Rapid Communication

Cloning of the Rat \( \alpha_{1C} \)-Adrenergic Receptor
From Cardiac Myocytes

\( \alpha_{1C}, \alpha_{1B}, \) and \( \alpha_{1D} \) mRNAs Are Present in Cardiac Myocytes
but Not in Cardiac Fibroblasts

Alexandre F.R. Stewart, D. Gregg Rokosh, Beth A. Bailey, Larry R. Kars, Kevin C. Chang,
Carlin S. Long, Ken-ichi Kariya, Paul C. Simpson

Abstract \( \alpha_{1} \)-Adrenergic receptor (AR) activation in cardiac muscle has several different physiological effects that might be mediated through different \( \alpha_{1} \)-AR subtypes. Two \( \alpha_{1} \)-AR subtypes have been cloned from the rat, the \( \alpha_{1A} \) and the \( \alpha_{1D} \); both are present in adult rat heart. A third subtype, the \( \alpha_{1C} \), cloned from the cow and human, was reported to be absent in the rat. However, we recently found \( \alpha_{1C} \) mRNA in adult rat heart by using a partial \( \alpha_{1C} \) cDNA. Thus, all three cloned \( \alpha_{1} \)-AR subtypes are present in the heart, but it is unknown whether each is expressed in cardiac myocytes or in cardiac fibroblasts. In the present study, the full-length rat \( \alpha_{1C} \)-AR was cloned from cultured neonatal cardiac myocytes. \( \alpha_{1C} \) mRNA transcripts of 3, 9.5, and 11 kb were present in adult rat heart by Northern blot analysis. \( \alpha_{1A}, \alpha_{1C}, \) and \( \alpha_{1D} \)-subtype mRNAs were each present in isolated adult and neonatal cardiac myocytes by RNase protection assay. In addition, cultured neonatal cardiac myocytes expressed the three \( \alpha_{1} \)-AR subtype mRNAs. In contrast, none of the \( \alpha_{1} \)-AR mRNAs was detected in cultured neonatal cardiac fibroblasts. In addition, \( \alpha_{1} \)-ARs were absent in fibroblasts by \(^{3}H\)prazosin binding and norepinephrine-stimulated \(^{3}H\)inositol phosphate production. The absence of \( \alpha_{1} \)-ARs in cardiac fibroblasts differs from \( \beta \)-adrenergic and angiotensin II receptors, which are present in both cardiac fibroblasts and cardiac myocytes. Three \( \alpha_{1} \)-AR subtypes in cardiac myocytes will need to be considered in future studies of the physiological effects of \( \alpha_{1} \)-AR activation in cardiac muscle. ([Circ Res. 1994;75:796-802.])

Key Words • \( \alpha_{1} \)-adrenergic receptors • cardiac myocytes • cardiac fibroblasts

In cardiac muscle, \( \alpha_{1} \)-adrenergic receptor (AR) activation has several different physiological effects, including control of energy production, increased and decreased inotropy and chronotropy, preconditioning against ischemic injury, reduction of myocardial stunning, and induction of cardiac myocyte hypertrophy and gene transcription. In addition, in the coronary arteries, \( \alpha_{1} \)-AR activation produces vasoconstriction. \( \alpha_{1} \)-ARs belong to the superfamily of G protein-coupled receptors with seven transmembrane domains. The other receptors for catecholamines—\( \beta \)-AR, \( \alpha_{2} \)-AR, and dopaminergic—\( \alpha_{1} \)-ARs constitute a multigene family. Three \( \alpha_{1} \)-AR subtypes have been cloned: the \( \alpha_{1A} \) from the hamster, dog, rat, and human; the \( \alpha_{1D} \) from the rat and human; and the \( \alpha_{1C} \) from the cow and human. In earlier studies, the mRNAs for two of these \( \alpha_{1} \)-AR subtypes were found in adult rat heart, the \( \alpha_{1A} \) and the \( \alpha_{1D} \). Recently, we have shown that \( \alpha_{1C} \) mRNA is also present in adult rat heart, contrary to prior reports. Thus, the mRNAs for all three cloned \( \alpha_{1} \)-ARs—\( \alpha_{1A}, \alpha_{1D}, \) and \( \alpha_{1C} \)—are expressed in adult rat cardiac muscle.

