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Effect of Alloxan-Diabetes on Gastrin-Releasing Peptide (GRP) Immunoreactivity in the Gastrointestinal Tract, of Sprague Dawley Rats and How This May Affect Some of the Diabetic Complications

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Abstract: Gastrin-releasing peptide (GRP) is the mammalian analogue of bombesin. Both neuroendocrine peptides have similar distribution, functions and immunoreactivity. The immunoreactivity for GRP has been demonstrated in the esophagus, stomach and small intestine. This peptide is reported to have various biological and pharmacological properties, which include the release of gastrointestinal hormones, control of satiety, gastrointestinal motility and stimulation of cellular proliferation which results in wound healing. It is also implicated in the proliferation of many gastrointestinal (GI), renal and prostatic tumours. Transient increase in GRP synthesis in the brain and serum concentration, which was later followed by decreased serum concentration, has been reported in hyperglycemic states. The aim of this investigation was to determine the effect of hyperglycemia on GRP secreting neurons in the sub mucosa of the GIT and how this may affect some of the GI complications of diabetes. Result showed decreased immunoreactivity to GRP in the sub mucosal neurons of the stomach and small intestine in alloxan-diabetic Sprague Dawley rats. We conclude that this could contribute to the hyperglycemia-induced GRP decrease in diabetes. This could contribute to reduced peristalsis, with resultant constipation in diabetics. It is also possible that this may also contribute to poor wound healing in diabetics.

Key words: GRP immunoreactivity in the GIT of alloxan-diabetic rats

INTRODUCTION

Gastrin-releasing peptide (GRP) is a mammalian analogue of bombesin, which was isolated from porcine pancreas and gastrointestinal tract[1]. It is synthesized in the suprachiasmatic nuclei neurons, where the GRP-containing cell bodies are localized[2, 3]. It is demonstrated in the sub mucosal plexus of the enteric nervous system in the esophagus, stomach, small intestine and colon, where numerous GRP-immunoreactive nerve fibres are distributed in the lamina propria and the muscular layer[4-6]. GRP is also expressed by epithelial cells lining most of the gastrointestinal tract except the colon[7].

Gastrin-releasing peptide has multiple stimulating effects on metabolism, release of regulatory peptides, as well as gastrointestinal and pancreatic secretions. High levels of GRP-receptors are found in the smooth muscle fibres in the GIT and gall bladder, as well as in the secretory glands of the pancreas[8,9]. In vitro experiments with GRP caused smooth muscle contraction in almost all kinds of peripheral tissue preparation, while in vivo studies in humans demonstrated that it stimulates gastric acid, biliary and pancreatic secretions, as well as gall bladder contraction. GRP receptors are expressed in the longitudinal muscle fibres of the intestine, where they are reported to regulate gastrointestinal motility[10].

GRP has been reported to be a potent, dose-dependent arteriolar vasodilator and bronchoconstrictor in the guinea pig in vivo, although the mechanism of its action is not known[11, 12]. In the GIT, it is reported to be selectively released from sub mucosal neurons in descending pathways during the peristaltic reflex[13]. It had also been reported to induce gall bladder contraction, partly by direct action on the smooth muscles of the gall bladder and partly by gastrin release[14]. It is also suggested to be involves in prejunctional cholinergic-, somatostatinergic-and VIP-ergic pathways[15].

It had been earlier reported to inhibit glucose-stimulated insulin release, although it stimulates insulin secretion in clonal insulinoma cells[16,17]. GRP is reported to provoke a dose-dependent release of glucagon-like peptide-1 (GLP-1) and to additively stimulate the release of glucose-insulinotropic polypeptide (GIP) *in vivo*[18, 19].

It has been reported that although endogenous GRP may be a physiological regulator of gastric acid secretion, gastrin release does not seem to be under its control. Injection of GRP into the cerebrospinal fluid (CSF) has been reported to inhibit acid secretion in rats

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and dogs and to induce integrated gastric response which includes: increase in bicarbonate and mucus secretion, inhibition of acid and pepsin secretions, inhibition of vegally mediated contractions, as well as enhancing the resistance of the mucosa to injury through autonomic pathways[20,21] . GRP is one of the biologically important regulatory peptides that influence the meal stimulated pancreatic secretion and the receptors have been demonstrated in pancreatic islets[22,23].

