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Hexose metabolism in pancreatic islets: preferential utilization of mitochondrial ATP for glucose phosphorylation

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The respective contribution of exogenous and intramitochondrially formed ATP to D-glucose phosphorylation by mitochondria-bound hexokinase was examined in both rat liver and pancreatic islet mitochondria by comparing the generation of D-glucose 6-[^{32}P]phosphate from exogenous [γ-^{32}P]ATP to the total rate of D-[U-^{14}C]glucose phosphorylation. In liver mitochondria, the fractional contribution of exogenous ATP to D-glucose phosphorylation ranged from 4 to 74%, depending on the availability of endogenous ATP formed by either oxidative phosphorylation or in the reaction catalyzed by adenylate kinase. Likewise, in islet mitochondria exposed to exogenous ATP but deprived of exogenous nutrient, about 60% of D-glucose phosphorylation was supported by mitochondrial ATP. Such a fractional contribution was further increased in the presence of ADP and succcinate, and suppressed by mitochondrial poisons. It is concluded that, in islet like in liver mitochondria, mitochondrial ATP is used preferentially to exogenous ATP as a substrate for D-glucose phosphorylation by mitochondria-bound hexokinase. This may favour the maintenance of a high cytosolic ATP concentration in glucose-stimulated islet cells.

Introduction

The release of insulin evoked by D-glucose in the pancreatic B-cell is thought to be causally linked to the hexose-induced increase in cytosolic ATP concentration (or cytosolic ATP/ADP ratio) leading to the closure of ATP-responsive K^+ channels, membrane depolarization and gating of voltage-sensitive Ca^{2+} channels [1]. In response to a rise in extracellular D-glucose concentration, the rapid equilibration of hexose concentrations across the plasma membrane and the participation of both hexokinase and glucokinase in the phosphorylation of the hexose represent critical features of the glucose-sensing device in the B-cell [2]. The relatively large binding of hexokinase isoenzymes to mitochondria in islet cells and the dynamic regulation of such a binding in a phenomenon of ambiquity may play a propitious role in this glucose-sensing process [3,4]. First, mitochondria-bound hexokinase is less sensitive than the free enzyme to inhibition by D-glucose 6-phosphate [3]. Second, the phosphorylation of D-glucose by mitochondria-bound hexokinase coincides with an increase in O_2 uptake, so that these coupled processes may prime the mitochondria to generate ATP through the oxidation of pyruvate formed from exogenous D-glucose [5]. Last, it is conceivable that bound hexokinase uses preferentially mitochondrial rather than cytosolic ATP to support hexose phosphorylation, allowing for the maintenance of a high cytosolic ATP concentration. The present work aims at exploring the validity of the latter proposal. For such a purpose, the phosphorylation of D-[U-^{14}C]glucose was compared to the formation of D-glucose 6-[^{32}P]phosphate from [γ-^{32}P]ATP in liver and islet mitochondria.

Materials and Methods

Pieces of liver removed from fed albino rats were minced, washed thrice and homogenized in a Hepes-KOH buffer (2.0 mM, pH 7.4) containing sucrose (70 mM) and mannitol (220 mM), using a mechanical homogenizer (Braun, Melsungen, F.R.G.) with six passes at 1250 U/min. The homogenate (1.0 g wet weight liver

Abbreviations: CAT, carboxyatractyloside; Ado_{P5}, P^1,P^5-di(adenosine-5′)pentaphosphate.

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per 2.0 ml of buffer) was then brought to a total volume of 10 ml with the same buffer, and centrifuged for 15 min at 780 × *g*. The supernatant was then again centrifuged for 15 min at 6800 × *g*, the resulting pellet being resuspended in 5.0 ml of the same buffer, homogenized in Potter-Elvehjem tubes (five strokes) and then centrifuged for 15 min at 9700 × *g*. The pellet was resuspended in 1.0 ml of the same buffer.

Islets were isolated by the collagenase technique [6] from the pancreas of fed albino rats. Groups of approx. 1500 islets each were homogenized in Potter-Elvehjem tubes (eight strokes) in 1.0 ml of a Hepes-KOH buffer (5.0 mM, pH 7.4) containing sucrose (60 mM), mannitol (190 mM) and EDTA (0.5 mM). After 5 min centrifugation at 780 × *g*, an aliquot (0.75 ml) of the supernatant was removed, the remaining material being mixed with 0.75 ml of the same buffer, again homogenized (eight strokes) and centrifuged for 5 min at 780 × *g*. The supernatant (0.75 ml) of this second centrifugation was pooled with the first one, and centrifuged for 10 min at 12000 × *g* [7]. The supernatant of this last centrifugation was removed and the pellet was resuspended in 0.95 ml of the same buffer.

