The Interaction of Catecholamines and Adrenal Corticosteroids in the Induction of Phosphopyruvate Carboxylase in Rat Liver and Adipose Tissue

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Catecholamines induced an increase in the activity of rat adipose tissue and liver phosphopyruvate carboxylases that was maintained for 48 h. The response of adipose tissue phosphopyruvate carboxylase was blocked by actinomycin D, corticosteroids and propranolol, whereas corticosteroids and propranolol did not affect the liver enzyme. Cortisol phosphate, like actinomycin D, interfered only with the initiation of the increase in enzyme activity caused by noradrenaline, but not with the process of enzyme accumulation. In contrast, cycloheximide was effective in blocking enzyme induction throughout the course of the catecholamine effect. Adrenocorticotrophic hormone caused a short-term induction of adipose tissue phosphopyruvate carboxylase, which could be blocked by propranolol. Hepatic phosphopyruvate carboxylase, but not the adipose tissue enzyme, was induced by dibutyryl adenosine 3':5'-cyclic monophosphate and by glucagon. Both nicotinic acid and nicotinamide decreased the normal induction of adipose tissue phosphopyruvate carboxylase caused by starvation, but only nicotinamide increased the activity of the liver enzyme.

Phosphopyruvate carboxylase (EC 4.1.1.32) plays a key role in gluconeogenesis in the liver (Scrutton & Utter, 1968) and in glyceroneogenesis in adipose tissue (Hanson *et al.*, 1970; Reshef & Shapiro, 1970) and its activity is subject to changes induced by a variety of treatments (Reshef *et al.*, 1969*a,b*, 1970; Foster *et al.*, 1966). In adipose tissue this enzyme was induced by starvation, by alloxan diabetes and by the removal of the adrenals (Reshef *et al.*, 1969*a,b*, 1970). Adrenalectomy, combined with either starvation or alloxan diabetes, caused an additive increase in phosphopyruvate carboxylase activity, whereas treating adrenalectomized diabetic rats with either insulin or corticosteroids decreased the activity of the enzyme. If these hormones were given together, an additive decrease in phosphopyruvate carboxylase activity was observed. This additive and independent decrease of enzyme activity indicates diverse points of attack by insulin and corticosteroids, both of which maintain phosphopyruvate carboxylase at a low activity. It is not clear, however, whether this is the only mechanism acting on the adipose tissue enzyme, or if other mechanisms exist by which it may be induced even in the presence of either insulin or adrenal hormones. One indication of a possible alternative mechanism is our finding that although insulin and corticosteroids act independently and the starvation-mediated induction of adipose tissue phosphopyruvate carboxylase is additive to adrenal-

ectomy, the administration of corticosteroids abolished both of these effects (Reshef *et al.*, 1969*b*). This suggests that the starvation effect is caused by another factor in addition to insulin removal.

As rat adipose tissue and liver cytosol phosphopyruvate carboxylases are immunochemically identical proteins (Ballard & Hanson, 1969), it is possible that factors which are known to regulate this enzyme in rat liver cytosol also affect the adipose tissue enzyme. Catecholamines, for example, have been shown to induce foetal rat liver phosphopyruvate carboxylase (Yeung & Oliver, 1968) and 3':5'-cyclic AMP (adenosine 3':5'-cyclic monophosphate) increases the enzyme activities in adult rat liver (Wicks *et al.*, 1969). It therefore seemed reasonable to test the effect of these substances on the rat adipose tissue enzyme. The present report deals with the induction of adipose tissue and liver phosphopyruvate carboxylase by catecholamines and the relationship of this effect with corticosteroids.

Experimental

Animals

Male Wistar rats 6–7 weeks of age were used throughout the experiments. Adrenalectomized animals were maintained on 1% (w/v) saline and used

between the 5th. and 10th. post-operative days. Noradrenaline, adrenaline (free base), ACTH* and glucagon were administered subcutaneously, and cortisol phosphate, triamcinolone (9 α -fluoro-11 β , 16 α , 17 α , 21-tetrahydroxypregna-1,4-diene-3,20-dione 16 α , 21-diacetate), dibutyryl 3':5'-cyclic AMP (6-N, 2'-O-dibutyryl adenosine 3':5'-cyclic monophosphate), propranolol, actinomycin D, nicotinic acid and nicotinamide were injected intraperitoneally.

