Molecular Basis for ADP-induced Platelet Activation

I. EVIDENCE FOR THREE DISTINCT ADP RECEPTORS ON HUMAN PLATELETS

James L. Daniel‡§, Carol Dangelmaier‡, Jianguo Jin¶, Barrie Ashby‡§, J. Bryan Smith‡§, and Satya P. Kunapuli§¶

From the §Departments of Pharmacology, ¶Physiology, and ¶¶Sherry Thrombosis Research Center, Temple University Medical School, Philadelphia, Pennsylvania 19150

(Received for publication, September 25, 1997)

Acting through cell surface receptors, ADP activates platelets resulting in shape change, aggregation, thromboxane A₂ production, and release of granule contents. ADP also causes a number of intracellular events including inhibition of adenyl cyclase, mobilization of calcium from intracellular stores, and rapid calcium influx in platelets. However, the receptors that transduce these events remain unidentified and their molecular mechanisms of action have not been elucidated. The receptor responsible for the actions of ADP on platelets has been designated the P2T receptor. In this study we have used ARL 66096, a potent antagonist of ADP-induced platelet aggregation, and a P2X ionotropic receptor agonist, α,β-methylene adenosine 5'-triphosphate, to distinguish the ADP-induced intracellular events. ARL 66096 blocked ADP-induced inhibition of adenyl cyclase, but did not affect ADP-mediated intracellular calcium increases or shape change. Both ADP and 2-methylthio-ADP caused a 3-fold increase in the level of inositol 1,4,5-trisphosphate over control levels which peaked in a similar fashion to the Ca²⁺ transient. The increase in inositol 1,3,4-trisphosphate was of similar magnitude to that of inositol 1,4,5-trisphosphate. α,β-Methylene adenosine 5'-triphosphate did not cause an increase in either of the inositol trisphosphates. These results clearly demonstrate the presence of two distinct platelet ADP receptors in addition to the P2X receptor: one coupled to adenyl cyclase and the other coupled to mobilization of calcium from intracellular stores through inositol trisphosphates.

ADP was the first low molecular weight agent recognized to cause platelet aggregation (1, 2). It is stored in the dense granules of human platelets and is an important platelet agonist as evidenced by the fact that patients with defective ADP storage have bleeding tendencies (3, 4). Activation of platelets by ADP follows a defined sequence. The first event, shape change, occurs when discoid shaped resting cells are rapidly converted to spiculated spheres. Shape change is followed by platelet aggregation and granule secretion which releases more ADP as well as many other substances (5). Acting extracellularly, ADP causes a number of intracellular events including rapid calcium influx (6, 7), mobilization of intracellular calcium stores (8), and inhibition of adenyl cyclase (9). An increase in intracellular Ca²⁺ may be due to an increase in inositol trisphosphate (10–13) but this finding remains controversial (14, 15). In addition, arachidonic acid, liberated from platelet membranes due to activation of phospholipase A₂, is converted to thromboxane A₂, itself a powerful platelet agonist. Despite this knowledge the exact identity of platelet ADP receptors responsible for functional responses of ADP are not fully defined and the mechanism by which aggregation occurs is still under investigation.

The idea that ADP's effects on platelets are receptor-mediated was indicated by the finding that ATP shows true competitive inhibition of ADP-induced aggregation and adenyl cyclase inhibition (16). Several other nucleoside triphosphates are also competitive antagonists of ADP and have similar activity ratios for inhibition of aggregation and for inhibition of ADP's effect on adenyl cyclase (17). Evidence that an ADP receptor couple to G-proteins is based on the fact that ADP stimulates the binding of GTPγS to platelet membranes in humans and rats (18). The receptors activated by extracellular nucleotides have been classified as P2 receptors and are divided into two subclasses: P2X intrinsic ion channels and P2Y metabotropic receptors coupled to heterotrimeric G-proteins (19). These receptor subtypes are numbered in the order of cloning and to date eight subtypes of P2X receptors and six subtypes of P2Y receptors have been cloned (20). Because of its pharmacologic profile, the identity of platelet ADP receptor(s) could not be assigned to any cloned member of the P2Y receptor family and hence has been designated P2T (21, 22). Several molecules have been proposed to be the platelet ADP receptor, including aggregin, a 100-kDa protein that covalently binds to 5'-p-fluorosulfonyl benzoyladenosine (23), and platelet glycoprotein IIb, a component of the fibrinogen receptor on platelets (24), but none has been conclusively demonstrated to be a platelet ADP receptor.

