Short-term regulation of glycolysis by vasoactive intestinal peptide in epithelial cells isolated from rat small intestine

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In epithelial cells isolated from rat small intestine, we have studied the influence of vasoactive intestinal peptide (VIP), a neurotransmitter which markedly increases enterocyte cyclic AMP, and of two cyclic AMP analogues (8-bromo cyclic AMP and *N*⁶,2'-*O*-dibutyl cyclic AMP) on the rate of glycolysis, fructose 2,6-bisphosphate concentration and 6-phosphofructo-2-kinase activity, as well as on the rate of 3-*O*-methyl-D-[¹⁴C]glucose uptake. Our results show that, without affecting the rate of 3-*O*-methyl-D-[¹⁴C]glucose accumulation, VIP and cyclic AMP analogues were able to inhibit glucose consumption and L-lactate formation by isolated rat enterocytes. These effects occurred parallel to a significant decrease in the cellular concentration of fructose 2,6-bisphosphate and to a partial inactivation of 6-phosphofructo-2-kinase. These findings support the hypothesis that VIP inhibits glycolysis in rat enterocytes through a cyclic AMP-dependent mechanism.

INTRODUCTION

Epithelial cells of rat small intestine or enterocytes show large energy demands, according to their digestive and absorptive functions. Among other substrates, they can oxidize fatty acids, ketone bodies and glutamine [1–6]. Glucose is also a relevant energy source for enterocytes, being oxidized or to a great extent converted into L-lactate, even under aerobic conditions [4–8].

According to experimental evidence [9,10], the reaction catalysed by 6-phosphofructo-1-kinase seems to be the key regulatory step in the control of enterocyte glycolysis. Moreover, enterocyte phosphofructokinase seems to be distinct from other mammalian isoenzymes previously described, and is designated phosphofructokinase D [11]. It has also been shown that this isoenzyme is activated by micromolar concentrations of Fru-2,6-*P*₂ [12]. This regulatory metabolite, which has been found in all mammalian tissues, including enterocytes [13,14], plays a relevant role in the control of glycolysis [13,15].

However, although many hormones and peptide neurotransmitters have been identified in the small intestine [16], little is known about the short-term hormonal regulation of glycolysis in intestinal epithelial cells. Recently, it has been shown that VIP, a peptide neurotransmitter which markedly increases cyclic AMP in enterocytes [17], induces an acute inhibition of glucose oxidation in epithelial cells isolated from rat small intestine [18].

In this work we have studied the influence of VIP and two cyclic AMP analogues, 8-Br-cAMP and Bt₂-cAMP, on the rate of glycolysis, Fru-2,6-*P*₂ levels and 6-phosphofructo-2-kinase activity, in enterocytes isolated from rat small intestine. We have also examined the effect of the above-mentioned agents on the rate of glucose uptake, measured by the ability of these cells to accumulate 3-*O*-methylglucose.

Our results show that VIP and the cyclic AMP analogues were able to decrease glucose consumption and L-lactate formation, possibly by decreasing the cellular concentration of Fru-2,6-*P*₂ through the partial inactivation of 6-phosphofructo-2-kinase. VIP and 8-Br-cAMP did not significantly affect the uptake of 3-*O*-methylglucose.

EXPERIMENTAL

Materials

All enzymes and coenzymes were purchased from Boehringer Mannheim (Mannheim, Germany). Fru-2,6-*P*₂ used as standard, 8-Br-cAMP, Bt₂-cAMP, phloerhizin and ouabain were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Synthetic pig VIP was from Peninsula Laboratories (Belmont, CA, U.S.A.). 3-*O*-Methyl-D-[¹⁴C]glucose was obtained from Amersham International (Amersham, Bucks., U.K.). All the remaining chemicals were of reagent grade and were purchased from Boehringer, Sigma or Merck (Darmstadt, Germany).