After catecholamine treatment, the animals appeared to be sick, but complete recovery was noted at about 10h after the treatment and mortality was less than 25%. There was no noticeable weight loss associated with this treatment and the blood glucose concentrations were unaffected. No abnormal manifestations were observed with other treatments. The animals were killed between 1000h and 1600h and no significant changes in enzyme activity were noted that were due to an inherent circadian rhythm (Wurtman, 1969) in the control animals killed at this time of the day.

* Abbreviation: ACTH, adrenocorticotrophic hormone.

Materials

Noradrenaline, dibutyryl 3':5'-cyclic AMP, cortisol phosphate, ACTH, cycloheximide, phosphopyruvate, IDP, malate dehydrogenase and NADH were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A. and actinomycin D (Lyovac Cosmogen) from Merck, Rahway, N.J., U.S.A. Triamcinolone was from Squibb Co., New York, N.Y., U.S.A., and propranolol hydrochloride from Ayerst, McKenna and Harrison, New York, N.Y., U.S.A. Glucagon was the generous gift of Dr. O. K. Behrens of Eli Lilly and Co., Indianapolis, Ind., U.S.A. NaH¹⁴CO₃ was purchased from The Radiochemical Centre, Amersham, Bucks., U.K.

Phosphopyruvate carboxylase (EC 4.1.1.32) assay

Epididymal adipose tissue was homogenized in 4 volumes and liver in 9 volumes of buffered 0.25M sucrose (Henning *et al.*, 1966). Supernatant fractions prepared by centrifugation at 105000g for 30min were used for assay of phosphopyruvate carboxylase.

Table 1. *Effect of various compounds on the induction of phosphopyruvate carboxylase by catecholamines*

Rats were injected subcutaneously with adrenaline or noradrenaline (0.4mg/100g body wt.). Actinomycin D (50 μ g/100g body wt.), cycloheximide (0.1mg/100g body wt.) and triamcinolone (5mg/100g body wt.) were injected intraperitoneally at the times indicated after noradrenaline injection. Results are the means $\pm$ S.E.M. for the number of animals indicated in parentheses.

Treatment	Duration of experiment (h)	Phosphopyruvate carboxylase activity		
		Adipose tissue		Liver
		(munits/g of tissue)	(munits/mg of protein)	(units/g of tissue)
None (75)	—	43 $\pm$ 1.6	4.6 $\pm$ 0.36	2.1 $\pm$ 0.09
Adrenaline (6)	5	131 $\pm$ 14.7		3.7 $\pm$ 0.25
Adrenaline (3)	14	281 $\pm$ 24.0		6.0 $\pm$ 1.00
Noradrenaline (42)	5	123 $\pm$ 5.7	10.1 $\pm$ 0.50	4.4 $\pm$ 0.21
Noradrenaline + actinomycin D at 0h (3)	5	59 $\pm$ 5.5		2.3 $\pm$ 0.3
Noradrenaline	7.5	121 $\pm$ 6.7	10.7 $\pm$ 0.85	
Noradrenaline + cycloheximide at 2h (3)	7.5	56 $\pm$ 6.1	4.9 $\pm$ 0.75	
Noradrenaline + triamcinolone at 2h (8)	7.5	80 $\pm$ 8.3	6.9 $\pm$ 0.55	
Noradrenaline (7)	16	141 $\pm$ 13.0	11.1 $\pm$ 1.9	
Noradrenaline + actinomycin D at 5h (3)	16	117 $\pm$ 19.1	8.3 $\pm$ 0.56	
Noradrenaline (11)	24	185 $\pm$ 22.7	21.2 $\pm$ 2.9	4.8 $\pm$ 0.29
Noradrenaline + actinomycin D at 7.5h (4)	24	79 $\pm$ 5.3		
Noradrenaline + cycloheximide at 14h (5)	24	51 $\pm$ 7.8	4.6 $\pm$ 0.53	2.3 $\pm$ 0.26
Noradrenaline + triamcinolone at 14h (3)	24	39 $\pm$ 4.1	4.1 $\pm$ 0.56	4.4 $\pm$ 0.09

Table 2. *Effect of actinomycin D on cortisol-induced alteration in phosphopyruvate carboxylase activity*

Rats were injected with noradrenaline (0.4mg/100g body wt.) and killed either 5h or 7.5h later. Actinomycin D (50 µg/100g body wt.) was injected 2h after noradrenaline. Cortisol (5mg/100mg body wt.) was given either together with the noradrenaline or 2h later as indicated in the Table. Results are the means ± s.e.m. for the number of animals indicated in parentheses.

