Effect of propranolol on platelet signal transduction

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Propranolol inhibits platelet secondary aggregation and secretion by mechanisms unrelated to its β -adrenergic-blocking activity. We previously reported that a major effect of the drug is perturbation of the physical microenvironment of the human platelet membrane. To explore further the molecular mechanisms underlying propranolol-mediated platelet inhibition, we studied protein kinase C activity, estimated from the phosphorylation of the substrate protein pleckstrin, in propranolol-treated human platelets. The drug inhibited activation of the enzyme in thrombin-stimulated platelets but not in platelets stimulated with

phorbol esters, indicating that its site of action might be upstream of protein kinase C. It also inhibited the activity of phospholipase C, determined from the extent of generation of inositol phosphates and phosphatidic acid, in platelets stimulated with thrombin as well as the non-hydrolysable GTP analogue guanosine 5'-[β , γ -imido]triphosphate in a dose-dependent manner. These data suggest that propranolol inhibits signal transduction in thrombin-stimulated platelets by interacting at the level of phospholipase C and exclude interaction of the drug with the downstream effector enzyme protein kinase C.

INTRODUCTION

Propranolol is a widely used drug in cardiac pathology, ranging from angina pectoris to hypertension, for its β -adrenergic-blocking effects [1]. Propranolol therapy was reported to bring the exaggerated aggregatory responses of the 'hyperactive' platelets in angina pectoris back to a normal level [2–7]. A series of *in vitro* studies [8–11] demonstrated the inhibitory effect of propranolol on platelet secondary aggregation, uptake and release of 5-hydroxy[14 C]tryptamine, adhesion to collagen, clot retraction and thromboxane A_2 generation, at a drug concentration similar to or a little higher than those achieved *in vivo* in clinical practice [10]. Inhibition of platelet function appeared to be unrelated to the β -adrenergic blockade because d(+)-propranolol (which lacks the β -blocking effect) was equipotent with l(–)-propranolol. Thus a direct membrane effect, similar to the local anaesthetic-like or the membrane-stabilizing activity, and not the β -blocking property, was suggested to be the mode of action of the drug on platelets [8–10]. We have recently demonstrated *in vitro* that propranolol, at therapeutic concentrations, decreases the equivalent microviscosity of the platelet membrane and increases the degree of disorder of the hydrocarbon chains in the lipid bilayer, ostensibly by affecting the packing throughout the membrane [12,13]. In another study [14] propranolol was shown to specifically bind to phosphatidylserine and phosphatidylinositol present on the inner face of the platelet membrane. Disturbance of the membrane physicochemical microenvironment may interfere with the molecular signal transduction across the platelet membrane, thus affecting the cellular response to agonists. However, the reports available so far provide little information on the effect of propranolol on platelet signal-response coupling. In this paper, we have investigated the effect of the drug on various components of platelet signal-transduction pathways in order to characterize the propranolol action at a molecular level.

EXPERIMENTAL

Materials

[32 P] P_i (carrier-free), 5-hydroxy[14 C]tryptamine binoxalate and *myo*-[3 H]inositol were purchased from Bhabha Atomic Research Centre, New England Nuclear and Amersham International respectively. Precoated silica-gel TLC plates (art. no. 5553) were from Merck. X-Omat AR films were the products of Eastman-Kodak. Dowex 1 anion-exchange resin (AG 1-X8; 100–200 mesh; formate form) was from Bio-Rad. All other chemicals were of analytical reagent grade purchased from Sigma.

A stock solution of thrombin (25 units/ml) was made in 150 mM NaCl/50 mM sodium citrate and stored at -20°C . Stock solutions of phorbol 12-myristate 13-acetate (PMA) (0.1 mM) and guanosine 5'-[β , γ -imido]triphosphate (p[NH]ppG) (1 mM) were prepared in DMSO and 10 mM Hepes (pH 7.5) respectively and stored at -70°C . A 10 mM aqueous solution of ($\pm$)-propranolol hydrochloride was stored at -70°C and appropriate dilutions were made immediately before use. Solutions of (–)-timolol and (–)-isoprenaline were prepared fresh in water.

