Comparison of Two Isozymes of Carbonic Anhydrase in the Rat Anterior Pituitary Gland and Pituitary Tumors

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SUMMARY

Two isozymes of carbonic anhydrase (EC 4.2.1.1.) were compared in the anterior pituitary gland of non-tumor-bearing rats and in the hormone-secreting pituitary tumors. In contrast to the pituitary gland, which contained 60 to 70% of the total carbonic anhydrase in the particulate subcellular fraction, three hormone-secreting pituitary tumors were devoid of the particulate (Triton X-100-solubilized) enzyme activity. Another pituitary tumor, 7315a, contained particulate carbonic anhydrase, but the activity was only 45% of the activity of normal pituitary gland.

During the development of the rat brain, the particulate (Triton X-100-solubilized) carbonic anhydrase activity was undetectable in preparations up to 21 days of age (body weight, 47 g). After that age, the carbonic anhydrase activity in the particulate fraction increased rapidly and reached the adult level at 37 days (body weight, 120 g), while the activity in the soluble fraction increased gradually after birth and then reached a plateau at 30 days (body weight, 81 g).

These data show that the isozyme pattern of carbonic anhydrase in pituitary tumor tissue resembles the pattern in fetal cells more than the pattern in adult tissue.

INTRODUCTION

We have recently reported that a low-activity CA \(^2\) (type B) (EC 4.2.1.1.) is associated with the particulate fraction, and a high-activity CA (type C) is localized in the soluble fraction of the anterior pituitary gland (3). Our data suggested that the activity of CA in the particulate fraction may be related to the secretion of prolactin. It is known that type-B CA of human erythrocyte is markedly depressed in thyrotoxicosis (2, 4, 14) and may be a regulatory enzyme whose activity is controlled by a hormone or other substances. Recently, Tashian et al. (12) suggested that CA isozyme B evolved more rapidly than did the C enzyme in primates and that the latter form might represent an older evolutionary type of CA.

The purpose of the present study was to investigate whether hormone-secreting pituitary tumors contain CA isozyme B in the particulate fraction and to determine the stage of development at which CA occurs in the particulate fraction of the rat brain.

MATERIALS AND METHODS

Animals. Mature female Wistar-Furth rats were inoculated with the pituitary tumors MtTW5, MtTW15, and StTW5 as previously described (6). Female Buffalo rats were implanted with pituitary tumor 7315a. All rats were routinely housed 4 to 5/cage at 22 to 23° and allowed water and Purina laboratory chow ad libitum.

Materials. Tris, \(\beta\)-naphthyl acetate, fast blue BB salt, acetazolamide, and polyvinylpyrrolidone powder were purchased from Sigma Chemical Co., St. Louis, Mo. Triton X-100 was purchased from Packard Instrument Co., Inc., Downers Grove, Ill.

Enzyme Preparation. The procedure of enzyme preparation used was essentially the same as described previously (3). Rats were killed by decapitation and exsanguinated. The anterior pituitary glands and tumor tissues were quickly removed and chilled in ice-cold 0.25 M sucrose. The connective and necrotic tissues were quickly removed from the tumor tissue. The isolated tumor tissues were chopped into small pieces and washed several times by decantation with ice-cold 0.25 M sucrose to eliminate the contamination of blood coagulants. Aliquots weighing 100 mg were homogenized in 1 ml of 0.25 M sucrose using a homogenizer fitted with a Teflon pestle. One-half of the homogenate was brought to a final concentration of 1% Triton X-100 and incubated at 0° for 10 min. Both the detergent-treated and the untreated portions of the homogenate were centrifuged at 105,000 \(\times\) g for 60 min, and the supernatant fraction was used for the determination of CO\(_2\) hydration activity.

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To study Ouchterlony double-diffusion analysis, the particulate (Triton X-100-solubilized) CA was prepared by the following procedure. The 105,000 \(\times\) g precipitate obtained from homogenates of pituitary tumor 7315a and rat brain was suspended in 20 volumes of ice-cold 0.25 M sucrose at 0° for 15 min and then centrifuged at 105,000 \(\times\) g for 60 min. The precipitate was resuspended in 2 ml of 0.25 M sucrose, incubated with 1% Triton X-100 at 0° for 20 min to solubilize the enzyme, and then centrifuged at 105,000 \(\times\) g for 60 min. The resultant supernatant fraction was

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\(^2\) The abbreviation used is: CA, carbonic anhydrase.

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dialyzed against 0.15 M NaCl for 15 hr and then concentrated 5 times by immersion in polyvinylpyrrolidone powder. The concentrated solution was used as the Triton X-100-solubilized CA in the particulate fraction. The 1st 105,000 × g supernatant fraction was dialyzed and concentrated by the same procedures as that of the 105,000 × g precipitate.

