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Biochemistry

Cloning and expression of GRK5: A member of the G protein-coupled receptor kinase family

(receptor desensitization/regulation/phosphorylation)

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ABSTRACT **Guanine nucleotide binding protein (G-protein)-coupled receptor kinases (GRKs) specifically phosphorylate the agonist-occupied form of G-protein-coupled receptors such as the β_2-adrenergic receptor and rhodopsin. The best characterized members of this family include the β-adrenergic receptor kinase (βARK) and rhodopsin kinase. To identify additional members of the GRK family, the polymerase chain reaction was used to amplify human heart cDNA using degenerate oligonucleotide primers from highly conserved regions unique to the GRK family. Here we report the isolation of a cDNA that encodes a 590-amino acid protein kinase, termed GRK5, which has 34.8% and 47.2% amino acid identities with βARK and rhodopsin kinase, respectively. Interestingly, GRK5 has an even higher homology with *Drosophila* GPRK-2 (71.0% identity) and the recently identified human IT11 (69.1% identity). Northern blot analysis of GRK5 with selected human tissues reveals a message of ≈3 kilobases with highest levels in heart, placenta, lung > skeletal muscle > brain, liver, pancreas > kidney. GRK5, overexpressed in Sf9 insect cells using the baculovirus system, was able to phosphorylate rhodopsin in a light-dependent manner. In addition, GRK5 neither contains a consensus sequence for isoprenylation like rhodopsin kinase nor is activated by G-protein $\beta\gamma$ subunits like βARK1. Thus, GRK5 represents a member of the GRK family that likely has a unique physiological role.**

Guanine nucleotide binding protein (G protein)-coupled receptors represent a diverse family of cell-surface proteins that transduce the binding of extracellular ligands (hormones, neurotransmitters, odorants, light, etc.) into intracellular signaling events (1, 2). Two of the best characterized G-protein-coupled receptors are the hormone-responsive β_2-adrenergic receptor (β_2AR), which mediates catecholamine stimulation of adenylyl cyclase, and the visual "light receptor" rhodopsin, which mediates phototransduction in retinal rod cells (3, 4). The β_2AR and rhodopsin share many structural and functional similarities including a conserved protein topology (e.g., seven transmembrane domains) as well as an ability to specifically interact with G proteins upon activation. The similarities between these receptors also extend to mechanisms involved in receptor regulation (3–5). In both systems, rapid receptor desensitization or loss of responsiveness is promoted by phosphorylation of the receptor. This phosphorylation is mediated by protein kinases that have the unique ability to recognize and phosphorylate their receptor substrates only when they are in their active conformations—i.e., when they have been stimulated and/or occupied by appropriate agonist ligands (6). The β-adrenergic receptor kinase (βARK) (7, 8) and rhodopsin kinase (9, 10) have been identified as the kinases involved in the agonist-specific phosphorylation of the β_2AR and rhodopsin, respectively. Additional lines of evidence suggest that other G-protein-coupled receptors may also be regulated by similar mechanisms. These receptors include the m2 muscarinic cholinergic (11, 12) and α_2-adrenergic receptors (13, 14), which inhibit adenylyl cyclase; the type α mating factor receptor of the yeast *Saccharomyces cerevisiae* (15); and the chemotactic cAMP receptor of the slime mold *Dictyostelium discoideum* (16, 17).

Sequence information on G-protein-coupled receptor kinases (GRKs) was initially provided by the isolation of a cDNA encoding bovine βARK (18). βARK is a protein of 689 amino acids (79.6 kDa) containing a central protein kinase catalytic domain flanked by large N- and C-terminal domains. Additional members of the GRK family were subsequently cloned including bovine βARK2 (19), bovine rhodopsin kinase (20), *Drosophila* kinases GPRK-1 and GPRK-2 (21), and the recently identified human IT11 (22). Common features of these kinases include a centrally localized catalytic domain of ≈240 amino acids, which shares significant amino acid identity (46–95%), an N-terminal domain of 161–197 amino acids (except for GPRK-2), and a variable length C-terminal domain of 100–263 amino acids.