Platelet preparation

Platelet-rich plasma was obtained from fresh human blood and incubated with aspirin (final concentration 1 mM) at 37°C for 15 min. Washed platelet suspension was obtained by differential centrifugation as described previously [15]. Propranolol treatment was carried out by incubating the washed platelets (5×10^8 cells in 1 ml) at 37°C with the required concentration of the drug for 3 min before the addition of agonists. Platelet aggregation (measured with a Chrono-log platelet ionized Ca^{2+} aggregometer; model 600) and release studies using 5-hydroxy[14 C]tryptamine were performed by standard procedures [16,17]. Release of ATP

Abbreviations used: DAG, sn-1,2-diacylglycerol; p[NH]ppG, guanosine 5'-[β , γ -imido]triphosphate; PKC, protein kinase C; PLC, phosphoinositide-specific phospholipase C; PMA, phorbol 12-myristate 13-acetate; PtdOH, phosphatidic acid.

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into the medium was measured by the luciferin–luciferase reaction (reagents from Chrono-log Corp.), according to the protocol supplied.

Radioactive labelling of platelets

For ^{32}P labelling, washed platelets were suspended in phosphate-free modified Tyrode/Hepes buffer (20 mM Hepes, pH 7.5, 140 mM NaCl, 2.7 mM KCl, 12 mM NaHCO_3 , 2 mM MgCl_2 , 5.5 mM glucose and 0.1 mM EGTA), fortified with 0.35 g/100 ml BSA (fraction V; Sigma A-2153) and 0.6 unit/ml apyrase (Sigma A-7646). Subsequently, the cells (5×10^8 in 1 ml) were incubated with 0.5 mCi of carrier-free ^{32}P P_i for 90 min at 37 °C, washed and resuspended in the modified Tyrode/Hepes buffer, pH 7.5, containing 0.36 mM NaH_2PO_4 but no EGTA and albumin. Final platelet count was adjusted to 1×10^9 /ml.

Platelets were labelled with ^3H inositol as detailed in [18]. Briefly, platelets were resuspended at 2×10^9 /ml in the modified Tyrode/Hepes buffer which contains 0.36 mM NaH_2PO_4 and in which 2 mM MgCl_2 and 5.5 mM glucose were replaced with 2 mM MnCl_2 and 0.55 mM glucose. This suspension was incubated with 20 μCi of *myo*- ^3H inositol/ml at 37 °C for 2 h. Under these conditions there was no change in platelet reactivity to thrombin, as assessed from thrombin-induced aggregation and secretion of dense-granule contents (ATP and 5-hydroxytryptamine). The labelled platelets were pelleted and finally resuspended at 2×10^9 /ml in the modified Tyrode/Hepes buffer containing 0.36 mM NaH_2PO_4 , 2 mM MgCl_2 and 5.5 mM glucose but no EGTA and albumin.

Pleckstrin phosphorylation in platelets

Protein kinase C (PKC) was stimulated by incubating the ^{32}P -labelled platelets at 37 °C with thrombin (0.25 unit/ml) or PMA (10 nM) with constant stirring. Reactions were stopped after the required period by the addition of electrophoresis sample buffer. Proteins were separated by SDS/PAGE [19] using a 4.5% stacking gel and an 11% resolving gel along with appropriate molecular-mass marker proteins. After overnight staining with Coomassie Brilliant Blue, gels were destained, dried down under vacuum and autoradiographed on Kodak X-Omat AR films. The location of the phosphorylated 47 kDa protein substrate pleckstrin was determined in the autoradiogram.

Lipid extraction and analysis

Phosphatidylinositol-specific phospholipase C (PLC) activity was stimulated in aliquots (0.5 ml) of ^{32}P -labelled platelets by treatment with thrombin (0.25 unit/ml) at 37 °C with constant stirring. For stimulation with p[NH]ppG, cells were permeabilized by preincubating the washed platelets with saponin (15 μg /ml) for 60 s before the addition of p[NH]ppG (20 μM) as described previously [20]. Reactions were terminated after 120 s stimulation by the addition of 1.88 ml of chloroform/methanol/conc. HCl (10:20:0.2, by vol.) at 0 °C [21], and lipids were extracted as described by Holmsen et al. [22]. Lipids present in the lower organic phase were separated by TLC on potassium oxalate-treated silica-gel plates using the solvent system chloroform/methanol/20% methylamine in water (60:36:10, by vol.) [23]. The radioactive fractions were visualized by overnight autoradiography and identified from co-migrating phospholipid standards (Sigma) stained with iodine vapour. The radioactive bands were scraped off and their ^{32}P content was measured by liquid-scintillation counting.

