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An L-arginine/nitric oxide pathway present in human platelets regulates aggregation

(collagen/cyclic GMP/nitric oxide synthase)

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ABSTRACT **Aggregation of human washed platelets with collagen is accompanied by a concentration-dependent increase in cyclic GMP but not cyclic AMP. N^G-Monomethyl-L-arginine (L-MeArg), a selective inhibitor of nitric oxide (NO) synthesis from L-arginine, reduces this increase and enhances aggregation. L-Arginine, which has no effect on the basal levels of cyclic GMP, augments the increase in this nucleotide induced by collagen and also inhibits aggregation. Both of these effects of L-arginine are attenuated by L-MeArg. The anti-aggregatory action of L-arginine is potentiated by prostacyclin and by M&B22948, a selective inhibitor of the cyclic GMP phosphodiesterase, but not by HL725, a selective inhibitor of the cyclic AMP phosphodiesterase. L-Arginine also inhibits platelet aggregation in whole blood in a similar manner, although the concentrations required are considerably higher. L-Arginine stimulates the soluble guanylate cyclase and increases cyclic GMP in platelet cytosol. This stimulation is dependent on NADPH and Ca^{2+} and is associated with the formation of NO. Both the formation of NO and the stimulation of the soluble guanylate cyclase induced by L-arginine are enantiomer specific and abolished by L-MeArg. Thus, human platelets contain an NO synthase which is activated when platelets are stimulated. The consequent generation of NO modulates platelet reactivity by increasing cyclic GMP. Changes in the activity of this pathway in platelets may have physiological, pathophysiological, and therapeutic significance.**

Platelets possess a soluble guanylate cyclase which, when activated, induces an increase in cyclic GMP (1). It was originally thought that the increase in this nucleotide which occurs in response to aggregating agents (2, 3) was responsible for aggregation (4, 5). The function of cyclic GMP, according to the yin yang hypothesis in vogue at the time (6), was to antagonize the actions of cyclic AMP, an increase in the levels of which was known to inhibit platelet aggregation (7, 8). In 1981, however, it was found that the nitrovasodilators and nitric oxide (NO), which stimulate the soluble guanylate cyclase and increase the levels of cyclic GMP, concomitantly inhibit aggregation. This led to a reassessment of the yin yang hypothesis and to the suggestion that the increase in cyclic GMP may rather be related to inhibition of platelet aggregation (9).

We have found that the vascular endothelium synthesizes NO from the terminal guanidino nitrogen atom(s) of L-arginine (10). This occurs through the action of a soluble enzyme, the NO synthase (11), which is inhibited by the L-arginine analogue N^G-monomethyl-L-arginine (L-MeArg) (12). This biochemical system, which we have called the L-arginine/NO pathway (13), is now known to exist in many other cells (14), and we have proposed that it is the endogenous transduction mechanism for the stimulation of the soluble guanylate cyclase (13).

In view of these findings and of the fact that L-arginine has been shown to inhibit platelet aggregation by an unknown mechanism (15, 16), we have investigated the existence and biological role of the L-arginine/NO pathway in human platelets.

MATERIALS AND METHODS

Washed Platelets. Blood was obtained from healthy volunteers who had not taken drugs for 10 days prior to the study. Prostacyclin-washed platelets were prepared from blood collected into a trisodium citrate/prostacyclin mixture, and they were finally resuspended in Tyrode's solution containing 2 mM Ca^{2+} as described previously (17). Aggregation was induced by collagen (0.1–10.0 μg/ml) and was monitored for 6 min in a Payton dual-channel aggregometer by the method of Born and Cross (18).

Intraplatelet cyclic GMP was measured in the presence of M&B22948 (1 μM). Platelet aggregation was terminated after 3 min by addition of 100 μl of 20% $HClO_4$; the samples were then neutralized with K_3PO_4 (1.08 M, 200 μl) and, after centrifugation (12,000 × *g* for 2 min), samples were assayed for cyclic GMP content by specific radioimmunoassay (19). Cyclic AMP in the neutralized sample was also assayed by specific radioimmunoassay using a commercially available kit (Amersham).