Two \( \alpha_{1} \)-AR subtypes have been defined by radioligand binding studies of native receptors in a variety of rat tissues including the heart: the \( \alpha_{1A} \) and the \( \alpha_{1B} \) (for a review, see Reference 25). The cloned \( \alpha_{1B} \) corresponds to the native \( \alpha_{1B} \)-AR. The cloned \( \alpha_{1D} \), thought originally to correspond to the native \( \alpha_{1D} \), in fact appears to represent a distinct subtype. The cloned \( \alpha_{1C} \), thought originally to be an \( \alpha_{1} \)-AR not recognized in studies of native receptors, now appears to be the molecular equivalent of the native classical \( \alpha_{1A} \). Different physiological effects of \( \alpha_{1} \)-AR activation in the heart might be due not only to the presence of different \( \alpha_{1} \)-AR subtypes but also to a distinct distribution of \( \alpha_{1} \)-AR subtypes between cardiac myocytes and fibroblasts. Precedent exists for the presence of catecholamine receptors on cardiac fibroblasts. For example, \( \beta \)-ARs are predominant on rat cardiac fibroblasts, whereas \( \beta \)-ARs predominate on cardiac myocytes. In cell culture, activation of cardiac fibroblast \( \beta \)-ARs induces fibroblast growth and production of a factor or factors that stimulate growth of cardiac myocytes. Similarly, angiotensin II receptors are present in both cardiac myocytes and cardiac fibroblasts in culture, and in both cell types, the \( \beta_{1} \) receptor subtype mediates a growth response. Thus, the physiological effects of \( \beta \)-AR agonists and angiotensin II in cardiac muscle might reflect, in part, activation of fibroblasts. However, it is unknown whether the physiological effects of \( \alpha_{1} \)-AR agonists might be mediated through \( \alpha_{1} \)-ARs in fibroblasts.

In the present study, we report the DNA sequence of the rat \( \alpha_{1C} \)-AR cloned from cardiac myocytes and show...
that the mRNAs for all three cloned α1-AR subtypes—
the α1C, the α1B, and the α1D—are expressed in cardiac myocytes. Cardiac fibroblasts, in contrast, do not express any α1-AR mRNA or protein. Thus, α1-ARs are expressed specifically in cardiac myocytes, in contrast with β-ARs and angiotensin II receptors. All three cloned α1-AR subtypes in cardiac myocytes will need to be considered in future studies of the physiological effects of α1-AR activation in cardiac muscle.

Materials and Methods
Preparation of Cardiac Myocytes and Fibroblasts
Neonatal cardiac myocytes were isolated from the ventricles of 1-day-old Sprague-Dawley rats, exactly as described previously.36 Cardiac fibroblasts were attached in preplates, and the still-suspended myocytes either were used immediately (isolated neonatal myocytes) or were cultured overnight in 100-mm dishes at ~150 cells per square millimeter in MEM with 5% calf serum and 0.1 mmol/L bromodeoxyuridine (cultured neonatal myocytes). Each of these myocyte preparations contained ~10% fibroblasts.

Neonatal cardiac fibroblasts were scraped from the preplates mentioned above, replated, and grown in 100-mm dishes in MEM with 5% calf serum (fibroblasts passaged once). To remove the small fraction of myocytes present in this preparation, these cells were pretreated and again were trypsinized (fibroblasts passaged twice). Neonatal rat cardiac fibroblast lines were established by transformation with simian virus (SV) 40 large T antigen (Carlin S. Long, unpublished data). In brief, cardiac fibroblasts passaged once were infected with a retroviral vector (PSV10) carrying the SV40 large T antigen under the control of the murine leukemia virus long terminal repeat and expressing the hygromycin resistance gene. Colonies resistant to hygromycin were tested for large T antigen expression by immunohistochemistry. Fibroblasts transformed by large T antigen were grown in MEM with 5% calf serum. Cultured rat cardiac fibroblasts do not express markers for striated or smooth muscle cells or endothelial cells.32-36

