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Striatal Tyrosine Hydroxylase: Comparison of the Activation Produced by Depolarization and Dibutyryl-cAMP

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SUMMARY

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Incubation of striatal slices in high potassium media or in media containing 1 m dibutyryl-cAMP results in an increase in the activity of tyrosine hydroxylase. Bot treatments require a period of time before maximal activation is attained, and onc activated, the activated tyrosine hydroxylase has a similar time course of inactivatio The activation of tyrosine hydroxylase produced by potassium depolarization is calciu dependent while the activation produced by dibutyryl-cAMP is independent of th presence of calcium in the medium. The activation produced by depolarization is additiv with the activation produced by dibutyryl-cAMP. The pH optima for the tyrosin hydroxylase isolated from the striatal slices exposed to high potassium differs substantiall from the tyrosine hydroxylase obtained from slices incubated with dibutyryl-cAMP. Th difference is not due to changes in the amount of dopamine present in the striatal extract These observations suggest that the activation of tyrosine hydroxylase produced b depolarization and that produced by cAMP probably occur by different processes.

INTRODUCTION

Tyrosine hydroxylase (E.C. 1.14.16.2; tyrosine-3-monooxygenase), the rate-limiting enzyme in catecholamine biosynthesis, is activated by brief periods (5 to 15 min) of increased neuronal activity (1-5), and the enzyme remains activated for a finite period of time following a return to normal neuronal activity (2, 6). The accelerated synthesis of catecholamines which occurs during periods of increased impulse flow was originally believed to be due primarily to the removal of tyrosine hydroxylase from endproduct inhibition as a result of i creased transmitter release (7, 8). Howeve the more recent studies cited above ha shown that increased neuronal activi brings about a change in the physical pro erties of tyrosine hydroxylase resulting an enzyme exhibiting an increased affini for pterin cofactor and a decreased affini for inhibitors with a catechol moiety (1-5 Dependent upon the conditions of assay a increase in the affinity of tyrosine hydro ylase for its substrate tyrosine can also observed (1-3). However, the physiologic significance of this latter observation is u certain because tissue levels of tyrosine a sufficient under most circumstances to sa urate tyrosine hydroxylase.

Similar kinetic changes in tyrosine h droxylase to those produced by nerve stir

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ulation can be produced by the addition of cyclic AMP in combination with Mg^{2+}, ATP and protein kinase to soluble preparation of the enzyme *in vitro* (9–14). The use of soluble preparations of tyrosine hydroxylase, however, makes it difficult to ascertain if the mechanisms involved in the activation of the enzyme by small molecules has any relevance to the *in vivo* activation produced by enhanced neuronal activity. Experiments utilizing either brain slices or synaptosomes have shown that both exposure to dB-cAMP[2] or depolarizing conditions result in an activation of tyrosine hydroxylase and alterations in the kinetic constants of the enzyme prepared from the treated tissue (10, 15–17). These *in vitro* studies suggested that the activity of tyrosine hydroxylase may be regulated *in vivo* by a similar mechanism, perhaps involving a cyclic nucleotide dependent phosphorylation process.

Utilization of a more intact preparation, such as brain slices, to study the mechanisms involved in the activation of tyrosine hydroxylase is advantageous in some respects since in the same preparation it is possible to activate tyrosine hydroxylase both by incubation of slices in the presence of dB-cAMP or under conditions of depolarization. The slice preparation thus allows comparison of the two activation processes. While a number of independent reports dealing with the activation of brain tyrosine hydroxylase have appeared, the mechanism by which alterations in impulse flow or neuronal depolarization regulates the activity of this enzyme remains to be elucidated. In the present study, we have investigated the effects of potassium depolarization and dB-cAMP on striatal tyrosine hydroxylase and have compared a number of properties of the enzyme activated by these two different procedures in an attempt to delineate any similarities or differences in the properties of the activated enzyme.

We elected to carry out these experiments on crude supernatant preparations of the enzyme since it is quite likely that regulatory factors are present in this tissue milieu. Furthermore, procedures used to effectively purify tyrosine hydroxylase alter the physical and kinetic properties of the enzyme. In fact, even the use of simple filtration procedures result in alterations in the kinetic properties of tyrosine hydroxylase (18, 19).

[2] Abbreviations used are: dB-cAMP, dibutyryl cyclic AMP; KRP, Krebs-Ringer phosphate; MES, morpholino-ethanesulfonic acid; MOPS, morpholino-propanesulfonic acid.

