0026-895X/86/010001-06$02.00/0

MOLECULAR PHARMACOLOGY, 29:1-6

Desensitization of Epinephrine-Initiated Platelet Aggregation Does Not Alter Binding to the α_2-Adrenergic Receptor or Receptor Coupling to Adenylate Cyclase

HARVEY J. MOTULSKY, SANFORD J. SHATTIL, NICOLAS FERRY, DAVID ROZANSKY, and PAUL A. INSEL

Division of Pharmacology, M-013 H, Department of Medicine (H.J.M., D.R., P.A.I.), University of California, San Diego, La Jolla, California 92093; Hematology/Oncology Section, Department of Medicine, and the Coagulation and Thrombosis Laboratory, Department of Pathology and Laboratory Medicine (S.J.S.), University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, 19104; and INSERM U-99 (N.F.), Creteil, France F94010

Received July 1, 1985; Accepted October 11, 1985

SUMMARY

Several investigators have shown that incubating unstirred platelets with epinephrine blunts subsequent aggregation when the platelets are stirred. Using aspirin-treated platelets, we further characterized this desensitization of α_2-adrenergic receptor-initiated aggregation. Desensitization occurred with a $t_{1/2}$ of 3–6 min and was maximal at 20–30 min, at which time the initial rate of aggregation and its maximal extent were about half that of control platelets. When we preincubated platelets with epinephrine, and then added phentolamine to block the α_2-receptors, ADP-initiated aggregation occurred normally. Thus, the desensitization of ephinephrine-initiated aggregation was not associated with a generalized impairment of aggregation. At concentrations too low to initiate aggregation, epinephrine is known to potentiate aggregation initiated by other agents. Clonidine also acts at α_2-receptors to potentiate aggregation initiated by other agents, but it does not initiate aggregation by itself. Preincubating clonidine with platelets for 30 min abolished its potentiating effect on ADP-initiated aggregation. Thus, the ability of α_2-receptors to both potentiate and initiate aggregation desensitizes after a brief preincubation with agonist. We performed several types of experiments to investigate the mechanism of this desensitization. Platelet α_2-receptors are coupled to an inhibition of adenylate cyclase. We found, however, that α_2-mediated inhibition of prostaglandin E_1-stimulated cAMP accumulation occurred normally in desensitized platelets. Similarly, epinephrine inhibited basal adenylate cyclase activity normally in membranes prepared from desensitized platelets. In membranes prepared from desensitized platelets, epinephrine competed normally for [^{3}H]rauwolscine binding, and this competition was modulated normally by guanine nucleotides. Thus, the properties of the α_2-receptors, as measured in radioligand binding experiments, were unchanged by densensitization. In conclusion, desensitization of α_2-adrenergic receptor-mediated aggregation occurs without change in the α_2-adrenergic receptors or in their coupling to an inhibition of adenylate cyclase.

After prolonged incubation of target cells with hormones or neurotransmitters, the cellular responses that are evoked by agonist occupancy of surface receptors are often blunted or desensitized. This desensitization is thought to be an important means by which cells regulate their responsivity. Many pharmacological studies of receptor desensitization have focused on receptors that stimulate adenylate cyclase, particularly β-adrenergic receptors (reviewed in Refs. 1 and 2), but few studies have investigated desensitization of receptors that inhibit adenylate cyclase. In the human platelet, α_2-adrenergic receptors initiate aggregation and secretion and are coupled to inhibition of adenylate cyclase (3). Several investigators have demonstrated that incubating unstirred platelets with epinephrine blunts subsequent aggregation when the platelets are stirred (4–6). The mechanism of this desensitization has not been explored. We report here that platelet desensitization to epinephrine is not accompanied by any detectable changes in the α_2-adrenergic receptors themselves or in the ability of these receptors to inhibit cAMP accumulation.

This work was supported by grants from the National Institutes of Health (HL 26523, HL 25457, and HL 29317), NATO, and the American Heart Association.

Materials and Methods

Platelet preparation. Blood was drawn from healthy and ambulatory men and women between the ages of 20 and 40 who had taken no medications during the previous week, and the blood was anticoagulated with 3.8% sodium citrate. PRP was obtained by centrifuging the blood in plastic tubes at 180 × *g* for 15 min. In some experiments, PRP was incubated initially with 1 mM aspirin for 20 min at 37° to inhibit platelet cyclooxygenase (7).