Treatment	Activity of adipose tissue phosphopyruvate carboxylase	
	(munits/g of tissue)	(munits/mg of protein)
5h Experiment		
Noradrenaline (11)	130 ± 13	10.6 ± 0.9
Cortisol at 0h (6)	78 ± 17	6.0 ± 0.7
Cortisol at 2h (6)	139 ± 11	13.7 ± 1.9
Actinomycin D (11)	127 ± 9	11.2 ± 1.1
Cortisol at 0h+actinomycin D (6)	91 ± 11	6.3 ± 0.7
Cortisol at 2h+actinomycin D (3)	129 ± 13	12.9 ± 2.2
7.5h Experiment		
Noradrenaline (9)	138 ± 17	10.5 ± 1.2
Cortisol at 0h	63 ± 9	4.9 ± 1.2
Cortisol at 2h (5)	109 ± 12	8.7 ± 0.8
Actinomycin D (12)	128 ± 15	10.1 ± 0.9
Cortisol at 0h+actinomycin D (6)	74 ± 7	6.6 ± 0.8
Cortisol at 2h+actinomycin D (6)	107 ± 6	8.0 ± 1.0

The assay was a minor modification (Ballard & Hanson, 1967) of the method of Chang & Lane (1966), which involves the fixation of ^{14}C -labelled NaHCO_3 in the presence of phosphopyruvate, IDP, MnCl_2 , GSH, NADH, malate dehydrogenase and tissue extract. One unit of activity is defined as the amount of tissue extract that will catalyse the fixation of $1\text{ }\mu\text{mol}$ of $\text{NaH}^{14}\text{CO}_3/\text{min}$ at 37°C . The protein concentration was determined by a minor modification of the biuret method (Rowsell, 1962).

Antibody titration of enzyme

Antisera against phosphopyruvate carboxylase was prepared by injecting rabbits with 2–4mg of purified enzyme mixed with equal volumes of Freund's adjuvant. The γ -globulin fraction was isolated by precipitation with 50% (w/v) $(\text{NH}_4)_2\text{SO}_4$. This antibody demonstrated a similar specificity for cytosol phosphopyruvate carboxylase from both rat liver and rat adipose tissue (Ballard & Hanson, 1969). A detailed description of the enzyme purification, antibody isolation and specificity has been published (Ballard & Hanson, 1969).

Titration of the hepatic and adipose tissue phosphopyruvate carboxylase was done by reacting a

constant amount of tissue supernatant with increasing amounts of antibody in a constant volume. The reaction mixture was incubated for 15 min at 37°C , was left for 60 min at 5°C , and was then centrifuged at 3000g and the activity of the enzyme in the supernatant was assayed. The number of μl of antibody that caused a 50% loss of activity was expressed as the ab_{50} .

Results

Effect of catecholamines on phosphopyruvate carboxylase in rat liver and adipose tissue

After a single injection of either noradrenaline or adrenaline, phosphopyruvate carboxylase activities of liver and adipose tissue were increased by 2- to 3-fold (Table 1). In both of these organs the increased activity of the enzyme was blocked by a relatively small dose of actinomycin D (50 µg/100g body wt.), if given simultaneously with the hormone. However, actinomycin D was not effective if injected 2h later, in which case the enzyme activity increased for up to 7.5h at the same rate as observed in tissues from animals that were not treated with this drug (Table 2). Cycloheximide, however, when injected

Table 3. *Antibody titration of phosphopyruvate carboxylase in tissue extracts*

Adipose tissue and liver cytosol were obtained from two non-treated rats or from three rats injected subcutaneously with adrenaline (0.4mg/100g body wt.) and killed 5 h later. A cytosol (100000g, 1 h) fraction from each of the tissues was incubated with specific phosphopyruvate carboxylase antibody for 15min at 37°C and then left for 60min at 5°C. After the precipitation of the antibody-antigen complex, the activity of phosphopyruvate carboxylase in the supernatant was assayed. The number of μ l of antibody required for 50% decrease in enzyme activity is expressed as the ab_{50} .

Tissue	Hormonal treatment	...	ab_{50}	
			None	Adrenaline
Adipose tissue			2.5	22
Liver			75	160

2h after noradrenaline (Table 1) blocked the hormone-induced rise in phosphopyruvate carboxylase activity. We may therefore assume that the accumulation of enzyme, which occurs even during the period after actinomycin D injection, is dependent upon protein synthesis *de novo*.

