus accumbens, hippocampus, hypothalamus, thalamus, and cerebellum, with slightly lower levels in the cortex and amygdala (Figure 5). In peripheral tissues the strongest signal was observed in samples from spleen, lung, uterus and testis; with moderate levels of the amplification product from the liver and bladder, and very low levels in the heart, aorta, stomach, jejunum, proximal colon, kidney and eye; while no detectable PCR product was observed with skin and skeletal muscle RNA.

Discussion

This paper describes the tissue distribution of mRNAs encoding the 4 cloned adenosine receptors in the rat. *In situ* hybridisation was used initially to define the tissue and cellular localisation of adenosine receptor gene expression, but failed to detect mRNA in some locations thought to possess the functional protein. We therefore employed the more sensitive
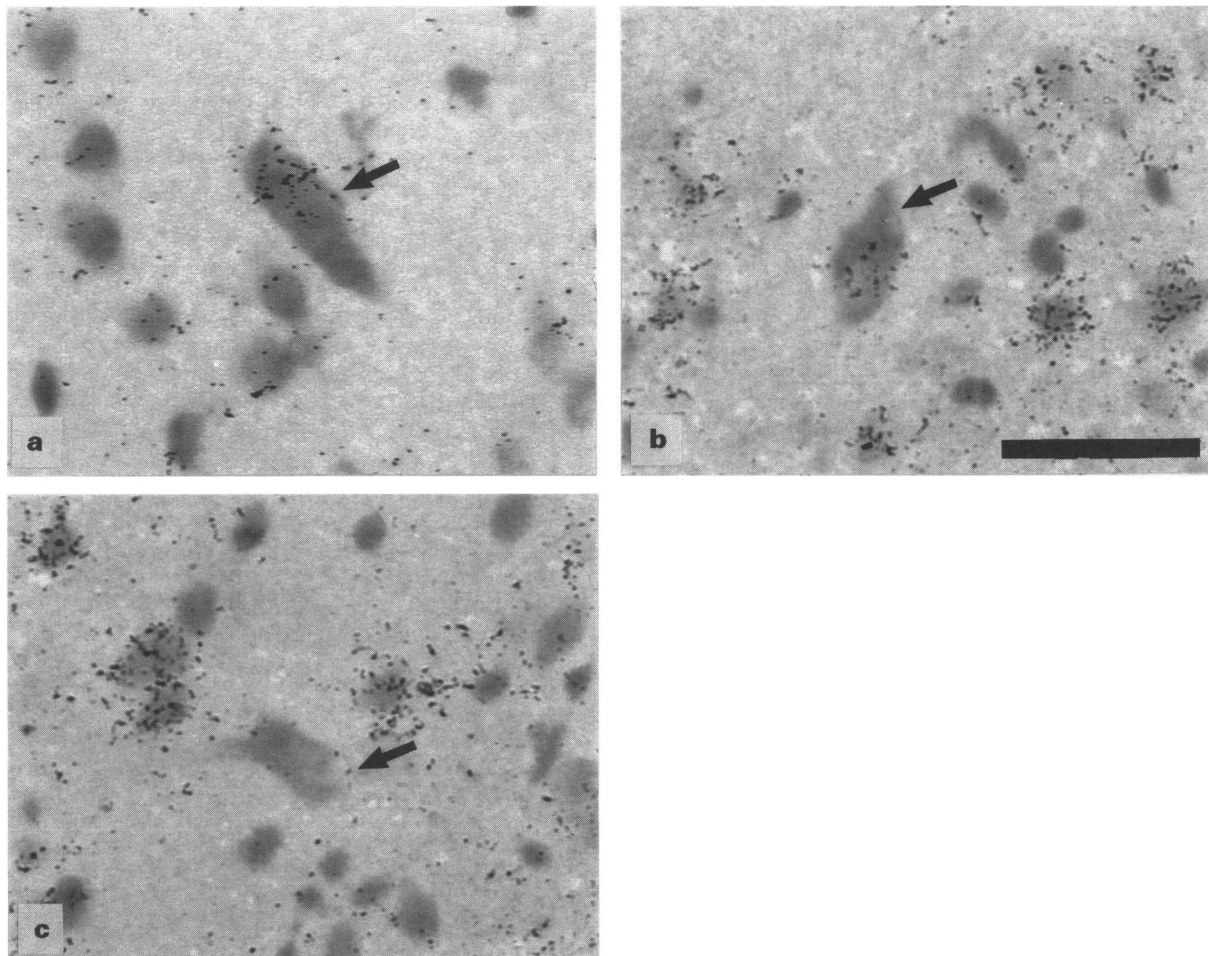

