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Review

Regulatory mechanism of tyrosine hydroxylase activity

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Abstract

Activity of tyrosine hydroxylase is regulated by feedback inhibition and inactivation by catecholamines, and activation by protein phosphorylation. In this article, reaction mechanisms for the conversion of tyrosine hydroxylase to an inactive/stable form by catecholamines, and activation of tyrosine hydroxylase by phosphorylation at Ser-40 are discussed. Inactivation may be induced by sub-stoichiometric amounts of catecholamines, and activation by phosphorylation of Ser-4 0 may require phosphorylation of three or all four subunits of a tyrosine hydroxylase molecule. Cooperative phosphorylation at Ser-40 in the subunits is also discussed.

Keywords: Tyrosine hydroxylase; Enzyme activity regulation; Protein phosphorylation; 14-3-3 protein; cAMP-dependent protein kinase; Ca^{2+}/calmodulin-dependent protein kinase; Catecholamine; Brain; Oxygenase; Cooperative phosphorylation

Tyrosine hydroxylase (TH) [L-tyrosine, tetrahydropteridine:oxygen oxidoreductase (3-hydroxylating), EC 1.14.16.2] catalyzes the conversion of L-tyrosine to DOPA, which is the initial and rate-limiting step in the biosynthesis of catecholamines such as dopamine, norepinephrine, and epinephrine [1,2]. Therefore, regulation of its activity is very important. Activity of TH can be regulated by two mechanisms: short-term direct regulation of enzyme activity and medium- to long-term regulation of gene expression [3]. In this article, mechanisms of the former direct regulation of TH are discussed largely on the basis of results obtained from in vitro studies in our laboratory.

TH activity is modulated by feedback inhibition by catecholamines, allosteric activation by heparin, phospholipids, polyanions, and RNA, and activation by protein phosphorylation (reviewed in [3–5]). Although polyanionic allosteric effects provide important experimental tools for characterizing the regulatory structure of TH, polyanions probably do not exert dominant regulatory effects in vivo [3]. Modulation of TH activity by catecholamines occurs through two different mechanisms: one involves the well-defined inhibition of TH by catecholamines being competitive with pterin cofactor [6,7], and the other involves inactivation of TH by catecholamines [8,9]. It has recently been proposed that inactivation occurs as a result of binding of catecholamines to Fe^{3+} in the active site of the enzyme, and that the catecholamines are bound to the inactive form of the enzyme [3–5], in contrast with our proposal that the catecholamines which have served to inactivate TH are not bound to the inactivated form of the enzyme [10]. In the first section of this article, we shall focus on inactivation of TH by catecholamines.

It has been reported that TH is phosphorylated at Ser-8, Ser-19, Ser-31, Ser-40, Ser-153, and Ser-404 by a variety of protein kinases [3–5]. Among them, phosphorylation of Ser-40 by cyclic AMP-dependent protein kinase (PKA) causes the most prominent and dramatic activation of TH [3]. Activation of TH phosphorylated at Ser-19 by another multifunctional protein kinase, Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II), requires the presence of 14-3-3 activator protein [3,4]. The second section of this article will focus on the phosphorylation of TH at Ser-40 and Ser-19.

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End-product feedback inhibition

TH activity is suppressed by end products, catecholamines, in two different ways, direct inhibition being competitive with pterin cofactor and inactivation. The former involves classic kinetic-mediated, readily reversible inhibition of the enzyme that acts as a sensor for local concentrations of catecholamines [3]. On the other hand, inactivation of TH by catecholamines is one of the principal mechanisms by which TH usually exists in an inactive form exhibiting no activity at physiological pH in the brain [8,11].

TH activity of a rat brain extract shows an acid pH optimum with a maximum at pH 5.4 but no activity at neutral pH, as shown in Fig. 1. Since intracellular pH of rat brain is 7.0–7.1 [12,13], TH usually has no activity under physiological conditions in the brain. When the enzyme is precipitated at pH 5 and separated from the acid-soluble fraction, high TH activity appears at pH values around 7, although activity at pH 5 is not altered.

