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MINIREVIEW

# High energy phosphate transfer by NDPK B/G$\beta\gamma$complexes – an alternative signaling pathway involved in the regulation of basal cAMP production

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**Abstract** The activation of heterotrimeric G proteins induced by G protein coupled receptors (GPCR) is generally believed to occur by a GDP/GTP exchange at the G protein $\alpha$-subunit. Nevertheless, nucleoside diphosphate kinase (NDPK) and the $\beta$-subunit of G proteins (G$\beta$) participate in G protein activation by phosphate transfer reactions leading to the formation of GTP from GDP. Recent work elucidated the role of these reactions. Apparently, the NDPK isoform B (NDPK B) forms a complex with G$\beta\gamma$ dimers in which NDPK B acts as a histidine kinase phosphorylating G$\beta$ at His266. Out of this high energetic phosphoamidate bond the phosphate can be transferred specifically onto GDP. The formed GTP binds to the G protein $\alpha$-subunit and thus activates the respective G protein. Evidence is presented, that this process occurs independent of the classical GPCR-induced GTP/GTP exchange und thus contributes, e.g. to the regulation of basal cAMP synthesis in cells.

**Keywords** Heterotrimeric G proteins · GDP/GTP exchange · NDPK · G$\beta$ phosphorylation · cAMP · Congestive heart failure

## Introduction

Heterotrimeric G proteins transduce information from a wide variety of extracellular signals to intracellular effector enzymes or ion channels. They are composed of three subunits (G$\alpha$, G$\beta$, G$\gamma$), with two functional units, the guanine nucleotide binding G$\alpha$-subunit and the G$\beta\gamma$ dimer (Hamm, 1998). In the inactive state, GDP is bound in the $\alpha$-subunit of the heterotrimer. According to current concepts (Wieland and Michel, 2005), G protein coupled receptors (GPCR) catalyze the release of GDP upon agonist binding. Thus GTP, which is present in much higher intracellular concentrations than GDP, binds to the empty nucleotide binding pocket in G$\alpha$. The binding of GTP induces conformational changes leading to the dissociation of the G protein into the GTP-liganded G$\alpha$ and G$\beta\gamma$, which both can directly regulate effector activities. After the hydrolysis of GTP to GDP and inorganic phosphate by the intrinsic GTPase activity present in G$\alpha$, the G protein returns to its inactive heterotrimeric state by re-association of G$\alpha$-GDP with G$\beta\gamma$.

Nucleoside diphosphate kinase (NDPK) is an ubiquitous enzyme, that catalyzes the transfer of $\gamma$-phosphate from nucleoside 5′-triphosphates (NTP) to nucleoside 5′-diphosphates (NDP) by a ping pong mechanism involving the formation of a high energy phosphate intermediate on His118 (Morera et al., 1995; Tepper et al., 1994). In mammalian tissues, the enzyme is a heterohexamer of 17–21 kDa subunits (Gilles et al., 1991; Janin et al., 2000) composed by different combinations of the three major isoforms, NDPK A, B and C, encoded by the human nm23 genes, nm23-H1, -H2 and DRnm23, respectively. In addition to nucleoside synthesis, the NDPK is involved in a variety of processes in cellular physiology, including tumor metastasis (Steeg et al., 1988), development (Rosengard et al., 1989), gene regulation (Postel, 2003), apoptosis (Fan et al., 2003) and endocytosis (Palacios et al., 2002).

A contribution of the plasma membrane-bound fraction of NDPK to the activation of heterotrimeric G proteins has been first postulated in the early 1980s by Kimura and co-workers

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(for review see Kimura, 1993) and has been supported by a large body of observations including data from our laboratory (for review see Piacentini and Niroomand, 1996; Otero, 1990). Nevertheless, a more specific role of NDPK than the simple replenishment of GTP from ATP and GDP has been thoroughly questioned (Otero, 1990). In this article we will therefore review recent work that describes the identification of a complex formed by G$\beta\gamma$ and the NDPK B isoform and discuss its role in the activation of heterotrimeric G proteins.

