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Regulation of human tyrosine hydroxylase activity

Effects of cyclic AMP-dependent protein kinase, calmodulin-dependent protein kinase II and polyanion

Sachiko Okuno, Yoshiharu Kanayama* and Hitoshi Fujisawa

Department of Biochemistry, Asahikawa Medical College, Asahikawa 078 and **Department of Internal Medicine, Osaka City University, School of Medicine, Osaka 545, Japan*

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To determine the regulatory mechanism for human tyrosine hydroxylase, we examined modulations of the activity of the enzyme from human pheochromocytoma by cyclic AMP-dependent protein kinase, calmodulin-dependent protein kinase II and polyanion. The most remarkable activation was observed when the enzyme was assayed at physiological pH (pH 7) after being subjected to phosphorylation by cyclic AMP-dependent protein kinase. Calmodulin-dependent protein kinase II and polyanion also modulated the enzyme activity. The results suggest that tyrosine hydroxylase may be regulated similarly in both human and rat.

Tyrosine hydroxylase; Protein kinase, cyclic AMP-dependent; Protein kinase II, calmodulin-dependent; Polyanion; (Human pheochromocytoma)

1. INTRODUCTION

Tyrosine hydroxylase (L-tyrosine, tetrahydropteridine:oxygen oxidoreductase (3-hydroxylating), EC 1.14.16.2) catalyzes the conversion of L-tyrosine to L-dopa, which is the initial and rate-limiting step in the biosynthesis of catecholamines in the nervous system [1,2] and, therefore, the regulation of its activity is particularly important. The regulatory mechanism for the rat or bovine enzyme has so far been extensively studied and a variety of mechanisms, including end-product feedback inhibition [1,3], inactivation by end-products [4], modulation by polyanions [5] or an anionic detergent such as SDS [6], and activation by protein kinases such as cAMP-dependent protein kinase [7–10] and calmodulin-dependent protein kinase II [11,12] have been demonstrated to modulate the enzyme activity reversibly.

In contrast to the rat or bovine enzyme, the human enzyme has recently been reported to be not significantly activated by cAMP-dependent protein kinase and calmodulin-dependent protein kinase II [13]. Thus, the question has been raised whether a regulatory mechanism similar to that for rat or bovine tyrosine hydroxylase can exert its effect on the activity of human tyrosine hydroxylase. In the present study, effects of cAMP-dependent protein kinase, calmodulin-dependent protein kinase II and polyanion on the activity of tyrosine hydroxylase from human pheochromocytoma have been examined and compared with those on the activity of the enzyme from rat pheochromocytoma cell, PC12. The results demonstrate for the first time that human tyrosine hydroxylase may be regulated by a mechanism similar to that of the rat enzyme.

Correspondence address: H. Fujisawa, Department of Biochemistry, Asahikawa Medical College, Asahikawa 078, Japan

Abbreviations: dopa, 3,4-dihydroxyphenylalanine; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

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2. MATERIALS AND METHODS

L-[1-^{14}C]Tyrosine (52 mCi/mmol) was purchased from Amersham. Catalase from bovine liver, phospho*enol*pyruvate and pyruvate kinase from rabbit muscle were from Boehringer. Dithiothreitol, *p*-chloromercuribenzenesulfonic acid, dexamethasone acetate, EGTA, Mes, Hepes and ATP were from Sigma. 6*R*-L-*erythro*-5,6,7,8-Tetrahydrobiopterin, a product of Suntory Biomedical Institute, Osaka, was a generous gift of Dr Osamu Hayaishi, Osaka Bioscience Institute, Osaka. Dextran sulfate was from Pharmacia. The clonal PC12 rat pheochromocytoma cell line [14] was kindly donated by Dr Takehiko Amano, Mitsubishi-Kasei Institute of Life Sciences, Tokyo. RPMI 1640 medium was from Nissui, Japan. Fetal calf serum and horse serum were from Gibco. Bio-Gel P-10 was from BioRad. Leupeptin, chymostatin and pepstatin were from Peptide Institute, Osaka.

The catalytic subunit of cAMP-dependent protein kinase was purified from bovine heart essentially according to the method of Sugden et al. [15] up to the second hydroxyapatite step.

Calmodulin-dependent protein kinase II [16] and activator protein [17] were purified from rat cerebral cortex as described previously.

Calmodulin was purified from rat testis by the method of Dedman et al. [18].

PC12 cells were cultured in RPMI medium supplemented with 5% fetal calf serum and 10% horse serum at 37°C. Dexamethasone (1 μM) was added to the medium 2 days before harvest to enhance the yield of tyrosine hydroxylase [19]. The harvested cells were stored at −80°C until use.

Frozen human pheochromocytoma (about 30 mg) and frozen PC12 cells (1.3 g) were homogenized in 0.3 ml and 4.4 ml, respectively, of 5 mM sodium phosphate buffer (pH 7.5) containing 20 μg/ml each of leupeptin, chymostatin and pepstatin in a Potter-Elvehjem homogenizer and each supernatant, obtained by centrifugation for 60 min at 100000 × *g*, was passed through a Bio-Gel P-10 column in 5 mM sodium phosphate buffer (pH 7.5) to remove low molecular mass substances. The resulting crude extracts were stored in aliquots at −80°C until use.