Adult cardiac myocytes were isolated by perfusion with collagenase. Hearts were excised from heparinized (1000 U IP) and anesthetized (ketamine, 100 mg IP) male Sprague-Dawley rats (350 to 400 g), arrested in cold isotonic saline containing 20 mmol/L KCl, and perfused retrogradically via the aorta at a constant pressure of 70 mm Hg by using a pH 7.4 Krebs-Henseleit (KH) solution containing (mmol/L) NaCl 138, KCl 4.7, CaCl2 1.5, MgSO4·7H2O 1.2, glucose 10, pyruvate 10, HEPES 5, and 20 U/L insulin. All solutions were at 37°C and were bubbled with 100% O2. After 10 minutes, the perfusate was changed to a nominally Ca2+-free pH 7.2 KH solution with 0.5 mg/mL albumin. After 10 minutes, the heart was perfused at a constant flow of 10 mL/min with KH solution containing 1 mg/mL collagenase B (Boehringer Mannheim), 25 μmol/L CaCl2, and 0.5 mg/mL albumin. After 30 to 45 minutes, the ventricles were minced in pH 7.2 Krafthüle (KB) buffer containing (mmol/L) potassium glutamate 70, KCl 25, KH2PO4 10, oxalic acid 10, taurine 10, glucose 11, pyruvate 2, KATP 2, phosphocreatine 2, HEPES 10, and MgCl2 5. After trituration with a Pasteur pipette, the cell suspension was filtered through a stainless-steel mesh and centrifuged at 5g for 5 minutes. This pellet was resuspended in 20 mL of 4% Ficoll-400 (Sigma Chemical Co), centrifuged again at 5g for 5 minutes, and resuspended in 5 mL KB buffer. Average myocyte yield was 7×106 to 8×106 per heart, >90% of which were rod-shaped.

Cloning of the Rat α1C-AR cDNA
As described previously,23 a partial rat α1C cDNA was obtained by reverse transcription (RT)—polymerase chain reaction (PCR) with total RNA from cultured neonatal rat cardiac myocytes and degenerate primers based on sequence conserved among known α1-ARs (primers A and B in Fig 1). This 530-bp cDNA was used to screen a random-primed cDNA library, constructed in AZAP II (Stratagene) from total RNA isolated from cultured neonatal rat cardiac myocytes according to the acid guanidinium isothiocyanate method.37 Since no positives were obtained by the filter-lift technique, a solution-phase library screen was used.19 Phage lysate from each of the 15 plates that composed the library was tested for the presence of α1C cDNA by PCR, using primers based on the sequence of the 530-bp α1C cDNA (primers C and D in Fig 1). The PCR product of one of the fifteen aliquots was positive by Southern blot analysis with the 530-bp α1C cDNA. This aliquot was replated at high density, and a clone containing the α1C cDNA was isolated by the conventional filter-lift method. This 1500-bp clone contained 1100 bp of coding sequence and 400 bp of the 3’ untranslated region (UTR) of the α1C cDNA but lacked ~350 bp of 5’ coding sequence.

To obtain the remaining 5’ coding sequence, genomic DNA was isolated from whole rat pups and cut with EcoRI. A 6.5-kb EcoRI fragment hybridized on Southern blot with the 530-bp α1C cDNA. This EcoRI fragment was isolated from an agarose gel, subcloned into Bluescript, and used in PCR with an α1C-specific primer (primer E in Fig 1) and a Bluescript T3 primer. The resulting 1.2-kb genomic fragment contained 695 bp of the α1C coding sequence.

At a length α1C coding sequence was constructed by using the three clones described above, as shown in Fig 1. All clones were sequenced on both strands by the deoxyribonucleotide method using Sequenase (USB) or on an automated fluorescent-sequencer at the University of California, San Francisco, Biomedical Resource Center.

Northern Blot Analysis
Twenty micrograms of polyA-enriched RNA (polyATract mRNA isolation kit, Promega) from heart, liver, and brain of adult male rats was fractionated on a formaldehyde/agarose gel, transferred to a nitrocellulose membrane in 10× SSPE, and subjected to high-stringency hybridization (65°C for 24 hours in 6× SSPE, 5× Denhardt’s solution, 1% sodium dodecyl sulfate [SDS]), and 100 μg/mL salmon sperm DNA in 20 mL with 1×104 cpm/mL and washing (twice for 5 minutes at 65°C in 2× SSPE and 0.1% SDS, and once for 30 minutes at 65°C in 0.2× SSPE and 0.1% SDS). Autoradiography was performed at −70°C for the times indicated in the legend to Fig 3. The following cDNAs, labeled with [α-32P]dCTP by random hexamer priming (Boehringer Mannheim) to a specific activity of at least 1×1010 cpm/μg, were used to detect the α1C-AR mRNAs: for the α1C, a 1649-bp Neo I fragment of the rat α1C described in the present study, encompassing the complete open reading frame and 200 bp of 3’ UTR (nucleotides 1 to 1648); for the α1B, a 937-bp EcoRI fragment of a rat α1B cDNA24 corresponding to nucleotides 323 to 1259 of the rat brain α1B cDNA; and for the α1D, a 1771-bp Nor 1–EcoRI fragment covering nucleotides 427 to 1297 of the rat brain α1D cDNA.19