## NDPK and G proteins

Since one of the major reactions catalyzed by NDPK is the phosphate transfer from ATP to GDP to maintain levels of GTP, NDPK, especially the membrane-bound fraction, may serve the synthesis of GTP, required for the activation of G proteins. Numerous *in vitro* studies have revealed G protein activation through the enzymatic activity of NDPK (reviewed by Otero, 1990; Kimura, 1993; Piacentini and Niroomand, 1996). Surprisingly, it was found that NDPK derived GTP or its stable analog GTP$\gamma$S is more potent in activating G proteins than exogenously added GTP or GTP$\gamma$S (Jakobs and Wieland, 1989; Wieland and Jakobs, 1992; Niroomand et al., 1997). Two possibilities to explain such data have been put forward: 1) Substrate channelling, which in this case means formation of GTP from ATP and GDP in immediate vicinity of the G protein (Kimura and Shimada, 1990; Otero et al., 1988; Jakobs and Wieland, 1989; Wieland et al., 1992) or 2) the transfer of the high energy phosphate on NDPK directly onto the GDP still bound to G$\alpha$ or monomeric GTPases (Kikkawa et al., 1990; Randazzo et al., 1991). Today it appears clear that the latter hypothesis was based on artefacts. GDP was released spontaneously from G proteins, served as a substrate for phosphotransfer by the NDPK and the formed GTP then bound back to the G protein (Randazzo et al., 1992; Lutz et al., 2002). Even an approach were GDP has been cross-linked to the monomeric GTPase Rad (Zhu et al., 1999) is questionable by its experimental design (Otero, 1990). As discussed before (Otero, 1990) structural considerations are also arguing against this possibility (see Fig. 1). Both, the phosphohistidine in the NDPK (Janin et al., 2000; Lascu and Gonin, 2000) as well as the GDP in the G protein $\alpha$-subunit (Lambright et al., 1994) are deeply buried in the respective structures making a direct transfer of the high energetic phosphate onto the bound GDP highly unlikely.

An effective channelling of GTP into the G protein would however require a close association, i. e. a complexation of at least a monomeric NDPK molecule with the heterotrimeric G protein or a subunit of it. While experimental data have been reported which support such a complex of membranous NDPK with the stimulatory G protein of adenylyl cyclase ($G_s$) (Kimura and Shimada, 1988; Kimura and Shimada, 1990) or the retinal G protein transducin ($G_t$) (Orlov et al., 1996; Klinker and Seifert, 1999) the contribution of local GTP formation to effector regulation was disputed or not detected in other systems (Xu et al., 1996; Sorota et al., 1998). With the enzymatic activity of NDPK as major read out for detection of the presence of the enzyme, the methodology in all these papers was prone to be suspected as an artefact. Due to its extremely high enzymatic activity contaminations with small amounts of NDPK, which are still present in G protein-enriched fractions, might have led to misinterpretations. Therefore, other methods to detect a productive interaction of NDPK with a G protein were absolutely required to substantiate the complexation hypothesis.

