Activation of Heterotrimeric G Proteins by a High Energy Phosphate Transfer via Nucleoside Diphosphate Kinase (NDPK) B and Gβ Subunits

SPECIFIC ACTIVATION OF Gα BY AN NDPK B-Gβγ COMPLEX IN H10 CELLS*

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Formation of GTP by nucleoside diphosphate kinase (NDPK)1 can contribute to G protein activation in vitro. To study the effect of NDPK on G protein activity in living cells, the NDPK isoforms A and B were stably expressed in H10 cells, a cell line derived from neonatal rat cardiomyocytes. Overexpression of either NDPK isoform had no effect on cellular GTP and ATP levels, basal cAMP levels, basal adenyl cyclase activity, and the expression of Gα and Gγ proteins. However, co-expression of Gα led to an increase in cAMP synthesis that was largely enhanced by the expression of NDPK B, but not NDPK A, and that was confirmed by direct measurement of adenyl cyclase activity. Cells expressing an inactive NDPK B mutant (H118N) exhibited a decreased cAMP formation in response to Gα. Co-immunoprecipitation studies demonstrated a complex formation of the NDPK with Gβγ dimers. The overexpression of NDPK B, but not its inactive mutant or NDPK A, increased the phosphorylation of Gβ subunits. In summary, our data demonstrate a specific NDPK B-mediated activation of a G protein in intact cells, which is apparently caused by formation of NDPK B-Gβγ complexes and which appears to contribute to the receptor-independent activation of heterotrimeric G proteins.

Nucleoside diphosphate kinase (NDPK) catalyzes the transfer of terminal phosphate groups from 5'-triphosphate to 5'-diphosphate nucleotides. In the cell, the major reaction is the phosphate transfer from ATP to other NTPs to maintain the levels of NTPs, especially the relatively high level of GTP. Only a small fraction of cellular NDPK binds to the plasma membrane, where it may serve the synthesis of GTP, required for the activation of G proteins (1–3). An activation of G proteins by NDPK has been disputed for more than 10 years. Although numerous in vitro studies (4–7) have shown G protein activation through the enzymatic activity of NDPK (synthesis of GTP from a nucleoside triphosphate and GDP), the specificity of this phenomenon has been questioned (8, 9). Particularly in the intact cell, where GTP concentrations are in the upper micromolar range, evidence for a mechanism beyond the sole synthesis of GTP appears mandatory to support this hypothesis. On the other hand, we have shown recently (10) that NDPK activates G proteins and regulates adenyl cyclase activity in canine cardiac sarcolemmal membranes. This activation required the catalytic activity of NDPK (synthesis of GTP) but was clearly distinct from the effect of exogenous GTP, suggesting a more direct interaction of NDPK and G proteins.

Evaluation of direct G protein activation through phosphate transfer by NDPK is associated with substantial methodological constraints. Mainly, GDP is released spontaneously from G proteins and may then serve as a free substrate for phosphorylation by the NDPK (8). Approaches to immobilize the bound GDP at the G protein (11) are associated with protein denaturation, which in turn may lead to unspecific protein phosphorylation by the NDPK (12). In addition, structural considerations make an interaction of the NDPK and the guanine nucleotide-binding Gα subunit unlikely. Access of the NDPK to the Gα subunit would require dramatic conformational changes, including the release of Gβγ dimers prior to the phosphorylation step. On the other hand, GTP formation by a phosphate transfer via immediately phosphorylated Gβ subunits has been observed in several tissues (13–18), and a complex formation of NDPK B with Gβγ is reported in the accompanying paper (19). Beyond the molecular mechanisms, the important question of whether NDPK can activate G proteins in intact cells has not been addressed, most likely because of the lack of specific activators and inhibitors of NDPK. To circumvent these problems, we took a pragmatic approach by co-expressing the human NDPK isoforms A (nm23-H1) and B (nm23-H2) and the α subunit of the Gα protein (Gα) and measured the effect on cAMP synthesis and phosphorylation of Gβ. We report that the Gα-mediated stimulation of cAMP formation increased with the expression level of NDPK B but not NDPK A. Moreover, evidence is provided that the increase in cAMP formation is dependent on the catalytic activity of the NDPK B isoform and a complex formation with Gβγ.

