Heparin uncouples α_2 -adrenoceptors from the G_i -protein in membranes of human platelets

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The influence of heparin was studied on the inhibitory regulation of adenylate cyclase in human platelet membranes. Heparin blocked the adrenaline-induced inhibition of adenylate cyclase and the stimulation of GTP hydrolysis with half-maximal and maximal efficiency at 0.3 and 1–3 $\mu\text{g}/\text{ml}$, respectively. The effect of heparin was reversed by washing the membranes. Heparin did not change the number of α -adrenoceptors. In contrast, the affinity of the α -adrenoceptor for adrenaline was decreased in the presence of heparin. The pertussis toxin-catalysed ADP-ribosylation of the inhibitory guanine nucleotide-binding G_i -protein was not altered by heparin. Heparin also abolished the inhibition of adenylate cyclase caused by GTP itself. The data indicate that heparin can impair the hormone-induced inhibition of adenylate cyclase and the stimulation of GTP hydrolysis and suggest that the effects of heparin are caused by an action at the G_i -protein of the adenylate cyclase system.

INTRODUCTION

Besides its anticoagulating effects and its effects on lipid metabolism, heparin and other glycosaminoglycans are evidently involved in a wide variety of physiological processes such as cell proliferation (Benitz *et al.*, 1986), aggregation (Thurn & Underhill, 1986), adhesion of cells and intercellular communication (Gallagher *et al.*, 1986). Whereas the effects of heparin on the coagulation cascade are reasonably well defined (de Prost, 1986), the understanding of the biochemical mechanisms involved in the effects of heparin on intact cells is still rudimentary. One likely candidate by which heparin elicits its effects on intact cells is the adenylate cyclase system. It has been reported that heparin and related glycosaminoglycans modulate the adenylate cyclase system in various cell types. Heparin was shown to decrease the activity of adenylate cyclase stimulated by hormonal factors like prostaglandin E_1 (Reches *et al.*, 1979), isoproterenol (Cutler & Christian, 1984) and follitropin (FSH) (Amsterdam *et al.*, 1978). Similarly, it has been reported that the activation of the enzyme by guanine nucleotides (Cutler & Christian, 1984; Salomon *et al.*, 1978) and forskolin (Cutler & Christian, 1984) is largely impaired by heparin. These studies almost exclusively focused on the heparin effect on the stimulatory regulation of the adenylate cyclase. As it has been shown during the past few years that the adenylate cyclase is bidirectionally regulated by hormonal factors (Jakobs *et al.*, 1985), we were prompted to study the effects of heparin on the inhibitory regulation of the enzyme.

Human platelets, which are paradoxically activated by heparin *in vitro* (Mohammad *et al.*, 1981), are a suitable model to study the inhibitory regulation of adenylate cyclase. The adenylate cyclase of human platelet membranes is inhibited by adrenaline via α_2 -adrenoceptors (Jakobs *et al.*, 1976). Additionally, ADP, vasopressin, platelet-activating factor and thrombin inhibit adenylate cyclase of human platelet membranes (Aktories & Jakobs, 1985). Recent studies have shown that an

inhibitory guanine nucleotide-binding G_i -protein is involved in the adenylate cyclase inhibition by these factors (Aktories & Jakobs, 1985). Although the exact mechanism of the hormonal inhibition of adenylate cyclase is not completely understood, it is generally accepted that the inhibitory hormonal factors activate the G_i -protein by facilitating the binding of GTP to the α -subunit, which may be followed by dissociation of the G_i -protein into its α and β/γ -components (Birnbaumer *et al.*, 1985). Both of these components are putative inhibitors of the adenylate cyclase (Bokoch *et al.*, 1985).

In this paper we report that heparin impairs the inhibition of the adenylate cyclase by adrenaline, presumably by affecting the inhibitory G_i protein.

MATERIALS AND METHODS

Materials

ATP, GTP, cyclic AMP, creatine kinase and creatine phosphate were obtained from Boehringer-Mannheim. PGE_1 , adrenaline and heparin (porcine mucosa; 178 USP units/mg; lot 34F-0742; M_r 6000–20000) were purchased from Sigma. Heparin (porcine mucosa; 169 USP units/mg; lot 406004; M_r 13500–15000) was also obtained from Calbiochem. Forskolin was a gift from Dr. H. Metzger (Hoechst AG, Frankfurt, Germany). [α - ^{32}P]ATP was prepared according to Walseth & Johnson (1979). [^{32}P]NAD was prepared according to Cassel & Pfeuffer (1978). [^3H]Rauwolscine was from New England Nuclear. All other reagents were obtained as previously described (Aktories & Jakobs, 1984).

