ROLE OF EXTRACELLULAR CALCIUM IN THE ACTION OF VASOPRESSIN ON HEPATIC GLYCOGENOLYSIS

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1. Introduction

Vasopressin (anti-diuretic hormone) has been shown to stimulate glycogen breakdown in perfused rat and mouse liver preparations [1–4] at concentrations which can occur in the intact animal (i.e. 0.1–1.0 mU/ml) especially during haemorrhagic shock [5]. In vivo, vasopressin (0.3 units) can produce hyperglycaemia, and this effect is more potent than that of glucagon [6].

It is clear that vasopressin does not act via cyclic-AMP [3,7] and it is therefore of interest to consider an alternative mechanism. Such a mechanism could be via cation-dependent events, as have been implicated in many types of cellular response to stimuli, including hormonal effects on hepatic gluconeogenesis, [8–11]. In the investigation reported here, hepatocyte suspensions have been employed to demonstrate that vasopressin acts on this population of liver cells, and also to characterise the dependence on extracellular calcium and potassium of vasopressin action. The data suggest that vasopressin action is markedly dependent on extracellular Ca^{2+}, to a greater extent than is the action of glucagon or adrenalin. Results obtained with the perfused liver are also presented which support this conclusion.

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2. Materials and methods

Hepatocyte suspensions were prepared [12] from livers of female Wistar rats, weighing about 200 g, which either had free access to a standard diet, or were meal-fed for 7 days [13]. To aid the retention of liver glycogen, the perfusate (Ca^{2+}-free) used in the preparation of cells contained 20 mM glucose. About 80 mg wet wt of cells were incubated in 25 ml conical flasks at 37°C for 40 min in a final volume of 4 ml Krebs–Henseleit medium containing 2.5% albumin. The gas phase was filled with 5% CO_2 in oxygen. In some experiments, $CaCl_2$ or K^+-salts (KCl and KH_2PO_4) were omitted and replaced by corresponding Na^+-salts in the perfusate, the cell washing medium, and the incubation medium. Cells were used immediately after preparation.

Liver perfusions were performed with 60 ml bicarbonate-buffered saline (sometimes Ca^{2+}-free) containing washed rat erythrocytes and an initial glucose concentration of 8–11 mM [1,3]. Vasopressin was added after 40 min, and glucose output followed for the next 40 min. The temperature of the perfusate was measured in the main reservoir and at the porta hepatis.

Chemicals were of AR grade. (8-arginine)-Vasopressin was from Sigma Ltd. (Grade VI). Glucose and glycogen were measured enzymically [14,15].

3. Results and discussion

In suspensions of hepatocytes from fed rats, vasopressin stimulated glucose output (table 1) over a concentration range of 0.1–1000 mU/ml, which includes the range effective in the perfused liver of fed rodents (0.1–1.0 mU/ml, refs. [1,2]). Glucose output in response to vasopressin was less marked in cells from meal-fed rats (table 1) as is also observed in the perfused liver (results not presented); this suggests that one factor in the excessive glycogen deposition due to meal-feeding may be an insensitivity to the catabolic actions of hormones.

If K^+ salts or Ca^{2+} salts were omitted from the incubation medium the basal glucose release was not much changed but the response to glucagon or adrenalin was slightly decreased (table 1). In a related study on gluconeogenesis similar results were obtained [8]. However glucose output due to vasopressin was markedly diminished in the absence of added K^+ or Ca^{2+} salts (table 1). In particular, when Ca^{2+}-salts were omitted from the incubation medium hepatocytes exhibited virtually no response to vasopressin, even at high concentrations (table 1).

Attempts were made to test the Ca^{2+}-dependence of vasopressin action in the perfused liver. During these experiments it emerged that hepatic glucose output was influenced by the temperature of the input perfusate. Therefore a comprehensive study of glucose output over the physiological range of temperature (measured at the porta hepatis) was carried out (fig.1). Despite the complexity of the processes of glucose metabolism, the data in fig.1 fitted linear plots relatively well (r values are 0.79 or higher), and so are presented as regression lines, for convenience. Alternative linear plots (abscissa T, or $^1/T$, or log T) gave lower r values. The data show that the temperature-dependence (i.e. slope in fig.1) of net glucose output was altered by perfusate Ca^{2+} (compare lines i and iii with ii and iv). No study of this phenomenon has yet been made with hepatocytes.

