Introduction

It was believed that aromatic L-amino acid decarboxylase (AADC) is localized in neurons that synthesize catecholamines (dopamine, norepinephrine, or epinephrine) or serotonin (5-HT). Such AADC-containing neurons that lack immunoreactivity to tyrosine hydroxylase (TH) and 5-HT were found in rat brain and were named D neurons. The presence of D neurons in the brain of vertebrates has recently been demonstrated by immunohistochemical techniques. However, there have been no histochemical studies on the distribution of AADC-only-positive cells like D neurons in the alimentary canal. The aim of this study was to confirm the presence of AADC-containing cells in the alimentary canal and to elucidate the relationship between AADC-only-positive cells in the alimentary canal and the amine precursor uptake and decarboxylation (APUD) system in the laboratory shrew, Suncus murinus. The laboratory shrews were treated pharmacologically to determine the changes in staining characteristics of two monoamine precursors, L-dihydroxyphenylalanine (L-DOPA) and 5-hydroxytryptophan (5-HTP), present in the mammals by immunohistochemical techniques using specific antibodies.

Materials and Methods

Animals

Male laboratory shrews (Suncus murinus) aged 15 to 20 weeks were used. They were bred in an air-conditioned room (room temperature: 24°C ± 1°C, humidity: 55% ± 5%) in the Education and Research Center of Animal Models for Human Disease at Fujita Health University. All animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals.
Pharmacological treatments

Adult laboratory shrews (30 to 45 g) were used and divided into three groups: the animals of group A were intraperitoneally (i.p.) injected with an isotonic sodium chloride solution (0.1 ml); the animals of group B were i.p. injected with a monoamine oxidase inhibitor (pargyline, 20 mg/dl), L-DOPA (100 mg/kg), and a peripheral AADC inhibitor (carbidopa, 20 mg/kg) for 30 min before sacrifice; the animals of group C received pargyline and 5-HTP (100 mg/kg) for 30 min before sacrifice.

Tissue preparation

The animals were anesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/kg) and were perfused through the ascending aorta with 0.15 M sodium chloride in 0.05 M sodium phosphate buffer (PBS, pH 7.4), followed by ice-chilled 4% paraformaldehyde in PBS. After sufficient fixation, the alimentary canal from the stomach to the large intestine was removed with scissors. All of the tissues were immersed in the same fixative for 24 h. The tissues were rinsed in PBS containing 10%, 20%, and 30% sucrose for 3 days. Frozen serial sections, 10 mm thick, were then prepared using a cryostat and were divided into two groups for hematoxylin-eosin (HE) and immunohistochemical staining.

Immunohistochemistry for examination

For immunoperoxidase labeling, endogenous peroxidase activity was blocked by incubation in 0.5% H₂O₂ in methanol for 30 min. After the incubation, the sections were then blocked with 2% normal goat serum in PBS for 30 min. Afterwards, the sections were incubated with anti-TH, anti-AADC, anti-DA, or anti-5-HT antibody (diluted

Fig. 1. HE and immunohistochemical staining of AADC, DA, and 5-HT in the mucous membrane of the stomach. (a) A section of the gastric foveolae. HE staining. GG: gastric gland. (b) DA antibody immunostaining. No pharmacological treatment. There are no DA-positive cells in the gastric gland. (c) 5-HT antibody immunostaining. No pharmacological treatment. There are no 5-HT-positive cells in the gastric gland. (d) AADC antibody immunostaining. No pharmacological treatment. AADC-positive cells are scattered in the gastric gland. (e) DA antibody immunostaining. After pharmacological treatment (L-DOPA intraperitoneal injection). DA-positive cells are scattered at the bottom of the gastric gland. (f) 5-HT antibody immunostaining. After pharmacological treatment (5-HTP intraperitoneal injection). 5-HT-positive cells are scattered at the bottom of the gastric gland.
Fig. 2. HE and immunohistochemical staining for TH, AADC, DA, and 5-HT in the mucous membrane of the small intestine, jejunum. (a) HE staining. A section of an intestinal crypt (IC). (b) AADC antibody immunostaining. No pharmacological treatment. AADC-positive cells are located at the bottom of the crypt. (c) TH antibody immunostaining. No pharmacological treatment. TH-positive cells are not observed. (d) DA antibody immunostaining. No pharmacological treatment. DA-positive cells are not observed. (e) 5-HT antibody immunostaining. No pharmacological treatment. 5-HT-positive cells are not observed. (f) AADC antibody immunostaining. After pharmacological treatment (L-DOPA or 5-HTP intraperitoneal injection). AADC-positive cells are present at the bottom of the crypt. (g) DA antibody immunostaining. After pharmacological treatment (L-DOPA intraperitoneal injection). DA-positive cells are present in the mucous membrane. (h) 5-HT antibody immunostaining. After pharmacological treatment (5-HTP intraperitoneal injection). 5-HT-positive cells are present in the mucous membrane.