Platelet Aggregation in Blood. Hirudin (200 units/ml) was used to prevent blood from clotting without altering the concentration of divalent cations (20). Blood samples were incubated in duplicate (37°C, stirring at 900 rpm) in a Payton dual-channel aggregometer for 3 min prior to addition of collagen (0.01–3.0 μg/ml). After a further 3 min, samples were taken and the free platelets were counted, using a whole blood platelet counter (Ultra Flo 100, Clay Adams) as previously described (21).

Preparation of Platelet Cytosol. Washed platelets (7–8 × 10^{10}) were resuspended in 5 ml of buffer (0.32 M sucrose, 10 mM Hepes, 1 mM DL-dithiothreitol, pH 7.4) and were homogenized by sonication twice for 5 s with a Soniprep (MSE). The homogenate was centrifuged at 150,000 × *g* for 30 min. The supernatant was passed through a 2-ml column of cation-exchange resin (AG 50W-X8) to remove endogenous arginine. The NO synthase in the platelet cytosol was determined either as activation of the soluble guanylate cyclase or as formation of NO.

Activation of the Soluble Guanylate Cyclase. In experiments in which NO synthase was assayed by activation of the soluble guanylate cyclase the column effluent was also

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Abbreviation: L(D)-MeArg, N^G-monomethyl-L(D)-arginine.

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passed through a 2-ml column of Sephadex G-25 to remove low molecular weight components. The resulting platelet cytosol was stored on ice and used within 30 min.

Incubations for cyclic GMP formation were initiated by addition of 100 μl of platelet cytosol (5 μg of protein) to prewarmed (37°C) buffer, to give (final concentrations) 25 mM Tris·HCl, 5 mM GTP, 5 mM $MgCl_2$, 1 mM 3-isobutyl-1-methylxanthine, pH 7.2 at 37°C. After 20 min of incubation the reaction was terminated and the cyclic GMP in the mixture was determined as described for washed platelets.

Spectrophotometric Determination of NO. Platelet cytosol was prepared from 1–2 × 10^{11} platelets as described above, except that the Sephadex G-25 step was omitted. In some experiments the Ca^{2+} concentration was controlled with Ca^{2+}/EGTA buffers such that the final free Ca^{2+} concentration was 0.03–3.0 μM as described previously (22). The cytosol was divided into two 3-ml samples (40.1 ± 1.7 mg of protein per ml, $n = 3$) and NO production was determined by the method of Feelisch and Noack (23). This method is based on the rapid and stoichiometric reaction of NO with oxyhemoglobin to yield methemoglobin and nitrate. Briefly, each 3-ml sample was incubated with oxyhemoglobin (5 μM) at 37°C in a dual-wavelength spectrophotometer (Shimadzu, Kyoto) and the rate of NO-induced formation of methemoglobin was measured for 15 min as the difference in absorbance between 401 and 411 nm (401 nm is the maximum and 411 nm is the isobestic point for the difference spectrum of methemoglobin vs. oxyhemoglobin). The rate of NO formation was linear over this time.

Reagents. Collagen (Hormon-Chemie, Munich), leech recombinant hirudin, L-arginine, D-arginine, L-homoarginine, L-lysine, NADPH, and DL-dithiothreitol were all from Sigma. Prostacyclin sodium salt, L-MeArg, and N^G-monomethyl-D-arginine (D-MeArg) were all from Wellcome. 2-*O*-Propoxyphenyl-8-azapurine-6-one (M&B22948, May & Baker, Dagenham, United Kingdom), 9,10-bimethoxy-3-methyl-2-mesitylimino-3,4,6,7-tetrahydro-2,4-pyrimido-(6,1-*A*)-isoquinoline-4-one hydrochloride (HL725, Hoechst), 3-isobutyl-1-methylxanthine (Aldrich), Sephadex G-25 (Pharmacia), and AG 50W-X8 (Bio-Rad) were obtained as indicated. Hemoglobin was prepared as described (24). Arginine analogues and hirudin were dissolved and diluted in distilled water and all the other compounds were made up as described previously (25). All reagents were incubated with washed platelets or blood for 3 min prior to the addition of the aggregating agent.

Statistics. All values are means ± SEM of n experiments. Values were compared by using the t test for unpaired data and $P < 0.05$ was considered as statistically significant.