Using α,β-MeATP as the agonist, MacKenzie et al. (7) demonstrated an ADP receptor in platelets, suggested to be the P2X1 receptor, that could cause a rapid calcium influx but not internal Ca²⁺ mobilization. However, it is unclear whether α,β-MeATP can activate any physiological platelet responses

* This work was supported in part by a grant-in-aid from the American Heart Association, Southeastern Pennsylvania Affiliate (to B. A.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† This work was performed during the tenure of an Established Investigator award in thrombosis from the American Heart Association and Genentech. To whom correspondence should be addressed: 3420 N. Broad St., Dept. of Physiology, Temple University, Philadelphia, PA 19150. Tel.: 215-707-4615; Fax: 215-707-4003; E-mail: kunapuli@sgi1.fels.temple.edu.

© 1998 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.

THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 273, No. 4, Issue of January 23, pp. 2024–2029, 1998

This paper is available on line at http://www.jbc.org

1 The abbreviations used are: GTPγS, guanosine 5'-3-O-thiotriphospho-
late; α,β-MeATP, α,β-methylene adenosine 5'-triphosphate; 2-MeSADP, 2-methylthio-adenosine 5'-diphosphate; ARL 66096, 2-propylthio-β,γ-di-
fluoromethylene adenosine 5'-triphosphate; IP₃, unspecified isomer of ino-
sitol trisphosphate; Ins₁,3,4IP₃, inositol 1,3,4-trisphosphate; Ins₁,4,5IP₃, ino-
sitol (1,4,5)-trisphosphate.
Three-receptor Model for ADP-induced Platelet Activation

α,β-MeATP were from Research Biologies, Inc. (Natick, MA).

Preparation of [32P]PO₄, Fura-2-labeled Platelets—Human blood was collected from informed healthy volunteers in acid/citrate/dextrose. Platelet-rich plasma was obtained by centrifugation at 180 × g for 15 min at ambient temperature and was recentrifuged (800 × g for 15 min, ambient temperature). The platelet pellet was resuspended in 0.5 volumes of autologous plasma and incubated with 32PO₄ (0.25 mCi/ml) at 37 °C. After 15 min, fura-2 (3 μM) and aspirin (1 μM) were added and the incubation at 37 °C continued for another 45 min. Platelets were isolated from the incubation medium by gel filtration as described previously (27). The final buffer consisted of 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 3.0 mM NaH₂PO₄, 5 mM glucose, 10 mM HEPES (pH 7.4), 0.2% bovine serum albumin, and 20 μg/ml appryase. The platelet count was adjusted to 2 × 10⁶ cells/ml All experiments were carried in buffers with no added Ca²⁺ unless otherwise noted.

Measurement of Ca²⁺ with Fura-2 and Platelet Activation—Platelets (1.0 ml) were activated in a Perkin-Elmer LS-5 spectrophotometer in a water jacketed cuvette maintained at 37 °C with stirring. Fura-2 fluorescence was monitored continuously using settings of 540 nm (excitation) and 510 nm (emission). Fura-2 fluorescence signals were calibrated as described by Pollock et al. (28). Reactions were stopped by the addition of 1:10 volume of ice-cold 6.6 x HClO₄ and kept on ice until further processing.