Measurement of inositol phosphates

^3H Inositol-labelled platelets (2×10^8 /ml) were stimulated for 120 s with either thrombin (0.25 unit/ml) or p[NH]ppG (20 μM) and subjected to lipid extraction as described above. The upper aqueous phase was neutralized with NaOH using Bromothymol Blue as an indicator, diluted to 5 ml with water and applied to the column containing 1.25 ml of AG 1-X8 anion-exchange resin. Inositol phosphates were isolated by stepwise elution, as previously described [18,24]. ^3H Inositol was first eluted with 3×5 ml of water, followed by elution of ^3H glycerophosphoinositol with 2×5 ml of 5 mM disodium tetraborate/60 mM sodium formate; after the elution of glycerophosphoinositol, InsP_1 , InsP_2 and InsP_3 were eluted together with 3×5 ml of 0.1 M formic acid/1.0 M ammonium formate. The fractions were counted for ^3H radioactivity.

Study of platelet lysis

The adenine nucleotides labelled with ^{32}P P_i in these experiments are exclusively present in the extragranular compartment [25], and their extracellular appearance is a measure of cell lysis [26–28]. Therefore 0.5 ml aliquots of ^{32}P -labelled platelets were incubated with different concentrations of propranolol for 3 min. The total ^{32}P count in the supernatant of experimental samples was measured and corrected for the residual counts in the control supernatants. Release of lactate dehydrogenase into the medium was studied by using a kit (Sigma 340-LD), according to the manufacturer's instructions.

Protein measurement

Protein was determined by the method of Lowry et al. [29], with BSA as the standard.

RESULTS

Inhibition of pleckstrin phosphorylation by propranolol in thrombin-stimulated platelets

It has been reported that propranolol inhibits various platelet responses when the cells are stimulated with agonists such as thrombin [10,11]. Thrombin-induced platelet activation is associated with stimulation of the enzyme PKC [30–32]. To probe the contribution of PKC in propranolol-mediated inhibition of platelets, we studied the activity of this enzyme in the presence of different concentrations of the drug.

As illustrated in Figure 1, thrombin treatment induced phosphorylation of the 47 kDa peptide substrate (pleckstrin) indicating PKC activation (lane 1). Addition of propranolol in increasing concentrations resulted in progressive inhibition of pleckstrin phosphorylation (Figure 1, lanes 2–4).

Effect of propranolol on pleckstrin phosphorylation in PMA-stimulated platelets

Phorbol esters are known to activate PKC directly, by-passing the receptor-mediated hydrolysis of inositol phospholipids [33,34]. In order to find out whether propranolol specifically inhibits this enzyme, propranolol-treated platelets were stimulated with a low dose of PMA (10 nM) for 45 s and the activity of PKC was monitored as described above. A representative autoradiogram of the results is presented in Figure 2. Treatment of platelets with PMA resulted in the phosphorylation of a 47 kDa peptide substrate. When propranolol was added in increasing concentrations ranging from 10 to 100 μM , no difference was observed in the extent of phosphorylation of the 47 kDa peptide band

Figure 1 Effect of propranolol on thrombin-induced phosphorylation of the 47 kDa peptide

Aliquots of ^{32}P -labelled platelets were separately incubated with propranolol (lane 1, no propranolol; lane 2, $20\ \mu\text{M}$; lane 3, $40\ \mu\text{M}$; lane 4, $60\ \mu\text{M}$) for 3 min, followed by stimulation with thrombin ($0.25\ \text{unit/ml}$) for 90 s at $37\ ^\circ\text{C}$. Control platelets without thrombin stimulation are shown in lane 5. SDS/PAGE was performed on an 11% resolving gel, and phosphorylated protein bands were visualized in the autoradiogram. The arrow indicates the position of the 47 kDa peptide. The results are from a single experiment, representative of four identical experiments performed on different days.

Figure 2 Effect of propranolol on PMA-induced phosphorylation of the 47 kDa peptide

Aliquots of ^{32}P -labelled platelets were separately incubated with $10\ \mu\text{M}$ (lane 3), $20\ \mu\text{M}$ (lane 4), $40\ \mu\text{M}$ (lane 5), $60\ \mu\text{M}$ (lane 6), $80\ \mu\text{M}$ (lane 7), $90\ \mu\text{M}$ (lane 8) and $100\ \mu\text{M}$ (lane 9) propranolol for 3 min, followed by PMA ($10\ \text{nM}$) stimulation for 45 s. Lanes 1 and 2 respectively represent control platelets without and with PMA treatment. The arrow indicates the position of the 47 kDa peptide in the autoradiogram. The results are from a single experiment, representative of three similar experiments performed on different days.

