Plasma Fibrinogen Concentrations after Intravenous Administration of Fibrinopeptides to Rats

By T. R. ITTYERAH, K. S. DODGSON and C. G. CURTIS

(Department of Biochemistry, University College, Cathays Park, Cardiff CF1 1XL, U.K.)

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Kropatkin & Izak (1968) demonstrated a twofold increase in plasma fibrinogen concentrations in rabbits 24h after the intravenous administration of the soluble breakdown products liberated by the action of thrombin on rabbit fibrinogen. They suggested that the fibrinopeptides A and B released by thrombin might stimulate fibrinogen synthesis in the liver. These findings appeared to provide a possible means of preparing ^{14}C -labelled fibrinogen of high specific radioactivity for studies on the mechanism of degradation of fibrinogen in the rat that were in progress in our own laboratories. The effects of rat fibrinogen breakdown products on rat plasma fibrinogen concentrations were therefore investigated.

Rat fibrinogen was prepared as described by Straughn & Wagner (1966) and purified by repeated precipitation with ethanol at 0°C . The purified material (20mg) was dissolved in 0.9% NaCl (12ml) and allowed to clot for 6h after the addition of purified bovine thrombin (2 N.I.H. units). The separated clot supernatant contained 0.21mg of protein/ml and was devoid of thrombin activity.

Three male rats (240-260g body wt.) were each injected intravenously with 1.25ml of the clot supernatant, an amount that approximates to the dosage used by Kropatkin & Izak (1968). Three litter mates were each injected with 0.9% NaCl (1.25ml) and used as controls. After 24h the rats were bled, and plasma fibrinogen was determined by two methods. In the first method fibrinogen was converted into

fibrin, which was then dissolved in 40% (w/v) urea in 0.2M-NaOH. The absorbance of this solution was then measured at 282nm as described by Blombäck & Blombäck (1956). The second method was based on the single radial immunodiffusion technique of Mancini *et al.* (1965) as modified for fibrinogen by John & Miller (1968). In contrast with the findings by Kropatkin & Izak (1968) in rabbits, no significant increase in plasma fibrinogen was observed in the rats (Table 1).

In a similar series of experiments purified rat fibrinogen (160mg) was dissolved in 0.9% NaCl (10ml) and allowed to clot for 6h after the addition of purified bovine thrombin (15 N.I.H. units). The separated clot supernatant contained 2.15mg of protein/ml and exhibited slight thrombin activity. Three male rats were each injected with 1.3ml of this clot supernatant, a dose in which the concentration of fibrinopeptides A and B is likely to be 10 times that in the dose used in the first set of experiments. Three litter mates, each injected with 1.3ml of 0.9% NaCl, served as controls. Again there was no appreciable increase in plasma fibrinogen after 24h (Table 1).

In further experiments the isolated rat liver was perfused with 110ml of heparinized rabbit blood from which the 'buffy' layer had been removed by centrifuging (Curtis *et al.*, 1970). The synthesis of fibrinogen by the liver was measured (by immunodiffusion) at 30min intervals for a period of 2.5h, after which

Table 1. Plasma fibrinogen concentrations in rats after the intravenous administration of clot supernatant

Fibrinogen concentration in plasma is expressed as the absorbance (at 282nm) corresponding to fibrin/ml of original plasma, and also as mg of fibrinogen/ml of plasma as measured by immunodiffusion.

Protein content of clot supernatant injected per animal (mg)	Injected animals			Control animals		
	Animal no.	E_{282} (corresponding to fibrin/ml of plasma)	Plasma concn. of fibrinogen (mg/ml)	Animal no.	E_{282} (corresponding to fibrin/ml of plasma)	Plasma concn. of fibrinogen (mg/ml)
0.26	1	1.30	—	1	1.06	—
	2	1.08	—	2	1.20	—
	3	1.00	—	3	1.08	—
2.80	1	1.09	3.42	1	0.82	2.86
	2	1.01	3.64	2	1.00	3.55
	3	0.94	3.00	3	0.91	2.93

time 4ml of the clot supernatant from the previous experiment (containing 2.15mg of protein/ml) was added to the perfusate. Fibrinogen synthesis was again measured at 30min intervals for a period of 1.5h. The appearance of rat fibrinogen in the perfusate increased linearly over the whole 4h period and was unaffected by the introduction of clot supernatant.

The collective results from these rat experiments contrast sharply with those obtained on rabbits by Kropatkin & Izak (1968). In the rat, either the fibrinopeptides have no effect on the plasma fibrinogen concentration or, alternatively, the rat differs markedly from the rabbit in the time-course of the response of plasma fibrinogen concentration to administered fibrinopeptides. In connexion with the first (and more likely) possibility it might be noted that the chemical structures of rabbit fibrinopeptides A and B are markedly different from those of the rat,

particularly with respect to fibrinopeptides B, where true homology exists in only four amino acids out of a possible total of 14 (13 for rabbit) (Blombäck *et al.*, 1966).

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