Membrane preparation

Preparation of human platelet membranes was performed in the presence of 5 mM-EDTA according to the procedure described by Aktories & Jakobs (1984). S49 mouse lymphoma cell membranes, prepared according to Aktories *et al.* (1983), were kindly donated by Dr. K. H. Jakobs (Heidelberg, Germany).

Abbreviations used: G_i , inhibitory guanine nucleotide-binding regulatory protein; PGE_1 , prostaglandin E_1 .

Adenylate cyclase and GTPase assay

Adenylate cyclase activity was determined essentially as described (Aktories & Jakobs, 1984). The reaction mixture contained 0.1 mM-EGTA, 1 mM-3-isobutyl-1-methylxanthine, 1 mM-dithiothreitol, 2 mM-MgCl₂, 0.1 mM-cyclic AMP, 50 μ M-ATP (0.2 μ Ci/tube), 5 mM-phosphocreatine, 0.4 mg of creatine kinase/ml, 2 mg of bovine serum albumin/ml and the additions indicated in 50 mM-triethanolamine/HCl, pH 7.5. Stimulation of basal adenylate cyclase activity was usually obtained by adding 30 μ M-forskolin. If differing forskolin concentrations or PGE₁ were added instead, it is specified in the individual experiments. GTPase activity was determined with the same reaction mixture with the exception that 0.5 μ M-[γ -³²P]GTP (0.1 μ Ci/tube) and 100 μ M unlabelled ATP were present. Incubation was carried out at 25 °C for 15 min after a preincubation period of 5 min, or as indicated. Reactions were started by adding human platelet membranes (5–30 μ g of protein/tube) or S49 lymphoma cell membranes (10–20 μ g of protein/tube) to the reaction mixture. Termination of the reaction and isolation and determination of cyclic AMP formed and P_i released were carried out as described (Jakobs *et al.*, 1976; Aktories & Jakobs, 1981).

Binding assay

Binding of the antagonist [³H]rauwolscine was performed in a medium containing the labelled antagonist at the indicated concentration, 2 mM-MgCl₂ and test substances as indicated in 50 mM-Tris/HCl, pH 7.5. Incubation was carried out for 30 min at 30 °C. The reactions were started by the addition of human platelet membranes (50–100 μ g of protein/tube) to the prewarmed reaction mixture. The reaction was stopped by adding 2 ml of ice-cold incubation buffer, followed by rapid filtration through Whatman GF/C glass fibre filters. The filters were washed with 12 ml of ice-cold incubation buffer. Unspecific binding was determined in the presence of 10 μ M unlabelled yohimbine.

Fig. 1. Impairment of the adrenaline-induced inhibition of human platelet adenylate cyclase by heparin

Adenylate cyclase activity was determined in the presence of forskolin (30 μ M) without (●) and with adrenaline (30 μ M, ■) at the indicated concentrations of heparin. [GTP] was 3 μ M.

Fig. 2. Inhibition of adenylate cyclase at increasing concentrations of adrenaline

In human platelet membranes adenylate cyclase activity stimulated by 30 μ M-forskolin was determined without (●) and with heparin (1 μ g/ml, ■) at the indicated concentrations of adrenaline. [GTP] was 3 μ M.

ADP-ribosylation assay

ADP-ribosylation of G_i was performed essentially as described (Aktories *et al.*, 1986). The reaction medium contained 0.5 mM-ATP, 10 mM-thymidine, 0.5 mM-MgCl₂, 1 mM-GDP, 0.1 μ M-[³²P]NAD (about 1 μ Ci/tube), 0.1 mg of Lubrol PX/ml, 100 mM-dithiothreitol, 10 μ g of preactivated pertussis toxin/ml and 50 mM-triethanolamine/HCl, pH 7.5. The reaction was started by the addition of platelet membranes (about 40 μ g/tube) and was carried out at 37 °C for 1 h in a total volume of 100 μ l. The reaction was stopped by the addition of 1 ml of trichloroacetic acid (20%, w/v). The labelled proteins were analysed by SDS/polyacrylamide-gel electrophoresis and autoradiography.

