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Expression of α_1-Adrenergic Receptor Subtype mRNA in Rat Tissues and Human SK-N-MC Neuronal Cells: Implications for α_1-Adrenergic Receptor Subtype Classification

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SUMMARY

At least three subtypes of α_1-adrenergic receptors (α_1ARs) have been identified using molecular techniques ($\alpha_{1a/d}$, α_{1b}, and α_{1c}), whereas two subtypes of α_1ARs have been identified pharmacologically (α_{1A} and α_{1B}); however, controversies exist regarding how these two classification schemes relate to each other. In an attempt to clarify some of the controversies regarding classification of α_1AR subtypes, we have re-evaluated the distribution of mRNA for the cloned α_1AR subtypes ($\alpha_{1a/d}$, α_{1b}, and α_{1c}) in various rat tissues thought to express α_1AR subtypes, as well as the human neuronal cell line SK-N-MC (a recently described model for pharmacologically defined α_{1A}AR and α_{1B}AR subtypes), using sensitive ribonuclease protection assay techniques. Total RNA extracted from various rat tissues and SK-N-MC cells was hybridized with rat and human α_1AR subtype-specific probes, respectively. In contrast to previously reported Northern blot analyses, α_{1c}AR mRNA is expressed in many rat tissues. Expression of α_{1c}AR mRNA is highest in those tissues that have been previously characterized by radioligand binding as expressing the classical, pharmacologically defined α_{1A}AR. Likewise, the human neuronal SK-N-MC cell line, classically thought to express pharmacological α_{1A}AR and α_{1B}AR subtypes, expresses both $\alpha_{1a/d}$AR and α_{1c}AR mRNA and no α_{1b}AR mRNA. Collectively, these data suggest that the cloned α_{1c}AR subtype may represent the pharmacological α_{1A}AR, and they have important implications for merging pharmacological and molecular classifications of α_1AR subtypes.

α_1ARs are G protein-coupled transmembrane receptors that mediate the physiological effects of norepinephrine and epinephrine. Initial pharmacological classification of α_1AR subtypes (α_{1A} and α_{1B})[1] demonstrated two distinct binding sites for α_1AR-selective agonists and antagonists, as well as differing sensitivity to inactivation by the alkylating agent CEC, in rat tissues (1, 2). The α_{1A} subtype is defined as having high affinity for the agonist oxymetazoline and the antagonists WB4101, phentolamine, 5-methylurapidil, and (+)-niguldipine, as well as insensitivity to inactivation by CEC; this subtype is present (as defined by ligand binding assays) in rat tissues as follows: hippocampus > vas deferens > kidney > cerebral cortex > heart (2–6). The α_{1B} subtype has low affinity for the same agonists and antagonists, is completely inactivated by CEC, and is present in the following rat tissues: spleen = liver > heart > cerebral cortex > kidney > vas deferens > hippocampus (2–6).

The pharmacological classification of α_1AR subtypes has been supported by recent molecular cloning studies in which three distinct cDNAs encoding α_1AR subtypes have been isolated. The first α_1AR subtype cDNA, cloned from hamster DDT$_1$MF-2 cells, was named the α_{1b}AR because, when expressed in various cell lines, it exhibits low affinity for WB4101, phentolamine, and oxymetazoline, as well as sensitivity to inactivation by CEC (7). Furthermore, the distribution in rat tissues of mRNA encoding this receptor, as assessed by Northern analysis, is consistent with that of the pharmacologically defined α_{1B}AR (8). A second α_1AR subtype cDNA, cloned from bovine cerebral cortex, demonstrates α_{1A}AR pharmacology (based on results with many subtype-selective agonists and antagonists) but is partially sensitive to inactivation by CEC; in addition, mRNA encoding this receptor subtype is not expressed (as determined by Northern analysis) in expected rat tissues (9). These results indicated that this receptor was a

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[1] In this manuscript we refer to pharmacologically defined α_1AR subtypes by upper case subscripts (e.g., α_{1B}) and to cloned α_1AR subtypes by lower case subscripts (e.g., α_{1b}), in analogy to current muscarinic nomenclature.

