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Localization of Messenger RNA for Three Distinct α_2-Adrenergic Receptor Subtypes in Human Tissues

Evidence for Species Heterogeneity and Implications for Human Pharmacology

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Background: α_2-Adrenergic receptor (α_2AR) agonists have become important adjuncts as anesthetic agents. They act by binding to α_2ARs on the surface of cell membranes and cause centrally mediated sedation and analgesia. α_2ARs also contribute to other aspects of physiologic regulation. Three subtypes of α_2ARs ($\alpha_{2\text{-C2}}$, $\alpha_{2\text{-C4}}$, and $\alpha_{2\text{-C10}}$) have been described using molecular and pharmacologic techniques. We recently demonstrated species heterogeneity in the distribution of α_1-adrenergic receptor subtypes, therefore making it imperative to analyze the distribution of α_2AR subtypes in human tissues. This information may have importance in the understanding of potential side effects of administration of α_2AR subtype-selective agonists for anesthesia in humans.

Methods: RNA extracted from human tissues was analyzed by using quantitative ribonuclease protection assays to determine α_2AR subtype messenger RNA (mRNA) expression in cardiovascular, central nervous system, and peripheral tissues.

Results: α_2AR mRNA is present in greatest concentrations in human kidney, followed by aorta > spleen > heart = lung. $\alpha_{2\text{-C4}}$ mRNA predominates in heart, lung, aorta, cerebral cortex, cerebellum, spleen, kidney, and adrenal gland; $\alpha_{2\text{-C2}}$ mRNA in liver; and $\alpha_{2\text{-C10}}$ mRNA in pancreas and small intestine. Hence α_2AR subtype mRNA distribution is tissue-selective and differs from that reported for rat.

Conclusions: (1) On comparison with previous research we find possible species heterogeneity in α_2AR subtype mRNA distribution (rat *vs.* human) for all three α_2AR subtypes. (2) We demonstrate the presence and subtype heterogeneity of α_2AR subtype mRNA in both brain and peripheral tissues. (3) Significant concentrations of α_2AR mRNA are present in adult human heart. These findings have important implications for our understanding of human adrenergic physiology, provide a possible explanation for the existence of pharmacologically similar yet distinct α_2AR subtypes, and may be important for the rational development of α_2AR subtype-selective anesthetics and other therapeutic agents for use in treating human diseases. **(Key words: Cardiovascular system: blood vessels. Heart: myocardium. Receptors: adrenergic; α-adrenergic. RNA: antisense; probes.)**

CATECHOLAMINES such as epinephrine and norepinephrine mediate their effects by binding to three different major families of adrenergic receptor (AR) subtypes.[1,2] ARs are encoded by different genes and are members of the larger family of guanine nucleotide–binding protein–coupled receptors.[3,4] α_2-Adrenergic receptor (α_2AR) activation leads to inhibition of adenylyl cyclase activity, downregulation of Ca^{2+} channels, and activation of K^+ channels, leading to hyperpolarization of the cell.[5] These effects have important consequences in cardiovascular physiology, smooth muscle contraction, neurotransmitter release, and modulation of sympathetic outflow from the central nervous system.[6] Whereas both βAR and α_1-adrenergic receptor (α_1AR) subtypes have been implicated in myocardial inotropy, α_2ARs appear to have a limited role in the heart, being generally localized to coronary arteries and prejunctional cardiac nerves but not postjunctional myocardium.[7,8] α_2ARs are also known to have important roles in peripheral tissues such as the liver (glycogenolysis and gluconeogenesis), pancreas (decreased secretion of insulin from islet β-cells), spleen (contraction of capsule), and kidney (yet undeter-

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mined role in water reabsorption in both proximal and distal tubules).[9] Finally, α_2ARs mediate potent sedative and hypnotic effects and modulate pain pathways at the level of the spinal cord.[10] Because of these effects, α_2AR agonists have become important adjuncts in the armamentarium of drugs used by the anesthesiologist. They are also increasingly becoming targets for intervention and therapy in various human diseases.

Three α_2AR subtypes have been described using molecular and pharmacologic techniques. Cloned α_2AR subtypes have been designated $\alpha_{2\text{-C10}}$, $\alpha_{2\text{-C4}}$, and $\alpha_{2\text{-C2}}$, according to the human chromosomal localization of the gene.[11-15] Cross-referencing these cloned α_2ARs with pharmacologic subtypes previously described by Bylund and colleagues[16-18] demonstrates that the $\alpha_{2\text{-C10}}$AR corresponds to the pharmacologic α_{2A} subtype (and represents the platelet α_2AR), the $\alpha_{2\text{-C4}}$AR corresponds to the pharmacologic α_{2C} subtype, and the $\alpha_{2\text{-C2}}$AR corresponds to the pharmacologically derived α_{2B} subtype.‖

