Effect of Alloxan-diabetes on the Immunoreactivity of Glicentin-producing L-cells in the Small Intestine of Sprague Dawley Rat

Edward O. Uche-Nwachi and Comille V. Mitchell

Department of Anatomy Unit, Faculty of Medical Sciences, University of the West Indies, St. Augustine, Trinidad and Tobago.

ABSTRACT

The L-cells of the small intestine synthesize a proglucagon molecule which is processed to form glicentin, oxyntomodulin, glucagon-like peptide 1 (GLP-1) and glucagon-like peptide 2 (GLP-2). Glicentin and oxyntomodulin are reported to inhibit gastric secretion, and to delay transit time through the stomach. Levels of GLP-1 and GLP-2, have been reported to vary in types 1 and 2 diabetes and in streptozotocin-induced diabetic rats. It was not clear which cell (α or L) was responsible for these variations. Since the products of L-cells are released synchronously on stimulation, it is believed that all the secretions of L-cells will be affected in these variations.
In this experiment the effect of alloxan diabetes on glicentin immunoreactivity in the L-cells of intestine was investigated. Results showed that the immunoreactivity of glicentin was significantly reduced in the L-cells of the small intestine of alloxan-diabetic rats.

Keywords: Glicentin, Immunoreactivity, Pancreatic α-cells.

INTRODUCTION

Masharani and Karam [1] reported that human proglucagon is processed differently in the pancreatic α-cell and in the intestinal L-cells. In the pancreas according to them, the major product is glucagon, whereas in the intestinal L-cells the major products released are glicentin, oxyntomodulin, glucagon-like peptide 1 (GLP1) and glucagon-like peptide 2 (GLP-2). This according to Dhanvantari, Seidah, and Brubaker [2] and Damholt, et al [3], is due to the presence of distinct prohormone convertase (PC) in α-cells of the pancreas and L-cell of the small intestine. In the L-cells, they reported that PC 1 and PC 3, convert the proglucagon to glicentin, oxyntomodulin, GLP-1 and GLP-2, while PC 2 converts proglucagon in the α-cells in the pancreas to produce glucagon and GLP-2 [2,3].

According to Thim and Moody [4] and Holst [5], glicentin consists of 69 amino acid residues. The sequence of glicentin [1-30] represents the glicentin-related pancreatic peptide [GRPP], while the sequence [33-61] represents the full sequence of glucagon and the sequence [64-69] is a C-terminal hexapeptide.

The products of L-cells are released synchronously in response to appropriate stimulation. Thus glicentin, oxyntomodulin, GLP-1 and GLP-2 are all released on the proper stimulation of the gut [5]. Naito et al [6] reported that glicentin is secreted from the small intestine in response to intraluminal glucose stimulation in humans.

Druker et al [7,8] had reported that glicentin stimulates intestinal growth in rodents and that administration of glicentin to rats or mice produced small bowel growth, although not to

the extent as the more potent GLP-2. Holst [5] later reported that glicentin inhibits gastric secretion, while Pellisseir et al. [9], recently reported that glicentin regulates digestion, and reduces gastric secretion and delays gastric emptying. Savage et al. [10], and Myojo et al. [11], reported that it stimulates insulin and gastric acid secretions, gut growth, and regulates gut motility. Recently Hashimoto et al [12] also reported that glicentin stimulates the growth of normal and atrophic bowel mucosa. Earlier Sasaki et al. [13] had reported that this trophic effect is limited to the small intestine but not the colon.

The existence of receptors, specific for glicentin and oxyntomodulin in smooth muscle cells, has been reported by Rodier et al [14]. In a different study however, Shibata et al [15] reported that glicentin had no effect on contraction in any part of the gut, and therefore does not contribute to gastroduodenal motility.

Glesson et al [16], Bloom [17], and Brubaker et al [18], reported that patients with enteroglucagonoma presented with severe constipation and delayed intestinal transit. These symptoms, according to these authors disappeared after surgical removal of the tumor.