All preceding manipulations were conducted at 4 °C. In all cases, aliquots (50 μl each) of the mitochondrial suspension were mixed with 150 μl of incubation medium to yield the following final concentrations: Hepes-KOH buffer (2.0 or 5.0 mM, pH 7.4), sucrose (70 or 60 mM), mannitol (220 or 190 mM), EDTA (0.5 mM), KH_2PO_4 (10 mM), $MgCl_2$ (5.5 mM) and fatty acid-free bovine serum albumin (0.2 mg/ml). Under these conditions, the material derived from 50 mg wet weight liver was eventually placed in 0.2 ml of incubation medium, the activity of glutamate dehydrogenase, measured as described elsewhere [8], being close to 0.2 μmol/min per sample. In experiments performed with islet mitochondria, the material derived from 50 to 80 islets (i.e., approx. 0.2–0.4 mg wet weight) was also placed in 0.2 ml of medium. The activity of glutamate dehydrogenase amounted to 0.3–0.5 nmol/min per sample and, as such, was about 500-times lower in islet than liver samples.

The activity of hexokinase was measured over 5 to 30 min incubation at 20 °C in the presence of 1.0 mM D-[U-^{14}C]glucose and 0.2 to 1.0 mM unlabelled ATP or 1.0 mM unlabelled D-glucose and 0.2 to 1.0 mM [γ-^{32}P]ATP. The reaction was halted by adding 0.3 ml of ethanol and 1.0 ml of iced water. The labelled hexose phosphates and, when required, [^{32}P]P_i were separated by anion-exchange chromatography [9]. All readings were corrected for the blank value measured in the absence of mitochondria.

All results are expressed as the mean (± S.E.) together with the number of individual observations (*n*). The statistical significance of differences between mean values was assessed by use of Student's *t*-test.

Results

ATP hydrolysis

A large fraction of the ATP added to the medium at the onset of incubation was apparently hydrolyzed in the presence of liver mitochondria. Thus, already over 10 min incubation, the amount of [^{32}P]P_i generated from [γ-^{32}P]ATP, relative to total radioactivity, averaged 64.7 ± 0.9% ($n = 6$) and 55.8 ± 3.0% ($n = 4$) when the initial concentration of ATP amounted to 0.2 and 1.0 mM, respectively, as distinct from a control value (no mitochondria present) of 2.7 ± 0.2% ($n = 9$). Preincubation of the mitochondria for 5 min in the presence of ADP (0.2 mM) and succinate (10.0 mM) slightly decreased ($P < 0.02$) the subsequent formation of [^{32}P]P_i to 83.1 ± 4.7% ($n = 6$) of the paired value found in mitochondria preincubated in the absence of ADP and succinate.

When the final incubation was extended to 30 min, the generation of [^{32}P]P_i from [γ-^{32}P]ATP (initial ATP concentration: 0.4 mM) was further increased to 74.9 ± 4.2% ($n = 8$) of total radioactivity, as distinct from a control value of 2.3 ± 0.3% ($n = 2$). Preincubation for 30 min with ADP (2.0 mM) and succinate (10.0 mM) again decreased the subsequent formation of [^{32}P]P_i to 72.8 ± 1.6% ($n = 3$) of the paired reading recorded in mitochondria preincubated in the absence of ADP and succinate. Inversely, the incorporation into the incubation medium of Ado_2P_5 (10 μM), an inhibitor of adenylate kinase [10], and/or CAT (12 μM) together with KCN (1.0 mM) and rotenone (10 μM) increased the apparent rate of [γ-^{32}P]ATP hydrolysis. For instance, the formation of [^{32}P]P_i was increased to 117.0 ± 3.0% ($n = 5$) and 110.4 ± 2.3% ($n = 15$) of the paired reference value in the presence, respectively, of Ado_2P_5 and CAT together with KCN and rotenone ($P < 0.005$ or less).

At an initial ATP concentration of 0.4 mM and after 30 min incubation, the total ATP content of the incubation medium and mitochondria, as measured by the luciferase method, was also decreased to 24.5 ± 7.3% ($n = 4$) of the control value recorded at the onset of incubation. The latter value was virtually identical to that found in the absence of mitochondria.

In one series of experiments, vanadate (0.2 mM) was added to the incubation medium as a potential inhibitor of ATPase [11,12]. Vanadate increased the phosphorylation rate of D-[U-^{14}C]glucose (1.0 mM) over 10 min incubation in the presence of exogenous ATP (0.2 or 1.0 mM), whether the mitochondria had been preincubated for 5 min in the absence or presence of ADP (0.2 mM) and succinate (10.0 mM). Thus, in the presence of vanadate, the rate of D-[U-^{14}C]glucose phosphorylation averaged 129.9 ± 5.3% ($n = 12$) of the paired reference value ($P < 0.001$). Likewise, vanadate increased by 11.7 ± 3.7% ($n = 4$) the generation rate of D-glucose 6-