The results obtained with the perfused liver confirm the finding in hepatocytes that vasopressin action is Ca^{2+}-dependent. This can be seen from comparison (1) of the two regressions lines where vasopressin was present with and without Ca^{2+} (i.e., i and ii) and (2) of the absolute increments in glucose output, due to vasopressin (as reflected in the differences between the parallel regression lines i and iii, ii and iv); this increment was greater for the two

Table 1
Effect of vasopressin on glucose release in hepatocytes

	Rats fed ad lib			Meal-fed rats
	Complete media	K^+-Free media	Ca^{2+}-Free media	Complete media
Control glucose release:	68 ± 7 (8)	61 ± 7 (3)	61 ± 5 (4)	50 ± 4 (3)
Initial glycogen (μmol glucose/g):	197 ± 14 (7)	196 ± 21 (3)	206 ± 18 (4)	388 ± 18 (3)
Increments in glucose release:				
Glucagon (5 μg/ml)	77 ± 8 (8)	56 ± 13 (3)	67 ± 5 (4)	–
Adrenalin (50 μM)	65 ± 10 (5)	40 ± 16 (3)	50 ± 6 (4)	–
Vasopressin (mU/ml):				
1000	46 ± 8 (3)	–	6 ± 2 (3)	–
100	48 ± 9 (4)	22 ± 7 (3)	2 ± 4 (3)	–
10	49 ± 8 (7)	22 ± 3 (3)	−4 ± 2 (4)	29 ± 2 (3)
5	–	–	−2 ± 2 (4)	30 ± 3 (3)
1	35 ± 6 (7)	–	–	16 ± 2 (3)
0.5	–	–	–	11 ± 2 (3)
0.1	9 ± 3 (3)	–	–	

Hormones were added to hepatocyte suspensions, at zero time, at the concentration indicated. Calcium (as chloride) was omitted from perfusate and incubation media in Ca^{2+}-free experiments. In experiments with K^+-free media, KCl and KH_2PO_4 were replaced by NaCl and NaH_2PO_4 in the perfusate during hepatocyte preparation and in incubation media. Other details are in the text. Results are expressed in μmol glucose formed/g wet wt for a 40 min incubation with mean ± SEM when there are three or more observations.

groups containing Ca^{2+} in the perfusate. The Ca^{2+}-dependence of vasopressin action was not as complete as that in hepatocyte suspensions, presumably because in the intact organ, the removal of extracellular Ca^{2+} was not complete. A similar consideration may explain the lack of Ca^{2+}-dependence of glucagon and adrenalin action in the perfused liver [16].

The dependence of glucose synthesis by liver cells on extracellular Ca^{2+} has been established [8–11]; indeed, if Ca^{2+}-chelators are employed, an obligatory requirement for Ca^{2+} can be demonstrated [17]. However, this aspect of glycogen metabolism in cells from fed animals has not been studied previously. Glucose output in the present conditions may be presumed to be largely due to glycogen breakdown, as no glucogenic substrates were added to incubation

