LIPOPHILIC β-ADRENOCEPTOR ANTAGONISTS AND LOCAL ANESTHETICS ARE EFFECTIVE DIRECT ACTIVATORS OF G-PROTEINS

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Abstract—We studied the effects of various β-adrenoceptor (βAR) antagonists and local anesthetics (LAs), i.e. substances possessing one basic and one lipophilic domain each, on activation of regulatory heterotrimeric guanine nucleotide-binding proteins (G-proteins). In membranes of differentiated HL-60 cells, propranolol activated high-affinity GTP hydrolysis with a half-maximal effect at 0.19 mM and a maximum at 1 mM. There was a close correlation between the log Q values (logarithm of the octanol: water partition coefficient) of βAR antagonists and the logarithm of their effectiveness at activating GTPase (EC 3.6.1.-) in HL-60 membranes. The lipophilic LA, tetracaine, was also an effective activator of GTPase in HL-60 membranes, whereas more hydrophilic LAs were less stimulatory (bupivacaine and lidocaine) or even inhibitory (procaine). Propranolol and tetracaine also stimulated binding of guanosine 5'-*O*-[3-thio]triphosphate (GTP[γS]) to HL-60 membranes, but their stimulatory effects on GTP[γS] binding were smaller than on GTP hydrolysis. The stimulatory effects of propranolol and tetracaine on GTPase and GTP[γS] binding were inhibited by pertussis toxin. Propranolol and tetracaine effectively activated GTP hydrolysis of a reconstituted mixture of bovine brain G_i/G_o-proteins, but the concentrations of substances needed for GTPase activation were higher than in HL-60 membranes. Procaine showed stimulatory effects on the GTPase of G_i/G_o-proteins. Our data show that βAR antagonists and LAs activate pertussis toxin-sensitive G-proteins, presumably through interaction with the C-terminus of their α-subunits. Apparently, the lipophilic domain of βAR antagonists and LAs is more important for G-protein activation than the basic domain. We discuss the possibility that activation of nucleoside diphosphate kinase by βAR antagonists and LAs contributes to their stimulatory effects on GTP hydrolysis in HL-60 membranes.

Key words: β-adrenoceptor antagonists; local anesthetics; lipophilicity; pertussis toxin; G-proteins; GTPase

Numerous intercellular signal molecules exert their effects through receptors which interact with G-proteins§ [1, 2]. G-protein-coupled receptors possess seven putative transmembrane domains three extracellular and three cytoplasmic loops. There is evidence to support the assumption that the second and third cytoplasmic loop are important for G-protein activation [2]. Recent studies have shown that cationic-amphiphilic substances such as compound 48/80 and the tetradecapeptide, mastoparan, activate PTX-sensitive G-proteins, i.e., G_i- and G_o-proteins, directly, presumably by acting as substitutes for the third cytoplasmic loop of receptors [3–7]. Studies with mastoparan and mastoparan derivatives have shown that lipophilicity of the peptides is important for G-protein activation [4].

βAR antagonists and LAs are cationic-amphiphilic substances, i.e. they possess one basic and one lipophilic domain each and show substantial

differences in lipophilicity [8, 9]. We therefore used βAR antagonists and LAs as model substances to learn more about the relative importance of lipophilic and basic domains of cationic-amphiphilic substances for G-protein activation. We show here that βAR antagonists and LAs directly activate PTX-sensitive G-proteins and that lipophilicity increases their effectiveness in this regard.

MATERIALS AND METHODS

Materials. βAR antagonists, (−)-isoproterenol, LAs, azolectine and mastoparan were obtained from the Sigma Chemical Co. (Deisenhofen, Germany). With the exception of (−)- and (+)-propranolol and (−)-timolol, racemates of βAR antagonists were employed. Unless stated otherwise, experiments were performed with racemic propranolol. Stock solutions of metroprolol tartrate, timolol maleate, hydrochlorides of the other βAR antagonists and hydrochlorides of LAs (30 mM each) were prepared in distilled water and were stored at −20°. [^{35}S]-guanosine 5'-*O*-[3-thio]triphosphate [^{35}S]GTP[γS]) (1000–1500 Ci/mmol) was obtained from Dupont/New England Nuclear (Bad Homburg, Germany). Sources of other materials have been described elsewhere [10–13].

