JCI The Journal of Clinical Investigation

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J Clin Invest. 1968;47(9):2133-2142. https://doi.org/10.1172/JCI105899.

Research Article

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Brain Metabolism in Uremic and Adenosine-Infused Rats

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ABSTRACT Analyses of nucleotides and glycolytic intermediates were performed on perchlorate extracts of blood and quick-frozen brain from rats nephrectomized 48 hr previously, and from rats infused for 6 hr with adenosine or AMP. Blood nucleotides of acutely uremic rats were normal. Uremic brain showed an increase of creatine phosphate (CP), ATP, and glucose with a corresponding decrease in creatine, ADP, AMP, and lactate. Other nucleotide triphosphates were increased, but total adenine nucleotide in brain was unchanged. Uremic brain failed to use ATP or produce ADP, AMP, and lactate at normal rates when subjected to the stress of ischemic anoxia. Although levels of cation responsive ATPase in extracts of uremic brain were normal, the inhibition of glycolysis in the intact brain appeared to be due to a failure of ATP hydrolysis (a diminished ATPase activity). Adenosine infusion produced mild azotemia, marked hyperglycemia, an increase in blood ATP, and an increase in total blood adenine nucleotide. Brain from rats infused with adenosine or AMP also had high levels of ATP, creatine phosphate, and glucose, whereas levels of ADP, AMP, and lactate were low. However these brains responded with normal use of ATP and normal production of lactate when stimulated by ischemic anoxia.

This work was presented in part at the meeting of the Academy of Neurology, April 1967, San Francisco, Calif.

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Received for publication 5 February 1968 and in revised form 13 May 1968.

INTRODUCTION

The pathogenesis of uremic encephalopathy remains obscure. In an effort to improve our understanding of this state, a study was made of brain and blood nucleotides in acutely uremic rats. The choice of this approach was influenced by reports of abnormally high nucleotide and sugar phosphate levels in erythrocytes of uremic patients (1). Since incubation of erythrocytes with adenine is also known to produce a net increase in total adenine nucleotide (2), it seemed possible that increased tissue levels of adenine or other precursors might accumulate during renal insufficiency and lead to high levels of nucleotide in brain. These elevated nucleotides might play some role in the pathogenesis of the uremic state. For this reason changes were also sought in brain and blood nucleotides after infusion of rats with adenosine or AMP. In these experiments the object was to produce a similar disturbance in nucleotide metabolism without uremia. Changes in glycolysis were also measured in brains of uremic and infused rats.

METHODS

100 g Fisher female rats were nephrectomized under ether and maintained on water ad lib. for 48 hr. Other unanesthetized rats received 0.5 mmole of adenosine or AMP,[1] i.p. by injection or by a 6 hr infusion with a flexible catheter and a Harvard Apparatus pump. Rats were guillotined so that the heads fell into more than

[1] Adenosine and AMP were purchased from Calbiochem, Los Angeles, Calif.; both were dissolved with warming in 10 ml of water and neutralized with IN NaOH. Adenosine partially reprecipitated during infusion.

20 volumes of liquid nitrogen while blood drained from the torso into a dish containing heparin. In selected animals, decapitated heads were held at room temperature for 30 sec before freezing. 1.0 ml of blood was mixed with 7.0 ml of ice-cold 0.6 M $HClO_4$ in 1.22 mM EDTA, stored on ice for 10 min, and centrifuged in the cold at 1600 *g* for 10 min. Supernatants were neutralized with 3.3 M K_2CO_3 containing 0.5 M KCl and were recentrifuged; these supernatants were then frozen at −70°C until analysis.

In a −20°C room, the cerebral hemispheres, cerebellum, and brain stem were removed with bone clippers and ground in a mortar under liquid nitrogen. 1.0 g of whole brain powder was weighed on a spiral spring balance (sensitivity: 0.002 g at −20°C) and homogenized with 2.0 ml of 6 M $HClO_4$. Homogenates were removed to a 4°C room, diluted with 18.0 ml of 1.22 mM EDTA and recentrifuged at 2900 *g* for 15 min. Supernatants were neutralized and stored as above.

ATPase was extracted from freshly dissected unfrozen brains with a solution containing 0.25 M sucrose, 0.03 M histidine, 0.005 M EDTA, and 0.1% sodium deoxycholate (3). These brains were ground with this solution in all glass homogenizers at 4°C. The homogenates were centrifuged at 10,000 *g*, and analysis was performed on the supernatants within 24 hr. 0.1 ml of supernatant was incubated at 37°C in the presence of 3 mM ATP, 3 mM $MgCl_2$ and 100 mM NaCl with and without 30 mM KCl in a final volume of 3.0 ml. Inorganic phosphate (P_i) was measured colorimetrically (4). The protein content of these extracts was measured with the Biuret reaction (5).

