CHAPTER 35

The Role of Cyclic Nucleotides in Platelets

D. C. B. MILLS

Overview

Cyclic AMP in blood platelets is regulated by several endogenous factors and can also be influenced by drugs. Elevation of platelet cAMP, either by inhibition of phosphodiesterase or by stimulation of adenylate cyclase, leads to the suppression of their responsiveness to stimulation, and their participation in haemostatic and thrombotic processes is reduced.

Platelet adenylate cyclase is regulated by specific receptors for prostaglandins I_2 and D_2, for adenosine ("P"- and "R"-type receptors) and for ADP and catecholamines, (α and β). PGI_2, PGE_1, PGD_2 adenosine and β-adrenergic agonists stimulate adenylate cyclase activity; ADP and α-adrenergic agonists inhibit the enzyme. Both receptor-mediated stimulation and inhibition are influenced by intracellular guanine nucleotides. The enzyme is also stimulated by histamine, cholera toxin and fluoride ion. Adenosine, acting on an intracellular "P" receptor, proaggregatory prostaglandins and endoperoxides inhibit the enzyme.

Platelets have three cyclic nucleotide phosphodiesterases, one relatively specific for cAMP (FI), one for cGMP (FIII) and a nonspecific, low affinity enzyme (FII). They differ in their physical characteristics, and in their susceptibility to inhibition by a variety of drugs.

Two cAMP-dependent protein kinases (type I and type II) have been detected, and an increase in platelet cAMP is associated with the phosphorylation of two membrane proteins, and with an increased ability of membrane vesicles to accumulate calcium. Many of the effects of cAMP may be mediated by the consequent reduction in intracellular free calcium ions.

Platelets have a powerful guanylate cyclase which, in contrast to the adenylate cyclase, is not membrane bound. The cGMP level in platelets is rapidly elevated during aggregation, and also when guanylate cyclase is stimulated by a variety of drugs, including inhibitors of aggregation (azide, nitroprusside), aggregating agents (fatty acids, calcium ionophore) and some compounds having no detectable effect on aggregation (ascorbic acid).

Elevation of cGMP level is a consequence rather than a cause of aggregation, and the function of this nucleotide remains a mystery.

Abnormalities of cAMP metabolism have been recognised in some conditions, Bartter's syndrome, essential thrombocythemia and acute thrombosis, in which platelet haemostatic function is abnormal, as well as others in which no functional abnormally has been detected. It is possible that the adenylate cyclase system of platelets may have a useful role as an indicator of events occurring in the brain or in other inaccessible organs.

J. W. Kebabian et al. (eds.), *Cyclic Nucleotides*

A. Introduction

I. Natural History of Platelets

The basic biochemistry, physiology and morphology of the blood platelet have been reviewed by HOLMSEN et al. (1979), MARCUS and ZUCKER (1965), MUSTARD and PACKHAM (1970), WHITE (1979) and by ZUCKER (1980). Platelets circulate as small cells, lacking a nucleus, that are formed by the splitting off of cytoplasmic fragments from their parent cell, the megakaryocyte. Human blood contains from 150,000–450,000 platelets per microliter, or $0.7–2.5 \times 10^{12}$ in the total blood volume. The average volume of a human platelet is 7fl, so the total volume of the circulating platelets is between 5 and 20 ml/70 kg. The platelet survives in the blood stream for 5–10 days, until it is removed by the reticuloendothelial system. The main function of platelets is to provide the bulk of the haemostatic plug, the major initial defense against bleeding from injured blood vessels, particularly those in the middle range of size. Formation of the haemostatic plug is accomplished by the platelet's ability to undergo an extremely rapid transition from its normal, mutually repellent state to a condition in which it adheres avidly to other platelets and also to other surfaces, and by the catalytic effect that activated platelets have on the plasma coagulation mechanism.

II. Aggregation and Secretion

The aggregation of platelets can be induced by a large number of physiological stimuli, among which some of the more important are thrombin, ADP and collagen fibers. Other physiological aggregating agents, reviewed by MILLS and MACFARLANE (1976), include catecholamines, antigen/antibody complexes, fatty acids, prostaglandin endoperoxides and thromboxanes, serotonin, vasopressin and also PAF ("platelet activating factor", PAF-acether, 1-0-alkyl-2-acetyl glycerol-3-phosphoryl choline), a novel phospholipid produced by stimulated leukocytes (DEMOPOULOS et al. 1980) and by platelets (VARGAFTIG et al. 1981). Dog platelets are aggregated by cholinergic agents acting on a muscarinic receptor, and pig platelets are aggregated by prostaglandin E_2. Aggregation can also be induced by unphysiological agents including flouride ion, triethyl tin, methyl mercury and phorbol myrisate acetate. Aggregation is regarded as an active process for the platelet, and is inhibited by disruption of energy metabolism, by simultaneous blockade of glycolysis and respiration. A distinct process, agglutination, that does not require the metabolic machinery of the cell, occurs in the presence of coagulation factor VIII and the antibiotic, ristocetin.

The platelets themselves, when stimulated to aggregate, can release aggregating agents. These agents are either formed *de novo* (i.e. thromboxane A_2) or released from specific storage organelles (i.e. ADP). In human platelets, ADP is stored along with 5-hydroxytryptamine and calcium ion in the "dense bodies" which closely resemble enterochromaffin- and adrenal medullary chromaffin granules. The platelets may also secrete lysosomal enzymes, and a variety of factors, mostly protein in nature, that are stored together in organelles known as alpha-granules. These factors include proteins that are immunologically specific to the platelet