MATERIALS AND METHODS

Preparation and incubation of rat striatal slices. Male Sprague-Dawley rats (Charles River Breeding Laboratories) were killed by decapitation and the brains rapidly removed to a petri dish pre-chilled on ice. The striata were immediately dissected, blotted and weighed. Slices (35–45 mg; 0.2 mm in thickness) were prepared with a Sorvall tissue chopper and transferred to 5.0 ml of incubation medium at 37°. Incubation was continued at 37° for 15 min (unless otherwise stated) under an atmosphere of 95% O_2/5% CO_2. At the end of the incubation period, the slices were separated from the medium by filtration through a nylon mesh (pore size, 35 microns) mounted on a filter paper disc. The nylon mesh containing the striatal slices was immediately frozen on dry ice until the time of assay (approximately 2 hr). In some experiments (deactivation studies), rather than immediately freezing the slices on dry ice, the slices were reincubated in the standard Krebs-Ringer phosphate medium for various periods of time. In these experiments, the incubation procedure was as described above for the typical experiments, except that the slices were gently shaken from the nylon mesh into 5.0 ml of fresh medium and further incubated at 37°. After this reincubation period, the slices were again filtered, frozen, and processed as described below for the assay of tyrosine hydroxylase. The KRP[2] media used had the following composition: NaCl, 128 mM; KCl, 4.8 mM; $CaCl_2$, 1.3 mM; $MgSO_4$, 1.2 mM; Na_2HPO_4 (pH 7.4), 15.8 mM; dextrose, 11.1 mM. Potassium enriched KRP (K^+-KRP) containing 60 mM KCl was prepared by replacing a portion of the NaCl with an equiosmolar amount of KCl.

Dopamine analysis. In some experiments a portion of the homogenate (0.4 ml) pre-

pared from striatal slices incubated under various conditions was acidified with 15% trichloroacetic acid and centrifuged at 10,000 rpm for 20 min. The supernate was purified by alumina chromatography and a portion of the acid eluate analyzed spectrofluorometrically for dopamine according to the procedure described by Walters and Roth (20). Data is expressed in terms of dopamine concentration relative to the protein concentration of the starting homogenate or dopamine concentration present in the tyrosine hydroxylase assay media.

Tyrosine hydroxylase assay. Frozen striatal slices were homogenized in 0.5 ml of 20 mM Tris acetate buffer, pH 7.0. The homogenates were centrifuged at 20,000 × *g* for 20 min at 4°, and the supernate served as the source of soluble tyrosine hydroxylase. Enzyme activity was assayed according to the method of Shiman *et al.* (21) with minor modifications (14).

The reaction was carried out in a total volume of 0.3 ml; 0.05 ml of supernate was added to a reaction mixture containing 4 μmoles acetate buffer, pH 7.0 (except for experiment in which the pH was varied), 3700 units of catalase (Boehringer Mannheim), 0.02 ml (*ca.* 0.45 mg protein) of partially purified sheep liver dihydropteridine reductase, 0.15 μmoles NADPH, and 0.075 μmoles of DL-6-methyl 5,6,7,8-tetrahydropterine HCl (6-MPH_4, Sigma Chemical Co.). After a 5 min preincubation at 37°, the reaction was initiated by the addition of 3 μCi of L-[(3,5)-^{3}H]tyrosine (1 Ci/mmole, Amersham) previously purified according to the method of Coyle (22) and evaporated to dryness just prior to use. Tyrosine hydroxylase assays were routinely conducted in the presence of subsaturating concentrations of tyrosine (10 mM) and 6 MPH_4 (250 mM). In experiments in which the pH and buffer type were varied the pH of each individual buffer was adjusted at 37° to the indicated pH on a pH meter prior to use. Subsequently the pH of the complete reaction mixture (minus the labeled tyrosine) at 37° was checked with narrow range pH paper to make certain that the pH remained unaltered. After a 15 min incubation at 37°, the reaction was terminated by the addition of 0.2 ml 10% TCA (W/V). Blank values were determined by running the assay in the absence of enzyme. Analysis of the tritiated water formed during the reaction was carried out by ion exchange chromatography using Dowex 50 × 8 (H^+) columns. Tyrosine hydroxylase activity is expressed as picomoles of dopa formed per min per mg wet weight of tissue or per mg protein. Protein was measured according to the method of Lowry *et al.* (23) using serum albumin as the standard. Statistical analyses were performed utilizing a one-way analysis of variance, followed by the Newman Keuls multiple comparison test. $p < 0.05$ was taken as the level of significance.

