SYNTHETIC POLYCATIONS AS ACTIVATORS OF THE ENDOGENOUS MECHANISM OF PLATELET AGGREGATION

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Synthetic nitrogenous cationic polymers possess the ability to aggregate platelets in citrated plasma in a concentration of 1.5-10 μg/ml. The action of polycations on platelets leading to their aggregation is brought about, if the cells are in a functionally active state, through activation of the endogenous aggregation mechanism.

KEY WORDS: platelet aggregation; synthetic polycations.

Evidence that platelets contain an endogenous mechanism, stimulation of which leads to cellular contraction, a change in the shape of the platelets, their aggregation, and a liberation reaction has recently been obtained [9, 13, 16-18]. It is assumed that the biogenic activators of this process (ADP, thrombin, collagen, catecholamines, serotonin) cause the intracellular liberation of calcium ions, which mediate the ATP-dependent effector mechanisms of the cell [4, 6]. The synthetic and natural polycations – basic polypeptides (polylysine, protamine, Trasylol), ionenes (Polybrene), DEAE-dextran, etc. – constitute a new class of stimulators of platelet aggregation [3, 5, 7, 8, 10-12, 14-16]. These compounds possess high membrane activity and a varied biological action. It is not clear, however, whether or not polycations induce platelet aggregation by activation of the endogenous mechanism or whether their action consists essentially of a physicochemical process of flocculation of the cells.

The object of this investigation was to shed light on this problem.

EXPERIMENTAL METHOD

The aggregating action of 20 water-soluble synthetic nitrogen-, sulfur-, and phosphorus-containing polymers of different structure on platelets was studied in experiments in vitro. Platelet-rich plasma was obtained from arterial blood of dogs anesthetized with pentobarbital sodium (25 mg/kg), stabilized with 3.8% sodium citrate solution. Aggregation of the platelets was recorded by a turbidimetric method [1, 2]. The ability of the compounds to cause aggregation was studied by comparison with ADP on their addition to platelet-rich plasma or to platelets washed once and suspended in Owren–Koller medinal buffer (pH 7.3). To analyze the mechanism of action of the polymers, ATP ($8 \cdot 10^{-5}$ M), aspirin (1 mg/ml), potassium cyanide (1 mg/ml), and EDTA (0.2 mg/ml) were used.

EXPERIMENTAL RESULTS

The experiments showed that some polymers of the strongly polybasic type are able to aggregate platelets in citrated plasma in concentrations of 1.5-10 μg/ml, which are comparable with the aggregating concentrations of ADP. At the same time, most of the polybases studied exhibited no such activity or their action was detectable only in doses of about 10 μg/ml. Characteristically, only the nitrogen-containing polycations possessed aggregating properties, whereas polysulfonium and polyphosphonium salts had no such action in

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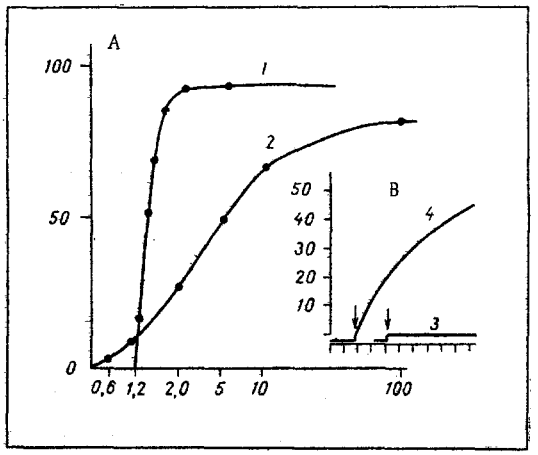

Fig. 1. Aggregation of platelets under the influence of PMP and ADP in citrated and EDTA plasma. A) Dose–effect curves of PMP (1) and ADP (2) in citrated platelet-rich plasma. Abscissa, concentrations of aggregation inducers, μg/ml; ordinate, aggregation, %; B) action of PMP on platelets in EDTA plasma before (3) and after (4) addition of calcium chloride in concentration of 0.5 mg/ml. Abscissa, time of aggregation, min; ordinate, aggregation, %. Arrows indicate times of addition of PMP.

concentrations not exceeding 100 μg/ml. Among the most active compounds were polymethylene piperidine (PMP), its quaternary N-diethyl analog, the aminoethyl ester of methacrylic acid, and polyvinylacetatepyridine-chloride, with a molecular weight of over 50,000.

