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THE ACTIVITY OF TYROSINE 3-MONOOXYGENASE IS USUALLY SUPPRESSED BY AN ENDOGENOUS FACTOR IN BRAIN

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SUMMARY: Tyrosine 3-monooxygenase activity of the crude extract from rat striatum had a sharp pH optimum at pH 5.6 and showed almost no activity at pH 7 and higher. When the crude extract was adjusted to pH 5.2, the resulting precipitate showed high activity in the pH range of 6 to 7.5. Incubation of the acid-precipitated fraction with the acid-soluble fraction resulted in a remarkable decrease in the enzyme activity, as assayed at a neutral pH. These findings suggest that there is an endogenous factor inhibiting the activity of tyrosine 3-monooxygenase in rat brain.

Tyrosine 3-monooxygenase is the rate-limiting enzyme in the biosynthesis of catecholamines such as dopamine, norepinephrine, and epinephrine in the nervous system (1,2). The activity of the enzyme is enhanced by a variety of conditions including enzymatic phosphorylation by cyclic AMP-dependent protein kinase (3,4) or calmodulin-dependent protein kinase II (5), partial proteolytic digestion (6), and the presence of polyanions (7) and an anionic detergent such as sodium dodecyl sulfate (8). These observations together with the fact that even purification resulted in an remarkable increase in the enzyme activity at a neutral pH (9) led us to postulate that the activity of tyrosine 3-monooxygenase is suppressed in a normal state in brain. We report here that there is an endogenous factor completely suppressing the activity of tyrosine 3-monooxygenase in rat brain.

The abbreviation used is: Mes; 2-(*N*-morpholino)ethanesulfonic acid.

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EXPERIMENTAL PROCEDURES

L-[1-^{14}C]Tyrosine (56 mCi/mmol) was purchased from Radiochemical Centre, Amersham. Mes was from Sigma. Microbial proteinase inhibitors (leupeptin, chymostatin, and pepstatin) were from the Peptide Institute, Osaka, Japan. Rabbit anti-mouse immunoglobulin was from DAKO-Immunoglobulins a/s, Copenhagen, Denmark. Bio-Gel P-10 was from Bio-Rad.

Frozen striata from adult Wistar rats were homogenized in 3 volumes of 5 mM sodium phosphate, pH 7.5, containing 20 µg each/ml of leupeptin, pepstatin, and chymostatin in a Potter-Elvehjem homogenizer and the supernatant obtained by centrifugation for 60 min at 100,000 X *g* was passed through a Bio-Gel P-10 column in 5 mM sodium phosphate, pH 7.5, to remove low molecular weight substances. The resulting crude extract was stored at -80°C until use.

Tyrosine 3-monooxygenase activity was determined essentially according to the method described previously (10). One unit of the activity was defined as the amount of enzyme which catalyzed the formation of 1 nmol of dihydroxyphenylalanine per min at 30°C.

The monoclonal antibody against tyrosine 3-monooxygenase was prepared by the use of technology originally described by Köhler and Milstein (11). The monoclonal anti-tyrosine 3-monooxygenase antibody was purified by affinity chromatography on rabbit anti-mouse antibody-substituted Sepharose 4B essentially as described previously (12).

Staphylococcus aureus (Cowan I strain) was cultured as described by Goding (13). The cells were collected and fixed with formaldehyde essentially as described by Kessler (14), and stored as a 10% (wet wt./volume) suspension at -80°C.

Immunotitration was carried out by incubating the enzyme (0.25 unit) with varying amounts of purified monoclonal antibody in a solution containing 0.05% Tween 80, 0.1 mM EDTA, and 5 mM sodium phosphate, pH 7.5, in a final volume of 78 µl, for 20 min at 24°C. To the mixture was added 3 µl of rabbit anti-mouse immunoglobulin and the mixture was incubated for 20 min at 24°C. To the mixture were furthermore added 30 µl of a 10% suspension of *Staphylococcus* in 5 mM sodium phosphate, pH 7.5, containing 25% glycerol, 0.05% Tween 80, and 0.1 mM EDTA, and the mixture was incubated at 24°C for 30 min with shaking and centrifuged at 20,000 X *g* for 10 min. An aliquot of the supernatant was withdrawn and assayed for the enzyme activity.

Protein was determined by the method of Lowry *et al.* (15), as modified by Peterson (16), with bovine serum albumin as a standard.

RESULTS AND DISCUSSION

The pH fractionation in our earlier purification procedure of tyrosine 3-monooxygenase resulted in a recovery of the enzyme activity of 160-240% (9), suggesting the possibility that an

endogenous inhibitory factor may be separated from the enzyme by acid treatment. Immunotitration of crude and purified tyrosine 3-monooxygenase indicated that purification resulted in a remarkable increase in the enzyme activity assayed at a neutral pH but such a remarkable activation was not observed at an acidic pH (9). When the crude extract from rat striata, where tyrosine 3-monooxygenase is known to exist abundantly (17,18), was adjusted to pH 5.2 and the resulting precipitate was washed in acidic buffer, the enzyme activity at pH 7.0 appeared and increased markedly up to 2nd washing as shown in Fig. 1. The activity at pH 5.0, presumably reflecting the recovery of the enzyme protein, decreased with increasing the number of washing. Thus, as expected, an endogenous factor suppressing the activity of tyrosine 3-monooxygenase assayed at pH 7.0, appeared to be separated from tyrosine 3-monooxygenase by acid treatment. In order to confirm the existence of the endogenous factor in the acid-soluble fraction, whether or not the acid-soluble fraction can suppress the enzyme activity of the acid-precipitated fraction was