RESULTS

Dose response relationship for the activation by potassium and dB-cAMP of striatal tyrosine hydroxylase. Initial studies evaluating the dose response relationship of the activation of tyrosine hydroxylase produced by incubation of striatal slices in high potassium media or media containing dB-cAMP indicated that maximal activation was produced by 60 mM K^+ and 5 mM dB-cAMP. Incubation of slices in K^+ concentrations as high as 100 mM and dB-cAMP concentrations as high as 10 mM did not result in a further significant increase in tyrosine hydroxylase activity. For this reason in the following experiments we routinely depolarized striatal slices with 60 mM K-KRP. However, mainly for reasons of economy, in studies in which we employed dB-cAMP we routinely used a concentration of 1 mM dB-cAMP, unless otherwise indicated. This concentration of dB-cAMP caused a very reproducible (>100% increase) increase in tyrosine hydroxylase activity.

Time course for the activation of striatal tyrosine hydroxylase by potassium and dB-cAMP. When striatal slices were incubated in the presence of 60 mM potassium (K^+-KRP) or 1 mM dB-cAMP for various periods of time, an activation of tyrosine hydroxylase was observed (Fig. 1). The increase in enzyme activity was maximal after a 15 min exposure in each case, resulting in an activation of approximately 100% and 130% for potassium and dB-cAMP, respec-

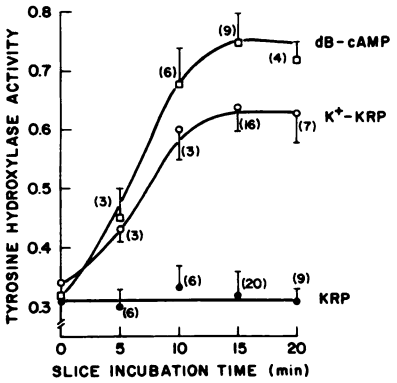

FIG. 1. *Time course for the activation of striatal tyrosine hydroxylase by potassium and dB-cAMP*

Striatal slices were incubated for various periods of time in KRP, K^+-KRP or KRP containing 1 mM dB-cAMP. Tyrosine hydroxylase was assayed in the 20,000 × *g* supernate. Tyrosine hydroxylase activity is expressed as picomoles dopa per min per mg tissue. The number in parentheses equals the number of individual experiments. Data is plotted as the mean and vertical bars illustrate the S.E.M.

tively. Tyrosine hydroxylase activity in the homogenates prepared from control slices was independent of the duration of slice incubation time and remained essentially constant over the entire 20 min incubation period (Fig. 1).

Additive effects of potassium depolarization and addition of dB-cAMP on tyrosine hydroxylase activity. Incubation of striatal slices in concentrations of potassium (60 mM) and dB-cAMP (10 mM) which alone produce a maximal activation of tyrosine hydroxylase resulted in more than an additive increase in enzyme activity (Table 1). When studied individually, incubation in K-KRP caused about a 90% increase in tyrosine hydroxylase activity and addition of dB-cAMP (10 μm) resulted in about a 250% increase in activity. When striatal slices were simultaneously incubated with K-KRP and dB-cAMP a 480% increase in tyrosine hydroxylase activity was observed. These results suggest that the mechanism of activation of tyrosine hydroxylase by potassium depolarization and treatment with dB-cAMP are different.

Effect of calcium on the potassium and dB-cAMP induced activation of striatal tyrosine hydroxylase. Since alterations in calcium flux are known to accompany depolarization, it was of interest to examine the calcium-dependence of the potassium induced increase in striatal tyrosine hydroxylase activity. The results presented in Fig. 2 show that when calcium ions were omitted from the control incubation medium, there was a slight but nonsignificant increase in tyrosine hydroxylase activity.

After incubation of the slices in the presence of 60 mM potassium or 1 mM dB-cAMP, significant increases in enzyme activity were observed, however, only the stimulation by potassium was found to require the presence of calcium ions in the incubation medium. Stimulation of the striatal enzyme by dB-cAMP was not dependent on the presence of calcium ions in the medium bathing the slices. Furthermore, the results show that when striatal slices were incubated in the presence of 60 mM potassium and 1 mM dB-cAMP together, the effects of these two treatments on tyrosine hydroxylase activity were additive as in the previous experiments in which a supramaximal concentration of dB-cAMP (10 mM) was employed.

