THE ACTIVATION OF LIVER GLYCOGEN PHOSPHORYLASE BY VASOPRESSIN

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1. Introduction

It has been shown [1] that vasopressin stimulates glycogenolysis in the rat liver at concentrations which can occur in vivo, especially during haemorrhagic shock. We report here that the glycogenolytic effect of vasopressin is due to the activation of glycogen phosphorylase, the rate limiting enzyme in glycogen degradation. The mechanism involved appears different from that of glucagon or adrenaline: it is apparently not mediated by cyclic AMP, since – in contrast with the two latter hormones – no increase in protein kinase activity is observed with vasopressin. A preliminary communication describes part of these results [2].

2. Materials and methods

2.1. *Experiments with intact rats*

The general procedure has been described [3]. Briefly, pentobarbital anesthetized fed rats of about 300 g were used; liver biopsies were taken before and after the intravenous injection of the hormone and quick-frozen.

2.2. *Analytical methods*

For the assay of protein kinase (EC 2.7.1.37), a portion of a frozen liver sample was homogenized with 9 vol of a solution containing 250 mM sucrose, 10 mM potassium–phosphate buffer pH 7.4, 4 mM EDTA and 2 mM dithiotreitol. After a 10-fold dilution with the same solution, a 25 μl aliquot of the homogenate was incubated at 30°C in a final vol of 125 μl, in the presence of 0.5 mM [γ-^{32}P]ATP and 5 mM magnesium acetate, with or without 10^{-6} M cyclic AMP; two histone fractions were used at the following concentrations: histone II_A (Sigma-type) at 0.6% and histone f_{2b} (nomenclature according to [4]) at 0.05%. The rate of phosphorylation was linear in time for about 6 min (see figs.2 and 3). After 0, 2, 4, 6 and 8 min, a 20 μl-aliquot was spotted on filter paper and processed according to [5]. The activity is expressed as nmol phosphate incorporated per min and per g tissue.

Glycogen phosphorylase *a* (EC 2.4.1.1.) and glycogen synthetase *a* (EC 2.4.1.11) were measured according to [3] except that the temperature was 25°C instead of 20°C.

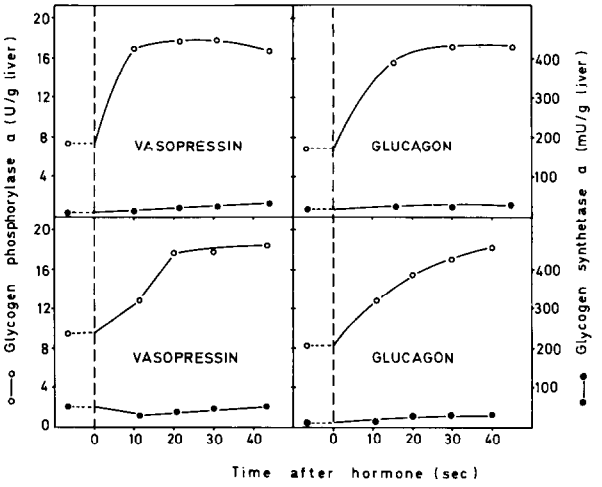

Fig. 1. The kinetics of the activation of liver glycogen phosphorylase by vasopressin and by glucagon. Vasopressin (20 mU) or glucagon (10 μg) was injected intravenously to an anaesthetized rat. In each experiment, liver samples were taken at various time intervals from the same rat.