The cloning of βARK, rhodopsin kinase, and additional members of the GRK family raises some interesting questions concerning the size of this gene family as well as the substrate specificities of the individual members. In this study, we have used PCR with degenerate oligonucleotide primers generated from highly conserved regions unique to the GRK family in an attempt to identify additional members of this family. Here we report the cloning, expression, and initial characterization of GRK5, a newly described member of the GRK gene family.†

MATERIALS AND METHODS

Materials. A human multiple tissue Northern blot and the BacPAK baculovirus expression system were purchased from Clontech. Wild-type *Spodoptera frugiperda* (Sf9) cells were obtained from American Type Culture Collection. A human heart cDNA library was obtained from Stratagene. Tissue culture reagents were purchased from Gibco and Sigma while frozen bovine retinas were from Hormel. *Taq* polymerase was purchased from Promega. Restriction endonucleases and other molecular biology reagents were from Boehringer Mannheim. [α-^{32}P]dCTP, dATP[α-^{35}S], and [γ-^{32}P]ATP were purchased from NEN.

PCR. PCR mixtures initially contained 200 ng of DNA template (human heart cDNA), 100 pmol of oligonucleotide primers, 50 mM KCl, 10 mM Tris·HCl (pH 8.4), 1.5 mM

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Abbreviations: βARK, β-adrenergic receptor kinase; G protein, guanine nucleotide binding protein; GRK, G-protein-coupled receptor kinase; ORF, open reading frame; ROS, rod outer segment(s).

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. L15388).

$MgCl_2$ in a total reaction vol of 25 μl. The samples were heated to 99°C for 5 min, cooled on ice for 3 min, and then heated to 72°C followed by the addition of 200 μM dNTPs and 2 units of *Taq* polymerase. The samples were then denatured at 95°C for 1 min, annealed at 40°C for 1 min, and extended at 72°C for 3 min for 5 cycles. This was followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 3 min. The sense PCR primer used was 5′-ACIGGIAARATGTAYGC-3′ encoding the amino acid sequence T(G/L)KMYA, while the antisense PCR primer was 5′-YTCIGGIGCCATRWAIC-3′ encoding G(Y/F)MAPE (I, inosine; R, A or G; Y, T or C; W, T or A).

Isolation of a Full-Length cDNA Clone. To isolate a full-length cDNA, the ≈450-bp PCR product was labeled with [^{32}P]dCTP by random priming and then used to probe a human heart cDNA library in Lambda ZAP (Stratagene). The cDNA library was plated at a density of 50,000 plaque-forming units per 150-mm dish (10^6 total clones), transferred to nitrocellulose filters, and hybridized with the labeled PCR probe for 48 hr at 37°C in buffer containing 25% formamide, 5× saline sodium citrate (SSC), 5× Denhardt's solution, 1% SDS, 0.1% sodium pyrophosphate, and 100 μg of denatured salmon sperm DNA per ml. The filters were initially washed in 2× SSC/0.1% SDS at 50°C for 1 hr followed by a high-stringency wash in 0.1× SSC/0.1% SDS at 65°C for 1 hr. The six clones identified by this procedure were isolated by repeated plating and screening with the labeled PCR product. The isolated clones were then rescued with a helper phage to yield the cDNAs as inserts in pBluescript SK. All six clones were restriction mapped and sequenced by the dideoxynucleotide chain-termination technique (23) using T3 and T7 primers and oligonucleotide primers synthesized to known regions of the sequence.

Northern Blot Analysis. Tissue distribution of the mRNA for GRK5 was analyzed on a human multiple tissue Northern blot containing 2 μg of poly(A)$^+$ RNA from human heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas. A 1157-bp *Pst* I open reading frame (ORF) fragment (bp 383–1540) from pGRK5 was isolated and labeled with [^{32}P]dCTP by random priming to a specific activity of 3.5×10^9 cpm/μg. This probe was hybridized with the human multiple tissue Northern blot for 24 hr at 42°C in a buffer containing 5× SSPE, 10× Denhardt's solution, 100 μg of salmon sperm DNA per ml, 50% formamide, and 2% SDS. The blot was washed with 2× SSC/0.1% SDS at room temperature for 1 hr followed by a high-stringency wash with 0.1× SSC/0.1% SDS at 65°C for 1 hr.

