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Activation of GTP Formation and High-Affinity GTP Hydrolysis by Mastoparan in Various Cell Membranes

G-PROTEIN ACTIVATION *VIA* NUCLEOSIDE DIPHOSPHATE KINASE, A POSSIBLE GENERAL MECHANISM OF MASTOPARAN ACTION

*Jan F. Klinker, Karl-Ludwig Laugwitz, Astrid Hagelüken and Roland Seifert**
INSTITUT FÜR PHARMAKOLOGIE, FREIE UNIVERSITAT BERLIN, THIELALLEE 69–73, D-14195 BERLIN, GERMANY

ABSTRACT. The wasp venom, mastoparan (MP), is a direct activator of reconstituted pertussis toxin-sensitive G-proteins and of purified nucleoside diphosphate kinase (NDPK) [E.C. 2.6.4.6.]. In HL-60 membranes, MP activates high-affinity GTPase [E.C. 3.6.1.-] and NDPK-catalyzed GTP formation, but not photolabeling of G-protein α-subunits with GTP azidoanilide; this suggests that the venom activates G-proteins in this system indirectly *via* stimulation of NDPK. Moreover, the MP analogue, mastoparan 7 (MP 7), is a much more effective activator of reconstituted G-proteins than MP, whereas with regard to NDPK and GTPase in HL-60 membranes, the two peptides are similarly effective. In our present study, we investigated NDPK- and G-protein activation by MP in membranes of the human neuroblastoma cell line, SH-SY5Y, the human erythroleukemia cell line, HEL, the rat basophilic leukemia cell line, RBL 2H3, and the hamster ductus deferens smooth muscle cell line, DDT_1MF-2. All these membranes exhibited high NDPK activities that were increased by MP. Compared to basal GTP formation rates, basal rates of high-affinity GTP hydrolysis in cell membranes were low. MP activated high-affinity GTP hydrolysis in cell membranes but did not enhance incorporation of GTP azidoanilide into G-protein α-subunits. As with HL-60 membranes, MP and MP 7 were similarly effective activators of NDPK and GTPase in SH-SY5Y membranes. Pertussis toxin inhibited MP-stimulated GTP hydrolyses in SH-SY5Y- and HEL membranes, whereas NDPK activations by MP were pertussis toxin-insensitive. Our data suggest that indirect G-protein activation *via* NDPK is not restricted to HL-60 membranes but is a more general mechanism of MP action in cell membranes. Pertussis toxin-catalyzed ADP-ribosylation of α-subunits may inhibit the transfer of GTP from NDPK to G-proteins. NDPK may play a much more important role in transmembrane signal transduction than was previously appreciated and, moreover, the GTPase of G-protein α-subunits may serve as GDP-synthase for NDPK. BIOCHEM PHARMACOL 51;3:217–223, 1996.

KEY WORDS. cell membranes; mastoparan; G-proteins; GTPase; GTP azidoanilide; nucleoside diphosphate kinase; pertussis toxin

Heterotrimeric G-proteins consist of an α-subunit and a βγ-complex and mediate transmembrane signal transduction from agonist-occupied heptahelical receptors to cellular effector systems [1, 2]. It is generally assumed that the release of GDP from α-subunits is the rate-limiting step in G-protein activation [1, 2]. In their activated state, G-protein α-subunits are GTP-liganded. Termination of G-protein activation is achieved by the high-affinity GTPase [EC 3.6.1.-] residing in α-subunits.

NDPK† [EC 2.6.4.6.] catalyzes the reaction $N_1TP + N_2DP \rightarrow N_1DP + N_2TP$ [3, 4]. By virtue of its function as a GTP synthase, NDPK has been suggested to play a role as GTP-supplying or regulatory enzyme in G-protein activation [3, 4]. In the early studies on G-protein regulation of adenylyl cyclase [EC 4.6.1.1.], it had already been observed that not only GTP, but also other purine- and pyrimidine base-containing NTPs, possess the ability to support agonist-induced activation of this effector system [5–7]. Much later, there arose a debate as to whether or not NDPK phosphorylates *free* GDP or GDP *bound* to G-protein α-subunits [8–11]. This debate was, however, not crucial for elucidating the *functional role* of NDPK in G-protein activation. In fact, substantial evidence has accumulated for such an involvement of NDPK in G-protein activation. For example, NDPK regulates agonist binding to chemoattractant receptors in membranes of HL-60 leukemia cells [12], receptor-mediated phospholipase C [EC 3.1.4.10.] activation [13], and arachidonic acid-stimulated NADPH oxidase [EC 1.6.99.6.] activity [14] in these membranes. In platelets, NDPK-mediated phosphotransfer reactions are involved in phospholipase D [EC 3.1.4.4.] stimulation [15] and in receptor-

