Evidence that noradrenaline increases pyruvate dehydrogenase activity and decreases acetyl-CoA carboxylase activity in rat interscapular brown adipose tissue *in vivo*

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The rate of fatty acid synthesis in interscapular brown adipose tissue of female cold-adapted rats, as measured by the incorporation of ^3H from $^3\text{H}_2\text{O}$ into tissue lipid, was decreased by about 70% after injection of noradrenaline. There was a similar decrease in the activity of acetyl-CoA carboxylase. In contrast, the proportion of pyruvate dehydrogenase in its active non-phosphorylated form was greatly increased after injection of noradrenaline. This finding suggests that the oxidation of glucose may be important in noradrenaline-induced thermogenesis in rat brown adipose tissue.

Brown adipose tissue is an important site of heat generation by non-shivering mechanisms in many animals, and most studies in the tissue have been concerned with this topic (for reviews see Nicholls, 1979; Cannon & Johansson, 1980; Nicholls & Locke, 1984; Rothwell & Stock, 1984). It has been rather generally assumed that the major substrate oxidized by this tissue is fatty acids (Nicholls & Locke, 1984; Rothwell & Stock, 1984). Although this seems very likely in many situations, especially in starved animals, in the rat fed on a typical high-carbohydrate laboratory diet glucose may also be a major substrate. This possibility was first suggested by the finding that brown adipose tissue from the interscapular region of the rat contains a high activity of pyruvate dehydrogenase (McCormack & Denton, 1977). Indeed, the amount of enzyme is probably greater when expressed in terms of tissue protein than in any other rat tissue. Subsequently, it has been shown that the tissue also contains high activities of the three key kinases in glycolysis, namely hexokinase (Cooney & Newsholme, 1982; Young *et al.*, 1984), phosphofructokinase (Cooney & Newsholme, 1984; Young *et al.*, 1984; E. Sale & R. M. Denton, unpublished work) and pyruvate kinase (Sale, 1985) and also has the ability to take up 2-deoxyglucose rapidly *in vivo* (Young *et al.*, 1984). Thus the tissue has the enzymic capacity to be able to convert glucose into acetyl-CoA at a rate sufficient to account for much of the oxygen consumption by the stimulated tissue (McCormack, 1982; Cooney & Newsholme, 1984). It is also well established that the tissue is capable of high rates of fatty acid synthesis (McCormack & Denton, 1977; Trayhurn, 1979; Agius & Williamson, 1981). This process is stimulated *in vivo* under conditions of high circulating concentrations of insulin (McCormack & Denton, 1977; Agius & Williamson, 1981), probably because the hormone is able to elicit the parallel activation of glucose transport, pyruvate dehydrogenase and acetyl-CoA carboxylase (McCormack & Denton, 1977).

In the present study, we have investigated changes in the rate of fatty acid synthesis, and in the degree of activation of pyruvate dehydrogenase and acetyl-CoA carboxylase, which accompany the increase in heat production by interscapular brown adipose tissue of cold-adapted rats injected intraperitoneally with noradrenaline. The results are consistent with the hormone stimulating glucose oxidation while inhibiting fatty acid synthesis.

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Methods

Female albino Wistar rats were transferred to a room at 5–8°C within 1 week of weaning and were cold-adapted for a period of 3–4 weeks. Rats were allowed free access to a stock laboratory diet (modified 41B; Oxoid, Basingstoke, Hants., U.K.) and weighed 180–220 g at use. Animals were removed from the cold-room into the laboratory (20–22°C) about 1 h before each experiment and then anaesthetized by interperitoneal injection of Sagatal (250 μl /rat, equivalent to 15 mg of sodium

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pentobarbitone). The rats remained anaesthetized throughout the experiments. Details of further intraperitoneal injections of $^3\text{H}_2\text{O}$, glucose and hormones are as given in Figure and Table legends.

Details of the removal and extraction of brown adipose tissue, and the methods employed for the measurement of rates of fatty acid synthesis, pyruvate dehydrogenase and acetyl-CoA carboxylase were as described by McCormack & Denton (1977) and Stansbie *et al.* (1976), unless otherwise stated. One unit of enzyme activity is defined as the amount that will catalyse the formation of $1\text{ }\mu\text{mol}$ of product/min at 30°C . Changes in temperature of interscapular brown adipose tissue were monitored with a small thermocouple (RS Digital Thermometer type K; RS Components Ltd., Corby, Northants., U.K.). The thermocouple was inserted between the brown adipose tissue and underlying muscle of the back and held in place with two clips. In some experiments a second thermocouple was used to monitor changes in rectal temperature.

