Altered K^+ Channel Subunit Composition following Hormone Induction of Kv1.5 Gene Expression†

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ABSTRACT: Diverse voltage-gated K^+ channels are produced by tetramerization of channel α subunits that can be encoded by multiple genes. In GH_3 pituitary cells, the Kv1.4 and Kv1.5 subunit genes are constitutively expressed, and dexamethasone selectively up-regulates expression of the latter gene. Here we use subunit-specific antibodies to determine the effect of Kv1.5 gene induction on expression of homomeric and heteromeric channels. In control cells, almost all detectable Kv1.4 proteins are associated with Kv1.5 protein, whereas only ∼30% Kv1.5 protein is present as Kv1.4/Kv1.5 heteromeric channels. Dexamethasone treatment doubled expression of Kv1.5 homomeric channels but only modestly up-regulated Kv1.4/Kv1.5 heteromeric complexes. The steroid treatment similarly increased total cellular and cell surface Kv1.5 protein without affecting Kv1.4 proteins in either fraction. Thus, hormone induction of one K^+ channel subunit gene differentially influences expression of cell surface homomeric and heteromeric channels leading to specific alteration of excitability.

Voltage-gated K^+ channels constitute a diverse group of membrane proteins. This diversity largely arises from the presence of a large repertoire of channel subunits and the combination of these subunits in multimeric protein assembly. Specifically, at least eight mammalian *Shaker* or Kv1 subfamily genes encoding K^+ channel α subunits have been identified (Chandy & Gutman, 1995), and a pore-forming channel is formed with four α subunits that may be encoded by more than one gene (Isacoff et al., 1990; Ruppersberg et al., 1990; Christie et al., 1990; McCormack et al., 1990; Sheng et al., 1993; Wang et al., 1993). This subfamily-specific assembly is also observed with the hydrophilic amino-terminal region of channel polypeptide that is highly conserved within the subfamily (Li et al., 1992; Shen et al., 1993; Xu et al., 1995; Pfaffinger & DeRubeis, 1995). Furthermore, protein synthesis studies suggest that α subunit assembly occurs rapidly and may be cotranslational (Shen et al., 1993; Deal et al., 1994). Therefore, formation of both heteromeric and homomeric K^+ channels appears to be efficient in heterologous expression systems.

Expression and assembly of native Kv1 subfamily homomeric and heteromeric channels may be more complex. Immunohistochemical studies indicate that different Kv1 subfamily channel subunits are segregated to discrete subcellular sites in single cells (Klumpp et al., 1995; Mi et al., 1995). Thus, in native cells, formation of homomeric but not heteromeric channels might preferentially occur. Alternatively, homomeric channels might be more stable than heteromeric channels in these cells. Furthermore, in Schwann cells, immunoreactivity of one Kv1 subfamily channel was only seen in intracellular compartments, whereas significant immunoreactivity of another was detected at the plasma membrane (Mi et al., 1995). Thus, cell surface expression of distinct Kv1 subfamily channel proteins may be differentially controlled. Hence, the rules that govern assembly and localization of distinct subunit compositions of native channels have not been elucidated. Yet understanding these rules is essential for predicting the impact of changes in subunit expression on the structure and function of these multimeric proteins.

Voltage-gated K^+ channel gene expression is rapidly regulated by many stimuli. For example, drug-induced seizure activity down-regulates Kv1.2 and Kv4.2 mRNAs in rat hippocampal neurons (Tsaur et al., 1992). Likewise, opiates influence Kv1.5 and Kv1.6 mRNA levels in striatal neurons and NG108 cells (Mackler & Eberwine, 1994). Furthermore, we have found that glucocorticoid hormones, the neuropeptide thyrotropin-releasing hormone (TRH), and membrane potential control Kv1.5 gene transcription in GH_3 pituitary cells (Levitan et al., 1991, 1995; Takimoto et al., 1993, 1995). These responses then alter channel mRNA and protein levels within hours. Concomitant changes in K^+ current density have been found with the glucocorticoid agonist dexamethasone and TRH (Takimoto et al., 1993, 1995). Dramatic regulation of Kv1.5 expression has also been detected *in vivo* in pituitary and heart (Attardi et al., 1993; Takimoto et al., 1994). Thus, expression of endogenous K^+ channel genes can be rapidly regulated by hormones, neurotransmitters, and electrical activity to produce long-term changes in excitability.

The finding that expression of K^+ channel subunit genes is rapidly regulated raises the possibility that extracellular stimuli may dramatically influence the structure of these multimeric proteins. GH_3 pituitary cells are an accessible system for addressing this issue. These cells constitutively express Kv1.4 and Kv1.5 subunits (Meyerhof et al., 1992; Takimoto et al., 1993, 1995). In heterologous expression systems, Kv1.4 and Kv1.5 homomeric channels carry

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transient and delayed rectifier K^+ currents, respectively, whereas heteromeric channels consisting of these two subunits exhibit intermediate current kinetics (Po et al., 1993; Lee et al., 1994). Our previous finding that the glucocorticoid agonist dexamethasone significantly increases the noninactivating, but not the transient, component of K^+ current (Takimoto et al., 1993) suggests that the hormone might differentially influence amounts of homomeric and heteromeric channels. Thus, this study was carried out to determine the effect of the glucocorticoid-induced increase in Kv1.5 subunit synthesis on K^+ channel subunit composition in GH_3 cells. We show here that Kv1.5 subunits are expressed on the cell surface as homomeric and heteromic channels. Furthermore, our results suggest that the synthesis ratio of the two Kv1 subfamily subunits determines the subunit compositions of channels, and thus, regulation of one channel subunit gene can differentially influence the expression of homomeric and heteromeric channels.

