Effects of Added Adenine Nucleotides on Renal Carbohydrate Metabolism

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1. The regulatory effects that adenine nucleotides are known to exert on enzymes of glycolysis and gluconeogenesis were demonstrated to operate in kidney-cortex slices and in the isolated perfused rat kidney by the addition of exogenous ATP, ADP and AMP to the incubation or perfusion media. 2. Both preparations rapidly converted added ATP into ADP and AMP, and ADP into AMP; added AMP was rapidly dephosphorylated. AMP formed from ATP was dephosphorylated at a lower rate than was added AMP, especially when the initial ATP concentration was high (10 mM). Deamination of added AMP occurred more slowly than dephosphorylation of AMP. 3. Gluconeogenesis from lactate or propionate by rat kidney-cortex slices, and from lactate by the isolated perfused rat kidney, was inhibited by the addition of adenine nucleotides to the incubation or perfusion media. In contrast, oxygen consumption and the utilization of propionate or lactate by slices were not significantly affected by added ATP or AMP. 4. The extent and rapidity of onset of the inhibition of renal gluconeogenesis were proportional to the AMP concentration in the medium and the tissue, and were not due to the production of acid or P_i or the formation of complexes with Mg^{2+} ions. 5. Glucose uptake by kidney-cortex slices was stimulated 30–50% by added ATP, but the extra glucose removed was not oxidized to carbon dioxide and did not all appear as lactate. Glucose uptake, but not lactate production, by the isolated perfused kidney was also stimulated by the addition of ATP or AMP. 6. In the presence of either glucose or lactate, ATP and AMP greatly increased the concentrations of C_3 phosphorylated intermediates and fructose 1,6-diphosphate in the kidney. There was a simultaneous rise in the concentration of malate and fall in the concentration of α -oxoglutarate. 7. The effects of added adenine nucleotides on renal carbohydrate metabolism seem to be mainly due to an increased concentration of intracellular AMP, which inhibits fructose diphosphatase and deinhibits phosphofructokinase. This conclusion is supported by the accumulation of intermediates of the glycolytic pathway between fructose diphosphate and pyruvate. 8. ATP or ADP (10 mM) added to the medium perfusing an isolated rat kidney temporarily increased the renal vascular resistance, greatly diminishing the flow rate of perfusion medium for a period of several minutes.

AMP is known to inhibit gluconeogenesis, mainly on account of inhibition of fructose 1,6-diphosphatase, and to promote the degradation of glucose, mainly on account of activation of phosphofructokinase (for review see Newsholme & Gevers, 1967). The experiments reported in this paper show that in rat kidney, a tissue in general more permeable to metabolites than other mammalian tissues, externally added AMP affects

gluconeogenesis and glycolysis in the expected direction. Externally added ATP was rapidly degraded, mainly to AMP, and affected metabolism in the same way as AMP. Both these nucleotides inhibited glucose synthesis and promoted glucose degradation. These findings apply equally to rat kidney-cortex slices and to the isolated perfused rat kidney.

EXPERIMENTAL

Incubation of kidney-cortex slices. Washed kidney-cortex slices from male rats were incubated as described by Weidemann & Krebs (1969), either in the phosphate-buffered

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medium of Krebs & de Gasquet (1964) or in the bicarbonate medium of Krebs & Henseleit (1932). Total respiratory CO_2 was collected and determined manometrically as described by Krebs, Hems, Weidemann & Speake (1966). For the determination of metabolites the medium was deproteinized with HClO_4 as described by Gevers & Krebs (1966).

Perfusion of isolated kidneys. Perfusions were carried out as described by Nishiitsutsuji-Uwo, Ross & Krebs (1967) except that, to approximate the conditions of circulation more closely to those *in vivo*, a pulsatile input to the right renal artery was generated with a roller-pump through a plastic distributor, instead of perfusing by hydrostatic pressure. The 'systolic' pressure was about 120 mm. Hg (measured with a sphygmomanometer gauge) and the 'diastolic' about 80 mm. Hg; pulse rate was 170–200/min. Samples of perfusion medium were taken every 5–15 min. and deproteinized and neutralized as described for slices. Metabolic processes usually attained a linear rate during the first 3–10 min. of a perfusion and, for both glycolysis and gluconeogenesis, remained linear for 1–2 hr. There was a loss of dry weight of tissue during perfusion amounting to up to 20%. This is similar to the losses observed when kidney-cortex slices are incubated in a saline medium (see Krebs, Speake & Hems, 1965). The loss was measured by comparing the weights of the right perfused and left non-perfused kidneys. The results are expressed as $\mu\text{moles/hr./g. final dry wt. of kidney}$.

For anaerobic experiments the gas mixture was replaced by $\text{CO}_2 + \text{N}_2$ (5:95), and 1 mM-HCN was added to the medium because quantitative elimination of O_2 from the perfusion system is difficult.