Expression of GRK5 Using the Baculovirus System. The GRK5 ORF was initially excised by restriction digestion of the full-length clone pGRK5 using *Nae* I and *Sma* I. The resulting 1906-bp fragment, including 27 bp of 5′ untranslated and 109 bp of 3′ untranslated sequence flanking the 1770-bp ORF, was isolated and ligated into the blunt-ended *Bam*HI site of pBacPAK1 to generate the construct pBacPAK-GRK5.

Monolayers of Sf9 cells (3×10^6 cells) were cotransfected with 1 μg of the pBacPAK-GRK5 construct and 0.25 μg of *Bsu*36I-digested BacPAK6 viral DNA using the calcium phosphate precipitation technique (24). The cells were incubated for 4 hr at 27°C and the medium was then replaced with complete medium (TNM-FH; 10% fetal bovine serum/0.25 mg of Fungizone per ml/50 μg of streptomycin per ml/50 μg of penicillin per ml). The cells were incubated for 6 days at 27°C. To obtain the isolated recombinant virus, 4×10^6 cells were plated on a 35-mm dish and the cells were overlaid with 1.5 ml of the diluted viral stock from the transfection. After 1.5 hr, the virus was removed and the cells were overlaid with 5 ml of 1% low-melting-point agarose in complete medium. The plates were incubated at 27°C and isolated plaques were observed after 4–6 days. To confirm the presence of the GRK5 cDNA in the recombinant virus, six isolated viruses were amplified and the viral DNA was extracted and analyzed by restriction digestion and Southern blot hybridization. One of these recombinant viruses was chosen for further amplification and characterization of the expressed kinase.

For the purpose of preliminary characterization of GRK5 and to compare it with βARK, the respective recombinant baculoviruses were used to infect a monolayer of Sf9 cells in a 100-mm dish. After a 48-hr infection, the cells were rinsed with phosphate-buffered saline and harvested by scraping and homogenizing in 1.5 ml of ice-cold buffer containing 20 mM Hepes (pH 7.2), 10 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 20 μg of leupeptin per ml, 200 μg of benzamidine per ml, 250 mM NaCl, and 0.02% Triton X-100. The cells were lysed with a Brinkmann tissue disrupter (30 sec at 30,000 rpm) and were centrifuged at 40,000 × *g* for 20 min. The supernatants were then assessed for the ability to phosphorylate urea-treated rod outer segments (ROS).

Preparation of G Protein βγ Subunits. The GTP binding proteins G_o and G_i were purified from bovine brain by successive chromatography on DEAE-Sephacel, Sephacryl S200, and heptylamine-Sepharose as described (25). The G-protein preparation, consisting primarily of G_i and G_o, was further purified by chromatography on a Mono Q column and then stored in 20 mM Tris·HCl, pH 8.0/1 mM EDTA/1 mM dithiothreitol/250 mM NaCl/0.05% Lubrol (buffer A) at −80°C. The βγ subunits were isolated by chromatography of the purified G proteins on heptylamine-Sepharose in the presence of AMF (30 μM $AlCl_3$/6 mM $MgCl_2$/10 mM NaF) (26). The AMF and cholate were removed from the βγ subunit preparation by anion-exchange chromatography on a Mono Q column.

Receptor Phosphorylation. Urea-treated ROS were prepared as described (27, 28). Phosphorylation reaction mixtures contained 5 μg of total protein from the Sf9 cell supernatants expressing either GRK5 or βARK, urea-treated ROS (80 pmol of rhodopsin), 20 mM Tris·HCl, pH 7.5/2 mM EDTA/6 mM $MgCl_2$/0.1 mM [γ-^{32}P]ATP, in a total reaction vol of 30 μl. When the effect of G-protein βγ subunits was assessed, the reaction mixtures also contained 0.25 mM dithiothreitol, 0.01% Lubrol, and 62 mM NaCl with or without 100 nM βγ subunits. Reaction mixtures were incubated at 30°C for 15 min and then stopped by the addition of 50 μl of SDS sample buffer. Samples were electrophoresed on a homogeneous SDS/10% polyacrylamide gel by the method of Laemmli (29). Gels were dried and autoradiographed at room temperature for 30–120 min. The rhodopsin bands in the gel were then cut and assayed in a scintillation counter.

