ACTION OF KCN ON INTACT AND REFRACTORY PLATELETS

I. L. Lisovskaya, R. I. Volkova,
E. Ya. Pozin, and R. A. Markosyan

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The action of potassium cyanide on ADP-induced aggregation of intact and refractory platelets was investigated. Cyanide ($5 \cdot 10^{-4}$ M) had virtually no effect on the aggregation of intact cells but stimulated aggregation of refractory platelets. The stimulating effect of the inhibitor on aggregation was not connected with liberation of further quantities of ADP from the cells. Differences in the sensitivity of the aggregating power of intact and refractory cells to partial depression of metabolism is discussed in terms of the "calcium" model of autoregulation of platelet aggregation suggested previously.

KEY WORDS: *Platelets; aggregation; refractoriness; ADP; cyanide.*

The ability of ADP to lower the sensitivity of platelets to aggregating agents has attracted considerable attention [10, 15]. So far, however, no general agreement has been reached regarding the nature and biological significance of refractoriness. The writers showed previously that, besides reduced ability to undergo ADP-induced aggregation, refractory platelets also differ from intact in their lower sensitivity to the deaggregating action of glucose and a lower rate of decrease of their aggregating power in the presence of additional doses of ADP [1, 3]. In accordance with the model suggested by the writers previously [4], the special characteristics of refractory platelets can be explained by an increase in the level of local ATP concentrations close to the active centers of the ATPases of the endoplasmic reticulum (the "calcium pump"), as a result of which the normal rate of pumping of intracellular calcium from the cytoplasm is reached and exceeded [14].

In this connection it is interesting to study how the aggregating power of intact and refractory platelets depends on the state of their metabolism.

EXPERIMENTAL METHOD

Platelet aggregation was recorded by a nephelometric method at room temperature [6]. Platelet-enriched plasma obtained by the standard method was used for the investigation [4]. KCN was dissolved in physiological saline. Refractory cells were obtained by preincubating the platelet-enriched plasma with ADP without mixing. Platelets were washed by Walsh's method [17] with certain modifications. The ATP concentration in platelet-enriched plasma and in the suspension of washed platelets was determined by a bioluminescence method [9] on a special apparatus [5].

EXPERIMENTAL RESULTS

The transition of the platelets into the refractory state was manifested, in particular, by a successive decrease in the degree of aggregation following repeated addition of equal doses of ADP to the plasma (Fig. 1). On the addition of potassium cyanide in a final concentration of $5 \cdot 10^{-4}$ M, further addition of ADP to the platelet-enriched plasma in the course of deaggregation led to aggregation, the degree of which was virtually the same as initially. KCN thus restores (at least partially) the sensitivity of platelets to ADP when depressed during interaction with ADP.

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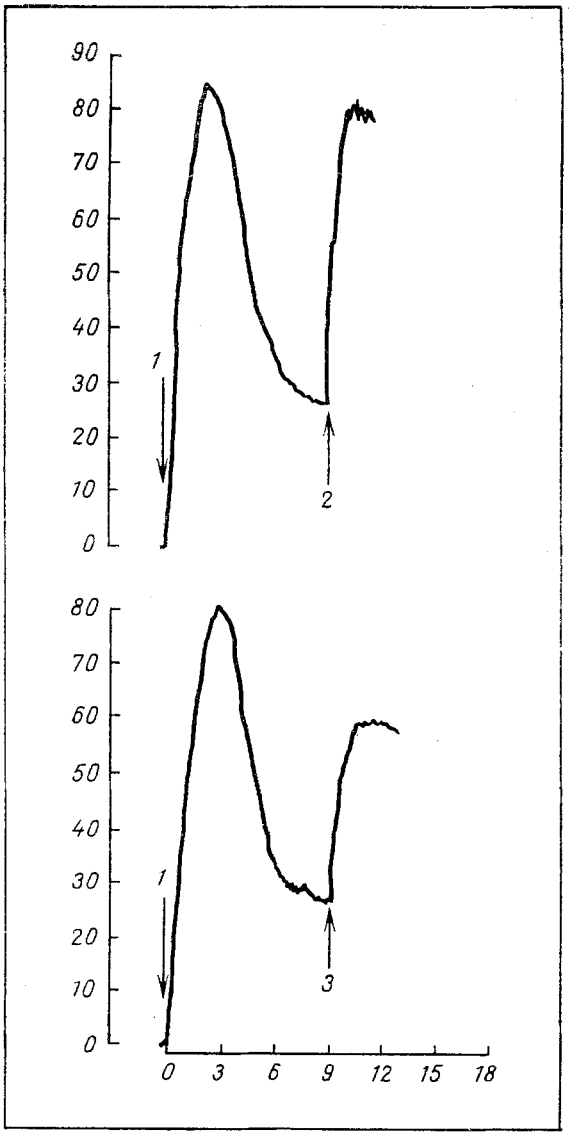

