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SUCCINIC ACID OXIDATION AS THE ONLY ENERGY SUPPORT OF INTENSIVE Ca^{2+} UPTAKE BY MITOCHONDRIA

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SUMMARY: On the addition of succinate, the Ca^{2+} capacity of mitochondria is greater by 5-7 times and Ca^{2+} retention is more than 10 times longer as compared with different NAD-Dependent substrates.

The great kinetic predominance of succinic acid over NAD-dependent substrates[1] in respect to oxidative phosphorylation and energy-dependent NAD reduction was discovered by Chance (1,2). This provides succinate monopolization in regulation of many energy-dependent and NAD-controlled processes in the respiratory chain. Ca^{2+} transport seems to be one of such processes. The effects of low concentrations of Ca^{2+} on the dynamics of respiration were first described also by Chance (3,4,5). These experiments indicated "...that respiratory activation by Ca^{2+} and oxidative phosphorylation of ADP involved the same energy conserving sites in the respiratory chain" (6). Considering these mechanisms succinate should be a much more effective support of Ca^{2+} transport than NDS. Succinate is usually used in current investigations of Ca^{2+} transport, however, no special comparison of its efficiency with NDS has been made under limited Ca^{2+} loading which does not damage mitochondria. Under massive Ca^{2+} loading in the presence of ATP and Mg^{2+}, its accumulation under β-hydroxybutyrate and ascorbate addition is 30-40% lower than that under succinate addition (7). As malonate was not used in those experiments the efficiency of NDS may be overestimated due to endogeneous succinate involvement (8,9).

Massive Ca^{2+} loading exceeds the range of physiological transport and is related to the damage of mitochondria. We investigated substrate dependence of Ca^{2+} uptake under more physiological conditions. Repeated small portion

[1] NDS, NAD-dependent substrates

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additions were used (5). Low concentrations of inorganic phosphate and Mg^{2+} were included in the media in order to avoid limitation of Ca^{2+} accumulation and to prevent the damage to mitochondrial membranes.

In order to compare energy support of Ca^{2+} uptake by different substrates more correctly, ATP was not added and β-hydroxybutyrate with malonate was used as NDS substrate. This combination is most appropriate for observation of "pure" NDS oxidation as it does not yield succinate in rat liver mitochondria, whereas endogeneous succinate input usually present in mitochondria (8,9) is abolished by malonate. When compared under such conditions succinate kinetic predominance over NDS in Ca^{2+} uptake is even more obvious than in oxidative phosphorylation.

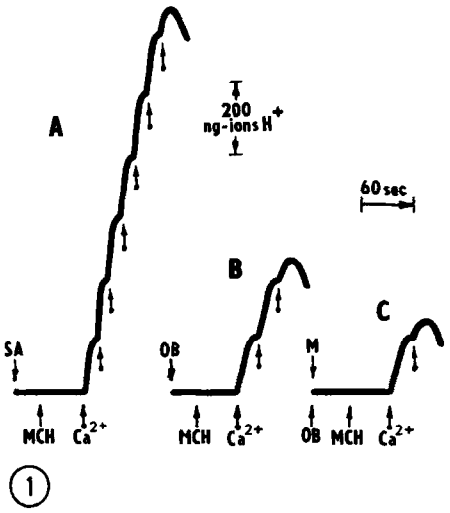
METHODS

Male Wistar albino rats (180-210 g in weight) were used in all experiments. Liver mitochondria were isolated from homogenate in media containing 0.25 M sucrose, 0.01MTris-HCl, pH 7.4. The 4500 g, 10 minute, fraction was used without washing. Mitochondrial protein was determined according to Lowry (10). Ca^{2+} capacity of mitochondria was measured as H^{+} output by hydrogen electrode. Respiration was registered polarographically with a rotating Pt electrode.

RESULTS

In order to characterize Ca^{2+} uptake under limited loading the following parameters were measured: Ca^{2+} capacity, Ca^{2+} input and output, Ca^{2+} retention. For Ca^{2+} capacity estimation, mitochondria were titrated with very small quantities of $CaCl_2$ (15 nmol/mg) up to spontaneous output (11). Data on Ca^{2+} capacity measurements given in Fig. 1 clearly demonstrate great difference in the power of Ca^{2+} uptake supported by succinate and β-hydroxybutyrate. Under succinate oxidation mitochondria completely accumulated 6 portions of Ca^{2+}, and Ca^{2+} output occurred only at the 7th addition. Under oxidation of hydroxybutyrate alone only 1 portion was accumulated and output occurred at the 2nd addition. Even very small amounts of endogeneous succinate elevated Ca^{2+} capacity two fold under addition of hydroxybutyrate; that is still only one third the capacity displayed under succinate addition. Similar results were obtained with α-oxoglutarate and pyruvate + malate.