RESULTS AND DISCUSSION

To initiate the search for additional members of the GRK family, degenerate oligonucleotide primers were designed to encode several highly conserved amino acid stretches found in all of the GRKs. These oligonucleotides were then used as primers in a PCR (30) with template DNA prepared from a human heart cDNA library. One set of primers yielded a PCR product of the expected size (≈450 bp) as assessed by electrophoresis on a 2% agarose gel. The 450-bp DNA product was then restriction digested with *Eco*RI and *Hin*dIII (sites present in the two PCR primers), subcloned into *Eco*RI/*Hin*dIII-digested pBluescript KS and sequenced using T3 and T7 primers. Of the 20 clones sequenced, 13 were identical and had 54% amino acid identity with bovine βARK, 64% identity with bovine rhodopsin kinase, and 83% identity with *Drosophila* GPRK-2. Notably, the PCR product also encoded the amino acid sequence DLG, a sequence that is highly conserved in the GRK family. The other 7 clones

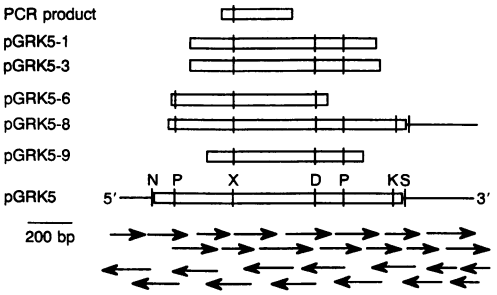

FIG. 1. Restriction map and sequencing strategy for the GRK5 clones. Restriction sites on the human heart pGRK5 clones are indicated for the enzymes *Nae* I (N), *Pst* I (P), *Xho* I (X), *Dra* II (D), *Kpn* I (K), and *Sma* I (S). Clone pGRK5 has an ORF of 1770 bp starting from the first in-frame ATG. DNA sequencing was performed by the dideoxynucleotide chain-termination technique (23). The extent and direction of the sequence reactions are shown by arrows. ORF is indicated as an open rectangle, while untranslated sequences are indicated as a line.

sequenced had no appreciable homology with the GRK family.

The GRK-related PCR product was then labeled with [^{32}P]dCTP by random priming and used as a probe to screen the human heart cDNA library. Six different cDNA clones were isolated by this method. The relative lengths, partial restriction maps, and sequencing strategy for these clones are shown in Fig. 1. Of the six clones isolated, pGRK5-1 and pGRK5-3 encode an ≈1.5-kb ORF fragment, while pGRK5-6 and pGRK5-9 encode ≈1.1 kb of the ORF. The clone pGRK5-8 is ≈2.2 kb long and appears to encode the 3′ end of the ORF as well as the entire 3′ untranslated sequence. The full-length clone pGRK5 is ≈2.55 kb long and contains an ORF of 1770 bp, which is flanked by 220 bp of 5′ untranslated and 567 bp of 3′ untranslated sequence. The 5′ untranslated sequence is relatively G+C-rich (73%), while the 3′ untranslated sequence ends with a poly(A) tail. While pGRK5 does not have a good Kozak consensus sequence (31) for translation initiation (TCAATGG instead of ACCATGG), the comparison of GRK5 with the other known GRKs helped to determine the initiator methionine. The predicted ORF, beginning at the first in-frame ATG and ending at the first in-frame stop codon, encodes a protein of 590 amino acids (Fig. 2).

