PHYSIOLOGY

SIGNIFICANCE OF HEPARIN IN THE PROCESS OF BLOOD COAGULATION

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Heparin is one of the most important anticoagulants of blood. The use of heparin as an anticoagulant presents many advantages over other preparations; it is readily soluble in water and physiological saline, its action is virtually instantaneous (peak effect after 5 minutes), it is rapidly eliminated from the organism, and it does not increase the osmotic pressure of blood. Yet, although heparin is so widely applied, the mechanism of its effect on blood clotting has not been adequately studied. Heparin is usually regarded as a physiological anticoagulant, which is always present in the blood, and which inhibits intravascular clotting by binding one [7, 11, 13, 14, 16] or more [9, 10] of the components of the blood clotting system.

The present paper is devoted to the study of the reaction between heparin and thrombotropin, which was shown by B. A. Kudryashov [4] in 1948 to be a new component of this system. Thrombotropin is the initiator of the process of blood clotting, its action on prothrombokinase being the first step of this process. The effect of heparin on this important stage of blood coagulation has not heretofore been investigated.

We also investigated the reactions of heparin with blood calcium, prothrombokinase, and thrombokinase; very diverse views as to the nature of these reactions have been reported, and highly contradictory opinions have been expressed by different authors.

EXPERIMENTAL METHODS AND RESULTS

We used the following methods: determination of blood prothrombin activity [5], of thrombotropin [4], of blood calcium [de Waard (3)], clotting time of normal and heparinized plasma (G. G. Bazazyan's method) after addition of thrombin. In a number of experiments we used plasma which had been treated with $Ca_3(PO_4)_2$, to remove prothrombin [15] and thrombotropin [1].

Heparin and thrombotropin: we studied the mutual relations of heparin and thrombotropin in blood clotting systems, in vivo, using albino rats, and in vitro, with thrombotropin preparations.

We examined the effect of thrombotropin on the activity of heparin added to $Ca_3(PO_4)_2$-treated rat plasma to which thrombin had been added to promote clotting. These experiments were repeated with fibrinogen solutions instead of plasma. Thrombotropin was added to the plasma or fibrinogen solutions simultaneously with 0.5% heparin.

The activity of heparin fell in the presence of physiological levels of thrombotropin activity; this was shown by a shortening of clotting time of thrombin-activated plasma, from 25 to 17.8 seconds. Thrombotropin alone had no effect on the clotting time of plasma after addition of thrombin.

Only active thrombotropin exerted any effect on the activity of heparin. If the thrombotropin was inactivated by heating at 48° for 5 minutes it did not lower the activity of heparin, and even somewhat lengthened the clotting time of plasma treated with thrombin.

The same effects were observed when 0.5% heparin and thrombotropin solutions were injected simultaneously

into the jugular vein of rats; active thrombotropin shortened the clotting time of heparinized plasma treated with thrombin, from 30.8 to 20.3 seconds, while inactivated thrombotropin somewhat lengthened clotting time, from 30.8 to 35.9 seconds (30.8 seconds is the mean clotting time found for thrombin-induced clotting of plasma from control rats injected with 0.5% heparin solution and physiological saline).

The effect of 0.5% heparin solution on the thrombotropin activity of rat plasma was studied in vitro and in vivo. The in vivo experiments, performed on 33 rats, showed a mean thrombotropin activity of 94.3% before injection of heparin, and of 25.1% after. The same volume of 0.85% NaCl was given to a group of 12 control rats, instead of heparin solution, and in this group thrombotropin activity fell very slightly, from 96.5 to 85.5%, i.e., it remained within the normal range.

It thus appears that heparin causes a marked fall in thrombotropin activity. The same effect was seen in experiments in which 0.5% heparin was added to rat plasma in vitro. Similar results were obtained when fibrinogen solutions were taken instead of plasma.

Effect of Prothrombokinase and Thrombokinase on the Activity of Heparin

The effect of heparin and thrombokinase taken together was studied separately for thrombokinase itself and for its precursor prothrombokinase. Prothrombokinase was activated by means of plasma thrombotropin, using B. A. Kudryashov's method [1].

Heparin was dissolved in prothrombokinase or thrombokinase solutions, to give a concentration of 0.5%, and 0.1 ml of the resulting solutions was added to 0.9 ml of $Ca_3(PO_4)_2$ -treated rat plasma.

Clotting of 0.2 ml portions of the mixtures was effected by adding an equal volume of thrombin solution, and clotting time was recorded. As a control, we added the same volume of heparin solution dissolved in 0.85% NaCl, instead of in prothrombokinase or thrombokinase solutions, to plasma.

It was found that prothrombokinase or thrombokinase somewhat lowered the activity of heparin, shortening the clotting time from the control value of 15 sec. to 9-10 sec.

There was no significant difference between the effects of prothrombokinase and thrombokinase.

Addition to the plasma of prothrombokinase or thrombokinase alone had no effect on the clotting time.

We used prothrombokinase prepared from rat brain, thromboplastin, and a preparation of prothrombokinase not contaminated with other proteins, called Hemostasin. Hemostasin was somewhat more active than thrombokinase or prothrombokinase prepared from rat brain.