RESULTS

Platelet Aggregation and Cyclic GMP Levels. Collagen (0.1–10.0 μg/ml) induced a concentration-dependent aggregation of washed platelets (Fig. 1), which was accompanied by an increase in intraplatelet cyclic GMP (Fig. 2) but not cyclic AMP (not shown).

The aggregation induced by collagen at 1 μg/ml was inhibited by L-arginine (Figs. 1 and 3) in a concentration-dependent manner with an IC_{50} of 8.9 ± 1.0 μM ($n = 5$). This anti-aggregating effect was not affected by HL725 (1 fM), but it was potentiated by a subthreshold concentration of prostacyclin (0.1 nM; not shown) or by M&B22948 (1 μM; Fig. 3), such that the IC_{50} values were 2.8 ± 0.5 μM and 2.3 ± 0.7 μM, respectively ($n = 5$).

L-Arginine (30 μM) did not affect basal cyclic GMP levels (0.9 ± 0.2 pmol per 10^8 platelets, $n = 5$). However, it significantly enhanced the rise in cyclic GMP induced by collagen (Fig. 2). L-MeArg (1–30 μM), in contrast, potentiated collagen-induced aggregation (Fig. 1) and decreased the collagen-induced rise in cyclic GMP (Fig. 2). The anti-aggregating effect and the enhancement of cyclic GMP levels induced by L-arginine were also inhibited by L-MeArg (Figs. 1 and 2). Hemoglobin (1 μM) or D-MeArg (100 μM) had no effect on either collagen-induced aggregation or rise in cyclic GMP ($n = 3$ for each).

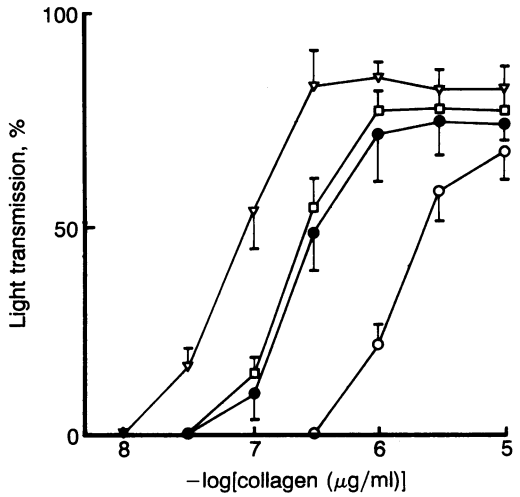

FIG. 1. Effect of L-arginine and L-MeArg on platelet aggregation induced by collagen. ●, Control, no additions. Platelet aggregation is enhanced by L-MeArg (10 μM; ▽) and inhibited by L-arginine (30 μM; ○). The inhibitory effect of L-arginine (30 μM) is prevented by L-MeArg (30 μM; □). Each value is the mean ± SEM from five separate experiments.

L-Homoarginine was a weak inhibitor of platelet aggregation. At 100 μM, it inhibited significantly platelet aggregation by 15% ± 5% ($n = 3$). This was increased to 55% ± 10% ($n = 3$, $P < 0.05$) in the presence of M&B22948 (1 μM). D-Arginine, D-MeArg, L-lysine (all at 100 μM, $n = 3$), and hemoglobin (1 μM; $n = 3$) had no effect on platelet aggregation.