Reproducibility of experiments

All experiments were performed in triplicate with an intra-assay variation of usually less than 5% of the means. All experiments shown herein were repeated several times with comparable results.

RESULTS

In the absence of heparin, 30 μ M-adrenaline inhibited the forskolin (30 μ M)-stimulated adenylate cyclase activity of human platelet membranes by about 45% (Fig. 1). The addition of increasing concentrations of heparin diminished the inhibitory effect of adrenaline in a dose-dependent manner. Inhibition of adenylate cyclase was largely reduced at 1 μ g of heparin/ml and completely abolished at 3 μ g/ml. In some experiments adrenaline-induced inhibition of adenylate cyclase was completely suppressed even at 1 μ g of heparin/ml. These effects were observed with heparin from different commercial sources. At 3 μ g/ml, heparin by itself reduced the forskolin-stimulated adenylate cyclase activity by 20–30%. As shown in Fig. 2, an increase in the concentration of adrenaline up to 300 μ M did not cause an inhibition of

Fig. 3. Influence of GTP on the heparin effects on adenylate cyclase inhibition by adrenaline

In human platelet membranes adenylate cyclase activity was determined using 30 μ M-forskolin without (●) and with adrenaline (100 μ M, ■), heparin (10 μ g/ml, ▲) and heparin plus adrenaline (▼) at the indicated concentrations of GTP.

Fig. 4. Influence of heparin on inhibition of prostaglandin E_1 -stimulated adenylate cyclase by adrenaline

Adenylate cyclase activity of human platelet membranes was determined in the absence (●) and in the presence of 10 μ M- PGE_1 without (■) and with 100 μ M-adrenaline (▲) at the indicated concentrations of heparin. [GTP] was 10 μ M.

adenylate cyclase in the presence of 3 μ g of heparin/ml. As the adrenaline-induced inhibition of adenylate cyclase depends absolutely on the presence of GTP (Jakobs *et al.*, 1978), we studied whether the effect of heparin could be overcome by an increase in the concentration of GTP. Fig. 3 shows that heparin (10 μ g/ml) blocked the adrenaline-induced inhibition even at very high concentrations of GTP. Similar results were obtained with 3 μ g of heparin/ml (results not shown).

Table 1. Reversal of the heparin effects on adenylate cyclase inhibition

Human platelet membranes were incubated without (control) and with heparin (3 μ g/ml) for 10 min at 25 °C. The membranes were pelleted and washed twice with 10 mM-triethanolamine/HCl, pH 7.5. Thereafter, the membranes were used for determination of adenylate cyclase activity. Numbers in parentheses indicate inhibition (%) by adrenaline. Means $\pm$ S.E.M. of triplicate values are given.

Additions to assay	Pretreatment ...	Adenylate cyclase activity (pmol of cyclic AMP/min per mg of protein)	
		Control	Heparin
None		430 $\pm$ 20	349 $\pm$ 14
Adrenaline (100 μ M)		315 $\pm$ 2 (27)	256 $\pm$ 11 (27)
Heparin (3 μ g/ml)		304 $\pm$ 3	260 $\pm$ 8
Adrenaline plus heparin		343 $\pm$ 12 (0)	285 $\pm$ 9 (0)

Fig. 5. Saturation analysis of specific [3 H]rauwolscine binding to human platelet membranes in the absence and presence of heparin

In human platelet membranes, binding of [3 H]rauwolscine (0.8–8 nM) was studied without (●) and with heparin (3 μ g/ml, ○) in the absence and presence of 10 μ M-yohimbine. The amount of [3 H]rauwolscine that can be displaced by yohimbine is given. The inset shows a Scatchard analysis of these data. In the absence of heparin the K_D was 4.2 nM and B_{max} was 423 fmol/mg of protein ($r = -0.96$). In the presence of heparin the K_D value was 4.6 nM and B_{max} was 445 fmol/mg of protein ($r = -0.97$).