The predicted molecular mass of GRK5 is 67.7 kDa, with a predicted pI of 8.75. GRK5 contains a centrally located protein kinase catalytic domain of 238 amino acids flanked by N-terminal and C-terminal regions of 193 and 159 amino acids, respectively. Comparison of the amino acid sequence of GRK5 with other known GRKs reveals a high degree of overall homology, particularly in the catalytic domain. Construction of a phylogenetic tree (32) of all the known GRKs demonstrates that GRK5 is most closely related to human IT11 and that GRK5, IT11, GPRK-2, and rhodopsin kinase form a distinct branch of the GRK family as compared to βARK, βARK2, and GPRK-1 (Fig. 3). GRK5 has an overall 69% amino acid identity and 82% similarity with human IT11. The major differences between these proteins include a 33-amino acid gap in IT11 near the N terminus, a divergent

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cagagggaggaagaagcggcggcgcggcggcggcgctcctctttgcagaggggaaactcttgggctgagagcaggaacaacgcggtaggcaaggcgggctgctggctccccggctccgg  122
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                                                                                                  M  E  L  E  N  I  V  A    8

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gctccaagaccagttttaaccaccacatagaaactcaaaccatgtcagctcgaactccaccggaagcagctagtttcggctctggcctccaagtccacagtggaaccagcccagaccctct  2042
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taaatgtagaaagtgatccatacttcaaaaaaaaa  2557
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FIG. 2. Nucleotide and deduced amino acid sequence of human GRK5. Nucleotide sequence of the full-length clone pGRK5 is shown. Nucleotides are numbered on the right beginning at the 5′ end and ending at the 3′ end of clone pGRK5. Predicted amino acid sequence is numbered on the right just below the nucleotide sequence, beginning at the first in-frame ATG (bp 221) and ending just before the first in-frame stop codon (bp 1990). The predicted initiator methionine is flanked by the nucleotide sequence TCAATGG.

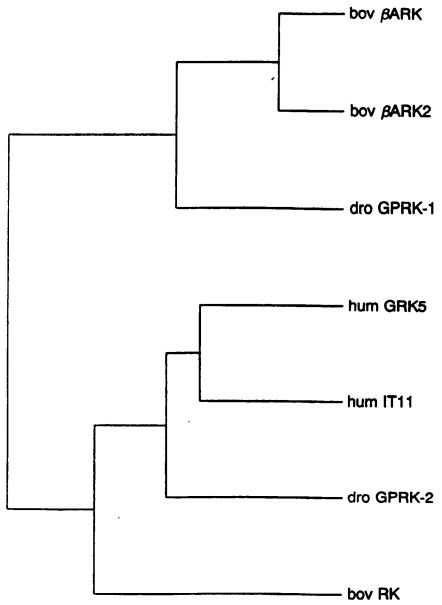

FIG. 3. Comparison of amino acid sequence of GRK5 with related GRKs by dendrogram analysis. The PILEUP program in the Wisconsin Genetics Computer Group software was used to align and compare the amino acid sequences of human (hum) GRK5 and IT11; bovine (bov) βARK, βARK2, and rhodopsin kinase; and *Drosophila* (dro) GPRK-1 and GPRK-2. Overall amino acid identities of GRK5 with the other GPRKs are 69% with human IT11, 71% with *Drosophila* GPRK-2, 47% with bovine rhodopsin kinase, 36% with bovine βARK and βARK2, and 35% with *Drosophila* GPRK-1. The PILEUP program uses a progressive pairwise alignment (32).

stretch in the central portion of the N terminus (only 19% identity and 47% similarity from amino acids 97–149), and a significant difference in the length of the C terminus (100 and 159 amino acids for IT11 and GRK5, respectively). GRK5 also has high homology with *Drosophila* GPRK-2, with 71% amino acid identity and 82% similarity. However, GPRK-2 has a very short N-terminal domain (28 amino acids) compared to all other GRKs (161–197 amino acids). GRK5 also has higher homology to rhodopsin kinase (68% amino acid similarity) than to βARK (58% similarity), in both amino acid identity and size. While the functions of the N-terminal and C-terminal domains of these kinases are largely unknown, the C-terminal tail of rhodopsin kinase is farnesylated, a post-translational modification that appears to be important for its translocation to the disc membrane (33, 34). By comparison, the C-terminal domain of βARK appears to interact with G-protein βγ subunits (35). Overall, GRK5, IT11, rhodopsin kinase, and GPRK-2 have significantly shorter C-terminal tails as compared to βARK, βARK2, and GPRK-1.