Rapid freezing of perfused kidneys. During the linear part of the time-course of glycolysis or gluconeogenesis, the renal vessels were cut and the kidney was rapidly clamped between aluminium tongs cooled in liquid N_2 (Wollenberger, Ristau & Schoffa, 1960). Delay between cutting the vessels and freezing was 2–3 sec. The frozen tissue, still in liquid N_2 , was ground in a mortar, weighed quickly and homogenized at 0° in 3 vol. of HClO_4 (6%, w/v)/g. of frozen tissue. The protein was removed and the extracts were neutralized as described for the medium. The content of metabolites in the kidney is expressed per g. fresh wt. 'before perfusion' on the following basis. During perfusion the wet weight of tissue (weighed when congested with blood or medium, immediately after severance of vessels) increased because of swelling. On average the increase was 22%, a value obtained from a comparison of weights of perfused right kidney and fresh left kidney. Fresh or dry weights of left and right kidneys did not differ significantly. On the assumption that this 22% of extra wet weight was perfusion medium containing no metabolites, and taking no account of loss of dry weight during perfusion, the concentration of metabolites in extracts was calculated for the fresh weight of kidney 'before perfusion'. This method slightly overestimates the intracellular concentration of those substances that are also found in the perfusion medium, but in no case does this affect the interpretation.

Analytical methods. Metabolites were determined spectrophotometrically by enzymic methods: glucose, pyruvate, lactate, triose phosphates, fructose diphosphate, α -glycerophosphate, hexose monophosphates, glutamate, aspartate, α -oxoglutarate, malate, citrate, ATP, ADP and AMP as given in Gevers & Krebs (1966); phosphoenolpyruvate, 2- and 3-phosphoglycerate by the method of

Czok & Eckert (1963); and acetoacetate and β -hydroxybutyrate by the method of Williamson, Mellanby & Krebs (1962).

P_i was determined by the method of Martin & Doty (1949) and NH_3 by the method of Kirsten, Gerez & Kirsten (1963). Propionate was determined by g.l.c. as described by Baumgardt (1964), with valeric acid as the internal standard.

Radiochemical methods. [^{14}C]Glucose was obtained from The Radiochemical Centre, Amersham, Bucks. The radioactivity of the respiratory CO_2 produced by slices was counted by adding samples (diluted 1:10) of NaOH solution from the centre wells directly to the scintillation medium as described by Weidemann & Krebs (1969).

RESULTS

Experiments with kidney-cortex slices

Effect of added adenine nucleotides on gluconeogenesis. The formation of glucose from lactate or propionate was inhibited by added ATP (5 mM) and to a smaller extent by added ADP (Tables 1 and 2). At 5 mM AMP had no effect on gluconeogenesis from propionate. The oxygen consumption of the tissue and the net removal of substrate were not affected by added ATP in a major way. This indicates that a proportion of the substrate normally converted into glucose was, in the presence of added ATP, either

Table 1. *Effects of added ATP (2.5 mM) on gluconeogenesis from lactate (5 mM) in kidney-cortex slices*

Kidney-cortex slices from 48 hr.-starved rats were incubated for 60 min. in phosphate-buffered saline. For other experimental details see the text.

	Metabolic changes ($\mu\text{moles/hr./g. dry wt. of tissue}$) in the presence of	
	Lactate (5 mM) + butyrate (5 mM) + ATP (2.5 mM)	Lactate (5 mM) + butyrate (5 mM)
O_2	– 1330	– 1560
Glucose	+ 138	+ 61
Lactate	– 334	– 370
Pyruvate	+ 36	+ 52
α -Glycerophosphate	+ 0	+ 12
Total ketone bodies	+ 68	+ 46
[β -Hydroxybutyrate] [Acetoacetate] ratio	0.34	0.61
[Lactate] [Pyruvate] ratio	39	34
Lactate removal not accounted for as glucose and pyruvate	22	196
Percentage contribution of lactate to fuel of respiration	5	38

oxidized in preference to endogenous fuels or converted into intermediates. The calculated percentage contribution of lactate to the fuel of respiration rose from 5% to 38% on the addition of 2.5mm-ATP, but there was also an increased release of α -glycerophosphate into the incubation medium. At 10mm-ATP two intermediates, lactate and α -glycerophosphate, were found in the medium in sufficient amounts to account for 18% of the propionate prevented by ATP from forming glucose (Table 3).

With propionate as substrate, 5mm-ATP inhibited glucose synthesis by more than 70% and at 10mm-ATP the inhibition was almost complete.

Table 2. *Effects of adenine nucleotides on gluconeogenesis from propionate (2.5mm) in kidney-cortex slices*

Kidney-cortex slices from 48hr.-starved rats were incubated for 60 min. in either the phosphate- or bicarbonate-buffered saline described in the text. The cups contained propionate (2.5mm) and DL-carnitine (4.0mm). The results are given as means $\pm$ S.E.M. (numbers of experiments in parentheses).

Nucleotide added (5mm)	Glucose formed (μ moles/hr./g. dry wt. of tissue)	
	In bicarbonate- buffered saline	In phosphate- buffered saline
None	84 $\pm$ 3 (6)	65 $\pm$ 3 (10)
ATP	37 $\pm$ 4 (3)	16 $\pm$ 1 (6)
ADP	55 $\pm$ 1 (3)	27 $\pm$ 5 (3)
AMP	97 (1)	54 (1)

Significant inhibitions were observed only when the AMP formed from ATP reached a final concentration of at least 1mm in the medium (see below).

In experiments with lactate (5mm) + butyrate (5mm) the rate of gluconeogenesis in the presence of ATP (2.5mm) fell rapidly after 20min. and net glucose synthesis ceased after 40min. (Fig. 1a). There was a simultaneous fall in the rate of formation of acetoacetate but not of β -hydroxybutyrate from butyrate (Fig. 1b). These changes coincided with the formation of AMP from ATP (see below).