Sources of materials were as given in Stansbie *et al.* (1976), and noradrenaline and propranolol were from Sigma Chemical Co., Poole, Dorset, U.K. Fresh stock solutions (1 mg/ml) in distilled water were prepared within 60 min of use.

Results

It is well established that the injection of rats with noradrenaline results in a rapid rise in the temperature of the interscapular brown adipose tissue (Flaim *et al.*, 1977; Doi & Kuroshima, 1982; Foster, 1984). Fig. 1 shows a typical time course of the changes in temperature that followed a single intraperitoneal injection of noradrenaline under the conditions used in the present study. Intraperitoneal injections of a glucose solution were given routinely in our studies to ensure high circulating concentrations of glucose and insulin. The mean increase in temperature after injection of noradrenaline as in Fig. 1 was $1.0 \pm 0.21^\circ\text{C}$ after 10 min and $1.9 \pm 0.31^\circ\text{C}$ after 20 min. The maximum increase of $3.1 \pm 0.6^\circ\text{C}$ was reached after 30–45 min (results are given as means $\pm$ S.E.M. for independent observations on five rats). Over this period the core temperature of the rats measured intrarectally increased much more slowly; increases after 45 min were in the range 0.5 – 1.0°C . The temperature of interscapular brown adipose tissue and the core temperature of animals injected with water alone or with the β -blocker propranolol declined slightly by up to 1.0°C over the same period (Fig. 1). A similar small decline was evident in animals injected with both noradrenaline and propranolol, indicating that little or no effect of noradrenaline was evident in the presence of a β -blocker (Foster,

Fig. 1. Changes in temperature of rat interscapular brown adipose tissue after injection of noradrenaline in the presence or absence of propranolol

A small thermocouple was placed under the interscapular brown adipose tissue of anaesthetized rats. Temperature recordings were started after 10–15 min and the four rats were injected with glucose (200 mg in 0.5 ml of water), followed 10 min later (at arrow) by (i) noradrenaline ($250\text{ }\mu\text{g}$ in 0.25 ml of water: $\circ$), (ii) water (0.25 ml : $\bullet$), (iii) propranolol ($300\text{ }\mu\text{g}$ in 0.3 ml of water: $\triangle$), or (iv) noradrenaline plus propranolol ($\blacktriangle$).

1984). We also found very similar changes in temperature in animals which were not pre-injected with glucose solution (results not shown).

Rates of fatty acid synthesis in brown adipose tissue were measured from the incorporation of $^3\text{H}_2\text{O}$ into tissue fatty acids over a 25 min period after injections of noradrenaline, water or propranolol. All animals were pre-injected with glucose solution (Table 1). The rate of fatty acid synthesis in the absence of either noradrenaline or propranolol was similar to that measured in our earlier studies in comparable conditions in conscious animals over a 1 h period (McCormack & Denton, 1977). Noradrenaline treatment resulted in a marked decrease in the rate of fatty acid synthesis, whereas after injection of propranolol there was a small, statistically insignificant, increase in rate, perhaps because propranolol was inhibiting the actions of endogenous catecholamines (Table 1). Previously, fatty acid synthesis in the brown adipose tissue of warm-adapted female rats has been reported to be both increased (Shimazu & Takahishi, 1980) and decreased (Agius & Williamson, 1980) after injection of the rats with noradrenaline.

Changes in the activities of pyruvate dehydrogenase and acetyl-CoA carboxylase measured 15 min after injection of noradrenaline or propranolol are given in Table 1.

For pyruvate dehydrogenase, activity was measured either in the fresh extract (initial activity) or after treatment of the extract with pyruvate dehydrogenase phosphate phosphatase in the presence of 25 mM-MgCl₂ and 1 mM-CaCl₂ for 15 min at 30°C. Under these latter conditions, the phosphorylated (inactive) form of the pyruvate dehydrogenase complex was converted into the active non-phosphorylated form, and thus 'total' activity of the enzyme can be determined (McCormack & Denton, 1977). In animals preinjected with glucose, it was evident that injection of noradrenaline caused a marked increase in 'initial' activity, whereas propranolol resulted in a small decrease. There were no statistically significant changes in 'total' activity. In all there was a near 10-fold difference in the proportion of pyruvate dehydrogenase in its active form between animals treated with noradrenaline (28% in active form) and those treated with propranolol (3% in active form). A significant difference was also evident in animals not pre-treated with glucose (Table 1).