The physiologic relevance of three distinct α_2AR subtypes is not yet known. Recently we have found species heterogeneity in the distribution of α_1AR subtypes,[19] making it imperative to analyze the localization of α_2AR subtypes in human tissues, in order to help answer questions about the physiologic function of receptor subtypes and their perturbations in human pathophysiologic states. Such information has important implications for understanding potential side-effects of administration of α_2AR subtype selective agonists for anesthesia in humans. In this report we describe the expression of α_2AR subtypes messenger RNA (mRNA) in human tissue using the ribonuclease (RNase) protection assay, a technique that is sensitive for quantitating specific mRNA species. Our results indicate that distribution of α_2AR subtype mRNA is somewhat tissue selective and species specific. Furthermore we demonstrate the presence of α_2AR mRNA in adult human heart. These findings have potentially important implications for expanding our understanding of human adrenergic physiology and may be important for the rational development of α_2AR subtype selective anesthetics and other therapeutic agents for use in various human diseases.

‖ When referring to the human cloned receptor subtypes, we will use the human chromosomal location classification ($\alpha_{2\text{-C2}}$, $\alpha_{2\text{-C4}}$, and $\alpha_{2\text{-C10}}$). When referring to the rat and other species homolog subtypes, as well as human protein binding data, we will use the pharmacologic classification (α_2A, α_2B, and α_2C). This is done to be strictly scientifically correct. $\alpha_{2\text{-C2}}$ = α_2B; $\alpha_{2\text{-C4}}$ = α_2C, and $\alpha_{2\text{-C10}}$ = α_2A.

Materials and Methods

Cell Culture

Chinese hamster ovary cells stably expressing individual human α_2ARs ($\alpha_{2\text{-C10}}$, $\alpha_{2\text{-C2}}$, $\alpha_{2\text{-C4}}$, ~1 pmol receptor/mg total protein)[9,10,13] used to test sensitivity and specificity of human α_2AR subtype mRNA probes, were generously provided by Robert Lefkowitz, of Duke University. These cells were grown as monolayers in Dulbecco's modified Eagle's medium (Gibco/BRL, Gaithersburg, MD) supplemented with 10% fetal calf serum, penicillin (100 U/ml), and streptomycin (100 μg/ml) in 5% CO_2 at 37°C. Selection was maintained by adding the antibiotic G418 (0.8 mg/ml) to the media.

Human Tissue Preparation and Messenger RNA Isolation

Normal adult human tissues were obtained either from rapid autopsy (within 1–3 h of death), from organ donor patients (within 30 min of death), or as discarded tissues during surgery (within 5 min of dissection) with appropriate institutional approval. Tissues were snap-frozen in liquid nitrogen and stored at −70°C. Total RNA was extracted from tissue samples by the guanidinium isothiocyanate–cesium chloride gradient method.[20] RNA samples were then quantitated using a spectrophotometer at 260 and 280 nm, aliquoted into 30-μg samples, and stored at −70°C as ethanol precipitates.

Human Complementary DNA Constructs and RNA Probes

The complementary (cDNA) $\alpha_{2\text{-C10}}$AR plasmid construct consists of a 0.324-kb *Bgl*II/*Acc*I (restriction endonucleases used to "cut" and allow "pasting" of DNA at unique sites) fragment in pSP72 (a transcription vector used to generate cRNA probe) (Promega, Madison, WI) (nucleotide 318–642), encoding the third through fifth transmembrane domains.[11] The $\alpha_{2\text{-C4}}$AR cDNA construct consists of a 0.348-kb SacI/AccI fragment in pGEM-3Z (Promega) (nucleotide 377–725), encoding the third transmembrane domain through the initial third intracellular loop.[12] The $\alpha_{2\text{-C2}}$AR cDNA construct consists of a 0.333-kb PstI/BamHI fragment in pGEM-3Z (Promega) (nucleotide 1395–1728),

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corresponding to the initial 3′ untranslated region of the receptor gene.[15] To compare concentrations of α_2AR mRNA with α_{1C}AR mRNA in human heart, a human α_{1C}AR probe was constructed from human cDNA using a 0.322-kb *Pvu*II/*Hin*dIII fragment inserted into pGEM-4Z (Promega) corresponding to the third extracellular loop through the initial carboxyl terminus of the human α_{1C}AR cDNA.[21] Control β-actin probe was received as a gift from Joanne M. Pyper, Johns Hopkins University School of Medicine (Baltimore, MD). This probe consists of a 104-bp fragment of the human β-actin gene cloned into the *Acc*I/*Hin*dII sites of the vector pGEM-4Z (Promega). Linearized cDNA constructs were used to make both single stranded sense (nonradiolabeled control) and antisense (radiolabeled) RNA probes using either T7 or SP6 RNA polymerase as previously described.[22] Probes were radiolabeled with ^{32}P-αCTP (New England Nuclear–DuPont). Due to varying lengths of polylinker sequence between RNA polymerase initiation sites and the constructs described above, predicted antisense (specific) probe lengths are as follows: $\alpha_{2\text{-C10}}$ = 362 nucleotides, $\alpha_{2\text{-C4}}$ = 368 nucleotides, $\alpha_{2\text{-C2}}$ = 364 nucleotides. Control sense/antisense RNA hybrids are slightly larger due to varying amounts of polylinker transcribed with sense strand RNA.