Fate of ATP added to kidney-cortex slices. The rates of formation of ADP and AMP from ATP by slices were dependent on the initial ATP concentration. AMP was the main product of the added ATP, but some ADP was also formed. In each case the sum of AMP, ADP and ATP recovered after 60min. was less than the amount of ATP added; the loss of total adenine nucleotides was 12% with 10mm-ATP, 25% with 5mm-ATP and 60% with 2.5mm-ATP. These results mean that, within the range of ATP concentration studied, the absolute amount of adenine nucleotides degraded beyond the stage of AMP was approximately constant.

The rapid fall in the ATP concentration after addition of 2.5mm-ATP was accompanied by formation of both ADP and AMP (Fig. 2). The ADP concentration increased for 20min., and then fell to a very low level, whereas that of AMP rose steadily.

Effects of acidification, P_i and Mg^{2+} on gluconeogenesis. Since the hydrolysis of ATP generates H^+ ions and P_i , the effects of changes of pH and of a high P_i concentration (15mm) on gluconeogenesis were tested. Neither pH changes in the range 6.5–7.5

Table 3. *Effects of variations of the initial ATP concentration on gluconeogenesis from propionate (2.5mm) in kidney-cortex slices*

Kidney-cortex slices from 48hr.-starved rats were incubated for 60 min. in phosphate-buffered saline, pH 7.4. The cups contained propionate (2.5mm) and DL-carnitine (4mm). The results are means of duplicates. For other experimental details see the text.

ATP added (mm)	...	...	...	...	Metabolic changes (μ moles/hr./g. dry wt. of tissue)					
					0	0.625	1.25	2.50	5.0	10.0
O ₂					– 1305	– 1290	– 1270	– 1250	– 1270	– 1170
Propionate					– 261	– 246	– 228	– 230	– 216	– 205
Glucose					+ 69	+ 70	+ 66	+ 49	+ 21	+ 4
Lactate					+ 13	+ 20	+ 25	+ 25	+ 27	+ 33
Pyruvate					+ 2.7	+ 2.7	+ 2.1	+ 2.3	+ 1.9	+ 2.2
[Lactate] [Pyruvate] ratio					4.8	7.4	11.7	10.9	14.2	14.9
α -Glycerophosphate					+ 2.0	+ 2.3	+ 3.0	+ 3.3	+ 4.6	+ 5.2
Propionate removed Glucose formed ratio					3.8	3.5	3.5	4.7	10.3	55.5
Percentage contribution of propionate to fuel of respiration					28	22	19	29	39	47
P _i produced (μ moles/cup)					—	5.5	12.4	22.9	36.3	55.2
Final pH of medium					7.2	7.1	7.1	6.8	6.6	6.5

Fig. 1. Effect of ATP on time-course of production of (a) glucose and (b) ketone bodies by kidney-cortex slices. Slices from 48hr.-starved rats were incubated with butyrate (5mM) and lactate (5mM) in phosphate-buffered saline at 37° for various times. When present, ATP was 2.5mM. Glucose formation: ○, no ATP added; ●, ATP added. Acetoacetate formation: △, no ATP added; ▲, ATP added. β -Hydroxybutyrate formation: □, no ATP added; ■, ATP added.

(i.e. of the order caused by the hydrolysis of ATP; see Table 3) nor addition of P_i had major effects on gluconeogenesis from propionate. The effect of pH on gluconeogenesis is shown in Fig. 3. In the experiments with P_i , DL-carnitine was added to counteract the specific inhibitory effect of P_i on gluconeogenesis from propionate (Weidemann & Krebs, 1969). Added magnesium chloride (20mM) had no effect on gluconeogenesis from propionate, either in the presence or in the absence of added ATP. This indicates that chelation of Mg^{2+} was not responsible for the inhibition of gluconeogenesis by ATP.

Effect of added adenine nucleotides on aerobic glycolysis. The normal low rate of aerobic lactate production from glucose by kidney-cortex slices was increased 50% by added 5mM-ATP (Table 4). Glucose uptake was also increased by ATP. Although the percentage increase was more marked with slices from starved rats, in both the fed and starved states the additional glucose uptake was of the same order of magnitude (15–16 μmoles/hr./g. dry wt. of tissue). In each case the mean increase in glucose uptake was greater than the extra lactate formation. Added AMP (10mM) had a similar effect on glucose uptake but increased lactate production more than 3-fold. ATP raised the

[lactate]/[pyruvate] ratio in the incubation medium about 2.5-fold. This does not imply that the ratio [free NAD^+]/[free $NADH$] changed, because ATP (by generation of P_i) also caused a change of pH. A fall in pH of 0.4 unit and a 2.5-fold rise of the [lactate]/[pyruvate] ratio would mean a constant [free NAD^+]/[free $NADH$] ratio.

