The Effects of Inhibition of Fatty Acid Oxidation in Suckling Newborn Rats

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Inhibition of fatty acid oxidation with pent-4-enoate in suckling newborn rats caused a fall in blood [glucose] and blood [ketone bodies] and inhibition of gluconeogenesis from lactate. Glucose utilization was not increased in newborn rats injected with pent-4-enoate. Active fatty acid oxidation appears to be essential to support gluconeogenesis and to maintain normal blood [glucose] in suckling newborn rats.

A profound hypoglycaemia develops in starved newborn rats (Haymond *et al.*, 1972; Wapnir *et al.*, 1973; Girard *et al.*, 1973, 1975), secondary to a deficiency of gluconeogenesis (Girard *et al.*, 1975). It has been suggested that gluconeogenesis may be limited during starvation by unavailability of gluconeogenic substrates (Haymond *et al.*, 1972; Girard *et al.*, 1975) and non-esterified fatty acids (Girard *et al.*, 1975). Starvation-induced hypoglycaemia is partially reversed by fat feeding, and is completely reversed by fat feeding in combination with injection of gluconeogenic substrates (Ferré *et al.*, 1977b). Suckling newborn rats maintain their blood [glucose] mainly by gluconeogenesis (Snell & Walker, 1973; Ferré *et al.*, 1977a). They obtain their non-esterified fatty acids from the milk, which is a high-fat low-carbohydrate diet (Luckey *et al.*, 1954). Pent-4-enoate inhibits fatty acid oxidation and produces hypoglycaemia when administered to food-deprived adult mice and rats (Corredor *et al.*, 1967; Senior & Sherratt, 1969). On the basis of studies *in vitro*, the hypoglycaemic effect of pent-4-enoate has been attributed to inhibition of gluconeogenesis, secondary to impairment of fatty acid oxidation (Ruderman *et al.*, 1970; Williamson *et al.*, 1970). In the present study, pent-4-enoate was used to investigate the effects of inhibition of fatty acid oxidation on glucose homeostasis in suckling newborn rats.

Experimental design

Each litter of 16h-old rats was divided into two groups. One was injected subcutaneously with sodium pent-4-enoate (pH 7.4) at a dose of 2mg/g body wt. and the other was injected with the same volume (50 μ l) of 0.9% NaCl. The pups were then immediately placed in a Humidicrib (Jouan S.A., Paris, France) in which the temperature was maintained at 37°C and the relative humidity at 70%.

In the experiments in which blood metabolites and hormones were determined, the newborn rats were killed 2.5h after injections of pent-4-enoate or NaCl. The maximum effects of pent-4-enoate were observed at this time.

In the gluconeogenesis studies, the newborn rats were injected intraperitoneally with labelled precursor (1 μ Ci of [U-¹⁴C]lactate or 1 μ Ci of [U-¹⁴C]glycerol) 2h after pent-4-enoate or NaCl injections, placed again in the Humidicrib and killed 30min after injection of the labelled precursor.

In the glucose-tolerance studies, the newborn rats were injected intraperitoneally with D-glucose (2mg/g body wt.) 30min after pent-4-enoate or NaCl injections, placed again in the Humidicrib and killed 30, 60, 120 or 180 min after injection of glucose.

Blood samples for metabolite and hormone assays were collected as described previously (Girard *et al.*, 1973, 1975).

Measurement of gluconeogenesis rates *in vivo* from ¹⁴C-labelled substrates

The technique used and the method for calculation of rate of gluconeogenesis *in vivo* were as described previously (Girard *et al.*, 1975, 1976; Ferré *et al.*, 1977a); lactate and glycerol spaces equal to the total body water were used, i.e. 80ml/100g body wt., and a glucose space equal to extracellular body water was used, i.e. 60ml/100g body wt. The separation of

Experimental

Animals

Rat pups of an albino Wistar strain were born between 15:00 and 18:00h on day 22 of gestation. They were left with the mother for 16h after delivery in a room in which external temperature was 22°C. The pups began to suckle between 1 and 2h after birth.