Ribonuclease Protection Assay

RNase protection assays were conducted as previously described by Zinn *et al.*[23] with the following modifications. In brief, 30 μg of dried total RNA (from ethanol precipitates) was dissolved in 30 μl of hybridization buffer (80% formamide, 40 mM piperazinediethanesulfonic acid, pH 6.4, 400 mM NaCl, 1 mM ethylenediaminetetraacetic acid) containing $\sim 5 \times 10^5$ cpm of the cRNA probe. The solution was then heated for 5 min. at 85°C, followed by an overnight incubation at 55°C (α_{1C} and β-actin) or 60°C (all three α_2 probes). RNase digestion was accomplished using 300 μl of RNase solution (RNase A [40 μg/ml, Sigma, St. Louis, MO] and RNase T1 [800 U/ml, Gibco/BRL] in 300 mM Na acetate and 5 mM ethylenediamine tetraacetic acid) at 30°C for 60 min and was terminated by addition of 10 μl 20% sodium dodecyl sulfate and 2.5 μl proteinase K (15 mg/ml) for 30 min at 37°C. After phenol–chloroform extraction, RNA was precipitated with 20 μg transfer RNA as a carrier. After resuspension in 4 μl loading buffer (80% formamide, 1 mM pH 8.0, 0.1% bromophenol blue, 0.1% xylene cyanol), the samples were separated by electrophoresis on a denaturing 8 M urea/6% acrylamide gel, followed by drying and exposure to XAR-5 (Kodak) film for 12–48 h at −70°C.

Quantitation

The quantitation of mRNA protected by the radiolabeled antisense probe was performed by a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). This technique converts ^{32}P-emitted radiation into phosphorescence. This technique is far more sensitive and linear over a longer range than autoradiography. Each gel was exposed for 24 h to PhosphorImager screens and volume integration of bands resulting from hybridization products was performed using gel image analysis computer software (Imagequant, Molecular Dynamics). Tenfold dilutions of single-strand sense RNA (100 ng to 1 pg) were included with each hybridization assay as controls. A standard curve was fit using saturation binding isotherms (InPlot; GraphPad, San Diego, CA), enabling interpolation of concentration of message in human tissue from protected hybridization bands.

Results

Specificity of α_2-Adrenergic Receptor RNA Probes

To ensure specificity of the synthesized radiolabeled antisense human α_2AR subtype selective probes, RNase protection assays were performed on total RNA extracted from Chinese hamster ovary cells stably expressing each cloned α_2AR subtype. As shown in figure 1, all three α_2AR subtype-specific probes protect a single predominant fragment without cross-hybridization.

RNA Isolation from Tissues

Total RNA was isolated from various human tissues including representative cardiovascular (heart, lung, and aorta), brain (cerebral cortex and cerebellum), and several clinically important peripheral tissues (liver, pancreas, spleen, kidney, and adrenal gland). To ascertain uniformity of RNA isolation procedures for each human tissue, RNase protection assays with β-actin probes were also performed in a representative number of samples; β-actin experiments demonstrated equivalent concentrations of β-actin mRNA in each tissue. In certain samples of pancreatic tissue the β-actin signal was decreased. This is not surprising because pancreas is known to contain large concentrations degrading enzymes.

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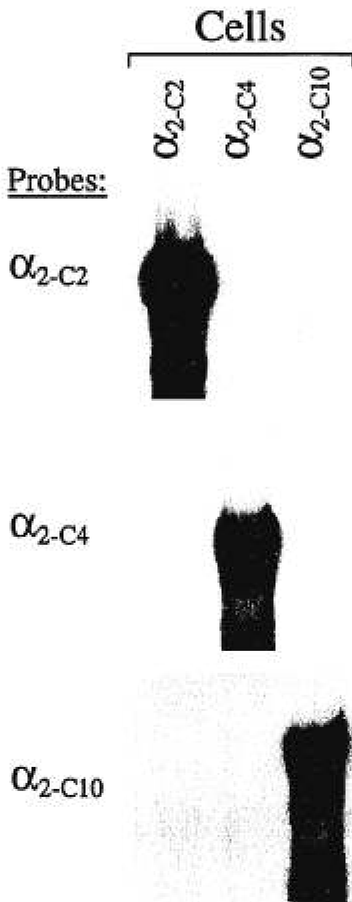

Fig. 1. Ribonuclease protection assays were performed with radiolabeled antisense α_2-adrenergic receptor (α_2AR) subtype-selective RNA probes on total RNA extracted from Chinese hamster ovary (CHO) cells stably expressing each cloned α_2AR subtype. All three α_2AR subtype probes protected a single predominant fragment without cross-hybridization.