TABLE I

Phosphorylation of D-[U-^{14}C]glucose by liver mitochondria

Expt.	Preincubation (min 0 to 5)	Incubation (min 6 to 10)	D-Glucose phosphorylation (pmol/min per mg liver)	
			Control	CAT (12 μM)
1	Nil	Nil	0.87 ± 0.08 (12)	
	Nil	ATP (0.2 mM)	6.82 ± 0.17 (45)	
	Nil	ATP (0.4 mM)	9.65 ± 0.63 (13)	
	Nil	ATP (1.0 mM)	16.82 ± 0.77 (12)	
	ADP (0.2 mM) + succinate (10.0 mM)	Nil	3.63 ± 0.10 (12)	
	ADP (0.2 mM) + succinate (10.0 mM)	ATP (0.2 mM)	8.22 ± 0.17 (42)	
	ADP (0.2 mM) + succinate (10.0 mM)	ATP (0.4 mM)	9.36 ± 0.53 (10)	
	ADP (0.2 mM) + succinate (10.0 mM)	ATP (1.0 mM)	17.23 ± 0.52 (11)	
2	Nil	Nil	0.70 ± 0.08 (11)	0.21 ± 0.05 (19)
	Nil	ATP (0.2 mM)	7.70 ± 0.12 (28)	4.14 ± 0.21 (24)
	ADP (0.2 mM) + succinate (10.0 mM)	Nil	4.24 ± 0.19 (34)	1.30 ± 0.11 (34)
	ADP (0.2 mM) + succinate (10.0 mM)	ATP (0.2 mM)	7.32 ± 0.11 (32)	4.83 ± 0.31 (31)

[^{32}P]phosphate by mitochondria exposed to [γ-^{32}P]ATP and unlabelled D-glucose ($P < 0.06$). However, vanadate decreased by only 6.0 ± 0.6% ($n = 4$) the generation of [^{32}P]P_i from [γ-^{32}P]ATP and failed to increase the ratio between the generation of D-glucose 6-[^{32}P]phosphate and D-[U-^{14}C] glucose 6-phosphate, respectively.

These findings indicate that the true concentration of exogenous ATP during incubation is much lower than the nominal or initial concentration of the nucleotide and document that the availability in endogenous ATP, formed either by oxidative phosphorylation or in the reaction catalyzed by adenylate kinase, modulates the rate of exogenous [γ-^{32}P]ATP hydrolysis.

D-Glucose phosphorylation

In a large series of experiments ($n = 21$), the rate of phosphorylation of D-[U-^{14}C]glucose (1.0 mM) in the presence of ATP (0.2 mM) by mitochondria preincubated for 5 min in the absence of any substrate or nucleotide averaged, over 5 min incubation and 20 °C, 7.53 ± 0.30 pmol/min per mg liver wet weight. As shown in Table I (Expt. 1), such a phosphorylation rate was about 8-times higher than that recorded, under the same experimental conditions, in the absence of exogenous ATP. When ATP was present during incubation at increasing concentrations (0.2 to 1.0 mM), the rate of phosphorylation was progressively increased, with an apparent K_m for ATP close to 0.6 mM (Fig. 1). A somewhat different picture was observed when the mitochondria were preincubated for 5 min with both ADP (0.2 mM) and succinate (10.0 mM). In these experiments, the concentration of ADP was low enough to allow its full conversion to ATP over the 5 min preincubation. The preincubation with ADP and succinate markedly increased ($P < 0.001$) the subsequent rate of D-[U-^{14}C]glucose phosphorylation, as measured in the absence of exogenous ATP. Even in the presence of a low concentration of ATP (0.2 mM) in the final incubation medium, a modest increase in hexose phosphorylation was observed, on occasion, when the mitochondria had been preincubated in the presence, rather than absence, of ADP and succinate. Such an increase faded out, however, at higher concentrations of ATP (0.4 and 1.0 mM). The preincubation with ADP and succinate did not suppress the dependency of D-glucose phosphorylation on exogenous ATP concentra-

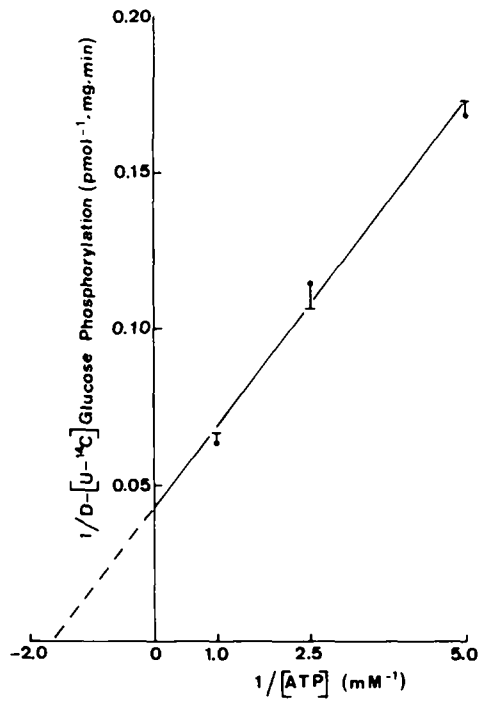

Fig. 1. Double-reciprocal plot for the phosphorylation of D-[U-^{14}]glucose by mitochondria preincubated in the absence of substrate and then exposed to both D-glucose (1.0 mM) and increasing nominal concentrations of ATP.

