Vol. 141, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 26, 1986 Pages 306-312

DEMONSTRATION OF IMMUNOHISTOCHEMICAL AND IMMUNOCHEMICAL CROSS-REACTIVITY OF L-HISTIDINE AND L-DOPA DECARBOXYLASES USING ANTIBODIES AGAINST THE TWO ENZYMES

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Received October 16, 1986

SUMMARY: Both rat L-histidine decarboxylase (HDC) and guinea-pig L-DOPA decarboxylase (DDC) were shown immunohistochemically and immunochemically to react with anti-rat HDC antibody . No cross-reaction was observed in immunoprecipitation experiments, but both anti-rat HDC antibody and anti-rat DDC antibody immunostained neurons in the substantia nigra, raphe nucleus and locus coeruleus of guinea-pig brain. Moreover, on immunoblotting, anti-rat HDC antibody recognized not only rat HDC but also guinea-pig DDC, but not rat DDC. However, anti-rat DDC antibody showed no immunohistochemical or immunochemical cross-reactivity with rat HDC.

Recently using antibody against L-histidine decarboxylase (HDC) purified from fetal rat liver, Watanabe et al. demonstrated histaminergic neurons in rat brain and showed that their cell bodies were restricted to the posterior hypothalamic area (4,5). However, antibody against rat HDC (anti-rat HDC Ab) stained cells that are known to contain DDC (1-3) as well as those containing HDC in guinea-pig brain (6). In this report we present evidence obtained by studies with anti-rat DDC antibody (anti-rat DDC Ab) and anti-rat HDC Ab for the immunohistochemical cross-reactivity between rat HDC and guinea-pig DDC.

Abbreviations: HDC; L-histidine decarboxylase (EC 4.1.1.22), DDC; L-DOPA decarboxylase (EC 4.1.1.28), anti-rat HDC Ab; anti-rat HDC antibody, anti-rat DDC Ab; anti-rat DDC antibody.

0006-291X/86 $1.50

MATERIALS AND METHODS

Assays of HDC and DDC Activities: HDC and DDC activities were assayed by condensation method of o-phthalaldehyde and ethylene diamine, respectively, as described previously (4,5,7).
Purification of rat DDC and preparation of polyclonal and monoclonal anti-rat DDC Ab: The purification of DDC and preparation of anti-DDC antibodies are described in detail elsewhere (Ando-Yamamoto et al. J.Biochem. in press). Briefly, DDC was purified from rat liver about 800-fold by successive chromatographic steps and the final preparation (specific activity, about 6 μmol/min/mg protein) gave a single band of protein 10% SDS-PAGE. The purified DDC (100 μg) was emulsified with Freund's complete adjuvant and injected into female rabbits and BALB/c mice with three booster injections of the same preparation at monthly intervals. Polyclonal anti-rat DDC Ab was obtained by bleeding the immunized rabbits two weeks after the final injection. Monoclonal anti-rat DDC Ab was obtained by the method of Köhler and Milstein (9). One monoclonal antibody, MA-1, belonged to the IgG_1 subclass and recognized rat and guinea-pig DDC's to the same extents.
Immunoprecipitation: Rat and guinea-pig brains were homogenized in three volumes of 0.1 M potassium phosphate buffer (pH 6.9) containing 1 mM dithiothreitol, 0.01 mM pyridoxal 5'-phosphate and 1% polyethylene glycol (Buffer A). The homogenate (50 μl) were incubated with various amounts of anti-rat DDC Ab or anti-rat HDC Ab at 37°C for 1 h. Then antibody-antigen complexes were precipitated with a 10% suspension of Protein A (50 μl). After brief centrifugation, the DDC and HDC activities of the supernatant were assayed as described previously (4-7).
Other procedures: Protein was determined by the method of Lowry et al.(8) with bovine serum albumin as a standard. Polyacrylamide gel (10%) electrophoresis in the presence of 0.1% SDS was performed as described by Laemmli (11).

