EFFECTS OF FASTING, ADRENALECTOMY AND STREPTOZOTOCIN-DIABETES ON SENSITIVITY OF HEPATIC CARNITINE ACYLTRANSFERASE TO MALONYL CoA

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1. Introduction

States of rapid ketogenesis such as fasting or diabetes are associated with increased hepatic oxidation of fatty acids. In the fed state malonyl CoA acts as a potent inhibitor of the overt form of carnitine acyltransferase in liver mitochondria. It has therefore been suggested that the level of this metabolite may be important in regulating ketogenesis by influencing the entry and oxidation of fatty acids in mitochondria [1]. Here, we have investigated the effect of prior nutritional and hormonal states upon the sensitivity of rat liver mitochondrial overt carnitine palmitoyltransferase (CPT_1) to malonyl CoA. Fasting is shown to considerably reduce the sensitivity to malonyl CoA. Surprisingly, streptozotocin-diabetes did not have a parallel effect. It is also shown that the presence of the adrenal glands is not required to elicit the decrease in sensitivity to malonyl CoA seen on fasting. During the course of this study it was reported that fasting reduces the inhibitory effect of malonyl CoA on ketogenesis from palmitate [2,3]. Our findings suggest that this results from a change in the regulatory properties of carnitine acyltransferase.

2. Materials and methods

Coenzyme A thioesters were from PL-Biochemicals and L-carnitine from Sigma. Male rats were of the Sprague-Dawley strain and, with the exception of adrenalectomized (ADX) or sham–ADX animals, were bred at University College London. In expt 1, rats of 165–175 g were selected 1 day prior to sacrifice and then either fed as usual or fasted for 24 h starting at ~10:00 h. In expt 2, adrenalectomy or sham adrenalectomy was performed by Charles River (Margate) at 130 g body wt and the animals sacrificed after 8–12 days. ADX rats were given 0.9% (w/v) NaCl in tap water to drink. Absence of the adrenal glands was established at the time of sacrifice. Fasting of these animals was as described above. At the time of sacrifice, body weights (g) were: fed sham–ADX, 194 ± 3; fed ADX, 177 ± 5; fasted sham–ADX, 183 ± 6; fasted ADX, 165 ± 8. In expt 3, rats of 130 g body wt were made diabetic by subcutaneous injection of streptozotocin (60 mg/kg) dissolved in 50 mM citrate, 0.15 M NaCl buffer (pH 4.0). Controls received injections of saline–citrate buffer alone. Suitably diabetic animals were selected on the basis of glucosuria. At sacrifice, 1 week later, diabetic rats (155 ± 8 g body wt) and their controls (168 ± 9 g body wt) had plasma glucose levels of 23 ± 2.0 mM and 7.7 ± 0.1 mM (means ± SEM of 6 and 4 animals, respectively).

Livers were homogenised and centrifuged to obtain mitochondria as in [4]. Homogenisation was in ice-cold 0.25 M sucrose containing 10 mM Tris–HCl (pH 7.4), 1 mM EGTA and defatted albumin (10 mg/ml). Washing of mitochondria was performed in the same buffer without albumin and final resuspension at ~5 mg mitochondrial protein/ml was in 0.3 M sucrose containing 10 mM Tris–HCl (pH 7.4), 1 mM EGTA.

Mitochondrial protein was measured [5] using bovine serum albumin as standard.

For assay of CPT_1, 50 μl fresh whole mitochondria were preincubated at 25°C in 1.0 ml containing 220 mM sucrose, 40 mM KCl, 10 mM Tris–HCl (pH 7.4), 1 mM EGTA, defatted albumin (1.3 mg/ml), 0.5 mM dithiothreitol, 40 μM palmitoyl CoA and the required [malonyl CoA]. The reaction was initiated by addition of 20 μl containing 0.4 μmol L-carnitine

and 1 μCi D,L-[*methyl*-^{3}H]carnitine (Radiochemical Centre, Amersham). After 4 min the reaction was terminated with 1 ml ice-cold 1.2 M HCl followed by 2 ml water-saturated butanol. After mixing and brief centrifugation, the butanol layer was washed with 5 ml butanol-saturated water and 1.0 ml butanol layer, containing acyl-[^{3}H]carnitine taken for liquid scintillation counting. The assay of overt carnitine octanoyltransferase (COT) was identical except that 40 μM octanoyl CoA was used in place of palmitoyl CoA. These assays were linear with time and amounts of mitochondria in the fed and fasted state and in the presence and absence of malonyl CoA. Zero-time blanks were measured and subtracted.