Measurement of Inositol Trisphosphates—After addition of HClO₄, 0.02 μg/ml Ins(1,4,5)P₃ and [3H]Ins(1,3,4)P₃ were added to each sample and the samples were centrifuged. Supernatants were adjusted to pH 7.5–8.0 by addition of 2 μl K₂CO₃ and the precipitate of KClO₄ was removed by centrifugation. The resulting supernatant was treated at 37 °C overnight with inorganic pyrophosphatase (10 units/ml) in the presence of 5 mM MgCl₂, 10 mM Tris-HCl (pH 8.0). Each sample was treated with 100 mg of charcoal (Darco, G-60) for 5 min on ice to remove nucleotides. Charcoal was removed by centrifugation (3 min at 5000 × g) followed by filtration through Rainin microfiltertubes. Separation of inositol phosphates was accomplished using a Rainin Rabbit HP HPLC and a Whatman Partisil 10 SAX column. The column was eluted at a flow rate of 1.5 ml/min with a 45-min gradient of water as initial buffer and 1.5 M ammonium formate adjusted to pH 3.7 with IPA as final buffer. Fractions were collected and counted in a LKB liquid scintillation counter with windows set to separate 3H and 32P. The [3H]Ins(1,4,5)P₃ and [3H]Ins(1,3,4)P₃ standards were used to indicate the positions of their 32P counterparts and to correct for recovery.

Platelet Aggregation—For aggregation experiments, aspirated platelets were isolated from plasma by centrifugation at 800 × g for 15 min and resuspended in the same buffer that was used for gel filtration. Platelet shape change and aggregation (0.5-ml samples) were measured in a Chronolog lumi-aggregometer with stirring at 37 °C. Fibrinogen (400 μg/ml) was added to all samples. The base line was set using the platelet suspension diluted 1:1 with platelet suspension buffer to increase the gain of the aggregometer output.

Measurement of Cyclic AMP Formation in Intact Platelets—Platelet-rich plasma was incubated with 2 μl/ml adenine and aspirin (1 μM) for 1 h at 37 °C (29). Platelets were isolated from plasma by centrifugation at 800 × g for 15 min and resuspended in the same buffer as was used for gel filtration. Reactions were stopped with 1 M HCl and 4000 disintegrations/min of [3H]cAMP as recovery standard. Cyclic AMP was determined by the method of Salomon (30) and expressed as percentage of total [3H]adenine nucleotides.

RESULTS

Effect of ADP and 2-MeSADP on Inositol Phosphate Metabolism in Platelets—The time course of ADP and 2-MeSADP-induced increases in [32P]Ins(1,4,5)P₃ and [3H]Ins(1,3,4)P₃ are compared with the changes in intracellular Ca²⁺ in Fig. 1, A and B, respectively. Both agonists caused a rapid increase in [32P]Ins(1,4,5)P₃ that reached peak levels at about 3-fold over basal at the earliest measured time of 2 s. [32P]Ins(1,3,4)P₃ also increased rapidly. Intracellular Ca²⁺ also increased rapidly and decreased to near control levels at 30 s.

Concentration Dependence of ADP and 2-MeSADP-induced Inositol Phosphate Formation—The concentration dependence of ADP and 2-MeSADP-induced inositol phosphate formation and intracellular [Ca²⁺] mobilization is shown in Fig. 2, A and B, respectively. Both agonists caused a concentration-dependent increase in the two isomers of IP₃. There was a good

**EXPERIMENTAL PROCEDURES**

Materials—Apyrase (Type V), ADP, ATP, fibrinogen, and bovine serum albumin (fraction V) were from Sigma. Fura-2 was from Molecular Probes (Eugene, OR). ARL 66096 was a gift from Fisons (now known as Astra Research Laboratories, Loughborough, United Kingdom). RGDS was from Bachem Bioscience Inc. (King of Prussia, PA). [32P]PO₄, [3H]1,3,4-IP₃, [3H]1,4,5-IP₃, [3H]adenine, and [3H]cAMP were from NEN Life Science Products (Boston, MA). 2-MeSADP and

In this report, we provide evidence that there are two separate receptors for ADP on the platelet surface; one coupled to inhibition of adenylyl cyclase, designated P2Tₐc, and a second coupled to mobilization of intracellular calcium stores through inositol phosphate production, designated P2TPₐc, in addition to the intrinsic ion channel P2X, coupled to rapid calcium influx previously demonstrated by MacKenzie et al. (7). ATP is an antagonist at both the receptor coupled to inhibition of adenylyl cyclase and that coupled to increases in inositol phosphates.