Additional experiments on the effects of calcium removal indicated that the addition

TABLE 1

Effect of optimal concentrations of dB-cAMP and potassium on tyrosine hydroxylase activity isolated from striatal slices

Slices for one striatum were incubated for 15 min and homogenized in 1.0 ml MES buffer, 10 mM, pH 6.0 with 0.2% Triton. TH assays were conducted at pH 7.0 in the presence of 10 μM tyrosine and 250 μM 6-MPH_4. Numbers in parentheses indicate the number of striata used. Results are expressed as mean ± S.E.M.

Incubation conditions of the slices	Tyrosine hydroxylase activity	Tyrosine hydroxylase activity
	(% control)	*(pmoles DOPA/min/mg protein)*
mKRP	100 ± 11.5 (6)	8.02 ± 0.92 (6)
KRP + dB-cAMP (10 mM)	357 ± 29 (4)	28.6 ± 2.30 (4)
KRP + K^+ (60 mM)	192 ± 28 (4)	15.40 ± 2.20 (4)
KRP + dB-cAMP (10 mM) + K^+ (60 mM)	580 ± 30 (4)	46.50 ± 2.4 (4)

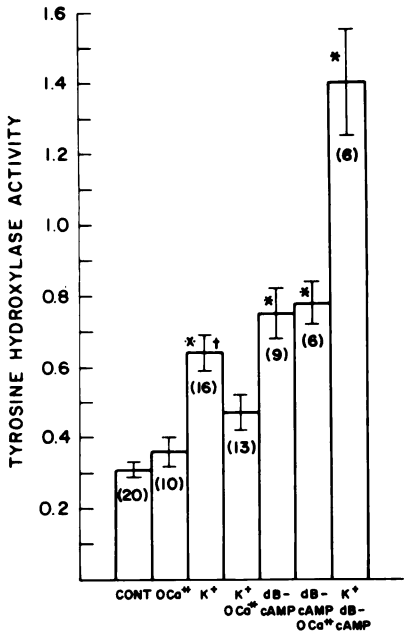

FIG. 2. *Effect of calcium on the potassium- and dB-cAMP-activation of striatal tyrosine hydroxylase*

Striatal slices were incubated for 15 min under the indicated conditions. Tyrosine hydroxylase was assayed in the 20,000 × *g* supernatant. Data is expressed as picomoles dopa per min per mg tissue ± S.E.M. The number in parentheses is number of individual experiments. * significantly different from control (KRP); † significantly different from K^+-KRP minus Ca^{++}; ‡ significantly different from K-KRP and from dB-cAMP. $p < 0.05$.

of the calcium chelator, EGTA, to a Ca^{2+}-free incubation medium resulted in a significant increase in tyrosine hydroxylase prepared from striatal slices incubated under these conditions (Table 2). Furthermore, the Ca^{2+}-dependence of the potassium-induced increase in tyrosine hydroxylase activity became more apparent when EGTA was included in the incubation medium.

Time course for deactivation of striatal tyrosine hydroxylase after activation by potassium or dB-cAMP. Based on the observation that striatal tyrosine hydroxylase remains activated for a finite period of time after cessation of electrical stimulation of the nigro-neostriatal pathway (2, 24), it was of interest to investigate the time course for deactivation after the *in vitro* activation elicited by potassium or dB-cAMP. The results presented in Fig. 3 show the time course for the decay of enzyme activity back to control values (deactivation) of the potassium- and dB-cAMP-activated enzymes. After the enzyme was activated by exposure of the slices to a potassium enriched medium (60 mM K^+), it required an additional 20 min of incubation in KRP before the activity returned to control values. When similar studies were carried out with the dB-cAMP-stimulated enzyme, only a 10 min reincubation period was required before the activity returned to control values. Thus, the activated form of tyrosine hydroxylase resulting from exposure of the slices to dB-cAMP shows a similar although significantly faster decline in activity than the enzyme which has been activated by prior exposure of the slices to a potassium enriched medium. Comparison of initial rates of "fall off" of enzyme activity indicates the dB-cAMP-activated enzyme activity declines 1.5 times faster than does the activity from the potassium-activated enzyme.

pH dependency of tyrosine hydroxylase isolated from striatal slices exposed to high potassium or dB-cAMP. In order to further compare the activated states of the enzyme after exposure to elevated potassium or dB-cAMP, tyrosine hydroxylase was assayed over the pH range of 5.5–8.0. In these experiments, 50 mM MES buffer was used in the pH range of 5.5 to 7.0 and 50 mM MOPS buffer was employed in the

TABLE 2

Effect of calcium removal on the potassium induced activation of striatal tyrosine hydroxylase

At the end of the incubation period slices were homogenized and tyrosine hydroxylase assayed in the high-speed supernate as described in Methods. Results are expressed as the mean ± S.E.M. Values in parentheses indicate the number of individual experiments.

