Effects of VIP on glucose and lactate metabolism in isolated rat liver cells

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1. INTRODUCTION

Vasoactive intestinal polypeptide (VIP) has been shown to exert a hyperglycemic effect in vivo and to induce glucogenolysis in rabbit liver slices [1] but the biochemical evidence supporting an effect of VIP on glucose metabolism in liver cells is scarce [2–3] except for a recently described effect [4]. This effect is thought to be cyclic adenosine monophosphate (cAMP)-dependent since numerous studies have shown that VIP can stimulate rat liver adenylate cyclase through the interaction with a specific membrane receptor [5–8]. VIP seems to share this membrane binding site with secretin but not with glucagon [6] or gastric inhibitory polypeptide (GIP) [3]. Although the VIP effect on membrane adenylate cyclase was clearly documented, the action of VIP on cAMP levels in isolated liver cells is still unclear [4,8]. The aim of this study was to systematically investigate the actions and mechanism of action of VIP, secretin and GIP, three glucagon-related polypeptides [9], on glucose and lactate metabolism in isolated rat liver cells and to compare them to the known, cAMP-mediated, effects of glucagon.

2. MATERIALS AND METHODS

Porcine VIP and secretin were obtained from Professor V. Mutt (Karolinska Institut, Stockholm) and porcine GIP from Professor J.C. Brown (Vancouver). Glucagon, bacitracin, bovine serum albumin (fraction V), histone 2b, and protein kinase were from Sigma, other enzymes and coenzymes from Boehringer, Mannheim. [γ-^{32}P]adenosine triphosphate and cyclic [^{3}H]AMP were obtained from Amersham.

Isolated liver cells were prepared from adult male rat (180–250 g) with the method of Berry and Friend [10] as described in [11]. One ml liver cells (8–15 mg/ml wet weight, 120 000 cells/mg wet weight) were distributed in 20 ml cylindrical tubes, continuously shaken and gassed (O_2 95%, CO_2 5%) and incubated with or without hormone in a Krebs–Ringer bicarbonate buffer containing albumin 1% and bacitracin 0.1% (w/v). Metabolites were measured according to enzymatic procedures in [12]. Pyruvate kinase was assayed spectrophotometrically with the method of Llorente [13] as described in [11] in 60% ammonium sulfate cell extract prepared by the method in [14]. cAMP-dependent protein kinase activity was measured in a 10 000 × *g* supernatant cell extract after homogenizing the liver cells as described in [15]. The assay was performed as described in [16] using histone 2b as substrate. The transfer of [γ-^{32}P]phosphate from adenosine triphosphate (spec. act. 30–40 cpm/pmol) on histone was measured during an incubation of 6 min at 30°C with or without 5 μM cAMP. One unit of activity is the amount of enzyme which catalyzes the transfer of 1 pmol of phosphate on histone per min at 30°C. The protein kinase activity ratio was calculated as activity minus cAMP/activity plus cAMP [15]. Protein concentration was measured with the method of Lowry [17] using bovine serum albumin as stan-

Abbreviations: VIP, Vasoactive intestinal polypeptide; GIP, gastric inhibitory polypeptide; cAMP, 3′,5′-cyclic adenosine monophosphate

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dard. cAMP was determinated by the protein kinase binding assay as described in [18]. Aliquots (1 ml) of cell suspension (35–50 mg cells/ml) were incubated as indicated in the presence of 1 mM theophylline for 3 min with or without hormone. The incubation was stopped with 500 μl of 15% cold trichloracetic acid. The supernatants were purified on Dowex AG 50 W-X4 (200–400 mesh hydrogen/vrm) as described in [19] ([^{3}H]cAMP (8000 cpm) was added to each sample in order to estimate the recovery of cAMP).

All experiments, performed in triplicate, were repeated at least three times with different cell batches in order to ensure that the results were reproducible. All the data are presented as the mean ± SD or SEM. Student's test was used for statistical analysis.

