*p*-CMB; 3:4-DHT formation is slower in absence of *p*-CMB attesting to the presence of the induction period.

A separate paper describing in more detail the work reported here is now in preparation.

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## Regulation of the uptake of glucose by the isolated rat diaphragm

The rate of utilisation of glucose by the isolated rat diaphragm is thought to be limited by the rate at which glucose is transferred from extracellular to intracellular water[1]. The uptake of glucose by the isolated rat diaphragm incubated *in vitro* in a balanced salt solution buffered with bicarbonate has been shown to be greater under anaerobic than aerobic conditions[2]. These observations suggest that the access of glucose to those enzymes in muscle which metabolise it is restricted by a process dependent upon oxidation. Further evidence in support of this view has been obtained by studying the uptake of glucose and release of potassium by isolated rat hemidiaphragms *in vitro* incubated in succession under aerobic and under anaerobic conditions, and also under aerobic conditions in the presence of 2:4-dinitrophenol, sodium arsenite and sodium cyanide.

Hemidiaphragms were obtained from male albino wistar rats of 100–150 g weight which had been fasted for 18–24 h. The conditions of incubation were as described previously[2]. The incubation medium was a balanced salt solution buffered with bicarbonate[3], and gassed either with 95% $O_2$, 5% $CO_2$ (aerobic conditions) or with 95% $N_2$, 5% $CO_2$ (anaerobic conditions). The uptake of glucose and release of potassium by the tissue during incubation were measured by the changes in the glucose and potassium contents of the medium, glucose being estimated by SOMOGYI's modification[4] of Nelson's method, and potassium by flame photometry. Glucose uptake was calculated as mg glucose disappearing from the medium/g of wet diaphragm/h of incubation and potassium release as $\mu$equiv. potassium appearing in the medium/g of wet diaphragm/h of incubation.

When diaphragms were incubated anaerobically under these conditions, both glucose uptake and release of potassium were greater than under aerobic conditions (Table I). This confirms earlier observations for both glucose[2] and potassium[5]. If diaphragms were incubated anaerobically for 20 min and then aerobically for a further 40 min, or incubated aerobically for 20 min and then anaerobically for a further 40 min, the overall uptake of glucose and release of potassium in either instance were both significantly less than the values for diaphragms incubated anaerobically for 60 min and significantly greater than those for diaphragms incubated aerobically for 60 min (Table I). In further experiments it was found that the enhanced uptake of glucose and release of potassium under anaerobic conditions could be readily discerned after only 5 min of incubation. Furthermore, when the conditions of incubation were changed after 20 min of incubation from anaerobic to aerobic or from aerobic to anaerobic the rates of uptake of glucose and release of potassium decreased in the former instance and increased in the latter within 10 min of changing the gas phase. Thus the changes which occur in the diaphragm under anaerobic conditions and which lead to increased rates of glucose uptake and potassium release can be reversed by restoration of aerobic conditions.

When diaphragms were incubated aerobically but in the presence of 2:4-dinitrophenol ($5 \cdot 10^{-5} M$ or $2.5 \cdot 10^{-4} M$), sodium arsenite ($10^{-3} M$) or sodium cyanide ($10^{-3} M$) the rates of uptake of glucose and release of potassium were very greatly increased (Table I).

TABLE I

UPTAKE OF GLUCOSE AND RELEASE OF POTASSIUM BY THE ISOLATED RAT DIAPHRAGM INCUBATED *in vitro* IN A BALANCED SALT SOLUTION BUFFERED WITH BICARBONATE

| *Experimental conditions* | *Period of incubation (min)* | *Glucose uptake. Mean ± S.E. of Mean. mg glucose/g of wet diaphragm/h of incubation* | *Potassium release. Mean ± S.E. of Mean. μequiv. potassium/g of wet diaphragm/h of incubation* |
|---|---|---|---|
| 1. Anaerobic | 0–60 | 5.2 ± 0.24 (18) | 37 ± 1.6 (12) |
| 2. Anaerobic | 0–20 | 4.2 ± 0.16 (18) | 21 ± 0.7 (12) |
| Aerobic | 20–60 | | |
| 3. Aerobic | 0–20 | 3.9 ± 0.11 (18) | 18 ± 1.8 (12) |
| Anaerobic | 20–60 | | |
| 4. Aerobic | 0–60 | 2.3 ± 0.10 (18) | 6 ± 1.3 (12) |
| Aerobic in the presence of: | | | |
| 5. Buffer alone | 0–60 | 2.0 ± 0.15 (12) | 14 ± 1.3 (12) |
| 6. 2:4-Dinitrophenol | | | |
| a. ($5 \cdot 10^{-5} M$) | 0–60 | 3.1 ± 0.15 (6) | 44 ± 1.8 (6) |
| b. ($2.5 \cdot 10^{-4} M$) | 0–60 | 5.0 ± 0.32 (6) | 47 ± 1.5 (6) |
| 7. Sodium arsenite ($10^{-3} M$) | 0–60 | 6.8 ± 0.33 (12) | 37 ± 2.7 (12) |
| 8. Sodium cyanide ($10^{-3} M$) | 0–60 | 8.4 ± 0.36 (12) | 33 ± 2.1 (12) |

For experiments 1–4, $P$ is not greater than 0.01 for differences between all means except for 3–2 where $P > 0.05$.

For experiments 5–8, $P$ is not greater than 0.01 except for (8–7, 6b–6a, 7–6a potassium) where $P > 0.05$.

Figures in parentheses – number of observations.

According to current views[6], potassium is retained in the muscle cell as a consequence of the selective extrusion of sodium by an active process: the present observations would not be at variance with this idea. The entry of glucose into the muscle cell has been envisaged as occurring either by an active process or by diffusion[1]. Our results suggest that the entry of glucose into the muscle cell is restrained directly or indirectly by an active process dependent upon oxidative phosphorylation.

The accelerated uptake of glucose by the isolated rat diaphragm which is brought about by insulin under aerobic conditions would appear to be distinct from that brought about by oxygen lack, 2:4-dinitrophenol, arsenite or cyanide in that it is not associated with an increased release of potassium[7]. Nevertheless, the possibility now has to be considered that insulin facilitates the uptake of glucose by the isolated rat diaphragm by inhibiting a process which restricts the entry of glucose into the cell. If insulin acts, for example, by dissociating phosphorylation (whether oxidative or glycolytic) from a process which otherwise restricts the entry of glucose into the cell, then such an action could make more ATP available for glycogen and peptide bond synthesis, reactions which are known to be promoted by insulin. Such an action of insulin would have an important element in common with that of the thyroid hormone, which is believed to uncouple oxidative phosphorylation in a specific manner (LARDY AND FELDOTT[8]).

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