(lanes 3–9). When platelets were stimulated with a still lower concentration of PMA ($5\ \text{nM}$) for different time periods up to 45 s, again no difference was observed in the extent of the phosphorylation in the presence of propranolol ($100\ \mu\text{M}$) (results not shown).

Figure 3 Effect of propranolol on the metabolism of [^{32}P]phosphoinositides in platelets stimulated with thrombin

Aliquots of ^{32}P -labelled platelets were preincubated with different concentrations of propranolol, followed by thrombin stimulation for 120 s. $\square$, + $60\ \mu\text{M}$ propranolol; $\blacksquare$, + thrombin; $\square$, + $20\ \mu\text{M}$ propranolol + thrombin; $\blacksquare$, + $40\ \mu\text{M}$ propranolol + thrombin; $\square$, + $60\ \mu\text{M}$ propranolol + thrombin. Lipids were extracted, separated by TLC and the radioactivity in individual fractions was quantified, as described in the Experimental section. Results are expressed as percentage of values obtained in control platelets (without propranolol and thrombin) and are means $\pm$ S.E.M. of three experiments.

Inhibition of phosphatidic acid generation by propranolol in thrombin-stimulated platelets

Interaction of thrombin with specific membrane receptors activates PLC through the mediation of putative G-proteins. Diacylglycerol (DAG) released by PLC-catalysed hydrolysis is rapidly and efficiently converted into PtdOH by a kinase and its level remains constant for at least 5 min before conversion into CDP-DAG [35]. The level of [^{32}P]PtdOH represents a highly sensitive index of stimulated phosphoinositide turnover [36–41]. The role of PLC in propranolol-induced inhibition of platelets was investigated in this experiment.

Figure 3 shows the effect of propranolol on PtdOH and different phosphoinositide fractions in thrombin-stimulated platelets. Thrombin induced a $40 \pm 5.5\%$ decrease in the radio-

Table 1 Effect of propranolol on the metabolism of [32 P]phosphoinositides in platelets stimulated with p[NH]ppG

Aliquots of 32 P-labelled platelets were preincubated with different concentrations of propranolol. The cells were permeabilized and stimulated with p[NH]ppG (20 μ M) for 120 s. Lipids were extracted, separated by TLC and the radioactivity in individual fractions was quantified, as described in the Experimental section. Results are expressed as percentage of values obtained in control platelets (without propranolol and agonist) and are means $\pm$ S.E.M. of three experiments.

[Propranolol] (μ M)	Metabolism (% of control)			
	PtdIns	PtdInsP	PtdInsP ₂	PtdOH
0	71 $\pm$ 7.9	205 $\pm$ 11.9	89 $\pm$ 10.3	1803 $\pm$ 48.8
20	74 $\pm$ 4.5	210 $\pm$ 9.8	95 $\pm$ 8.7	1590 $\pm$ 68.2
40	68 $\pm$ 8.6	208 $\pm$ 14.3	90 $\pm$ 8.5	1202 $\pm$ 72.5
60	75 $\pm$ 8.9	201 $\pm$ 8.8	90 $\pm$ 9.8	832 $\pm$ 53.9

Table 2 Effect of propranolol on inositol phosphate production in platelets stimulated with thrombin (0.25 unit/ml) or p[NH]ppG (20 μ M)

myo-[3 H]inositol-labelled platelets were stimulated for 120 s as indicated in the Experimental section, and inositol phosphates produced were isolated by anion-exchange chromatography and quantified. Results are expressed in d.p.m. of inositol phosphates (InsP₁ + InsP₂ + InsP₃) produced and are means $\pm$ S.E.M. of three experiments.

[Propranolol] (μ M)	[3 H]inositol phosphates (d.p.m.)		
	Control	Thrombin	p[NH]ppG
0	978 $\pm$ 90	2445 $\pm$ 225	2735 $\pm$ 248
20	—	2113 $\pm$ 190	2250 $\pm$ 210
40	—	1479 $\pm$ 110	1530 $\pm$ 150
60	—	1122 $\pm$ 105	1108 $\pm$ 189

activity of PtdIns and 89 $\pm$ 7.9% and 35 $\pm$ 6.6% increase in the radioactivities of PtdInsP and PtdInsP₂ respectively (mean $\pm$ S.E.M., n = 3). The radioactivity of PtdOH was 25-fold higher in thrombin-stimulated platelets. Preincubation with propranolol (at 20, 40 and 60 μ M concentrations) had no significant effect on the levels of PtdIns, PtdInsP and PtdInsP₂ in thrombin-stimulated platelets. However, the drug progressively inhibited the generation of [32 P]PtdOH in a dose-dependent manner. Propranolol alone (60 μ M) had no effect on the levels of different phospholipids studied (P > 0.1, Student's t test) (Figure 3).