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labelled glucose, lactate and charged compounds was performed by ion-exchange chromatography (Kreisberg *et al.*, 1972). Labelled glucose was separated from labelled glycerol as described by Pilkis *et al.* (1976). A portion of the $\text{Ba}(\text{OH})_2/\text{ZnSO}_4$ filtrate was treated with glycerol kinase to convert the remaining $[\text{U}-^{14}\text{C}]\text{glycerol}$ into $[\text{U}-^{14}\text{C}]\text{glycerol phosphate}$. After neutralization, the samples were treated with Dowex 1 (X8) and Dowex 50 (X8) ion-exchange resins. The resin treatment separated labelled glycerol phosphate from labelled glucose.

Assays

Enzymic assays were performed for glucose, lactate, pyruvate, glycerol, alanine, acetoacetate and 3-hydroxybutyrate as previously described (Girard *et al.*, 1973). Plasma non-esterified fatty acids were measured by the radioactive nickel method of Ho (1970).

Hormones

Plasma insulin and glucagon were determined by radioimmunoassay as previously described (Girard *et al.*, 1973, 1975). Glucagon was assayed by using 30K antiserum (Faloona & Unger, 1974), which is considered to be specific for pancreatic glucagon.

Statistics

Results are expressed as means $\pm$ S.E.M.; significant differences were determined by using the Wilcoxon rank-order test (Wilcoxon, 1947).

Special chemicals

Enzyme and coenzymes were from Boehringer Corp. (Paris), 75006 Paris, France. Pent-4-enoic acid was purchased from K & K Laboratories, Plainview, NY, U.S.A. $[\text{U}-^{14}\text{C}]\text{Lactate}$ and $[\text{U}-^{14}\text{C}]\text{glycerol}$ were from The Radiochemical Centre, Amersham, Bucks., U.K.

Results

Blood metabolites

The injection of pent-4-enoate (2mg/g subcutaneously) in 16h-old suckling rats resulted in a marked decrease in blood [acetoacetate] and [3-hydroxybutyrate] of 0.29mm and 2.38mm respectively, and in a significant increase in plasma [non-esterified fatty acids] of 0.2mm after 2.5h (Table 1). This suggests that pent-4-enoate strongly inhibited fatty acid oxidation in suckling newborn rats.

The injection of pent-4-enoate induced a dramatic fall in blood [glucose] of 3.7mm after 2.5h (Table 1).

Table 1. *Blood and plasma metabolite concentrations after injection of NaCl or pent-4-enoate into suckling newborn rats*

	Metabolite concn. ($\mu\text{mol/ml}$)	
	NaCl	Pent-4-enoate
Blood [glucose]	5.35 ± 0.25	$1.65 \pm 0.13^*$
Blood [pyruvate]	0.09 ± 0.01	0.08 ± 0.01
Blood [lactate]	1.60 ± 0.10	$2.10 \pm 0.20^*$
Blood [glycerol]	0.30 ± 0.04	$0.17 \pm 0.02^*$
Plasma [non-esterified fatty acid]	0.58 ± 0.04	$0.78 \pm 0.03^*$
Blood [acetoacetate]	0.43 ± 0.02	$0.14 \pm 0.01^*$
Blood [3-hydroxybutyrate]	3.09 ± 0.34	$0.71 \pm 0.04^*$
Total [ketone bodies]	3.52	0.85
Blood [alanine]	0.41 ± 0.01	0.44 ± 0.02

It also produced an increase in blood [lactate] of 0.50mm and a decrease in blood [glycerol] of 0.13mm (Table 1). The blood [pyruvate] and [alanine] did not change significantly.

Rate of gluconeogenesis in vivo

The rate of conversion of $[\text{U}-^{14}\text{C}]\text{lactate}$ into glucose decreased from $11 \pm 1 \mu\text{mol/h}$ per 100 g body wt. in NaCl-injected newborn rats ($n=6$) to $1.8 \pm 0.1 \mu\text{mol/h}$ per 100g body wt. in newborn rats injected with pent-4-enoate ($n=6$) ($P<0.01$). There was no significant difference in the rate of conversion of $[\text{U}-^{14}\text{C}]\text{glycerol}$ into glucose between the NaCl-injected newborn animals [$3.5 \mu\text{mol/h}$ per 100 g body wt. ($n=6$)] and the newborn rats injected with pent-4-enoate [$4.4 \pm 0.7 \mu\text{mol/h}$ per 100 g body wt. ($n=6$)].