**FIG. 1. Time course comparing intracellular Ca²⁺ mobilization to IP₃ formation**—Gel-filtered human platelets at 37 °C with constant stirring were activated with either 10 μM ADP (Panel A) or 10 μM 2-MeSADP (Panel B). Samples were stopped at the indicated times by addition of 0.1 volume of 6.6 x HClO₄. Symbols indicate the agonist-induced changes in intracellular free Ca²⁺ in nM (•), the 32P disintegrations/min associated with Ins(1,3,4)P₃ (□), and 32P disintegrations/min associated with Ins(1,4,5)P₃ (■). Data are the average and standard deviation of three determinations from the same platelets and are representative of five experiments.

(7). Léon et al. (25) detected mRNA for the P2Y1 receptor, a metabotropic receptor, in platelets. They expressed this receptor in Jurkat cells and demonstrated that ATP is an antagonist at this receptor and postulated that the P2Y1 receptor is the platelet ADP receptor coupled to adenylyl cyclase inhibition (25, 26).

In this report, we provide evidence that there are two separate receptors for ADP on the platelet surface; one coupled to inhibition of adenylyl cyclase, designated P2Tₐc, and a second coupled to mobilization of intracellular calcium stores through inositol phosphate production, designated P2TPₐc, in addition to the intrinsic ion channel P2X, coupled to rapid calcium influx previously demonstrated by MacKenzie et al. (7). ATP is an antagonist at both the receptor coupled to inhibition of adenylyl cyclase and that coupled to increases in inositol phosphates.
correlation between the concentration dependence of Ca\(^{2+}\) mobilization and IP\(_3\) metabolism for both agonists. The EC\(_{50}\) for ADP was about 1 mM, which is comparable with published results for induction of shape change and Ca\(^{2+}\) mobilization (21). The EC\(_{50}\) for 2-MeSADP was about 4-fold lower which is in agreement with the published activity of 2-MeSADP for induction of platelet Ca\(^{2+}\) mobilization (21).

Effect of \(\alpha,\beta\)-MeATP on Inositol Phosphate Formation and Adenylyl Cyclase Inhibition—\(\alpha,\beta\)-MeATP induced a small Ca\(^{2+}\) transient similar to that shown by MacKenzie et al. (7) but only if the suspension buffer contained 1 mM Ca\(^{2+}\). However, it did not induce a significant increase in Ins(1,4,5)P\(_3\) formation (Table I). In addition, \(\alpha,\beta\)-MeATP did not inhibit forskolin-stimulated adenylyl cyclase activity. An increase in external Ca\(^{2+}\) from nominal (approximately 10 \(\mu\)M) to 1 mM had no effect on the amount of Ins(1,4,5)P\(_3\) or cyclic AMP formed when platelets were stimulated by ADP (Table I).

Effect of ARL 66096 on Platelet Aggregation and Shape Change—ARL 66096 is an ATP analog that is a highly potent and selective inhibitor of ADP-induced platelet aggregation (31). As shown in Fig. 3, ARL 66096 was an effective inhibitor of ADP-induced platelet aggregation, resulting in complete inhibition of aggregation at 100–300 nM. However, ARL 66096 failed to block shape change even when a concentration of 1 \(\mu\)M was used (Fig. 3).