EXPERIMENTAL PROCEDURES

Antibodies. Anti-Kv1.5 antibody was produced by injection of a bacterial glutathione *S*-transferase fusion protein containing a part of the amino-terminal intracellular region of rat Kv1.5 polypeptide corresponding to amino acids 2−97 (Swanson et al., 1990; K41N). Anti-Kv1.4 antibody was obtained by injection of a keyhole limpet hemocyanin conjugated with a part of the amino-terminal rat Kv1.4 polypeptide corresponding to amino acids 13−37 (Stuhmer et al., 1989), following the method described previously (Sheng et al., 1992). Anti-Kv1.5 antibody was affinity-purified by acid elution of bound antibodies from nitrocellulose membrane to which the fusion protein had been nonspecifically attached. Affinity-purified anti-Kv2.1 antibody (anti-GST-*drk1*, Sharma et al., 1993) was supplied by Dr. J. S. Trimmer (State University of New York, Stony Brook, NY).

Transfections. Rat K^+ channel cDNAs (Kv1 for Kv1.5, Swanson et al., 1990; RK3 for Kv1.4, Roberds & Tamkun, 1991) were subcloned into pRc/CMV-neo expression vector (Invitrogen, San Diego, CA). CHO K1 cells grown on 100 mm dishes were transfected with 10 μg of channel cDNA/CMV-neo plasmid or vector without insert by the calcium phosphate precipitation method (Transfinity, Gibco-BRL, Gaithersberg, MD). For coexpression of Kv1.4 and Kv1.5 channels, Kv1.4/CMV-neo and Kv1.5/CMV-neo plasmid DNAs were mixed at a 1:1 ratio. The transfected cells were selected with 400 μg/mL neomycin. Expression of K^+ channel subunits was tested by RNA blot hybridization for mRNA and patch clamp recording for K^+ current.

Immunoblot Analysis. Cells grown on plastic dishes were washed with Tris-buffered saline and collected with the same buffer containing 1 mM EDTA. Cells were lysed in buffer containing 20 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1 mM iodoacetamide, and 1 mM phenylmethanesulfonyl fluoride. Total membrane fraction was obtained by centrifugation of the cell lysate, followed by washing the pelleted membranes with the same lysis buffer. Protein concentration was determined with Bio-Rad protein assay solution (Bio-Rad, Hercules, CA) using human IgG as a control.

Proteins were separated on a 7.5% SDS−polyacrylamide gel and transferred to a nitrocellulose membrane. The membrane was coated with 5% nonfat dry milk in phosphate-buffered saline containing 0.1% Tween 20. The coated membrane was probed with a 1/500 dilution of affinity-purified anti-K41N (anti-Kv1.5) or 1 μg/mL anti-Kv1.4 antibody in the coating solution. Bound antibodies were detected with anti-rabbit goat antibody conjugated with peroxidase, followed by the enhanced chemiluminescence method (Amersham, Arlington Heights, IL).

Immunoprecipitation. Triton extract was prepared by suspending total membrane fraction at ∼5 mg of protein/mL in the lysis buffer supplemented with 0.15 M NaCl and 1% Triton X-100. The suspension was stirred for 1 h at 4 °C and then centrifuged at 20000*g* for 20 min to remove Triton-insoluble materials. This procedure solubilized ∼80% of total proteins and almost all K^+ channel immunoreactivities from the membrane fraction of GH_3 or channel cDNA-transfected CHO cells.

Anti-K41N (anti-Kv1.5) and control IgGs were isolated and conjugated with CNBr-activated Sepharose CL-4B (Pharmacia Biotech, Piscataway, NJ) at ∼4 mg of IgG/(mL of wet resin). Immunoprecipitation was performed with Triton extracts and either anti-Kv1.4 IgG or anti-Kv1.5 IgG-Sepharose in the lysis buffer at a final sample protein concentration of ∼2 mg/mL. Briefly, a total of 0.2 mL of IgG-Sepharose suspension (50% slurry) consisting of various ratios of anti-Kv1.5 and control IgG-Sepharoses was mixed with Triton extract in a final volume of 0.4 mL for 2 h. Anti-Kv1.4 or control IgG was similarly mixed with Triton extract, except that, after 1 h of incubation, formaldehyde-fixed protein A-carrying bacteria was added. After centrifugation, obtained supernatant (unbound) or precipitated (bound) proteins were analyzed by immunoblot analysis. For bound proteins, the precipitated materials were washed four times with the Triton and NaCl-containing lysis buffer. The bound proteins were then eluted by boiling in 2 × SDS sample buffer.

Molecular Sieve Chromatography. CHAPS and Zwittergent extracts were prepared as described above for Triton extraction, except that 1% CHAPS (Sigma, St. Louis, MO) or Zwittergent 3−12 (Calbiochem, San Diego, CA) was used in the place of Triton X-100. Detergent extract (1 mL) was separated on a Bio-gel A5m (Bio-Rad, Hercules, CA) column (1.4 × 22 cm) in 20 mM Tris-HCl (pH 7.4), 1 mM EDTA, 0.15 M NaCl, and 0.5% of the corresponding detergent. Each fraction (0.4 mL) was collected and examined for channel immunoreactivities and protein concentration.

To estimate the molecular mass of K^+ channel proteins, molecular size markers (Pharmacia) were run on the column under identical conditions: thyroglobulin, 669 kDa; ferritin, 440 kDa; catalase, 232 kDa; and aldolase, 158 kDa. Most of the marker proteins and Blue Dextran 2000 (fraction 19) were eluted as single peaks at the identical positions in either detergent-containing buffer, except that ferritin was eluted significantly slowly in Zwittergent buffer.

