Evidence for the involvement of a cyclic AMP-independent protein kinase in the activation of soluble tyrosine hydroxylase from rat striatum*

(tyrosine monooxygenase/enzyme phosphorylation/kinetics)

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ABSTRACT Activation of rat striatal tyrosine hydroxylase [TyrOHase; tyrosine monooxygenase; L-tyrosine, tetrahydropteridine:oxygen oxidoreductase (3-hydroxylating), EC 1.14.16.2] by ATP/Mg²⁺ and endogenous protein kinase can be produced without the addition of cAMP. This activation is not due to endogenous free catalytic subunit derived from cAMP-dependent protein kinase. In the presence of amounts of protein kinase inhibitor sufficient for complete inhibition of striatal cAMP-dependent protein kinase and the cAMP-mediated activation of TyrOHase, addition of ATP/Mg²⁺ results in an enhancement of TyrOHase activity. Enzyme activation does not occur when the nonhydrolyzable form of ATP, adenylyl imidodiphosphate, is substituted for ATP. When TyrOHase is assayed in the presence of ATP/Mg²⁺ and different concentrations of either tyrosine or 6-methyltetrahydropterin cofactor, a 2-fold increase in enzyme $V_{\max}$ is demonstrable, with no change in the K_m for either substrate or cofactor. In contrast, in the presence of cAMP and ATP/Mg²⁺, both an increase in $V_{\max}$ and an enhanced affinity for pterin cofactor are demonstrable. In the latter circumstance, the 2-fold increase in $V_{\max}$ can be attributed entirely to the action of cAMP-independent protein kinase. The addition of either EGTA or CaCl₂ does not modify the effect seen in the presence of ATP, suggesting that the effect of ATP/Mg²⁺ is not mediated by a Ca²⁺-dependent protein kinase. These data support the existence of a cAMP-independent striatal protein kinase that can catalyze the activation of TyrOHase.

In striatum, as in peripheral adrenergic tissues, the enzyme tyrosine hydroxylase [TyrOHase; tyrosine monooxygenase; L-tyrosine, tetrahydropteridine:oxygen oxidoreductase (3-hydroxylating), EC 1.14.16.2] catalyzes the first and rate-limiting step in the biosynthesis of the neurotransmitter dopamine (1–4). cAMP and analogs of this cyclic nucleotide are able to increase the activity of the enzyme in crude tissue preparations in the presence of added ATP and Mg²⁺ (ATP/Mg²⁺) (5–11), and this activation is associated with either an increased affinity of enzyme for cofactor (5, 7, 11, 12) or a reduction of feedback inhibition by dopamine or norepinephrine (7, 13). Presumably, cAMP activates cAMP-dependent protein kinase, releasing free catalytic subunit which in turn activates TyrOHase. In recent studies (14–18) it has been shown that the free catalytic subunit of cAMP-dependent protein kinase activates TyrOHase by directly phosphorylating the enzyme.

In many of the earlier studies on the activation of TyrOHase by protein kinase, crude tissue extracts were used, and the constituents of the system responsible for the activation of the enzyme in the presence of cAMP and ATP/Mg²⁺ were not rigorously defined. In a number of studies (7–10), activation of TyrOHase by ATP/Mg²⁺ alone has been reported, and it had

been assumed that this activation was attributable to the presence of free catalytic subunit of cAMP-dependent protein kinase, thus accounting for the lack of an absolute requirement for cAMP to produce this effect.

In the present study, using purified heat-stable protein kinase inhibitor, we have attempted to demonstrate TyrOHase activation in crude striatal extracts when the cAMP-dependent protein kinase is completely inhibited. Under these conditions, TyrOHase is activated significantly and this activation is dependent on the presence of ATP/Mg²⁺. Furthermore, the kinetics of this activation differ from those seen with cAMP-mediated activation of the enzyme. These results support the existence of a cAMP-independent protein kinase in striatal tissue which is capable of activating TyrOHase.

MATERIALS

Materials were obtained as follows: L-tyrosine, 2-(N-morpholino)ethanesulfonic acid (Mes), N-[tris (hydroxymethyl)methyl]-aminoethanesulfonic acid (Tes), ATP, cAMP, NADPH, histone 2- α , dithiothreitol, and adenylyl imidodiphosphate (p[NH]ppA) from Sigma; 2-amino-4-hydroxy-6-methyl-5,6,7,8-tetrahydropteridine (6-MeH₄Pter) from Calbiochem; [γ -³²P]ATP tetra-(triethylammonium) salt (10–40 Ci/mmol; 1 Ci = 3.7×10^{10} Bq), and L-[3,5-³H]tyrosine (40–60 Ci/mmol) from New England Nuclear. cAMP-dependent protein kinase (HK1), and the two cAMP-independent kinases, growth-associated histone kinase and histone kinase 2 (HK₂), and calf thymus histone H1 were prepared according to Langan (19).

