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Research Report

Effects of nitric oxide synthase blockade on dorsal vagal stimulation-induced pancreatic insulin secretion

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ABSTRACT

We and others have previously shown that the dorsal motor nucleus of the vagus (DMV) is involved in regulation of pancreatic exocrine secretion. Many pancreatic preganglionic neurons within the DMV are inhibited by pancreatic secretagogues suggesting that an inhibitory pathway may participate in the control of pancreatic exocrine secretion. Accordingly, the present study examined whether chemical stimulation of the DMV activates the endocrine pancreas and whether an inhibitory pathway is involved in this response. All experiments were conducted in overnight fasted isoflurane/urethane-anesthetized Sprague Dawley rats. Activation of the DMV by bilateral microinjection of bicuculline methiodide (BIM, $GABA_A$ receptor antagonist, 100 pmol/25 nl; 4 mM) resulted in a significant and rapid increase in glucose-induced insulin secretion (9.2±0.1 ng/ml peak response) compared to control microinjection (4.0±0.6 ng/ml). Activation of glucose-induced insulin secretion by chemical stimulation of the DMV was inhibited (2.1±1.1 ng/ml and 1.6±0.1 ng/ml 5 min later) in the presence of the muscarinic receptor antagonist atropine methonitrate (100 μg/kg/min, i.v.). On the other hand, the nitric oxide (NO) synthesis inhibitor L-nitroarginine methyl ester (30 mg/kg, i.v.) significantly increased the excitatory effect of DMV stimulation on glucose-induced insulin secretion to 15.3±3.0 ng/ml and 16.1±3.1 ng/ml 5 min later. These findings suggest that NO may play an inhibitory role in the central regulation of insulin secretion.

1. Introduction

The role of the dorsal motor nucleus of the vagus (DMV) in the regulation of pancreatic secretion (PS) has been confirmed by several studies (Buijs et al., 2001; Ionescu et al., 1983; Love et al., 2006; Mussa and Verberne, 2008; Viard et al., 2007). The DMV is the site of origin of vagal efferent neurons that innervate both the endocrine and exocrine pancreas (Berthoud and Powley, 1991; Jansen et al., 1997; Rinaman and Miselis, 1987). The compact formation of the nucleus ambiguus also contains vagal motor neurons but these mainly innervate the upper GI tract including the striated muscles of the soft palate, pharynx,

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Abbreviations: AMN, atropine methonitrate; BIM, bicuculline methiodide; CCK-8S, cholecystokinin sulphated octapeptide; DMV, dorsal motor nucleus of the vagus; L-NAME, L-nitroarginine methyl ester; NO, nitric oxide; NANC, nonadrenergic-noncholinergic; PBG, phenylbiguanide; PS, pancreatic secretion; RVLM, rostral ventrolateral medulla

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esophagus and larynx while cardiovagal motor neurons are found in the loose formation of the nucleus ambiguus (Loewy and Spyer, 1990). We have previously shown that chemical activation of the DMV induced by blockade of local $GABA_A$ receptors produced profound excitatory effects on pancreatic exocrine secretion. These effects were sensitive to the muscarinic acetylcholine receptor antagonist atropine methonitrate, emphasizing that the DMV influences pancreatic exocrine secretion via a cholinergic pathway (Mussa and Verberne, 2008). In view of these findings, we wished to determine whether chemical stimulation of the DMV also modulates pancreatic endocrine secretion via a cholinergic pathway.

It is well-documented that DMV vagal efferents synapse onto cholinergic and non-cholinergic postganglionic neurons in the pancreas. Stimulation of these efferents directly influences both endocrine and exocrine secretions (Bergman and Miller, 1973; Berthoud and Powley, 1991; Roze, 1991). A recent investigation of DMV-pancreatic preganglionic neurons has shown that the discharge rate of these neurons was differentially affected by the pancreatic secretagogues, cholecystokinin sulfated octapeptide (CCK-8S) and the 5-HT_3 receptor agonist phenylbiguanide (PBG) (Mussa et al., 2010). Although some of the DMV-pancreatic preganglionic neurons were activated by CCK-8S and PBG, the majority were inhibited. Despite their differential responsiveness to CCK or PBG these neurons could not be distinguished on the basis of other physiological properties *e.g.* axonal conduction velocity. These findings suggest that the link between the DMV and PS is more complex than previously believed and raises the possibility that an inhibitory pathway is implicated in regulation of PS (Mussa et al., 2010). An inhibitory vagal pathway arising from the DMV is not a novel concept since it has been previously proposed that parallel excitatory and inhibitory vagal efferent pathways control gastric function (Browning and Travagli, 2010; Hornby, 2001; Owyang and Logsdon, 2004; Travagli et al., 2003; Travagli et al., 2006).