Isolation of Cell Surface Glycoproteins. Cell surface glycoproteins were biotinylated with biotin-LC-hydrazide (Pierce, Rockford, IL) following the previously described method (Deal et al., 1994). Triton extract was prepared from the treated cells, as described above. The extract (200 μL, ∼1 mg of protein/mL) was then mixed with streptavidin-Sepharose (200 μL, 50% slurry) in the lysis buffer supplemented with 1% Triton X-100 and 0.15 M NaCl for 2 h at 4 °C (Lantz & Holmes, 1995). After being washed four times with the same solution, bound materials were eluted

by boiling in 2 × SDS sample buffer. This procedure recovered ≥90% of biotinylated proteins, as judged by measuring biotinylated proteins with streptavidin peroxidase following SDS−polyacrylamide gel electrophoresis of extract and unbound preparations.

RESULTS

K^+ Channel α Subunit Assembly Requires Coexpression of Subunits. We have previously detected channel subunit proteins in GH_3 cells using antibodies specific for Kv1.4 or Kv1.5 channel subunit polypeptides (Takimoto et al., 1993 Takimoto & Levitan, 1994). However, the anti-Kv1.5 antibodies directed against a part of the carboxyl terminus or the extracellular loop of the rat Kv1.5 polypeptide were ineffective at immunoprecipitating channel proteins. Therefore, we generated another anti-Kv1.5 antibody against a part of the amino terminus of the rat Kv1.5 polypeptide. This antibody as well as anti-Kv1.4 antibody specifically recognized channel subunit polypeptides present in CHO cells transfected with the corresponding rat channel subunit cDNAs (Figure 1A). Similar sizes of immunoreactive proteins were also seen in a membrane fraction of GH_3 cells. In both transfected CHO and GH_3 cells, Kv1.4 proteins are present as two distinct size forms (96 and 83 kDa). *N*-Glycosidase, but not *O*-glycosidase or neuraminidase, reduced both sizes of the Kv1.4 proteins to ~80 kDa (data not shown). In contrast, anti-Kv1.5 antibody detected a single size band with a molecular mass of ~76 kDa in both transfected CHO and GH_3 cells. The size of this Kv1.5 protein was also slightly reduced after *N*-glycosidase treatment (data not shown). Thus, the anti-Kv1.5 and anti-Kv1.4 antibodies specifically detect the corresponding exogenous and endogenous channel subunit proteins.

We then examined whether these antibodies were able to immunoprecipitate channel subunit proteins. Triton extracts were prepared from channel cDNA-transfected CHO cells and subjected to immunoprecipitation (Figure 1B). To facilitate immunoprecipitation, the anti-Kv1.5 antibody was conjugated with CNBr-activated Sepharose resin. As can be seen in Figure 1B, the anti-Kv1.5 antibody-Sepharose and anti-Kv1.4 antibody immunoprecipitated their targeted channel subunit proteins. No significant reduction in the nontargeted subunit immunoreactivity was detected in the antibody-unbound supernatant fractions after immunodepletion. Thus, immunoprecipitations with these antibodies are specific.

Furthermore, we found that anti-Kv1.4 and Kv1.5 antibodies each coprecipitated Kv1.5- and Kv1.4-immunoreactive proteins from the extract of Kv1.4/Kv1.5-cotransfected cells. In contrast, when channel subunits were individually expressed, solubilized, and then mixed, neither antibody significantly co-immunoprecipitated the other channel protein. Thus, assembly of heteromeric K^+ channels requires coexpression of subunits.

Endogeneous K^+ Channel α Subunits Multimerize Efficiently. Since we wished to determine glucocorticoid-induced change in homomeric and heteromeric channels, it was essential to examine how much of the native channel subunits were present as multimeric forms in GH_3 cells. It was previously shown that Triton or CHAPS extraction retains multimeric forms of channels, whereas Zwittergent extraction dissociates multimeric forms of channels to monomers (Sheng et al., 1993; Shen et al., 1993). Thus, cell extracts were prepared with either CHAPS or Zwittergent