Figure 3 Bright-field emulsion autoradiographs illustrating adenosine A_1 and A_{2A} receptor gene expression in the rat striatum. (a) Silver grains demonstrate A_1 receptor mRNA associated with large sized (indicated by arrow), but not medium sized cells of the striatum; (b) A_{2A} receptor probe hybridisation to both large sized and medium sized cells of the striatum; (c) A_{2A} receptor probe hybridisation to medium sized, but not large cells of the striatum. Scale bar = 50 μm.

technique of RT-PCR to investigate the expression of this important family of receptors.

The distribution of the A₁ receptor has been examined previously by *in situ* hybridisation (Reppert *et al.*, 1991), receptor autoradiography (Goodman & Snyder, 1982; Alexander & Reddington, 1989; Weber *et al.*, 1990) and, more recently, by immunohistochemistry (Rivkees *et al.*, 1995). Results of the current *in situ* hybridisation study were very similar to those previously obtained, confirming the accepted central pattern of expression for the adenosine A₁ receptor gene. In the caudate putamen previous *in situ* hybridisation studies failed to detect A₁ receptor mRNA (Reppert *et al.*, 1991), although the A₁ receptor protein has been detected by immunohistochemistry

in 38% of neurones in this brain region (Rivkees *et al.*, 1995). Interestingly, we detected specific labelling to the large (30– 50 μm) neurones in the caudate putamen (Figure 3), and since these large neurones correspond to the cholinergic interneurones (Bolam *et al.*, 1984), this is consistent with the ability of the A₁ receptor to inhibit striatal acetylcholine release (Richardson *et al.*, 1987).

The results of the RT-PCR presented here show A₁ receptor gene expression throughout the CNS and also in a wide variety of peripheral tissues. In addition to those tissues found to express the A₁ receptor gene by Northern blot analysis (brain, spinal cord, epididymis plus vas deferens, testis white adipose tissue, stomach, spleen, pituitary and adrenal, Reppert *et al.*, 1991), we detected A₁ receptor mRNA in the heart, aorta, kidney, liver, eye and bladder. Functional effects of the A₁ receptor in the periphery have been observed in the heart (Mullane & Williams, 1990), the spleen (Fozard & Milavec-Krizman, 1993), the liver (Ohigashi *et al.*, 1993), the eye (Blazynski, 1990), the aorta (Conti *et al.*, 1993), the stomach (Christofi *et al.*, 1992), the colon (Jarvis *et al.*, 1994) and the bladder (Foster & Ferguson, personal communication). A₁ receptor mRNA has also been previously found in the testis (Rivkees, 1994), spleen and liver (Stehle *et al.*, 1992). Very low levels of A₁ receptor amplification were also observed in the lung which is consistent with the low functional activity of this receptor in the non-allergic lung (Ali *et al.*, 1994). The RT-PCR also suggested the presence of low levels of this receptor transcript in the kidney, where the A₁ receptor controls blood flow and renin secretion (Churchill & Bidani, 1990). A similar low level (or absence) of A₁ receptor mRNA in the kidney and small intestine was shown by Reppert *et al.*, (1991) and Stehle *et al.* (1992). It is interesting that we failed to detect A₁ receptor mRNA in the jejunum even though an A₁-like receptor has been shown to be active in this tissue (Hancock & Coupar, 1995). Thus, the tissue distribution of the A₁ mRNA transcript has been extended by RT-PCR primarily to those tissues in which a functional A₁ receptor has been reported.

