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IMMUNOCHEMICAL CROSS REACTIVITY OF THE ANTIBODY ELICITED AGAINST L-HISTIDINE DECARBOXYLASE PURIFIED FROM THE WHOLE BODIES OF FETAL RATS WITH THE ENZYME FROM RAT BRAIN

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SUMMARY

L-Histidine decarboxylase was stabilized with 1% polyethylene glycol and purified to a homogeneous state from the whole bodies of fetal rats. Antibody was raised in a rabbit against the purified enzyme. The antibody strongly inhibited histidine decarboxylase activity from rat brain as well as that from whole fetal rats, but on Ouchterlony's double difusion it gave a single precipitin line against the fetal enzyme that fused with the line against the brain enzyme with spur formation.

There is much circumstantial evidence that histamine may be a neurotransmitter in the mammalian central nervous system (1-5). However, histaminergic pathways have not been demonstrated unequivocally, although Schwartz et al. suggested the presence of ascending histaminergic neurons through the median forebrain bundle from results in degeneration experiments (6). One reason for the uncertainity about whether histamine is a neurotransmitter is that, unlike catecholamines and serotonin (7), it does not form any specific reaction product with fluorescence which can be used in histochemical studies. Therefore, we are attempting to develop an immunofluorescent technique using antibody against the enzyme forming histamine, L-histidine decarboxylase (HDC)*. For this, we

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Abbreviations used ; HDC, L-Histidine decarboxylase [E.C.4.1.1.22] DDC, L-DOPA decarboxylase [E.C.4.1.1.28].

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first tried to obtain a preparation of HDC of sufficient purity for use in production of antibody against the enzyme.

As a source of enzyme, brain is poor because its HDC activity is extremely low (0.1-1.0 pmole/min/mg protein) (8), and therefore, we used whole bodies of fetal rats, one of the richest sources of HDC (9). Previously (10), we partially purified HDC from whole fetal rats, but we could not obtain a pure preparation because the purified enzyme was very unstable. This paper describes the purification of HDC from whole bodies of fetal rats and the immunochemical cross-reactivity of the antibody elicited against fetal HDC with brain HDC.

MATERIALS AND METHODS

Assays of HDC and DDC*: HDC and DDC were assayed by condensation with o-phthalaldehyde (11) and ethylene diamine (12), respectively, as described previously (10,13).
Purification of HDC from Whole Bodies of Fetal Rats and Rat Brain: HDC was purified from the whole bodies of fetal rats (16-20 days of gestation) as described previously (10) except that 1 % polyethylene glycol (average molecular weight, 300) was included throughout the purification. HDC from rat brain was purified in the same way as fetal HDC (H. Fukui et al. to be published) and the fraction from Bio-Gel A-0.5m was used as purified enzyme.
Polyacrylamide Gel Electrophoresis: Polyacrylamide gel electrophoresis was carried out by the method of Ornstein and Davis (14) with some modifications as described in the legend to Fig. 1.
Immunization of Rabbits with Fetal HDC: HDC (2,000 pmoles/min, assumed to correspond to 10-20 μg of pure protein) was injected with complete Freund's adjuvant into 100 sites in the skin of the abdomen of a rabbit under pentobarbital anesthesia 4 times every 10 days. The IgG fraction was obtained by the method of Kekwick (15) and was stored at -20°C.
Chemicals: Sheep anti-rabbit IgG was obtained from Calbiochem. All other chemicals used were standard commercial products.

RESULTS AND DISCUSSION

Attempts to Stabilize HDC: As described previously (10), highly purified HDC was too unstable for further purification, especially on freezing and thawing. Therefore, we tested the abilities of various agents to stabilize the activity. As shown in Table 1, polyethylene glycol (average molecular weight, 300) was the most

Table 1 Effects of Various Agents on Stability of HDC

Agent	Concentration	Activity (%) -20°C	4°C
None		19	73
Polyethylene glycol	1 %	100	100
Ethylene glycol	1 %	74	79
Acetone	1 %	10	67
Sucrose	0.32M	83	59
Glycerol	10 %	16	16
EDTA	1 mM	34	65
Imidazole	1 mM	37	68

Two hundred U of HDC (specific activity ; 4500 U/mg protein) was stored in 0.1 M potassium phosphate buffer, pH 6.8 containing 0.2 mM dithiothreitol and 0.01 mM pyridoxal 5'-phosphate in the presence of the reagents listed above for 3 days in a total volume of 1 ml. Then aliquots were assayed for HDC activity. An activity of 200 U/ml was taken as 100 %.
U, pmole product/min.

effective of the agents tested, stabilizing the activity completely at both -4 and -20°C.

