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Activation of Pyruvate Dehydrogenase in the Perfused Rat Liver by Vasopressin

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The proportion of pyruvate dehydrogenase in its active form is doubled in rat liver within 5 min of addition of vasopressin to the perfusing medium.

Two groups of hormones can exert catabolic effects on liver metabolism, namely those that act via cyclic AMP (glucagon and β-adrenoceptor stimuli) and those that do not, such as α-adrenoceptor stimuli, vasopressin, oxytocin and angiotensin (see Hems, 1977, for review).

Several lines of evidence indicate that vasopressin-induced glycogen breakdown may be associated with an acceleration of glycolysis in the liver. Net glycogen loss in the presence of vasopressin is more than sufficient to account for the glucose released (Hems & Whitton, 1973; Hems *et al.*, 1978). At the lowest effective concentrations of vasopressin glycogen phosphorylase is activated, but there is no increase in glucose release (Hems *et al.*, 1976, 1978). In the absence of added extracellular Ca^{2+}, vasopressin stimulates glycogen breakdown, but not glucose or lactate release (Hems *et al.*, 1978). Since vasopressin seems to have little or no effect on lactate output (Hems & Whitton, 1973; Hems *et al.*, 1978), it follows that the hormone probably increases the rate of pyruvate oxidation. The hormone is known to stimulate the rate of O_2 uptake by the liver (Hems *et al.*, 1978) whereas it inhibits or does not affect the synthesis of fatty acids (Ma & Hems, 1975; Hems *et al.*, 1975).

The pyruvate dehydrogenase complex from mammalian tissues including liver is inactivated by phosphorylation of the α-subunits of the pyruvate decarboxylase component by a tightly bound ATP-linked kinase (Linn *et al.*, 1969*a*,*b*; Barrera *et al.*, 1972; see also Denton *et al.*, 1975, 1978, for reviews). Reactivation is brought about by a specific Mg^{2+}-requiring phosphatase that is stimulated by micromolar concentrations of Ca^{2+} (Denton *et al.*, 1972; Severson *et al.*, 1974). In muscle and liver, alterations in the rate of pyruvate oxidation associated with the utilization of fat fuels or the long-term effects of diabetes and starvation can be largely explained in terms of changes in the proportion of the complex in its active form (Kerbey *et al.*, 1976, 1977). In adipose tissue, it is well established that the short-term effects of insulin and adrenaline on the conversion of pyruvate into acetyl-CoA and thence into fatty acids are brought about by changes in the phosphorylation of pyruvate dehydrogenase (Jungas, 1971; Coore *et al.*, 1971; Denton *et al.*, 1975).

In the present study, we have examined the effect of vasopressin on pyruvate dehydrogenase activity in liver and report that the hormone rapidly causes a 2-fold increase in the active form of pyruvate dehydrogenase.

Experimental

Liver perfusion

Livers from fed Wistar albino rats were perfused with 60 ml of bicarbonate-buffered medium gassed with O_2/CO_2 (19:1, v/v) (Krebs & Henseleit, 1932) containing bovine serum albumin (25 mg/ml) and washed rat erythrocytes (Hems & Whitton, 1973). Initially the perfusing medium contained glucose at 5 mM; after 20 min the glucose concentration in the medium stabilized at a concentration between 8 and 10 mM.

After perfusion for 1 h, an initial liver sample (the median lobe) was removed after tying with thread and within 3 s was frozen between tissue clamps previously cooled to the temperature of liquid N_2 (Hems *et al.*, 1976). Perfusions were then continued for a further 5 or 10 min with or without addition of [Arg^8]vasopressin [grade IV; Sigma (London) Chemical Co., Poole, Dorset BH17 7NH, U.K.]. The second (final) sample (the left lateral lobe) was removed and immediately frozen with the precooled tissue clamps. The frozen liver samples were stored at −70°C for up to 10 days before assay of pyruvate dehydrogenase activity; no changes in pyruvate dehydrogenase activity were found over this period.

