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The regulation of the oxidation of fatty acids and other substrates in rat heart mitochondria by changes in the matrix volume induced by osmotic strength, valinomycin and Ca^{2+}

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1. The rate of ADP-stimulated respiration with various substrates and the matrix volume of rat heart mitochondria were measured over a range of osmolarities of the medium. 2. The rate of oxidation of palmitoylcarnitine (in the presence of malate) was stimulated 7-fold by increasing the matrix volume from 0.6 to 1.0 μl/mg of protein. Oxidation of octanoate showed a similar sensitivity to the matrix volume, whereas oxidation of other substrates showed little sensitivity until the volume fell below 0.7 μl/mg of protein. 3. The matrix volume of heart mitochondria incubated under physiological conditions was about 0.8 μl/mg of protein. 4. Low concentrations of valinomycin added to mitochondria incubated under such physiological conditions could activate the rate of ADP-stimulated palmitoylcarnitine oxidation by at least 100%. 5. Decreasing the matrix volume increased the reduction of the electron-transferring flavoprotein (ETF), suggesting an effect on electron flow between ETF and ubiquinone, as has been observed for liver mitochondria [Halestrap & Dunlop (1986) Biochem. J. **239**, 559–565]. 6. A rapid decrease in light-scattering by heart mitochondria incubated in State 4 was induced by addition of Ca^{2+}, reaching 50% of the maximal effect after about 30 s at 30 °C and with $K_{0.5}$ for Ca^{2+} of 0.3 μM. This was not associated with a change in matrix volume, and is discussed in terms of a conformational change whose identity remains to be determined. 7. However, incubation of heart mitochondria at 37 °C in the presence of 0.65 μM-Ca^{2+} for 4 min did increase the matrix volume significantly, by 0.181 ± 0.029 μl/mg of protein ($n = 7$, $P < 0.001$), similar to the Ca^{2+}-induced changes observed with liver mitochondria [Halestrap, Quinlan, Whipps & Armston (1986) Biochem. J. **236**, 779–787]. 8. The possible significance of these results in the co-ordinate regulation of fatty acid oxidation and the citric acid cycle in the heart responding to increased work load or hormonal stimulation is discussed.

INTRODUCTION

The regulation of fatty acid β-oxidation in the liver has been studied in some detail (see Sugden & Williamson, 1981, 1982; McGarry & Foster, 1983), and it has been concluded that control of carnitine acyltransferase 1 by malonyl-CoA is of major importance. In addition, work from our laboratory has provided evidence for intramitochondrial regulation of β-oxidation through changes in the matrix volume (Halestrap & Dunlop, 1986). Such increases in matrix volume have been shown to occur in liver cells in response to hormonally induced increases in cytoplasmic [Ca^{2+}] (Quinlan *et al.*, 1983; Halestrap *et al.*, 1986). In the heart, low activities of acetyl-CoA carboxylase have been detected (Scholte *et al.*, 1986) and the carnitine acyltransferase 1 is very sensitive to inhibition by malonyl-CoA (Saggerson, 1986). However, there is no evidence that fatty acid β-oxidation in the heart is regulated by this means.

The only function of β-oxidation in the heart is the production of acetyl-CoA for further oxidation by the citric acid cycle. Its regulation might therefore be expected to be linked to that of the citric acid cycle. When glucose rather than fatty acids is the respiratory fuel, the supply of acetyl-CoA from pyruvate is regulated in such a way. Thus a build-up either of acetyl-CoA itself or of NADH will inhibit pyruvate dehydrogenase both through a direct feedback inhibition and by increasing the phosphorylation of the enzyme (Randle, 1978). Latipaa *et al.* (1986) have demonstrated that [NADH]/[$NADH^+$] ratios regulate β-oxidation by inhibiting 3-hydroxyacyl-CoA dehydrogenase, whereas changes in [acetyl-CoA]/[CoA] ratios were without significant effect.

In addition to these feedback effects, isocitrate dehydrogenase, 2-oxoglutarate dehydrogenase and pyruvate dehydrogenase may be regulated by physiological concentrations of Ca^{2+} (see Denton & McCormack, 1985; Hansford, 1985). Thus, when glucose is the substrate for the heart, hormones and increased workload, acting through an increase in cytoplasmic and therefore mitochondrial [Ca^{2+}], are able to stimulate the co-ordinated production of acetyl-CoA and its oxidation around the citric acid cycle (McCormack & England, 1983). Such a mechanism would allow stimulation of the respiratory chain through the supply of NADH as well as through changes in the [ATP]/[ADP] ratio. Changes in the latter might well be undesirable for a tissue which seeks to maintain its [ATP]/[ADP] ratio in order to drive contraction. Indeed, there is considerable evidence to suggest that respiration can be stimulated in the working heart without a significant fall in the [ATP]/[ADP] ratio (Opie *et al.*, 1971; Neely *et al.*, 1972; From *et al.*, 1986). However, if such a Ca^{2+}-mediated stimulation of NADH supply is to occur when fatty acids are the major

Abbreviation used: ETF, electron-transferring flavoprotein.

respiratory fuel for the heart, there must be a Ca^{2+}-dependent means of stimulating acetyl-CoA production from β-oxidation in order to service the stimulated citric acid cycle. A Ca^{2+}-induced increase in matrix volume and consequent stimulation of fatty acid oxidation such as is observed in liver mitochondria would provide such a mechanism. In this paper I demonstrate that such a mechanism can operate in heart mitochondria.