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§ Abbreviations: βAR, β-adrenoceptor; G-protein, regulatory heterotrimeric guanine nucleotide-binding protein; GTP[γS], guanosine 5'-*O*-[3-thio]triphosphate; LA, local anesthetic; NDPK, nucleoside diphosphate kinase; PTX, pertussis toxin.

Cell culture and membrane preparation. HL-60 cells were grown in suspension culture at 37° and were differentiated towards neutrophil-like cells with dibutyryl cAMP (0.2 mM) for 48 hr [13]. Membranes were prepared as described [10]. PTX (100 ng/mL) or its carrier (control) were added to cell cultures 24 hr before membrane preparation. Under these conditions, >95% of G_i-protein α-subunits were ADP-ribosylated (data not shown).

Purification and reconstitution of G-proteins. A mixture of heterotrimeric G_i/G_o-proteins was purified from bovine brain membranes [12]. Briefly, cholate extracts of membranes were subjected to chromatography on a DEAE-Sepharose Fast Flow column (Pharmacia, Freiburg, Germany), followed by an AcA 34 gel filtration column (Serva, Heidelberg, Germany) and a Heptylamine-Sepharose column. Fractions were analysed for GTP[γS] binding and immunoreactivity using specific antibodies [11, 12]. Purity of pooled heterotrimeric G-proteins was estimated by SDS–PAGE and silver-staining and was >90%. The purified mixture contained predominantly G_{o1}, substantial amounts of G_{o2}, G_{i1} and G_{i2} and traces of G_{i3}.

Reconstitution of purified G-proteins into phospholipid vesicles was performed essentially as described by Tomita *et al.* [6] with slight modifications. In brief, a purified mixture of bovine brain G_i/G_o proteins (25–30 pmoles) was mixed with azolectine (0·1%, w/v), sodium cholate (1%, w/v) in a buffer consisting of 100 mM NaCl, 2 mM $MgCl_2$, 1 mM EDTA and 20 mM HEPES/NaOH, pH 8.0, 4°, and loaded onto a 10 mL AcA 34 gel filtration column (25 cm × 8.5 mm) equilibrated with the above buffer. Liposomes eluted in the void volume. Association of G-proteins with liposomes was confirmed by GTP[γS] binding. Pooled fractions were then used for measurement of GTP hydrolysis.

Measurement of the activity of GTPase (EC 3.6.1.-). GTP hydrolysis was determined as described [13]. For determination of GTP hydrolysis in membranes from dibutyryl cAMP-differentiated HL-60 cells, reaction mixtures (100 μL) contained 3.0–7.0 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. Reactions were conducted for 15 min at 25°. For determination of the GTPase activity of reconstituted G-proteins, reaction mixtures (100 μL) contained 0.4–0.6 pmoles of G_i/G_o-proteins, 50 nM [γ-^{32}P]GTP (0.1 μCi/tube), 1 mM $MgCl_2$ and 0.5 mM EDTA. The other conditions were as described above.

Assay for GTP[γS] binding in HL-60 membranes. [^{35}S]GTP[γS] binding in HL-60 membranes was assessed according to Wieland *et al.* [14] with modifications. In brief, reaction mixtures (100 μL) contained membranes from dibutyryl cAMP-differentiated HL-60 cells (3.0–5.0 μg of protein/tube), 0.4 nM [^{35}S]GTP[γS], 5 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol and 0.2% (w/v) bovine serum albumin in 50 mM triethanolamine/HCl, pH 7.4. Reactions were conducted for 60 min at 0°. Reactions

were terminated by rapid filtration through cellulose nitrate BA 85 filters (Schleicher & Schuell, Dassel, Germany) followed by two washes with 5 mL of buffer (0°) consisting of 5 mM $MgCl_2$ and 50 mM triethanolamine/HCl, pH 7.4. Filter-bound radio-activity was determined in a liquid scintillation counter. Non-specific binding was determined in the presence of 10 μM GTP[γS] and was less than 1% of total binding.

Miscellaneous. Protein was determined according to Lowry *et al.* [15]. [γ-^{32}P]GTP was prepared as described [16]. Data shown in Figs 1, 3, 4 and 5 and Table 1 are the means of assay quadruplicates of a representative experiment. Similar results were obtained in at least three independent experiments. The SD values were generally <5% of the means. The statistical significance of the stimulatory effects of pindolol, nadolol and atenolol on GTPase in HL-60 membranes (Fig. 1) was assessed using the Wilcoxon test. The regression line shown in Fig. 2 was calculated using the program, Sigmaplot 4.0 (Jandel, Erkrath, Germany).

