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The apparent failure of L-carnitine to prevent the hypoglycaemia and hypothermia caused by hypoglycin or by pent-4-enoic acid in mice

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HYPOGLYCIN, the active principle of the ackee *Blighia sapida*, and a related simple fatty acid pent-4-enoic acid, cause hypoglycaemia by a mechanism probably depending on impaired gluconeogenesis secondarily to inhibition of fatty acid oxidation.[1,2] Hypoglycin poisoning may result from eating unripe ackee fruits (see Sherratt[1,2]) and pent-4-enoic acid has been used experimentally to inhibit β-oxidation.[3,4] It is important, therefore, to understand the detailed mechanism of action of these compounds. Bressler, Corredor and Brendel[5] concluded that they sequester cellular CoA, necessary for β-oxidation,[6] as non-metabolizable CoA-derivatives of their metabolites. However, other non-metabolizable simple fatty acids which extensively acylate CoA do not inhibit β-oxidation strongly and are not hypoglycaemic.[1,2,7–12]

Entman and Bressler[13] and Corredor, Brendel and Bressler[14] administered L-carnitine (6–12 mg/mouse) to mice also given hypoglycin (7·5 or 15 mg/mouse) or pent-4-enoic acid (7·5 mg/mouse) and they reported that this greatly decreased their hypoglycaemic effects. With pent-4-enoic acid this effect was transient since the onset of hypoglycaemia was only delayed for about 30 min[14] while with hypoglycin blood glucose concentrations had only fallen slightly after 90 min.[13] It was also claimed that L-carnitine antagonized inhibition of palmitate oxidation by hypoglycin or by pent-4-enoic acid in tissue homogenates.[13,14] These results were explained by the formation of acylcarnitines from the reaction of acyl-CoA derivatives of metabolites of these hypoglycaemic compounds with exogenous L-carnitine, catalysed by the carnitine acyltransferases, releasing sufficient of the sequestered CoA to enable an adequate rate of oxidative metabolism to occur to maintain gluconeogenesis, in apparent support of the CoA-sequestration hypothesis.[5] It was therefore considered essential to try to repeat these experiments of Entman and Bressler[13] and of Corredor, Brendel and Bressler[14] on the effects of L-carnitine on the hypoglycaemia caused by these compounds, particularly since we

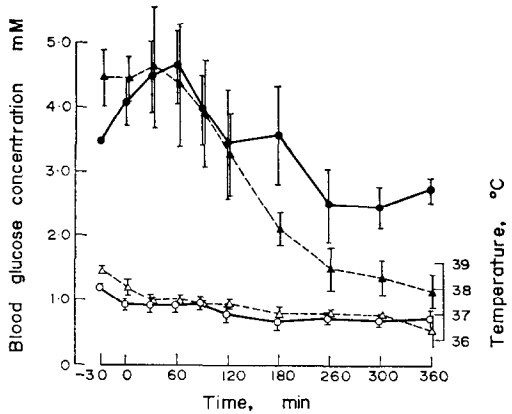

FIG. 1. Effect of L-carnitine on blood glucose concentrations and body temperature in the mouse. Male albino mice 19 weeks old (ca. 30 g) were starved for 24 hr and food was withheld during the experiment. Glucose was determined using glucose oxidase (EC 1.1.3.4) by a scaled down version of the procedure of Senior and Sherratt[12] in serial 20 μl blood samples from the tail vein. The animals were put in a restriction cage each time a blood sample was taken and for the measurement of rectal temperature with a thermistor probe and a digital thermometer. The ambient temperature was 23°. Six mice were given 0·14 M–NaCl (3·5 ml/kg body wt) intraperitoneally at time 0: (●) blood glucose concentration, (○) rectal temperature. Six mice received three doses of L-carnitine chloride (Koch–Light Laboratories Ltd., Colnbrook, Bucks), each of 100 mg/kg body wt, at times −30, 0 and 60 min, given subcutaneously as a 0·15 mM solution so as to delay absorption and to maintain the blood concentration of L-carnitine for as long as possible, and 0·14 M–NaCl intraperitoneally at time 0: (▲) blood glucose concentration, (△) rectal temperature. Each point represents a mean value and the vertical bars the S.E.M. The initial hyperglycaemia observed in this experiment is a response of the animals to handling.

have repeatedly failed to modify the inhibition of fatty acid oxidation in rat or mouse liver mitochondria by pent-4-enoic acid with L-carnitine.[7,10] We also determined the effects of all these compounds on body temperature since we have shown that hypothermia closely parallels hypoglycaemia caused by pent-4-enoic acid or by hypoglycin.[2]