Effect of ARL 66096 on Ca\(^{2+}\) Mobilization, IP\(_3\) Metabolism, and Adenylyl Cyclase—Since ARL 66096 inhibited ADP-induced platelet aggregation, we expected that it would be equally effective as an inhibitor of ADP-induced Ca\(^{2+}\) mobilization and IP\(_3\) metabolism. However, concentrations known to effectively inhibit aggregation had no effect on either Ca\(^{2+}\) mobilization (Fig. 4) or IP\(_3\) formation (Table II). Since inhibition of adenylyl cyclase is another major intracellular effect of ADP on platelets, we investigated the ability of ARL 66096 to block the ADP-induced inhibition of forskolin-stimulated adenylyl cyclase activity (Fig. 5). ARL 66096 inhibited this response in the same concentration range as it inhibited aggregation. In contrast to ARL 66096, ATP blocked ADP-induced

### Table I

| Agonist                  | \[^{3}H\]cAMP (% total \[^{3}H\]adenine) | Disintegration/min \[^{32}P\]Ins(1,4,5)P\(_3\) |
|--------------------------|------------------------------------------|---------------------------------------------|
| Basal                    | 3.17 ± 0.06                              | 127 ± 24                                    |
| ADP (10 \(\mu\)M)        | 1.38 ± 0.11\(^a\)                       | 317 ± 98\(^a\)                              |
| ADP (10 \(\mu\)M) + 1 mM CaCl\(_2\) | 1.50 ± 0.04\(^a\)                       | 338 ± 110\(^a\)                            |
| \(\alpha,\beta\)-MeATP (10 \(\mu\)M) + 1 mM CaCl\(_2\) | 2.98 ± 0.03\(^a\)                       | 150 ± 47                                    |

\(^a\) Significantly different than control by analysis of variance (Fisher’s test). Control and \(\alpha,\beta\)-MeATP were not significantly different from each other.

---

**Fig. 2. Comparison of concentration dependence of intracellular Ca\(^{2+}\) mobilization to IP\(_3\) formation.** Samples were taken after stimulation with indicated concentrations of either ADP (Panel A) or 2-MeSADP (Panel B). Symbols and conditions are as indicated Fig. 1 with the exception that Ins(1,3,4)P\(_3\) and Ins(1,4,5)P\(_3\) data are expressed as change from basal. The curves represent hyperbolae that best fit the data using the program Kaleidagraph (Synergy Software, Reading, PA).

**Fig. 3. The effect of ARL 66096 on ADP-induced platelet aggregation and shape change.** Platelet aggregation was measured as described. The ordinate represents apparent changes in optical density due to light scattering by the platelets. The first arrow indicates the addition of ARL 66096 or solvent; the second arrow indicates the addition of 10 \(\mu\)M ADP. This scale was set to give a full response for control ADP-induced aggregation.
The stopping solution was added. Forskolin-stimulated cyclic AMP level in indicated concentrations of ARL 66096 were added 30 s prior to ADP.

The same concentration range (data not shown).

Kaleidagraph (Synergy Software, Reading PA).

represents the hyperbola that best fit the data using the program Ca$_2^+$ mobilization, shape change, and aggregation within the same concentration range (data not shown).

Ca$_2^+$ mobilization, shape change, and aggregation within the same concentration range (data not shown).

FIG. 5. The effect of ARL 66096 on the inhibition of adenylyl cyclase by ADP. Washed platelets were stirred at 37 °C. Forskolin (100 µM) was added 1 min 30 s prior to the addition of ADP (10 µM). The indicated concentrations of ARL 66096 were added 30 s prior to ADP. Incubations were continued for 2 min after ADP addition after which stopping solution was added. Forskolin-stimulated cyclic AMP level in the absence of ADP was 4.2% of total [³²P]adenine nucleotide. The curve represents the hyperbola that best fit the data using the program Kaleidagraph (Synergy Software, Reading PA).