Platelet aggregation. Aggregation was monitored as a change in light transmittance through PRP using a platelet aggregometer (Chronolog Corp., Broomall, PA). As described for each experiment, unstirred PRP was incubated for various periods of time at 37° with one or more

ABBREVIATIONS: PRP, platelet-rich plasma; EDTA, ethylenediaminetetraacetate; IBMX, isobutylmethylxanthine; PGE_1, prostaglandin E_1.

agonists. Aggregation was initiated by adding a stir bar to the cuvette and stirring at 1000 rpm. Both the initial rate and the extent of aggregation were measured. The initial rate of platelet aggregation was expressed in arbitrary units, and the extent of aggregation was taken as the percentage of platelet aggregation 3 min after adding the stir bar. Light transmittance through untreated PRP was set at 0% and that through platelet-poor plasma at 100%.

cAMP accumulation. In most experiments we measured cAMP generation by monitoring the conversion of [^{3}H]ATP to [^{3}H]cAMP using a procedure modified from published reports (8, 9). PRP was incubated with 1 μCi/ml of [^{3}H]adenine for 45 min at room temperature and then centrifuged at 2000 × g for 10 min. The platelet pellets were resuspended in 100 mM NaCl, 50 mM Tris-Cl, 5 mM EDTA at pH 7.5, washed twice, and then resuspended in the same buffer containing 0.2 mM IBMX, an inhibitor of cAMP phosphodiesterase, 0.1 mg/ml of ascorbic acid (to block oxidation of epinephrine), and 1 μM (−)-propranolol to block β-adrenergic receptors. The protocols used for preincubating the platelets and for stimulating adenylate cyclase activity are given in the figure legends. Reactions (1 ml each) were terminated by adding 0.2 ml of 22% perchloric acid containing 1000–2000 cpm of [^{14}C]cAMP, and centrifuging at 1000 × g for 10 min. cAMP in the supernatant was isolated by sequential Dowex and alumina chromatography (10), and both ^{3}H and ^{14}C were counted in a scintillation counter. The results were corrected for column efficiency (determined from the recovery of [^{14}C]cAMP) and the spillover between ^{3}H and ^{14}C in the scintillation counter. The final results are expressed as the fraction of the ^{3}H incorporated into the cells that was converted to [^{3}H]cAMP. In some experiments, adenylate cyclase activity in platelet membranes was measured directly (11).

Radioligand binding. As indicated in each particular experiment, platelets were isolated from PRP either by gel filtration (12) or by centrifugation and washing (13). Platelet membranes were prepared by freeze-thawing and homogenizing washed platelets as reported previously (11). The buffers used are described in the figure legends. The platelets or membranes were incubated with radioligands and other additions in a total volume of 0.5 ml. The two radioligands used ([^{3}H]rauwolscine and [^{3}H]yohimbine) yielded indistinguishable results. The reaction was terminated by adding 10 ml of buffer and washing over glass fiber filters with a further 10 ml of buffer. Nonspecific binding was defined as binding that occurred in the presence of 100 μM epinephrine. This binding was indistinguishable from binding observed in the presence of 10 μM phentolamine.

Results

Desensitization of epinephrine-initiated platelet aggregation. The experiments in Figs. 1 and 2 demonstrate that platelet aggregation initiated by epinephrine is subject to desensitization. Aspirin was added to PRP to block cyclooxygenase and the second wave of aggregation, and the PRP was preincubated for various lengths of time at 37° in the presence or absence of epinephrine. The platelets did not aggregate during this preincubation because the PRP was not stirred. Aggregation was initiated by adding a stir bar. In Fig. 1, PRP was preincubated with 10 μM epinephrine for various amounts of time, and aggregation was then assessed. Desensitization was half-maximal by 3–6 min and maximal by 20–30 min. In Fig. 2, PRP was preincubated with or without various concentrations of epinephrine for 30 min at 21°. The samples preincubated with epinephrine were then stirred, and aggregation was monitored. Epinephrine was then also added to stirred control platelets that had not been preincubated with the agonist. Both the initial rate and the maximal extent of aggregation were reduced in the PRP that had been preincubated with epinephrine compared to PRP that had been preincubated without epinephrine. Similar data were obtained with platelets that had not been treated with aspirin, when the preincubation was performed at 37°, and when 1 μM propranolol was included in the incubation; desensitization was not due to destruction of the epinephrine because epinephrine-preincubated platelets were poorly responsive even when a second dose of epinephrine

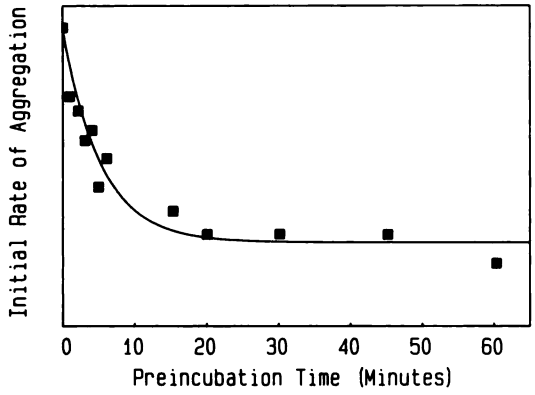

Fig. 1. Time course of epinephrine-initiated desensitization. PRP was incubated with aspirin for 20 min to block the second wave of aggregation. The PRP was then incubated with 10 μM epinephrine at 37° for various lengths of time without stirring. At the times indicated the PRP was stirred and the rate of platelet aggregation was measured.

