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Stimulation of glycolysis and accumulation of a stimulator of phosphofructokinase in hepatocytes incubated with vasopressin

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Vasopressin stimulates glycolysis in hepatocytes prepared from fed rats, or from starved rats when incubated with glucose. It causes the stimulation of phosphofructokinase activity and the accumulation of a stimulator of phosphofructokinase, which is probably fructose 2,6-bisphosphate, the recently discovered stimulator of phosphofructokinase.

In isolated hepatocytes, glucose and glucagon influence recycling between fructose 6-phosphate and fructose 1,6-bisphosphate, as well as the activity of phosphofructokinase (Rognstad & Katz, 1976; Castano *et al.*, 1979; Kagimoto & Uyeda, 1979; Pilkis *et al.*, 1979; Van Schaftingen *et al.*, 1980*a*). It has been shown that changes in phosphofructokinase activity caused by glucose and glucagon were best explained by variations in the intracellular concentration of a low-molecular-weight stimulator which has been purified and tentatively identified as fructose 2,6-bisphosphate, and synthesized chemically (Van Schaftingen *et al.*, 1980*a*,*b*; Van Schaftingen & Hers, 1980). Incubation of hepatocytes with increasing glucose concentrations gave rise to an accumulation of this potent stimulator, and the addition of glucagon abolished this accumulation (Van Schaftingen *et al.*, 1980*a*).

Vasopressin has been shown to stimulate gluconeogenesis when isolated liver preparations from starved rats are perfused or incubated with gluconeogenic precursors such as lactate (Hems & Whitton, 1973; Whitton *et al.*, 1978). In the fed state, vasopressin stimulates the formation of radioactive glucose from [^{14}C]lactate or [^{14}C]pyruvate (Chan & Exton, 1978; Hue *et al.*, 1978); there is, however, no clear evidence for a net uptake of lactate under these conditions (Hems *et al.*, 1978*a*). A stimulation of the lactate production by vasopressin has been observed in hepatocytes prepared from fed rats and incubated with oleate (Williamson *et al.*, 1980). In the present paper we report the effect of vasopressin on the glycolytic flux, phosphofructokinase activity and the concentration of the above-mentioned stimulator of phosphofructokinase in hepatocytes.

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Methods

Methods for preparation and incubation of hepatocytes have been previously described (Hue *et al.*, 1978). After 30 min of preincubation at 37°C the various agents were added to the cell suspension and the incubation was continued for 15–30 min. For the measurement of the phosphofructokinase stimulator, 2 ml of cell suspension, corresponding to about 100 mg of liver, was briefly (15 s) centrifuged (200 *g*) and the cell pellet was immediately frozen in liquid N_2. The frozen pellets were thawed in 0.9 ml of 50 mM-Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid]/0.1 M-KF/15 mM-EGTA, pH 7.1. After centrifugation for 5 min in an Eppendorf centrifuge, the supernatants were heated for 5 min at 80°C. The extracts were centrifuged again and 50 μl of the supernatant, corresponding to 5 mg of liver, was added to the phosphofructokinase assay mixture in a final volume of 1 ml. The source of enzyme was a Sephadex G-25 filtrate prepared from whole liver of fed rats (Van Schaftingen *et al.*, 1980*a*). Phosphofructokinase activity was assayed at low (0.25 mM) and saturating (5 mM) concentrations of fructose 6-phosphate as described else-

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where (Castano *et al.*, 1979; Van Schaftingen *et al.*, 1980*a*) and is expressed as activity ratio ($v_{0.25}/V_5$). The activity of the stimulator is expressed as the increase in this ratio, the ratio being about 0.1 without addition.

To measure the phosphofructokinase activity of hepatocytes, extracts were prepared as described above, but with no heat-step; the activity was assayed on 50 μl samples.

Lactate was measured enzymically (Hohorst, 1963) in neutralized perchlorate extracts. The flux through phosphofructokinase can be evaluated by the measurement of ^3H_2O release from [3-^{3}H]-glucose added to the cell suspension (Katz & Rognstad, 1976; Hue, 1980); ^3H_2O was separated from radioactive glucose by passing deproteinized cell extracts through columns of Dowex AG 1 (X8; borate form), which retained glucose (Bontemps *et al.*, 1978). All enzymes and biochemical reagents were purchased from Boehringer.