Hematocrits were done in Wintrobe tubes. Blood urea nitrogen was measured with diacetyl (6) and blood creatinine with alkaline picrate (7).

Enzymic analyses were based on the optical density change at 340 mμ due to oxidation or reduction of pyridine nucleotides. The details of these methods are shown in Table I. The specificity of phosphoglycerate kinase and hexokinase methods for nucleotide triphos-

TABLE I

*Analytic Conditions**

Substance	Buffer	Enzymes	Other additions†	Sample	Total volume
				ml	*ml*
1 ATP	Triethanolamine *144 μmoles*; pH 7.5.	Yeast hexokinase, *20 μg* Glucose-6-phosphate dehydrogenase, *4 μg*	$MgCl_2$, *12 μmoles* NADP, *0.65 μmole* Glucose, *20 μmoles*	0.4	2.24
2 ADP	Triethanolamine *50 μmoles*; pH 7.5	Muscle lactic dehydrogenase, *2 μg* Pyruvate kinase, *20 μg*	$MgSO_4$, *11 μmoles* KCl, *92 μmoles* Phospho-enol-pyruvate, *1.5 μmole* NADH, *0.35 μmole* ATP, *0.02 μmole*	1.50	2.20
3 AMP	Same as 2	Same as 2, plus adenylate kinase *10 μg*	Same as 2	1.50	2.20
4 ATP, GTP, ITP	Triethanolamine *151 μmoles*; pH 7.5	Glyceraldehyde-3-phosphate dehydrogenase, *80 μg* Phosphoglycerate kinase, *80 μg*	Hydrazine sulfate, *6.2 μmoles* $MgSO_4$, *6.2 μmoles* 3-Phosphoglycerate, *20 μmoles* NADH, *0.28 μmole*	0.4	2.24
5 Creatine phosphate	Same as 1	Same as 1, plus creatine phosphokinase, *20 μg*	Same as 1, plus ADP, *0.2 μmole*	0.4	2.30
6 Creatine	Glycine *155 μmoles*; pH 9.0	Muscle lactic dehydrogenase, *22 μg* Pyruvate kinase, *55 μg* Creatine phosphokinase, *1000 μg*	$MgCl_2$, *1.1 μmoles* Phospho-enol-pyruvate, *2.1 μmoles* NADH, *0.33 μmole* ATP, *1.1 μmoles*	0.2	3.31
7 Glucose	Triethanolamine *84 μmoles*; pH 7.5	Yeast hexokinase, *20 μg* Glucose-6-phosphate dehydrogenase, *4 μg*	$MgCl_2$, *12 μmoles* NADP, *0.65 μmole* ATP, *10 μmoles*	1.0	2.24
8 ‡Glucose-6-phosphate	Tris *30 μmoles*; pH 7.5	Glucose-6-phosphate dehydrogenase, *0.1 μg*	NADP, *0.065 μmole*	0.3	3.00
9 Dihydroxyacetone, glyceraldehyde phosphates	Triethanolamine *60 μmoles*; pH 7.5	Glycerophosphate dehydrogenase/triosephosphate isomerase, *20 μg*	NADH, *0.28 μmole*	1.50	2.14
10 Fructose diphosphate	Same as 9	Same as 9, plus aldolase, *40 μg*	Same as 9	1.50	2.14
11 Lactate	Glycine *1500 μmoles*; pH 9.7	Beef heart lactic dehydrogenase, *200 μg*	Hydrazine sulfate, *600 μmoles* EDTA, *7.5 μmoles* NAD, *6 μmoles*	0.4	2.10

* All reagents expressed as amount per cuvette. All enzymes purchased from Boehringer except beef heart lactic dehydrogenase which was purchased from Worthington.

‡ In this instance analysis performed in Farrand fluorimeter with excitation at 357 mμ and emission at 450 mμ.

phates is indicated in Table II. The presumed absence of ITP from brain (8) together with the specificity of hexokinase for ATP indicate that the difference between hexokinase and phosphoglycerate kinase levels of triphosphate provides an indirect measure of brain GTP. In blood this difference would include ITP. These differences are referred to in the following as nonadenine nucleotide triphosphate. All values are reported as means ± standard error.