TABLE II

Formation of D-[U-^{14}C]glucose 6-phosphate and D-glucose 6-[^{32}P]phosphate by liver mitochondria

Expt.	Preincubation (min 0 to 5)	Incubation (min 6 to 15)	Phosphorylation rate (pmol/min per mg)	
			D-[U-^{14}C]glucose 6-*P*	D-glucose 6-[^{32}P]*P*
1	nil	Nil	1.06 ± 0.15 (6)	
	nil	ATP (0.2 mM)	3.85 ± 0.09 (13)	0.23 ± 0.01 (8)
	ADP (0.2 mM) + succinate (10.0 mM)	Nil	2.91 ± 0.02 (6)	
	ADP (0.2 mM) + succinate (10.0 mM)	ATP (0.2 mM)	4.03 ± 0.08 (13)	0.26 ± 0.01 (4)
2	nil	Nil	0.88 ± 0.12 (5)	
	nil	ATP (1.0 mM)	4.06 ± 0.05 (9)	0.71 ± 0.07 (6)
	ADP (0.2 mM) + succinate (10.0 mM)	Nil	4.84 ± 0.08 (5)	
	ADP (0.2 mM) + succinate (10.0 mM)	ATP (1.0 mM)	4.29 ± 0.09 (9)	0.88 ± 0.01 (3)

tion. Although these experiments indeed illustrate that the rate of hexose phosphorylation tightly depends on the availability of exogenous ATP, they also clearly document that endogenous ATP formed during preincubation in the presence of ADP and succinate, can also serve as a substrate in supporting D-[U-^{14}C]glucose phosphorylation.

The presence of CAT (12 μM) in the final incubation medium severely decreased ($P < 0.001$) the rate of D-[U-^{14}C]glucose phosphorylation under all experimental conditions (Table I, Expt. 2). The relative extent of such an inhibition was much higher ($P < 0.001$), however, when the final incubation was carried out in the absence of exogenous ATP (69.5 ± 5.2 percent inhibition) rather than presence of ATP (39.5 ± 2.9 percent inhibition). In this respect, there was no obvious difference between mitochondria preincubated in either the absence or presence of ADP and succinate. Control experiments indicate that CAT tested at the same concentration of 12 μM failed to affect significantly the phosphorylation of D-[U-^{14}C]glucose in either crude liver homogenates or the hepatic postmitochondrial supernatant. For instance, in the latter case, the reaction velocity averaged 46.7 ± 1.1 and 43.8 ± 1.1 pmol/min per mg liver wet weight in the absence and presence of CAT, respectively ($n = 9$ in both cases). These findings indicate that the phosphorylation of D-glucose by intact mitochondria is largely dependent on the integrity of ATP translocation across the inner mitochondrial membrane, even in the presence of exogenous ATP.

In order to distinguish between the contribution of endogenously formed ATP and exogenous ATP, respectively, the generation of D-glucose 6-phosphate from D-[U-^{14}C]glucose and unlabelled ATP was compared to that from unlabelled D-glucose and [γ-^{32}P]ATP. As shown in Table II, the contribution of exogenous [γ-^{32}P]ATP accounted for only a minor fraction of the overall rate of D-glucose phosphorylation. The relative extent of such a contribution was virtually identical in mitochondria preincubated in the absence or presence of ADP and succinate. It averaged 6.07 ± 0.31 percent ($n = 12$) at a low concentration of exogenous ATP (0.2 mM), and was increased ($P < 0.001$) to 18.05 ± 1.44 per cent ($n = 9$) at a higher ATP concentration (1.0 mM). Incidentally, the rates of D-[U-^{14}C]glucose phosphorylation recorded in the presence of ATP were lower, especially at a high initial concentration of the nucleotide, in this second series of experiments, probably as a result of the longer incubation period and, hence, further fall in exogenous ATP concentration.

If it is assumed that the difference between the generation of D-[U-^{14}C]glucose 6-phosphate and D-glucose 6-[^{32}P]phosphate reflects the contribution of endogenously formed ATP to hexose phosphorylation, it may be observed that such a contribution, expressed in absolute terms, was higher in the mitochondria incubated in the presence, rather than absence, of exogenous ATP, at least when the preincubation was carried out in the absence of ADP and succinate. Thus, in the latter case, the amount of endogenous ATP used for the phosphorylation of D-glucose represented, in the absence of exogenous ATP, only 27.8 ± 3.2% ($n = 11$) of that used in the presence of exogenous ATP. A different situation was found in mitochondria preincubated with ADP and succinate. In such a case, the ratio between the utilization of endogenous ATP in the absence/presence of exogenous ATP was higher, and increased from 77.2 ± 2.2% to 141.9 ± 3.5% as the concentration of exogenous ATP was raised from 0.2 to 1.0 mM.