Glucose-tolerance test

In order to estimate whether glucose utilization was altered by pent-4-enoate, intraperitoneal glucose tolerance was tested. The results are shown in Fig. 1. A significant decrease in blood [glucose] ($P<0.01$) was already observed 30min after pent-4-enoate injection, before the glucose-tolerance test was started. Although the peak in glycaemia was higher in NaCl-injected newborn rats, the disappearance rate was identical, and blood [glucose] returned to initial values 3h after glucose injection.

The rate of glucose disappearance calculated from the slope of the semi-logarithmic plot of blood glucose expressed as $\%/min$ was 0.54 in NaCl-

Fig. 1. Variations in blood glucose concentration after intraperitoneal injection of glucose into suckled newborn rats previously injected with NaCl (●) or pent-4-enoate (○). Glucose (2mg/g body wt.) was injected 30min after the injection of NaCl or pent-4-enoate. Values are means $\pm$ S.E.M. of six to twelve determinations for each time.

injected controls and 0.61 in pent-4-enoate-injected rats. No glucose intolerance was demonstrated by this technique after pent-4-enoate injection.

Plasma insulin and glucagon

Plasma insulin decreased from $18 \pm 1 \mu\text{i.u./ml}$ in NaCl-injected controls to $10 \pm 1 \mu\text{i.u./ml}$ ($P < 0.01$), in association with hypoglycaemia, in the newborn rat injected with pent-4-enoate. A significant increase in plasma glucagon was also observed: $906 \pm 43 \text{ pg/ml}$ for the NaCl-injected controls versus $1907 \pm 189 \text{ pg/ml}$ for the newborn rats injected with pent-4-enoate ($P < 0.01$). Each value was the mean of six determinations.

Discussion

In the present study, pent-4-enoate was used to examine the metabolic effects of a blockage of fatty acid oxidation in suckling newborn rats. This compound has been reported to inhibit *in vitro* the oxidation of long-chain fatty acids in several tissues, particularly the liver [see Sherratt & Osmundsen (1976) for a review]. *In vivo*, pent-4-enoate raised the plasma [non-esterified fatty acid] and caused marked ketosis in starved rats (Senior & Sherratt, 1969). In suckling newborn rats, pent-4-enoate produced a fall in blood [ketone bodies] and a rise in plasma [non-esterified fatty acid], which suggests inhibition of fatty acid oxidation. In adult rats, ketosis has been

attributed to greater inhibition of the peripheral utilization of ketone bodies than their production by the liver. The decrease in the rate of ketone-body utilization could be secondary to hypothermia produced by pent-4-enoate injection into adult rat maintained at an environmental temperature less than 26°C (Marley & Sherratt, 1973). This interpretation did not apply to suckling newborn rats maintained at an ambient temperature of 37°C .

The development of profound hypoglycaemia in suckling rats occurred in spite of the high plasma [non-esterified fatty acid], but concomitantly with a fall in blood [ketone bodies]. A possible increase in glucose utilization, secondary to the fall in blood [ketone bodies], can be ruled out, since (1) the rate of glucose disappearance after an intra-peritoneal glucose injection was not increased by pent-4-enoate and (2) the fall in plasma [insulin] that occurred in newborn rats injected with pent-4-enoate would contribute to a decrease rather than an increase in glucose utilization.

The hypoglycaemic effect of pent-4-enoate in suckling newborn rats resulted from inhibition of gluconeogenesis at a site between pyruvate and glyceraldehyde 3-phosphate, since it decreased the rate of gluconeogenesis from lactate by 84% but not that from glycerol *in vivo*. This is in agreement with previous observations showing that pent-4-enoate inhibited gluconeogenesis from pyruvate, alanine and aspartate but not from glycerol or fructose in isolated perfused rat liver (Ruderman *et al.*, 1970; Williamson *et al.*, 1970). The 30% rise in blood [lactate] when gluconeogenesis is inhibited suggests that lactate is the most important substrate for gluconeogenesis in newborn rats, as postulated previously (Girard *et al.*, 1975; Ferré *et al.*, 1977a). Glycerol did not appear to make a major contribution to blood-glucose regulation in suckling newborn rats, since hypoglycaemia occurred even though pent-4-enoate did not inhibit gluconeogenesis from glycerol. Moreover, the fall in blood [glycerol] after pent-4-enoate injection suggests increased utilization of glycerol for gluconeogenesis. This can explain why the rate of gluconeogenesis from glycerol is not decreased by pent-4-enoate despite the 2-fold decrease in glycerol-pool size. The absence of a rise in blood [alanine] when gluconeogenesis was inhibited suggests again that alanine is not a quantitatively important substrate for gluconeogenesis in suckling newborn rats (see also Ferré *et al.*, 1977a).