TABLE II

*Substrate Specificity of Phosphoglycerate Kinase and Yeast Hexokinase Assays**

Substrate	Δ OD‡	
	Phosphoglycerate kinase	Yeast hexokinase
0.1 μmole/ml reaction mixture		
ATP	0.111	0.114
GTP	0.112	0.002
ITP	0.111	0.025*
UTP	0.015*	0.000

* Enzymes from Boehringer; Substrates from Calbiochem, except ITP which was from Pabst Lab.

‡ OD recorded occurs over 90 sec, at which time all reactions are complete except those marked with an asterisk which remain incomplete at 8 min.

RESULTS

The values reported here for normal young adult female Fisher rat blood and whole brain have no precise counterpart in published reports. Table III compares the present data with comparable published data. It is likely that the difference between the present results and those of Lowry et al (9–11) are largely due to the smaller head size in the mouse and differences in freezing technique.

Rats killed 48 hr after nephrectomy were tremulous and excessively responsive to loud noise, whereas spontaneous activity was slightly reduced; righting responses were normal. Infusions of adenosine and AMP appeared to have a slight "taming" effect. Blood urea nitrogen rose from 18 ±1 mg/100 ml in normal rats to levels of 240 ± 8 mg/100 ml in acutely uremic rats. After adenosine or AMP infusion the BUN was 53 ± 4 mg/100 ml. In acutely uremic rats the pH of blood collected from the torso was 7.16 ± 0.02 as opposed to 7.53 ± 0.03 in normal controls. AMP-infused rat blood pH was 7.36 ± 0.06.

Alterations of blood nucleotides in uremic and infused rats are compared in Fig. 1. Acutely uremic rats did not show significant changes in blood adenine nucleotides. There was a significant ($P < 0.001$) increase in the fraction designated as nonadenine nucleotide triphosphate (Table IV). Adenosine and AMP infusion did produce a significant ($P < 0.001$) rise in each of the individual and total adenine nucleotides. The small rise of nonadenine nucleotide triphosphate in response to adenosine infusion was not significant (Table IV).

Brain nucleotide changes in these same groups are shown in Fig. 2. Infused and uremic rats all show significant increases in ATP (all $P <$ 0.005). However, in each instance there is a statistically significant ($P < 0.001$) decline in both ADP and AMP so that total adenine nucleotide

TABLE III

Comparison of Present Data with Previous Reports

	Present study*	Mouse (9–11)	Rat (8)	Rat (12)	Rat (13)	Rat (14)
Brain, *μmoles/g*						
Creatine	8.5 ± 0.2					7.9
Phosphocreatine	1.5 ± 0.1	2.4				3.2
ATP	2.1 ± 0.1	2.4	1.4	2.1	2.2	2.6
ADP	0.8 ± 0.1	0.9	0.2			1.0
AMP	0.5 ± 0.1	0.2	0.03			0.5
Glucose	0.6 ± 0.1	0.6–1.5				
Fructose diphosphate	0.17 ± 0.01			0.10		
Blood, *μmoles/ml of erythrocytes*						
ATP	1.0 ± 0.1			1.0‡		

* Normal rats, means ± SE.

‡ Recalculated with assumed hematocrit of 50.

TABLE IV

*Nonadenine Nucleotide Triphosphate: Calculated As Difference between Phosphoglycerate Kinase and Hexokinase Assays**

	Brain	Blood
	μmoles/g	*μmoles/ml of erythrocytes*
Normal (23)	0.56 ± 0.05	0.28 ± 0.02
Uremic (18)	0.69 ± 0.06	0.48 ± 0.05
Adenosine infused (8)	0.76 ± 0.11	0.40 ± 0.09
AMP infused (4)	0.90 ± 0.08	0.22 ± 0.04

Values represented are ± SE.

* Results represent predominantly GTP in brain and GTP + ITP in blood.

is unchanged. Nonadenine nucleotide triphosphate is also increased in uremic and infused brains but these changes are not statistically significant ($P < 0.2$) (Table IV). Creatine phosphate in brain is increased ($P < 0.001$) in uremic and infused brains with a corresponding decline in free creatine ($P < 0.01$) (Fig. 2). Brain lactate is low and brain glucose is high in uremic and infused groups (Fig. 2). Increases in brain glucose in uremic rats were not due to hyperglycemia; blood glucose was 100 ± 5 mg/100 ml in both normal and uremic rats. By contrast, infusion of 0.5 mmole of adenosine over 6 hr in fasting rats led to blood sugar levels of 307 and 435 mg/100 ml.

