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Kininogen and kininogenase synthesis by the liver of normal and injured rats

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Isolated livers from normal rats or from others at 2 days after subcutaneous injection of turpentine have been perfused with a simplified medium. Estimation of kininogen, kininogenase and 2 plasma proteins in the perfusates thus obtained indicate that both kininogen and kininogenase are synthesized in the liver. Furthermore, because twice as much kininogen and kininogenase was synthesized by the livers from the rats which had been injected with turpentine as by those from the normal rats, these two proteins must be considered to be members of the group of plasma proteins known as acute phase reactants.

Acute inflammatory stimuli such as injection of croton oil or acetic acid have been shown by Zach & Werle (1972, 1973) to lead to increased concentration of kininogen and certain other plasma constituents. During chronic inflammation, e.g. adjuvant arthritis in the rat, increased plasma concentrations of kininogen have also been found (Von Arman & Nuss, 1969).

In the present work rats were stimulated by subcutaneous injection of turpentine. As expected, plasma kininogen increased rapidly and was at a maximum approximately two days after the stimulus. At this time, the concentration of kininogenase was also greatly increased. If fully confirmed, these findings imply that both kininogen and kininogenase belong to the group of plasma proteins usually known as acute phase reactants (APRs) (Gordon, 1970). These, together with most other plasma proteins, are known to be synthesized almost entirely by the liver (Koj, 1974).

To confirm this hypothesis and to exclude the possibility that the observed changes of kininogen and kininogenase concentration might be due primarily to altered rates of catabolism, the livers from both normal and stimulated rats have been perfused and the rates of synthesis of kininogen and kininogenase measured. To serve as a basis for comparison, the rates of synthesis of one normal plasma protein and one belonging to the APR group have also been measured.

MATERIALS

The following materials were used: polybrene; hexadimethrine bromide (Aldrich Chemical Co.); trypsin (L. Light & Co. Ltd., Colnbrook, England); bradykinin triacetate (Sigma); L-lysyl-β-naphthylamide carbonate (Koch–Light Laboratories, Colnbrook, England); atropine sulphate (BDH); α_1-acute-phase globulin (α_1 globulin) was prepared according to Gordon & Dykes (1972); α_2-acute-phase globulin (α_2M) was prepared according to Ganrot (1973); rat urinary kallikrein purified according to Nustad & Pierce (1974) was a gift from Dr. J. A. Guimaraes; Hageman-deficient human plasma was obtained from Dr. R. Biggs (Oxford) and also from Prof. E. A.

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Loeliger (Leiden), to both of whom we are most grateful. Hooded rats of the strain maintained at N.I.M.R. weighing between 200 and 250 g were used both as liver donors and for the *in vivo* experiments.

METHODS

Turpentine injection. Certain of the rats received 0·5 ml turpentine oil subcutaneously at each of two sites on either side of the abdomen.

Kininogen assay

The assay method using trypsin was essentially that of Diniz & Carvalho (1963) and that using an excess of rat urinary kallikrein (RUK) was performed according to Webster & Prado (1970). The liberated kinin was tested against bradykinin freshly diluted in 10^{-3}M oxalic acid, on the isolated guinea-pig ileum suspended in atropinized (1 μg^{-1} ml) Tyrode solution at 37° aerated with a mixture of 5% CO_2 in oxygen. When the perfusates were assayed, the Tyrode also contained 1 $\mu g\ ml^{-1}$ of chlorpheniramine maleate (Piriton) and 0·5 $\mu g\ ml^{-1}$ methysergide bimaleate. One milliunit (mU) of kininogen is the amount of substance yielding one nmol of bradykinin equivalent, in the presence of excess enzyme. A molecular weight of 50 000 for kininogen was assumed in order to calculate the synthesis rate.

Kininogenase assay

This was carried out essentially as described by Horton (1959). To 2·0 ml of fresh rat plasma 0·2 ml of 3 × 10^{-2}M 1,10-phenanthroline and 0·4 ml of N HCl were added; after 15 min incubation at 37° the mixture was cooled to 0° and 0·4 ml of N NaOH was added. To 0·2–0·5 ml of this mixture, an excess of rat plasma previously treated for 3 h at 56° followed by dialysis overnight against 5 mM EDTA was added (final volume 2·0 ml). Aliquots taken after ½, 1 and 2 h incubation at 37° were tested on the guinea-pig ileum preparation. The enzyme unit corresponds to the liberation of one μg of bradykinin h^{-1}. Perfusates were assayed by addition of 0·04 ml N HCl to each sample (1·5 ml). Then after 15 min at 37°, 0·04 ml of N NaOH, 0·2 ml Tyrode solution and 0·2 ml of 3 × 10^{-2}M 1,10-phenanthroline were also added. The mixture was incubated at 37° with 0·5 ml of rat plasma which had been previously treated for 3 h at 56° followed by overnight dialysis against 5 mM EDTA. Aliquots taken after 5 and 7½ h of incubation were tested as above.

Rat plasma albumin, α_1 globulin and α_2 globulin ($\alpha_2 M$)

These estimations were carried out according to Mancini, Carbonara & Heremans (1965).

Perfusion of isolated livers

The livers were cannulated via the portal and hepatic veins and transferred to an oil bath maintained at 37°. Before transfer sufficient of the perfusion medium was allowed to flow through the liver to remove all but the last traces of plasma. In addition, the exterior of the liver was carefully washed with Tyrode solution.