* Corresponding author: Roland Seifert, Department of Molecular and Cellular Physiology, Howard Hughes Medical Institute, Beckman Center for Molecular and Genetic Medicine, Stanford University Medical Center, Stanford, CA 94305-5428, U.S.A. Tel. 001-415-7237069; FAX 001-415-4985092.

† *Abbreviations:* DADLE, D-Ala^2,D-Leu^5-enkephalin; MP, mastoparan (INLKALAALAKKIL); MP 7, mastoparan 7 (INLKALAALAKALL); NDPK, nucleoside diphosphate kinase; PTX, pertussis toxin.

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regulation of adenylyl cyclase [16, 17]. Additionally, NDPK is involved in activation of potassium channels by receptor agonists in the guinea pig atrium [18], activation of exocytosis in PC12 pheochromocytoma cells [19], and regulation of agonist binding to cholecystokinin receptors in pancreatic membranes [20]. Taken together, it appears that NDPK is of general importance in the regulation of G-protein activity. Unfortunately, studies on this issue have been hampered by the nonavailability of NDPK activators. Evidence for regulation of NDPK by heptahelical receptors is scarce [16, 21].

Recently, the cationic-amphiphilic tetradecapeptide from wasp venom, MP, has been shown to be an activator of purified NDPK and of NDPK in HL-60 membranes and rat islet homogenates [22–24]. The MP analogue, MP 7, is a similarly effective NDPK activator [23, 24]. However, MP also activates purified G-proteins and low-molecular mass GTP-binding proteins [25, 26]. Unlike NDPK, MP 7 is much more effective than MP at activating reconstituted pertussis toxin (PTX)-sensitive G-proteins [24, 25]. Thus, despite certain limitations, MP and MP 7 are useful experimental tools for studying NDPK involvement in G-protein activation.

In our recent study, we presented evidence that MP activates PTX-sensitive G-proteins in HL-60 membranes in a largely indirect manner (i.e. *via* stimulation of NDPK [24]). This hypothesis is supported by the fact that MP effectively activates high-affinity GTP hydrolysis, whereas it is ineffective in enhancing incorporation of the photoreactive GTP analogue, GTP azidoanilide, into G-protein α-subunits. Most important, under experimental conditions very similar to those employed for measurement of GTP hydrolysis, MP effectively increases NDPK-catalyzed GTP formation in HL-60 membranes.

To address the question as to whether or not G-protein activation *via* NDPK is a more general mechanism of MP action, we studied the effects of the venom on GTP formation and GTP hydrolysis in various cell membranes.

MATERIALS AND METHODS

Materials

All cell culture media were obtained from Biochrom (Berlin, Germany). MP and MP 7 were from Saxon Biochemicals (Hannover, Germany). DADLE was obtained from Sigma Chemie (Deisenhofen, Germany). Stock solutions of MP, MP 7, and DADLE (1 mM each) and dilutions were prepared in 1 mM sodium acetate, pH 5.0, and stored at −20°. Sources of other materials have been described elsewhere [14, 24, 27–29].

Cell Culture and Membrane Preparation

All cell lines were cultured under the conditions described by Hagelüken *et al.* [27]. PTX (25 ng/mL, SH-SY5Y cells; 100 ng/mL, HEL cells) or its carrier (control) was added to cell cultures 24 hr before membrane preparation. Under these conditions, virtually all α-subunits of PTX-sensitive G-proteins were ADP-ribosylated as assessed by subsequent ADP-ribosylation of membranes with activated PTX and [^{32}P]NAD (data not shown). Cell membranes were prepared as described [28]. Before experiments, membranes (100–500 μg of protein) were resuspended in 1.5 mL of 10 mM triethanolamine/HCl, pH 7.4, and were centrifuged for 10 min at 30.000 × g at 4° to remove residual cytosolic NDPK. Thereafter, membranes were resuspended at various protein concentrations in the above buffer and immediately used for experiments.