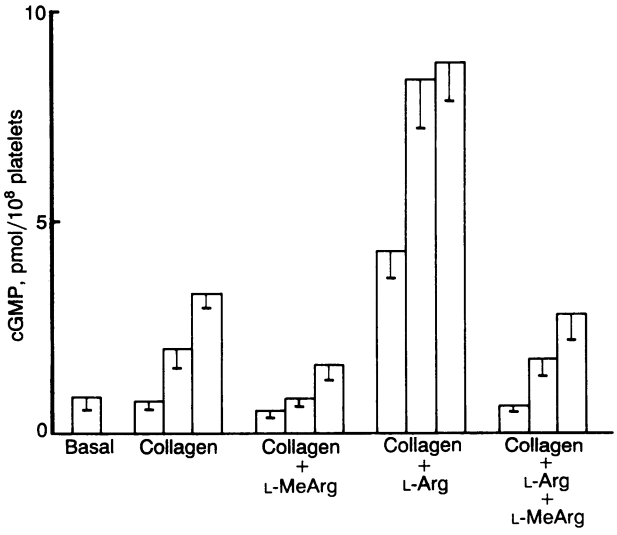

FIG. 2. Effect of L-arginine and L-MeArg on cyclic GMP (cGMP) formation induced by collagen. Collagen (0.3, 1.0, and 3.0 μg/ml) results in a dose-dependent increase in the formation of cyclic GMP above basal levels. This effect is inhibited by L-MeArg (10 μM). In contrast, L-arginine (30 μM) enhances the formation of cyclic GMP induced by collagen. This effect of L-arginine is abolished by L-MeArg (30 μM). All incubations were carried out in the presence of M&B22948 (1 μM). Each value is the mean − SEM from five separate experiments.

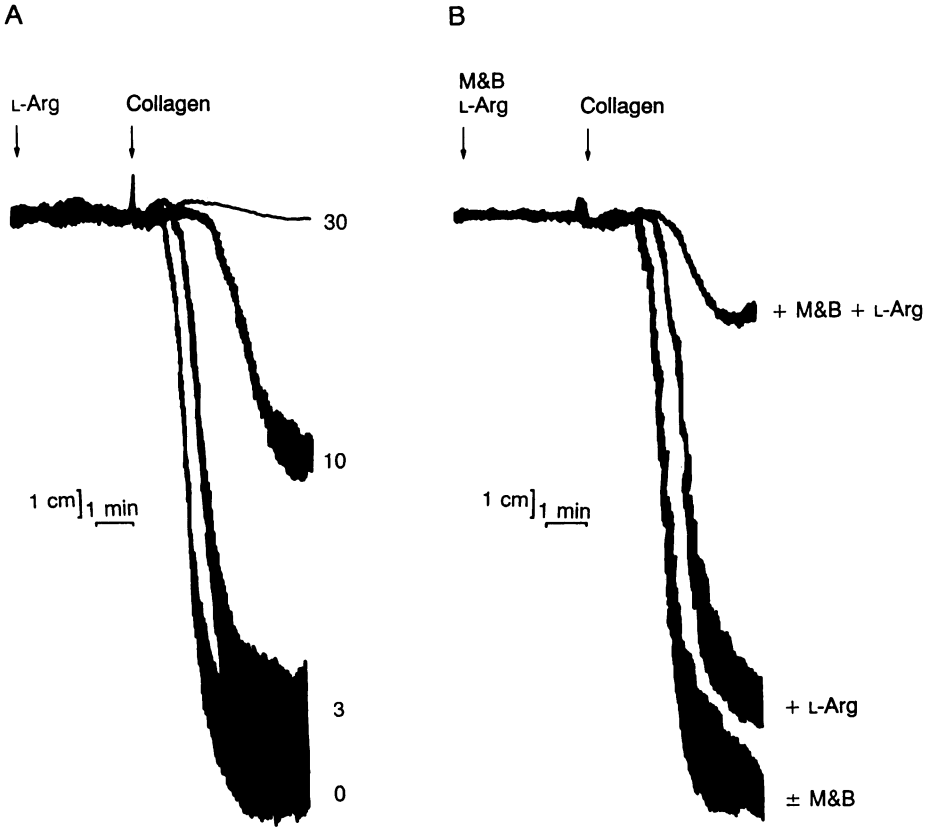

FIG. 3. Effect of L-arginine on collagen-induced platelet aggregation. (*A*) L-Arginine (3–30 μM) inhibits platelet aggregation induced by collagen (1 μg/ml) in a dose-dependent manner. The numbers beside the tracings show concentrations of L-arginine in μM. (*B*) The aggregation of platelets induced by collagen (1 μg/ml) is only slightly inhibited by L-arginine (3 μM; + L-Arg). This effect of L-arginine is potentiated by M&B22948 (1 μM; + M&B + L-Arg). M&B22948 does not affect aggregation on its own. Tracings are representative of five similar experiments.

