Effects of Ischemia on the Concentration of Adenine Nucleotides in the Kidney of Anesthetized Dogs[1] (34950)

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Adenosine triphosphate (ATP), a major product of both aerobic and anaerobic metabolism, is essential to the transformation of chemical energy to biological work. Although the rates of production and consumption of ATP cannot be measured by available techniques, the quantitation of ATP in tissues is possible and informative. ATP is extremely labile in some tissues, including the renal cortex of rats (1, 2). The medulla of rat kidney, on the other hand, maintains its ATP for as long as 60 sec after extirpation or interruption of blood flow (2). These observations suggest a preponderance of oxidative metabolism in the renal cortex and of anaerobic glycolysis in the medulla.

The present study was designed to compare the effects of ischemia on the adenine nucleotide concentration in the cortex and medulla of dog kidney. We have found important similarities and differences between the published results of ischemia in rat kidney in comparison with dog kidney. In both species, cortical ATP degrades rapidly during ischemia whereas medullary ATP concentration is slightly reduced or not affected. Kidney cortex of the fasting dog contains less ATP than that of the rat. The distribution of the adenine nucleotides in dog cortex after ischemia is apparently determined by the reaction catalized by the enzyme adenylate kinase. Approximately one quarter of the ATP in the intact cortex is dependent upon a continuing flow of blood.

Methods. Renal tissue was obtained from fasting mongrel dogs anesthetized with pentobarbital. Each animal received a liter of isotonic saline intravenously to augment the rates of urine flow and glomerular filtration. This was administered during the 60- to 90-min period needed to complete the surgical preparation. The procedure consisted of catheterization of the ureters at the site of their junction with the urinary bladder and exteriorization of the left kidney through a flank exposure of the retroperitoneal space. Only those animals with rates of urine flow from the exteriorized kidney that were within ± 10% of the flow rate of the *in situ* kidney were utilized in this study. In studies reported elsewhere (3) we have established that this preparation, with the above qualification, results in equivalent renal function of the two kidneys.

When the rates of urine flow had equalized, tissue was obtained for quantitation of the adenine nucleotides. The renal pedicle was clamped with a hemostat at "zero time." Freezing of the tissue was accomplished by one of two methods. In studies at "zero" and at 2 sec, the left kidney was clamped between two 1500-g aluminum blocks chilled to the temperature of liquid nitrogen. A 1-mm thick sample of tissue that was in direct contact with the aluminum blocks was removed and analyzed. In studies at other time intervals, the kidney was sliced with a triple-bladed knife, and the tissue was dropped into liquid nitrogen. The times listed include the estimate of 5 sec needed to freeze tissue so obtained (3).

The adenine nucleotides of both cortical and inner medullary tissues were measured by column chromatography using Dowex-1 resin in the chloride form as previously reported from this laboratory (3). All analyses were performed in duplicate with a precision

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TABLE I. Effect of Ischemia on the Concentration of Cortical Nucleotides.

		Nucleotide concentration (μmoles/g)			Adenylate kinase ratio
Time (sec)	N	AMP	ADP	ATP	$\frac{[AMP]\cdot[ATP]}{[ADP]^2}$
0	6	0.160 ± 0.02	0.565 ± 0.02	1.13 ± 0.05	0.57
2	6	0.210 ± 0.02	0.566 ± 0.04	1.00 ± 0.06	0.62
8	10	0.405 ± 0.04	0.859 ± 0.03	0.763 ± 0.05	0.42
30	3	0.913 ± 0.11	1.01 ± 0.10	0.527 ± 0.08	0.48
45	3	1.00 ± 0.03	1.00 ± 0.09	0.465 ± 0.06	0.47
60	3	1.10 ± 0.16	1.02 ± 0.07	0.385 ± 0.01	0.42

of ± 4%. The data are presented as μmoles/g wet weight of tissue.