Plasma insulin decreased and plasma glucagon increased during pent-4-enoate-induced hypoglycaemia. Hypoglycaemia of the same magnitude was observed after inhibition of gluconeogenesis by 3-mercaptopicolinate and was associated with a decrease in plasma [insulin] but no change in plasma [glucagon] (Ferré *et al.*, 1977a). *In vitro*, ketone bodies have been shown to inhibit glucagon

release from neonatal rat pancreas (Marliss *et al.*, 1973). In the present studies, the dramatic fall in blood [ketone bodies] induced by pent-4-enoate might have contributed to the increase in plasma [glucagon].

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References

- Corredor, C., Brendel, K. & Bressler, R. (1967) *Proc. Natl. Acad. Sci. U.S.A.* **58**, 2299–2306
- Faloon, G. R., & Unger, R. H. (1974) in *Methods of Hormone Radioimmunoassay* (Jaffe, B. M. & Behrman, R., eds.), pp. 317–330, Academic Press, New York
- Ferré, P., Pégorier, J. P. & Girard, J. (1977a) *Biochem. J.* **162**, 209–212
- Ferré, P., Pégorier, J. P., Girard, J. R. & Marliss, E. B. (1977b) *Biochem. Soc. Trans.* **5**, 982–983
- Girard, J. R., Cuendet, G. S., Marliss, E. B., Kervran, A., Rieutort M. & Assan, R. (1973) *J. Clin. Invest.* **52**, 3190–3200
- Girard, J. R., Guillet, I., Marty, J. & Marliss, E. B. (1975) *Am. J. Physiol.* **229**, 466–473
- Girard, J. R., Guillet, I., Marty, J., Assan, R. & Marliss, E. B. (1976) *Diabetologia* **12**, 327–337
- Haymond, M., Karl, I., Pagliara, A. & Kipnis, D. M. (1972) *J. Clin. Invest.* **51**, 43a
- Ho, R. J. (1970) *Anal. Biochem.* **36**, 105–114
- Kreisberg, R. A., Siegal, A. M. & Owen, W. C. (1972) *J. Clin. Endocrinol. Metab.* **34**, 876–883
- Luckey, T. D., Mende, T. J. & Pleasants, J. (1954) *J. Nutr.* **54**, 345–359
- Marley, J. & Sherratt, H. S. A. (1973) *Biochem. Pharmacol.* **22**, 281–284
- Marliss, E. B., Wollheim, C. B., Blondel, B., Orci, L., Lambert, A. E., Stauffacher, W., Like, A. A. & Renold, A. E. (1973) *Eur. J. Clin. Invest.* **3**, 16–26
- Pilkis, S. J., Riou, J. P. & Claus, T. H. (1976) *J. Biol. Chem.* **251**, 7841–7852
- Ruderman, N. B., Toews, C. J., Lowy, C., Vreeland, I. & Shafir, E. A. (1970) *Am. J. Physiol.* **219**, 51–57
- Senior, A. E., & Sherratt, H. S. A. (1969) *J. Pharm. Pharmacol.* **21**, 85–92
- Sherratt, H. S. A. & Osmundsen, H. (1976) *Biochem. Pharmacol.* **25**, 743–750
- Snell, K. & Walker, D. G. (1973) *Enzyme* **15**, 40–81
- Wapnir, R. A., Tildon, J. T. & Cornblath, M. (1973) *Am. J. Physiol.* **224**, 596–599
- Wilcoxon, F. (1947) *Biometrics* **3**, 119–122
- Williamson, J. R., Rostand, S. G. & Peterson, M. J. (1970) *J. Biol. Chem.* **245**, 3242–3251