RNase Protection Assay
Total RNA from the cardiac myocyte and fibroblast preparations described above was used in RNase protection assay with rat α1-AR antisense cRNA probes labeled to a high specific activity (1.2×1010 to 1.8×1010 cpm/μg) with [α-32P]UTP (800 Ci/mmol, Amersham), as described in detail recently.25 RNA loading was assessed by using a cRNA probe for glyceraldehyde phosphate dehydrogenase (GAPDH).25 Each antisense α1-AR cRNA probe recognizes only its cognate sense RNA.23 The level of each α1-AR subtype mRNA was estimated by counting the protected fragment excised from the gel and taking into account the number of radioabeled uridine residues present in each protected fragment (for α1B, 101; for α1C, 77; and for α1D, 41).

Radioligand Binding
Cultured myocytes and fibroblasts were scraped from the dishes in ice-cold 0.25 mol/L sucrose, 30 mmol/L histidine, 1
mmol/L EDTA, and 0.1 mmol/L phenylmethylsulfonyl fluoride (PMSF) and centrifuged (60 minutes at 5°C and 90 000g). The pellet was homogenized (Polytron) and resuspended at ≈0.5 μg protein (Bradford) per microliter in assay buffer containing (mmol/L) Tris-HCl 50 [pH 7.4], EDTA 1, and PMSF 0.1. Tripleclic samples containing 100 μg protein (equivalent to ≈200 000 cells) in a total volume of 1 mL assay buffer were incubated for 60 minutes at 30°C with five concentrations of [7-methoxy-[3H]prazosin (50 to 300 pmol/L, 71.8 Ci/mmoll, New England Nuclear), then mixed with 3 mL ice-cold assay buffer diluted 10-fold, filtered through a polyethyleneimine-treated Whatman GF/C filter in a Brandel Harvester, washed four times with 4 mL diluted assay buffer, and counted. In assays on myocytes, there were at least 10-fold more total bound counts than filter blanks; nonspecific binding, defined by 10 μmol/L phenolamine, was <10% of total binding. The number (B_max) and affinity of [3H]prazosin binding sites (α₁-ARs) were estimated by Scatchard analysis.38,39

**Phosphatidylinositol Hydrolysis**

Fibroblasts passed once were grown in 35-mm dishes. Phosphatidylinositolts were radiolabeled by incubation for 48 hours in MEM with 5% calf serum, 0.1 mmol/L bromodeoxyuridine, and 1 μCi/mL myo-[2-3H]inositol (3.9 Ci/mmoll, Amersham). Cells were rinsed and treated in serum-free MEM with 2 μmol/L l-norepinephrine HCl (Sigma), the vehicle for norepinephrine (100 μmol/L l-ascorbic acid, Sigma), or 10% calf serum. After 1 hour at 37°C, total cellular [3H]inositol phosphates were extracted with trichloroacetic acid and separated by anion exchange chromatography, as described previously.36

**Results**

A composite DNA encoding a rat cardiac α₁c-AR was constructed from three clones (Fig 1): a 530-bp cDNA obtained by RT-PCR from neonatal cardiac myocytes,23 a 1500-bp cDNA obtained by solution-phase screening of a rat cardiac myocyte cDNA library, and a 1200-bp genomic fragment amplified by PCR. The deduced amino acid sequence of the rat α₁c was 92% and 90% identical to the human and bovine α₁c-ARs, respectively (Fig 2). Only four of the amino acid substitutions among the different species were nonconservative (indicated by an asterisk in Fig 2).

