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FUNCTIONAL COUPLING BETWEEN THE ACTIVE TRANSPORT OF GLUCOSE AND THE SECRETION OF INTESTINAL NEUROTENSIN IN RATS

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SUMMARY

1. In this study, the mechanisms involved in the release of neurotensin-like immunoreactivity (NTLI) by glucose were investigated with the isolated, vascularly perfused rat jejunoileum preparation.

2. Luminal infusion of glucose (1–250 mM) produced a sharp and sustained release of NTLI in the intestinal venous effluent. The first significant response was observed with 5 mM glucose and the release reached a maximum under 250 mM glucose with a plateau secretion at 500% of basal.

3. There was no significant difference in the ability of galactose and 3-*O*-methylglucose to release NTLI when compared to glucose, but α-methylglucose, mannose, 2-deoxyglucose and fructose did not stimulate NTLI release.

4. Luminal infusion of 5 mM phloridzin reduced the glucose-induced release of NTLI by 90%. Intra-arterial infusion of glucose (25 mM) or of phloretin (20 μM) had no significant effect on the glucose-evoked NTLI secretion.

5. Intra-arterial infusion of ouabain (1 mM) produced a dramatic increase (at about 1500% of basal) in portal NTLI although it drastically reduced intestinal absorption of glucose.

6. Intra-arterial infusion of tetrodotoxin (1 μM), atropine (10 μM), verapamil (50 μM) or nifedipine (50 μM) did not modify the glucose-induced NTLI secretion.

7. Intra-arterial infusion of forskolin (2–20 μM) evoked a prompt and well-sustained secretion of NTLI which was increased to a mean value of 800% of basal with the highest dose tested. 3-Isobutyl-1-methylxanthine (IBMX, 10–100 μM) also stimulated the secretion of NTLI (maximal increase at 725% of basal at 100 μM). In contrast, intra-arterial infusion of 4-β-phorbol 12-myristate, 13-acetate (PMA, 0·05–0·5 μM) had no effect on NTLI release.

8. IBMX (10–100 μM) synergistically enhanced NTLI responses induced by 250 mM glucose; the integrated response of NTLI release was 3- to 5-fold higher

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than the sum of individual responses produced by the same stimulants given separately.

9. It is concluded that the carbohydrate-induced NTLI release is related to the active, sodium-dependent hexose transport, but not to the carbohydrate catabolic pathway. Furthermore, the intramural nerves and L-type calcium channels are not involved in the glucose-induced NTLI secretion. Finally, the secretory activity of the intestinal N cell seems to be mainly stimulated through a cAMP-dependent pathway.

INTRODUCTION

Neurotensin (NT) is a tridecapeptide originally isolated from bovine hypothalamus (Carraway & Leeman, 1973). In the gastrointestinal tract, neurotensin-like immunoreactivity (NTLI) is present in open-type endocrine cells (N cells) which are located in the lower part of the small intestine (Carraway & Leeman, 1976). Paracrine and endocrine effects of NT on several gut functions have been assumed (Ferris, 1989).

Luminal nutrients have been shown to stimulate NTLI release in various experimental conditions, including: (i) ingestion of carbohydrate-rich meals in pigs (Cuber *et al.* 1990*b*) or fat-rich meals in humans (Mashford, Nilsson, Rôkaeus & Rosell, 1977; Go & Demol, 1981; Flaten & Hanssen, 1982; Hammer, Carraway & Leeman, 1982); (ii) jejunal infusion of fatty acids in man and dogs (Read *et al.* 1984; Gallavan *et al.* 1986); and (iii) luminal infusion of glucose, fatty acids and bile salts in isolated porcine and rat ileum models (Gill *et al.* 1984; Holst-Pedersen, Knuthsen, Bernabei, Orskov & Holst, 1988; Cuber *et al.* 1990*a*). On the other hand, several studies pointed to a control of N cells by neurotransmitters and peptides: β-adrenergic agonists (Barber, Buchan, Walsh & Soll, 1986), bombesin and gastrin-releasing peptide (Rôkaeus, Yanaihara & McDonald, 1982; Gill *et al.* 1984; Holst-Pedersen *et al.* 1988; Cuber *et al.* 1990*a*), and substance P (Herrmann *et al.* 1991; Herrmann, Cuber, Bernard & Chayvialle, 1992) stimulated NTLI release. Cholinergic agonists inhibited the release of NTLI from the isolated canine N cell in culture (Barber *et al.* 1986), while stimulatory effects were reported in rats, using the isolated vascularly perfused ileum model (Gill *et al.* 1984; Cuber *et al.* 1990*a*). Somatostatin was inhibitory in both species (Rôkaeus, 1984; Barber, Gregor & Soll, 1987).