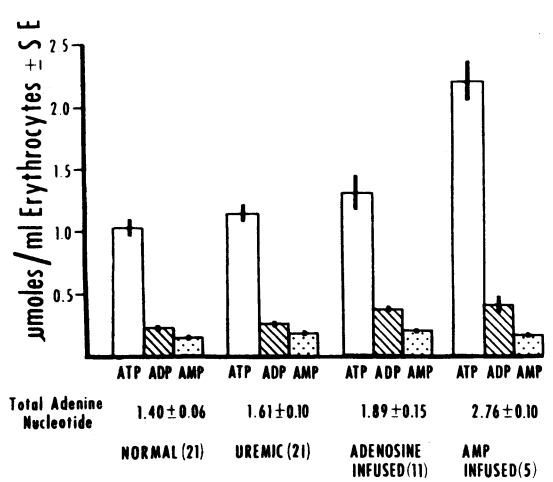

FIGURE 1 Erythrocyte adenine nucleotides in normal, uremic, and infused rats. (No. of rats in parentheses.)

The response of brain to 30 sec of ischemic anoxia achieved by delayed freezing of the severed head is presented in Fig. 3. Each point represents

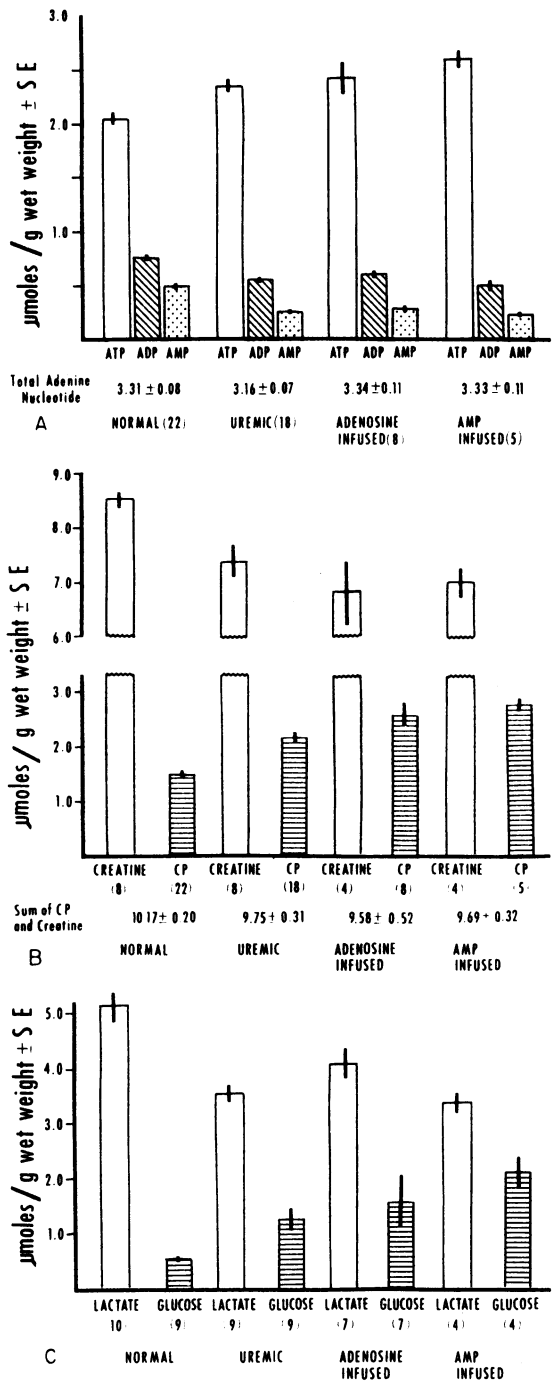

FIGURE 2 Brain levels of adenine nucleotides, creatine phosphate, creatine, glucose, and lactate in normal, uremic, and infused rats. (No. of rats in parentheses).