We were unable to prevent in our strain of mice, by administration of L-carnitine (300 mg/kg body wt. in divided doses), either the hypoglycaemia or the hypothermia caused by intraperitoneal injection of pent-4-enoic acid (350 mg/kg) or of our hypoglycin preparation (500 mg/kg) (Figs. 1–3). Similar results were obtained in several other experiments. These doses were similar to those used by Bressler and associates;[13,14] although these workers gave pent-4-enoic acid or hypoglycin intravenously there is little difference in the hypoglycaemic response with different routes of parenteral administration.[1] Unexpectedly, L-carnitine alone with our dose schedules caused delayed hypoglycaemia although it did not affect body temperature (Fig. 1) and this effect was partly superimposed on the hypoglycaemia caused by pent-4-enoic acid (between about 120–240 min) (Fig. 2). The experiment shown in Fig. 2 illustrates the difficulties in assessing the significance of any delay in the onset of hypoglycaemia after administration of L-carnitine. There was some apparent delay (between 30 and 90 min) when the mean values for the blood glucose concentrations of mice given both pent-4-enoic acid and L-carnitine were compared with those of mice given pent-4-enoic acid alone although this difference was not significant ($P = 0{\cdot}3$), yet by contrast the former mice tended to have lower body temperatures. Hypothermia caused by pent-4-enoic acid or by hypoglycin could be due to impairment of oxidative metabolism. That caused by pent-4-enoic acid might also be partly due to an effect depending on interference with the action of central transmitters since related short-chain fatty acids, including *n*-pentanoic acid in large doses, induce a sleep- or anaesthetic-like state in animals;[15] although *n*-pentanoic acid is less toxic than pent-4-enoic acid in mice and is not hypoglycaemic in

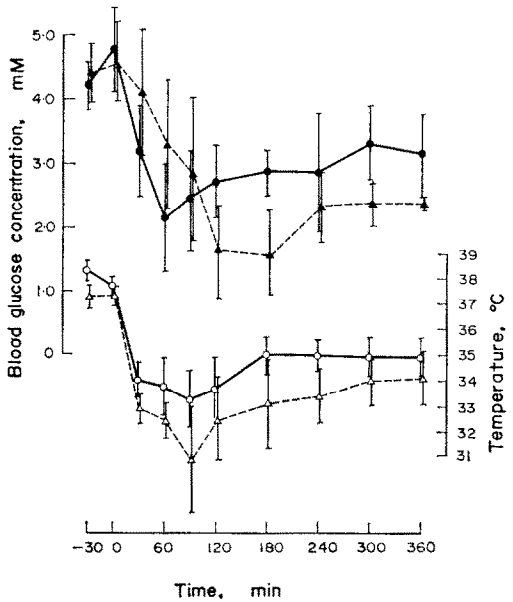

FIG. 2. Effect of L-carnitine on the hypoglycaemia and hypothermia caused by pent-4-enoic acid in mice. Six mice were given pent-4-enoic acid (Fluka A.G. Buchs, Switzerland) 350 mg/kg body wt, adjusted to pH 7·4 with NaOH, intraperitoneally at time 0: (●) blood glucose concentration, (○) rectal temperature. Six mice were given pent-4-enoic acid 350 mg/kg body wt, pH 7·4 intraperitoneally at time 0 and three doses of L-carnitine chloride, each of 100 mg/kg body wt, at times −30, 0 and 60 min subcutaneously: (▲) blood glucose concentration, (△) rectal temperature. Each point represents a mean value and the vertical bars the S.E.M. These mean values for blood glucose concentrations partly obscure the strong hypoglycaemia obtained in both groups because minimum values were recorded in individual animals at different times: for mice given pent-4-enoic acid; after 30 min (1·06 mM), 60 min (0·15 and 0·18 mM), 90 min (1·16 mM), 120 min (0·96 mM) and after 360 min (2·07 mM) with a mean minimum value of 0·92 ± 0·32 mM; for mice given pent-4-enoic acid and L-carnitine; after 60 min (0·48 mM), 90 min (0·02 mM), 120 min (1·02 and 2·39 mM) and after 180 min (undetectable and 1·02 mM) with a mean minimum value of 0·82 ± 0·40 mM. Other details are given in the legend to Fig. 1.