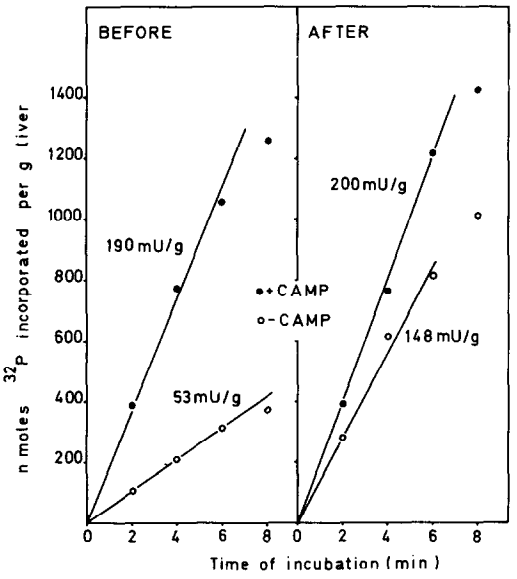

Fig. 2. Influence of a glucagon treatment on liver protein kinase. Two liver samples were taken from the same rat, one before and the other 1 min after the intravenous injection of glucagon (10 μg). Protein kinase was assayed with histone f_{2b} as the substrate, either without or with 10^{-6} M cyclic AMP. Phosphorylase activity was 7.8 U/g before and 17.5 U/g after glucagon.

2.3. *Chemicals*

[8-arginine]vasopressin (type VI) was obtained from Sigma Chemical Co. (St. Louis), glucagon from Novo-laboratories (Copenhagen) and adrenaline bitartrate from Federa (Brussels). Histones, ATP and cyclic AMP were from Sigma Chemical Co.

3. Results

Vasopressin (20 mU) caused a rapid activation of glycogen phosphorylase, clearly evident after 10 sec and essentially complete within 30 sec (fig.1). This rate of activation was at least as rapid as with 10 μg of glucagon (fig.1) and both hormones activated the enzyme to the same level (fig.1 and table 1) In these (fig.1) and other experiments (not shown) glycogen synthetase remained at a low level of activity.

The question arises whether vasopressin activates glycogen phosphorylase through an increased protein kinase activity, as it is generally accepted for glucagon and adrenaline (see [6]). Fig.2 shows a typical experiment illustrating the effect of glucagon on the activity of protein kinase: one min after the intravenous injection of glucagon there was an almost trebling of the protein kinase activity assayed without added cyclic AMP (cyclic AMP independent protein kinase); no significant change in the total activity as measured with 10^{-6} M cyclic nucleotide was recorded.

The effect of glucagon on glycogen phosphorylase and on protein kinase assayed with two different histone fractions is shown in table 1. On the average, glycogen phosphorylase was activated 2.6-fold; with histone f_{2b} as the substrate, cyclic AMP independent protein kinase activity was increased 2.7-fold and with histone II_A 3.6-fold. Total protein kinase was not significantly influenced by the glucagon treament (table 1). Smaller amounts of glucagon, down to 0.125 μg, were still clearly able to increase both the phosphorylase *a* level and the cyclic AMP independent protein kinase acting on histone f_{2b} (not shown).

Unlike glucagon, which can elevate the cyclic AMP level up to 60-fold, adrenaline raises this level at the most 2 to 4 times [7]. Therefore we have examined

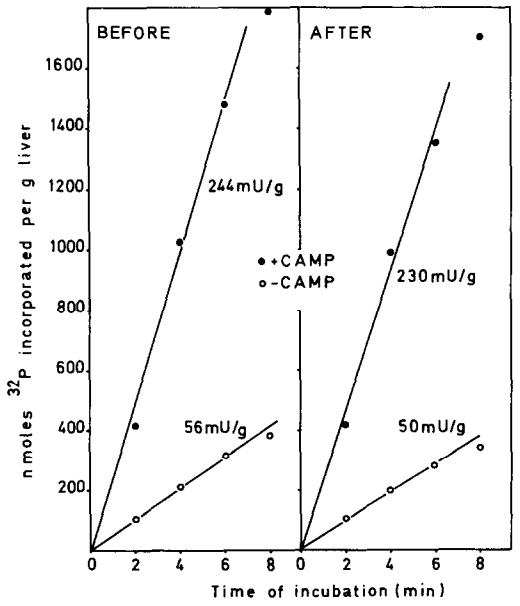

Fig. 3. Influence of a vasopressin treatment on liver protein kinase. Same procedure as in fig.2. The rat received 20 mU of vasopressin. Phosphorylase activity was 8.4 U/g before and 16.4 U/g after treatment.