Extraction and assay of pyruvate dehydrogenase activity

This was based on methods used in previous studies (Coore *et al.*, 1971; Stansbie *et al.*, 1976*a*,*b*). Frozen

samples of liver were extracted at 0°C with 5ml of 100mM-potassium phosphate buffer (pH7.0)/g wet wt. containing 2mM-EDTA, 1mM-dithiothreitol and rat serum (50μl/ml) in a Polytron PT20 tissue homogenizer for 30s. Rat serum was added to inhibit the high activity of a proteinase present in liver extracts and that rapidly and irreversibly degrades pyruvate dehydrogenase (Lynen *et al.*, 1978). Extracts were immediately frozen in liquid N_2 and stored for up to 2h. After thawing and centrifugation of the extracts for 30s in an Eppendorf 3200 Microfuge, the initial activity of pyruvate dehydrogenase was measured in samples (50–100μl) of the supernatant (Stansbie *et al.*, 1976*a*). Total activity of pyruvate dehydrogenase was taken as the activity present in extracts after incubation with pig heart pyruvate dehydrogenase phosphate phosphatase in the presence of 25mM-$MgCl_2$ and 1mM-$CaCl_2$ (Stansbie *et al.*, 1976*b*). One unit of pyruvate dehydrogenase activity is the amount that catalyses the oxidation of pyruvate at the rate of 1μmol/min at 30°C.

Results and Discussion

The time course of the effect of [Arg^8]vasopressin in the perfused liver is shown in Fig. 1. At hormone concentrations of both 5×10^{-8}M and 5×10^{-9}M, the initial activity of pyruvate dehydrogenase was significantly increased within 5min of addition of vasopressin to the perfusing medium. The proportion of pyruvate dehydrogenase in its active form increased from about 25 to 50% of the total. Vasopressin had no effect on the total activity of pyruvate dehydrogenase. This was 1.15±0.05 (27) units/g wet wt. of tissue for control samples and 1.20±0.07 (23) units/g wet wt. for samples taken after addition of the hormone (values are given as means ±S.E.M. for the numbers of observations in parentheses). Since there was no change in the initial activity of pyruvate dehydrogenase in the control perfusions (Fig. 1), it is evident that the effect of vasopressin cannot be explained in terms of differences between the lobes of liver. Also, in separate perfusions, both lobes were removed simultaneously either before or after addition of vasopressin; no appreciable differences in initial activity of pyruvate dehydrogenase were observed between lobes and similar increases in initial activity in both main lobes were apparent after addition of the hormone.

It has proved difficult to observe clear-cut rapid hormone effects on pyruvate dehydrogenase activity in isolated-liver preparations. Effects of insulin have been reported by Topping *et al.* (1977). However, others have not been able to find any changes in activity (Patzelt *et al.*, 1973; Mukherjee & Jungas, 1975). Moreover, no change in liver pyruvate dehydrogenase was found *in vivo* after manipulation of circulating insulin by injections of anti-insulin serum

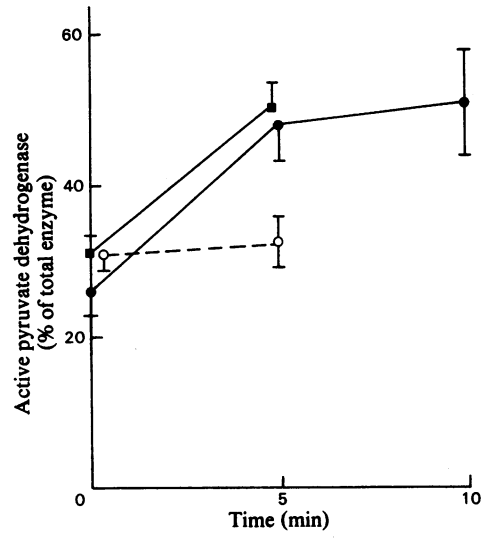

Fig. 1. *Time course of activation of pyruvate dehydrogenase by vasopressin*

Livers were perfused with Krebs–Ringer bicarbonate buffer containing serum albumin, erythrocytes and 8–10mM-glucose. After a 60min stabilization period, an initial liver sample was freeze-clamped. Then vasopressin was added to 5×10^{-9}M (■) or 5×10^{-8}M (●), and a second (final) sample was freeze-clamped after 5 or 10min. In control perfusions (○) no vasopressin was added. Results are means±S.E.M. (bars) for 3 (■, ○) or five to eight (●) perfusions. All values after vasopressin addition are significantly different from initial or control values ($P<0.01$: two-tailed t test for differences between pairs or groups, as appropriate).

