Biochemical Pharmacology, Vol. 18, pp. 1585–1591. Pergamon Press. 1969. Printed in Great Britain

RELEASE OF DIAMINE OXIDASE BY HEPARIN IN THE RAT

YUTAKA KOBAYASHI, JACOB KUPELIAN and DAVID V. MAUDSLEY

Worcester Foundation for Experimental Biology, Shrewsbury, Mass 01545, U.S.A.

(*Received* 5 *October* 1968; *accepted* 13 *December* 1968)

Abstract—Heparin produces a substantial rise in diamine oxidase activity in rat plasma. An increase in the plasma is evident within 10 min of an intravenous (i.v.) injection of heparin and a peak is observed between 30 and 60 min. The rise in the plasma can be accounted for by the release of diamine oxidase from the intestine, since the enzyme activity in this tissue is markedly reduced when the plasma level is at its peak. Only a slight increase in the plasma level is observed when heparin is given to eviscerated animals, suggesting that the contribution from tissues other than the intestine is small. As the rise in plasma activity is evident even with normal anticoagulant doses of heparin, care should be exercised in whole animal experiments when potential substrates for diamine oxidase, such as histamine and putrescine, are being studied.

DIAMINE oxidase is an oxidative deaminating enzyme widely distributed in many species and it metabolizes a variety of substrates such as histamine, putrescine and cadaverine.[1] Although the common identity of histaminase with diamine oxidase has not been established in all cases, for the purposes of the present work the two enzymes are assumed to be identical.

An increase in the level of blood histaminase has been observed in different animal species immediately after anaphylactic shock.[2–5] This increase is dependent on a release of the enzyme from tissues. In the guinea pig the liver is the most important source of the enzyme and its release seems mediated through the concomitant release of heparin.[6–8] Heparin has also been shown to release histaminase from the isolated perfused liver into the blood.[9] Recent reports[10–12] have shown that heparin causes a marked rise in plasma diamine oxidase in humans. The present investigation was carried out to determine the effect of heparin on the plasma diamine oxidase in the albino rat; a preliminary report has been published.[13]

METHODS

Diamine oxidase was determined by using a minor modification of the method of Okuyama and Kobayashi.[14] Briefly, the assay involves incubating plasma or tissue extract with ^{14}C-putrescine as the substrate. After stopping the reaction, the end product, Δ^1-pyrroline, is extracted directly into a toluene–PPO* counting solution and counted in a scintillation counter. The modification centers around the manner of stopping the reaction. In the original procedure the reaction was stopped by saturating the reaction mixture with sodium bicarbonate, but it was subsequently found that if the extraction was delayed the reaction continued. In the present method a mixture

* PPO = 2,5-diphenyloxazolyl.

of aminoguanidine sulphate (227 μg) and sodium bicarbonate (200 mg) is added to the reaction mixture. The presence of aminoguanidine prevents further enzyme action and does not interfere with the extraction of the reaction product. For the assay of intestine, 1 g tissue was homogenized in an Omnimixer in 24 ml of 0·1 M borate buffer, pH 7·8, and then centrifuged at 10,000 *g* for 30 min. The supernatant solution was decanted and diluted 1:1 with buffer to a final dilution of 1:50. Two ml of this solution was taken for analysis. For other tissues, preliminary experiments indicated that the diamine oxidase activity was low compared to the intestines. Accordingly, stomach, lung, liver, kidney and spleen were diluted to a final concentration of 1:10.

Heparin was injected intraperitoneally (i.p.) or intravenously (i.v.) through the jugular vein under ether anesthesia. Blood was withdrawn by heart puncture or draining through the vena cava under ether anesthesia. Both methods gave essentially the same results. Whole blood was spun down at 1000 *g* for 15 min and the plasma was used for assay.

Evisceration was carried out under ether anesthesia. The esophagus just above the cardiac sphincter, the mesenteric vessels, and the intestine just above the cecum were ligated prior to removal of the stomach and intestines. Control animals were treated similarly, but the vessels were not ligated nor were the intestines removed. All animals were allowed 1 hr of postoperative recovery before further treatment.