Animals

Fed male Wistar rats (200–300 g) from our inbred colony were used. They were fed with a standard chow (A.40; Panlab S.L., Barcelona, Spain) and water *ad libitum*. Care was taken to use rats free from infection with protozoal parasites, such as *Hexamita* or *Giardia*, in the intestine. To prevent this kind of infection, levamisole hydrochloride [Veterin-Vermisol (L) 7.5, Laboratorios Andreu, Barcelona, Spain] was added to drinking water (2 ml/l) for 4 days. Rats were used at least 15 days after this treatment was completed.

Isolation and incubation of intestinal epithelial cells

Epithelial cells from the small intestine of rat were

Abbreviations used: VIP, vasoactive intestinal peptide; Fru-2,6-*P*₂, fructose 2,6-bisphosphate; 8-Br-cAMP, 8-bromo cyclic AMP; Bt₂-cAMP, *N*⁶,2'-*O*-dibutyl cyclic AMP.

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isolated by the method described by Watford *et al.* [5]. This method, which allows the isolation of metabolically competent enterocytes, is based on EDTA and gentle mechanical treatment of small-intestine mucosa. The isolated cells were suspended in Krebs–Henseleit medium supplemented with 2.5% (w/v) dialysed bovine serum albumin. The suspension contained approx. 2–9 mg dry wt. of tissue/ml. Cell incubations were carried out in stoppered 25 ml glass Erlenmeyer flasks, previously silicone-treated, containing 2 ml of cell suspension together with the corresponding additions. The flasks were shaken (90–100 oscillations/min), at 37 °C. The gas space was filled with O₂/CO₂ (19:1). At selected times, samples of the cell suspensions were taken and processed as indicated below, for biochemical analysis.

The isolated cells were predominantly (more than 85%) enterocytes of columnar shape, the brush border being clearly visible under phase-contrast microscopy. Goblet cells and some enteroblasts were identified among the remaining cells. It must be mentioned that 1 g wet wt. of packed cells corresponded to 85 mg dry wt.

Analytical methods

Samples of cell incubations were deproteinized in HClO₄ (5%, w/v) and subsequently neutralized [19]. Glucose, glucose 6-phosphate, L-lactate, ATP, ADP and AMP were determined spectrophotometrically in these extracts by enzymic methods [20]. For the measurement of Fru-2,6-P₂, samples of cell suspension were frozen in liquid N₂ and processed as previously described [19]. Fru-2,6-P₂ was estimated by its ability to activate PP_i:fructose-6-phosphate 1-phosphotransferase from potato tubers, by the method of Van Schaftingen *et al.* [21]. 6-Phosphofructo-2-kinase activity was measured by the method reported by Bartrons *et al.* [22]. As described by Hue *et al.* [23], the metabolic flux through the reaction catalysed by 6-phosphofructose-1-kinase was estimated by the rate of ³H₂O formation from [3-³H]glucose. The ³H₂O was separated from [³H]glucose as described by Bontemps *et al.* [24].

Accumulation of 3-O-methyl-D-[¹⁴C]glucose by intestinal epithelial cells

Uptake of 3-O-methylglucose by enterocytes was monitored by the filtration technique reported by Kimich [3]. Enterocytes were suspended in a medium containing 4.75 mM-KCl, 118 mM-NaCl, 1.18 mM-KH₂PO₄, 1.18 mM-MgSO₄, 2.5 mM-CaCl₂, 2.5% bovine serum albumin and 20 mM-Hepes, at pH 7.4. Samples of this suspension (2 ml) were incubated in the presence of a mixture of lactate (4 mM) and pyruvate (0.4 mM) at 37 °C, with agitation and under a gas phase of O₂/CO₂ (19:1), in previously silicone-treated stoppered 20 ml glass vials. Then 3 min later 1 mM-3-O-methyl-[¹⁴C]glucose (0.4 μ Ci/ μ mol) was incorporated into the incubation medium, together with VIP, 8-Br-cAMP, phlorrhizin or ouabain. At the indicated times, 200 μ l samples of these incubations were taken and pipetted into 6 ml of ice-cold NaCl (0.9%). These diluted cell suspensions were immediately filtered under vacuum (25 cmHg) through a 0.65 μ m-pore-size filter (Millipore Ibérica S.A., Madrid, Spain) held in a sintered-glass filter holder. The cell pellet was rinsed twice with the ice-cold NaCl solution, and the radioactivity incorporated on the filters was measured in a liquid-scintillation counter.