RESULTS

Immunoprecipitation: As shown in Fig.1A, anti-rat DDC Ab immunoprecipitated the rat and guinea-pig DDC activities, but not the HDC activity of either species. Conversely, anti-rat HDC Ab

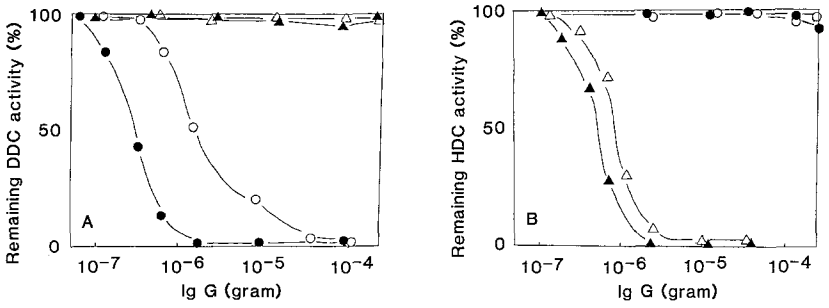


Fig. 1. Immunoprecipitation of DDC and HDC activities by anti-rat DDC Ab and anti-rat HDC Ab. Rat (●,▲) and guinea-pig (○,△) brain homogenates were incubated with anti-rat DDC Ab (●,○) or anti-rat HDC Ab (▲,△), and remaining DDC activities (A) and HDC activities (B) were assayed as described in MATERIALS AND METHODS.

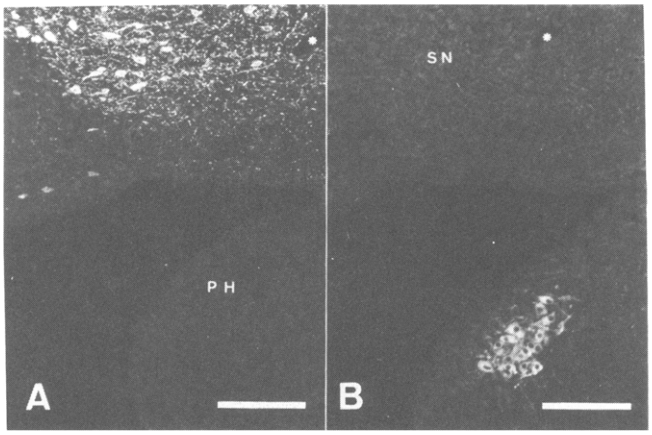


Fig. 2. Immunohistochemical staining of rat brain with anti-rat DDC Ab (A) and anti-rat HDC Ab (B). Rats were perfused with Zamboni's fixative (2% paraformaldehyde and 0.21% picric acid in 0.1 M sodium phosphate buffer, pH 7.4) (13). Their brains were then removed and immersed in the same fixative for 24 h-48 h and then transferred to 30% sucrose in 0.1 M phosphate buffer (pH 7.4) for 48 h. The specimens were frozen at -20°C and cut into serial sections of 5 μm thickness on a cryostat, and the sections were processed for indirect immunofluorescence staining as described by Coons (14). The sections were treated with anti-rat DDC Ab (A) or anti-rat HDC Ab (B) first and then with fluorescein isothiocyanate-conjugated goat anti-rabbit IgG and examined with a fluorescence microscope. SN, substantia nigra; PH, posterior hypothalamus; ✱; the same blood vessel. (Scale bar: 200 μm)

immunoprecipitated rat and guinea-pig HDC's, but not DDC's (Fig.1B). Higher concentrations of anti-rat HDC Ab (> $5x10^{-4}$g of IgG) inhibited DDC activity, but at these concentrations IgG from nonimmunized rabbits also showed nonspecific inhibition.

Immunohistochemical studies on rat brain: Figure 2 shows serial sections of rat brain including the substantia nigra and posterior hypothalamus. Anti-rat DDC Ab stained cells in the substantia nigra, which are dopaminergic neurons, but not those in the posterior hypothalamus (Fig.2A), whereas anti-rat HDC Ab stained cells in the posterior hypothalamic area but not those in the substantia nigra (Fig.2B).