The relationship between carnitine acyltransferase activity and malonyl CoA concentration shown in the figures could be linearised in a plot of the form: 100 × (% inhibition by malonyl CoA)$^{-1}$ *vs* [malonyl CoA]$^{-1}$. In all cases, the linear correlation coefficients (r) obtained by linear regression analysis were very close to unity and are shown in the figures. The ordinate intercept was therefore used to determine the proportion of carnitine acyltransferase that is inhibitable by infinite [malonyl CoA]. Concentrations of malonyl CoA giving 50% inhibition ([malonyl CoA]$_{0.5}$) refer to those inhibiting this 'malonyl CoA-sensitive' carnitine acyltransferase activity by 50%.

3. Results and discussion

In all cases CPT measurements were made with fresh mitochondria and are presumed to represent mainly the overt form (CPT_1) rather than the latent (CPT_2) activity. It has been concluded that, whereas CPT_1 is sensitive to malonyl CoA, CPT_2 is insensitive to this effector [6]. Furthermore, it has been suggested [6] that malonyl CoA sensitivity of CPT_1 requires membrane association. All of the experimental treatments (e.g., fasting, adrenalectomy, diabetes) slightly decreased the proportion of the measured CPT that is malonyl CoA-sensitive. This could either be attributed to increased mitochondrial breakage leading to exposure of some CPT_2 or to some detachment of CPT_1, from membranes. The method used for calculation of [malonyl CoA]$_{0.5}$ corrects for either of these possibilities.

3.1. *The effect of fasting (expt 1)*

Fig.1a shows that fasting increased the [malonyl CoA]$_{0.5}$ for CPT by 6-fold. There is evidence for a medium-chain carnitine acyltransferase (COT) that is distinct from the long-chain carnitine acyltransferase and from carnitine acetyltransferase [7,8]. It is therefore noteworthy that COT activity (fig.1b) had a