In order to clarify whether the effects of heparin were also observed with a hormonally stimulated enzyme, we studied its influence on the PGE_1 -stimulated adenylate cyclase. PGE_1 (10 μ M) stimulated the adenylate cyclase about 20-fold. At increasing concentrations of heparin, the PGE_1 -stimulated adenylate cyclase activity was dose-dependently reduced by heparin (Fig. 4). At 100 μ g of heparin/ml the control activity was inhibited by about 40%. Adrenaline (100 μ M) inhibited the stimulated enzyme by about 35%. Similarly, as found with the

Fig. 6. Influence of heparin on agonist binding to α_2 -adrenoceptors in human platelet membranes

[3 H]Rauwolschic (4 nM) binding was measured in the absence (●) and presence (○) of 3 μ g of heparin/ml at increasing concentrations of adrenaline. Control [3 H]-rauwolschic binding was 120 fmol/mg of protein in the absence and presence of heparin.

forskolin-activated enzyme, the inhibitory effect of adrenaline was completely abolished at 1–3 μ g of heparin/ml.

To test the reversibility of the heparin effect on the adrenaline-induced inhibition, we treated platelet membranes for 10 min in the presence of 3 μ g of heparin/ml. Thereafter, the membranes were washed and used for the adenylate cyclase assay. As shown in Table 1, washing the heparin-treated membranes restored the adrenaline-induced inhibition of adenylate cyclase.

At least four molecular levels are possible sites for the heparin-induced impairment of the adenylate cyclase inhibition: (i) adrenaline itself, (ii) the α_2 -adrenoceptor, (iii) the inhibitory G_i -protein and (iv) the cyclase. In order to exclude a possibly direct interaction of heparin with adrenaline, we studied the β -adrenoceptor-mediated stimulation of adenylate cyclase by adrenaline in membranes of S49 lymphoma cells. At concentrations up to 10 μ g/ml, heparin did not decrease but rather slightly increased the stimulation of adenylate cyclase by adrenaline. Even at 100 μ g of heparin/ml, a concentration which largely reduced the basal activity of adenylate cyclase, in S49 lymphoma cell membranes, adrenaline still clearly stimulated the enzyme (not shown). Secondly, we studied whether the binding of adrenergic agents was somehow altered by heparin treatment of platelet membranes. The saturation experiment shows that the binding of the α_2 -adrenoceptor antagonist [3 H]-rauwolschic was not affected by heparin (3 μ g/ml) (Fig. 5). Furthermore, also in the presence of heparin (3 μ g/ml), adrenaline competed with [3 H]rauwolschic for the α_2 -adrenoceptor binding site (Fig. 6). However, heparin apparently lowered the affinity for the agonist.

In order to clarify whether heparin acted on the G_i -protein we studied the influence of the glycosaminoglycan on the adrenaline-sensitive G_i -associated GTPase activity. As shown in Fig. 7, adrenaline (30 μ M) stimulated

Fig. 7. Influence of heparin on adrenaline-induced GTP hydrolysis

In human platelet membranes GTP hydrolysis was determined in the absence (●) and presence (■) of adrenaline (30 μ M) at increasing concentrations of heparin. Data are given as means $\pm$ S.E.M. ($n = 3$).

Fig. 8. GTP hydrolysis at increasing concentrations of adrenaline

In human platelet membranes GTP hydrolysis was determined without (●) and with heparin (1 μ g/ml, ■) at increasing concentrations of adrenaline. Data are given as means $\pm$ S.E.M. ($n = 3$).

the GTP hydrolysis by about 55%. The addition of increasing concentrations of heparin impaired the adrenaline-stimulated GTP hydrolysis dose-dependently. At a concentration of 1–3 μ g of heparin/ml the stimulatory effects of adrenaline on GTP hydrolysis were completely abolished. Also the basal GTP hydrolysis was largely reduced even at low concentrations of heparin. Similarly, as found for adenylate cyclase inhibition, an increase in the concentration of adrenaline did not overcome the heparin impairment of GTPase stimulation (Fig. 8).

As the heparin-induced reduction in GTPase activity

Fig. 9. Lineweaver-Burk plot of GTP hydrolysis

In human platelet membranes GTP hydrolysis was determined without (○) and with heparin (0.3 $\mu\text{g/ml}$, ●; 1 $\mu\text{g/ml}$, □; 10 $\mu\text{g/ml}$, ■) at various concentrations of GTP (0.1, 0.15, 0.2, 0.3, 0.5 and 1 μM). The apparent K_m value for GTP was about 0.3 μM .

could be caused by a decrease in the affinity for GTP, we studied whether the K_m value of the GTPase was changed in the presence of heparin. As shown in the Lineweaver-Burk plot (Fig. 9), increasing concentrations of heparin up to 10 $\mu\text{g/ml}$ reduced the V_{max} , but apparently did not change the K_m value for GTP.