In blood, collagen (0.01–3.0 μg/ml) also induced platelet aggregation, an effect shown as a concentration-dependent decrease in the number of free platelets to 5% ± 3% (n = 5) of the initial count at the highest concentration. This aggregation was inhibited by L-arginine with an IC_{50} of 234 ± 43 μM (n = 5). The anti-aggregatory effect of L-arginine was reversed by L-MeArg (30–1000 μM) and significantly potentiated by subthreshold doses of prostacyclin (0.1 nM) or by M&B22948 (1 μM), such that the IC_{50} values were 108 ± 9 and 122 ± 15 μM, respectively (n = 4). L-Homoarginine was also a weak inhibitor of platelet aggregation in blood, causing a significant inhibition of 13% ± 5% (n = 3) at 1000 μM. This was increased to 38% ± 9% (n = 3) in the presence of M&B22948 (1 μM). D-Arginine, D-MeArg, and L-lysine, at concentrations up to 1000 μM, had no effect on collagen-induced aggregation in blood.

Activation of the Soluble Guanylate Cyclase. The basal rate of cyclic GMP formation in platelet cytosol from which the low molecular weight components had been removed was 0.10 ± 0.06 nmol/min per ml. This was significantly elevated to 2.2 ± 0.5 nmol/min per ml by addition of sodium nitroprusside (30 μM) (Fig. 4).

L-Arginine alone (3–300 μM) did not affect the rate of formation of cyclic GMP (n = 5). However, in the presence of NADPH (300 μM), it caused a significant increase in the rate of formation of cyclic GMP (Fig. 4). All further determinations of guanylate cyclase activity in platelet cytosol were carried out in the presence of NADPH at 300 μM, which was found to be maximally effective (data not shown). Cyclic AMP formation was not observed in these incubations (<0.01 nmol/min per ml, n = 3). L-Homoarginine (300 μM) was approximately 1/5th as effective as L-arginine in stimulating guanylate cyclase activity in platelet cytosol, while D-arginine (300 μM) had no effect (Fig. 4).

The stimulation of guanylate cyclase by L-arginine (300 μM) was inhibited in a concentration-dependent manner by hemoglobin (IC_{50} = 0.22 ± 0.06 μM; n = 5) and L-MeArg (IC_{50} = 35.3 ± 7.1 μM; n = 5).

NO Synthase. The basal rate of NO formation in platelet cytosol from which the low molecular weight fraction had not been removed was 8.0 ± 2.7 fmol/min per mg of protein (n = 3). This was abolished (<2 fmol/min per mg of protein; n = 3) by L-MeArg (100 μM). Addition of L-arginine (10–100 μM), but not D-arginine (up to 100 μM), resulted in a concentration-dependent increase in the rate of formation of NO. Addition of L-MeArg (100 μM) reduced the rate of NO

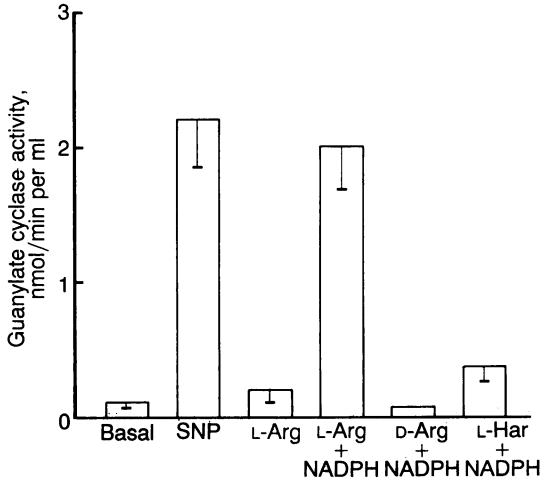

FIG. 4. NO synthase activity assayed by activation of guanylate cyclase in platelet cytosol. The basal activity of guanylate cyclase is enhanced by sodium nitroprusside (30 μM; SNP). L-Arginine alone (300 μM) has no significant effect on the rate of formation of cyclic GMP, but it induces significant enhancement in the presence of NADPH (300 μM). D-Arginine (300 μM) + NADPH has no effect, whereas L-homoarginine (300 μM; L-Har) + NADPH is a weak activator. All incubations were carried out in the presence of 3-isobutyl-1-methylxanthine (1 mM). Each value is the mean − SEM of five separate experiments.