GTPase Assay

High-affinity GTP hydrolysis was determined as described [24]. Reaction mixtures (100 μL) contained 5.0–15.0 μg of membrane protein/tube, 0.5 μM [γ-^{32}P]GTP (0.1 μCi/tube) 0.5 mM $MgCl_2$, 0.1 mM EGTA, 0.1 mM ATP, 1 mM adenosine 5′-[β,γ-imido]triphosphate, 5 mM creatine phosphate, 40 μg of creatine kinase, 1 mM dithiothreitol, and 0.2% (w/v) bovine serum albumin in 50 mM triethanolamine/HCl, pH 7.4, and substances at various concentrations. Reactions were conducted at 25° for 15 min.

Assay for Photolabeling of Membrane Proteins

Cell membranes (50 μg of protein in a total volume of 60 μL) were incubated at 30° in a buffer consisting of 0.1 mM EDTA, 10 mM $MgCl_2$, 10 μM GDP, and 30 mM Hepes/NaOH, pH 7.4. Following exposure to various substances for 5 min, samples were incubated for another 3 min with 10 nM [α-^{32}P]GTP azidoanilide (1 μCi/tube). Stopping of reactions, irradiation of samples, and autoradiography were performed according to Laugwitz *et al.* [29].

Assay for [^{3}H]GTP Formation

For determination of [^{3}H]GTP formation in cell membranes, reaction mixtures (50 μL) contained 0.5 μg of membrane protein/tube, 0.5 μM [^{3}H]GDP (1 μCi/tube), 10 μM NTP, 0.5 mM $MgCl_2$, 0.1 mM EGTA, 1 mM adenosine 5′-[β,γ-imido]-triphosphate, 5 mM creatine phosphate, 40 μg of creatine kinase, 1 mM dithiothreitol, and 0.2% (w/v) bovine serum albumin in 50 mM triethanolamine/HCl, pH 7.4 [24]. Reactions were conducted for 10 min at 25°. Stopping of reactions, separation of nucleotides by TLC, and nucleotide elution from TLC plates were performed as described [14].

RESULTS

First, we studied the effects of MP and MP 7 on [^{3}H]GTP formation from [^{3}H]GDP and GTP and on high-affinity GTP hydrolysis in membranes of the human neuroblastoma cell line, SH-SY5Y. Under the experimental conditions employed, basal [^{3}H]GTP formation rates were 13-fold higher than the rates of GTP hydrolysis (Table 1). MP (10 μM) increased [^{3}H]GTP formation by 36.6–76.1% (see Tables 1–4). The corresponding values for MP 7 (10 μM) were 47.2–80.1% (see Tables 1 and 3). PTX had no effect on basal [^{3}H]GTP formation and did not reduce the stimulatory effect of MP on [^{3}H]GTP formation (see Table 2). MP and MP 7 (10 μM each) increased GTP hydrolysis by 68% and 75%, respectively (see Table 1). Unlike what occurred with [^{3}H]GTP formation,

TABLE 1. Effects of various substances on [^{3}H]GTP formation and GTP hydrolysis in cell membranes

Addition	[^{3}H]GTP formation (pmol mg^{-1} min^{-1})	GTP hydrolysis (pmol mg^{-1} min^{-1})
SH-SY5Y membranes		
Solvent (basal)	327 ± 15	25.2 ± 1.4
MP (10 μM)	474 ± 28	42.3 ± 0.6
MP 7 (10 μM)	493 ± 25	44.1 ± 0.7
DADLE (10 μM)	314 ± 23	38.6 ± 0.9
HEL membranes		
Solvent (basal)	441 ± 35	24.3 ± 3.6
MP (10 μM)	551 ± 17	31.3 ± 1.1
RBL 2H3 membranes		
Solvent (basal)	227 ± 9	6.7 ± 0.3
MP (30 μM)	465 ± 34	13.6 ± 1.0
DDT_1MF_2 membranes		
Solvent (basal)	204 ± 18	11.7 ± 0.7
MP (30 μM)	288 ± 17	16.1 ± 0.5

[^{3}H]GTP formation and high-affinity GTP hydrolysis were determined in the presence of various substances at the indicated concentrations as described in Materials and Methods. Data shown are the means ± SD of assay quadruplicates. Similar results were obtained in 3–6 independent experiments.

