Effects of Vasoactive Intestinal Peptide on Glycogenolysis in Cultured Liver Cells

MITSUHIRO MATSUMURA, HIROTO AKIYOSHI*, SHIRO SAITO**,
AND HIROYOSHI MORI

*Department of the Second Internal Medicine, School of Medicine,
Tokushima University, Kuramoto-cho, Tokushima, Tokushima 770*

Synopsis

When isolated rat liver cells were incubated in the presence of vasoactive intestinal peptide at the concentrations ranging from 0.2 μg to 2 μg per ml, glycogenolysis was maximally stimulated within 15 min. However, somatostatin inhibited the liver glycogenolysis. The combined addition to the incubation medium showed that insulin and somatostatin inhibited the stimulated glycogenolysis induced by vasoactive intestinal peptide, while vasoactive intestinal peptide plus secretin showed no additive effect on glycogenolysis, as compared with single the addition of vasoactive intestinal peptide. On the other hand, the addition of glucagon to vasoactive intestinal peptide showed additive effects on glycogenolysis. These results suggest that the receptor site for vasoactive intestinal peptide may be distinguishable from that for glucagon. Extracellular calcium ions were demonstrated to play an important role in the modulation of vasoactive intestinal peptide-induced glycogenolysis.

The evidence presented in this paper indicates that glucose metabolism may be partly regulated by the direct action of vasoactive intestinal peptide on hepatocytes, which is referred to as an enterohepatic axis and that the axis is inhibited by insulin and somatostatin.

Vasoactive intestinal peptide (VIP) was isolated from the porcine small intestine (Said and Mutt, 1970) and shown to have an amino acid sequence related to that of secretin, glucagon and gastric inhibitory polypeptide (Grossman, 1974). VIP has a wide spectrum of biological activities, including a positive inotropic action on the heart (Said *et al.*, 1972), smooth muscle relaxation (Piper *et al.*, 1970), stimulation of pancreatic secretion (Said and Mutt, 1972), inhibition of gastric secretion (Schorr *et al.*, 1974), lipolysis (Frandsen and

Moody, 1973) and glycogenolysis (Kerins and Said, 1973). With respect to carbohydrate metabolism, it has been reported that VIP elevates serum glucose levels by inducing insulin and glucagon release through the enteroinsular axis (Unger *et al.*, 1976) and stimulates glycogenolysis in rabbit liver slices (Kerins and Said, 1973).

Desbuquois *et al.* (1973) and Bataille *et al.* (1974) demonstrated that the specific receptor sites for VIP and secretin were identical and located on liver cell membranes, but differed from those for glucagon. Although this receptor has not been found in the isolated hepatocytes, these findings suggest that VIP and secretin may have some direct effects on the glucose metabolism in hepatocytes.

The results of the present work has raised

Received August 9, 1978.

* Department of Enzyme Physiology, Institute for Enzyme Research, School of Medicine, Tokushima University, Tokushima 770.

** Department of Laboratory Medicine, School of Medicine, Tokushima University, Tokushima 770.

a possibility that serum glucose levels are not only regulated by the enteroinsular axis, but also by the enterohepatic axis through direct actions of VIP, secretin and glucagon on glucose synthesis in the liver. This paper also demonstrates results which suggest that the biological receptor site for VIP may be distinguished from that for glucagon and that calcium ions may play an important role in VIP-induced glycogenolysis.

Materials and Methods

Materials

Male rats of the Wistar-King strain, weighing ca. 250 g, were used for the preparation of single liver cells. Glucagon and collagenase were obtained from Sigma Chemical Co., U.S.A. Synthetic materials such as vasoactive intestinal peptide (VIP) and secretin were the generous gifts from Prof. N. Yanaihara, Shizuoka College of Pharmacy, Shizuoka. Synthetic somatostatin was purchased from the Protein Research Foundation, Osaka.

