INCORPORATION OF ^{35}S-HEPARIN INTO RAT MAST CELLS AND ITS RELEASE INTO THE BLOOD STREAM

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Despite the extensive clinical use of heparin many aspects of its storage, site of synthesis, and processes of release remain unexplained. In the opinion of most investigators, heparin is released by exocytosis from the mast cells, its principal producers. However, there is no general agreement regarding the role of heparin in mast cells in the regulation of hemostasis. For instance, as well as the data published by Hatanaka [8] and Kitamura [11], explaining the tendency toward thrombus formation in W/W^V mice by their inability to produce heparin as a result of a mast cell deficiency, Marcum [14] is doubtful about the role of the heparin of mast cells in the maintenance of the anticoagulant potential of the blood.

In previous publications the writers showed that release of heparin from the mast cells is a component of the anticoagulant effect arising during excitation of the anticlotting system by thrombin. This is shown by the high degree of correlation between the morphometric data on the secretory status of the mast cells and biochemical blood analyses, reflecting the anticoagulant activity of heparin [4].

Information in the literature showing that, besides secreting heparin, the mast cells can also take up and store heparin [2] is of particular interest.

In the light of these facts, the following question must be asked: Can mast cells function as it were "in both directions" – secrete heparin when the anticoagulant potential of the blood is raised, and assimilate heparin when it is present to excess in the blood stream? In the present investigation this problem was studied with the use of ^{35}S-labeled heparin. By this approach it was possible to obtain a direct answer to the question whether mast cells do in fact secrete the heparin which they accumulated from the blood stream in the case of hypercoagulation induced by injection of thrombin.

EXPERIMENTAL METHOD

Heparin (activity 130 U/mg) was obtained from "Sigma" (USA), ^{35}S-heparin from "Amersham" (England), and α-thrombin was obtained by the method in [5] and had a specific activity of 2000 NIH units/mg protein. Experiments were carried out on noninbred male albino rats weighing 180-200 g. The preparations were injected into the jugular vein in a volume of 1 ml. ^{35}S-heparin (1 μCi, biological activity 1.5 U) was injected together with unlabeled heparin in an amount so that the final dose of the mixture was 15 U/200 g. To determine heparin clearance, blood samples were taken 1, 10, 20, 30, 60, 120, and 240 min after injection of the label. The concentration of ^{35}S-heparin in the subcutaneous cellular tissue, kidney and liver tissue, and in the mast cells from peritoneal fluid was determined during the first 60 min of the experiment. The animals were given an injection of thrombin in a dose of 8 NIH units/200 g 30 and 60 min after the label. Animals of the control group received equal volume of physiological saline. The reaction of thrombin was studied 7 min after its injection. Samples of blood, mast cells, and the test tissues were treated by the method in [3] and the radioactivity of the samples was determined on a "Mark III" counter ("Nuclear Chicago," USA). Specific radioactivity of blood, mast cell, and tissue samples was calculated relative to radioactivity of the blood 1 min after injection of ^{35}S-heparin, which was taken as 100%.

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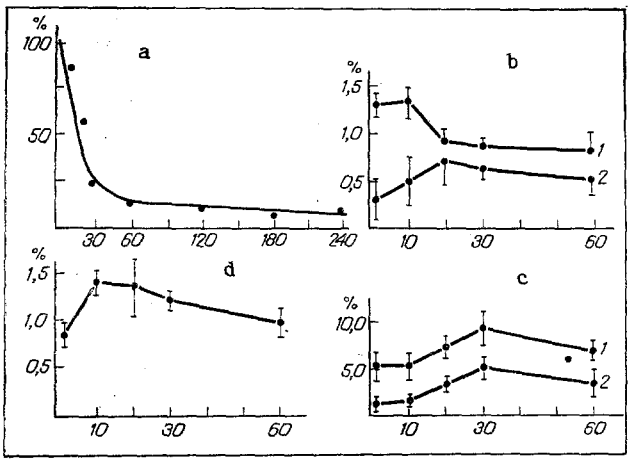

Fig. 1. Changes in specific radioactivity of blood, mast cells, and tissues after injection of ^{35}S-heparin. Radioactivity of blood 1 min after injection of ^{35}S-heparin taken as 100%. Abscissa, time (in min); ordinate, specific radioactivity. a) Blood, b) subcutaneous cellular tissue (1) and mast cells (2), c) kidneys (1) and liver (2), d) vessel wall.