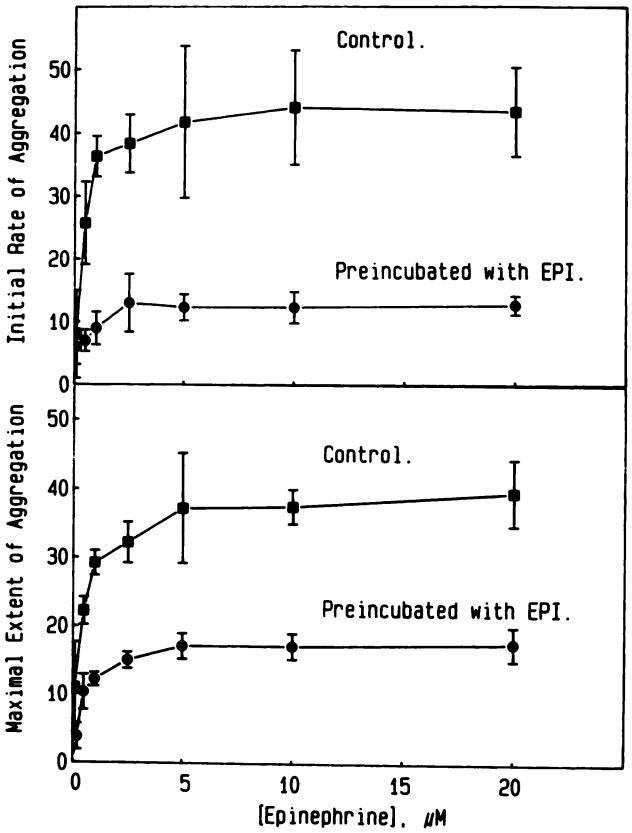

Fig. 2. Dose response for desensitization of epinephrine (*EPI.*)-initiated platelet aggregation. PRP was incubated with aspirin for 20 min to block the second wave of aggregation. The PRP was then preincubated for 90 min at 22° in the presence (●) or absence (■) of epinephrine. After 90 min the PRP was warmed to 37°; various concentrations of epinephrine were also added to the control PRP that had not been preincubated with epinephrine. The platelets were then stirred and aggregation was monitored as a change in light transmittance. Both the initial rate (*top*) and the ultimate extent of aggregation (*bottom*) were reduced in the PRP that had been preincubated with epinephrine for 90 min.

was added at 90 min (data not shown). We also observed desensitization of epinephrine-initiated aggregation in PRP that was not pretreated with aspirin; this was most pronounced when we added creatine phosphokinase (0.5 μg/ml) and phosphocreatine (12 mM) to the PRP to block the effects of released ADP.

Fig. 3 shows that desensitization by epinephrine is receptor specific. PRP was preincubated for 30 min at 37° in the presence or absence of 10 μM epinephrine. This preincubation desensitized epinephrine-initiated aggregation. Phentolamine (10 μM final) was then added to block the α_2-adrenergic receptors. Control experiments showed that 10 μM phentolamine completely blocked aggregation initiated by 10 μM epinephrine. One minute later, ADP (1 or 10 μM) was added and aggregation was monitored. ADP-initiated aggregation occurred identically in PRP preincubated with and without epinephrine. Identical conclusions were obtained when platelets were not preincubated with aspirin. Thus, the desensitization caused by incubating PRP with epinephrine is not a generalized desensitization of platelet aggregation, but is a specific desensitization of aggregation initiated by α_2-adrenergic receptors.

By itself, epinephrine aggregates platelets only at concentrations exceeding 0.5 μM. However, at lower concentrations it potentiates aggregation initiated by other agents, such as ADP (14). We found that we could best study desensitization of this potentiation response by using clonidine, a partial α_2-agonist that does not initiate aggregation by itself (15). Preincubating the platelets with 10 μM clonidine for 15 min desensitized the ability of clonidine to potentiate ADP-initiated aggregation (Fig. 4).

Adenylate cyclase activity in desensitized platelets. We next asked whether incubating platelets with epinephrine would desensitize α_2-receptor-mediated inhibition of adenylate cyclase activity, the generally recognized second messenger system for α_2-receptors in platelets. Platelets were incubated for 90 min at 37° with 10^{-4}–10^{-10} M epinephrine. At 90 min, forskolin (20 μM final) was added to stimulate adenylate cyclase activity (11, 16) and epinephrine was simultaneously added to control platelets that had not been preincubated with epinephrine. After 2 min, the reaction was stopped by addition of perchloric acid, and cAMP generation was quantitated. In control platelets, forskolin stimulated cAMP production, and epinephrine blocked this stimulation with an IC_{50} of 10 nM (Fig. 5). The ability of epinephrine to inhibit cAMP production was not altered by a 90-min preincubation of the platelets with the epinephrine.