Saturating concentrations of hormones were used.

Results

Effects of vasopressin and glucagon on the glycolytic flux

The effect of these agents on the net production of lactate and on the flux through phosphofructokinase is shown in Table 1. In hepatocytes from fed rats, vasopressin caused a small, but significant, increase in lactate output, whereas glucagon had the reverse effect. Measurement of flux through phosphofructokinase was only applied to hepatocytes from starved rats, since in fed rats the glycogenolytic effect of vasopressin would cause an isotopic dilution of the hexose 6-phosphate pool, which would render the interpretation of results very difficult. In hepatocytes from overnight-starved rats, vasopressin caused an increase in flux, whereas glucagon decreased it.

Effects of vasopressin, glucose and glucagon on the activity of phosphofructokinase and on the phosphofructokinase stimulator

It was of interest to know whether vasopressin could also influence the activity of phosphofructokinase as well as the concentration of the stimulator of phosphofructokinase, in view of the changes in flux reported above. Results are shown in Table 2. In cells from fed rats, vasopressin and glucose caused a similar increase in both the enzyme activity and the concentration of the stimulator. In cells obtained from starved rats and incubated without glucose, there was no effect of hormones, whereas, when 10 mM-glucose was added, vasopressin could again produce an increased activity of phosphofructokinase and an accumulation of the stimulator. Its effect was, however, smaller than in the fed state, and quite variable. By contrast, and in agreement with previous results (Van Schaftingen *et al.*, 1980*a*), glucagon caused an inactivation of phosphofructokinase and a decrease in the concentration of the stimulator in cells from fed or from starved rats and incubated with 10 mM-glucose.

To test whether the stimulator had properties similar to those reported for the recently discovered stimulator of phosphofructokinase (Van Schaftingen *et al.*, 1980*a*,*b*), heat-treated extracts prepared from cells incubated with vasopressin or glucose were passed through columns of anion exchanger [Dowex 1 (X8; Cl^- form)]: the stimulator from glucose- or vasopressin-treated cells was eluted between 0.2 and 0.25 M-NaCl. Furthermore, when the fractions containing the stimulator were treated with alkaline phosphatase or brought to pH 2 for 20 min at 20°C, the stimulating activity completely disappeared and fructose 6-phosphate was formed; treatment with purified fructose bisphosphatase had no effect (results not shown). All these properties are identical with those described for the stimulator (Van Schaftingen *et al.*, 1980*a*,*b*).

Table 1. *Effects of vasopressin and glucagon on the production of lactate and on the flux through phosphofructokinase in rat hepatocytes*

After a 30 min preincubation with no addition or with 10 mM-glucose when indicated, the cells were further incubated for 30 min (lactate production) or 20 min (release of ^3H_2O) in the presence of the various agents, and the reaction was stopped by the addition of 1 vol. of 1 M-$HClO_4$. The concentration of lactate before adding the hormones to hepatocytes from fed rats was 0.56 ± 0.06 mM. Negative production of lactate means a net utilization of lactate. Results shown are means ± S.E.M. for three cell preparations and are expressed per g wet wt. of cells. The statistical significance of the difference calculated by the paired *t* test is indicated as $^*P < 0.05$.

Additions	Lactate production in hepatocytes from fed rats (μmol of lactate/min per g)	Release of ^3H_2O from [3-^{3}H]glucose in hepatocytes from starved rats + 10 mM glucose (μmol of glucose/min per g)
None	0.24 ± 0.01	0.15 ± 0.01
10 nM-vasopressin	0.38 ± 0.10*	0.22 ± 0.03*
1 μM-glucagon	−0.24 ± 0.02*	0.03 ± 0.01*

Table 2. *Effects of vasopressin, glucagon and glucose on the activity of phosphofructokinase and on the concentration of its stimulator in hepatocytes*

After a 30min preincubation with no addition or with 10mM-glucose when indicated, the various agents were added to the cell suspension, which was further incubated for 15min. Values shown are means ± S.E.M. for three to five different cell preparations, except for the activity of phosphofructokinase in cells from starved rats with no glucose added, where the means for two cell preparations are shown. The statistical significance of the difference calculated by the paired *t* test is indicated as $*P < 0.05$.