RESULTS

The effects of βAR antagonists on high-affinity GTP hydrolysis, i.e. the enzymatic activity of G-protein α-subunits, in HL-60 membranes were studied. HL-60 cells contain mostly the G-protein, G_{i2}, and, to a lesser extent, G_{i3} and G_s, and are a widely used model system for the analysis of G-protein-mediated signal transduction processes [17]. Propranolol activated GTPase with an EC_{50} of 0.19 mM and a plateau at 1–3 mM (Fig. 1). At maximally effective concentrations, propranolol increased GTP hydrolysis by 85–90%. Stimulation of GTP hydrolysis by propranolol was due to an increase in V_{max} without a change in K_m (data not shown), indicating that the substance increased the catalytic rate of GTP turnover. In membranes from PTX-treated cells, the stimulatory effect of propranolol on GTPase was almost prevented (Table 1). (+)-Propranolol and (−)-propranolol activated GTPase with a very similar potency and effectiveness as racemic propranolol (see Fig. 1).

Other βAR antagonists activated GTP hydrolysis in HL-60 membranes in a concentration-dependent manner as well, but, their stimulatory effects did not reach saturation until 3 mM (see Fig. 1). The order of effectiveness of βAR antagonists at activating GTP hydrolysis was propranolol > alprenolol > metoprolol > timolol > pindolol > nadolol > atenolol (Fig. 2). There was a close correlation ($r = 0.98$) between the log Q values, i.e. the logarithm of the octanol:water partition coefficient of βAR antagonists [8], and the logarithm of their effectiveness to activate GTPase (see Fig. 2).

In marked contrast to βAR antagonists, the βAR agonist, (−)-isoproterenol (1–100 μM), did not stimulate GTP hydrolysis in HL-60 membranes (data not shown). Mastoparan at a maximally effective concentration (10 μM) increased GTPase activity in HL-60 membranes by 85% (data not shown).
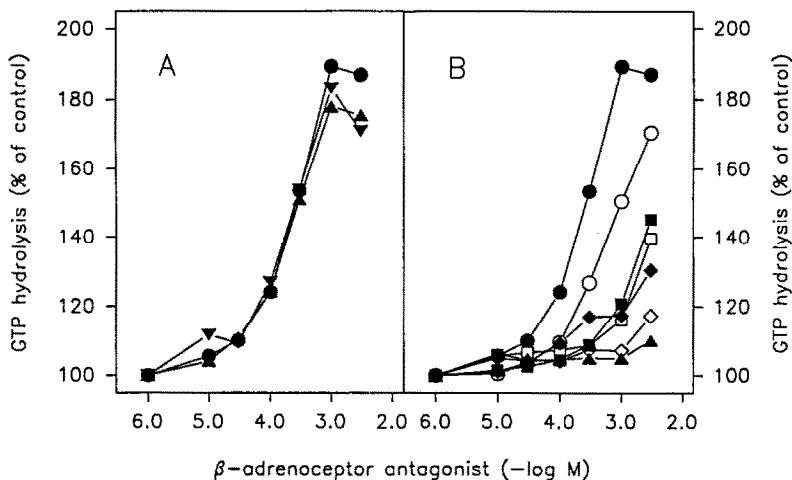
The effects of some LAs on GTP hydrolysis in

Fig. 1. Concentration–response curves for various βAR antagonists on high-affinity GTP hydrolysis in membranes from dibutyryl cAMP-differentiated HL-60 cells. GTP hydrolysis was determined as described in Materials and Methods. Reaction mixtures contained βAR antagonists at the indicated concentrations. Panel A: racemic propranolol (●), (−)-propranolol (▼), (+)-propranolol (▲). Panel B: propranolol (●), alprenolol (○), metoprolol (■), timolol (□), pindolol (◆), nadolol (◇), atenolol (▲). Basal GTP hydrolysis rate was 15.3 ± 0.5 pmol/mg/min. The stimulatory effects of pindolol were significant (P < 0.05) at a concentration of 0.3 mM and above, and those of nadolol and atenolol were significant (P < 0.05) at 3 mM.