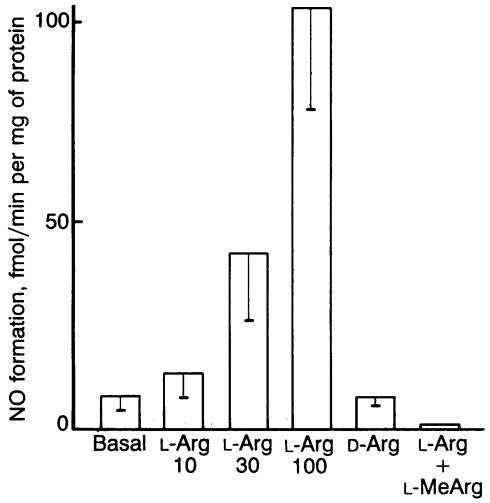

FIG. 5. NO formation in platelet cytosol. The basal rate of NO formation is enhanced in a concentration-dependent manner by L-arginine (10, 30, 100 μM) but not by D-arginine (100 μM). NO formation induced by L-arginine (100 μM) is abolished by L-MeArg (100 μM). Each value is the mean ± SEM of three separate experiments.

formation in the presence of L-arginine (100 μM) to below the limit of detection (Fig. 5). In the absence of Ca^{2+} (1 mM EGTA) the L-arginine (100 μM) -induced formation of NO was not detectable (<2 fmol/min per mg of protein). The addition of Ca^{2+} (0.03–3.00 μM) caused a concentration-dependent increase in the rate of NO formation (Table 1) to a maximum of 110 ± 11 fmol/min per mg of protein (n = 3).

DISCUSSION

Platelet aggregation induced by collagen was accompanied by an increase in cyclic GMP. L-MeArg, an inhibitor of the NO synthase in other tissues (14), inhibited this increase in cyclic GMP and potentiated aggregation. Furthermore, L-arginine, the substrate for NO generation (10), enhanced the increase in cyclic GMP and inhibited aggregation. Both of these effects of L-arginine were inhibited by L- but not D-MeArg and were potentiated by M&B22948, a selective inhibitor of the platelet cyclic GMP phosphodiesterase (26), but not by HL725, a selective inhibitor of the cyclic AMP phosphodiesterase (27). Furthermore, prostacyclin, which synergizes with NO to inhibit platelet aggregation (25, 28), also synergized with L-arginine in this respect. All these effects are consistent with the formation of NO from L-arginine by the platelets. Furthermore, the NO formed activates soluble guanylate cyclase to elevate intraplatelet cyclic GMP levels, leading to down-regulation of platelet reactivity. Whether the NO formed also influences platelet reactivity by a cyclic GMP-independent mechanism, such as by stimulation of ADP-ribosylation (29), remains to be established.

Support for the existence of the L-arginine/NO pathway in the platelets comes from experiments with platelet cytosol. In these an increase in cyclic GMP was observed not only with sodium nitroprusside, a compound that releases NO (23), but also with L-arginine and to a lesser extent with L-homoarginine. The effect of L-arginine was enantiomer specific, inhibited by L-MeArg, and dependent on the presence of NADPH, indicating the enzymatic nature of this reaction. In addition, measurements in the platelet cytosol demonstrated an L-arginine- and NADPH-dependent formation of NO which was inhibited by L-MeArg, providing conclusive evidence for the existence of the L-arginine/NO pathway in the platelets.

Table 1. Effect of Ca^{2+} on NO synthesis by platelet cytosol

Free Ca^{2+}, μM	NO formation, fmol/min per mg protein
0.03	ND
0.10	22 ± 4
0.30	110 ± 18
3.00	102 ± 14

Results are mean ± SEM (n = 3). ND, not detectable.

The formation of NO from L-arginine in platelet cytosol was dependent on the free Ca^{2+} concentration over the range 0.10–3.00 μM, indicating that the NO synthase in platelets is Ca^{2+} dependent. These concentrations of Ca^{2+} are similar to those over which the brain NO synthase is activated and are comparable to the changes in intracellular Ca^{2+} concentration in platelets after stimulation (30). It is therefore likely that, as we have previously proposed for the brain NO synthase (22), it is this rise in Ca^{2+} that activates the NO synthase in platelets.

