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Renal Function after Intravascular Coagulation in the Rat Kidney

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Summary. The renal function was studied 24 h after a 2 h thrombin infusion in rats with inhibition of the fibrinolytic system. The functional changes found were decreased osmolar and negative free water clearances, increased serum osmolarity, increased rejection of Na and elevated Na/K quotient in the urine. The glomerular filtration rate was markedly reduced.

Key words: Renal function, intravascular coagulation, thrombin infusion, fibrin deposits

Intravascular fibrin deposits in the kidneys have been observed in a number of renal diseases such as acute and chronic glomerulonephritis (Kincaid-Smith et al., 1970), toxaemia of pregnancy (Morris et al., 1964), microangiopathic haemolytic anemia (Brain et al., 1962) and in rejection of renal transplants (Busch et al., 1969). In recent times such deposits have also been shown to occur regularly in post-traumatic renal failure (Clarkson et al., 1970; Larsson et al., 1971).

Despite the widespread occurrence of fibrin deposition in the kidneys the pathogenetic importance of this factor in the development of the renal failure is largely unknown. In rats however thrombin-induced fibrin deposition in the kidneys during fibrinolysis inhibition has been found to give rise to tubular necrosis (Margaretten et al., 1964). The present investigation was performed to study if these lesions are associated with any functional impairment and if so, which aspect of renal function is affected.

Material and Methods

Experimental animals. Fifteen female Sprague-Dawley rats (Anticimex Farm, Stockholm) weighing 200 ± 5 g were used. The animals were allowed free access to tap water and food (Anticimex rat pellets).

Thrombin infusion. Fifteen minutes before the thrombin infusion tranexamic acid (AB Kabi), 10 mg/100 g body weight in 0.5 ml of saline, was given into a tail vein. Bovine thrombin (Topostasin®, Roche) was dissolved shortly before injection. 100 NIH units/100 g body weight in 2.4 ml of saline were constantly infused for 2 h into a tail vein under ether anaesthesia. Control rats were given tranexamic acid and were infused with saline.

Renal function. The measurements were performed 24 h after the termination of the thrombin infusion. The rats were anaesthetized by an intraperitoneal injection of 3 ml of 0.8% α-chloralose (Merck) solution. The rectal temperature was kept at 38 ± 1° C by manual regulation of an infrared warming lamp placed above the animal. Free airways were ensured by tracheotomy, one catheter was inserted into the left carotid artery for blood sampling and another catheter into the right common jugular vein for infusion. For urine collection a catheter was inserted into the bladder through the urethra and sutured firmly to the skin. The bladder was emptied by repeated air insufflations. About 0.3 ml of blood was collected 2 min before midpoint of the clearance periods. This procedure has been shown not to influence the acid-base condition of the animals (Rammer, 1972). Of a saline solution containing 50 mg polyethylene glycol (PEG) 1000 (Union Carbide) 1 ml was given in 5 min and 1.2 ml/h as a constant infusion. After about 45 min infusion the bladder was emptied and urine collected for 30 min. After that diuresis was induced by injection of 1 ml of Mannitol 10% (AB Pharmacia) over 5 minutes. The bladder was emptied 10 min later and urine collected for 15 min. PEG 1000

was analyzed by a turbidimetric method as described by Berglund (1964). Osmolarity was determined with a Knauer osmometer and Na and K by flamephotometry. The glomerular filtration rate (GFR) was calculated as the clearance of PEG 1 000. Osmolar and free water clearances were calculated in the conventional way and tubular rejection of Na as the clearance of Na/GFR. The statistical evaluation of the results was performed by Student's t-test.

After the experiment the kidneys were fixed in 10% neutral formalin. Five μ thick paraffin sections were stained with haematoxyline-eosin and by Mallory's PTAH method for the demonstration of fibrin.

Results

The results of the functional studies are shown in Table 1.

The urine flow and the diuresis after injection of Mannitol were similar in the rats 24 h after the thrombin infusion and in the control rats.

The GFR values on the other hand were markedly reduced in the thrombin infused rats, the mean value being 0.28 ml/min, 100 g body weight compared to 1.54 ml/min, 100 g in the control rats. During Mannitol diuresis the GFR fell to about 2/3 in both groups.

The urine osmolarity in the thrombin infused rats was about 700 mosm/l, compared to approximately 1500 mosm/l in the control group. The serum osmolarity was increased: the mean value was about 325 compared to about 305 mosm/l in the control rats. Occasional thrombin infused rats had a serum osmolarity of about 340 mosm/l. The osmolar clearance and the negative free water clearance were markedly reduced in the thrombin infused rats and their increase during Mannitol diuresis was smaller than in the control rats.