When the preincubation with epinephrine was extended from 90 min to 5 hr, desensitization of adenylate cyclase inhibition was still not observed (Fig. 6). In this particular experiment, PGE_1, rather than forskolin, was used to stimulate adenylate cyclase activity. Furthermore, we also found no evidence for desensitization of the adenylate cyclase response when we replaced the NaCl/Tris-EDTA incubation buffer with Dulbecco's modified Eagle's cell culture medium supplemented with 1

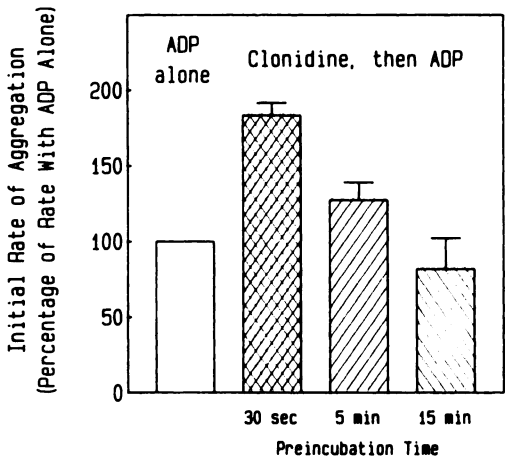

Fig. 4. Desensitization of clonidine-mediated potentiation of platelet aggregation. Unstirred PRP was incubated for 30 min at 37° with or without 10 μM clonidine. The PRP was then placed in the aggregometer and 10 μM clonidine was added to tubes preincubated without clonidine. ADP (1 μM) was added to both tubes, the samples were stirred, and the initial rate of aggregation was monitored. Clonidine alone did not cause aggregation. Data are means ± SE of three experiments.

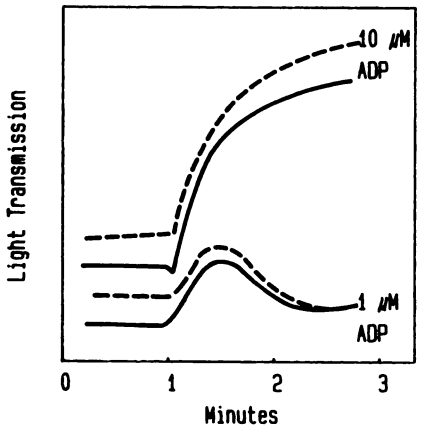

Fig. 3. Desensitization by epinephrine is receptor specific. We incubated aspirin-treated PRP with (——) or without (– – –) 10 μM epinephrine for 30 min and confirmed that epinephrine-initiated aggregation was desensitized as in Fig. 1. Phentolamine (10 μM) was then added at time 0 to block the α_2-adrenergic receptors. One minute later, ADP (1 or 10 μM) was added and aggregation monitored. In a control experiment we demonstrated that 10 μM phentolamine was sufficient to entirely block aggregation initiated by 10 μM epinephrine. The starting positions on the *y* axis are arbitrary, and fine oscillations in the tracing were filtered out.

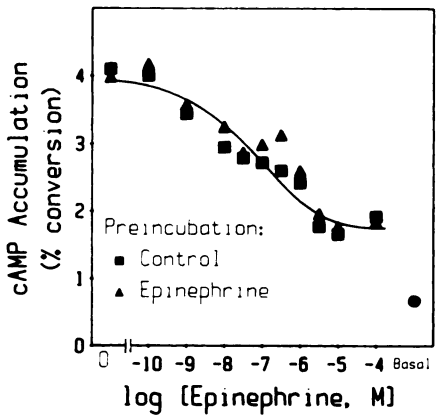

Fig. 5. Dose response for epinephrine-mediated inhibition of cAMP accumulation in human platelets. Washed platelets were incubated for 1 hr at 37° either in the presence (curve A, ▲) or absence (curve B, ■) of various concentrations of epinephrine. The platelets were then transferred to tubes containing 20 μM forskolin. For curve B these tubes also contained various amounts of epinephrine. After incubating for 2 min, the reaction was terminated and the conversion of [^{3}H]adenine to [^{3}H]cAMP was determined. The *solid circle* represents the basal level of cAMP in platelets not stimulated with forskolin.

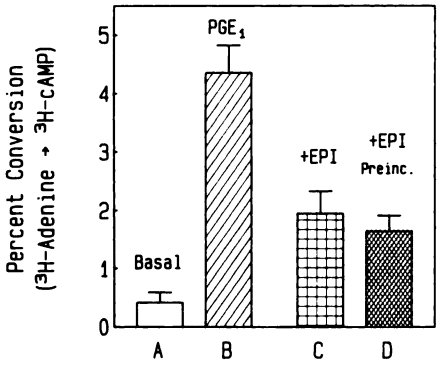

Fig. 6. Lack of desensitization in platelet α_2-receptors after incubation with epinephrine for 5 hr. Washed platelets were prelabeled with [^{3}H] adenine and then incubated for 5 hr alone (A–C) or with 100 μM epinephrine (D). The platelets were then added to tubes containing 20 μM PGE_1 (B–D), with epinephrine also present in C. The reaction was terminated after 2 min, and cAMP generation was quantitated.