Tyrosine hydroxylase was assayed radiometrically based on $^{14}CO_2$ evolution through coupled non-enzymatic decarboxylation of L-[1-^{14}C]dopa formed from L-[1-^{14}C]tyrosine according to the method described previously [4,20], except that Mes (pH 5.0–7.0) and Hepes (pH 7.0–7.5) in place of Hepes (pH 7.0), 1 mM 6*R*-L-*erythro*-5,6,7,8-tetrahydrobiopterin in place of 1 mM 2-amino-4-hydroxy-6-methyl-5,6,7,8-tetrahydropteridine, and 10 μM tyrosine in the place of 200 μM tyrosine were used.

When the enzyme activity was measured after exposure to enzymatic phosphorylating conditions, the incubation mixture for the phosphorylation by cAMP-dependent protein kinase contained 5 mM sodium phosphate buffer (pH 7.5), 0.1 mM EGTA, 3 mM phospho*enol*pyruvate (pH 7), 10 μg of pyruvate kinase, 0.1 mM ATP, 5 mM magnesium acetate, 4.3 μg of the catalytic subunit of cAMP-dependent protein kinase and suitable amounts of the crude extract from the human pheochromocytoma or the crude extract from the PC12 cells, in a final volume of 100 μl. For the incubation mixture for the reaction of calmodulin-dependent protein kinase II, the catalytic subunit was replaced by 1.05 μg of calmodulin-dependent protein kinase II, 3.5 μg of calmodulin, 4.5 μg of activator protein and 0.12 mM $CaCl_2$. After incubation for 3 min at 30°C, tyrosine hydroxylase activity was measured as described above. One unit of the activity of tyrosine hydroxylase was defined as the amount of enzyme that catalyzed the formation of 1 nmol of dopa/min at 30°C.

3. RESULTS AND DISCUSSION

Effects of cAMP-dependent protein kinase, calmodulin-dependent protein kinase II, or polyanion on the activity of human tyrosine hydroxylase were examined and compared with those on the activity of the rat enzyme as shown in fig.1. Since only about 30 mg of human pheochromocytoma was available for the experiment, the assay conditions were chosen so that the sensitivity of the assay was as high as possible. cAMP-dependent protein kinase markedly activated the human enzyme as well as the rat enzyme, and the activated enzymes both exhibit pH optima at the physiological pH (pH 7). Calmodulin-dependent protein kinase II did not cause an appreciable increase in the activity measured at a neutral pH but it caused an increase in the activity measured at an acidic pH of both the human and rat enzymes. A polyanion such as dextran sulfate caused an increase in the activity measured over the pH range 6–7 and a decrease in the activity measured at an acidic pH of both the enzymes. Thus, the modes of the modulations of the human and rat enzymes by cAMP-dependent protein kinase, calmodulin-dependent protein kinase II and polyanion appeared to be essentially identical. The pH profile of the enzyme activity of the crude extract from human pheochromocytoma was apparently distinct from that of the crude extract from PC12 cells. Since treatment of the enzyme with trypsin [21,22] or Ca^{2+}-dependent neutral protease, calpain [23], was reported to result in an increase in the activity measured at a neutral pH, the apparent difference may be due to partial proteolysis in the human sample.

From the results obtained from the in vitro studies with rat tyrosine hydroxylase, we have proposed a molecular mechanism for the regulation of tyrosine hydroxylase that calmodulin-dependent protein kinase II is involved in the depolarization-induced activation and cAMP-dependent protein kinase is involved in the autoreceptor-mediated activation of tyrosine hydroxylase in the nerve ter-

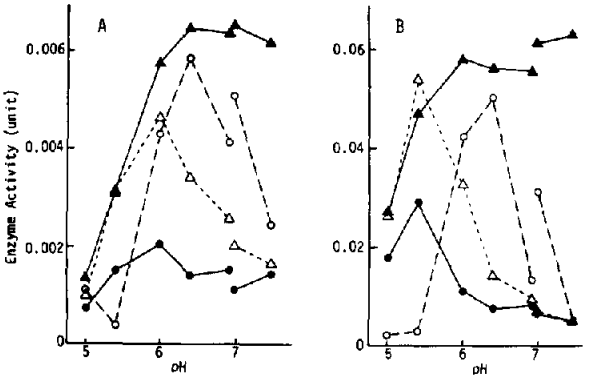

Fig.1. Effects of cAMP-dependent protein kinase, calmodulin-dependent protein kinase II and polyanion on pH profile of the activity of human and rat tyrosine hydroxylase. Tyrosine hydroxylase activity of the crude extract from human pheochromocytoma (A) and rat PC12 cells (B) was measured as described in section 2 in the absence (●) or presence (○) of 100 μg/ml dextran sulfate, after activation by cAMP-dependent protein kinase (▲), and after activation by calmodulin-dependent protein kinase II (△).

minal [24,25]. Recent studies conducted in rat striatal slices suggest the possible involvement of calmodulin-dependent protein kinase II in the depolarization-induced activation of tyrosine hydroxylase [26–28], providing strong support for our proposed mechanism. Richtand et al. [29] have reported that heparin is effective in stabilizing tyrosine hydroxylase and accelerates the phosphorylation of the enzyme by cAMP-dependent protein kinase and further suggested that polyanions may act as a physiological effector of tyrosine hydroxylase in concert with cAMP-dependent protein kinase. The present study suggests that human tyrosine hydroxylase also may be regulated by the regulatory mechanism similar to that for rat tyrosine hydroxylase.

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