METHODS

Tissue Preparation. Male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 150–250 g were stunned by a blow to the head and decapitated, and the brains were removed. Striata were dissected according to the procedure of Glowinski and Iversen (20) and frozen at –70°C until assayed. Tissue was homogenized in 5 vol of 0.32 M sucrose/20 mM Tris/0.2% Triton X-100, pH 6.2. The homogenate was centrifuged at 20,000 $\times g$ for 20 min at 4°C, and the supernatant served as the enzyme source.

Purification of Protein Kinase Inhibitor. Protein kinase inhibitor was prepared from whole bovine brain through the

Abbreviations: TyrOHase, tyrosine hydroxylase; 6-MeH₄Pter, 2-amino-4-hydroxy-6-methyl-5,6,7,8-tetrahydropteridine; p[NH]ppA, 5'-adenylyl imidodiphosphate.

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phosphocellulose step according to the procedure of Demaille *et al.* (21). The peak fractions were pooled, dialyzed against distilled water overnight, lyophilized, and stored at -20°C .

Protein Kinase Assay. Supernatant enzyme (25 μl) was added to the incubation system which contained, in a final volume of 100 μl (pH 6.2): 75 mM Mes, 12 mM potassium phosphate, 154 μg of dithiothreitol, 5 mM NaF, 60 μM EGTA, 800 μM theophylline, 5 mM magnesium acetate, 100 μg of histone, and 800 μM [γ - ^{32}P]ATP (0.25 μCi per tube). Test agents included 125 μM cAMP and various concentrations of protein kinase inhibitor. Values for protein kinase blanks were obtained by using tubes lacking histone substrate and containing amounts of protein kinase inhibitor sufficient for full inhibition of cAMP-dependent histone kinase. The reaction was initiated by the addition of enzyme to the mixture and was allowed to proceed for 5 min at 37°C , after which it was terminated by the addition of 2.25 ml 28% (wt/vol) trichloroacetic acid. Samples were allowed to stand for 15 min and then mixed on a Vortex shaker, and the contents of each tube were filtered separately through type HAWP Millipore filters to trap phosphorylated histone. Filters were dried under a heat lamp, and assayed for radioactivity in 4 ml of an Omnifluor scintillation cocktail. Counting efficiency was 85–90%.

Protein Phosphorylation Reaction. The activation of TyrOHase and the subsequent assay of the enzyme was carried out by a two-step incubation procedure. A 5-min incubation was carried out under essentially the same conditions as the protein kinase assay; histone was omitted because it interfered with the activation of TyrOHase, presumably by competition of substrates for the kinase. This phase of the assay was terminated by adding 10 μl of 1 M potassium phosphate, pH 7.2/25 mM EDTA containing 2 mg of bovine serum albumin and placing the tubes in an ice bath. The EDTA chelates Mg^{2+} , thus inhibiting any further protein kinase activity. This was verified in control tubes by the addition of EDTA to the incubation mixture prior to the initiation of the reaction: values for TyrOHase activity in the presence of cAMP and ATP/Mg^{2+} plus EDTA were not different from either control activity or activity obtained with the addition of EDTA alone, in agreement with the observation by Morgenroth *et al.* (8).

TyrOHase. Assay I. The second step in the procedure, during which TyrOHase activity is assayed, was adapted from the procedure of Coyle (22). The following components were added (final concentrations) to bring the final incubation volume to 170 μl : 6-MeH₄Pter (800 μM), NADPH (1 mM), catalase (1,660 units), a previously determined optimal amount of pteridine reductase prepared through the DEAE-cellulose step according to the procedure of Craine *et al.* (23), and [$3,5$ - ^3H]tyrosine (200 μM ; 0.1–0.4 μCi). The final pH of the TyrOHase assay was 6.6. Blanks consisted of the complete enzyme mixture to which was added 200 μM 3-iodotyrosine which inhibits TyrOHase completely (11). The TyrOHase reaction was initiated by placing tubes into a 37°C shaking water bath. The reaction was allowed to proceed for 20 min and was terminated by the addition of 5 ml of 400 mM perchloric acid containing carrier L-dopa at a concentration of 1 $\mu\text{g}/\text{ml}$. Tubes were centrifuged at $5,000 \times g$ for 5 min to remove protein. The supernatant was added to vials containing 6.5 ml of 55 mM EDTA/44 mM sodium metabisulfite/80 mM KH_2PO_4 and titrated to pH 8.5 with NaOH. Samples were then poured over 400 mg of neutral alumina (Brockman, grade I) in Kontes Chromaflex columns that had been previously washed with 2 ml of 400 mM HCl followed by 25 ml of distilled water. After the samples passed through the columns, the columns were washed with 25 ml of distilled water, and catecholamines were eluted with 2 ml of 400 mM HCl. [^3H]Catecholamines were measured in 10 ml of a Triton X-100/