Inhibition of phosphatidic acid generation by propranolol in p[NH]ppG-treated platelets

Non-hydrolysable GTP analogues are known to cause secretion and PtdOH formation in permeabilized platelets by directly interacting with the G-protein that regulates PLC activity [20,42–46]. In order to study further whether the effect of propranolol resides at the level of PLC-catalysed inositol phospholipid hydrolysis or at any stage upstream of it, we examined the action of the drug on PLC activity in p[NH]ppG-stimulated platelets. p[NH]ppG induced an increase in the radioactivities of PtdInsP and PtdOH, and a decrease in the radioactivities of PtdIns and PtdInsP₂ fractions. Propranolol treatment (20–60 μ M) was found to inhibit PtdOH generation in these platelets in a dose-dependent manner whereas the drug had no effect on the levels of PtdIns, PtdInsP and PtdInsP₂ (Table 1).

Inhibition of inositol phosphate production by propranolol in thrombin- or p[NH]ppG-activated platelets

Inhibition of PtdOH accumulation could be due to inhibition of activities of PLC and/or DAG kinase. To discriminate between these two possibilities we have measured the production of inositol phosphate in myo-[3 H]inositol-prelabelled platelets, stimulated with either thrombin or p[NH]ppG. Agonist addition induced an increase in the radiolabelled inositol phosphates (Table 2). Propranolol decreased the production of inositol phosphates in a dose-dependent manner, suggesting inhibition of agonist-induced PLC activation in the platelets. However, we cannot formally exclude a possible effect of propranolol on DAG kinase. The effects of two other compounds, an antagonist [(–)-timolol] and an agonist [(–)-isoprenaline] of the β -adrenergic receptor, were also studied. Neither of these compounds (each at 100 μ M) was found to inhibit aggregation, secretion, phosphorylation of the 47 kDa protein or inositol phosphate production in platelets stimulated with thrombin (results not shown).

Propranolol does not permeabilize platelet membrane under the conditions used

Propranolol is a cationic amphiphilic molecule that may have many properties in common with phenothiazines. The latter compounds permeabilize platelet membrane at concentrations above 40 μ M, causing leakage of low-molecular-mass substances (ATP, glycolytic intermediates) but not lactate dehydrogenase from the cytoplasm [26–28]. Propranolol is known to induce physical changes in the platelet membrane [12]. In order to ensure that the inhibitory effects of propranolol were not due to depletion of cytosolic ATP by possible membrane permeabilization by the drug, we measured ATP leakage under the experimental conditions used. Incubation of 32 P-labelled platelets with propranolol (20, 40, 60, 100 and 125 μ M concentrations) caused no net release of 32 P radioactivity into the medium, thus ruling out any permeabilizing effect of the drug at these concentrations. However, at propranolol concentrations of 150 and 200 μ M, 10 $\pm$ 0.7 and 18 $\pm$ 0.9% increases respectively in the radioactivity in the supernatant were observed (mean $\pm$ S.D., n = 4). These data are in agreement with a previous report [10] suggesting that propranolol concentrations below 100 μ M do not induce membrane permeabilization. Leakage of lactate dehydrogenase from control platelets was 1% of total. We did not observe any increase in lactate dehydrogenase activity in the medium at propranolol concentrations up to 200 μ M, thus excluding the possibility of platelet lysis under these conditions.