By Northern blot analysis of polyA-enriched RNA, three α₁c mRNA transcripts of ≈3, 9.5, and 11 kb were present in adult rat heart (Fig 3). α₁c mRNAs of the same size were present in adult rat brain, although at lower levels, whereas α₁c mRNA was not detected in adult rat liver. Expression of α₁c mRNA in adult rat heart and brain, but not in liver, is observed also by RNase protection assay.23 α₁c mRNA has been detected previously by Northern analysis only in rabbit liver24 and rabbit hepatocytes40 by using bovine α₁c DNA as a probe.20 The ≈9.5- and 11-kb α₁c transcripts found here were not seen in rabbit liver or hepatocytes24,40 and might reflect a species and/or tissue difference in α₁c mRNA synthesis or processing. No consensus polyadenylation site was present in ≈400 bp of rat α₁c 3' UTR (Fig 1), nor is one present in ≈1000 bp of bovine 3' UTR20 or ≈300 bp of human 3' UTR.23 The bovine α₁c also contains ≈750 bp of 5' UTR.20 Thus, long UTRs can account at least for the 3-kb α₁c transcript and might also explain the 9.5- and 11-kb transcripts (Fig 3), although the latter might also reflect incomplete mRNA processing.

α₁b mRNA was most abundant in adult rat heart, followed by liver and then brain, whereas α₁d mRNA was present at higher levels in brain than in heart but was not detected in liver (Fig 3). An identical distribution of these mRNAs by RNase protection23 indicates the specificity of the Northern analysis. The α₁b and α₁d transcripts were smaller (≈2.2 kb) than those for the α₁c (Fig 3) and were in the size range reported in prior...
All mRNAs in rat a1c-ARs. FIG 3. Northern blot analysis of a1-adrenergic receptor mRNAs in adult rat heart. Twenty micrograms of polyA-enriched RNA from adult male rat heart, liver, and brain were used for Northern blot analysis (see "Materials and Methods"). The membrane was probed consecutively for the a1c, the a1D, and the a1B mRNAs. Film exposure times were 48 hours for the a1c, 4 hours for the a1B, and 15 hours for the a1D. The mobility of cRNA size markers is indicated on the left and of ribosomal RNA, on the right.

The mobility of cRNA size markers is indicated on the left and of ribosomal RNA, on the right.

Fig 2. Comparison of the deduced amino acid sequence of the rat a1c-adrenergic receptor (AR) with that of the human and bovine a1c-ARs. The derived amino acid sequence of the rat a1c-AR is compared with the human (hum) and bovine (bov) sequences. Amino acid identity with the rat sequence is indicated by a dot. The dash in the bovine sequence at position 19 indicates a missing amino acid. The positions of nonconservative amino acid substitutions are indicated by an asterisk. The seven transmembrane (TM) domains are underlined. Potential N-terminal glycosylation sites include asparagine residues 7, 13, and 22; a consensus site for phosphorylation by protein kinase C is present in the third intracellular loop at threonine 224.

studies of adult rat heart.15,22 However, a longer a1B transcript (≈3.3 kb) noted by others in rat brain and liver23 was not seen.

RNase protection assay was used to detect the a1-AR mRNAs in rat cardiac myocytes and fibroblasts (Fig 4). All three a1-AR mRNAs were present in cardiac myocytes isolated from the adult and neonatal rat heart (Fig 4, lanes 5, 6, and 8) and in cultured neonatal cardiac myocytes (Fig 4, lane 7). In contrast, none of the a1-AR mRNAs was detected in primary or transformed cultured neonatal cardiac fibroblasts (Fig 4, lanes 1 through 3). In the myocytes, the abundance of each a1-AR mRNA subtype was distinctly lower in the isolated neonatal cells compared with both the isolated adult myocytes and the cultured neonatal myocytes (Fig 4, lane 6 versus lanes 5 and 7). The lower abundance in isolated neonatal cells might have reflected the longer time that these myocytes remain in suspension before RNA preparation (≈6 hours), since the levels of all three a1-AR mRNAs were higher in the intact neonatal hearts from which the myocytes were isolated (Fig 4, lane 8 versus lanes 9 and 10). a1B mRNA, and to a lesser extent a1D mRNA, appeared to be upregulated after culture of the myocytes for 1 day in serum-containing medium (Fig 4, lane 7 versus lane 6).