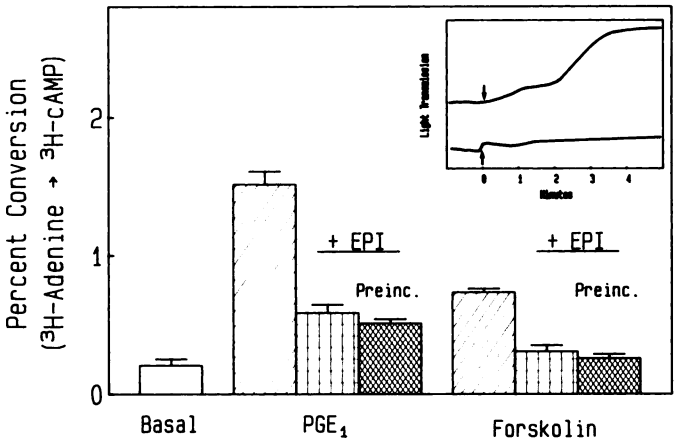

Fig. 7. Lack of desensitization of α_2-mediated inhibition of cAMP accumulation in platelets suspended in plasma. Whole blood was incubated in the presence or absence of 10 μM epinephrine (*EPI.*) for 30 min at 37°. The PRP was then isolated. Some of the PRP was used to quantitate aggregation: as shown in the *inset*, aggregation was diminished in the plasma isolated from blood preincubated with epinephrine. The remainder of the PRP was incubated at 37° for 30 min with [^{3}H]adenine (1 μCi/ml), IBMX (0.2 mM), and propranolol (1 μM). The PRP was then placed into tubes containing 20 μM forskolin or PGE_1. In addition, some tubes that had not been preincubated with epinephrine were now supplemented with 10 μM epinephrine. After a further 2-min incubation, the reaction was stopped and [^{3}H]cAMP accumulation was determined.

mg/ml of bovine serum albumin and 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, when the reaction time was decreased to 0.5 or 1 min, or when IBMX was omitted from the incubation. In addition, we also measured the ability of epinephrine to inhibit adenylate cyclase activity in platelet membranes. Again we found no evidence for desensitization: 30 μM epinephrine inhibited basal adenylate cyclase activity by 57 ± 1% (mean ± standard error, $n = 3$) in control membranes and by 59 ± 3% in membranes prepared from platelets that had been preincubated with 10 μM epinephrine for 1 hr.

These data demonstrate that preincubating platelets with epinephrine desensitizes the ability of the α_2-receptors to initiate platelet aggregation, but does not alter the ability of these receptors to inhibit cAMP accumulation. However, because the aggregation studies were conducted in plasma whereas the cAMP studies employed washed platelets, we thought it important to perform both types of experiments in plasma. Whole blood was incubated either with or without 10 μM epinephrine for 30 min, and PRP was then prepared. A portion was placed into aggregometer tubes and the tracings shown in the *inset* to Fig. 7 were generated: a marked desensitization of aggregation was noted. [^{3}H]Adenine (1 μCi/ml), IBMX (0.2 mM), and propranolol (1 μM) were then added to the PRP, which was incubated 30 min at 37°, and then added to tubes containing 20 μM PGE_1 or 20 μM forskolin. After 2 min, the reaction was stopped and cAMP accumulation was measured. The degree of inhibition of cAMP generation (stimulated by either PGE_1 or forskolin) was the same in platelets that had been preincubated with the epinephrine and in platelets to which the agonist was added simultaneously with the PGE_1 or forskolin. These data substantiate the conclusion that the ability of epinephrine to inhibit cAMP accumulation does not desensitize, whereas the ability of this agonist to stimulate platelet aggregation does desensitize.

α_2-Receptors on desensitized platelets. We next asked whether desensitization might directly modify the α_2-receptors on platelets. Radioligand binding was used to examine the receptors in two types of experiments—one using platelet membranes, the other using intact platelets.

Desensitization is known to alter β-adrenergic receptors on many cells in a manner that can be recognized in radioligand binding experiments. The apparent affinity of the receptors for agonists is reduced because the receptors are "uncoupled" from guanine-nucleotide coupling proteins. α_2-Receptors on platelets are coupled to the guanine nucleotide regulatory protein, G_i (17, 18). We prepared platelet membranes from control and desensitized platelets, and examined the competition between epinephrine and [^{3}H]rauwolscine for binding to α_2-receptors (Fig. 8). The results for control and desensitized platelets were indistinguishable. In addition, the α_2-receptors on desensitized platelets displayed a normal rightward shift in the competition curve in the presence of 100 μM guanosine 5′-(β,γ-imido)triphosphate indicating that they were still coupled to G_i.