These data indicate that a large fraction of the ATP used to support D-glucose phosphorylation is derived from endogenously formed ATP, even in the presence of exogenous ATP. They also suggest that the generation of ADP from exogenous ATP (e.g., as catalyzed by ATPase) conditions the regeneration of ATP from ADP either by oxidative phosphorylation or in the reaction catalyzed by adenylate kinase. However, when the mitochondria were loaded with endogenous ATP through preincubation with ADP and succinate, the role of exogenous ATP during incubation as a source of ADP faded out. At a high concentration of exogenous ATP, the prevailing effect of the nucleotide even re-

TABLE III

Effect of mitochondrial poisons upon the fractional contribution of exogenous ATP to D-glucose phosphorylation

Preincubation (min 0 to 5)	Incubation (min 6 to 35)	Phosphorylation rate (pmol/min per mg)		Exogenous ATP (%) [b]	
		D-[U-^{14}C]glucose 6-*P*	D-glucose 6-[^{32}P]*P*		
Nil	ATP (0.4 mM)	4.46 ± 0.06 (3)	0.33 (1)	7.4	(1)
ADP (2.0 mM) + succinate (10.0 mM)	ATP (0.4 mM)	5.21 ± 0.01 (3)	0.22 (1)	4.2	(1)
Nil	ATP (0.4 mM) + poisons [a]	1.59 ± 0.01 (3)	0.53 ± 0.01 (2)	33.1 ± 0.3	(2)
ADP (2.0 mM) + succinate (10.0 mM)	ATP (0.4 mM) + poisons	2.32 ± 0.02 (3)	0.38 ± 0.04 (2)	16.2 ± 1.6	(2)

[a] CAT (12 μM) + KCN (1.0 mM) + rotenone (10 μM)

[b] Fractional contribution of exogenous ATP to the overall rate of D-glucose phosphorylation.

sulted in a sparing action on the utilization of endogenously formed ATP for the purpose of D-glucose phosphorylation.

Contribution of exogenous and endogenous ATP to glucose phosphorylation

The two last series of experiments performed with liver mitochondria aimed at altering the respective contributions of exogenous and endogenous ATP to glucose phosphorylation through environmental changes during preincubation and/or incubation.

The initial ADP concentration was first raised to 2.0 mM and the final incubation period increased from 5 to 30 min (Table III). Despite the longer incubation, the rate of D-[U-^{14}C]glucose phosphorylation in the absence of ADP and succinate but presence of ATP (0.4 mM) was close to that otherwise found over 10 min incubation and the fractional contribution of exogenous ATP remained quite low (7.4 %). The presence of metabolic poisons (KCN 1.0 mM and rotenone 10 μM) and CAT (12 μM) during incubation decreased the total rate of D-[U-^{14}C]glucose phosphorylation, whether ADP (2.0 mM) and succinate (10.0 mM) were present or not in the medium at the onset of preincubation. However, the metabolic poisons markedly increased both the absolute and fractional contribution of exogenous [γ-^{32}P]ATP to hexose phosphorylation. Moreover, in the presence of metabolic poisons, (pre)incubation with ADP and succinate reduced by about half ($P < 0.01$) the fractional contribution of exogenous ATP to D-glucose phosphorylation.

TABLE IV

Effect of various agents upon the phosphorylation of D-[U-^{14}C]glucose (1.0 mM) during preincubation (30 min) of liver mitochondria in the absence of exogenous ATP

Ado_2P_5 (μM)	CAT (μM)	KCN (mM)	Rotenone (μM)	D-[U-^{14}C]Glucose phosphorylation (pmol/min per mg)
–	–	–	–	0.65 ± 0.06 (12)
10	–	–	–	0.45 ± 0.03 (3)
500	–	–	–	0.41 ± 0.03 (3)
–	12	1.0	10	0.07 ± 0.01 (12)
10 to 500	12	1.0	10	0.08 ± 0.01 (6)

In order to further decrease the fractional contribution of endogenous ATP to glucose phosphorylation, the mitochondria were then preincubated for 30 min in the presence of D-glucose (1.0 mM) with the view of depleting endogenous nutrients and ATP. During such a preincubation, the rate of phosphorylation of D-[U-^{14}C]glucose, as measured in absence of exogenous ATP, averaged 0.65 ± 0.06 pmol/min per mg. During the preincubation, the phosphorylation of D-[U-^{14}C]glucose was inhibited by about 30% ($P < 0.01$) in the presence of 10 μM Ado_2P_5, no further decrease in phosphorylation rate being observed at a much higher concentration (500 μM) of the adenylate kinase inhibitor (Table IV). An inhibition of about 90% ($P < 0.001$) was recorded in the presence of CAT (12 μM) together with KCN (1.0 mM) and rotenone (10 μM), in which case Ado_2P_5 failed to cause a further decrease in hexose phosphory-

TABLE V

Effect of a preincubation with D-glucose upon the fractional contribution of exogenous ATP to D-glucose phosphorylation

Preincubation (min 0 to 30)	Incubation (min 31 to 60)	Phosphorylation rate (pmol/min per mg)		Exogenous ATP (%) [b]
		D-[U-^{14}C]glucose 6-*P*	D-glucose 6-[^{32}P]*P*	
D-Glucose (1.0 mM)	ATP (0.4 mM)	0.81 ± 0.17 (7)	0.28 ± 0.02 (7)	44.3 ± 6.5 (7)
D-Glucose (1.0 mM)	ATP (0.4 mM) + poisons [a]	0.78 ± 0.08 (10)	0.41 ± 0.01 (4)	62.6 ± 1.4 (4)
D-Glucose (1.0 mM) + poisons	ATP (0.4 mM) + poisons	0.96 ± 0.04 (6)	0.50 ± 0.01 (4)	55.6 ± 3.9 (4)

[a] CAT (12 μM) + KCN (1.0 mM) + rotenone (10 μM).

