EFFECT OF HIGH CONCENTRATIONS OF GLUCOSE ON PLATELET AGGREGATION

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The effect of high concentrations of glucose (0.01-0.1 M) on ADP-induced platelet aggregation was investigated in adult rabbits. In these concentrations glucose significantly inhibited the aggregation of intact platelets highly sensitive to ADP, but did not change the aggregation of refractory cells, i.e., those preincubated with ADP. Replacing glucose by deoxyglucose gave similar results. It is postulated that the effect observed may be linked with the the conversion of the platelets into a refractory state during incubation with glucose or deoxyglucose.

KEY WORDS: platelets – aggregation; adenosine diphosphate; glucose.

It has recently been shown that platelets are actively metabolizing cells obtaining their energy by both respiratory and glycolytic phosphorylation [5, 9, 15, 18]. All the main types of functional activity of platelets (aggregation [13], absorption of serotonin [6], clot retraction [15], transmembrane K^+ transport [10], and the liberation reaction [11]) require the expenditure of energy. Inhibition of metabolism [12] or a deficiency of substrates [17] leads to the partial or total loss of functional activity by these cells. In particular, low concentrations of glucose (10^{-3} M) have been shown to prevent the loss of the ability of washed platelets to aggregate and to take part in the liberation reaction under the influence of ADP, thrombin, collagen, or antigen–antibody complexes [7, 13, 14]. Similar results have been obtained in experiments with dialysis of thrombocytic plasma [17]. On the other hand, evidence has been obtained of the inhibitory action of glucose on aggregation and other functions of platelets [16].

The object of the present investigation was to study the effect of glucose in high concentrations (10^{-2}-10^{-1} M) on the aggregation of rabbit platelets in plasma in vitro.

EXPERIMENTAL METHOD

Platelet-enriched plasma was obtained from rabbits by the usual way [2]. Aggregation was measured by a nephelometric method at 18.5°C. Glucose and deoxyglucose were dissolved in modified (not containing Ca^{++} and Mg^{++}) Tyrode's solution and added to platelet-deprived plasma in the ratio of 1:8. In control experiments the plasma was treated with the corresponding volume of modified Tyrode's solution.

EXPERIMENTAL RESULTS AND DISCUSSION

Previous investigations [1, 2] showed that preincubation with glucose can strongly inhibit the ADP-induced aggregation of platelets. Aggregation curves after treatment with various doses of ADP are given in Fig. 1: control curves on the left, curves obtained after preincubation of the platelets with glucose in a final concentration of 0.1 M for 5 min on the right. As Fig. 1A shows, in the presence of glucose the aggregation of the platelets was about halved. The addition of glucose to platelet-enriched plasma, in which

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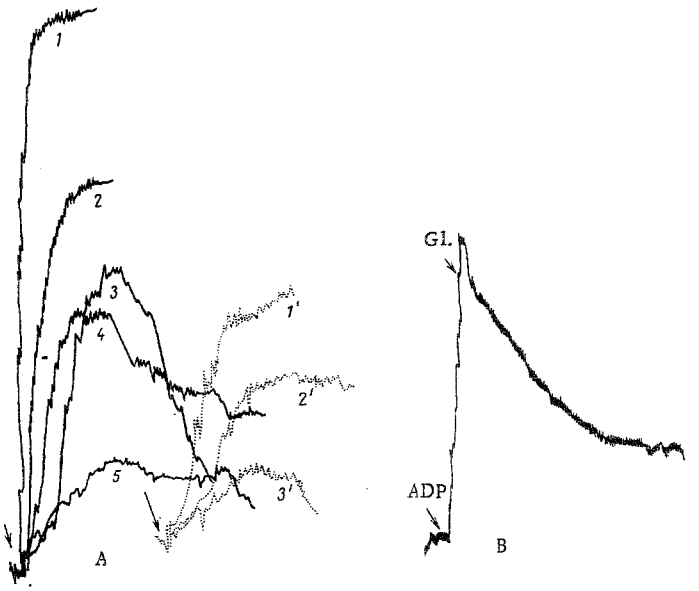


Fig. 1. Inhibitory effect of glucose on ADP-induced platelet aggregation: A) decrease in degree of platelet aggregation after incubation for 5 min with glucose in a final concentration of 0.1 M. Final ADP concentrations without glucose: 1) $2 \cdot 10^{-6}$ M, 2) $3 \cdot 10^{-6}$ M, 3) $4 \cdot 10^{-7}$ M, 4) $2 \cdot 10^{-7}$ M, 5) $1 \cdot 10^{-7}$ M, in the presence of glucose: 1') $2 \cdot 10^{-6}$ M, 2') $4 \cdot 10^{-7}$ M, and 3') $2 \cdot 10^{-7}$ M. B) Outset of disaggregation on the addition of glucose solution to platelet-enriched plasma. Arrows indicate times of addition of ADP(final concentration $2 \cdot 10^{-7}$ M) and glucose (final concentration 0.1 M).