Immunohistochemical studies on guinea-pig brain: Figure 3 shows serial sections of the region of the substantia nigra in guinea-pig brain stained with anti-rat DDC Ab (Fig.3A) and

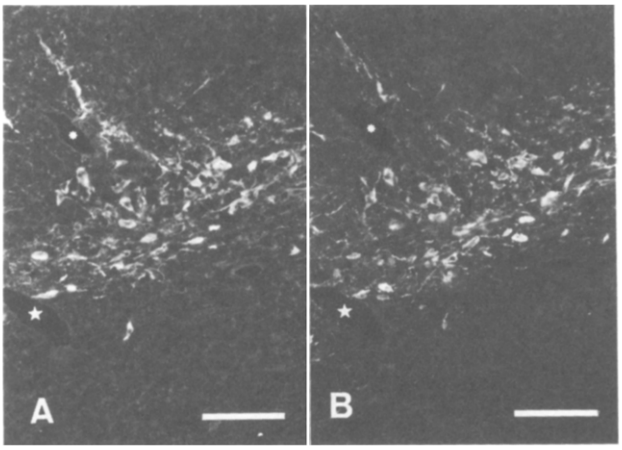
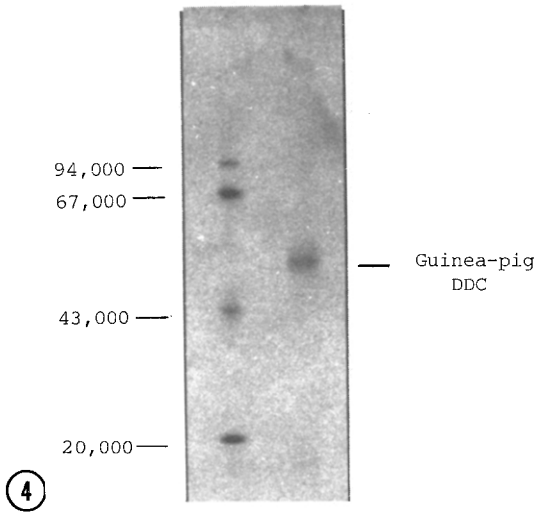


Fig. 3. Immunohistochemical staining of guinea-pig brain with anti-rat DDC Ab (A) and anti-rat HDC Ab (B). Two serial sections of the substantia nigra of guinea-pig brain were prepared and immunostained with anti-rat DDC Ab and anti-rat HDC Ab as described in the legend of Fig.2. Note most of the cells in the substantia nigra that were stained by anti-rat DDC Ab (A) were also stained with anti-rat HDC Ab (B). ✱★; the same blood vessels. (Scale bar: 100 μm)

anti-rat HDC Ab (Fig.3B). Comparison of stained cells in the two sections shows that most cells were stained with both antibodies. In other regions, such as the raphe nucleus and locus coeruleus, the same cells were also stained with anti-rat HDC Ab and anti-rat DDC Ab.

Purification of guinea-pig DDC on an immunoaffinity column with monoclonal anti-DDC Ab as a ligand: DDC was purified from guinea-pig liver as described in the legend of Fig.4. The eluate gave a single band with a molecular weight of 50,000 on SDS-PAGE.

Immunoblotting: Immunoblotting was carried out to confirm the above immunohistochemical results. As shown in Fig.5A, anti-rat DDC Ab as a probe detected the bands of rat DDC (lane 1) and guinea-pig DDC (lane 2) but not that of rat HDC (lane 3). On the other hand, as shown in Fig.5B, anti-rat HDC Ab as a probe detected guinea-pig DDC (lane 2) and rat HDC (lane 3), but not rat DDC (lane 1).