These data show that the platelet enzyme is similar to that in the endothelium (10), the brain (22), and the adrenal gland (31), for all of them are Ca^{2+} dependent and do not utilize L-homoarginine effectively as a substrate. These two characteristics are not, however, shared by the macrophage (32) and neutrophil (33) enzymes. Further evidence for the platelet NO synthase being of the endothelial type comes from the finding that it requires only NADPH for activity, unlike the macrophage enzyme, which requires other low molecular weight cofactors (34, 35).

Hemoglobin, a powerful inhibitor of the actions of NO (25), did not reverse the effects of L-arginine in washed platelets. However, it was the most effective inhibitor of the increase in cyclic GMP induced by L-arginine in platelet cytosol, indicating that hemoglobin did not penetrate the platelet membrane efficiently. Thus, the L-arginine/NO pathway acts as an intraplatelet negative feedback mechanism to regulate reactivity. This is different from the vascular smooth muscle, which relaxes in response to NO generated in a different cell, the endothelium.

Interestingly, L-arginine did not increase the basal levels of cyclic GMP in unstimulated platelets but did so when they were activated with collagen. This shows that this NO synthase can utilize exogenous substrate only once it is activated, a fact that we have previously observed in the neutrophil (33). Whether this is a function of the uptake of arginine into the cell or of the regulation of the NO synthase by the substrate is not yet known. In this context it is worth noting that L-arginine was 1/20th to 1/30th as active as an inhibitor of platelet aggregation in blood as in washed platelets. This may be a reflection of a down-regulation of L-arginine uptake into the platelets by the high concentrations (48–140 μM; refs. 36 and 37) of L-arginine in blood.

The relationship between intracellular and extracellular arginine and its biological relevance remains to be established. It is possible that a decrease in the levels of L-arginine may enhance platelet reactivity and be prothrombotic. In contrast, an increase in dietary L-arginine might result in a decrease in platelet reactivity and therefore provide an antithrombotic effect. Since L-arginine will be utilized not only by the platelets but also by endothelial cells and phagocytes, the potential biological consequences of this intervention might be significant for the prevention or treatment of diseases other than arterial thrombosis.

Finally, the description of the formation of NO from L-arginine in the platelets further exemplifies the widespread distribution and the biological relevance of the L-arginine/NO pathway as the endogenous activator of the soluble guanylate cyclase (13).