toluene scintillation cocktail. Counts per minute were corrected for column recovery (consistently 60%) and counting efficiency (20%).

Assay II. During the course of these studies, it was found that 1 mM ascorbate served as a more effective reducing agent than the NADPH/pteridine reductase reducing system, allowing for a more sensitive assay and obviating the need for a relatively crude preparation of pteridine reductase, in agreement with the earlier results of Lerner *et al.* (24). With the use of 1 mM ascorbate, the incubation time was decreased to 15 min. Where designated, 25 mM NaF was used instead of 5 mM as in assay I. All other details of the assay were carried out as in assay I.

Protein Assay. Protein was assayed by the method of Lowry *et al.* (25) with bovine serum albumin as standard.

RESULTS

Specificity of Protein Kinase Inhibitor. Assay of histone kinases in the presence of different concentrations of protein kinase inhibitor revealed complete inhibition of histone phosphorylation by cAMP-dependent protein kinase at 3 μg of protein kinase inhibitor, whereas 100 μg of the inhibitor had no effect on histone phosphorylation catalyzed by either of two cAMP-independent histone kinases.

Titration of Protein Kinase Inhibitor Against Protein Kinase and TyrOHase. Additions of up to 100 μg of protein kinase inhibitor failed to modify either basal protein kinase or basal TyrOHase activity (Fig. 1). These results indicate that there is no free endogenous catalytic subunit of cAMP-dependent protein kinase in our preparations of crude rat striatal supernatant. Basal protein kinase activity (that found in the absence of cAMP or in the presence of excess protein kinase inhibitor) represented one-third of the total histone kinase activity in crude striatal supernatant in the presence of cAMP, reflecting the presence of a cAMP-independent histone kinase(s).

Addition of 25 μg of protein kinase inhibitor caused complete inhibition of cAMP-dependent histone kinase activity but produced only a 75% inhibition of the activation of TyrOHase seen with cAMP and ATP/Mg^{2+} . This residual component of increased TyrOHase activity in the presence of cAMP and ATP/Mg^{2+} persisted unchanged in the presence of 100 μg of protein kinase inhibitor. The apparent K_m for the effect of ATP on the cAMP-independent protein kinase-mediated activation of TyrOHase was determined to be approximately 0.3 mM (Fig. 1B).

Time Course of the Reaction Catalyzed by Activated TyrOHase. In these studies, the protein phosphorylation reaction (step 1) was carried out for 5 min in the presence of: (a) cAMP and ATP/Mg^{2+} ; (b) ATP/Mg^{2+} ; (c) ATP/Mg^{2+} , cAMP, and protein kinase inhibitor; (d) ATP/Mg^{2+} and protein kinase inhibitor; (e) p[NH]ppA and Mg^{2+} ; and (f) no additions. Then, excess EDTA was added and TyrOHase was assayed for different periods of time, ranging from 2.5 to 40 min.

Basal TyrOHase activity in the presence of 800 μM 6-MeH₄Pter was linear for at least 40 min of incubation (Fig. 2). After incubation of TyrOHase in the presence of cAMP and ATP/Mg^{2+} for 5 min, the activity of the enzyme was about 8-fold higher during the first 10 min of incubation. Enzyme activity then declined progressively during the next 30 min. After incubation of TyrOHase in the presence of ATP/Mg^{2+} a more modest activation was elicited, but this activation persisted undiminished throughout the 40 min of incubation. The degree of activation by ATP/Mg^{2+} and the time course of the reaction were identical in the presence or absence of protein kinase inhibitor, which was added in a concentration previously shown