The effect of diabetes on the levels and/or the response of GLP-1 and GLP-2 to mixed meal has been varied. Lugari et al [19] reported that in type 2 diabetics, fasting GLP-1 was similar to controls. However Mannucci et al [20], reported that GLP-1 amide baseline concentrations are reduced in type 2 diabetics, while Vilsboil et al [21], reported that late intact GLP-1 postprandial response was strongly reduced in type 2 diabetics. Vilsboil et al [22], reported that type 2 diabetic patients have decreased plasma concentration of GLP-1 after ingestion of normal mixed meal when compare with control while Vilsboil et al [23], reported that the decreased GLP-1 secretion may contribute to impaired insulin secretion in type 2 diabetics. They reported also that the secretion was normal in type 1 diabetics. Naito et al. [6] had reported a minor decrease of plasma glicentin levels in diabetic patients when compared with control. However, Orskov, et al [24], had reported that increased GLP-1 immunoreactivity from pancreatic α-cells in STZ-diabetic rats. Hiroyoshi et al [25] also reported that the plasma GLP-1 levels were significantly elevated after oral glucose in diabetics. Hartman et al [26], reported elevated endogenous GLP-2 in streptozotocin diabetic rats. Nie et al [27] reported that hyperglycemic rats had increased prohormone converting enzymes in islet alpha cells, leading to increase in amidiated GLP-1.

According to Holst [5], the products of L-cell secretion are released synchronously, in response to appropriate stimulation. Although Mannucci et al. [20], Vilsboll et al. [21,22, 23] reported decreased levels of GLP-1 in diabetics, and Naito et al. [6], had reported a minor decrease of plasma glicentin levels in diabetics, there has been little done to determine the cellular origin of these enteroglucagon derivatives.

The aim of this experiment was to determine the effect of hyperglycemia on the immunoreactivity of glicentin in the L-cells of the intestine. This may also help to determine whether the variations of GLP-1 and GLP-2, reported in diabetics, are due to intestinal L-cells, or to pancreatic α-cells.

MATERIALS AND METHODS

Thirty Sprague Dawley rats (fifteen males and fifteen females), weighing between 250-300g were selected from the Animal House of the Faculty of the Medical Sciences, University of the West Indies. They were given the normal rat feed and water ad libitum for two weeks before the commencement of the experiment. Their caging and care was strictly under the stipulated guidelines of the Faculty of Medical Sciences guideline. Fifteen of the animals were made diabetic by intraperitoneal injection of alloxan monohydrate 150mg/kg body weight. Control animals were injected similar volumes of physiologic saline. Their blood glucose levels were measured twice daily, using One Touch Profile Glucose Meter (Johnson and

Johnson, Trinidad) and the rats with blood glucose levels of between 250-600mg/dl were considered diabetic. Control rats had blood glucose ranging between 60-70mg/dl. Diabetic rats were main-tained in the diabetic range by oral adminis-tration of Gliclazide solution (80mg/70kg body weight) prepared by dissolving 80mg of the tablet in 10ml of water. The animals were maintained in the diabetic blood range for 12 weeks.

At the end of 12 weeks, twenty rats, (ten diabetic and ten control) were sacrificed by direct blow to the head. Their entire small intestine was dissected and specimens were obtained from the jejunum and the ileum.

Immunohistochemistry

Paraffin sections 5 microns thick were pre-incubated with 5% non-fat milk in phosphate buffered saline for 30 minutes at room temperature. The sections were then incubated for 60 minutes in a humidified chamber with rat glicentin (Novocastra Laboratories Ltd, United Kingdom) at 25^0C. The slides were then rinsed in Tris NaC1 pH 7.4. The sections were then incubated with secondary antibody, goat biotinylated anti-rabbit antibody diluted in TRIS-NaC1 buffer for an hour. After rinsing with TRIS/NaC1 buffer, Avidin Biotin Complex was applied at a concentration of 10µ1 of A and 10µ1 of B per ml of the TRIS/NaC1 for one hour. The sections were then rinsed in TRIS and then placed in sodium acetate buffer 0.1M pH 6.0. 1.5gr Ammonium Nickel Sulphate was dissolved in 50ml of sodium acetate buffer 0.2M pH 6.0. This was left to stir into solution. 50mg diaminobenzidine, dissolved in 50ml distilled water, was then poured into the ammonium nickel sulphate solution. The following salts were then added to the solution in this order: 200mg glucose, 40mg ammonium chloride, and 1mg β glucose oxidase. This solution was stirred briefly and poured onto the sections. The sections were then rinsed, dehy-drated, cleared in Xylene, cover slipped and mounted in protex.

RESULTS

The immunoreactivity for glicentin was significantly reduced in the jejunum and the ileum in the diabetic animals when compared with control (Fig. 1a and 1b and 2a and 2b).

