Effects of Spontaneous Ischemia on Aerobic Metabolism of Cortical and Medullary Homogenates of Dog Kidney[1] (35101)

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Acute cortical ischemia of the kidney has been reported to be an outgrowth of neurogenic and/or cardiovascular events (1). We recently reported a high incidence of acute renal cortical ischemia in 12 of 30 dogs anesthetized with sodium pentobarbital (2). The ischemia was not induced intentionally, but appeared to be a by-product of acute surgical procedures required for nephrectomy and/or anesthesia. Thus we have classified this phenomenon as spontaneous cortical ischemia. It appeared that dogs which were overly excited prior to or during administration of anesthesia were more likely to have blanched or ischemic kidneys than the more relaxed dogs.

Associated with the ischemic condition was a low activity of an anerobic energy-yielding reaction in the renal cortex. The cortex of ischemic kidneys had a lower activity of the reaction: α-ketoglutarate + oxaloacetate + GDP + inorganic phosphate → succinate + malate + GTP + CO_2 than that of nonischemic kidneys (2). This particular reaction is localized in the cortex of the kidney (3).

We are now reporting on the effect of spontaneous cortical ischemia on the aerobic metabolism of washed homogenates of cortical and medullary tissue. In the cortex, no reduction in the rates of oxygen consumption ($\dot{Q}O_2$), α-ketoglutarate (α-KG) utilization of inorganic phosphate (P_i) disappearance results from spontaneous cortical ischemia. In contrast, each of the above rates is significantly lower in homogenates of ischemic medullary tissue than in nonischemic medullary tissue.

Certain changes have taken place within ischemic cortical tissue since the activities of malic dehydrogenase (MDH)[2] and isocitric dehydrogenase (ICDH) are significantly lower in ischemic tissue than in nonischemic tissue. In addition, preincubation of cortical homogenates of ischemic tissue significantly reduced the rates of the measured variables of aerobic metabolism below that of controls.

Methods. All experiments were done on kidneys of fasted, mongrel dogs of either sex weighing between 12 and 20 kg. The dogs were anesthetized with sodium pentobarbital (30 mg/kg) by an intravenous injection. The kdneys were exposed by means of two dorsolateral incisions, removed, and placed quickly in ice-cold 0.9% NaCl. Nephrectomy was accomplished within 30–60 min after induction of anesthesia.

The ice-cold kidneys were then decapsulated and separated into cortical and medullary segments, and washed homogenates of cortical and medullary tissue were prepared as described previously (3). Aerobic metabolic studies were done on washed homogenates prepared from kidneys having evidence of ischemia and from kidneys having no evidence of ischemia. All experiments were carried out in Warburg flasks at 30° in air. In each experiment the rates of oxidative phosphorylation, $\dot{Q}O_2$ and α-KG utilization were

[1] This study was supported in part by grants from the Louisiana Heart Association and the National Institutes of Health, HE-11987-01. A preliminary report of this work has been presented to the FASEB, Atlantic City, New Jersey, 1970.

[2] The following enzyme abbreviations are used in this report as recommended by the Enzyme Commission of the International Union of Biochemistry (12): malate dehydrogenase (MDH): L-malate: NAD^+ oxidoreductase (EC 1.1.1.37); isocitrate dehydrogenase (ICDH): L-isocitrate: NADP oxidoreductase (EC 1.1.1.42).

TABLE I. Effect of Ischemia on Rates of O_2 Consumption, α-KG Utilization and P_i Disappearance in Fresh Homogenates.[a]

Preparation		(μmoles/100 mg of dry wt • min)[b]		
		$\dot{Q}O_2$	$\dot{Q}\alpha$-KG	P_i disappearance rate
Cortex	Nonischemic	0.660 ± 0.040 (6)	0.861 ± 0.049 (6)	2.62 ± 0.165 (6)
	Ischemic	0.667 ± 0.008 (5)	0.889 ± 0.026 (5)	2.82 ± 0.093 (5)
Medulla	Nonischemic	0.743 ± 0.040[c] (4)	0.841 ± 0.065[d] (4)	3.53 ± 0.102[e] (4)
	Ischemic	0.446 ± 0.092 (5)	0.548 ± 0.105 (5)	1.83 ± 0.59 (5)