FIG. 1. (A) Endogenous protein kinase activity in crude rat striatal homogenate. ○, Control; ●, activity in the presence of 125 μM cAMP. (B) Endogenous TyrOHase activity in the same homogenate as A. Assay I was performed. ▲, No additions; ■, 125 μM cAMP and 800 μM ATP; □, 125 μM cAMP and 300 μM ATP. Concentration of 6-MeH₄Pter, 800 μM ; tyrosine, 200 μM . Values represent the mean $\pm$ SEM for at least three separate observations, with significance of difference from control established by Student's two-tailed *t* test. **P* < 0.01; †*P* < 0.02; ‡*P* < 0.05.

to inhibit cAMP-dependent protein kinase completely.

p[NH]ppA, the nonhydrolyzable form of ATP, failed to activate TyrOHase, an observation in agreement with the findings of Simon *et al.* (10) with TyrOHase from striatum, adrenal medulla, and pheochromocytoma. In addition, in the absence of Mg^{2+} , ATP was unable to activate TyrOHase (data not shown), also in agreement with Simon *et al.* (10).

FIG. 2. Time course of TyrOHase activity after various conditions of activation. Activation of striatal supernatant was for 5 min in the presence of: no additions (×); p[NH]ppA (○); 125 μM cAMP and 800 μM ATP/Mg²⁺ (●); 800 μM ATP/Mg²⁺ (■); 125 μM cAMP, 800 μM ATP/Mg²⁺, and a saturating concentration of protein kinase inhibitor (PKI) (○); or 800 μM ATP/Mg²⁺ and a saturating concentration of protein kinase inhibitor (□). Then, EDTA was added and TyrOHase was assayed for different time periods (assay I) with 800 μM 6-MeH₄Pter and 200 μM tyrosine. The reactions were terminated at the designated time points. 3-Iodotyrosine blanks and EDTA controls were included for each experimental condition. A saturating concentration of protein kinase inhibitor was considered that concentration which completely inhibited cAMP-dependent protein kinase in a concomitant protein kinase assay. Generally, the amount used was 100 μg . Values represent the mean $\pm$ SEM for at least three separate observations.

Yamauchi and Fujisawa (14) recently reported that TyrOHase from rat brainstem, cerebral cortex, and adrenal gland can be activated by a brainstem protein kinase that is dependent on calcium and calmodulin. It was therefore of interest to determine whether CaCl_2 had any potentiating effect on the ATP-mediated activation of striatal TyrOHase in our assay system. The addition of 100 μM CaCl_2 to the preincubation medium was not associated with an increase in TyrOHase activity above that achieved with ATP alone, irrespective of cofactor concentration or the presence of 100 μM EGTA in the homogenizing buffer.

Kinetics of TyrOHase Activation Under Phosphorylating Conditions. Kinetic analysis of rat striatal TyrOHase suggested that two forms of the native enzyme exist, one with an apparent low K_m for 6-MeH₄Pter cofactor and one exhibiting a high K_m

FIG. 3. Lineweaver-Burk analysis of TyrOHase activity [nmol of [^3H]dopa/hr per mg of protein (assay II)] with different 6-MeH₄Pter cofactor concentrations (0.05–4.0 mM) and 200 μM tyrosine. ●, Control; ○, ATP/Mg²⁺; △, cAMP and ATP/Mg²⁺. Values represent the means $\pm$ SEM for at least three separate observations. Lines were constructed by linear regression analysis; each line represents a goodness-of-fit value of at least *r* = 0.98. Y intercept values $\pm$ SEM were derived from linear regression analysis (see text).

(100 μ M and 2.5 mM, respectively) (Fig. 3). This finding agrees with those of Weiner *et al.* (26) in crude striatal supernatant as well as those of Masserano and Weiner (27) in crude adrenal supernatant. In the previous studies, in which the effect of cAMP and ATP/Mg²⁺ on enzyme activity was examined, no change in enzyme $V_{\max}$ was noted. However, the range of cofactor concentrations used in these studies would not be expected to permit an accurate estimate of the $V_{\max}$ for the striatal enzyme because a modest proportion of the enzyme in the high K_m form would be difficult to detect.

The addition of ATP appeared to cause an activation of both the low K_m and high K_m forms of the enzyme with an apparent $V_{\max}$ increase ($V_{\max}$: control, 5.9 ± 3.8 nmol of [³H]dopa/hr per mg of protein; ATP, 14.1 ± 7.9) and no change in the proportion of the two forms of the enzyme. The addition of cAMP caused an increase in TyrOHase activity above that seen with ATP, due to a transformation of the high K_m form of the enzyme to the low K_m form (from 2.5 mM to 100 μ M 6-MeH₄Pter).