[b] Fractional contribution of exogenous ATP to the overall rate of D-glucose phosphorylation.

lation. The subsequent rate of D-[U-^{14}C]glucose phosphorylation by mitochondria preincubated in the sole presence of unlabelled D-glucose and then incubated in the presence of the ^{14}C-labelled hexose and exogenous ATP (0.4 mM) did not exceed 0.81 ± 0.17 pmol/min per mg (Table V), being lower than that seen under comparable conditions of incubation in mitochondria not exposed to D-glucose during preincubation (see Table III). The rate of D-[U-^{14}C]glucose phosphorylation was now comparable to that found in mitochondria exposed either during incubation or during both preincubation and incubation to CAT and metabolic poisons (Table V). In the absence of these agents, but after preincubation with D-glucose, the relative contribution of exogenous ATP to the phosphorylation of the hexose was 2- to 6-times higher than that recorded in previous experiments (see Tables II and III) when the mitochondria had been preincubated in the absence of D-glucose and incubated in the presence of 0.2 to 1.0 mM ATP. When CAT and metabolic poisons were incorporated into the final incubation medium, the production of D-glucose 6-[^{32}P]phosphate was increased ($P < 0.001$) to $169.4 \pm 4.5\%$ ($n = 7$) of the paired value found in the absence of these agents, resulting in a concomitant increase ($P < 0.01$) of the fractional contribution of [γ-^{32}P]ATP to the total rate of hexose phosphorylation. Essentially similar results were recorded when CAT and the metabolic poisons were present during both the preincubation and incubation period. Incidentally, the fractional contribution of [γ-^{32}P]ATP to hexose phosphorylation was further increased to $73.8 \pm 5.7\%$ ($n = 3$) when Ado_2P_5 (10 μM) was present together with CAT and the metabolic poisons in the final incubation medium (Fig. 2).

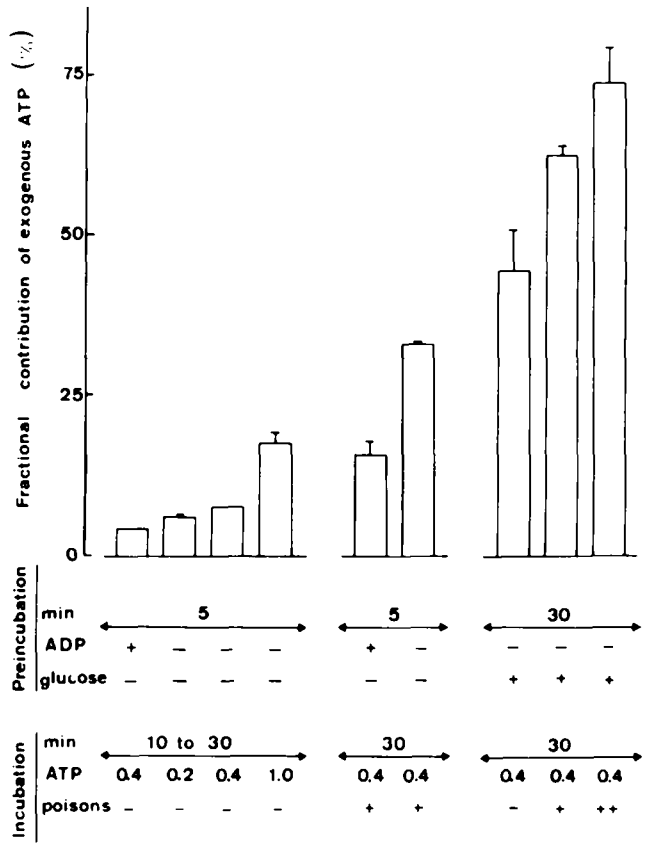

Fig. 2. Changes in the fractional contribution of exogenous ATP to the phosphorylation of D-glucose (1.0 mM) by liver mitochondria, as induced by environmental manipulations during preincubation and/or incubation. The length of both the preincubation and incubation periods (min), the presence of either ADP (together with succinate) or D-glucose during preincubation, and the nominal concentration of ATP (expressed as mM) and presence of poisons during incubation are shown in each case. Metabolic poisons included CAT (12 μM), KCN (1.0 mM) and rotenone (10 μM) in the concomitant absence (+) or presence (+ +) of Ado_2P_5 (10 μM).

Taken as a whole, these findings indicate that the availability of endogenous nutrients plays a critical role in regulating the relative contribution of exogenous and endogenous ATP to hexose phosphorylation. This interpretation is supported by the finding that the relative contribution of exogenous [γ-^{32}P]ATP to D-glucose phosphorylation during the final incubation period was directly related to the extent of hexose phosphorylation and, hence, endogenous substrate utilization during the preincubation period (Fig. 3).

