Acetoacetyl-Coenzyme A Thiolases in Rat Brain during Postnatal Development

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Rat brain at all stages of development has been shown by chromatography on DEAE-cellulose and by isoelectric focusing to contain two separate acetoacetyl-CoA-specific thiolases. These resemble the cytoplasmic and mitochondrial acetoacetyl-CoA thiolases detected in rat liver (Middleton, 1971). The two brain enzymes have pI values 5.1 and 8.3. The low-pI thiolase is cytoplasmic and has the same K_m for acetoacetyl-CoA as the rat liver cytoplasmic enzyme. The high-pI thiolase is particulate in brain, and like the rat liver mitochondrial thiolase it is activated by K^+ ions.

The absolute amounts of these two acetoacetyl-CoA-specific thiolases have been determined in rat brain during postnatal development by calculation from the observed thiolase rates measured in the presence and in the absence of K^+ ions. These calculated values agreed well with those determined directly by separation of the enzymes on DEAE-cellulose. The cytoplasmic enzyme was found to decline slowly in activity from $2.8 \mu\text{mol/min per g}$ of brain at birth to the adult value of $1 \mu\text{mol/min per g}$. The mitochondrial acetoacetyl-CoA thiolase activity increased rapidly from $0.9 \mu\text{mol/min per g}$ at birth to a maximum of $4 \mu\text{mol/min per g}$ at 28 days when the rats were weaned. After weaning the mitochondrial thiolase activity slowly declined to the adult value of $1.8 \mu\text{mol/min per g}$. Thus the total brain acetoacetyl-CoA thiolase activity remained approximately constant over the first 40 days from birth, as has been found by other investigators (Lynen, 1957; Page, Krebs & Williamson, 1971; Dierks-Ventling & Cone, 1971). This constant total activity is, however, made up from the changing activities of two different acetoacetyl-CoA thiolases.

The distinctive activity pattern of the mitochondrial thiolase closely resembles the patterns of development of 3-hydroxybutyrate dehydrogenase (Klee & Sokoloff, 1967) and 3-oxo acid CoA-transferase (Page *et al.* 1971; Tildon, Cone & Cornblath, 1971) in rat brain during suckling. This suggests that the mitochondrial high-pI K^+ -activated thiolase is involved in ketone-body utilization in rat brain and possibly in all relevant extrahepatic tissues. The cytoplasmic acetoacetyl-CoA thiolase in brain may provide acetoacetyl-CoA for the synthesis of cholesterol *de novo*. It is noteworthy that the rate of this process is high during the suckling period and very low in mature brain (Srere, Chaikoff, Treitman & Burstein, 1950).

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Quantitative Aspects of Assimilation of [$U-^{14}C$]Glucose Carbon in various Rat Tissues *in vivo*

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A group of four adult male rats was injected subcutaneously with 0.320 mCi of [$U-^{14}C$]glucose (3 mCi/mmol). The animals were decapitated 4 h later and samples of the following tissues were obtained: pancreas, serum, ileum and jejunum, colon and rectum, spleen, brain, thymus, stomach, skin, testes, long bone (femur), fat tissue, kidney, liver, lung, heart, skeletal muscle and formed elements of blood. The following parameters were measured: (1) specific radioactivity of tissue proteins; (2) quantity of ^{14}C incorporated into acid-insoluble substances per g fresh wt. of tissue; (3) percentage of [$U-^{14}C$]glucose carbon assimilated per whole organs. Experimental methods have been described elsewhere (Vrba, 1966, 1967; Vrba & Cannon, 1970; Vrba & Winter, 1971).

More ^{14}C was incorporated into the protein fractions than into the lipid fractions of all investigated tissues with the exception of adipose tissue. Ileum and jejunum, which account for less than 4% of the body weight of our experimental animals, nevertheless assimilated more than half the amount of glucose carbon assimilated by the whole mass of body muscles, although the latter represent approx. 38% of the body weight (Curtis-Prior, Trethewey, Stewart & Hanley, 1969). There is a striking difference between the high rate of incorporation of ^{14}C from glucose into serum proteins and the low rate of incorporation into the proteins of formed elements of blood.