Methods

Hepatocytes were isolated from the liver of well-fed rats by the method of Berry and Friend (1969) with some modifications. The rat liver was perfused through the portal vein with 50 ml of Hanks solution. Then the liver was removed, cut into slices with a razor in a cold room and suspended in 15 ml of digestive solution composed of 0.05 g/ml of collagenase and 2 g/ml of bovine albumin in calcium-free Hanks solution containing 10 mM Hepes (pH 8.0). The suspension was incubated in a 200 ml Erlenmeyer flask for 30 min at 37° under 95% O₂ and 5% CO₂ with constant shaking. After incubation the detached cells were collected and centrifuged at 50×g for 30 s. Hepatocytes in the pellet were washed twice with Hanks solution and finally suspended so as to have the cell density of 2×10⁶/ml in Krebs-Ringer bicarbonate buffer. Cell viability was monitored by the trypan blue staining of the dead cells.

For the study of glycogenolysis, the cell suspension (1 ml) was incubated at 37° for the indicated period with constant and mild shaking with or without the addition of various hormones. After incubation the cell suspension was sonicated to disrupt cell membranes. Glucose content of the reaction mixture was measured by the glucose oxidation method. Values in the tables indicate the means ±S.E.M. of the four experiments. The data were

analysed by the Student's *t* test, the criterion for significance being $p < 0.05$ (Hill, 1967).

Results

Time course of glycogenolysis induced by vasoactive intestinal peptide in cultured liver cells

As shown in Fig. 1, the liver glycogenolysis reached a maximum level about 15 min after incubation with 2.0 µg/ml of VIP. Therefore, the incubation time of 15 min was adopted for the further experiments.

glycogenolysis in response to VIP doses in cultured liver cells

When liver cells were incubated for 15 min in the presence of VIP, the increases of glucose production were observed (Table 1). VIP showed a stimulatory effect on glycogenolysis at the concentrations ranging from 0.2 µg/ml to 2.0 µg/ml with the increase rates of 20 to 67%.

Fig. 1. Time course of glycogenolysis induced by vasoactive intestinal peptide in cultured liver cells.

Additive effects of VIP, secretin, and glucagon on glycogenolysis

As shown in Table 2, the single addition of 0.2 $\mu\text{g/ml}$ VIP, 2.0 $\mu\text{g/ml}$ secretin, and 0.2 $\mu\text{g/ml}$ glucagon stimulated glycogenolysis by 29%, 30% and 45%, respectively. On the other hand, a combination of the same concentrations of VIP and secretin caused a 34% increase in glucose synthesis. A combination of VIP and glucagon at the same concentrations caused a 69% increase.

Effects of insulin and somatostatin on VIP-induced glycogenolysis

Table 3 shows that somatostatin alone was inhibitory for glycogenolysis and that slight but significant suppression of VIP-induced glycogenolysis was also observed with addition of somatostatin (about 13% inhibition). Insulin was found to be a strong inhibitor for both basal and VIP-induced glycogenolysis.

Calcium ion dependency of VIP-induced glycogenolysis

We examined the effect of Verapamil, an inhibitor of Ca^{2+} transport (Davis *et al.*, 1975), on VIP-induced glycogenolysis. As shown in Table 4, glycogenolytic activity of VIP in the presence of Ca^{2+} was 33%, whereas the activity did not appear in the absence of the ions. The glycogenolytic activity evoked by calcium ions was suppressed with the addition of Verapamil or EDTA.

Table 1. Stimulation of glycogenolysis in response to VIP doses in cultured liver cells

VIP concentration ($\mu\text{g/ml}$)	Increase in glucose production (%)
0	100 $\pm$ 2
0.2	120 $\pm$ 10
1.0	132 $\pm$ 6
2.0	167 $\pm$ 6

2.2 μmoles of glucose were produced without the addition of VIP, and taken as 100%. In all cases $p < 0.001$.

Table 2. Combination effects of VIP, secretin and glucagon on glycogenolysis in cultured liver cells

Exp.	Addition	Concentration ($\mu\text{g/ml}$)	Increase in glucose production (%)
I.	None		100 $\pm$ 3
	Glucagon	0.2	141 $\pm$ 4
	VIP	0.2	129 $\pm$ 7
	VIP plus glucagon		169 $\pm$ 6
II.	None		100 $\pm$ 1
	VIP	0.2	120 $\pm$ 2
	Secretin	2.0	130 $\pm$ 4
	VIP plus secretin		134 $\pm$ 4

A rate of 100% indicates the amount of glucose produced in the absence of the hormones, that is, 2.4 μmoles in Exp. I and 2.6 μmoles in Exp. II ($p < 0.001$ in all cases).

