Bombesin Immunoreactivity in the Stomach and Small Intestine of Alloxan-Diabetic Sprague Dawley Rats

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Abstract

Bombesin is a neuroendocrine peptide found in the submucosal nerve endings in the esophagus, stomach and small intestine. It is reported to stimulate the release of gastrointestinal hormones, control satiety, stimulate gastrointestinal motility, and also stimulate cellular proliferation which results in wound healing. Transient increases in bombesin concentration in the brain and serum (later followed by decrease in serum concentrations) have been reported in hyperglycemic states. The aim of this investigation is to determine the effect of hyperglycemia on bombesin secreting neurons in the sub mucosa of the GIT and the possible contribution of such changes to some diabetic complications. Result showed decreased immunoreactivity to bombesin in the sub mucosal neurons of the stomach and small intestine of alloxan-diabetic Sprague Dawley rats. We conclude that the reduced immunoreactivity of bombesin in these sub mucosal neurons, may contribute to the reduced paracrine-induced peristalsis, observed in diabetics. It may also contribute to poor wound healing in diabetics.

Key words: Bombesin, immunoreactivity, GIT alloxan-diabetic rats.

Introduction

Bombesin was originally isolated from the skin of the frog, Bombina bombina. It was later isolated from porcine pancreas and gastrointestinal tract in mammals. It is synthesized in some nuclei of suprachiasmatic neurons, where the bombesin-containing cell bodies are localized. Its is also demonstrated in the sub mucosal plexus of the enteric nervous system in the esophagus, stomach, small intestine and colon, where numerous bombesin-immunoreactive nerve fibres are distributed in the lamina propria and the muscular layer. Bombesin is also expressed by epithelial cells lining most of the gastrointestinal tract except the colon.

Bombesin stimulates gastrointestinal and pancreatic secretions, and high levels of bombesin-receptors are found in the smooth muscle fibres in the GIT and bladder, as well as in the secretory glands of the pancreas. In vitro studies demonstrated that bombesin caused smooth muscle contraction in almost all kinds of peripheral tissue preparation. Humans demonstrated that it stimulates gastric acid, biliary and pancreatic secretions. It also showed that it stimulates gall bladder contraction. The receptors are expressed in the longitudinal muscle fibres of the intestine, where they are reported to regulate the motility of the GIT. The effects of bombesin on smooth muscle fibres have been varied. Guinea pig experiments have shown that bombesin was a potent dose-dependent arteriolar vasodilator and a bronchoconstrictor. In the GIT, it is reported to be selectively released from sub mucosal neurons in descending pathways during the peristaltic reflex. Its effect on the gall bladder is reported to be partially due to direct action on the smooth muscle fibres and partly due to gastrin release.
Bombesin reactivity in Alloxan diabetic rats

Bombesin inhibits glucose-stimulated insulin release (although it stimulates insulin secretion in insulinoma cells\(^{16, 17}\)), provokes a dose-dependent release of GLP-1, and additively stimulates the release of glucose-insulinotropic polypeptide (GIP) \textit{in vivo}\(^{18, 19}\). Intrathecal injection of bombesin inhibits acid secretion in rats and dogs\(^{20}\). It induces integrated gastric response which includes increase in bicarbonate and mucus secretion, inhibition of acid and pepsin secretions, inhibition of vagus mediated contractions, and enhanced resistance (through autonomic pathways) of the mucosa to injury \(^{21}\). Bombesin is one of the biologically important regulatory peptides that influence the stimulation of pancreatic secretions by meals, and its receptors have been demonstrated in pancreatic islet cells\(^{22, 23}\).

Bombesin has been reported to play an important role in the control of food intake, and may contribute to dietary disruptions associated with anorexia and bulimia and it has been implicated in some eating disorders such as bulimia nervosa \(^{24, 25, 26}\). It induces a reduction in food intake and body weight in rodents and humans in a dose-dependent manner\(^{27}\). The effect of bombesin on satiety is reported to be due to the inhibition of feeding through the regulation of satiety and appetite\(^{28, 29, 30, 31}\). Synthetic bombesin fragment 6-14 show a reducing effect on food intake in male Wister rats \(^{32}\). A high density of bombesin receptors has been demonstrated in the central part of amygdala, which is essentially involved in the regulation of feeding and body weight\(^{30}\), and may include the termination of meals in mice\(^{33}\). A high oral dose (1.0nmol/kg) of Bombesin produces a significant suppression of feeding in man\(^{34}\), while injection of bombesin into the cerebral ventricles decreased food intake in a dose-dependent manner in male Wistar rats fasted for 17 hours\(^{35}\).