To substantiate further that the heparin-induced impairment of adenylate cyclase inhibition by adrenaline was due to an interaction with the inhibitory G_i -protein, we studied the effect of heparin on adenylate cyclase inhibition caused by GTP. Similarly, as found in adipocyte membranes (Aktories *et al.*, 1980) GTP, but not GDP, inhibits the platelet adenylate cyclase via G_i without the addition of any inhibitory hormonal factor when the enzyme activity is determined in the absence of a regenerating system (Marquetant *et al.*, 1987). As shown in Fig. 10, GTP inhibited the forskolin-stimulated adenylate cyclase maximally by about 40%. This inhibitory effect by GTP was completely abolished in the presence of 3 μg of heparin/ml. The time course in Fig. 11 shows that under these conditions the formation of cyclic AMP was linear for at least 15 min. GTP (10 μM) decreased the adenylate cyclase activity by about 25% in the absence of heparin. The addition of 1 μg of heparin/ml completely abolished the inhibition of adenylate cyclase by GTP. The amount of adenylate cyclase inhibition caused by GTP varied to some degree with each individual platelet membrane preparation. In any case, however, inhibition of adenylate cyclase was abolished by heparin.

Pertussis toxin has been shown to ADP-ribosylate the α -subunit of the G_i -protein (Bokoch *et al.*, 1983). This ADP-ribosylation depends on the associated heterotrimeric structure of G_i (Tsai *et al.*, 1984). Studying the

Fig. 10. Impairment of the GTP-induced inhibition of platelet adenylate cyclase by heparin

Forskolin (30 μM)-stimulated adenylate cyclase of human platelet membranes was determined in the absence of phosphocreatine and creatine kinase without (●) and with heparin (3 $\mu\text{g/ml}$, ■) at increasing concentrations of GTP.

Fig. 11. Influence of heparin on the GTP-induced inhibition of adenylate cyclase

The formation of cyclic AMP in human platelet membranes was determined in the absence of phosphocreatine and creatine kinase without (○) and with heparin (1 $\mu\text{g/ml}$, □), GTP (10 μM , ●) and heparin plus GTP (■) at the indicated time points.

effect of heparin on the ADP-ribosylation of G_i by pertussis toxin with [^{32}P]NAD, we did not find any difference in the labelling of G_i whether heparin (10 $\mu\text{g/ml}$) was present or not.

DISCUSSION

It is a well-known phenomenon that heparin decreases the basal adenylate cyclase activity and impairs its stimulation by hormonal factors, fluoride, stable GTP analogues and forskolin (Amsterdam *et al.*, 1978; Salomon *et al.*, 1978; Reches *et al.*, 1979; Cutler &

Christian, 1984). In this paper we report that heparin also potently impairs the adrenaline-induced inhibition of the adenylate cyclase of human platelet membranes. Our studies indicate that these two heparin effects may be achieved by distinct mechanisms, since much lower concentrations of heparin were necessary for the impairment of the adrenaline-induced inhibition (EC_{50} about $0.3 \mu\text{g/ml}$) than for the reduction of platelet adenylate cyclase stimulation by PGE_1 ($EC_{50} > 3 \mu\text{g/ml}$).

