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Vasopressin and angiotensin control the activity of liver phosphodiesterase

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Vasopressin and angiotensin are able to lower the glucagon-induced increase of cyclic AMP levels in isolated hepatocytes. Results presented are in favour of an enhanced phosphodiesterase activity to account for this cyclic AMP lowering effect. In particular, vasopressin prevents exogenous cyclic AMP from activating glycogen phosphorylase: in the presence of phosphodiesterase inhibitors, the hormone becomes unable to decrease glucagon-induced cyclic AMP levels. This anti-glucagon effect of vasopressin and angiotensin might be physiologically more important than their glycogenolytic effect; indeed, the latter is very transient in nature and, in addition, requires higher hormone concentrations [Bréant, Keppens & De Wulf (1981) *Biochem. J.* **200**, 509–514] than those needed for the anti-glucagon effect, as reported here.

Angiotensin has been shown to inhibit glucagon-stimulated adenylate cyclase in purified rat liver plasma membranes (Jard *et al.*, 1981). This inhibition has been suggested to account for the angiotensin-induced decrease in the accumulation of cyclic AMP in isolated hepatocytes stimulated by glucagon (Cardenas-Tanus *et al.*, 1982; Crane *et al.*, 1982). Vasopressin, which cannot inhibit glucagon-stimulated adenylate cyclase in isolated plasma membranes (Jard *et al.*, 1981) likewise appeared without effect on hepatocytes *in vivo* (Cardenas-Tanus *et al.*, 1982). However, Crane *et al.* (1982) reported briefly that vasopressin produced an inhibition of glucagon-stimulated cyclic AMP accumulation in isolated liver cells. More recently, Morgan *et al.* (1983*a*) showed that angiotensin and vasopressin caused significant inhibition of the cellular cyclic AMP response to glucagon; since angiotensin was unable to inhibit phosphorylase activation elicited by exogenous cyclic AMP, it was suggested that its action must be at the level of cyclic AMP generation. Similarly, Morgan *et al.* (1983*b*) concluded from their experiments that the inhibition by vasopressin of glucagon-induced cyclic AMP accumulation in liver may involve direct inhibition of adenylate cyclase. We have reinvestigated this problem and present results which are more consistent with a (direct?) stimulation of phosphodiesterase.

Abbreviations used: Ro 20-1724, 4-(3-butoxy-4-methoxybenzyl)-2-imidazole-dione.

Experimental

Methods

We have used male Wistar strain albino rats (200–250g body wt.) which were fed *ad libitum*. Liver cells were prepared as described previously (Vandenheede *et al.*, 1976) and incubated in Krebs–Henseleit bicarbonate buffer equilibrated with O_2/CO_2 (19:1) (Krebs & Henseleit, 1932). Cells (5×10^6 cells/ml) were suspended in this medium and preincubated for 20–30min with 10mM-glucose and 14mM-bacitracin at 37°C in closed plastic 20ml vials with continuous shaking. The sampling procedure and assay of phosphorylase *a* activities were as described (Vandenheede *et al.*, 1976). For the determination of cyclic AMP, aliquots of the cell suspension were frozen in liquid N_2; later on, cyclic AMP was determined with a competitive protein-binding technique (Gilman, 1970) by using an assay kit from The Radiochemical Centre (Amersham, Bucks., U.K.). Protein concentration was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Chemicals

[Arg^8]Vasopressin (grade VI) and 3-isobutyl-1-methylxanthine were from Sigma. Glucagon was purchased from Novo Laboratories, Copenhagen, Denmark and angiotensin from Schwarz-Mann, Orangeburg, NY, U.S.A. Ro 20-1724, a product of Hoffman-La Roche Co., Nutley, NJ, U.S.A. and 1-isoamyl-3-isobutylxanthine were kindly donated

by Dr. C. Erneux (Université Libre de Bruxelles, Belgium).