Table 1. Effect of pertussis toxin on stimulations of GTP hydrolysis and GTP[γS] binding by propranolol and tetracaine in membranes from dibutyryl cAMP-differentiated HL-60 cells

Addition	GTP hydrolysis (pmol/mg/min)		GTP[γS] binding (pmol/mg)	
	Control	PTX	Control	PTX
None (basal)	15.2 ± 0.4	7.0 ± 0.2	1.12 ± 0.05	0.85 ± 0.07
Propranolol (1 mM)	28.0 ± 0.4	7.3 ± 0.3	1.52 ± 0.04	0.83 ± 0.06
Tetracaine (1 mM)	24.0 ± 0.3	7.2 ± 0.5	1.55 ± 0.03	0.87 ± 0.01

Pretreatment of HL-60 cells with PTX or carrier (control) was as described in Materials and Methods. GTP hydrolysis and GTP[γS] binding in HL-60 membranes were also determined as described in Materials and Methods.

HL-60 membranes are shown in Fig. 3. Tetracaine, bupivacaine and lidocaine activated GTPase in a concentration-dependent manner. As was the case for most βAR antagonists (see Fig. 1), the stimulatory effects of LAs did not reach saturation until 3 mM. The order of effectiveness of LAs at activating GTPase was tetracaine > bupivacaine > lidocaine. With respect to these LAs, high lipophilicity was associated with high effectiveness at activating GTP hydrolysis (Table 2). Among the LAs studied, procaine was the most hydrophilic one (see Table 2) [9], and this LA reduced GTP hydrolysis up to 20% below basal values in HL-60 membranes (see Fig. 3). Tetracaine (3 mM) and propranolol (1 mM) were similarly effective at stimulating GTP hydrolysis (see Figs 1 and 3).

We also studied the effects of some βAR antagonists on the GTPase activity of a mixture of reconstituted Gi/Go-proteins. In accordance with the results obtained for HL-60 membranes (see Figs 1 and 2), propranolol was much more effective than pindolol at activating GTP hydrolysis of reconstituted G-proteins (Fig. 4). Stimulation of the GTPase of Gi/Go-proteins by propranolol did not reach saturation, even at a concentration as high as 5 mM. At this high concentration, propranolol increased GTP hydrolysis by 130%. Metoprolol and pindolol were similarly effective activators of the GTPase of Gi/Go-proteins. Mastoparan (100 μM) increased GTP hydrolysis of the reconstituted G-proteins by 60% (data not shown).

Tetracaine also activated the GTPase of reconstituted Gi/Go-proteins in a concentration-dependent manner (Fig. 5). Similar to propranolol, the concentrations of tetracaine required to activate Gi/Go-proteins were higher than those needed for activation of GTP hydrolysis in HL-60 membranes (compare Figs 1 and 4 and Figs 3 and 5). Similar to
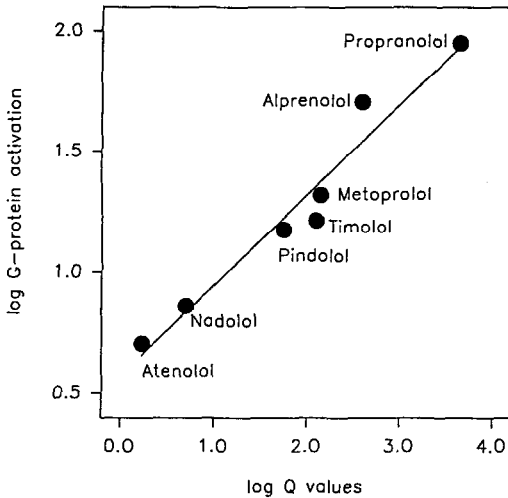
Fig. 2. Correlation between the log Q values of βAR antagonists and their effectiveness at activating GTP hydrolysis in HL-60 membranes. The log Q values for βAR antagonists were taken from Ref. 8. The stimulatory effects of βAR antagonists (1 mM each) on GTP hydrolysis in HL-60 membranes are the means of five independent experiments. The increase in GTP hydrolysis caused by propranolol was defined as 100%. The stimulatory effects of the other βAR antagonists were related to this value. Log Q values were plotted against the logarithm of the effectiveness of βAR antagonists to activate GTPase.
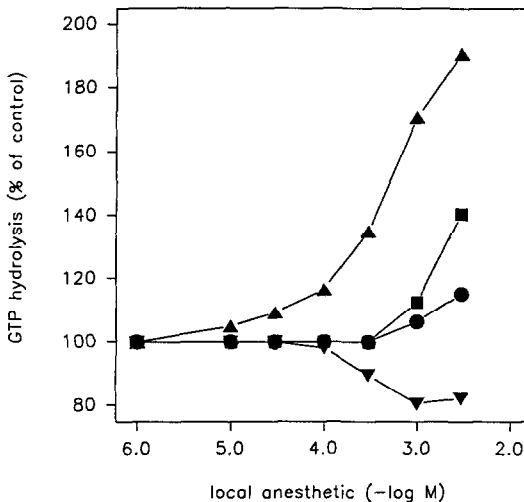