Desensitization of β-adrenergic receptors has also been demonstrated in radioligand binding experiments using intact cells: initially the β-adrenergic receptors show a high apparent affinity for catecholamine agonists, and this affinity appears to rapidly decrease as the receptors desensitize (2, 19). The initial high affinity "state" is detected in competitive radioligand binding assays with agonists by terminating the reaction at early time points. We wondered whether the apparent affinity of the platelet α_2-receptor agonists similarly changes. Fig. 9 shows the competition of epinephrine for [^{3}H]yohimbine binding to intact platelets at 1 and 30 min. The data points were obtained experimentally, whereas the curves are computer-drawn predictions for competition of radioligand and competitor for receptor binding based on the law of mass action with no "time-dependent" change in affinity (20). These calculated curves show that the law of mass action predicts the rightward shift of the competitive binding curve as a greater fraction of the receptors are occupied by radioligand; the shift does not reflect a change in the affinity of the receptors. Thus, the affinity of the platelet α_2-adrenergic receptor for epinephrine does not detectably change between 1 and 30 min.

Discussion

Desensitization is an important mechanism by which the responsiveness of cells to hormones and neurotransmitters is

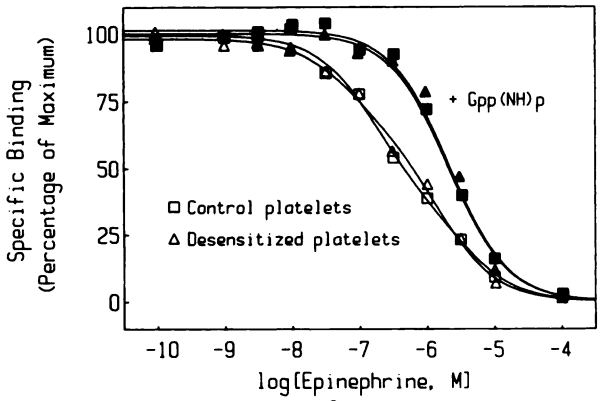

Fig. 8. Competition of epinephrine for [^{3}H]rauwolscine binding to platelet membranes. PRP was incubated for 60 min at 37° in the presence (*triangles*) or absence (*squares*) of 10 μM epinephrine. The platelets were washed at 4°, and membranes were prepared and suspended in 50 mM Tris-HCl, 8 mM $MgCl_2$, 0.5 μM EDTA, and 0.8 mM ascorbic acid at pH 7.5. The membranes were incubated for 45 min at 25° with 4.9 nM [^{3}H] rauwolscine and various concentrations of epinephrine, with no addition (*open symbols*), or with 100 mM guanosine 5′-(β,γ-imido)triphosphate [*Gpp(NH)p*] (*solid symbols*). The membranes were then filtered and washed, and specific binding was determined.

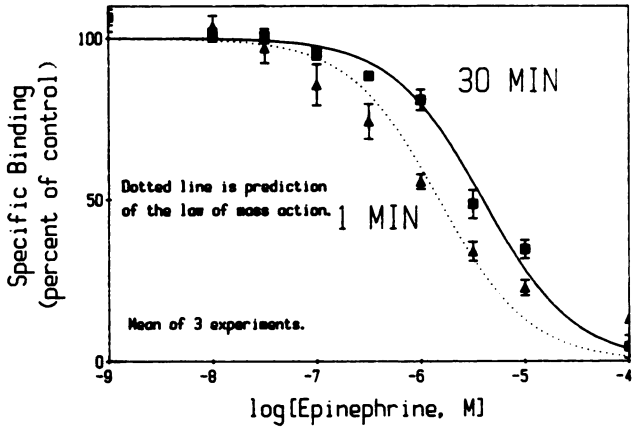

Fig. 9. Competition by epinephrine for [^{3}H]yohimbine binding sites on intact platelets at 1 and 30 min. Platelets were prepared from PRP by gel filtration into a Tyrode's buffer, warmed to 37°, and then added to tubes containing 8 nM [^{3}H]yohimbine and various concentrations of epinephrine. The reactions were terminated by filtration on glass fiber filters at 1 min (●) or 30 min (■). The curves were drawn by a computer programmed with equations describing the law of mass action (20). The dissociation constant for epinephrine in 30-min experiments is 1.1 ± 0.1 μM (mean ± SE; n = 3). The IC_{50} of epinephrine in 1-min experiments is 1.42 ± 0.2 μM.

regulated. Desensitization of epinephrine-initiated platelet aggregation was first reported in 1964 by O'Brien (4) and has since been confirmed by several other investigators (e.g., Refs. 5 and 6). We have confirmed and extended those findings by demonstrating desenitization in aspirin-treated platelets, where only primary aggregation can occur in response to epinephrine. Thus, desensitization does not appear to require the synthesis of prostaglandin endoperoxides or thromboxane A_2. Using the α_2-adrenergic partial agonist clonidine, we have also demonstrated that a second α_2-adrenergic receptor function is also subject to desensitization: the potentiation of ADP-induced aggregation. Moreover, we have found that desensitization to epinephrine is α_2-receptor specific. Thus, the aggregation response to epinephrine is reduced while the primary response to the unrelated agonist ADP is not.

