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Calcium/phospholipid-dependent protein kinase (protein kinase C) phosphorylates and activates tyrosine hydroxylase

(tyrosine 3-monooxygenase/catecholamines/cAMP-dependent protein kinase/calcium/calmodulin-dependent protein kinase)

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ABSTRACT **Protein kinase C, purified to homogeneity, was found to phosphorylate and activate tyrosine hydroxylase that had been partially purified from pheochromocytoma PC 12 cells. These actions of protein kinase C required the presence of calcium and phospholipid. This phosphorylation of tyrosine hydroxylase reduced the K_m for the cofactor 6-methyltetrahydropterine from 0.45 mM to 0.11 mM, increased the K_i for dopamine from 4.2 μM to 47.5 μM, and produced no change in the K_m for tyrosine. Little or no change in apparent V_{max} was observed. These kinetic changes are similar to those seen upon activation of tyrosine hydroxylase by cAMP-dependent protein kinase. Two-dimensional phosphopeptide maps of tyrosine hydroxylase were identical whether the phosphorylation was catalyzed by protein kinase C or by the catalytic subunit of cAMP-dependent protein kinase. Both protein kinases phosphorylated serine residues. The results suggest that protein kinase C and cAMP-dependent protein kinase phosphorylate the same site(s) on tyrosine hydroxylase and activate tyrosine hydroxylase by the same mechanism.**

Nerve stimulation and neurotransmitters have been demonstrated to accelerate catecholamine biosynthesis in central and peripheral nervous tissue, adrenal medulla, and pheochromocytoma cells (1–4). This acceleration is thought to result from an increase in the catalytic activity of tyrosine hydroxylase (tyrosine 3-monooxygenase, EC 1.14.16.2) (cf. ref. 5), the rate-limiting enzyme in the biosynthesis of catecholamines (6). Several lines of evidence have suggested that cAMP-dependent phosphorylation may be one means of activation of tyrosine hydroxylase *in situ* (cf. ref. 7 and references therein). Analogues of cAMP have been reported to accelerate catecholamine biosynthesis in intact tissue preparations (8–11). In broken cell preparations, tyrosine hydroxylase has been shown to be activated, in the presence of ATP and Mg^{2+}, by cAMP or cAMP-dependent protein kinase (e.g., see refs. 8 and 12). Finally, purified tyrosine hydroxylase has been shown to be phosphorylated and activated by purified cAMP-dependent protein kinase (13–15).

Recent evidence has suggested a role for calcium as well as cAMP in the activation of tyrosine hydroxylase *in situ* (16, 17). It therefore seemed important to analyze the potential role of calcium-dependent protein kinases in the regulation of tyrosine hydroxylase activity. We report here that purified protein kinase C phosphorylates and activates partially purified tyrosine hydroxylase, in a manner similar to cAMP-dependent protein kinase.

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MATERIALS AND METHODS

Materials. Fresh bovine brains were obtained from a local slaughterhouse. The PC 12 clonal rat adrenal medullary pheochromocytoma line (18, 19) was grown as previously described. The catalytic subunit of cAMP-dependent protein kinase (20) and calcium/calmodulin-dependent protein kinase I (21) were purified as described. Calcium/calmodulin-dependent protein kinase II was a gift from Yvonne Lai and Teresa McGuiness. Calmodulin was purified as described (22). Walsh inhibitor was purified by a modification of the published procedure (23).

Other reagents and enzymes were obtained from the following commercial sources: H1 histone (III-S), catalase, bovine serum albumin, bovine brain L-α-phosphatidyl-L-serine, diolein, dithioerythritol, EGTA, Tris, and ATP (Sigma); leupeptin (Chemicon, El Segundo, CA); DL-6-methyl-5,6,7,8-tetrahydropterine (6-MePH$_4$) and dopamine (Calbiochem–Behring); L-tyrosine and 20 × 20 cm cellulose thin-layer chromatography plates (Eastman); EDTA and 2-mercaptoethanol (Baker); Ag 50W-X8 cation-exchange resin (200–400 mesh, dry, hydrogen form), and Bio-Rex 9 anion-exchange resin (200–400 mesh, dry, chloride form) (Bio-Rad); trypsin and chymotrypsin (Worthington); L-[3,5-^{3}H]tyrosine and [γ-^{32}P]ATP (New England Nuclear). All other chemicals used were reagent grade.