Fig. 3. Concentration–response curves for various LAs on high-affinity GTP hydrolysis in membranes from dibutyryl cAMP-differentiated HL-60 cells. GTP hydrolysis in HL-60 membranes was determined as described in Materials and Methods. Reaction mixtures contained tetracaine (▲), bupivacaine (■), lidocaine (●) and procaine (▼) at the indicated concentrations. Basal GTP hydrolysis rate was 16.2 ± 0.6 pmol/mg/min.

the results obtained with HL-60 membranes, an increase in lipophilicity of LAs (lidocaine < bupivacaine < tetracaine) resulted in an increase in effectiveness of substances at activating the GTPase of reconstituted G_i/G_o-proteins (see Fig. 5 and Table 2). In contrast to HL-60 membranes, procaine showed stimulatory effects on GTP hydrolysis by G_i/G_o-proteins, and this LA was even slightly more effective than lidocaine (see Figs 3 and 5 and Table 2).

In addition to stimulation of high-affinity GTP hydrolysis, stimulation of GTP[γS] binding is a parameter reflecting G-protein activation [14]. In HL-60 membranes, both propranolol and tetracaine stimulated GTP[γS] binding in a PTX-sensitive manner (see Table 1). However, compared to GTP hydrolysis, the stimulatory effects of propranolol and tetracaine on GTP[γS] binding in HL-60 membranes were smaller (84% vs 36% stimulation in the case of propranolol and 58% vs 38% stimulation in the case of tetracaine) (see Table 1).

DISCUSSION

In order to learn more about the properties of cationic-amphiphilic substances needed for direct G-protein activation we employed βAR antagonists and LAs as model substances. We found that βAR antagonists and LAs increased GTP hydrolysis and GTP[γS] binding in HL-60 membranes in a PTX-sensitive manner, indicating that they activate G_i-proteins (see Figs 1 and 3 and Table 1). In accordance with this, the substances also activated the GTPase of reconstituted G_i/G_o-proteins (see Figs 4 and 5).

Unlike βAR antagonists, the βAR agonist, (−)-isoproterenol, did not stimulate high-affinity GTP hydrolysis in HL-60 membranes. In addition, the stimulatory effects of propranolol on GTPase and GTP[γS] binding in HL-60 membranes were almost or completely inhibited by PTX (see Table 1). Moreover, (+)- and (−)-propranolol were similarly potent and effective at activating GTPase although they possess different affinities to βARs (see Fig. 1) [18]. Thus, it is unlikely that activation of βARs and the PTX-insensitive G-protein, G_s [1], substantially contributed to the stimulatory effects of βAR antagonists on high-affinity GTPase in HL-60 membranes.

As βAR antagonists and LAs may change physical membrane properties [18–20] the question arises whether they activate PTX-sensitive G-proteins by altering this parameter. An argument in favor of this assumption may be the finding that (+)- and (−)-propranolol were similarly potent and effective at activating GTP hydrolysis in HL-60 membranes (see Fig. 1). However, the data obtained with membranes from PTX-penetrated HL-60 cells do not support this view. PTX catalyses the ADP-ribosylation of a cysteine residue near the C-terminus of G_i-protein α-subunits [1] and, thereby, inhibits the stimulatory effects of mastoparan and compound 48/80 on GTP hydrolysis of, and GTP[γS] binding to, reconstituted G_i/G_o-proteins [3,5,6]. If the stimulatory effects of βAR antagonists and LAs on GTP hydrolysis and GTP[γS] binding in HL-60 membranes were due to changes in physical

Table 2. Comparison of the log Q values of LAs and their effectiveness at activating GTP hydrolysis in HL-60 membranes and of reconstituted G_i/G_o-proteins

| LA | Log Q value | Log G-protein activation | |
		HL-60 membranes	G_i/G_o-proteins
Procaine	−0.22	—*	1.13
Lidocaine	0.46	1.18	1.02
Bupivacaine	1.45	1.64	1.34
Tetracaine	1.90	2.00	2.00

The log Q values for LAs were taken from Ref. 7. The stimulatory effects of LAs (3 mM each) on GTPase in HL-60 membranes were taken from Fig. 3, and those of LAs (5 mM each) on GTP hydrolysis of reconstituted G_i/G_o-proteins were taken from Fig. 5. The increase in GTP hydrolysis caused by tetracaine was defined as 100%. The stimulatory effects of the other LAs were related to this value. The logarithm of the effectiveness of LAs at activating GTPase was calculated.

