Interaction of GLP-1 with NPY, VIP and Galanin

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Abstract
Many central neurotransmitters are involved in the control of feeding behaviour and disturbed metabolism of these neuropeptides contribute to hyperphagia and feeding related disorders of diabetes. Glucagon like peptide 1 (GLP-1) had been found to decrease food intake when administered intracerebro ventricular (ICV). It is not known whether this effect is direct or through modulation of other feeding regulatory peptides like Neuro Peptide Y (NPY) and galanin. The present study was conducted to know the interaction of GLP-1 with NPY, Vasoactive Intestinal Peptide (VIP) and galanin by measuring changes in contents of these peptides in hypothalamus, brain stem, intestine and pancreas in normal and diabetic rats which were infused with 32 nmol/kg body wt/day GLP-1 or saline (controls) for one week.

GLP-1 infusion significantly decreased NPY, VIP and galanin contents in normal and diabetic rats in intestine and hypothalamus while no significant changes in brain stem were observed. A significant decrease in pancreatic NPY and VIP was also observed in diabetic rats.

It is concluded that GLP-1 acts centrally and peripherally directly and via humoral and nervous factors. NPY, VIP and galanin have autocrine and paracrine role in CNS, pancreas and intestine. GLP-1 acts by modulation of these peptides in these tissues.

Keywords: Glucagon like peptide-1, Neuropeptides, Galanin, NPY, VIP, Interaction

1. Introduction
Many central neurotransmitters are involved in the control of feeding behaviour and disturbed metabolism of these neuropeptides in diabetes contribute to hyperphagia and other feeding related disorders of diabetes. GLP-1 had been found to decrease the food intake when administered ICV. (1 & 2). It is not known whether this effect is direct or through modulation of other feeding regulatory peptides like NPY and galanin. At present central role of GLP-1 and its interaction with NPY, VIP and galanin is unclear. We suggested that GLP-1 may regulate neuropeptides like NPY, galanin and VIP concerned with feeding and glucose homeostasis. Altered metabolism of NPY, VIP and galanin has been found in diabetes mellitus (3-5). We proposed that abnormal metabolism of these peptides may contribute in the development of acute and chronic complications of diabetes mellitus and GLP-1 may help to control these complications by normalising the concentration of these peptides.

GLP-1 induced NPY gene expression and secretion in pancreatic B cells lines (6). The effect of GLP-1 on NPY in B cells is required to be tested in pancreas in-vivo. Interaction of GLP-1 (7-37) with galanin was reported by (7). They observed that galanin inhibits GLP-1 induced proinsulin gene expression. It means negative correlation occurs between GLP-1 and galanin. Little is known about the interaction of GLP-1 with VIP which has role in central and peripheral regulation of glucose homeostasis (8, 9). Therefore the present study was aimed to know the effect of GLP-1 on tissue contents of NPY, galanin and VIP in hypothalamus, brain stem and pancreas.

2. Materials and methods
Male wister rats weighing 240-280 g were obtained. Sixteen rats were made diabetic by i/v injection of 60 mg /kg body weight of streptozotocin (Sigma Chemicals Co. USA) Another group of normal rats (8 control and 8 GLP-1 infused) were also included in the study. After one week of streptozotocin injection eight diabetic and eight normal rats were treated with 32 nmol/kg body weight of intraperitoneal (IP) GLP-1 injection. Therefore study included.

1) Normal control rats, n=8 (0.3 ml IP saline injection)
2) Normal treated rats, n=8 (0.3 ml IP GLP-1, 32 nmol/kg body weight/day)
3) Diabetic control rats, n=8 (0.3 ml IP saline injection)
4) Diabetic treated rats, n=8 (0.3 ml IP GLP-1, 32 nmol/kg body weight/day)

One rats from normal control, two from normal treated rats, three from diabetic control and two from diabetic treated rats died during the study.

The rats were treated with GLP-1 for one week and their blood glucose was measured on every alternate day. On the final day of treatment rats were anaesthetized and killed by exanguination by cardiac puncture after 45 minutes of last day GLP-1 injection. The pancreas, intestine (5-6 cm), hypothalamus and brain stem were taken and immediately transferred to liquid nitrogen and than stored at -70°C for peptide extraction.