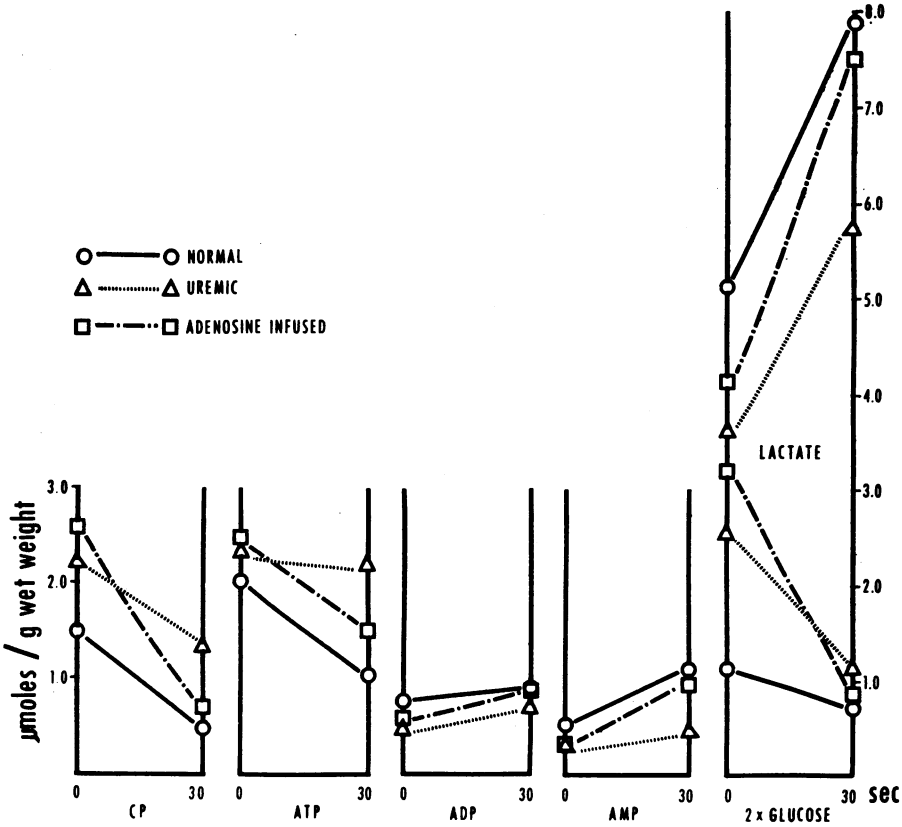

FIGURE 3 Glucose is plotted as twice the observed value to facilitate comparison with lactate changes.

the average of at least five rats. In normal rats there is a sharp fall in creatine phosphate and ATP, whereas AMP, ADP, and free creatine (not shown) rise. Lactate increases but the already low value of glucose changes little. In uremic rats, although creatine phosphate falls rapidly, ATP is maintained near the initial level with only a slight rise in AMP and ADP. Production of lactate is less than normal but the glucose consumption, initiated at a much higher glucose level, is increased. Rats injected with adenosine respond to ischemic anoxia with changes that resemble those of the normal rat; the only notable differences are that brain glucose is initially high and the magnitude of the fall in brain glucose during ischemia is unusually large. The percentage of lactate produced that can be accounted for as glucose consumed is 14% in the normal, 90% in the uremic, and 65% in the adenosine-infused rats. There is little doubt that the lactate which does not originate from glucose is derived from glycogen (9).

The rate at which glucose and labile phosphate ($\backsim$ P) are utilized after decapitation may be used to calculate a metabolic rate of brain. Gatfield et al. (11) have calculated the metabolic rate of mouse brain in similar circumstances with the formula:

$$\Delta \backsim P = 2\,\Delta ATP + \Delta ADP + \Delta CP + 1.45\,(\Delta lactate - 2\,\Delta glucose) + 2\,\Delta glucose.$$

They give a value of 5.4 μmoles of $\backsim$ P/g per 30 sec for unanesthetized mice. Application of this formula to the present data gives a value of 7.09 ± 0.70 μmoles of $\backsim$ P/g per 30 sec for normal Fisher rats. In the uremic rat this value fell to 3.94 ± 0.60 μmoles of $\backsim$ P/g per 30 sec ($P < 0.005$). In the adenosine injected rats it was 7.02 ± 1.80 μmoles of $\backsim$ P/g per 30 sec.

Brain levels of triose phosphate, fructose diphosphate, and glucose-6-phosphate are given in Table V. Fructose diphosphate is slightly increased in

TABLE V

Glycolytic Intermediates in Various Experimental Groups

	Brain					
	Triosephosphate		Fructose diphosphate		Glucose-6-phosphate	
	Immediate	30-sec Ischemia	Immediate	30-sec Ischemia	Immediate	30-sec Ischemia
	μmoles/g		*μmoles/g*		*μmoles/g*	
Normal	0.12 ± 0.01	0.10 ± 0.01	0.17 ± 0.01	0.18 ± 0.02	0.06 ± 0.02	0.06
Uremic	0.12 ± 0.01	0.11 ± 0.01	0.20 ± 0.01	0.15 ± 0.01	0.08	0.07
Adenosine infused	0.14 ± 0.02	0.15 ± 0.02	0.18 ± 0.02	0.18 ± 0.02	0.07	
AMP infused	0.14		0.21		0.08	

Values represented are ± SE.
(Values with standard errors based on 5–8 samples; values without errors are averages of 2–4 samples.)

uremic rat brain and falls in response to ischemia only in the uremic rat.