EXPERIMENTAL PROCEDURES

Cell Culture and Preparation of Cell Lysates and Membranes—Neonatal rat heart myocytes, immortalized with a temperature-sensitive SV40 T antigen (H10 cells) (20), were cultured at 33 °C in DMEM supplemented with 10% fetal calf serum, 2 mM l-glutamine, and 10 μg/ml gentamycin. For cell lysis, cells were washed twice with ice-cold phosphate-buffered saline, scraped off in Buffer A (10 mM Tris-HCl, pH 7.2) and...
In H10 cells either the EGFP or one of the listed isoforms of NDPK were stably expressed. C1, C2, and C3 differ in their expression level of NDPK B (nm23-H2). M118 expresses the catalytically inactive H118N mutant of nm23-H2. CH1 denotes the NDPK A (nm23-H1)-expressing cell line. Basal adenylyl cyclase (AC) activity was determined in the absence of Mg\(^{2+}\) and guanine nucleotides and with 10 mM free Mn\(^{2+}\) as co-substrate. ND, not detected.

### TABLE I

| Transfected cDNA | H10 | C-GFP | C1 | C2 | C3 | M118 | CH1 |
|------------------|-----|-------|----|----|----|------|-----|
| NDPK activity\(a\) |     |       |    |    |    |      |     |
| cell lysate      |     |       |    |    |    |      |     |
| 449.4 ± 107.7    |     |       |    |    |    |      |     |
| 455.5 ± 32.1     |     |       |    |    |    |      |     |
| Basal AC activity\(b\) |     |       |    |    |    |      |     |
| membrane         |     |       |    |    |    |      |     |
| 29.4 ± 5.0       |     |       |    |    |    |      |     |
| 28.5 ± 2.0       |     |       |    |    |    |      |     |
| 
| NDPK B\(b\)      |     |       |    |    |    |      |     |
| 0.1              |     |       |    |    |    |      |     |
| 0.1              |     |       |    |    |    |      |     |
| Basal AC activity\(b\) |     |       |    |    |    |      |     |
| 50.2 ± 7.4       |     |       |    |    |    |      |     |
| 53.3 ± 4.3       |     |       |    |    |    |      |     |
| 52.4 ± 4.3       |     |       |    |    |    |      |     |
| NDPK B\(b\)      |     |       |    |    |    |      |     |
| 1.1              |     |       |    |    |    |      |     |
| 1.1              |     |       |    |    |    |      |     |
| Basal Ac activity |     |       |    |    |    |      |     |
| 41.5 ± 4.4       |     |       |    |    |    |      |     |
| 41.6 ± 3.1       |     |       |    |    |    |      |     |
| 24.5 ± 7.7       |     |       |    |    |    |      |     |

| ATP\(b\) |     |       |    |    |    |      |     |
| 8.2 ± 1.5       |     |       |    |    |    |      |     |

| ATP/GTP |     |       |    |    |    |      |     |
| 5.6     |     |       |    |    |    |      |     |

7.4, 0.1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride), and homogenized with two 10-s bursts applied by a Polytron (Kinematica) at a setting of 20,000 rpm. Cell lysates were then centrifuged at 100,000 x g for 30 min. Pellets were resuspended and centrifuged three more times in Buffer A to obtain the membrane fraction.