Statistical analysis

Values are means $\pm$ S.E.M. of several experiments. Student's paired *t* test was applied for statistical analysis; differences were considered statistically significant when *P* was equal to or less than 0.05.

RESULTS

Enterocyte viability

Different criteria have been applied to assess the viability of isolated enterocytes. Cytological examinations indicated that approx. 90% of intestinal epithelial cells excluded Trypan Blue stain. Furthermore, the content of adenine nucleotides in isolated enterocytes incubated with 1 mM-glucose for 10 min (see below) was similar to that reported by Towler *et al.* [4] and Watford *et al.* [5] for metabolically competent enterocytes isolated from rat jejunum. The calculated energy charge [25] of our cell population was 0.81, a much better value than those estimated by these last two groups [4,5] for their enterocyte preparations (respectively 0.57 and 0.73). Also, isolated enterocytes incubated in the presence of glucose showed a linear rate of glucose consumption and L-lactate production during the first 20 min of incubation; thereafter, the glycolytic flux was progressively decreased and the percentage of stained enterocytes was significantly increased. According to these findings, cell incubations did not last more than 20 min. Finally, we have demonstrated the ability of isolated enterocytes to accumulate 3-O-methyl[¹⁴C]glucose by a mechanism sensitive to phlorrhizin and ouabain. Thus, after 10 min of incubation, the amount of 3-O-methyl[¹⁴C]glucose present in enterocytes incubated with either phlorrhizin (300 μ M) or ouabain (200 μ M) was about 50% lower than that measured in control incubations (results not shown).

Enterocyte glycolysis and Fru-2,6-P₂ levels

Incubation of enterocytes with increasing concentrations of glucose (0.5–20 mM) markedly raised the rate of glucose consumption, as well as the rate of L-lactate production (Fig. 1); the calculated glucose concentrations corresponding to the half-maximal effects were 0.85 and 0.72 mM respectively. This last value was very similar to that of 0.76 $\pm$ 0.2 mM reported by Vidal *et al.* [18] for the concentration of glucose giving half-maximal increase in the rate of lactate formation in isolated rat enterocytes. Moreover, according to those authors [18], the maximal rate of lactate production was already obtained at 5 mM-glucose.

The presence of glucose in the incubation medium caused a significant increase in the cellular concentration of Fru-2,6-P₂ (11.2 $\pm$ 0.94 and 16.6 $\pm$ 0.6 nmol/g dry wt. respectively, for enterocytes incubated without or with 5 mM-glucose; *n* = 6 experiments, *P* < 0.05), it being possible to establish a close direct correlation between enterocyte levels of Fru-2,6-P₂ and either the rate of L-lactate formation (*r* = 0.94; *P* < 0.01; *n* = 6) or glucose consumption (*r* = 0.89; *P* < 0.01; *n* = 6). Also, about 70% of the metabolized glucose was converted into lactate, a value very similar to that reported by other groups [4,5,18].

The presence of VIP or 8-Br-cAMP in the incubation medium caused a significant decrease in the rate of glucose utilization and lactate formation (Table 1 and Fig. 2). As shown in Fig. 2, the decrease in lactate production caused by the cyclic AMP analogue (22–28%

Fig. 1. Effect of different concentrations of glucose on Fru-2,6- P_2 levels and on the rates of glucose consumption and L-lactate production in isolated rat enterocytes

Enterocytes were incubated in Krebs–Henseleit medium in the presence of the indicated glucose concentrations. Fru-2,6- P_2 was measured in samples of the cell suspensions taken after 7 min of incubation. Values are means $\pm$ S.E.M. of six experiments.