Fig. 1. Course of aggregation of platelets during repeated addition of equal doses of ADP to platelet-enriched plasma. To 0.7 ml platelet-enriched plasma 0.1 ml of ADP solution in a concentration of $2 \cdot 10^{-6}$ M was added; 9 min later a further 0.1 ml of the same ADP solution was added simultaneously with KCN solution (final concentration $5 \cdot 10^{-4}$ M) (above) or with Tyrode solution (below). Abscissa, time (in min); ordinate, ΔD (in conventional units). Arrows indicate: 1) time of addition of ADP; 2) of ADP + KCN; 3) of ADP + Tyrode solution.

When the ADP-induced aggregation of intact platelets and platelets preincubated with ADP (i.e., refractory) was investigated it was found that incubation with KCN, in a concentration of $5 \cdot 10^{-4}$ M, which does not affect the aggregating power of intact cells, caused a marked increase in aggregation of the refractory platelets (Fig. 2). The degree of increase in aggregation by cyanide depended on the depth of refractoriness: the lower the sensitivity of the refractory cells to ADP, the more marked the increase in aggregation in the presence of cyanide. During prolonged incubation the ADP in the plasma was destroyed and the platelets reverted to their original (nonrefractory) state, insensitive to the aggregation-stimulation action of KCN.

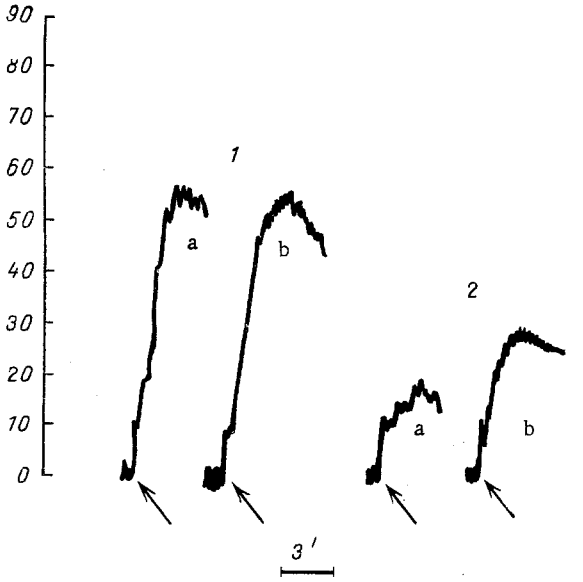

Fig. 2. Effect of KCN on ADP-induced aggregation of intact (1) and refractory (2) platelets. Refractory cells obtained by preincubation with ADP in a final concentration of $2 \cdot 10^{-6}$ M for 14 min. KCN ($5 \cdot 10^{-4}$ M) incubated with samples of platelet-enriched plasma for 3 min (b). In control (a) KCN replaced by physiological saline. Aggregation of intact and refractory platelets induced by adding ADP solution in a concentration of $9 \cdot 10^{-7}$ M in a ratio of 1:10. Mixing began simultaneously. Ordinate, here and in Fig. 3: ΔD (in conventional units).

If added to platelet-enriched plasma or a suspension of washed platelets at the stage of complete aggregation or the beginning of deaggregation, KCN quickly induced marked additional aggregation of the cells (Fig. 3a, b). Cyanide was also able to induce aggregation of platelets which had completely lost their power of aggregation as a result of preincubation with ADP (Fig. 3c).