Table 1
The effect of glucagon and vasopressin on liver glycogen phosphorylase and protein kinase

Enzyme	cAMP	Glucagon Before	Glucagon After	Vasopressin Before	Vasopressin After
Phosphorylase *a* (U/g liver)		6.4 ± 0.8	16.6* ± 0.3	8.5 ± 0.8	16.6* ± 0.5
Protein kinase $+f_{2b}$ (mU/g liver)	−	49 ± 4	130* ± 7	49 ± 3	47 ± 2
	+	182 ± 4	185 ± 10	238 ± 3	233 ± 6
$+II_A$	−	22 ± 5	81* ± 10	32 ± 2	27 ± 4
	+	108 ± 5	98 ± 3	105 ± 9	120 ± 11

Two liver samples were taken from the same rat, one before and the other 1 min after the intravenous injection of glucagon (10 μg) or of vasopressin (20 mU). Protein kinase was assayed with and without added cyclic AMP (10^{-6} M). Values shown are means ± S.E.M. for 4–7 rats. Statistical significance was calculated from paired data.
* $P<0.005$

whether such a moderate increase in the level of the cyclic nucleotide would be reflected in an enhanced protein kinase activity. One minute after the injection of 3 μg of adrenaline, the activation of glycogen phosphorylase was as complete as with glucagon and accompanied with a 1.6-fold increase in the cyclic AMP independent kinase activity assayed with histone f_{2b}.

The situation with vasopressin is quite different: indeed, the activation of phosphorylase is not associated with an increase in the cyclic AMP independent protein kinase activity. Fig.3 illustrates how a one minute treatment with 20 mU of the hormone failed to enhance the cyclic AMP independent protein kinase activity as measured with histone f_{2b}. Neither were higher amounts of vasopressin (up to 85 mU) able to increase the hepatic protein kinase activity (not shown).

Table 1 summarizes our data obtained with 20 mU of vasopressin: no increase of cyclic AMP independent protein kinase activity with neither histone fraction was apparent one minute after the application of the hormone, yet phosphorylase *a* had been doubled and brought to the same level as with glucagon. Total protein kinase remained essentially constant.

4. Discussion

Our results show that the glycogenolytic effect of vasopressin is due to the activation of liver glycogen phosphorylase, the rate limiting enzyme in glycogen degradation. The activation of phosphorylase by vasopressin is at least as rapid as with glucagon and is as complete as with glucagon or adrenaline, the two well known glycogenolytic hormones. However, the mechanism involved appears different.

Glucagon and adrenaline are known to act by their ability to stimulate the adenyl cyclase system: the increase in intracellular cyclic AMP activates the protein kinase and this will ultimately lead to the conversion of glycogen phosphorylase *b* to *a* [6]. We have found indeed that both glucagon and adrenaline activate liver protein kinase. The rise in kinase activity is more marked with glucagon than with adrenaline; this is in agreement with the different increases in cyclic AMP concentrations known to be produced by these hormones, glucagon being much more efficient than adrenaline [7]. The degree of kinase activation with glucagon is similar to that reported recently by Sudilovsky [8].

Vasopressin however does not enhance the cyclic AMP independent protein kinase activity in the liver. Yet, our assay is sensitive enough to detect a 2 to 4-fold increase in the level of cyclic AMP, the maximal raise which can be obtained with adrenaline [7]. The lack of effect of vasopressin on protein kinase suggests therefore that it may activate liver glycogen phosphorylase by a mechanism different from that of glucagon and adrenaline, i.e. not mediated by cyclic AMP. This conclusion is supported by the corroborative data of Kirk and Hems [9] who reported very recently that vasopressin (up to 100 mU per rat) fails to raise the cyclic AMP level in rat liver.

In summary, the glycogenolytic action of vasopressin in the liver is explained by a prompt activation of glycogen phosphorylase; this activation, apparently not mediated by cyclic AMP nor by an increased protein kinase activity, seems to be due to a novel mechanism.

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