Because reactants and products of the creatine phosphokinase, adenylate kinase, and phosphofructokinase reactions were measured, the ratios of these were calculated (Table VI). The adenylate kinase ratio, $(ATP)(AMP)/(ADP)^2$, was normal in uremic blood and brain but fell in blood and rose in brain in response to precursor infusion. In accord with previous reports this ratio fell in response to ischemic anoxia. The creatine phosphokinase ratio, $(ATP)(C)/(ADP)(CP)$, was similar at zero time in normal and infused brains; in both instances the ratio rose sharply in response to anoxia. The uremic brain has a normal ratio at zero time and this ratio remains absolutely unchanged during ischemic anoxia. Table VI also records the ratio of reactants and products for the phosphofructokinase reaction. In the normal rat ischemia provokes a sharp increase in this ratio; in the uremic rat the initial ratio is depressed and rises only slightly in response to ischemia. With adenosine injection the initial ratio is low as in the uremic rat but rises strikingly in response to ischemia as it does in the normal rat.

Adenosine infusion was carried out for 6 hr before sacrifice in a few rats who had been nephrectomized 42 hr previously. The effects are

TABLE VI

Ratio of Reactants and Products of Adenylate Kinase, Creatine Phosphokinase, and Phosphofructokinase in Normal, Uremic, Infused, and Ischemic Rats

	$\frac{(ATP)(AMP)}{(ADP)^2}$		$\frac{(ATP)(C)}{(CP)(ADP)}$		$\frac{(FDP)(ADP)}{(G6P)(ATP)}$	
	Immediate	30-sec Ischemia	Immediate	30-sec Ischemia	Immediate	30-sec Ischemia
Brain						
Normal	1.7	1.5	15.2	24.2	1.04	2.57
Uremic	2.0	1.6	14.4	14.5	0.59	0.73
Adenosine infused	1.9	1.8	11.0	20.1	0.63	2.16
AMP infused	2.5		13.5			
Blood						
Normal	3.2					
Uremic	3.1					
Adenosine infused	2.0					
AMP infused	2.2					

TABLE VII

Blood and Brain Composition of Rats 48 hr after Nephrectomy and after 6 hr Infusion of 0.5 mM Adenosine

BUN, *mg/100 ml*	207 (168–226)
Blood nucleotides, *μmoles/ml of erythrocytes*	
Nonadenine nucleotide triphosphate	1.26 (0.99–1.76)
ATP	2.48 (2.29–3.12)
ADP	0.44 (0.29–0.74)
AMP	0.25 (0.21–0.34)
Brain constituents, *μmoles/g*	
CP	2.75 (2.28–3.00)
Nonadenine nucleotide triphosphate	1.13 (0.22–1.68)
ATP	2.14 (1.68–2.58)
ADP	0.58 (0.37–0.71)
AMP	0.28 (0.23–0.32)
Lactate	2.04 (1.87–2.28)
Glucose	1.64 (1.33–2.10)
Triose phosphate	0.08 (0.14–0.20)
Fructose diphosphate	0.18 (0.14–0.20)

Values are average of four rats with range in parentheses.

shown in Table VII. In blood, the rise in ATP and nonadenine nucleotide triphosphate exceeds the expected additive effects of acute uremia and adenosine infusion. In this small group of animals the increase in brain ATP is not significant. Creatine phosphate and brain glucose were increased. Brain lactate was lower than might have been expected from the additive effects of uremia and adenosine infusion. Brain triose phosphate was depressed, whereas fructose diphosphate was normal. Brain nonadenine nucleotide triphosphate was markedly increased.

Cation responsive ATPase values for a 10,000 *g* supernatant fraction of uremic and normal brain showed no significant differences (Table VIII).

DISCUSSION

Erythrocytes from uremic patients show an increase in total adenine nucleotide (1). This of necessity requires net synthesis of adenine nucleotide from phosphate, ribose, and adenine, or smaller precursors of the purine ring. That this nucleotide should appear as ATP is to be expected in the presence of active glycolysis. In contrast to the situation in the human, acute uremia in the rat failed to produce a significant change in erythrocyte adenine nucleotide. The infusion of adenosine into normal or acutely uremic rats did result in an increase in erythrocyte ATP and total adenine nucleotide. The response of the acutely uremic rat to adenosine infusion is especially marked and indicates an at least normal potential for the generation of erythrocyte ATP in uremia. Conceivably the hypothesis that a retained adenine nucleotide precursor leads to high erythrocyte ATP in the human is correct; in the acutely uremic rat it is obviously wrong. More chronic experiments (to be published) do show an increase in erythrocyte ATP and total adenine nucleotide. It may be that the duration of uremia and its effects on the average age of circulating erythrocytes determine these differences between acute and chronic uremia.