RESULTS

When a high dose of heparin (4000 U/kg) is given i.p., a marked rise in the plasma diamine oxidase activity is observed which reaches a peak around 3 hr followed by a rapid return to normal values by about 8 hr (Fig. 1). The peak represents an increase in activity of approximately 50-fold, although this may vary markedly in individual animals. When the heparin is given i.v., the peak occurs much earlier, at about 30 min, but the decline in enzyme activity follows essentially the same pattern as that

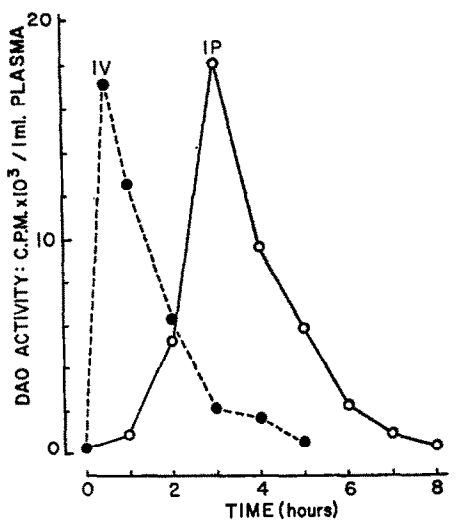

FIG. 1. Plasma diamine oxidase activity after an i.p. or i.v. injection of heparin (4000 U/kg). Each point represents the mean of three animals.

observed when the heparin is given i.p. (Fig. 1). The values obtained with an i.v. injection were more reproducible than with an i.p. injection, and in all subsequent experiments described in this paper intravenous injections were used throughout.

Figure 2 shows the effect of different doses of heparin on the diamine oxidase activity in the plasma. A linear dose–response curve was obtained between 400 and 4000 U/kg. Higher doses of heparin increase the plasma diamine oxidase still further, but the values are variable and the reproducibility between animals is not as good as with the lower doses. However, values as high as 400 times the control levels have occasionally been observed. Even the lowest dose of heparin used produced a 10-fold increase in diamine oxidase activity. The increase in the plasma could be detected 10 min after injection and is still detectable 3 hr after injection. In view of the levels of activity found in the plasma, the increase may be detectable earlier than 10 min, but this was not tested.

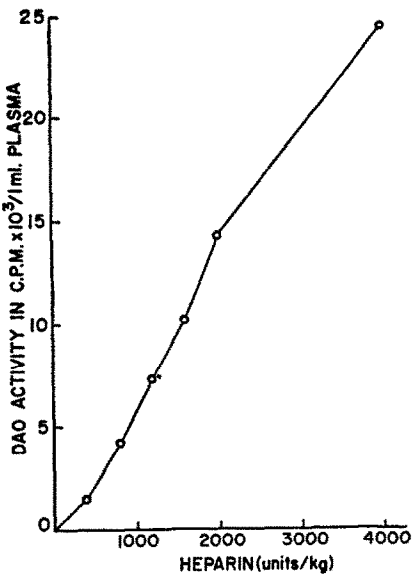

FIG. 2. Plasma diamine oxidase after different i.v. doses of heparin. Blood samples were obtained 30 min after injection. Each point is the mean of three animals.

In an attempt to determine the source of the diamine oxidase occuring in the plasma, a high dose of heparin (4000 U/kg) was given and the enzyme activity of several tissues was determined 60 min later. The results given in Table 1 show that in animals not given heparin the enzyme activity in the intestine is much higher than in any of the other tissues tested. This is in accord with previously published data showing that the intestine is the richest source of this enzyme in the rat.[15] Furthermore, the results also show that heparin significantly reduces the diamine oxidase in the intestine. The activity in the spleen is also reduced, but since the activity in tissues other than the intestine is comparatively low, the contribution to the plasma is probably small.

TABLE 1. DIAMINE OXIDASE ACTIVITY IN PLASMA (cpm/1·0 ml) AND TISSUES (cpm × 10/g) 1 hr AFTER AN I.V. DOSE OF HEPARIN (4000 U/kg)*

Tissue	Control	Heparin
Plasma	167	6180
Intestine	78, 570	32, 330
Stomach	10	30
Lung	28	28
Kidney	18	24
Spleen	115	30
Liver	85	95

* Each result represents the mean of three animals.

TABLE 2. DIAMINE OXIDASE ACTIVITY (cpm × 10^2/g) IN THE INTESTINE 15 min AFTER AN I.V. INJECTION OF HEPARIN (4000 U/kg)

Experiment No.	Control	Heparin
1	6547	2811
2	3914	2867
3	6507	2433
4	6925	2049
5	6279	2251
Mean ± S.D.	6034 ± 1208	2482 ± 353

TABLE 3. EFFECT OF EVISCERATION ON THE PLASMA DIAMINE OXIDASE (cpm/1·0 ml) RESPONSE TO HEPARIN (4000 U/kg)*

Experiment No.	Sham-operated	Eviscerated
1	13,143	105
2	8389	785
3	10,972	322
4	15,372	490
5	5100	314
6	14,323	256
Mean ± S.D.	11,217 ± 3901	379 ± 234

* Blood samples were withdrawn 30 min after injection.