DISCUSSION

ADP has been known to be a platelet agonist for more than three decades and the ADP-induced physiological responses and several intracellular responses are well characterized. However, the molecular mechanisms of the effect of ADP on platelets remain obscure. It is well accepted that the effects of ADP in platelets are receptor-mediated and this receptor has been designated P2T receptor. However, several observations have remained unexplained. First, 2-MeSADP is at least 100-fold more potent than ADP in causing platelet aggregation and adenylyl cyclase inhibition, but only 4-fold more potent in mobilization of calcium from intracellular stores (21). Second, the thienopyridine analogs ticlopidine and clopidogrel when used in vivo are potent and specific inhibitors of ADP-induced platelet aggregation and antagonize ADP inhibition of prostaglandin E$_1$-activated adenylyl cyclase in intact platelets (21). However, these compounds do not block ADP-induced shape change or intracellular calcium mobilization (32) nor do they affect ADP-induced phosphorylation of myosin light chain and pleckstrin in platelets (33). Third, a congenital defect in platelet function attributed to an ADP receptor defect has been described by Nurden et al. (34). Platelet aggregation was abnormal and ADP-induced inhibition of adenylyl cyclase activity was abolished. However, ADP-induced shape change was unaltered. Finally, several megakaryocytic cell lines and human erythroleukemia cells have been reported to express platelet ADP receptors (35–37) but ADP does not inhibit adenylyl cyclase in these cell lines (38).

Hourani and Hall (22) have proposed a two-receptor model to explain the actions of ADP on platelet function. In this model, rapid calcium influx is mediated by a receptor-operated Ca$_2^+$ channel while adenylyl cyclase inhibition and intracellular Ca$_2^+$ mobilization are modulated by the P2T receptor through multiple G-proteins. A similar model was advocated by Gachet and co-workers (26), who proposed that the P2Y1 receptor is the P2T receptor mediating inhibition of adenylyl cyclase. They propose that calcium entering through P2X receptor activation could cause mobilization of calcium from intracellular stores by a calcium-induced calcium release mechanism. Severe deficiencies of the latter model are first that the P2X receptor agonist, α,β-MeATP, does not produce either the extent or duration of Ca$_2^+$ mobilization that ADP produces (7) and second that ADP can cause intracellular Ca$_2^+$ mobilization in the absence of external Ca$_2^+$.

Although phospholipase C activation and inositol phosphate formation have been shown to be important in mobilization of calcium from intracellular stores in many cellular systems, this intracellular effect of ADP-induced platelet activation has been controversial. In this paper, we have confirmed our earlier findings that ADP causes an increase in inositol trisphosphates using high performance liquid chromatography to separate inositol triphosphate isomers and show that there is a significant, 3-fold increase in Ins(1,4,5)P$_3$, which is the isomer that causes release of intracellular stores of Ca$_2^+$ (39). Overall the increases in Ins(1,4,5)P$_3$ correlate well with changes in intracellular Ca$_2^+$. However, Ca$_2^+$ levels start to decline at 10 s while IP$_3$ levels remain relatively high. This suggests that the initial release of Ca$_2^+$ may trigger resequestration mechanisms that rapidly lower intracellular Ca$_2^+$ levels. Levels of intracellular Ca$_2^+$, even through much lower than the peak concentration, remain above basal for 30 s (Fig. 1), especially in the presence of 1 mM external Ca$_2^+$ (7).

We were surprised to find that ARL 66096 had no effect on ADP-induced intracellular Ca$_2^+$ mobilization, in the range where it is known to inhibit ADP-induced platelet aggregation (31). The possibility that the effects of ADP on platelets are mediated through at least two different receptors has long been the subject of conjecture (21, 22). Using ARL 66096, we can clearly separate two distinct responses of platelets to ADP. ARL 66096 was able to completely inhibit ADP-induced aggregation at a concentration as low as 100 nM while it had no effect platelet shape change at concentrations over 10-fold higher. ARL 66096 also did not inhibit ADP-induced Ca$_2^+$ mobilization or IP$_3$ production in this range.

