tive treatments was seen in all cell-retained preparations, but never found with the secreted preparations. The molecular basis for this novel property is not clear at present.

Taken overall, our data indicate that granule-enriched cultures are active in the synthesis and secretion of chondroitin sulphate and heparan sulphate proteoglycans. Since the secreted proteoglycans differ in size distribution and composition from the cell-retained material, it would appear that either secretion of proteoglycans is a selective process in these cells, or that modification occurs on secretion. However, there is no significant change in the nature of cell-retained and secreted proteoglycans during the first 9 days of culture development. Our tissue culture data suggest that granule cells are likely to contribute significantly to cell-

retained and extracellular matrix proteoglycans in the cerebellum.

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A simple method for the purification of carnitine palmitoyl transferase 2 from rat liver

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Introduction

Carnitine palmitoyl transferase (CPT) catalyses the transfer of acyl groups between CoA and carnitine to facilitate the transfer of activated fatty acids into mitochondria. Functional studies have illustrated that two differently localized carnitine-dependent CPTs are required for the oxidation of fatty acyl groups. In intact mitochondria two recently clarified locations have been identified for the two forms of the CPT: an overt form (CPT1), now localized to the inner side of the outer mitochondrial membrane (Murthy & Pande, 1987), and a latent form (CPT2) which resides on the inner side of the inner mitochondrial membrane (Bieber & Farrell, 1983; Hoppel & Brady, 1985).

We have used detergents to solubilize the membranes under optimal conditions for the purification of malonyl-CoA-insensitive CPT.

Materials and methods

Rat liver mitochondria were isolated essentially as described by Parvin & Pande (1979). Briefly, rat liver was homogenized in the isolation buffer (210 mm-mannitol, 70 mm-sucrose, 1 mm-EDTA, 10 mm-Tris/HCl, pH 7.4, containing 0.1% (w/v) phenylmethanesulphonylfluoride at 7–10 ml/1 g of tissue). The homogenate was centrifuged at 1700 rev./min using a Sorvall centrifuge for 5 min. The supernatant was re-centrifuged at 2500 rev./min for a further 5 min. The mitochondrial were then brought down by centrifugation at 9000 rev./min for 10 min. They were washed at least twice by resuspension and precipitation. Mitochondrial membranes were prepared by resuspending the mitochondrial pellets in 20 mm-potassium phosphate buffer, pH 7.2, and sonicating for a total of 1 min at 10 μm in 15 s bursts. The membranes were collected by centrifugation at 115 000 g, for 45 min. They were washed several times and were resuspended in the phosphate buffer. The membranes were enzymically tested for the presence of both inner and outer mitochondrial membrane markers.

Succinate dehydrogenase was used as a marker enzyme for the inner mitochondrial membranes as described by Jenkins & Peters (1978). Monoamine oxidase was used as a marker enzyme for outer mitochondrial membranes as previously described by Weisshack et al. (1960).

CPT was assayed spectrophotometrically essentially as described by Saggerson (1982), except that 4,4′-dithiodipyridine was used as the thiol reagent at 324 nm with the pH of the buffer at 6.8. Under these conditions it was possible to detect the malonyl-CoA sensitivity of the liver CPT enzyme spectrophotometrically.

Purification of CPT

Mitochondrial membranes were thawed out and diluted with 20 mm-phosphate buffer (pH 7.2) to approx. 10 mg protein/ml and were continuously stirred on ice. Tween-20 at ratio of 2:1 (μl of detergent to mg of protein) was added to the membranes. The mixture was sonicated at 10 min intervals placed in ice-cold water for 1 min using a Kerry sonicator. After 40 min incubation, the mixture was centrifuged at 115 000 g, for 45 min. The resulting supernatant was carefully removed for further treatment. To this polyethylene glycol (PEG) was added to a 15% saturation from a

Fig. 1. Purified CPT eluted from chromatofocusing column was run on a 10% (w/v) SDS/PAGE

Lanes 1–9 correspond to the fractions of the CPT peak. The CPT molecule gives a band with molecular mass of 68 000.