Purification of HDC from the Whole Bodies of Fetal Rats : HDC was purified from the whole bodies of fetal rats as described previously (10), except that 1 % polyethylene glycol was present through out the purification. This inclusion in all the buffers did not affect the purification procedures and permitted the preparation of sufficiently pure HDC for use in raising antibody.

On polyacrylamide gel electrophoresis, our final preparation gave two protein bands, only one of which had HDC activity, as shown in Fig. 1(a). The preparation was applied to a series of gels, and the portions corresponding to the activity were cut out and extracted with buffer. The combined extracts, when resubjected to electrophoresis, gave a single protein band (Fig. 1 (b)). This preparation of HDC was used to raise antibody.

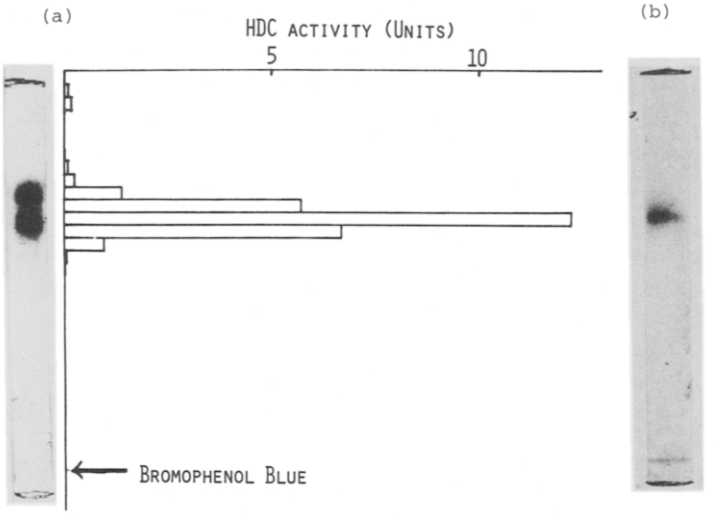

Fig. 1. Polyacrylamide Gel Electrophoresis of Purified HDC from the Whole Bodies of Fetal Rats. HDC was purified from whole fetal rats as described previously (10), except that 1 % polyethylene glycol (average molecular weight, 300) was included in all buffers used. Polyacrylamide gel electrophoresis was carried out as described by Ornstein and Davis using 7.5 % acrylamide. (a) After electrophoresis for 6 hours at 1 mA/tube at 4°C, a gel was stained with 0.01 % Coomassie Brilliant Blue in 5 % methanol and 5 % acetic acid for 2 hours and then destained with the same solvent (left). A duplicate gel was cut into 2-mm slices and each slice was assayed for HDC activity as described previously (right). (b) Replicate gels were subjected to electrophoresis as in (a) and the portion of each gel corresponding to the HDC activity was cut out and extracted with 0.02 M potassium phosphate buffer, 0.2 mM dithiothreitol, 0.01 mM pyridoxal 5'-phosphate, and 1 % polyethylene glycol. Polyacrylamide gel electrophoresis was carried out as in (a) and gels were stained for protein.

Immunochemical Cross Reactivity of the Anti-Fetal HDC Antibody with Rat Brain HDC : The HDC activity of fetal rats was strongly inhibited by the antibody raised against the fetal rat enzyme, as shown in Fig. 2, but not by a non-immunized rabbit serum. The DOPA decarboxylase activity of the crude extract of fetal rats was not inhibited by this antibody, confirming the idea that HDC and DDC are distinct enzymes. Rat brain HDC was also inhibited by the antibody produced against the fetal enzyme, although less than the fetal enzyme (Fig. 2).