Quantitation of α_2-Adrenergic Receptor Subtype Messenger RNA in Human Tissues

As seen in figure 2, the $\alpha_{2\text{-C4}}$AR probe protects a 348-bp fragment, the $\alpha_{2\text{-C2}}$ probe a 333-bp fragment, and the $\alpha_{2\text{-C10}}$ probe a 324-bp fragment in a variety of human tissues. It is important to note that radiolabeled antisense RNA probes are slightly larger than final protected fragments due to varying amounts of polylinker RNA present in the probe and not in the mRNA product. In order to quantitate expression of each α_2AR subtype in human tissues, known concentrations of unlabeled single-strand sense RNA (100 ng to 1 pg) were included as standards with each hybridization assay. A standard curve fit to protected sense strand RNA was used to interpolate the concentration of message in human tissues from protected fragments. RNA standards saturate at approximately 10 ng (range 1–10 ng) sense RNA. The lower limits of resolution of this assay are 0.5–1 pg α_2AR mRNA/30 μg total RNA. α_2AR mRNA ranged from 0–50 pg/30 μg total RNA for all human tissues studied.

Expression of α_2-Adrenergic Receptor Subtype Messenger RNA in Human Tissues

Table 1 shows the quantitative distribution of α_2AR subtype mRNA in human tissues. Note that RNA encoding all three α_2AR subtypes is present in both human brain and peripheral tissues. Total α_2AR mRNA levels are highest in human kidney, aorta, spleen, and heart (table 1). With the exception of liver, small intestine, and pancreas, the $\alpha_{2\text{-C4}}$ subtype represents the majority of α_2AR mRNA present in all human tissues studied (table 1). In particular, $\alpha_{2\text{-C4}}$AR mRNA predominates in human cardiovascular tissues (heart and aorta). Cardiovascular tissues express $\alpha_{2\text{-C2}}$ subtype mRNA in lower concentrations whereas $\alpha_{2\text{-C10}}$ mRNA is virtually absent in human myocardium but does represent some of α_2AR mRNA in human aorta and lung, respectively.

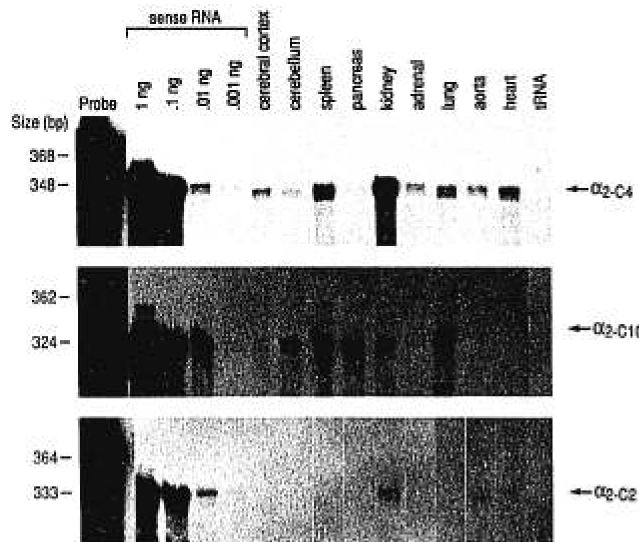

Fig. 2. Representative autoradiograms from ribonuclease protection assays performed with a radiolabeled $\alpha_{2\text{-C4}}$ (α_{2C}), $\alpha_{2\text{-C10}}$ (α_{2A}), and $\alpha_{2\text{-C2}}$ (α_{2B}) (adrenergic receptor antisense probes) in a variety of human tissues. Exposure times depicted are 24 ($\alpha_{2\text{-C4}}$), 36 ($\alpha_{2\text{-C10}}$), and 12 ($\alpha_{2\text{-C2}}$) h. Autoradiograms were used only for screening because quantitation was performed using 24-h exposure to PhosphorImager screens, a process more sensitive than autoradiography for quantitation (see table 1 for semiquantitative results). Transfer RNA was the negative control.

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Table 1. Distribution of α_2-Adrenergic Receptor Subtype mRNA in Human Tissues

	$\alpha_{2\text{-}C2}$ (α_{2B})	$\alpha_{2\text{-}C4}$ (α_{2C})	$\alpha_{2\text{-}C10}$ (α_{2A})	n
Cardiovascular				
Heart (ventricle)	++	+++	−	2–3
Lung	+	+	++	2
Aorta	++	++++	++	3–4
Brain				
Cerebral cortex	±	++	+	2
Cerebellum	±	+	+	3
Other peripheral tissues				
Spleen	++	+++	++	3
Pancreas	±	+	++	4
Liver	++	+	−	3
Kidney	+	+++++	+	4
Adrenal	+	+	±	2
Small intestine	+	+	++	2

Semiquantitative analysis of the distribution of α_2-AR subtypes in human tissues in pg/30 μg of total RNA. The following represents the scale used: <1 pg, ±; 1–4 pg, +; 5–9 pg, ++; 10–19 pg, +++; 20–40 pg, ++++; >40 pg, +++++.

