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# Effects of hydrazine on liver and brown adipose tissue lipogenesis in 24 h-starved rats

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Hydrazines have been widely used industrially, despite toxic side effects which include the production of fatty livers (see e.g. Cooling et al 1979; Lamb & Banks 1979). Hydrazine poisoning results in raised hepatic triacylglycerol concentrations which may be the result of increased hepatic triacyglycerol synthesis (Lamb & Banks 1979), associated with increased phosphatidate phosphohydrolase activity (Cooling et al 1979). The present work demonstrates that, in addition, hydrazine increases the de novo synthesis of fatty acids in livers of 24 h-starved rats. Such increased hepatic lipogenesis could contribute to the production of fatty livers. In contrast to its stimulatory effects on hepatic lipogenesis, hydrazine did not increase lipogenesis in brown adipose tissue (BAT). The effects of hydrazine on hepatic and BAT lipogenesis in 24 h starved rats after glucose-feeding are also reported since glucose-feeding can depress the accumulation of citrate cycle intermediates which occurs in livers of hydrazine-treated animals (Ray et al 1970). We have previously found that glucose-feeding of 24 h-starved rats increases in-vivo rates of lipogenesis in BAT but not in liver (Sugden et al 1981, 1982).

## *Materials and methods*

*Materials.* Enzymes and coenzymes were from BCL Ltd, Lewes, East Sussex, U.K. Hydrazine sulphate was a gift from Dr D. Myles, Glaxo Group Research Ltd, Ware, Herts., U.K.

*Studies in-vivo.* Female Albino Wistar rats (150–180 g) were subjected to a 12 h light: 12 h dark cycle, the light period starting at 0800 h and were used after 24 h starvation in grid-bottomed cages. Water was freely available. Diabetes was induced by the intravenous administration of streptozocin (60 mg $kg^{-1}$ in 0·1 M sodium citrate buffer, pH 4·5) and confirmed by whole-blood glucose concentrations greater than 15 mM in the fed rats. Experiments were initiated on the third day after streptozocin injection.

Experiments were started between 0900 h and 0930 h. Fluids were given intragastrically via a plastic tube, with the rats under light ether anaesthesia. Hydrazine sulphate (20 mg/100 g) was administered as a suspension in either 1·5 ml water or 1·5 ml glucose solution (2 mmol/100 g), containing 0·5% methyl cellulose. Water or glucose were administered similarly. Methyl cellulose had no effect on any parameter measured. The rate of lipogenesis in-vivo was measured by the incorporation of $^3H$ from $^3H_2O$ into tissue fatty acid, as described by Stansbie et al (1976). This method measures lipogenesis from all precursors. $^3H_2O$ (5 mCi in 0·5 ml of 0·15 M NaCl) was injected 1 h after the intragastric load.

The rats were dissected 1 h later (i.e. 1 h after injection of $^3H_2O$ and 2 h after the gastric load) as described by Agius & Williamson (1980). An arterial blood sample was withdrawn at the time of killing for determination of blood metabolites by enzymic methods (see Sugden et al 1981).

*Statistical analysis.* Statistical significance of differences was assessed using Student's unpaired *t*-test. Results are given as means ± s.e.m. with the number of observations in parentheses.

## *Results*

Intragastric glucose-feeding of 24 h-starved, control or hydrazine-treated rats was without effect on hepatic lipogenesis but BAT lipogenesis was increased (Table 1). Glucose-feeding did not increase BAT lipogenesis if the rats were streptozocin-diabetic [water-fed, 18·5 ± 0·03 (5); glucose-fed, 20·3 ± 4·0 (10)]. Hydrazine-treatment slightly attenuated the increase in BAT lipogenesis observed after glucose-feeding (increases of 191% and 132% in BAT of control and hydrazine-treated rats respectively). Hydrazine increased hepatic fatty acid synthesis by 196% in water-fed 24 h starved rats and by 126% in glucose-fed 24 h-starved rats (Table 1). Hydrazine-treatment decreased fatty acid synthesis in BAT (by 33% and 47% in water-fed and glucose-fed rats respectively).