Since luminal nutrients are potent stimulants of NTLI release, the question could be raised whether nutrient absorption and metabolism is mandatory, or simple contact with the apical pole of N cells is sufficient for stimulation. In addition, the contribution of intrinsic neural pathways and the effects of cAMP accumulation or of calcium channel blockade on the nutrient-induced NTLI release are not known. Some of these points were addressed here using an isolated rat jejunoileum model for the analysis of glucose-induced NTLI response.

METHODS

Materials

β-D-Galactose, D-mannose, 3-*O*-methyl-α-D-glucoside, α-methyl-D-glucoside, 2-deoxyglucose, D-fructose, tetrodotoxin, atropine sulphate, nifedipine, verapamil, phloretin, ouabain, forskolin,

3-isobutyl-1-methylxanthine (IBMX), 1-ethyl-3(3-diethyl-aminopropyl) carbodiimide HCl (ECl) and 4β-phorbol 12-myristate, 13-acetate (PMA) were obtained from Sigma Chemical Co. (St Louis, MO, USA). α-D-Glucose was obtained from Merck (Darmstadt, Germany). Bovine serum albumin (BSA; fraction V) was obtained from Biomerieux (Charbonnieres, France). Azonutril 25, a mixture of amino acids (3·4% (w/v) isoleucine, 9·3% leucine, 8·5% lysine, 6·3% methionine, 8·3% phenylalanine, 3·4% threonine, 1·7% tryptophan, 8·4% valine, 2·7% aspartic acid, 3·4% glutamic acid, 6·4% alanine, 16·8% arginine, 1% cysteine, 6% glycine, 3·4% histidine, 5·4% proline, 0·9% serine, 0·2% tyrosine, 2% citrulline and 1·5% ornithine) was obtained from Laboratoire Roger Bellon (Paris, France). The total amino acid content was 14·8 g/100 ml of mixture. [^{14}C]D-Glucose (2·3 mCi/mmol) was purchased from New England Nuclear (Dupont de Nemours, Paris, France).

Surgical preparation

The complete description of the surgical steps and functional viability of the isolated vascularly perfused rat jejunoileum were previously reported in detail (Cuber *et al.* 1990*a*). Male Wistar rats (250–350 g) were anaesthetized with pentobarbitone sodium (50 mg/kg I.P.) and the abdomen was opened with a mid-line incision. The right and middle colic veins and arteries were tied and cut between ligatures near the serosa of the colon in order to free the upper small intestine from the hindgut. A Silastic cannula was inserted and tied into the terminal ileum and a similar cannula was inserted into the jejunum 20 cm proximal to the first. The gut lumen was flushed twice with 10 ml of isotonic saline, then the remaining upper jejunum and the duodenum were removed after their respective arteries and veins were ligated. A metal cannula and a Silastic one were quickly inserted (within 30 s) in the mesenteric artery and portal vein, respectively. The arterial perfusion was started immediately at a rate of 2·5 ml/min with a Krebs–Henseleit solution (pH 7·40) containing 25% washed bovine erythrocytes, 3% BSA, glucose 5 mM and Azonutril 1% (v/v). This mixture was continuously gassed with 95% O_2–5% CO_2 and warmed to 37 °C. The perfusion pressure was about 50–70 mmHg. The jejunoileal block was removed and transferred to a plastic box filled with isotonic saline maintained at a temperature of 37 °C. Venous blood effluent was collected as 2 min fractions in glass tubes chilled in ice and containing 250 μl of 200 mM EDTA. The supernatant was rapidly separated by centrifugation and then treated with two volumes of ethanol. The ethanol extracts were dried and kept at −30 °C for the subsequent determination of NT.