In marked contrast with the substantial activation of pyruvate dehydrogenase, the activity of acetyl-CoA carboxylase in interscapular brown adipose tissue was markedly decreased after injection of the rats with noradrenaline (Table 1). The decrease was evident in the activity measured both in fresh extracts and after incubation of extracts with citrate. This treatment results in activation of acetyl-CoA carboxylase, probably

because of the promotion of polymerization of the enzyme (Halestrap & Denton, 1974; Brownsey *et al.*, 1979). The conditions used in the present study were incubations with 30 mM-potassium citrate for 30 min at 30°C; this gave maximum activation. No further enhancement in activity was found if the assay medium was supplemented with further citrate. Addition of citrate only to the assay medium resulted in increases in activity, but they were appreciably less than those found as a result of the pretreatment of extracts with citrate. Little or no change in activities was apparent if the extracts were preincubated in the absence of citrate unless MgCl₂ and CaCl₂ were added. Under these conditions, the activity of acetyl-CoA carboxylase in extracts prepared from the tissue of noradrenaline-treated animals increased, so that differences in activity compared with control extracts were no longer apparent (results not shown). All the above measurements were made with extracts prepared with phosphate-based medium (0.1 M-potassium phosphate, pH 7.0, containing 2 mM-EDTA, 1 mM-dithiothreitol and 50 µl of rat serum/ml). Some extractions were also carried out in a sucrose-based medium containing albumin (250 mM-sucrose, 10 mM-Tris, 20 mg of albumin/mg, 7.5 mM-reduced glutathione, 2 mM-EDTA, pH 7.4). Very similar changes in activity both in fresh extracts and after treatment with citrate were found (results not shown).

Discussion

Adrenaline and other catecholamines, apparently acting through β -receptors, inhibit fatty acid

Table 1. *Effects of noradrenaline and propranolol on rates of fatty acid synthesis and activities of pyruvate dehydrogenase and acetyl-CoA carboxylase in rat interscapular brown adipose tissue*

For measurements of fatty acid synthesis, rats were anaesthetized and then injected with a glucose solution, followed after 1 min by noradrenaline (250 µg) or propranolol (300 µg), as given in the legend to Fig. 1, and after a further 0.5 min by 5 mCi of ³H₂O in 0.5 ml of water. Tissue was removed 25 min after injection of ³H₂O, frozen in liquid N₂, and blood samples were taken for determination of specific radioactivity of ³H₂O [see Stansbie *et al.* (1971) for further details]. For measurement of pyruvate dehydrogenase and acetyl-CoA carboxylase activities, anaesthetized rats were injected with glucose, noradrenaline and/or propranolol as given in the legend to Fig. 1. Tissue was rapidly removed and frozen in liquid N₂, 15 min after completion of the injections. Results are given as means $\pm$ S.E.M. for the numbers of observations given in parentheses: **P* < 0.01 versus appropriate value for animals treated with propranolol; ††*P* < 0.01, †*P* < 0.05, versus value for animals injected with glucose only.

Treatment	Rate of fatty acid synthesis (µg-atoms of 'H' incorporated/h per g wet wt.)	Activities (units/g wet wt.) of:			
		Pyruvate dehydrogenase		Acetyl-CoA carboxylase	
		Initial	Total	Initial	After citrate treatment
Glucose	71 $\pm$ 12.0 (4)	0.6 $\pm$ 0.18 (8)	6.5 $\pm$ 0.83 (8)	0.21 $\pm$ 0.052 (8)	0.97 $\pm$ 0.17 (8)
Glucose and propranolol	84 $\pm$ 14.1 (8)	0.2 $\pm$ 0.05† (10)	7.9 $\pm$ 0.52 (10)	0.26 $\pm$ 0.024 (6)	1.02 $\pm$ 0.13 (6)
Glucose and noradrenaline	23 $\pm$ 3.6*†† (12)	2.5 $\pm$ 0.42*†† (13)	8.8 $\pm$ 0.77 (13)	0.08 $\pm$ 0.007*†† (9)	0.50 $\pm$ 0.05*†† (9)
Propranolol	—	0.16 $\pm$ 0.07 (6)	6.7 $\pm$ 1.0 (6)	0.30 $\pm$ 0.012 (6)	1.29 $\pm$ 0.14 (6)
Noradrenaline	—	1.22 $\pm$ 0.27* (6)	10.7 $\pm$ 1.5 (6)	0.10 $\pm$ 0.024* (6)	0.47 $\pm$ 0.09* (6)