1:1000) at room temperature for 2 h. After washing with PBS, the sections were incubated with peroxidase-conjugated goat anti-mouse IgG (Vectastain Elite ABC Kit; Vector Laboratories, Burlingame, CA, USA) followed by incubation with the enzyme substrate, 0.04% 3,3'-diaminobenzidine tetrahydrochloride (DAB; Merck KGaA, Darmstadt, Germany), with 0.01% H$_2$O$_2$ at room temperature for 10 to 15 min, and these sections were
mounted on slides by a conventional method.

**Results**

In the control (group A), distinctive AADC-immunoreactive cells, but not TH-, DA- or 5-HT-immunoreactive cells, were distributed in the epithelial lining from the stomach (Fig. 1b, 1c, 1d) to the intestine (Fig. 2b, 2c, 2d, 2e). These AADC-immunoreactive cells were small and spindle in shape; their size ranged from 8 to 10 mm, and they possessed processes. They were localized to the gastric glands in the stomach (Fig. 1d) as well as the bottom of intestinal crypts in the small and large intestine (Fig. 2b).

In the experimental group B (pargyline + carbidopa + L-DOPA), small cells with short processes showed immunoreaction with not only anti-AADC antibody (Fig. 2f), but also with anti-DA antibody (Fig. 1e, 2g). In the experimental group C (pargyline + 5-HTP), small cells with short processes also showed immunoreaction with anti-5-HT antibody in the stomach (Fig. 1f) and in the small (Fig. 2h) and large intestine.

**Discussion**

Pearse\(^4\) reported the existence of the APUD group of cells, which take up and decarboxylate amine precursors for the synthesis of amines, and many findings have since been reported on this group of cells\(^3\). The existence of APUD cells in the brain of vertebrates of lower orders, mainly in circumventricular organs, has also been reported\(^5\).

Jaeger et al.\(^1\) reported that TH-negative and AADC-positive neurons existed in the brain of mammals. With respect to the physiological functions of AADC-only-positive neurons, which were named D neurons, and they synthesize trace amines, such as phenylethylamine and tyramine\(^1\).

In the current study, we demonstrated the presence of AADC-only-immune-positive cells in the alimentary canal and speculated on the functions of these cells through an experiment involving intraperitoneal injections of monoamine precursors DA-positive cells were recognized in the epithelial layer of the alimentary canal after i.p. injection of L-DOPA. As DA is not present in the alimentary canal under normal conditions, the cells likely became DA-positive due to the L-DOPA injection. After L-DOPA and 5-HTP treatment, some cells became positive for both AADC and 5HT. L-DOPA was decarboxylated and changed into DA by AADC in the AADC-positive cells of the alimentary canal; this suggests that these neurons are APUD system neurons. From the results of our experiments, it is strongly suggested that D neuron-like cells, which are widely distributed in the alimentary canal of the laboratory shrew, take up L-DOPA and decarboxylate it into DA.

Among reports using amine precursors, Sakamoto et al.\(^6\) reported that 5-HT antibody-stained cells were distributed around the hypothalamus in an experiment involving intracranial injection of 5-HTP in rats and cats; these results suggested that the neurons were APUD system neurons, which are currently considered to be AADC-containing neurons. However, at the time of the experiment of Sakamoto et al.\(^6\) the distribution of D neurons had not yet been clarified and no immunohistochemical studies using AADC antibodies had been published. In the present study, when L-DOPA was i.p. injected into laboratory shrew, AADC-immunoreactive cells that were stained with DA antibody in the alimentary canal were found to be distributed from the stomach to the large intestine. From these results, it is evident that AADC-only-immunoreactive cells have similar distinguishing characteristics as D neurons, suggesting that they belong to the APUD system cells group.

These findings revealed for the first time that APUD cells exist widely in the alimentary canal of the laboratory shrew, in contrast to a previous report in which APUD cells were found only in the cerebrospinal fluid contacting neurons of lower order vertebrates. Therefore, the present study suggests an endocrine-like function for AADC-only-positive cells: when amine precursors are administered, uptake of precursors into the cells occurs via capillaries, which are abundantly present around the cells, and after the cells synthesize amines, the amines are then released into the capillaries.

Finally, the reason for the use of laboratory shrew, *Suncus murinus*, in this study was because, based on systematic zoology, this animal is the oldest animal classified under class Mammalia; therefore, the laboratory shrew was chosen for the purpose of comparative study with higher mammals.

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