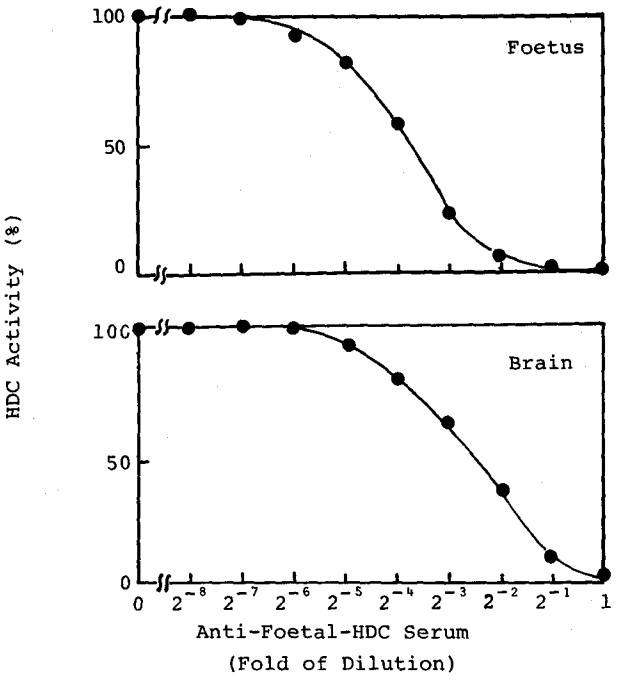

Fig. 2. Inhibition of Fetal Rat and Brain HDC Activities by Antiserum Against HDC from Whole Bodies of Fetal Rats. The antibody was produced by injecting HDC (2,000 pmoles product/min) obtained from the gels together with complete Freund's adjuvant into 100 sites in the abdomen of a rabbit (3 kg) four times every 10 days. Blood was obtained from ear veins and the IgG fraction was purified by the method of Kekwick (15). For inhibition studies, 20 µl of fetal or brain HDC at Step 6 in the purification procedure (Bio-Gel A-0.5 m) was incubated with 20 µl IgG in phosphate buffered saline (0.01 M potassium phosphate buffer, pH 6.8-0.9 % NaCl) supplemented with 0.2 mM dithiothreitol, 0.01 mM pyridoxal 5'-phosphate and 1.0 % polyethylene glycol for 24 hours at 4°C. 10.4 and 3.7 units (pmoles histamine per min) of fetal and brain enzymes were used respectively. The antigen-antibody complex was cosedimented by incubation with 60 µl of sheep anti-rabbit-IgG for 24 hours at 4°C. The control mixture without anti-HDC antibody was treated similarly. 50 µl aliquots of the supernatant were assayed for HDC as described previously.

On Ouchterlony double diffusion, the antibody gave a single precipitin line against fetal HDC, and this line fused with spur formation with the precipitin line against the brain enzymes, as shown in Fig. 3, indicating that the two enzyme share some antigenic determinants. This is consistent with the finding that HDC from rat brain was inhibited to some extent by the antibody against fetal HDC, but less than the fetal enzyme.

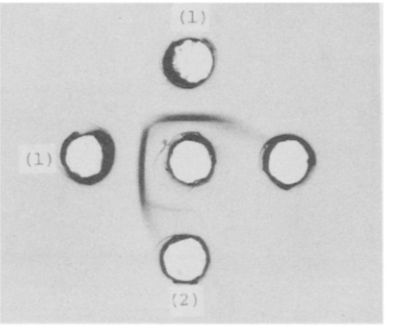

Fig. 3. Ouchterlony Double Diffusion of the Antibody against HDC from Whole Bodies of Fetal Rats toward HDC's from Rat Fetuses and Brain. The central well contained antibody; Wells (1) and (2) contained HDC's from whole fetal rats and brain, respectively. Agar gel (1 %) was prepared in 0.9 % NaCl containing 0.01 M potassium phosphate buffer, pH 6.8, with 0.01 % NaN_3.

These results suggest that there are at least two kinds of HDC in rat tissues. Immunochemically, the HDC's from rat fetuses and brain are very similar, but not identical. They catalyze the same reaction, and have similar Km values for L-histidine and similar molecular sizes, but their isoelectric points are slightly different (T. Watanabe et al., to be published). Probably the HDC's from whole fetal rats and rat brain are isozymes of HDC. These isozymes may be fetal and adult types of HDC, like hemoglobin F and A, and they may be derived from different cells. With regard to the second possiblility, the fetal and brain enzymes could be mast cell and non-mast cell types of HDC respectively, because whole fetuses are rich in mast cells for several days before birth (16), whereas the brain is rich in non-mast cells, although it has a few mast cells, too (17). To clarify these points, we are now examining HDC from various tissues of rats and from mutant mice deficient in mast cells (18).

Irrespective of the nature of these two types of HDC, the present studies indicate that the antibody raised against fetal rat HDC could be used to develop an immunofluorescent technique for use in studies on histaminergic pathways in rat brain.

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