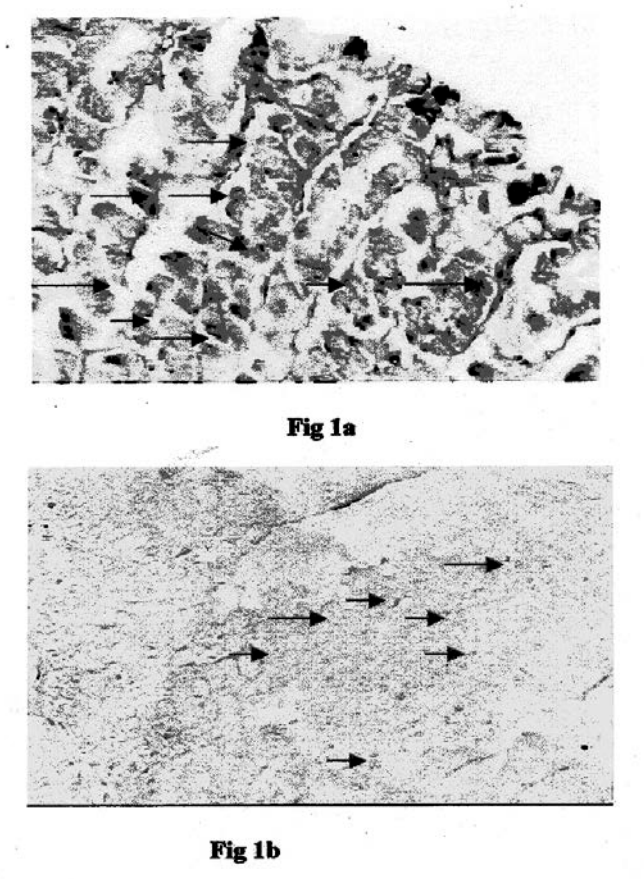

Fig. 1. Photomicrograph of small intestine (jejunum) of Sprague Dawley rats stained to demonstrate the immunoreactivity of glicentin in L-cells. Control rats showed more immunoreactivity (a) than experimental rats (b) x40

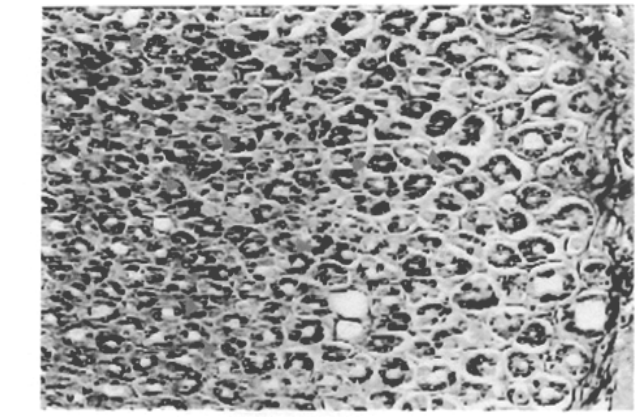

Fig 2a

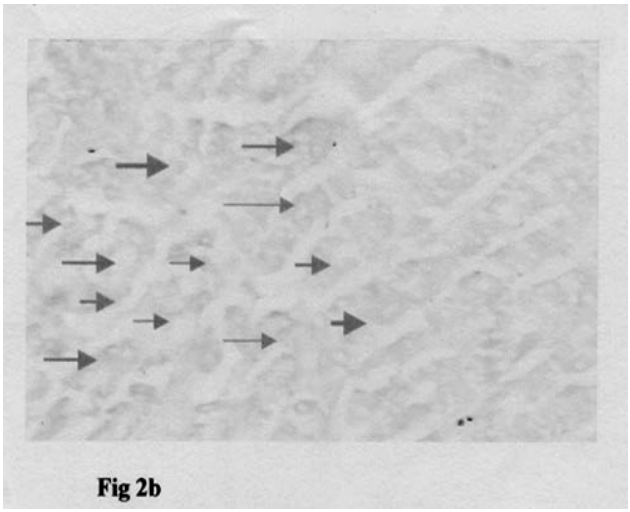

Fig 2b

Fig. 2. Photomicrograph of small intestine (ileum) of Sprague Dawley rats stained to demonstrate the immunoreativity of glicentin in L-cells. Control rats showed more immuno reativity (a) than experimental rats (b) x40