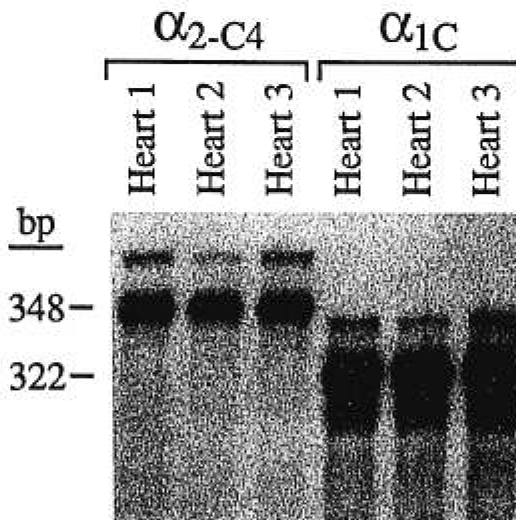

Fig. 3. To compare the quantities of α-adrenergic receptor (αAR) messenger RNA (mRNA) in human myocardium, ribonuclease protection assays were performed with probes recognizing mRNA for the most abundant myocardial α_2AR ($\alpha_{2\text{-}C4}$) and α_1AR (α_{1C}). A single predominant protected fragment was seen for each αAR (348-bp $\alpha_{2\text{-}C4}$ and 322-bp α_{1C}); the lighter band above each protected fragment represents excess undigested probe. Quantitation of these radiolabeled protected fragments is reported in table 2.

Although α_1ARs have been documented to have positive inotropic effects in human and other mammalian ventricular tissue, α_2ARs are not well described in adult human myocardium. Therefore, the presence of two α_2AR subtype mRNA subtypes ($\alpha_{2\text{-}C4} > \alpha_{2\text{-}C2}$) in relatively high concentrations in human left ventricular myocardium is surprising and novel. In order to investigate the relative abundance of α_2AR compared to α_1AR mRNA in human left ventricle, the highest expressing myocardial α_2AR subtype ($\alpha_{2\text{-}C4}$) was compared with the previously described highest expressing myocardial α_1AR subtype (α_{1C}).[24] Figure 3 and table 2 demonstrates that $\alpha_{2\text{-}C4}$ mRNA concentrations (15–19pg/30 μg total RNA, n = 3) are approximately 30-fold lower than α_{1C} mRNA (461–607 pg/30 μg total RNA, n = 3) in human myocardial (left ventricular) tissue. Myocardial mRNA expression levels are remarkably consistent between patients for both α_2 and α_1AR mRNA (table 1).

In addition to cardiovascular tissues, α_2ARs are known to be present and have important physiologic roles in brain and peripheral tissues. Two human brain regions were examined in this study—cerebral cortex and cerebellum (table 1). Although present in fairly low concentrations overall, all three α_2AR subtype mRNAs are present in each tissue. In these human brain regions, $\alpha_{2\text{-}C4}$AR mRNA represents the majority of α_2AR mRNA, followed by $\alpha_{2\text{-}C10}$AR mRNA, and only a small percentage of $\alpha_{2\text{-}C2}$AR mRNA.

In peripheral tissues, kidney and spleen have the highest levels of α_2AR mRNA. Although possibly underestimated according to β-actin controls, pancreas and liver contain far less α_2AR mRNA overall than spleen or kidney, as do small intestine, adrenal, and retina. In peripheral tissues the pattern of α_2AR subtype mRNA distribution differs from brain or cardiovascular tissues. For example the vast majority of α_2AR mRNA in human liver is of the $\alpha_{2\text{-}C2}$ subtype. In pancreas and small intestine, where α_2ARs are known to have important physiologic functions, the $\alpha_{2\text{-}C10}$ subtype predominates. However, in human kidney, spleen and adrenal, the

Table 2. Human Myocardial α-Adrenergic Receptor Subtype mRNA Expression: Comparison of the Predominant α_1-AR Subtype (α_{1C}) with the Predominant α_2-AR Subtype (α_2-C4) in Ventricular Tissue

	$\alpha_{2\text{-}C4}$AR (pg/30 μg total RNA)	$\alpha_{1\text{-}C}$AR (pg/30 μg total RNA)	$\alpha_{1\text{-}C}/\alpha_{2\text{-}C4}$ (ratio)
Heart 1	19	461	24
Heart 2	15	494	33
Heart 3	17	607	36
Mean ± SEM	17 ± 1	520 ± 24	

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$\alpha_{2\text{-C4}}$ subtype prevails, although other α_2AR subtype mRNAs are present to varying degrees (table 1).

