Kininogenase in Urine Produced by Isolated Perfused Rat Kidneys

Although several reports show that urinary kallikrein differs from plasma kallikrein and resembles renal kininogenase[1-4], no definitive evidence has been provided as to whether the urinary enzyme represents the clearance of a blood kininogenase or is a product elaborated and/or stored by the kidneys. Experiments here described show the occurrence of kallikrein in the urine produced by isolated rat kidneys, perfused with adequate fluids which do not contain either kininogenase or its precursors.

Methods. Adult rats (300–400 g) were anesthetized with diethyl-barbiturate. After the injection of heparin (10 mg in 0.1 ml), polyethylene catheters connected with the perfusion system, were introduced into the cava vein and aorta. Both vessels and their branches were rapidly ligated, leaving free the short segment corresponding to the renal arteries and veins emergences in order to irrigate both kidneys with the perfusion fluid. The kidney circulation was interrupted for only 1–2 min (time required for ligature) and then artificial circulation was set in motion. The perfusion system, which can be used as a closed or open model, was set up in a similar way as that described by KRAHÈ et al.[5]. A rotating flask where a mixture of O_2 (95%) and CO_2 (5%) was continuously flowing was used as fluid reservoir and oxygenator. Two different perfusion fluids were used: 1. Tyrode-dextran solution (1 l contained 8 g NaCl, 0.2 g KCl, 0.24 g $CaCl_2$, 1 g $MgCl_2$ 6 H_2O, 1 g $NaHCO_3$, 1 g D-glucose) and 40 g dextran (m.w. 60,000) plus rat red cells (TDR) 2. called TAR was similar to TDR, but dextran was substituted by 5% human albumin (U.S.P. Squibb and Sons). Either to dextran or albumin fluids, washed rat red cells were added in order to obtain a final hematocrit of 30–35%. During the first 10–15 min of perfusion, oxygenated fluid purposely lacking red cells was pumped to the kidneys in order to wash out the blood remaining in these organs. From then on washed rat red cells were added to the perfusing fluid. The pulsatil perfusion pump was regulated so as to maintain a mean blood pressure of 90–120 mm Hg (systolic pressure of 120–140 mm Hg) on the arterial side, which provided a flow of 5–8 ml of perfusate per min. The perfusion pressure was continuously recorded through a Statham transducer P23 connected to a Grass polygraph. The outflow was checked every 15–20 min measuring the fluid volume draining from the kidneys. With that flow, a drop of 8–12% O_2 saturation was recorded between arterial and venous sides. Urine produced during the experiment was collected through an endwelling bladder catheter after a thoroughly washing the cavity with saline; 17 experiments using the closed system and 3 using open system were performed. In each experiment, lasting from 60 to 180 min, 2 or 3 samples of urine (0.2 ml to 0.8 ml/h) were collected.

The occurrence of a kallikrein-like enzyme in the urine was investigated by chemical and biological approach, according to the method already described for renal kininogenase identification[6].

Results and discussion. Both with TAR or TDR, the following results were obtained: 1. The urine samples display direct oxytocic effect upon rat uterus, as normally urine does. This activity does not diminish with dialysis. Effect equivalent to 10 ng of bradykinin were recorded with 0.05–0.15 ml of urine, when introduced into a bath of 20 ml capacity, where the isolated rat uterus was immersed. 2. Kininogenase activity was easily demonstrated incubating for 2 min, 0.02 to 0.08 ml of urine either with kininogen II (Figure) or kininogen I at pH 7.4. These substrates were prepared from rat plasma by the method of JACOBSEN[7]. The substance produced during incubation induces rat uterus contraction similarly to kinins; its oxytocic activity is destroyed by chymotrypsin, but not by pepsin. 3. The addition to the urine of 200–500 IU of aprotinin (Trasylol, Bayer) blocks its kininogenase activity. 4. DFP added to the urine (final concentration 0.25 *M*) produced a striking inhibition of

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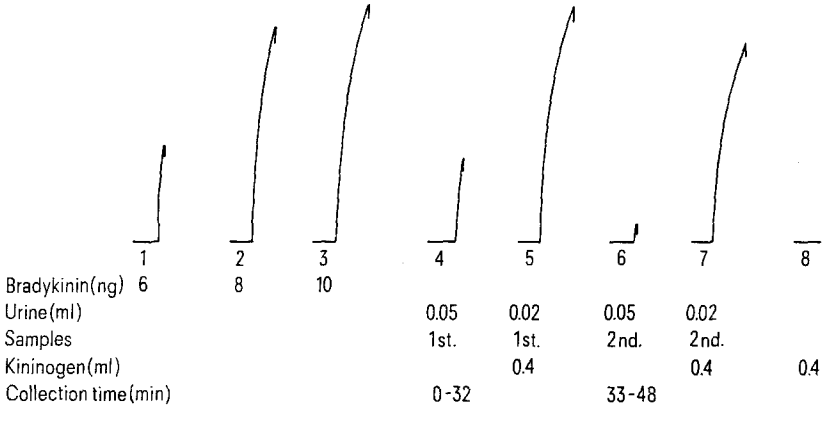