Because the pterin cofactor concentrations used in the earlier studies (0.05–4.0 mM) (Fig. 3) did not permit a precise determination of either the apparent K_m or the apparent $V_{\max}$ for the low-affinity form of the enzyme, we determined the activity of the enzyme in the presence of higher cofactor concentrations. In these studies it was noted that the activity of TyrOHase was linear for only 8 min, after which it declined. This was especially prominent in the presence of cAMP and ATP/Mg²⁺ and may account for the failure to demonstrate a $V_{\max}$ effect under these conditions when longer incubation times were used. For the studies in which pterin cofactor concentrations >5 mM were used, the duration of the assay was decreased to 6 min.

Fig. 4 depicts the Lineweaver–Burk plot of TyrOHase activity at the higher 6-MeH₄Pter concentrations in the presence and absence of ATP/Mg²⁺ and cAMP ($V_{\max}$: control, $9.71 \pm$

FIG. 4. Lineweaver–Burk analysis of the effects of ATP/Mg²⁺ and cAMP plus ATP/Mg²⁺ on striatal TyrOHase activity (nmol of [³H]dopa/hr per mg of protein) at high cofactor concentrations (0.4–12.0 mM). The values represent the mean $\pm$ SEM of at least three separate experiments. TyrOHase was measured in assay II. In the presence of 5, 10, and 12 mM 6-MeH₄Pter the assays were carried out for only 6 min because it was found that, at the high cofactor concentrations, enzyme activity was not linear beyond 8 min. Lines and Y intercept values $\pm$ SEM were derived from linear regression analysis (see text). The 12 mM 6-MeH₄Pter point was deleted for clarity but was included in the regression analysis. ●, Control; ○, ATP/Mg²⁺; △, cAMP plus ATP/Mg²⁺.

0.66 nmol of [³H]dopa/hr per mg of protein; ATP, 17.24 ± 1.2 ; cAMP, 15.87 ± 0.76). The K_m for 6-MeH₄Pter in the presence of cAMP and ATP/Mg²⁺ was determined to be 100 μ M.

Kinetic analysis of the effect of ATP/Mg²⁺ and cAMP plus ATP/Mg²⁺ on TyrOHase activity in the presence of 12 mM 6-MeH₄Pter and different concentrations of tyrosine was also performed. The Lineweaver–Burk plot indicated an ATP/Mg²⁺-mediated increase in $V_{\max}$ over control from 16.9 ± 0.3 to 29.4 ± 0.9 nmol of [³H]dopa/hr per mg of protein. The addition of cAMP caused a similar increase in $V_{\max}$ over control to 35.7 ± 1.3 nmol of [³H]dopa/hr per mg of protein. This increase in $V_{\max}$ in the presence of cAMP and ATP/Mg²⁺ most likely was due to the independent effect of ATP/Mg²⁺ and probably did not represent a cAMP-dependent phenomenon because it was not significantly greater than that obtained with ATP/Mg²⁺ alone.

DISCUSSION

The data in the present study support the idea that the catalytic subunit of cAMP-dependent protein kinase activates TyrOHase because, when assessed in the presence of cAMP, the activations of both protein kinase and TyrOHase are sensitive to inhibition by protein kinase inhibitor. In addition, these data suggest the existence of a new protein kinase in crude striatal supernatant which increases the reaction velocity of TyrOHase by a cAMP-independent mechanism. The activation of TyrOHase by ATP/Mg²⁺ is proportional to the concentration of ATP and is unaffected by the presence of protein kinase inhibitor. The involvement of a protein kinase is supported by the observation that p[NH]ppA, which has a nonhydrolyzable γ -phosphate moiety, cannot activate TyrOHase. The latter observation precludes the possibility that ATP is exerting a non-specific effect such as anionic activation (28, 29). Further evidence against an ionic or other noncovalent effect are the observations that ATP does not activate TyrOHase either in the presence of 25 mM EDTA or when Mg²⁺ is absent, and the effect of ATP persists after (NH₄)₂SO₄ fractionation of the activated enzyme (data not shown).