3. RESULTS

The effects of GIP, secretin, VIP and glucagon on glucose and lactate production were first measured in liver cells from fed rats (fig.1). Neither GIP nor secretin affected these metabolic events. Many attempts to unmask a biological effect of these peptides were unsuccessful (data not shown). Only VIP and glucagon significantly affected both glucose and lactate production. The effects of VIP were additive with a submaximal dose of glucagon (fig.1) but not with the maximal effective dose of glucagon (data not shown). Fig.2 shows that the effect of VIP on glucose production was dose-dependent and the half maximum effect of the hormone occured at about 2.10^{-8} M. The inset shows that glucagon was more potent than VIP and acted at a much lower concentration (half maximum stimulation 3.10^{-11} M). Fig.3 shows that the effect of VIP could be detected as soon as 3 min after hormone addition and persisted linearly for at least 20 min. In cells from 24 h-fasted rats, VIP increased gluconeogenesis from lactate or dihydroxyacetone, but decreased it from 10 mM pyruvate, as did glucagon (table 1). These effects were small but reproducible and statistically significant.

This glucagon-like effect of VIP on glucose pro-

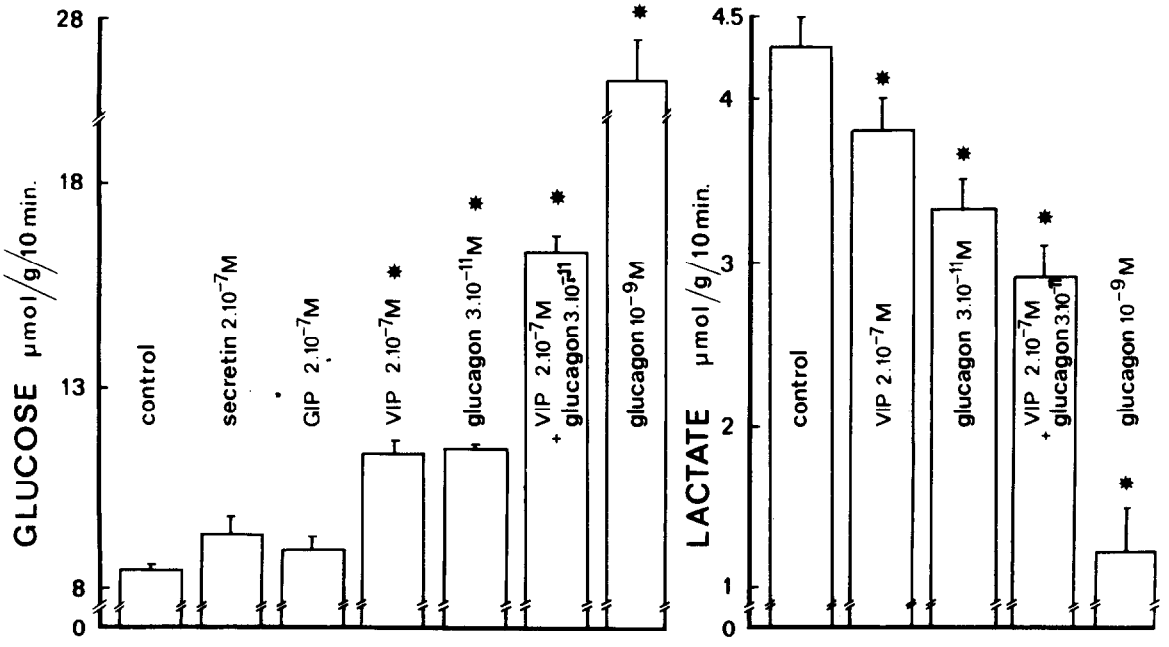

Fig.1. Effects of glucagon and glucagon-related polypeptides on glucose and lactate production ($\bar{x}$ ± SD, $n=3$) in isolated liver cells from fed rats. Cells were incubated for 10 min as indicated in section 2 and glucose and lactate were measured in cell extract as indicated. *$P<0.01$.