Experimental protocol

Tests based on luminal perfusions included a 20 min basal period, followed by 30 min stimulation and 10 min recovery. Glucose (1, 5, 25, 125 and 250 mM) or other carbohydrates and glucose analogues (250 mM) were administered as an initial volume of 4 ml over 1 min, followed by a slow infusion at a rate of 200 μl/min for 29 min. The lumen was then flushed out first with air, followed by isotonic saline at a rate of 200 μl/min. The osmolarity of each test solution was adjusted to 300 mosm/l as required, by adding the appropriate amounts of sodium chloride. The final osmolarity was verified with an osmometer (Vogel Roebling, Germany). Solutions of [^{14}C]glucose (1·25 μCi, specific activity 2·3 mCi/mmol) plus glucose (25 mM), and of phloridzin (5 mM) plus glucose (5 and 250 mM) were administered in volumes of 2·5 ml. Stock solutions of forskolin, IBMX, nifedipine and PMA were prepared by dissolving appropriate amounts of the compounds in 70% ethanol. Phloretin was prepared in 100% DMSO (dimethyl sulphoxide). The final concentration of DMSO in the arterial line never exceeded 0·25%. In control experiments, intra-arterial infusion of 0·25% DMSO alone in isotonic saline had no effect on NTLI release. For vascular infusion, forskolin (final concentration 2 and 20 μM), IBMX (10 and 100 μM), PMA (0·05 and 0·5 μM), phloretin (20 μM), ouabain (1 mM), TTX (1 μM), atropine (10 μM), verapamil (50 μM) and nifedipine (50 μM) were diluted in Krebs–Henseleit buffer supplemented with 3% BSA and delivered at a rate of 0·25 ml/min via a catheter close to the vascular inflow.

Radioimmunoassay of NT

NTLI in the portal effluent was measured with an antiserum specific for intact NT, as previously described (Cuber *et al.* 1990*a*, *b*). Briefly, antiserum 29G was obtained from a rabbit after repeated injection of NT conjugated to bovine albumin through ECl condensation and used in the assay at a final dilution of 1:250000. The radioactive ligand was monoiodo-[^{125}I]-(Tyr3)-NT labelled and purified as described by Holst-Pedersen, Stadil & Fahrenkrug (1983). The plasma ethanol extract was reconstituted in assay buffer (50 mM, pH 7·5 containing 5 mM EDTA and 2% porcine serum) on the day of the assay. The sensitivity and ID_{50} were 0·6 and 4·0 fmol per tube, respectively. The

recovery of exogenous NT (2·5–50 fmol/ml plasma prior to ethanol extraction) was 75·2±6·8% ($n = 24$). Antiserum 29G requires the biologically active part of the NT molecule for recognition and does not cross-react significantly with N-terminal fragments of NT (Cuber *et al.* 1990*a*). High-performance liquid chromatography (HPLC) analysis of portal supernatants followed by radioimmunoassay of NT with antiserum 29G revealed a single peak co-eluting with NT 1–13 (Cuber *et al.* 1990*a*).

Calculations

Data are presented as means±S.E.M. and are expressed as femtomoles per 2 minutes. The integrated responses of immunoreactive material released by a given stimulus were calculated by subtraction of the basal immunoreactivity produced during a given period from the immunoreactivity released upon stimulation during the same period. For statistical analysis of data, the Wilcoxon test for paired values and the Mann–Whitney test for unpaired values were used. $P < 0·05$ was considered to be significant.

RESULTS

Luminal factors involved in the release of NTLI

Stimulation of NT release by luminal glucose

Luminal administration of glucose produced a dose-dependent release of NTLI (Fig. 1). The integrated responses (IR) were (in fmol/30 min) 42±18 ($n = 4$), 81±67 ($n = 4$), 183·3±65·6 ($n = 6$), 337·2±105·5 ($n = 5$), 468·0±103·4 ($n = 5$) and 505·3±90·5 ($n = 18$), for saline and glucose at 1, 5, 25, 125 and 250 mM, respectively. The kinetic study showed that infusion of glucose (250 mM) caused a prompt and sustained release of NTLI with a plateau value of 500% of basal (Fig. 2).

Effects of phloridzin on glucose-induced release of NTLI

Luminal infusion of phloridzin (5 mM) completely abolished the glucose-induced NTLI secretion (Fig. 2). The same effect of phloridzin was observed on luminal administration of glucose 5 mM (IR: 9·9±5·5 fmol/30 min, $n = 4$, *vs.* 183·3±65·6 fmol/30 min, $n = 6$, for control with luminal glucose alone).

Effects of various carbohydrates on NTLI secretion

Integrated responses (IR) of NTLI during luminal infusion of various carbohydrates (250 mM) are shown in Fig. 3. Solutions containing glucose, galactose or 3-*O*-methylglucose all produced a significant stimulation of NTLI (IR: 505·3±90·5 fmol/30 min, $n = 18$, 465·0±124·9 fmol/30 min, $n = 5$, 303·1±108·7 fmol/30 min, $n = 8$, respectively). In contrast, there was no significant increase of NTLI level under infusion of α-methylglucose, fructose, mannose or 2-deoxyglucose (IR: 80·1±29·9 fmol/30 min, $n = 9$, 45·6±21·1 fmol/30 min, $n = 5$, 35·8±3·4 fmol/30 min, $n = 4$, 20·0±8·6 fmol/30 min, $n = 4$, respectively).