Discussion

In this study, α_2AR subtype selective mRNA distribution is examined in various human tissues. The physiologic significance of three distinct α_2ARs is not yet known, but elucidating receptor subtype distribution may play an important role in ultimately understanding mechanisms of receptor function and their physiologic role in health and disease. It is important to note in interpreting the data, that mRNA levels do not necessarily reflect expressed, functional receptor protein. However, in general, the presence or absence of mRNA correlates with the presence or absence of receptor protein in a given tissue and hence may be important in conjunction with receptor protein studies in ultimately understanding the complexity of the AR system. It is also important to emphasize that although the RNase protection assay technique is extremely is extremely sensitive and can give you quantitative information about general receptor subtype mRNA in homogenized tissue, it gives no information about the cellular location of receptors within tissue, information that can only be gleaned from *in situ* hybridization. With this in mind, this study has three important findings: (1) species heterogeneity in α_2AR subtype mRNA distribution; (2) relative organ selectivity in α_2AR subtype mRNA distribution; and (3) the presence of relatively large concentrations of for α_2AR in human heart.

Species heterogeneity of ARs is a complex issue. With regard to α_2ARs, a number of studies have addressed the question of the distribution of α_2AR subtype mRNA in rat tissues. The literature is, however, somewhat confusing and lacks consensus. Lorenz *et al.*,[25] using Northern analysis with human probes and rat tissues found α_{2B} (also called $\alpha_{2\text{-C2}}$) mRNA expressed only in rat liver and kidney, α_{2C} ($\alpha_{2\text{-C4}}$) mRNA expressed only in the brain and not in peripheral tissues, and α_{2A} ($\alpha_{2\text{-C10}}$) mRNA expressed in both brain and peripheral tissues. Voigt *et al.*,[26] after the cloning of the rat homologue of the α_{2C} (88% sequence homology with human $\alpha_{2\text{-C4}}$AR), demonstrated its expression in high-stringency Northern blot analysis in rat brain, kidney, a small quantity in heart, and no expression in pancreas, liver, and lung. Lanier *et al.*[27] also isolated two α_2AR rat clones, corresponding to the α_{2C} ($\alpha_{2\text{-C4}}$) and α_{2A} ($\alpha_{2\text{-C10}}$), and demonstrated using Northern Blot analysis mRNA expression of α_{2C} ($\alpha_{2\text{-C4}}$) only in rat brain but not in heart and kidney as Voigt *et al.*[26] had demonstrated. Differences between investigators in the localization of α_2AR subtype mRNA in rat tissues may be accounted for by differences in Northern analysis methods including stringency of hybridization and possibly the use of human probes with rat tissues. Handy *et al.*[28] have recently investigated the distribution of rat α_2AR subtypes expression using RNase protection assays. Their data is summarized in table 3 and shows expression of α_{2A} ($\alpha_{2\text{-C10}}$) mRNA in rat brain, kidney and spleen and a small amount in rat lung; α_{2B} ($\alpha_{2\text{-C2}}$) mRNA expressed highly in rat kidney with weak hybridization in liver, adult and neonatal lung, and heart; and α_{2C} ($\alpha_{2\text{-C4}}$) mRNA expressed in rat brain with weak hybridization in rat kidney. Because RNase protection assay experiments are more sensitive and specific than Northern analysis, and correspond to our current methods, this data will be used in drawing conclusions on species heterogeneity. Differences in the distribution of α_2AR subtype mRNA distribution between human and rat are immediately apparent (tables 1 and 3). In human cardiovascular tissues, α_{2C} ($\alpha_{2\text{-C4}}$) mRNA clearly predominates, whereas in rat α_{2B} ($\alpha_{2\text{-C2}}$) mRNA predominates. However in brain tissues, rat and human have similar α_2AR subtype mRNA distribution with α_{2C} ($\alpha_{2\text{-C4}}$) and α_{2A} ($\alpha_{2\text{-C10}}$) mRNA predominating. Human kidney contains almost exclusively α_{2C} subtype mRNA, whereas rat kidney contains predominantly α_{2B} mRNA. Liver, however, contains mostly α_{2B} subtype mRNA in both human and rat. Species differences in the location of α_2AR subtypes have potentially important consequences for human physiology; thus it is important to define α_2AR subtype distribution in human tissues.

Table 3. Summary of the Distribution of α_2-AR Subtype mRNA in Rat Tissues

	α_{2B} ($\alpha_{2\text{-C2}}$)	α_{2C} ($\alpha_{2\text{-C4}}$)	α_{2A} ($\alpha_{2\text{-C10}}$)
Heart	+	−	−
Adult lung	++	−	+
Brain	+	+	+++
Liver	++	−	−
Kidney	++++++++	+	++
Spleen	−	−	++++

Compiled from previously published data using RNase protection assay data.[28] Note that the range is an arbitrary scale from − to ++++++++ as presented by the original authors.

