Influence of Substrate Composition on *in vitro* Oxygen Consumption of Lung Slices

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SUMMARY

The endogenous oxygen consumption of lung, liver and spleen slices is only slightly increased by glucose in an SRP medium compared with its effect on heart and kidney slices.

Individual substrates which induced a highly significant increase in oxygen uptake of lung tissue were succinate, acetate, pyruvate and glucose, whereas citrate and fumarate gave a non-significant response. When glucose and α-ketoglutarate were simultaneously used as substrates the rate of oxygen uptake was significantly higher than with glucose alone. Acetate, pyruvate and glutamate in combination with glucose had no significant effect.

Acetate and succinate in combination with pyruvate induced a significantly higher rate of oxygen uptake than pyruvate alone. The changes observed with pyruvate + α-ketoglutarate and pyruvate + citrate were not significant.

Albumin complexes of palmitate, stearate and β-hydroxybutyrate did not affect the endogenous oxygen uptake. Similarly, these complexes in combination with carnitine had no stimulating effect. However, fatty acid complexes in the presence of adenosine triphosphate (ATP) gave a highly significant increase, followed by a further increase in oxygen uptake when a combination of ATP and carnitine was used.

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The lung is an organ of extreme metabolic complexity. It is concerned with external gas exchange, acts as a filter for smaller particles, maintains a stable level of circulating leucocytes,[1] functions as a blood reservoir[2] and is the site of intrathoracic reflexes.[3]

The lung is of major importance in combating respiratory infections.[4] The macrophages are not only phagocytic but have bacteriolytic properties, while the bronchial secretions contain immunoglobulins, with IgA predominant.[5]

The lung is one of the richest sources of co-factors that either promote or inhibit blood coagulation.[1] It is a source of thromboplastin which converts prothrombin to thrombin; of an activator to convert plasminogen to plasmin;[6] of heparin synthesised by the mast cells;[7] of histamine and slow-reacting substances (SRS);[8] and of lipoprotein lipase.[9]

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The lung is also concerned with the inactivation of serotonin,[10] bradykinin[11] and the removal of prostaglandins from the circulation[12] and it activates the angiotensin system by converting angiotensin I to angiotensin II.[13] It also participates in the *de novo* synthesis of fatty acids,[14] proteins[15] and of phospholipids (surfactant).[14]

The oxygen consumed by the lung is used not only for its own basal metabolic needs but for additional metabolic reactions, as indicated above. Owing to the heterogeneous composition of the lung the oxygen uptake by the different cellular components is still unknown, but it would appear that the alveolar macrophages, epithelial and mast cells might be responsible for most of this oxygen uptake.

The rate of oxygen consumption of lung during *in vitro* perfusion experiments is but little affected by the addition of lactate, glucose or epinephrine to the perfusion fluid.[16] Glucose appeared to be an important nutrient and lactic, pyruvic, acetic and palmitic acids are used for the biosynthesis of various components of lipids.[1]

Information regarding the rate of oxygen consumption of lung tissue and the major substrates needed for metabolic processes is still fragmentary. Experiments were therefore designed to investigate these aspects.

MATERIALS AND METHODS

Male albino rats (*Rattus norvegicus,* Wistar Institute) weighing 200 ± 10 g were used. The animals were fed on a balanced standard ration and prior to killing they were fasted for 12 hours. The rats were stunned by a blow on the head and then killed by severing the blood vessels of the neck. Special care was taken to prevent blood entering the lungs. The lungs and other organs were immediately removed and chilled in Petri dishes on ice before processing.

Slices from lung and other organs were cut free-hand, blotted, weighed (±80 mg) and then transferred into the main compartments of Warburg flasks each containing 2,7 ml medium, 0,3 ml substrate in the side-arm and 0,2 ml 10% KOH in the centre well, for the absorption of CO_2.

The oxygen uptake was measured according to the direct technique of Warburg in a Braun's Warburg apparatus, Model V at 37°C. Air was used as gas phase and the V_f was 3,2 ml.

Two suspension media were used: (*a*) Krebs-Ringer-phosphate (KRP) consisting of 100 ml NaCl (0,9%), 4 ml KCl (1,15%), 3 ml $CaCl_2$ (1,22%), 1 ml $MgSO_4.7H_2O$ (3,82%) and 12 ml phosphate buffer [$Na_2HPO_4.2H_2O$ (0,15M) plus N HCl, pH = 7,31; (*b*) Sorensen-Ringer-phosphate (SRP) consisting of 49 ml Ringer's solution, 26

ml NaCl (0,9%) and 25 ml buffer [$Na_2HPO_4.2H_2O$ (0,15M) plus KH_2PO_4 (0,15M), pH = 7,3].

All solutions were made with boiled de-ionised water. The concentration of the water-soluble substrates was 0,1M for single substrates or 0,05M when two substrates were investigated.