By using specific antibodies against phosphopyruvate carboxylase, we were able to demonstrate that the induction of both the adipose tissue and liver enzyme by catecholamines was due to an increase in the amount of enzyme protein. Table 3 shows that the amount of antibody needed to decrease the activity of phosphopyruvate carboxylase by 50% (ab_{50}) increased from 2.5 and 75 μ l for adipose tissue and liver enzyme respectively to 22 and 160 μ l for adipose tissue and liver of animals given noradrenaline for 5h. There was no altered or modified reaction of antibody and enzyme caused by noradrenaline because, over a wide range of enzyme activities, 1 μ l of antibody always inhibited the same amount of enzyme activity (1.2munits) by 50%.

Time-course of the noradrenaline-mediated induction of phosphopyruvate carboxylase

Liver phosphopyruvate carboxylase was induced by corticosteroids (Foster *et al.*, 1966; Reshef *et al.*, 1969b) and mannoheptulose (Shrago *et al.*, 1963), but the response was rapid and transient. Unlike these inducers, both noradrenaline (Fig. 1) and adrenaline (Table 2) caused a longer-lasting increase in phosphopyruvate carboxylase activity. There is (Fig. 1) an apparent lag period of 2h after which the activity of phosphopyruvate carboxylase increased rapidly and reached a peak between 5 and 10h; a high enzyme activity was still evident 48h after injection of the hormone (Fig. 1).

In adipose tissue the noradrenaline-mediated increase in phosphopyruvate carboxylase activity consisted of two sequential phases, with the second phase starting approx. 7.5h after the administration of the

Fig. 1. *Time-course of the noradrenaline-mediated response of phosphopyruvate carboxylase activity in adipose tissue and liver*

Animals were injected with noradrenaline (0.4mg/100g body wt.) at zero time. At the times indicated groups of animals were killed and enzyme activities in liver (●) and adipose tissue (○) were assayed. Each point represents means from 3–15 animals and the bars represent S.E.M.

hormone (Fig. 1). The resolution of this effect into two phases was clearer when the animals were injected with actinomycin D at various times after enzyme induction. Actinomycin D completely abolished the noradrenaline effect when given together with the hormone, whereas when given 2h later, actinomycin D did not prevent the initial rise in enzyme activity from continuing up to about 7.5h (Fig. 2a). However, actinomycin D given at any time between 2 and 7.5h after noradrenaline administration completely abolished the second phase of enzyme induction (i.e., the increase in enzyme activity taking place after 7–10h) (Fig. 2a). These findings indicate that the process initiated by noradrenaline was completed in 2h and that this initial stimulus apparently

Fig. 2. Effect of actinomycin D and triamcinolone on the induction of adipose tissue phosphopyruvate carboxylase by noradrenaline

Groups of animals (3–18 animals/group) were injected with noradrenaline at zero time (●). (a) Actinomycin D (50 µg/100g body wt.) was injected together with noradrenaline (□—□), 2h later (□—□) or 7h later (Δ). Groups of animals (3–18 animals/group) were killed and enzyme activity was measured. The bars represent s.e.m. (b) Triamcinolone (5mg/100g body wt.) was injected 2h or 14h (Δ) after noradrenaline (●). Groups of animals (15–42 animals/group) were killed at various times after the injection of noradrenaline and enzyme activity was measured. The bars represent s.e.m.

caused the release of a second, new stimulus resulting in a continuous long-term induction of phosphopyruvate carboxylase.

The mechanism of noradrenaline induction of liver phosphopyruvate carboxylase is not clear, as this hormone causes a long-term maintenance of an elevated enzyme activity rather than a continuous accumulation of the enzyme (Fig. 1). Owing to the relatively short-lasting effect of actinomycin D in liver (Reshef *et al.*, 1969b), the resolution of a possible second phase of enzyme induction was experi-

Fig. 3. Effect of cortisol on the induction of adipose tissue phosphopyruvate carboxylase by noradrenaline

Animals were injected with noradrenaline (●) at zero time. Cortisol (5mg/100g body wt.) was injected together with noradrenaline (Δ) or 2h later (□). At the times indicated groups of 5–10 rats were killed and enzyme activity was measured. The bars represent the s.e.m. for an average of seven animals.

mentally impossible. Nevertheless, even the long-term maintenance of an increased activity of liver phosphopyruvate carboxylase was due to a continuous synthesis of the enzyme, as giving cycloheximide at 14h after noradrenaline caused a decay of enzyme activity that was similar for both the liver and adipose tissue (Table 1).