PTX reduced basal GTP hydrolysis and abolished the stimulatory effect of MP on GTPase (see Table 2).

NDPK does not show strict base specificity [3]. Therefore, we assessed the effectiveness of various NTPs as phosphoryl group donors for [^{3}H]GTP formation in SH-SY5Y membranes. In agreement with the fact that purine nucleotides are better substrates for NDPK than pyrimidine nucleotides [30, 31], basal [^{3}H]GTP formation rates with GTP and ATP were higher than with CTP and UTP (see Table 4). With all NTPs studied, MP effectively enhanced [^{3}H]GTP formation.

For comparison with MP, we studied the effect of DADLE, a μ- and δ opoid receptor agonist, on [^{3}H]GTP formation and GTP hydrolysis in SH-SY5Y membranes. DADLE (10 μM) did not increase [^{3}H]GTP formation, but was as effective an activator of hydrolysis as MP (10 μM) (see Table 1). The stimulatory effect of DADLE on GTP hydrolysis in SH-SY5Y membranes was abolished by PTX treatment (data not shown).

To corroborate the hypothesis that parallel NDPK- and GTPase-stimulations by MP occur generally in cell membranes, we studied the effects of MP on [^{3}H]GTP formation and high-affinity GTP hydrolysis in membranes of three additional cell lines: the human erythroleukemia cell line, HEL, the rat basophilic leukemia cell line, RBL 2H3, and the hamster ductus deferens smooth muscle cell line, DDT_1MF-2. These membranes showed NDPK activities within the same order of magnitude as that in SH-SY5Y membranes (see Table 1). Compared to basal [^{3}H]GTP formation rates, those of high-affinity GTP hydrolysis in HEL-, RBL 2H3-, and DDT_1-MF_2 membranes were 17–34-fold lower. In HEL-, RBL 2H3-, and DDT_1MF-2 cells, MP stimulated [^{3}H]GTP formation by 23.9–59.4%, 37.9–104.8%, and 19.6–47.4%, respectively (see Tables 1 and 3). Similar to SH-SY5Y membranes (see Table 4), MP stimulated [^{3}H]GTP formation in HEL-, RBL 2H3, and DDT_1MF-2 membranes with various NTPs as phosphoryl group donors (data not shown). In these cell membranes, MP stimulated GTP hydrolysis by 29%, 102%, and 38%, respectively (see Table 1). Similar stimulatory effects of MP on GTP hydrolysis in various cell membranes as in our present study were reported in our recent paper [27]. The stimulatory effect of MP on [^{3}H]GTP formation in HEL membranes was PTX-insensitive, whereas stimulation by MP of GTP hydrolysis was PTX-sensitive (see Table 2).

To determine whether or not MP interacts with G-protein

TABLE 2. Effect of PTX on basal and MP-stimulated [^{3}H]GTP formation and GTP hydrolysis in SH-SY5Y- and HEL membranes

	[^{3}H]GTP formation (pmol mg^{-1} min^{-1})		GTP hydrolysis (pmol mg^{-1} mg^{-1})	
Addition	Control	PTX	Control	PTX
SH-SY5Y membranes				
Solvent (basal)	313 ± 35	328 ± 19	24.9 ± 1.5	9.5 ± 0.8
MP (10 μM)	428 ± 39	443 ± 27	41.1 ± 10.0	10.0 ± 0.5
HEL membranes				
Solvent (basal)	432 ± 18	405 ± 25	19.9 ± 2.5	17.0 ± 1.8
MP (10 μM)	535 ± 25	514 ± 32	25.5 ± 1.3	16.9 ± 0.7

[^{3}H]GTP formation and high-affinity GTP hydrolysis were determined in the absence or presence of MP as described in "Materials and Methods." Treatment of cells with carrier (control) or PTX was performed as described in "Materials and Methods." Data shown are the means ± SD of assay quadruplicates. Similar results were obtained in three independent experiments.