Experiments in islet mitochondria

All experiments so far presented were conducted in liver mitochondria. In experiments performed with islet mitochondria, the hydrolysis of [γ-^{32}P]ATP (0.4 mM) increased from $11.2 \pm 0.2\%$ of total radioactivity ($n = 7$) after 5 min incubation to $34.8 \pm 2.6\%$ ($n = 16$) after 30 min incubation, as compared to a control value (no mitochondria) of $2.3 \pm 0.1\%$ ($n = 29$). As already observed in liver mitochondria, the relative extent of such a hydrolytic process appeared modulated by the generation rate of mitochondrial ATP. Thus, when ADP (0.2 to 2.0 mM) and succinate (10.0 mM) were added to the (pre)incubation medium, the amount of [^{32}P]P_i formed averaged $65.6 \pm 5.5\%$ ($n = 16$) of the paired reference value. Inversely, the presence of Ado_2P_5 (10 μM), KCN (1.0 mM), rotenone (10 μM) and CAT (12 μM) increased by $10.7 \pm 3.6\%$ ($n = 9$; $P < 0.02$) the rate of [γ-^{32}P]ATP hydrolysis.

In a series of five experiments, the rate of D-[U-^{14}C]glucose 6-phosphate formation over 30 min incubation in the sole presence of D-glucose (1.0 mM) and ATP (0.4 mM) averaged 0.88 ± 0.10 pmol/min per islet-equivalent. The formation of D-glucose 6-[^{32}P]-phosphate accounted for $41.7 \pm 1.6\%$ of such a total phosphorylation rate.

In experiments designed to study the effect of ADP and succinate upon the respective contribution of endogenous and exogenous ATP to hexose phosphorylation, it was first noticed, as already observed in other experimental models [13], that ADP, in high concentra-

tion, inhibited the formation of D-[U-^{14}C]glucose 6-phosphate. The relative extent of such an inhibition decreased from 64.6 ± 3.4% to 15.2 ± 2.0% as the concentration of ADP was lowered from 2.0 to 0.2 mM ($n = 6$ in both cases). Likewise, in the islet post-mitochondrial supernatant, the phosphorylation of D-[U-^{14}C]glucose (1.0 mM) in the presence of 0.4 mM ATP averaged 96.7 ± 4.8% and 41.0 ± 4.5% of the paired reference value (no ADP) in the presence of 0.2 and 2.0 mM ADP, respectively ($n = 6$ in each case). In order to make allowance for such an inhibitory action of ADP upon hexokinase activity, the results collected in the presence of ADP and succinate were analyzed in terms of the fractional contribution of exogenous [γ-^{32}P]ATP to the total rate of D-[U-^{14}C]glucose phosphorylation. Whether over 5 or 30 min incubation, the presence of ADP (0.2 to 2.0 mM) and succinate (10.0 mM) significantly decreased ($P < 0.01$) such a fractional contribution (Fig. 4).

When unlabelled D-glucose was present in the medium during preincubation and when Ado_2P_5 (12 μM), KCN (1.0 mM), rotenone (10 μM) and CAT (12 μM) were present in the incubation medium, the total rate of D-[U-^{14}C]glucose phosphorylation was decreased by 53.2 ± 3.1% ($n = 9$). The rate of D-glucose 6-[^{32}P]phosphate formation was not significantly affected, however, and became virtually identical to the total rate of D-[U-^{14}C]glucose phosphorylation (0.29 ± 0.03 vs. 0.28 ± 0.03 pmol/min per islet-equivalent; $n = 9$ in both cases).

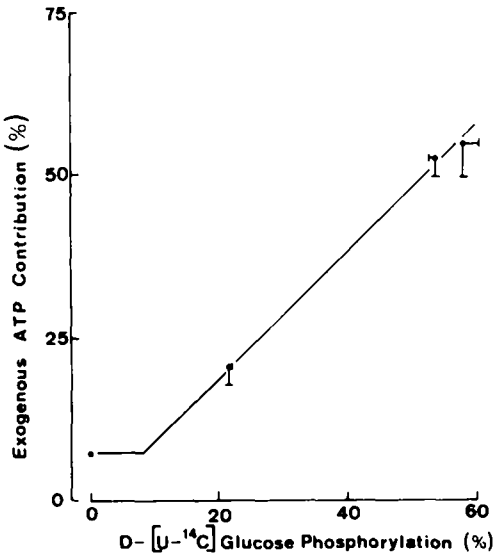

Fig. 3. Relationship between the fractional contribution of exogenous [γ-^{32}P]ATP (0.4 mM) to the total rate of hexose phosphorylation by liver mitochondria incubated in the presence of D-glucose (1.0 mM) and the amount of D-glucose phosphorylated over 30 min preincubation at the same hexose concentration expressed relative to the total rate of hexose phosphorylation during both preincubation and incubation (each for 30 min). The data without hexose phosphorylation during preincubation refer to mitochondria preincubated in the absence of D-glucose.