inhibition as compared with control enterocytes) was observed at all the assayed glucose concentrations. As shown in Fig. 3, the decrease in glucose consumption and lactate formation caused by VIP was dose-dependent; for both processes, the maximal inhibition was obtained at 1 μ M-VIP, the concentration of neurotransmitter responsible for the half-maximal inhibitory effects ranging

Fig. 2. Effect of 8-Br-cAMP on the rate of L-lactate production in isolated rat enterocytes

Enterocytes were incubated in Krebs–Henseleit medium with different glucose concentrations, in the absence (○) or in the presence (●) of 8-Br-cAMP (1 mM). Values are means $\pm$ S.E.M. of 11 experiments: * P < 0.05, ** P < 0.01 versus the corresponding saline incubation.

from about 0.03 to 0.06 mM. Simultaneously, VIP caused a dose-dependent decrease in enterocyte Fru-2,6- P_2 from 14.8 ± 2.4 nmol/g dry wt. in enterocytes incubated under control conditions to 9.1 ± 2.0 in enterocytes incubated in the presence of 1 μ M-VIP (Fig. 3).

As shown in Table 2, the decrease in enterocyte Fru-2,6- P_2 provoked by VIP was accompanied by a decrease in the amount of 6-phosphofructo-2-kinase in active form, without affecting total activity of this enzyme. According to these findings, it must be mentioned that VIP (1 μ M), in enterocytes incubated in the presence of 10 mM- $[^3\text{H}]$ glucose (0.05 $\mu\text{Ci}/\mu\text{mol}$), significantly decreased the metabolic flux through the reaction catalysed by 6-phosphofructo-1-kinase as compared with that measured in control enterocytes (152 ± 16 versus 136 ± 17 μmol of ^3H released/15 min per g dry wt; P < 0.05; n = 8 experiments).

The inhibition of the glycolytic flux caused by VIP or 8-Br-cAMP was not due to a decrease in the viability of enterocyte. In fact, these two agents did not significantly change the cellular concentrations of adenine nucleotides, as compared with those measured in control incubations

Table 1. Effect of VIP and 8-Br-cAMP on glucose consumption and L-lactate production in isolated rat enterocytes

Isolated enterocytes were incubated in Krebs–Henseleit medium in the presence of either 1 mM- or 5 mM-glucose, together with VIP, 8-Br-cAMP or saline. Portions of cell incubations were taken before and 15 min after the addition of the different agents to the incubation medium. Values are means $\pm$ S.E.M. for five experiments. * P < 0.05, ** P < 0.01 versus the corresponding saline incubation. N.D., not determined.

Glucose concn.	Other treatment	Glucose consumption		L-Lactate production	
		($\mu\text{mol}/\text{min}$ per g dry wt.)	% inhibition	($\mu\text{mol}/\text{min}$ per g dry wt.)	% inhibition
5 mM	Saline	23.1 ± 1.6	–	37.4 ± 2.9	–
	VIP (1 μM)	$18.7 \pm 1.6^*$	19	$33.0 \pm 1.6^{**}$	12
1 mM	Saline	9.9 ± 0.5	–	N.D.	–
	VIP (1 μM)	$7.6 \pm 0.6^*$	22	N.D.	N.D.
	8-Br-cAMP (1 mM)	$7.4 \pm 0.1^*$	26	N.D.	N.D.

Fig. 3. Effect of different concentrations of VIP on the rates of glucose consumption and lactate production and on Fru-2,6-P₂ levels, in isolated rat enterocytes

Enterocytes were incubated in Krebs–Henseleit medium in the presence of 1 mM-glucose for 15 min. Fru-2,6-P₂ was measured in samples of cell suspensions taken after 7 min of incubation. Data are the means $\pm$ S.E.M. of four to six experiments, and are expressed as percentage of basal values (○): glucose consumption, 101 ± 16.7 μ mol/15 min per g dry wt.; lactate production, 146 ± 25 μ mol/15 min per g dry wt.; Fru-2,6-P₂, 14.8 ± 2.0 nmol/g dry wt.

