HEPATIC ACTION OF VASOPRESSIN: LACK OF A ROLE FOR ADENOSINE-3',5'-CYCLIC MONOPHOSPHATE

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1. Introduction

Vasopressin (anti-diuretic hormone) can stimulate glycogen breakdown and gluconeogenesis in the rat liver, at concentrations (0.1–1.0 ng/ml) which can occur in the intact rat [1]. This is a third major systemic action of vasopressin in rats; its concentration–dependence [1] resembles that of the pressor action, rather than the anti-diuretic effect. The hepatic action of vasopressin therefore has significance in the intact animal, in conditions where plasma vasopressin levels are high, such as haemorrhagic shock [2], and warrants further investigation.

The question arises of the mechanism of the hepatic action of vasopressin. A simple theory would be that adenosine cyclic-3',5'-monophosphate (cyclic AMP) is implicated. To investigate this possibility, concentrations of cyclic – AMP have been measured in the liver of intact rats, and in the medium during perfusion, in conditions where glucogon and adrenalin produced a rise in concentration of this nucleotide [3–5].

In this report, it is shown that vasopressin does not produce an increase in cyclic AMP in the liver or perfusate, in any of the conditions tested. Thus the hepatic effect of vasopressin provides a clearcut example of a short-term hormonal effect on mammalian metabolic processes, which is not mediated by cyclic AMP.

2. Materials and methods

2.1. *Perfusion of rat liver*

Livers of fed rats (170–190 g) were perfused with 50 ml recirculating bicarbonate buffered medium containing albumin and washed rat erythrocytes [1]. After 40 min, during which a steady perfusate glucose concentration was established, hormones were added to the perfusate, to give the following initial concentrations; glucagon, 10 ng/ml; adrenalin, 10^{-6} M; vasopressin, 100 mU/mlitre. Over the next 30 min, samples of the dripping effluent medium were collected for analysis of cyclic AMP. Glucose was measured in samples taken from the reservoir of perfusion medium. Adrenalin and vasopressin produced transient decreases in flow rate.

2.2. *Experiments with intact rats*

Rats were anaesthetised with Nembutal. The abdomen was opened, to permit injection of hormones (in 0.25 ml 0.9% Na Cl) into the hepatic portal vein; glucagon, 1.0 μg; adrenalin 1.5×10^{-8} mol; vasopressin 10 or 100 mU. Control injections were 0.9% Na Cl. After various times, the liver was removed for measurement of cyclic AMP. During this period, the abdomen was covered with tissue moistened with 0.9% Na Cl, and the rat kept warm by a nearby bulb. Only one sample was taken from each rat. In preliminary experiments, it was established that steady basal (lowest) hepatic cyclic AMP concentrations were attained 15–20 min after opening the abdomen. At earlier times, the concentration was about 50% higher. Therefore a delay of 20 min after opening the abdomen was routinely employed before hormone injection.

2.3. *Analytical methods*

Cyclic AMP was measured by a radioisotope saturation assay [6]. Samples of liver were quick-frozen in liquid N_2, and extracted in 10 vols (w/v) of 6% (w/v) TCA (trichloroacetic acid); 200–400 μl of extract was washed 4 times with water-saturated diethyl ether to

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remove the TCA, dried and taken up in buffer containing theophylline and mercapto–ethanol for assay [6]. Perfusion medium was spun to remove erthrocytes, and mixed with an equal volume of buffer, heated at 100°C for 3 min and centrifuged to remove protein. Standard samples (0.5–5 pmol), taken through the same procedure, gave reproducible standard curves (range of counts bound 50–90% of that in the absence of cyclic AMP); Recovery of added cyclic AMP from liver extracts was 95–115%; Separate experiments showed that vasopressin did not affect the extraction or binding of cyclic AMP. Glucose was measured by a glucose oxidase method.

2.4. *Chemicals*

L-adrenalin bitartrate and [8-arginine] vasopressin were from Sigma Chemical Co., and glucagon was kindly donated by Eli-Lilly Ltd. (courtesy of Dr. M. Root). Cyclic AMP was from C. F. Boehringer Corp. Ltd., or Radiochemical Centre, Amersham, UK (^{3}H-labelled). Other materials for perfusion and analysis were of the highest grade commercially available.