Results and discussion

We first report data showing that the capacity of vasopressin and angiotensin to lower cyclic AMP levels induced by glucagon stimulation may be physiologically more relevant than their ability to increase phosphorylase *a* levels. Indeed, the former effect is obtained at lower hormone concentrations than those needed for glycogenolysis and is, in contrast with the glycogenolytic action (Bréant *et al.*, 1981; Keppens & De Wulf, 1982) not transient in nature. Figs. 1(*a*) and 1(*b*) illustrate, for angiotensin and vasopressin, the dose-dependency of both phosphorylase activation and of the prevention of the glucagon-induced rise of cyclic AMP levels. The latter effect is half-maximally obtained at approx. 2-fold (angiotensin) and 5-fold (vasopressin) lower concentrations than those required for the half-maximal glycogenolytic effect. A similar greater sensitivity in the case of vasopressin has also been observed by Morgan *et al.* (1983*b*). In contrst with the very transient nature of phosphorylase activation (Bréant *et al.*, 1981; Keppens & De Wulf, 1982), we find that the ability of vasopressin to decrease cyclic AMP levels is as clearly evident whether the hormone is added together with glucagon or 1 min after glucagon (Fig. 2) or 20 min before glucagon (results not shown).

We next designed experiments to test whether this cyclic AMP lowering effect is due to a decreased activity of adneylate cyclase or to an increased activity of phosphodiesterase(s). The kinetic experiment depicted in Fig. 2 possibly suggests an involvement of phosphodiesterase since the whole of the decrease in the cyclic AMP level is obtained within 30 s after the addition of vasopressin (or of angiotensin; results not shown), reaching the level obtained in the simultaneous presence of glucagon and vasopressin. The experiment illustrated in Fig. 3 is more suggestive of a role for phosphodiesterase. Phosphorylase activation by exogenous cyclic AMP is clearly diminished by vasopressin; in this case, vasopressin was added 20 min earlier so as to allow for the transiently increased phosphorylase *a* levels to return to the control level (Bréant *et al.*, 1981). Thirdly, in the presence of each of three phosphodiesterase inhibitors, the ability of vasopressin to prevent