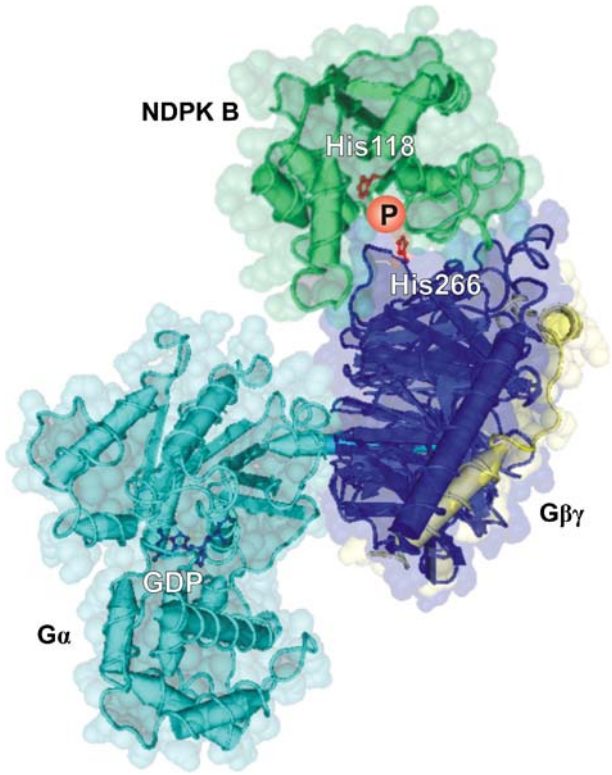


**Fig. 1** Hypothetical three-dimensional structure of NDPK B complexed with a heterotrimeric G protein. The three-dimensional structure of a NDPK B monomer (Webb et al., 1995) is superimposed onto the three-dimensional structure of the $G\alpha_{i1}\beta_1\gamma_2$ heterotrimeric G potein (Wall et al., 1995). Ribbon diagrams of $G\alpha_{i1}$-GDP (light blue), $G\beta_1$ (magenta) and $G\gamma_2$ (yellow) combined with NDPK B (green). The likely structural vicinity of the histidine residues of NDPK B (His118) and $G\beta_1$ (His266) is illustrated. The GDP molecule in the nucleotide binding site in G$\alpha$ is depicted in dark blue.

## Phosphorylated G protein $\beta$ subunits as GTP forming enzyme intermediate

In the early 1990s, it was detected that the $\beta$-subunit of G proteins can carry a high energetic phosphate. This phosphate was also found to be a phosphoamidate on a histidine residue, which was transferable onto GDP thus leading to

GTP formation, G protein activation and regulation of effector activity (Wieland et al., 1991; 1992, 1993). In contrast to NDPK however, GDP was the only NDP substrate for that phosphate transfer reaction and even more important, in contrast to phospho-NDPK, phospho-G$\beta$ is not a product of an "autophosphorylation" but required a "histidine kinase" activity. The phosphorylation of G$\beta$ has been detected in a variety of mammalian tissues (Nürnberg et al., 1996), including rat pancreatic $\beta$ cells (Kowluru et al., 1996) and human platelets (Hohenegger et al., 1996), but the identity of the proposed membrane-bound histidine kinase to catalyse this reaction remained elusive (Kowluru, 2002). Similar as for the NDPK-mediated activation of G proteins the phosphorylation of GDP bound to G$\alpha$ was a matter of debate (Wieland et al., 1993; Kaldenberg-Stasch et al., 1994; Hohenegger et al., 1996; Kowluru et al., 1996; Niroomand et al., 1997). As discussed below structural constrains (see Fig. 1) as well as some experimental data (Hohenegger et al., 1996) are arguing against such a reaction.

## Complex formation of NDPK B with G$\beta\gamma$ dimers

A progress in the search of the histidine kinase which phosphorylates G$\beta$ was made by recent work (Cuello et al., 2003) in which the G$\beta$ phosphorylating activity was enriched from $G_t$ preparations out of bovine rod outer segments membranes and preparations of the inhibitory G proteins of adenylyl cyclase ($G_{i/o}$) out of bovine brain membranes. The fractions in which histidine phosphorylation of G$\beta$ was observed has been obtained by completely different purification protocols out of both sources. Nevertheless, all of them contained G$\beta\gamma$ dimers and NDPK as detected by its autophosphorylation, its enzymatic activity and specific antibodies. Isoform-specific antibodies revealed a selective enrichment of NDPK B, whereas NDPK A, although abundantly expressed in the source tissues, was not detected. By size exclusion chromatography the molecular mass of the kinase activity was estimated to be 60–70 kDa. A similar size was reported for the G$\beta$ phosphorylating "histidine kinase" activity of rat pancreatic $\beta$ cells, which contain membraneous NDPK B but not NDPK A (Kowluru et al., 2002). This histidine kinase activity can be stimulated by the known NDPK activator mastoparan (Kowluru, 2002). The molecular mass (G$\beta$ 35–36 kDa, G$\gamma$ 5–12 kDa, NDPK 17–21 kDa) as well as the sensitivity for mastoparan suggested that this "histidine kinase" might be a complex formed by G$\beta\gamma$ and a monomeric NDPK B. Indeed the complex formation could be shown by co-immunoprecipitation experiments (Cuello et al., 2003). Using a G$\beta$- and a NDPK B-specific antiserum phosphorylated NDPK B and phosphorylated G$\beta$ was co-immunoprecipitated from the retinal NDPK/G$\beta\gamma$-containing fractions, respectively. However, it has to be pointed out that the majority of G$\beta\gamma$ is not complexed with NDPK B and that *in vitro* reconstitution of purified G$\beta\gamma$ with purified cytosolic NDPK was not sufficient to achieve complex formation and phosphorylation of G$\beta$. These data indicate that an additional scaffold protein might be required for complexation of G$\beta\gamma$ with NDPK B.