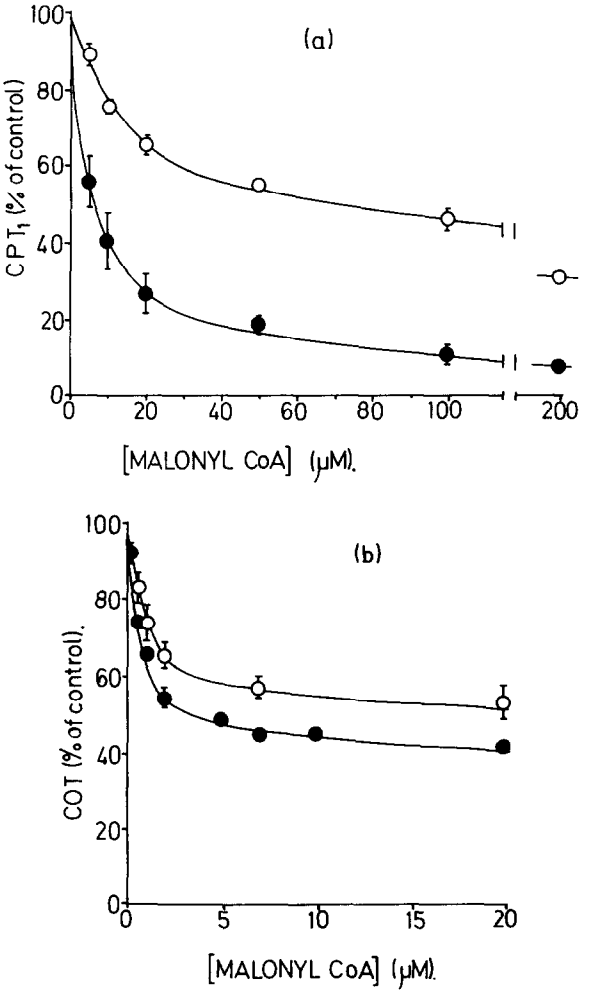

Fig.1. Effect of malonyl CoA on carnitine acyltransferase activities in liver mitochondria from fed and fasted (24 h) rats. The values are means ± SEM for 4 expt in each case: (•) fed; (○) fasted. (a) Fed: at [malonyl CoA] = O, CPT_1 activity = 2.90 ± 0.35 nmol . min^{-1} . mg protein^{-1}; 93% is malonyl CoA-sensitive; [malonyl CoA]$_{0.5}$ = 5.4 μM; r = 0.999. Fasted: at [malonyl CoA] = O, CPT_1 activity = 4.67 ± 0.11 nmol . min^{-1} . mg protein^{-1}; 83% is malonyl CoA-sensitive; [malonyl CoA]$_{0.5}$ = 32 μM; r = 0.986. (b) Fed: at [malonyl CoA] = O, overt COT activity = 3.87 ± 0.18 nmol . min^{-1} . mg protein^{-1}; 59% is malonyl CoA-sensitive; [malonyl CoA]$_{0.5}$ = 0.7 μM; r = 0.999. Fasted: at [malonyl CoA] = O, overt COT activity = 5.84 ± 0.12 nmol . min^{-1} . mg protein^{-1}; 50% is malonyl CoA-sensitive; [malonyl CoA]$_{0.5}$ = 0.9 μM; r = 0.999.

smaller proportion of malonyl CoA-sensitive activity than CPT and had a considerably lower [malonyl CoA]$_{0.5}$ which was unaffected by fasting. The physiological significance of this finding is unclear at present.

3.2. *The effects of adrenalectomy and fasting (expt 2)*

Fig.2 shows that adrenalectomy had no appreciable effect upon the sensitivity of CPT_1 to malonyl CoA. It would seem therefore that a change in sensitivity to this effector is unlikely to contribute to the elevation in ketogenesis seen after adrenalectomy [9]. Fig.2 also shows that sensitivity of CPT_1 to malonyl CoA is decreased by fasting to a similar extent in ADX and sham–ADX animals, implying that adrenal secretions are not necessary for this response to the fasted state. The [malonyl CoA]$_{0.5}$ in the fed sham–ADX case (11.7 μM) was higher than that found in the fed state in expt 1. It is not known whether this

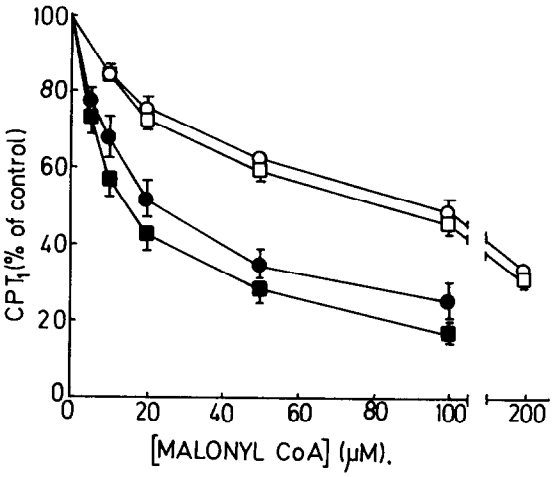

Fig.2. Effect of fasting (24 h) on sensitivity ot CPT_1 to malonyl CoA in liver mitochondria from adrenalectomized and sham-adrenalectomized rats. The values are means ± SEM for 5 expt in each case: (•) fed ADX; (○) fasted ADX; (▪) fed sham-operated; (□) fasted sham-operated. Fed ADX: at [malonyl CoA] = O, CPT_1 activity = 4.45 ± 0.03 nmol . min^{-1} . mg $protein^{-1}$; 81% is malonyl CoA-sensitive; [malonyl CoA]$_{0.5}$ = 13.2 μM; r = 0.997. Fasted ADX: at [malonyl CoA] = O, CPT_1 activity = 4.99 ± 0.26 nmol . min^{-1} . mg $protein^{-1}$; 70% is malonyl CoA-sensitive; [malonyl CoA]$_{0.5}$ = 36 μM; r = 0.998. Fed sham-operated: at [maloyl CoA] = O, CPT_1 activity = 3.30 ± 0.24 nmol . min^{-1} . mg $protein^{-1}$; 91% is malonyl CoA-sensitive; [malonyl CoA]$_{0.5}$ = 11.7 μM; r = 0.999. Fasted sham-operated: at [malonyl CoA] = O, CPT_1 activity = 4.51 ± 0.17 nmol . min^{-1} . mg $protein^{-1}$; 75% is malonyl CoA-sensitive; [malonyl CoA]$_{0.5}$ = 37 μM; r = 0.998.