Previous *in situ* hybridisation studies have demonstrated that the A₂ₐ receptor mRNA is concentrated in the striato-pallidal GABAergic projection neurones of the striatum (Schiffman *et al.*, 1991; Fink *et al.*, 1992), consistent with the overall pattern of hybridisation observed in this study (Figure 3b,c). However, the earlier studies failed to detect A₂ₐ expression in the large striatal cholinergic interneurones and therefore it is of particular interest that 13 out of 50 of these cells examined here, contained A₂ₐ mRNA (Figure 3b). This is concordant with the control of striatal acetylcholine release by the A₂ₐ receptor (Brown *et al.*, 1990; Kirk & Richardson, 1993; 1994; Jin *et al.*, 1993; Kurokawa *et al.*, 1994). Our *in situ* hybridisation studies failed to detect A₂ₐ receptor mRNA in any brain areas other than the striatum, nucleus accumbens and olfactory tubercle (Figure 2) despite functional evidence for the presence of this receptor in the hippocampus (Cunha *et al.*, 1994a,b), the cortex (O'Regan *et al.*, 1992; Simpson *et al.*, 1992; Kirk & Richardson, 1995) and the nucleus tractus solitarius (Barraco *et al.*, 1991). Therefore, the much more sensitive technique of RT-PCR was employed to investigate the distribution of A₂ₐ receptor gene expression in the rat CNS. As expected the highest levels of amplification product were observed in samples from the striatum and nucleus accumbens but in addition lower levels of amplification product were observed in all other brain regions examined (Figure 5). The low levels of amplification product detected in samples from the cortex are consistent with previous ligand binding results which suggested that the abundance of this receptor in the cortex was less than 20% of that in the striatum (Kirk & Richardson, 1995). The widespread expression of the A₂ₐ receptor transcript is similar to that seen in the developing rat brain (Weaver, 1993). These data suggest that this receptor should no longer be viewed as having a restricted distribution in the CNS and that the observed extra-striatal effects of A₂ₐ receptor ligands could be due to their action on the cloned receptor.
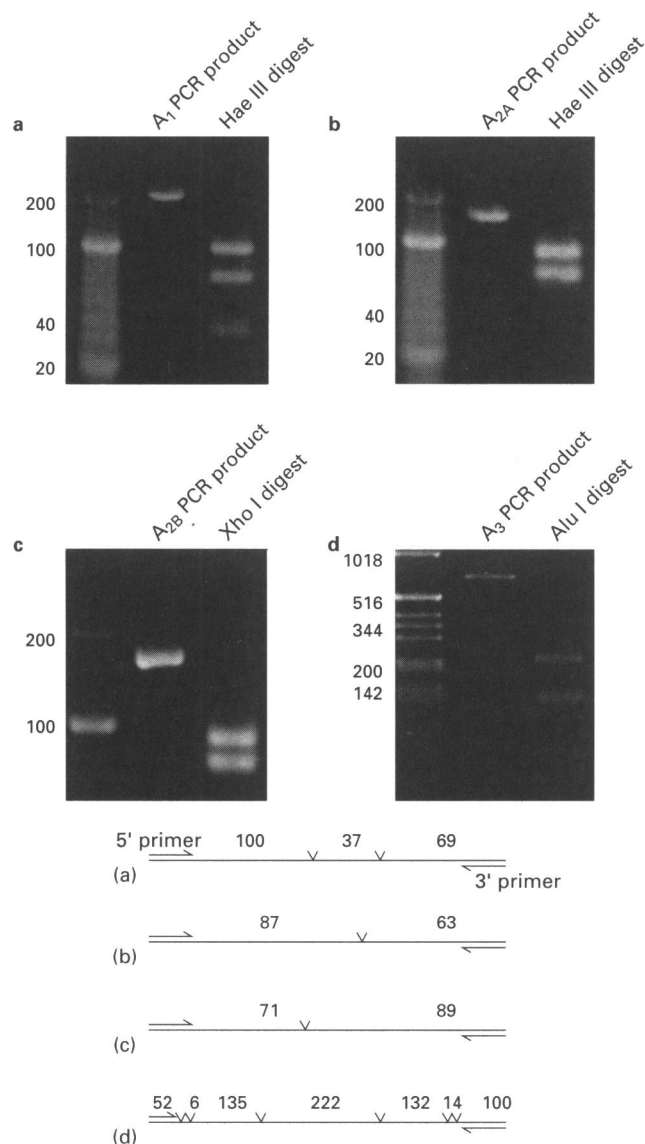

