MORPHOLOGICAL FEATURES OF TYROSINE HYDROXYLASE IMMUNOREACTIVE CELLS IN THE MOUSE ISLETS OF LANGERHANS

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ABSTRACT

The current immunohistochemical study used the antibody against tyrosine hydroxylase (TH) to observe the immunoreactive elements in the mouse pancreas. The results indicated the presence of immunoreactive nerve fibers and endocrine cells. The immunopositive nerve fibers appeared as thick and thin bundles; thick bundles were seen to run along the blood vessels giving out fine fibers to the wall. Varicose nerve fibers were seen in the islets of Langerhans and also in close association with the exocrine endpieces. The TH immunoreactive cells were oval-round in shape and some showed the central non-staining area and the dense staining peripheral zone. More than 80% of the islets of Langerhans contained the immunoreactive cells. Individual islet showed between 3-10 immunopositive cells and a few contained 1-2 cells. The TH immunopositive cells were widely distributed in the islets; they were seen in the centre, at the intermediate position and at the periphery of the islets. The exact role of the TH immunoreactive cells in the islets of Langerhans is not known. It is possible that they secrete tyrosine hydroxylase that may have some paracrine influence to the endocrine cells. Wide distribution of these cells in the islets indicates that they may regulate the entire population of the islets cells.

Keywords: Balb/c mouse, Pancreas, Islet of Langerhans, Tyrosine Hydroxylase, Immunohistochemistry

INTRODUCTION

Tyrosine hydroxylase (TH) also known as tyrosine 3-monooxygenase is an important enzyme that is involved in the biosynthesis of catecholamines. The enzyme catalyzes the conversion of the amino acid L-tyrosine to dihydroxyphenylalanine (DOPA), which is a precursor for adrenaline and noradrenaline (Levit *et al*. 1965, Fujita *et al*. 1988). The TH imunoreactivity has been observed in many tissues such as the adrenal glands, pancreas, liver, intestines, stomach, heart, brain, autonomic ganglia and pineal gland (Teitelman *et al*. 1981; Sternini and Brecha, 1985; Goehler and Sternini, 1991; Oomori *et al*., 1991, 1994, Zhang *et al*. 1991, Persson-Sjogren *et al*. 1998, Milner 2004), and it is considered to regulate the blood flow and secretory activities in these tissues.

The pancreas is a mixed gland with both the endocrine and exocrine cells and its interstitium contains the blood vessels, ducts for the exocrine gland, the autonomic nerve fibers and ganglion cells (Beckman 1866, Gesase and Satoh, 2006). TH immunoreactivity in the pancreas has been studied mainly in the mice, rats, cow, guinea pigs and birds and the immunopositive elements appeared to be the nerve fibers, ganglion cells and endocrine cells (Kirchgessner and Pintar 1991; Salkaji *et al*. 1992, Persson-Sjogren *et al*. 1998). However, there are differences in the staining pattern among the animals that have been studied. In the birds and cow the immunoreactive elements appeared to be the nerve fibers and ganglion cells (Kitamura *et al*. 1999, Mensah-Brown *et al*. 2000,

Mensah-Brown and Pallot 2000). In the rats TH immunoreactivity was found to be in the nerve fibers and the small intensely fluorescence cells and not the ganglion cells (Oomori *et al*. 1994, Kitamura *et al*. 1999). TH immunoreactivity in mice has been studied during development and there is limited information on TH immunoreactivity in the adult mice. TH immunopositive elements in mice during development appeared to be in the nerve fibers and some cells of the islets of Langerhans (Teitelman *et al*. 1987, Teitelman and Lee 1987, Hashimoto *et al*. 1988). But, little is known on the distribution and location of the TH immunoreactive cells among the endocrine cells. To this end the antibody against tyrosine hydroxylase was used to describe the TH immunoreactive elements in the adult mice pancreas.

MATERIALS AND METHODS

The study was carried out with twenty one male mice (Balb/c, about 39g body weight). The animals received commercial food and water and were kept under constant conditions in animal house at Muhimbili University College of Health Sciences.