Aggregation of platelets suspended in plasma produced by polycations differs significantly in its kinetics and character of its dose–effect curve from the process induced by ADP. For instance, over a wide range of concentrations (from 100 to 5 μg/ml), polycations produced maximal aggregation and a decrease in dose led only to an increase in the latent period of the reaction, the duration of which could vary from a few seconds to 3 min or more. If the aggregation had begun, as a rule it continued until the end at the same rate as in higher concentrations of the polymer. Only within a narrow range of concentrations, for example, between 2.05 and 1.42 μg/ml for PMP, was a gradual dependence found between dose and aggregating effect. The corresponding switch from minimal to maximal degree of platelet aggregation for ADP required a tenfold increase in the concentration of the inducer in the medium (Fig. 1A).

The process of platelet aggregation induced by polycations, unlike that of ADP aggregation, is not inhibited by ATP and aspirin. Meanwhile EDTA and potassium cyanide blocked the action of both polycations and ADP (Table 1). The addition of calcium chloride to EDTA plasma in a dose of 0.5 mg/ml, which did not cause spontaneous fibrin formation or platelet aggregation, restored the effect of the polymer (Fig. 1B). Polycations differed from ADP by inducing aggregation of washed platelets suspended in Owren–Koller buffer. In this case the aggregating effect of the polycations was distinctly dependent on dose, and the dynamics of the aggregation process, unlike that of platelets suspended in plasma, was biphasic in character: After the first wave of decrease in optical density, recorded after 15-20 sec, the velocity of the process began to decrease, and this was followed by a second wave of aggregation (Fig. 2).

It can be concluded from the analysis of these data that the action of polycations on platelets, leading to their aggregation, is exerted on cells in a functionally active state through the activation of the endogenous mechanism of aggregation. This conclusion is supported by evidence of the existence of a latent period of aggregation, which depends on the dose of the polymer, the sensitivity of the process to cyanide, an inhibitor of cell respiration, and requiring calcium ions. This conclusion is also confirmed by the fact that the dynamics of polycation-induced aggregation of platelets in Owren–Koller buffer is biphasic in character, just as during the action of biogenic inducers of aggregation (ADP, collagen, thrombin) on platelets in plasma. The appearance of the second wave of aggregation of platelets suspended in plasma is known to be due to the formation of endogenous aggregation factors, especially ADP, or of one of the intermediate products of prostaglandin E_2 synthesis, namely cyclic endoperoxide [18], and their liberation from the platelets. Meanwhile, the action of polycations on platelets is evidently not connected with these agents, for neither ATP (an inhibitor of ADP aggrega-

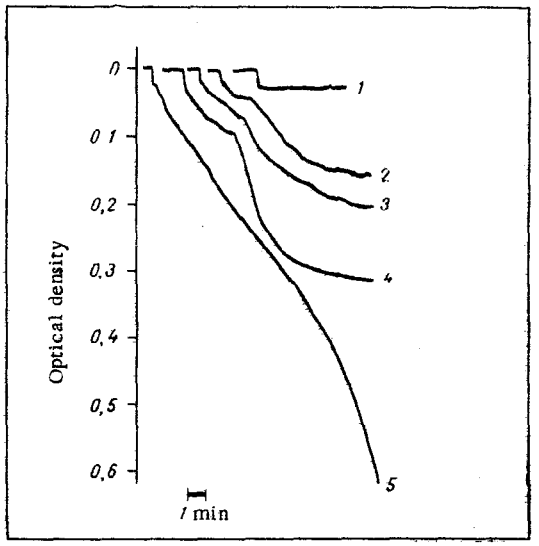

Fig. 2. Aggregation of platelets suspended in Owren–Koller buffer (pH 7.3) as a function of PMP concentration (in g/ml). Concentration of PMP, g/ml: 1) $0.31 \cdot 10^{-5}$; 2) $0.62 \cdot 10^{-5}$; 3) $1.25 \cdot 10^{-5}$; 4) $2.5 \cdot 10^{-5}$; 5) $1.0 \cdot 10^{-4}$.

TABLE 1. Effect of ATP, Aspirin, and Potassium Cyanide on Platelet Aggregation Induced by PMP and ADP

Aggregation inducer	Concentration, μg/ml	Aggregation (in %) in medium containing			
		0.9% NaCl solution	ATP	aspirin	KCN
PMP	3,1	88	86	90	30
ADP	10,0	87	22	40	8

tion) nor aspirin (an inhibitor of prostaglandin synthesis) blocks the action of polycations. The mechanism of aggregation of platelets by polycations can probably be reduced to their ability to stimulate intracellular liberation of calcium ions, which play the role of endogenous activators of aggregation. This property of the polycations is due not only to the presence of multiple positive charges in the macromolecule, but also to its structural features. This latter fact may perhaps explain why some polybases, notably DEAE-dextran, protamine sulfate, polylysine, and Polybrene, aggregate platelets in EDTA plasma and in the presence of inhibitors of cell respiration [3, 5, 12].

Synthetic polycations can thus be regarded as activators of physiological processes in secretory cells, in which category platelets are known to belong.

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