rats.[12] Other workers have not apparently considered hypothermia as a factor in interpreting the action of compounds related to hypoglycin nor have they apparently controlled the ambient temperature in their experiments. Temperature dependent changes in the absolute rates of glucose utilization and of gluconeogenesis may affect the time course of hypoglycaemia in an unpredictable way (cf. Ref. 2). Interpretation of these results is further complicated by the fact that L-carnitine (and its acetyl derivative which may be formed *in vivo*) have a variety of weak pharmacological effects[16] and the possibility that large amounts of L-carnitine potentiates hypothermia in animals given pent-4-enoic acid cannot be excluded. Further, L-carnitine is a permanent cation and the extent to which it enters cells following parenteral administration is unknown and we have shown that it is rapidly excreted via the kidneys. The toxicity of pent-4-enoic acid decreases with the age of the mice,[1,12] all our mice (ca. 30 g) starved for 24 hr survived a dose of pent-4-enoic acid of 350 mg/kg while its LD_{50} in 20–25 g mice starved for 22 hr was 315 (237–437) mg/kg.[12] Pretreatment with DL-carnitine (800 mg/kg) in single or in divided doses does not affect the LD_{50} of pent-4-enoic acid.[12]

In our experiments mice given 500 mg/kg of 70 per cent pure hypoglycin invariably died, while the data given in Fig. 6 of Entman and Bressler[13] implies that all their (starved) animals survived a dose of 15 mg/mouse (equivalent to 600 mg/kg in a 25 g mouse) since no deaths were reported. Yet hypoglycin can cause marked hypoglycaemia in mice with a dose of only 100 mg/kg.[17] The toxicity of

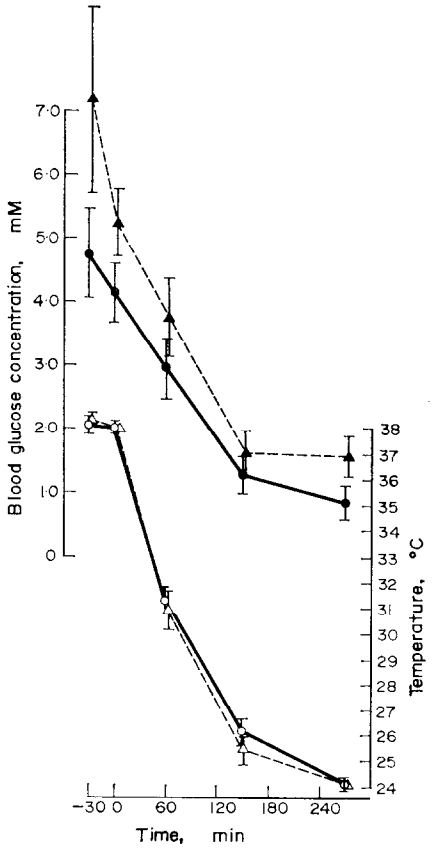

FIG. 3. Effect of L-carnitine on the hypoglycaemia and hypothermia caused by hypoglycin in mice. Hypoglycin was prepared from ackee seeds by a method said to yield leucine-free hypoglycin,[18] however our preparation contained 30 per cent of leucine. Five mice were given hypoglycin (500 mg/kg body wt intraperitoneally) at time 0: (●) blood glucose concentration. (○) rectal temperature. Six mice were given hypoglycin, 500 mg/kg body wt intraperitoneally at time 0 and three doses of L-carnitine chloride, each of 100 mg/kg body wt at times −30, 0 and 60 min subcutaneously: (▲) blood glucose concentration, (△) rectal temperature. Each point represents a mean value and the vertical bars the S.E.M. Other details are given in the legend to Fig. 1.

hypoglycin and the failure of L-carnitine to modify its hypoglycaemic effects in our experiments are in total disagreement with the report of Entman and Bressler.[13] Hypoglycin is notoriously difficult to free from contamination with leucine[18] and Entman and Bressler[13] gave no data about the purity of their preparation. Riboflavin has been reported to antagonize specifically the chronic toxicity of hypoglycin in mice[19] and we have been able to confirm this observation.*

The failure of L-carnitine in our experiments to antagonize the hypoglycaemic effects of hypoglycin and of pent-4-enoic acid is entirely consistent with our extensive *in vitro* biochemical work which has provided strong evidence for a specific inhibition of β-oxidation, probably at the stage catalyzed by 3-oxyacyl-CoA thiolase (EC 2.3.1.16).[1,2,7–11] It is therefore difficult to explain the discrepancy between the results of Entman and Bressler[13] and Corredor, Brendel and Bressler[14] and our investigations, and to accept the conclusions of these authors[5,13,14] about the mechanism of inhibition of β-oxidation by hypoglycin or pent-4-enoic acid.

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