Basolateral factors involved in the release of NTLI

Effect of intra-arterial infusion of glucose and of phloretin on glucose-induced release of NTLI

Intra-arterial infusion of glucose (25 mM) did not provoke a significant increase in portal NTLI (IR: 67·3±21·3 fmol/20 min, $n = 6$, *vs.* 45·6±19·3 fmol/20 min, $n = 6$, for control with isotonic saline). Administration of arterial glucose (25 mM) in

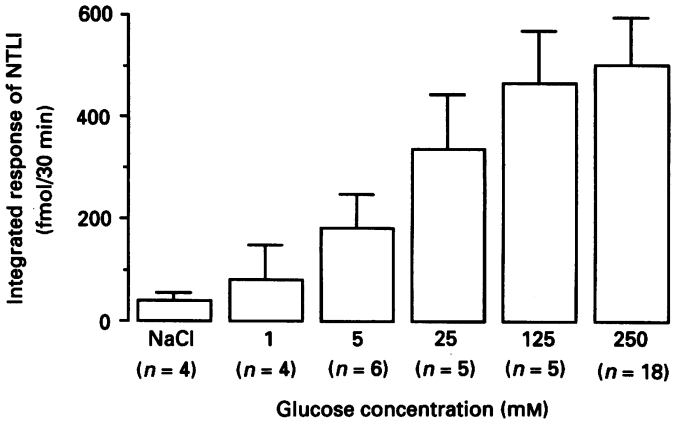

Fig. 1. Integrated response of NT-like immunoreactivity (NTLI) during luminal infusion of increasing concentration of glucose (fmol/30 min ± S.E.M.).

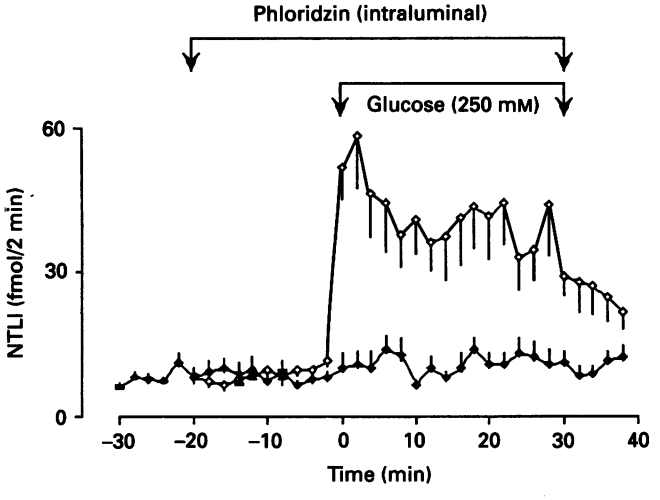

Fig. 2. Effect of luminal infusion of phloridzin ($n = 11$) on the release of NTLI induced by luminal administration of glucose ($n = 18$). Results are expressed as fmol immunoreactive material per 2 min ± S.E.M. ◇, glucose alone; ◆, +phloridzin (5 mM).

combination with luminal infusion of glucose (250 mM) produced a release of NTLI similar to that produced by luminal glucose alone (IR: 453·0 ± 174·2 fmol/30 min, $n = 6$, *vs.* 505·3 ± 90·5 fmol/30 min, $n = 18$, for luminal glucose alone).

Intra-arterial infusion of phloretin (20 μM) had no significant effect on glucose-induced release of NTLI. The IR (777·2 ± 111·1 fmol/30 min, $n = 6$) was not significantly different from control value under glucose (250 mM) alone (505·3 ± 90·5 fmol/30 min, $n = 18$).

Effect of ouabain on release of NTLI and absorption of glucose

The time course of ^{14}C in the portal vein effluent during the luminal infusion of glucose (25 mM) plus trace amounts of [^{14}C]glucose under ouabain (1 mM) is shown in Fig. 4. In loops infused with glucose alone, there was a rapid increase in the amount

