Glucocorticoid Induction of Kv1.5 K⁺ Channel Gene Expression in Ventricle of Rat Heart

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Abstract  Multiple voltage-gated K⁺ channels contribute to the repolarization phases of the cardiac action potential and are targets of several antiarrhythmic drugs. The Kv1.5 K⁺ channel gene is expressed in the heart, and heterologous expression of this gene generates a slowly inactivating K⁺ current. Previously, we found that glucocorticoids specifically upregulate pituitary Kv1.5 gene expression. To test whether these steroids might also induce Kv1.5 gene expression in the heart, cardiac channel mRNA and protein were measured by RNase protection assay and by immunoblotting with antibody specific for the extracellular domain of Kv1.5 polypeptide. Kv1.5 mRNA and immunoreactive protein appeared to be more abundant in rat ventricle than atrium. Reduction of endogenous glucocorticoids by adrenalectomy decreased ventricular Kv1.5 mRNA by ~8-fold, which was estimated by using cyclophilin mRNA as an internal control. Kv1.5 immunoreactive protein also decreased ~6-fold. Injection of dexamethasone into adrenalectomized rats acted within a day to increase ventricular Kv1.5 mRNA and immunoreactive protein ~50-fold and ~20-fold, respectively. In contrast, atrial Kv1.5 mRNA expression was unaffected by either adrenalectomy or injection of the glucocorticoid agonist. Furthermore, dexamethasone-induced upregulation was specific for Kv1.5, since whole-heart Kv1.4 and Kv2.1 mRNA levels, as well as ventricular Kv2.1 mRNA expression, were unchanged. Thus, dexamethasone specifically upregulates Kv1.5 K⁺ channel gene expression in rat ventricle but not atrium. Glucocorticoids may affect excitability of ventricular myocytes and the efficacy of clinically useful drugs by changing the expression of the Kv1.5 K⁺ channel. (Circ Res. 1994;75:1006-1013.)

Key Words  • voltage-gated K⁺ channels  • gene regulation  • adrenalectomized rats  • dexamethasone

Multiple voltage-gated K⁺ channels participate in the various repolarization phases of the cardiac action potential. Molecular biological studies have shown that at least seven distinct voltage-gated K⁺ channel genes are expressed in rat cardiac tissues.¹,³ These include four Shaker-related or Kv1 subfamily genes: Kv1.1, Kv1.2, Kv1.4, and Kv1.5.¹,² Studies with heterologous expression systems have revealed that Kv1.1, Kv1.2, and Kv1.5 genes encode slowly inactivating channels,²,⁴,⁵ whereas the Kv1.4 gene encodes a rapidly inactivating channel.⁶,⁷,⁸ Furthermore, these channel gene products can form heteromeric K⁺ channels that exhibit current kinetics and pharmacological properties distinct from the original homomeric channels.⁸,¹³ For example, coexpression of Kv1.4 and Kv1.5 in Xenopus oocytes generates heteromeric K⁺ channels with intermediate inactivation kinetics.¹⁴,¹⁵ Hence, the diversity of voltage-gated K⁺ currents in cardiac tissues may be due to expression of multiple gene products that assemble into a variety of homomeric and heteromeric channels.

The existence of multiple K⁺ channel genes may also allow for differential control of channel expression. It is known that the expression of K⁺ channel mRNAs varies between regions of rat heart: significant amounts of transcripts for Kv1.1, Kv1.2, Kv1.4, Kv1.5, and Kv2.1 are detected in the rat atrium, whereas only the last two channel mRNAs are abundant in the ventricle.¹ Moreover, the expression of these mRNAs is differentially regulated during cardiac development: Kv1.5 mRNA expression is significant in neonatal and adult heart, whereas transcripts for the other four channel genes are nearly undetectable in prenatal heart and increase during heart development.¹⁶ Finally, K⁺ channel gene transcription may also be differentially regulated by extracellular signals, such as hormones and neurotransmitters. Previously, we have shown that glucocorticoid hormones specifically activate transcription of the Kv1.5 gene, leading to a rapid upregulation of Kv1.5 immunoreactive protein in GH3 clonal pituitary cells.¹⁷,¹⁸ This upregulation of Kv1.5 gene expression also occurs in normal pituitary cells in vitro and in vivo.¹⁹ Thus, K⁺ channel gene expression is not static; it is cell type-specific and influenced by extracellular signals.