3. Peptides extraction

Tissues were weighed immediately on harvesting and weight was recorded. Tissue was placed in polypropylene tube immediately to minimise evaporation. Approximately 10 X tissue weight volume of 0.5 M acetic acid was added to the tubes. The tubes were then placed with loosely fitted cap in vigorously boiling water bath (Keeping topping up level of water bath). The tubes were allowed to stand in boiling water bath for water in the tube to reach 100°C. Then they were left at 100°C for 10 minutes and after that were allowed to cool for 10 minutes. Tubes were labelled and tightly packed and stored at –20°C. For calculation of results weight of tissue and volume of 0.5 M acetic acid added were recorded (10).

4. Radioimmunoassay (RIA)

Samples from tissues extracts (2-20 ul) were assayed in duplicate for galanin (11), neuropeptide Y (12) and vasoactive intestinal peptide (13). Briefly all assays were performed in a total volume of 0.8 ml of phosphate buffer (pH 7.4), containing 10 mmol/1 EDTA, 1% (w/v) BSA. The sensitivity of an assay increases with incubation time reaching a maximum after about 5 days. Incubation was carried out at 4°C to help prevent bacterial growth and minimize proteolytic degradation and evaporation. After incubation antibody bound label was separated from free label by adding 250 ul of a suspension containing 4-8 mg charcoal (Norit GS, Hopkins and Williams) coated with clinical grade dextran (1:10 g charcoal, average mol wt 70,000, Sigma). The tubes were centrifuged at 1600 g for 20 minutes at 4 °C, followed by immediate separation of the supernatant. All the radioimmunoassay were performed.

5. Results

GLP-1 infusion decreased NPY contents in intestine in diabetic (12.3 ± 1.2 in GLP-1 infused vs 25.2 ± 1.6 in controls; p <0.001) and in non diabetic rats (16.0 ± 1.0 in GLP-1 infused vs 23.5 ± 0.8 in controls; p < 0.001). In brain stem no significant effect on NPY contents were observed in both the groups (8.1 ± 0.7 in GLP-1 infused vs 9.1 ± 0.8 in controls in diabetic and 9.0 ± 0.6 vs 9.6 ± 1.0 in controls in non diabetic rats). A well marked reduction in NPY contents were seen in hypothalamus in diabetic and normal rats by GLP-1 (141.8 ± 13.7 in GLP-1 infused vs 315.8 ± 16.6 in control in case of diabetic; p < 0.001) and 121.9 ± 11.2 vs 285.4 ± 20.7 in controls in non diabetic rats; p < 0.001). A decrease in pancreatic NPY was also observed in pancreas in diabetic rats (4.0 ± 0.3 in GLP-1 infused vs 6.0 ± 0.4 controls; p<0.001) (figure 1). NPY contents were more in diabetic as compared to non diabetic control rats in hypothalamus indicating increased NPY production in diabetic rats. These results showed that GLP-1 interacts with NPY in all the tissue and it is a negative modulator of NPY.

GLP-1 has been found to decrease food intake in rats and it is suggested that GLP-1 act through inhibition of NPY contents in hypothalamus. GLP-1 may be helpful to reduce hyperphagia in diabetics by reducing the content of NPY. Suppressive effect of GLP-1 in pancreas on NPY is important because NPY decrease insulin secretion and increase glucagon production in isolated islets. The GLP-1 has opposite action on these two hormones. It is suggested that GLP-1 may affect islet directly as well through inhibition of production of NPY and decreased NPY caused increased secretion of insulin. This was the first study in which effect of GLP-1 on tissue contents of NPY was observed and this study generated valuable insights regarding the mechanisms of action of GLP-1 in brain and on peripheral tissues.