A potential inhibitory neurotransmitter in this pathway is nitric oxide (NO). Although NO has been implicated in neurally mediated PS, there is a lack of consensus in regard to the exact role of this agent (Holst et al., 1994; Vaquero et al., 1998). In addition, neural regulation of pancreatic exocrine secretion has been investigated in greater detail than neural control of pancreatic endocrine secretion. Therefore, in the present study, we have examined the effects of chemical stimulation of the DMV on glucose-induced insulin secretion and the role of NO in these responses.

2. Results

2.1. *Effects of chemical stimulation of the DMV on glucose-induced secretion*

At baseline, glucose levels were 7.2±0.4 mM and insulin levels were 2.1±0.2 ng/ml and in all experiments similar basal glucose and insulin levels were observed. Intravenous administration of glucose produced a substantial, immediate and short-lasting elevation in insulin levels (from 2.1±0.2 to 9.8±0.7 ng/ml, $n=6$, $P<0.0001$). The results of the first group of experiments showed that chemical stimulation of the DMV by bilateral microinjection of BIM produced significant and rapid increases in glucose-induced insulin secretion compared to control microinjection (Fig. 1). Insulin levels were measured twice at 5 min intervals after bilateral microinjection of BIM and after control (vehicle) microinjection. After the first 5 min period insulin levels were 8.9±1.2 ng/ml and 5.5±0.7 ng/ml after BIM and control microinjections, respectively. After the second 5 min period insulin levels were 9.2±1.0 ng/ml after BIM and 4.0±0.6 ng/ml after control microinjections, respectively ($n=6$, $^{*}P<0.05$, and $^{***}P<0.001$) (Fig. 1). Furthermore, there were no further changes in insulin levels when the BIM or control microinjections were repeated 40 min later. As shown in Fig. 1B, basal levels of glucose increased rapidly after intravenous administration of glucose (from 7.2±0.4 mM to 30.2±0.1 mM, $n=6$; $P<0.0001$) but this increase lasted only for 5 min. No changes in glucose levels were observed after microinjection of BIM into the DMV and glucose levels continued to fall until the end of the experiment. Histological analysis of the microinjection sites showed that control microinjection of BIM outside the DMV or microinjection of ACSF inside the DMV did not affect insulin levels emphasizing that insulin secretion occurred only after microinjection of BIM into the DMV (Figs. 1 and 4B).

In addition, the increase in insulin secretion occurring in response to chemical activation of the DMV was inhibited after infusion of AMN compared to infusion of saline (Fig. 2A). In the saline treated group, insulin levels after bilateral microinjection of BIM were 8.9±1.2 ng/ml and 9.2±0.9 ng/ml 5 min later ($n=6$) whereas those after AMN were 2.1±1.1 ng/ml and 1.6±0.1 ng/ml, respectively ($n=3$ and $P<0.05$). As shown in Fig. 2B, neither infusion of AMN or saline affected the blood glucose levels and, as noted previously, glucose levels were only increased after intravenous administration of glucose and returned approximately to baseline at the end of the experiment.

2.2. *Chemical stimulation of the DMV and glucose-induced insulin secretion: effects of NO inhibition*

In the second group of experiments, the hypothesis that nitrergic nerves are involved in regulation of glucose-induced insulin secretion was tested. Fig. 3 shows that increased insulin secretion in response to chemical activation of the DMV was significantly enhanced (from 8.9±1.2 ng/ml to 15.3±3.0 ng/ml and from 9.2±0.1 ng/ml to 16.1±3.1 ng/ml 5 min later, $n=6$, $^{*}P<0.05$) in the presence of the nitric oxide synthesis inhibitor L-NAME. The enhanced secretion appeared to be due to NO synthase inhibition induced by L-NAME since infusion of saline did not alter the secretory response to chemical stimulation of the DMV (Fig. 3A). Fig. 3B shows that the glucose levels declined more rapidly ($n=6$, $^{**}P<0.01$, and $^{***}P<0.001$) after infusion of L-NAME compared to that observed after infusion of normal saline. Fig. 4 summarizes the microinjection sites of BIM within the DMV during infusion of saline (Fig. 4A), AMN (Fig. 4C) and L-NAME (Fig. 4D) and microinjection sites of ACSF within the DMV and BIM microinjections sites outside the DMV (Fig. 4B).