Our previous finding that dexamethasone directly activates Kv1.5 gene transcription in clonal pituitary cells¹⁸ raised the possibility that the glucocorticoid agonist may also upregulate the channel gene expression in other tissues. Kv1.5 mRNA is highly expressed in rat cardiac tissues¹,² and may encode a channel that is sensitive to several therapeutic drugs.²⁰,²⁴ Therefore, we tested whether glucocorticoids might upregulate cardiac Kv1.5 gene expression in vivo by using adrenalectomized rats. In the present study, we report that dexamethasone specifically and markedly increases Kv1.5 mRNA and immunoreactive protein in the rat ventricle but not the atrium.

Materials and Methods

Animals

Female Sprague-Dawley rats weighing 200 to 225 g (7 to 8 weeks old) were obtained from Zivic-Miller (Zelienople, Pa). For examining the effect of glucocorticoids, rats were either
sham-operated or bilaterally adrenalectomized (Zivic-Miller) and kept in plastic cages with free access to food and drinking water. Adrenalectomized rats were given 0.9% NaCl as drinking water. Seven days after the operation, the rats were injected intraperitoneally with 10 mg dexamethasone in 0.5 mL sesame oil or with vehicle alone. Twenty-four hours after injection, the rats were anesthetized by inhalation of methoxyflurane and decapitated. The atria and ventricles were dissected and immediately frozen on dry ice.

Transfections

The full-length cDNAs encoding rat K⁺ channels from Kv3.2 (Kv1.3), RK1 (Kv1.4), and Kv1.5 (Kv1.5) were subcloned into pRc/CMV (Invitrogen). Chinese hamster ovary (CHO) cells were transfected with one of these plasmid DNAs or with the parental plasmid pRc/CMV by the calcium phosphate precipitation method (Transfintein, BRL). Transfected cells were selected with 0.2 mg/mL neomycin. After three rounds of subclonings, cloned cells were tested for expression of K⁺ channel mRNA by Northern blot hybridization, and for K⁺ currents by the standard whole-cell patch-clamp method.

RNA Isolation and Measurements

Total RNAs were isolated from a part of the frozen tissues by a one-step method with acid phenol guanidine thiocyanate with additional extractions. Briefly, the obtained RNA pellet was dissolved in solution D and extracted repeatedly with an equal volume of phenol-chloroform-isomylalcohol (25:24:1) until an intermediate layer of proteins became invisible. RNA concentrations were determined spectrophotometrically by using absorbance at 260 nm (A260)=40 mg RNA.

For Northern blotting, an aliquot of total RNA was electrophoresed on 1% formaldehyde-agarose gel and transferred to nylon membrane by capillary action. Prehybridization and hybridizations were performed as described previously by using random priming-labeled rat cDNA probes: EcoRI-HindIII fragment of Kv1.2 for Kv1.5, HindIII-BamHI fragment of dhr27 for Kv2.1, Pst I-BamHI fragment of pBluescript for cyclophilin, Pst I-Pst I fragment of pGAPDH29 for glyceraldehyde phosphate dehydrogenase, and Pst I-Pst I fragment of pANF1-6 for atrial natriuretic factor. Densitometric measurements of autoradiograms (LKB 2400 Gel ScanXL, version 1) were used to quantify hybridization signals. K⁺ channel mRNA levels were normalized by reprobing blots with cyclophilin cDNA probe.

For RNase protection assay, parts of rat Kv1.5 cDNA and cyclolphilin cDNA were subcloned into pGEM1 (Promega). The 5'-end EcoRI-blunt-ended Bgl II fragment of rat Kv1.5 cDNA (pGEM-A-Kv1.5) corresponding to the sequence −295 to 4 was subcloned into the EcoRI-Sma I site of pGEM1 (pKv1 EB300). The 5'-end Pst I-Asp 700 fragment of rat cyclolphilin cDNA (pBluescript) corresponding to the sequence −6 to 104 was subcloned into the Pst I-Sma I site of pGEM1 (pCyc PA100). RNA probes were prepared from EcoRI-digested pKv1 EB300 (the 5'-end Kv1.5 probe), HindIII-digested pCyc PA100 (internal control probe), or Xba I-digested pGEM-A-Kv1.5 (the 3'-end Kv1.5 probe) by using either SP6 or T7 polymerase. Solution hybridization was performed with 5×10⁶ cpm of one of the Kv1.5 probes and cyclolphilin probe, as described previously. RNase digestion, recovery of protected RNAs, and running a denaturing gel were performed as described previously. Kv1.5 mRNA levels were determined by densitometric measurements of autoradiograms (LKB 2400 Gel ScanXL, version 1) using cyclolphilin mRNA levels as internal controls.