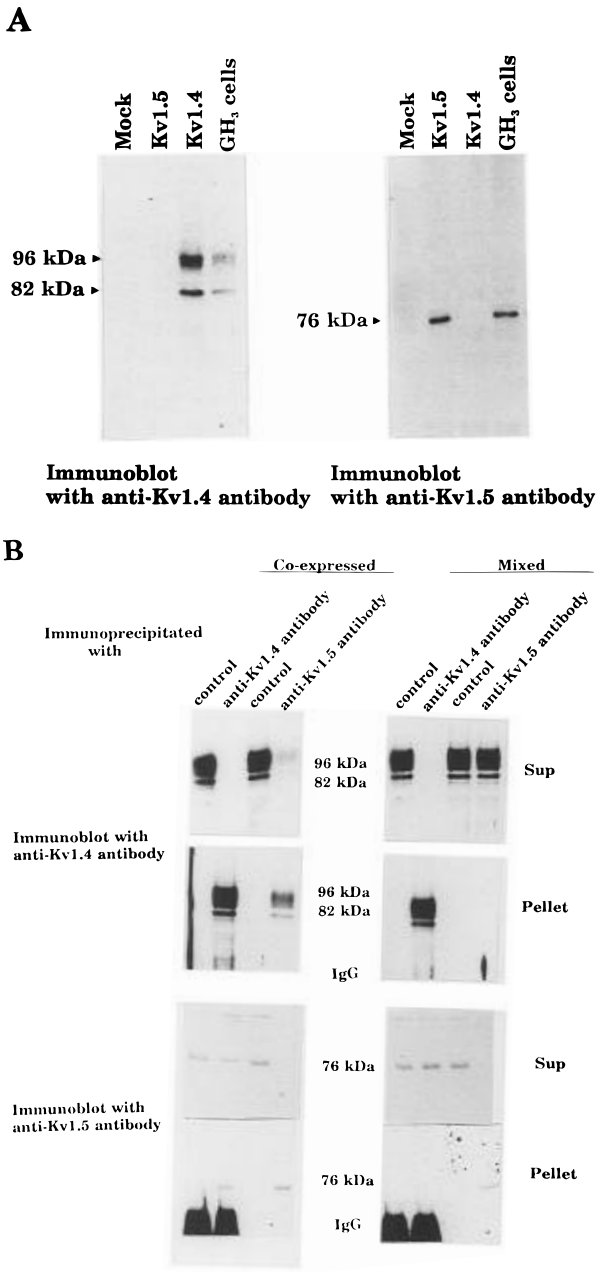

FIGURE 1: K^+ channel α subunit assembly requires coexpression of channel subunits. Specificities of antibodies were examined by immunoblot analysis with extracts from GH_3 cells and channel cDNA- or mock-transfected CHO cells (A). Membrane fractions of GH_3 and transfected CHO cells (50 μg of protein) were subjected to immunoblot analysis with anti-Kv1.4 antibody (1 μg/mL, left) or affinity-purified anti-Kv1.5 antibody (1/500 dilution, right). Coassembly of channel subunits was tested by immunoprecipitation, followed by immunoblot analysis (B). Triton extracts were prepared from membrane fractions of CHO cells individually transfected with rat Kv1.4 or Kv1.5 cDNA, solubilized, and then mixed (Mixed), and of CHO cells cotransfected with the two cDNAs (Co-expressed). Control IgG (first lane), anti-Kv1.4 antibody, control IgG-Sepharose (third lane), or anti-Kv1.5 antibody-Sepharose was used for immunoseparation, as described in Experimental Procedures. Unbound supernatant and the precipitated materials were subjected to immunoblot analysis with anti-Kv1.4 antibody (top) or affinity-purified anti-Kv1.5 antibody (bottom). Note that the two antibodies each co-immunoprecipitated channel subunits along with their targeted proteins from cotransfected cell extracts but not from the mixture of individually transfected cell extracts.

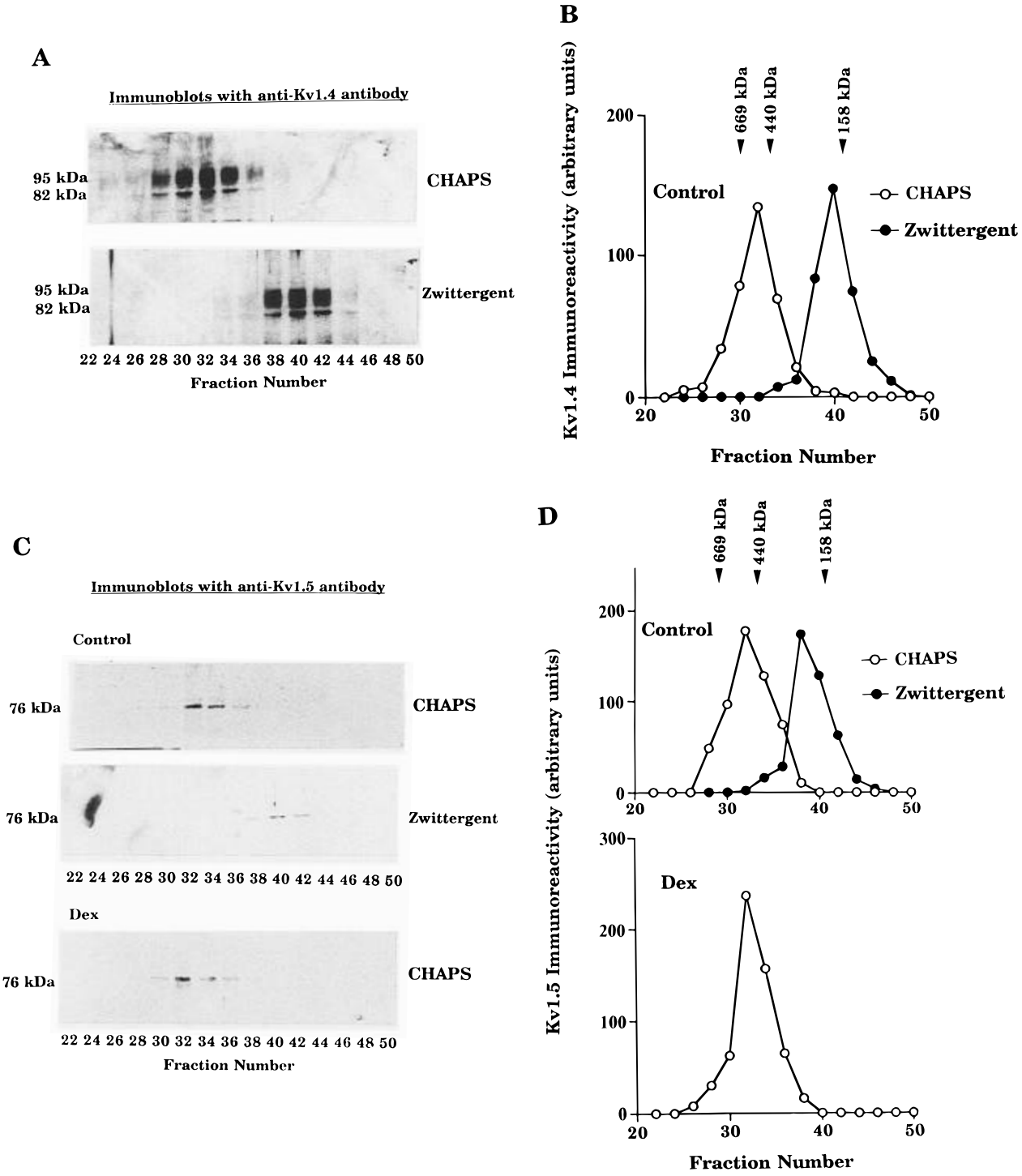

FIGURE 2: K^+ channel α subunits are present as high-molecular weight multimeric forms in GH_3 cells. Detergent extracts were prepared from a membrane fraction of GH_3 cells untreated (Control) or treated with 1 μM dexamethasone for 24 h (Dex). The extracts were size-fractionated on a Bio-gel A5m column, and eluants were examined for Kv1.4 (A) and Kv1.5 (C) channel subunit immunoreactivities. Densitometric measurement of blots was used to quantitate immunoreactivities in each fraction (B and D).