Fatty acid-albumin complexes were prepared according to Milstein and Driscoll[17] and were used in combination with carnitine (10^{-3}M) and adenosine triphosphate (3×10^{-4}M).[18]

The results are expressed as Q_{O_2} -wet weight or Q_{O_2} -dry weight. (Q_{O_2} = μL O_2 per mg tissue wet or dry weight per hour.)

RESULTS AND DISCUSSION

Rate of Oxygen Uptake of Various Tissues of the Rat, Obtained under Standardised Conditions in a SRP Medium (Table I)

The results obtained in a glucose-free medium indicate that the basal rate of oxygen consumption of lung and heart slices was of the same magnitude within narrow limits, that of spleen and liver slightly higher, whereas kidney slices consumed nearly three times more oxygen than lung and more than twice as much as spleen and liver.

In a glucose-containing medium (0,1M) the oxygen uptake of lung increased by 4,42%, liver by 6,08% and spleen by 6,71%. In contrast with these tissues the oxygen consumption of kidney and heart slices increased by 26,66% and 34,85%, respectively.

The major uptake of oxygen in cells occurs as a result of oxidative reactions in the mitochondria. Judged by the relatively high increase in oxygen consumption of heart and kidney slices in a glucose medium it would appear that exogenous glucose makes an important contribution to the energy needs of these tissues.

TABLE I. OXYGEN CONSUMPTION OF LUNG, HEART, SPLEEN, LIVER AND KIDNEY SLICES IN AN SRP MEDIUM *IN VITRO*

	Q_{O_2} dry weight*		
Tissue	Without glucose	With glucose (0,1M)	% increase
Lung	**5,66**	**5,91**	**+ 4,42**
Heart	**5,94**	**8,01**	**+34,85**
Spleen	**7,15**	**7,63**	**+ 6,71**
Liver	**7,40**	**7,85**	**+ 6,08**
Kidney	**15,53**	**19,67**	**+26,66**

* $Q_{O_2}{}^{dw}$ = μL O_2/mg dry tissue/h.

Effects of Various Substrates on the Oxygen Uptake of Lung Slices in a KRP Medium (Table II)

The substrates investigated can be divided into two groups. Glucose, pyruvate, lactate and acetate are normal extramitochondrial substrates, whereas citrate, α-ketoglutarate, succinate and fumarate are mostly components of the citric acid cycle in the mitochondria.

The results indicate that glucose, acetate, α-ketoglutarate, lactate and succinate increased the oxygen uptake of lung slices significantly ($P<0,01$) compared with the basal metabolic rate. Pyruvate caused a less significant increase ($P<0,05$), whereas citrate and fumarate gave an insignificant effect ($P>0,05$).

Why a relatively high concentration of extramitochondrial pyruvate gave a less significant increase in oxygen uptake than either glucose or acetate, is difficult to explain. Pyruvate may be used either for gluconeogenesis or for energy production. Under basal conditions the acetyl-CoA derived from β-oxidation of fatty acids may activate pyruvate carboxylase and thus stimulate gluconeogenesis.[19] The concentration of ATP may also determine whether the pyruvate dehydrogenase enzyme complex, which is necessary for the conversion of pyruvate to acetyl-CoA, is in an active or inactive state. If the ATP production is relatively high during the experiment, it may inhibit the activity of the pyruvate dehydrogenase complex directly.[20]

The rate of oxygen uptake may also be affected by the diffusion rate of the substrates across the mitochondrial membrane or by the relative concentrations of co-enzymes in the cell. If these mechanisms are involved, it would appear that acetate, α-ketoglutarate and succinate are readily transported into the mitochondria. Apparently the ability of fumarate to diffuse into the mitochondria of lung cells is very limited. No information is available regarding the relative concentrations of the co-dehydrogenases of lung tissue.

TABLE II. *IN VITRO* EFFECT OF VARIOUS SUBSTRATES ON THE OXYGEN CONSUMPTION OF LUNG SLICES IN A KRP MEDIUM

Substrate (0,1M)	Q_{O_2}wet weight* ± SE	% deviation	*P* values
No substrate	**0,79 ± 0,03**		
Glucose	**0,85 ± 0,02**	**+ 7,06**	**<0,01**
Pyruvate	**0,85 ± 0,03**	**+ 7,06**	**<0,05**
Acetate	**1,03 ± 0,07**	**+23,30**	**<0,01**
Citrate	**0,83 ± 0,04**	**+ 4,82**	**>0,05**
α-ketoglutarate	**0,85 ± 0,02**	**+ 7,06**	**<0,01**
Succinate	**1,07 ± 0,07**	**+26,17**	**<0,01**
Fumarate	**0,77 ± 0,02**	**− 2,60**	**>0,05**
Lactate	**0,88 ± 0,01**	**+11,39**	**<0,01**

* $Q_{O_2}{}^{ww}$ = μL O_2/mg wet tissue. Each Q_{O_2} value represents the mean of 9 determinations.