Ca^{2+} accumulation in mitochondria is the result of two simultaneous processes - electrogenic transport into mitochondria and electroneutral



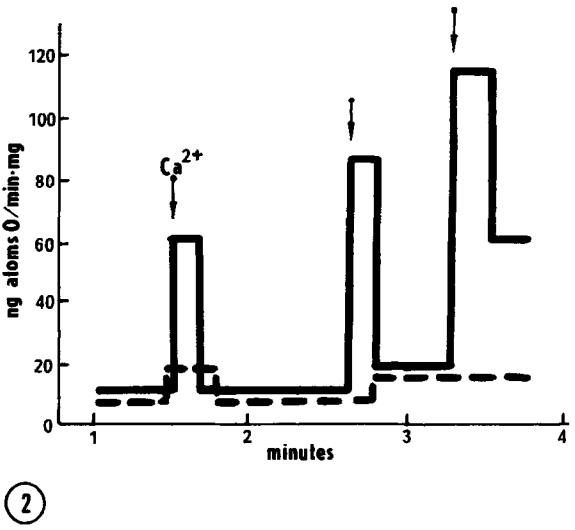


Fig. 1 Substrate dependence of Ca^{2+} capacity of mitochondria. A-SA (succinate) 6 mM, B - OB (β-hydroxybutyrate) 6 mM, C - OB + M (malonate) 3 mM. Medium: 100 mM KCl, 0.7 mM KH_2PO_4, 3 mM tris-HCl pH 7.5. $CaCl_2$ by portions of 15 nmol/mg. MCH(mitochondria)-2.2 mg protein/ml.

Fig. 2 Ca^{2+}-induced respiration of mitochondria under addition of different substrates.
Thick line - SA 6 mM, dotted line - OB 6 mM. Abbreviations as in Fig. 1. Medium 100 mM sucrose, 100 mM KCl, 2 mM KH_2PO_4, 5 mM tris-HCl pH 7.5. $CaCl_2$ by portions of 42 nmol/mg. MCH - 6.0 mg pr/ml.

exchange (12) pumping Ca^{2+} out. Therefore, the small Ca^{2+} capacity of mitochondria during hydroxybutyrate oxidation may be due to either weak Ca^{2+} input or high Ca^{2+} output. As Ca^{2+} input is limited by respiration the intensity of Ca^{2+} transport may be evaluated from the rate of oxygen consumption. These data are given in Fig. 2. The rate of Ca^{2+} induced respiration with succinate is considerably higher than that with hydroxybutyrate. Succinate oxidation increases progressively under consecutive Ca^{2+} additions because succinate dehydrogenase is activated by Ca^{2+} (13). In order to measure the Ca^{2+}/H^{+} exchange, mitochondria were loaded with moderate amounts of Ca^{2+} (35 nmol/mg) and then transport was blocked by ruthenium red. Although the comparison was made under hydroxybutyrate oxidation with endogeneous succinate participation (without malonate) the rate of Ca^{2+} output is 5 times higher in the presence of hydroxybutyrate than under succinate addition (Fig. 3). That is related to the maximal level of NAD reduction by succinate, as Ca^{2+} output is greater under higher NAD oxidation (14).