The Na/K quotient in the urine averaged 0.13 in the control rats and 0.34 in the thrombin infused rats. After Mannitol injection these values increased to 0.78 and 1.58 respectively. The tubular rejection of sodium was markedly increased in the rats infused with thrombin, especially during Mannitol diuresis.

Morphological examination of the kidneys showed moderate patchy necrosis of the tubular epithelium, non-collabated tubules, abundant hyaline casts but only rare deposits of fibrin in the glomerular capillaries.

Discussion

Previous studies on the effect of disseminated intravascular coagulation upon renal function have

Table 1. Results of the renal function studies in control rats and in rats 24 h after injection of tranexamic acid and a 2 h infusion of thrombin.

	Controls (n = 7)		Tranexamic acid + thrombin (n = 8)	
		Mannitol		Mannitol
Urine flow, μ l/min, 100 g	4.20 ± 0.80	24.50 ± 5.30	3.50 ± 2.40	22.20 ± 9.20
GFR, μ l/min, 100g	1.56 ± 0.38	0.98 ± 0.33	0.28 ± 0.28[a]	0.19 ± 0.12[a]
Osm Se, mosm/l	307 ± 9	304 ± 8	322 ± 17	330 ± 13[a]
Osm U, mosm/l	1480 ± 90	670 ± 170	680 ± 320[a]	450 ± 60
Closm, μ l/min, 100 g	20.50 ± 4.10	52.50 ± 5.10	6.90 ± 4.30[a]	31.30 ± 14.40[a]
-C1 H_2O, μ l/min, 100 g	16.30 ± 3.40	27.50 ± 8.10	3.40 ± 3.30[a]	9.20 ± 6.50[a]
Na/K	0.13 ± 0.03	0.78 ± 0.54	0.34 ± 0.17[a]	1.58 ± 0.40
Tubular rej. of Na, %	0.60 ± 0.40	8.60 ± 3.0	6.75 ± 9.22	78.00 ± 61.10[a]

Mean ± S. D.

a = significantly different from control rats ($p < 0.05$).

only been concerned with the acute changes during coagulation. Reduced urine production and impaired concentrating capacity during the infusion of endotoxin (Beller et al., 1969) or thrombin (Thiess et al., 1970) were found in the rabbit. In dogs increased vascular resistance and reduced urine production were found during the infusion of thrombin (Rådegran et al., 1970; Høie and Schenck, 1972). In the present investigation the function of the kidney that had been injured by a previous episode of fibrin deposition in the kidneys was studied.

The experimental model used, an infusion of thrombin for two hours in rats with inhibition of the fibrinolytic system, gives rise to heavy fibrin deposits in the kidneys mainly located in the glomerular capillaries (Rammer et al., 1972). At the time studied, 24 h after termination of the thrombin infusion, the fibrin had almost completely disappeared from the kidneys.

The results of the present investigation have shown that an episode of intravascular clotting in the kidneys gives rise not only to morphological changes but also to a functional impairment of different parts of the nephron. Thus the reduction of the osmolarity of the urine and of the osmolar and negative free water clearance indicated an impaired capacity to eliminate osmoles and to preserve water.

The increase in tubular rejection of sodium and the elevated Na/K quotient especially during mannitol diuresis indicated a decreased reabsorbtion of sodium both in the proximal and distal tubules. These functional changes had a morphological counterpart in the focal necroses of the tubular epithelium seen in the thrombin infused rats.

The GFR was markedly reduced in the rats after thrombin infusion. The urine flow, however, was normal probably due to the impairment of reabsorbtion indicated above. The reduction in GFR was not a result of obstruction of the glomerular capillaries by fibrin since the fibrin had disappeared almost completely from the kidneys at this time point after the thrombin infusion.

The severe dehydration with increased serum osmolarity, as a result of inability of the damaged kidney to preserve water, probably gives rise to an increased activity of the sympathetic nervous system with contraction of the afferent arterioles. Another possible explanation of the result is an increase in the renin concentration in the region of the juxtaglomerular apparatus with resultant reduction of the glomerular perfusion. The juxtaglomerular apparatus might be activated through the increased Na concentration of the distal tubular fluid as a result of the coagulation induced tubular injury (Thurau and Schneerman, 1965). To differentiate between these mechanisms microperfusion experiments are required.

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