Antibody Production and Immunoblot Analysis

Polyclonal anti-Kv1.5 antisera were generated by immunization of rabbits (HTI Bio-Products, Ramona, Calif.) with a fusion protein containing a part of the extracellular loop between S1 and S2 domains of rat Kv1.5 polypeptide (amino acids 272 to 312, K41E) and bacterial glutathione S-transferase (GST).

The frozen ventricles (0.5 g) were thawed on ice and homogenized in 10 vol of a solution (0.25 mol/L sucrose, 1 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, and 1 mmol/L iodoacetamide). Nuclear debris and undisrupted tissues were removed by centrifugation of the homogenate at 1000g for 10 minutes. The low-speed supernatant was then centrifuged at 100 000g for 1 hour. The resulting pellet was washed with a solution containing (mmol/L) Tris-HCl 20 (pH 7.4), EDTA 1, phenylmethylsulfonyl fluoride 1, and iodoacetamide 1 and referred to as post–nuclear membrane fraction. Cultured cells were lysed in a hypotonic solution, and the lysed cells were centrifuged at 15 000g for 20 minutes. The post–nuclear membrane fraction was then prepared by homogenization and differential centrifugation of the pellet total membrane fraction, as described for the ventricular tissues. Protein concentrations were determined by using Bio-Rad protein assay solution with human immunoglobulin as a standard. The post–nuclear membrane proteins were separated on a 7.5% sodium dodecyl sulfate–polyacrylamide gel and transferred to nitrocellulose membrane. The nitrocellulose membrane was washed with 0.1% Tween 20 and 0.1% dodecyl phosphatase, and incubated in phosphate-buffered saline containing 0.1% Tween 20. The coated membrane was probed with 1/2000 dilution of anti-Kv1.4 or 1/200 dilution of affinity-purified anti-Kv1.4 by the coating solution. Bound antibodies were detected with sheep anti-rabbit IgG conjugated with horseradish peroxidase and visualized with enhanced chemiluminescence reagents (ECL, Amersham). Kv1.5 immunoreactive proteins were measured by densitometry using enhanced laser scanning (LKB 2400 Gel ScanXL, version 1), as described previously.

Results

Kv1.5 K⁺ Channel Expression in Rat Heart

It has been shown that Kv1.5 mRNA is significant in both rat atrium and ventricle by Northern blot analysis. To obtain more quantitative information about cardiac Kv1.5 mRNA expression, we used RNase protection assays with the two distinct regions of the rat Kv1.5 cDNA as probes (Fig 1). Various amounts of total RNAs isolated from young (7- to 8-week-old) adult female atria and ventricles were used to compare the abundance of Kv1.5 mRNA in these two tissues. The two probes detected significant signals of Kv1.5 mRNA in both the atrium and ventricle (Fig 1). Densitometric measurements of the signals indicate that the two probes gave consistent results, suggesting that no alternatively spliced transcripts for Kv1.5 mRNA are present in the rat heart. The steady state concentration of Kv1.5 mRNA measured in two independent experiments was estimated to be 18-fold higher in the ventricle than in the atrium. Therefore, Kv1.5 mRNA expression varies between the atrium and ventricle.