EXPERIMENTAL

Materials

Rats. Male Wistar rats (300–400 g body wt.) were used and were allowed free access to food (Breeding Diet, from Oxoid, Basingstoke, Hants., U.K.) and water.

Chemicals. The sources of all chemicals, biochemicals and radiochemicals were as given in Halestrap *et al.* (1986) and Halestrap & Dunlop (1986).

Methods

Preparation of isolated heart mitochondria. Rats were anaesthetized by intraperitoneal injection with Sagatal (1 ml/kg body wt.) and left for 20 min before removal of hearts and preparation of mitochondria by using Nagarse, by a modification of the method of Tyler & Gouse (1976). Hearts were chopped finely in medium (300 mM-sucrose, 2 mM-EGTA and 10 mM-Tris/HCl, pH 7.4) and incubated at 0 °C for 10 min in the presence of 0.17 mg of Nagarse (Teikoku Chemical Industry Co., Osaka, Japan)/ml before homogenization at 0 °C in fresh medium containing 5 mg of defatted albumin/ml (approx. 5 ml of medium/g wet wt. of heart). The initial homogenate was centrifuged at full speed in an M.S.E. Centaur centrifuge for 90 s at 4 °C, and the pellet was then rehomogenized in the same volume of medium and centrifuged again. Both supernatants were combined and centrifuged at 10000 *g* for 5 min at 4 °C, the resulting supernatants discarded and the mitochondrial pellets resuspended with a 'cold finger' in fresh medium to give a final protein concentration of approx. 15 mg of protein/ml. Percoll was then added to the mitochondrial suspension (final concn. 19%, v/v). After centrifugation at 14000 *g* for 10 min, the supernatant (which contains contaminating membrane fractions) was removed and the mitochondrial pellet resuspended in fresh medium. Low-speed and high-speed centrifugation was performed in the same manner as for the original homogenate, and a final mitochondrial pellet was obtained, which was largely free of red blood cells and contaminating membranes. This was resuspended to a final protein concentration of approx. 50 mg/ml.

Mitochondrial incubations. Mitochondria were incubated in buffer containing 10 mM-Mops, 5.5 mM-Tris, 3 mM-$MgCl_2$, 2.5 mM-potassium phosphate, 0.5 mM-EGTA, 0.5 mM-ATP, 5 mg of defatted bovine serum albumin/ml, 10 mM-NaCl (except where stated) and 15 mM-KCl, pH 7.2. Additional osmotic support was provided by the addition of either KCl or sucrose as indicated in the Figure legends. The same incubation buffer was used for oxygen-electrode studies and for the measurements of changes in light-scattering, flavoprotein reduction and matrix volumes. Details of these techniques may be found elsewhere (Halestrap *et al.*, 1986; Halestrap & Dunlop, 1986), but brief descriptions are given in the legends to the Figures. Where Ca^{2+} was added to mitochondrial incubations, the final free $[Ca^{2+}]$ was calculated by using a computer program, utilizing the dissociation constants for Mg^{2+} and Ca^{2+} of ATP, P_i, EGTA and any respiratory substrates present as described elsewhere (Thomas *et al.*, 1986). Corrections were made for the small pH changes apparent on Ca^{2+} addition. The exact concentration of the EGTA present

