別紙様式第４

学　位　論　文　要　旨

博士課程 (甲)・乙	第16号	氏　名	MOIN ABU SALEH MD

［論文題名］ Neuroendocrine regulatory peptide-2 stimulates glucose-induced insulin secretion *in vivo* and *in vitro*

［要　旨］

Introduction: A great number of peptide hormones and neuropeptides are involved in the control of highly integrated metabolic pathways in the systemic organs. Most bioactive peptides are synthesized as longer pre-proteins and undergo proteolysis by a class of proteases called prohormone convertases (PCs) to generate mature peptides. Our novel approach to discover bioactive peptides involved targeting post translational modifications of peptides secreted from endocrine cells. Previously we profiled two C-terminally amidated VGF derived peptides designated neuroendocrine regulatory peptide (NERP)-1 and NERP-2 (Yamaguchi *et. al.*, J Biol Chem., 2007). NERP-1 (PESA-25, VGF281–306) and -2 (QAEA-38, VGF310–347) are 25-amino acid and 38-amino acid peptides with C-terminal amidation, respectively and are abundant in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) in rat hypothalamus. In the PVN and SON, NERPs were colocalized frequently with vasopressin (AVP), but rarely with oxytocin. NERPs dose-dependently suppressed AVP release induced by intracerebroventricular (icv) injection of high salt or angiotensin II (AII) *in vivo*. Moreover, NERP-2 also localized in the rat lateral hypothalamus (LH) and dorsomedial perifornical hypothalamus (DMH) of brain and regulated feeding behavior (Toshinai *et. al.*, Am J Physiol Endocrinol Metab., 2010). In peripheral system NERPs (NERP-1 and NERP-2) coexpressed with pancreatic hormones in human pancreas (T. Matsuo *et. al.*, Regul Pept., 2012).

Objectives of the study: Previously we reported that NERPs colocalized with insulin in human pancreatic β cells. But the role of NERPs in pancreas was yet to be identified. I hypothesized that NERPs might act on pancreatic β cells as an autocrine or paracrine manner. I tried to identify cellular localization of NERPs in rodent pancreatic islets by immunohistochemical study. I also performed *in vitro* and *in vivo* experiments in cultured β cells and in rodents, respectively to identify the role of NERPs on β cells.

Results: In my current study I investigated the localization and functional analyses of NERP-1 and NERP-2 in rodent pancreatic β cells. By immunohistochemical study, I identified that both NERP-1 and NERP-2 were localized in mouse pancreatic islets and NERP-2 colocalized with insulin in pancreatic β cells. *In vitro* study revealed that NERP-2 enhanced glucose stimulated insulin secretion (GSIS) in both pancreatic β cell line (MIN6 cells) and isolated mouse islets in a dose dependent manner with 10^{-7}M NERP-2 as the lowest effective dose. NERP-2 showed insulinotropic effect only in presence of high glucose concentration. Neither NERP-1 nor NERP-2-Gly (non amidated form of NERP-2) enhanced GSIS in pancreatic β cells. Plasma insulin level was increased after administration of NERP-2 along with glucose in mice and rats. The elevated level of Fura2-AM (intracellular Ca^{2+} indicator) ratio (340-to-380 fluorescence ratio) in MIN6 cells upon continuous perfusion of NERP-2 with glucose indicated that NERP-2-induced insulin release was mediated by intracellular Ca^{2+} influx.

Discussion: To investigate the biological activity of NERPs in the islets, we studied their expression pattern and our data suggested that their localization were confined in the β cells of pancreatic islets. NERP-2, but not NERP-1, stimulated insulin secretion both *in vitro* and *in vivo*. NERP-2 is not itself a secretagogue, and its effect on insulin secretion is evident only in the presence of hyperglycemia. This is also true for other insulinotropic peptides including GLP-1, glucose-dependent insulinotropic peptide, vasoactive intestinal polypeptide, and pituitary adenylate cyclase-activating polypeptide.

The insulin secretion pattern after intraperitoneal (IP) administration of NERP-2 in mice suggested a rapid elevation of plasma insulin secretion. On the other hand in rats, large initial peak followed by return to basal level of insulin indicated NERP-2 potentiated the monophasic induction of insulin secretion in case of intravenous (IV) administration. Intracellular signaling mechanism of NERP-2 was also investigated. NERP-2 increased intracellular $[Ca^{2+}]_i$ in pancreatic β cells and the profile of NERP-2-induced calcium influx is quite different from that of GLP-1 (another insulinotropic peptide that was used as a positive control). The monophasic increase of intracellular calcium level after administration of NERP- 2 suggested that this peptide stimulated intracellular calcium entry via receptor-operated channels rather than voltage-gated calcium channels. Taken together my data reveals that NERP-2 is a novel bioactive peptide and a regulator of insulin secretion and glucose homeostasis.

備考　論文要旨は、和文にあっては 2，000 字程度、英文にあっては 1，200 語程度とする。