MacKenzie et al. (7) have shown a receptor of the P2X family, P2X1, on platelets that is coupled to rapid Ca$_2^+$ influx. α,β-MeATP, a P2X receptor agonist, while causing a small tran-
ADP is an agonist (20). However, since both the P2TPLC and P2TAC receptors respond to ADP and are not cloned at this stage, the name P2YADP would not distinguish the two receptors that we have defined. After cloning, these receptors will be designed with standard P2Y numbering. The accompanying paper (40) describes the molecular identity of the P2TPLC receptor and its functional role in platelet activation. The fact that P2Y1 has been shown to couple through a G-protein-dependent mechanism to phospholipase C activation, IP3 formation, and Ca2+ mobilization (25, 41, 42) is fully consistent with our observations that ADP produces IP3 through activation of this receptor on platelets.

Acknowledgments—We thank Drs. David C. B. Mills, A. Koneti Rao, and Robert W. Colman, The Sol Sherry Thrombosis Research Center, for helpful discussions.

REFERENCES

1. Hellern, A. (1960) Scand. J. Clin. Invest. 12, 1–17
2. Gaarder, A., Jonsen, A., Laland, S., Hellem, A. J., and Owren, P. (1961) Nature 192, 531–532
3. Holmsen, H., and Weiss, H. J. (1979) Ann. Rev. Med. 30, 119–134
4. Holmsen, H., and Weiss, H. J. (1970) Br. J. Haematol. 19, 643–649
5. Mills, D. C. B., Robb, I. A., and Roberts, G. C. K. (1968) J. Physiol. 195, 715–729
6. Sage, S. O., and Rink, T. J. (1986) Biochem. Biophys. Res. Commun. 136, 1124–1129
7. MacKenzie, A. B., Mahaut-Smith, M. P., and Sage, S. O. (1990) J. Biol. Chem. 275, 2879–2881
8. Hallam, T. J., and Rink, T. J. (1985) J. Physiol. 368, 131–146
9. Mills, D. C. B., and Smith, J. B. (1972) Acta. N. Y. Acad. Sci. 201, 391–399
10. Daniel, J. L., Dangelmaier, C. A., Selak, M., and Smith, J. B. (1986) FEBS Lett. 206, 299–303
11. Heemskerk, J. W. M., Vis, P., Feijge, M. A. H., Hoyland, J., Mason, W. T., and Sage, S. O. (1993) J. Biol. Chem. 268, 356–363
12. Obrich, C., Aepfelbacher, M., and Siess, W. (1989) Cell. Signalling 1, 483–492
13. Raha, S., Jones, G. H., and Gear, A. R. L. (1990) Biochem. J. 262, 643–646
14. Vickers, J. D., Kinlough-Rathbone, R. L., Packham, M. A., and Mustard, J. F. (1990) Eur. J. Biochem. 193, 521–528
15. Vickers, J. D. (1993) Eur. J. Biochem. 216, 231–237
16. Macfarlane, D. E., and Mills, D. C. B. (1975) Blood 46, 309–320
17. Casadevall, J., and Hourani, S. M. (1982) Br. J. Pharmacol. 76, 221–227
18. Gachet, C., Cazenave, J. P., Ohlmann, P., Pfiff, G., Wieland, T., and Jacobs, K. H. (1992) Eur. J. Biochem. 207, 259–263
19. Fredholm, B. B., Abbrachio, M. P., Burnstock, G., Daly, J. W., Harden, T. K., Jacobson, K. A., Leff, P., and Williams, M. (1994) Pharmacol. Rev. 46, 143–150
20. Fredholm, B. B., Abbracheio, M. P., Burnstock, G., Duhl, G. K., Harden, T. K., Jacobson, K. A., Schwabe, U., and Williams, M. (1997) Trends Pharmacol. Sci. 18, 43–48
21. Mills, D. C. (1996) Thromb. Haemost. 76, 835–856
22. Hourani, S. M., and Hall, R. A. (1994) Trends Pharmacol. Sci. 15, 103–108
23. Colman, R. W. (1992) News Physiol. Sci. 7, 274–278
24. Greco, N. J., Tandon, N. N., Jackson, B., and Jamieson, G. A. (1995) Biochem. Biophys. Acta 1236, 142–148
25. Léné, C., Hechler, B., Vial, C., Leray, C., Cazenave, J. P., and Gachet, C. (1997) FEBS Lett. 401, 36–30