To examine cardiac Kv1.5 protein expression, we generated a polyclonal antibody specific for the Kv1.5 polypeptide (anti-Kv1.4). This antibody was produced against a fusion protein containing a portion of the extracellular loop between S1 and S2 transmembrane domains of the Kv1.5 polypeptide corresponding to amino acids 272 to 312, which is unique for Kv1.5 channel (Fig 2A). The specificity of the antibody was tested by immunoblot analysis with the post–nuclear membrane fractions prepared from CHO cells stably transfected with one of three rat Shaker-related K⁺ channel genes (Kv1.3, Kv1.4, and Kv1.5; Fig 2B). These transfected cells express significant amounts of the
Fig 1. RNase protection assay of rat ventricular and atrial RNAs with Kv1.5 probes. Total RNAs were isolated from the ventricles and atria of adult rats. Various amounts of total RNAs (2, 5, and 10 μg) as well as 10 μg of yeast RNA (negative control) were hybridized with either the 3'-end (nucleotides 1803 to 2227) or the 5'-end (nucleotides ~295 to 4) Kv1.5 RNA probe and cyclophilin probe (nucleotides ~6 to 104, internal control). After RNase digestion, the protected RNAs were isolated and separated on a 4% polyacrylamide gel containing 7.8 mol/L urea. Undigested RNA probes were also applied to the gel (probe). The gels were dried and exposed to an X-ray film for various times to quantify the signals. The probes consist of the sequences corresponding to channel cDNA and a part of the vector: the 3'-end Kv1.5 probe, 445 (424+21) bases; the 5'-end Kv1.5 probe, 357 (299+58) bases; and the cyclophilin probe, 166 (111+55) bases. The lengths of the protected fragments were consistent with the cDNAs inserted in the vector. Smaller fragments seen in the figure were mostly due to shorter (not fully extended) RNA probes. Kv1.5 mRNA in the ventricle was estimated to be ~18-fold more abundant than that in the atrium (the average of the two independent experiments with the two different probes).

corresponding K⁺ channel mRNA as well as voltage-gated outward K⁺ currents (50 to 500 pA/pF at +50-mV test potential; data not shown). The antibody shows a strong reaction with an ~76-kD protein present only in Kv1.5-transfected cells but not in mock-transfected or Kv1.3- or Kv1.4-transfected cells (Fig 2B). Preadsorption of the antiserum with 50 μg of the fusion protein (GST-K41E) but not with the parental protein (GST) abolished this reaction with the 76-kD protein (data not shown). This antibody also exhibits strong binding to a 76-kD membrane protein from many rat tissues, including the skeletal muscle, atrium, ventricle, and brain, as well as from GH₂ clonal pituitary cells (Fig 2B). The antibody against a part of the C-terminus of Kv1.5 polypeptide (anti-K41C) reacts with the identical size of the membrane protein in the Kv1.5-transfected cells and various tissues (data not shown). Thus, the antibody against the extracellular domain (anti-K41E) specifically recognizes Kv1.5 K⁺ channel protein.

To compare the abundance of Kv1.5 immunoreactive protein in the atrium and ventricle, two different amounts of the post-nuclear membrane proteins from the two tissues were subjected to immunoblot analysis with the anti-K41E antibody (Fig 2C). Densitometric measurements of the 76-kD Kv1.5 immunoreactive protein indicate that this protein is approximately eightfold more abundant in the ventricle than the atrium from two independent experiments. Thus, the expression of Kv1.5 immunoreactive protein also varies between the two regions of rat heart.

Glucocorticoid Induction of Kv1.5 mRNA Expression

Three groups of animals were used for experiments: sham-operated and vehicle-injected (sham), bilaterally adrenalectomized and vehicle-injected (Adex), and bilaterally adrenalectomized and dexamethasone-injected (Adex+Dex). Seven days after the operation, the rats were injected with either 10 mg dexamethasone or vehicle alone. Twenty-four hours after injection, the rats were killed. First, we measured steady state levels of voltage-gated K⁺ channel mRNAs in whole hearts of these animals by Northern blot analysis (n=3 for each