GLP-1 decreased VIP contents in intestine (166.1 ± 17.9 vs 288.8 ± 34.9 in controls in diabetes; p<0.01 and 162.9 ± 5.2 vs 234.2 ± 13.1 in controls in non diabetic rats; p<0.001). In brain stem no significant effect on VIP of GLP-1 was observed. In hypothalamus GLP-1 had marked effect on VIP contents (15.9 ± 2.3 in GLP-1 infused vs 27.5 ± 1.9 in controls in diabetes; p < 0.01 and 10.3 ± 1.1 in GLP-1 infused vs 15.1 ± 1.3 in controls in non diabetic rats; p <0.05). Similarly significant suppressive effect of GLP-1 on tissue contents of VIP in pancreas in diabetic rats was observed (2.4 ± 0.5 in GLP-1 infused vs. 7.8 ± 2.2 in diabetic controls; p <0.05) (figure 2). The VIP contents in intestine and hypothalamus in diabetic control rats were more as compared to non diabetic control rats indicating increased contents of VIP diabetic rats.
Galanin contents were significantly decreased in intestine (42.6 ± 6.1 GLP-1 infused vs 87.9 ± 16.5 in controls; p < 0.05 in diabetic rats and 50.8 ± 3.7 GLP-1 infused vs 88.0 ± 6.4 in control in normal rats; p<0.001). In hypothalamus galanin was significantly depressed by GLP-1 infusion (80.3 ± 6.1 in GLP-1 infused vs 105.7 ± 9.8 in non diabetic control rats; p<0.05 and 64.9 ± 13.7 in GLP-1 infused vs 104.7 ± 10.0 in diabetic control rats; p < 0.05). In brain stem galanin contents were decreased (4.9 ± 0.3 in GLP-1 infused vs 5.0 ± 0.5 in diabetic control rats and 5.0 ± 0.4 in GLP-1 infused vs 5.1 ± 0.9 in non diabetic control rats) but statistically non significant (figure 3).

It was the first study in which effects of GLP-1 on NPY, VIP and galanin has been observed and the results of this study will be helpful in understanding of hormonal regulation of these peptides.

6. Discussion

GLP-1 is synthesized in the intestinal L-cell and released into the circulation following a meal (14). Systemic administration of GLP-1 stimulates insulin release from pancreatic β-cells (15). Other effects of systemically administered GLP-1 include delayed gastric emptying and inhibition of gastric acid (16). GLP-1 has been shown to be present in the rat hypothalamus (17 & 18) and a similar distribution of specific hypothalamic GLP-1 receptors has been identified in both man and rat (18-20).

From these finding we suggested that GLP-1 performs its central and peripheral functions by binding to its receptor and may also modulate neuro-peptides which may synergise the action of GLP-1. To date, a physiological role of central and peripheral GLP-1 on the regulation of NPY, VIP and galanin has not been known.

Tissue contents of NPY decreased in intestine, hypothalamus and pancreas by GLP-1. It has been observed previously that icv GLP-1 administration inhibited the food intake in fasted rats (1 & 2). Neuropeptide Y is the most powerful stimulant of feeding known when injected intracerebro ventricular (21). Intracerebro ventricular administration of extendin (9-39) (GLP-1 antagonist) immediately prior to NPY significantly increased food intake as compared to treatment with NPY alone (21). This indicated that GLP-1 may act on hypothalamus by decreasing endogenous NPY concentrations in hypothalamus. Our findings are consistent with these observations because decreased contents of NPY by administration of GLP-1 have been observed in the present study.

Decreased NPY by GLP-1 may be also being indirectly mediated through increased insulin levels caused by GLP-1 and insulin in turn suppressed the NPY. Suppression of NPY by insulin has been observed by (22). Therefore it is suggested that GLP-1 may inhibits NPY by direct binding to its receptors and also indirectly by increasing insulin levels. Increased contents of NPY in diabetic control rats as compared to normal control rats explain the pathophysiology of hyperphagia in diabetic patients and it is suggested that hyperphagia in diabetics is due to increased NPY in specific hypothalamic regions of diabetic rats. This finding is consistent to (3, 10, 22) because they also observed increased hypothalmic neuropeptide Y concentrations in diabetic rats. It is suggested that GLP-1 may correct diabetic hyperphagia by reducing NPY in hypothalumus.