Nevertheless, the data obtained by Cuello et al. (2003) indicate that NDPK B in this complex acts as protein histidine kinase phosphorylating G$\beta$. This interpretation was strengthened by data obtained in immortalized neonatal rat cardiac myocytes (H10 cells) stably overexpressing NDPK A, NDPK B or its catalytically inactive mutant NDPK B-H118N (H118) (Hippe et al., 2003). Only in membranes of cells overexpressing NDPK B, but not H118 or NDPK A, the phosphorylation of G$\beta$ was increased about 2-fold. Moreover, in membranes of NDPK B-overexpressing cells an increase in the content and activity of NDPK B as well as the formation of NDPK B/G$\beta\gamma$ complexes was detected. Thus, an increase in functional NDPK B/G$\beta\gamma$ complexes apparently increases the amount of intermediately phosphorylated G$\beta$ subunits.

The possibility to reconstitute the phosphorylation of G$\beta$ with purified G$\beta\gamma$ dimers from bovine brain, $G_t\beta\gamma$ and the G$\beta$ phosphorylating activity from bovine brain (Cuello et al., 2003) raised the possibility to identify the phosphorylated histidine residue in G$\beta$. Proteolytic digest with endoproteinase Glu-C or trypsin followed by Erdman degradation of the phosphorylated peptides revealed that histidine 266 of G$\beta_1$ is specifically phosphorylated. This histidine is conserved in the mammalian G$\beta_1$-G$\beta_4$ isoforms but not in G$\beta_5$. As shown in three-dimensional structure of G$\alpha\beta\gamma$ heterotrimers (Wall et al., 1995), only the imidazolyl side chain of His266 is exposed on the surface of the G protein and can therefore be the target of protein phosphorylation by a histidine kinase, whereas the seven other highly conserved histidines in G$\beta$ subunits are part of the 7 blade propeller structure and thus not accessible to kinases. Moreover, by superimposing the three dimensional structure of an NDPK B monomer (Webb et al., 1995) (Fig. 1), it appears structurally feasible that His118 of the NDPK B and His266 of G$\beta_1$ can come in close contact to allow a phosphate transfer from one His to the other, a reaction for example involved in recognition of chemotactic stimuli in bacteria (for review see Falke et al., 1997). We recently substantiated this interpretation by functional data, which demonstrated that adenovirus-mediated overexpression of G$\beta_1\gamma_2$ dimers in NDPK B-overexpressing H10 cells further increased G$\beta$ phosphorylation by about 2-fold (H.J. Hippe, T. Wieland, unpublished observations). In contrast, overexpressed G$\beta_1\gamma_2$ dimers in which His266 of G$\beta_1$ was mutated to leucine (G$\beta_1$H266L$\gamma_2$) were no longer substrate for the NDPK B-mediated phosphorylation.