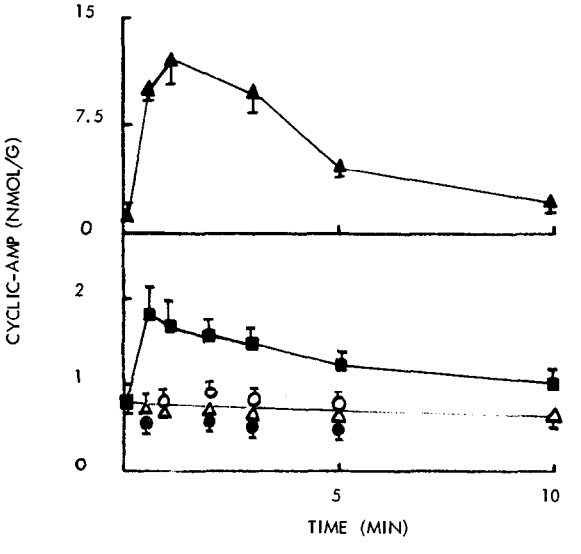

Fig. 1. Effect of hormones on hepatic cyclic AMP in vivo. Fed rats were anaesthetized; the hepatic content of cyclic AMP was determined at various times after the injection, into the hepatic portal vein, of: glucagon, 1 μg (▲); adrenalin, 1.5 × 10^{-8} mol (■); vasopressin (10 mU, ●; 100 mU, ○); or 0.9% (w/v) NaCl (△). Other details are in the text. Results are means of 3–6 measurements; bars indicate S.E.M.

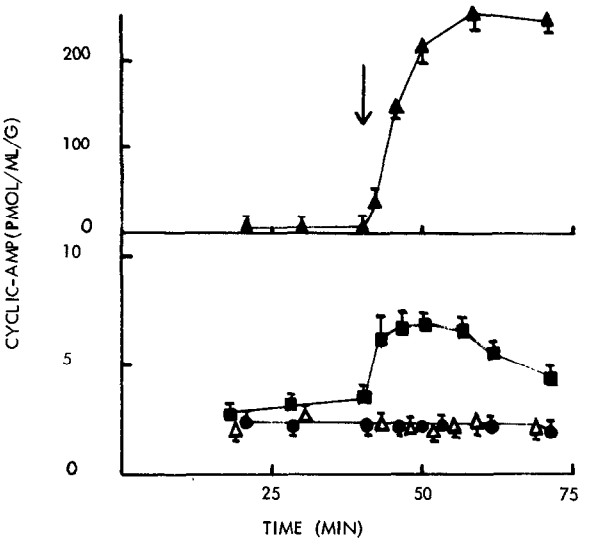

Fig. 2. Effect of hormones on venous effluent cyclic AMP in the perfused liver. Livers of fed rats were perfused with recirculating bicarbonate buffered saline, and cyclic AMP was measured in the dripping effluent medium; hormones were added, after 40 min, to the following initial concentrations: glucagon, 10 ng/ml (▲); adrenalin, 10^{-6} M (■); vasopressin, 100 mU/ml (●). No additions: (△). Other details are in the text. Results are means from 3 perfusions; bars indicate S.E.M.

3. Results

3.1. *Experiments in vivo*

In intact rats, intravenous glucagon and adrenalin produced the expected rise in the hepatic concentration of cyclic AMP (fig. 1). These effects were of the same order as those previously reported [3–5]. Vasopressin (10 mU or 100 mU) produced no detectable increase in hepatic cyclic AMP (fig. 1).

3.1. *Experiments with the perfused liver*

In the perfused liver, as in vivo, glucagon and adrenalin caused an increase in the concentration of cyclic AMP in the effluent (fig. 2: see refs. [3–5,7]). Again, vasopressin did not bring about an increase in the perfusate cyclic AMP (fig. 2), despite causing glucose output which was of the same order as that previously observed [1], and that produced by adrenalin (fig. 3).

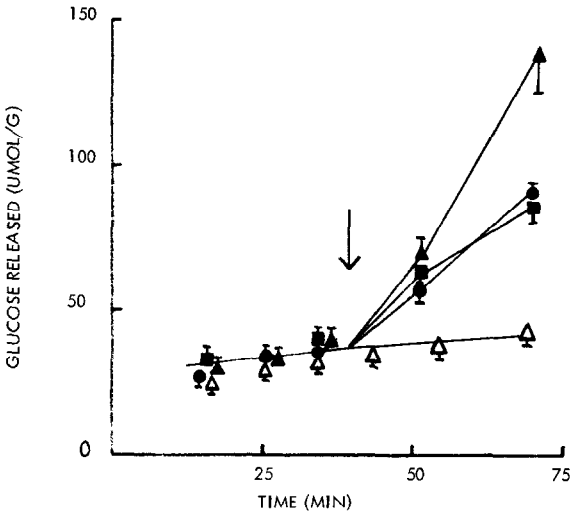

Fig. 3. Effect of hormones on hepatic glucose output. Livers were perfused as described in fig. 2. Glucose was measured in the main reservoir of medium being initially about 5 mM. The extra glucose released during perfusion was calculated. Other details are as described in fig. 2.