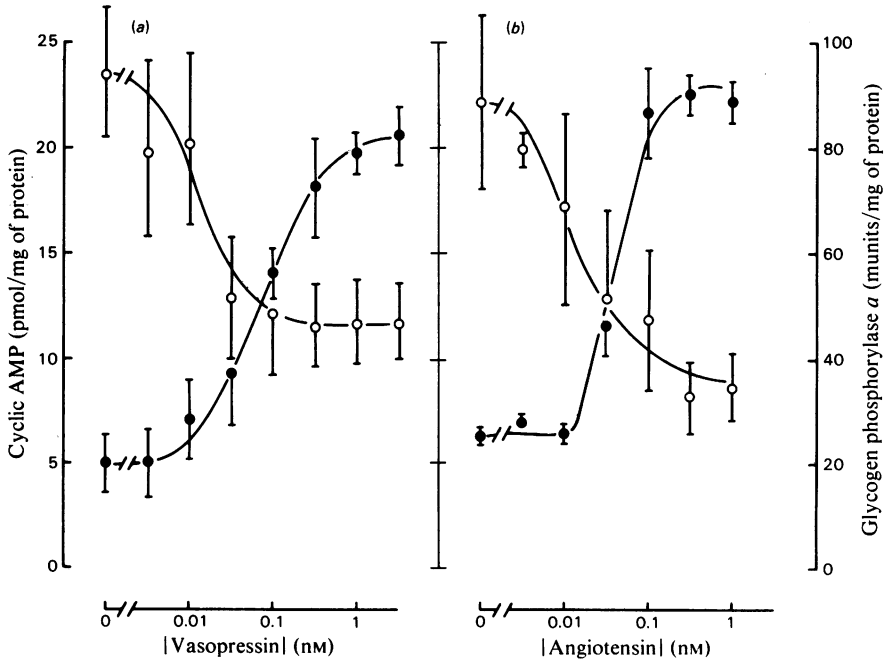

Fig. 1. *Dose-dependent activation of glycogen phosphorylase and lowering of cyclic AMP levels by vasopressin and angiotensin*
Hepatocytes were incubated with increasing amounts of vasopressin (*a*) or angiotensin (*b*). Phosphorylase was assayed 90 s after the addition of these hormones (●). Cyclic AMP (○) was assayed 2 min after the addition of 5 nM-glucagon given 1 min after either vasopressin (*a*) or angiotensin (*b*). Values are given as means ± S.E.M. for three (●) or six (○) experiments. Basal levels of cyclic AMP were 2.6 ± 0.9 pmol/mg of protein ($n = 6$).

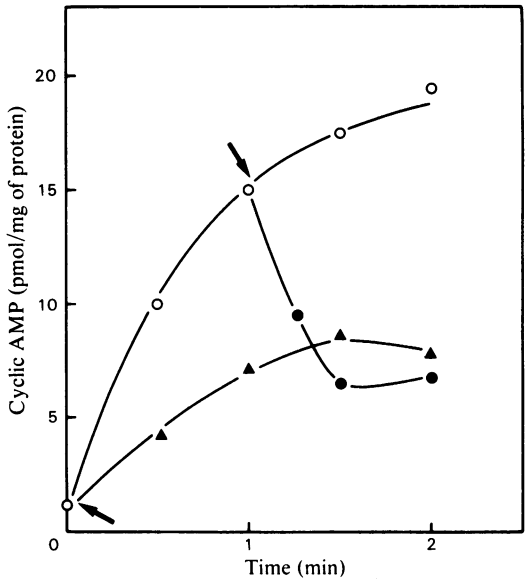

Fig. 2. *Effect of vasopressin on the glucagon-induced accumulation of cyclic AMP*
Hepatocytes were incubated with 5nM-glucagon (○); 25nM-vasopressin was added at the times indicated (closed symbols). This Figure shows results that are representative of several similar experiments.

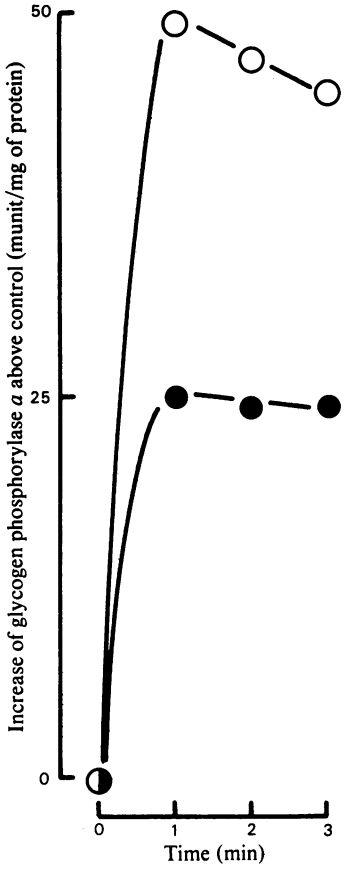

Fig. 3. *Effect of vasopressin on the cyclic AMP-induced activation of glycogen phosphorylase*
Hepatocytes were incubated with (●) or without (○) 25nM-vasopressin; 20min later, 0.1mM-cyclic AMP was added and phosphorylase was assayed at the times indicated. This Figure shows results that are representative of several similar experiments.

glucagon-induced cyclic AMP levels is lost (Table 1). A similar result was obtained for angiotensin in the presence of 10^{-5}M-3-isobutyl-1-methylxanthine (results not shown).

Conclusions

Two conclusions can be drawn from our data.

First, the counteraction exerted by vasopressin and angiotensin of the effect of glucagon on cyclic AMP levels is probably of greater physiological significance than the glycogenolytic effect of these hormones.