The data presented herein suggest that heparin affected adenylate cyclase inhibition most probably by a process involving the inhibitory guanine nucleotide-binding regulatory component G_i . This conclusion can be drawn from the following arguments. First, a direct interaction of adrenaline with the macromolecular compound heparin can be excluded by the finding that in S49 lymphoma cell membranes adrenaline-induced stimulation of adenylate cyclase via β -adrenoceptors was not at all diminished by heparin concentrations up to $30 \mu\text{g/ml}$. Second, the heparin effects are most probably not due to an alteration of the α_2 -adrenoceptor. The binding of the labelled antagonist rauwolsine to the α_2 -adrenoceptor was not affected by the glycosaminoglycan. Furthermore, also in the presence of heparin, adrenaline competed with labelled rauwolsine for the α_2 -adrenoceptor binding site. Third, inhibition of platelet adenylate cyclase by GTP itself, which can be observed with the forskolin-stimulated enzyme in the absence of any ATP-regenerating system (Marquetant *et al.*, 1987), was completely blocked by heparin. Finally, heparin not only impaired the adrenaline-induced inhibition of the adenylate cyclase but also the GTP hydrolysis stimulated by adrenaline. As there is no evidence that the catalytic subunit of adenylate cyclase is involved in the regulation of the GTPase activity of G-proteins (Brandt & Ross, 1986), these findings argue against the catalyst as the target of heparin. Thus, the data presented suggest that heparin somehow impairs the activation of the G_i -protein by GTP and the inhibitory hormone, adrenaline. In addition to the effects on adenylate cyclase inhibition, heparin decreased the basal, PGE_1 - and forskolin-stimulated adenylate cyclase of human platelet membranes at concentrations above $1 \mu\text{g/ml}$. At these high concentrations heparin probably affects the stimulatory G_s -protein and/or the catalyst. This dose-dependence of the heparin action is a likely explanation for the previously reported biphasic effects of heparin on dopamine-stimulated adenylate cyclase in rat caudate nucleus membranes (Amsterdam *et al.*, 1978).

The precise mechanism of the interaction between heparin and G_i is not known at present. The reversal of the heparin effect after washing the membranes argues against a covalent alteration in the structure of the G_i -protein. Whether the effects of the polyanion heparin are mainly charge-related has to be ruled out. Studies with differently charged glycosaminoglycans are in progress in our laboratory. It has been shown that heparin inhibits a membrane-bound tyrosine kinase by competition with ATP (Boivin *et al.*, 1985). The effects of heparin on adenylate cyclase inhibition were evidently not due to a competition at the GTP-binding site of the G_i -protein, because an increase in the concentration of GTP did not restore the inhibitory effect of adrenaline and the K_m value of the high-affinity GTPase for GTP was not altered by heparin up to $10 \mu\text{g/ml}$. Another likely explanation for the heparin effects is a stimulation of the

G_i -associated GTPase activity, which apparently terminates the active state of G_i . However, we found that the activity of the high-affinity GTPase is decreased rather than increased with heparin. G_i served as substrate of pertussis toxin even in the presence of heparin. This shows that the associated heterotrimeric structure of G_i , which is a prerequisite of a significant ADP-ribosylation (Tsai *et al.*, 1984), is formed in the presence of heparin. On the other hand it is important to note that heparin reduced the affinity of the adrenoceptor for adrenaline. Such a shift in the affinity of the receptor for its specific agonist is typical for an uncoupling of the GTP-binding protein from the receptor. Thus, the data shown may suggest that heparin alters or even prevents the interaction of G_i with the inhibitory receptor.

Recent studies have shown that several homologous GTP-binding proteins exist, which are probably involved in different regulatory processes (Sternweis & Robishaw, 1984; Gierschik *et al.*, 1986). It would be of major interest to study whether these G-proteins are also altered by heparin or not. In this context it is notable that we have carried out studies in our laboratory showing that in platelet membranes the thrombin-stimulated GTP hydrolysis, which is evidently caused by at least two distinct GTPases (Grandt *et al.*, 1986), was much less affected by heparin (B. Willuweit & K. Aktories, unpublished work).

In intact platelets, heparin has been shown to facilitate the adrenaline-induced aggregation (Mohammad *et al.*, 1981). This well-established observation contrasts with the impairment of adrenaline-induced inhibition of adenylate cyclase, an effect which is widely accepted as a stimulus-response-coupling mechanism for α_2 -adrenoceptors. However, much evidence suggests that inhibition of adenylate cyclase is not the only mechanism responsible for induction of aggregation by adrenaline (Kerry & Scrutton, 1985). Furthermore, heparin binds to at least two platelet glycoproteins, with molecular masses of 170 kDa (glycoprotein Ib) and 207 kDa (reduced 60 kDa), which are apparently not related to any subunit of the adenylate cyclase system (Wolf *et al.*, 1986). Most importantly, we have studied the influence of heparin on adenylate cyclase inhibition in platelet membranes but not in intact platelets. Thus, the physiological significance of the heparin effects on adenylate cyclase regulation in intact human platelets is still obscure. Further studies performed with other cell types and with intact cells which focus on a possible interaction of heparin with the inhibitory pathway of adenylate cyclase or with other G-protein-mediated signal-transduction processes would be very helpful to clarify the physiological role of the glycosaminoglycans.

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