Detection of Histidine Decarboxylase (HDC) in Paraffin Sections of Formalin Fixed Rat Stomach Using Avidin-biotin-peroxidase-complex Method

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Immunohistochemical localization of histidine decarboxylase (HDC) in rat stomach was studied. Formalin fixed, paraffin sections were prepared and immunohistochemical localization of HDC was performed by the avidin-biotin-peroxidase-complex method (ABC method). As a result, HDC was clearly detected in the basal portion of the oxyntic glands. The shape of many HDC-positive cells were flasklike. No reaction products were seen in chief cells or parietal cells. These findings are in agreement with the results reported by many workers. Therefore, ABC method of HDC is thought to be a useful technique for the detection of enterochromaffin-like cells using paraffin sections.

(Key Words: Histidine Decarboxylase (HDC), Rat, Stomach, Avidin-Biotin-Peroxidase-Complex Method, Paraffin section)

INTRODUCTION

It is a well documented fact that enterochromaffin-like cells were present in rat stomach using immunohistochemical method for histidine decarboxylase (HDC) (5-7). Many workers have described an immunohistochemical method using fluorescence by frozen sections (5-7). Although many reports have been published, HDC has not been observed in paraffin sections of formalin fixed rat stomach. In the present study, attempts were made to localized the HDC immunohistochemically in paraffin sections of formalin fixed rat stomach.

MATERIALS AND METHODS

Animals and their tissue preparation
Male Wistar rats weighing 180 to 200g were used. Each rat was killed by decapitation and the stomach was removed immediately. Then the stomach was opened along the greater curvature, washed in saline, pinned to a cork plate, and subsequently fixed in 10% neutral buffered formalin. Specimens were taken from gastric corpus and embedded in paraffin and prepared for 4μm paraffin sections. After the sections were de-paraffinized and rehydrated, immunohistochemical staining was performed by the avidin-biotin-peroxidase-complex method (8).

Immunohistochemical staining
Rabbit antiserum against histidine decarboxylase (HDC) was kindly provided by prof. Hiroshi Wada, Department of Pharmacology, Osaka University School of Medicine, Nakanoshima, Osaka, Japan. This antiserum at a 1:2,000 dilution was incubated on the sections at room temperature for 30 minutes. As an immunologic negative control, 1:100 diluted nonimmune rabbit serum was used. After the incubation was completed, the sections were incubated with biotinylated anti-mouse Ig rabbit Ig (secondary antibody, 1:100, Vector Lab.)
Fig. 1  Immunohistochemical localization of HDC in paraffin section of formalin fixed rat stomach. HDC is predominantly observed in the basal portion of the oxyntic glands.
Avidin-biotin-peroxidase-complex method, × 120

Fig. 2  Intracellular localization of HDC by a higher magnified view. HDC is evenly distributed in the flasklike cytoplasm. No reaction products are seen in chief cells.
Avidin-biotin-peroxidase-complex method, × 600

Fig. 3  Histological appearance of the basal portion of the oxyntic glands.
H&E, × 600

Fig. 4  By normal rabbit serum, no immunohistochemical reaction is observed in rat stomach.
Avidin-biotin-peroxidase-complex method, × 120
RESULTS AND DISCUSSION

Immunohistochemical localization of HDC was predominantly observed in the basal portion of the oxyntic glands (Fig. 1). Within the cells, HDC was diffusely distributed in the cytoplasm of the cells but not in chief cells or parietal cells. At high magnification, the shape of many HDC-positive cells were flakelike structures and they were closely related to the adjacent chief cells (Figs. 2,3). The control serum (NRS) was negative for immunohistochemical localization of HDC in the rat stomach (Fig. 4).

These findings are in agreement with the results reported by Taguchi et al. (7), who studied frozen sections of Zamboni’s fixed stomach using immunofluorescent histochemical method. In addition, immunohistochemical localization of HDC in rat stomach has only been demonstrated in frozen sections by many workers (5–7).

In the present study using the avidin-biotin-peroxidase-complex method, HDC was clearly recognized in paraffin sections of formalin fixed stomach. Therefore, by this method, the localization of HDC can be determined in paraffin sections of formalin fixed stomach. Furthermore, enterochromaffin-like cells are argentophil in that they stain with the Grimelius and the Sevier-Munger silver staining method using paraffin sections. In many species, they seem to contain histamine and also the histamine-forming enzyme, histidine decarboxylase (HDC) (2–4). In this respect, ABC method of HDC is thought to be a very useful technique detecting enterochromaffin-like cells using paraffin sections.

REFERENCES