To determine whether release of diamine oxidase from the intestine could account for the rapid rise in plasma activity, the intestinal diamine oxidase was measured 15 min after an i.v. dose of heparin. As shown in Table 2, even at this time there is a substantial reduction in diamine oxidase in the tissue. In order to detect the decline in intestinal diamine oxidase it is necessary to use large doses of heparin. Although

small doses of heparin produce a significant rise in the plasma, the amount released is only a small proportion of that present in the intestine and may therefore go undetected.

The results thus far suggest that the rise in plasma diamine oxidase is due primarily to a release of the enzyme from the intestine. This was tested further by observing the effect of evisceration 1 hr prior to an injection of heparin. At 30 min after a dose of 4000 U/kg of heparin, blood was withdrawn and estimated for diamine oxidase. Evisceration drastically reduced the rise in plasma diamine oxidase produced by heparin, as shown in Table 3, suggesting that the enzyme in the intestine is the main contributor to the raised plasma levels.

DISCUSSION

The release of diamine oxidase by heparin in the rat is similar to that which occurs in guinea pigs[8] and humans.[12] In the guinea pig the source of the enzyme is mainly the liver, but the enzyme is located in substantial quantities elsewhere. In humans it is obviously difficult to trace the source of the enzyme and the particular advantage of the rat is that the enzyme is located primarily in a single tissue, the intestine, which is relatively easy to study.

The release of diamine oxidase induced by heparin is very rapid and in many ways analogous to the effect of heparin on lipoprotein lipase.[16] In humans and cats, much of the diamine oxidase appearing in the plasma is transported by the lymph.[12, 17] While this may also be true in rats, it was not tested directly. Furthermore, the rapid appearance of substantial amounts of the enzyme in the plasma suggests that at least some of it may have a more direct access to the blood stream.

The mechanism of the release of diamine oxidase is obscure and the effect is not restricted to heparin. Other polyanions have been shown to release diamine oxidase in guinea pigs.[18] Under certain conditions, protamine, in doses sufficient to inhibit the anticoagulant activity of heparin, prevents the release of diamine oxidase in guinea pigs,[19] but in experiments which are still in progress we have not yet confirmed this in the rat.

The present study lends support to the suggestion that heparin may be the agent responsible for the raised plasma levels of diamine oxidase which occur during anaphylactic shock.[8] The concomitant release of histamine and heparin from mast cells and the subsequent release of diamine oxidase by heparin provide the basis of a simple regulatory control for limiting the deleterious effects of histamine, and heparin has been reported useful in the treatment of certain allergic conditions in man.[20] Nevertheless, the involvement of histamine as the prime causative agent in anaphylaxis in the rat is doubtful[21] and, since diamine oxidase attacks a variety of substrates, it may be unwise to assume that its function is simply to metabolize histamine. Recent and growing interest in the metabolism and functions of polyamines such as putrescine and their involvement in rapidly growing tissues opens up new areas of speculation.[22, 23] The procedure described here provides a convenient test system for manipulating diamine oxidase levels in the plasma and for studying the effect of these changes on the metabolism of substrates of the enzyme such as histamine and putrescine.

Another well documented condition in which the diamine oxidase is markedly changed is pregnancy and increased amounts of the enzyme have been reported in the

plasma of rats,[24] guinea pigs[25] and humans.[26] Pregnancy plasma is characterized by sustained high levels of diamine oxidase which decline rapidly after parturition. Diamine oxidase activity in the plasma after a single dose of heparin also declines rapidly, indicating that once released into the plasma the enzyme is short-lived. In conjunction with the raised tissue levels of diamine oxidase occuring in pregnancy, it seems reasonable to suppose that the sustained plasma levels of the enzyme during the latter part of gestation are indicative of a continuous release of the enzyme from the tissues. This, in turn, implies that the enzyme-releasing factor is either very potent or produced at a high rate. There is, however, little justification at the present time for suggesting that the agent responsible is heparin or a heparin-like substance.