synthesis in white adipose tissue (Denton & Halperin, 1968; Saggerson & Greenbaum, 1970). There is much evidence that the mechanism involves activation of cyclic AMP-dependent protein kinase, and hence increased phosphorylation of acetyl-CoA carboxylase, which results in the inactivation of this enzyme (Lee & Kim, 1978; Brownsey *et al.*, 1979; Brownsey & Denton, 1982; Witters *et al.*, 1983; Holland *et al.*, 1985). This inactivation is observed in initial extracts of tissue previously exposed to adrenaline and after citrate treatment of the extracts, but is lost after treatment of extracts with Mg^{2+} and Ca^{2+} ; this treatment is known to result in partial dephosphorylation of the enzyme (Halestrap & Denton, 1974; Brownsey *et al.*, 1979). The results of the present study indicate that noradrenaline causes inhibition of fatty acid synthesis in brown adipose tissue and that this is probably largely brought about by the inactivation of acetyl-CoA carboxylase. Since the changes in kinetic properties are similar to those found with the enzyme in white adipose tissue after exposure to adrenaline, it seems likely that the inhibition is the result of increased phosphorylation by cyclic AMP-dependent protein kinase, but further studies are required to substantiate this.

The marked increase in pyruvate dehydrogenase activity in the brown adipose tissue of rats treated with noradrenaline is strong evidence that increased oxidation of glucose may occur when thermogenesis in this tissue is stimulated. The proportion of acetyl-CoA derived from pyruvate which is converted into fatty acids under these conditions is likely to be very small. In animals injected with noradrenaline, the initial activity of acetyl-CoA carboxylase is only about 3% of that of pyruvate dehydrogenase (Table 1).

Catecholamines increase pyruvate dehydrogenase activity in heart, acting through β -receptors (Hiraoka *et al.*, 1980; McCormack & Denton, 1981), and in liver, acting through α -receptors (Assimakopoulos-Jeannet *et al.*, 1983; Oviyasu & Whitton, 1984). In contrast, exposure of white adipose tissue or fat-cells to adrenaline leads, at least in the presence of insulin, to inhibition of pyruvate dehydrogenase activity (Coore *et al.*, 1971; Martin *et al.*, 1972). The stimulatory effects of catecholamines on the activity of pyruvate dehydrogenase in both heart and liver probably result from the activation of pyruvate dehydrogenase phosphate phosphatase by an increase in the intramitochondrial concentration of Ca^{2+} (McCormack & Denton, 1984; McCormack, 1985). Present studies are concerned with investigating the possible role of Ca^{2+} in the activation of brown-adipose-tissue pyruvate dehydrogenase by noradrenaline. An increase in the intramitochondrial concentration of Ca^{2+} concentration would

also be expected to result in the activation of NAD-isocitrate dehydrogenase and oxoglutarate dehydrogenase (McCormack & Denton, 1980; Denton & McCormack, 1980), and hence the observed increased rate through the citrate cycle which occurs when brown adipose tissue is stimulated by noradrenaline. It should be noted that the activities of pyruvate dehydrogenase, NAD-isocitrate dehydrogenase and oxoglutarate dehydrogenase may also be enhanced by increases in the concentration ratios of ADP/ATP and/or NAD^+ /NADH in the mitochondria (Denton & McCormack, 1980). However, there is no indication of any such increases occurring in rat brown adipose tissue exposed to noradrenaline *in vivo*, on the basis of whole-tissue concentrations of ATP, ADP and AMP (Ma & Foster, 1984) and tissue NAD(P)H fluorescence (Seydoux *et al.*, 1984). Indeed, this last parameter increases markedly (Seydoux *et al.*, 1984).

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