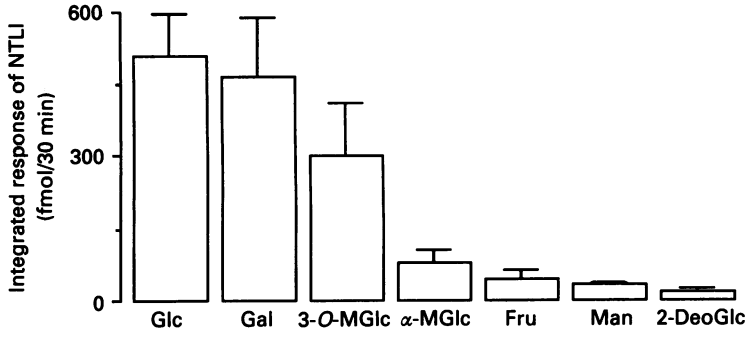

Fig. 3. Integrated response of NTLI (fmol/30 min, means ± S.E.M.) during luminal administration of glucose (Glc) ($n = 18$), galactose (Gal) ($n = 5$), 3-*O*-methylglucoside (3-*O*-MGlc) ($n = 8$), α-methylglucose (αMGlc) ($n = 9$), fructose (Fru) ($n = 5$) mannose (Man) ($n = 4$), or 2-deoxyglucose (2-DeoGlc) ($n = 3$). All carbohydrates were administered at a final concentration of 250 mM.

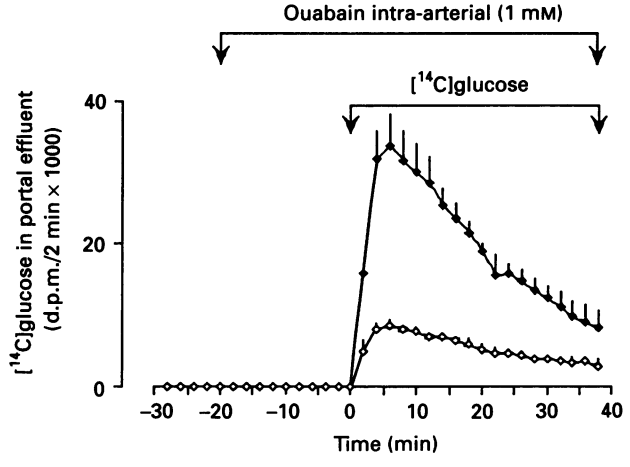

Fig. 4. Effect of intra-arterial infusion of ouabain ($n = 3$) on absorption of [^{14}C]glucose. A solution containing 1·25 μCi [^{14}C]Glc plus 25 mM unlabelled glucose was administered as a bolus of 2·5 ml for 40 min ($n = 4$). Vascular ouabain was infused for 60 min starting at 10 min ($n = 3$). Results are expressed as the total radioactivity (in d.p.m.) of each 2 min consecutive fraction of portal effluent (means ± S.E.M.). ◆, glucose alone; ◇, + ouabain.

of label appearing in the portal effluent (peak of 37802 ± 5274 d.p.m./2 min); the absorption then decreased to a value of 13186 ± 1758 d.p.m./2 min. In loops infused with glucose (25 mM) plus ouabain (1 mM), the absorption of glucose was greatly diminished (peak of 8790 ± 439 d.p.m./2 min and plateau of 4395 ± 439 d.p.m./2 min, $P < 0·05$).

Upon vascular infusion of 1 mM ouabain, portal NTLI rose to a peak value of 228·3 ± 52·8 fmol/2 min from a basal value of 6·6 ± 0·6 fmol/2 min. Luminal administration of glucose (250 mM) further increased the portal concentration of NT (peak at 279·1 ± 56·0 fmol/2 min and subsequent mean plateau level at 189·6 ± 39·6 fmol/2 min) (Fig. 5).

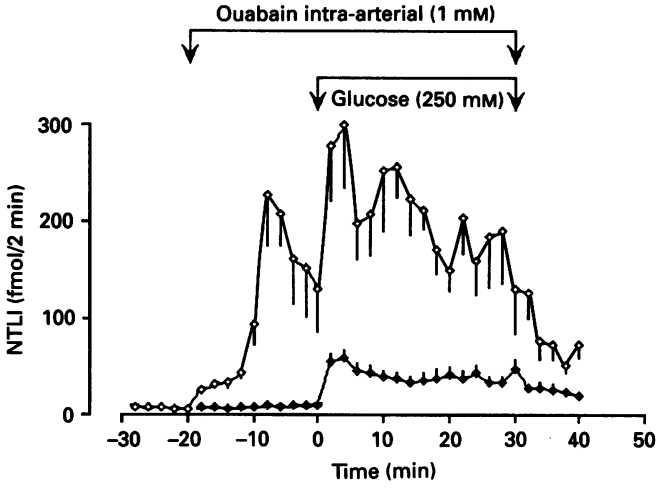

Fig. 5. Effects of luminal administration of glucose ($n = 18$) alone (◆) or in combination with intra-arterial infusion of 1 mM ouabain ($n = 7$) (◇) on NTLI concentration in portal effluent (fmol/2 min ± S.E.M.).