* Procaine did not stimulate but rather inhibited GTP hydrolysis in HL-60 membranes.
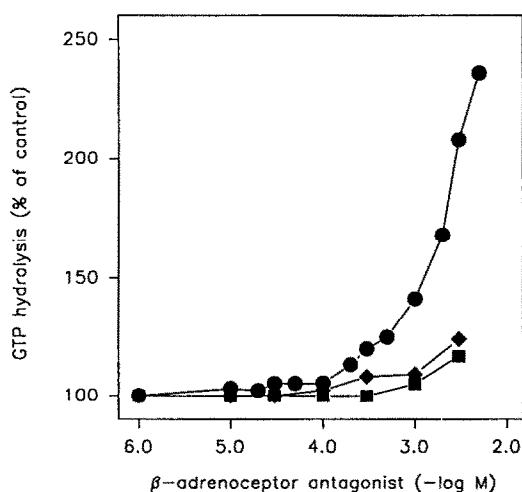

Fig. 4. Concentration–response curves for some βAR antagonists on high-affinity GTP hydrolysis of a reconstituted mixture of G_i/G_o-proteins. G-proteins purified from bovine brain were reconstituted into phospholipid vesicles and assayed for GTP hydrolysis as described in Materials and Methods. Reaction mixtures contained propranolol (●), metoprolol (◆) or pindolol (■) at the indicated concentrations. Basal GTP turnover was $0.085 \, min^{-1}$.

membrane properties, PTX would not be expected to greatly affect the stimulatory effects of these substances. However, the toxin strongly inhibited the stimulatory effects of propranolol and tetracaine on GTPase and GTP[γS] binding (see Table 1). These data suggest that the stimulatory effects of βAR antagonists and LAs are attributable to interaction of these substances with C-terminal portions of G_i-protein α-subunits. It remains to be clarified inasmuch as βγ-complexes are involved in the interaction of βAR antagonists and LAs with G-proteins and of whether these substances affect the anchoring of G-protein α-subunits to the plasma membrane.

There is a close correlation between the lipophilicity of βAR antagonists and their effectiveness to activate G_i-proteins in HL-60 membranes (see Fig. 2). In addition propranolol, the most lipophilic βAR antagonist studied, was more effective than less lipophilic substances of this class of drugs to activate the GTPase of reconstituted G_i/G_o-proteins (see Fig. 4). Moreover, lipophilic LAs were more effective than hydrophilic ones at activating the GTPase in HL-60 membranes and of reconstituted G_i/G_o-proteins (see Figs 3 and 5 and Table 2). These data suggest that the lipophilic domain of βAR antagonists and LAs is more important than the basic one for effective G-protein activation. With respect to mastoparan and mastoparan derivatives, lipophilicity of peptides is also important for effective G-protein activation [4].