The animals were anaesthetized with ether, thoracotomized, and then perfused via the left cardiac ventricle with 30 ml of physiological saline followed by 50 ml of phosphate-buffered saline (PBS; 0.1 M, pH 7.4) containing 4% paraformaldehyde at 4ϒ C. The pancreas was removed and cut into small pieces and stored in the same fixative at a temperature of 4ϒ C for 2 h. After rinsing with PBS, the specimens were left overnight in PBS containing 30% sucrose at 4ϒC. The pancreatic tissue were frozen and cut about 12µm thick using a cryostat, and mounted on glass slides coated with poly-**L**-lysine (Sigma, St. Louis, Mo., USA).

The sections were incubated with a rabbit antiserum to tyrosine hydroxylase (Eugene Technology International, Allendale, N.J., USA) for 24 h at room temperature, followed by incubation in goat biotinylated anti-rabbit IgG and avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, Calif., USA) for 1 h at room temperature. The antigen-antibody reaction sites were made visible by incubating the sections with diaminobenzidine tetrahydrochloride (DAB) and 0.01% hydrogen peroxide in 2.5 mM Tris-HCl buffer (pH 7.6) for 10 min at room temperature. The specificity of the immunohistochemical staining was confirmed by replacing the primary antiserum with a normal rabbit or mouse serum and by using diluted antiserum pre-treated with TH purified from the rat adrenal gland (courtesy of Prof. H. Fujisawa, Department of Biochemistry, Asahikawa Medical College, Japan).

RESULTS

TH-immunoreactivity was demonstrated in the mouse pancreas and the tissues that stained positively included a few endocrine cells in the islet of Langerhans and the nerve fibers (Figure 1a). The TH-immunoreactive cells were oval-round in shape and close examination revealed that some of the immunoreactive cells contained a dense stained peripheral part and the central non-staining zone (Figure 1b; c). The immunopositive cells occupied different positions in the islets; some were centrally placed, others were in the intermediate position and at the periphery of the islets. More than 80% of the islets population contained the TH immunoreactive cells. In most cases the individual islets contained between 3-10 immunoreactive cells and rarely there were 1-2 immunopositive cells. The TH immunoreactive cells frequently united with each other via short cytoplasmic processes (Figure 1b) and in some cases they united to form a circle-like structure in the islet of Langerhans (Figure 2b). The immunoreactive cells did not appear to be associated with TH immunoreacted nerve fibers and there was no immunopositive cells that were associated with the exocrine pancreas, ganglion cells or that appeared in the interstitial space of the gland. All

immunoreactive cells were associated with the islets of Langerhans.

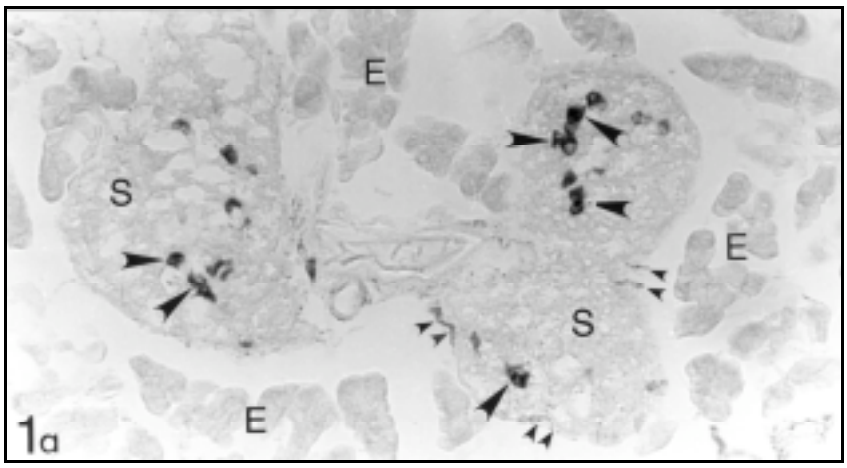

Figure 1 (a): Light micrographs of tyrosine hydroxylase (TH) immunoreactivity in the mouse pancreas. **a**. Shows the section of the pancreas containing the portions of the exocrine (*E*) and endocrine pancreas or islets of Langerhans (*S*). TH immunoreactive endocrine cells (*large arrowheads*) were located in the islets of Langerhans (*S*). The immunoreactive nerve fibers (*small arrowheads*) appeared to enter the islets (*S*). x160

