necrosis to the zone of ischemia, i.e., the value which was most stable and least dependent on fluctuations in the size of the zone of ischemia, was calculated as a percentage. Investigations conducted by the method described above showed that the zone of necrosis, 1 h after ligation of the coronary artery, was 25 ± 3.7% of the zone of ischemia, and after 2 and 4 h it was 41 ± 4.5 and 68 ± 4.3% respectively. Intravenous injection of propranolol (1 mg/kg) 12 min and 2.5 h after creation of the infarct caused a decrease in size of the zone of necrosis, which was 52 ± 2.7% of the area of ischemia ($p < 0.05$) 4 h after occlusion of the coronary artery. These figures are close to the results obtained by other workers who conducted similar investigations [4].

The differential indicator method of determining the dimensions of the zones of ischemia and necrosis in myocardial infarction is easily reproducible, accurate, and sufficiently informative and has high throughput capacity, thus making it promising for use in experimental cardiac pharmacology.

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DEPENDENCE OF PLATELET AGGREGATION RESPONSE ON ARGINYL-GLYCYL-ASPARAGINE TRIPEPTIDE

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Fibronectin (FN) is the term given to a class of structurally and immunologically related polyfunctional high-molecular-weight glycoproteins, which are involved in various functions of cells, including adhesion of cells, formation of the extracellular matrix, and changes in the cytoskeleton, cell mobility, phagocytosis, differentiation, and neoplastic transformation [6]. The role of the blood plasma FN in functions of the reticuloendothelial system, in regenerative processes, and in the mechanism of hemostasis, of which the key components are platelet aggregation and the formation of the primary platelet plug, has frequently been described [2, 3, 5, 9]. For the platelet aggregation response (PAR) to develop, not only is an inducer (aggregant) necessary [8], but so also are bivalent cations (Ca^{++}) and protein cofactors, which may include fibrinogen, FN, and Willebrandt's factor [1].

This paper describes a study of the action of exogenously added FN on PAR and also the effect of binding of endogenous FN with specific antibodies (AB), and the action of the synthetic tripeptide arginyl-glycyl-asparagine (AGA), which is an amino-acid sequence found in the region (domain) of the FN molecule which interacts with the cell receptors of platelets and other cells [7], on PAR.

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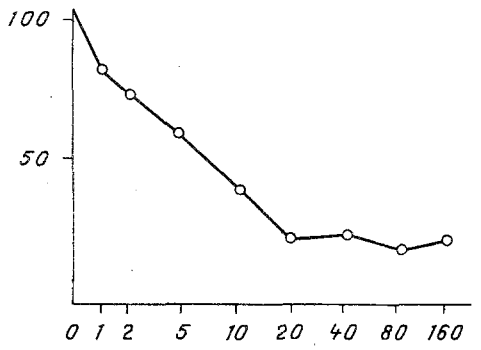

Fig. 1. Dependence of level of PAR (induced by 10 μM ADP) in PEP on quantity of AB to FN added. Abscissa, concentration of AB to FN (in μg/ml); ordinate, level of platelet aggregation (in %).