Results and Discussion. The results obtained in 31 studies of cortical nucleotides are summarized in Table I. Mean values and ± 1 Standard Error of the Mean (SEM) are tabulated. When tissue was obtained with minimum delay ("zero time"), ATP concentration was 1.13 μmoles/g, equivalent to 61% of the total nucleotide content. Half as much ADP and even smaller amounts of AMP were present in cortical tissue. This distribution of nucleotides is in agreement with previously reported data for rat kidney cortex (1, 2). However, the total nucleotide content of dog kidney is about half that of rat kidney.

ATP concentration declines rapidly after ischemia. After 60 sec only one third of the original ATP remains in cortical tissue. Concomitant with the decrease in ATP, concentrations of AMP and ADP increase. The relationships between the concentration of the three nucleotides as altered by ischemia are presented graphically in Figs. 1, 2, and 3. The curves labeled "A" represent changes in concentration in units of percentage of total nucleotides. The vertical bars represent ± SEM.

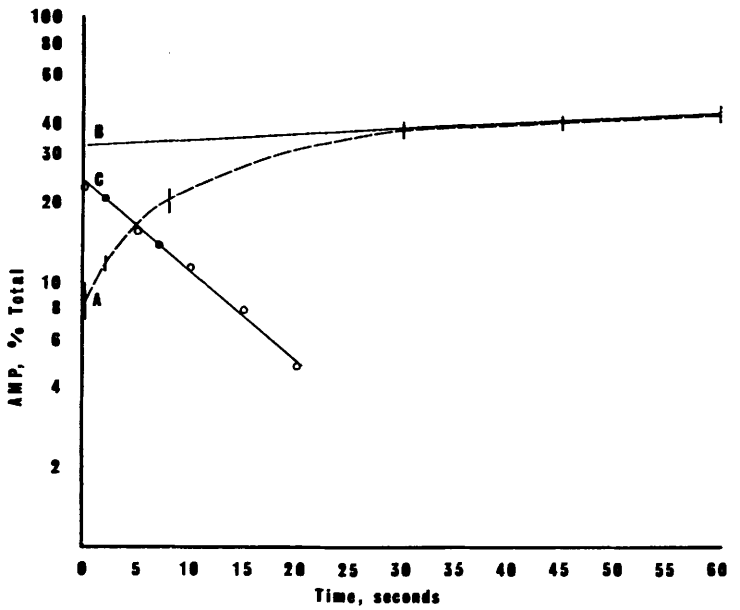

FIG. 1. The change in concentration of AMP after interruption of blood flow. "A" is the original curve. "B" is the slower rate component extrapolated to zero. "C" is the calculated rapid rate component.

Graphical analysis was performed for the purpose of identifying the major rate processes (4). The three "A" curves are linear between 30 and 60 sec. Extrapolation of the linear part of these curves to zero on the time axis permits calculation of slopes that are equivalent to the rates of change in concentration. The slower rate process is labeled