ABBREVIATIONS: α_1AR, α_1-adrenergic receptor; CEC, chloroethylclonidine; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; kb, kilobase(s).

newly described α_1AR subtype, and hence it was named the α_{1c}AR. A third α_1AR cDNA, cloned from both rat genomic DNA and cerebral cortex, was reported (8). This cDNA encodes a receptor that initially exhibited α_{1A}-like pharmacology (high affinity for initial α_{1A}-selective compounds such as WB4101 and phentolamine) and is resistant to inactivation by CEC, and expression of its mRNA in rat tissues is consistent with that of the pharmacologically described α_{1A}AR. Hence, this receptor was named the α_{1a}AR (8). However, designation of this receptor as the α_{1a} subtype has been controversial, because Perez *et al.* (10) cloned a virtually identical cDNA (differing in only two nucleotides) but designated it the α_{1d}AR based on lower than expected affinity for newer α_1AR-selective compounds such as 5-methylurapidil and (+)-niguldipine and partial sensitivity to inactivation by CEC. It has been suggested that, until this controversy is resolved, this receptor should be referred to as the $\alpha_{1a/d}$AR (11).

Despite detailed descriptions of the pharmacologically defined and cloned α_1AR subtypes, uncertainty still exists regarding the unification of the two classification systems. In an attempt to clarify this controversy, Esbenshade *et al.* (12) performed detailed pharmacological and molecular (Northern blot) analysis of α_1AR subtypes in human SK-N-MC neuronal cells, a potential model for coexisting α_1AR subtypes. That study concluded that SK-N-MC cells contain two subtypes of α_1ARs, which resemble the pharmacologically defined α_{1A}- and α_{1B}AR subtypes (12). To further correlate pharmacological and molecular biological α_1AR subtypes, we have explored the expression of α_1AR subtype mRNA in various rat tissues and human SK-N-MC cells using the more sensitive and specific RNase protection assay. This study demonstrates that SK-N-MC cells express mRNA for $\alpha_{1a/d}$ and α_{1c} but not α_{1b} subtypes; furthermore, a very different pattern of distribution for α_{1c}AR mRNA is identified, compared with that previously reported in rat tissues. Collectively, these findings suggest that the cloned α_{1c}AR may encode the pharmacological α_{1A}AR, and they have important ramifications for the classification of α_1AR subtypes as well as for the possible merging of pharmacological and molecular classification schemes.

Materials and Methods

Cell culture. As controls, rat-1 fibroblasts stably expressing cloned human $\alpha_{1a/d}$-, α_{1b}-, and α_{1c}ARs were grown as monolayers in Dulbecco's modified Eagle's medium (GIBCO) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin, in 5% CO_2 at 37°. Selection was maintained by adding the antibiotic G418 (400 μg/ml) to the medium. SK-N-MC cells were grown in Eagle's minimum essential medium with nonessential amino acids, sodium pyruvate, and Earle's balanced salt solution, supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin, in 5% CO_2 at 37°.

Rat tissues. Tissues were obtained from euthanized male Fisher 344 rats (300 g; Charles River Laboratories, Raleigh, NC), with institutional Animal Care Committee approval. Rat tissues (brain, heart, lung, liver, spleen, intestine, kidney, bladder, prostate, vas deferens, testis, epididymis, and penis) were rapidly harvested, immersed in liquid nitrogen, and stored at −70°. Rat cerebral cortex, hippocampus, corpus striatum, submaxillary gland, and submaxillary node/parotid glands were obtained from Zivic-Miller Laboratories (Zellenople, PA).

RNA isolation. Total RNA was extracted from cells expressing human α_1AR subtypes, SK-N-MC cells, and rat tissues by using the RNazol method (Teltest Inc., Friendswood, TX). Each RNA sample was quantitated spectrophotometrically at 260 and 280 nm and was stored at −70° as an ethanol precipitate for later use.