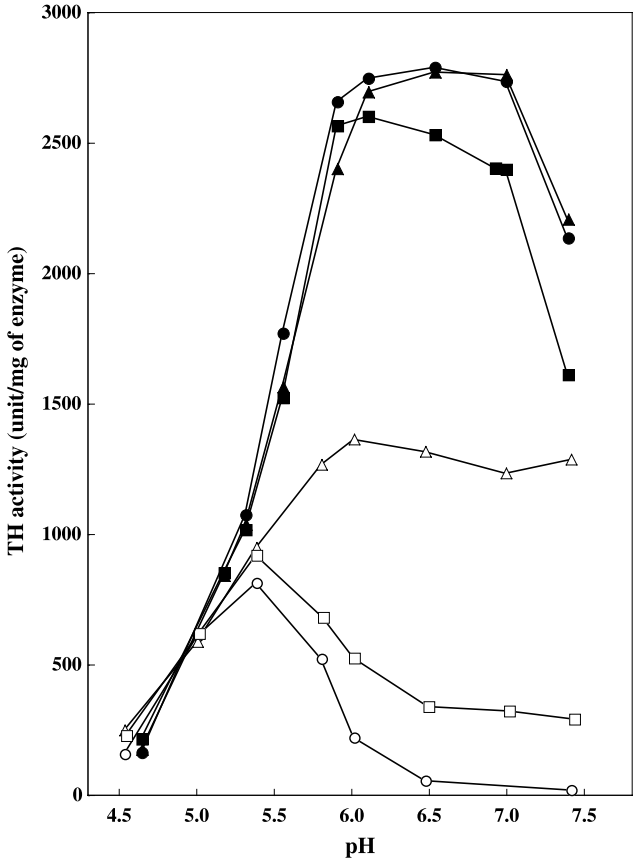

Fig. 1. Effects of pH on activity of tyrosine hydroxylase. TH activity of a rat brain extract (○, ●), the acid (pH 5)-precipitated fraction of the extract (△, ▲), and the acid-precipitated fraction which had been incubated with dopamine (□, ■) were measured at various pH values before (○, △, □) and after (●, ▲, ■) activation by PKA. Results are expressed as enzyme activity per milligram of enzyme protein, estimated by immunotitration. Details of the procedures are given in [8].

When the "acid-precipitated fraction" is incubated with the "acid-soluble fraction" or catecholamines such as dopamine, enzyme activity at neutral pH is markedly decreased and reveals pH optimum at 5.4, again. Several lines of evidence indicate that the endogenous factor converting TH from an active form to an inactive form contained in the "acid-soluble fraction" of the brain is a catecholamine such as dopamine since: (1) the endogenous factor is dialyzable, (2) it is particularly abundant in the striatum and adrenal medulla, (3) it is adsorbed by alumina, (4) its activity is labile to alkaline pH, (5) it can be replaced by catecholamines such as dopamine, norepinephrine, and epinephrine for inactivating TH, (6) pH profile of activity of TH inactivated by the "acid-soluble fraction" is very similar to that of the enzyme inactivated by dopamine, and (7) HPLC analysis showed that the "acid-soluble fraction" contained reasonable amounts of dopamine. Thus, TH usually exists as an inactive form in the brain, showing no activity at physiological pH, which has been produced by the action of catecholamines, and inactivation can be eliminated by acid treatment. Inactivation of TH by catecholamines and recovery of activity by acid treatment can be repeated with no significant loss of activity [10]. Both the inactive form of TH produced by catecholamines and the form whose catecholamine inactivation has been eliminated by acid treatment are remarkably activated by PKA, and show similar high activities at physiological pH values, as shown in Fig. 1. Of interest, catecholamines not only inactivate but also stabilize TH. When purified TH was incubated in the presence or absence of dopamine at 30 °C, a time-dependent loss of activity measured at pH 5, where both the active and inactive forms of the enzyme showed similar activities as described above (Fig. 1), was markedly prevented in the presence of dopamine [10,14]. Thus, TH is converted to an inactive/stable form by its end products.

We investigated the nature of the inactivation reaction of TH by dopamine and found that: (1) it was a time- and temperature-dependent reaction; (2) it was reversible; (3) it occurred under anaerobic conditions as well as under aerobic conditions; (4) dopamine was not consumed during the reaction; (5) dopamine was not bound to the inactive form of the enzyme [15]. When TH was incubated with dopamine in equimolar amounts with the subunit of the enzyme, and subjected to gel filtration on Bio-Gel P-10, enzyme activity was eluted in the void volume but only the same amount of dopamine (15% of radioactivity of added dopamine) as that eluted in a control experiment carried out without TH was eluted, as shown in Fig. 2, indicating that added dopamine was not bound to the enzyme. Dopamine eluted in the void volume may be nonspecifically bound to bovine serum albumin that is added for stabilization of the enzyme. Recovery of