All perfusions were carried out for 1 h using either Eagles B.H.K. medium or Tyrode solution saturated with 5% CO_2 in oxygen at 37°. Perfusion was at a rate of 7–10 ml min^{-1}. The perfusate which was not recirculated was collected in a plastic vessel

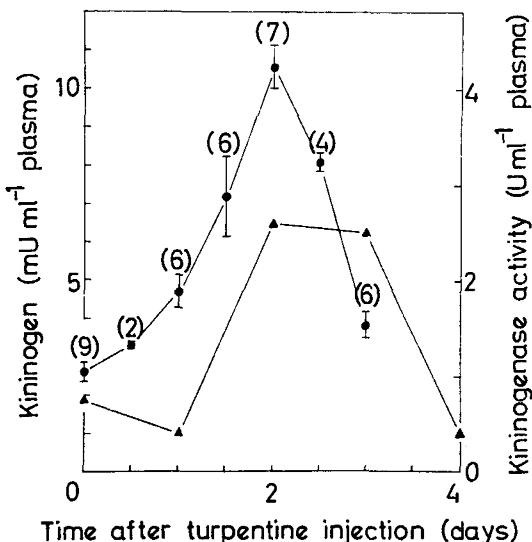

FIG. 1. Plasma kininogen (●) and kininogenase (▲) at various times after subcutaneous injection of turpentine into rats. Kininogen was estimated using trypsin incubation (see Methods) of the plasma from individual rats. The bars show + or − one s.e.m. The values for n are shown in parentheses. In a separate experiment, kininogenase was estimated in pools of plasma, each from 3–6 rats. For both assays different groups of rats were used for each time point to avoid rebleeding.

containing 1·0 g EDTA and 400 mg polybrene. Under the conditions employed rather little exudate passed through the capsule of the liver. Only the small volume formed in the second 30 min was transferred to the main pool of perfusate which was then concentrated by ultrafiltration (PM-10 Amicon membrane).

RESULTS

Plasma protein concentration

Plasma from the normal rats estimated by the method of Hartree (1972) was found to contain 52 mg protein ml^{-1}. At two days after subcutaneous injection of turpentine this value increased to 63 mg protein ml^{-1}.

Plasma constituents after stimulus

Fig. 1 shows the time curves of both plasma kininogen and kininogenase after subcutaneous injection of turpentine. When RUK was used instead of trypsin in the assay of kininogen somewhat lower values were obtained. Using pools of plasma from 3 rats for each time point these were for normal rats 0·35 mU ml^{-1} and for those at 2 and 4 days after stimulus 0·78 and 0·35 mU ml^{-1} respectively.

Following turpentine injection neither plasma arylamidase, kininase nor Hageman factor activity showed any change. On the other hand at two days after the subcutaneous injection of turpentine the plasma concentration of albumin had declined to 75% of its initial value and that of α_2M had reached its maximum value of 1·5 mg ml^{-1}.

Synthesis of proteins by perfused livers

As shown in Table 1, approximately twice as much kininogen was produced during perfusion of the livers of the rats which had previously been injected with turpentine

Table 1. *Kininogen, albumin and α_1 globulin synthesis rates by the isolated perfused liver from normal and stimulated rats.*

Perfusion	h ex turpentine	Synthesis rates (μg g^{-1} liver h^{-1}) Kininogen* RUK	TRY	albumin	α_1 globulin
1	0	0·6	3·1	124	0
2	0	0·6	2·9	120	0
3	0	0·5	0·9	121	0
4	42	0·9	5·2	106	44
5	42	1·0	7·7	102	15

* Kininogen in the perfusate was determined both by the use of rat urinary kallikrein (RUK) and trypsin (TRY); a mol. wt of 50 000 for kininogen was assumed.

as compared with that from the livers of normal rats. In addition, two livers from a stimulated rat and a normal rat produced kininogenase at rates of 3·1 and 1·4 mU g^{-1} liver h^{-1} respectively.

As expected, the livers from the stimulated rats but not those from the normals produced α_1 globulin (Gordon & Koj, 1968). The rate of synthesis of albumin by the livers from the normal rats was on average 122 μg h^{-1} g^{-1} liver or approximately 33% of the rate of synthesis of albumin observed by Hoffenberg, Gordon & Black (1971) who perfused the livers of the same strain of rats with rabbit blood.

DISCUSSION

Before the present investigation very little evidence, except of an indirect kind, existed on the site of synthesis of kininogen. However, the possible importance of the liver was already apparent because as a result of hepatectomy in rabbits Diniz & Carvalho (1963) had reported a marked reduction of plasma kininogen. More recently, Ryan, Bryan & Niemeyer (1971) obtained evidence that detectable amounts of [^{14}C]arginine and [^{14}C]proline are incorporated into kininogen during perfusion of the liver from a nephrectomized, stilbeostrol treated rat.

Now, in order to obtain direct evidence on the site of synthesis of kininogen in physiologically normal animals, livers from untreated rats have been investigated by perfusion with Tyrode solution. Recirculation of the perfusate could not be employed because under these conditions, even if added initially, kininogen was found to disappear. Fortunately if recirculation was avoided and the perfusate was allowed to collect in a vessel containing polybrene as stabilizer sufficient kininogen was produced to make possible its assay as described above. As shown in Table 1 most kininogen and kininogenase appeared in the perfusate when livers from rats at two days after subcutaneous injection of turpentine were used. Although the few experiments so far carried out do not permit a definite conclusion the increased rates of synthesis of kininogen may explain both the finding of Zach & Werle (1973) that after similar treatment the concentration in the plasma of kininogen approximately doubled and the present results for plasma kininogen and kininogenase.

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