### Subcloning of Human NDPK A, NDPK B, and NDPK B H118N

Original cDNA clones were obtained from Dr. N. Kimura, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan and Dr. M.-L. Lacomb, Facula de Medicine Saint-Antoine, Paris, France (NDPK B H118N in pCDNA3). After verifying the cDNA sequences by automated DNA sequencing, specific primers with 5'-EcoRI restriction sites were designed for amplification of NDPK A and NDPK B coding sequences (NDPK A, 5'-AGGAGAATCTGACCTTCAATCAATGACAAGCAG and 5'-ACTGATTACCATCGATAGTACGATGTTTCCT-3'; NDPK B, 5'-AGGATATATCTCTTCTGTCTCCAGC-3' and 5'-ACTGAAATGATGTTTCCTGTCCAGC-3'). The PCR products were cleaved with EcoRI, purified by gel extraction, and then ligated in EcoRI-linearized pRSETrpu vector (Clontech). The NDPK B H118N cDNA was isolated from pcDNA3 vector by an EcoRI/XhoI digest. The cohesive ends were blunted with Klenow fragment and ligated into the EcoRV-linearized pRSETrpu vector. The constructs were confirmed by restriction digestion analysis and automated DNA sequencing.

### Stable Transfection of H10 Cells

The day before transfection, H10 cells were seeded at a density of 7 x 10⁵ cells per 60-mm dish in 5 ml of DMEM with 10% fetal calf serum. For transfection, 8 pmol of DNA of pcDNA3 vector by an pIRESpuro vector (Clontech). The NDPK B H118N in pcDNA3). After verifying the cDNA sequences by automated DNA sequencing.

### Western blotting

Western blotting—5 to 20 µg of membrane-enriched or cytosolic fractions were suspended in SDS sample buffer for SDS-PAGE. The separated proteins were transferred electrophoretically to nitrocellulose membranes, and immunodetection was carried out using antibodies against Gₛα and anti-NDPK (T-20; Santa Cruz Biotechnology, Inc.) antibodies.

### Measurement of cAMP Levels

Cells were cultured in 12-well plates and serum-starved for 4 h. Accumulation of cAMP was assayed in serum-free medium containing 20 mM HEPES, pH 7.4, 100 µM propranolol, and 1 mM isobutylmethylxanthine (IBMX) for 30 min at 33°C. Then, the medium was removed, and 400 µl of ice-cold 0.1 N HCl were added. Lysates were centrifuged for 15 min at 4°C and 20,000 x g, and 100 µl of the supernatant were used for the competitive enzyme immunoassay for cAMP, according to the manufacturer's protocol (R&D Systems). The cell pellets were neutralized with 0.1 N NaOH and used for the determination of protein concentrations with the Bradford Bio-Rad dye-binding assay and bovine serum albumin as standard.

### Enzymatic Activities

Adenylyl cyclase activity was determined by measuring the conversion of [α-32P]ATP to [α-32P]cAMP (21). The assay volume was 100 µl containing 0.1 mM ATP with 0.5-5 x 10⁶ cpm [α-32P]ATP (3000 Ci/mmol), 3 mM MgCl₂ or 11 mM MnCl₂, 0.1 mM cAMP, 1 mM IBMX, 1 mM EDTA, 0.5 mM dithiothreitol, and 75 mM triethanolamine hydrochloride, pH 7.6. The membranes (10-25 µg of protein) were pre-incubated with alamethicin for 20 min at 4°C in a 1:1 ratio (w/w) to unmask latent adenylyl cyclase activity. This peptide ionophore increases the accessibility of substrates to the adenylyl cyclase in sealed membrane vesicles without affecting the functional coupling to receptors (22). The adenylyl cyclase reaction was started by the addition of membrane protein and conducted for 10 min at 37°C.

Under these conditions, enzyme activity was stable during the entire incubation period.

### NDPK Activity

NDPK activity was determined, using [3H]GDP (100 µCi, 0.1 Ci/mmol) as substrate, under the conditions used for measurement of adenylyl cyclase activity, with 0.2-1 µg of protein and incubation for 10 min at 37°C. Reactions were stopped by the addition of 5 µl of 10% (w/v)
SDS. Aliquots of 10 μl (in 2-μl steps) were spotted onto polyethyleneimine cellulose F thin layer chromatography plates (E. Merck, Darmstadt, Germany). A mixture of GTP/GDP/GMP (3 mM each, 10 μl) was run in parallel and used as marker. The nucleotides were identified under UV light, the polyethyleneimine cellulose was scraped off, and radioactivity was measured by liquid scintillation counting. The purity of all nucleotides was analyzed by thin layer chromatography.