	Activity of phosphofructokinase ($v_{0.25}/V_5$) in hepatocytes from		
		Overnight-starved rats	
Additions	Fed rats	No glucose	With 10mM-glucose
None	0.21 ± 0.01	0.08	0.22 ± 0.05
10nM-vasopressin	0.44 ± 0.07*	0.09	0.39 ± 0.09
0.1μM-glucagon	0.08 ± 0.01*	0.09	0.05 ± 0.01*
50mM-glucose	0.50 ± 0.09*	0.59	0.47 ± 0.03*

	[Stimulator of phosphofructokinase] (increase in activity ratio) in hepatocytes from		
		Overnight-starved rats	
	Fed rats	No glucose	With 10mM-glucose
None	0.14 ± 0.02	0.08 ± 0.01	0.14 ± 0.02
10nM-vasopressin	0.34 ± 0.06*	0.11 ± 0.02	0.26 ± 0.03*
0.1μM-glucagon	0.08 ± 0.01*	0.07 ± 0.01	0.04 ± 0.01*
50mM-glucose	0.33 ± 0.05*	0.32 ± 0.05*	0.35 ± 0.04*

Discussion

The increased flux through phosphofructokinase as well as the increased lactate output observed in hepatocytes after vasopressin strongly suggests that glycolysis was stimulated under these conditions. This confirms previous observations of an increase in glycolytic flux by vasopressin (Hue *et al.*, 1978) and a stimulation of lactate production by vasopressin (Williamson *et al.*, 1980). It should, however, be pointed out that the extent of stimulation of glycolysis that we have observed is probably underestimated. Indeed, our data give no information about the further utilization of lactate. Relevant to this point is the reported activation of pyruvate dehydrogenase by vasopressin (Hems *et al.*, 1978*b*). Furthermore, the actual increase in flux through phosphofructokinase by vasopressin might be larger than what we have observed, since this agent could have increased the concentration of hexose 6-phosphate causing, by isotopic dilution, an underestimation of our measurement of flux.

What is the mechanism responsible for the stimulation of glycolysis by vasopressin in hepatocytes from fed rats? One can propose that it results from (*a*) the well-known stimulation of glycogen breakdown (for a review, see Hems & Whitton, 1980), which will provide glucose 6-phosphate for the glycolytic pathway, and (*b*) from a stimulation of phosphofructokinase. This stimulation of enzyme activity may result from the accumulation of a stimulator of phosphofructokinase. Furthermore, the stimulator found after vasopressin treatment is probably the same as that formed after glucose treatment, since it displays identical properties. We can therefore propose that vasopressin, as well as glucose, causes an activation of phosphofructokinase by the accumulation of a stimulator, which is probably fructose 2,6-bisphosphate.

In hepatocytes from starved rats, an effect of vasopressin on phosphofructokinase and its stimulator was only observed when cells were incubated with 10mM-glucose, i.e. above the physiological concentration. This effect has probably little physiological meaning, since hyperglycaemia is not expected to occur during starvation *in vivo*. Our results, however, indicate that the formation of the stimulator is glucose-dependent. It therefore seems that the occurrence of a stimulation of liver glycolysis by vasopressin will probably depend on the availability of substrates such as glucose or

glycogen, which are plentiful in the fed state, and on the accumulation of fructose 2,6-bisphosphate, the stimulator of phosphofructokinase.

Pyruvate kinase is another enzyme at which the glycolytic pathway is controlled by hormones in liver (for a review, see Hue, 1980). Since vasopressin caused an increase in net lactate output by hepatocytes, it would seem that its effect on pyruvate kinase is too small to inhibit significantly the flux through this enzyme. This confirms previous observations that the actual activity of pyruvate kinase was little affected by vasopressin (Hue *et al.*, 1978), although others have reported that it is slightly inactivated (Chan & Exton, 1978; Garrison *et al.*, 1979).

The decrease in lactate output caused by glucagon may result from inhibition of both phosphofructokinase and pyruvate kinase activities.

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