The amount of each a1-AR subtype mRNA was estimated by counting the protected fragments excised from the gel and taking into account the number of radiolabeled uridine residues in each probe, which was least for the a1D. In the cultured neonatal myocytes, the ratio of a1B/a1C/a1D was 1:1:1; and there was =0.1 amol of each mRNA per microgram total RNA (mean of five experiments as shown in Fig 4, lane 7) or =0.5 molecules of each subtype mRNA per myocyte, when assuming =10 pg total RNA per cell. By comparison, there were an estimated 50 molecules of GAPDH per myocyte. In the adult myocytes (Fig 4, lane 5), the ratio of a1B/a1C/a1D was 1:4:0.6; in the neonatal whole.
heart (Fig 4, lanes 9 and 10), the ratio of $\alpha_{1B}$: $\alpha_{1C}$: $\alpha_{1D}$ was 1:2:4:0.3.

To confirm the absence of $\alpha_1$-ARs in cardiac fibroblasts, functional $\alpha_1$-AR proteins were assayed by radioligand binding and stimulation of phosphatidylinositol hydrolysis. In the cultured cardiac myocytes, saturation binding of $[\text{H}]$prazosin produced linear Scatchard plots (data not shown), with a $K_d$ of $256\pm20$ fmol/mg protein and a $B_{\text{\tiny max}}$ of $68\pm8$ pmol/L (mean$\pm$SEM, $n=4$). In contrast, specific binding of $[\text{H}]$prazosin was not detected in cultured cardiac fibroblasts (two experiments with fibroblasts passed once, one experiment with transformed fibroblasts). Activation of phosphatidylinositol hydrolysis through an $\alpha_1$-AR in this cultured myocyte preparation has been reported by us previously.$^{42}$ In cardiac fibroblasts (passed once) labeled with $[\text{H}]$inositol, norepinephrine (2 $\mu$mol/L for 1 hour) caused no change in total cellular $[\text{H}]$inositol phosphates compared with vehicle, whereas calf serum (10%, 1 hour) stimulated a fourfold and sevenfold increase (two experiments). Taken together, these data indicated that cultured rat cardiac fibroblasts did not express $\alpha_1$-ARs.

Discussion

There are two main findings of the present study. First, specific $\alpha_{1C}$-AR mRNA transcripts are present in adult rat heart and brain by Northern blot analysis with an $\alpha_{1C}$-AR cDNA cloned from cultured neonatal rat cardiac myocytes. These data provide conclusive evidence that the $\alpha_{1C}$-AR is expressed in the rat and, specifically in rat heart, in contrast to prior reports,$^{15,20,24}$ Second, $\alpha_{1A}$, $\alpha_{1C}$, and $\alpha_{1D}$ mRNAs are each present in cardiac myocytes, both adult and neonatal, whereas none of these mRNAs is detected in cardiac fibroblasts. Furthermore, $\alpha_1$-ARs are absent in cardiac fibroblasts by $[\text{H}]$prazosin binding and norepinephrine-stimulated $[\text{H}]$inositol phosphate production. Thus, we conclude that all three cloned $\alpha_1$-ARs are expressed in rat cardiac myocytes and that none is expressed in cardiac fibroblasts.

The absence of detectable $\alpha_1$-ARs and their mRNAs in cardiac fibroblasts differs importantly from $\beta$-ARs and angiotensin II receptors, which are present in both cardiac myocytes and cardiac fibroblasts. This finding provides a molecular explanation for the observation that $\alpha_1$-AR agonists do not stimulate growth of cultured rat cardiac fibroblasts,$^{43,44}$ whereas both a $\beta$-AR agonist$^{31}$ and angiotensin II$^{32,33}$ do stimulate growth of cultured fibroblasts. In addition, $\alpha_1$-AR effects in heart cell culture preparations, which are inevitably contaminated by cardiac fibroblasts, are not likely to be mediated by regulatory molecules released from the fibroblasts, a paracrine effect that has been suggested for $\beta$-AR stimulation.$^{31}$ Rather, $\alpha_1$-AR agonists would appear to act exclusively through interaction with receptors in cardiac myocytes.

We have not been able to obtain a pure cardiac fibroblast preparation without the use of culture and thus cannot state with certainty that $\alpha_1$-ARs are also absent in cardiac fibroblasts in vivo. However, it does not seem likely that $\alpha_1$-ARs would be expressed by cardiac fibroblasts in vivo yet be lost with culture in serum-containing medium or after transformation. On the other hand, it does seem likely that all three $\alpha_1$-AR mRNAs are present in rat cardiac myocytes in vivo, since they are present in intact hearts and the myocytes isolated from them.