TABLE 3. Stimulatory effects of MP and MP 7 on [^{3}H]GTP formation in cell membranes: comparison of the results obtained in various experiments

Experiment no.	[^{3}H]GTP formation (% stimulation) SH-SY5Y membranes MP	MP 7	HEL membranes MP	RBL 2H3 membranes MP	DDT_1MF_2 membranes MP
1	76.1	80.1	23.9	104.8	41.2
2	44.9	50.7	59.4	96.6	19.6
3	36.6	47.2	24.9	37.9	47.4
4				72.7	
5				47.3	
6				64.7	

The stimulatory effects of MP and MP 7 on [^{3}H]GTP formation in various cell membranes were determined as described in "Materials and Methods." In experiments performed with SH-SY5Y and HEL membranes, the concentration of MP and MP 7 was 10 μM each. In experiments performed with RBL 2H3 and DDT_1MF_2 membranes, the concentration of MP was 30 μM. Values for basal [^{3}H]GTP formation in cell membranes are given in Tables 1, 2, and 4.

α-subunits or low-molecular mass GTP-binding proteins, we studied the incorporation of the photoreactive GTP analogue, GTP azidoanilide, into cell membrane proteins. Figure 1 shows the effects of MP, MP 7, and DADLE on photolabeling of G-protein α-subunits in SH-SY5Y membranes. DADLE substantially increased photolabeling of proteins with apparent molecular masses of 41.5 kDa (corresponding to the α-subunits of G_{i1} and G_{i3}), 40/40.5 kDa (corresponding to the α-subunits of G_{i2} and G_{o1}) and 39 kDa (corresponding to the α-subunit of G_{o2}) [29]. By contrast, MP and MP 7 had no stimulatory effects on photolabeling of G-protein α-subunits in SH-SY5Y membranes. Additionally, no stimulatory effects of MP and MP 7 on labeling in the 20 kDa region (i.e. where labeling of low-molecular mass GTP-binding proteins would have been expected [26], were evident. As in SH-SY5Y membranes, MP did not enhance incorporation of GTP azidoanilide into G-protein α-subunits in membranes of HEL-, RBL 2H3-, and DDT_1MF_2 membranes (data not shown). Under experimental conditions similar to those employed by us, Fields *et al.* [32] reported on a stimulatory effect of MP on the labeling of G-protein α-subunits with GTP-azidoanilide in bovine brain membranes. Except for the fact that different membranes were employed, we have no explanation for the divergent results.

DISCUSSION

Membranes of SH-SY5Y-, HEL-, RBL 2H3-, and DDT_1MF_2 cells exhibit high NDPK activities as assessed by [^{3}H]GTP formation from [^{3}H]GDP and NTP (see Tables 1 and 4 and Fig. 2A). Under experimental conditions similar to those employed by us, Kowluru and Metz [23] reported comparably high NDPK activities in homogenates of rat and human pancreatic islet cells and insulin-secreting cell lines (294–560 pmol mg^{-1} min^{-1}). MP increased [^{3}H]GTP formation in SH-SY5Y-, HEL-, RBL 2H3-, and DDT_1MF_2 membranes by 19.6–104.8%, depending on the specific experiment and type of membrane

TABLE 4. [^{3}H]GTP formation with various NTPs as phosphoryl group donors in SH-SY5Y membranes: stimulatory effect of MP

NTP	GTP formation (pmol mg^{-1} min^{-1}) basal	MP
GTP	315 ± 21	555 ± 24
ATP	270 ± 18	479 ± 19
CTP	191 ± 6	359 ± 30
UTP	200 ± 14	410 ± 5

[^{3}H]GTP formation from [^{3}H]GDP and various NTPs (10 μM each) in SH-SY5Y membranes was determined as described in "Materials and Methods." The concentration of MP was 10 μM. Data shown are the means ± SD of assay quadruplicates. Similar results were obtained in three independent experiments.