Table 3. Effects of insulin and somatostatin on VIP-induced glycogenolysis in cultured liver cells

Exp.	Addition	Increase in glucose production (%)
I.	None	100 $\pm$ 1
	VIP (1 $\mu\text{g/ml}$)	133 $\pm$ 10 ($p < 0.001$)
	VIP plus insulin (200 $\mu\text{U/ml}$)	89 $\pm$ 6
II.	None	100 $\pm$ 2
	Somatostatin (2 $\mu\text{g/ml}$)	88 $\pm$ 7
	VIP (2.0 $\mu\text{g/ml}$)	167 $\pm$ 6
	VIP plus somatostatin	154 $\pm$ 3 ($p < 0.05$)

The basal amounts of glucose produced in the absence of the hormones (100%) were 2.9 μmoles in Exp. I and 2.2 μmoles in Exp. II.

Table 4. Calcium ion dependency of VIP-induced glycogenolysis in cultured liver cells

Addition	Increase in glucose production (%)
None	100 $\pm$ 2
VIP 1.0 $\mu\text{g/ml}$	133 $\pm$ 10
VIP plus Verapamil (1 mM)	102 $\pm$ 1
VIP (minus Ca^{2+})	94 $\pm$ 8
VIP plus EDTA (4 mM)	100 $\pm$ 2

(minus Ca^{2+}) indicates omission of CaCl_2 from the reaction mixture.

Discussion

VIP is a highly basic peptide which resembles secretin, glucagon, and gastric inhibitory polypeptide in the amino acid sequence and biological actions, thus these hormones have been classified into the secretin-glucagon family (Grossman, 1974). With respect to the effects of VIP on carbohydrate metabolism, it has been reported that VIP had effects on serum glucose levels by inducing either insulin or glucagon release *in vivo* (Schebalin *et al.*, 1974). This mechanism is consistent with the findings formed as the enteroinsular axis by Unger *et al.* (1967). Kerins and Said (1973) first reported that VIP had a stimulatory effect on glycogenolysis in *in vitro* experiments. The present study showed that VIP maximally stimulated glucose synthesis in cultured liver cells within 15 min, but that insulin and somatostatin inhibited VIP-induced glycogenolysis. Since VIP stimulated glucose production in hepatocytes, it might be considered that blood glucose levels were influenced at least in part by a direct action of VIP on liver cells in addition to the enteroinsular axis. This effect could be referred to as the entero-hepatic axis.

It has been reported that VIP activates adenylyl cyclase (Desbuquois *et al.*, 1973), and thus subsequently synthesized cyclic AMP may act as a second messenger in VIP-mediated glycogenolysis. Receptor sites for VIP on liver cell membranes were found to be identical with those for secretin but different from those for glucagon (Desbuquois *et al.*, 1973; Bataille *et al.*, 1974). As shown in Table 2, the combined use of VIP and glucagon indicates additive effect on glycogenolysis, but no additive effect was observed in the combined use of VIP and secretin. These findings may support the results that VIP and secretin have the same receptor sites but differ

from that for glucagon.

VIP stimulates insulin secretion (Ohneda *et al.*, 1977) and insulin concentration in the portal vein was found to be twenty-fold higher than in the peripheral vein (Blackard and Nelson, 1970). The results shown in Table 3 suggest that the decrease of glucose levels after the addition of insulin is partly caused by the inhibitory action of insulin on the VIP effect. Table 3 also demonstrates that somatostatin was inhibitory for both basal and VIP-induced glycogenolysis. Since somatostatin concentration in the portal vein was three-fold higher than in the peripheral vein (Arimura, 1978), it is also suggested that the decrease of serum glucose levels after addition of somatostatin was caused by the direct inhibitory effect of somatostatin on the liver glycogenolysis.

Verapamil is thought to block the channels responsible for the calcium influx into myocardial and smooth muscle cells (Devis *et al.*, 1975). The present work shows that VIP-induced glycogenolysis was suppressed with addition of Verapamil or with the removal of calcium ions. This indicated that extracellular Ca^{2+} may play an important role in the modulation of VIP-induced glycogenolysis.

Acknowledgement

We are indebted to Prof. N. Yanaihara for the gifts of synthetic VIP and secretion.

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