Effect of 2 urine samples upon isolated rat uterus. In 1, 2 and 3, 6, 8 and 10 ng bradykinin were introduced in the bath; in 4, 0.05 ml of urine (first sample collected between 0–32 min of perfusion); in 5, a mixture incubated for 2 min (pH 7.4) of 0.02 ml of the first urine sample plus 0.4 ml of kininogen II; 6, 0.05 ml of the second urine sample (collected between 33–48 min; in 7, an incubated mixture of 0.02 ml of urine plus 0.4 ml kininogen II; in 8, 0.4 ml kininogen II.

both the direct oxytocic effect and kininogenase activity of the urine. 5. Soyabean antitrypsin also inhibits at a high concentration (200–500 µg for 0.1 ml of urine) this kininogenase activity, but ovomucoid (Sigma Chemicals Co.) does not. 6. Pepstatin, a polypeptide which inhibits renin but not kallikrein activity, does not hinder kininogenase effects. The kallikrein activity of the urine produced during the perfusion period was 10–30-fold lower compared with the urine voided by the rat before the experiment. During the experiment, O_2 consumption and vascular resistance were not significantly changed, but a progressive decrease in kallikrein concentration was observed in the formed urine. On this question further studies will be required to establish whether some biochemical impairment or other factors are involved. The small amount of urine collected (not greater than 0.6 to 1.8 ml in the experimental period) limited the possibility of a chemical purification of the enzyme. The results tend to support the assumption that kallikrein found in the normal urine is also produced by the kidney and is not an enzyme cleared from the circulating blood.

Résumé. L'urine produite par les reins isolés du rat perfusés à pression normale pendant 60–180 min avec des liquides oxygenés ayant en suspension des globules rouges contient une kininogénase qui a les mêmes propriétés que la kallikréine de l'urine produite en conditions physiologiques.

J. ROBLERO, H. R. CROXATTO, R. GARCÍA and J. CORTHORN

Laboratorio de Fisiología, Instituto de Ciencias Biológicas, Universidad Católica de Chile, Casilla 114-D, Santiago (Chile), 12 November 1973.

Effect of Starvation on Blood Glucose and Nonprotein Nitrogen Levels of the Fish *Clarias batrachus*

Starvation effects the normal body metabolism and prolonged starvation may even cause death of the animal. A decline in various body constituents of fish, following experimental starvation, have been reported by various authors[1–3]. Studies, following starvation, on the blood glucose and nonprotein nitrogen (NPN) levels, etc. have also been made[4–10]. This paper deals with the results obtained for the fresh-water cat fish *Clarias batrachus*, following starvation up to 150 days.

Materials and methods. Normal, well fed *Clarias batrachus*, fully acclimatized to laboratory conditions were used. Right from the beginning of the experiment the starving fish were kept in separate aquaria and were not given any food for the entire period of starvation. Even aquatic plants were removed and nothing except pebbles and bed of river-sand was left in the aquaria. Water was changed twice a week throughout the experimentation period and this possibly removed even the naturally developing micro-fauna and flora and also accumulating toxic waste products of the fish. Control fish were kept in separate aquaria and were given minced goat liver, earthworms and snails on alternate days.

The experiment was started in the month of November and concluded in the month of April. The starvation was prolonged up to 150 days and observations were made on 1st, 10th, 30th, 90th and 150th day of starvation. On the first day only 5 fish of the control batch were examined. In subsequent periods, 5 fish of control group were also examined along with the starved fish, to counter the effects of seasonal variations, etc. Thus 25 fish of the control group and 36 starved fish were examined in this experiment.

For taking blood, the fish was carefully taken out of the aquarium with the help of a small hand net, immersed in a jar containing 1.5% paraldehyde solution. It took about 2 min to make a fish senseless. Immediately the fish was taken out, wiped dry with a turkish towel and put on a disection tray. Its caudal vein exposed just behind the anal region and blood drawn in a syringe fitted with a 20 gauze needle. Exactly 1 ml blood was deproteinized using zinc hydroxide-barium-sulphate procedure (OSER[11]). Blood glucose and NPN determinations were made following the Nelson-Somogyi and Folin-Wu methods, respectively (OSER[11]). After the blood was drawn, gut contents and viscera of both the control and starved group of fish were examined. No mortality of these fishes occurred during the entire period of starvation.

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Effect of starvation on blood glucose and NPN levels of the fish *Clarias batrachus*

Status and No. of observations	Glucose and standard deviation (mg/100 ml)	NPN and standard deviation (mg/100 ml)
Control (25)	63.3 ± 11.7	38.9 ± 7.7
Post starvation		
10th day (8)	59.8 ± 13.2	35.6 ± 6.0
30th day (8)	53.5 ± 11.7	31.1 ± 7.7
90th day (10)	44.3 ± 12.5	27.3 ± 7.3
150th day (10)	32.0 ± 9.1	20.5 ± 4.8