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1. Bohme, E., Jung, R. & Mechler, I. (1974) *Methods Enzymol.* **38,** 199–202.
2. Haslam, R., Davidson, M. M., Davies, T., Lynham, J. A. & McClenaghan, M. D. (1978) *Adv. Cyclic Nucleotide Res.* **9,** 533–552.
3. Steer, L. M. & Salzman, E. W. (1980) *Adv. Cyclic Nucleotide Res.* **12,** 71–92.
4. Chiang, T. M., Dixit, S. N. & Kang, A. H. (1976) *J. Lab. Clin. Med.* **88,** 215–221.
5. Glass, D. B., Gerrard, J. M., Townsend, D., Carr, D. W., White, J. G. & Goldberg, N. D. (1977) *J. Cyclic Nucleotide Res.* **3,** 37–44.
6. Goldberg, N. D., Haddox, M. K., Nicol, S. E., Glass, D. B., Sanford, C. H., Kuehl, Jr., F. A. & Estensen, R. (1975) *Adv. Cyclic Nucleotide Res.* **5,** 307–330.
7. Marcus, A. J. & Zucker, M. B. (1965) *Physiology of Blood Platelets* (Grune & Stratton, London), pp. 42–56.
8. Mills, D. C. & Smith, J. B. (1971) *Biochem. J.* **121,** 185–196.
9. Mellion, B. T., Ignarro, L. J., Ohlstein, E. H., Pontecorvo, E. G., Hyman, A. L. & Kadowitz, P. J. (1981) *Blood* **57,** 946–955.
10. Palmer, R. M. J., Ashton, D. S. & Moncada, S. (1988) *Nature (London)* **333,** 664–666.
11. Palmer, R. M. J. & Moncada, S. (1989) *Biochem. Biophys. Res. Commun.* **158,** 348–352.
12. Palmer, R. M. J., Rees, D. D., Ashton, D. S. & Moncada, S. (1988) *Biochem. Biophys. Res. Commun.* **153,** 1251–1256.
13. Moncada, S., Palmer, R. M. J. & Higgs, E. A. (1989) *Biochem. Pharmacol.* **38,** 1709–1715.
14. Moncada, S. & Higgs, E. A., eds. (1990) *Nitric Oxide from L-Arginine: A Bioregulatory System* (Elsevier, Amsterdam), in press.
15. Caren, R. & Corbo, L. (1973) *Proc. Soc. Exp. Biol. Med.* **143,** 1067–1071.
16. Houston, D. S., Gerrard, J. M., McCrea, J., Glover, S. & Butler, A. M. (1983) *Biochim. Biophys. Acta* **734,** 267–273.
17. Radomski, M. & Moncada, S. (1983) *Thromb. Res.* **30,** 383–389.
18. Born, G. V. R. & Cross, M. J. (1963) *J. Physiol.* **168,** 178–195.
19. Garthwaite, J. & Gilligan, G. J. (1984) *Neuroscience* **11,** 125–138.
20. Lages, B. & Weiss, H. J. (1981) *Thromb. Haem.* **45,** 173–179.
21. O'Grady, J., Hedges, A., Whittle, B. J. R., Al-Sinawi, L., Mekki, Q. K., Burke, C., Moody, S. G., Moti, M. J. & Hassan, S. (1984) *Br. J. Clin. Pharmacol.* **18,** 921–933.
22. Knowles, R. G., Palacios, M., Palmer, R. M. J. & Moncada, S. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 5159–5162.
23. Feelisch, M. & Noack, E. A. (1987) *Eur. J. Pharmacol.* **139,** 19–30.
24. Paterson, R. A., Eagles, P. A., Young, D. A. & Beddell, C. R. (1976) *Int. J. Biochem.* **7,** 117–118.
25. Radomski, M. W., Palmer, R. M. J. & Moncada, S. (1987) *Br. J. Pharmacol.* **92,** 181–187.
26. Lugnier, C., Schoefter, P., Le Bec, A., Strouthou, E. & Stoclet, J. C. (1986) *Biochem. Pharmacol.* **35,** 1743–1751.
27. Ruppert, D. & Weithmann, K. U. (1982) *Life Sci.* **31,** 2037–2043.
28. Radomski, M. W., Palmer, R. M. J. & Moncada, S. (1987) *Br. J. Pharmacol.* **92,** 639–646.
29. Brune, B. & Lapetina, E. G. (1989) *J. Biol. Chem.* **264,** 8455–8458.
30. Ware, J. A., Johnson, P. C., Smith, M. & Salzman, E. W. (1986) *J. Clin. Invest.* **77,** 878–886.
31. Palacios, M., Knowles, R. G., Palmer, R. M. & Moncada, S. (1989) *Biochem. Biophys. Res. Commun.* **165,** 803–809.
32. Marletta, M. A., Yoon, P. S., Iyengar, R., Leaf, C. D. & Wishnok, J. S. (1988) *Biochemistry* **27,** 8706–8711.
33. McCall, T., Palmer, R. M. J., Boughton-Smith, N., Whittle, B. J. R. & Moncada, S. (1990) in *Nitric Oxide from L-Arginine: A Bioregulatory System*, eds. Moncada, S. & Higgs, E. A. (Elsevier, Amsterdam), in press.
34. Tayeh, M. A. & Marletta, M. A. (1989) *J. Biol. Chem.* **264,** 19654–19658.
35. Kwon, N. S., Nathan, C. F. & Stuehr, D. J. (1989) *J. Biol. Chem.* **264,** 20496–20501.
36. Glass, R. E., Goode, A. W., Houghton, B. J. & Rowell, L. W. (1986) *Gut* **27,** 844–848.
37. Matsumoto, M., Kishikawa, H. & Mori, A. (1976) *Biochem. Med.* **16,** 1–8.