Table 1

Effects of VIP (10^{-7} M) and glucagon (10^{-9} M) on gluconeogenesis

	Lactate 10 mM	DHA 10 mM	Pyruvate 10 mM
Control	13.0±0.1	20.4±0.2	11.6±0.2
VIP 10^{-7} M	14.5±0.2*	22.8±0.1*	10.9±0.3*
Gluc 10^{-9} M	16.8±0.2*	27.2±0.3*	6.9±0.2*

* $P<0.05$

Cells from 24 h-fasted rats were incubated for 20 min with 10 mM lactate, pyruvate or dihydroxyacetone. Glucose production was expressed as μmol/g of cells/20 min ($\bar{x}$ ± SD, $n=4$)

duction in cells from fed as well as fasted rats was also substantiated by the measurement of some intermediary metabolites in cells from fed rats incubated with 5 mM dihydroxyacetone (table 2). VIP addition, to a lesser extent than glucagon, increased glucose-6-phosphate, fructose-6-phosphate, and phosphoenol pyruvate and decreased fructose-1,6-diphosphate and pyruvate concentrations. This profile of the amounts of glycolytic intermediates suggests an effect of VIP at both the Fru. 6 P⇌Fru. 1–6 P and P-enolpyruvate⇌pyruvate steps. The hypothesis that VIP was actually