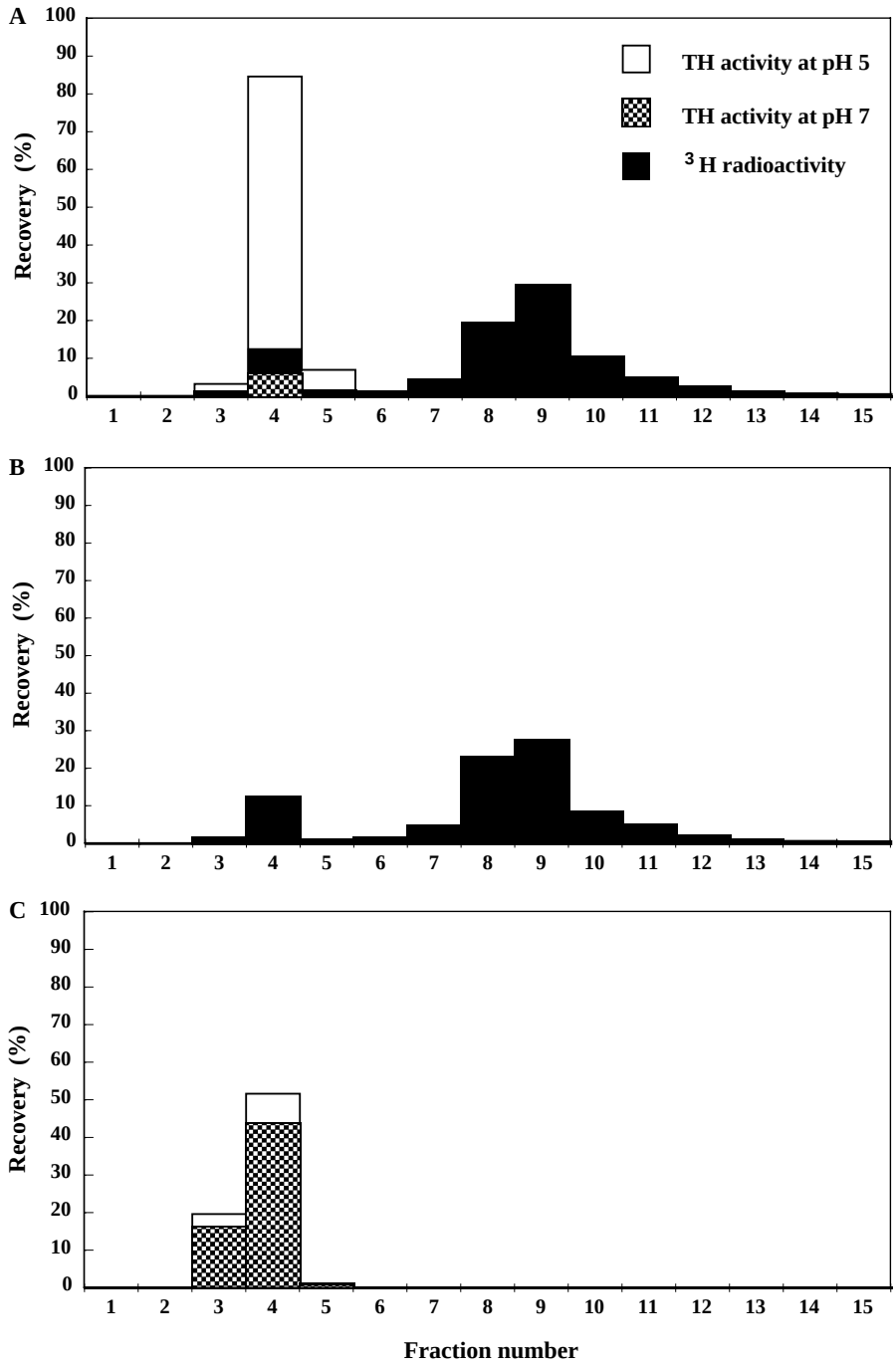

Fig. 2. Gel filtration of tyrosine hydroxylase after inactivation with [^{3}H]dopamine. Purified TH (4.0 μg, 68 pmol of subunit of the enzyme) was inactivated by incubation with (A) or without (C) 69 pmol of [^{3}H]dopamine in the presence of 1 mg/ml bovine serum albumin added for stabilization of TH and then subjected to gel filtration on Bio-Gel P-10. TH activities at pH 5 (□) and at pH 7 (▣), and ^{3}H radioactivity (■) of each fraction were measured. A control experiment was done without TH (B). Details of the procedures are given in [10,14].