It was postulated that the stimulation of platelet aggregation observed in the presence of KCN was the result of the appearance of further quantities of ADP in the plasma or suspension. To test this hypothesis, control samples of platelet-enriched plasma or of platelet suspension, or samples preincubated in the presence of inhibitor were fixed with EDTA in a final concentration of 13 mM immediately after the end of recording aggregation, and their content of extracellular ATP was determined by a bioluminescence method. On incubation of the platelets with KCN the appearance of appreciable quantities of adenine nucleotides in the plasma was not observed, although the bioluminescence method used could record as little as 2×10^{-7} M ATP under the experimental conditions. The liberation reaction was thus evidently not the direct cause of the increase in platelet aggregation produced by the action of KCN.

Data in the literature on the action of KCN on ADP-induced platelet aggregation are contradictory [2, 8, 11, 16, 20]. The results of the present experiments indicate that this could be due to differences in the initial state of the platelets. According to the observations of Hampton et al. [7], the action of KCN on the electrophoretic mobility of platelets is also manifested only if the cells examined are refractory.

The stimulating effect of KCN could be connected with the inhibition of oxidative phosphorylation, which makes a substantial contribution to the energy balance of platelets. There are indications that hydrolysis of the ATP formed during oxidative phosphorylation can play a special role in the energy supply for certain functions (for example, the liberation reaction) of platelets [13].

During the simultaneous and total inhibition of glycolysis and oxidative phosphorylation platelets lose their power of aggregation, adhesion, the liberation reaction, and so on [11,

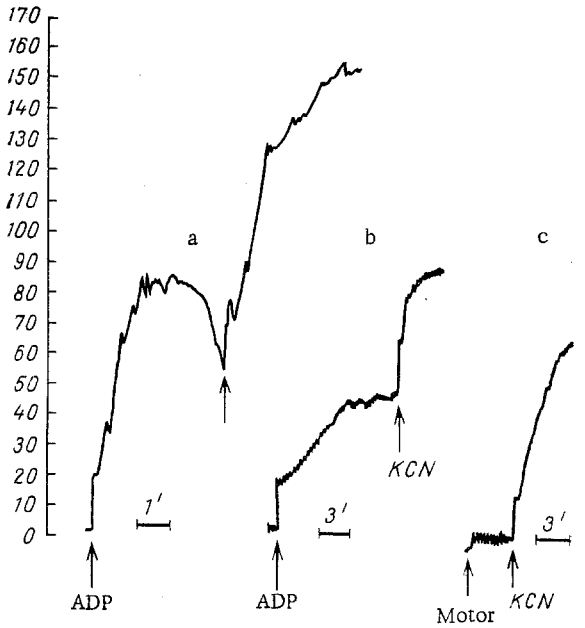

Fig. 3. Stimulation of additional aggregation of platelets in platelet-enriched plasma or suspension of platelets by cyanide. KCN (final concentration $5 \cdot 10^{-4}$ M) added: a) to platelet-enriched plasma at deaggregation stage; b) to platelet suspension after reaching maximal aggregation; c) to platelet-enriched plasma in which, as a result of prolonged preincubation with ADP, no aggregation was observed during mixing.

12]. However, partial depression of synthesis of metabolically active ATP can lead to an increase in the aggregating power of some cells [18].

The different behavior of intact and refractory platelets in the presence of cyanide can be explained from the standpoint of the writers' model of autoregulation of the aggregating power of platelets [4]. The metabolic activity of platelets is evidently high enough to provide for the functions of the intact cells even in the presence of an inhibitor, i.e., under conditions of partial depression of metabolism. However, under those circumstances the platelets may be unable to maintain the increased level of local ATP concentrations close to the pump ATPases characteristic of refractory platelets. This would lead to delay in the pumping of intracellular calcium outward by the endoplasmic reticulum, to stimulation of contractile structures and, correspondingly, to increased aggregation of refractory platelets, as was observed experimentally. On the other hand, the possibility that the effect of cyanide in stimulating aggregation may be due to its direct action on the cell membranes of the platelets [19] cannot be ruled out.

Whatever the mechanism of the action of cyanide on platelet aggregation, the difference in the sensitivity of intact and refractory platelets to cyanide can be used as an additional criterion for the evaluation of the functional state of platelets.