Purification of Tyrosine Hydroxylase. Tyrosine hydroxylase was partially purified as described (13), from the cytosol of cultured PC 12 pheochromocytoma cells, by two cycles of $(NH_4)_2SO_4$ fractionation (80% and 25–35% saturation) and Sepharose 4B chromatography. At this stage of purification, the M_r 62,000 subunit of tyrosine hydroxylase constituted approximately 75% of the total protein present (estimated from Coomassie blue protein stain of a NaDodSO$_4$/8% polyacrylamide gel) (Fig. 1*A*). An M_r 60,000 band, a proteolytic product of the M_r 62,000 subunit, was also present. The specific activity of the fresh preparation, when measured at pH 6.0, was 0.12 μmol of dopa formed per min per mg of protein. The protein was stored at −70°C for up to 3 months before use.

Purification of Protein Kinase C. Protein kinase C was purified from bovine cerebral cortex, using a modification (unpublished results) of the procedure of Kikkawa *et al.* (24) by chromatography on DEAE-cellulose, hydroxylapatite, blue agarose, and phenyl-Sepharose. The final peak of enzyme activity ran as a single protein band (M_r, 87,000) on NaDodSO$_4$/8% polyacrylamide gel electrophoresis. The specific activity with H1 histone as substrate was 2.87 μmol of ^{32}P incorporated per min per mg of protein, and the kinase was stored in 25% (vol/vol) ethylene glycol under liquid nitrogen.

Abbreviation: 6-MePH$_4$, 6-methyltetrahydropterine.

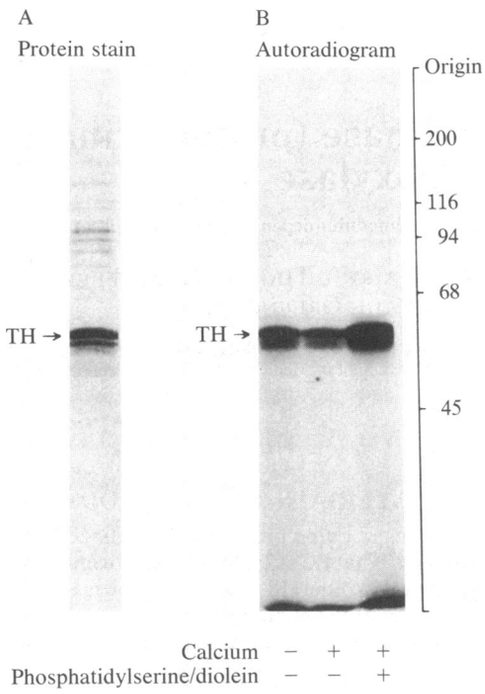

FIG. 1. (*A*) Coomassie blue protein stain of the partially purified tyrosine hydroxylase preparation (9.2 μg). (*B*) Autoradiogram showing calcium/phospholipid-dependent phosphorylation of tyrosine hydroxylase by protein kinase C. The standard phosphorylation reaction mixture was used with the following modifications: 21.6 μg of tyrosine hydroxylase and 2.4 ng of protein kinase C were used, and calcium (0.5 mM free) and phosphatidylserine (5 μg/100 μl)/diolein (0.1 μg/100 μl) were present as indicated. The reaction was initiated by the addition of [γ-^{32}P]ATP (final concentration, 10 μM; specific activity, 15×10^3 cpm/pmol) and carried out for 2 min. TH, tyrosine hydroxylase. Positions of size markers are shown in kilodaltons.