Human and rat α_1AR cDNA constructs and probe synthesis. The human $\alpha_{1a/d}$AR cDNA construct consists of a 0.375-kb (*Eco*RI/*Stu*I) fragment in pSP72 (Promega) corresponding to nucleotides 449–824 of the previously reported cloned human α_{1a}AR cDNA (13). The human α_{1b}AR construct consists of a 0.670-kb (*Bam*HI/*Xho*I) fragment in pGEM-4Z (Promega) and is identical to the recently cloned human α_{1b}AR cDNA (nucleotides 91–761) (14). The human α_{1c}AR construct consists of a 0.323-kb (*Hind*III/*Pvu*II) insert in pGEM-4Z (Promega) and is identical to the recently cloned human α_{1c}AR cDNA (nucleotides 1392–1715) (15). The control human β-actin cDNA construct, obtained from Dr. Joanne M. Pyper (Johns Hopkins University School of Medicine, Baltimore, MD), consists of a 0.104-kb (*Acc*I/*Hind*II) insert in pGEM-4Z (Promega) that is identical to the cloned human β-actin gene (nucleotides 1858–2064) (16). The rat $\alpha_{1a/d}$AR cDNA construct consists of a 0.276-kb (*Bgl*I/*Bam*HI) fragment in pGEM-4Z (Promega) corresponding to nucleotides 1104–1380 of the previously reported cloned rat α_{1a}AR cDNA (8). The rat α_{1b}AR construct consists of a 0.306-kb (*Bam*HI/*Pst*I) fragment in pGEM-3Z (Promega) and is identical to the reported rat α_{1b}AR cDNA sequence from nucleotide 469 to nucleotide 775.[2] Both $\alpha_{1a/d}$AR and α_{1b}AR cDNA constructs were obtained from Dr. James E. Faber (University of North Carolina School of Medicine, Chapel Hill, NC). The rat α_{1c}AR construct, obtained from Dr. Carina Tan (Merck, Rahway, NJ), consists of a 0.304-kb (*Spe*I/*Xba*I) insert in pGEM-9zf (Promega) that corresponds to nucleotides 556–860 of the bovine α_{1c}AR cDNA (9). The control rat GAPDH cDNA construct consists of a 0.172-kb (*Nco*I/*Sca*I) insert in pBKS (Stratagene) that corresponds to nucleotides 1–172 (17). Linearized cDNA constructs were used to synthesize high specific activity, radiolabeled, antisense RNA probes, using T3, T7, or SP6 RNA polymerase and [α-^{32}P]CTP (New England Nuclear-DuPont), as described previously (18).

RNase protection assay. Hybridization of RNA probes to total cellular rat and human RNA was performed as previously described by Zinn *et. al.* (19), with modifications; human α_1AR experiments were conducted as described previously (20), whereas experiments using probes generated from the rat α_1AR cDNA construct were performed as follows. In brief, 30 μg of total RNA were hybridized with 3×10^5 cpm of α_1AR riboprobe and 1.5×10^5 cpm of simultaneously loaded radiolabeled GAPDH riboprobe, for 16 hr at 48°. RNase A (5 μg/ml) and T1 (100 units/ml) were added to each sample and incubated at 37° for 40 min. After digestion, RNA samples were precipitated with ethanol, resuspended in gel loading buffer, heated to 95° for 5 min, and fractionated on a denaturing sequencing gel at 60 W for 2–3 hr. Gels were dried on Whatmann filter paper (Whatmann Inc., Maidstone, UK) and placed on a PhosphorImager screen for analysis.

Results

Total RNA isolated from a series of rat tissues was analyzed by RNase protection assays using antisense radiolabeled RNA probes specific for each α_1AR subtype mRNA. Experimental data shown in Fig. 1 demonstrate differential expression of mRNA for each of the α_1AR subtypes in various rat tissues. $\alpha_{1a/d}$AR mRNA is present most abundantly in vas deferens, hippocampus, and cerebral cortex; α_{1b}AR mRNA most abundantly in rat heart, liver, and cerebral cortex; and α_{1c}AR mRNA most abundantly in vas deferens and cerebral cortex. Simultaneously loaded GAPDH probe was used to control for RNA integrity (data not shown). Gels were quantitated by PhosphorImager analysis (Molecular Dynamics, Sunnyvale, CA). Transformation from PhosphorImager counts for protected fragments to an arbitrary scale of +1 to +5 for each rat tissue and α_1AR subtype is described in Table 1.