Phosphorylation of Gβ and NDPK in H10 Cell Membranes—The indicated amounts of H10 cell membranes were phosphorylated with 10 nM [γ-32P]GTP (PerkinElmer Life Sciences) for the indicated periods of time at 30°C in a reaction buffer containing 50 mM triethanolamine hydrochloride, pH 7.4, 150 mM NaCl, 2 mM MgCl2, 1 mM EDTA, and 1 mM dithiothreitol in a total volume of 20 μl. The reaction was terminated by the addition of 10 μl of 3-fold concentrated sample buffer, followed by incubation at room temperature for 1 h. Proteins were separated by discontinuous SDS-PAGE on gels containing 10–12% (w/v) acrylamide and autoradiographed. Immunoprecipitates were subjected to SDS-PAGE, transferred to nitrocellulose, and immunostained with antibodies against Gα and Gβ.

Measurement of ATP and GTP Levels—Cellular ATP and GTP levels were assessed by HPLC as described (23). Briefly, the cells were deproteinized by adding 60% (v/v) ice-cold acetonitrile and subsequently homogenized using a Branson sonifier. Denaturated proteins were pelleted by centrifugation, and the supernatant was injected into the chromatograph in an appropriate dilution. The system consisted of an Amersham Biosciences gradient pump, a Spark autosampler, and a C18 column (inner diameter 4 mm, length 20 mm; Ziemer, Mannheim, Germany), which was equipped with a 30-mm guard column of the same diameter. The UV detector was set to 206 nm. The detected metabolites were normalized to protein content. The latter was assessed using standard protocols, following solubilization of the denaturated proteins in NaOH at 50°C.

Data Analysis—All experiments were carried out in triplicate and were repeated at least three times. Values are given as means ± S.D. For statistical analysis, one-way analysis of variance, followed by the Tukey-Kramer post test, was performed with GraphPad PRISM 3 software.

RESULTS

Characterization of H10 Cells Stably Expressing NDPK Isoforms—Untransfected H10 cells and a stable transfectant expressing EGFP (C-GFP) were compared with stable transfectants, expressing different levels of NDPK B, its catalytically inactive mutant, NDPK B-H118N, or NDPK A. The NDPK activity was determined in cell lysates and membrane fractions. Importantly, differences in total cellular activity were reflected by similar changes in membrane-associated activity. The factors that determine the intracellular distribution of NDPK, particularly binding to membranes, are unknown. The amount and activity of NDPK were increased 1.5-, 2-, and

![Image](108x144)

**Fig. 1.** Overexpression of NDPK B enhances Gα-mediated cAMP accumulation in H10 cells. **A**, the cAMP content was determined in H10 cell clones stably expressing EGFP (C-GFP) or NDPK B at different expression levels and increasing expression levels of Gα. C1, C2, and C3 represent cell clones with a 1.5-, 2.1-, and 2.7-fold overexpression of NDPK B, respectively. Representative immunoblots of NDPK B content are shown in the insets. Expression of Gα achieved by infection with a recombinant adenovirus at different multiplicities of infection (MOI) was quantified by immunoblotting. A representative immunoblot is shown. B, integration of overexpressed Gα into heterotrimeric G proteins. Gα was immunoprecipitated with a specific antibody and protein G-Sepharose from lysates of infected cells. Immunoprecipitates were subjected to SDS-PAGE, transferred to nitrocellulose, and immunostained with antibodies against Gα and Gβ.
3-fold in the clones C1, C2, and C3, respectively (Table I). The amount of NDPK in cells expressing the inactive mutant H118N (M118) was also increased 3-fold, whereas activity remained at the level found in untransfected cells (see Table I and Fig. 2). In H10 cells, the content of NDPK B (1.1 ng per µg of total protein) exceeded the content of NDPK A by a factor of 10, as determined by quantitative immunoblots using isoform-specific antibodies and recombinant proteins as standard (see Figs. 2 and 3). In cells expressing NDPK A (CH1), the amount of NDPK A was increased 6-fold, leading to a 1.6-fold increase of combined NDPK A/B (see Fig. 3). This was not associated with an increase in whole cell or membrane-associated NDPK activity. The amount of Gs and Gi subunits, as well as basal, G protein-uncoupled adenylyl cyclase activity (with 10 mM free Mn2+ as co-substrate), and cAMP content were not affected by overexpression of the NDPK isoforms (Table I). An increase of NDPK activity could change cellular levels of ATP and GTP, which might cause direct or G protein-mediated effects on adenylyl cyclase activity. However, we could not detect any significant changes in the ATP and GTP contents or in the ATP/GTP ratio in cells transfected with the NDPK isoforms (Table I).