Measurement of Tyrosine Hydroxylase Phosphorylation. The standard reaction mixture (final volume 100 μl) contained 20 mM Tris·HCl at pH 7.4, 10 mM magnesium acetate, 1.0 mM EGTA, 1.5 mM $CaCl_2$, 5 μg of phosphatidylserine/0.1 μg of diolein, 2 mM dithioerythritol, 50 μg of bovine serum albumin, 10 μg of leupeptin, and 18 μg of tyrosine hydroxylase, in the absence or presence of 72 ng of protein kinase C. The reaction was initiated by the addition of [γ-^{32}P]ATP (final concentration, 500 μM; specific activity, 175 cpm/pmol) and carried out for 15 min at 30°C. The reaction was terminated by the addition of 20 μl of $NaDodSO_4$ stop solution [20% (vol/vol) glycerol/10% (wt/vol) $NaDodSO_4$/10% (wt/vol) 2-mercaptoethanol/0.25 M Tris·HCl, pH 6.7, containing a trace of bromophenol blue] and heating in a boiling water bath for 5 min.

The samples were electrophoresed on $NaDodSO_4$/8% polyacrylamide gels by the method of Laemmli (25). The gels were stained, destained, dried, and subjected to autoradiography as described (26). The phosphorylated M_r 62,000/60,000 tyrosine hydroxylase doublet was located by autoradiography and excised, and the radioactivity was quantitated by liquid scintillation counting.

Measurement of Tyrosine Hydroxylase Activity. Tyrosine hydroxylase activity was measured by a two-step assay. In the first step (preincubation), the standard phosphorylation reaction was carried out as described above, except that nonradioactive ATP was used and that the phosphorylation reaction was terminated by the addition of 10 μl of 250 mM EDTA. To initiate the second step (incubation) (27), two aliquots (50 μl each) of the stopped phosphorylation mixture were immediately added to tubes containing the standard reaction mixture for the measurement of tyrosine hydroxylase activity. This reaction mixture (final volume, 110 μl) contained (final concentrations) 200 mM sodium phosphate buffer (pH 7.0), 0.1 mM 6-$MePH_4$, 100 μM L-[3,5-^{3}H]tyrosine (specific activity, 1 μCi/4 nmol), 800 units of catalase, and 90 mM NaF. The reaction was carried out for 5 min at 37°C and terminated by the addition of 200 μl of 0.1 M NaOH. The stopped reaction mixture was passed through an ion-exchange column (0.1 × 1.5 cm) consisting of equal volumes of anion-exchange resin (Bio-Rex 9, upper layer) and cation-exchange resin (Ag 50W-X8, lower layer). The column was washed with 0.5 ml and 1.0 ml of water. Tritiated water was measured in the flow-through by liquid scintillation counting.

Additional Methods. Two-dimensional peptide mapping was performed as described (28), except that chymotrypsin (50 μg/ml) was used in addition to trypsin (75 μg/ml). Protein determination was performed by the method of Peterson (29) with bovine serum albumin as standard.

RESULTS

Phosphorylation of Tyrosine Hydroxylase by Protein Kinase C. In the presence of calcium, tyrosine hydroxylase was phosphorylated by protein kinase C in a phosphatidylserine/diolein-dependent manner (Fig. 1*B*). The maximum stoichiometry achieved with this preparation of tyrosine hydroxylase was about 0.5 mol of ^{32}P per mol of M_r 62,000/60,000 doublet (data not shown). The phosphorylation of tyrosine hydroxylase was compared with that of H1 histone, one of the most effective substrates for protein kinase C (30). Under identical conditions, incorporation of ^{32}P into H1 histone was about twice that into tyrosine hydroxylase (data not shown).

Measurement of Phosphorylation and Activity of Tyrosine Hydroxylase in the Absence and Presence of Protein Kinase C. The phosphorylation and activity of tyrosine hydroxylase were measured in parallel by the standard two-step assay, in which phosphorylation was carried out in the first step and tyrosine hydroxylase activity was measured in the second step. In the presence of protein kinase C, the phosphorylation of tyrosine hydroxylase increased with time and reached a plateau after about 20–30 min (Fig. 2*A*). Addition of a second aliquot of protein kinase C at 40 min resulted in a further increase in phosphorylation, indicating that the first plateau was attributable to inactivation of the kinase. In experiments in which basal phosphorylation of tyrosine hydroxylase was measured, it constituted less than 20–25% of the phosphorylation seen in the presence of protein kinase C during the first 15 min of preincubation; this basal phosphorylation was not affected by the addition of calcium, phospholipid, calmodulin, or Walsh inhibitor, an inhibitor of cAMP-dependent protein kinase (data not shown).