and glucose, although there were marked alterations in the activity of pyruvate dehydrogenase in epididymal adipose tissue of the same rats (Stansbie *et al.*, 1976*a*). Glucagon also appears to have little or no effect on pyruvate dehydrogenase activity (Patzelt *et al.*, 1973; Claus & Pilkis, 1977).

The activation of pyruvate dehydrogenase by vasopressin could be of physiological significance. The lower of the two concentrations used in these studies (5×10^{-9}M, corresponding to about 2munits/ml or 5ng/ml) is of the same order as that observed in hypovolaemic rats (Ginsburg & Heller, 1953; Forsling *et al.*, 1971; see also the Discussion section by Hems *et al.*, 1976). It is also sufficient to cause near-maximal stimulation of glycogen breakdown and glucose release (Hems & Whitton, 1973; Hems *et al.*, 1976, 1978). On the other hand, the concentrations used in the present study are below those that can cause appreciable vasoconstriction in rat liver (Hems *et al.*, 1976); thus it seems unlikely that the activation of pyruvate dehydrogenase is the

result of a decrease in mitochondrial ATP/ADP ratio caused by hypoxia. Certainly there are no effects of vasopressin on the concentrations of ATP, ADP and AMP in freeze-clamped whole liver under the conditions of these studies. Measured values for ATP, ADP and AMP were 2451±218, 940±72 and 188±24nmol/g of fresh liver respectively in control tissue, and 2503±85, 1137±79 and 181±31nmol/g in livers perfused with vasopressin (C. J. Kirk & D. A. Hems, unpublished work; results are means±S.E.M. for three observations in each case).

Any extra acetyl residues formed from pyruvate in response to vasopressin stimulation in rat liver are not likely to be converted into fatty acid or cholesterol, as lipogenesis is inhibited by vasopressin in mouse liver (Ma & Hems, 1975), and not affected in rat liver (Hems *et al.*, 1975). Conversion into CO_2 is the most likely fate of any extra acetyl residues formed during vasopressin-stimulated glycogenolysis, in accord with the fact that vasopressin stimulates O_2 uptake (Hems *et al.*, 1978).

Both pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphate phosphatase have rather complex regulatory properties (for brief review see Denton *et al.*, 1978) and thus a large number of mechanisms could be suggested for the observed effect of vasopressin. Possibilities include inhibition of pyruvate dehydrogenase kinase by a decrease in the mitochondrial ratio of NADH/NAD^+ or an increase in the mitochondrial concentration of pyruvate. However, neither of these possibilities seems likely as the concentration ratio of 3-hydroxybutyrate/acetoacetate in whole freeze-clamped liver remains unchanged, and the concentration of pyruvate in whole tissue is halved by vasopressin under the conditions used in this study (C. J. Kirk & D. A. Hems, unpublished work). The effects of vasopressin on glucose release and glycogen breakdown are dependent on extracellular Ca^{2+} and are thought to involve an increase in the cytoplasmic concentration of Ca^{2+}, which results in activation of phosphorylase *b* kinase (Stubbs *et al.*, 1976; Keppens *et al.*, 1977; Hems *et al.*, 1978). Pyruvate dehydrogenase phosphate phosphatase in mammalian mitochondria, including those from liver, is also activated by Ca^{2+} (Denton *et al.*, 1972, 1978; Severson *et al.*, 1974; H. T. Pask & R. M. Denton, unpublished work). It is therefore possible that vasopressin may bring about the increase in pyruvate dehydrogenase activity through an increase in the mitochondrial concentration of Ca^{2+}, parallel, and perhaps secondary to, an increase in the cytoplasmic concentration.

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