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Hepatocyte swelling increases inositol 1,4,5-trisphosphate, calcium and cyclic AMP concentration but antagonizes phosphorylase activation by Ca^{2+}-dependent hormones

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Swelling of hepatocytes increases the concentration of inositol 1,4,5-trisphosphate, Ca^{2+} and cAMP, without activating glycogen phosphorylase. In these hepatocytes, the activation of phosphorylase by suboptimal concentrations of vasopressin or angiotensin II was partly antagonized.

Calcium; cAMP; Hepatocyte; Phosphorylase; Vasopressin; Swelling

1. INTRODUCTION

In isolated hepatocytes from overnight-fasted rats, glycogen synthesis is directly proportional to cell swelling, whether the latter was induced by hypotonic media or by Na^+-transported amino acids, such as glutamine [1]. Thus, cell swelling might be regarded as a stimulatory signal for glycogen synthesis. In this work we have investigated the mechanism by which swelling could control glycogen synthesis. From what is known on the control of glycogen metabolism, a possibility is that such a mechanism could lead to a decrease in cAMP or intracellular Ca^{2+} concentrations which are well-known signals of the glycogenolytic cascade. By contrast, studies on modifications induced by cell swelling in various types of cells have shown that the concentrations of metabolic signals such as cAMP and Ca^{2+} are indeed increased [2–7], i.e. the opposite of what is expected to stimulate glycogen synthesis. Therefore, we studied the effect of swelling on the concentrations of cAMP and Ca^{2+} in hepatocytes. We also studied whether swelling could interfere with the activation of phosphorylase brought about by Ca^{2+}-dependent hormones such as vasopressin or angiotensin II.

2. MATERIALS AND METHODS

The method for preparation and incubation of isolated hepatocytes from overnight-fasted rats (200–250 g) has been described [8]. Intracellular calcium was measured with Quin-2, a fluorescent probe, by following the changes in fluorescence in a spectrofluorometer (excitation wavelength: 342 nm; emission wavelength: 492 nm) [9]. After 30 min of incubation with or without 10 mM glutamine, the hepatocytes were loaded with the acetoxymethyl ester of Quin-2 during 5 min, the cells were then collected by centrifugation and their fluorescence was monitored. To measure inositol 1,4,5-trisphosphate, the cells were pre-incubated for 90 min with radioactive inositol (10 μCi/ml) [10] to label the phosphoinositide pool, and the various phosphoinositides, including inositol 1,4,5-trisphosphate, were then separated on columns (1 × 3 cm) of Dowex AG 1-X8 (formate form) by batch-wise elution with formate solution as described [10]. Another method based on specific binding of inositol 1,4,5-trisphosphate to protein was used to measure inositol 1,4,5-trisphosphate and was carried out as described by the manufacturer (Amersham International plc). cAMP [3], phosphorylase *a* activity [11] and cell volume [1] were measured as described. One unit of enzyme activity corresponds to 1 μmol of product formed per min under the assay conditions.

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3. RESULTS

The activation of glycogen synthase that results from cell swelling induced by Na^+-transported amino acids or hypotonic media could be due to a decrease in the concentration of cytosolic Ca^{2+}. An increase in Ca^{2+} concentration as it occurs after treatment with hormones such as vasopressin or angiotensin II is indeed known to stimulate glycogen breakdown by activation of glycogen phosphorylase and inactivation of glycogen synthase [12]. One might expect that a decrease in the concentration of Ca^{2+} has the opposite effect and activates glycogen synthase as a result of a decreased inhibition of synthase phosphatase by Ca^{2+} [13,14].

The effect of amino acids and hypotonic media on the activation of glycogen phosphorylase by Ca^{2+}-dependent hormones such as vasopressin or angiotensin II was first studied. Hepatocytes were incubated for 30