DISCUSSION

In this experiment the immunoreactivity of glicentin secreting L-cells was significantly reduced in alloxin-diabetic Sprague Dawley rats when compared with control (Figs 1a, 1b and Figs 2a, 2b). This is consistent with the findings of Naito et al [6]. According to Manuccci et al. [20], Vilsboil et al. [21,22], there is a decrease in GLP-I and GLP-2 secretions in diabetes. The implication of this finding is that all the secretions of the intestinal L-cells are decreased in diabetics. This finding further reinforces the findings of Holst [5], who reported that the products of L-cells are released synchronously on appropriate stimulation. This also suggests that the decrease in the concentrations of GLP-1 and GLP-2, reported in diabetics by Mannucci et al [20], Vilsboil et al [21], and Vilsboil et al [22] and Vilsboil et al [23], is probably of L-cell origin. Thus the major products of L-cell processing of proglucagon; glicentin, oxyntomodulin, GLP-1, and GLP-2 are reduced in diabetes.

Glesson et al [16], Bloom [17] and Brubaker et al [18], reported severe constipation and delayed intestinal transit in patients with enteroglucagonoma, while Pellisseir et al. [9], reported that glicentin reduces the duration of postprandial myoelectrical activity of the duodenum and the jejunum by 63% and 65% respectively. According to Vanik et al. [28], the most common lower G1 symtom of diabetes is constipation. The implication of this finding is that glicentin probably does not contribute to this symptom.

REFERENCES

1. Masharani U and Karam JH (2001) Pancreatic hormones and diabetes mellitus. In Basic and Clinical Endocrinology. Greespan FS and Gardner DG Eds. New York: McGraw-Hill, Pp623-698.
2. Dhanvantari S, Seidah NG, and Brubaker PL (1996) Role of prohormone convertase in the tissue-specific processing of proglucagon. Mol Endocrinol 10:342-355.
3. Damholt AB, Buchan AM, Holst JJ, Kofod H (1999) Proglucagon processing profile in canine L-cells expressing endogenous prohormone convertase 1/3 and prohormone convertase 2. Endocrinology; Oct; 140(10): 4800-8.
4. Thim L and Moody AJ (1981) The primary structure of porcine glicentin (proglucagon). Regul Pept. May; 2(2): 139-50.
5. Holst JJ (1997) ENTEROGLUCAGON; Annual Review of Physiology March; 59: 257-271.
6. Naito H, Ohneda A, Kojima R, Sato T, Sasaki K, Funyama Y; Jukishima K; Shibata C; Matsuno S; and Sasaki I (1999) Plasma glicentin in diabetic and gastrectomized Patients; Regul Pept Jan 1; 79(1): 55-6.

