The Effect of ATP and Carnitine on the Endogenous Respiration of Mitochondria from Brown Adipose Tissue

Brown adipose tissue has a very high oxygen consumption in vitro[1] and electron microscopic data show its cells to possess numerous mitochondria with many intramitochondrial cristae[2]. The main role of brown adipose tissue is to produce heat[3]. The lipid droplets surrounding the mitochondria[2] indicate that this heat may be produced to a large extent from lipids. Yet there are very few data on the oxidation of fatty acids by mitochondria from brown adipose tissue.

Since brown adipose tissue is particulary important for heat production in the new-born period[3], the aim of this work was to define the optimum conditions for fatty acid oxidation and to search for developmental changes in the oxidation of fatty acids.

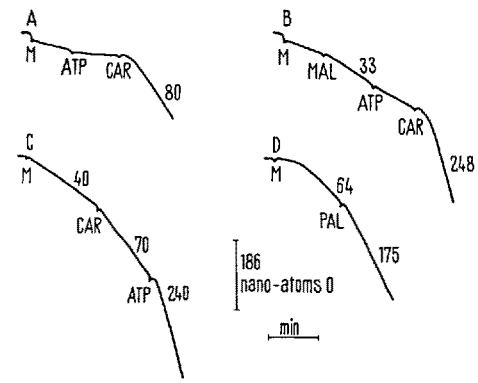
Mitochondria from brown adipose tissue of rats were isolated in 0.25 *M* saccharose, 0.01 *M* *Tris*-HCl pH 7.4, and 0.001 *M* EDTA pH 7.4 in the usual way[4]. Oxygen consumption by these mitochondria was determined with a Clark platinum electrode. The total volume was 3 ml, temperature 30 °C, mitochondrial protein 1–2 mg/ml. Proteins were determined according to Lowry et al.[5], fatty acids according to Novák[6]. $C^{14}O_2$ was absorbed on a mixture of methyl-celosolve and ethanolamine (2:1) and radioactivity determined in a liquid scintillation counter.

In agreement with the work of Smith et al.[7] and Lindberg et al.[8], we have not observed phosphorylation coupled to the respiratory chain oxidation in brown-fat mitochondria isolated from 6- to 30-day-old rats. In contrast to liver mitochondria, maximum respiratory rates were found after substrate addition (succinate or ketoglutarate) and no further activation was observed after addition of ADP or 2,4-dinitrophenol.

Exogenously supplied palmitate alone or together with malate, carnitine or CoA had no effect on the endogenous respiration of these uncoupled mitochondria. If, however, ATP and carnitin were added, the respiratory rate was increased considerably (Figure A). This activation was much more pronounced if malate was also added (Figure B). The slight activating effect of carnitin alone (Figure C) might indicate the amount of fatty acids present as acyl-CoA esters in brown-fat mitochondria. Addition of palmitate in the presence of ATP and carnitin was without any effect on the respiratory rate.

The fact that ATP and carnitin alone increase the respiratory rate of mitochondria from brown adipose tissue indicates that these substances make endogenous fatty acids available for oxidation. This conclusion is supported by the effect of serum albumin on the ATP-carnitin activated respiration. Addition of bovine serum albumin decreases the high respiratory rate induced by ATP and carnitin from 248 to 64 nano-atoms of oxygen per min/mg (Figure B and D) suggesting that exogenous fatty acids are bound to that substance. Palmitate added to the medium in the presence of albumin has an activation effect on the respiratory rate (Figure D). Palmitate added in the absence of serum albumin evidently enters the endogenous fatty acid pool and is oxidized without further increase of overall fatty acid oxidation. This is indicated by the fact that exogenously added palmitate-1-C^{14} is oxidized to $C^{14}O_2$, this oxidation being ATP-carnitin dependent. When 0.12 m*M* palmitate-1-C^{14} was incubated with mitochondria from 8-day-old rats, 1700 c.p.m./mg protein were found as $C^{14}O_2$, compared with the 12,500 c.p.m./mg in the presence of 3.3 m*M* ATP and 1.7 m*M* carnitin.

During postnatal development, maximum values of respiration after addition of ATP and carnitin were found in 6- to 30-day-old rats, i.e. 205–280 nano-atoms of oxygen/min/mg protein. In new-born and old rats this activation was much smaller. In 6- to 30-day-old rats



The effect of ATP, carnitine (CAR), malate (MAL), palmitate (PAL) and serum albumine on respiration of mitochondria from brown adipose tissue of 10-day-old rats. The following was added to the incubation mixture without malate (see Table): (A) ATP 3.3 m*M* and carnitine 1.66 m*M*; (B) K-malate 3.3 m*M*, ATP and carnitin; (C) K-malate added before mitochondria and carnitine and ATP as indicated; (D) K-malate, ATP, carnitine and bovine serum albumin 0.2% added before mitochondria and palmitate 0.12 m*M* as indicated. M indicates addition of mitochondria. The numerals indicate respiration expressed as nano-atoms of oxygen/min/mg protein.