Tyrosine hydroxylase activity in the presence of protein kinase C increased along with phosphorylation up to 15 min (Fig. 2*B*), after which there was a sharp decline in activity. Addition of a second aliquot of protein kinase C at 40 min did not restore activity. A decrease in activity with increasing time of phosphorylation has also been reported in studies with cAMP-dependent phosphorylation of tyrosine hydroxylase (e.g., see ref. 31) and has been attributed to an increased susceptibility to denaturation of the phospho form of the enzyme relative to the dephospho form (32). There was little or no change in tyrosine hydroxylase activity as a function of time of preincubation in the absence of protein kinase C (Fig. 2*B*).

Effect of Phosphorylation of Tyrosine Hydroxylase on Its Affinity for 6-$MePH_4$. The K_m for 6-$MePH_4$ was determined for tyrosine hydroxylase in the absence and presence of protein kinase C by the standard two-step assay except that the concentration of 6-$MePH_4$ was varied from 0.1 mM to 1.0

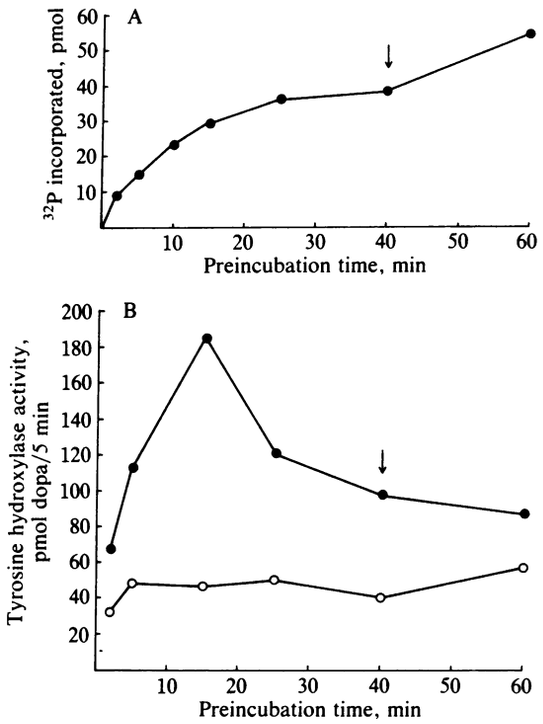

FIG. 2. Time course of phosphorylation (*A*) and activation (*B*) of tyrosine hydroxylase by protein kinase C. (*A*) The first step of the standard assay (the preincubation step to phosphorylate tyrosine hydroxylase) was carried out with [γ-^{32}P]ATP (specific activity, 175 cpm/pmol) at 30°C for the times indicated and was terminated by the addition of 20 μl of $NaDodSO_4$ stop solution. After gel electrophoresis, the M_r 62,000/60,000 tyrosine hydroxylase doublet, located by autoradiography, was excised, and the radioactivity was quantitated by liquid scintillation counting. (*B*) The first step (preincubation) of the standard assay was carried out with nonradioactive ATP at 30°C for the times indicated, followed by the second step (incubation) at 37°C for 5 min. Each point represents the mean of duplicate samples. ●, In the presence of protein kinase C; ○, in the absence of protein kinase C; ↓, addition of a second aliquot of protein kinase C.

mM. The K_m for 6-$MePH_4$ was 0.45 mM in the absence of protein kinase C and 0.11 mM in the presence of protein kinase C (Fig. 3). There was no change in V_{max}.

