Stimulatory effect of the intestinal peptide PHI on glycogenolysis and gluconeogenesis in isolated rat hepatocytes

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The newly isolated peptide PHI provoked a dose-dependent stimulation of glycogenolysis and gluconeogenesis in isolated rat hepatocytes; at $1\text{ }\mu\text{M}$ -PHI, both processes were increased 1.6-fold as compared with basal values. These PHI-mediated effects were accompanied by the activation of glycogen phosphorylase and the inactivation of pyruvate kinase. PHI ($1\text{ }\mu\text{M}$) also caused a 2-fold increase in hepatocyte cyclic AMP.

PHI is a novel peptide isolated by Tatemoto & Mutt (1980) from pig upper intestinal tissue. It consists of 27 amino acid residues, whose sequence is markedly homologous to those of VIP, secretin, glucagon and gastric inhibitory peptide (Tatemoto & Mutt, 1981).

Concerning the biological actions of PHI *in vivo* (Dimaline & Dockray, 1980) and *in vitro* (Jensen *et al.*, 1981), studies have shown that it stimulates exocrine pancreatic secretion, as well as release of insulin and glucagon (Szecowska *et al.*, 1980). It has been demonstrated that PHI increases the cyclic AMP content of fat-cells, intestinal epithelial cells, gastric glands and pancreatic acini (Bataille *et al.*, 1980; Jensen *et al.*, 1981). Furthermore, this peptide activates adenylate cyclase of rat liver membranes, apparently by interacting with VIP receptors (Bataille *et al.*, 1980).

Since PHI is considered a new member of the glucagon–secretin family (Tatemoto & Mutt, 1981), we have investigated its possible influence on glucose production in isolated rat hepatocytes. For this purpose, we have studied the effect of PHI on glycogenolysis and gluconeogenesis, as well as on glycogen phosphorylase *a* and pyruvate kinase activities, enzymes respectively implicated in the short-term hormonal regulation of the above-mentioned pathways (Hers, 1976; Felíu *et al.*, 1976). In addition, the influence of PHI on hepatocyte cyclic AMP concentrations was examined.

Experimental

Materials

Natural pig PHI was kindly provided by Dr. V. Mutt and Dr. K. Tatemoto. It contains no measur-

Abbreviations used: PHI, peptide (P) having N-terminal histidine (H) and C-terminal isoleucine (I) amide; VIP, vasoactive intestinal peptide.

able glucagon and less than 0.02% VIP (K. Tatemoto, personal communication). Synthetic pig VIP was obtained from Peninsula Laboratories (San Carlos, CA, U.S.A.). Ox/pig glucagon was purchased from Novo Industri A/S (Copenhagen, Denmark). [^{14}C]Pyruvate was supplied by The Radiochemical Centre (Amersham, Bucks., U.K.). The remaining reagents were of analytical grade from Boehringer Mannheim G.m.b.H. (Mannheim, Germany), Merck (Darmstadt, Germany) and Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Methods

Fed male Wistar rats weighing 200–300 g were used. Hepatocytes were isolated by perfusion of the liver with collagenase, as described by Felíu *et al.* (1976). The cells were incubated in Krebs–Henseleit bicarbonate medium containing bacitracin (1 mg/ml) in order to retard the degradation of hormones (Desbuquois, 1974), and also 10 mM-glucose. The viability of the isolated hepatocytes was evaluated by the Trypan Blue test; usually 90–95% of the cells excluded the stain. PHI, VIP, glucagon, saline and [^{14}C]pyruvate were added to hepatocytes after 30 min preincubation of the cell suspension.

Gluconeogenesis was measured by the conversion of 2 mM-[^{14}C]pyruvate (0.25 Ci/mol) into glucose (Felíu *et al.*, 1982). Glycogenolysis was evaluated by the rate of net glucose production (Felíu *et al.*, 1982); in the later experiments the incubation medium contained 2 mM-glucose, with no addition of gluconeogenic precursor.

For the determination of enzyme activities, 0.1 ml samples of the incubated cell suspension were taken 5 min after addition of hormones and subsequently frozen in liquid N_2 . Glycogen phosphorylase *a* (EC 2.4.1.1) and pyruvate kinase (EC 2.7.1.40) activities were assayed as described previously (Felíu *et al.*,

1976; Hue *et al.*, 1978). One unit of enzymic activity is defined as the amount of enzyme that transforms 1 μmol of substrate/min under the conditions of the assay.