Secondly, the effect of vasopressin (and by analogy that of angiotensin) on the cyclic AMP levels in the presence of glucagon can be attributed to an enhanced phosphodiesterase activity rather than to a decreased activity of adenylate cyclase. This latter conclusion is in agreement with the lack of effect of vasopressin on adenylate cyclase (Cantau *et al.*, 1980; Jard *et al.*, 1981) and is not in contradiction with the very modest (±15%) inhibition by angiotensin of the glucagon-stimulated adenylate cyclase (Jard *et al.*, 1981; Crane *et al.*, 1982). Admittedly, angiotensin is able to inhibit by up to about 40% the activity of basal adenylate cyclase (Jard *et al.*, 1981; Crane *et al.*, 1982), but angiotensin does not lower basal levels of cyclic AMP in hepatocytes (Cardenas-Tanus *et al.*, 1982; Morgan *et al.*, 1983*a*).

Interestingly, this cyclic AMP lowering effect of vasopressin and angiotensin bears similarities to the anti-glucagon effect of insulin. In particular, it has been shown (reviewed by Houslay *et al.*, 1983) that insulin lowers cyclic AMP levels in hepatocytes previously challenged by glucagon and that this effect is obtained through an activation (by phosphorylation) of a phosphodiesterase. Further work is required to find out whether a similar effect underlies the mechanism of action of vasopressin and angiotensin in their anti-glucagon action.

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Table 1. *Effect of phosphodiesterase inhibitors on the ability of vasopressin to reduce the glucagon-induced accumulation of cyclic AMP*

Hepatocytes were incubated in the presence of 10^{-5}M of a phosphodiesterase inhibitor or of 1% dimethyl sulphoxide, which was used as solvent; 5min later, 5nM-glucagon with or without 25nM-vasopressin was added. Cyclic AMP was assayed 2min later. Values given are means ± S.E.M. for 13 experiments. The statistical significance of the effect of vasopressin was estimated by the paired *t*-test.

	Cyclic AMP (pmol/mg of protein)			
Condition	Control	Glucagon	Glucagon + vasopressin	*P*
Dimethyl sulphoxide	3.6 ± 0.4	46 ± 4.6	32 ± 3.8	< 0.001
3-Isobutyl-1-methylxanthine	6.2 ± 0.5	128 ± 13	125 ± 10	> 0.1
Ro 20-1724	5.8 ± 0.6	148 ± 12	133 ± 10	> 0.1
1-Isoamyl-3-isobutylxanthine	8.1 ± 1.1	165 ± 15	150 ± 15	> 0.1

References

Bréant, B., Keppens, S. & De Wulf, H. (1981) *Biochem. J.* **200**, 509–514

Cantau, B., Keppens, S., De Wulf, H. & Jard, S. (1980) *J. Receptor Res.* **1**, 137–168

Cardenas-Tanus, R. J., Huerta-Bahena, J. & Garcia-Sáinz, J. A. (1982) *FEBS Lett.* **143**, 1–4

Crane, J. K., Campanile, C. P. & Garrison, J. C. (1982) *J. Biol. Chem.* **257**, 4959–4965

Gilman, A. G. (1970) *Proc. Natl. Acad. Sci. U.S.A.* **67**, 305–312

Houslay, M. D., Wallace, A. V., Marchmont, R. J., Martin, B. R. & Heyworth, C. M. (1983) *Adv. Cyclic Nucleotide Res.* **16**, 159–176

Jard, S., Cantau, B. & Jakobs, K. H. (1981) *J. Biol. Chem.* **256**, 2603–2606

Keppens, S. & De Wulf, H. (1982) *Biochem. J.* **208**, 317–322

Krebs, H. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Morgan, N. G., Exton, J. H. & Blackmore, P. F. (1983*a*) *FEBS Lett.* **153**, 77–80

Morgan, N. G., Shipp, C. C. & Exton, J. H. (1983*b*) *FEBS Lett.* **163**, 277–281

Vandenheede, J. R., Keppens, S. & De Wulf, H. (1976) *FEBS Lett.* **61**, 213–217