Three important categories of human tissues have been considered in this study—brain, cardiovascular

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tissues, and non-cardiovascular peripheral organs; each of these will be discussed in order. α_2ARs subserve a number of functions in the central nervous system important in integrating autonomic, visceromotor, and affective information. α_2ARs are involved in central modulation of blood pressure, control of affect, sedation, nociception, and interact with opiate receptors to suppress opiate withdrawal. Perala *et al.*,[29] who investigated the distribution of the $\alpha_{2\text{-C4}}$ and $\alpha_{2\text{-C10}}$ in various human and fetal brain regions, found $\alpha_{2\text{-C10}}$ to be the predominant α_2AR subtype mRNA in adult cerebral cortex and cerebellum. Ordway *et al.*,[30] using radioligand-binding studies, have demonstrated the α_{2A} ($\alpha_{2\text{-C10}}$) to predominate in most brain regions with the α_{2C} ($\alpha_{2\text{-C4}}$) being expressed in high concentrations in the caudate nucleus. Although we demonstrate the presence of $\alpha_{2\text{-C10}}$ in human cerebral cortex, we find greater expression of $\alpha_{2\text{-C4}}$ mRNA in both cerebral cortex and cerebellum in adult brain. Zeng *et al.*,[14] using Northern blot analysis in rat tissues, found the α_{2A} ($\alpha_{2\text{-C10}}$) subtype to be prominent in the midbrain and brainstem, with the cortex having roughly equal amounts of α_{2A} ($\alpha_{2\text{-C10}}$) and α_{2C} ($\alpha_{2\text{-C4}}$). The α_{2C} ($\alpha_{2\text{-C4}}$) subtype predominated in the cerebellum. Rosin *et al.*,[31] using an antibody specific for the α_{2A} ($\alpha_{2\text{-C10}}$) AR subtype, demonstrated the presence of receptor protein in brainstem and spinal cord predominantly in areas involved in the control of autonomic function. In a comprehensive study of the localization of α_2AR subtype mRNA in the rat brain using *in situ* hybridization, Nicholas *et al.*[32] demonstrated α_{2A} ($\alpha_{2\text{-C10}}$) mRNA in rat brain areas containing central nonadrenergic and adrenergic cell groups (locus ceruleus, reticular thalamic nucleus, as well as deep cerebellar nuclei), α_{2C} ($\alpha_{2\text{-C4}}$) expressed in cerebral cortex, cerebellar cortex, and deep cerebellar nuclei, and α_{2B} ($\alpha_{2\text{-C2}}$) only weakly labeling a small area of thalamus. Because deeper nuclei appear to contain distinct α_2AR subtype mRNA compared with more superficial layer of cortex in rat,[32] the discrepancy in our data and that of Perala *et al.*[29] may possibly reflect the lack of deeper nuclei present in our cerebral cortical and cerebellar brain samples.

We clearly demonstrate the presence of α_2AR mRNA in adult human left ventricular myocardium. Only $\alpha_{2\text{-C4}}$ (78%) and $\alpha_{2\text{-C2}}$ (22%) subtype mRNAs are present in human heart. When compared to the predominant α_1AR subtype mRNA in human myocardium, the α_{1C}AR,[33] $\alpha_{2\text{-C4}}$ mRNA is present in approximately 30-fold lower concentrations. However, overall α_2AR mRNA concentrations in human myocardium are some of the highest determined in this study, suggesting that these receptors may possibly be physiologically important. Whereas postsynaptic myocyte βARs predominate in mediating inotropy and chronotropic responses, and α_1ARs mediate inotropic and arrhythmogenic responses,[34] postsynaptic α_2ARs have never been well described in human myocardium. In adult rat, low levels of α_{2A} ($\alpha_{2\text{-C10}}$) mRNA do appear to be present by Northern blot analysis.[25] Transient relatively high expression of α_2ARs (up to 85 fmol/mg total protein) as assessed by ligand binding techniques,[35] occurs in fetal rat heart; however rat myocardial α_2AR concentrations decrease dramatically by birth. In functional assays, Housmans *et al.*[36] found dexmedetomidine to have no inotropic actions in isolated ferret papillary muscle, and Flacke *et al.*[37] reported no direct effect of dexmedetomidine in isolated dog hearts. Because α_2ARs have important presynaptic roles, speculation regarding a potential postsynaptic role for myocardial α_2ARs must await further studies (*i.e.*, *in situ* hybridization to determine if the α_2AR mRNA is localized in the myocardial cells themselves). Thus α_2AR mRNA detected in the heart may represent coronary vascular α_2AR, presynaptic α_2AR regulating cardiac sympathetics, or postsynaptic α_2ARs. In other cardiovascular tissues, $\alpha_{2\text{-C4}}$AR mRNA predominates in human aorta. This is in striking contrast to rat aorta which expresses only α_{2A} ($\alpha_{2\text{-C10}}$) AR mRNA.[25,38,39] In this regard, it will be important to ascertain whether other human vascular beds, particularly smaller resistance vessels, also contain predominantly the $\alpha_{2\text{-C4}}$AR mRNA subtype. The last cardiovascular tissue we studied was the lung. Ligand binding and functional data demonstrate the presence of tracheobronchial α_1 and α_2ARs which may modulate bronchoconstriction in many species.[40] In addition, neonatal rat lung is the prototypic tissue used in the definition of the α_{2B} ($\alpha_{2\text{-C2}}$) AR subtype. Although we demonstrate some $\alpha_{2\text{-C2}}$ (α_{2B}) AR mRNA in the lung, the predominant subtypes are $\alpha_{2\text{-C4}}$ and $\alpha_{2\text{-C10}}$. α_2AR species differences in cardiovascular tissues may be important in the design of drugs for treatment of human cardiovascular diseases, as well as predicting side-effects of future α_2AR subtype selective drugs for use in anesthesia and analgesia.