Cyclic AMP was determined in cells plus medium by using a radioimmunological method [Cyclic AMP (^{125}I)RIA Kit, from New England Nuclear, Boston, MA, U.S.A.] (Feliu *et al.*, 1983).

Data presentation

Data represent the means ($\pm$ S.E.M.) of duplicate cell incubations from four or five cell preparations. The paired Student's *t* test was applied for statistical analysis.

Results and discussion

As shown in Fig. 1(a), PHI stimulated the rate of hepatic glycogenolysis in a dose-dependent manner; at 1 μM , the highest PHI concentration tested, glycogenolysis was increased 1.7-fold as compared with the basal value ($P < 0.05$). In parallel incubations, VIP and glucagon (0.1 μM) augmented glucose production 1.6- and 3-fold respectively

($P < 0.02$). Fig. 1(b) demonstrates that the stimulation of glycogenolysis by PHI correlated with the activation of glycogen phosphorylase ($P < 0.02$). VIP and glucagon (each 0.1 μM) raised the basal activity of this enzyme 1.8- and 4.8-fold respectively ($P < 0.01$).

Fig. 2 shows that PHI stimulated gluconeogenesis from pyruvate in a dose-dependent manner and, simultaneously, inactivated pyruvate kinase. At 1 μM -PHI, the basal gluconeogenic flux was increased 1.6-fold ($P < 0.01$) and pyruvate kinase activity was decreased by 30% ($P < 0.01$). VIP (0.1 μM) stimulated gluconeogenesis 1.8-fold ($P < 0.01$) and caused a 30% inactivation of pyruvate kinase ($P < 0.001$). Glucagon, at the same concentration, induced much more marked effects on the above-mentioned parameters: 4.4-fold increase of gluconeogenesis and 80% inactivation of pyruvate kinase.

The stimulatory effect of PHI on hepatocyte glucose output cannot be attributed to the VIP

Fig. 1. Effect of different concentrations of PHI (●) on the rate of glycogenolysis (a) and on glycogen phosphorylase activity (b) in isolated rat hepatocytes. Cell incubations were performed as indicated in the Experimental section. ■, Hepatocytes incubated in the presence of 0.1 μM -VIP; ▲, hepatocytes incubated in the presence of 0.1 μM -glucagon.

Fig. 2. Effect of different concentrations of PHI (●) on the rate of gluconeogenesis (a) and on pyruvate kinase activity (b) in isolated rat hepatocytes. Cell incubations were performed as indicated in the Experimental section. ■, Hepatocytes incubated in the presence of 0.1 μM -VIP; ▲, hepatocytes incubated in the presence of 0.1 μM -glucagon.

content (less than 0.02%) of the PHI preparation that we used. Even at the highest PHI concentration tested ($1\mu\text{M}$), the contaminating VIP is insufficient to stimulate either glycogenolysis or gluconeogenesis in

isolated rat hepatocytes (Souquet *et al.*, 1982; Feliu *et al.*, 1983).

Fig. 3 describes the time-course effect of equimolar concentrations ($1\mu\text{M}$) of PHI, VIP and glucagon on

Fig. 3. Time-course effect of equimolar concentrations ($1\mu\text{M}$) of PHI (●), VIP (■) and glucagon (▲) on cyclic AMP concentrations in isolated rat hepatocytes
Cell incubations were performed as indicated in the Experimental section. ○, Hepatocytes incubated without any hormone.

glucagon on cyclic AMP concentrations in isolated hepatocytes. At all times tested, these three peptides induced significant increases in cell cyclic AMP content, compared with basal values ($P < 0.05$). Maximal effects were obtained 4 min after peptide addition. At this incubation time, PHI, VIP and glucagon provoked 2-, 3- and 23-fold increases of basal cyclic AMP concentrations respectively.

The relative high concentration of PHI needed to obtain significant effects on liver cells, together with the small stimulation of hepatic glucose output given by this peptide as compared with glucagon, suggest that PHI does not play a physiological role on hepatic glucose metabolism. This suggestion is reinforced by the apparent lack of PHI-specific receptors in liver cell membranes (Bataille *et al.*, 1980). However, definitive evaluation of the physiological sense of our findings must await information about plasma concentrations and tissue distribution of PHI.

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