The mechanism of the rapid desensitization of human platelets is as yet unknown. "Down-regulation" of receptor number explains desensitization in many other receptor systems, but we have previously shown that the number of α_2-adrenergic receptors on platelets does not decrease following incubation with epinephrine (13). Platelet α_2-receptors are coupled to an inhibition of adenylate cyclase. However, our data demonstrate that the ability of these receptors to inhibit adenylate cyclase activity is not subject to desensitization. Thus, α_2-initiated platelet aggregation must become desensitized either at a step distal to inhibition of adenylate cyclase, or at some as yet undefined step that links receptor occupancy to aggregation without involving adenylate cyclase. Platelet aggregation in response to epinephrine requires 1) the binding of extracellular fibrinogen to specific figrinogen receptors on the platelet surface membrane, and 2) stirring to enhance platelet-to-platelet interactions. The fibrinogen receptor has been localized to a heterodimer complex of the integral platelet membrane glycoproteins, IIb and IIIa. Fibrinogen receptors are not expressed in resting platelets. However, about 50,000 fibrinogen receptors per platelet are expressed when platelets are stimulated by epinephrine or other agonist (21, 22). Nothing is known about the intramembrane or intracellular processes linking occupancy of α_2-adrenergic receptor to expression of fibrinogen receptors, and we are currently investigating the possibility that desensitization of epinephrine-induced aggregation occurs at that level.

Receptors that stimulate adenylate cyclase typically desensitize in two phases: first, within several minutes after incubation with agonist, the function of the receptors is desensitized although the number of receptors is changed; then over the next few hours the number of detectable receptors decreases. We found that α_2-mediated inhibition of adenylate cyclase, at least as measured by the conversion of [^{3}H]adenine to [^{3}H] cAMP, was not decreased in desensitized platelets. Relatively little evidence exists for rapid desensitization of "inhibitory" receptors. In one study, Green and Clark (23) found that the ability of the muscarinic cholinergic agonist carbachol to inhibit cAMP accumulation in cultured fibroblasts desensitizes with a $t_{1/2}$ of 11 min. Other investigators have presented data demonstrating a lack of desensitization of inhibitory receptors. For example, α_2-adrenergic receptor-mediated inhibition of human adipocyte adenylate cyclase does not desensitize when cells are incubated *in vitro* with epinephrine for 3 hr (24), nor do hamster adipocyte α_2-receptors desensitize when animals are injected with epinephrine for up to 6 days (25). Other reports of desensitization of inhibitory receptors (muscarinic cholinergic, and somatostatin) have involved incubations with agonists for at least several hours (26–28), and it is possible that changes of receptor number, rather than desensitization of receptor function, explain these results.

Elevated levels of cAMP are known to block platelet activation. α_2-Receptors inhibit adenylate cyclase and thus decrease platelet cAMP. Several pieces of data, however, suggest that depression of cAMP levels may not be the mechanism mediating α_2-receptor action in platelets (8, 29). For example, Salzman et al. have shown that whereas the compound SQ22536 inhibits adenylate cyclase in platelets, it does not activate platelets (29). More recently, Clare *et al.* (15) identified several agonists that initiate aggregation (presumably through α_2-receptors) without inhibiting cAMP accumulation, and other compounds that inhibit cAMP accumulation without initiating aggregation. Our desensitization data support the notion that

different mediators (or perhaps different receptor subtypes) mediate epinephrine-initiated aggregation and inhibition of adenylate cyclase. Likely candidates for the mediator of α_2-mediated aggregation are stimulation of Na^+/H^+ exchange, phosphoinositide metabolism, and Ca^{2+} mobilization. Determining whether these pathways are coupled to α_2-receptors in platelets and, if so, whether desensitization occurs seems a fruitful direction for further work.