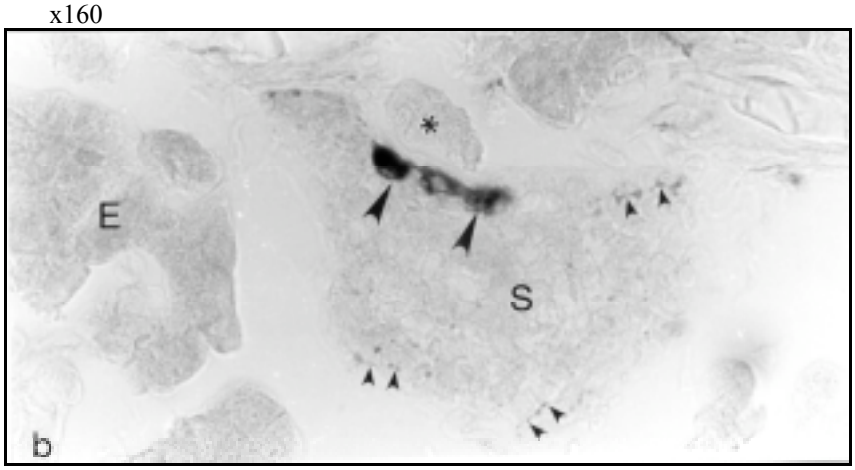

Figure 1(b): The micrograph shows the immunoreactive endocrine cells (*large arrowheads*) at the periphery of the islet of Langerhans (S) and the immunoreactive nerve fibers (*small arrowheads*). Note the presence of the exocrine pancreas (E) and the immunonegative ganglion cells (*asterisk*) in the interstitial space. x240

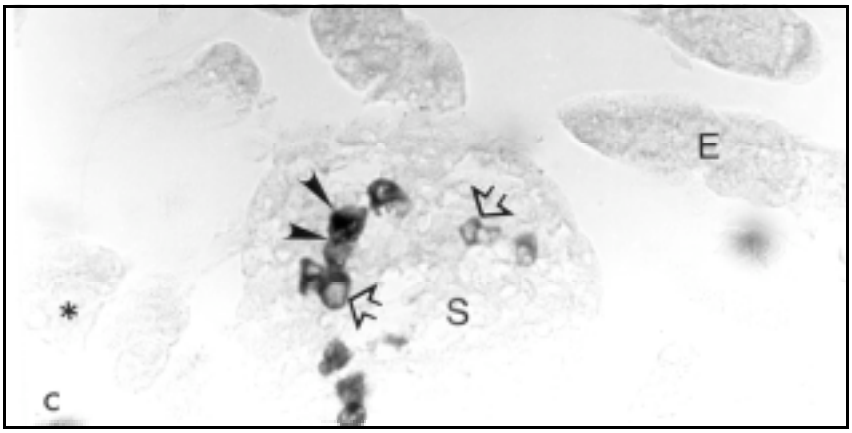

Figure 1(c): Shows the islet of Langerhans (S) containing the TH immunoreactive cells with non-staining central part and deeply staining peripheral zone (*open arrows*). Other imunoreactive cells stained homogenously (*large arrowheads*). Note the exocrine pancreas (E) and the immunonegative ganglion cells (*asterisk*). x240