 Isoform-specific Effect of NDPK B on cAMP Synthesis—As overexpression of NDPK may lead to activation of both stimulatory (Gs) and inhibitory (Gi) G proteins, expressed in H10 cells at a 1:2 ratio (2.0 ng/µg of Gs versus 4.0 ng/µg of Gi), their effects on cAMP synthesis may be neutralized. We therefore co-expressed Gs in NDPK transfectants with a recombinant adenovirus (Fig. 1). The functional integration of overexpressed Gs into trimeric G proteins was assessed by co-immunoprecipitation of Gα dimers. Immunoprecipitation of Gs from cells with increasing levels of expression was associated with a parallel increased co-precipitation of Gβ (Fig. 1B). Overexpression of Gs, up to 10-fold above the endogenous protein content, led to a linear increase in cAMP accumulation, measured in the presence of the phosphodiesterase inhibitor, IBMX, and the inverse β-adrenoreceptor agonist, propranolol (24). Most important, the increase in cAMP accumulation induced by overexpression of Gs was strongly enhanced in cells overexpressing the NDPK B isoform. In cells with an ~3-fold higher expression of NDPK B (C3), the increases in cAMP in response to Gs were ~4-fold higher at each level of Gs compared with H10 cells stably expressing EGFP (C-GFP; see Fig. 1A) or untransfected H10 cells (H10; see Fig. 2A). In cells with lower expression of NDPK B (C1 and C2), intermediate levels of cAMP accumulation were observed. In contrast, cells expressing the same amount of the inactive mutant of NDPK B, H118N (M118), exhibited a lower cAMP content than...
untransfected H10 cells at any Gs level (Fig. 2). Furthermore, in cells with a 6-fold overexpression of NDPK A (CH1), the cAMP content was not altered compared with untransfected H10 cells at any level of Gs (Fig. 3).

Because the stimulatory effect of NDPK B was dependent on Gs, stimulation of adenylyl cyclase is the most likely mechanism for the increase in cAMP content observed in the NDPK B transfectants. This was supported by direct measurements of adenylyl cyclase activity in membranes of cells with increased expression levels of NDPK B and Gs. Whereas activity of the adenylyl cyclase uncoupled from regulatory influences, measured with 10 mM Mn2+ as co-substrate, was not altered by overexpression of NDPK B (Table I) in the presence of Mg2+ and specifically upon the addition of GDP, adenylyl cyclase activity was increased in membranes of C3 cells, and this increase was enhanced largely by overexpression of Gs (Fig. 4). In contrast, when GDP was replaced by its analog, guanosine 5’-O-(2-thio)diphosphate (GDPβS), which, similar to GDP, binds to G proteins but is a poor substrate for NDPK, the NDPK B-induced increase in adenylyl cyclase activity was diminished.

G Protein Activation by Phosphate Transfer II

**Fig. 3.** Lack of effect of NDPK A on Gs-mediated cAMP accumulation. A, the cAMP content was determined in untransfected H10 cells and a cell clone with a stable, 6-fold overexpression of NDPK A (CH1) and increasing expression levels of Gs, induced by adenoviral infection at different multiplicities of infection. B, the amount of expressed NDPK A was quantified by immunoblotting with an isoform-specific antibody. The recombinant human NDPK A migrates at an apparent molecular mass of 21 kDa, whereas endogenous rat NDPK A is detected at 17 kDa. The indicated amounts of recombinant NDPK A were used as standard. C, membranes of untransfected H10 cells and of the CH1 clone were phosphorylated with [γ-32P]GTP (10 nM). Autophosphorylated NDPK A and NDPK B are indicated.