The kinetic properties of TyrOHase activated in the presence of ATP/Mg²⁺ are distinctly different from the kinetics of TyrOHase after cAMP activation, manifesting a $V_{\max}$ increase over control with no change in apparent K_m for pterin cofactor. In fact, the activation of TyrOHase by ATP/Mg²⁺ appears to be additive with cAMP. The kinetic data generated in the presence of the lower range of cofactor concentrations (Fig. 3) suggest that ATP activates both high and low K_m forms to an equal extent without markedly shifting the proportion of the high K_m and low K_m forms of the enzyme. This would suggest a second phosphorylation site on the TyrOHase molecule which, upon phosphorylation, would increase reaction velocity of both high and low K_m forms of the enzyme. The kinetics of enzyme activity in the presence of a higher range of 6-MeH₄Pter concentrations (0.4–12.0 mM) were also examined (Fig. 4). In this concentration range, the low K_m form of the enzyme is already maximally activated and therefore the full activity of the low K_m form of the enzyme is being expressed, irrespective of cofactor concentration. In contrast, the activity of the low-affinity high K_m population of TyrOHase molecules, which have not been covalently modified by endogenous cAMP-dependent phosphorylation, increases as pterin cofactor is increased from 0.4 mM to 12 mM. It is under these circumstances that an increase in the apparent $V_{\max}$ of the enzyme becomes obvious. This effect is presumably related to enzyme phosphorylation by ATP/Mg²⁺ at a site distinct from that phosphorylated by cAMP plus ATP/Mg²⁺. Concomitant addition of cAMP and ATP allows for contemporaneous phosphorylation at two separate sites and a

"hybrid" kinetic state which includes both an ATP/Mg²⁺-mediated $V_{\max}$ shift and a cAMP-ATP/Mg²⁺-mediated K_m shift for pterin cofactor.

It is conceivable that cAMP-dependent protein kinase catalyzes the phosphorylation of two sites on TyrOHase, one of which results in the K_m shift and the other results in the $V_{\max}$ increase. It is also possible that the second phosphorylation site is the same site phosphorylated by cAMP-independent protein kinase. Only studies on the stoichiometry and sites of the phosphorylation mediated by these two kinases will resolve these possibilities. It should be noted that the cAMP-independent protein kinase does not appear to be present in rat adrenal medulla. In this tissue, addition of cAMP plus ATP/Mg²⁺ to adrenal supernatant produces only a K_m shift which is blocked by protein kinase inhibitor. No $V_{\max}$ effect is elicited in this tissue unless striatal supernatant enzyme is also added (unpublished data). These results suggest that cAMP-dependent protein kinase does not phosphorylate a site on the enzyme that results in an increase in the $V_{\max}$ of TyrOHase.

Preliminary observations have demonstrated direct phosphorylation of purified pheochromocytoma TyrOHase added to rat striatal supernatant under conditions identical to those required to produce TyrOHase activation by ATP/Mg²⁺. After enzyme phosphorylation in the presence of cAMP and [γ -³²P]-ATP/Mg²⁺, tryptic digestion of the immunoprecipitated protein yields several phosphopeptides. One phosphopeptide has been shown to be the product of cAMP-dependent phosphorylation of TyrOHase and the other phosphopeptides are apparently derived from the actions of cAMP-independent protein kinase(s) which catalyze the phosphorylation of other sites on TyrOHase (unpublished data).

There is suggestive evidence that a cAMP-independent protein kinase may be present in peripheral adrenergic nerve terminals. Takimoto and Weiner (30) have observed an increase in the apparent $V_{\max}$ of TyrOHase from rabbit portal vein, after decapitation, which does not seem to be related to cAMP-dependent protein phosphorylation and appears to occur in vascular tissue but not in the adrenal medulla. Using the assay procedures described in this manuscript, we have been unable to achieve activation of TyrOHase prepared from rat adrenal gland with ATP/Mg²⁺ alone (unpublished data). In addition to our earlier observations on the presence of a cAMP-independent protein kinase in rat striatum (31, 32), other workers have obtained evidence to suggest the existence of a cAMP-independent protein kinase capable of activating TyrOHase (33, 34).

cAMP-independent protein kinase activity and protein phosphorylation stimulated by ⁴⁵Ca²⁺ influx have been reported to occur in the absence of any change in levels of cAMP in synaptosomes prepared from rat brain. Furthermore, cyclic nucleotides and several putative neurotransmitters were reported to be without effect on protein phosphorylation in these synaptosome preparations (35). In addition, potassium has been shown to stimulate TyrOHase activity by a means which is additive with dibutyl cAMP-induced activation in pheochromocytoma cells (36, 37), suggesting the possibility that a cAMP-independent protein kinase may be involved and that this mechanism of TyrOHase activation may be of physiological significance.

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