GLP-1 induced NPY gene expression and secretion in INS-1 cells which have characteristics remarkably similar to those of normal B cells (6). But, in-vitro in present study suppressive effect of GLP-1 on NPY content in the pancreas has been observed. Waeger et al (6) observed effect of GLP-1 on NPY gene expression in a cell line. The results observed in cell line should be interpreted with reservation to compare them with in-vivo due to transformed form of the cells. Secondly, in-vivo various other hormonal and nervous factors are also involved and they participate in the out come of action of GLP-1. Further studies are needed to clarify this in-vivo and in-vitro contradiction observed in present study and Waeger et al (6). Decreased NPY in intestine due to GLP-1 infusion was observed and it is suggested that action of GLP-1 on gut e.g. delayed gastric emptying and inhibition of gastric acid secretion may be modulated partly through changes in NPY in gut by GLP-1.

VIP contents were decreased by GLP-1 in intestine, hypothalamus and pancreas but no change in VIP in brain stem was observed. It has been shown that in rats, the neurons in hypothalamic suprachiasmatic nucleus are involved in the regulation of glucose metabolism. VIP containing neurons in suprachiasmatic nucleus regulate the glucose metabolism (8). Injection of VIP in lateral cerebral ventricle caused hyperglycaemia and hyperglucagonemia. These facts suggested that VIP is involved in the regulation of glucose metabolism governed by suprachiasmatic nucleus and increased VIP in Non insulin dependent Diabetes Mellitus (NIDDM) may contribute in the development of hyperosmolar coma. It is suggested that GLP-1 is also involved in the central regulation of glucose metabolism governed by suprachiasmatic nucleus and it does so by decreasing the contents of VIP in hypothalamus significantly as observed in present experimental model. Stimulation of glycogenolysis and gluconeogenesis in isolated rat hepatocytes by VIP has been observed previously (9) and
inhibition of insulin stimulated glucose transport by VIP in rat adipocytes had been reported (23). GLP-1 has opposite action on these tissues as observed by VIP. We explain these peripheral effects of GLP-1 partly by decreasing VIP contents in these tissues by GLP-1. VIP affects endocrine secretions of pancreas and stimulates insulin and glucagon (24 & 25) and somatostatin (26). Very high amounts of VIP are required to stimulate glucagon and insulin secretion as compared to those to stimulate somatostatin (26). Therefore it is suggested that under physiological condition GLP-1 inhibit VIP contents in pancreas which in turn suppresses somatostatin and due to inhibition of somatostatin net outcome is increased insulin secretion. Inhibition of somatostatin by GLP-1 has been observed (27). It is proposed that this inhibition of somatostatin by GLP-1 may be due to direct action of GLP-1 on D cells and also through reduction in contents of VIP as observed in present study. Therefore actions of GLP-1 on endocrine pancreas may be partly modulated by reduction of VIP in pancreas by GLP-1. Further studies will clarify role of GLP-1 on VIP inhibition and its applications in physiological and pathological conditions.

Galanin contents were significantly reduced in intestine and hypothalamus by GLP-1 but no effect in brain stem was observed. Negative interaction of GLP-1 with galanin has been reported previously (7 & 28). We confirmed these findings in present study and reported that GLP-1 decreases galanin in intestine and hypothalamus. GLP-1 is a insulinotropic hormone while galanin inhibit insulin secretion. It is therefore suggested that GLP-1 acts on islet cells directly and via humoral and nervous factors and that galanin is one of peptide through which GLP-1 may affect islet cells secretions.

It is concluded that GLP-1 acts centrally and peripherally directly by binding to its receptors and via humoral and nervous factors. NPY, VIP and galanin are peptides which have autocrine and paracrine role in CNS and peripherally in pancreas and intestine. GLP-1 acts by decreasing contents of these peptides in hypothalamus, intestine and pancreas.

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References


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Figure 1. Tissue contents of NPY in diabetic and normal rats infused with GLP-1 and saline (control). *p<0.01 & ** p<0.001. Units for hypothalamus are 10 pmol/g tissues
Figure 2. Tissue contents of VIP in diabetic and normal rats infused with GLP-1 and saline (control). *p<0.05, **p<0.01 & ***p<0.001. Units for intestine are 10 pmol/g tissues.

Figure 3. Tissue contents of Galanin in diabetic and normal rats infused with GLP-1 and saline (control). *p<0.05 & **p<0.01.