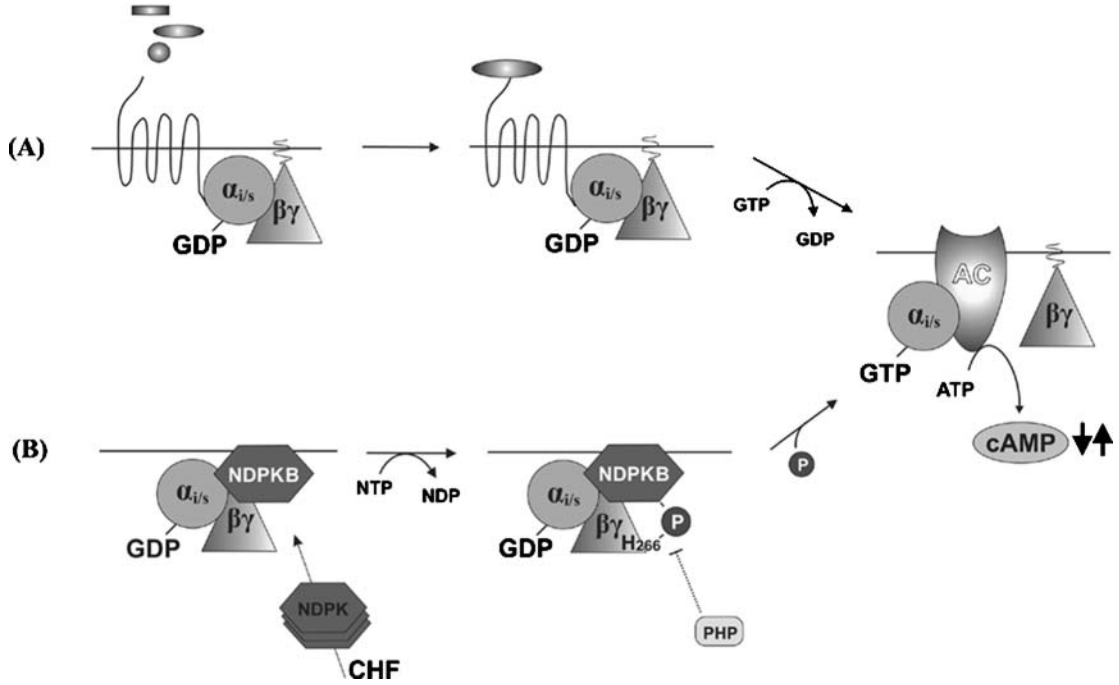


**Fig. 2** Model of classic receptor-dependent and alternative NDPK B-dependent G protein activation. (A) In the classical model, agonist binding to a G protein coupled receptor (GPCR) triggers GDP/GTP exchange on G$\alpha$ and release of G$\beta\gamma$, leading to effector response. (B) In the subpopulation of heterotrimeric G proteins complexed with NDPK B, a phosphotransfer form NTP, preferably ATP, to His118 in NDPK B and subsequently onto His266 of G$\beta$, results in a high energetic phosphate which promotes the formation of GTP, leading to GPCR-independent G protein activation and, in turn, regulation of basal adenylyl cyclase (AC) activity. Two possible mechanisms, modulating the extent of G$\beta$ phosphorylation are included. In chronic heart failure (CHF), plasma membrane content of NDPK is increased. Phosphohistidine phosphatase (PHP), which is capable to specifically dephosphorylate G$\beta$, is a potential counter regulator of NDPK B in this pathway.

Most interestingly, in a recent report (Mäurer et al., 2005) it was described that the first identified mammalian phosphohistidine phosphatase (PHP) (Ek et al., 2002; Klumpp et al., 2002) is able to dephosphorylate G$\beta$, but not NDPK B, in reconstituted systems and in H10 cells membranes. Its stable overexpression in living H10 cells also interferes with G$\beta$ phosphorylation. Therefore, PHP might be a candidate for an endogenous regulator of basal NDPK-dependent G protein activation targeting the same specific step in the activation, which is artificially suppressed by mutating His266 in G$\beta_1$ (see Fig. 2).

## Regulation of basal, receptor-independent cAMP production by the NDPK B/G$\beta\gamma$ complex

The results reported so far point to a role of the NDPK B/G$\beta\gamma$ complex in the activation of heterotrimeric G proteins by high energy phosphate transfer. The cell clones of stable transfected H10 cells which overexpress NDPK B at different levels as well as H118 or NDPK A enabled us to address the question whether G protein activation by NDPK B can be observed in intact cells (Hippe et al., 2003). This was of outmost importance as previously reported data concerning transphosphorylation reactions by NDPK or by G$\beta$ based on studies in cell membranes have been suspected to be the result of artificial conditions and their relevance for signal transduction in the living cell has therefore been questioned (Hohenegger et al., 1996; Clapham and Neer, 1997). Cellular GTP and ATP levels, basal cAMP formation, and the expression of G$\alpha_s$, G$\alpha_i$ and G$\beta\gamma$ were not different between the individual cell clones. Large differences in basal, receptor-independent cAMP accumulation were however evident when increasing levels of G$\alpha_s$ were expressed in the individual cell clones by means of adenoviral gene transfer (Hippe et al., 2003). In all cell clones cAMP accumulation increased linearly with the amount of overexpressed G$\alpha_s$. The slope of this linear increase however was strictly dependent on the amount of overexpressed NDPK B in the respective cell clone. Thus, in the cell clone with the 3-fold higher activity of NDPK B the increase in cAMP levels in response to G$\alpha_s$ expression was about 4-fold higher at each level of G$\alpha_s$ compared to control H10 cells. Overexpression of NDPK A was without effect on cAMP synthesis. The activation of G$\alpha_s$ required specifically the catalytic activity of the NDPK B. Most interestingly, the cell clone 3-fold expressing the inactive NDPK B mutant H118, displayed even a reduced cAMP accumulation (by about