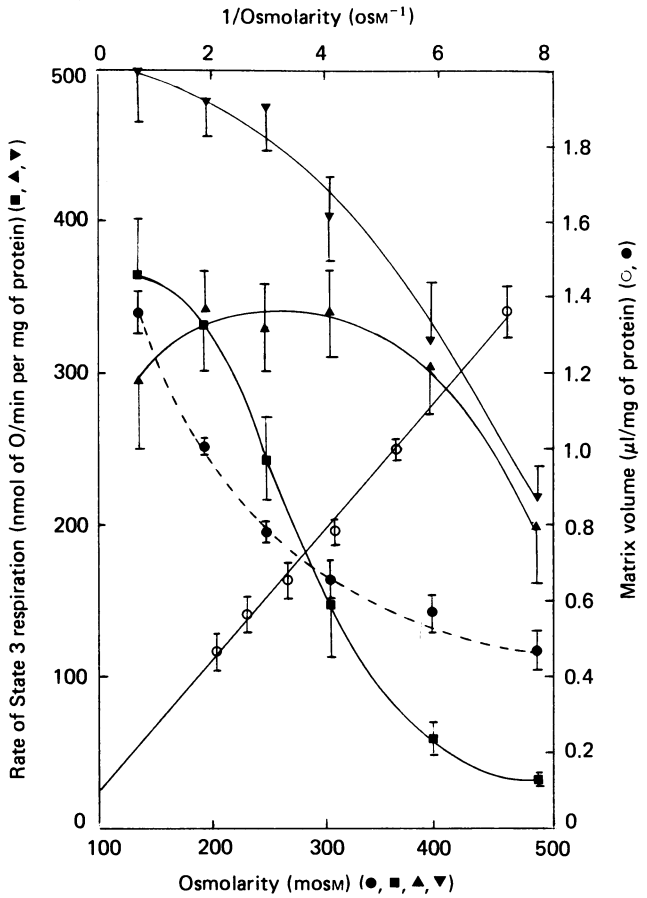

Fig. 1. Effects of osmolarity of the medium on the matrix volume of rat heart mitochondria and their rate of ADP-stimulated oxidation of various substrates

Rates of oxygen uptake were measured at 30 °C in the incubation medium described in the Experimental section but supplemented with 10 μg of cytochrome *c*/ml, 1 mM-ADP, sucrose to adjust the osmolarity to the required value and the relevant substrates. These were 5 mM-succinate in the presence of 0.1 μg of rotenone/ml (▼), 5 mM-glutamate + 1 mM-malate (▲), or 50 μM-palmitoylcarnitine + 1 mM-malate (■). Oxygen uptake was initiated by addition of approx. 0.7 mg of mitochondrial protein into 1 ml of medium, and the initial rate of oxygen uptake was measured. Mitochondrial volumes (●, ○) were measured at 0 °C in the same buffer without added substrate and in the presence of 1 μg of antimycin/ml, 0.1 μCi of [^{14}C]sucrose/ml and 1 μCi of ^3H_2O/ml. Values are plotted against both the osmolarity (broken line, ●) and its reciprocal (○). Details are given in the Experimental section. All values are given as the means ± S.E.M. (error bars) for five experiments on separate mitochondrial preparations.

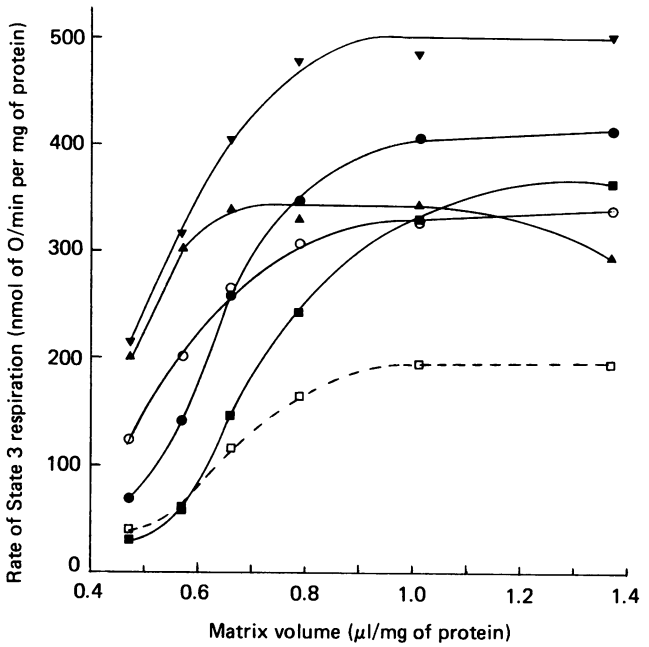

Fig. 2. Relationship between mitochondrial matrix volume and the rate of ADP-stimulated oxidation of various substrates by isolated rat heart mitochondria

Data are taken from Fig. 1 (■, ▲, ▼), with the same symbols as for that Figure, or from parallel experiments where the rate of oxidation of 5 mM-pyruvate ($n = 3$; ○), 10 mM-2-oxoglutarate ($n = 3$; ●) or 1 mM-octanoate ($n = 1$; broken line, □), all in the presence of 1 mM-malate and 1 mM-ADP, were measured. Error bars are not shown for clarity, but can be estimated by reference to Fig. 1.