C: Standard molecular masses.
100% (w/v) PEG solution. After thorough mixing the precipitate formed was removed by centrifugation at 30000 g for 30 min, using the Sorvall centrifuge. The resulting supernatant was made to 50% PEG saturation and the precipitate formed was pelleted as before. This pellet was solubilized in 20 mM-phosphate buffer containing 0.5% (v/v) Tween-20. The solubilized pellet was treated with 50% (NH₄)₂SO₄ to 40% saturation. The precipitate was discarded by centrifugation and the supernatant was then made to 50% (NH₄)₂SO₄ saturation. The precipitate was collected by centrifugation and the pellet was dissolved in the phosphate, Tween-20 buffer and dialysed against the same buffer under vacuum overnight. The dialysed, concentrated sample of approx 20 mg/ml was applied to a f.p.1.c. mono Q 5/5 column previously equilibrated with 20 mM-bis Tris propane pH 7.0 containing 0.5% (v/v) Tween-20. The enzyme was eluted with a linear gradient system formed from the same buffer to approx. 5-7 ml against phosphate Tween-20 buffer. This was applied on to a chromatofocusing column as previously described by Brady & Brady (1986) except that Tween-20 at 0.5% (v/v) was used instead of n-octylglucoside. The purity of the CPT enzyme was tested by SDS/polyacrylamide-gel electrophoresis (PAGE) (Laemmli, 1970) yielding only one band of molecular mass 68000 (Fig. 1).

Results and discussion

Although malonyl-CoA-insensitive CPT2 has been purified by others (Brady & Brady, 1986; Miyazawa et al., 1983; Ramsay et al., 1987), the procedures used are expensive and often time consuming. The method described here is inexpensive and relatively quick. The purified enzyme yields a single band of molecular mass 68000 on SDS/PAGE (Fig. 1) and in accordance with the CPT enzyme purified by other workers, this is believed to be the CPT2 enzyme.


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Comparison of two cyclic-nucleotide-independent acetyl-CoA carboxylase kinases from lactating rat mammary gland: identification of the kinase responsible for acetyl-CoA carboxylase inactivation in vivo

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Acetyl-CoA carboxylase (EC 6.4.1.2) is widely recognized as an important regulatory enzyme in the pathway of fatty acid synthesis, where its activity is hormonally regulated by reversible phosphorylation [1]. In parallel with the inhibition of fatty acid synthesis, acetyl-CoA carboxylase is phosphorylated and inactivated in vivo in the lactating rat mammary gland in response to 24 h-starvation and high-fat feeding [1, 2]. This results in a decrease in the V₅₅ (75% and 65%, respectively) and an increase in the concentration of citrate required for half-maximal activation Kₐ₅₅ (citrate) (75% and 35%, respectively) of the purified enzyme, accompanied by an increased phosphate content of approximately 1 mol/mol of enzyme subunit. Purified mammary gland acetyl-CoA carboxylase can be phosphorylated and inactivated in vivo by cyclic-AMP-dependent protein kinase [3]. However, it appears that cyclic-AMP-dependent protein kinase is not responsible for the phosphorylation and inactivation in vivo described above. We have shown that although phosphorylation of acetyl-CoA carboxylase by cyclic-AMP-dependent protein kinase significantly increases (100%) the A₅₅ (citrate) of the enzyme, it produces only a minimal decrease (13%) in V₅₅ [14]. Hormones which in other tissues act via increased cyclic AMP concentrations have no effect on fatty acid synthesis or acetyl-CoA carboxylase activity in mammary gland [5, 6] and pharmacological increases in cyclic AMP concentrations in mammary cells induced by β-agonists and phosophodiesterase inhibitors have no effect on the rate of fatty acid synthesis or the phosphorylation state and activity of acetyl-CoA carboxylase [7, 8]. Furthermore, there is no significant difference between the activity ratios of cyclic-AMP-dependent protein kinase in the mammary glands of 24 h-starved and chow-fed lactating rats (K. A. Ottey & R. A. Clegg, unpublished work). This evidence infers that a cyclic-nucleotide-independent protein kinase must be responsible for the increased acetyl-CoA carboxylase phosphorylation in response to 24 h-starvation or high-fat feeding. We have previously identified a cyclic-nucleotide-independent protein kinase in lactating rat mammary gland termed acetyl-CoA carboxylase kinase-2 (ACK2) [3], and a further acetyl-CoA carboxylase kinase termed acetyl-CoA carboxylase kinase-3 (ACK3), or AMP-activated protein kinase, has been identified in rat liver [9, 10], where it also inactivates 3-hydroxymethylglutaryl-CoA reductase, the rat-liming enzyme of cholesterol biosynthesis.

We have identified and partially purified both ACK2 and ACK3 from the lactating rat mammary gland. ACK2 was partially purified approximately 350-fold from lactating rat mammary gland using a combination of 35-60% (w/v) ammonium sulphate precipitation of a crude post-mitochondrial fraction followed by chromatography on phosphocellu-