Effect of corticosteroids

Cortisol prevented the noradrenaline-mediated increase of adipose tissue phosphopyruvate carboxylase activity. When cortisol was administered together with noradrenaline the activity of the enzyme did not increase during a period of up to 7.5h. Conversely, when cortisol was given 2h after noradrenaline the accumulation of phosphopyruvate carboxylase could not be prevented (Fig. 3). Cortisol may affect protein synthesis by a mechanism similar to actinomycin D or alternatively cortisol may induce the destruction of phosphopyruvate carboxylase. These alterations were tested by giving a combined treatment of cortisol and actinomycin D. Whether actinomycin D was given together with or after cortisol, it did not significantly alter the effect caused by cortisol (Table 2).

The use of a synthetic, long-acting glucocorticoid (triamcinolone) allowed us to test the effect of this

hormone on the later phase of the phosphopyruvate carboxylase response to noradrenaline treatment. The second phase of noradrenaline induction of the enzyme could be blocked by triamcinolone given either 2h or 14h after the injection of the hormone (Fig. 2b). However, the first phase of enzyme accumulation, although slightly lowered, was still substantial even in the presence of triamcinolone. Although these observations indicate that actinomycin D and triamcinolone affect the activity of phosphopyruvate carboxylase by a similar mechanism, the decrease in enzyme activity caused by triamcinolone was quantitatively larger and began before the effect caused by the administration of actinomycin D. However, hepatic phosphopyruvate carboxylase induction by noradrenaline was not decreased by triamcinolone (Table 1).

Effect of other hormones

As catecholamines are known to stimulate 3':5'-cyclic AMP formation (Exton & Park, 1968; Robinson *et al.*, 1967), which can in turn induce hepatic phosphopyruvate carboxylase (Wicks *et al.*, 1969), we tested the effect of glucagon, ACTH and dibutyryl 3':5'-cyclic AMP on the induction of this enzyme. In adipose tissue these hormones are lipolytic (Vaughn & Steinberg, 1963), an effect presumably mediated by 3':5'-cyclic AMP (Jefferson *et al.*, 1968; Butcher, 1970). Table 4 shows that liver phosphopyruvate carboxylase was induced to the same extent by glucagon and dibutyryl 3':5'-cyclic AMP. Unlike the slow course of induction by catecholamines, both glucagon and dibutyryl 3':5'-cyclic

AMP caused a rapid increase of enzyme activity, which was evident at 2h after treatment (Fig. 4). However, adipose-tissue phosphopyruvate carboxylase did not respond to glucagon or to dibutyryl 3':5'-cyclic AMP treatment (Table 4). ACTH did induce the adipose-tissue enzyme, but, unlike the action of catecholamines, the effect of ACTH disappeared by 24h (Table 4). The possibility that ACTH acted through the adrenals could be excluded, because in adipose tissue corticosteroids act as depressors of phosphopyruvate carboxylase activity (Reshef *et al.*, 1969b).

The response of adipose tissue phosphopyruvate carboxylase to both ACTH and noradrenaline was blocked when propranolol, a β -adrenergic blocking agent, was administered concomitantly with the hormones, which indicated the involvement of these receptors as mediators of the effects of noradrenaline and ACTH (Table 4). Unlike adipose tissue, the response of liver phosphopyruvate carboxylase to noradrenaline was not blocked by propranolol (Table 4). A similar situation was described for the induction of liver tyrosine aminotransferase by noradrenaline, where the effect was not blocked by propranolol (Black & Axelrod, 1970).

Effect of nicotinic acid and nicotinamide on the induction of phosphopyruvate carboxylase by starvation

Despite the failure of dibutyryl 3':5'-cyclic AMP to induce phosphopyruvate carboxylase in adipose tissue from intact rats, it is possible that the induction of phosphopyruvate carboxylase by starvation or

Table 4. *Effect of dibutyryl 3':5'-cyclic AMP, glucagon, ACTH and propranolol on phosphopyruvate carboxylase*

The dibutyryl 3':5'-cyclic AMP (6mg/100g body wt.) was injected intraperitoneally. Glucagon (0.5mg/100g body wt.), noradrenaline (0.4mg/100g body wt.) and ACTH (5 μ g/100g body wt.) were injected subcutaneously, whereas propranolol (0.2mg/100g body wt.) was injected intraperitoneally. Animals were killed 3h after treatment unless otherwise indicated in the Table. Results are the means $\pm$ S.E.M. for the number of animals indicated in parentheses.