Effect of Phosphorylation of Tyrosine Hydroxylase on Its Affinity for Tyrosine. The K_m for tyrosine was determined for tyrosine hydroxylase in the absence and presence of protein kinase C by the standard two-step assay except that the concentration of tyrosine was varied from 10 to 100 μM, and the second step was carried out for 6 min. Protein kinase C did not significantly alter the K_m value for tyrosine, but it did cause a 30% increase in apparent V_{max} (data not shown). This small increase in V_{max} probably resulted from the increased affinity for 6-$MePH_4$ (see above), which was subsaturating in these assays.

Effect of Phosphorylation of Tyrosine Hydroxylase on Its Susceptibility to Inhibition by Dopamine. The K_i for dopamine inhibition of tyrosine hydroxylase activity in the absence and presence of protein kinase C was determined by the standard two-step assay with the following exceptions: the first step was carried out for 13 min and the second step for 6 min; dopamine concentration was varied from 2 to 80 μM; two concentrations of 6-$MePH_4$ (0.1 and 1.0 mM) were used; and the second step was carried out at pH 6.0 rather than 7.0, because tyrosine hydroxylase activity at pH 7.0 in the absence of protein kinase C was too low to obtain a reliable inhibition curve. A Dixon plot of the data yielded straight lines, except for the plus kinase data at 0.1 mM 6-$MePH_4$, which could be resolved into two straight lines (Fig. 4). In the absence of protein kinase C, the K_i for dopamine was calculated to be 4.2 μM. On the basis of the data obtained with the higher dopamine concentrations (Fig. 4 *Lower*, extrapolated broken line), the K_i was calculated to be 47.5 μM in the presence of protein kinase C. On the basis of the data obtained with the lower concentrations of dopamine, the K_i was calculated to be 4.7 μM in the presence of protein kinase C. We attribute these latter data to the presence of the dephospho form of tyrosine hydroxylase in the protein kinase C-treated preparation of the enzyme.

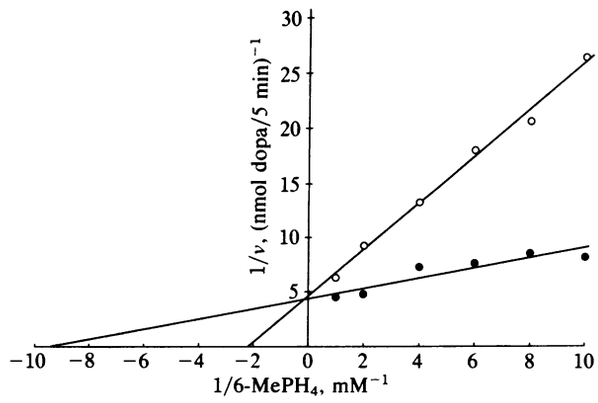

FIG. 3. Lineweaver–Burk plot of tyrosine hydroxylase activity as a function of 6-$MePH_4$ concentration, in the presence (●) and in the absence (○) of protein kinase C. Each point represents the mean of duplicate samples.