Topical application of GRP has been reported to accelerate wound healing in burns, injuries, chronic ulcers and skin graft donor sites through the enhancement of keratinocyte growth and spreading[24]. It has been reported to be effective in promoting the healing process of chronic gastric ulcers in rats[25] and also found to improve intestinal barrier function and oxidative stress in experimental jaundiced rats[26]. There is recent evidence that GRP is a potent gastroprotective agent. This is achieved through activation of sensory neurons located in the gastric mucosa. Such activation causes increased production of nitric oxide through activation of constitutive nitric oxide synthase, with resultant increase in gastric mucosal blood flows, which renders the stomach less susceptible to damage from luminal irritants[27]. It has been reported to stimulate pancreatic beta cell function in rats with experimental diabetes[28].

The immunoreactivity of GRP is also reported to increase in the supraoptic and paraventricular nuclei in the second week following streptozotocin-induced diabetes. This later decreased in the fifth week. Thus it is now believed that the initial increase was a compensatory reaction directed on the activation of the central mechanisms of feeding restriction and stimulation of insulin synthesis[29].

The aims of this investigation was to determine the effect of alloxan-induced hyperglycemia on the immunoreactivity of GRP, in the submucosal neurons of the stomach and small intestine and to suggest the possible role of such findings on some diabetic complications.

MATERIALS AND METHODS

Animals: Forty Sprague Dawley rats, 20 males and 20 females, weighing 250-300gm were selected from the Animal Holding of the Faculty of Medical Sciences, University of the West Indies. Their fasting blood glucose levels were measured with One Touch Profile Glucose Meter (Johnson and Johnson Trinidad), before alloxan monohydrate (150mg/kg) was administered intraperitoneally to ten males and ten females. These represented the experimental animals. The rest were kept as control. Rats with blood glucose levels of 250-600mg/dl were considered to be diabetic. The rats were maintained on the diabetic fasting glucose range with gliclazide (80mg/kg body weight) orally every 24 hours. After 8 weeks the rats (control and experimental) were sacrificed by direct blow to the head. Their stomach and small intestines were dissected out for paraffin sections.

Immunohistochemistry: Paraffin sections 5 microns thick were pre-incubated with non-fat milk in phosphate buffered saline for 30 minutes at room temperature. The sections were then incubated overnight in a humidified chamber with rabbit bombesin at 25^0C. They were then rinsed in Tris-NaCl (pH 7.4). The sections were again incubated with secondary antibody: goat biotinated anti-rabbit antibody diluted in Tris-NaCl buffer for one hour. Avidin Biotin Complex was then applied for one hour. The sections were then rinsed in water, dehydrated cleared and mounted in protex.

RESULTS AND DISCUSSION

Stomach: The immunoreactivity to GRP was reduced in the submucosal neurons of the stomach of the diabetic rat (Arrows in Fig. 1b), when compared with the control rats (Arrows in Fig. 1a).

Small intestine: There is reduced immunoreactivity to GRP in the submucosal neurons of the diabetic rats (Arrows in Fig. 2b) when compared with control rats (Arrows in Fig. 2a).

The effect of gut hormones in glucose homeostasis as well as the paracrine effects of some of these hormones on gut motility has been reported[30].

GRP is reported to have direct paracrine action on smooth muscle preparations. Longitudinal muscle fibres showed concentration-dependent increase in rhythmic activity while circular muscle fibres had a little decrease in tone. The combined effect is the control of ileocolonic transit[31]. It had been reported that GRP is involved in regulating the motility of the gut and that only GRP receptor is expressed in human intestine, where the highest concentration is found in the longitudinal muscle fibres and the myenteric plexus of the Colon[10].

Constipation is reported to be one of the commonest lower GI complications of diabetes. Part of this is associated with the diabetic autonomic neuropathy[32]. Intrathecal administration of is reported to induce integrated gastric response to food, which included vegally mediated contractions, while its role in peristalsis has been reported[13, 21, 33, 34].

Diabetic autonomic neuropathy is reported to be the commonest cause of GI dysfunction, which usually presents as vagally controlled impaired motility. It was also reported that the dysfunction of intrinsic enteric neurons may contribute to this as well[35].

In this experiment, the immunoreactivity of GRP was reduced in submucosal neurons in the stomach and small intestine (Fig. 1b and 2b).