[a] All values are means ± SE; numbers of experiments are shown in parentheses. Experiments were carried out in Warburg flasks containing 3.0 ml of the following: 1 m*M* ADP, 0.5 m*M* NAD^+, 5 m*M* $MgCl_2$, 10 m*M* K-phosphate buffer (pH 7.4), 9 m*M* KF, 0.02% cytochrome C, and either cortical or medullary homogenate (~10 mg of protein/ml) in the main compartment; 0.2 ml of 20% KOH in the centerwell; 8 m*M* α-KG, 19 m*M* glucose, and 0.02% hexokinase in sidearm no. 1. 0.3 ml of 33% $HClO_4$ was in sidearm no. 2 for termination of experiment.

[b] p values for differences between the mean rates in nonischemic vs ischemic preparations are: [c] $p < 0.025$; [d] $p < 0.05$; and [e] $p < 0.005$.

studied in both cortical and medullary homogenates. A complete description of all experimental protocols, materials and methods can be found in our earlier report (4).

Spontaneous cortical ischemia was identified by a blanched or white zone at the cortico-medullary border which radiates outward toward the surface of the cortex. Kidneys not having this blanched zone but having pink cortices were considered to be nonischemic. All kidneys, ischemic or nonischemic, were studied; none were discarded.

Two basic types of experiments were done: (i) the rates of O_2 consumption, α-KG utilization and P_i disappearance were studied in nonischemic and ischemic kidney cortex and medulla; and (ii) the effects of aging or preincubation was studied in nonischemic and ischemic tissue in which the above rates were determined. Aging was accomplished by preincubating an aliquot of homogenate at 37° for 30 min in a Dubnoff metabolic shaker. In addition the activities of MDH and ICDH were determined in cortex and medulla of both types of experiments.

Results. Spontaneous ischemia was observed in 8 of 18 dogs. Of the 18, 11 were used for metabolic studies. All 18 were used for enzyme activity determinations.

A. Effect of acute spontaneous cortical ischemia on oxidative phosphorylation, O_2 consumption and α-KG utilization of fresh cortical and medullary homogenates. Ischemia had no effect on the rates of O_2 consumption, α-KG utilization or P_i disappearance in fresh cortical homogenates as indicated in Table I. Thus spontaneous cortical ischemia did not alter either the potential capacity of the cortex to carry out aerobic metabolism or the potential capacity of the cortex to produce high energy phosphates. In contrast, spontaneous ischemia did alter the capacity of medullary tissue to produce high energy phosphate compounds (Table I). The rates of O_2 consumption, α-KG utilization and P_i disappearance were all significantly lower in ischemic homogenates than in nonischemic homogenates. This greater sensitivity of the medulla to spontaneous cortical ischemia is consistent with our findings of induced ischemia of 1-hr duration resulting from renal venous (RV) occlusion (4).

B. Effect of spontaneous ischemia on the activities of MDH and ICDH in fresh homogenates of cortex and medulla. We reported previously that the activities of MDH and ICDH were reduced prior to any reduction in the rate of aerobic metabolism in cortical homogenates of the occlusive ischemic kidneys. Thus it did not appear that the activity of either enzyme was limiting the rate of aerobic metabolism in the ischemic preparation. Similar findings were observed for cortical homogenates of spontaneous, nonocclusive

TABLE II. Effect of Ischemia on the Activities of MDH and ICDH in Fresh Homogenates.

Preparation		(IU/mg of protein)[a] MDH	ICDH
Cortex	Nonischemic	2.96 ± 0.10[b] (11)	0.059 ± 0.004[c] (11)
	Ischemic	2.34 ± 0.21 (7)	0.048 ± 0.006 (6)
Medulla	Nonischemic	2.89 ± 0.32[d] (7)	0.074 ± 0.018[e] (4)
	Ischemic	2.28 ± 0.30 (4)	0.060 ± 0.03 (2)

[a] p values for differences between the mean rates of nonischemic and ischemic preparations are: [b] $p < 0.012$; [c] $p < 0.05$; [d] $p > 0.10$; and [e] $p > 0.10$.

ischemic kidneys (4).