Effect of tetrodotoxin (TTX), atropine and calcium channel blockers on glucose-induced release of NTLI

Vascular infusion of TTX (1 μM) had no significant effect on the NTLI release induced by glucose (IR: 513·8 ± 129·3 fmol/30 min, $n = 6$, *vs.* 505·3 ± 90·5 fmol/30 min, $n = 18$, for control). After arterial infusion of atropine (10 μM) in combination with glucose, the mean integrated values were higher than in controls but the difference was not significant (616·9 ± 133·6 fmol/30 min, $n = 7$). Similarly, vascular verapamil (50 μM) or nifedipine (50 μM) did not modify the glucose-stimulated NTLI secretion. (IR: 618·0 ± 221·5 fmol/30 min, $n = 5$, 678·9 ± 103·8 fmol/30 min, $n = 7$, respectively).

Postreceptor activation of NT release

Effect of forskolin and PMA on NTLI secretion

Intra-arterial infusion of forskolin (2 μM) brought about a rise of portal NTLI to a maximal value of 28·8 ± 1·7 fmol/2 min from a basal value of 6·4 ± 2·0 fmol/2 min at the start of infusion ($n = 5$) (Fig. 6). Forskolin (20 μM) produced a sharp increase of portal NTLI (peak of 90·0 ± 18·5 fmol/2 min) followed by a plateau at a mean value of 42·8 ± 9·8 fmol/2 min (Fig. 6). On intra-arterial infusion of PMA (0·05–0·5 μM), portal NTLI remained at basal value till the end of the session (not shown).

Infusion of glucose in combination with IBMX or PMA

Vascular infusion of 100 μM IBMX alone produced an increase of portal NTLI release (IR, 442·0 ± 106·5 fmol/20 min). The IR of NTLI was 4184·0 ± 1253·7 fmol/20 min upon combined infusion of 100 μM IBMX (vascular) and 250 mM glucose (luminal). This was about 5-fold higher than the sum of individual responses

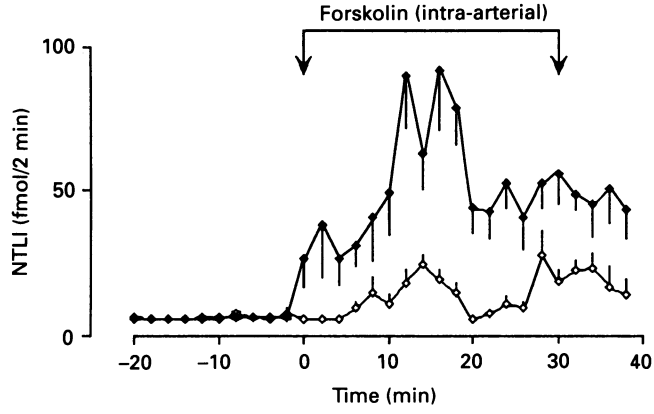

Fig. 6. Effects of intra-arterial infusion of forskolin on release of NTLI. Results are expressed as fmol/2 min ± S.E.M. Five experiments were performed with 20 μM of forskolin (◆) and six with 2 μM (◇).

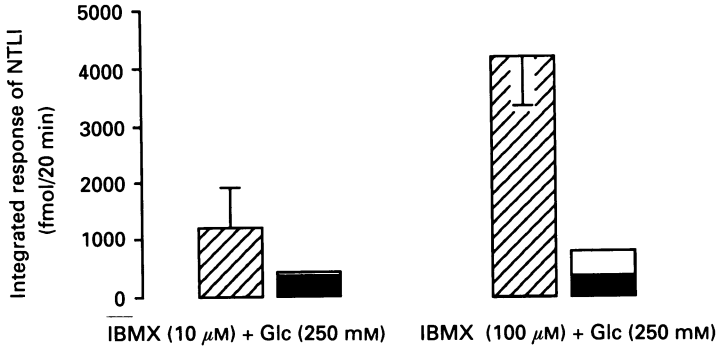

Fig. 7. Integrated responses of NTLI (fmol/20 min) during combined infusion of IBMX with glucose (hatched bars) as compared to the sum of responses to IBMX (open bars) and glucose (filled bars) given separately.

produced by the same stimulants given separately (Fig. 7). The effect of glucose (250 mM) was also potentiated by 10 μM IBMX (IR: 1207·3 ± 704·1 fmol/20 min). In contrast, PMA (0·05 μM) did not increase the NTLI response induced by glucose alone (IR: 368·7 ± 81·1 fmol/30 min as compared to 505·3 ± 90·5 fmol/30 min for control with glucose alone).