Respiration of brown-fat mitochondria (nano-atoms of oxygen per min/mg protein)

Age (days)	No. of experiments	No additions	ATP carnitin	ATP carnitin SA[a]	Palmitate ATP carnitin SA[a]
1–2	3	19 ± 2	154 ± 12	138 ± 8	162 ± 22
6–10	5	32 ± 4	280 ± 53	66 ± 12	133 ± 30
30	5	21 ± 5	205 ± 42	75 ± 16	118 ± 38
60	2	9 ± 4	66 ± 3	62 ± 3	55 ± 1

Brown adipose tissue from 8–16 rats was used for 1 experiment. The incubation medium contained: 56 m*M* KCl, 6.7 m*M* $MgCl_2$, 9 m*M* *Tris*-HCl pH 7.4, 15.6 m*M* K-phosphate pH 7.4, 1 m*M* EDTA, 3.3 m*M* K-malate, 8 m*M* saccharose, 3.3 m*M* ATP, 1.66 m*M* carnitin, 0.2% SA (bovine serum albumin) and 0.12 m*M* palmitate added where indicated. [a] Bovine serum albumin.

[1] C. D. Joel, J. biol. Chem. *241*, 841 (1966).
[2] L. Napolitano and D. Fawcett, J. biochem. biophys. Cytol. *4*, 685 (1958).
[3] M. J. R. Dawkins and D. Hull, J. Physiol. *172*, 216 (1964).
[4] W. C. Schneider and N. Y. Hogeboom, J. biol. Chem. *183*, 123 (1950).
[5] H. O. Lowry, N. Y. Rosebrough, A. L. Farr and A. L. Randall, J. biol. Chem. *193*, 265 (1951).
[6] M. Novák, J. Lipid Res. *6*, 431 (1965).
[7] R. E. Smith, J. C. Roberts and K. J. Hittelman, Science *154*, 635 (1966).
[8] O. Lindberg, J. DePierre, E. Rylander and B. A. Afzelius, J. Cell Biol. *34*, 293 (1967).

albumin decreases the respiratory rate and added palmitate returns it to higher levels.

In new-born and adult rats neither albumin nor palmitate has any effect (Table).

The age difference cannot be explained by a different fatty acid content because the amount of free fatty acids/mg of mitochondrial protein in new-born animals is about 60% higher than in 6- to 30-day-old rats (120 to 140 nmmole/mg protein) and adult animals have approximately the same amount of fatty acids bound to the mitochondria as 6- to 30-day-old rats.

Our results show that for maximum activation of the respiratory rate in uncoupled brown-fat mitochondria both ATP and carnitin are necessary. The inhibitory effect of serum albumin indicates that endogenous fatty acids are oxidized. Evidently the endogenous fatty acids are bound to the external mitochondrial membrane, where they are activated[9] and transported as carnitine esters into the mitochondria. Apparently, in our experiments, mitochondria contain such a large amount of endogenous fatty acids that addition of palmitate has no further activating effect on mitochondrial respiration.

Zusammenfassung. Nachweis der endogenen Atmungsaktivierung von Mitochondrien im braunen Fettgewebe durch ATP und Karnitin. Eine maximale Aktivierung wird bei 6–30 Tage alten Ratten gefunden, während sie in neugeborenen und erwachsenen Tieren kleiner ist. Nur bei den 6–30 Tage alten Tieren hemmt Albumin die durch ATP und Karnitin erhöhte Atmung.

Z. DRAHOTA, EVA HONOVÁ and P. HAHN

Institute of Physiology, Czechoslovak Academy of Sciences, Praha (Czechoslovakia),
21 September 1967.

[9] K. R. NORUM, M. FARSTAD and J. BREMER, Biochem. Biophys. Res. Comm. *24*, 797 (1966).

Über die Hydrierung im Ringsystem ungesättigter Steroide mit Mycobacterium phlei

In unseren früheren Arbeiten[1,2] wurden zahlreiche Oxydationsprozesse beschrieben, die mit Hilfe eines Mycobakteriumstammes durchgeführt werden können. Durch Zugabe von 8-Hydroxychinolin kann der Abbau des Ringsystems verhindert werden; dadurch häufen sich Δ1,4-3-Keto-Verbindungen mit Androstangerüst an.