Effects of Substrate Combinations on the Oxygen Uptake of Lung Slices (Table III)

Comparing the effects of glucose in combination with other substrates with that of glucose alone, it is evident that only the glucose + α-ketoglutarate combination increases the oxygen uptake significantly ($P < 0,05$). The glucose + acetate mixture even causes a slight decrease in the Q_{O_2}-value of lung slices.

When pyruvate with other substrates is used, the pyruvate + acetate and pyruvate + succinate combinations

TABLE III. *IN VITRO* EFFECT OF COMBINATIONS OF SUBSTRATES ON THE OXYGEN CONSUMPTION OF LUNG SLICES IN A KRP MEDIUM

Substrates (0,1M)	Qo_2* wet weight ± SE	% deviation	*P* values
Glucose	0,85 ± 0,02		
Glucose + acetate	0,82 ± 0,03	− 3,66	>0,05
Glucose + pyruvate	0,91 ± 0,05	+ 6,59	>0,05
Glucose + α-ketoglutarate	1,00 ± 0,06	+15,00	<0,05
Glucose + glutamate	0,89 ± 0,05	+ 4,49	>0,05
Pyruvate	0,85 ± 0,03		
Pyruvate + acetate	0,96 ± 0,04	+11,46	<0,05
Pyruvate + citrate	0,90 ± 0,06	+ 5,56	>0,05
Pyruvate + α-ketoglutarate	0,91 ± 0,04	+ 6,59	>0,05
Pyruvate + succinate	0,95 ± 0,03	+10,53	<0,01

* Each Qo_2 value represents the mean of 9 determinations.

TABLE IV. *IN VITRO* EFFECT OF ATP, CARNITINE, FATTY ACIDS AND BETAHYDROXYBUTYRATE ON THE OXYGEN UPTAKE OF LUNG SLICES

Substrates (0,1M)	Qo_2 wet weight ± SE	% deviation	*P* values
Na-palmitate			
Basal rate	0,82 ± 0,007		
Na-palmitate	0,82 ± 0,02		>0,05
Na-palmitate + carnitine	0,83 ± 0,02		>0,05
Na-palmitate + ATP	0,95 ± 0,01	+15,38	<0,01
Na-palmitate + ATP + carnitine	1,03 ± 0,01	+25,37	<0,01
Na-stearate			
Basal rate	0,82 ± 0,001		
Na-stearate	0,82 ± 0,005		>0,05
Na-stearate + carnitine	0,82 ± 0,007		>0,05
Na-stearate + ATP	0,90 ± 0,01	+ 8,95	<0,01
Na-stearate + ATP + carnitine	0,92 ± 0,01	+12,38	<0,01
Na β-hydroxybutyrate			
Basal rate	0,82 ± 0,007		
β-hydroxybutyrate	0,81 ± 0,005		>0,05
β-hydroxybutyrate + ATP	0,90 ± 0,009	+ 8,95	<0,01
β-hydroxybutyrate + ATP + carnitine	0,92 ± 0,01	+12,41	<0,01

* Each Qo_2 value represents the mean of 6 determinations.

increased the oxygen uptake significantly. The changes observed with pyruvate + citrate and pyruvate + α-ketoglutarate as substrate were non-significant.

From these observations it is concluded that (*i*) the basal rate of oxygen uptake of lung tissue is increased by all substrates and substrate combinations investigated except with the glucose + acetate combination; (*ii*) the glucose + α-ketoglutarate, pyruvate + acetate and pyruvate + succinate combinations gave significant increases of the oxygen uptake; (*iii*) glucose, acetate, α-ketoglutarate and succinate are the best individual substrates for lung tissue; (*iv*) acetate in combination with glucose inhibited the oxidation of glucose, resulting in a lowering of the oxygen uptake below the values obtained with either glucose or acetate.

Effect of ATP, Carnitine, Fatty Acids and Beta-Hydroxybutyrate on the Oxygen Uptake of Lung Slices (Table IV)

The following conclusions can be drawn: (*i*) lung cells cannot utilise fatty acid complexes and ketone bodies for energy purposes in the absence of ATP and carnitine from the suspension medium; (*ii*) ATP caused a significant increase in oxygen uptake when fatty acid complexes and ketone bodies were used as substrates; (*iii*) carnitine alone did not facilitate the metabolism of fatty acid complexes and ketone bodies; and (*iv*) carnitine in combination with ATP gave a significant increase in oxygen consumption, much more marked than the effect of ATP by itself.

From these observations it would appear that the function of carnitine in facilitating acyl group transfer across mitochondrial membranes or its stimulating effect on fatty acid oxidation[21] in lung cells is ATP-dependent. However, palmitate is presumably a better substrate than stearate and β-hydroxybutyrate. It is known that palmitate, apart from being oxidised, can also be incorporated into the phospholipid fraction of lecithin,[22] one of the major components of surfactant.

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