A comparison of fresh cortical homogenates revealed that the activities of both MDH and ICH were significantly lower in ischemic than in the nonischemic preparation (Table II). A similar difference was observed in medullary homogenates although the difference was not significant at the 5% level. These data indicate that specific physical changes of some nature have taken place within cortical tissue of ischemic kidneys despite the unchanged metabolic pattern. Changes in structure or in concentration of some metabolic intermediate became more evident when cortical homogenates were subjected to a secondary stress such as aging the homogenate.

C. Effect of aging on the rates of O_2 consumption, α-KG utilization and P_i *disappearance of homogenates of ischemic and nonischemic kidneys.* We reported earlier that preincubation of cortical homogenates at 37° uncovered latent metabolic changes observed in the occlusive ischemic kidney (4). Similar findings were observed in spontaneous ischemic kidneys. In the cortex, the $\dot{Q}O_2$, α-KG utilization, and P_i disappearance were all significantly lower in aged ischemic than in aged nonischemic homogenates (Table III). In medullary tissue, each of the measured variables of aerobic metabolism of both nonischemic and ischemic tissue were severely reduced by aging. Thus the stress was of too severe a magnitude in medullary tissue to demonstrate a differential effect. This finding is consistent with the concept that the aerobic metabolism of medullary tissue is more sensitive to the stress of ischemia than that of cortical tissue.

D. Effect of aging on the activities of MDH and ICDH in cortex and medulla of ischemic and nonischemic kidneys. The activities of MDH and ICDH did not limit the rate of aerobic metabolism of either cortical or medullary tissue of fresh homogenates as indicated in Tables I and II. Since the rate of aerobic metabolism is reduced in ischemic cortical homogenates by aging it was of interest to determine the activities of MDH and ICDH as a function of aging. No difference in activity was observed for either MDH or

TABLE III. Effect of Aging on the Rates of O_2 Consumption, α-KG Utilization and P_i Disappearance in Homogenates of Ischemic and Nonischemic Kidneys.

Preparation		Change (%) from paired fresh control[a,b] $\dot{Q}O_2$	$\dot{Q}_\alpha$-KG	P_i disappearance
Cortex	Nonischemic	−21.7 ± 5.18[c] (5)	−27.7 ± 6.06[d] (6)	−35.2 ± 4.77[e] (6)
	Ischemic	−73.0 ± 8.04 (5)	−70.1 ± 6.96 (5)	−87.4 ± 6.49 (5)
Medulla	Nonischemic	−75.6 ± 7.35 (4)	−70.9 ± 9.80 (4)	−94.1 ± 5.88 (4)
	Ischemic	−79.9 ± 2.01 (5)	−77.6 ± 4.58 (5)	−98.7 ± 0.90 (5)

[a] % change from paired control is [(aged−fresh/fresh) × 100].

[b] p values for difference between mean % change of nonischemic and ischemic preparations are: [c] $p < 0.001$, [d] $p < 0.001$, and [e] $p < 0.001$.

TABLE IV. Effect of Aging on the Activities of MDH and ICDH of Homogenates of Nonischemic and Ischemic Kidneys.

Preparation		Change (%) from paired fresh controls	
		MDH	ICDH
Cortex	Nonischemic	−9.93 ± 1.91[a] (9)	+0.98 ± 7.42 (8)
	Ischemic	−4.52 ± 5.56 (6)	+26.1 ± 7.02 (5)
Medulla	Nonischemic	−9.87 ± 3.04 (6)	+4.10 ± 8.01 (3)
	Ischemic	−4.38 ± 9.08 (4)	−13.0 ± 0.55 (2)

[a] p values for difference between mean % change of MDH in nonischemic and ischemic cortex preparations is >0.15.

ICDH of ischemic and nonischemic tissue as a function of aging as shown in Table IV.

Discussion. Blanching of the inner cortex of dog kidneys appears to be a reflection of renal ischemia which can alter the potential metabolic capacity of the kidney. The blanching phenomenon may be a result of sympathetic discharge over the renal nerves or the release of adrenaline or noradrenaline into the blood brought on by excitement during administration of anesthesia or by surgical trauma. Cortical blanching or ischemia may result in some degree of intrarenal hypoxia. Since stimulation of the renal nerves or infusion of adrenaline or noradrenaline reduces the rate of total renal blood flow which reflects a parallel reduction in rate of both cortical and medullary flow (5) the reduction in PO_2 within the cortex and medulla should be similar. Thus, a selective reduction in the rate of aerobic metabolism in the medulla indicates that medullary tissue is physically changed to a greater extent than cortical tissue following a stress that should be of similar magnitude in both regions of the kidney.