The radioactivity of the blood was calculated per ml, and that of the tissues per 100 mg. At the end of the experiment the presence of label in the heparin fraction of the blood was verified, this fraction being isolated by the method described previously. In some cases (indicated in the text) the specific radioactivity of the tissues also was calculated relative to radioactivity of the blood at the time when the samples were taken, this being taken as 100%. The specific radioactivity of samples of mast cells from peritoneal fluid was calculated per 10^7 cells. Samples of mast cells and tissues were taken immediately after sacrifice of the animals. Mast cells were obtained from peritoneal fluid by the method in [12].

EXPERIMENTAL RESULTS

Considering that heparin isolated in vivo is distributed among many compartments, the experimental animals were injected with ^{35}S-heparin together with unlabeled heparin in a total dose of 15 U/200 g body weight, this amount of heparin being considered to be sufficient for their saturation.

In accordance with the aim of the investigation, the first step was to examine how quickly the injected heparin is removed from the blood. Data characterizing clearance of ^{35}S-heparin in the blood over a period of 240 min after its injection are given in Table 1. As will be clear from Fig. 1a, the specific radioactivity of the blood fell rapidly during the first 30 min after injection of the label (by 79%), and thereafter it fell more slowly – after 60 min there remained 11% of the injected radioactivity, but only 3.7% after 240 min. These data on the high intensity of the initial stages of heparin clearance are in agreement with data in the literature [1, 10].

Since our discovery that heparin from the blood stream enters the mast cells, special attention was paid to samples of mast cells from the peritoneal fluid and samples of subcutaneous cellular tissues, which is the tissue richest in mast cells [9]. It was found that only 1 min after injection of the label, ^{35}S-heparin was present in these samples, their specific radioactivity being 0.27 and 1.28% respectively. During the next 30 min, against the background of intensive clearance of the heparin in the blood, changes observed in the specific radioactivity of these samples were not significant, and until the 60th minute their radioactivity remained virtually constant (Fig. 1b).

Considering the sharp decrease in the intensity of clearance of heparin in the blood toward the 60th minute after injection of the label and the relative stability of the ^{35}S-heparin concentration in the tissues tested until the same time, we considered that the best time to inject thrombin was 60 min after injection of the ^{35}S-heparin. It is evident that changes in specific radioactivity of the blood due to release of ^{35}S-heparin in response to

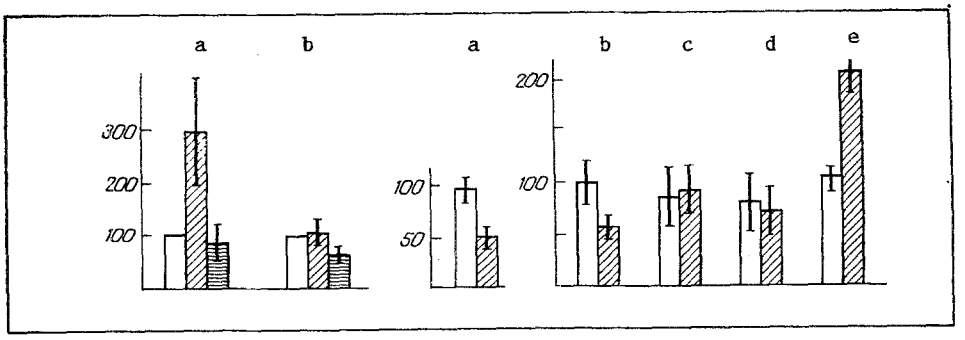

Fig. 2

Fig. 3

Fig. 2. Changes in radioactivity of blood after injection of thrombin and of physiological saline. a) Injection 60 min after injection of ^{35}S-heparin, b) 30 min after injection. Oblique shading – injection of thrombin, horizontal shading – injection of physiological saline. Radioactivity of blood before injection of preparations (unshaded columns) taken as 100%.

Fig. 3. Radioactivity of mast cells and tissues when thrombin and physiological saline were injected 60 min after injection of ^{35}S-heparin. a) Mast cells; b) subcutaneous cellular tissue; c) liver; d) kidney; e) vessel wall. Unshaded columns – injection of physiological saline, shaded – injection of thrombin. Radioactivity of mast cells (per 10^7 cells) and tissues (per 100 mg weight) before injection of preparations taken as 100%.

the injection of thrombin cannot be detected before this time. Meanwhile, as the tests showed, the label remained bound to the heparin fraction of the blood. Having chosen this time to inject thrombin, for comparison we injected thrombin into another group of animals 30 min after the injection of ^{35}S-heparin.

The results of the series of experiments in which radioactivity of the blood was measured in animals receiving thrombin (8 NIH units) 30 and 60 min after injection of ^{35}S-heparin, and of control animals receiving an injection of an equal volume of physiological saline, are given in Fig. 2.