Clearly, the increase in brain nucleotides originally predicted in uremia and after adenosine infusion did not occur and could not account for the central nervous system symptoms of uremic rats. Nonetheless, notable alteration of brain nucleotide distribution and of brain metabolism was found in acutely uremic and in precursor-infused rats. The most obvious changes in uremic rat brain were a marked decrease in lactate formation, a 50% decrease in calculated high energy phosphate utilization, and a marked attenuation of the nucleotide response to acute ischemic anoxia. In the response of normal brain to ischemia, ATP breakdown occurs much more rapidly than it can be generated from creatine phosphate or through adenylate kinase and glycolytic reactions from AMP or ADP; AMP therefore accumulates (Fig. 3). If glycolysis itself were inhibited by uremia, the breakdown of creatine phosphate and ATP in response to ischemia would be even more extensive in uremic than in normal brain because the regeneration of ATP by glycolysis would be impaired. Instead a decreased breakdown of ATP and a failure of ADP and AMP to accumulate is

TABLE VIII

Cationic Sensitive ATPase in 10,000 × g of Supernatant of Brain Homogenates

	100 mM Na^+	100 mM Na^+ 30 mM K^+	Δ
No.	*μmoles of Pi/mg of protein per hr*		
Normal (6)	15.81 ± 0.79	23.11 ± 0.41	7.31 ± 0.74
Uremic (6)	16.44 ± 0.44	24.11 ± 0.89	7.83 ± 1.05

observed in uremic brain (Fig. 3). It is concluded that the disordered brain function of acute uremia is associated with a defect in energy transformation manifested by a failure to use ATP. A similar failure to use ATP during ischemic anoxia has been reported during barbiturate anesthesia ([9] Figs. 2 and 3).

Direct evidence of altered ATPase activity could not be obtained from measurement of total and cation sensitive ATPase in brain homogenates. This does not exclude diminished activity of ATPase in vivo. Direct evidence that the Na- and K-sensitive ATPase of the red cell membrane is diminished in uremia has been presented by Welt, Sachs, and McManus (16).

It is necessary to consider the possibility that the acidosis of uremia may inhibit brain glycolysis, whatever the site of metabolic control. Acidosis is known to inhibit erythrocyte glycolysis (17). If, as suggested by Lowry et al (9), the ratio of reactants and products of the creatine phosphokinase reaction is an indicator of brain pH, then uremic brain was not acidotic at zero time and was less acidotic after 30 sec of ischemia than was either the normal or the precursor-infused rat brain. The hydrolysis of each μmole of ATP to ADP and P_i releases 1 μmole of H^+ ions as does the formation of each μmole of lactic acid. Because both these processes are diminished in uremic brain, failure of brain pH to fall may have been the result rather than the cause of diminished glycolysis. A normal intracellular pH in the presence of extracellular metabolic acidosis is quite consistent with present concepts of acid-base regulation (18).

The observed decrease of glycolysis in uremic brain appears to be a consequence rather than the cause of the reduced use of ATP. The control of glycolysis by the availability of ADP, AMP, and P_i has been recognized since the fundamental work of Meyerhof (19). This is also clear from the work of Lowry and his associates working with an experimetnal design similar to the one used in the present study. They found that ischemic anoxia leads to an accumulation of ADP, AMP, and presumably P_i; these compounds activate phosphofructokinase and lead to a burst of glycolysis. Their data clearly establishes phosphofructokinase as the major control point in glycolysis under these conditions (9). In the present study the ratio of reactants and products of the phosphofructokinase reaction, (FDP)(ADP)/(G6P)(ATP), (Table VII) may give an indication of the behavior of this enzyme. In the uremic rat this ratio is 60% of normal at zero time and does not rise in response to ischemia. In control rats the ratio more than doubles in response to ischemia. This change, the normal response to anaerobiosis, was due principally to the fall in ATP and the rise in ADP. ATP inhibits whereas AMP and ADP activate this reaction (9). The observed failure of this ratio to increase in uremia is the expected result of decreased ATP breakdown. The observation that the level of fructose diphosphate fell during ischemia in uremic rat brain also suggests a failure to supply fructose diphosphate to the subsequent reactions of glycolysis at the rate required. Because detailed studies of this reaction and of other control points of glycolysis during the early part of ischemia were not done in the present study, the relative importance of the phosphofructokinase reaction in the over-all decrease of glycolysis cannot be stated. When the above ratio is calculated from the data of Lowry et al. (9) for anesthetized mice and is compared with that found in normal mice, it appears that this ratio is also markedly decreased by anesthesia. It is likely therefore that the inhibition of glycolysis in uremia and in anesthesia occurs at the level of the phosphofructokinase reaction and is a direct consequence of the failure of brain to consume ATP at a normal rate. The metabolic rate of brain as calculated from the rate of use of high energy phosphate during ischemic anoxia is reduced in uremic brain. This metabolic rate has been shown to closely reflect the metabolic rate of brain in vivo (9). Our data therefore strongly suggest a depression of energy metabolism in uremic brain during life. A depression of cerebral oxygen consumption in vivo in uremia has been previously reported (15).