Fig 2. Anti-K41E (anti-Kv1.5) antibody detects a 76-kD membrane protein in transfected cells and many tissues. A. Amino acid sequences between S1 and S2 domains of rat Shaker subfamily K⁺ channels. Dashes indicate the sequences identical to Kv1.5 polypeptide. The fusion protein used to produce antisera contains the amino acid 272-312 of Kv1.5 polypeptide. B. Immunoblot analyses with anti-K41E antisera. Proteins (50 μg) from the post-nuclear membrane fractions of transfected Chinese hamster ovary (CHO) cells and rat tissues and protein (10 μg) from the post-nuclear membrane fraction of GH₂ cells were subjected to immunoblot analysis. C. Two different amounts of proteins from the post-nuclear membrane fractions of normal rat ventricles and atria were applied to the gel to compare the abundance of Kv1.5 immunoreactivity in these two tissues. The 76-kD immunoreactive protein was estimated to be approximately eightfold more abundant in the ventricle than the atrium (the average of the two independent experiments).
group; Fig 3). Kv1.5 probe exhibits a single band of ≈3.5 kb on a Northern blot, whereas Kv1.4 and Kv2.1 probes detect multiple bands (Fig 3A). The 3.5-kb Kv1.5 mRNA was significantly decreased by adrenalectomy (Adex versus sham, \( P < .05 \)) by two-tailed Bonferroni test and increased by injection of dexamethasone into the adrenalectomized rats (Adex+Dex versus Adex, \( P < .01 \); Fig 3B). In contrast, the larger sizes of mRNAs detected by the latter two probes (≈14 kb for Kv1.4 and ≈12 kb for Kv2.1; Fig 3B) as well as the smaller ones (≈4.5 kb for Kv1.4 and ≈4 kb for Kv2.1; data not shown) were unaffected. Hence, glucocorticoids specifically induce the expression of Kv1.5 mRNA without affecting Kv1.4 and Kv2.1 mRNA levels in rat heart.

To test whether this regulation of Kv1.5 mRNA expression might occur in both the ventricle and atrium, total RNAs were isolated from the ventricles and atria of another set of animals, and RNase protection assays were performed (n=4 for each group; Fig 4). In the ventricle, reduction of endogenous glucocorticoids by adrenalectomy decreased the steady state level of Kv1.5 mRNA ≈8-fold, estimated by using cyclophilin mRNA levels as internal controls (Adex versus sham, \( P < .01 \) by two-tailed Bonferroni test; Fig 4A and 4C). Furthermore, injection of dexamethasone in the adrenalectomized rats caused an increase in ventricular Kv1.5 mRNA level ≈50-fold within 24 hours (Adex+Dex versus Adex, \( P < .01 \)). Since ventricular cyclophilin mRNA expression might be affected by these operations, we compared cyclophilin mRNA levels with glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA expression, another constitutively expressed gene, by Northern blot analysis. The results indicate that the expression of cyclophilin mRNA is ≥30% higher in the Adex group than in the sham and Adex+Dex groups on the basis of GAPDH mRNA levels (data not shown). Thus, with GAPDH mRNA expression assumed to be constant, Kv1.5 mRNA levels as decreased by ≈6-fold by adrenalectomy and increased by ≈38-fold by injection of dexamethasone.

Although the expression of whole-heart Kv1.4 and Kv2.1 mRNA levels was unchanged, these channel mRNA levels might be regulated only in the ventricle. To test this possibility, we attempted to measure ventricular Kv1.4 and Kv2.1 mRNA levels. We found that ventricular Kv2.1 mRNA expression was unaffected by either adrenalectomy or injection of dexamethasone (Fig 5) and that Kv1.4 mRNA was nearly undetectable in the ventricle from all three groups of animals (data not shown). Thus, Kv1.5 mRNA expression in the ventricle is specifically and dramatically upregulated by glucocorticoids.

In contrast to the dramatic changes in ventricular Kv1.5 mRNA expression, neither adrenalectomy nor dexamethasone injection significantly affected atrial channel mRNA expression (Fig 4B and 4C). This failure of the glucocorticoid agonist to regulate atrial Kv1.5 mRNA expression is not due to a lack of the functional steroid receptors, since an upregulation of atrial natriuretic factor (ANF) mRNA expression\(^{31-35}\) was seen in the same RNA samples (data not shown). ANF mRNA was decreased by =30% by adrenalectomy (Adex compared with sham) and increased =2.2-fold by the subsequent injection of dexamethasone (Adex+Dex com-
pared with Adex) with the GAPDH mRNA level used as a control. Hence, glucocorticoids specifically upregulate Kv1.5 mRNA expression in the ventricle but not the atrium.