References

1. Harden, T. K. Agonist-induced desensitization of the β-adrenergic receptor-linked adenylate cyclase. *Pharmacol. Rev.* **35**:1–32 (1983).
2. Hertel, C., and J. P. Perkins. Receptor-specific desensitization of β-adrenergic receptor function. *Mol. Cell. Endocrinol.* **37**:245–256 (1984).
3. Steer, M. L., and E. W. Salzman. Cyclic nucleotides in hemostasis and thrombosis. *Adv. Cyclic Nucelotide Res.* **12**:71–92 (1980).
4. O'Brien, J. R. Variability in the aggregation of human platelets by adrenaline. *Nature (Lond.)* **202**:1188–1189 (1964).
5. Cooper, B., R. I. Handin, L. H. Young, and R. W. Alexander. Agonist regulation of the human platelet alpha-adrenergic receptor. *Nature (Lond.)* **274**:703–706 (1978).
6. Hollister, A. S., G. A. Fitzgerald, J. H. J. Nadeau, and D. Robertson. Acute reduction in human platelet α_2-adrenoreceptor affinity for agonist by endogenous and exogenous catecholamines. *J. Clin. Invest.* **72**:1498–1505 (1983).
7. Burch, J. W., N. Stanford, and P. W. Majerus. Inhibition of platelet prostaglandin synthase by oral aspirin. *J. Clin. Invest.* **61**:314–319 (1978).
8. Connolly, T. M., and L. E. Limbird. The influence of Na^+ on the α_2-adrenergic receptor system of human platelets. A method for removal of extra platelet Na^+: Effect of Na removal on aggregation, secretion, and cAMP accumulation. *J. Biol. Chem.* **258**:3907–3912 (1983).
9. Macfarlane, D. E., and D. C. B. Mills. The effects of ATP on platelets: evidence against the control role of released ADP in primary aggregation. *Blood* **46**:309–332 (1975).
10. Salomon, Y. Adenylate cyclase assay. *Adv. Cyclic Nucleotide Res.* **10**:35–55 (1979).
11. Insel, P. A., D. Stengel, N. Ferry, and J. Hanoune. Regulation of adenylate cyclase of human platelet membranes by forskolin. *J. Biol. Chem.* **257**:7485–7490 (1982).
12. Motulsky, H. J., and P. A. Insel. The influence of sodium on the alpha$_2$-adrenergic receptor system of human platelets: role for intraplatelet Na^+ in receptor binding. *J. Biol. Chem.* **258**:3913–3919 (1983).
13. Karliner, J. S., H. J. Motulsky, and P. A. Insel. Apparent "down-regulation" of human platelet alpha$_2$-adrenergic receptors is due to retained agonists. *Mol. Pharmacol.* **21**:36–43 (1982).
14. Mills, D. C. B., and G. C. U. Roberts. The effects of adrenaline on human blood platelets. *J. Physiol. (Lond.)* **193**:443–453 (1967).
15. Clare, K. A., M. C. Scrutton, and N. T. Thompson. Effects of α_2-adrenoceptor agonists and related compounds on aggregation of, and on adenylate cyclase activity in, human platelets. *Br. J. Pharmacol.* **82**:467–76 (1984).
16. Siegle, A. M., J. W. Daly, and J. B. Smith. Inhibition of aggregation and stimulation of cyclic AMP generation in intact human platelets by the diterpene forskolin. *Mol. Pharmacol.* **21**:680–687 (1982).
17. Katada, T., J. K. Northrup, and G. M. Bokoch. The inhibitory guanine-nucleotide binding regulatory component of adenylate cyclase. *J. Biol. Chem.* **259**:3578–3585 (1984).
18. Motulsky, H. J., and P. A. Insel. ADP- and epinephrine-elicited release of [^{3}H]guanylylimidodiphosphate from platelet membranes: implication for receptor-N_i stoichiometry. *FEBS Lett.* **164**:13–16 (1983).
19. Insel, P. A., L. C. Mahan, H. J. Motulsky, L. M. Stoolman, and A. M. Koachman. Time-dependent decreases in binding affinity of agonists for β-adrenergic receptors of intact S49 lymphoma cells. *J. Biol. Chem.* **259**:13597–13605 (1983).
20. Mahan, L., and H. J. Motulsky. The kinetics of competitive radioligand binding predicted by the law of mass action. *Mol. Pharmacol.* **25**:1–9 (1984).
21. Bennett, J. S. and G. Vilaire. Exposure of platelet fibrinogen receptors by ADP and epinephrine. *J. Clin. Invest.* **64**:1393–1401 (1979).
22. Plow, E. F. and G. A. Marguerie. Induction of fibrinogen receptor on human platelets by epinephrine and the combination of epinephrine and ADP. *J. Biol. Chem.* **255**:10971–10977 (1980).
23. Green, P. A. and R. B. Clark. Comparison of the muscarinic-cholinergic and lysophosphatate inhibition of fibroblast adenylate cyclase demonstrating desensitization to the cholinergic stimulus. *Life Sci.* **29**:1629–39 (1981).
24. Burns, T. W., P. E. Langley, B. E. Terry, and D. B. Bylund. Studies on desensitization of adrenergic receptors on human adipocytes. *Metabolism* **31**:288–293 (1982).
25. Pecquery, R., M. C. Leneveu, U. Giudicelli. In vivo desensitization of the β-, but not the α_2-adrenoceptor-coupled adenylate cyclase system in hamster white adipocytes after administration of epinephrine. *Endocrinology* **114**:1576–1583 (1984).
26. Galper, J. B. and W. Smith. Properties of muscarinic acetylcholine receptors in heart cell cultures. *Proc. Natl. Acad. Sci. USA* **75**:5831–35 (1978).
27. Champion, S. and J. Mauchamp. Muscarinic cholinergic receptors on cultured styroid cells. II. Carbachol-induced desensitization. *Mol Pharmacol.* **21**:73–77 (1982).
28. Resine, T. and J. Axelrod. Prolonged somatostatin pretreatment desensitizes somatostatin's inhibition of receptor-mediated release of adrenocorticotropin hormone and sensitizes adenylate cyclase. *Endocrinology* **113**:811–813 (1983).
29. Salzman, E. W., D. E. MacIntyre, M. L. Steer, and J. L. Gordon. Effect on platelet activity of inhibition of adenylate cyclase. *Thromb Res.* **13**:1089–1101 (1978).

Send reprint requests to: Dr. Harvey J. Motulsky, Division of Pharmacology, M-013, Department of Medicine, University of California, San Diego, La Jolla, CA 92093.