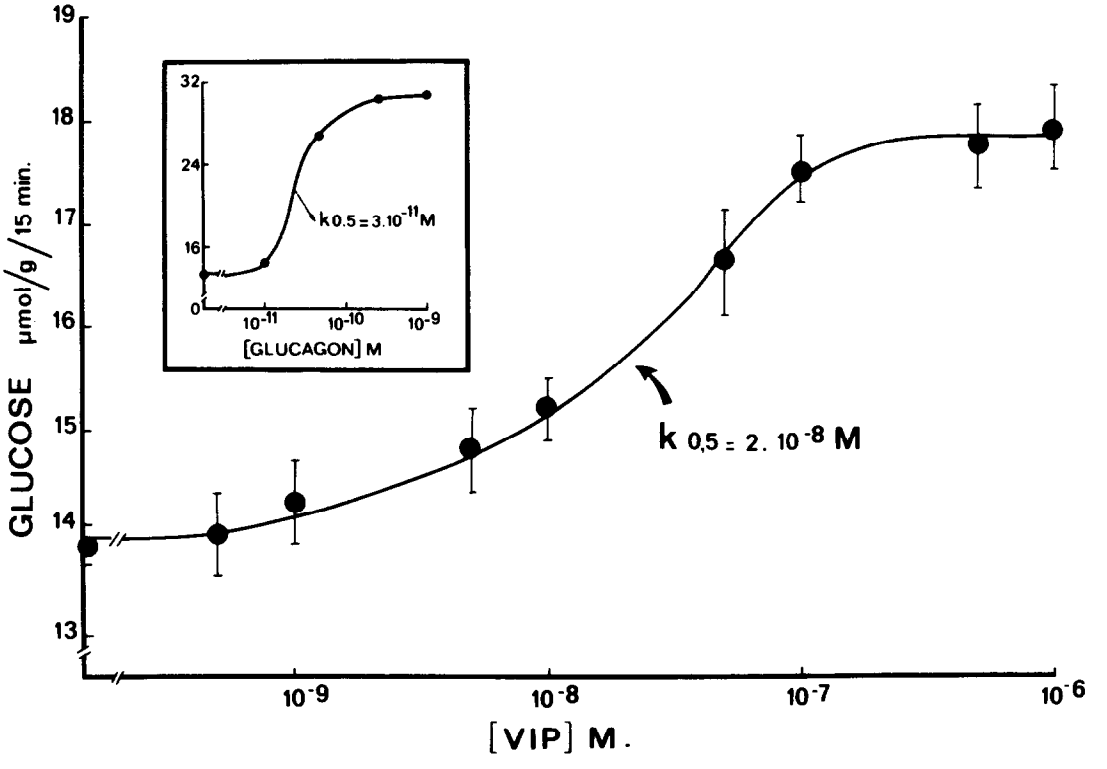

Fig.2. Dose–response curve of the glucose production induced by VIP and glucagon (inset). Cells from fed rats were incubated for 15 min as indicated in section 2 ($\bar{x}$ ± SD, $n=6$).

Table 2

Effects of VIP (10^{-6} M) and glucagon (10^{-9} M) on some intermediary metabolites

	ATP μmol/g	G6P nmol/g	F6P nmol/g	F-1,6-P nmol/g	DHAP nmol/g	PEP nmol/g	Pyruvate μmol/g
Control	2.0±0.1	126±13	51±8	39±1	181±18	138±10	5.4±0.2
VIP	2.0±0.1	176±13*	80±6*	34±3	174±4	161±12*	4.5±0.1*
Glucagon	2.0±0.1	370±27*	133±9*	26±5*	178±12	201±22*	3.9±0.1*

* $p<0.05$

Liver cells (50 mg/ml) from fed rats were incubated as indicated with 5 mM dihydroxyacetone for 8 min with or without hormones ($\bar{x}$ ± SEM, $n=3$).

Abbreviations: G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; F-1,6-P, fructose-1,6-diphosphate; DHAP, dihydroxyacetone phosphate; PEP, phosphoenol pyruvate.

Table 3

Effects of VIP (10^{-6} M) and glucagon (10^{-9} M) on cAMP level and cAMP-dependent protein kinase activity.

	cAMP (nmol/g	cAMP-dependent protein kinase (U/mg protein)		cAMP-dependent protein kinase activity ratio – cAMP/ + cAMP
		– cAMP	+ cAMP (5 μM)	
Control	0.53±0.09	96±8	313± 7	0.31±0.05
VIP 10^{-6} M	1.64±0.43	137±6	294±11	0.47±0.02
Glucagon 10^{-9} M	14.6 ±0.6	216±8	256±15	0.85±0.04

cAMP was measured as indicated in purified trichloroacetic acid extracts from cells from fed rats incubated for 3 min with or without hormone. Protein kinase activity was measured as indicated within 30 min after homogeneizing the cell pellet. Liver cells (50 mg/ml) were incubated with or without hormone as indicated, for 8 min. Results are expressed as $\bar{x}$ ± SEM of triplicate determination in 3 different cell batches ($n=3$).

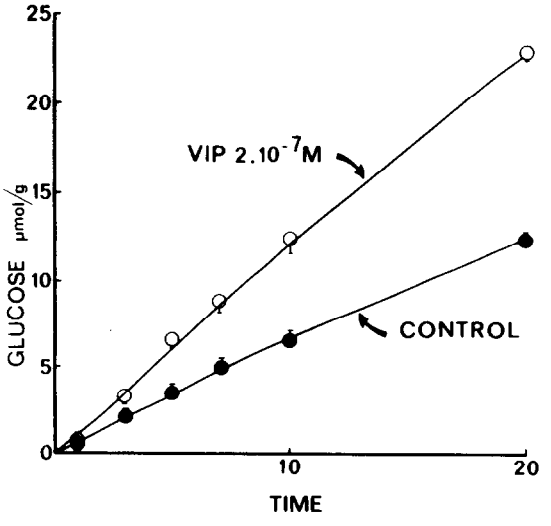

Fig.3. Time course of VIP action on glucose production in cells from fed rats. Liver cells were incubated in 125 ml conical flask in the buffer indicated in section 2 and 1 ml aliquots were sampled at the indicated time for glucose measurement ($\bar{x}$ ± SD, $n=3$).