Increased hepatic lipogenesis after hydrazine-treatment was also observed if the 24 h-starved rats were streptozocin-diabetic (control (6), 2·49 ± 0·15; + hydrazine (3), 18·02 ± 2·21 μg atoms of $^3H$ incorporated into fatty acid $h^{-1}$ $g^{-1}$ wet wt liver; $P < 0·001$). The increase in hepatic lipogenesis observed was of a similar order of magnitude to that found after hydrazine treatment of non-diabetic starved rats. Hydrazine did not inhibit BAT lipogenesis if the rats were diabetic (control (5), 18·5 ± 3·0; + hydrazine (4), 16·7 ± 3·4 μg atoms $^3H$ incorporated into fatty acid $h^{-1}$ $g^{-1}$ wet wt; n.s.) The lack of effect of hydrazine presumably is related to the very low rates of lipogenesis observed in BAT of diabetic rats. This lack of effect shows that

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Table 1. Effects of hydrazine on in-vivo rates of lipogenesis in liver and brown adipose tissue and on blood metabolite concentrations in 24 h-starved rats.

| Control | Lipogenesis (μg atoms $^3H$ incorporated into fatty acid $h^{-1}$ $g^{-1}$ wet wt tissue) Liver | BAT | Blood metabolite concentrations (mM) Glucose | Lactate |
|---|---|---|---|---|
| Water-fed | 5·0 ± 1·0 (10) | 34·1 ± 4·0 (6) | 6·21 ± 0·11 (7) | 0·80 ± 0·13 (7) |
| Glucose-fed | 5·8 ± 0·6 (8) | 99·4 ± 9·9 (4)††† | 7·60 ± 0·60 (8) | 0·90 ± 0·10 (6) |
| Hydrazine-treated | | | | |
| Water-fed | 14·8 ± 2·8 (4)*** | 22·9 ± 3·0 (4)* | 3·67 ± 0·17 (4)*** | 2·33 ± 0·64 (4)* |
| Glucose-fed | 13·1 ± 1·24 (6)*** | 53·1 ± 5·6 (6)††** | 6·30 ± 1·00 (6)††† | 1·95 ± 0·20 (6)*** |

For experimental details see the text. Statistical significance of differences between control and hydrazine-treated groups are given by: *$P$ <0·05; **$P$ <0·01; ***$P$ <0·001. Statistical significance of differences between the water-fed and glucose-fed animals in either group are given by: ††$P$ <0·01; †††$P$ <0·001.

insulin secretion in the streptozocin-diabetic rats after glucose-feeding is insufficient to increase rates of BAT lipogenesis, although this tissue is very sensitive to insulin (see Agius & Williamson 1980).

Hydrazine-treatment of water-fed, 24 h-starved rats decreased blood glucose concentrations by 40·9% (Table 1). Blood glucose concentrations were not affected by hydrazine-treatment of 24 h-starved rats if the rats were fed glucose (Table 1). In the former, but not the latter, group of animals, the maintenance of blood glucose concentrations is dependent on gluconeogenesis. Thus the hypoglycaemic action of the drug is primarily a consequence of inhibition of gluconeogenesis (see also Fortney et al 1967).

*Discussion*

Our work demonstrates that administration of hydrazine to 24 h-starved rats increases the rate of hepatic de novo fatty acid synthesis. The effect of hydrazine is not insulin-mediated since increases are also observed if the rats are diabetic. In support of this, administration of hydrazine in-vivo does not increase, or may decrease, blood insulin concentrations, (Cooling et al 1979). The mechanism for the stimulatory effect of hydrazine on hepatic lipogenesis is not known. Blood concentrations of lactate (a major precursor for both hepatic lipogenesis and gluconeogenesis in the rat) are markedly (191%) increased by hydrazine treatment (Table 1) and it is possible that hydrazine increases hepatic lipogenesis because of this.

The oral administration of glucose together with hydrazine to 24 h-starved rats did not inhibit the action of hydrazine to promote hepatic lipogenesis (Table 1) since glucose administered orally at the same time as hydrazine suppresses the accumulation of citrate which occurs after hydrazine-treatment (Ray et al 1970). It is therefore unlikely that increased citrate concentrations contribute to the drug's effect on lipogenesis. The decreased blood ketone-body concentrations (control (7) 0·63 ± 0·01 mM; + glucose (6), 0·10 ± 0·03 mM, $P$ <0·001) and increased blood glucose concentrations and BAT lipogenesis (Table 1) observed in glucose-fed hydrazine-treated rats, suggest that glucose absorption and insulin secretion in response to the glucose-load occurred.

Hydrazine did not increase BAT lipogenesis; instead a decrease was observed (Table 1). It is unlikely that this inhibitory action is secondary to decreases in blood concentrations of glucose (although glucose is a major lipogenic precursor for BAT, Sugden et al 1981) since inhibition was still observed after glucose-feeding. Lipogenesis in white adipose tissue is inhibited by quinolinate and 3-mercaptopicolinate, which, like hydrazine, inhibit phosphoenol pyruvate carboxy kinase (MacDonald & Grewe 1981). In this tissue, inhibited lipogenesis is secondary to inhibition of glyceroneogenesis from lactate and pyruvate (MacDonald & Grewe 1981) and the inhibition of BAT lipogenesis in-vivo by hydrazine may also be a consequence of this.

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