TH activity at pH 5 was 94 % but that at pH 7 was only 7 % (Fig. 2A), indicating that most of the enzyme in the mixture was converted to the inactive form. In contrast, in the control experiment performed without dopamine (Fig. 2C), recoveries of activity at pH 5 and 7 were 72% and 64%, respectively, indicating that most of the enzyme remained unchanged. The enzyme only lost 6% of its activity at pH 5 after incubation with dopamine, but lost 28% without dopamine, consistent with the contention that dopamine serves to stabilize the enzyme. Dopamine added to the mixture was completely recovered as dopamine after incubation with TH [10,14]. On the basis of studies described herein, we propose that dopamine converts TH to an inactive/stable form, acting as a kind of catalyst as shown in Fig. 3 [10,14].

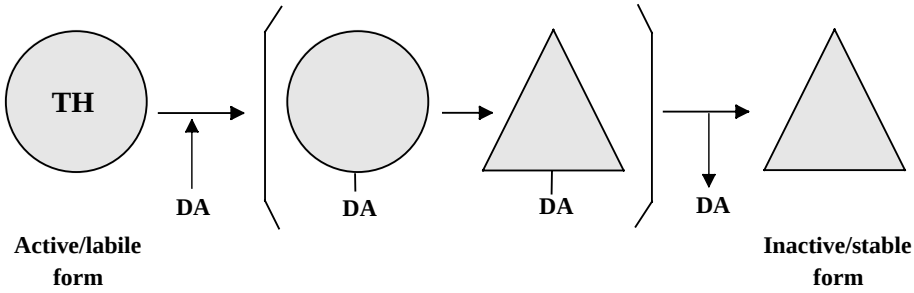

Fig. 3. Proposed mechanism for conversion of tyrosine hydroxylase to its inactive/stable form by dopamine (DA).

In contrast to our proposal that TH inactivated by incubation with catecholamines does not retain catecholamines added to the inactivation mixture [14], it is generally accepted that dopamine tightly binds to ferric iron in the active site of TH to produce an inactive "blue-green" form [9] of the enzyme [3–5]. However, observations showing that inactive form of TH produced by incubation with equimolar amounts of [^{3}H]dopamine binds no significant amount of [^{3}H]dopamine as shown in Fig. 2, and that [^{3}H]dopamine binds to TH with very low stoichiometry (0.08 mol/mol subunit of the enzyme) in the binding assay [16] suggest that the dopamine involved in inactivation of TH is not bound to the enzyme. On the other hand, the facts that our TH preparation purified from rat adrenal medulla contained 0.03 mol of norepinephrine, 0.10 mol of epinephrine, and 0.08 mol of dopamine/mol subunit of the enzyme (a total of 0.21 mol of catecholamines/mol subunit of the enzyme), and that TH preparations purified from bovine adrenal medulla contain 0.11 mol of norepinephrine and 0.25 mol of epinephrine/mol subunit of the enzyme (a total of 0.36 mol of catecholamine/mol subunit of the enzyme) [9] suggest that significant amounts of catecholamines are tightly bound to the enzyme. Since rat TH is a homotetramer composed of identical subunits [17], and each polypeptide possesses an active site [3–5], the number of tightly bound catecholamines is much fewer than that of the active site of the enzyme. Although roles of the tightly bound catecholamines in regulation of TH activity are still not clear, it is unlikely that they be involved in the inactivation of TH by catecholamines as described above. Of interest, Kaufman and his co-workers [16] speculated that only one of the monomers in the tetrameric enzyme binds dopamine with high affinity, either because of the conformation of the tetramer or because of negative cooperativity. Cooperative phosphorylation of the subunits in TH is discussed in the next section.