(control enterocytes: ATP, 5.1 ± 0.4 ; ADP, 1.8 ± 0.1 ; AMP, 0.49 ± 0.1 ; enterocytes incubated with 1 μ M-VIP: ATP, 4.8 ± 0.3 ; ADP, 2.2 ± 0.2 ; AMP, 0.69 ± 0.3 . Enterocytes incubated with 1 mM-8-Br-cAMP: ATP, 5.6 ± 0.6 ; ADP, 2.1 ± 0.4 ; AMP, 0.59 ± 0.3 ; values are the means $\pm$ S.E.M. of eight experiments, and are given in μ mol/g dry wt. of cells. Adenine nucleotides were measured in samples of cell suspensions taken after 10 min of incubation).

Uptake of 3-O-methyl-D-[¹⁴C]glucose

We have also examined the influence of VIP and 8-Br-cAMP on the ability of enterocytes to accumulate 3-O-methylglucose. Neither VIP nor 8-Br-cAMP significantly affected the rate of accumulation of this non-metabolized glucose analogue (control enterocytes, 1.94 ± 0.24 ; enterocytes incubated with 1 μ M-VIP, 1.87 ± 0.24 ; enterocytes incubated with 1 mM-8-Br-cAMP, 1.74 ± 0.19). Values are means $\pm$ S.E.M. for six to ten experiments, and are given in nmol of 3-O-methylglucose incorporated into the cells/mg dry wt. after 4 min of incubation). These findings indicate that the modulation of enterocyte glycolysis by these two agents does not occur at the level of glucose transport. The fact that the cellular concentration of glucose 6-phosphate was not significantly modified by the presence of 1 mM-8-Br-cAMP in the incubation medium (0.51 ± 0.02 versus 0.49 ± 0.02 μ mol/g dry wt., for enterocytes, incubated for 5 min with 1 mM-glucose, respectively, in the absence or in the presence of the cyclic AMP analogue) also seems to rule out glucose phosphorylation as one of the possible sites of regulation of glycolysis by a short-term cyclic AMP-dependent mechanism.

DISCUSSION

It has been demonstrated that, even under aerobic conditions, enterocytes show high glycolytic activity [3–6,18]. However, little is known about the short-term hormonal regulation of the enterocyte glycolytic pathway. This lack of information is notable, considering the great number of new hormones and peptidic neurotransmitters identified in the small intestine during recent years [16]. One of them is VIP, for which rat enterocytes have specific receptors [17]. Binding of this neurotrans-

Table 2. Effect of VIP, 8-Br-cAMP and Bt₂-cAMP on Fru-2,6-P₂ levels and on 6-phosphofructo-2-kinase activity of isolated rat enterocytes

Isolated enterocytes were incubated in Krebs–Henseleit medium in the presence of 1 mM-glucose, together with VIP, 8-Br-cAMP, Bt₂-cAMP or saline. Fru-2,6-P₂ measurements and enzyme assays were carried out on samples of cell suspensions taken 6 min after the addition of the different agents. Values are means $\pm$ S.E.M. for six experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, (NS), not significant, saline incubation.

Treatment	6-Phosphofructo-2-kinase			Fru-2,6-P ₂ (nmol/g dry wt.)
	Active form (munits/g dry wt.)	Total activity (munits/g dry wt.)	Active/total	
Saline	1.77 ± 0.19	3.35 ± 0.74	0.54 ± 0.06	20.1 ± 2.3
VIP (1 μ M)	$1.15 \pm 0.24^{***}$	3.33 ± 0.56 (NS)	$0.35 \pm 0.03^*$	$12.6 \pm 2.9^*$
8-Br-cAMP (1 mM)	$1.35 \pm 0.24^{**}$	3.60 ± 0.85 (NS)	$0.38 \pm 0.02^*$	$13.6 \pm 2.1^*$
Bt ₂ -cAMP (1 mM)	$1.10 \pm 0.18^{***}$	3.15 ± 0.43 (NS)	$0.35 \pm 0.02^*$	$14.6 \pm 2.9^*$

mitter stimulates enterocyte adenylate cyclase and markedly increases cellular cyclic AMP [18,27]. As reported by others [18,27], $1\text{ }\mu\text{M}$ -VIP is able to provoke a maximal accumulation of cyclic AMP in isolated rat enterocytes. On the other hand, the 'physiological' concentration of this neurotransmitter at the submucous plexus is not known, but seems to be far above the concentration of VIP in circulating plasma [28].