**Subcellular Fractionation.** The pituitary tumor 7315a tissue (2.78 g), prepared as described above, was homogenized in 9 volumes (w/v) of 0.25 M sucrose. Subcellular fractionation of the tumor was done by the same procedure as described previously (3).

**Assay of CA Activity.** The hydration of CO₂ was measured by the method of Nyman (8). One unit of hydrase activity is defined as the amount producing a decrease in absorbance of 1.0/min at 276 nm.

**Protein Determination.** Protein concentration was determined by the method of Lowry et al. (5).

**Immunological Procedures.** CA isozyme B antibody was prepared by the procedure as described (3). An immunological study was performed by Ouchterlony double-diffusion analysis (9).

**Polyacrylamide Gel Electrophoresis.** Polyacrylamide gel electrophoresis was carried out in 5% gels at pH 8.7 by the method of Davis (1). Esterase activity was detected using β-naphthyl acetate as a substrate, according to the method of Tashian (11).

**RESULTS**

The CA activities in the pituitary glands from non-tumor-bearing and tumor-bearing rats are compared in Table 1. The data show that enzyme activity was unchanged in the glands from rats bearing growth hormone-secreting tumors StW5 and MtTW15, as compared with the gland of Wistar-Furth controls. The pituitary glands of rats with prolactin-secreting tumor MtTW5 obtained less CA activity in the soluble fraction than did the pituitary glands of controls. The activity of Triton X-100-solubilized (particulate) CA was significantly less in glands of rats bearing the prolactin-secreting pituitary tumor 7315a than in glands from Buffalo controls.

The CA activity in pituitary tumor tissue was compared with that in the pituitary gland after treatment of whole tissue homogenates with Triton X-100 and centrifugation at 105,000 × g. This treatment resulted in solubilization of the particulate enzyme in the pituitary gland preparations. Most pituitary tumor homogenates, however, did not increase in total enzyme activity following Triton X-100 treatment. Treatment of the homogenate of pituitary tumor 7315a with the detergent increased the total enzyme activity 2- to 3-fold. However, the total enzyme activity in the presence of Triton X-100 is only 53% of the activity in pituitary, and the particulate enzyme (Triton X-100-solubilized) activity in tumor 7315a is 45% of the activity in nontumor tissue.

Enzymatic activities in the 105,000 × g precipitate of untreated preparations and preparations treated with Triton X-100 were also measured in this experiment. Treatment with Triton X-100 only solubilized the enzyme and was without effect on the CA activity per se. Triton X-100 itself had no effect on nonenzymatic hydration of carbon dioxide. The activities in the pituitary gland of normal and tumor-bearing rats and of pituitary Tumor 7315a were very similar to the activity of the Triton X-100-solubilized enzyme, while the precipitates of the Triton X-100-treated homogenates had no demonstrable activity. These results indicate that the particulate preparations of the pituitary gland and pituitary tumor 7315a contain comparable activity of CA, but the other pituitary tumors, MtTW15, MtTW5, and StTW5, lack this bound form of the enzyme.

Experiments utilizing Ouchterlony double-diffusion analysis and disc gel electrophoresis were performed to determine whether pituitary tumors contain CA isozyyme B. As shown in Chart 1, a single precipitin band was observed between the antibody against the CA isozyyme B from rat erythrocytes and the Triton X-100-solubilized CA obtained from the particulate fraction of tumor 7315a. No precipitin bands were observed between the antibody and the 105,000 × g supernatant enzyme from any pituitary tumor.

The electrophoretic pattern of CA activity using β-naphthylacetate as substrate is shown in Chart 2. The soluble enzyme from each pituitary tumor yielded 1 band that corresponded to CA isozyyme C from the normal pituitary gland. No band corresponding to CA isozyyme B was found, but the preparation had 3 other esterase bands. When 1 mM acetazolamide, a specific inhibitor of CA was added to the β-naphthylacetate, the esterase activity associated with CA isozyymes B and C was completely inhibited, but the other esterase bands were not affected. These results indicate that CA isozyyme B exists in the particulate fraction of the pituitary gland from normal female rats and pituitary tumor 7315a but is undetectable in pituitary tumors StW5, MtTW15, and MtTW5.

CA activity in rat brain was measured during various intervals of development (Chart 3). Brain homogenates untreated with detergent and centrifuged at 105,000 × g precipitate and 105,000 × g supernatant fraction, respectively (3). The distribution of CA activity was examined in the subcellular fractions of the homogenate of pituitary tumor 7315a (Table 2). The data show that the subcellular distribution pattern of tumor 7315a was almost identical to that of normal pituitary glands (3). Treatment of the individual tumor subcellular fractions with Triton X-100 caused no change in CA activity.
Comparison of CA activities in the soluble and particulate fractions of the anterior pituitary gland of normal and tumor-bearing rats and pituitary tumors

Enzyme preparations and assays were conducted as described under "Materials and Methods."