In der vorliegenden Arbeit berichten wir über einige Beobachtungen der enzymatischen Hydrierung des Ringsystems ungesättigter Steroide. In der Literatur fanden wir Angaben über die mikrobielle Sättigung der Doppelbindungen $\Delta 1^3$, $\Delta 4^{4,5}$, $\Delta 6^6$, $\Delta 7^{1,7}$, über die Umwandlung von Δ16-20-Ketosteroiden in 17-iso-Steroide[8] und über den Seitenkettenabbau der letztgenannten Verbindungen[9,10].

Wir untersuchten den Metabolismus einiger im Ring «B» ungesättigter, bzw. Δ16-20-Ketosteroide unter aeroben Bedingungen. Die Umsetzungen wurden mit einer in wässriger 0,4prozentiger Natriumchlorid-Lösung suspendierter Züchtung von Mycobacterium phlei bei 37°C durchgeführt, welche vorher in Maisquellwasser-Glycerin-Medium anwuchs und anschliessend vom Medium getrennt wurde. Die Substrat- und 8-Hydroxychinolin-Konzentrationen betrugen 400 μg/ml, bzw. 100 μg/ml. Nach 48 h Inkubation wurden die Kulturfiltrate der einzelnen Ausgangsmaterialien mit Chloroform extrahiert und das 8-Hydroxychinolin mit verdünnter, wässriger Oxalsäure entfernt. Schliesslich wurden die Extrakte im Vakuum eingedampft. Im Rückstand konnten wir Androsta-1,4-dien-3,17-dion und geringe Mengen von Androst-4-en-3,17-dion als Umsetzungsprodukte nachweisen. Durch Kristallisation bzw. präparative Dünnschichtchromatographie wurde aus den folgenden Substraten Androsta-1,4-dien-3,17-dion erhalten. Ausbeuten (in % der Theorie): Androsta-4,6-dien-3,17-dion (60%), Pregna-4,6-dien-3,20-dion (46%), Cholesta-4,6-dien-3-on (32%), Ergosta-4,6,22-trien-3-on (31%), 5α-Cholest-7-en-3β-ol (25%), Cholesta-5,7-dien-3β-ol (26%), 5α-Ergosta-7,22-dien-3β-ol (29%), Ergosta-5,7,22-trien-3β-ol (26%), Pregna-5,16-dien-3β-ol-20-on (75%). Auch die Ester (Acetate, Benzoate) der letztgenannten 5 Verbindungen wurden in Androsta-1,4-dien-3,17-dion übergeführt.

Wie man auf Grund der Versuche feststellen kann, ist es möglich, die Doppelbindungen Δ6 und Δ7 unter aeroben Umständen mit Mycobacterium phlei zu sättigen, sowie Δ16-20-Keto-Steroide in 17-Ketosteroide zu überführen. Das aus den verschiedenen Ausgangsmaterialien isolierte Androsta-1,4-dien-3,17-dion ist ein wertvoller Ausgangsstoff für die Östron- und Norsteroid-Synthesen.

Summary. Androsta-1,4-diene-3,17-dione was obtained from Δ6-, Δ7- and Δ-16-steroids by Mycobacterium phlei. The breakdown of the steroid nucleus was inhibited by 8-hydroxyquinoline added to the culture medium.

G. AMBRUS, E. TÖMÖRKÉNY und K. G. BÜKI

Institut für Arzneimittelforschung, Budapest 4 (Ungarn),
13. November 1967.

[1] G. WIX, K. G. BÜKI, E. TÖMÖRKÉNY und G. AMBRUS, Ungarisches Patent No. 153831 (1965).
[2] G. WIX, K. G. BÜKI, E. TÖMÖRKÉNY und G. AMBRUS, Steroids, im Druck.
[3] A. BUTENANDT, H. DANNENBERG und L. A. SURÁNYI, Chem. Ber. *73*, 818 (1940).
[4] L. MAMOLI, R. KOCH und H. TESCHEN, Z. physiol. Chem. *261*, 287 (1939).
[5] L. MAMOLI und G. SCHRAMM, Chem. Ber. *71*, 2698 (1938).
[6] Y. Y. TSONG, K. C. WANG und CH. J. SIH, Biochim. biophys. Acta *93*, 398 (1964).
[7] R. DEGHENGHI, S. RAKHIT, K. SINGH, C. VÉZINA und CH. J. SIH, Steroids *10*, 313 (1967).
[8] P. D. MEISTER, D. H. PETERSON, H. C. MURRAY, S. H. EPPSTEIN, L. M. REINEKE, A. WEINTRAUB und H. M. LEIGH, J. Am. chem. Soc. *75*, 55 (1953).
[9] G. WIX und M. RADOS, Schweizer Patent, No. 376499 (1959).
[10] O. EL-TAYEB, S. G. KNIGHT und CH. J. SIH, Biochim. biophys. Acta *93*, 411 (1964).