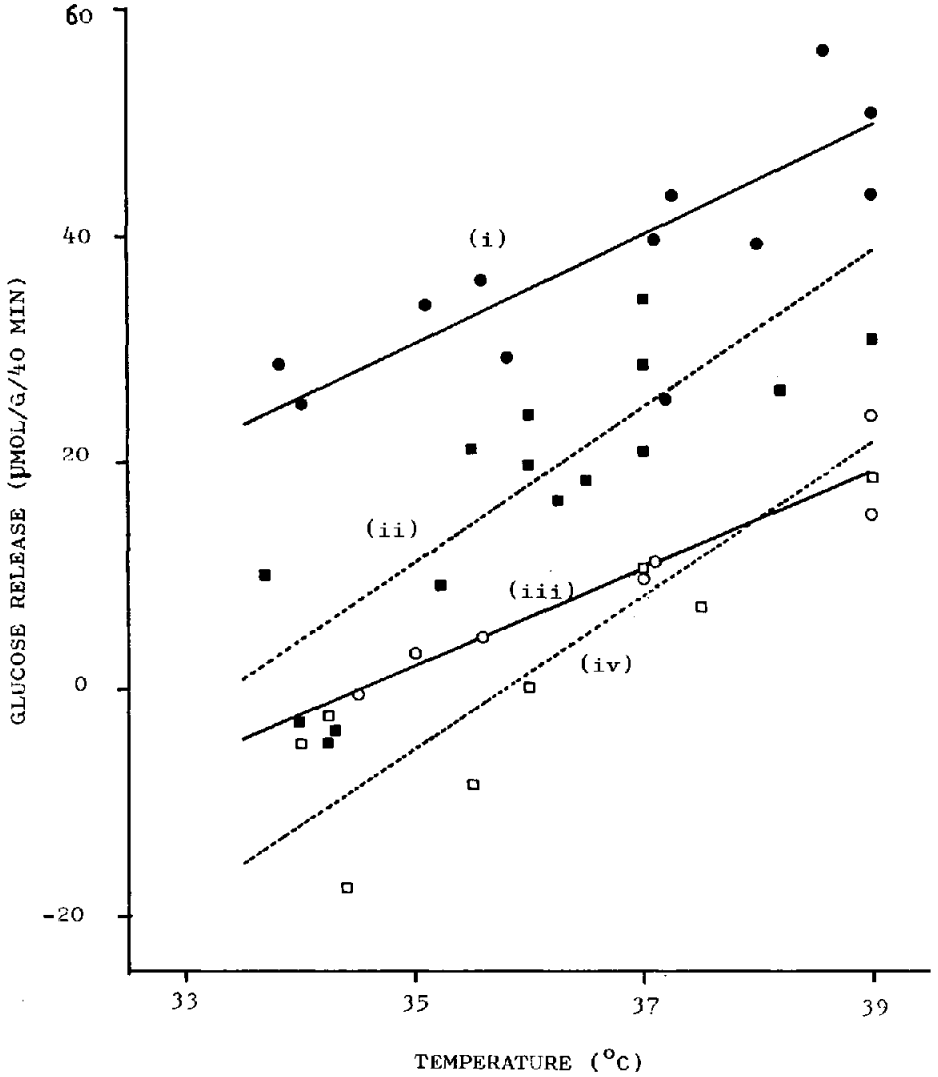

Fig.1. Livers from fed rats were perfused with bicarbonate-buffered saline, as described in the text. Glucose was initially 8–10 mM (remaining approximately constant in the absence of added hormones at 36°C). Glucose output was followed between 40 and 80 min. In Ca^{2+}-free perfusion (□, no vasopressin; ■, plus vasopressin,), Ca^{2+} was replaced by NaCl, from the start of perfusion. Vasopressin (5 mU/ml) was added at 40 min. (■, no Ca^{2+}; ●, plus Ca^{2+}). Control perfusions (○) contained Ca^{2+} but no vasopressin. Each point represents on perfusion. Correlation coefficients [5] are : (i) 0.79, (ii) 0.88, (iii) 0.95, (iv) 0.90.

media; studies with the perfused liver show that glycogen breakdown is rapid in the absence of added glucose or glucogenic precursors (e.g., refs. [18,19]). In the experiments with hepatocytes, lactate was released during the incubation, so that the concentration changed from about 0.2–0.8 mM in control incubations, in the absence or presence of Ca^{2+} in the medium. Lactate release was decreased by glucagon or adrenalin, but was not altered by vasopressin (results not presented in table 1). Taken together, the data show that basal glycogen breakdown is not markedly dependent on exogenous Ca^{2+}, (although a more extensive Ca^{2+}-dependence might emerge if chelators or leaching procedures were used). The absence of Ca^{2+}-dependence of glucose release, even in the presence of glucagon and adrenalin, (see also ref. [20]), provides a marked contrast to the sensitive Ca^{2+}-dependence of vasopressin action.

The main conclusions to be drawn from this study are: (1) the action of vasopressin on hepatic glycogen breakdown occurs in hepatocyte suspensions over about the same concentration range as in the intact organ [1,2]. Thus the parenchymal cells are the main locus of action of the hormone on glycogen breakdown and receptors are not damaged during cell preparation, (2) the action of vasopressin is very sensitive to extracellular Ca^{2+}, and is also partially diminished in the absence of extracellular K^+. Therefore cations, and Ca^{2+} in particular, are likely to be implicated in vasopressin action on liver glycogen metabolism, e.g., through the potent action of Ca^{2+} on hepatic glycogen phosphorylase kinase [21].

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