These data show that the radioactivity of the blood rose almost threefold ($p < 0.05$) during 7 min in the experimental animals receiving thrombin 60 min after injection of ^{35}S-heparin (Fig. 2a), whereas in the control animals it fell by 14.8%. Considering the fall in radioactivity of the blood in the control animals, due to heparin clearance, it can be tentatively suggested that the real increase in radioactivity of the blood after injection of thrombin was greater still. This effect was virtually absent when thrombin was injected 30 min after the label (Fig. 2b), and this can evidently be explained by the still considerable saturation of the blood at this time with exogenous heparin and the intensive course of its clearance. The writers showed previously that heparin blocks the ability of thrombin to intereact with vessel wall receptors and to activate the function of the anticlotting system [6].

The increase in radioactivity of the blood discovered when thrombin was injected 60 min after the radioactive label is evidence that in this case the ^{35}S-heparin accumulated by the tissues was released. In fact, the radioactivity of the mast cells from peritoneal fluid and samples of subcutaneous cellular tissue fell significantly under these circumstances. For instance, whereas the concentration of ^{35}S-heparin (per 10^7 cells) in samples of mast cells from peritoneal fluid after the control injection of physiological saline was virtually unchanged, in the corresponding samples after injection of thrombin it fell by 52.2% ($p < 0.001$; Fig. 3a).

The concentration of ^{35}S-heparin in the subcutaneous cellular tissue, calculated per 100 mg of tissue, also fell after injection of thrombin to reach 55.5% of its initial level ($p > 0.2$; Fig. 3b). The fact that this fall was not significant can probably be explained by the wide scatter of the sample. If the specific radioactivity of samples of subcutaneous cellular tissue was calculated relative to radioactivity of the blood at a given moment of time (67 min after injection of the label), in animals receiving thrombin it was $13.1 \pm 5.1\%$,

whereas in those receiving physiological saline it was 45.9 ± 9.3% ($p < 0.01$). There is thus every reason to suppose that in response to injection of thrombin, ^{35}S-heparin is released from the mast cells of subcutaneous cellular tissue also.

The radioactivity of the mast cells and subcutaneous cellular tissue was virtually identical when thrombin was injected 30 min after the label in both experimental and control animals, i.e., release of ^{35}S-heparin from these tissues was not observed. This correlates fully with the character of the change in specific radioactivity of the blood when thrombin was injected at this time (Fig. 2b).

So far as samples of liver and kidney tissue, which contain mast cells in their parenchyma, are concerned the specific radioactivity after injection of the label was an order of magnitude higher throughout the period of observation than samples of subcutaneous cellular tissue and mast cells from peritoneal fluid (Fig. 1c), and this is probably due to their role in metabolic and excretory processes. No significant changes in specific radioactivity of liver and kidney samples were observed throughout the period of observation, and they did not respond by release of ^{35}S-heparin to injection of thrombin (Fig. 3c, d).

The question whether heparin accumulated by the vessel wall can be released in response to injection of thrombin is particularly interesting. The dynamics of uptake of ^{35}S-heparin by the vessel wall is shown in Fig. 1d. These data show that during the first 10 min accumulation of ^{35}S-heparin proceeded more rapidly than in the other tissues, the difference being close to statistical significance ($p < 0.02$), evidently because of the ability of the vessel wall to bind heparin [13]. After injection of thrombin the amount of ^{35}S-heparin bound by the vessel wall increased considerably (Fig. 3e). This increase correlated with an increase in the specific radioactivity of the blood after injection of thrombin, and is evidence of the ability of the endothelium to bind ^{35}S-heparin additionally. Different points of view are expressed in the literature about the character of this binding, which can be explained both by the polydispersed nature of heparin and by its interaction with the wall of different parts of the vascular bed. Barzu and co-workers [7], who studied binding of labeled heparin with endothelial cells of the umbilical vein in culture, showed that the binding sites of these cells are not specific heparin receptors. Moreover, nearly all the anticoagulant activity of heparin (antifactor Xa and antithrombin) is neutralized after binding with the endothelium. Besides the low-affinity binding of heparin with endothelial cells in vitro (K_d = 10^{-6} M) the presence of relatively high-affinity binding also has been demonstrated (K_d = 10^{-7} M).

The results thus confirm the view that heparin of the mast-cell population is involved in the regulation of hemostasis, but that the mast cells themselves are not only to produce heparin, but also to store it and to release it in response to activation of the anticlotting system. Heparin bound with endothelium, however, evidently makes its own contribution to protection against thrombus formation by supporting the nonthrombogenic properties of the vessel wall.

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