[2] J. W. Lomasney. Rat ADRA1B. GeneBank accession number M60655 (1992).

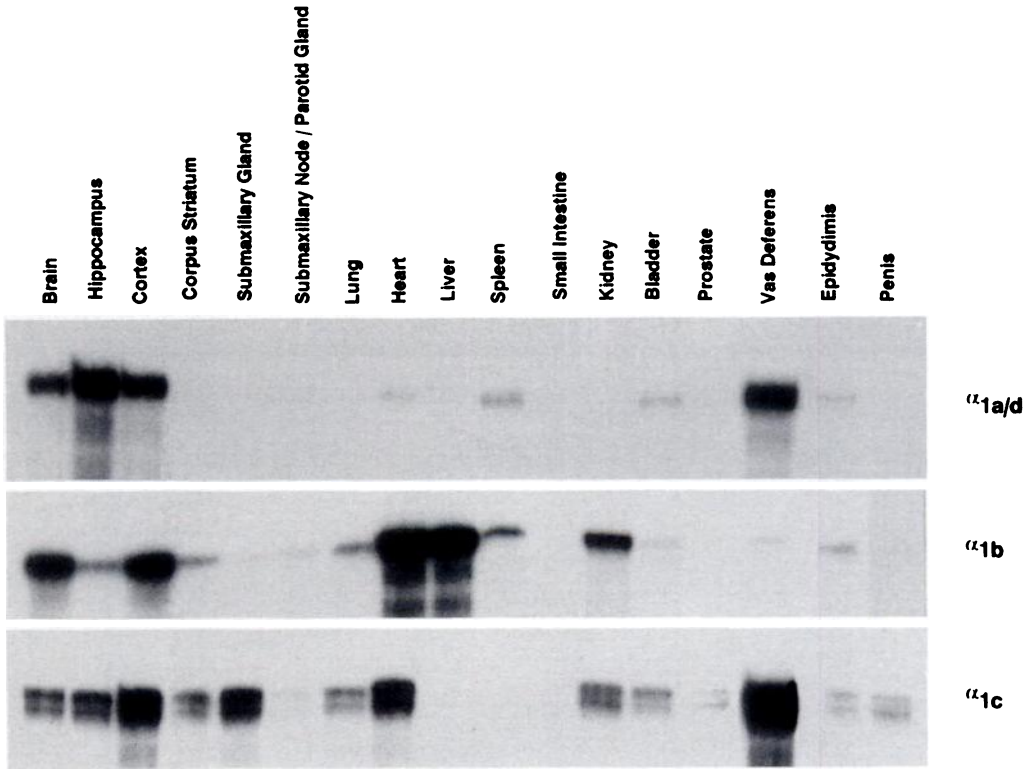

Fig. 1. RNase protection assay with selected rat tissues. This is a 36-hr autoradiograph, which demonstrates results from a representative RNase protection assay in which 30 μg of total RNA, extracted from several rat tissues, were hybridized with each of the radiolabeled antisense RNA probes ($\alpha_{1a/d}$, α_{1b}, and α_{1c}). tRNA served as a negative control (not shown).

TABLE 1

Relative quantitation and distribution of α_1AR mRNA transcripts in rat tissues

Data from two to four separate experiments are summarized. Transformation from Phosphorimager counts for protected fragments to a scale of + to +++++ was performed using the following equation: (total counts/10,000) × 1.6 = arbitrary units, from 1 to 100. +++++, defined as 81–100 arbitrary units; ++++, 61–80 units; +++, 41–60 units; ++, 21–40 units; and +, 2–20 units; +/−, the Phosphorimager detected a low signal (≤2 units) even when the autoradiograph was negative; −, lack of signal on both the Phosphorimager and the autoradiograph. Hence, the difference between +/− and +++++ is approximately 100-fold.