7. Druker DJ,Ehrlich P Asa SL, Brubaker PL Induction of intestinal epithelial proliferation by glucagons-like peptide 2. Proc Natl Acad Sci USA; 93:7911-7916.
8. Druker DJ, Yusta B, Boushey RP, Deforest L, and Brubaker PL (1996) Human Gly-2-GLP-2 reduces the severity of colonic injury in murine model of experimental colitis. Am J Physiol 276: G 79-G91.
9. Pellissier S, Sasaki D, Le-Nguyen D, Bataille (2004) Oxyntomodulin and glicentin are potent inhibitors of the fed motility pattern of small intestine. Neurogastroenterology and Motility, Aug, 16(4): 455-459.
10. Savage AP, Matthewa JL, Ghatei MA, Cooke T, Bloom SR (1987) Enteroglucagon and experimental carcinogenesis in the rat. Gut. Jan; 28(1): 33-339.
11. Mayojo S, Tsujikawa M et al (1997). Tropic effect of glicentin on rat small intestinal mucosa in vivo and in vitro. J. Gastroenterol. 32:300-305.
12. Hashimoto N, Ohyanagi H, Kazuyuki S (2003) Effect of glicentin on gut mucosal growth in rat with jejunal and ileal Thiry-Vella fistula. J Pediatr Surg; Apr; 38(4): 579-84.
13. Sasaki M, Fitzgerald AJ, Mandir N, Sasaki K, Wright NA, Goodlad RA (2001) Glicentin an active enteroglucagon, has a significant tropic role on the small intestine but not on the colon in rats. Aliment Pharmacol Ther; Oct; 15(10) 1681-6.
14. Rodier G, Magous R, Mochizuki T, Le Nguyen D, Martinez J, Bali JP, Bataille D, Jarrousse C, and Genevieve R (1999) Glicentin and oxyntomodulin modulate both the phosphoinositide and cyclic adenosine monophosphate signaling pathways in gastric myoctes. Endocrinology; Jan; 140(1): 22-28.
15. Shibata C, Naito H, Jin XL, Ueno T, Junayama Y, JukushimalK, Hashimoto A, Matsuno S, Sasali I (2001) Effect of glucagons, glicentin, glucagons-like Peptide 1 and 2 on interdigestive gastroduodenal motility in dogs with a vagally denervated gastric pouch; Scan J Gastroenterol; Oct; 36(10): 1049-55.
16. Glesson MH, Bloom SR, Polak JM, Henrey K and Dowling RH (1971) Endocrine tumour in kidney affecting small bowel structure, motility and absorptive function. Gut 12; 773-782.
17. Bloom SR (1972) An Enteroglucagon tumour. Gut 13: 520-523.
18. Brubaker PL, Druker DJ, Asa SL, Swallow C, Redston M and Greenberg. GR (2002). Prolonged Gastrointestinal Transit in a patient with Glucagon-like Peptide (GLP)-1 and -2 producing Neuroendocrine Tumour. The Journal of Clinical Investigation and Metabolism. Vol.87 No.7; 3078-3083.
19. Lugari L, Dell' Anna C, Ugolotti D, Dei Cas A, Barilli AL, Zandomeneghi R, Marani B, Iotti M, Orlandini A, and Gnudi A (2000) Effect of nutrients ingestion on glucagons-like peptide 1 (7-36 amide) secretion in human type 1 and 2 diabetes. Horm Metab Res; Oct; 32(10): 424-8.
20. Mannucci E, Ognbene A, Cremasco F, Bardini G, Mencucci S, Pierazzuoli E, Ciani S, Fnelli A, Messeri G, and Rotella CM (2000) Glucagon-like peptide (GLP)-1 and leptin concentrations in obese patients with type 2 diabetes mellitus. Diabet Med; Oct; 17(10): 713-9.
21. Vilsboll T, Krarup T, Deacon CJ, Madsbad S, and Holst JJ (2001) Reduced postprandial concentrations of intact biologically active glucagons-like peptide 1 in type 2 diabetic patients. Diabetes; Mar; 50(3): 609-13.
22. Vilsboll T, Agerso H, Krarup T, and Holst JJ (2003) Similar elimination rates of glucagons-like peptide-1 in obese type 2 diabetic patients and healthy subjects. J Clin Endocrinol Metab; Jan; 88(1): 220-4.
23. Vilsboll T, Krarup T, Sonne J, Madsbad S, Volund S, Juul AG, and Holst JJ (2003b) Incretin secretion in relation to meal size and body weight in health subjects and people with type 1 and type 2 diabetes mellitus. J Clin Endocrinol Metab; Jun; 88(6): 2706-13.
24. Orskov C, Jeppesen J, Madsbad S, and Holst JJ(1991) Proglucagon products in plasma of noninsulin-dependent diabetics and nondiabetic controls in the fasting state and after oral glucose and intravenous arginine. J. Clin. Invest. 87:415-423.
25. Hiroyoshi M, Tateishi K, Yasunami Y, Maeshiro K, Ono J, Matsuoka Y, and Ikeda S (1999) Elevated plasma levels of glucagon-like peptide-1 after oral glucose ingestion in patients with pancreatic diabetes. Am J Gastroenterol; Apr; 94(4): 976-81.
26. Hartman B, Thulesen J,Hard KJ, Kissow H, Orskov C, Poulsen SS, and Holst JJ (2002) Immunoneutralization of endogenous glucagons-like peptide-2 reduces adaptive intestinal growth

in diabetic rats. Regul Pept: May 30; 105(3): 173-9.
27. Nie Y, Nakashima M, Brubaker PL, Qiao-Li PR, Jansen E, Zambre Y, Pipeleers D and Friedman TC (2000) Regulation of pancreatic PC1 and PC2 associated with increased glucagons-like peptide 1 in diabetic rats J Clin Invest; April; 105(7) 955-65.
28. Vanik AL, Maser RE, Mitchell BD and Freeman R (2003) Diabetic autonomic neuropathy. Diabetes Care, May 26(5): 1553-76.