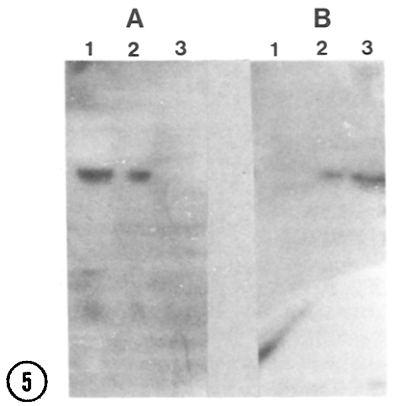


Fig. 4. SDS-PAGE of immunoaffinity-purified guinea-pig DDC. An immunoaffinity column was prepared by the method of Adachi and Hatanaka(10). Briefly, 150 mg of Protein A-Sepharose CL-4B was incubated with rabbit anti-mouse IgG (Fc) IgG (2 mg) in PBS at 4°C for 8 h. The gel was then washed extensively with PBS, mixed with monoclonal anti-rat DDC Ab, MA-1,(4 mg IgG) and incubated at 4°C overnight. Then the gel was washed repeatedly with PBS and equilibrated with 0.1 M borate buffer (pH 8.0). The gel was then incubated in borate buffer containing 5 mg/ml dimethyl suberimidate dihydrochloride at 20°C for 30 min. It was then washed as described above, stood for 2 h at 4°C in 0.1 M acetate buffer (pH 2.9) containing 0.15 M NaCl, equilibrated with PBS, and packed in a mini-column.

DDC was purified from guinea-pig liver as follows: 20 g of guinea-pig liver was homogenized in three volumes of Buffer A and centrifuged at 10,000 g for 1 h. The supernatant was dialyzed against 0.01 M potassium phosphate buffer (pH 6.9), and applied to the DEAE-cellulose column preequilibrated with the same buffer. The column was washed and then DDC activity was eluted with Buffer A. The eluted DDC was dialyzed against PBS, applied to the immunoaffinity column and incubated overnight at 4°C with shaking. Then the column was washed extensively with PBS. DDC was eluted from the column with 0.1 M acetate buffer (pH 2.9) containing 0.15 M NaCl. The pH of the eluate was promptly adjusted to pH 7.0 by adding 1 M Tris-HCl (pH 9.0). The eluate was concentrated and subjected to 10% SDS-PAGE with standard proteins. The gel was stained with Coomassie Brilliant Blue.

Fig. 5. Immunoblotting of rat DDC, guinea-pig DDC and rat HDC with anti-rat DDC and anti-rat HDC Ab's. Purified rat DDC (1 μg, lane 1), immunoaffinity-purified guinea-pig DDC (1 μg, lane 2) and purified rat HDC (2.5 μg, lane 3) were separated by 10% SDS-PAGE and transferred to nitrocellulose as described by Towbin et al. (12). The nitrocellulose was then incubated sequentially with anti-rat DDC (A) or anti-rat HDC (B) Ab's, with goat anti-rabbit IgG and with rabbit peroxidase-antiperoxidase complex, and washed several times with 50 mM Tris-HCl (pH 8.0) containing 0.05% Tween 20 between each step. 4-Chloro-1-naphthol was used as a substrate of the peroxidase reaction. Note that in A, anti-rat DDC Ab recognized rat DDC (lane 1) and guinea-pig DDC (lane 2), but not rat HDC (lane 3), while in B, anti-rat HDC Ab recognized not only rat HDC (lane 3) but also guinea-pig DDC (lane 2), but not rat DDC (lane 1).

DISCUSSION

Anti-rat DDC and anti-rat HDC Ab's are useful for detecting cells containing these respective enzymes in rat brain, as shown in Fig.2. However, anti-rat HDC Ab stained cells containing DDC as well as those containing HDC in guinea-pig brain. Previously Taguchi et al. reported staining of guinea-pig brain with anti-rat HDC Ab and suggested the cross-reactivity of the two enzymes(6). In the present work, by staining serial sections we found that anti-rat HDC Ab immunostained the cells recognized by anti-rat DDC Ab in the substantia nigra (Fig.3), raphe nucleus and locus coeruleus of guinea-pig brain. Moreover, we found in immunoblotting experiments that anti-rat HDC Ab recognizes immunoaffinity-purified guinea-pig DDC as well as rat HDC, thus obtaining direct evidence for the cross-reactivities of these two enzymes. However, in these experiments, anti-rat DDC Ab did not recognize rat HDC. Thus unlike guinea-pig DDC, rat DDC seems to differ immunologically from rat HDC. Alternatively, anti-rat HDC Ab may happen to recognize similar structures in the two enzymes, whereas anti-DDC Ab does not.

The present data suggest the presence of similar antigenic recognition sites inside the native molecules of the two decarboxylases that are exposed when the enzymes are denatured. Studies with various monoclonal antibodies against the two enzymes may be a good approach to detection of such common structures.

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