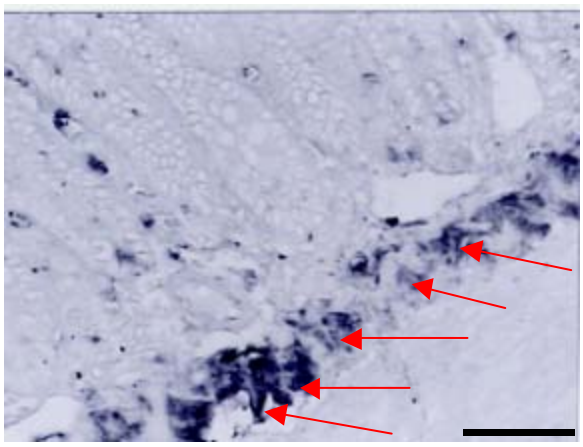

Fig. 1a

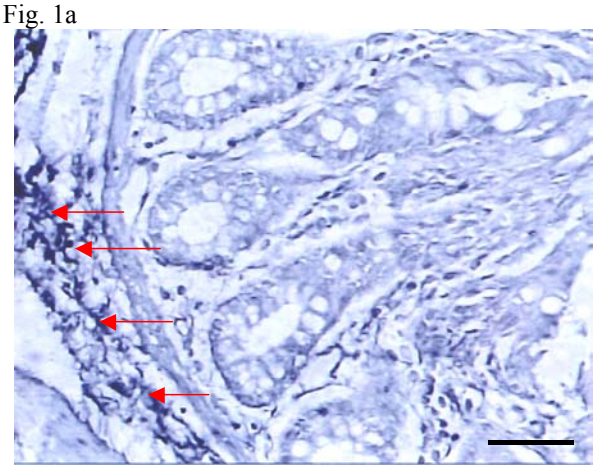

Fig. 1b

Fig. 1: Immunohistochemical staining for GRP in the stomach of control (Fig 1a) and alloxan-diabetic Sprague Dawley rats (Fig 1b). There is reduced immunoreactivity in the diabetic rat (Fig 1b), when compared with control (Fig 1a). Scale bar=3.8µm

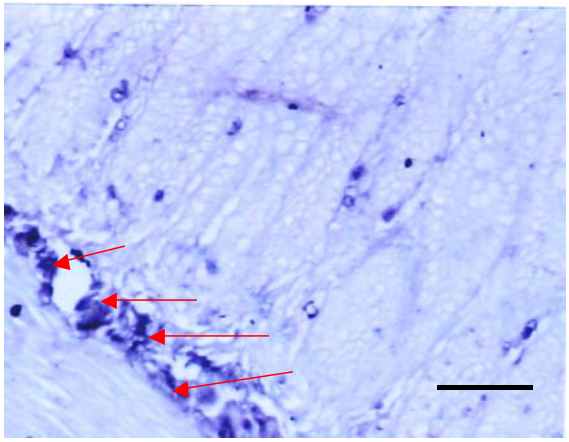

Fig. 2a

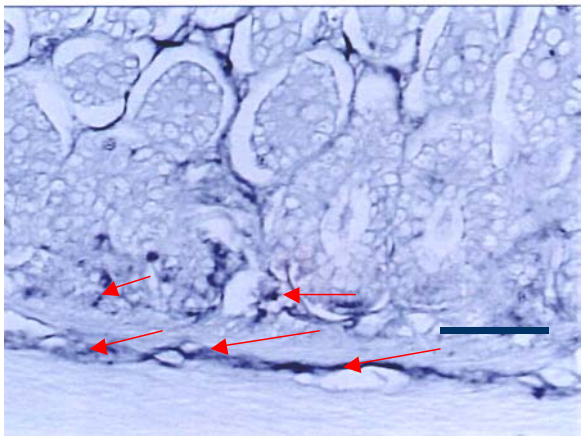

Fig. 2b

Fig. 2: Immunohistochemical staining for GRP in the duodenum of control (Fig. 2a), and alloxan-diabetic Sprague Dawley rats (Fig. 2b). There is reduced reactivity in the diabetic rat (Fig. 2b), when compared with control (Fig. 2a). Scale bar. a=4.3µm, b=5µm

This implies that, the paracrine effects of GRP on longitudinal muscle fibres in the GIT will be reduced. The resultant effect of this will be constipation, which is reported as one of the commonest GI complication in diabetics.

GRP has been reported to accelerate wound healing, in burns, injuries and chronic ulcers, through the enhancement of keratinocyte growth and spreading and also to be effective in promoting the healing process of gastric ulcers[24,25,36]. One of the common complications of diabetes is chronic leg ulcers, resulting from a combination of peripheral neuropathy and microangiopathy[33-37]. In this experiment, the immunoreactivity of GRP was reduced in the submucosal neurons of the GIT. This will affect the serum concentration, which has been reported to be reduced in diabetics[29]. This could contribute to delayed wound healing in diabetics.

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