DISCUSSION

In order to understand the molecular mechanisms underlying propranolol-mediated platelet inhibition, we studied the effect of ($\pm$)-propranolol (the clinically used form of the drug) on the activity of PKC as judged by phosphorylation of the 47 kDa substrate pleckstrin. The enzyme was found to be inhibited by propranolol in a dose-dependent manner when platelets were stimulated with thrombin. In order to find out whether the decreased PKC activity was due to specific inhibition of the enzyme by the drug or inhibition at any step proximal to the enzyme, we studied the effect of propranolol on platelets stimulated with phorbol esters. PMA is a strong and persistent stimulant of PKC and therefore might overcome any possible inhibition of the enzyme by the drug. Therefore we used this

agonist at low concentration (10 nM) to stimulate the enzyme. Various positive feedback mechanisms were blocked by pre-treatment of cells with apyrase (which eliminated ADP) and aspirin (which prevented generation of thromboxane A_2 or prostaglandin endoperoxides). At the different concentrations tested (10–100 μ M), propranolol was found to have no effect on the enzyme activity in PMA-stimulated platelets. In another experiment, when platelets were stimulated with a still lower dose of PMA (5 nM) for different periods of time, the drug (100 μ M) did not have any effect on the activity of PKC. These observations suggest that the inhibitory effect of propranolol might reside at a step upstream of PKC. In a study by Sozzani et al. [47], propranolol was shown to inhibit the phorbol ester-induced activation of PKC in neutrophils. However, the concentrations of propranolol used by these workers to elicit the inhibition were considerably higher than those employed by us. We have chosen a propranolol concentration in the range 20–60 μ M in our study which is close to the peak plasma levels of the drug attained after normal therapeutic doses [1].

To test the hypothesis that propranolol inhibits platelet activation by interacting at a step proximal to PKC, we studied its effect on PLC activity in platelets stimulated with thrombin or the non-hydrolysable GTP analogue, p[NH]ppG. Propranolol inhibited PtdOH formation in these platelets in a dose-dependent manner. It also inhibited inositol phosphate production in platelets treated with thrombin or p[NH]ppG. These data suggest that propranolol modulates signal transduction in platelets by interacting at the level of G-protein–PLC coupling and/or PLC itself. Inhibition of the enzyme DAG kinase by the drug is unlikely because, in that event, propranolol treatment would result in a persistent stimulation of platelet PKC activity as a result of an increase in DAG concentration inside the cell. However, we do not formally rule out any effect of the drug on DAG kinase or at the level of thrombin receptor.

Interestingly phenothiazines, cationic amphiphilic drugs that also display membrane-perturbing behaviour [48], have distinctly different effects on platelet phosphoinositide metabolism. As extensively demonstrated by Holmsen and his group [49], trifluoperazine enhances thrombin-induced PtdOH production in platelets possibly by inhibition of the phosphohydrolase. At non-permeabilizing concentrations, chlorpromazine increases incorporation of 32 P into PtdIns and PtdIns P_2 [27,50], in resting as well as thrombin-stimulated platelets, by interfering with the kinase and phosphohydrolase reactions [51]; however, the drug has no effect on thrombin-induced PtdOH generation [27] or pleckstrin phosphorylation [50].

In other studies [52,53], propranolol has been implicated in the inhibition of phospholipase A_2 activity and decreased thromboxane generation in platelets, which could be one of the mechanisms of inhibition of ADP- or adrenaline-induced secondary platelet responses by this drug [10]. We have used aspirin-treated platelets in our experiments in which thromboxane production is blocked; hence propranolol-mediated platelet inhibition in our study could not be attributed to its inhibitory effect on the phospholipase A_2 pathway. The direct dose–response relationship between concentrations of propranolol and the graded suppression of various platelet responses reflects the specific nature of the drug action. Propranolol has also been reported to suppress phosphatidate phosphohydrolase activity in rat brain cells [54,55]. The inhibitory effects of propranolol on platelet secondary aggregation and release reaction have been demonstrated at concentrations of the drug that are similar to or a little higher than those achieved *in vivo* in clinical practice [10]. We have reported that a similar range of drug concentrations (20–60 μ M) disturbs membrane microviscosity [12], as well as

inhibiting PLC and PKC activities in thrombin-stimulated platelets (present results). Therefore it may be surmised that the drug, when administered therapeutically, affects membrane microviscosity and signal-transducing systems of circulating platelets *in vivo*, resulting in their inhibition [2–7]. These effects of propranolol are in addition to its well documented β -adrenergic-blocking properties and may be related to its lipid-solubility/membrane-interaction ability [14]. Propranolol penetrates and specifically binds to phosphatidylserine and phosphatidylinositol present on the inner face of the platelet membrane [14] and perturbs the membrane microenvironment [12]. These properties may be related to its ability to inhibit PLC activation and/or G-protein–PLC coupling. Being a very widely used drug in clinical settings ranging from hypertension to angina pectoris, where platelets are reported to remain hyperactive, elucidation of the direct inhibitory effects of propranolol on platelets at a molecular level is of potential therapeutic significance.

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