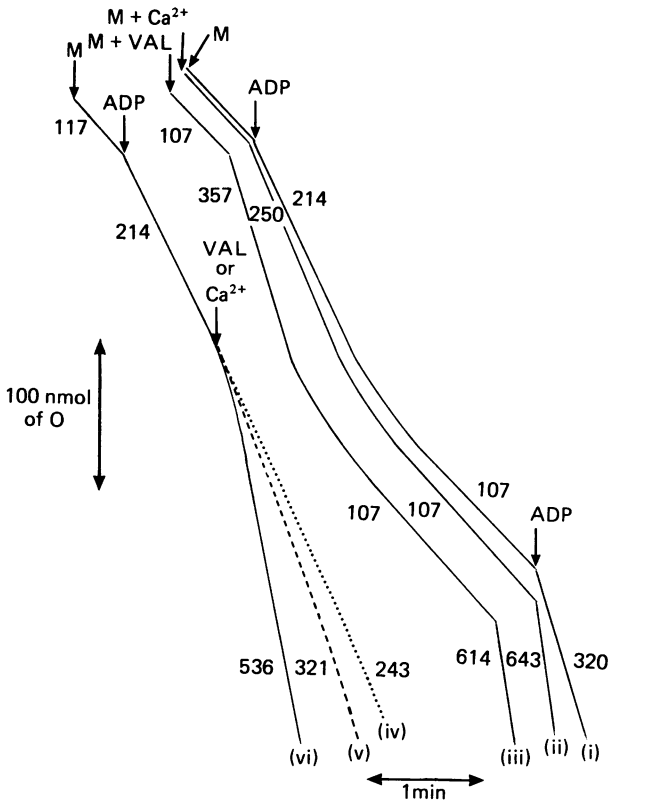

Fig. 3. Effects of valinomycin and Ca^{2+} on the oxidation of palmitoylcarnitine by rat heart mitochondria

Experiments were performed as described in the legend to Fig. 1 but at 37 °C in buffer containing 150 mM-KCl as osmotic support and initially no ADP. In addition the buffer contained 0.5 mM-ATP, 1 mM-malate and 50 μM-palmitoylcarnitine. Oxygen uptake was initiated by addition of 0.7 mg of mitochondrial protein (M), and ADP was added at 0.25 mM (iv–vi) or 1 mM (i–iii) as indicated. At the times shown, further additions were made as follows: 0.65 μM-Ca^{2+} (traces ii and v); 0.5 nM-valinomycin (VAL, traces iii and vi). Rates of oxygen uptake, in nmol of O/min per mg of protein, are given alongside each trace. The data shown are typical of three such experiments.

was calculated by titrating the EGTA in the incubation buffer containing mitochondria and Ruthenium Red (3 μg/ml) with standardized Ca^{2+} in the presence of Arsenazo-III (Halestrap *et al.*, 1986).

RESULTS

Effects of the mitochondrial matrix volume on the rate of ADP-stimulated oxidation of various substrates

Data are shown in Fig. 1 of the effects of varying the osmolarity of the incubation medium on the mitochondrial matrix volume and the rate of ADP-stimulated oxidation of various respiratory substrates. The matrix volume is also plotted against the reciprocal of the osmolarity in order to demonstrate the expected linear relationship for a perfect osmometer with an osmotically inactive space of about 0.1 μl/mg of protein (Halestrap & Quinlan, 1983). In one experiment [^{14}C]mannitol was used in place of [^{14}C]sucrose, and no significant difference was found between the results. This is in contrast with results obtained with liver mitochondria (Halestrap & Quinlan, 1983; Whipps & Halestrap, 1984) and may well reflect the use of Percoll in the present experiments to remove most of the contaminating membrane fractions. The latter could form vesicles which exclude sucrose but not mannitol or KCl. In Fig. 2 the rates of State 3 oxidation are plotted against the matrix volume. As was apparent for liver mitochondria (Armston *et al.*, 1982; Halestrap & Dunlop, 1986), all substrates showed some inhibition of oxidation when the matrix volume was decreased, but the oxidation of palmitoylcarnitine or octanoate was particularly sensitive. The oxidations of glutamate+malate and of succinate were only inhibited appreciably when the matrix volume fell below 0.6 μl/mg of protein, at which volume fatty acid oxidation was almost totally inhibited. The oxidation of 2-oxoglutarate and pyruvate showed intermediate sensitivity to matrix volume.

The effects of increasing the matrix volume by using valinomycin on the rate of palmitoylcarnitine oxidation are shown in the oxygen-electrode traces of Fig. 3. In these experiments an osmolality in the medium of 300 mosmolal (calculated from standard tables) was achieved by using 150 mM-KCl as osmotic support in an attempt to mimic the physiological situation in the heart. Either preincubation of the mitochondria in State 4 with 0.5 nM-valinomycin (trace iii versus trace i) or addition of 0.5 nM-valinomycin during State 3 oxidation (trace vi versus trace iv) led to substantial increases in the rate of