In the experiments described in the preceding paragraph, [$U\text{-}^{14}C$]glucose was employed as substrate, to ascertain the effect of ATP on oxidation of glucose to carbon dioxide. When no ATP was added, the specific radioactivity of the carbon dioxide (c.p.m./μg. atom of C) was 23% and 10% of that of the [$U\text{-}^{14}C$]glucose in the well-fed and starved states respectively (Table 5; i.e. almost identical with the calculated percentage contribution of glucose to the respiratory fuel). On addition of ATP (5mM), $^{14}CO_2$ production from [$U\text{-}^{14}C$]glucose was depressed to less than half and the dilution of specific radioactivity of the carbon dioxide carbon no longer agreed with the calculated net contribution of glucose to the oxygen consumption (Table 5). Thus ATP addition apparently did not increase the complete oxidation of glucose, in which case the extra carbon of glucose that was removed in the presence of ATP and that was not accounted for as lactate and carbon dioxide (see also the next

Fig. 2. Time-course of adenine nucleotide changes in medium during incubation of kidney-cortex slices. Kidney-cortex slices from 48hr.-starved rats were incubated with ATP (initially 2.5mM) in phosphate-buffered saline at 37° for various times. ○, ATP; ▲, ADP; □, AMP; ●, total adenine nucleotide.

section) must have accumulated in the form of other compounds in tissue or medium. This conclusion receives support from measurements of the concentrations of intermediates in the perfused kidney (see below).

Experiments with isolated perfused rat kidney

Effect of added adenine nucleotides on flow of perfusion medium through the kidney. When ATP or ADP (10mM) was added to the perfusion medium during an experiment, there was a diminution in the flow rate from 20ml./min./g. fresh wt. to less than 4ml./min./g. The increase in vascular resistance occurred rapidly (within 2–3min.) and was main-

Fig. 3. Effect of pH on gluconeogenesis from propionate (2.5mM) + DL-carnitine (4mM) by kidney-cortex slices. Kidney-cortex slices from 48hr.-starved rats were incubated in phosphate-buffered saline at the pH indicated for 1 hr. at 37°.

tained for several minutes. Within 10min. the flow rate returned to near normal, as the concentration of nucleotide in the perfusion medium fell. With AMP (10mM) there was a very transient decrease in the flow rate from 25ml./min. to about 12ml./min. Added sodium phosphate buffer, pH 7.4 (15mM), or 6-*N*-2'-*O*-dibutyryl-3',5'-(cyclic)-AMP (2.5mM) did not affect the flow rate. In a perfusion experiment in which adenosine (10mM) was present from the beginning, there was no major effect on flow rate, although the attainment of the maximal rate was slower than usual.

Effect of added adenine nucleotides on gluconeogenesis. As with slices, gluconeogenesis from lactate (10mM) was inhibited by the addition of ATP or AMP (10mM) in the perfused kidney (Fig. 4). ADP (10mM) also caused an inhibition, but there was a 15min. delay (Fig. 4), by which time the AMP concentration in the perfusion medium had reached approx. 5mM. Lower initial concentrations of added ATP (2.5mM) and AMP (5mM) inhibited gluconeogenesis by less than 20%. In experiments in which 10mM-adenosine or 2.5mM-6-*N*-2'-*O*-dibutyryl-3',5'-(cyclic)-AMP was added there was no major inhibition of gluconeogenesis from 10mM-lactate in perfused kidneys from 48hr.-starved rats.

Fate of added adenine nucleotides. As with kidney-cortex slices, ATP (10mM) was removed rapidly from the perfusion medium, generating some ADP and more AMP and P_i ; the time-course of these changes was similar to that with slices (shown in Fig. 2). The concentration of circulating ATP fell from 10mM to 5mM within 6–12min. in aerobic or

Table 4. *Effect of added ATP (5mm) on [U-¹⁴C]glucose uptake and lactate production by kidney-cortex slices*

Kidney-cortex slices were incubated for 60 min. in phosphate-buffered saline under standard conditions. The specific radioactivity of [U-¹⁴C]glucose was 9.6×10^4 c.p.m./ μ g. atom of C and the total radioactivity added per cup was 6×10^6 c.p.m. The results are given as means $\pm$ s.e.m. The isotope-distribution data from these experiments are given in Table 5.

		Metabolic changes (μ moles/hr./g. dry wt. of tissue)									
		Well-fed rats					48 hr.-starved rats				
Additions	...	None	ATP (5mm)	[U- ¹⁴ C]Glucose (2.5mm)	[U- ¹⁴ C]Glucose (2.5mm) + ATP (5mm)		None	ATP (5mm)	[U- ¹⁴ C]Glucose (2.5mm)	[U- ¹⁴ C]Glucose (2.5mm) + ATP (5mm)	
No. of observations		3	3	5	5		3	3	5	5	
O ₂		-920 $\pm$ 23	-890 $\pm$ 45	-1050 $\pm$ 70	-1010 $\pm$ 55		-975 $\pm$ 24	-970 $\pm$ 59	-1035 $\pm$ 21	-990 $\pm$ 35	
CO ₂		+765 $\pm$ 19	+797 $\pm$ 42	+920 $\pm$ 60	+945 $\pm$ 60		+755 $\pm$ 40	+875 $\pm$ 52	+945 $\pm$ 29	+980 $\pm$ 100	
Glucose		+5.3 $\pm$ 2.3	+5.4 $\pm$ 1.0	-52.5 $\pm$ 3.1	-67.9 $\pm$ 5.4		+17.0 $\pm$ 3.0	+9.9 $\pm$ 1.8	-28.0 $\pm$ 2.8	-44.6 $\pm$ 9.5	
Lactate		+3.4 $\pm$ 2.4	+7.2 $\pm$ 0.9	+12.5 $\pm$ 0.7	+19.1 $\pm$ 1.4		+6.7 $\pm$ 1.5	+17.6 $\pm$ 1.3	+18.4 $\pm$ 1.9	+28.9 $\pm$ 2.8	
Pyruvate				+1.4 $\pm$ 0.3	+0.9 $\pm$ 0.3				+3.2 $\pm$ 0.3	+2.0 $\pm$ 0.6	
[Lactate] [Pyruvate] ratio				8.9 $\pm$ 1.2	21.2 $\pm$ 3.5				5.8 $\pm$ 0.8	14.5 $\pm$ 3.7	
Percentage contribution of glucose to fuel of respiration				26.0 $\pm$ 3.4	34.4 $\pm$ 4.6				10.0 $\pm$ 1.4	17.6 $\pm$ 5.3	