The tissue distribution of GRK5 was analyzed by Northern hybridization using a human multiple tissue Northern blot containing 2 μg of poly(A)$^+$ RNA from eight different human tissues. A randomly primed 1157-bp *Pst* I ORF fragment from pGRK5 was labeled with [^{32}P]dCTP and used as a probe. This revealed a message of ≈3.0 kb, with highest levels in the human heart, placenta, lung > skeletal muscle > brain, liver, pancreas > kidney (Fig. 4). This tissue distribution is in contrast to βARK, which has a message of ≈3.8 kb, with highest levels in the brain, skeletal muscle > pancreas > heart, lung, placenta, kidney > liver (unpublished observation). Previous studies have demonstrated that IT11 has highest message levels in testis but is also found at low levels in a wide variety of tissues (22). *Drosophila* GPRK-2 also appears to be expressed in a variety of tissues (21). In contrast, rhodopsin kinase is more specifically localized in the ROS of the retina, with low levels also observed in the

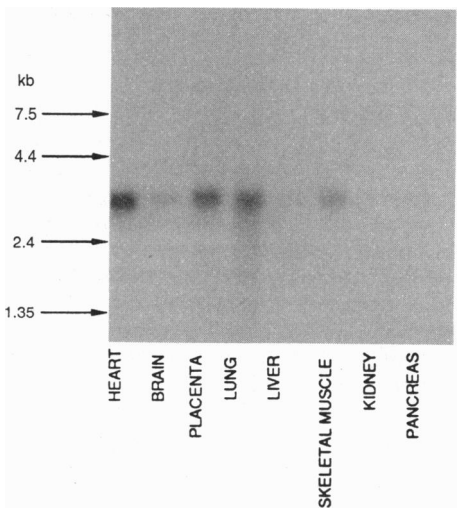

FIG. 4. Northern blot analysis of human mRNA from various tissues. Poly(A)$^+$ RNA (2 μg) from human heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas on a multiple tissue Northern blot was hybridized with a 1157-bp *Pst* I ORF fragment from the clone pGRK5 as described. The blot was washed in 0.1× SSC/0.1% SDS at 60°C followed by autoradiography at −80°C for 24 hr.

pineal gland (20). The rather unique tissue distribution of GRK5, as compared to the other GRKs, suggests that GRK5 likely has a unique substrate specificity and physiological role within the cell.

To initially characterize the activity of GRK5, the ORF from pGRK5 was excised by restriction digestion with *Nae* I and *Sma* I and then subcloned into the baculovirus expression vector pBacPAK1. Viral DNA and the pBacPAK-GRK5 construct were then cotransfected into Sf9 cells to obtain an isolated recombinant virus. The recombinant virus was amplified and then used to infect a monolayer of Sf9 cells. As shown in Fig. 5*A*, GRK5 expressed in Sf9 cells phosphorylates rhodopsin in a light-dependent manner. The ability of GRK5 to phosphorylate rhodopsin was also compared to βARK (Fig. 5*A Right*). Overall, while both GRK5 and βARK phosphorylated rhodopsin well above the basal βARK-like

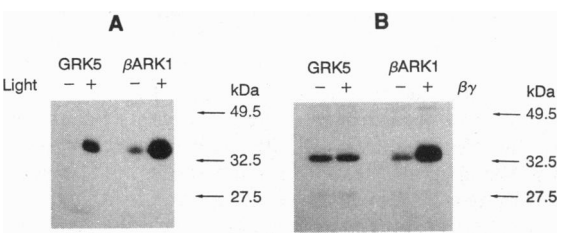

FIG. 5. Expression of GRK5 in Sf9 insect cells. (*A*) GRK5- and βARK-containing recombinant baculoviruses were used to infect a monolayer of Sf9 cells. After a 48-hr infection, the cells were harvested, lysed in 1.5 ml of buffer, and then centrifuged at 40,000 × *g* for 20 min. Supernatants were then used to phosphorylate urea-treated ROS in the presence or absence of room light. Reactions were stopped by addition of 50 μl of SDS sample buffer and samples were then electrophoresed on an SDS/10% polyacrylamide gel. The gel was dried and autoradiographed for 30 min at room temperature and rhodopsin bands were then cut and assayed. The level of light-dependent rhodopsin phosphorylation in this experiment was 7 pmol·min^{-1}·mg^{-1} with supernatant from wild-type Sf9 cells (data not shown), 1120 pmol·min^{-1}·mg^{-1} by Sf9 cells expressing GRK5, and 2470 pmol·min^{-1}·mg^{-1} by Sf9 cells expressing βARK. (*B*) Sf9 cell supernatants were also used to phosphorylate urea-treated ROS in the presence or absence of G-protein βγ subunits. Reactions were stopped followed by electrophoresis and autoradiography for 120 min at room temperature as described above.