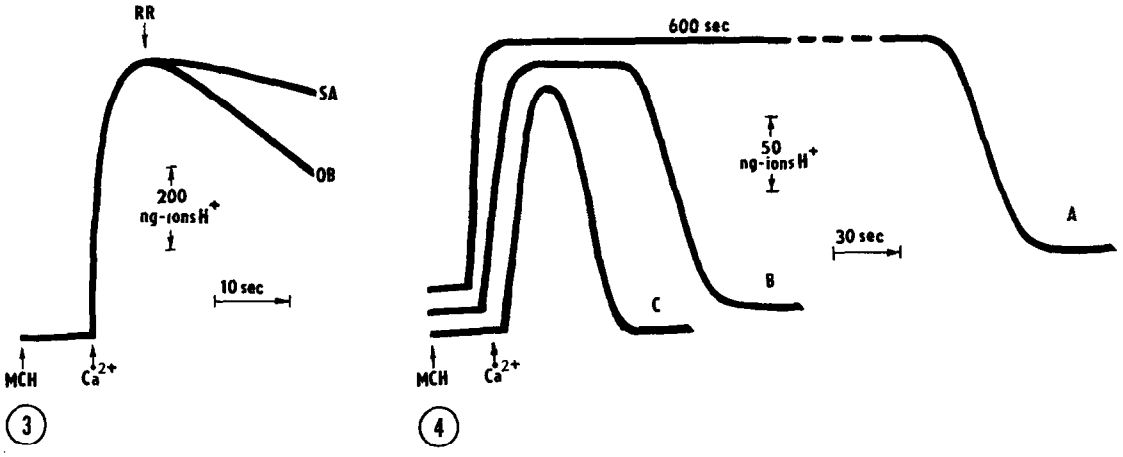


Fig. 3 Comparison of Ca^{2+}/H^{+} exchange rate under succinate and β hydroxybutyrate oxidation. Conditions as in Fig. 1. 0.01 mM RR (ruthenium red), $CaCl_2$ 35 nmol/mg.
MCH - 2.2 mg pr/ml.

Fig. 4 Substrate dependence of Ca^{2+} retention time by mitochondria.
A-SA 6 mM, B-OB 6 mM, C-OB+M 3 mM.
Medium: 170 mM sucrose, 50 mM KCl, 1 mM KH_2PO_4, 3 mM tris-HCl pH 7.4.
$CaCl_2$ 30 nmol/mg.
MCH - 5.1 mg pr/ml.

Succinate oxidation predominance as an energy support of Ca^{2+} uptake by mitochondria was also clearly demonstrated under moderate loading (30 nmol/mg) which is somewhat higher than in Fig. 1. Time of Ca^{2+} retention (15) was measured (Fig. 4). Under hydroxybutyrate oxidation in the presence of malonate spontaneous efflux of the chosen Ca^{2+} amount occurred immediately after input, whereas when succinate was oxidized this portion of Ca^{2+} was retained by mitochondria for 10 minutes. Under hydroxybutyrate oxidation, when endogeneous succinate is involved (without malonate) mitochondria retained Ca^{2+} for 60 sec.

Ca^{2+} retaining by mitochondria is related to its cycling through the membrane (12). The cycling induces damage of membrane due to Mg release (16). Mg^{2+} addition prevented and restored configuration changes and uncoupling in mitochondria induced by large amounts of Ca^{2+} - 100 nmol/mg (17). In our experiments with considerably smaller Ca^{2+} loading - 15 nmol/mg (Fig. 1) 1 mM Mg^{2+} did not increase Ca^{2+} uptake and Ca^{2+}-induced respiration under NDS oxidation. Consequently the lower Ca^{2+} uptake in the presence of NDS should not be attributed to the damage of mitochondria but to a poor energy supply.

Our conclusion that NDS are considerably less effective in Ca^{2+} uptake seems to contradict some earlier observations. In particular, rotenone was shown to abolish Ca^{2+}-induced changes in mitochondria supported by endogeneous substrates (17). However it may be due to rotenone inhibition of fatty acid and glutamate oxidation yielding succinate which oxidation serves as an immediate support of Ca^{2+} uptake. In this case Ca^{2+}-induced changes should be also malonate sensitive as was shown in our experiments for Ca^{2+} transport. It is relevant that amytal has been found to abolish most rapid uptake of very small concentrations of Ca^{2+} (5). Amytal dependence may be explained by a weak uncoupling effect of amytal in concentrations higher than 1 mM.

DISCUSSION

The great predominance of succinate over NDS as energy support for Ca^{2+} uptake by mitochondria, described here, is more pronounced than in the case of oxidative phosphorylation. This is due to higher kinetic demands of Ca^{2+} transport as compared with ADP phosphorylation. Inhibition of endogeneous succinate oxidation with malonate showed that pure oxidation of NDS may provide transport of only small amounts of Ca^{2+} close to its content in mitochondria of intact quiet animals, whereas succinate oxidation provides Ca^{2+} accumulation and retaining to a considerably greater extent. (It can not be excluded that small amounts of Ca^{2+} uptake under NDS addition is also supported by endogeneous succinate which oxidation may still not be inhibited in the presence of 3 mM malonate.

Ca^{2+} amount in cells and mitochondria increases considerably under physiological excitation and stress due to persistent adrenaline stimulation. Our observations provide evidence that, at least, forced Ca^{2+} uptake under physiological activity may be effectively supported only by succinate oxidation; moreover the latter may be additionally stimulated by Ca^{2+} (12). All this correlates well to the concept that physiological activity and stress are based on a preferential increase of succinate formation and oxidation (18,19,20) that produces a hyperactive metabolic state of mitochondria.

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