EXPERIMENTAL METHOD

PAR was recorded in platelet-enriched plasma (PEP) and in a suspension of washed platelets (SWP) on an ELVI-840 two-channel aggregometer (Italy). PEP was obtained from freshly prepared citrated blood from healthy donors (1 volume of 3.8% sodium citrate solution to 9 volumes of donated blood) by centrifugation at 160g for 15 min. Platelet-deprived plasma (for adjusting the instrument) was prepared from PEP by sedimenting the platelets at 1500g for 10 min. SWP was obtained from PEP, layered on an equal volume of Ficoli–Paque (Pharmacia, Sweden) and centrifuged at 290g for 30 min. The isolated platelets were added to 1 ml of washing buffer [136.75 mM NaCl, 2.68 mM KCl, 11.90 mM $NaHCO_3$, 0.42 mM NaH_2PO_4, 12.00 mM citric acid, and 5.50 mM dextrose (Serva, West Germany), pH 6.5] and recentrifuged for 15 min at 400g. The sedimented cells were washed twice with the same buffer and centrifuged for 10 min at 360g. The washed platelets were resuspended in working buffer (136.75 nM NaCl, 2.63 mM KCl, 11.90 mM $NaHCO_3$, 0.42 mM NaH_2PO_4, pH 7.4), making allowance for the fact that 50% of the cells are lost in the course of isolation. Commercial solutions were used as stimulators of PAR: ADP (100 μM), adrenalin (50 μM), and collagen (1 mg/ml), and also lyophilized thrombin (Kaunas, USSR), a working solution of which (5 U/ml) was prepared in phosphate-buffered saline (PBS), pH 7.4 (Serva). Standard solutions from the kit marketed by Boehringer (West Germany) for turbidimetric determination of the FN concentration in biological fluids served as the FN preparations. AB and FN were obtained from Calbiochem (USA), and the synthetic tripeptide AGA from the Laboratory of Peptide Synthesis (Head, Professor M. I. Titov), All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. All dilutions were prepared in PBS (pH 7.4). PAR was induced in a suspension of platelets (450 μ) in constant-temperature aggregometer cuvettes with constant mixing (1000 rpm), by the addition of 50 μl of a solution of the aggregation inducer.

EXPERIMENTAL RESULTS

In the course of the experiments the optimal doses of PAR stimulators were found to be as follows (final concentrations of aggregants in the cuvette): 1) for platelet aggregation in PEP: 10 μM ADP, 5 μM adrenalin, 100 μg/ml collagen, 0.5 U/ml thrombin: 2) for aggregation in SWP: 5 μM ADP (with 1 μM Ca^{++} and 1 mg/ml fibrinogen), 0.05 U/ml thrombin, 100 μg/ml collagen.

Incubation (1-2 min) of PEP with specific AB to FN in a dose of 10 μg/ml led to marked (on average by 40-50%) inhibition of ADP-induced PAR. The degree of platelet aggregation was found to depend precisely on quantity of added AB to FN (Fig. 1).

Although according to data in the literature [10], FN in higher concentrations inhibits platelet aggregation induced in vitro, we found that FN itself, without the addition of any other aggregant, stimulates the development of PAR in SWP and in PEP, diluted 1:4 with PBS (Fig. 2). The experimental data are evidence that the presence of free functionally active FN in the incubation medium is absolutely necessary for development of the normal PAR.

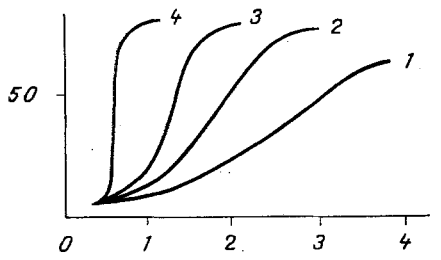

Fig. 2. PAR induced by FN in SWP. Concentration of FN added: 1-4) 3, 7, 14, and 28 μg/ml respectively. Abscissa, time (in min); ordinate, level of aggregation (in %).

TABLE 1. Inhibitory Action of AB to FN and Synthetic Tripeptide AGA on PAR in PEP

Inducer of aggregation (concentration)	Inhibitor of aggregation			
	C	AB to FN (5 μg/ml)	C	AGA (1 mg/ml)
ADP (5 μM)	65	22 (67)	51	12 (76)
Adrenalin (2.5 μM)	63	58 (8)	48	11 (77)
Thrombin (0.5 U/ml)	69	5 (93)	64	2 (97)
Collagen (5 μg/ml)	75	13 (83)	60	6 (90)

Legend. Mean level of aggregation shown (in %): C) control aggregation (induced by the given inducer without addition of inhibitor); degree of inhibition PAR by action of inhibitor shown (in %) in parentheses.