Rat tissue	Distribution		
	$\alpha_{1a/d}$	α_{1b}	α_{1c}
Whole brain	++	++	+
Hippocampus	++++	+	++
Cerebral cortex	+++	+++	+++
Corpus striatum	+/−	+	+
Submaxillary gland	−	+	++
Submaxillary node/parotid gland	−	+	+
Lung	+/−	+	+
Heart	+	++++	++
Liver	−	++++	−
Spleen	+	+	−
Intestine	−	−	+/−
Kidney	+/−	++	+
Bladder	+	+	+
Prostate	−	−	+
Vas deferens	++++	+	+++++
Epididymis	+	+	+
Penis	−	+	+

Total RNA extracted from human SK-N-MC cells and cells stably expressing human α_1AR subtypes was analyzed by the RNase protection assay using antisense radiolabeled human RNA probes specific for each α_1AR mRNA. Cells expressing α_1AR subtypes demonstrate specificity for human α_1AR subtype RNA probes (Fig. 2), with each antisense probe protecting a full length fragment ($\alpha_{1a/d}$, 375 nucleotides; α_{1b}, 670 nucleotides; α_{1c}, 323 nucleotides) without cross-hybridization. Fig. 2 also demonstrates the expression of only $\alpha_{1a/d}$ and α_{1c} mRNA in SK-N-MC cells. Simultaneously loaded β-actin probe was used to control for RNA integrity and equivalent concentrations of RNA in SK-N-MC samples (data not shown).

Discussion

Results from RNase protection assays performed in this study clearly demonstrate differential patterns of α_1AR gene expression in rat tissues. Whereas the distribution of $\alpha_{1a/d}$AR and α_{1b}AR subtype mRNAs generally agrees with previous Northern analyses, distribution of α_{1c}AR mRNA is significantly different from that reported previously. Initial descriptions of α_{1c}AR mRNA limited its distribution to only rabbit liver and selected regions of human brain (9, 21); however, several recent studies have identified mRNA for the α_{1c}AR subtype in many human tissues using RNase protection assays (20, 22) and in selected rat tissues using polymerase chain reaction techniques (23). The reason for the inability to localize mRNA for this receptor subtype in rat tissues by Northern analysis is unknown but may reflect the fact that Northern blots were performed using the bovine α_{1c}AR cDNA as a probe against rat tissue RNA; differences in α_{1c}AR nucleotide sequences may have played a role under the stringent conditions used, in spite of the fact that hybridization occurred under the same conditions in rabbit tissues. Similar negative results using the bovine