anaerobic conditions. In spite of these rapid changes there was no measurable loss of total adenine nucleotides (sum of ATP + ADP + AMP) during 30 min. of perfusion with 10mm-ATP. This is in contrast with the 30–60% loss after 60 min. of incubation of kidney-cortex slices with 2.5mm-ATP (see Fig. 2).

In view of the rapid fall in ATP concentration in the perfusion medium, control experiments were carried out to test for adenosine triphosphatase activity in the medium, both before and after perfusion of the kidney. No such activity was observed. Thus the degradation of ATP occurred within the kidney. ADP (10mm) added to the perfusion medium was removed in 30 min.; the concentration of AMP rose to about 5mm and no ATP was released into the medium. Added AMP (10mm) was completely dephosphorylated in 45 min. This contrasts with the stability of AMP formed from 10mm-ATP, which reached 20–30 mm. after the addition of ATP. The reasons for this stability of AMP derived from ATP are apparently inhibition of both 5'-nucleotidase and adenylate deaminase by P_i, and of the former enzyme by ATP (see the first section of the Discussion for references).

Degradation of adenine nucleotides was also followed by measuring ammonia production in the perfusion medium. On adding AMP (10mm) the ammonia concentration in the medium increased from 0.2mm to 1.7mm over 45 min., whereas after addition of ATP (10mm) ammonia concentration rose from 0.2 to 0.6mm over 30 min. Ammonia was also released from added adenosine (10mm), reaching 1.8mm after 120 min. of perfusion.

Effects of acidification and P_i on gluconeogenesis. Checks analogous to those carried out with slices refuted the possibility that the effects of ATP might be caused by the release of P_i and acid. Neither 15mm-sodium dihydrogen phosphate nor sufficient hydrochloric acid to change the pH of the medium from 7.4 to 7.1 affected glucose formation from lactate. Addition of sodium hydroxide to maintain the pH at about 7.4 did not prevent the inhibition of gluconeogenesis from lactate by ATP. It is also relevant that the effect of AMP (10mm) was at least as rapid in onset and extensive as that of ATP (although AMP generated less P_i and no acid) and that the effects of both ATP and AMP on gluconeogenesis were almost immediate, whereas dephosphorylation of nucleotides was gradual.

Effect of added adenine nucleotides on glycolysis in the perfused kidney. ATP (10mm) added after 30 min. of aerobic perfusion with glucose (1mm) increased the rate of removal of glucose from the medium. The increase was accompanied by an inhibition of lactate formation (Table 6 and Fig. 5). These effects occurred immediately after the addition of ATP. AMP (10mm) had a similar effect on glucose

Table 5. *Effect of ATP addition on ¹⁴CO₂ production from [U-¹⁴C]glucose (2.5 mM)*

The isotope incorporation into ¹⁴CO₂ was calculated as described in the text. The incubations were those for which the net changes are reported in Table 4. The results are given as means ± S.E.M. of five observations.

Substrates added	Well-fed rats		48 hr.-starved rats	
	[U- ¹⁴ C]Glucose (2.5 mM)	[U- ¹⁴ C]Glucose (2.5 mM)+ATP (5 mM)	[U- ¹⁴ C]Glucose (2.5 mM)	[U- ¹⁴ C]Glucose (2.5 mM)+ATP (5 mM)
10 ⁻³ × Radioactivity in ¹⁴ CO ₂ (c.p.m.)	331 ± 31	252 ± 19	137 ± 4	115 ± 7
10 ⁻⁴ × Sp. radioactivity of ¹⁴ CO ₂ (c.p.m./μg.atom of C)	2.17 ± 0.3	1.60 ± 0.2	0.97 ± 0.2	0.72 ± 0.1
Sp. radioactivity of ¹⁴ CO ₂ × 100	22.6	16.7	10.1	7.5

Fig. 4. Effect of adenine nucleotides on time-course of gluconeogenesis from lactate (10 mM) in isolated perfused kidney. Kidneys from 48 hr.-starved rats were perfused with bicarbonate-buffered saline as described in the text. ATP or AMP was added at the time indicated by the arrow. ○, Glucose formation before addition of adenine nucleotide. Filled-in symbols represent glucose formation after addition, to the initial concentration indicated, of: ■, ATP (10 mM); ▲, ADP (10 mM); ●, AMP (10 mM); ▼, P_i (15 mM).

uptake. No α-glycerophosphate or glycerol appeared in the medium in these experiments. Under anaerobic conditions ATP (10 mM or 2.5 mM) caused no alteration in the rate of lactate production from 1 mM- or 5 mM-glucose or in the rate of uptake of 1 mM-glucose. The anaerobic glucose uptake was always greater than the aerobic rate

Table 6. *Effect of ATP on glycolysis in the isolated perfused kidney*

Kidneys from well-fed rats were perfused with bicarbonate-buffered saline, and ATP (10 mM) was added after 20–30 min. of perfusion. The metabolic rate after addition of ATP was the average rate over the next 15 min. The results are given as means ± S.E.M. (numbers of observations in parentheses).