LITERATURE CITED

1. R. I. Volkova, S. A. Koroleva, I. L. Lisovskaya, et al., Fiziol. Zh. SSSR, No. 2, 218 (1974).
2. K. M. Lakin, V. A. Fel'dbaum, and A. A. Lebedeva, in: Problems in Neurohumoral Regulation of the Blood Clotting Process under Normal and Pathological Conditions [in Russian], Chita (1971), pp. 83-88.
3. I. L. Lisovskaya, R. I. Volkova, and R. A. Markosyan, Byull. Éksp. Biol. Med., No. 10, 24 (1974).
4. I. L. Lisovskaya, A. A. Markosyan, and R. I. Volkova, Fiziol. Zh. SSSR, No. 5, 789 (1973).

5. E. Ya. Pozin, in: Morpho-Functional Peculiarities of the Growing Organism [in Russian], Moscow (1974), p. 90.
6. G. V. R. Born and M. Hume, Nature, 215, 1027 (1967).
7. I. R. Hampton, D. G. Nicolas, and J. R. A. Mitchell, Thromb. Diath. Haemorrh., 28, 419 (1972).
8. M. J. G. Harrison, P. R. Emmons, and J. R. A. Mitchell, Thromb. Diath. Haemorrh., 16, 130 (1966).
9. H. Holmsen, I. Holmsen, and A. Bernhardsen, Anal. Biochem., 18, 456 (1966).
10. H. Holmsen and M. C. Rosenberg, Acta. Med. Scand., Suppl. No. 525, 113 (1970).
11. S. Karpatkin, J. Clin. Invest., 46, 406 (1967).
12. R. L. Kinlough-Rathbone, M. A. Packham, and J. F. Mustard, J. Lab. Clin. Med., 75, 780 (1967).
13. S. Matsui, Y. Watanabe, and B. Kobayashi, Thromb. Diath. Haemorrh., 32, 441 (1974).
14. E. H. Mürer, Biochim. Biophys. Acta, 222, 197 (1970).
15. J. R. O'Brien, M. Etherington, and S. Jamieson, Lancet, 2, 741 (1971).
16. E. W. Salzman, in: Physiology of Hemostasis and Thrombosis (Symposium), (ed. by S. A. Johnson et al.), Springfield (1967), pp. 140-151.
17. P. N. Walsh, Brit. J. Haematol., 22, 205 (1972).
18. D. E. Wooley and P. C. T. Jones, J. Cell. Physiol., 76, 191 (1970).
19. P. D. Zeive and H. M. Solomon, J. Clin. Invest., 45, 1251 (1966).
20. M. B. Zucker and Z. Jerushalmy, in: Physiology of Hemostasis and Thrombosis (Symposium), ed. by S. A. Johnson et al., Springfield (1967), pp. 249-263.

IODINE METABOLISM IN THE THYROID GLAND IN CHRONIC URANIUM POISONING

A. F. Malenchenko, V. V. Seregin, and T. S. Kuz'mina

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Metabolism of radioactive and stable iodine in the thyroid gland and blood of rats with chronic uranium poisoning was studied for 21 days after administration of ^{131}I. In uranium poisoning the number of iodide-transport loci in the gland and the size of the intrathyroid iodine pool are reduced and the concentration of stable iodine in the thyroid tissue is lowered. The compensatory reaction of the gland takes the form of an increase in its mass and in the rate of thyroid metabolism.

KEY WORDS: *Thyroid gland; uranium; iodine.*

When it enters the body, uranium disturbs thyroid function [1, 3-5]. However, the mechanisms of this effect have not been explained.

In this investigation the kinetics of metabolism of radioactive and stable iodine in the thyroid tissue and blood was studied at widely spaced time intervals during chronic uranium poisoning.

EXPERIMENTAL METHOD

Experiments were carried out on 80 Wistar rats weighing 170-220 g. The animals were given drinking water containing 0.05% (group 1) and 0.5% (group 2) uranium nitrate for 2 months. The experiments with the animals of group 1 were carried out in September and October, and those with group 2 in November and December 1974-1975. A single dose of 20 μCi

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