All brain proteins that could be separated by gel-filtration chromatography (Sephadex G-200) had very similar specific radioactivities. This is in

striking contrast with serum proteins. The specific radioactivity of the serum proteins was highest in the globulin fraction and decreased in correlation with the decrease of molecular weights of the serum proteins in the globulin and albumin fractions.

The results indicate that the proteins of tissues that display a rapid rate of cell proliferation and/or rapid synthesis of proteins, hormones and antibodies (gastrointestinal epithelium, pancreas, thymus, spleen, liver) show a high affinity for assimilation of glucose carbon, in sharp contrast with the relatively low rates of assimilation of glucose carbon by heart or skeletal muscles. However, the brain proteins had a higher specific radioactivity than did the liver proteins.

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Activation of Membrane Adenosine Triphosphatase by Chelating Reagents and Phospholipids

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Solubilization of a microsomal fraction from brain tissue with deoxycholate removes most or all of the ouabain-inhibited ATPase* activity and subsequent addition of certain phospholipid dispersions, notably phosphatidylserine, partly restores this activity, suggesting that the ATPase consists of a phospholipid-protein complex (Tanaka & Abood, 1964; Fenster & Copenhaver, 1967; Wheeler & Whittam, 1970). However, this interpretation was rendered questionable by the observation that phosphatidylserine also activates the ouabain-sensitive ATPase of the particulate enzyme, untreated with deoxycholate (Wheeler & Whittam, 1970). An alternative interpretation is that phosphatidylserine activates by virtue of its ability to chelate endogenous ions, such as Ca^{2+} or Fe^{2+} , that inhibit the ATPase. To test this possibility the effects of both EDTA and EGTA on similar preparations from rabbit kidney have been examined and compared with the effect of phosphatidylserine.

In the absence of added phospholipid the mean ouabain-sensitive ATPase activities (in μmol of P_i

liberated/min per g of protein) of the particulate and solubilized preparations were 172 and 31 respectively, and both of those rates increased, to 273 and 278, when 0.2mm-phosphatidylserine was added. However, addition of 0.2mm-EGTA to the particulate enzymes similarly stimulated the ATPase, to 314; and with both EGTA and phosphatidylserine the activation was virtually identical, giving a value of 295. Thus EGTA effectively mimicked the activating effect of the phospholipid. In contrast, 0.2mm-EGTA increased the ouabain-sensitive ATPase activity of the solubilized enzymes from 31 to only 58, whereas the further addition of the same concentration of phosphatidylserine raised the activity to 332. Almost identical results were obtained when EDTA was substituted for EGTA, and when these reagents were added at a final concentration of 2mm, compared with only 0.2mm-phosphatidylserine.

Phosphatidylserine therefore appears to activate the membrane ATPase both by its chelating ability and, presumably, by forming a phospholipid-protein complex. Further, the chelating action accounts completely for its activation of the particulate ATPase, but not of the solubilized enzyme, so that the phospholipid-protein-complex concept of the ATPase system remains tenable. This conclusion is not affected if the chelators and the phosphatidylserine produce their effects by some direct action on the enzyme, rather than by chelating endogenous metal ions.

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Some Catalytic Properties of IRC-50 Arvin

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Arvin, an enzyme fraction isolated from the venom of *Agkistrodon rhodostoma* (Esnouf & Tunnah, 1967), is useful for producing controlled defibrination in patients requiring anticoagulant therapy. Arvin catalyses a limited proteolysis of fibrinogen to yield a readily dispersible fibrin clot (Ewart, Hatton, Basford & Dodgson, 1970); it has no effect on other clotting factors (Bell, Pitney & Goodwin, 1968; Sharp, Warren, Paxton & Allington, 1968; Bell, Bolton & Pitney, 1968). Chromatography of

* Abbreviations: ATPase, adenosine triphosphatase; EGTA, ethanedioxybis(ethylamine) - NNN'N' - tetraacetate.