Our results clearly demonstrate that, in isolated rat enterocytes, VIP causes a dose-dependent and statistically significant decrease in the rates of glucose consumption and lactate formation. The inhibition of the glycolytic flux occurred without significant modification of the levels of adenine nucleotides, ruling out a possible non-specific toxic effect of this neurotransmitter on isolated enterocytes. Furthermore, the effect of VIP on glucose utilization seems to be independent of either glucose uptake or glucose phosphorylation.

The inhibition of glycolysis elicited by VIP was accompanied by a significant decrease in the cellular levels of Fru-2,6- P_2 and in the amount of 6-phosphofructo-2-kinase present in the active form. These findings, together with the fact that similar metabolic effects were also elicited by two cyclic AMP analogues, $\text{Bt}_2\text{-cAMP}$ and 8-Br-cAMP, support the hypothesis that the inhibition of the glycolytic flux was the consequence of a cyclic AMP-mediated decrease in enterocyte Fru-2,6- P_2 by the partial inactivation of 6-phosphofructo-2-kinase. This enzymic effect could be due to phosphorylation of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase by the cyclic AMP-dependent protein kinase, as occurred with the liver enzyme [13,15]. However, to our knowledge, it has not been established what type of 6-phosphofructo-2-kinase isoenzyme [15] is present in rat enterocytes.

Considering that 1 g wet wt. of cells corresponds to 85 mg dry wt. and that the cytosolic space represents 50 % of the total volume [14], one can calculate, according to our measurements, that the intracellular concentration of Fru-2,6- P_2 is between 0.24 and $0.6\text{ }\mu\text{M}$. These values, similar to those previously reported [14], are in the range of the K_a ($0.6\text{ }\mu\text{M}$) for Fru-2,6- P_2 determined by Jamal *et al.* [12] in the presence of 0.5 mM-fructose 6-phosphate and 2.5 mM-ATP for 6-phosphofructo-1-kinase of rat small-intestine mucosa. Taking into account these data, and in accordance with Louis *et al.* [14], we can postulate that, in isolated rat enterocytes, Fru-2,6- P_2 might play a regulatory role in the control of 6-phosphofructo-1-kinase activity and in the modulation of the glycolytic flux. In fact, a close direct correlation could be established between cellular Fru-2,6- P_2 concentration and the rate of both glucose consumption and lactate formation. On the other hand, the metabolic flux through the reaction catalysed by 6-phosphofructo-1-kinase was significantly decreased in enterocytes incubated in the presence of VIP, in which Fru-2,6- P_2 levels were diminished.

Recently, Vidal *et al.* [18] have shown that VIP can decrease glucose oxidation in preparations of isolated rat enterocytes. This effect occurred without significant modification of either glucose utilization or lactate production, and it seemed to be related to a VIP-induced stimulation of fatty acid oxidation. These results are discrepant with ours, but we have no clear explanation for the differences. However, we can speculate that an increased lipolytic rate induced by VIP could collaborate, raising enterocyte citrate concentration, to decrease 6-phosphofructo-2-kinase activity and Fru-2,6- P_2 levels

further, as well as 6-phosphofructo-1-kinase activity, and in this way facilitate the partial blockade of the glycolytic flux.

Finally, we must mention the possible physiopathological significance that a cyclic AMP-mediated decrease in the glycolytic flux, together with a partial blockade of glucose oxidation [18], could have in order to gain more insight into the metabolic abnormalities which occur in the epithelial intestinal cells of patients suffering from VIPomas or cholera-toxin effects [29,30].