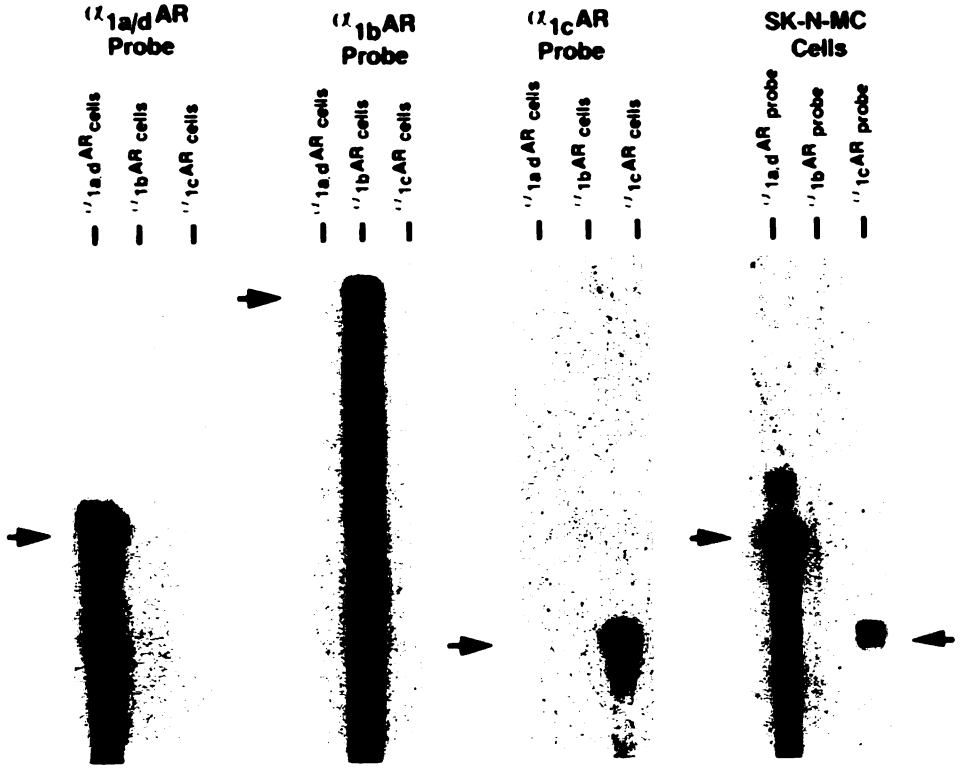

Fig. 2. RNase protection assay on stably transfected cell lines and human SK-N-MC cells. This is a 24-hr autoradiograph, which demonstrates specificity of human α_1AR riboprobes and expression of mRNA for the α_1AR subtypes in human SK-N-MC cells. Five micrograms of total RNA extracted from cells expressing each α_1AR subtype, or 30 μg of total RNA extracted from SK-N-MC cells, were hybridized with each of the radiolabeled antisense RNA probes ($\alpha_{1a/d}$, α_{1b}, and α_{1c}). *Arrows*, specific protected fragments. Bands appearing above protected fragments represent excess probe from the assay (slightly larger due to plasmid polylinker sequence present in the probe but not in the protected fragment).

α_{1c}AR cDNA probe in Northern blots have been reported by other laboratories (12). Nevertheless, RNase protection assay data presented in this manuscript clearly demonstrate the presence of all three cloned α_1AR subtype mRNAs in rat tissues, a fact that refutes the original Northern blot experiments.

Tissue distribution studies in whole animals can be complicated by many factors. Therefore, to further explore the relationship between pharmacologically defined and cloned α_1AR subtypes, we next examined α_1AR mRNA in the human SK-N-MC neuronal cell line previously characterized pharmacologically as expressing both α_{1A}AR and α_{1B}AR subtypes (12). Data from RNase protection assays performed on RNA extracted from SK-N-MC cell lines, presented in this manuscript, demonstrate mRNA expression of cloned α_{1c}AR and $\alpha_{1a/d}$AR and not α_{1b}AR subtypes; cross-hybridization between probes did not occur (Fig. 2). Although mRNA levels do not always correlate with protein expression, the absence of α_{1b}AR mRNA in SK-N-MC cells suggests that pharmacological characterization of this cell line may be inaccurate. These results differ from those presented by Ramarao *et al.* (14), where primer extension analysis and RNase protection assays were used to demonstrate the presence of α_{1b}AR mRNA in human brain and SK-N-MC cells. Because a primer of only 17 nucleotides was used for primer extension experiments, it is possible that cross-hybridization occurred in those studies. RNase protection assays performed by the same group, using a 67-base pair probe from the 5′ untranslated region of the human α_{1b}AR gene, resulted in multiple protected fragments.[14] Because the largest of those fragments was only 35–40 nucleotides in length, this also raises the possibility of cross-hybridization. Another explanation is that α_{1b}AR mRNA might be present in fairly low concentrations that could be detected using 100 μg of total RNA, as shown by Ramarao *et al.* (14), but not in our experiments using 30 μg of total RNA from SK-N-MC cells. Our results also differ from those presented by Esbenshade *et al.* (12) using Northern blot analysis. Close examination of the Northern blots presented in their manuscript (12) reveals a much larger transcript in SK-N-MC cells (5.0 kb), compared with α_{1b}AR transcripts in previous studies (2.5–3.0 kb) (14). This strongly suggests that cross-hybridization with other α_1AR subtypes may be occurring. However, in support of our findings, Esbenshade *et al.* (12) demonstrated a one-site fit for WB4101 binding in SK-N-MC cells, consistent with the presence of $\alpha_{1a/d}$AR and α_{1c}AR and inconsistent with original descriptions of $\alpha_{1a/d}$AR and α_{1b}AR subtypes. In the final analysis, RNase protection assays using species-specific probes of ≥100 nucleotides remain one of the most sensitive and specific methods of detecting the presence of mRNA in various tissues.