Figure 4 Restriction endonuclease digest of adenosine receptor RT-PCR products. Adenosine receptor specific RT-PCR was performed on rat whole brain total RNA as described in the Methods section. cDNA products (15 μg) were digested with the restriction enzyme indicated, separated on 2.5% agarose gels and stained with ethidium bromide. Sizes of PCR and digest products are given in base pairs. (a) A₁ receptor product digested with Hae III; (b) A₂ₐ receptor product digested with Hae III; (c) A₂ᵦ receptor product digested with Xho I; (d) A₃ receptor product digested with Alu I. Expected sizes of digest fragments are indicated below in schematic diagrams and Table 1. ᵛ Represents a restriction site on the amplified sequence.
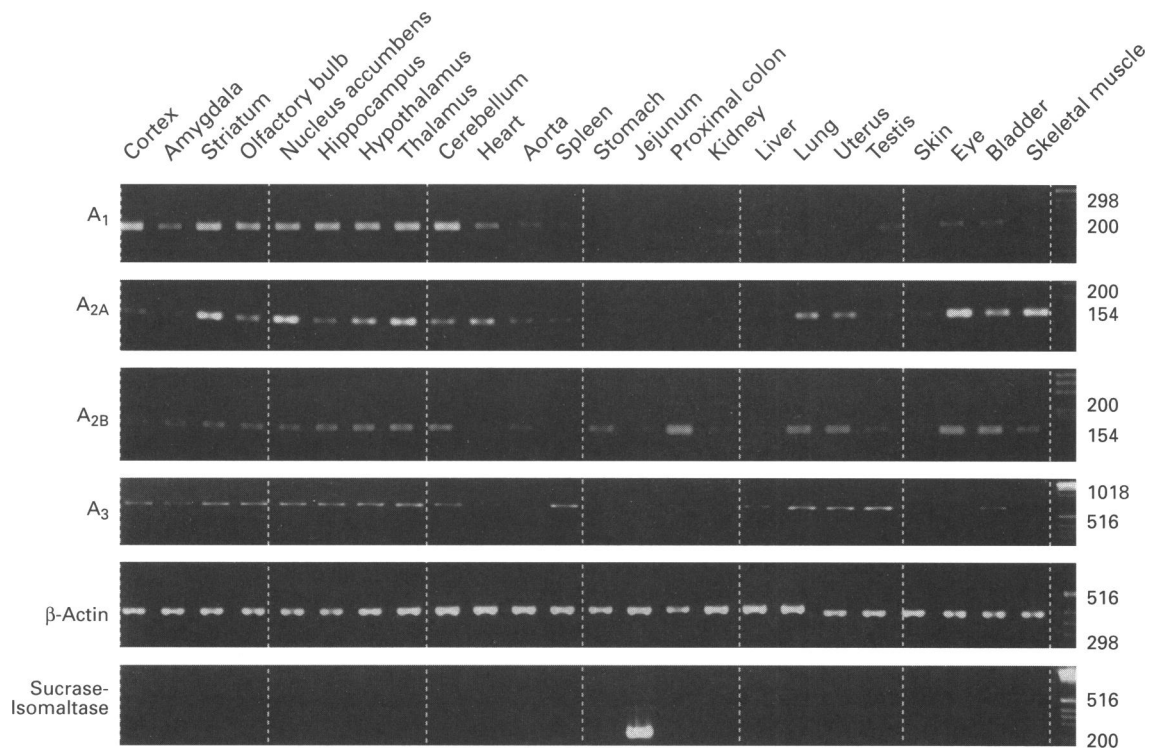
Figure 5 Tissue distribution of adenosine receptor mRNA expression as examined by RT-PCR. RT-PCR was performed using 10 μg of total RNA extracted from a wide range of rat tissues as described using the 5' and 3' primers shown in Table 1. RT-PCR of β-actin and sucrase-isomaltase mRNAs serve as controls. PCR products were separated on a 2.5% agarose gel and visualised with ethidium bromide. Sizes of PCR products are given in base pairs.

In the periphery, A_{2A} receptor function has been detected in the heart (Martin et al., 1993), retina (M'Intosh & Blazynski, 1994), aorta (Conti et al., 1993), and a variety of blood cells (Cronstein & Hirschhorn, 1990). The data presented here extend the distribution of this receptor to include the testis, where previous in situ hybridisation studies had failed to detect this transcript (Rivkees, 1994), spleen, lung, uterus, skin, bladder and skeletal muscle. This is in addition to the thymus, vas deferens, epididymal fat pad, oesophagus and liver (Stehle et al., 1992) and supports the presence of the A_{2A} receptor in many peripheral tissues.