**Fig. 4.** Stimulatory effect of NDPK B on adenylyl cyclase activity. Adenylyl cyclase activity was determined in membranes from untransfected H10 cells (empty bars) and from cells with a 3-fold overexpression of NDPK B (C3; filled bars) as described under “Experimental Procedures.” Activity was determined with 3 mM Mg2+ as co-substrate, and, as shown from left to right, in the absence (C) and presence of 100 μM GDP or 50 μM GDPβS (GβS). * denotes measurements in membranes from cells overexpressing equal amounts of Gs as determined by immunoblot (see inset).

**Fig. 5.** Phosphorylation of Gβ by NDPK and formation of Gβγ-NDPK complexes. A, membranes (200 μg of protein) of H10 cells 3-fold overexpressing human NDPK B (C3) were subjected to immunoprecipitation with an anti-NDPK antiserum or a nonspecific IgG and subjected to SDS-PAGE. Precipitated Gβ subunits were detected by Western blotting. B, H10 cell membranes (200 μg of protein) were phosphorylated with [γ-32P]GTP and subjected to immunoprecipitation with the anti-Gβ antibody (T-20). Purified IgG was used as a control. C, Membranes (5 μg of protein) of non-transfected H10 cells (H10) or H10 cells 3-fold overexpressing wild-type NDPK B (C3) or its catalytically inactive mutant (M118) were phosphorylated with [γ-32P]GTP for 1 min at 30 °C. D, membranes (2.5 μg of protein) of non-transfected H10 cells (H10) and H10 cells 1.5-fold overexpressing wild-type NDPK B (C1) or 6-fold NDPK A/CH1 were phosphorylated with [γ-32P]GTP for 5 min at 30 °C. Autoradiographs after SDS-PAGE are shown.

**Phosphorylation of Gβ by NDPK B in H10 Cell Membranes**—In the accompanying paper (19), a complex formation of NDPK B with Gβγ has been described. Therefore, we studied whether NDPK B forms complexes with Gβγ in H10 cells, as well. Membranes of the C3 clone with 3-fold overexpression of NDPK B were solubilized at low detergent and subjected to immunoprecipitation with the anti-NDPK antiserum (C-20). As shown in Fig. 5A, co-precipitated Gβ was detected by Western blot...
analysis. Next, we studied whether phosphorylation of Gβ occurs in H10 cells. Phosphorylation of H10 cell membranes with \(^{32}\)P[GTP disclosed the presence of a 36-kDa phosphoprotein that could be immunoprecipitated with the Gβ-specific antiserum (Fig. 5B). We therefore analyzed whether the overexpression of NDPK isoforms alters the phosphorylation of Gβ in H10 cells. An ∼2- and 3-fold increase in Gβ phosphorylation was observed in membranes of H10 cells with 1.5-fold (C1) and 3-fold (C3) overexpression of NDPK B, respectively. Concurrently, the overexpression of NDPK B could be detected by its autophosphorylation (Fig. 5, C and D). In contrast, no increase in Gβ phosphorylation was detected in membranes from cells 3-fold overexpressing the catalytic inactive NDPK B mutant, H118N (M118) (Fig. 5C), or 6-fold overexpressing NDPK A (CH1; see Fig. 5D).