33%) in response to $G\alpha_s$ compared to control H10 cells. An even stronger reduction in $G\alpha_s$-dependent cAMP accumulation was obtained when $G\beta_1H266L\gamma_2$ was adenovirally overexpressed in H10 cells. Compared to the overexpression of wild type $G\beta_1\gamma_2$, cells overexpressing comparable levels of $G\alpha_s$ and $G\beta_1H266L\gamma_2$ exhibited about 55% lower cAMP levels (H.J. Hippe, T. Wieland, unpublished observations). The combined stimulatory effect of NDPK B and $G\alpha_s$ overexpression on cAMP formation was confirmed by measurements of adenylyl cyclase activity in membranes of the H10 cell clones (Hippe et al., 2003). Most interestingly, in this *in vitro* assay, the NDPK B and $G\alpha_s$ dependent stimulation of adenylyl cyclase was largely suppressed when the phosphate acceptor substrate GDP was replaced by its analog, guanosine 5′-O-(2-thio)diphosphate (GDP$\beta$S), which similar to GDP binds to G proteins, but is a poor substrate for NDPK.

Therefore, all this functional data provide evidence for a physiological relevance of NDPK-mediated G protein activation in the regulation of basal cAMP synthesis. They agree with a model (see below) in which this alternative pathway of G protein activation contributes to the GPCR-independent, basal activation of G proteins in living cells.

## Model of receptor-independent, NDPK B/G$\beta\gamma$-mediated G protein activation

The data on the interaction of NDPK B and $G\beta\gamma$ accumulated so far can be summarized in a novel mechanism for the regulation of the basal, receptor-independent activity of heterotrimeric G proteins (Fig. 2). Whereas the majority of G proteins are not complexes with NDPK B and thus can be activated only by classical pathway of GPCR-induced GDP/GTP exchange, a fraction of the heterotrimeric G proteins contain $G\beta\gamma$ dimers, which are complexed with NDPK B. At least one additional protein (most likely the limiting one) appears to be required as scaffold for this complex. In this context, it has to be noted that complex formation with NDPK has been reported for a variety of proteins, e.g. dynamin I, phocein, ICAP$\alpha$ or KSR (for recent review see Lombardi and Mileo, 2003). This G protein subpopulation is able to be activated by phosphate transfer: A nucleoside triphosphate, in the living cell most likely ATP, binds to the NDPK B in that complex and its $\gamma$-phosphate is transferred onto His118 in the NDPK B. Acting as protein histidine kinase, this high energetic phosphate is transferred onto His266 in G$\beta$. Out of that phosphoamidate bond, the phosphate is transferred specifically onto GDP, and the formed GTP leads to receptor-independent G protein activation. At present the experimental data support such a model for $G_s$, $G_t$ and most likely other members of the $G_i$ family. Whether NDPK-mediated G protein activation occurs at members of the $G_q$ or $G_{12}$ families of G proteins has not been addressed so far.

As already discussed above, experimental evidence as well as the three-dimensional structure of a heterotrimeric G Protein (Fig. 1) does not support a direct transfer onto GDP bound to G$\alpha$. The distance between His266 and the bound GDP molecule within the G$\alpha$-subunit is far too large to allow such a transfer. Nevertheless, the model would allow for the "old" hypothesis of GTP channelling. It might be speculated that a GDP bound in G$\alpha$, which dissociates with a certain frequency from the G$\alpha$ binding pocket can come in close proximity to that phosphate. The newly formed GTP can bind with high affinity to the still empty binding pocket and thus G$\alpha$ gets activated.

