THE ACTIVATION OF LIVER GLYCOGEN PHOSPHORYLASE BY ANGIOTENSIN II

S. KEPPENS and H. DE WULF

Afdeling Biochemie, Faculteit Geneeskunde, Katholieke Universiteit te Leuven, Dekenstraat 6, B-3000 Leuven, Belgium

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1. Introduction

Vasopressin, in amounts likely to be produced in haemorrhagic shock, causes glycogenolysis in the liver [1,2] through a prompt activation of liver glycogen phosphorylase (EC 2.4.1.1) [3,4]. This activation, unlike that produced by glucagon or adrenaline, is not mediated by an enhanced activity of protein kinase (EC 2.7.1.37) [3] and neither does vasopressin increase the concentration of cyclic AMP in rat liver [5].

A haemorrhage results in an increase of the blood levels of angiotensin II (for recent reviews see [6,7]) and the question arises naturally whether angiotensin II shares the glycogenolytic properties of vasopressin. Conflicting reports can be found in the literature concerning an effect of angiotensin II on blood sugar level, which has been reported to rise [8–10], to remain unaltered [11–13] or even to decline [14] after the injection of the octapeptide. It should however been noted that those authors who did not find a hyperglycemic response to angiotensin II [11–14] have been using fasting animals or subjects, so that the lack of a systemic hyperglycemia can conceivably stem from unsufficient liver glycogen stores. It has indeed been shown [8] that the hyperglycemia caused by angiotensin II is accompanied with a progressive loss of liver, but not of muscle, glycogen. Furthermore, two groups of investigators have presented evidence for a direct glycogenolytic effect of angiotensin II on isolated rat liver preparations [8,10]. We report here that, as vasopressin, angiotensin II causes a direct activation of liver glycogen phosphorylase, the rate limiting enzyme for glycogenolysis; likewise, the mechanism involved appears to be cyclic AMP independent. Preliminary communications describe part of the data [15,16].

2. Materials and methods

Angiotensin II and its hexapeptide were obtained from Schwarz-Mann (Orangeburg, N.Y.) 1-Sar-8-Ile-angiotensin II was a gift of Dr. F. M. Bumpus (Cleveland Clinic, Cleveland).

All techniques including the experiments with intact rats with isolated hepatocytes and the enzyme assays have been described or referred to [3,17,18].

3. Results

3.1. *Experiments with intact anaesthetized rats*

Angiotensin II (0.2 or 0.4 μg per rat) caused a prompt activation of liver glycogen phosphorylase which was already evident after 20 sec and reached plateau levels between 30 and 60 sec (fig.1). This rate of activation compares favourably with those obtained with either vasopressin or with glucagon [3]. The hexapeptide derived from angiotensin II is endowed with much less pressor activity (see [6,7]); likewise, its glycogenolytic potency is also less marked: fig.1 shows that 0.4 μg of the hexapeptide did not activate liver glycogen phosphorylase.

The mode of action of angiotensin II resembles that of vasopressin [3,5]; indeed, no change occurred in the activity of the protein kinase measured with histone f_{2b} or II_A as the substrate and without added cyclic AMP (cyclic AMP independent activity); neither was there, as expected, an increase in total protein

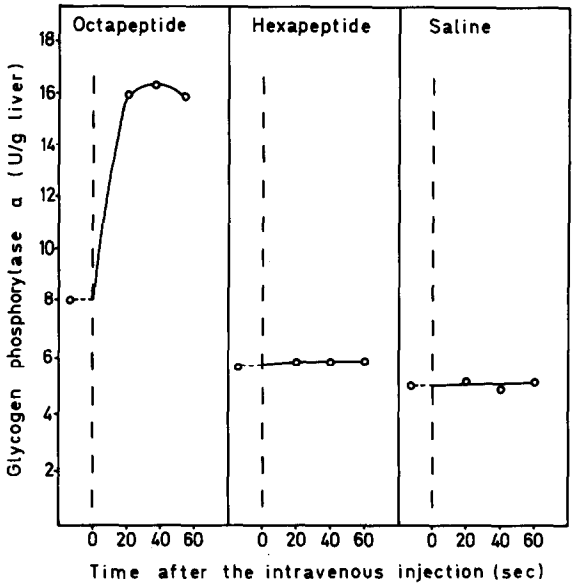

Fig.1. The influence of angiotensin II on the level of liver glycogen phosphorylase. The octapeptide (0.4 μg), the hexapeptide (0.4 μg) or the corresponding saline solution (1.5 ml) was injected intravenously into an anaesthetized rat. In each experiment, liver biopsies from the same animal were taken at various time intervals.

kinase activity, as assayed with 10^{-6}M cyclic nucleotide (table 1). We conclude therefore that, like vasopressin [3,5], angiotensin activates liver glycogen phosphorylase by a mechanism which appears not to be mediated by cyclic AMP.

3.2. *Experiments with isolated liver cells*

The rapidity of onset in vivo of the activation of liver glycogen phosphorylase by angiotensin II (fig.1) clearly suggests a direct action on the liver. We corroborate this proposal by showing in fig.2 that isolated

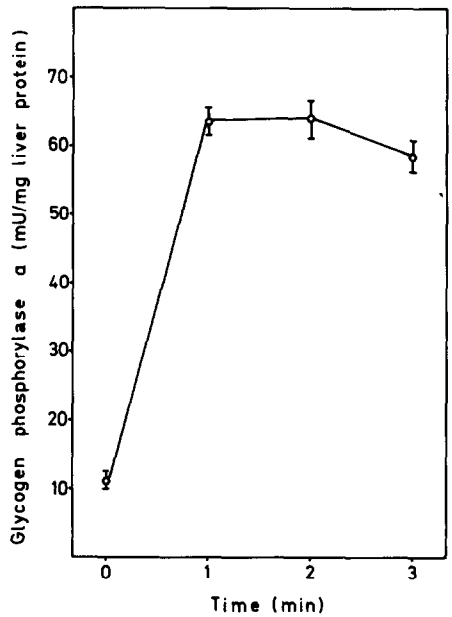

Fig.2. Kinetics of the activation of glycogen phosphorylase by angiotensin II in isolated liver cells. Liver cells (10^7/ml) were preincubated for 30–45 min with 20 mM glucose; phosphorylase *a* levels were measured in aliquots taken at the indicated time intervals after the addition of angiotensin II (20 ng/ml). Results shown are the means of 8 experiments ± S.E.M.