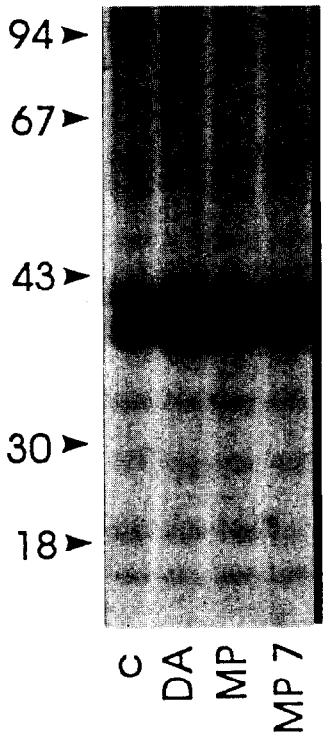

FIG. 1. Effects of MP, MP 7, and DADLE on incorporation of GTP azidoanilide into G-protein α-subunits in SH-SY5Y membranes. Photolabeling of membrane proteins was performed as described in "Materials and Methods." The autoradiography of an SDS gel containing 6 M urea and 9% (w/v) acrylamide is shown. Lane 1, solvent (control) (c); lane 2, DADLE (10 μM) (DA); lane 3, MP (10 μM); lane 4, MP 7 (10 μM). Numbers on the left, molecular masses of marker proteins (kDa). The autoradiography is representative for three independent experiments.

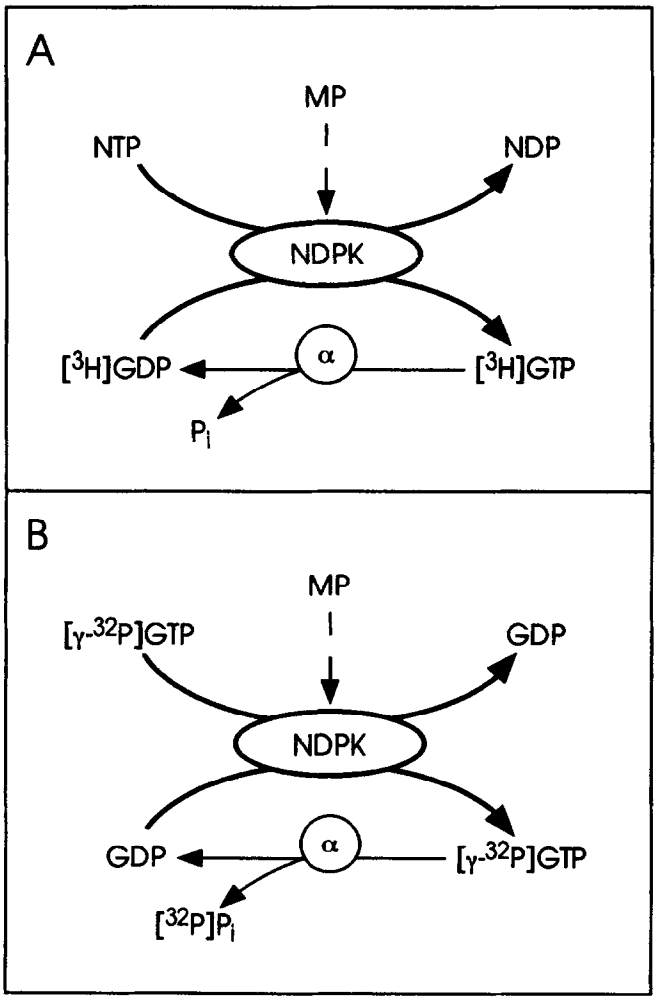

FIG. 2. Schematic presentation of the functional interaction of NDPK with G-protein α-subunits in cell membranes. (A) Reactions taking place in the NDPK assay (measurement of [^{3}H]GTP formation from [^{3}H]GDP and NTP). Accumulation of [^{3}H]GTP and its stimulation by MP is assessed. Degradation of [^{3}H]GTP to [^{3}H]GDP and P_i does not mask the stimulatory effect of MP on NDPK. (B) Reactions taking place in the GTPase assay (measurement of GTP hydrolysis). In cell membranes, no direct interaction of MP with G-protein α-subunits can be detected, as assessed by photolabeling with GTP azidoanilide. Thus, stimulation by the venom of GTPase presumably occurs indirectly *via* NDPK activation and not *via* the classical GDP/GTP exchange. According to the latter model, GDP is first phosphorylated to [γ-^{32}P]GTP with [γ-^{32}P]GTP as phosphoryl group donor. This new [γ-^{32}P]GTP, after transfer to G-protein α-subunits, is cleaved to GDP and [^{32}P]P_i. For further explanations and interpretations, see Discussion. α, G-protein α-subunit.

studied (see Table 3). Stimulatory effects of MP on NDPK activity were also reported for HL-60 membranes [24, 33] and homogenates of insulin-secreting cells [23]. These data show that stimulation of NDPK by MP is a process occurring in all cell types studied until now.