Treatment	Phosphopyruvate carboxylase activity		
	Adipose tissue		Liver
	(munits/g of tissue)	(munits/mg of protein)	(units/g of tissue)
None (13)	44 $\pm$ 3	5.6 $\pm$ 0.8	2.1 $\pm$ 0.24
Dibutyryl 3':5'-cyclic AMP (9)	26 $\pm$ 5	2.9 $\pm$ 0.8	4.2 $\pm$ 0.25
Glucagon (5)	56 $\pm$ 11	6.5 $\pm$ 1.5	3.9 $\pm$ 0.37
ACTH (6)	123 $\pm$ 24	12.7 $\pm$ 2.0	
ACTH+propranolol (6)	68 $\pm$ 10	5.0 $\pm$ 1.1	
ACTH (24h) (4)	32 $\pm$ 6		
Noradrenaline (5h) (6)	123 $\pm$ 6	10.1 $\pm$ 0.6	4.5 $\pm$ 0.33
Noradrenaline+propranolol (5h) (4)	50 $\pm$ 8	5.2 $\pm$ 1.0	4.2 $\pm$ 0.28

adrenalectomy involves the participation of 3':5'-cyclic AMP. This was tested by treating animals with nicotinic acid, which was reported to cause a decrease of 3':5'-cyclic AMP concentrations in adipose tissue (Kupiecki & Marshall, 1968; Butcher *et al.*, 1968; Blecher *et al.*, 1968). Both nicotinic acid and nicotinamide (5 mmol/kg body wt.) decreased the

phosphopyruvate carboxylase activity in adipose tissue, whereas the liver enzyme activity was increased by treatment with nicotinamide (Table 5). The concentration of nicotinic acid and nicotinamide used in this study was optimum over a dose range of 1.0–10 mmol/kg body wt. After nicotinamide treatment, a rapid decay of adipose tissue enzyme activity occurred (half-life $t_{\frac{1}{2}}$ = 7.0h) (Table 6). This value is comparable with the decay time observed with insulin (Reshef *et al.*, 1969a). The effect of nicotinamide was similar in tissues from intact and adrenalectomized starved rats, indicating that it was not mediated by adrenocorticosteroids released in response to this treatment (Blake *et al.*, 1967).

Discussion

In studies of the regulation of rat adipose tissue phosphopyruvate carboxylase (Reshef *et al.*, 1969a,b), we observed that adrenal corticosteroids decreased the activity of this enzyme. However, in those studies the enzyme was continuously induced and maintained at an increased activity either by adrenalectomy or by starvation. Because this higher enzyme activity represented a balance between enzyme synthesis and degradation, it was difficult to interpret the underlying mechanism of the corticosteroid effect. The use of catecholamines provides a valuable tool to study the initial phases leading to the induction of phosphopyruvate carboxylase, as it is possible to follow the time-course of induction. We are therefore able to isolate the phase of phosphopyruvate carboxylase induction that is decreased by corticosteroids, and perhaps indicate a possible mechanism for this effect.

The response of phosphopyruvate carboxylase activity in adipose tissue to a single injection of noradrenaline can be divided into three phases:

1. A lag period of 2h, in which the increase in enzyme activity is minimal.
2. An initial, but short, period of rapid accumulation of enzyme activity

Fig. 4. Effect of dibutyryl 3':5'-cyclic AMP and glucagon on liver phosphopyruvate carboxylase

Animals were injected with noradrenaline (●), with glucagon (○) or with dibutyryl 3':5'-cyclic AMP (□). At the times indicated groups of five rats were killed and the enzyme activity was measured. The bars indicate S.E.M.

Table 5. Effect of the nutritional state of the animals on the response of phosphopyruvate carboxylase to nicotinamide or nicotinic acid

Nicotinamide or nicotinic acid (5 mmol/kg body wt.) was injected intraperitoneally into 24h-starved or fed rats. After 5h the animals were killed and the activity of phosphopyruvate carboxylase in the liver and adipose tissue was measured. Values (units/g of tissue) are expressed as the mean $\pm$ S.E.M. for six animals. 'With': experimental animals; 'without': control, untreated animals.