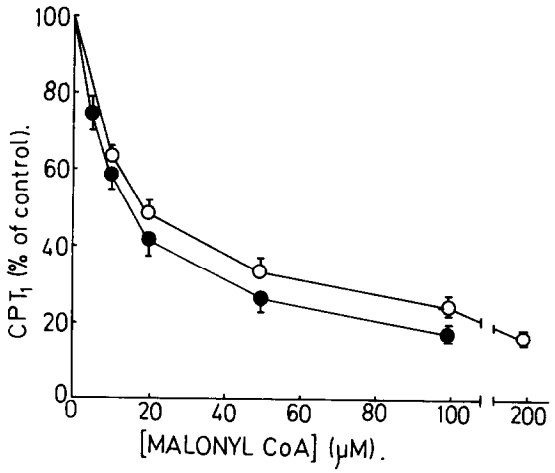

Fig.3. Effect of diabetes on sensitivity of CPT_1 to malonyl CoA. The values are means ± SEM for 6 diabetic and 4 control animals. (•) control; (○) diabetic. Control: at [malonyl CoA] = O, CPT_1 activity = 4.50 ± 0.35 nmol . min^{-1} . mg $protein^{-1}$; 95% is malonyl CoA-sensitive; [malonyl CoA]$_{0.5}$ = 13.4 μM; r = 0.999. Diabetic: at [malonyl CoA] = O; CPT_1 activity = 4.90 ± 0.13 nmol . min^{-1} . mg $protein^{-1}$; 86% is malonyl CoA-sensitive; [malonyl CoA]$_{0.5}$ = 13.4 μM; r = 0.999.

could be attributed to differences in the sources of animals (see section 2) or to the trauma of the sham operation. Adrenalectomy and fasting both resulted in increases in the overt CPT activity expressed/mg mitochondrial protein. However, the total liver mitochondrial protein decreased in these conditions with the result that the overt CPT activity/liver was not significantly altered.

3.3. *The effect of streptozotocin-diabetes (expt 3)*

It was most surprising to find that the sensitivity of CPT_1 to malonyl CoA was unchanged in the diabetic state (fig.3). These animals were clearly diabetic on the basis of plasma glucose measurements (see section 2). In many instances fasting and diabetes exert similar effects upon carbohydrate and lipid metabolism. This is not so in this instance and suggests that different mechanisms may be responsible for the increase in the ketogenic capacity of the liver in fasting and diabetes.

4. Conclusion

The 6-fold change in the [malonyl CoA]$_{0.5}$ for CPT_1 seen after fasting suggests that this physiologi-

cal state is associated with a striking change in the regulatory properties of this enzyme with the result that the onset of fasting should be associated with an extensive deinhibition of CPT_1 activity brought about by a fall in hepatic malonyl CoA content [1] and reinforced by a diminution in sensitivity to this effector. The signal, hormonal or otherwise, responsible for this change in sensitivity is unknown at present. Adrenal secretions do not appear to be involved nor is insulin implicated otherwise a similar change in sensitivity would have been expected in diabetes.

References

[1] McGarry, J. D. and Foster, D. W. (1980) Ann. Rev. Biochem. 49, 395–420.
[2] Cook, G. A., Otto, D. A. and Cornell, N. W. (1980) Biochem. J. 192, 955–958.
[3] Ontko, J. A. and Johns, M. L. (1980) Biochem. J. 192, 959–962.
[4] Harper, R. D. and Saggerson, E. D. (1975) Biochem. J. 152, 485–494.
[5] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[6] McGarry, J. D., Leatherman, G. F. and Foster, D. W. (1978) J. Biol. Chem. 253, 4128–4136.
[7] Kopec, B. and Fritz, I. B. (1971) Can. J. Biochem. 49, 941–948.
[8] Solberg, H. E. (1971) FEBS Lett. 12, 134–136.
[9] Klausner, H. and Heimberg, M. (1967) Am. J. Physiol. 212, 1236–1246.