<table>
<thead>
<tr>
<th>Tissue</th>
<th>CA activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated (unit/g wet wt)</td>
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<tr>
<td>Pituitary gland</td>
<td></td>
</tr>
<tr>
<td>Non-tumor-bearing rats</td>
<td></td>
</tr>
<tr>
<td>Wistar-Furth (4)</td>
<td>139 ± 4b</td>
</tr>
<tr>
<td>Buffalo (4)</td>
<td>68 ± 7</td>
</tr>
<tr>
<td>Tumor-bearing rats</td>
<td></td>
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<tr>
<td>StW5 in Wistar-Furth (2)</td>
<td>132</td>
</tr>
<tr>
<td>MtTW15 in Wistar-Furth (4)</td>
<td>134 ± 2</td>
</tr>
<tr>
<td>MtTW5 in Wistar-Furth (4)</td>
<td>109 ± 5b</td>
</tr>
<tr>
<td>7315a in Buffalo (4)</td>
<td>78 ± 10</td>
</tr>
<tr>
<td>Pituitary tumor</td>
<td></td>
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<tr>
<td>StW5 in Wistar-Furth (4)</td>
<td>181 ± 28b</td>
</tr>
<tr>
<td>MtTW15 in Wistar-Furth (4)</td>
<td>156 ± 20</td>
</tr>
<tr>
<td>MtTW5 in Wistar-Furth (2)</td>
<td>121</td>
</tr>
<tr>
<td>7315a in Buffalo (4)</td>
<td>50 ± 5b</td>
</tr>
</tbody>
</table>

a ΔA236 nm/min.
b Numbers in parentheses, number of preparations used.
c Mean ± S.E.
d The difference between the activity of the enzyme treated with Triton X-100 and untreated enzyme is significant at p < 0.01.
e p < 0.05, compared to corresponding mean value for non-tumor-bearing rats.

particulate CA increases rapidly and reaches the adult level at 37 days (body weight, 120 g).

Ouchterlony double-diffusion analyses were carried out to determine the immunological identity of the Triton X-100-solubilized CA obtained from the particulate fraction of the adult rat brain (Chart 1). A single precipitin band was observed between the antibody against the CA isozyme B from rat erythrocytes and the Triton X-100-solubilized CA obtained from the particulate fraction of rat brain.

Chart 1. Ouchterlony double-diffusion analysis of CA B antibody against the Triton X-100-solubilized CA from pituitary tumor 7315a and brain. The center well contained CA isozyme B antibody; Well 1, the Triton X-100-solubilized CA from brain; Well 2, the soluble CA from brain; Well 3, the Triton X-100-solubilized CA from tumor 7315a; Well 4, the soluble CA from tumor 7315a. Each well contained 0.5 to 1.2 units of CA.

Chart 2. Disc gel-electrophoretic patterns of CA activities in pituitary gland and pituitary tumors. In A, 20 μl of the erythrocyte CA (31.5 units/ml) treated with chloroform-ethanol, and 100 μl of the 105,000 x g supernatant fraction (4.5 units/ml) of homogenate from the pituitary gland, and 50 μl of 105,000 x g precipitate (11.5 units/ml) solubilized with Triton X-100 were applied to origin, and gels were stained against β-naphthylacetate after electrophoresis. 1, erythrocyte CA; 2, particulate CA; 3, soluble CA. In B, 100 μl of 105,000 x g supernatant fraction (0.5 to 1.9 units) of homogenate from the pituitary tumors were applied to origin. 1, 7315a; 2, MtTW5; 3, StW5; 4, MtTW15.
Table 2
Subcellular distribution of CA in pituitary tumor 7315a

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Untreated</th>
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<tr>
<td></td>
<td>Unit*/fract. % total</td>
<td>Unit/mg protein % total</td>
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<tr>
<td>Homogenate</td>
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<tr>
<td>800 x g precipitate</td>
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<td>36.9</td>
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<td>13,000 x g precipitate</td>
<td>8.3</td>
<td>8.1</td>
</tr>
<tr>
<td>105,000 x g precipitate</td>
<td>6.9</td>
<td>6.7</td>
</tr>
<tr>
<td>105,000 x g supernatant</td>
<td>49.7</td>
<td>48.3</td>
</tr>
<tr>
<td>Recovery</td>
<td>84.7</td>
<td>82.3</td>
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<td></td>
<td>136.9</td>
<td>0.83</td>
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<tr>
<td></td>
<td>39.3</td>
<td>34.9</td>
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<tr>
<td></td>
<td>8.4</td>
<td>7.4</td>
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<tr>
<td></td>
<td>5.9</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>59.1</td>
<td>52.5</td>
</tr>
<tr>
<td></td>
<td>49.7</td>
<td>48.3</td>
</tr>
</tbody>
</table>

*ΔA276 nm/min.

The authors thank Ronald C. Pace for his excellent technical assistance.

REFERENCES


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