Tissue	Nutritional state	Nicotinamide		Nicotinic acid	
		With	Without	With	Without
Liver	Fed	5.2 $\pm$ 0.5	3.3 $\pm$ 0.4	3.01 $\pm$ 0.7	4.02 $\pm$ 0.4
	Starved	9.7 $\pm$ 1.1	7.4 $\pm$ 0.7	6.63 $\pm$ 0.5	6.73 $\pm$ 1.4
Adipose tissue	Fed	0.055 $\pm$ 0.01	0.083 $\pm$ 0.02	0.073 $\pm$ 0.002	0.053 $\pm$ 0.01
	Starved	0.11 $\pm$ 0.01	0.21 $\pm$ 0.03	0.100 $\pm$ 0.04	0.210 $\pm$ 0.03

Table 6. *Effect of nicotinamide on phosphopyruvate carboxylase in adipose tissue and liver from starved and adrenalectomized starved rats*

Animals were starved for 24h, then were injected with 5mmol/kg body wt. of nicotinamide and were killed at the time-intervals indicated; phosphopyruvate carboxylase was assayed in liver and adipose tissue. Values (units/g of tissue) are the means $\pm$ S.E.M. for six rats.

Duration of experiment (h)	Intact rats		Adrenalectomized rats	
	Adipose tissue	Liver	Adipose tissue	Liver
0	0.21 $\pm$ 0.03	7.4 $\pm$ 0.68	0.51 $\pm$ 0.07	7.9 $\pm$ 0.6
2	0.18 $\pm$ 0.02	8.0 $\pm$ 0.43	0.44 $\pm$ 0.04	9.1 $\pm$ 0.3
3	0.18 $\pm$ 0.02	8.2 $\pm$ 0.31	0.40 $\pm$ 0.04	11.2 $\pm$ 0.6
4	0.13 $\pm$ 0.01	9.8 $\pm$ 0.74	0.33 $\pm$ 0.05	11.4 $\pm$ 0.7
5	0.11 $\pm$ 0.01	9.7 $\pm$ 1.0	0.36 $\pm$ 0.07	9.7 $\pm$ 0.3
6	0.11 $\pm$ 0.01	10.3 $\pm$ 1.1	0.32 $\pm$ 0.03	10.2 $\pm$ 0.4
7	0.09 $\pm$ 0.004	9.5 $\pm$ 0.9	0.24 $\pm$ 0.05	7.0 $\pm$ 0.9
8	0.09 $\pm$ 0.01	8.6 $\pm$ 1.1	0.26 $\pm$ 0.06	6.8 $\pm$ 0.5

lasting 2–7h after noradrenaline injection. 3. A later period of continued elevation] in enzyme activity lasting for more than 24h. Unlike the first two phases, the last phase is prevented by the administration of actinomycin D, whether injected at 2h or at 7.5h after the noradrenaline.

The evidence for the mechanism of induction of phosphopyruvate carboxylase in adipose tissue suggests the following points. First, RNA synthesis is involved in the noradrenaline-mediated stimulation of phosphopyruvate carboxylase, as the increase in enzyme activity can be blocked by actinomycin D (Greengard, 1967; Csanyi *et al.*, 1968; Greengard *et al.*, 1963; Tata, 1970). Secondly, the primary effect triggered by noradrenaline is completed before 2h, with the message for the synthesis of phosphopyruvate carboxylase being stable for at least 3h. Thirdly, the fact that actinomycin D blocks phase 3, if given 2–7.5h after noradrenaline, indicates that this phase of the response could be due to a secondary event caused by the injection of catecholamine. It is possible, for example, that the decreased rate of insulin secretion known to occur after noradrenaline administration (Porter *et al.*, 1966; Milner & Hales, 1969) has a direct, but delayed, effect on phosphopyruvate carboxylase activity.

Corticosteroids are most effective in decreasing the effect of noradrenaline on phosphopyruvate carboxylase during the first 2h (phase 1) and not during the subsequent period of enhanced enzyme production (phase 2). This suggests that corticosteroids and actinomycin D share a common site of action during both phase 1 and 2, probably at the level of transcription. The effect of cortisol on phase 3 of the noradrenaline-mediated increase in phosphopyruvate carboxylase is less clear. Corticosteroids caused a faster rate of decay of adipose tissue phospho-

pyruvate carboxylase than when actinomycin D was injected (Reshef *et al.*, 1969a). A similar difference in the effect of corticosteroids and actinomycin D on the rate of enzyme decay during phase 3 of noradrenaline induction is shown in Fig. 2. It is possible, therefore, that corticosteroids act by two different mechanisms. One is similar to actinomycin D during phases 1 and 2 but the other is quite different from actinomycin D and occurs during the longer-term induction of phase 3. Alternatively, the corticosteroid effect could be due to a single mechanism operating on all three phases of the induction process, such as an enhanced destruction of the specific message for phosphopyruvate carboxylase. This would result in a faster decay rate of the enzyme than that caused by the administration of actinomycin D. This suggestion is supported by the fact that triamcinolone, given at 2h, slightly diminished the rapid increase in enzyme activity characteristic of phase 2. This contrasts with the virtual elimination of phase 2 caused by cycloheximide injection (Table 1). Triamcinolone may therefore cause a labilization of the specific message required for the continued (phase 3) synthesis of phosphopyruvate carboxylase after noradrenaline administration. Proof of this point would require a measurement (using immunochemical techniques) of the rate of enzyme synthesis during phase 2.