These results from isolated and cultured cells might be relevant to certain observations on myocardial hypertrophy in vivo. Chronic subpressor infusion of norepinephrine in the dog induces cardiac myocyte hypertrophy without fibrosis.$^{45}$ In contrast, infusions of isoproterenol$^{46}$ and angiotensin II$^{47,48}$ in the rat both induce myocardial hypertrophy with fibrosis. The absence of fibrosis with norepinephrine and its presence with isoproterenol and angiotensin II might be related in part to differences in receptor expression in cardiac fibroblasts.

Our analysis did not include cardiac smooth muscle and endothelial cells, both of which might contribute to $\alpha_1$-AR expression when the intact heart is taken for assay. Indeed, $\alpha_1$-ARs mediate coronary vasoconstriction,$^{10,11}$ and $\alpha_1$-ARs are present on resistance and conductance arteries by autoradiography with $[\text{H}]$prazosin, although at a much lower density than the adjacent myocytes.$^{49,50}$ Rat aorta and vena cava contain all three $\alpha_1$-AR subtype mRNAs.$^{23}$

The mechanism of cardiac myocyte–specific expression of $\alpha_1$-AR subtype mRNAs could involve regulation of mRNA synthesis or processing. Whatever the mechanism of cell-specific expression of $\alpha_1$-AR subtype mRNAs, species differences in cell-specific expression are found in certain organs. For example, $\alpha_{1A}$ mRNA is abundant in human$^{51}$ and rabbit liver$^{24,40}$ but is absent in rat liver (present data and Reference 23). In the aorta and kidney, in contrast, $\alpha_{1C}$ mRNA is just detectable in humans$^{51}$ but is abundant in the rat.$^{23}$ In the human heart, $\alpha_{1C}$ is the most abundant $\alpha_1$-AR mRNA.$^{51}$

It is not yet possible to quantify exactly the relative proportions of the $\alpha_1$-AR subtypes in the cardiac myocytes. Radioligand binding studies in adult rat heart indicate that the relative proportion of $\alpha_1$-ARs is 80% $\alpha_{1A}$ and 20% $\alpha_{1D}$ (eg, see Reference 52). In contrast, the present data with RNase protection suggest that $\alpha_{1B}$ and $\alpha_{1C}$ mRNAs are present at approximately equal levels in adult cardiac myocytes, with $\alpha_{1D}$ mRNA somewhat less. Thus, most $\alpha_{1B}$-ARs appear to be recognized by radioligand binding in adult rat heart than would be predicted by the relative level of $\alpha_{1B}$ mRNA. $\beta$-ARs in cardiac myocytes also show a dissociation between receptor mRNA and protein levels. Specifically, $\beta_2$-AR mRNA is approximately fourfold more abundant than $\beta_1$-AR mRNA, whereas $\beta_2$-ARs are approximately fourfold more abundant than $\beta_2$-ARs as assayed by radioligand binding.$^{30}$ Inhibition of $\beta_2$-AR mRNA translation by the peptide product of a $5^\prime$ leader citron may explain this disparity,$^{53}$ and $\alpha_1$-AR subtype abundance might also be subject to translational regulation. An additional problem with $\alpha_1$-AR quantification to date is that the $\alpha_{1D}$ has been recognized in only a few radioligand binding studies.$^{27,54}$ Precise quantification of the three $\alpha_1$-AR proteins in various myocyte populations is a challenge for future work.

Assignment of a physiological effect to a specific $\alpha_1$-AR subtype is also likely to prove difficult, given the limited selectivity of the available antagonists$^{18,27}$ as well as the possibility that additional subtypes remain to be identified. For example, hypertrophic growth in cultured rat cardiac myocytes has been attributed to the $\alpha_{1D}$. How-
ever, the affinity of 5-methyl-urapidil to inhibit hypertrophy (pK, 8.0) is intermediate to pK, values reported for the cloned α1A and α1D.

How different α1-AR subtypes might mediate specific physiological effects is an additional, interesting problem. One possibility is coupling to different second-messenger pathways. However, all three α1-AR subtypes couple to phosphatidylinositol hydrolysis, cAMP accumulation, and arachidonic acid release when over-expressed in cell lines. A future goal is to determine if each α1-AR subtype is associated with a unique second-messenger pathway in cardiac myocytes or has some other unique biological property.

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References

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