Concn. of glucose (mM)	Concn. of added ATP (mM)	Gas phase (95:5)	Metabolic rates (μmoles/hr./g. dry wt.)	
			Glucose removal	Lactate formation
—	0	O ₂ + CO ₂	—	< 15 (2)
1	0	O ₂ + CO ₂	38 ± 3 (4)	71 ± 3 (6)
1	10	O ₂ + CO ₂	95 ± 8 (3)	16 ± 5 (3)
1	0	N ₂ + CO ₂	157 ± 15 (6)	233 ± 19 (6)

even when the latter was measured in the presence of ATP or AMP (Table 6). In anaerobic experiments the concentration of α-glycerophosphate in the medium rose to 0.015 mM in the absence of ATP, and to 0.1 mM in its presence. The former concentration, reached after 30 min., represents an average rate of α-glycerophosphate formation of about 10 μmoles/hr./g. dry wt.

Concentrations of intermediates related to glycolysis and the tricarboxylic acid cycle in the rapidly frozen kidney. Kidneys were rapidly frozen 15–20 min. after addition of ATP or AMP (10 mM) to the perfusion medium. The major effect of either nucleotide in the presence of lactate (10 mM), and of ATP in the presence of glucose (1 mM), was to raise the concentrations of the C₃ phosphorylated intermediates (α-glycerophosphate, triose phosphates, 3- and 2-phosphoglycerate and phosphoenolpyruvate) and fructose diphosphate (Table 7). The increase in concentration of triose phosphates, fructose diphosphate and α-glycerophosphate was more marked with lactate than with glucose as substrate (Table 7). The concentrations of hexose

Fig. 5. Effect of adenine nucleotides on time-course of glycolysis (initial concn. of glucose 10 mM) in isolated perfused kidney. Kidneys from well-fed rats were perfused with bicarbonate-buffered saline as described in the text. ATP or AMP was added at the time indicated by the arrow. O, Metabolic changes before addition of adenine nucleotide. Filled-in symbols: addition, to an initial concentration of 10 mM, of ●, ATP; ■, AMP. The continuous lines represent glucose removal, and the broken lines lactate production.

monophosphates fell slightly in the presence of lactate when either ATP or AMP was added, but rose slightly in the presence of glucose when ATP was added. The addition of sodium dihydrogen phosphate (15 mM) instead of nucleotides during perfusion with lactate caused a small increase in the tissue concentrations of phosphoenolpyruvate and 3-phosphoglycerate, but had no other effect (Table 7). This shows that the effects of adenine nucleotides on the intermediates were not due to formation of P_i or acid.

With both lactate and glucose ATP caused a striking fall in the α -oxoglutarate concentration (from 1 mM to 0.08 mM) and a 100% increase in the concentration of malate. A similar fall occurred when AMP was added during perfusion with lactate. The concentration of citrate fell on addition of both nucleotides (Table 7).

The concentrations of adenine nucleotides and P_i in the rapidly frozen tissue reflected their concentrations in the perfusion medium (compare Table 7 and Fig. 2).

DISCUSSION

Fate of added adenine nucleotides. The effects of added adenine nucleotides on renal carbohydrate metabolism that are dependent on the intracellular concentration of AMP imply that at least the AMP added as such and the AMP formed from ATP can penetrate into the cytoplasm of the cortical cells.

Table 7. *Content of intermediate metabolites and of adenine nucleotides in kidney perfused with adenine nucleotides*