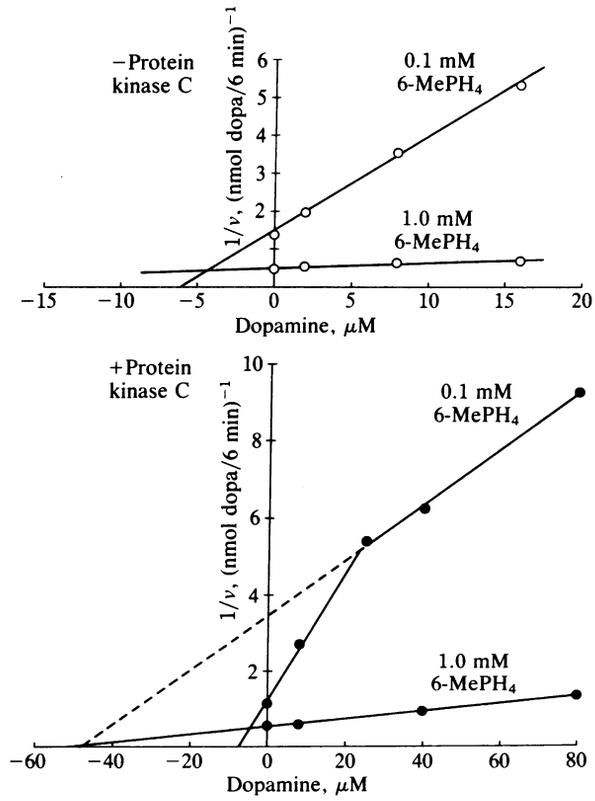

FIG. 4. Dixon plots of tyrosine hydroxylase activity as a function of dopamine and 6-$MePH_4$ concentrations, in the absence (upper panel) and presence (lower panel) of protein kinase C. The standard assay was carried out at the indicated concentrations of dopamine and 6-$MePH_4$ for 6 min. Each point represents the mean of duplicate samples.

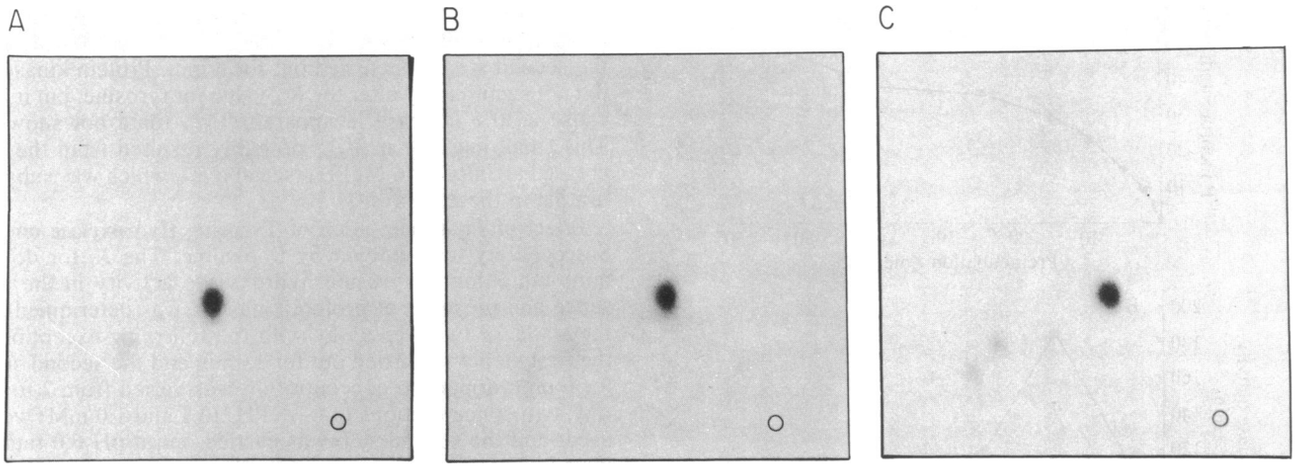

FIG. 5. Autoradiograms showing tryptic/chymotryptic phosphopeptide maps of tyrosine hydroxylase phosphorylated by the catalytic subunit of cAMP-dependent protein kinase (40 ng) (*A*); by protein kinase C (72 ng) (*B*); and by the catalytic subunit of cAMP-dependent protein kinase plus protein kinase C (*C*). The standard two-step conditions were used with the following exceptions: both steps were carried out for 10 min; bovine serum albumin and catalase were omitted; [γ-^{32}P]ATP (specific activity, 175 cpm/pmol) and nonradioactive tyrosine were used; the second step was terminated by the addition of $NaDodSO_4$ stop solution. After gel electrophoresis, the M_r 62,000/60,000 tyrosine hydroxylase doublet, located by autoradiography, was excised and subjected to tryptic/chymotryptic digestion followed by separation on cellulose plates, first by electrophoresis in the horizontal direction (negative pole left, positive pole right), and then by ascending chromatography in the vertical direction. o, Origin.