**Glucocorticoid Induction of Kv1.5 Immunoreactive Protein Expression**

To test whether the upregulation of ventricular Kv1.5 mRNA might be associated with changes in Kv1.5 protein, Kv1.5 immunoreactivity was measured by using antibody specific for the extracellular loop of Kv1.5 polypeptide (anti-K41E). Post-nuclear membrane fractions were prepared by homogenization and differential centrifugation of the ventricles (the same tissues used for channel mRNA measurement in Fig 4, n=4 for each group). The membrane proteins were then subjected to immunoblot analysis (Fig 6A and 6B). Densitometric measurements of the 76-kD Kv1.5 immunoreactive protein revealed that this protein decreased ~6-fold by adrenalectomy (Adex versus sham, P<.05 by two-tailed Bonferroni test). Moreover, dexamethasone injection into the adrenalectomized rats increased the Kv1.5 immunoreactive protein ~20-fold (Adex+Dex versus Adex, P<.01). Qualitatively similar results were also seen when Kv1.5 immunoreactivity was measured from whole heart (n=3 for each group). Therefore, glucocorticoids act within a day to dramatically increase the expression of Kv1.5 immunoreactive protein in rat ventricle.

**Discussion**

Many hormones and neurotransmitters produce acute effects on excitability by modulating the gating of ion channels already present in the plasma membrane. In addition, these extracellular stimuli may cause long-term changes in excitability by regulating the expression of ion channels. It has been shown that treatment of primary cultured neonatal cardiac myocytes with high KCl, Bay K 8644, or phorbol ester increases Kv1.4 mRNA expression. Furthermore, significant changes in Kv1.4 and Kv1.5 mRNA levels are seen in hypertro-
Moreover, we demonstrate that glucocorticoid agonist upregulated Kv1.5 mRNA expression in the ventricle but not the atrium. This finding could not be due to a defect in atrial myocyte glucocorticoid action in our experiments, because we verified that ANF mRNA expression was increased by dexamethasone in the same samples. Hence, glucocorticoid induction of Kv1.5 mRNA expression differs between the atrium and ventricle. A similar differential hormone effect in the two regions of the heart is seen with thyroid hormone induction of myosin heavy chain expression: thyroid hormones markedly upregulated the expression of α- and β-myosin heavy chains in the ventricle but not the atrium.\(^9\) In addition to the heart, we also found that dexamethasone markedly increases the channel mRNA expression in the pituitary\(^9\) and skeletal muscle (K. Takimoto, unpublished data), whereas only a small change is seen in the hypothalamus and the lung (K. Takimoto and B.R. Pitt, unpublished data). Moreover, the changes in ventricular Kv1.5 mRNA produced by adrenalectomy or injection of dexamethasone are larger than those in the pituitary\(^9\) and skeletal muscle. Therefore, the glucocorticoid agonist might also indirectly affect Kv1.5 gene expression in the ventricle, in addition to directly inducing channel gene transcription. These findings suggest that the hormone induction of Kv1.5 gene might require an additional factor whose expression is confined in certain tissues. Alternatively, some tissues might possess a factor that represses hormone responsiveness. In any case, our findings demonstrate that glucocorticoids differentially regulate atrial and ventricular K\(^+\) channel gene expression.

Previous studies from several laboratories have shown that voltage-gated K\(^+\) channel mRNA expression is regionally and developmentally regulated in rat heart. However, there are some disagreements concerning the expression of Kv1.5 mRNA: one group reported that the expression of Kv1.5 mRNA is dramatically regulated in the ventricle during development and aging,\(^5,36\) whereas the other group showed relatively constant levels of Kv1.5 mRNA throughout early development.\(^6\) Furthermore, the results from the two groups also differ concerning the levels of ventricular Kv1.4 mRNA: one group detected substantial amounts of Kv1.4 mRNA in the ventricle,\(^36\) whereas the other showed a nearly undetectable level of channel message.\(^1\) Our results indicate that Kv1.5 mRNA and immunoreactive protein are much more abundant in the ventricle than the atrium of young (7- to 8-week-old) adult rats. We also found that Kv1.4 mRNA is abundant in the atrium but not the ventricle. These discrepancies might be due to different experimental conditions that may influence channel mRNA levels. Indeed, recent findings\(^10,19,36,37\) as well as findings in the present study suggest that expression of K\(^+\) channel mRNA is not static but is dynamically regulated under various conditions. For example, in adrenalectomized animals, Kv1.5 mRNA levels were comparable in the atrium and ventricle. Moreover, the regulation of channel mRNA expression might be regionally confined, as our results suggest. Further studies including detailed immunohistochemis-

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**A**

Adex | Sham | Adex+Dex
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![Image](http://circres.ahajournals.org/)

**B**

Kv1.5 Immunoreactivity relative to Sham

![Image](http://circres.ahajournals.org/)