Table 1
The effect of angiotensin II on liver glycogen phosphorylase and protein kinase in intact anaesthetized rats

Enzyme		cAMP	Before	After
Glycogen phosphorylase (U/g liver)			7.0 ± 0.7	16.0[a] ± 0.7
Protein kinase (mU/g liver)	with f_{2b}	–	45 ± 4	43 ± 4
		+	204 ± 3	197 ± 7
	with II_A	–	30 ± 3	30 ± 3
		+	87 ± 4	79 ± 3

Two liver samples were taken from the same rat, one before and the other 1 min after the intravenous injection of 0.4 μg angiotensin II. Protein kinase was assayed with and without added cyclic AMP (10^{-6}M), with histones f_{2b} or II_A as the substrate [3]. Values shown are means of 9 experiments (± S.E.M.). Statistical significance was calculated from paired data.
[a] $P < 0.005$.

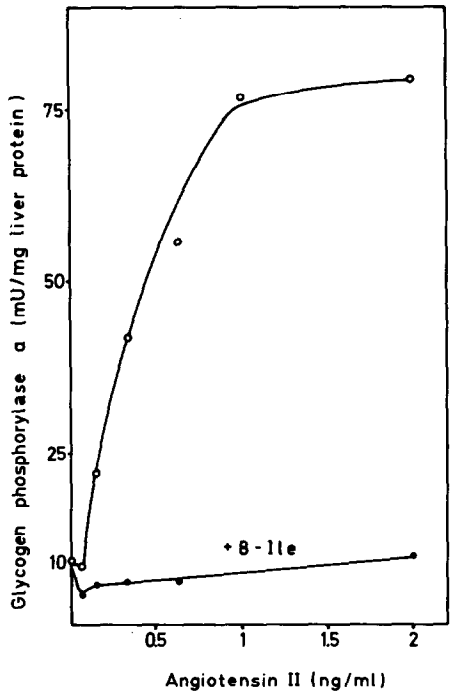

Fig.3. Dose-response curve for the activation of liver glycogen phosphorylase by angiotensin II. Same procedure as in fig.2; samples were taken before and 1 min after the addition of angiotensin II at the indicated concentrations (o) without or (•) with 1-Sar-8-Ile-angiotensin II (8-Ile) at 200 ng/ml.

rat liver parenchymal cells reacted to the addition of angiotensin II by a prompt activation of glycogen phosphorylase. This enzymic conversion occurred as rapidly as with glucagon ($2X10^{-9}$ M) and to approximately the same extent [18]. A typical dose effect curve (fig.3) showed that half-maximal activation was obtained with about 0.3 ng/ml.

A known inhibitor of angiotensin II action, 1-Sar-8-Ile [19] was able to prevent the activation of glycogen phosphorylase by increasing concentrations of angiotensin II, while having no activating effect on the enzyme by itself (fig.3).

In confirmation and extension of the data obtained in vivo, angiotensin II did not activate liver protein kinase, as measured with histone f_{2b} and without added cyclic AMP (table 2).

4. Discussion

The in vivo and in vitro experiments reported here show that angiotensin II shares the glycogenolytic properties of vasopressin as previously reported both for the intact rat and for perfused liver [1–4]. As was the case with the antidiuretic hormone [3], no increase in the cyclic AMP dependent protein kinase was induced by angiotensin II which therefore stimulates the conversion of phosphorylase *b* to *a* in a cyclic AMP independent way. Whether vasopressin and angiotensin II share a common mode of action remains to be elucidated.

The amounts of angiotensin II required to obtain this activation of glycogen phosphorylase are likely to be produced in haemorrhagic conditions. It has been estimated that in the dog a moderate haemorrhage (the withdrawal of 14–26 ml blood/kg) results in increases of the concentration of angiotensin II of 0.33 ng/ml blood [20]; a more severe haemorrhage (between 28 and 37 ml/kg) yields

Table 2
The effect of angiotensin II on glycogen phosphorylase and protein kinase in isolated rat liver cells

Enzyme activity (mU/mg liver protein)	cAMP	Before	After
Glycogen phosphorylase		15.7 ± 1.8	63.8[a] ± 7.0
Protein kinase	–	0.14 ± 0.03	0.15 ± 0.02
	+	0.82 ± 0.05	0.78 ± 0.07

Two aliquots were taken, one before and the other 1 min after the addition of 20 ng angiotensin/ml. Protein kinase was assayed with and without cyclic AMP (10^{-6}M) with histone f_{2b} as the substrate [3,18]. Values shown are means of 6 experiments ± S.E.M. Statistical significance was calculated from paired data.
[a] $P < 0.005$.

concentrations of nearly 2 ng/ml [21]. Similar values were obtained by other authors [22–24]. Since a half-maximal activation of glycogen phosphorylase was obtained with about 0.3 ng angiotensin II per ml cell suspension (fig.3), we conclude that the amounts of angiotensin II found to affect liver glycogen phosphorylase are likely to be encountered in haemorrhagic shock.

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