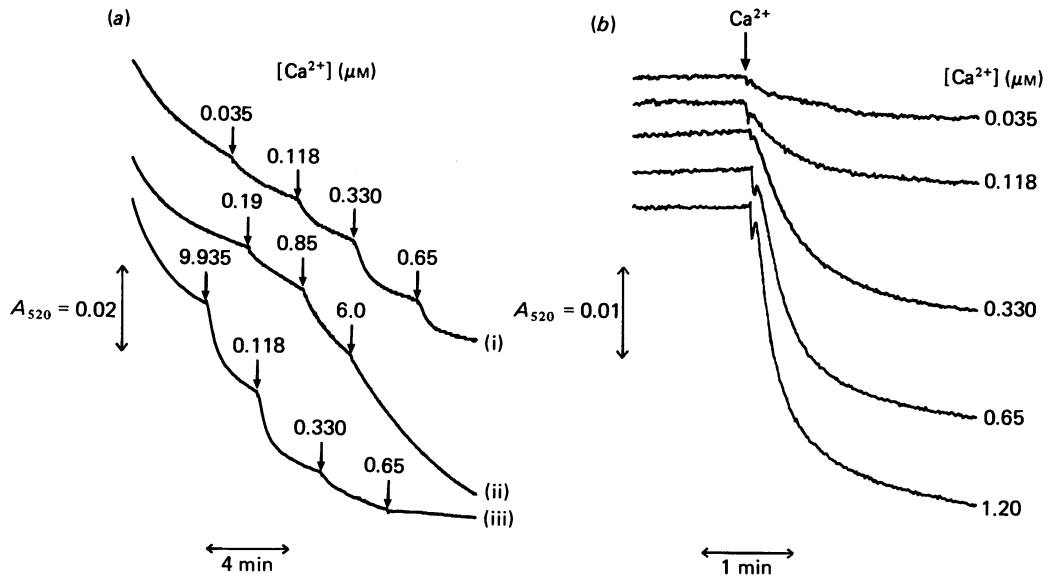

Fig. 4. Effects of Ca^{2+} on the light-scattering by rat heart mitochondria

Mitochondria (approx. 3 mg of protein/ml) were incubated at 30 °C with constant overhead stirring in a split-beam spectrophotometer with an atmosphere of 100% O_2. Standard incubation buffer was used containing 125 mM-KCl as osmotic support and 5 mM-glutamate + 1 mM-malate as substrate. In addition 10 mM-NaCl was present in the experiments of Fig. 4(*a*)(i) and throughout Fig. 4(*b*), and in Fig. 4(*a*)(ii) 3 μg of Ruthenium Red/ml was present. Light-scattering was monitored at 520 nm either with mitochondrial suspension only in the sample cuvette and with a metal grid of 1.0 absorbance in place of the reference cuvette (*a*) or with mitochondrial suspension in both cuvettes (*b*). Ca^{2+} was added to the sample cuvette as indicated to give the calculated free $[Ca^{2+}]$ shown.

palmitoylcarnitine oxidation. Parallel experiments (results not shown) showed no effects on the oxidation of glutamate + malate or succinate under these conditions.

Mitochondria incubated for 6 min in State 4 under the conditions of Fig. 3 had a matrix volume (mean ± S.E.M. for three separate mitochondrial preparations) of 0.798 ± 0.022 μl/mg of protein. If this can be taken as an estimate of the volume of the mitochondrial matrix *in situ*, then inspection of Fig. 2 shows that regulation of fatty acid β-oxidation by mitochondrial volume could occur under physiological conditions. After 4 min incubation in the oxygen-electrode chamber under the conditions of Fig. 3, the rate of oxygen uptake in the presence of palmitoylcarnitine (320 nmol of O/min per mg of protein at 37 °C) was similar to the value predicted from Fig. 2 for a matrix volume of this order (250 nmol/min per mg at 30 °C), when allowance is made for the difference in temperature between the experiments.

Effects of the matrix volume on flavoprotein reduction

In isolated liver mitochondria the inhibition of palmitoylcarnitine oxidation by decreasing the matrix volume can be localized between the electron-transferring flavoprotein (ETF) and ubiquinone (Halestrap & Dunlop, 1986). This also seems to be the case in heart mitochondria oxidizing palmitoylcarnitine in the presence of ADP. In two separate experiments the reduction state of ETF measured by changes in $A_{460-480}$ as described previously (Halestrap & Dunlop, 1986) increased from 15.7% and 24.6% to 35.7% and 42.8% respectively when the osmolarity of the incubation medium was increased from 160 to 500 mosmolar. The effect of matrix volume on the oxidation of octanoate, which does not require a specific transport process or the operation of carnitine acyltransferase for its oxidation, is shown in Fig. 2 and shows a similar sensitivity to matrix volume to that of palmitoylcarnitine oxidation. This also supports an intramitochondrial locus for the action of matrix volume on fatty acid oxidation in heart mitochondria.