The induction of phosphopyruvate carboxylase by the various substances tested is organ-specific. It is noteworthy that the response of the tissue generally correlates with the pattern observed when changes in tissue 3':5'-cyclic AMP concentrations are measured (Exton & Park, 1968; Jefferson *et al.*, 1968). For example, glucagon is most effective in increasing hepatic 3':5'-cyclic AMP concentrations, whereas in adipose tissue both catecholamines and ACTH are more effective than glucagon. Exton &

Park (1968) showed that the increased 3':5'-cyclic AMP concentration caused by glucagon was instantaneous and required a continuous presence of this hormone. This implies that if a hormonal induction is mediated by 3':5'-cyclic AMP, the response to either hormone or to exogenously administered 3':5'-cyclic AMP should follow a similar time-course. The fact that the time-course of the liver phosphopyruvate carboxylase induction by glucagon was similar to that observed with dibutyryl 3':5'-cyclic AMP (Fig. 4) indicates that 3':5'-cyclic AMP is a possible modulator of the liver enzyme. However, the failure of propranolol, a β -adrenergic inhibitor, to block the response of the liver enzyme to noradrenaline and the different time-course of its action excludes an involvement of 3':5'-cyclic AMP as mediator of the noradrenaline effect. Conversely, in adipose tissue propranolol blocked the induction of phosphopyruvate carboxylase by noradrenaline. Hence, despite the failure of dibutyryl 3':5'-cyclic AMP to induce the adipose tissue enzyme, the involvement of tissue cyclic 3':5'-AMP as modulator of this enzyme is still possible. This is supported by the fact that both insulin (Reshef *et al.*, 1969a) and nicotinic acid (Table 5) decrease the activity of adipose tissue phosphopyruvate carboxylase. The alternative possibility that β -adrenergic receptors mediate an unrelated increase of both 3':5'-cyclic AMP and phosphopyruvate carboxylase activity should also be considered.

A number of studies have linked nicotinic acid and nicotinamide with the metabolism of adipose tissue (Hanson & Ziporin, 1967; Carlson, 1963; Pereira, 1967; Carlson & Oro, 1962). Nicotinic acid is a potent inhibitor of lipolysis *in vitro* and it decreases free fatty acid concentrations in rats (Carlson, 1963), dogs (Pereira, 1967) and man (Carlson & Oro, 1962). The decrease in plasma free fatty acid concentrations has been attributed to an effect of nicotinic acid on the noradrenaline-induced lipolysis of adipose tissue (Carlson, 1963). Carlson & Oro (1962) have reported that nicotinic acid caused an initial decrease in plasma free fatty acid concentrations followed by an increase to concentrations exceeding those in control animals. Pereira & Mears (1971) have shown that these bi-phasic effects are due to two separate and opposing effects on the rate of adipose tissue free fatty acid release. Corticosteroids, which increase in concentration after nicotinic acid administration, are not directly related to the nicotinic acid-mediated decrease in plasma free fatty acid concentrations or to the subsequent 'rebound' of free fatty acid concentration 2-3h later (Pereira, 1967). Because both nicotinic acid and nicotinamide lower the activity of phosphopyruvate carboxylase in adipose tissue from starved rats, it is possible that both of these compounds affect free fatty acid release by altering the rate of glyceroneogenesis. The time-course of the nicotinic

acid effect on free fatty acid rebound (2-3h) corresponds generally to the decrease in adipose tissue phosphopyruvate carboxylase activity noted in the present study. Also, corticosteroids are not required for the nicotinamide-mediated decrease in phosphopyruvate carboxylase activity, a finding consistent with the observation of Pereira (1967) on the effect of nicotinic acid on plasma free fatty acid in adrenalectomized rats. It seems clear that alteration in adipose tissue phosphopyruvate carboxylase activity may be directly related to the rate of free fatty acid release by that tissue.

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