Enzymuria Following Acute Renal Ischemia in Dogs

A variety of enzymes have been reported in the normal urine[1-4] and enzymuria has been found to increase in renal disease or injury in dogs[2] and other species[5,6]. Thus, increased alkaline phosphatase excretion was described in rats after renal ischemia[5]. High urinary levels of glutamic oxaloacetic transaminase (GOT) followed renal ischemia[6] and infarction[7] in man. Increase in lactic acid dehydrogenase followed ischemia and infarction of kidneys in dogs[2] and renal infarction in man[7]. A rise in leucine-amino-peptidase was noted in patients who had renal ischemia[6].

The purpose of this work was to study the excretion of urinary enzymes with bradykinolitic activity, also named kininases[8], after acute renal ischemia in dogs and to relate this enzymatic complex with GOT and protein excretions.

Material and methods. Eight normally hydrated healthy dogs weighing 15–20 kg were used for these acute experiments. The dogs were anaesthetized with 10 mg/kg sodium pentobarbital i.v. An infusion of 5% glucose in water (3 ml/min) was started in the right brachial vein and maintained until the end of the experiment. The abdomen was opened through a mid-line incision and both ureters were catheterized with number PE 240 catheters, threaded into the upper portions of the ureters. Through a left flank incision the left renal artery was exposed. After a control collection period was made, the left renal artery was clamped with a shod curved clamp during 20 min. After release of the clamp, urine collections were made for 2 more periods.

The enzymes were measured in fresh or fresh frozen, undialysed urines.

GOT was determined by the method of REITMAN and FRANKEL[9], urinary bradykinolitic activity by the method used by FERREIRA and ROCHA E SILVA[10] and proteinuria by the method of EXTON[11].

Results. The results are summarized in the Table. After 20 min of complete renal ischemia, urine flow from the injured kidney resumed within the first post-ischemia period. In the animals enzymuria increased on the left, immediately after release of the clamp on the left renal artery. The same occurred with the levels of proteinuria. Contralateral urine flow, enzymuria or proteinuria did not change significantly during or after the clamping. The Figure illustrates our findings.

Discussion. Our results indicate that the canine kidney reacts to short periods of ischemia by immediate increases in excretion of kininases, GOT and total protein. The mechanism and location of the protein leak (from glo-

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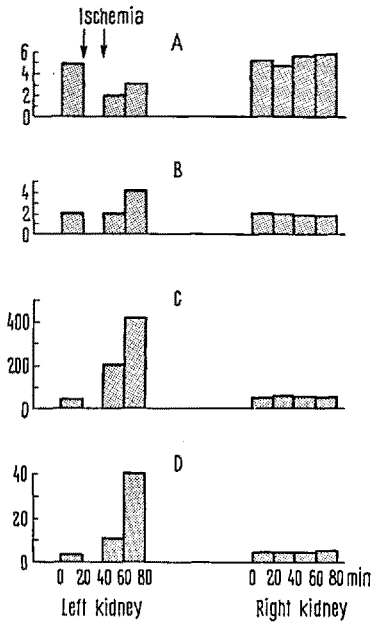

Effect of acute unilateral renal ischemia on: (A) urinary volume (ml/20 min); (B) proteinuria (mg/20 min); (C) glutamic oxaloacetic transaminase (GOT) excretion (units REITMAN and FRANKEL/20 min); (D) bradykinolitic activity excretion (γ-bradykinin destroyed/20 min per min of incubation).

Urinary volumes, proteinuria, bradykinolitic activity and GOT excretion in dogs following acute unilateral renal ischemia (mean value of 8 dogs)

Period	Urinary volume ml/20 min		Proteinuria mg/20 min		Bradykinolitic activity[a]		GOT excreted units REITMAN and FRANKEL per 20 min	
	Left kidney	Right kidney	Left kidney	Right kidney	Left kidney	Right kidney	Left kidney	Right kidney
1	4.8	4.9	1.95	1.99	2.8	2.5	48.1	44.9
2	–	4.4	–	1.77	–	2.5	–	52.2
3	1.7 ± 0.2	5.4 ± 0.65	1.92 ± 0.2	1.85 ± 0.17	10.2 ± 1.3	2.4 ± 0.2	199.1 ± 23.4	56.6 ± 4.2
4	3.0 ± 0.5	5.6 ± 0.6	4.09 ± 0.54	1.74 ± 0.18	41.3 ± 6.8	2.6 ± 0.23	421.2 ± 58.3	50.8 ± 5.3

[a] γ-Bradykinin destroyed/20 min per min of incubation.

merulus and/or tubulus) which occurs in the rapid response to ischemia of kidney, remains to be elucidated. Even the localization of the different enzymes within the kidney has to be well clarified.