and then size-fractionated with gel filtration column chromatography (Figure 2A,B). Kv1.4-immunoreactive proteins in CHAPS extract were eluted as a single peak at the position between two molecular mass markers (669 and 440 kDa), whereas those in Zwittergent extract were eluted near the 158 kDa marker (Figure 2A). Thus, most of the endogenously expressed Kv1.4 proteins are high-molecular mass multimeric forms in GH_3 cells. This was also the case for Kv1.5 protein; all the detectable Kv1.5-immunoreactive protein in CHAPS extract was eluted at the position between the two larger size markers in either control untreated cells or dexamethasone-treated cells (1 μM, 24 h, Figure 2B). Therefore, basal and dexamethasone-induced Kv1.5 proteins are present as high-molecular mass complexes. Thus, the endogenously expressed Kv1 subfamily channel subunits multimerize efficiently.

Dexamethasone-Induced Kv1.5 Channel Subunits Mostly Form Homomeric Channels. PCR analysis suggested that Kv1.4 and Kv1.5 were the only members of the Kv1 subfamily channel subunits found in GH_3 cells (Meyerhof et al., 1992). Our RNA blot hybridization also failed to detect other Kv1 subunit messages with the exception of a

very low level of Kv1.3 mRNA (<5% of Kv1.4 or Kv1.5 mRNA estimated with the probe lengths and film exposure times, data not shown). Thus, the vast majority of Kv1.4 and Kv1.5 channel subunits are present as either homomeric channels or Kv1.4/Kv1.5 heteromeric channels. The fact that only two Kv1 channel subunits are abundant, that these channel subunits multimerize efficiently, and that the multimeric complexes are stable during solubilization and immunoprecipitation allowed us to quantitate separately homomeric and heteromeric channels in GH_3 cells.

First, Triton extracts were prepared from control untreated and dexamethasone-treated (1 μM for 24 h) GH_3 cells and subjected to immunoprecipitation with anti-Kv1.5 antibody. Anti-Kv1.5 antibody-Sepharose should immunoprecipitate all the channels containing Kv1.5 subunits. Thus, finding an appreciable amount of Kv1.4 protein in the supernatant would be indicative of the presence of Kv1.4 homomeric channels. However, we found that anti-Kv1.5 antibody-Sepharose effectively and proportionally co-immunoprecipitated Kv1.4 proteins ($N \geq 4$ for each condition, Figure 3A,B). Amounts of anti-Kv1.5 antibody-Sepharose which depleted ~85% of Kv1.5 protein from the extract (100−200 μL of the antibody-Sepharose) co-immunoprecipitated Kv1.4 proteins in nearly identical percentages. In contrast, Kv2.1 immunoreactivity (Figure 3A) as well as other proteins judged by protein staining (data not shown) did not decrease in the supernatant, indicating that the immunoprecipitation was specific. Thus, at the limitation of detection, it appears that all of Kv1.4 proteins are associated with Kv1.5 subunits in GH_3 cells.

A similar strategy was used to measure Kv1.5 homomeric channels. Channels containing Kv1.4 subunits were removed with anti-Kv1.4 antibody, and remaining Kv1.5 proteins in the supernatant were measured. Under the conditions where almost all the detectable Kv1.4 proteins were removed from the extract (0.2−0.4 μg of anti-Kv1.4 IgG), significant Kv1.5-immunoreactive protein still remained in the supernatant (Figure 3C,D). Densitometric measurement of the immunoblots revealed that ~30% of total Kv1.5 protein was present in association with Kv1.4 proteins in control cells (see the Figure 3 legend for the estimation). Dexamethasone treatment (1 μM, 24 h) only slightly increased Kv1.4 protein-associated Kv1.5 protein (~40% of the total control Kv1.5 protein). In contrast, the steroid treatment doubled the amount of Kv1.5 homomeric channel protein. These results suggest that the native Kv1.5 protein is present as Kv1.4/Kv1.5 heteromeric and Kv1.5 homomeric channels and that most of the dexamethasone-induced Kv1.5 protein forms homomeric channels. Hence, selective induction of channel subunit expression can differentially influence amounts of homomeric and heteromeric channels.

Dexamethasone Increases Cell Surface Kv1.5 Channel Expression. We determined the effect of dexamethasone treatment on channel subunit assembly. However, it was possible that those homomeric and heteromeric channels might be differentially transported to the cell surface to form functional channels. To correlate dexamethasone-induced changes in homomeric and heteromeric channels to electrical activity, we measured cell surface expression of channel subunits. We used mild oxidation and biotinylation of cell surface glycoproteins, followed by isolation of the biotinylated proteins with streptavidin-Sepharose (Deal et al., 1994; Lantz & Holmes, 1995), for separation of surface K^+ channel proteins. Total cellular and the isolated cell surface proteins were then examined for Kv1.4 and Kv1.5 proteins by immunoblot analysis (Figure 4A,B). The isolation procedure recovered two distinct sizes of Kv1.4 proteins in the cell surface preparation, with a slight increase in the ratio of the larger band (96 kDa) to the smaller band (82 kDa). In control cells, the cell surface glycoprotein isolation yielded ~30% of the total cellular 96 kDa Kv1.4 protein ($N = 4$ for each preparation). Although the isolation procedure may not have been completely efficient, increasing the concentration of reagents or the amount of avidin-Sepharose did not increase this value. Nevertheless, the presence of substantial cell surface Kv1.4 proteins along with our finding that nearly all Kv1.4 proteins are associated with Kv1.5 protein indicates that heteromeric channels are expressed at the plasma membrane of GH_3 cells.