TABLE III

Resolution of the P2T receptor into three distinct receptors based on the pharmacological properties

| P2T receptor | P2TAC receptor | P2TPLC receptor | P2X receptor |
|-------------|----------------|-----------------|-------------|
| Rapid calcium influx | No | Yes | Yes |
| Mobilization of intracellular calcium stores | No | Yes | No |
| Inhibition of adenyl cyclase | Yes | No | No |
| Stimulation of IP3 formation | No | Yes | No |
| ATP an antagonist | Yes | Yes | No |

Three-receptor Model for ADP-induced Platelet Activation

![Diagram of the model](image)
Three-receptor Model for ADP-induced Platelet Activation

26. Gachet, C., Hechler, B., Léon, C., Vial, C., Leray, C., Ohlmann, P., and Cazenave, J. P. (1997) Thromb. Haemost. 77, 271–275
27. Daniel, J. L., Dangelmaier, C. A., and Smith, J. B. (1987) Biochem. J. 246, 109–114
28. Pollock, W. K., Rink, T. J., and Irvine, R. F. (1986) Biochem. J. 235, 869–877
29. Mao, G.-F., Jin, J.-G., Bastepe, M., Ortiz-Vega, S., and Ashby, B. (1996) Prostaglandins 52, 175–185
30. Salomon, Y. (1979) Adv. Cyclic Nucleotide Res. 10, 35–55
31. Humphries, R. G., Tomlinson, W., Ingall, A. H., Cage, P. A., and Leff, P. (1994) Br. J. Pharmacol. 113, 1057–1065
32. Mills, D. C. B., Puri, R. N., Hu, J., Minnitti, C., Granna, C., Freedman, M., Colman, R. F., and Colman, R. W. (1992) Atheroscler. Thromb. 12, 430–436
33. Savi, P., Artcanathurry, V., Bornia, J., Grelac, F., Maclouf, J., Levytoledano, S., and Herbert, J. M. (1997) Br. J. Haematol. 97, 185–191
34. Nurden, P., Savi, P., Heilman, E., Bihour, C., Herbert, J. M., Maffrand, J. P., and Nurden, A. (1995) J. Clin. Invest. 95, 1612–1622
35. Akbar, G. K., Dasari, V. R., Steth, S. B., Ashby, B., and Mills, D. C. K. (1996) J. Receptor Signal Transd. Res. 16, 209–224
36. Murgo, A. J., Contrera, J. G., and Sistare, F. D. (1994) Blood 83, 1258–1267
37. Shi, X-P., Yin, K-C., and Gardell, S. J. (1995) Thromb. Res. 77, 235–247
38. Vittet, D., Mathieu, M. N., Launay, J. M., and Chevillard, C. (1997) Exp. Hematol. 20, 1129–1134
39. Berridge, M. J., and Irvine, R. F. (1989) Nature 341, 197–205
40. Jin, J., Daniel, J. L., and Kunapuli, S. P. (1997) J. Biol. Chem. 273, 2030–2034
41. Schachter, J. B., Li, Q., Boyer, J., Nicholas, R. A., and Harden, T. K. (1996) Br. J. Pharmacol. 118, 167–173
42. Janssens, R., Communi, D., Piroton, S., Samson, M., Parmentier, M., and Boeynaems, J. M. (1996) Biochem. Biophys. Res. Commun. 221, 588–593