Activation by protein phosphorylation

TH is known to be activated via phosphorylation at Ser-40 by PKA, but TH is also phosphorylated at Ser-40 by a number of protein kinases such as PKC, CaM kinase II, cyclic GMP-dependent protein kinase, MAPK-activated protein kinases, p38-regulated/activated kinase, and mitogen- and stress-activated protein kinase [5]. Interestingly, both PKA and PKC can phosphorylate TH only at Ser-40 (Table 1) [18], but TH is markedly activated by PKA but not activated by PKC at all. As given in Table 1, maximal extents of phosphorylation of TH by PKA, PKC, and CaM kinase II are approximately 0.78, 0.43, and 1.19 mol of phosphate/mol of subunit of the enzyme, and PKA and PKC among the three kinases exclusively phosphorylate Ser-40 of the enzyme, although CaM kinase II phosphorylates both Ser-40 and Ser-19 at a phosphorylation ratio of Ser-40 to Ser-19 of approximately 0.5. These results indicate that PKA can phosphorylate all subunits of TH at Ser-40, but PKC and CaM kinase II can phosphorylate only two of four subunits at Ser-40, although CaM kinase II can phosphorylate all subunits at Ser-19. Thus, phosphorylation at Ser-40 of two subunits in a TH molecule completely blocks phosphorylation at Ser-40 of the other two subunits by PKC or CaM kinase II, indicating the presence of negative cooperativity of phosphorylation of Ser-40 of the subunit of TH by the two protein kinases.

Since phosphorylation of TH at Ser-40 by PKA results in marked activation of the enzyme, but phosphorylation of two of the four subunits at Ser-40 by PKC or CaM kinase II causes no activation [18], phosphorylation of the third or fourth subunit at Ser-40 may be involved in the activation. Fig. 4 shows the relationship between phosphorylation of TH at Ser-40 and its activation by PKA and PKC. When phosphorylation of TH by PKA is plotted against the resulting

Table 1
Maximal phosphorylation of TH by PKA, PKC, and CaM kinase II

Phosphorylation site	mol/mol subunit of the enzyme		
	PKA	PKC	CaM kinase II
Ser-40	0.78	0.43	0.43
Ser-19	NS[a]	NS	0.76
Others	NS	NS	NS

Details of the experimental procedures are given in [18].
[a] NS, not significant.

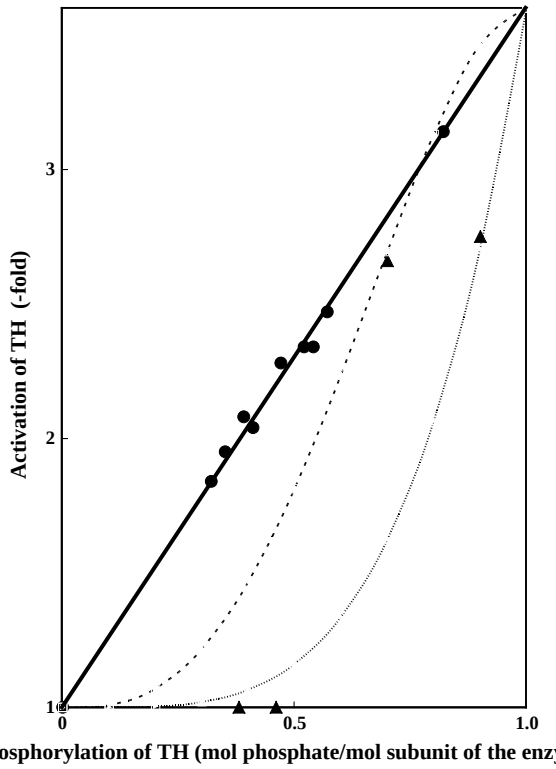

Fig. 4. Relationship between phosphorylation of Ser-40 and activation of tyrosine hydroxylase. TH was phosphorylated by PKA for an appropriate period, and the phosphorylation and activation of the enzyme were measured (●, a solid line). TH was maximally phosphorylated by PKC, then phosphorylated by PKA, and phosphorylation and activation of the enzyme were measured (▲, a dash line). Two theoretical curves obtained on the basis of the assumptions that any subunit of TH is similarly susceptible to phosphorylation by PKA, and that phosphorylation of all four subunits of the enzyme molecule causes enzyme activation (given by p^4, where p represents the proportion of phosphorylated subunit of TH) (a dotted line), or that phosphorylation of three subunits causes activation (given by $4p^3(1-p)-p^4$) (a short dash line) are shown. Details of the procedures are given in [18].