Considerable uncertainty regarding the relationship between pharmacologically defined α_1AR subtypes (α_{1A} and α_{1B}) and cloned α_1AR subtypes ($\alpha_{1a/d}$, α_{1b}, and α_{1c}) exists. Although it is generally agreed that the cloned α_{1b}AR encodes a protein with characteristics of the pharmacologically defined α_{1B}AR subtype, this is not the case for the cloned $\alpha_{1a/d}$AR and α_{1c}AR subtypes, because neither of these subtypes has all of the characteristics of the pharmacologically defined α_{1A}AR. For instance, mRNA for the cloned $\alpha_{1a/d}$AR has tissue distribution characteristic of the α_{1A}AR and has high affinity for some α_{1A}AR-selective compounds (WB4101 and phentolamine); however, the $\alpha_{1a/d}$AR has lower than expected affinity for several other α_{1A}AR-selective compounds [oxymetazoline, 5-methylurapidil, and (+)-niguldipine] (10, 11). In addition, although it was originally described as being resistant to CEC inactivation (8), others have recently reported that the $\alpha_{1a/d}$AR is sensitive to CEC inactivation (10) and we have recently confirmed this in our laboratory.[3] Hence, the cloned $\alpha_{1a/d}$AR does not appear to

encode a receptor with characteristics of the α_{1A}AR subtype.

In contrast, the cloned α_{1c}AR encodes a receptor protein that demonstrates high affinity for every α_{1A}AR subtype-selective compound available to date (9, 11). The only exception is (+)-niguldipine, which has higher absolute affinity in rat tissues than for any of the cloned α_1AR subtypes (4, 6). In spite of the match in pharmacology between the cloned α_{1c}AR and the pharmacologically described α_{1A}AR, this receptor was named the α_{1c}AR (and not the α_{1A}AR) because of its lack of mRNA expression in characteristic rat tissues and its partial sensitivity to CEC. In the present study, however, a more sensitive and specific technique for demonstrating mRNA expression reveals that α_{1c}AR mRNA is expressed in all rat tissues that have been pharmacologically characterized as expressing the classical α_{1A}AR subtype (vas deferens, heart, hippocampus, cerebral cortex, submaxillary salivary gland, and prostate) (2–6, 24). Therefore, based on the characteristic high binding affinity for α_{1A}AR-selective ligands and the tissue distribution pattern demonstrated at the mRNA level in this manuscript, the cloned α_{1c}AR may actually represent the pharmacologically defined α_{1A}AR.

Before redefining the α_{1c}AR as the pharmacological α_{1A}AR, three important points must be addressed, i.e., 1) the issue of partial sensitivity of the cloned α_{1c}AR to CEC, 2) discrepancies reported for absolute K_i values for (+)-niguldipine between the cloned bovine α_{1c}AR and the α_{1A}AR in rat tissues, and 3) the definition of the pharmacologically defined α_{1A}AR itself. Each of these points is discussed in order. Existing literature concerning the use of CEC is not consistent, in that it is well recognized that CEC sensitivity is dependent on time, temperature, CEC concentration, and protein concentration, as well as buffer ion concentration; it has also been suggested that the degree of alkylation caused by CEC may be dependent on local tissue environmental factors (6). In experiments conducted on rat tissues, conditions for CEC treatment of membranes have been defined as 10 μM CEC for 10 min in hypotonic buffer, at 37°; however, a review of the literature suggests that these conditions are not generally followed (8–10, 21, 25). Intact whole cells expressing the α_1AR subtypes are more resistant than membrane preparations to CEC inactivation (21). Additionally, in CEC experiments conducted in our laboratory on membranes collected from stably transfected cell lines, the cloned human α_{1c}AR is much more resistant to CEC inactivation than is either the human $\alpha_{1a/d}$AR or α_{1b}AR subtype.[4] Species differences in CEC sensitivity for the α_1AR have also been reported recently, with rat and human homologs being less sensitive to inactivation by CEC (18–19% inactivated) than is the bovine α_{1c}AR (45%) in whole cells (26). Although the existing literature with regard to CEC sensitivity is confusing, lesser CEC sensitivity of the rat α_{1c}AR, compared with other cloned subtypes, supports the proposition that the α_{1c}AR may represent the pharmacological α_{1A}AR subtype.