DISCUSSION

Nutrients have been shown to be potent stimuli to NT release in various species (Rosell & Rôkaeus, 1979; Go & Demol, 1981; Read *et al.* 1984; Gallavan *et al.* 1986; Holst-Pedersen *et al.* 1988; Cuber *et al.* 1990*b*). This is taken to suggest that crucial events are occurring in its release at the apical side of N cells. We here confirmed the stimulatory effect of glucose, at luminal concentrations as low as 5 mM. This effect is most probably physiological, since luminal concentrations of glucose over the range 0·2–48 mM were recorded in rats (Ferraris, Yasharpour, Lloyd, Mirzayan & Diamond, 1990).

Intestinal absorption of glucose is an active process that involves a Na^+–glucose co-transporter (SGLT1), located in the brush border and blocked by the glycoside phloridzin (Hediger, Coady, Ikeda & Wright, 1987). The mechanism is specifically used by hexoses with a pyranose ring and a C^2 OH group in α-configuration (Crane, 1968). We thus tested whether glucose-induced NT release requires active absorption and metabolism. Mannose, a hexose with a C^2 OH group in β-configuration, and 2-deoxyglucose were unable to stimulate NT-LI release. Since both hexoses are poorly transported by the active process involved in glucose absorption, the data suggest that active transport (here for glucose, galactose and 3-*O*-methylglucose) is required for stimulation of N cells. This was confirmed by the drastic inhibition of NT release in the presence of phloridzin.

Intracellular metabolism does not appear to be a limiting factor, because 3-*O*-methylglucose, a non-metabolizable derivative of glucose, was able to increase NTLI secretion. Surprisingly, α-methyglucose, a non-metabolized substrate which possesses an equatorial hydroxyl group on carbon-2 did not stimulate the release of NTLI. Since this carbohydrate is handled by SGLT1 but not by the basolateral facilitative glucose transporter, it could be argued that the activation of the latter transporter is also required for NTLI secretion. However, stimulation of the basolateral facilitative glucose transporter by increasing arterial glucose concentration up to 25 mM did not induce any release of NTLI in the basal state (i.e. isotonic saline in the lumen) nor did it enhance the NTLI secretion induced by luminal glucose. In the experiments with phloretin administered at the largest possible concentration in the present system (20 μM), NTLI secretion under luminal infusion of glucose showed no tendency to decrease. Taken together, these data suggest that the facilitated glucose transport at the basolateral pole is not a critical factor for N cell stimulation. As a consequence, the lack of effect of α-methylglucose on NTLI release cannot be explained by its inability to stimulate the basolateral glucose transporter. The possibility that a Na^+–glucose co-transporter insensitive to α-methylglucose is implicated in the NTLI release may be raised. The existence of at least two Na^+-dependent glucose transporters was suggested by Brot-Laroche and co-workers (Brot-Laroche, Supplisson, Delhome, Alcalde & Alvarado, 1987; Brot-Laroche *et al.* 1988). The Na^+-dependent system 2 described by these authors would be a candidate, since α-methylglucose is not a substrate for this system. However, the fact that a facilitated transport of glucose is involved in this system 2 argues against its involvement in the release of NTLI, since phloridzin drastically inhibited the NTLI secretion in our study.

Although the crucial event of the glucose-induced NTLI secretion is the activation of the apical Na^+–glucose co-transporter, as judged from experiments with luminal phloridzin, the possibility that an apical facilitative transporter was involved as well in the release of NTLI was investigated. The recently discovered glucose transporter GLUT5 (Kayano *et al.* 1990) has been localized to the luminal surface of mature absorptive epithelial cells in humans (Davidson *et al.* 1992). This transporter was shown to exhibit selectivity for fructose in human small intestine (Burant, Takeda, Brot-Laroche, Bell & Davidson, 1992). Although the structure, localization and function of the rat GLUT5 have not yet been identified, the effect of luminal fructose on NTLI release was investigated in the present study. Fructose

did not elicit any release of NTLI, indicating that the release of NTLI is not coupled to the activation of the facilitative glucose transporter GLUT5 unless the distribution and function of the transporter are species specific.