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REFERENCES

1. Parsons, D. S. (1975) in *Intestinal Absorption and Malabsorption* (Csáky, T. S., ed.), pp. 9–36, Raven Press, New York
2. Lamers, J. M. J. & Hülsmann, W. C. (1974) *Biochim. Biophys. Acta* **343**, 215–225
3. Kimmich, G. A. (1975) in *Methods in Membrane Biology* (Korn, E. D., ed.), vol. 5, pp. 51–115, Plenum Press, New York
4. Towler, C. M., Pugh-Humphreys, G. P. & Porteous, J. W. (1978) *J. Cell Sci.* **29**, 53–75
5. Watford, M., Lund, P. & Krebs, H. A. (1979) *Biochem. J.* **178**, 589–596
6. Mallet, R. T., Kelleher, J. K. & Jackson, M. J. (1986) *Am. J. Physiol.* **250**, C191–C198
7. Porteous, J. W. (1978) *Biochem. Soc. Trans.* **6**, 534–539
8. Porteous, J. W. (1980) *Biochem. J.* **188**, 619–632
9. Jamal, A. & Kellett, G. L. (1983) *Biochem. J.* **210**, 129–135
10. Jamal, A. & Kellett, G. L. (1983) *Diabetologia* **25**, 355–359
11. Khoja, S. M. & Kellett, G. L. (1983) *Biochem. J.* **215**, 335–341
12. Jamal, A., Kellett, G. L. & Robertson, J. P. (1984) *Biochem. J.* **218**, 459–464
13. Van Schaftingen, E. (1987) *Adv. Enzymol.* **59**, 315–395
14. Louis, J., Philippe, B. & Hue, L. (1985) *Biochem. J.* **232**, 622–623
15. Hue, L. & Rider, M. H. (1987) *Biochem. J.* **245**, 313–324
16. Yamada, T. (1987) in *Physiology of the Gastrointestinal Tract* (Johnson, L. R., ed.), 2nd edn., vol. 1, pp. 131–142, Raven Press, New York
17. Laburthe, M., Prieto, J. C., Amiranoff, B., Dupont, Ch., Hui Bon Hoa, D. & Rosselin, G. (1979) *Eur. J. Biochem.* **96**, 239–248
18. Vidal, H., Comte, B., Beylot, M. & Riou, J. P. (1988) *J. Biol. Chem.* **262**, 9206–9211
19. Monge, L., Mojena, M., Ortega, J. L., Samper, B., Cabello, M. A. & Feliú, J. E. (1986) *Diabetes* **35**, 89–96
20. Bergmeyer, H. U. (1974) *Methods of Enzymatic Analysis*, 3rd edn., vols. 1–4, Academic Press, New York
21. Van Schaftingen, E., Lederer, B., Bartrons, R. & Hers, H. G. (1982) *Eur. J. Biochem.* **129**, 191–195
22. Bartrons, R., Hue, L., Van Schaftingen, E. & Hers, H. G. (1983) *Biochem. J.* **214**, 829–837
23. Hue, L., Sobrino, F. & Bosca, L. (1984) *Biochem. J.* **244**, 779–786
24. Bontemps, F., Hue, L. & Hers, H. G. (1978) *Biochem. J.* **174**, 603–611

25. Atkinson, D. E. (1968) *Biochemistry* 7, 4030–4034
26. Reference deleted
27. Prieto, J. C., Laburthe, M. & Rosselin, G. (1979) *Eur. J. Biochem.* 96, 239–248
28. Fahrenkrug, J. (1981) in *Gut Hormones* (Bloom, S. R. & Polak, J. M., eds.), 2nd edn., pp. 385–391, Churchill Livingstone, London
29. Welbourne, R. B., Wood, S. M., Polak, J. M. & Bloom, S. R. (1981) in *Gut Hormones* (Bloom, S. R. & Polak, J. M., eds.), 2nd edn., pp. 547–554, Churchill Livingstone, London
30. Binder, H. J. (1989) in *Gastrointestinal Disease* (Sleisenger, M. H. & Fordtran, J. S., eds.), 4th edn., pp. 1022–1045, W. B. Saunders, Co., Philadelphia

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