**DISCUSSION**

The aim of the present study was to evaluate the possible interaction of NDPK and G proteins in living cells. Our experimental setting offers two major advantages when compared with prior approaches performed in membranes or reconstituted in vitro systems. First, we circumvent the technical problems associated with a proof of phosphate transfer to G protein-bound GDP or GTP channeling (9), and second, we address the issue of a physiologically relevant mechanism by studying the effects in intact cells. The results of the combined overexpression of NDPK isoforms and Gα in H10 cells can be summarized as follows. The stimulation of cAMP synthesis by NDPK required Gα, and much of the effect of Gα was dependent on NDPK activity. The effects of both NDPK and Gα were dependent on and linear with the expression of the respective proteins. The activation of Gα by NDPK required the catalytic activity of the enzyme. It was not seen with its inactive mutant. Furthermore, the effect was specific for the NDPK B isoform. Orlov et al. (25) recently found a transducin-mediated isofrom-specific binding of NDPK to rod outer segment membranes. In their study, the transducin-mediated binding of NDPK B to the membrane exceeded that for the A isoform 100-fold, indicating a specific interaction of the G protein with NDPK B.

Cellular cAMP is influenced by several factors that had to be controlled to confine our results to NDPK activity. To block influences of β-adrenoceptors and phosphodiesterases, cAMP assays were performed in the presence of propranolol and IBMX, respectively. Furthermore, expression of NDPK had no influence on the expression levels of the adenylyl cyclase-regulating G proteins, the basal adenylyl cyclase activity, or cellular ATP and GTP contents. Finally, direct measurements of adenylyl cyclase activity in vitro exhibited an NDPK B- and Gα-dependent activation in the presence of GDP and ATP.

Evidence of G protein activation by NDPK in cells does not resolve the problem of its mechanism. Previous studies had demonstrated an activation of G proteins (13–15) and regulation of adenylyl cyclase activity (14) by phosphorylated Gβ subunits. In H10 cell membranes, the extent of Gβ phosphorylation was dependent upon the level, activity, and isoform of NDPK. In addition, Gβ subunits co-precipitated with an antibody against NDPK. Furthermore, the accompanying paper (19) shows a highly selective co-purification of NDPK B and Gβ dimers with different isolation protocols. A reciprocal co-immunoprecipitation of NDPK with transducin Gβy further confirms a complex formation. Most important, the phosphorylation of Gβ subunits could be reconstituted by the addition of NDPK-enriched co-factor fractions to purified Gβy dimers from different origin. The identification of a phosphorylated histidine residue (His-266 in Gβ1) that is exposed at the surface of the Gβ molecule raises the possibility of a phosphate transfer from His-118 of NDPK B to His-266 in Gβ (19). The enhanced phosphorylation of Gβ in membranes of cells overexpressing NDPK B and the lack of such an effect in membranes of cells overexpressing its catalytic inactive mutant H118N (Fig. 5) substantiates this hypothesis.

Assuming that the NDPK B-dependent effects on cAMP formation are mediated by NDPK B-Gβy complexes, our data suggest that the activation of G proteins by GTP formation via intermediately phosphorylated NDPK B-Gβy complexes is a mechanism to regulate the basal, receptor-independent activation of heterotrimeric G proteins (Fig. 6). In line with this
hypothesis, an at least 3-fold increase of the NDPK content and activity in sarcolemmal plasma membranes from failing human myocardium was accompanied by a higher basal activation of G proteins (26). As G protein expression is also increased in heart failure (27, 28), the higher NDPK and G content may contribute to the well known decreased response to β-adrenergceptor agonists (29) and diminished basal cAMP formation (30).

If NDPK B is a regulator of G protein activity, an important question is whether its contribution is regulated in cells. Recently, we found a 3-fold elevated plasma membrane-associated NDPK in hearts from patients with severe congestive heart failure (26). This elevation was diminished in patients treated with a β-adrenergceptor antagonist. Furthermore, chronic treatment of rats with the β-adrenergceptor agonist, isoproterenol, induced an increase in plasma membrane-bound NDPK (31), suggesting chronic β-adrenergic activation as a mechanism for regulation of the NDPK membrane content. Therefore, it is a reasonable assumption that an NDPK B-Gβγ complex, as a receptor-independent activator of G proteins, may have the potential to regulate a broad spectrum of cellular functions. Studies are in progress to further substantiate this hypothesis.

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