activation of the enzyme, an approximately linear relationship is obtained, as shown by the solid line in Fig. 4. The fact that PKC and CaM kinase II phosphorylate two of the four subunits of TH but produce no activation of TH indicates that activation of TH by PKA is caused by phosphorylation of three subunits or all four subunits of the enzyme molecule. If all subunits were equivalent in undergoing phosphorylation by PKA, relationship between phosphorylation and activation would give a sigmoid curve as shown by the short dash line in Fig. 4, when phosphorylation of three of the four subunits causes activation; or would give a curve shown by the dotted line in Fig. 4, when phosphorylation of all four subunits causes activation. Thus, linear relationship between phosphorylation and activation of TH by PKA indicates that phosphorylation of one subunit of TH may make the other subunits susceptible to phosphorylation by PKA, suggesting the presence of positive cooperativity of phosphorylation of Ser-40 of the subunit of TH by PKA, in contrast to the negative cooperativity of the phosphorylation by PKC and CaM kinase II. When TH which has been maximally phosphorylated by PKC is further phosphorylated by PKA, the relationship between phosphorylation and activation appears to produce a straight line (the long dash line) with a slope two times greater than that obtained without PKC (the solid line), suggesting that PKC (and also CaM kinase II) can indirectly contribute to activation by helping the action of PKA, although it cannot directly activate TH by phosphorylation of Ser-40.

As shown in Table 1, CaM kinase II phosphorylates Ser-40 of two of the four subunits and Ser-19 of all four subunits of TH without significant activation. However, phosphorylated TH is activated by addition of 14-3-3 protein [19–21]. It is well accepted that 14-3-3 protein regulates diverse cellular processes by interaction with a variety of phosphoproteins [22]. Activations of TH and tryptophan hydroxylase phosphorylated by CaM kinase II by 14-3-3 protein [19,20] are the first demonstrations of the function of 14-3-3 protein as a

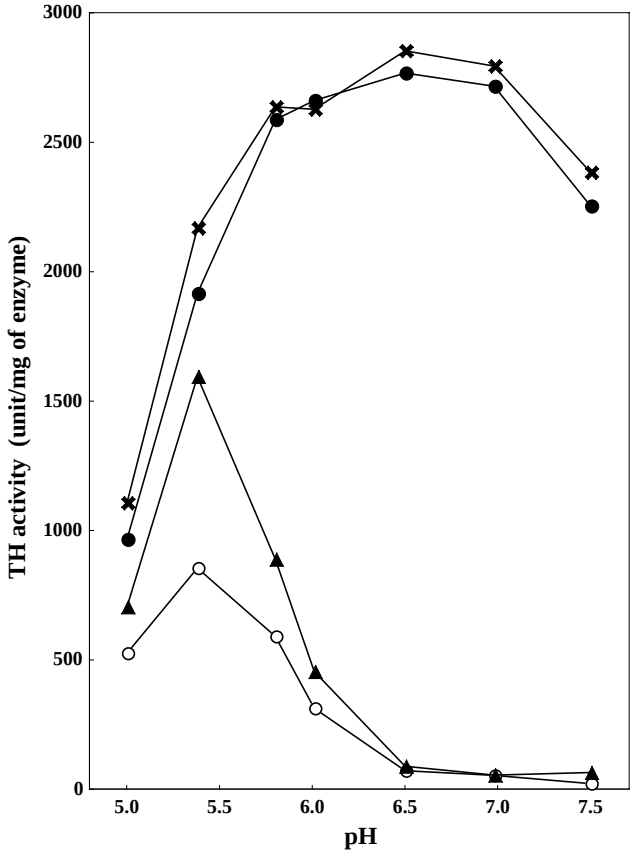

Fig. 5. Effects of PKA and CaM kinase II on pH profile of activity of tyrosine hydroxylase. TH activity of a rat brain extract was measured at various pH values without treatment (○), after activation by PKA (●) or CaM kinase II (▲), and after activation by both PKA and CaM kinase II (×). Details of the procedures are given in [15].

phosphoprotein modulator. Since the enzyme which has been phosphorylated by PKC is not activated by 14-3-3 protein, this activation of TH is thought to result from phosphorylation of Ser-19 followed by binding of 14-3-3 protein to the phosphorylated TH. Fig. 5 shows pH dependency of activity of TH which has been activated by PKA, by CaM kinase II and 14-3-3 protein, or both. Unlike PKA, which strongly stimulates the activity of TH at physiological pH, CaM kinase II (together with 14-3-3 protein) shows appreciable stimulatory effects on enzyme activity measured at an acidic pH but no significant effect on enzyme activity at physiological pH, suggesting that activation of TH by CaM kinase II together with 14-3-3 protein does not exert dominant regulatory effects.

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