It is worthwhile to point out that the 2 enzymes studied had an equally rapid and simultaneous increase in the urine which may suggest a very near site of localization within the renal parenchima. The kininases, which have been described in normal urine, followed the same general pattern, namely, increased excretion after renal ischemia.

Measurements of serum enzymes were not made. However it is well known that increases in plasma GOT occur after renal infarction[7,12,13]. It indicates a leakage of enzymes through the plasma-cell barrier, besides the possible release of enzymes toward the tubular end of cell.

A similar study should be done concerning the serum bradykinolitic activity during and after renal ischemia. It might reveal whether or not the phenomenon of higher serum enzymes concentration following kidney injury is characteristic of some specific enzymes[14,15].

Resumen. Fue observado en los perros, nítido aumento en la excreción de transaminase glutamico-oxalo-acética, cininases y proteínas, inmediatamente después de una isquemia renal unilateral de 20 min. No fue observada ninguna alteración en la enzimuria o en la proteinuria en el riñon de control.

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The Bundle of Schütz and its Relation to the Regulation of Food Intake

The experimental evidence for the presence of glucoreceptors in the ventromedial area of the hypothalamus (VMA) has recently been reviewed[1]. Briefly summarized, MARSHALL, BARRNETT and MAYER[2] have shown that mice injected with goldthioglucose (GTG) will develop lesions in VMA. These lesions will occur only if the glucose moiety is attached to the gold by the sulfur bridge. Other goldthio compounds, including goldthiogalactose, goldthiosorbitol, goldthiomalate, goldthioglycerol and goldthiocaproic or goldthiocapric acids will not cause VMA lesions[3]. Simultaneous injections of GTG with sodium thioglucose[3] or 2 glucose analogues[4] do not result in VMA lesions or hyperphagia and obesity, presumably because the injected glucose analogues compete with GTG for the sites of action. Mice made diabetic and subsequently injected with GTG also do not develop VMA lesions nor do they become hyperphagic and obese[5]. In the VMA, GTG-induced lesions are not due to a general increase in permeability in the blood-brain barrier[6].

Unlike electrolytically induced lesions in VMA, lesions made with GTG seem to be specific to the function of food intake regulation with minimal impairment shown to other functions. For example, GTG-obese mice, unlike mice made obese by electrolytic lesions do not show: (a) a range response[6], (b) gonadal atrophy but the mice can mate and rear their young[7], and (c) a water imbalance[8].

Recently, RIDLEY and BROOKS[9] demonstrated that destruction of VMA also eliminates the gastroacidity response to insulin hypoglycemia. In a previous study using rats, MAYER and SUDSANEH[10] had shown that destruction of VMA eliminates the inhibition of gastric hunger contractions by injections of glucagon and the consequent hyperglycemia and increase in glucose utilization. That gastric hunger contractions are not refractory to local agents is shown by the fact that they still respond to epinephrine and norepinephrine. On the basis of their results, MAYER and SUDSANEH[10] hypothesized that glucoreceptors in the ventromedial region of the hypothalamus, which may also include a portion of the periventricular system, involve fiber pathways that connect VMA to the lateral hypothalamic area (LHA) and the dorsal longitudinal fasciculus (bundle of Schütz) which is the primary efferent pathway from the hypothalamus to the mesencephalon and nucleus of the vagus.

Through the use of the FINK-HEIMER method[11] which is a stain for secondary degenerated fibers, AREES and MAYER[12] demonstrated connection from VMA to LHA. The purpose of the study reported here was to determine whether the bundle of Schütz would similarly degenerate when GTG was administered to animals.

Fifteen Charles River (CD-1) female mice weighing between 20–25 g were injected i.p. with goldthioglucose in concentrations equal to 0.5 mg/g body weight. 5 other mice were used as controls and injected with 0.5 mg/g body weight of glucose. 4 days after injection all mice were sacrificed, their brains removed and fixed in a manner similar to that described by AREES and MAYER[12]. Histological examination using the FINK-HEIMER method showed degeneration of the bundle of Schütz in 13 of the

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