activity observed in Sf9 cells, GRK5 was less active than βARK at phosphorylating rhodopsin. When the supernatant fractions from the GRK5- and βARK-infected cells were analyzed by SDS/PAGE and Coomassie blue staining, comparable levels of GRK5 and βARK were observed (data not shown). This suggests that the difference in activity is not due to a difference in the expression levels of the two kinases. However, a more detailed analysis of the substrate specificity of GRK5 and comparison with βARK will have to await further purification of GRK5.

The two most extensively studied GRKs, rhodopsin kinase and βARK, appear to be regulated by different mechanisms. Farnesylation of rhodopsin kinase at its C terminus appears to be important for translocation of rhodopsin kinase to the disc membrane (33, 34). βARK, on the other hand, may associate with membranes via its specific interaction with G-protein βγ subunits (35). Since GRK5 does not contain a consensus sequence for isoprenylation (CAAX) at its C terminus, we tested the ability of G-protein βγ subunits to modulate the activity of GRK5. As previously demonstrated, βARK is significantly activated in the presence of G-protein βγ subunits (Fig. 5*B Right*). In contrast, the ability of GRK5 to phosphorylate rhodopsin is not affected by G-protein βγ subunits (Fig. 5*B Left*). Thus, GRK5 may utilize a novel mechanism of regulation and/or cellular localization as compared to βARK and rhodopsin kinase.

These studies have identified another member of the G-protein-coupled receptor kinase family. In vertebrates, this family presently consists of βARK, βARK2, rhodopsin kinase, IT11, and the newly isolated GRK5. Two *Drosophila* kinases termed GPRK-1 and GPRK-2 have also been identified. Among all the GRKs, rhodopsin kinase appears to be the most appropriately named given its selected tissue distribution and ability to specifically phosphorylate photolyzed rhodopsin (4, 20). Since rhodopsin kinase was the first identified G-protein-coupled receptor kinase (9, 10), it might well also be termed GRK1. By comparison, βARK appears to play a major role in phosphorylation and desensitization of the agonist-activated β_2-adrenergic receptor, although it is a ubiquitous protein whose expression does not correlate with any particular receptor (18, 19). In fact, *in vitro* studies have demonstrated that βARK can phosphorylate numerous receptors including the β_2-adrenergic (7), α_2-adrenergic (13, 14), and m2 muscarinic cholinergic receptors (11, 12). Thus, βARK may well be more appropriately named GRK2 since it was the second member of this family to be identified. The third member of this family βARK2 (19) could be termed GRK3, while the recently identified IT11 (22) could be appropriately represented as GRK4. In this paper, we describe the cloning of GRK5, the fifth member of the GRK family. We propose that additional members of this family be named by using a consecutive numbering system.

At present, nothing is known about the substrate specificity of the *Drosophila* GPRK-2 or the recently identified human GRK, IT11. Thus, GRK5 is the only member of this group of three similar protein kinases so far that has been demonstrated to phosphorylate a G-protein-coupled receptor in a stimulus-dependent manner. The substrate specificity of human GRK5 has yet to be established for receptors other than rhodopsin, due in part to the need to further purify GRK5 as well as the unavailability of purified preparations of other receptors. However, the initial differences between βARK and GRK5 in phosphorylating rhodopsin as well as their distinct tissue distributions suggest that the two kinases have distinct substrate specificities. Future studies will need to involve a more rigorous approach to addressing specificity by both *in vivo* and *in vitro* analyses. The involvement of specific GRKs in stimulus-dependent protein phosphorylation may well serve as a general mechanism for regulating many G-protein-coupled receptors. Addressing questions about the number, diversity, substrate specificity, and biological roles of GRKs will serve as a major focus of future investigations in this area.

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