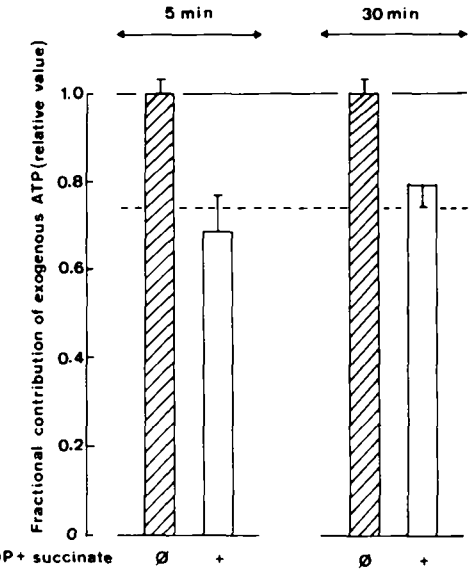

Fig. 4. Effect of ADP (0.2 to 2.0 mM) and succinate (10.0 mM) upon the fractional contribution of exogenous ATP to D-glucose phosphorylation by islet mitochondria over 5 min (left) or 30 min (right) incubation in the presence of D-glucose (1.0 mM) and ATP (0.4 mM). The results recorded in the presence of ADP and succinate (open columns) are expressed relative to the corresponding mean control value (shaded columns). The horizontal solid and dashed lines refer to the overall mean value for control and experimental data. Mean values (± S.E.) are derived from six or seven individual measurements.

These findings indicate that islet mitochondria are also able to derive the major fraction of ATP used to support D-glucose phosphorylation from mitochondrial ATP, even in the presence of exogenous ATP.

Discussion

It is well established that mitochondria-bound hexokinase can use ATP generated in the mitochondria as a substrate for D-glucose phosphorylation [14,15]. To our knowledge, however, the respective contributions of exogenous and intramitochondrially formed ATP to hexose phosphorylation in mitochondria exposed to exogenous ATP were never directly measured. Instead, available data are largely based on the use of selective inhibitors of either ATP formation or ADP translocation. In the present study, the parallel measurement of D-[U-^{14}C]glucose phosphorylation and D-glucose 6-[^{32}P]phosphate formation from [γ-^{32}P]ATP made it possible to directly assess the respective contribution of exogenous and endogenous ATP to hexose phosphorylation by liver and islet mitochondria. The initial concentration of ATP (usually 0.4 mM) was much lower than that of inorganic phosphate (10.0 mM) in order to minimize the reincorporation of [^{32}P]P_i, generated by hydrolysis of [γ-^{32}P]ATP into ATP formed by oxidative phosphorylation. Exogenous [γ-^{32}P]ATP indeed underwent hydrolysis under the present experimental conditions, the relative extent of [^{32}P]P_i formation depending

on such factors as the amount of mitochondria, length of incubation and generation rate of mitochondrial ATP. The process of ATP hydrolysis should not be overlooked if reference needs to be made to the true, rather than nominal or initial, concentration of exogenous ATP.

A salient finding in the present study consisted in the fact that, under conditions aiming at simulating close-to-physiological conditions, namely in the concomitant presence of exogenous ATP, ADP and succinate, the contribution of mitochondrial ATP to D-glucose phosphorylation largely exceeded that of exogenous ATP. The contribution of endogenous ATP to hexose phosphorylation was only partially (liver mitochondria) or completely (islet mitochondria) suppressed by the combination of various procedures aiming at either the exhaustion of endogenous nutrients (preincubation with D-glucose) or inhibition of oxidative phosphorylation (in the presence of KCN and rotenone), adenylate kinase (in the presence of Ado_2P_5) and ADP–ATP translocation (in the presence of CAT). In this respect, our results confirm the recent finding recorded in hepatoma mitochondria that ATP generated both by oxidative phosphorylation and in the reaction catalyzed by adenylate kinase may contribute to D-glucose phosphorylation [16]. Moreover, the present experiments indicate that the extramitochondrial concentration of ATP also modulates the contribution of endogenous ATP to hexose phosphorylation.

The relative contribution of exogenous ATP to total hexose phosphorylation was higher in islet than liver mitochondria, even when measured in the presence of ADP and succinate, which is a suitable metabolic substrate for islet cells [17,18]. Several factors may account for such a difference. First, relative to the amount of mitochondrial material (as judged from the activity of glutamate dehydrogenase), the rate of D-glucose phosphorylation was at least two orders of magnitude higher in islet than liver mitochondria and, hence, might correspond to a much larger fraction of the total rate of ATP generation. Second, the lesser hydrolysis of [γ-^{32}P]ATP in islet than liver samples, itself coinciding with the lower amount of mitochondrial material in the former than latter samples, may be expected to decrease the fractional contribution of endogenous ATP to hexose phosphorylation, in the same manner as here observed in liver mitochondria exposed to increasing concentrations of exogenous ATP. Third, the procedure and time required to separate pancreatic islets from the acinar tissue and to prepare mitochondria from such islets may conceivably modify their oxidative capacity relative to hexokinase activity. Moreover, the activity of mitochondria-bound hexokinase relative to that of either soluble enzyme or mitochondria-bound glucokinase may well differ in islet and liver samples, respectively [4].

With these considerations in mind, the present results unambiguously document that, in both liver and islet mitochondria, the major fraction of D-glucose phosphorylation by bound hexokinase is accounted for by the utilization of mitochondrial rather than exogenous (i.e., cytosolic) ATP. In islet cells, this situation may favour the coupling between metabolic and cationic events during the initial phase of glucose-stimulated insulin release by both helping to maintain a high concentration of cytosolic ATP and priming the mitochondria to produce ATP in response to an increase in pyruvate availability.

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