A significant amount of Kv1.5 protein was also detected in the cell surface preparation. In control cells, ~25% of the total cellular Kv1.5 protein was found in the cell surface preparation. Dexamethasone treatment proportionally increased total cellular and cell surface Kv1.5 proteins by 74 ± 28 and 68 ± 34% (the mean ± SE, $N = 4$ for each preparation), respectively. In contrast, dexamethasone treatment did not affect total cellular or cell surface Kv1.4 proteins. Thus, the steroid increases expression of Kv1.5 protein at the plasma membrane without significantly affecting Kv1.4 proteins. Taken together with the results in the previous sections and our previous electrophysiological data (Takimoto et al., 1993), it is likely that dexamethasone preferentially up-regulates cell surface expression of Kv1.5 homomeric channel.

DISCUSSION

In the present study, the subunit composition of endogenous Kv1 subfamily voltage-gated K^+ channels in GH_3 cells was examined. These cells constitutively express the two Kv1 subfamily genes, Kv1.4 and Kv1.5 (Meyerhof et al., 1992; Takimoto et al., 1993, 1995), whose homomeric channels carry transient and delayed rectifier K^+ currents, respectively. We report that specific up-regulation of Kv1.5 protein expression by dexamethasone (Takimoto et al., 1993) doubled the expression of Kv1.5 homomeric channels. In contrast, only a minimal increase in Kv1.4/Kv1.5 heteromeric channels was detected. The steroid also similarly increased total cellular and cell surface Kv1.5 protein without affecting Kv1.4 protein in either preparation. The presented results do not completely rule out the possibility that the glucocorticoid agonist might cause a small change in cell surface Kv1.4 protein. Also, the steroid might slightly alter the ratio of the two channel subunits in plasma membrane. However, the most likely explanation for our results is that dexamethasone preferentially increases the number of functional Kv1.5 homomeric channels in the cell surface. This is also supported by our previous finding (Takimoto et al., 1993) that the steroid significantly increases delayed rectifier K^+ current density in GH_3 cells. Hence, changing the expression of one K^+ channel subunit gene differentially affects homomeric and heteromeric channels, leading to specific alteration of electrical properties.

Our results indicate that almost all the detectable Kv1.4 and Kv1.5 subunits are present as high-molecular mass multimeric forms in GH_3 cells. We also found no significant