The A_{2B} receptor cloned by Stehle et al. (1992) has not been as well studied as the other adenosine receptors, although it has been shown (by Northern blot analysis) to be present in the caecum, large intestine and bladder; with lower levels in the brain, spinal cord, lung, vas deferens and pituitary (Stehle et al., 1992). In situ hybridisation studies failed to detect a specific pattern of A_{2B} receptor mRNA distribution in the brain; however, hybridisation levels did appear to be slightly above background (Figure 2). RT-PCR studies suggest a more widespread distribution of the A_{2B} receptor gene expression than found previously, some degree of amplification being observed in every tissue studied, highest in the proximal colon and lowest in the liver (Figure 5). The transcript was also detected in every brain area examined, supporting the suggestion that this receptor is present throughout the brain (Daly et al., 1983). Such a ubiquitous yet low level of expression throughout the CNS would explain our inability, and that of Stehle et al. (1992), to detect a pattern of expression by in situ hybridisation in the CNS. It is possible that adenosine A_{2A} and A_{2B} receptor expression in vascular endothelial and smooth muscle cells may have contributed to the expression pattern of these genes. However, it is evident from the variable amount of amplification product from each tissue sample, negative and trace through to strongly positive, that the contribution of signal obtained from these sources to the overall pattern of expression is relatively small in comparison to amplification of A_2 mRNAs from other cell types in those tissues.

The rat A_3 receptor was originally detected in the testis with low levels in the brain (Zhou et al., 1992). Some recent functional studies have suggested that the A_3 receptor may also be involved in the regulation of cerebral blood flow (von Lubitz et al., 1994), the induction of hypotension (Fozard & Carruthers, 1993), mast cell activation (Ramkumar et al., 1993), contraction of the spleen (Linden, 1994) and behavioural depression in mice (Jacobson et al., 1993). However, in our initial studies we were only able to detect A_3 receptor mRNA by in situ hybridisation in the testis (data not shown), perhaps because it is concentrated in a small subset of cells (i.e. the spermatocytes and spermatids) in this tissue. In contrast, RT-PCR revealed the expression of the A_3 receptor gene in every brain region studied (Figure 5). In the periphery, moderate amounts of amplification product were observed from the spleen, liver, lung, uterus, testis, bladder, with lower levels from the heart, jejunum, proximal colon, kidney and eye. A_3 receptor mRNA was not detected in skin or skeletal muscle. This extends considerably the distribution found by Zhou et al. (1992). The strong expression in the spleen is consistent with the suggestion that A_3 receptors may be involved in contraction in this tissue (Linden, 1994), while that in the lung is of interest since the A_3 receptor may be involved in mast cell activation (Ramkumar et al., 1993) and may be a suitable target for anti-asthmatic drugs (Linden, 1994). The widespread expression of the adenosine A_3 receptor in rat tissues, is similar to that observed for this receptor in human and sheep tissues (Linden, 1994). This suggests that the previously observed species-specific pattern of A_3 receptor gene expression may be due to quantitative differences in the abundance of this transcript between species and tissues, rather than the perceived qualitative variation.

The results presented herein consolidate and expand the current understanding of adenosine receptor distribution in the rat. A_1 receptor gene expression was observed, in addition to previously identified tissues, in the heart, aorta, liver, eye and bladder. A_{2A} receptor mRNA was detected in all brain regions tested, demonstrating that expression of this receptor is not restricted to the basal ganglia. In the periphery, A_{2A} receptor

mRNA was also found to be more widely distributed than previously recognised. The ubiquitous expression of the A_{2B} receptor was shown for the first time, with A_{2B} receptor mRNA detected in all rat tissues studied. A_3 receptor mRNA was detected throughout the CNS and in many peripheral tissues. This pattern of expression is similar to that observed in the human and sheep, suggesting that the adenosine A_3 receptor ought no longer to be considered to have a species-specific distribution. These observations should be taken into consideration in future studies into the pharmacology and physiology of this important family of receptors.

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