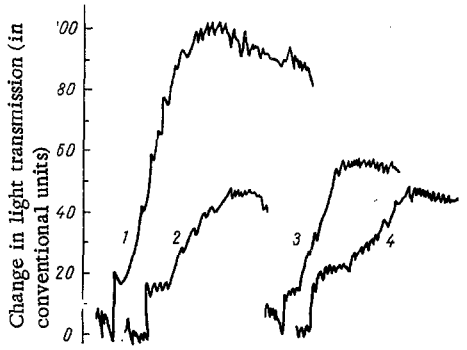
ADP-induced aggregation takes place, often led to the cessation of aggregation and to disaggregation of the platelets (Fig. 1B). The maximal inhibitory action of glucose was observed when its concentration in the plasma was 0.05-0.1 M, but sometimes a considerable effect could be seen in a concentration of 0.01 M.

It was shown previously [1] that platelets most sensitive to ADP are at the same time most sensitive to glucose. During prolonged incubation of platelet-enriched plasma at a raised temperature parallel decreases are observed in the aggregating power of the platelets and their sensitivity of the inhibitory action of glucose. The low aggregating power of the platelets in this case is evidently explained by their refractoriness as a result of the liberation of the intracellular ADP into the plasma. The writers accordingly postulated that refractory platelets, i.e., those preincubated with ADP, lose their sensitivity to the inhibitory action of glucose on aggregation. To test this hypothesis the action of glucose was compared on ADP-induced aggregation of intact platelets (Fig. 2, curves 1 and 2) and of refractory platelets obtained as a result of incubation with ADP for 5 min (Fig. 2, curves 3 and 4). Just as was postulated, the sensitivity of the platelets refractory to the inhibitory action of glucose was much lower than the sensitivity of the intact cells.

The substance 2-deoxyglucose, a competitive inhibitor of glycolysis, also had an inhibitory effect on ADP-induced platelet aggregation. The simultaneous presence of glucose and deoxyglucose in the plasma potentiated this effect (Fig. 3).

The phenomenon of inhibition of ADP-induced platelet aggregation by glucose thus observed could be the result of an increase in the energy production of the cells through an increase in the level of the principal substrate of platelet metabolism [2, 19]. However, this hypothesis is contradicted by several facts. The original glucose level in rabbit plasma is sufficiently high [3], so that the limitation of the rate of glycolysis by the substrate concentration is unlikely. Moreover, 2-deoxyglucose, a competitive inhibitor of glycolysis [4], not only did not reduce the inhibitory action of glucose on aggregation, but in isomolar concentrations it had the same effect itself.

On entering the platelets, deoxyglucose like glucose undergoes hexokinase phosphorylation, coupled with the dephosphorylation of ATP to ADP. The product of this reaction, deoxyglucose phosphate, ac-



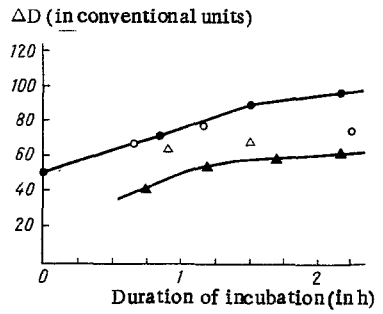


Fig. 2

Fig. 3

Fig. 2. Action of glucose (final concentration 0.06 M) on aggregation of intact and refractory platelets induced by ADP ($4 \cdot 10^{-7}$ M): 1) control; 2) incubation for 5 min with glucose (0.06 M); 3) incubation for 5 min with ADP ($2 \cdot 10^{-7}$ M) and glucose (0.06 M); 4) incubation for 5 min with ADP ($2 \cdot 10^{-7}$ M).

Fig. 3. Aggregation power of platelets as a function of duration of their incubation at 22°C in the control and in the presence of glucose and (or) deoxyglucose. Empty circles represent glucose (0.06 M); open triangles – deoxyglucose (0.06 M); filled triangles – deoxyglucose (0.06 M) and glucose (0.06 M); filled circles – control.

cumulates in the platelets rapidly, so that after incubation for 5 min it reaches a constant level several hundreds of times higher than the normal glucose phosphate level in these cells. At the same time the level of intracellular ATP falls to two-thirds of its initial value and the corresponding amount of ADP is formed [8].

The following hypothesis can be put forward on the basis of data in the literature and the results of the present experiments. Incubation with high concentrations of glucose and deoxyglucose, increasing the intracellular ADP level, may induce a refractory state in the platelets similar to that arising during their incubation with exogeneous ADP. This hypothesis is supported by the reduced sensitivity to glucose of platelets preincubated with ADP.

On the one hand, therefore, during exhaustion of the substrate, incubation with low concentrations of glucose (0.001-0.01 M) restores the decreased aggregating power and other functions of the platelets to their initial level [7, 13, 14], whereas on the other hand, incubation of intact platelets with high concentrations of glucose (0.01-0.1 M) sharply reduces their power of aggregation. These two phenomena evidently differ in nature. Whereas the first is connected with an increase in the energy supply of the cells in the presence of the exogenous substrate of metabolism, the second evidently cannot be explained in this way. The hypothesis that the hexokinase reaction plays an important role in the mechanism of the inhibitory action of high concentrations of glucose on ADP-induced platelet aggregation is in accordance with the available experimental data, but further confirmation is necessary.

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