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**Fig. 6.** Glucocorticoids upregulate ventricular Kv1.5 immunoreactive protein. Sham indicates sham-operated and vehicle-injected rats; Adex, adrenalectomized and vehicle-injected rats; and Adex+Dex, adrenalectomized and dexamethasone-injected rats. A, Immunoblot analysis of ventricular post-nuclear membrane proteins with anti-Kv1.5E (anti-Kv1.5) serum. Post-nuclear membrane fractions were prepared from the ventricles (the same tissues used for Fig 4), and 50 μg of protein from the membrane fractions was subjected to immunoblot analysis. B, Bar graph showing Kv1.5 immunoreactivity determined by measuring the signal of the 76-kD protein by densitometry. Columns represent the immunoreactivities relative to the mean of the sham group with error bars indicating SEM (n=4). Kv1.5 immunoreactivity in the Adex group is significantly lower than that in the sham group (P<.05 by two-tailed Bonferroni test), and that in the Adex+Dex group is significantly higher than that in the Adex group (P<.01).
try and in situ hybridization are needed to resolve these issues.

Expression of the Kv1.5 gene in Xenopus oocytes generates a very slowly inactivating outward K⁺ current.5-7 Since rat and human atrial myocytes exhibit a prominent delayed rectifying outward K⁺ current, Kv1.5 has been proposed to constitute the atrial delayed rectifier.8,9 However, our results demonstrate that Kv1.5 mRNA and immunoreactive protein are more abundant in rat ventricle than atrium. Thus, Kv1.5 protein is likely to contribute to the ventricular voltage-gated outward K⁺ current. There are two main components of ventricular voltage-gated K⁺ current in the rat: a tetraethylammonium (TEA)-sensitive delayed rectifier and a TEA-insensitive 4-amino pyridine-sensitive transient current. The fact that the heterologously expressed Kv1.5 channels differ from these native ventricular currents may be due to the association of Kv1.5 channel subunit with other proteins. Such proteins might be other Shaker subfamily K⁺ channel subunits. For example, Kv1.4 encodes a channel that rapidly inactivates in heterologous expression systems.10-12 Therefore, Kv1.5 protein might be present in an association with Kv1.4 protein to form the transient K⁺ current in rat ventricle. However, a previous study13 and our results indicate that transcripts for this gene are nearly undetectable in rat ventricle. Another possibility is suggested by the recent findings that brain dendrotoxin-binding voltage-gated K⁺ channels are associated with auxiliary β subunits14 and that the association of certain β subunits affects inactivation kinetics.15 Thus, it is possible that the ventricular Kv1.5 channel may encode the transient K⁺ current by associating with a β subunit. Resolving this issue will require purification and biochemical identification of cardiac K⁺ channels.

The dramatic upregulation of Kv1.5 immunoreactive protein in rat ventricle may produce many physiological and pharmacological effects. We have previously found that the dexamethasone-induced upregulation of Kv1.5 immunoreactive protein is associated with an increase in voltage-gated K⁺ current density in GH3 cells.16 Thus, the upregulation of ventricular Kv1.5 immunoreactive protein might also lead to an increase in the number of functional K⁺ channels. In patients with long QT syndrome, congestive heart failure,43 or chronically infarcted regions of the ventricle,44 action potential repolarization mechanisms are compromised. Guanidinocoid induction of Kv1.5 channel expression might be a novel therapeutic treatment to compensate for the loss of normal repolarization mechanisms. In addition to influencing basal electrical activity, hormone regulation of Kv1.5 gene expression might also affect responsiveness to hormones, autonomic inputs, and clinically useful drugs. For example, β-adrenergic agonists suppress the delayed rectifying K⁺ current in rat ventricular myocytes.45 Likewise, several clinically useful drugs, especially class III antiarrhythmic drugs, act on voltage-gated K⁺ channels to produce their therapeutic effects. Heterologously expressed Kv1.5 K⁺ channels are found to be sensitive to quinidine,20 verapamil,21 clofilium,22 tetradsamil,23 and an antihistamine.24 Hence, regulation of K⁺ channel expression may influence basal electrical activity, its modulation by natural extracellular signals, and the efficacy of therapeutic drugs.
Glucocorticoid induction of Kv1.5 K+ channel gene expression in ventricle of rat heart.
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