Comparison of Phosphopeptide Maps of Tyrosine Hydroxylase Phosphorylated by Protein Kinase C and by the Catalytic Subunit of cAMP-Dependent Protein Kinase. Tyrosine hydroxylase, after phosphorylation by protein kinase C, the catalytic subunit of cAMP-dependent protein kinase, or both, was subjected to tryptic/chymotryptic digestion and subsequent two-dimensional separation on cellulose plates. The pattern in each case was identical (Fig. 5), indicating that the two kinases phosphorylated the same site(s) on tyrosine hydroxylase. Furthermore, analysis of phosphorylated amino acids indicated that only serine residues were phosphorylated (data not shown).

DISCUSSION

The present results support and extend the data of Raese *et al.*, which suggested that protein kinase C might phosphorylate and activate tyrosine hydroxylase (33). We found that phosphorylation and activation of tyrosine hydroxylase by protein kinase C was characterized by a decrease in the K_m for the 6-$MePH_4$ cofactor, an increase in the K_i for dopamine, no change in the K_m for tyrosine, and little or no change in apparent V_{max} under any of the conditions tested. Similar effects on the kinetics of tyrosine hydroxylase were found previously when the enzyme was phosphorylated *in vitro* by cAMP-dependent protein kinase (e.g., see refs. 14 and 34). Moreover, the cAMP-dependent activation of tyrosine hydroxylase *in situ* has also been characterized by a decrease in K_m for the cofactor with no change in K_m for tyrosine and no change in V_{max} (e.g., see refs. 8 and 9). The phosphopeptide maps of tyrosine hydroxylase were identical whether *in vitro* phosphorylation was catalyzed by protein kinase C, the catalytic subunit of cAMP-dependent protein kinase, or both (Fig. 5). Thus, the similar kinetic effects of the two kinases on tyrosine hydroxylase activity may be attributable to phosphorylation of the same site(s) on this substrate.

There is evidence that calcium-dependent protein phosphorylation may be involved in the *in situ* activation of tyrosine hydroxylase (16, 17, 33, 35). However, it is not yet possible to attribute the calcium-dependent activation of tyrosine hydroxylase seen in intact cells to any one of the known calcium-dependent protein kinases. In a study of calcium-dependent activation of tyrosine hydroxylase *in vitro* (36, 37), the activation was characterized by a decrease in the K_m for cofactor and a small, statistically insignificant, increase in V_{max}. These results are consistent with the present study. In another study, however, the *in situ* calcium-dependent, depolarization-induced activation of tyrosine hydroxylase was characterized by an increase in V_{max}, with no change in K_m for the cofactor (17).

Calcium/calmodulin-dependent protein kinase(s) might be involved in the *in situ* calcium-dependent activation of tyrosine hydroxylase (34). However, in the present study, calcium/calmodulin-dependent protein kinase I (21) did not phosphorylate or activate tyrosine hydroxylase, and calcium/calmodulin-dependent protein kinase II phosphorylated but did not activate tyrosine hydroxylase (data not shown). It had been reported previously that calcium/calmodulin-dependent protein kinase II phosphorylated tyrosine hydroxylase but did not activate it unless a protein activator was present (35). Clearly, further work will be necessary to identify unequivocally the kinase(s) responsible for the calcium-dependent activation of tyrosine hydroxylase *in situ* and to determine the physiological significance of the activation of tyrosine hydroxylase by protein kinase C.

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1. Ip, N. Y., Perlman, R. L. & Zigmond, R. E. (1982) *J. Pharmacol. Exp. Ther.* **223,** 280–283.
2. Weiner, N., Lee, F.-L., Dreyer, E. & Barnes, E. (1970) *Life Sci.* **22,** 1197–1216.
3. Murrin, L. C. & Roth, R. H. (1976) *Mol. Pharmacol.* **12,** 463–475.
4. Greene, L. A. & Rein, G. (1978) *J. Neurochem.* **30,** 549–555.
5. Weiner, N. (1970) *Annu. Rev. Pharmacol.* **10,** 273–290.
6. Nagatsu, T., Levitt, B. G. & Udenfriend, S. (1964) *J. Biol. Chem.* **239,** 2910–2917.
7. Haycock, J. W., Meligeni, J. A., Bennett, W. F. & Waymire, J. C. (1982) *J. Biol. Chem.* **257,** 12641–12648.
8. Goldstein, M., Bronaugh, R. L., Ebstein, B. & Roberge, C. (1976) *Brain Res.* **109,** 563–574.