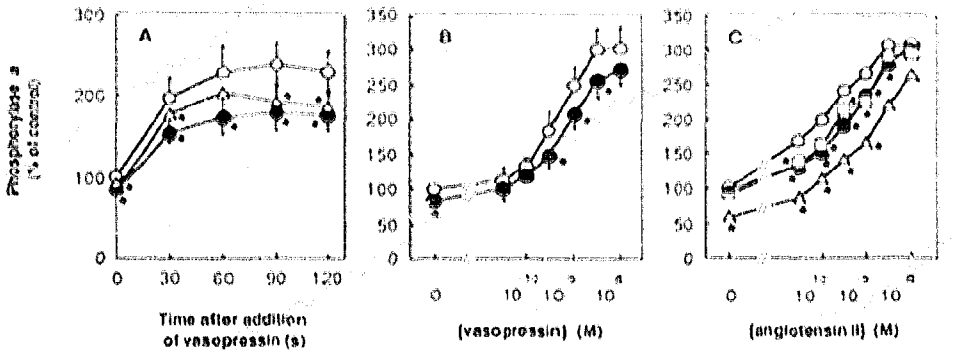

Fig. 1. Inhibition of phosphorylase activation by Ca^{2+}-dependent hormones in hepatocytes incubated with amino acids or in hypotonic medium. Hepatocytes were incubated for 30 min in the presence of 20 mM glucose and without (○) or with 10 mM glutamine (●), or 10 mM proline (□), or in a hypotonic medium (△) (85 mM Na^+) before the addition of the hormones. In (A), 0.56 nM vasopressin was added and samples were taken at the times indicated. In (B) and (C) various concentrations of vasopressin (B) or angiotensin II (C) were added and samples were taken after 2 min of incubation. Values of phosphorylase *a* in the absence of hormones were 2.36 ± 0.17 (A), 2.87 ± 0.42 (B) and 3.34 ± 0.35 (C) U/g of cells. The values are means ± SE for 4 (C) or 7 (A,B) different cells preparations.

* Significantly different ($P < 0.05$) from corresponding values in the absence of amino acids in a normal medium.

min with 20 mM glucose with or without 10 mM amino acid or in a medium made hypotonic by decreasing the Na^+ concentration from 145 to 85 mM; the hepatocytes were then challenged with the hormones (Fig. 1). The basal value of phosphorylase was, if anything, slightly decreased by amino acids or hypotonic media (Fig. 1 and Table I). The time-course of the effect of 0.56 nM vasopressin on phosphorylase activation (Fig. 1A) indicates that, in the presence of glutamine or in hypotonic media, the extent of phosphorylase activation was significantly decreased from 30 s and onwards. The dose-response curves of phosphorylase activation by vasopressin (Fig. 1B) or angiotensin II (Fig. 1C) measured at 2 min after the addition of the hormone also indicate that glutamine, proline or hypotonic media could antagonize the effect of suboptimal concentrations of the hormones. Thus hepatocyte swelling renders the cells less sensitive to these hormones and the concentrations of hormones required for half-maximal activation were at least doubled in swollen hepatocytes (Fig. 1B and C). The fact that a similar antagonism was observed when hepatocytes were challenged either with vasopressin or angiotensin II suggests that inhibition of hormone binding was probably not responsible for the inhibition of phosphorylase activation by glutamine. This conclusion is confirmed by the study of inositol 1,4,5-trisphosphate release (see below).

The experiments described above suggest that intracellular Ca^{2+} decreases in response to cell swelling. However, and quite unexpectedly, measurement of inositol 1,4,5-trisphosphate and Ca^{2+} showed that this was not the case. Inositol 1,4,5-trisphosphate is an intracellular signal, which is produced by phospholipase C from plasma membrane phosphatidylinositol-4,5-bis-

Table I

Cell volume, phosphorylase *a* activity, inositol 1,4,5-trisphosphate (IP_3) and cAMP content in hepatocytes incubated with amino acids or in hypotonic medium

Hepatocytes were incubated for 30 min with 20 mM glucose and with or without 10 mM amino acid in normal medium, or in the absence of amino acids in a medium made hypotonic (Hypo) by decreasing Na^+ from 145 to 85 mM. For inositol 1,4,5-trisphosphate measurement, the cells were preincubated for 90 min with 20 mM glucose and [2-^{3}H]inositol (10 μCi/ml) with or without 10 mM amino acid or in a hypotonic medium, and then incubated for 10 min under the same conditions but without radioactive inositol and with 10 mM LiCl. The values are means ± SE for the indicated number (*n*) of different cell preparations.

	Cell volume (% of control)	Phosphorylase *a* (U/g of cells)	IP_3 content (% of control)	cAMP content (nmol/g of cells)
Control	100 (4)	3.35 ± 0.39 (10)	100 (6)	0.47 ± 0.10 (4)
Gln	115.0 ± 2.1* (4)	3.60 ± 0.39 (10)	187 ± 30* (6)	0.66 ± 0.11* (4)
Pro	107.8 ± 0.4* (3)	3.07 ± 0.26 (5)	152 ± 13* (5)	0.61 ± 0.09* (4)
Leu	99.1 (2)	2.80 ± 0.90 (3)	107 ± 7 (4)	0.36 (2)
Hypo	116.2 ± 2.4* (3)	2.26 ± 0.28* (6)	117 ± 6* (5)	0.55 ± 0.08 (4)

* Significantly different ($P < 0.05$) from control values.