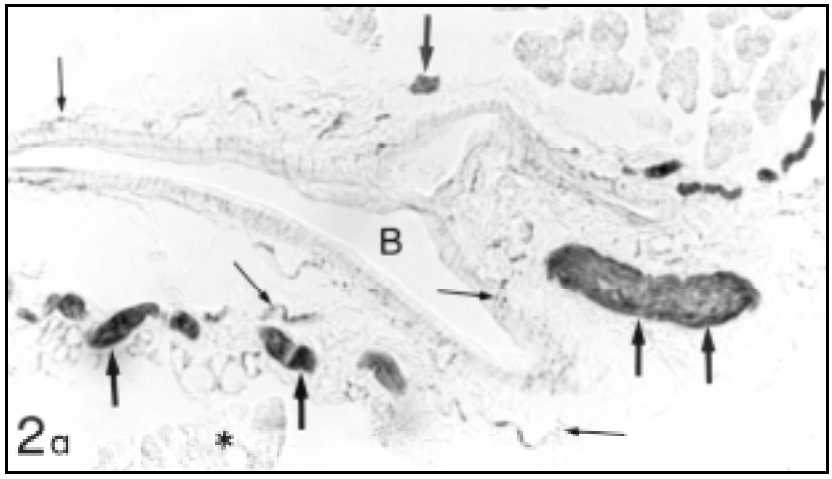

Figure 1(a): Light micrographs of tyrosine hydroxylase (TH) immunoreactivity in the mouse pancreas. **a**. Shows the thick (*thick arrows*) and thin (*thin arrows*) immunoreactive nerve fibers and the immunonegative ganglion cells (*asterisk*) in the interstitial space of the pancreas. Note the immunoreactive fibers in the wall of blood vessels (B). x160

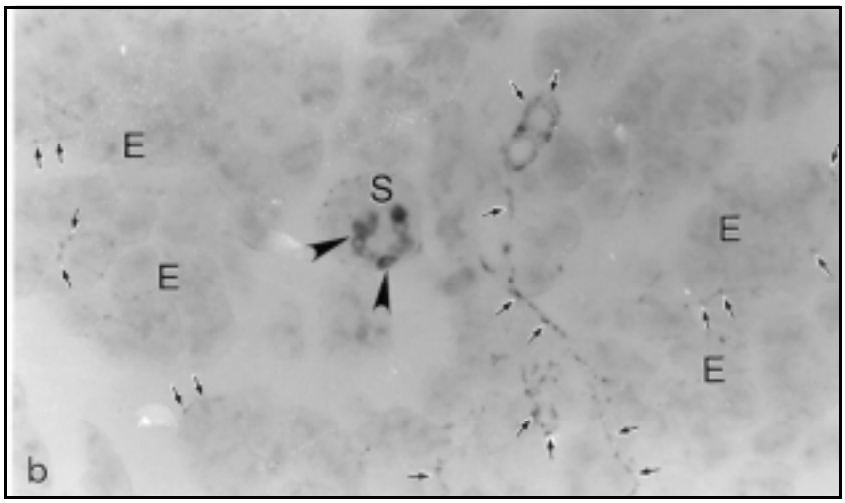

Figure (b): The photograph showing fine immunoreactive nerve fibers (*small arrows*) in the exocrine pancreas and the immunopositive endocrine cells (*arrowheads*) in the islet of Langerhans. x160

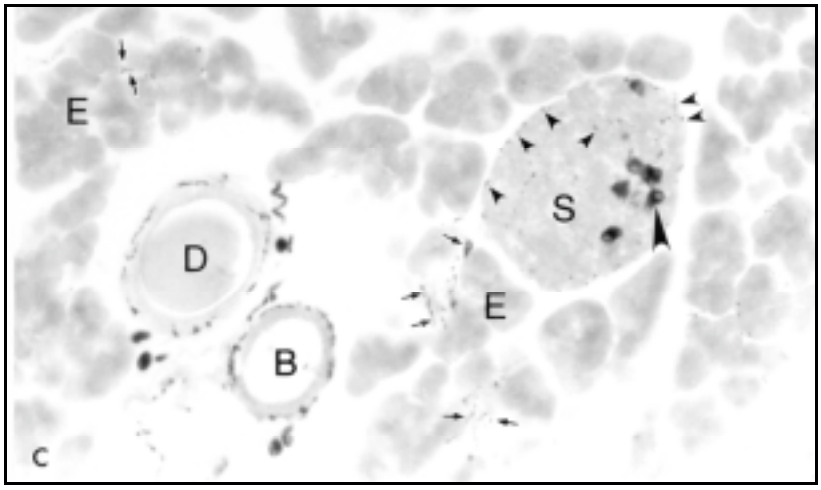

Figure (c): Shows the immunoreactive nerve fibers (*small arrowheads*) in the islet of Langerhans (S) and in the exocrine (*small arrows*) pancreas (E). The immunopositive nerve fibers were located around the blood vessel (B) and the glandular duct (D). x160