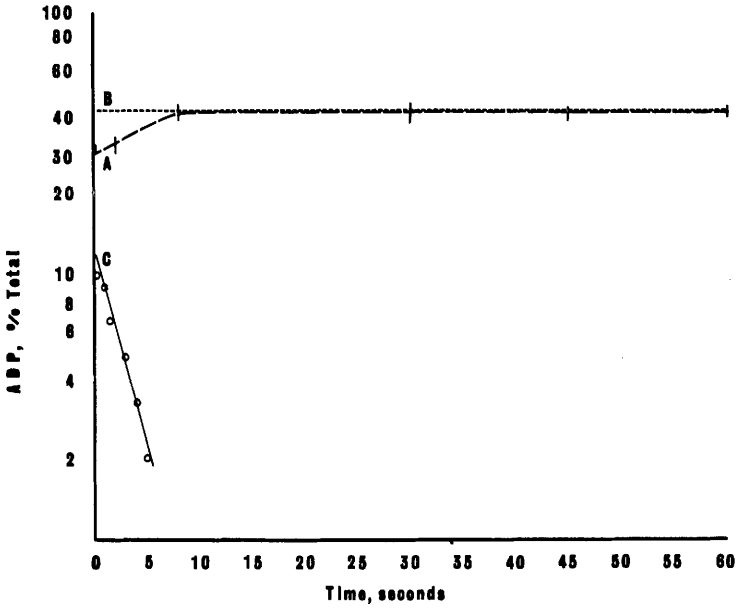

FIG. 2. The change in concentration of ADP after interruption of blood flow. "A" is the original curve. "B" is the slower rate component extrapolated to zero. "C" is the calculated rapid rate component.

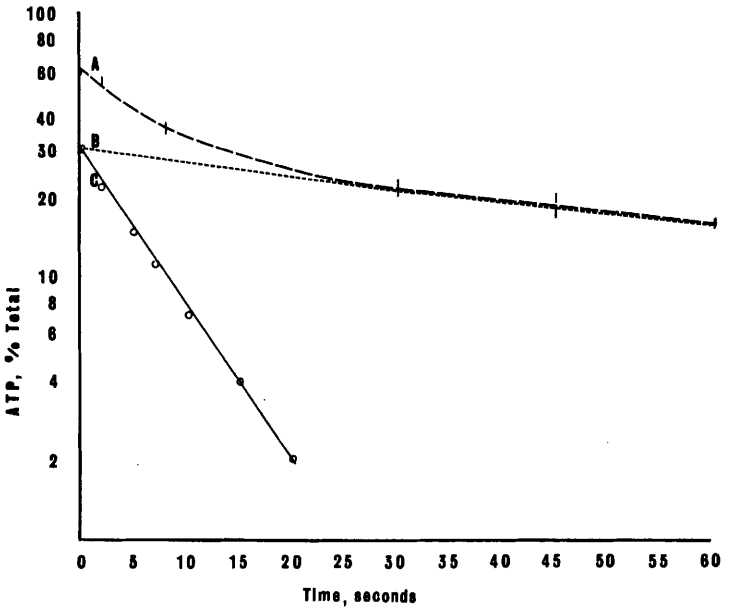

FIG. 3. The change in concentration of ATP after interruption of blood flow. "A" is the original curve. "B" is the slower rate component extrapolated to zero. "C" is the calculated rapid rate component.

"B." The concentration half-time ($t_{1/2}$) of these rates of AMP, ADP, and ATP measured +130, ∞ and −63 sec, respectively. In other words, AMP concentration doubled in 130 sec and ATP concentration was halved in 63 sec. Note that the $t_{1/2}$ for AMP is twice that for ATP and that after 30 sec, ADP remains constant with a slope of zero. This observation lends support to the idea that the adenylate kinase (AMP–ATP phosphotransferase) reaction is a major determinant of the distribution of cortical nucleotides during ischemia. According to this reaction:

$$2ADP \rightleftharpoons AMP + ATP. \quad (1)$$

Simultaneous with this, ATP degrades to ADP and inorganic phosphate (P_i) according to this reaction.

$$ATP \rightleftharpoons ADP + P_i. \quad (2)$$

Thus, as ATP degrades during ischemia, it forms ADP which, in turn, by reaction (1), forms AMP and ATP. The newly formed ATP from (1) cycles back to form additional ADP. According to this sequence, the rate of ATP degradation should be and actually measures to be twice that of AMP formation.

The above evidence for adenylate kinase activity is spported additionally by the calculated ratios presented in Table I. According to Eggleston and Hems (5), the product of $[AMP] \cdot [ATP]/[ADP]^2$ equals 0.45 when equilibrium is reached in reaction (1). After 8 sec of ischemia the values for this ratio *in vivo* are the same as the valves observed *in vitro*.