The difference in intrarenal sensitivity of aerobic metabolism may be a consequence of stress in general rather than a specific effect of renal ischemia. We reported previously that 1 hr of RV occlusion reduced the rate of aerobic metabolism of medullary homogenates while no change was observed in cortical homogenates (4). In addition, aging of cortical homogenates prepared from RV-occluded kidneys had a rate of aerobic metabolism which was significantly lower than that of control kidneys. Thus, the results of RV-occluded kidneys parallel those of spontaneous cortical ischemia reported above.

A similar intrarenal separation in aerobic metabolism can be found in the report of Kean *et al.* (6). Mitochondria prepared from medullary tissue of dogs were reported to have a reduced rate of aerobic metabolism when incubated in a hypertonic environment while the rate of metabolism of cortical mitochondria remained unchanged. Thus, the capacity of medullary tissue to carry out aerobic metabolism can be reduced to a greater extent than cortical tissue by: cortical blanching, RV occlusion, and a hypertonic environment. Perhaps other types of stress can also selectively alter the metabolism of the medulla before significantly altering cortical metabolism.

Exactly how ischemic conditions precipitate changes in the rate of aerobic metabolism of the medulla is not known. Perhaps the studies done in cortical tissue may be of some value in understanding this phenomenon. Spontaneous ischemia as indicated above had no overt effect on the rate of aerobic metabolism in cortical tissue. However, cortical tissue could be made to respond to spontaneous ischemia in a manner similar to that of medullary tissue by stressing the cortical homogenates secondarily by aging. The exact change in the homogenates responsible for the observed results is not known. One possible explanation is that both aging and ischemia reduce the number of SH groups in the mitochondria of renal tissue.

Vignais and Vignais (7) have reported that aging reduces the number of SH groups in mitochondria. The activity of a number of Krebs cycle enzymes is dependent on SH

groups. Stressed medullary tissue may have a greater loss of substances such as glutathione which are thought to protect essential SH groups on enzymes. SH groups are necessary for maintaining an intact cell membrane (8). SH groups may also be of importance in maintaining the integrity of the outer membrane of mitochondria, the locus of the Krebs cycle enzymes.

The changes in activity of 2 Krebs cycle enzymes, MDH and ICDH, brought on by spontaneous ischemia, do not appear to be responsible for the changes observed in aerobic metabololism. Spontaneous renal ischemia reduced the activity of both enzymes, the magnitude of which was similar in both cortex and medulla. The activities were not reduced further by aging. Thus, the changes in activity of these particular enzymes are not likely to be responsible for the observed changes in aerobic metabolism caused by either spontaneous ischemia or aging. However, the reduction in activities may be an early indicator of subsequent metabolic changes.

The relative sensitivity of aerobic metabolism of the medulla may be of importance in explaining certain functional changes which are known to occur in the kidney following changes in renal hemodynamics. Reduction in GFR (9), hypotension (10), and severe exercise (11) reduce the capacity of the kidney to elaborate a maximally concentrated urine. Each of these phenomena could be explained by a reduced rate of aerobic metabolism.

Summary. Spontaneous ischemia of the kidney has been reported in dogs subsequent to anesthesia and/or surgical trauma. This phenomenon can be characterized as having a differential effect on intrarenal aerobic metabolism. Washed homogenates of cortical and medullary tissue were prepared from nonischemic and ischemic kidneys. The rates of O_2 consumption, α-KG utilization, and P_i disappearance were the same in cortical homogenates of ischemic and nonischemic kidneys. Each of these rates was significantly lower in medullary homogenates of ischemic kidneys than in nonischemic kidneys. Cortical homogenates that were stressed secondarily by acute aging had rates of O_2 consumption, α-KG utilization, and P_i disappearance that were below that of fresh homogenates. The changes in metabolism cannot be explained by changes that were observed in the activities of MDH and ICDH.

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Received June 8, 1970. P.S.E.B.M., 1970, Vol. 135.