Effects of Ca^{2+} on the matrix volume of heart mitochondria and on their light-scattering

In Fig. 4 I present data on the effects of submicromolar $[Ca^{2+}]$ on the light-scattering by isolated heart mitochondria. In Fig. 4(*a*) a single-beam technique was used to show that when incubated in State 4 the mitochondria show a decrease in light-scattering, just as do liver mitochondria (Halestrap *et al.*, 1986). A rapid further decrease in light-scattering occurs on addition of submicromolar $[Ca^{2+}]$, and this process is more sensitive to $[Ca^{2+}]$ in the absence of Na^+ and less sensitive in the presence of Ruthenium Red. These data imply that Ca^{2+} must enter the mitochondria to exert its effect, just as it does in liver mitochondria. The sensitivity to $[Ca^{2+}]$ of this response to Ca^{2+} under conditions approximating to physiological are shown by using a split-beam spectrophotometer in Fig. 4(*b*). In Fig. 5 a plot is given of $[Ca^{2+}]$ against the change in A_{520} observed in experiments on four different mitochondria preparations; a hyperbolic curve is obtained. Regression analysis of this curve by least-squares fit was used to calculate a $K_{0.5}$ for $[Ca^{2+}]$ of 0.32 μM (95% confidence limits 0.24–0.40 μM).

The light-scattering response seen in liver mitochondria on addition of submicromolar $[Ca^{2+}]$ was shown to reflect an increase in matrix volume (Halestrap *et al.*, 1986). However, when samples were taken from the

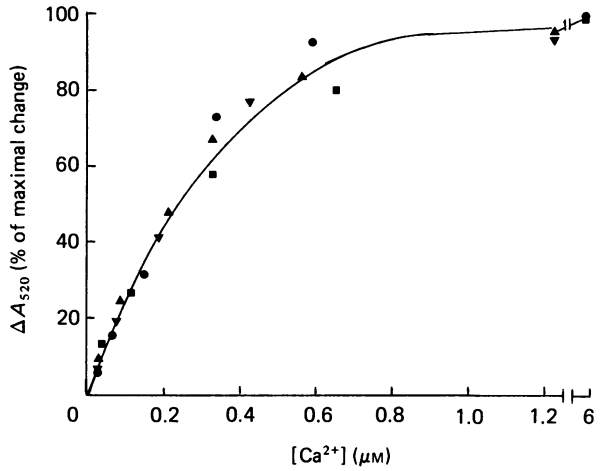

Fig. 5. Ca^{2+}-sensitivity of light-scattering in isolated heart mitochondria

Data are taken from four experiments similar to that shown in Fig. 4(*b*) and using different mitochondrial preparations as indicated by the symbols. The final change in light-scattering (ΔA_{520}), expressed as a percentage of the value at 6 μM-Ca^{2+}, after Ca^{2+} addition is plotted against the free [Ca^{2+}]. Statistical analysis of the plot is given in the text.

light-scattering experiments such as those shown in Fig. 4(*b*), no change in matrix volume was detected in four separate experiments. Thus the light-scattering changes seen must represent some conformational change of the mitochondria rather than a volume change. The adenine nucleotide transporter is known to exist in two conformations, and there is a light-scattering change when interconversion occurs, such as on addition of ADP or atractylate (Scherer & Klingenberg, 1974). However, addition of neither of these compounds produced a similar effect to that of Ca^{2+}. Nor did uncoupler, respiratory-chain inhibitors or anaerobiosis (results not shown).

It should be noted that the light-scattering changes seen here are considerably faster than those observed in liver mitochondria, and yet a temperature of 30 °C rather than 37 °C was used, again supporting a conformational change rather than a volume change being responsible for effects. The lower temperature was found to be important to avoid too rapid uptake of oxygen and anaerobiosis under the conditions used in the spectrophotometer. In order to establish whether volume changes might be observed if heart mitochondria were incubated with Ca^{2+} for longer and at 37 °C, experiments were performed in an orbital shaking waterbath and an atmosphere of 100% O_2. Mitochondria were incubated in State 4 under the same conditions as used for light-scattering for 6 min in the absence of Ca^{2+} or for 2 min in the absence and then 4 min in the presence of 0.65 μM-Ca^{2+} before addition of ^3H_2O and [^{14}C]sucrose and removal of 1 ml samples for the measurement of matrix volume. In seven separate experiments on different mitochondrial preparations the presence of 0.65 μM-Ca^{2+} increased the matrix volume (±S.E.M.) by 0.181 ± 0.022 μl/mg of protein from a basal value of 0.896 ± 0.064 μl/mg. In three such experiments the increase in volume caused by 2 nM-valinomycin was 0.300 ± 0.062 μl/mg of protein. It should be noted that the standard buffer used in these experiments is 300 mosmolar rather than 300 mosmolal. By using standard tables to correct for divergence from ideality for KCl, the osmolality can be calculated to be about 260 mosmolal and would account for the slightly higher value of matrix volume found in these experiments than those described above, where the osmolality was increased to 300 mosmolal.