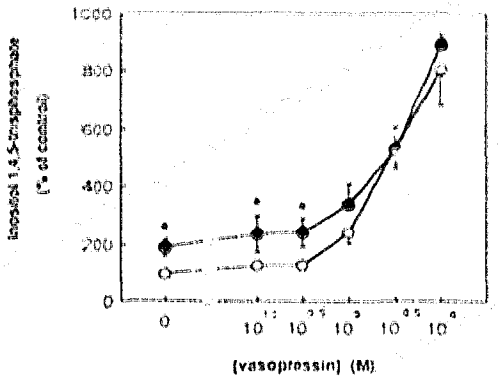

Fig. 2. Increase in inositol 1,4,5-trisphosphate formation in hepatocytes incubated with vasopressin and glutamine. After 90 min of pre-incubation with 20 mM glucose and [2-^{3}H]inositol (10 μCi/ml) in the absence (○) or in the presence (●) of 10 mM glutamine, hepatocytes were incubated for 10 min under the same conditions but without [2-^{3}H]inositol and with 10 mM LiCl before being further incubated for 5 min with the indicated concentrations of vasopressin. The values are means ± SE for 6 different cell preparations. * Significantly different ($P < 0.05$) from the corresponding values in the absence of glutamine.

phosphate, and which mobilizes Ca^{2+} from the endoplasmic reticulum [15,16]. The formation of radioactive inositol 1,4,5-trisphosphate was measured in hepatocytes which had been pre-incubated with radioactive inositol to label the phosphoinositide pool [10]. Fig. 2 shows that, in the absence of vasopressin, inositol 1,4,5-trisphosphate content in glutamine-treated cells was 2-fold higher than in control cells, and that the difference persisted in hepatocytes treated with suboptimal doses of vasopressin. The difference in inositol 1,4,5-trisphosphate formation was confirmed by measurement of inositol 1,4,5-trisphosphate concentration with a different method based on a binding assay (results not shown). The results presented in Fig. 2 also demonstrate that inositol 1,4,5-trisphosphate formation was not impaired in glutamine-treated cells suggesting that hormone-binding and subsequent signal transduction were unaffected by glutamine.

Cell volume, phosphorylase activity, inositol 1,4,5-trisphosphate formation, and cAMP concentration were measured in hepatocytes incubated either with glutamine or proline, or in hypotonic media (Table I). Incubation of hepatocytes in the presence of leucine was taken as a control since it is known that this amino acid is not transported via a Na^+-dependent system and that it does not increase cell volume and thus does not stimulate glycogen synthesis [1]. Table I confirms the increase in inositol 1,4,5-trisphosphate in hepatocytes treated with glutamine and shows a similar effect with proline but a smaller increase when hepatocytes were incubated in hypotonic media although the change in volume was similar to that obtained with glutamine (Table I). The table further shows that the increase in inositol 1,4,5-trisphosphate concentration was not accompanied by an activation of phosphorylase. As expected, leucine was without effect.

Intracellular Ca^{2+} was measured in control and glutamine-treated cells using Quin-2, a fluorescent probe. Ca^{2+} increased from 210 ± 16 nM ($n = 6$) in the absence of glutamine to 496 ± 45 nM ($n = 6$) in its presence ($P < 0.05$) as expected from the measured increase in inositol 1,4,5-trisphosphate concentration.

Moreover and in agreement with results published in the literature [3,7], we also found that swelling resulting from glutamine or proline treatment induced a 30–40% increase in cAMP concentration (Table I). The difference, however, was not significant in hepatocytes incubated in hypotonic medium. Again it is remarkable that the increase in the concentration of this glycogenolytic signal did not result in phosphorylase activation.

4. DISCUSSION

From our results it appears that cell swelling increases cAMP and Ca^{2+} concentration. The latter probably comes from the endoplasmic reticulum stores since swelling also increased inositol 1,4,5-trisphosphate concentration. Previous observations showed that a rise of intracellular Ca^{2+} as well as an increase in cAMP concentration were associated with cell swelling in various types of cells such as dissociated epithelial cells [4], lymphoma cells [3,7] and pituitary tumor-derived cells [5].

In swollen hepatocytes, the rise in Ca^{2+} and cAMP did not result in phosphorylase activation. Moreover, the activation of phosphorylase brought about by suboptimal concentrations of vasopressin or angiotensin II was even partly antagonized. This suggests that cell swelling induces the formation of a compound or factor which is able to overcome, at least in part, the effect of Ca^{2+} and cAMP on phosphorylase activation. Whether the antagonism occurs at the level of Ca^{2+}/calmodulin or phosphorylase kinase activity itself is not known. One may also speculate that this swelling-induced compound should also be able to stimulate synthase phosphatase in order to activate synthase and stimulate glycogen synthesis.

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