# New perspectives of vesicular monoamine transporter 2 chemical characteristics in mammals and its constant expression in type 1 diabetes rat models

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**Vesicular monoamine transporter 2 (VMAT2) has been exploited as a biomarker of $\beta$-cell mass in human islets. However, a current report suggested no immunoreactivity of VMAT2 in the $\beta$ cells of rat islets. To investigate the cellular localization of VMAT2 in islets further, the pancreatic tissues from monkeys and humans were compared with those of rats and mice. The study was performed using among-species comparisons and a type 1 diabetes model (T1DM) for rats by Western blotting, double-label immunofluorescence, and confocal laser scanning microscopy. We found that VMAT2-immunoreactivity (IR) was distributed peripherally in the islets of rodents, but was widely scattered throughout the islets of primates. Consistent with rodent islets, VMAT2-IR did not exist in insulin (INS)-IR cells but was abundantly present in glucagon (GLU)-IR and pancreatic polypeptide (PP)-IR cells in monkey and human islets. VMAT2-IR had no colocalization with INS-IR in any part of the rat pancreas (head, body, and tail). INS-IR cells were reduced dramatically in T1DM rat islets, but no significant alteration in the proportion of VMAT2-IR cells and GLU-IR cells was observed. Furthermore, a strong colocalization of VMAT2-IR with GLU-IR was distributed in the peripheral regions of diabetic islets. For the first time, the current study demonstrates the presence of VMAT2 in $\alpha$ cells and PP cells but not in $\beta$ cells in the islets of monkeys and humans. This study provides convinced morphologic evidence that VMAT2 is not present in $\beta$ cells. There needs to be studies for new markers for $\beta$ cell mass. (Translational Research 2014;163:171–182)**

**Abbreviations:** GLU = Glucagon; INS = Insulin; IR = Immunoreactivity; PET = Positron emission tomography; PP = Pancreatic polypeptide; SOM = Somatostatin; T1DM = Type 1 diabetes model; VMAT2 = Vesicular monoamine transporter

Pancreatic islets are composed of multiple cell types, including glucagon (GLU)-secreting $\beta$ cells, insulin (INS)-secreting $\beta$ cells, somatostatin (SOM)-secreting $\beta$ cells and pancreatic polypeptide (PP)-secreting PP cells.[1] The islets' cytoarchitecture varies from species to species, although the 4 cell types are present in the islets in all different species. In rodent islets, most of the $\beta$ cells

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Conflicts of Interest: All authors have read the journal's policy on conflicts of interest and have no financial, consultant, institutional, or other relationships that might lead to bias or conflict of interest.

This work was supported by the National Natural Science Foundation of China (81170346 and 81370482) and the Beijing Natural Science Foundation (7132017 and 7121003).

Submitted for publication August 10, 2013; revision submitted September 23, 2013; accepted for publication October 1, 2013.

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1931-5244/$ - see front matter



http://dx.doi.org/10.1016/j.trsl.2013.10.001

## AT A GLANCE COMMENTARY

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#### Background

Vesicular monoamine transporter (VMAT) is a monoamine transport protein integrated in the membrane of intracellular vesicles. Currently, the localization of the VMAT2 site in pancreatic islets is being followed with interest for exploring an efficient biomarker of β-cell mass, but many controversial interpretations exist.

#### Translational Significance

The current study demonstrates that VMAT2 is distributed significantly in α cells and pancreatic polypeptide cells, but not in β cells in primate islets (monkey and human). This study provides convincing morphologic evidence that supports that VMAT2 is not present in β cells. There needs to be more research for new markers for β-cell mass.

are clustered in the core of a generally round islet, surrounded by a mantle of $\alpha$, $\delta$, and PP cells. However, human islets do not show obvious subdivisions, with all cell types scattered throughout the entire islet.[2]

