Effect of the carbonic anhydrase inhibitor acetazolamide on lipid synthesis in the locust

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The zinc metalloenzyme carbonic anhydrase (CA; EC 4.2.1.1.), which catalyses the hydration of CO₂ to HCO₃⁻, plays a number of physiologically important roles, for example in respiration, acid–base regulation and several secretory processes [for reviews see Ann. N.Y. Acad. Sci. (1984) 429]. This wide diversity of important functions is reflected in its relative abundance in a broad range of tissues and cells (Tashian et al., 1984). Interest has recently been focused on the possible function of CA in the provision of HCO₃⁻ for essential carboxylation reactions in metabolism. There is evidence for its involvement in ureogenesis (Dodgson et al., 1984; Haussinger et al., 1985) and in gluconeogenesis (Herbert et al., 1983; Dodgson et al., 1984; Metcalf et al., 1985). Moreover, Herbert & Coulson (1984) have suggested that it may play a role in fatty acid synthesis in reptilian liver. We have examined the possible involvement of CA in lipogenesis in the fat body of the desert locust Schistocerca gregaria. This animal is an excellent model for studying fat metabolism. It has been known since biblical times that the locust undertakes long migratory flights (Exodus). For these it uses fat as the principal energy source. The necessary fat reserves are largely contained within a specialized organ, the fat body (Weiss-Fogh, 1952; Kilby, 1965).

Incorporation of radioactive label from [¹⁴C]acetate into total lipid in fat body preparations from the adult locusts of different ages was monitored over a 1 h period in the presence and absence of the potent CA inhibitor acetazolamide (1.6 mM). For each experiment, the fat bodies from at least five locusts of the same age group were pooled, passed through a nylon mesh to disrupt the tissue into fragments, and suspended in incubation buffer (Hepes, 30 mM; NaCl, 10 mM; KCl, 12 mM; MgSO₄, 2 mM; K₂HPO₄, 1 mM; CaCl₂, 1 mM; glucose, 10 mM; sucrose, 50 mM; 2% (w/v) bovine serum albumin, adjusted to pH 7.2 with NaOH). Each reaction mixture (total volume 3.6 ml) contained 0.8 μCi of [¹⁴C]acetate and was incubated with shaking at 34°C for 1 h.

Samples were transferred at timed intervals into chloroform/methanol (1:2, v/v) and placed on ice. The lipid was removed by chloroform extraction, evaporated to dryness with nitrogen and redissolved in scintillation fluid for measurement of radioactivity. Carbonic anhydrase activity was measured in the direction of bicarbonate dehydration employing a pH-stat assay system (30 mM-NaHCO₃, pH 7.1, 2°C) as described by Chegwidden et al. (1984).

The Figure shows that maximum incorporation of radioactive label from [¹⁴C]acetate into total lipid in the fat body of the adult locust occurred 4–6 days after the last moult, indicating that the major period of lipogenesis occurred at this stage during development. This result is in broad agreement with the data of Walker & Bailey (1970) and Gokuldas et al. (1988). The high level of lipogenesis over the 4–6 day period of development of the adult locust was inhibited by the CA inhibitor acetazolamide to a level similar to that of the mature insect.

Fat body homogenates (0.05 M-Hepes, pH 7.1) from locusts both in the first 3 days of adult life and in the 4–6 day age range were shown to possess CA activity which was totally inhibited by 0.1 mM-acetazolamide.

Rous & Favarger (1963, 1964) demonstrated an inhibitory effect of acetazolamide on fatty acid synthesis in mice. Furthermore, acetazolamide, at concentrations of either 8 mm or 16 mm, did not inhibit fatty acid synthetase, but did inhibit acetyl-CoA carboxylase, even in the presence of high concentrations of bicarbonate (Cao & Rous, 1978). However, in the present study the inhibition of lipogenesis

**Abbreviation used:** CA, carbonic anhydrase.
was achieved at an acetazolamide concentration of 1.6 mM. Studies by Cao & Rous (1978) on the mouse acetyl-CoA carboxylase showed a maximum inhibition of this enzyme of only 14% by 2 mM-acetazolamide.

The data presented here suggest a role for CA in the provision of HCO$_3^-$ for lipogenesis in the fat body of the desert locust, with the possibility of a regulatory involvement at the high levels of lipogenesis evident in the developing adult insect.

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Exodus 10:13 In The Old Testament

Active-site modification of chicken carbonic anhydrase III

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Carbonic anhydrase (CA; EC 4.2.1.1) catalyses the reversible hydration of CO$_2$ to HCO$_3^-$ and also possesses some esterase activity towards a number of ‘synthetic’ substrates such as 4-nitrophenyl acetate (Pocker & Sarkanen, 1978). Carbonic anhydrase III (CA III), which is abundant in red skeletal muscle, is strikingly different from the other two characterized cytosolic isoenzymes, CA I and CA II, in terms of activities, inhibition parameters and active-site structure (Tashian et al., 1983). This low activity muscle isoenzyme, uniquely among these three, possesses three basic active-site residues at positions 64, 67 and 91. Positions 67 and 91 are occupied by either lysine or arginine in chicken CA III (tryptic cleavage has been observed between residues 67–68 and 91–92) and by arginine in CA III from all other species examined. Position 64 is occupied by arginine in CA III from horse and chicken (Wendorff et al., 1985; Chegwidden et al., 1986a) and by lysine in other species. Intriguingly, Koester et al. (1981) demonstrated a low acid phosphatase activity in CA III from several species.

Previous studies showed that incubation of carbonic anhydrase isoenzymes with 2,3-butanedione, under conditions known to modify arginine residues, enhanced both the bicarbonate dehydrogenation and esterase activities of human CA III, but only the esterase activity of chicken CA III, and was without effect on CA I and II. Moreover the $I_{50}$ of both human and chicken CA III for the potent CA inhibitor acetazolamide was greatly reduced. A detailed study of the structural modification of human CA III by butanedione revealed that both arginines at positions 67 and 91 had been modified, while initial studies on chicken CA III showed that position 67 remained unaffected (Chegwidden et al., 1984). In the present study, investigation of the modification of chicken CA III was extended in an attempt to relate these modifications of CA III from both species to the observed activity changes.

Carbonic anhydrase was isolated from chicken muscle essentially as described by Carter et al. (1984). Arginine modification was performed in the dark at 25°C by incubation...