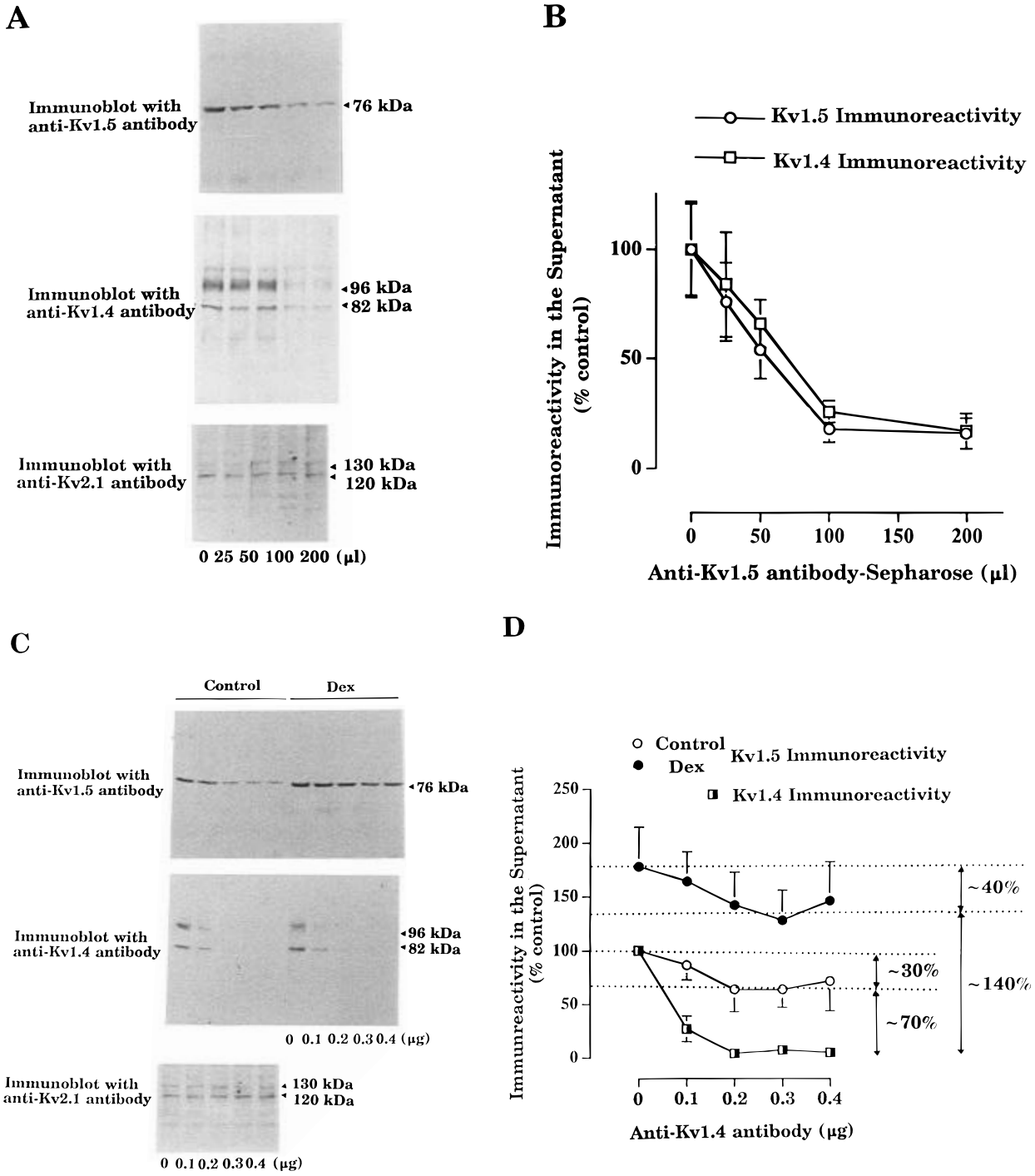

FIGURE 3: Dexamethasone differentially affects amounts of homomeric and heteromeric channels in GH_3 cells. Triton extracts were prepared from GH_3 cells untreated (Control) or treated with 1 μM dexamethasone for 24 h (Dex). Immunoprecipitation was performed with either anti-Kv1.5 antibody-Sepharose (A and B) or anti-Kv1.4 antibody (C and D). Total amounts of IgG-Sepharose (A and B) or antibody (C and D) were kept constant by adding control IgG-Sepharose or antibody. The supernatant of each immunoprecipitation was examined for channel proteins by immunoblot analysis (A and C). Densitometric measurement of 76 kDa Kv1.5 and 96 kDa Kv1.4 immunoreactivities was used to quantitate channel subunit immunoreactivities (C and D). Each point and error bar indicate the mean and standard error compared to precipitation with control IgG precipitation in control untreated cells ($N \geq 4$ for each condition): open circles, Kv1.5 immunoreactivity in control untreated cells; filled circles, Kv1.5 immunoreactivity in dexamethasone-treated cells; open squares, Kv1.4 immunoreactivity in control untreated cells; and filled squares, Kv1.4 immunoreactivity in dexamethasone-treated cells. In panel D, Kv1.4 immunoreactivities in control and dexamethasone-treated cells were overlapped and are presented as half-filled squares. Dexamethasone treatment increased total cellular Kv1.5 immunoreactivity to 184 $\pm$ 31% of control (the mean $\pm$ SE). Because the three amounts of anti-Kv1.4 antibody (0.2, 0.3, and 0.4 μg) removed $\geq$95% of Kv1.4 immunoreactivity from the extract, the averages of Kv1.5 immunoreactivity obtained with the three anti-Kv1.4 antibody amounts were used to estimate Kv1.5 protein associated and unassociated with Kv1.4 (dotted lines in D). Thus, the differences between these lines indicate amounts of Kv1.5 unassociated (~70% for Control and ~140% for Dex) and associated (~30% for Control and ~40% for Dex) with Kv1.4 proteins.

monomers of Kv1.4 or Kv1.5 subunit protein in transfected CHO cells. These findings are consistent with previous *in vitro* translation (Shen et al., 1993) and heterologous expression studies (Deal et al., 1994). Therefore, the tetramerization of endogeous and exogenous K^+ channel α subunits is rapid and efficient, forming a striking contrast to