The rank order of potency for all α_{1A}AR-selective compounds suggests that the α_{1c}AR is the pharmacologically defined α_{1A}AR; however, absolute K_i values obtained with (+)-niguldipine are lower than would be expected for the pharmacological α_{1A}AR. This raises the possibility of an additional α_1AR subtype with even higher affinity for this compound than observed for the cloned α_{1c}AR. An alternative explanation would be a species homolog, where the rat α_{1c} subtype has higher affinity for (+)-niguldipine than do currently available cloned subtypes. In support of this position, a single amino acid substitution across species for the α_{2A}AR subtype significantly alters receptor affinity for an antagonist (27). In fact, higher affinity for (+)-niguldipine of the rat α_{1c}AR, compared with the bovine homolog, has recently been demonstrated (26), further supporting the premise that the α_{1c}AR may encode the pharmacological α_{1A}AR.

Finally, results of this study also bring into question the definition of the pharmacological α_{1A}AR subtype. α_1AR heterogeneity (α_{1A} and α_{1B}) was originally defined by Morrow and Creese (1), using [^{3}H]prazosin and [^{3}H]WB4101 as labeling ligands and WB4101 and phentolamine as competing ligands, in rat cortex and hippocampus membranes. Data from this manuscript clearly demonstrate the presence of mRNA for all three cloned subtypes in both rat cortex and hippocampus, bringing into question the original biphasic competition curves. With the introduction of CEC and other highly selective ligands, the pharmacological α_{1A}AR is currently defined as having high affinity for oxymetazoline, WB4101, phentolamine, 5-methylurapidil, and (+)-niguldipine, as well as insensitivity to inactivation by CEC. It is this current definition of the α_{1A}AR that we use when stating that the cloned α_{1c}AR may encode the pharmacological α_{1A}AR. In support of this premise, we present evidence that rat submaxillary gland, previously reported to contain only the α_{1A}AR (5), expresses predominantly mRNA for the cloned α_{1c}AR. Even more compelling are results with rat prostate, which has been characterized pharmacologically as expressing only α_{1A}ARs (24); in this study mRNA for the cloned α_{1c}AR is the only subtype present in rat prostate. Although mRNA expression may not equal protein expression, these data raise the possibility that α_1AR subtype-selective ligands developed to date may not be sensitive enough to determine the presence of more than two α_1AR subtypes in a given tissue and must be augmented by molecular techniques.

In summary, using RNase protection assays this study evaluated the expression of α_1AR mRNA in various rat tissues, including many tissues that have been traditionally thought to express only the pharmacologically defined α_{1A}AR subtype. We identified α_{1c}AR mRNA expression in every tissue and cell line where the classical, pharmacologically defined α_{1A}AR has been demonstrated. Collective analysis of these data, combined with the fact that the cloned α_{1c}AR has a characteristic rank order of potency for α_{1A}AR-selective ligands and the fact that the rat α_{1c}AR has higher affinity for (+)-niguldipine and more resistance to CEC, compared with the bovine α_{1c}AR homolog, suggests that the cloned α_{1c}AR may actually represent the pharmacologically defined α_{1A}AR as it is currently defined. More selective compounds are needed to corroborate receptor protein expression with mRNA expression presented in this manuscript. The possibility also remains that additional α_1AR subtypes may yet be identified. However, given the new data presented in this manuscript, existing pharmacological ligands, and sensitive molecular tools, many of the existing controversies in α_1AR pharmacology can be currently addressed and a merger of classification schemes initiated.

[3] D. A. Schwinn, unpublished observations.

[4] D. A. Schwinn, S. O. Page, K. Wilson, S. Campbell, G. I. Johnston, M. J. Mosley, N. P. Worman, M. D. Fidock, L. M. Furness, D. Parry-Smith, B. Peter, and D. S. Bailey. Cloning and pharmacologic characterization of human α_1-adrenergic receptors: sequence corrections and direct comparison with other species homologs. Submitted for publication.

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