A study of the effect of the tripeptide AGA on PAR showed that this amino-acid sequence (in a dose of 0.5 to 1 mg/ml) significantly inhibits platelet aggregation in PEP and SWP, stimulated by various inducers. Table 1 gives the results of a comparative study of the inhibitory action of the tripeptide AGA and of AB to FN on PAR in PEP.

It can thus be concluded that AGA, which is a common amino-acid sequence of a number of adhesive proteins [4], such as fibrinogen, FN, collagen, Willebrandt's factor, thrombin, etc., not only inhibits their binding with the surface of platelets [7], and thereby reduces platelet adhesion to various substrates, but also inhibits platelet aggregation, in all probability for the same reason. This is evidence of the need for protein aggregation cofactors (including FN) to participate in this process.

The tripeptide AGA can be used in the future to prepare surfaces to which adhesion of adherent cells is undesirable (for example, surgical vascular prostheses, etc.), and also to inhibit excessive connective tissue formation during wound healing.

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PLATELET ACTIVATING FACTOR AND ENDOTOXIN-INDUCED PLATELET ACTIVATION

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KEY WORDS: endotoxin-induced platelet activation; platelet aggregation

The possibility that platelet activating factor (PAF) participates in endotoxin-induced platelet activation has virtually not been studied. Yet its generation and release from activated leukocytes and zones of inflammation suggest that the presence of PAF in the blood may play a definite role in platelet activation in the thrombohemmorrhagic syndrome, which accompanies infectious diseases. Meanwhile the endotoxinemia in infectious pathology creates special conditions for activation of the blood cells, for it has been shown that endotoxins are inducers of aggregation and secretion of platelets [1, 2, 3]. The high sensitivity of platelets to PAF [7] and to endotoxins [4] may contribute to the fact that during their interaction subthreshold concentrations, which themselves do not give rise to any significant activation of cells, become sufficient to stimulate cell functions. Interaction of this kind between PAF and arachidonic acid and between PAF and collagen has been demonstrated experimentally [6, 7].

To elucidate the role of PAF in endotoxin-induced platelet aggregation, experiments were carried out in vitro in which endotoxin-induced platelet aggregation was recorded before and after incubation of platelets with PAF and also during exposure to the combined action of PAF and endotoxin.

EXPERIMENTAL METHOD

Experiments were carried out on platelet-enriched plasma (PEP) obtained from blood donors. Blood stabilized with 3.8% sodium citrate solution was centrifuged for 10 min at 1500 rpm. The number of platelets in 1 μl of plasma was counted and their number adjusted to 250,000-300,000 /μl with platelet-deprived plasma. The aggregating capacity of the platelets [5] was recorded by means of an aggregometer (made by the experimental workshops of the Academy of Medical Sciences of the USSR). ADP (10^{-3} %), PAF (in concentrations of 10^{-14} to 10^{-6} M), menigococcal B lipopolysaccharide (LPS) (from 0.1 to 1 μg/ml), and *Salmonella typhimurium* LPS (10-20 μg/ml) were used to induce aggregation. The meningococcal LPS was obtained in the Laboratory of the Central Research Institute of Epidemiology, Ministry of Health of the USSR, the *S. typhimurium* LPS from "Sigma" (USA), and the PAF from "Serva" (West Germany). Addition of the aggregation inducers to PEP in different orders was carried out with continuous mixing and recording of the aggregation process, so that the effect of each of them and also the possibility of their interaction, on platelet activity could be studied. In control experiments, instead of aggregation inducers, corresponding volume of physiological saline was added. The degree of irreversible ADP-induced platelet aggregation served as the control value (100% aggregation). On the addition of one of the inducers, the aggregation process was recorded until the curve flattened out on a plateau, and only when this had occurred was the next added.

EXPERIMENTAL RESULTS

According to data obtained by several workers [6-8], PAF potentiates the action of other inducers (ADP, thrombin, collagen) in weak concentrations, but in high concentrations it does not affect the response of the cells to these inducers.

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