The other TH immunoreactive elements of the mice pancreas included the nerve fibers (Figure 2). The interstitial space contained numerous immunoreactive nerve fibers; thick and thin nerve fibers were seen in association with the exocrine and endocrine pancreas and also in contact with the blood vessels and glandular ducts (Figure 2a). The thick TH immunoreactive nerve fibers were seen to run parallel with the blood vessels

and giving out thin immunoreactive fibers that appeared to encircle the vascular wall (Figure 2b). Some nerve fibers that were associated with blood vessels appeared to have varicosities. Numerous varicose nerve fibers unassociated with blood vessels were also in close association with the exocrine pancreas and islets of Langerhans (Figure 2c; d). The TH immunoreactive fibers appeared in the peripheral and central parts of the islets. No immunopositive ganglion cells was associated with the immunoreactive nerve fibers.

DISCUSSION

TH immunoreactive cells in the pancreas show great variations among different species. Observations made in the rat pancreas indicated that TH immunoreactive cells were the small intensely fluorescence cells (SIF) that were located both in the interstitial space close to the immunonegative ganglion cells and at the periphery of the islets of Langerhans (Oomori *et al*. 1994). In the cow and birds the immunoreactive cells appeared to be the ganglion cells (Oomori *et al*. 1994, Kitamura *et al*. 1999; Mensah-Brown *et al*. 2000). In the current study the TH immunoreactive cells were the endocrine cells and were randomly distributed within the islets. The distribution pattern of the TH imunoreactive cells in the mice islets points to the possibility that it may influence the physiology of the entire endocrine cell population in the islet. Putting together the current and previous findings it indicates that the physiological activities of tyrosine hydroxylase in the pancreas are differently regulated in different animal species. The unifying feature is that the TH immunoreactive cells appear to release TH. It remains to be determined whether TH secreted by the SIF, ganglion cells and endocrine cells can have similar physiological influence to the pancreas. Variations in the TH regulation among different animals are also seen during development. Studies that have been made during development reported TH immunoreactivity in the epithelial cord of the developing pancreas on day 11 and during this time the TH immunoreactive cells also co-expressed glucagons and insulin (Teitelman *et al*. 1981). However, as the pancreas matures the glucagons and insulin secreting cells loose the TH immunoreactivity and the TH immunoreactive cells remain as terminally differentiated cells that do not express glucagons or insulin (Teitelman *et al*. 1981). The authors suggested that the TH immunopositive progenitor cells present during early development may differentiate into glucagons and insulin secreting cells of the endocrine pancreas. The observation that was done in the rat pancreas during development indicated the presence of TH immunoreactive cells in the endocrine cells, but unlike in the mice pancreas these cells did not express glucagons or insulin (Hashimoto *et al*. 1988). This may indicate that there may be differences in the ontogeny of pancreas in these two rodent species. Such findings call for more studies to characterise the exact role of TH during pancreatic development.

The TH immunoreactive fibers have been described in the pancreatic tissue of many species and in the rat the immunoreactive fibers were seen to associate with blood vessels, glandular ducts and the glandular cells (Oomori *et al*. 1994). Similar findings have been described in the current study; TH immunopositive nerve fibers were seen to associate with vessels and glandular cells. These nerve fibers may be involved in the regulation of blood flow and secretory processes of both the exocrine and endocrine glandular cells. TH immunoreactive fibers are considered to be extrinsic in origin, arising from the cell bodies in the celiac ganglia (Beckman 1986). Observations made in the rat have showed that neurones in the celiac ganglia that innervate the pancreas are immunopositive for TH (Hökfelt *et al*. 1977; Schultzberg *et al*. 1979). TH immunopositive nerve fibers are among the many nerves that innervate the pancreas such

as neuropeptide Y, galanin, substance P, vasoactive intestinal peptide, methionine-enkephalin, calcitoni gene related peptide and serotonin and each appears to have its own role (Kirchgessner and Pintar 1991; Baltazar *et al*. 2000; Myojin *et al*. 2000). It will be interesting in future to see how TH interacts with the neuropeptides in the regulation of the pancreatic cell physiology and blood flow.

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