The cortex of dog differs from that of the rat with respect to adenylate kinase activity. Gerlach *et al.* (1) in a study of the effect of ischemia on nucleotide concentration in the rat kidney reported a rate of ATP decline similar to the present data, but with adenylate kinase ratios that all measure greater than 1.0. Apparently, this enzyme is less dominant as a determinant of adenine nucleotide distribution in rat kidney than is evident from the present study in dog.

The arithmetic difference between the original curves "A" and the extrapolated curves "B" yields a series of points on the graphs that form straight lines labeled "C." The slopes of these lines describe rapid rate processes for each of the three nucleotides. From these, the amount of ATP that is sensitive to ischemia can be calculated. The $t_{1/2}$ for AMP, ADP, and ATP are 9.0, 2.5, and 5.3 sec, respectively. Loss of ATP via reaction (2) is offset in part by contributions from reaction (1). This addition of ATP is exactly equal to increments in AMP from the same reaction. The $t_{1/2}$ for ATP adjusted for this addition equals 3.3 sec.[2] Using this value, the cortex loses 0.171 μmoles/g/sec. Assuming a cortex weight of about 40 g, the ATP loss because of ischemia is 410 μmoles/kidney/min.

TABLE II. Effect of Ischemia on the Concentration of Medullary ATP.

Time (sec)	ATP (μmoles/g)
10	0.387
15	0.360
30	0.371
60	0.401

Total ATP production in the kidney can be calculated from the average renal O_2 consumption and a P:O = 3:1. Average O_2 consumption of the whole kidney of the saline-loaded dog is approximately 0.25 moles/min (7). Assuming that all of the O_2 is used in ATP production, 1500 μmoles/kidney/min is produced. If the above assumptions are correct, approximately 27% (410/1500) of the turnover of ATP is dependent upon a continuing flow of blood.

ATP in the medulla, in contrast, is insensitive to ischemia. An example of the effect of interruption of blood flow on medullary ATP concentration is shown in Table II. This confirms in dog the observations of Jones and Welt (2) obtained in rat kidney. Clamping the renal artery did not affect the concentra-

[2] It is of interest that the $t_{1/2}$ for the rapid-phase uptake of inorganic radiophosphate by ATP in the kidney cortex equals 4.3 sec (6). This is perhaps only a coincidental finding but is consistent with the notion of pool of ATP that turns over with rapidity and which is sensitive to ischemia.

tion of ATP. This finding is appropriate in a tissue such as the medulla in which blood flow is low and in which anaerobic metabolism predominates.

Summary. Ischemia causes a rapid decline in the concentration of ATP in the kidney cortex of anesthetized dogs. Within 8 sec of ischemia distribution of adenine nucleotides is determined by activity of adenylate kinase. Nucleotide metabolism of the cortex of dog is distinguished from that of rat by the observation that concentrations are lower and by the evidence of activity of adenylate kinase. We estimate that blood-flow dependent ATP turnover in the cortex is 27% of that calculated on the basis of renal O_2 consumption. The medulla, in contrast to the cortex, maintains its ATP concentration during 60 sec of interruption of blood flow. Studies of nucleotide metabolism in the kidney must include consideration of differences in adenylate kinase activity between cortex and medulla and between different mammalian species.

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1. Gerlach, E., Bader, W., and Schwoerer, W., Pfluegers Arch. **272**, 407 (1961).

2. Jones, N. F., and Welt, L. P., Amer. J. Physiol. **212**, 939 (1967).

3. Urbaitis, B. K., and Kessler, R. H., Nephron **6**, 217 (1969).

4. Borghgraef, R. R. M., Kessler, R. H., and Pitts, R. F., J. Clin. Invest. **35**, 9, 1055 (1956).

5. Eggleston, L. V., and Hems, R., Biochem. J. **52**, 156 (1952).

6. Kessler, R. H., Landwehr, D., Quintanilla, A., Weseley, S. A., Kaufmann, W., and Arcilia, H., Nephron **5**, 474 (1968).

7. Fujimoto, M., Nash, F. D., and Kessler, R. H., Amer. J. Physiol. **206**, 1327 (1964).

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