Topical application of bombesin accelerates wound healing through the enhancement of keratinocyte growth and spread, in patients with burn injuries, chronic ulcers and skin graft donor sites\(^{36}\). It has also been reported to be effective in promoting the healing process in chronic gastric ulcers in rats\(^{37}\).

Bombesin together with other hormones has modulatory effects on learning and memory\(^{38}\). Bombesin-R immunoreactivity has been reported in the GABAAergic neurons of the limbic region, which provides support for the idea that the bombesin/bombesin-R system mediates memory performance by modulating neurotransmitter release in the local GABAAergic network\(^{39}\).

Pancreatic beta cell function in experimental diabetic rats is stimulated with bombesin\(^{40}\), and the immunoreactivity of bombesin increases in the supraoptic and paraventricular nuclei in the second and fifth week (respectively) following streptozotocin-induced diabetes in rats. The initial increase is believed to be a compensatory reaction directed at the activation of the central mechanisms of feeding restriction and stimulation of insulin synthesis\(^{41}\).

The aim of this investigation is to determine the effect of alloxan-induced hyperglycemia on the immunoreactivity of bombesin in the submucosal neurons of the stomach and small intestine, and the possible implication of this effect on some of gastrointestinal complications of diabetes.

MATERIALS AND METHODS

Animals:

Sixty Sprague Dawley rats (30 males and 30 females), weighing 250-300gm were selected from the Laboratory animal facilities of the Faculty of Medical Sciences, the University of the West Indies. Their fasting blood glucose levels were measured with One Touch Profile\(^{8}\) Glucose Meter (Johnson and Johnson Trinidad), before streptozotocin solution (65mg/kg) was administered intraperitoneally to ten males and ten females. The remaining rats were used as controls. Rats with blood glucose levels of 250-600mg/dl were considered to be diabetic. 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 for 30 minutes at room temperature with non-fat milk in phosphate buffered saline. The sections were then incubated with rabbit bombesin overnight at 25°C in a humidified chamber. They were then rinsed in Tris-NaCl (pH 7.4). The sections were again incubated for one hour with secondary antibody - goat biotinated anti-rabbit antibody diluted in Tris-NaCl buffer. Avidin Biotin Complex was then applied for one hour. The sections were then rinsed in water, dehydrated cleared and mounted in protex.

**Results**

**Stomach**  Bombesin immunoreactivity was reduced in the submucosal neurons of the stomach of the diabetic rat (Arrows in Fig 1b), when compared with the control rats (Fig 1a)

**Small intestine:** The immunoreactivity of bombesin in the submucosal neurons was reduced in the diabetic rat (Fig 2b) when compared with control (Fig 2a)

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

**Fig 2:** Immunohistochemical staining for bombesin 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 (fig2a). Scale bar. a=4.3μm, b=5μm
Discussion

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. Bombsin is reported to have direct paracrine action on smooth muscle fibres. 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. Bombsin is also involved in regulating the motility of the gut, and only GRP receptors, which are the mammalian analogue of bombsin is expressed in human intestine, where the highest concentration is found in the longitudinal muscle fibres and the myenteric plexus of the colon. One of the most common lower GI complications of diabetes is constipation. Its pathogenesis is reported to be partly due to diabetic autonomic neuropathy. Diabetic autonomic neuropathy is the commonest cause of GI dysfunction, which usually presents as vagally controlled impaired motility, although dysfunction of intrinsic enteric neurons may contribute to this as well.

Intrathecal administration of bombsin is reported to induce integrated gastric response to food, which included vagally mediated contractions, with resultant peristalsis.

In this study, the immunoreactivity of bombsin was reduced in submucosal neurons in the stomach and small intestine, as shown in Figs 1b, and 2b. This reduction implies that the paracrine effects of bombsin on longitudinal muscle fibres of the GIT will be reduced, contributing to the constipation, which is reported as one of the commonest GI complication in diabetics.

Bombsin has also been reported to accelerate wound healing in burns, injuries, chronic ulcers, through the enhancement of keratinocyte growth and spreading, and also to be effective in promoting the healing process of gastric ulcers. Chronic leg ulcers, which are usually reported to be due to a combination of peripheral neuropathy and microangiopathy, are common complication of diabetes. In this study the immunoreactivity of bombsin was reduced in the submucosal neurons of the GIT. It is suggested that this may contribute to the reduce serum concentration, which has been reported in diabetics. It is suggested that this can also contribute to delayed wound healing in diabetics.

Acknowledgement

We are grateful for the technical assistance from the Laboratory staff of Anatomy Unit of the Pre-Clinical Sciences Department.

References