Intriguingly, the product formed in the NDPK reaction, [^{3}H]GTP, is not stable but may be cleaved to [^{3}H]GDP and P_i by the GTPase of G-protein α-subunits (see Fig. 2A). The fact that stimulation of [^{3}H]GTP formation by MP in cell membranes was detected, although concomitant GTP hydrolysis took place, indicates that the activity of NDPK is higher than that of GTPase (see Fig. 2A). In fact, under the conditions employed, basal [^{3}H]GTP formation rates were 13–34-fold higher than basal GTP hydrolysis rates (see Table 1). In future studies, detailed analyses of the kinetic properties of GTPase and NDPK in cell membranes under exactly the same experimental conditions will have to be performed. It should be noted that, as in HL-60 membranes [24, 33], MP-induced NDPK stimulations in SH-SY5Y-, HEL-, RBL 2H3-, and DDT_1MF_2 membranes were observed in the presence of a NTP-regenerating system consisting of creatine kinase and creatine phosphate (see Materials and Methods). This indicates that GTP formation by NDPK takes place in a microcompartment deeply buried in the plasma membrane and unaccessible to the regenerating system.

MP and MP 7 not only stimulated [^{3}H]GTP formation in the various cell membranes studied, but also stimulated high-affinity GTP hydrolysis (see Tables 1 and 2) [24, 33]. In SH-SY5Y- and HEL membranes, we studied the effect of PTX on GTP hydrolysis. In both cell membranes, the stimulatory effects of MP on GTPase were abolished by the toxin (see Table 2). The PTX substrates in SH-SY5Y cells are G_i- and G_o-proteins (see Fig. 1) [29] and, in HEL cells, the toxin substrates are G_i-proteins [34]. These data show that in SH-SY5Y membranes, MP activates the GTPase of G_i- and G_o-proteins and, in HEL membranes, MP stimulates the GTPase of G_i-proteins.

The question arises as to whether these GTPase activations are achieved by direct interaction of MP with G-protein α-subunits or indirectly *via* NDPK stimulation. According to the latter model (see Fig. 2B), MP would first stimulate the conversion of endogenous GDP (e.g. GDP released from G-protein α-subunits) and [γ-^{32}P]GTP to new [γ-^{32}P]GTP and GDP. The newly synthesized [γ-^{32}P]GTP would then be transferred to, and cleaved by, G-protein α-subunits, resulting in the formation of GDP and [^{32}P]P_i. The latter product is measured in the GTPase assay. To discriminate between these two possibilities, we studied the effects of MP and MP 7 on incorporation of GTP azidoanilide into G-protein α-subunits in cell membranes. This GTP analogue, like GTP, is exchanged for GDP and can be covalently attached to α-subunits following UV irradiation of membranes [24, 29]. Agonists at heptahelical receptors cause parallel increases in GTP hydrolysis (reflecting GDP/GTP exchange) and incorporation of the photoreactive GTP analogue into PTX-sensitive G-proteins in HL-60 and SH-SY5Y membranes (see Table 1 and Fig. 1) [24]. However, MP failed to stimulate incorporation of GTP-azidoanilide into G-protein α-subunits in SH-SY5Y-, HL-60, HEL-, RBL 2H3-, and DDT_1MF_2 membranes, although the venom induced substantial increases in GTP hydrolysis (see Table 1 and Fig. 1). As is the case for HL-60 membranes [24], MP and MP 7 are similarly effective activators of GTPase on the one hand and of NDPK, on the other, in SH-SY5Y membranes (see Tables 1 and 3). With respect to purified NDPK and NDPK in homogenates of insulin-secret-

ing cells, MP and MP 7 are also similarly effective stimuli [22–24]. Moreover, MP 7 failed to stimulate photolabeling of G_i- and G_o-protein α-subunits in SH-SY5Y membranes although MP 7 is a very effective activator of these G_i- and G_o-proteins in a reconstituted system (see Fig. 1) [24, 25]. Taken together, all these data indicate that MP and MP 7 activate high-affinity GTPase in cell membranes indirectly *via* NDPK stimulation (see Fig. 2). Thus, not only GDP release but also GTP availability may be a rate-limiting factor in G-protein activation.