4. Discussion

Present results confirm the action of vasopressin in stimulating glucose output in the rat liver [1]. Several considerations suggested that this effect might be mediated by cyclic AMP. Thus the hepatic action of glucagon (for which the minimally effective dose is also about 0.1 ng/ml) and the renal action of vasopressin, appear to involve cyclic AMP ([3–5,8] respectively). More generally, it appears that most hormones which exert clearcut rapid effects on metabolic processes can influence the concentration of cyclic AMP in the target tissue [9].

However, in the present study, no increase in cyclic AMP were detected in liver or perfusate, in response to vasopressin concentrations that were maximal or supramaximal in regard to their effect on glucose output [1]. This negative result was not a consequence of any limitation in the conditions of the experiments, or in the determination of cyclic AMP, since glucagon and adrenalin produced the expected increases in cyclic AMP.

It has been reported that the activity of the adrenalin-responsive hepatic adenyl cyclase is increased after adrenalectomy [10], and that basal and hormone-stimulated cyclic AMP levels are increased in fasted rats perfused with theophylline [11]. In view of these facts, cyclic AMP was assayed in the perfusate during perfusion of the liver of the 48 hr starved, adrenalectomised rat, in the presence of 1 mM theophylline: the basal cyclic AMP level was elevated 2–3-fold, but there was no increase in response to vasopressin (100 mU/ml: C. J. Kirk, unpublished experiments).

The above results strongly suggest that the hepatic actions of vasopressin are not mediated by cyclic AMP. In this respect, there is a partial resemblance to short-term adrenalin action on the liver, where the associated small increases in hepatic cyclic AMP do not appear to be relevant to the stimulation of gluconeogenesis [12,13] or to an α-receptor route for stimulation of glycogenolysis [14].

With regard to cyclic AMP dependent glycogenolysis, it has been proposed that hormones stimulate the formation of cyclic AMP in a particular hepatic pool of free nucleotide, of which effluent venous cyclic AMP provides a sensitive index [3,4]. There was no sign that the content of cyclic AMP in this pool was increased by vaso pressin.

The lack of an effect of vasopressin on hepatic cyclic AMP concentrations, observed in the present experiments, is in agreement with the lack of effect of vasopressin on hepatic adenyl cyclase (reported in ref. [15] and confirmed: separate personal communications from Rodbell and Hanoune).

In summary, the glycogenolytic action of vasopressin on the liver appears to be unique among short-term 'catabolic' hormone effects in mammals (reported so far) in being associated with no discernible alteration in tissue cyclic AMP concentration.

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References

[1] Hems, D. A. and Whitton, P. D. (1973) Biochem. J. 136, 705.

[2] Ginsburg, M. (1968) in: Handbook of Experimental Pharmacology, Vol. 23, (Berde, B., ed.), p. 286 Springer-Verlag, 1968.
[3] Exton, J. H., Robison, G. A. and Sutherland, E. W. (1972) in: Handbook of Physiology, Section 7 (Endocrinology, Greep, R. O. and Astwood, E. B., eds.), Vol. I. p. 425, American Physiological Society.
[4] Exton, J. H. and Park, C. R. (1972) in: Handbook of Physiology, Section 7 (Endocrinology, Greep, R. O. and Astwood, E. B., eds.), Vol. 1, p. 437, American Physiological Society.
[5] Exton, J. H., Lewis, S. B., Ho, R. J. Robison, G. A. and Park, C. R. (1971) Ann. N.Y. Acad. Sci. 185, 85.
[6] Brown, B. L., Albano, J. D. N., Ekins, R. P., Sgherzi, A. M. and Tampion, W. (1971) Biochem. J. 121, 561.
[7] Kuster, J., Zapf, J. and Jakob, A. (1973) FEBS Letters. 32, 73.
[8] Beck, N. P., Kaneko, T., Zor, U., Field, J. B. and Davis, B. B. (1971) J. Clin. Invest. 50, 2461.
[9] Robison, G. A., Butcher, R. W. and Sutherland, E. W. (1968) Ann. Rev. Biochem. 37, 149.
[10] Bitensky, M. W., Russell, V. and Blanco, M. (1970) Endocrinology, 86, 154.
[11] Exton, J. H., Robison, G. A., Sutherland, E. W. and Park, C. R. (1971) J. Biol. Chem. 246, 6166.
[12] Tolbert, M. E. M., Butcher, F. R. and Fain, J. N. (1973) J. Biol. Chem. 248, 5686.
[13] Tolbert, M. E. M. and Fain, J. N. (1974) J. Biol. Chem. 249, 1162.
[14] Sherline, P., Lynch, A. and Glinsmann, W. (1972) Endocrinology, 91, 680.
[15] Pohl, S. L., Birnbaumer, L. and Rodbell, M. (1971) J. Biol. Chem. 246, 1849.