The oxygen-electrode experiments recorded in Fig. 3 are fully consistent with the light-scattering data and matrix-volume measurements described above. Thus addition of 0.65 μM-Ca^{2+} to the mitochondria gave little initial stimulation of oxygen uptake (traces ii and v), but incubation in State 4 for 2 min with the same [Ca^{2+}] (or valinomycin) led to stimulation of oxygen uptake (trace iii) of the order predicted from the volume changes measured. Incubation even in the absence of [Ca^{2+}] gave a small stimulation of palmitoylcarnitine oxidation, which could be caused by a slight swelling during incubation in KCl medium as is seen with liver mitochondria (Halestrap *et al.*, 1986; Halestrap & Dunlop, 1986). The light-scattering changes reported in Fig. 4(*a*) might suggest that considerable swelling occurs, but direct measurement of the matrix volume confirmed that the volume only increased by 0.043 ± 0.014 μl/mg of protein (mean±S.E.M. for six separate mitochondrial preparations). This suggests that even these initial light-scattering changes are in large part due to some factor other than an increase in matrix volume, just as were effects of Ca^{2+} on light-scattering discussed above.

DISCUSSION

The data presented in this paper show that fatty acid β-oxidation in heart mitochondria can be regulated through changes in the matrix volume in the same manner as has been observed for liver mitochondria (Halestrap & Dunlop, 1986). The volume-sensitive step appears to be the transfer of electrons from ETF to flavoprotein, most probably through an effect on ETF dehydrogenase. Oxidation of other substrates may also be inhibited at low matrix volume, as they are in liver mitochondria, but significant effects at physiological matrix volumes would seem unlikely. Slater & Cleland (1953) also reported that incubation of a crude heart mitochondrial preparation in hyper-osmotic media inhibited the rate of succinate and 2-oxoglutarate oxidation. It is of interest that in liver mitochondria the relationship between matrix volume measured with [^{14}C]mannitol and the rate of palmitoylcarnitine oxidation is similar to that shown in Fig. 2 for heart mitochondria, where [^{14}C]sucrose was used. It seems likely that differences between the matrix volumes of liver mitochondria measured with the two extramitochondrial markers are a consequence of the presence of a non-mitochondrial contaminant in liver mitochondrial preparations, such as lysosomes, peroxisomes or endoplasmic-reticulum vesicles. Since Percoll-treated heart mitochondria were used in the present studies, and lysosomes and peroxisomes are less prevalent in heart, it would seem fair to compare the present results with the relationship between the rate of palmitoylcarnitine oxidation and the ^3H_2O/[^{14}C]mannitol-derived matrix volumes of liver mitochondria.

In the liver, hormonally induced Ca^{2+}-mediated changes of matrix volume have been detected which, when compared with the experiments *in vitro*, would lead to increased rates of β-oxidation (Quinlan *et al.*, 1983; Quinlan & Halestrap, 1986; Halestrap *et al.*, 1986; Halestrap & Dunlop, 1986). The 20% increase in matrix volume of heart mitochondria observed with addition of 0.65 μM-Ca^{2+} could well be of importance when hearts respond to adrenaline or increased workload by increasing their rate of respiration. It has been proposed that an increase in cytoplasmic, and hence mitochondrial-matrix, Ca^{2+} under these conditions will cause an activation of pyruvate dehydrogenase, isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase, leading to increased citric acid cycle activity (Denton & McCormack, 1985; Hansford, 1985). This would result in increased NADH production to fuel the increased respiratory demand. Although this is an attractive hypothesis, two factors are not obviously compatible with it. Firstly, both adrenaline and increased workload cause a decrease rather than an increase in mitochondrial NADH, as measured by fluorescence (Williamson & Jamieson, 1966; Moravec *et al.*, 1974; Illingworth *et al.*, 1975). This may reflect the change in [ATP]/[ADP][P_i] ratio that has been measured in some experiments (Illingworth *et al.*, 1975; Neely *et al.*, 1976; Soboll & Bunger, 1981; Bunger & Soboll, 1986) and the consequent stimulation of the respiratory chain. However it should be pointed out that several studies, including those using n.m.r., have failed to detect significant changes in whole-tissue ratios of these metabolites with increasing work (Opie *et al.*, 1971; Neely *et al.*, 1972; From *et al.*, 1986) or in the mitochondrial ratio after adrenaline treatment (Soboll & Bunger, 1981). It is possible that the Ca^{2+} effects on the mitochondrial dehydrogenases prevent NADH concentrations falling to values at which respiration is limited by the supply of NADH. Alternatively the fall in NADH could reflect volume-mediated stimulation of electron flow through the respiratory chain, as is thought to occur in liver mitochondria (Halestrap, 1982; Armston *et al.*, 1982; Quinlan & Halestrap, 1986), but the data of Fig. 2 would make this unlikely.