Procaine reduced GTP hydrolysis in HL-60 membranes, but it slightly increased that of reconstituted G_i/G_o-proteins (see Figs 3 and 5). In addition, metoprolol (3 mM) was more effective than pindolol (3 mM) at activating GTP hydrolysis in HL-60 membranes, but, were similarly effective with respect to G_i/G_o-proteins (see Figs 1 and 4). These data suggest that the mechanisms by which βAR antagonists and LAs activate G_i-proteins in HL-60 membranes and reconstituted G_i/G_o-proteins are not identical. Activation of NDPK (EC 2.7.4.6) by mastoparan may play an important role in its stimulatory effects on GTP hydrolysis in HL-60 membranes, and mastoparan is only a poor activator of GTP[γS] binding in this system [21]. Similarly, propranolol and tetracaine were more effective activators of GTP hydrolysis than of GTP[γS] binding in HL-60 membranes (see Table 1). In addition, mastoparan is a considerably more potent activator of purified NDPK than of reconstituted G_o-proteins [22]. Intriguingly, the concentrations of propranolol and tetracaine required to activate GTP hydrolysis of reconstituted G_i/G_o-proteins were also higher than those needed for GTPase activation in HL-60 membranes (compare Figs 1 and 4 and Figs 3 and 5). Thus, by analogy to mastoparan, a part of the stimulatory effects of βAR antagonists and LAs on GTP hydrolysis in HL-60 membranes may be
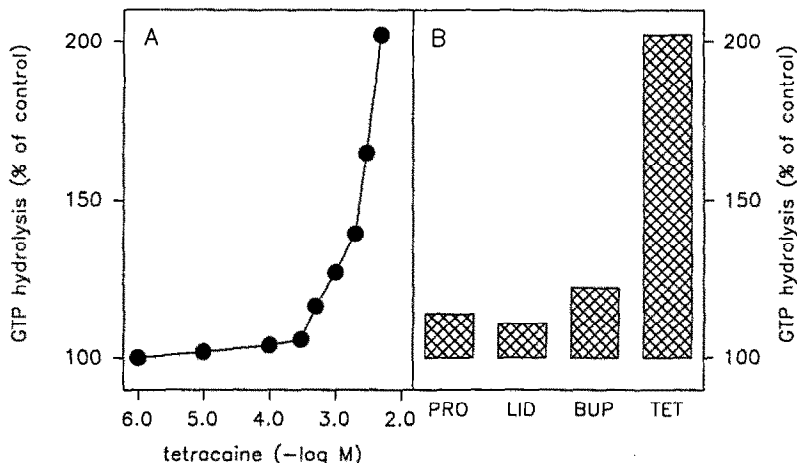
Fig. 5. Effects of various LAs on high-affinity GTP hydrolysis by a reconstituted mixture of G_i/G_o-proteins. G-proteins purified from bovine brain were reconstituted into phospholipid vesicles and assayed for GTP hydrolysis as described in Materials and Methods. Panel A: concentration–response curve for tetracaine. Panel B: comparison of the stimulatory effects of various LAs (5 mM each) on GTP hydrolysis. PRO, procaine; LID, lidocaine; BUP, bupivacaine; TET, tetracaine. Basal GTP turnover was $0.085 \, min^{-1}$.

mediated through activation of NDPK by these substances.

Our data also suggest that the structure–activity relationships of βAR antagonists and LAs for interaction with G-proteins and NDPK may be different, e.g. metoprolol may be a more effective activator of NDPK than pindolol (see Figs 1 and 4), and procaine may be a weak activator of G-proteins but not of NDPK (see Figs 3 and 5). If nucleoside diphosphate kinase is, in fact, involved in G-protein activation by βAR antagonists and LAs in situ, the PTX-sensitivity of the stimulatory effects of propranolol and tetracaine on GTPase implies that ADP-ribosylation interferes with the channeling of GTP from NDPK to the G-protein (see Table 1).

The concentrations of propranolol required to activate G-proteins are substantially higher than those obtainable in vivo (see Figs 1 and 4) [23]. Thus, it is most unlikely that G-protein activation induced by βAR antagonists contributes to their therapeutic effects in the treatment of cardiac arrhythmias, angina and hypertension [23]. However, the concentrations of LAs inducing local anesthesia and/or cardiac and neuronal toxicity are within the same order of magnitude as those required for activation of G-proteins (see Figs 3 and 5) [24, 25]. Although it is generally assumed that LAs exert their therapeutic and toxic effects via inhibition of voltage-dependent sodium channels, additional, yet unknown mechanisms are likely to be involved [9, 24]. Interestingly, the anesthetic potency of LAs is related to their lipophilicity, and lipophilic LAs are more effective G-protein activators than hydrophilic ones (see Figs 3 and 5 and Table 2) [9]. Moreover, various types of ion channels are regulated by G-proteins [1]. From all these findings the intriguing question arises inasmuch as activation of PTX-sensitive G-proteins by LAs, mediated either

directly or indirectly through stimulation of NDPK, contributes to their therapeutic and/or toxic effects. Testing of this hypothesis will require, for example, assessment of the effect of PTX on the actions of LAs in isolated neurons and cardiac tissue and in intact animals.

In conclusion, we have shown that lipophilic βAR antagonists and LAs are effective direct activators of PTX-sensitive G-proteins in situ and in reconstituted systems. Apparently, the lipophilic domain of these substances is more important for G-protein activation than the basic one. Activation by βAR antagonists and LAs of the GTPase of G_i-proteins in HL-60 membranes may involve NDPK.

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