Kidneys from 48 hr.-starved rats were perfused with lactate (10mM) and those from well-fed rats with glucose (1 mM). Adenine nucleotide, to an initial concentration of 10 mM, or P _i (15 mM) was added after 30–40 min., and after a further 15–20 min. kidneys were rapidly frozen as described in the text. Results are means of three perfusions $\pm$ s.e.m. Abbreviations: Lact, lactate; Pyr, pyruvate; PEP, phosphoenolpyruvate; 2PG, 2-phosphoglycerate; 3PG, 3-phosphoglycerate; Triose-P, sum of triose phosphates; α GP, α -glycerophosphate; FDP, fructose 1,6-diphosphate; F6P, fructose 6-phosphate; G6P, glucose 6-phosphate; Glu, glutamate; Asp, aspartate; Oxo, α -oxoglutarate; Cit, citrate; P _i , inorganic phosphate.																							
Nutritional state	Additions to medium	Content of intermediates (nmol/g. wt. of kidney)											Content of intermediates (nmol/g. wt. of kidney)										
		Lact	Pyr	PEP	2PG	3PG	Triose-P	α GP	FDP	F6P	G6P	Malate	NH ₄ ⁺	Glu	Asp	Oxo	Cit	P _i	ATP	ADP	AMP		
Well-fed	Glucose (1 mM)	501 $\pm$ 112	<10	<10	<10	32 $\pm$ 1	11 $\pm$ 8	168 $\pm$ 40	<10	<10	33 $\pm$ 2	80 $\pm$ 30	1430 $\pm$ 90	2090 $\pm$ 70	530 $\pm$ 50	990 $\pm$ 110	260 $\pm$ 20	3920 $\pm$ 230	980 $\pm$ 110	380 $\pm$ 30	100 $\pm$ 10		
Well-fed	Glucose (1 mM) + ATP (10 mM)	720 $\pm$ 171	33 $\pm$ 6	116 $\pm$ 36	28 $\pm$ 4	241 $\pm$ 45	61 $\pm$ 24	458 $\pm$ 83	21 $\pm$ 8	<10	44 $\pm$ 7	160 $\pm$ 80	2470 $\pm$ 430	1200 $\pm$ 20	590 $\pm$ 70	80 $\pm$ 30	100 $\pm$ 20	19700 $\pm$ 3540	3300 $\pm$ 780	5600 $\pm$ 50	6010 $\pm$ 580		
Starved	Lactate (10 mM)	8380 $\pm$ 232	89 $\pm$ 11	13 $\pm$ 2	<10	26 $\pm$ 5	21 $\pm$ 2	604 $\pm$ 80	<10	14 $\pm$ 3	54 $\pm$ 18	330 $\pm$ 40	1110 $\pm$ 220	2750 $\pm$ 150	480 $\pm$ 60	1070 $\pm$ 150	690 $\pm$ 100	4790 $\pm$ 420	960 $\pm$ 60	640 $\pm$ 40	250 $\pm$ 20		
Starved	Lactate (10 mM) + ATP (10 mM)	7490 $\pm$ 1180	66 $\pm$ 22	97 $\pm$ 21	21 $\pm$ 7	193 $\pm$ 39	144 $\pm$ 29	1315 $\pm$ 180	158 $\pm$ 53	11 $\pm$ 4	40 $\pm$ 14	680 $\pm$ 90	2220 $\pm$ 300	2070 $\pm$ 350	600 $\pm$ 150	100 $\pm$ 30	200 $\pm$ 20	18520 $\pm$ 3100	2880 $\pm$ 610	3710 $\pm$ 460	5880 $\pm$ 1140		
Starved	Lactate (10 mM) + AMP (10 mM)	8600 $\pm$ 976	139 $\pm$ 10	231 $\pm$ 37	37 $\pm$ 5	425 $\pm$ 74	99 $\pm$ 31	1516 $\pm$ 403	108 $\pm$ 28	15 $\pm$ 4	40 $\pm$ 3	530 $\pm$ 50	4390 $\pm$ 220	1920 $\pm$ 140	540 $\pm$ 30	110 $\pm$ 40	310 $\pm$ 80	9060 $\pm$ 3280	2490 $\pm$ 680	2450 $\pm$ 480	2710 $\pm$ 310		
Starved	Lactate (10 mM) + P _i (15 mM)	8650 $\pm$ 345	96 $\pm$ 14	21 $\pm$ 3	<10	56 $\pm$ 4	17 $\pm$ 5	501 $\pm$ 55	<10	10 $\pm$ 3	30 $\pm$ 6	310 $\pm$ 40	—	2130 $\pm$ 180	—	800 $\pm$ 170	—	18700 $\pm$ 1900	710 $\pm$ 170	570 $\pm$ 70	330 $\pm$ 60		
Nutritional state	Additions to medium																						
Well-fed	Glucose (1 mM)	80 $\pm$ 30	1430 $\pm$ 90	2090 $\pm$ 70	530 $\pm$ 50	990 $\pm$ 110	260 $\pm$ 20	3920 $\pm$ 230	980 $\pm$ 110	380 $\pm$ 30	100 $\pm$ 10	100 $\pm$ 10	100 $\pm$ 10	100 $\pm$ 10	100 $\pm$ 10	100 $\pm$ 10	100 $\pm$ 10	100 $\pm$ 10	100 $\pm$ 10	100 $\pm$ 10	100 $\pm$ 10		
Well-fed	Glucose (1 mM) + ATP (10 mM)	160 $\pm$ 30	2470 $\pm$ 430	1200 $\pm$ 20	590 $\pm$ 70	80 $\pm$ 30	100 $\pm$ 20	19700 $\pm$ 3540	3300 $\pm$ 780	5600 $\pm$ 50	6010 $\pm$ 580	6010 $\pm$ 580	6010 $\pm$ 580	6010 $\pm$ 580	6010 $\pm$ 580	6010 $\pm$ 580	6010 $\pm$ 580	6010 $\pm$ 580	6010 $\pm$ 580	6010 $\pm$ 580	6010 $\pm$ 580		
Starved	Lactate (10 mM)	330 $\pm$ 40	1110 $\pm$ 220	2750 $\pm$ 150	480 $\pm$ 60	1070 $\pm$ 150	690 $\pm$ 100	4790 $\pm$ 420	960 $\pm$ 60	640 $\pm$ 40	250 $\pm$ 20	250 $\pm$ 20	250 $\pm$ 20	250 $\pm$ 20	250 $\pm$ 20	250 $\pm$ 20	250 $\pm$ 20	250 $\pm$ 20	250 $\pm$ 20	250 $\pm$ 20	250 $\pm$ 20		
Starved	Lactate (10 mM) + ATP (10 mM)	680 $\pm$ 90	2220 $\pm$ 300	2070 $\pm$ 350	600 $\pm$ 150	100 $\pm$ 30	200 $\pm$ 20	18520 $\pm$ 3100	2880 $\pm$ 610	3710 $\pm$ 460	5880 $\pm$ 1140	5880 $\pm$ 1140	5880 $\pm$ 1140	5880 $\pm$ 1140	5880 $\pm$ 1140	5880 $\pm$ 1140	5880 $\pm$ 1140	5880 $\pm$ 1140	5880 $\pm$ 1140	5880 $\pm$ 1140	5880 $\pm$ 1140		
Starved	Lactate (10 mM) + AMP (10 mM)	530 $\pm$ 50	4390 $\pm$ 220	1920 $\pm$ 140	540 $\pm$ 30	110 $\pm$ 40	310 $\pm$ 80	9060 $\pm$ 3280	2490 $\pm$ 680	2450 $\pm$ 480	2710 $\pm$ 310	2710 $\pm$ 310	2710 $\pm$ 310	2710 $\pm$ 310	2710 $\pm$ 310	2710 $\pm$ 310	2710 $\pm$ 310	2710 $\pm$ 310	2710 $\pm$ 310	2710 $\pm$ 310	2710 $\pm$ 310		
Starved	Lactate (10 mM) + P _i (15 mM)	310 $\pm$ 40	—	2130 $\pm$ 180	—	800 $\pm$ 170	—	18700 $\pm$ 1900	710 $\pm$ 170	570 $\pm$ 70	330 $\pm$ 60	330 $\pm$ 60	330 $\pm$ 60	330 $\pm$ 60	330 $\pm$ 60	330 $\pm$ 60	330 $\pm$ 60	330 $\pm$ 60	330 $\pm$ 60	330 $\pm$ 60	330 $\pm$ 60		