Vesicular monoamine transporter (VMAT) is a monoamine transport protein integrated into the membrane of intracellular vesicles in monoaminergic neurons.[3] It is widely distributed in the central and peripheral nervous systems,[4] autonomic nervous system,[5] hematopoietic system, and neuroendocrine system.[6] The main function of VMAT is to transport monoamines from the cytoplasm into the secretory vesicles. Two family members of VMAT—VMAT1 and VMAT2—have been characterized in rodent and human tissues.[7,8] In the human pancreas, VMAT1 is expressed exclusively in the endocrine cells of the pancreatic duct system, and VMAT2 is present predominantly in the pancreatic islets.[9] On April 10, 2012, the U.S. Food and Drug Administration approved Amyvid (18F-AV-45) for use in positron emission tomography of Alzheimer's disease and other causes of cognitive decline because it displays a high binding affinity and specificity to amyloid-$\beta$ plaques. 18F-AV-133 (a potential VMAT2 imaging agent) was used as a biomarker of $\beta$-cell mass in the pancreas[10,11] based on a study of the pancreas from patients with pancreatic adenocarcinoma, which showed that the insulin-secreting $\beta$ cells manifested VMAT2-immunoreactivity (IR).[9] Meanwhile, other proteins have been reported to be highly expressed in $\beta$ cells, such as protein phosphatase 1 regulatory subunit 1A and the 65-kDa glutamic acid decarboxylase.[12] These proteins are being used to monitor the number of $\beta$ cells in islet and could be potential markers of $\beta$ cells. Histologic studies have suggested near-complete $\beta$-cell mass depletion in patients with long-standing type 1 diabetes,[13] but the VMAT2 binding potential was reduced only modestly in those patients,[14] indicating an unparalleled variation between the VMAT2 binding signal and $\beta$-cell mass in patients with type 1 diabetes. Moreover, VMAT2-IR was distributed predominantly in the $\beta$ and PP cells but was absent in the $\beta$ cells in rat islets,[15] which is different from reports of human islets.[9,13] The location of the different islet cells manifested species differences, with $\beta$ cells clustered in the core and $\alpha$, $\delta$, and PP cells localized in the periphery of the islets in rodents, whereas the $\beta$ cells intermingled with $\alpha$, $\delta$, and PP cells in primate islets.[2] Species differences in the distribution of VMAT2 sites have also been observed in rat and human pancreases.[13,15] All these controversial results prompted us to determine the exact *in situ* cellular localization of VMAT2 in the pancreatic islets of different species. Pancreatic tissues from humans, monkeys (*Macaca fascicularis*), rats, and mice were collected to investigate the distribution of VMAT2 in the $\alpha$, $\beta$, $\delta$, and PP cells in the pancreatic islets by double-label immunohistochemistry, confocal laser microscopy, and Western blotting. To investigate further the localization of VMAT2 and to assess the relationship between VMAT2 and $\beta$-cell mass, alloxan-induced type 1 diabetic rats were used as the $\beta$-cell injury model. The current study provides experimental evidence for the exploration of VMAT2 as a biomarker for pancreas cell masses and additional functional investigation of pancreatic endocrinology.

## MATERIALS AND METHODS

**Tissue preparation.** Adult male Sprague-Dawley rats (Laboratory Animal Services Center, Capital Medical University, Beijing, China), ranging from 220 to 250 g in weight, were maintained in a pathogen-free facility under a 12-hour light/dark cycle and were given free access to food and water. The rats were sacrificed by decapitation, and their pancreases were removed quickly after sacrifice. Six paraffin-embedded monkey pancreases were collected from 2 male *Macaca fascicularis* from Guangxi Grandforest Scientific Primate Company Ltd (Guangxi, China). The monkeys were sacrificed by euthanasia, and their pancreatic tissues for immunofluorescence staining were placed in 4% paraformaldehyde/phosphate-buffered saline (pH 7.4) for 24 hours at 4°C, infiltrated sequentially with 15% sucrose and 30% sucrose, and embedded in optimal cutting temperature solution (Sakura Finetek).