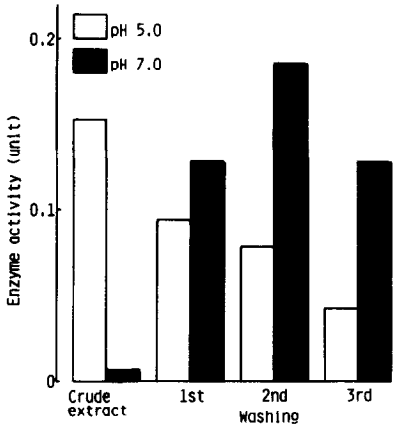
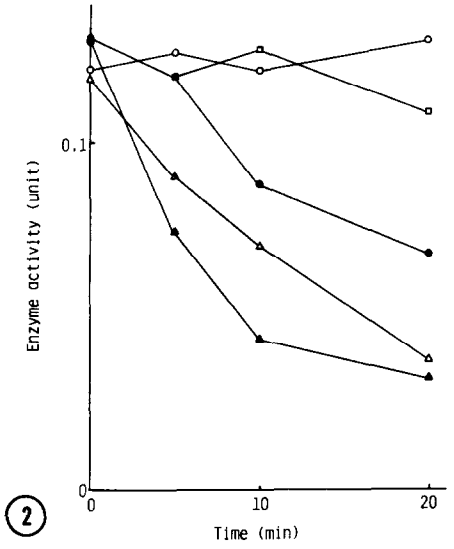


Fig. 1. Effect of acid treatment on the activity of tyrosine 3-monooxygenase. The pH of the crude extract from rat striata was adjusted to 5.2 with 0.1 M acetic acid. After standing for 15 min in ice, the resulting precipitate was collected by centrifugation for 10 min at 20,000 X *g*. The precipitate was washed three times in 1 mM Mes, pH 5.2 and the activity of tyrosine 3-monooxygenase of each precipitate from 1st, 2nd, and 3rd washing was determined at pH 5.0 and pH 7.0 essentially as described previously (10).



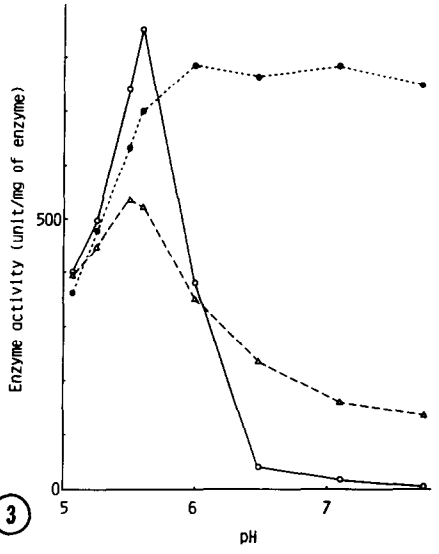


Fig. 2. Time course of inactivation of tyrosine 3-monooxygenase by incubation with varying amounts of the acid-soluble fraction. The pH of the crude extract from rat striata was adjusted to 5.2 with 0.1 M acetic acid. After standing for 15 min in ice, the acidified extract was centrifuged and the supernatant was neutralized with 0.5 M Na_2HPO_4 (acid-soluble fraction). The resultant precipitate was washed twice in 1 mM Mes, pH 5.2 and dissolved in one-fifth of the volume of the original crude extract (acid-precipitated fraction). The tyrosine 3-monooxygenase activity of 10 µl of the acid-precipitated fraction was measured after incubation at 30°C for the times indicated without (○) or with 25 µl (●), 50 µl (△) and 100 µl (▲) of the acid-soluble fraction or 50 µl (□) of boiled acid-soluble fraction.

Fig. 3. Effect of pH on the activity of tyrosine 3-monooxygenase. The enzyme activity of the crude extract (-○—○-), the acid-precipitated fraction (-●--●-) and the acid-precipitated fraction which had been preincubated for 20 min at 30°C with the acid-soluble fraction (100 µl to 10 µl of the acid-precipitated fraction) (-△—△-) was measured in 0.1 M Mes buffer. The results are expressed as the enzyme activity (unit) per mg of the enzyme protein. The amounts of the enzyme protein were estimated by immunotitration with the monoclonal antibody against the enzyme.

examined as shown in Fig. 2. Incubation of the acid-precipitated fraction with the acid-soluble fraction at 30°C resulted in a decrease in the enzyme activity assayed at pH 7.0. The inactivation occurred relatively slowly and it increased as the amounts of the acid-soluble fraction increased. Boiling of the acid-soluble fraction for 5 min caused an almost complete loss of the inhibitory activity. Fig. 3 shows the pH profiles for the tyrosine 3-monooxygenase activity of the crude extract, the pH-

precipitated fraction and the pH-precipitated fraction which was preincubated with the pH-soluble fraction for 20 min at 30°C. The enzyme activity of the crude extract exhibited a relatively sharp pH optimum at pH 5.6, in agreement with our earlier observation (17), but the activity of the acid-precipitated fraction showed a broad pH optimum over the pH range of 6.0-7.5, the pH profile of which is very similar to that of the activity of the purified enzyme (9). When the acid-precipitated fraction was incubated with the acid-soluble fraction, the enzyme activity was markedly decreased at pH values around neutrality and showed a pH optimum at pH 5.5 again. The results, taken together, indicate that the endogenous factor, which is separable from tyrosine 3-monooxygenase by acid treatment, may almost completely suppress the tyrosine 3-monooxygenase activity at a neutral pH in the crude extract from rat striata. Since the intracellular pH of rat brain has been reported to be 7.0-7.1 (19,20), it is conceivable that the endogenous factor may play its physiologically important role in the regulation of catecholamine biosynthesis in brain tissues.

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