The results obtained so far do not argue for a contribution of the NDPK B/G$\beta\gamma$ complexes in GPCR-induced G protein activation. Recent data (H.J. Hippe, T. Wieland, unpublished observations) in which we compared wild type $G\beta_1\gamma_2$ with $G\beta_1H266L\gamma_2$ in their ability to support the $\beta$-adrenoceptor-induced activation of $G\alpha_s$ by measurement of isoproterenol-stimulated GTP$\gamma$S binding in membranes of baculovirus infected Sf9 insect cells (Kühn et al., 2002), revealed no difference between wild type and mutant G$\beta\gamma$ in this regard. Nevertheless, a variety of data accumulated by different laboratories in the early days of investigations on NDPK and G protein activation (reviewed in Otero, 1990; Piacentini and Niroomand, 1996) as well as some data on G protein activation by phosphorylated G$\beta$ subunits (Kaldenberg-Stasch et al., 1994) raised speculations about that possibility. Although that question is not finally answered until now, a direct stimulation of NDPK B/G$\beta\gamma$-mediated phosphate transfer by agonist-liganded GPCRs can be excluded at least by our data.

## Clinical implications

Studies concerning clinical aspects of the NDPK B-mediated G protein activation have so far been focussed on heart disease, in particular on congestive heart failure (CHF), and diabetes.

The basic important finding, linking NDPK and heart disease, was that the amount and activity of NDPK are increased at least 3-fold in human failing hearts as compared to non-failing controls (Lutz et al., 2001; Zhou and Artman, 2001). These changes were only seen in the small pool of membrane-associated NDPK, when sarcolemmal membranes from human myocardium were purified, and were not observed in either homogenate, cytosol or other remaining fractions, suggesting a translocation from the cytosol to the plasma membrane in cardiac myocytes. Furthermore, an up-regulation of all three major isoforms (NDPK A, B and C) was detectable (Lutz et al., 2004), which is probably due to

the composition of NDPK in heterohexamers (Gilles et al., 1991). As a consequence of elevated NDPK, a substantial receptor-independent inhibition of adenylyl cyclase activity up to 50% in failing hearts was demonstrated. These data therefore support the above outlined role of NDPK B as modulator of basal cAMP production via G$\beta$ phosphorylation. However, the net effect of the NDPK B mediated G protein activation obviously depends on the relative prevalence of G$\alpha_s$ and G$\alpha_i$, since these G$\alpha$ subunits are presumably non-selectively activated by NDPK B/G$\beta\gamma$ complexes. Whereas in sarcolemmal membranes of canine hearts, NDPK stimulates adenylyl cyclase via G$\alpha_s$ and increases cAMP (Niroomand et al., 1997), in human CHF, the well documented up-regulation of G$\alpha_i$ proteins (Neumann et al., 1988) most likely counts for the predominant inhibitory effect of NDPK on adenylyl cyclase and, in turn, on cAMP production. Thereby, this mechanism might contribute to the well known diminished cAMP signaling observed in failing hearts.

The elevation of the membrane-associated NDPK was partially prevented in patients with chronic heart failure treated with $\beta$-adrenoceptor blockers, which are meanwhile accepted agents of standard therapy in CHF (Lohse et al., 2003). In addition, the progression of cardiac hypertrophy, studied by chronic treatment of rats with the $\beta$-adrenoceptor-agonist isoproterenol, was paralleled by the increase in membrane-associated NDPK, which, in contrast, was not observed, when hypertrophy was induced with thyroid hormone. Consistent with recent reports showing NDPK translocation by stimulation of GPCRs (Gallagher et al., 2003; Rochdi et al., 2004), these data suggest that sustained stimulation of $\beta$-adrenergic receptors increases association of NDPK with the plasma membrane.

The notion that G protein activation via NDPK B/G$\beta\gamma$ complexes is altered in diabetic prancreatic $\beta$ cells is based on findings in rat model of human type 2 diabetes, i.e. the Goto-Kakizaki rats, which exhibit impaired insulin secretion in response to glucose. Compared to control wistar rats, these animals display an about 50% reduction in NDPK activity (Metz et al., 1999), NDPK autophosphorylation and histidine kinase activity (Kowluru, 2003). Interestingly and in accordance with the role of NDPK/G$\beta\gamma$ complexes in insulin secretion, it was recently shown that some polyunsaturated fatty acids, which are well known stimulators of insulin secretion, increase NDPK activity, NDPK autophosphorylation and G$\beta$ phosphorylation in rat pancreatic islet cells (Kowluru 2004).

In light of these findings, the physiological and pathophysiological relevance of the NDPK B-mediated G protein activation via G$\beta$ phosphorylation at His266 needs to be further evaluated, preferably in transgenic animal models, which enable specific targeting of key regulators in this pathway.

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