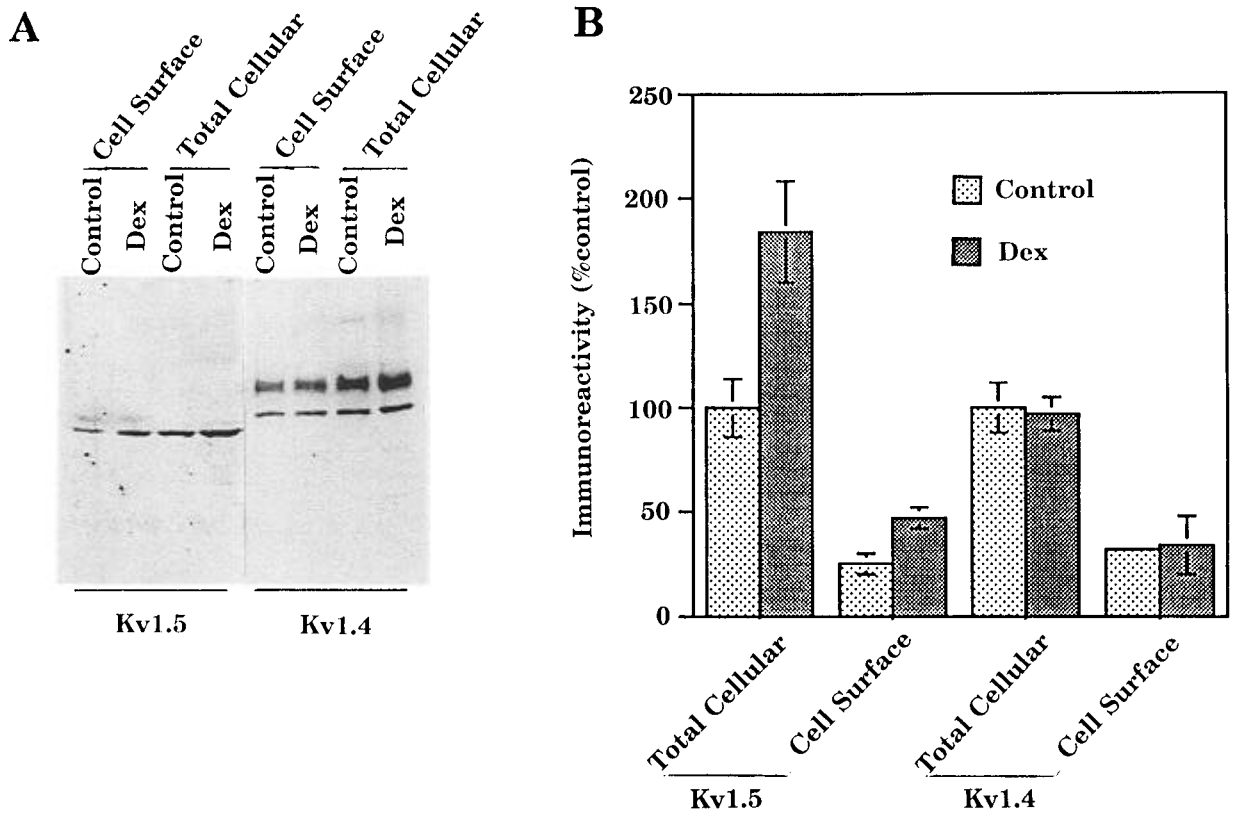

FIGURE 4: Dexamethasone increases cell surface expression of Kv1.5 protein in GH_3 cells. Cell surface-glycosylated proteins were isolated from cells untreated (Control) or treated with 1 μM dexamethasone for 24 h by mild oxidation and biotinylation of glycosyl residues, followed by precipitation with streptavidin-Sepharose. Total cellular and the isolated cell surface preparations were examined for Kv1.4 and Kv1.5 immunoreactivities (A). Densitometric measurements of 76 kDa Kv1.5 protein and 96 kDa Kv1.4 protein were used to quantitate amounts of channel subunits (B). $N = 4$ for each treatment.

the pentamer formation of nicotinic acetylcholine receptor subunits (Green & Millar, 1995).

While heterologous expression experiments have demonstrated that Kv1 subfamily channel subunits encoded by distinct genes can be assembled to form heteromic channels, some groups have found differential distribution of channel subunit-immunoreactive proteins in native single cells (Mi et al., 1995; Klumpp et al., 1995). In rat Schwann cells, Kv1.5 immunoreactivity was found in the cell membrane, whereas Kv1.1 immunoreactivity was seen in the perinuclear, intracellular compartment (Mi et al., 1995). Likewise, Kv1.1, Kv1.2, and Kv1.3 immunoreactivities were detected in distinct subcellular sites of mouse rod bipolar cells (Klumpp et al., 1995). Thus, formation or stabilization of homomeric, but not heteromeric, channels might preferentially occur in these cells. In contrast to these observations, we found significant Kv1.4/Kv1.5 heteromeric channels in GH_3 cells. Moreover, our findings that almost all the detectable Kv1.4 proteins are associated with Kv1.5 protein and that significant Kv1.4 proteins are detected in the cell surface exclude the possibility that heteromeric channels are not expressed at the plasma membrane. Hence, both endogenous homomeric and heteromeric channels are efficiently formed and transported to the plasma membrane in GH_3 cells.

Our results suggest that the synthesis ratio of Kv1.4 and Kv1.5 subunits might control channel subunit compositions in GH_3 cells. The determined ratios of distinct subunit compositions in control and dexamethasone-treated cells can be accounted for by random assembly of channel subunits; if Kv1.5 subunits are in excess over Kv1.4 subunits, almost all Kv1.4 subunits would associated with Kv1.5 subunits, and Kv1.5 subunits would also form homomeric channels. Furthermore, up-regulating Kv1.5 subunit synthesis would result in a large increase in Kv1.5 homomeric channels. Finally, because of the large deviation associated with our measurements, we were unable to detect a significant difference in Kv1.5 protein in heteromeric channels between control and the steroid-treated cells. Nevertheless, our results are consistent with the possibility that the steroid might also alter the stoichiometry of channel subunits in heteromeric channels. In other words, increasing Kv1.5 subunit synthesis might shift the ratio of the two subunits in heteromeric complexes. Hence, the synthesis ratio of Kv1 channel α subunits is likely to determine the cell surface expression of multiple channels with distinct subunit compositions in GH_3 cells.

Our findings provide new insights into the mechanisms that govern the assembly and functional expression of native K^+ channels. First, our results suggest that one K^+ channel α subunit gene product can participate in forming both homomeric and heteromeric channels in single cells. We found both channel forms in the cell surface. Thus, they both are likely to be functional. Second, our results indicate that hormone regulation of one K^+ channel subunit gene can differentially influence expression of homomeric and heteromeric channels. Thus, unlike voltage-gated Na^+ or Ca^+ channels, each of whose pore-forming subunits is encoded by single gene products, electrophysiological consequences of altering the expression of a K^+ channel subunit are particularly complex. We also suggest that the effect of

regulating K^+ channel subunit expression depends on context. For example, we have previously found that glucocorticoids up-regulate Kv1.5 gene expression in several tissues, such as pituitary, heart, and skeletal muscle (Attardi et al., 1993; Takimoto & Levitan, 1994). Because these tissues constitutively express different sets and amounts of Kv1 subfamily genes, the steroid induction of Kv1.5 subunit may result in tissue-specific changes in channel subunit compositions. Finally, many stimuli, including hormones, neurotransmitters, and electrical activity, regulate expression of K^+ channel proteins (Takimoto et al., 1993, 1995; Levitan et al., 1995). Our previous results suggest that these effects are rapid, because K^+ channel proteins can turn over within hours (Takimoto et al., 1993). Hence, regulation of K^+ channel subunit gene expression may be a general mechanism for producing dramatic changes in the structure and function of these multimeric proteins.

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