A final point arising from the present study is that the rise in plasma diamine oxidase is evident even with doses of heparin which are normally used for anti-coagulant purposes in whole animal experiments. The resultant raised levels of diamine oxidase should, therefore, be considered when studying the effects of potential substrates for the enzyme, such as histamine. We have noticed, for example, that the gastric secretory response to histamine, using the continuous infusion method of Ghosh and Schild,[27] was markedly impaired when a high dose of heparin was inadvertently injected as an anticoagulant. Although this was originally thought to be due to complexing of histamine and heparin, in view of the present results it may be due to metabolism of histamine by the increased diamine oxidase in the plasma.

Acknowledgements—This work was supported in part by Contract AT (30-1) 2085 with the U.S Atomic Energy Commission.

REFERENCES

1. F. BUFFONI, *Pharmac. Rev.* **18**, 1163 (1966).
2. B. ROSE and J. LEGER, *Proc. Soc. exp. Biol. Med.* **79**, 379 (1952).
3. C. F. CODE, D. T. CODY, M. HURN, J. C. KENNEDY and M. J. STRICKLAND, *J. Physiol., Lond.* **156**, 207 (1961).
4. G. B. LOGAN, *Proc. Soc. exp. Biol. Med.* **107**, 466 (1961).
5. G. B. LOGAN, *Proc. Soc. exp. Biol. Med.* **111**, 171 (1962).
6. W. BERNAUER, H. GIERTZ, F. HAHN, W. SCHMUTZLER, G. SESEKE and B. U. SIEVERS, *Naturwissenschaften* **51**, 412 (1964).
7. W. SCHMUTZLER, H. GIERTZ and G. SESEKE, *Arch. exp. Path. Pharmak.* **250**, 173 (1965).
8. H. GIERTZ, F. HAHN, P. KRULL and U. ALBERT, *Int. Archs Allergy appl. Immun.* **33**, 306 (1968).
9. H. GIERTZ, F. HAHN, W. SCHMUTZLER and G. SESEKE, *Klin. Wschr.* **15**, 1034 (1964).
10. N. TRYDING, *Scand. J. clin. Lab. Invest.* **17**, Suppl. 86, 196 (1965).
11. R. HANSSON, C. G. HOLMBERG, G. TIBBLING, N. TRYDING, H. WESTLING and H. WETTERQVIST, *Acta med. scand.* **180**, 533 (1966).
12. O. DAHLBÜCK, R. HANSSON, G. TIBBLING and N. TRYDING, *Scand. J. clin. Lab. Invest.* **21**, 17 (1968)
13. D. V. MAUDSLEY and Y. KOBAYASHI, *Fedn Proc.* **27**, 808 (1968).
14. T. OKUYAMA and Y. KOBAYASHI, *Archs Biochem. Biophys.* **95**, 242 (1961).
15. G. VALLETTE, Y. COHEN and W. BURKARD, *Pharm Acta Helv.* **31**, 282 (1956).
16. P. HAHN, *Science, N.Y.* **98**, 19 (1943).
17. A. CARLSTEN, G. KAHLSON and F. WICKSELL, *Acta physiol. scand.* **17**, 370 (1949).
18. W. SCHMUTZLER, F. HAHN, O. GOLDSCHMIDT and G. SESEKE, *Arch. exp. Path. Pharmak.* **255**, 344 (1966).
19. F. HAHN, W. SCHMUTZLER, G. SESEKE, H. GIERTZ and W. BERNAUER, *Biochem. Pharmac.* **15**, 155 (1966).
20. S. DAHL, *Ann. Allergy* **22**, 303 (1964).
21. W. DAWSON, M. S. STARR and G. B. WEST, *Br. J. Pharmac. Chemother.* **27**, 249 (1966).

22. J. JANNÉ, *Acta. physiol. scand.* suppl. **300**, 1 (1967).
23. D. H. RUSSELL and S. H. SNYDER, *Fedn Proc.* **27**, 642 (1968).
24. Y. KOBAYASHI, *Nature, Lond.* **203**, 146 (1964).
25. CZ. MASLINSKI and A. NIEDZIELSKI, *Experientia* **20**, 372 (1964).
26. I. MARCOU, E. ATHANASIU-VENGU, D. CHICICCANU, G. COSMA, N. GINGOLD and C. C. PARHON, *Presse méd.* **46**, 371 (1938).
27. M. N. GHOSH and H. O. SCHILD, *Br. J. Pharmac. Chemother.* **13**, 54 (1958).