acting at the P-enolpyruvate ⇌ pyruvate step was tested by measuring the kinetics of L-pyruvate kinase, which catalyzes this metabolic event. The app. $K_{0.5}$ of P-enolpyruvate for L-pyruvate kinase was significantly increased by VIP (2.10^{-7} M) glucagon (10^{-9} M) addition ($K_{0.5}$ PEP mM $\bar{x}$ ± SD: control 0.40 ± 0.02, n = 10; VIP 0.55 ± 0.02, n = 3; glucagon 0.82 ± 0.05, n = 10). L-Pyruvate kinase has been shown to be regulated by a cAMP-dependent phosphorylation mechanism [11,20].

Therefore the VIP-promoted inhibition of L-pyruvate kinase strongly suggests that VIP acts by increasing cAMP levels. This hypothesis was tested by measuring both the cAMP level and the cAMP-dependent protein kinase activity ratio in extracts of liver cells incubated with and without hormones. Table 3 shows that VIP addition slightly increased the cAMP level, although the difference was not statistically significant. The reality of a slight rise in cAMP was confirmed by a significant increase in the cAMP-dependent protein kinase activity ratio which has been shown to be an accurate stimulation of small changes in cAMP level [15]. The effect of glucagon on both parameters was much more pronounced.

4. DISCUSSION

The present report clearly confirms that glucose and lactate metabolism are strongly modified by VIP but not by GIP and secretin which also belong to the glucagon polypeptide family [9]. The stimulation of glucose production in cells from fed rats was dose- and time-dependent (figs. 2,3). It can be related to the stimulation of glycogenolysis since VIP activates phosphorylase activity in rat liver cells [4]. The inhibition of lactate production (fig.1) induced by VIP can be related, at least in part, to an inhibition of L-pyruvate kinase. Effectively, the enzyme activity was decreased by VIP when measured at physiological substrate concentration while lactate and pyruvate concentrations were reduced and phosphoenolpyruvate increased by VIP addition (table 2). VIP also affects glucose metabolism in cells from 24 h-fasted rats. Although small (table 1) the effect was reproducible and similar but smaller to the effect of glucagon. The relatively small effect of both peptides on gluconeogenesis is not comparable to data in the literature using isotopic tracer methods [21] but is similar to what has been reported on the effect of glucagon on gluconeogenesis from unlabelled lactate and DHA [22]. The inhibition of gluconeogenesis from 10 mM pyruvate by glucagon (table 1) has already been reported [23,24].

All these biochemical effects are probably related, at least partially, to a cAMP-dependent mechanism, since VIP increases the cAMP level and the cAMP-dependent protein kinase activity (table 3) and since both phosphorylase and pyruvate kinase activities are modulated by a cAMP-dependent phosphorylation [20].

All the effects of VIP, so far tested, were similar to the effects of glucagon but the magnitude of the effects were smaller and the sensitivity to the peptide was lower, at least when tested on glucose production. Similar results have been obtained when tested on glycogen metabolism [4]. The small amplitude of the VIP effect compared to glucagon may be related to a smaller activation of adenylate cyclase since in isolated membrane preparation the activation of adenylate cyclase was also small [5–8]. These data tend to support the hypothesis that VIP acts through the glucagon receptor to which it would bind with a lower affinity constant. In fact this hypothesis seems very unlikely since VIP and glucagon have separate binding sites in liver [5,8] and since their maximal effects on membrane adenylate cyclase were additive [5]. Surprisingly, the relatively low sensitivity of liver cells to VIP (2.10^{-8} M) did not correlate with the high affinity of VIP for its receptor which has been shown to be in the 10^{-10} molar range [8]. These discrepancies have been tentatively related to a poor coupling of the VIP receptor to the N_2 GTP binding protein of the adenylate cyclase system [25]. Further studies are needed to elucidate these discrepancies.

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