Heparin and Calcium

In the first series of experiments we examined the effect of heparin on the concentration of calcium salts in rat blood, both in vivo and in vitro, while in the second series we examined the effect of adding calcium chloride on the activity of heparin.

In the first series of in vitro experiments blood was taken from the jugular vein of rats, into a syringe containing 0.1 ml of 0.5% heparin solution, and it was then centrifuged, and the calcium content of the heparinized plasma was determined. The mean value found was 7.62 mg %. Blood had been taken previously from the same rats, with 0.85% NaCl in the syringe instead of heparin solution, and the calcium content of the serum was found to be 7.32 mg %.

It follows that the calcium content of blood is unaffected by heparin in vitro.

The same result was obtained in experiments in which heparin was injected into a vein, in various amounts, and blood samples were taken after injection.

We examined the effect of calcium chloride on the activity of heparin, using whole oxalated rat plasma, $Ca_3(PO_4)_2$ -treated rat plasma, and fibrinogen solutions. We hoped in this way to avoid the contradictory results obtained by workers using one type of experimental material only – plasma or fibrinogen.

For the in vitro experiments we added to 0.9 ml of plasma or fibrinogen 0.1 ml of 0.5% heparin solutions in 0.025 M calcium chloride, and clotting of the mixture was effected by means of thrombin solution. The control systems consisted of 0.9 ml of the same plasma or fibrinogen with 0.1 ml of heparin dissolved in 0.85% NaCl.

Our experiments showed, as did those of M. A. Ukolova (6), that calcium chloride causes considerable lowering of the activity of heparin (clotting time was shortened from 20.9 to 12.6 seconds for whole plasma, from 23.4 to 12.3 seconds for $Ca_3(PO_4)_2$-treated plasma, and from 12.0 to 6.7 seconds for fibrinogen solution). Addition of calcium chloride alone to the same plasma or fibrinogen solution had no effect on clotting time after addition of thrombin.

In the in vivo experiments heparin was injected, followed after 5 minutes by 0.025 M $CaCl_2$ solution. The same volume of physiological saline was in injected into animals of the control series. Blood samples were taken 5 minutes after injecting calcium chloride or saline solution, the plasma was separated by centrifugation, and was coagulated by means of thrombin. It was found that the clotting time of heparinized plasma was shortened from 78.6 seconds after saline injection to 29.2 seconds after calcium chloride injection. Injection of calcium chloride alone had no effect on the clotting time of thrombin-treated plasma.

DISCUSSION OF RESULTS

We conclude from our results that heparin and thrombotropin are antagonists. Addition to rat blood, in vivo or in vitro, of thrombotropin preparations, at levels close to the physiological ones, lowers the activity of heparin added to the same systems. This property of thrombotropin is abolished by thermal inactivations. Introduction of heparin into the blood lowers the activity of added thrombotropin. Heparin has no effect on the calcium content of blood.

Thrombotropin, prothrombokinase, thrombokinase, and calcium do not, by themselves, shorten the clotting time of oxalated rat plasma to which thrombin has been added. This confirms the findings of Conley (8) and Lyttleton (12) that free heparin is either absent from plasma, or present only in very insignificant amount; binding of free heparin by the above-named substances could cause significant shortening of clotting time of thrombin-treated plasma, as has been shown above.

It appears that calcium and active thrombotropin present in blood may exercise a sort of buffer action, preventing heparin from exerting its action in normal physiological states of the organism (i.e., when small amounts of heparin may be entering the blood stream).

The presence of large amounts of heparin in the blood in certain pathological states may be interpreted as a defense reaction of the organism, whereby heparin acts in the first place by binding plasma thrombotropin, so preventing intravascular clotting.

It may thus be concluded that heparin does not play any definitive part in the normal physiological process of blood clotting. In our opinion, the maintenance of the fluid state of blood is ascribable to the absence of thromobokinase from the plasma, but not to the inhibitory action of heparin on prothrombin or other blood constituents, as is generally supposed.

The basic physiological importance of heparin appears to be that it is secreted into the blood stream in large amounts when thrombogenic components appear, and could directly lead to intravascular clotting; heparin powerfully inhibits their activity.

Consideration should be given to the treatment of hyperheparinemia by administration of calcium chloride, which powerfully inhibits the activity of heparin. In using heparin therapeutically account should be taken not only of prothrombin, but also of thrombotropin, activity.

SUMMARY

The relationship of anticoagulant heparin was investigated with thrombotropin, prothrombokinase, thrombokinase and calcium.

It was demonstrated that heparin and thrombotropin are in antagonistic relationship.

Prothrombokinase and thromhokinase reduce the activity of heparin to the same extent.

Calcium chloride greatly decreases the activity of heparin introduced into the bloodstream.

Heparin introduced into the blood has no effect on the concentration of calcium salts in the blood.

The author considers that heparin exerts a powerful anticoagulant effect with compound action only in a pathological condition of the body when thrombogenic components which may directly cause the intravascular blood coagulation enter the bloodstream.

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