It is not surprising that such a decrease in metabolic rate should be associated with altered substrate concentrations in brains frozen immediately after decapitation. It has seemed plausible to relate the high brain glucose in uremic rats to the reduced utilization of high energy phosphate postulated to occur in these animals during life. This assumes, as does the work of Lowry et al. (9), that the utilization rates immediately after decapitation reflect high energy phosphate use in

life and that glucose metabolism is the source of the high energy phosphate. According to this formulation, the low brain glucose in the normal rat brain is due to rapid glucose consumption compared with the rate of delivery across the blood-brain barrier during life. The high brain glucose in the uremic rat merely reflects the fact that a slower rate of glucose consumption will permit brain glucose to more closely approach blood glucose during life. A marked increase of brain glucose is also seen in anesthesia and the extent of this increase has led others to suggest that, in addition to a decreased glucose consumption in anesthesia, there may be a primary increase of transport of glucose into brain (10, 11). An increase in the extracellular fluid volume of brain in anesthesia and uremia could also produce a high brain glucose. Neither the rate of glucose transport into brain nor extracellular volume of brain was measured in the present study.

Having shown that glycolysis is slowed in uremic brain, it is necessary to explain why consumption of glucose per se during the first 30 sec after decapitation is greater in the uremic than in the normal rat. Ischemic brain consumes glucose in preference to glycogen so that glucose is very quickly depleted from previously normal brain after decapitation and lactate production must then depend on glycogen (9). In uremic brain there is a much higher level of glucose at the time of decapitation, so that during the 30 sec period of ischemia the consumption of glucose per se exceeds that which is possible in the already glucose-depleted normal brain. In spite of this the actual total production of lactate and high energy phosphate utilization during this interval is decreased in uremic brain. The high glucose consumption observed after adenosine infusion indicates that a high glucose concentration in the brain does not itself inhibit glycolysis or high energy phosphate utilization.

It is difficult to ascribe the changes that follow adenosine infusion to the mild renal failure seen in these rats or to relate this minimal renal failure to the 2,8-dioxyadenine toxicity observed in chronic experiments by Philips, Thiersch, and Bendich (20). The elevation of nucleotide triphosphate at the expense of ADP and AMP, the high levels of cerebral glucose, and the large contributions of free glucose to lactate formation are phenomena shared by uremia and the response to adenosine infusion. On the other hand the rate of high energy phosphate utilization was diminished in uremia and was normal in adenosine-infused rats. It is possible that the high ATP/ADP and ATP/AMP ratios and the high brain glucose in the adenosine-infused rat can be shown to reflect a high potential for the generation of high energy phosphate compared with the potential for its hydrolysis. According to such an hypothesis, the changes in uremia are due to decreased hydrolysis of ATP in the presence of a normal capacity for generation of ATP. The same changes after nucleoside or nucleotide administration are due to an increased potential for ATP generation in the presence of a normal hydrolytic potential.

It is of interest to compare these observations with the known effects of anesthesia (10). A high level of labile phosphate sustained after decapitation and a diminished glycolytic response to ischemia are features of both uremia and anesthesia. Although the corresponding behavioral states seem markedly different, it is recognized that the irritability of the uremic rat appears on a background of diminished spontaneous activity and represents a stage in the evolution of uremic coma. However no sharp proportionality of consciousness and levels of labile phosphate can be discerned. Until the adenosine and AMP infusion effects were found, it seemed reasonable to accept a rough relationship of depressed brain function to high levels of labile phosphate and to low rates of high energy phosphate utilization. However, the adenosine data clearly show that an increase in labile phosphate may occur with little change in behavior and without depression of the response to ischemia anoxia. The more fundamental correlation is between depression of the central nervous system and a decrease in ATP utilization for energy transformation.

ACKNOWLEDGMENTS

This work was supported by grant 507 from The Kidney Foundation of Ohio and grants HD00669, FR 5410-06, and 642-5170 from the U. S. Public Health Service, National Institutes of Health.

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