The fact that no adenosine triphosphatase activity was detectable in the perfusion medium indicates that the degradation of added ATP occurs within the kidney tissue. It is likely that the cortical cells are permeable to all three adenine nucleotides because added ATP and AMP caused an increase of the intracellular concentrations of all three adenine nucleotides, with AMP reaching the highest concentrations (Table 7).

The striking observation of a greater stability of AMP formed from added ATP compared with the stability of the added AMP can be explained on the basis of the known reactions of AMP and the properties of the enzymes involved. AMP can be converted into adenosine, IMP and inosine by the following two reaction sequences (see Burger & Lowenstein, 1967):

In (1) deamination precedes and in (2) it follows dephosphorylation. In the perfused kidney added AMP formed P_1 much more rapidly than ammonia. Thus sequence (2) was the main pathway. Both 5'-nucleotidase and adenylate deaminase are inhibited by P_1 (Kizer, Cox, Lovig & de Estrugo, 1963; Segal & Brenner, 1960), and 5'-nucleotidase is also inhibited by ATP (Baer, Drummond & Duncan, 1966; Burger & Lowenstein, 1967). This can account for the greater stability of AMP when formed from ATP than when added as such.

Effects of adenine nucleotides on renal carbohydrate metabolism. The inhibition of gluconeogenesis by adenine nucleotides added to sliced or perfused kidney in the presence of lactate can be satisfactorily explained by a rise in the intracellular concentration of AMP. The time-course of the action of adenine nucleotides shows that ATP and AMP acted more quickly than ADP and that the effects were approximately parallel to the concentration of AMP in the perfusion medium and in the kidney (see Table 7). The rise in the concentrations of the intermediates of gluconeogenesis between phosphoenolpyruvate and fructose diphosphate during perfusion with lactate on addition of ATP or AMP are in agreement with the assumption that the inhibition of gluconeogenesis occurred at the step converting fructose diphosphate into fructose 6-phosphate. This conclusion is supported by the fall in the concentrations of the hexose monophosphates. The fructose diphosphate that

accumulated because of the inhibition of fructose diphosphatase by AMP (Taketa & Pogell, 1963; Newsholme, 1963) could further increase the inhibition of fructose diphosphatase (Mendicino & Vasarhely, 1963).

When lactate was replaced by glucose in the perfusion medium the effects of the adenine nucleotides on the concentrations of renal intermediates were similar, although the mechanism responsible for the changes must have been different. The accumulation of the C_3 phosphorylated intermediates can be accounted for by the activation of phosphofructokinase by AMP. The extra glucose taken up on addition of adenine nucleotides was thus probably due to an increased rate of cortical glycolysis. The rise in the concentration of glucose 6-phosphate in the presence of ATP also indicates an increased rate of cortical glucose utilization.

Changes in the concentrations of intermediates of the tricarboxylic acid cycle and of related metabolites. Addition of the adenine nucleotides caused a striking fall (10-fold) in the concentration of α -oxoglutarate, a smaller fall in the concentrations of citrate and glutamate and a rise in the concentrations of malate, phosphoenolpyruvate and ammonia. No satisfactory explanation can be offered that co-ordinates these changes.

Effects of added adenine nucleotides on blood flow through the kidney. Adenine nucleotides are known to cause a fall in systemic blood pressure that is taken to be due at least partly to peripheral vasodilatation (Green & Stoner, 1950). In the dog ATP and ADP have been reported to dilate and AMP to constrict the renal vessels on injection into the renal artery (Hashimoto & Kumakura, 1965). The increase in vascular resistance brought about by ATP or ADP in the present experiments shows that the above findings do not generally hold.

It is not likely, in view of the transience of the vasoconstrictor effect of ATP, that the more prolonged effects of ATP on carbohydrate metabolism in the kidney are due to decreased blood flow. This also follows from the facts that ATP inhibited gluconeogenesis in slices, where oxygenation was not affected, and that AMP inhibited gluconeogenesis in the perfused kidney, although the vasoconstrictor effect was negligible.

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