Dissociations between stimulations of GTP hydrolysis and labeling of G-protein α-subunits with GTP-azidoanilide are not restricted to MP. Specifically, synthetic lipopeptides derived from bacterial lipoprotein stimulate the GTPase of G_i-proteins, but not photolabeling of G_i-protein α-subunits in HL-60 membranes [33, 35]. Thus, by analogy to MP, NDPK activation presumably accounts for the stimulatory effects of lipopeptides on GTP hydrolysis in HL-60 membranes [33].

The receptor agonist, DADLE, substantially stimulated GTPase but was, unlike MP, without effect on [^{3}H]GTP formation (see Table 1). These findings, however, do not rule out the possibility that stimulation of opioid receptors induces NDPK activation. Possibly, receptor agonists stimulate the formation of as much GTP as is subsequently hydrolyzed by GTPase. Under such conditions, a net stimulation of NDPK is very difficult to detect. In this context, it should be emphasized that MP is a venom and does not mimic G-protein activation by heptahelical receptors [24]. Thus, one could imagine that the clearly measurable NDPK stimulation by MP reflects a toxic effect and is not physiological. It is also possible that we did not employ the correct assay conditions for detecting a stimulatory effect of DADLE on NDPK. Specifically, we used GTP as phosphoryl group donor (see Table 1). Wieland and Jakobs [16] observed NDPK stimulation by prostaglandin E_1 in platelet membranes with adenosine 5′-0-(3-thiotriphosphate) as thiophosphoryl group donor. In *Dictyostelium*, receptor-stimulation of NDPK is readily observed with ATP as substrate [21]. Thus, much more effort is required to understand regulation of NDPK by heptahelical receptors.

In marked contrast to MP-stimulated GTP hydrolyses, MP-induced GTP formations in SH-SY5Y- and HEL membranes were completely PTX-insensitive (see Table 2). In addition, we failed to obtain evidence for a direct interaction of MP with G-protein α-subunits in the photolabeling experiments (see Fig. 1). From these findings, it may be concluded that PTX-catalyzed ADP-ribosylation of G_i- and G_o-protein α-subunits inhibits the transfer of GTP from NDPK to G-proteins.

Our present data and those of our recent studies [24, 33, 35] raise a number of important questions as to the role of NDPK in transmembrane signal transduction. Specifically, is NDPK an enzymatically active "MP receptor" whose functional interaction with PTX-sensitive G-proteins, like that of heptahelical receptors, is inhibited by ADP-ribosylation of α-subunits? Is NDPK a novel G-protein-regulated effector system that, in turn, regulates the activity of G-proteins? Interestingly, heptahelical receptors possess the ability to mediate activation of effector systems in *Dictyostelium* in the absence of G-proteins [36] and, in these cells, stimulation of NDPK by heptahelical receptors has been reported [21]. Thus, can NDPK mediate coupling of receptors to effector systems (i.e. act as substitute for G-proteins)? It is generally assumed that NDPK plays a role in G-protein activation, but is it not possible that G-proteins play a role in NDPK activation? According to such a model, the GTPase of G-protein α-subunits would function as GDP-synthase for NDPK, and the end point of a signal transduction cycle ("NDPK cycle") would be GTP formation rather than GTP hydrolysis (see Fig. 2). We cannot exclude the possibility that the GTPase cycle and the NDPK cycle operate in parallel and have distinct functions in transmembrane signal transduction.

In conclusion, our results suggest that indirect activation of G-proteins by MP *via* stimulation of NDPK is a general mechanism of venom action in cell membranes. PTX-catalyzed ADP-ribosylation may interfere with the transfer of GTP from NDPK to G-protein α-subunits. The role of NDPK in transmembrane signal transduction processes may be much more complex than was previously appreciated, and the GTPase of G-protein α-subunits may serve as GDP-synthase for NDPK.

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