9. Meligeni, J. A., Haycock, J. W., Bennett, W. F. & Waymire, J. C. (1982) *J. Biol. Chem.* **257**, 12632–12640.
10. Chalfie, M., Settipani, L. & Perlman, R. L. (1979) *Mol. Pharmacol.* **15**, 263–270.
11. Ikeno, T., Dickens, G., Lloyd, T. & Guroff, G. (1981) *J. Neurochem.* **36**, 1631–1640.
12. Morgenroth, V. H., III, Hegstrand, L. R., Roth, R. H. & Greengard, P. (1975) *J. Biol. Chem.* **250**, 1946–1948.
13. Markey, K. A., Kondo, S., Shenkman, L. & Goldstein, M. (1980) *Mol. Pharmacol.* **17**, 79–85.
14. Vulliet, P. R., Langan, T. A. & Weiner, N. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 92–96.
15. Edelman, A. M., Raese, J. D., Lazar, M. A. & Barchas, J. D. (1981) *J. Pharm. Exp. Ther.* **216**, 647–653.
16. Haycock, J. W., Bennett, W. F., George, R. J. & Waymire, J. C. (1982) *J. Biol. Chem.* **257**, 13699–13703.
17. El Mestikawy, S., Glowinski, J. & Hamon, M. (1983) *Nature (London)* **302**, 830–832.
18. Warren, S. & Chute, R. (1972) *Cancer* **29**, 327–331.
19. Greene, L. A. & Tischler, A. S. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2424–2428.
20. Kaczmarek, L. K., Jennings, K. R., Strumwasser, F., Nairn, A. C., Walter, U., Wilson, F. D. & Greengard, P. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7492–7496.
21. Nairn, A. C. & Greengard, P. (1983) *Soc. Neurosci. Abstr.* **9**, 1029.
22. Grand, R. J. A., Perry, S. V. & Weeks, R. A. (1979) *Biochem. J.* **177**, 521–529.
23. McPherson, J. M., Whitehouse, S. & Walsh, D. A. (1979) *Biochemistry* **18**, 4835–4844.
24. Kikkawa, U., Takai, Y., Minakuchi, R., Inohara, S. & Nishizuka, Y. (1982) *J. Biol. Chem.* **257**, 13341–13348.
25. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
26. Ueda, T. & Greengard, P. (1977) *J. Biol. Chem.* **252**, 5155–5163.
27. Nagatsu, T., Levitt, M. & Udenfriend, S. (1964) *Anal. Biochem.* **9**, 122–127.
28. Huttner, W. B. & Greengard, P. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5402–5406.
29. Peterson, G. L. (1977) *Anal. Biochem.* **83**, 346–356.
30. Iwasa, Y., Takai, Y., Kikkawa, U. & Nishizuka, Y. (1980) *Biochem. Biophys. Res. Commun.* **96**, 180–187.
31. Vrana, K. E., Allhiser, C. L. & Roskoski, R., Jr. (1981) *J. Neurochem.* **36**, 92–100.
32. Vrana, K. E. & Roskoski, R., Jr. (1983) *J. Neurochem.* **40**, 1692–1700.
33. Raese, J. D., Edelman, A. M., Makk, G., Bruckwick, E. A., Lovenberg, W. & Barchas, J. D. (1979) *Commun. Psychopharmacol.* **3**, 295–301.
34. Ames, M., Lerner, P. & Lovenberg, W. (1978) *J. Biol. Chem.* **253**, 27–31.
35. Yamauchi, T. & Fujisawa, H. (1981) *Biochem. Biophys. Res. Commun.* **100**, 807–813.
36. Iuvone, P. M. & Butler, B. K. J. (1983) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **42**, 379 (abstr.).
37. Iuvone, P. M. (1984) *J. Neurochem.*, in press.