The second factor to be considered in relation to the involvement of Ca^{2+} activation of dehydrogenases in the regulation of respiration of the heart is that stimulating the citric acid cycle alone, without also increasing the supply of acetyl-CoA, would be ineffective. When glucose is the respiratory substrate, Ca^{2+}-dependent activation of pyruvate dehydrogenase can provide co-ordinated regulation of both processes. However, no such regulation of acetyl-CoA supply from β-oxidation has been described when fatty acids are the major fuel of the heart. The data in the present paper provide evidence that such a link may exist through Ca^{2+}-induced increases in matrix volume causing the required stimulation of fatty acid β-oxidation.

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REFERENCES

Armston, A. E., Halestrap, A. P. & Scott, R. D. (1982) Biochim. Biophys. Acta **681**, 429–439
Bunger, R. & Soboll, S. (1986) Eur. J Biochem. **159**, 203–213
Denton, R. M. & McCormack, J. G. (1985) Am. J. Physiol. **249**, E543–E554
From, A. H. L., Petein, M. A., Michurski, S. P., Zimmer, S. D. & Ugurbil, K. (1986) FEBS Lett. **206**, 257–261
Halestrap, A. P. (1982) Biochem. J. **204**, 37–47
Halestrap, A. P. & Dunlop, J. L. (1986) Biochem. J. **239**, 559–565
Halestrap, A. P. & Quinlan, P. T. (1983) Biochem. J. **214**, 387–393
Halestrap, A. P., Quinlan, P. T., Whipps, D. E. & Armston, A. E. (1986) Biochem. J. **236**, 779–787
Hansford, R. G. (1985) Rev. Physiol. Biochem. Pharmacol. **102**, 1–72
Illingworth, J. A., Ford, W. C. L., Kobayashi, K. & Williamson, J. R. (1975) Recent Adv. Stud. Card. Struct. Metab. **8**, 271–290
Latipaa, P. M., Karki, T. T., Hiltunen, J. K. & Hassinen, I. E. (1986) Biochim. Biophys. Acta **875**, 293–300
McCormack, J. G. & England, P. J. (1983) Biochem. J. **214**, 581–585
McGarry, J. D. & Foster, D. W. (1983) Handb. Exp. Pharmacol. **66**, 383–398
Moravec, J., Corsin, A., Owen, P. & Opie, L. H. (1974) J. Mol. Cell. Cardiol. **6**, 187–200
Neely, J. R., Denton, R. M., England, P. J. & Randle, P. J. (1972) Biochem. J. **128**, 147–159
Neely, J. R., Whitmer, K. M. & Mochizuki, S. (1976) Circ. Res. **38**, Suppl. 1, 22–30
Opie, L. H., Mansford, K. R. L. & Owen, P. (1971) Biochem. J. **124**, 475–490
Quinlan, P. T. & Halestrap, A. P. (1986) Biochem. J. **236**, 789–800
Quinlan, P. T., Thomas, A. P., Armston, A. E. & Halestrap, A. P. (1983) Biochem. J. **214**, 395–404
Randle, P. J. (1978) Trends Biochem. Sci. **3**, 217–223
Saggerson, D. (1986) Biochem. Soc. Trans. **14**, 679–681
Scherer, B. & Klingenberg, M. (1974) Biochemistry **13**, 161–170
Scholte, H. R., Luyt-Houwen, I. E. M., Dubelaar, M. & Hulsmann, W. C. (1986) FEBS Lett. **198**, 47–50
Slater, E. C. & Cleland, K. W. (1953) Biochem. J. **53**, 557–567
Soboll, S. & Bunger, R. (1981) Hoppe-Seyler's Z. Physiol. Chem. **362**, 125–132
Sugden, M. C. & Williamson, D. H. (1981) in Short-Term Regulation of Liver Metabolism (Hue, L. & Van de Werve, G., eds.), pp. 291–309, Elsevier Biomedical Press, Amsterdam
Sugden, M. C. & Williamson, D. H. (1982) in Compartmentation (Sies, H., ed.), pp. 287–315, Academic Press, London
Thomas, A. P., Diggle, T. A. & Denton, R. M. (1986) Biochem. J. **238**, 83–91
Tyler, D. D. & Gouse, J. (1976) Methods Enzymol. **10**, 75–77
Whipps, D. E. & Halestrap, A. P. (1984) Biochem. J. **221**, 147–152
Williamson, J. R. & Jamieson, D. (1966) Mol. Pharmacol. **2**, 191–205

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