Further experiments were based on ouabain. This glucoside inhibits the extrusion of Na^+ co-transported with glucose into enterocytes, and consequently glucose absorption, by blocking the basolateral Na^+–K^+-ATPase. In the present study, ouabain indeed inhibited glucose transport to the portal venous effluent. Unexpectedly, however, ouabain clearly stimulated NTLI release by itself, and this effect was boosted by subsequent luminal infusion of glucose. These results raise the hypothesis that the secretory activity of N cells may depend on Na^+–K^+ basolateral exchange, or, possibly, on intracellular sodium concentration.

The available data indicate that N cells are under the control of various neurotransmitters and neuropeptides (Barber *et al.* 1986, 1987; Herrmann *et al.* 1991). The intrinsic properties of the isolated jejunoileum model (i.e. preserved polarization of epithelium and intramural innervation) allowed us to test whether the glucose-induced NTLI response was mediated by neural pathways. TTX (1 μM) or atropine (10 μM) did not significantly modify the glucose-evoked NTLI secretion. Earlier experiments performed with TTX and atropine at the same concentrations in the same model clearly demonstrated the effectiveness of these agents as blockers of nervous conduction and muscarinic receptor antagonist, respectively (Cuber, Vilas, Charles, Bernard & Chayvialle, 1989; Herrmann *et al.* 1991). The lack of effect of atropine and TTX thus argues against the hypothesis of neurally mediated release of NTLI by luminal glucose.

Accumulating evidence indicates that calcium is important in the coupling of secretory events in the G cell and D cell of the antral mucosa (Sugano, Park, Soll & Yamada, 1986, 1987). Administration of calcium channel blockers strongly reduced the bombesin-induced release of gastrin in the isolated vascularly perfused stomach preparation (Guo, Thompson & Singh, 1988). Similarly, the cholecystokinin secretion was strongly dependent on calcium in the isolated vascularly perfused rat duodenojejunum preparation since calcium channel blockers of the phenylalkylamine and dihydropyridine families reduced the peptone-induced cholecystokinin secretion (Cuber, Aucouturier, Bernard & Chayvialle, 1992). The same compounds, perfused in the present study at the same concentrations as in that previous report, were unable to reduce the glucose-evoked NTLI secretion. Voltage-dependent calcium channels thus do not appear to contribute to the NTLI response to glucose.

The diterpene forskolin and the inhibitor of phosphodiesterase (IBMX), which both elevate intracellular cAMP levels via distinct pathways, stimulated the release of NTLI from the rat jejunoileal preparation. This confirms an earlier observation, on isolated canine N cells, showing an increase in NTLI secretion along direct activation of the adenylate cyclase system by forskolin (Barber *et al.* 1986). In contrast, intra-arterial infusion of PMA did not stimulate the secretion of NTLI in the present work. Although the effect of the activation of protein kinase C by PMA on NTLI release was not tested in the partially purified N cell preparation, it was shown that staurosporin, a relatively specific inhibitor of protein kinase C, reduced the release of NTLI stimulated by fatty acids by 60% (Barber, Cacace, Raucci & Ganz, 1991). From data gathered in two studies performed with the partially purified

N cell system (Barber *et al.* 1986, 1991), it appears that the maximal release of NTLI secretion induced through activation of adenyl cyclase is 3- to 6-fold higher than that produced through activation of protein kinase C. Overall, the secretion of intestinal NTLI seems to be mainly governed by a cAMP-dependent mechanism. The reason why IBMX potentiated glucose-induced NTLI release in the present study is not known. An additive or greater-than-additive action of forskolin on noradrenaline-induced NTLI release was observed in a primary culture of canine endocrine N cells (Barber *et al.* 1986). Since noradrenaline-stimulated NTLI release is mediated by a β-adrenergic receptor generally coupled to the adenyl cyclase system, it was speculated that noradrenaline and forskolin stimulated NTLI release by acting at different steps of the cAMP-dependent pathway. The same mechanism might well apply to the synergistic effect of IBMX on glucose-induced NTLI secretion here observed, suggesting that glucose stimulates NTLI release through a cAMP-dependent cell-activating mechanism. This hypothesis is supported by the fact that PMA did not potentiate the release of NTLI produced by luminal glucose. Further experiments with N cells purified to homogeneity are required to elucidate this point.

In conclusion, glucose stimulation of N cells appears to require active, stereospecific and Na^+-dependent apical transport of the hexose; intracellular metabolism is not mandatory, nor is facilitated hexose transport at the basolateral pole. The response to glucose is probably mediated by a cAMP-dependent pathway. Finally, calcium channels and intramural neurone pathways play no crucial role in glucose-induced NTLI secretion.

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