α_2ARs also have important roles in non-cardiovascular peripheral organs. α_2ARs are clearly important in mediating gastrointestinal function such as endocrine function of the pancreas;[34,41] specifically, epinephrine inhibits the release of insulin stimulated by glucose via α_2ARs. Phentolamine (a nonspecific αAR antagonist)

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and yohimbine (a specific α_2AR antagonist) increase plasma insulin immunoreactivity in mice, whereas phenoxybenzamine and prazosin (specific α_1AR antagonists) have no such effect;[42] radioligand binding assays confirm these results.[43] Our data demonstrate that the $\alpha_{2\text{-C10}}$ subtype predominates in human pancreas, followed by $\alpha_{2\text{-C4}}$. *In situ* hybridization will be needed to ascertain which of these two α_2AR subtype mRNAs is restricted to specific locations such as β-cells of pancreatic islets where insulin release occurs. In other gastrointestinal tissues such as the small intestine, α_2ARs possess antisecretory properties, therefore α_2AR agonists have recently been proposed to be a promising new class of antidiarrheal drugs in humans.[44] Our data from the small intestine of only one patient demonstrates that $\alpha_{2\text{-C10}}$ mRNA predominates (although $\alpha_{2\text{-C4}}$ subtype mRNA is also present) in this portion of the gastrointestinal tract in humans, and confirms the RNase protection assay receptor protein data of Valet *et al.*[45] In the kidney, $\alpha_{2\text{-C4}}$ mRNA clearly predominates, being present in both the cortex and medulla (data not shown). Because α_2ARs may be involved in either regulation of blood flow associated with renal arterioles or may play a role in the regulation of sodium and water homeostasis, $\alpha_{2\text{-C4}}$ARs may be involved in these functions. It is interesting that although the gene encoding the $\alpha_{2\text{-C4}}$AR was originally cloned from a human kidney library,[12] this receptor subtype is conspicuously absent in rat kidney where both α_{2B} ($\alpha_{2\text{-C2}}$) and α_{2A} ($\alpha_{2\text{-C10}}$) AR mRNA are found instead.[25] In contrast to kidney, the overwhelming majority of α_2AR mRNA located in human liver appears to be the $\alpha_{2\text{-C2}}$ subtype Of interest, α_{2B} mRNA is the only α_2AR subtype present in rat liver as well—one of the few peripheral tissues where rat and human α_2AR subtype mRNA distribution is identical. This finding is in agreement with the lack of $\alpha_{2\text{-C4}}$ and $\alpha_{2\text{-C10}}$AR subtype mRNA found in human liver by Perala *et al.*[29] It is important that during the preparation of this manuscript, Eason and Liggett[46] published the distribution of α_2AR mRNA in a number of peripheral human tissues using the method of reverse-transcription polymerase chain reaction, a technique which detects mRNA transcripts by reverse transcription into DNA template and then polymerase chain reaction amplification. Our data differ from those of this study, but quantitative comparisons are not possible in this case because the study by Eason and Liggett[46] contained no internal controls for efficiency of different primers or for different conditions used for each α_2AR subtype mRNA amplification in these polymerase chain reactions.

In conclusion, this study demonstrates (1) species heterogeneity in α_2AR subtype mRNA distribution for all three α_2AR subtypes (rat *vs.* human); (2) the presence of all three α_2AR subtype mRNAs in both human brain and peripheral tissues; and (3) the previously undescribed presence of relatively large concentrations of α_2AR mRNA in adult human heart. Although this study generally defines the distribution of α_2AR subtype mRNA in human tissues, it is clearly not the final answer in defining the distributions and functions of α_2AR subtypes. *In situ* hybridization studies are needed to localize α_2AR subtype mRNA to specific cells within human tissue samples and hence should facilitate interpretation of possible α_2AR subtype selective functions. Studies of α_2AR receptor protein (using highly selective ligands when they become available, and antibodies which have recently been developed) will be required to ascertain whether an α_2AR subtype is physiologically important in various human tissues. These studies may also provide a possible explanation for the existence of pharmacologically similar, yet distinct α_2AR subtypes, which may in turn be important for the development of α_2AR subtype selective therapeutic agents for use in various human diseases. Finally, and perhaps of most interest to anesthesiologists, the elucidation of α_2AR subtype distribution in human tissues provides a rational basis upon which to develop highly selective α_2AR agonists for use as anesthetics and analgesics.

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