Incubation conditions	Tyrosine hydroxylase activity
	(pmoles/min/mg protein)
Control (KRP)	15.9 ± 1.1 (5)
O Ca^{2+} + EGTA (0.1 mM)	22.1 ± 2.2 (5)*
K^+-KRP	27.4 ± 2.1 (3)*
K^+-KRP, O Ca^{2+} + EGTA	18.7 ± 0.6 (3)

* $p < 0.05$, compared to control.

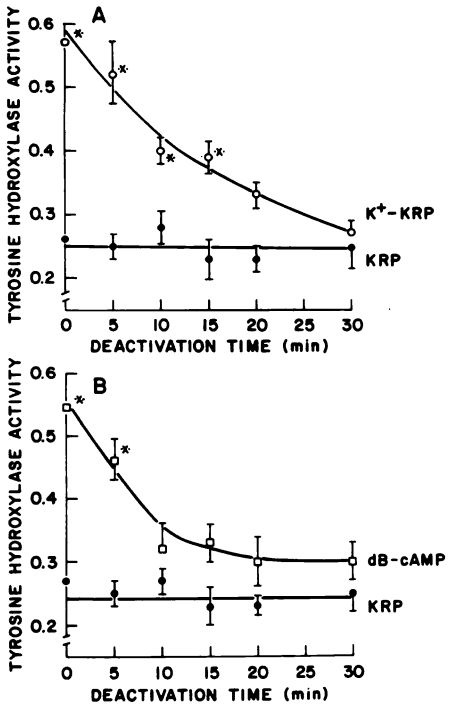

FIG. 3. *Time course for "de-activation" of striatal tyrosine hydroxylase after activation by potassium or dB-cAMP*

Striatal slices were incubated for 15 min in KRP, K^+-KRP, or KRP contianing 1 mM dB-cAMP. After filtration of the slices, as described under METHODS, the slices were reincubated for various periods of time in normal KRP. Slices were removed, homogenized and tyrosine hydroxylase assayed in the 20,000 × *g* supernatant. Data are expressed as picomoles dopa per min per mg tissue. Values represent the mean ± S.E.M. of 4–6 determinations. * significantly different from control; $p < 0.05$. The initial rate of inactivation of the enzyme activated by dB-cAMP is significantly faster than the inactivation following potassium depolarization.

pH range of 6.8–8.0. The pH optimum for tyrosine hydroxylase obtained from slices incubated in normal KRP or K^+-KRP was 6.0, and marked decreases in enzyme activity were observed at both higher and lower pH values (Fig. 4). The pH optimum for tyrosine hydroxylase obtained from striatal slices incubated in the presence of dB-cAMP was 7.5, but the pH profile for this enzyme preparation appeared to be somewhat more complex than that observed for the control and potassium-stimulated enzymes (see Fig. 4). Not only did the potassium- and dB-cAMP-stimulated enzymes exhibit different pH optima, but the pH for maximal percent stimulation was also different. Thus, the pH at which one observes the maximum percent stimulation was 7.0 for the potassium-stimulated enzyme and 7.5 for the dB-cAMP-stimulated enzyme.

In a similar series of experiments in which the pH optima of tyrosine hydroxylase isolated from potassium depolarized slices and slices incubated in dB-cAMP was examined over a narrower pH range in the presence of Tris-acetate buffer similar; less dramatic changes were observed, however (Fig. 5). The tyrosine hydroxylase isolated from slices incubated in normal KRP or high K-KRP had maximal activity in this buffer system at pH 6.0 while the tyrosine hydroxylase prepared from slices incubated with dB-cAMP had maximal activity at a pH of 7.0 to 7.4. The maximal percentage activation of tyrosine hydroxylase following potassium depolarization occurs in a pH range of 6.8–7.4 while maximal activation of the enzyme prepared from the dB-cAMP treated tissue occurs at an assay of 7.0–7.4.

Effect of potassium depolarization or incubation with dB-cAMP on the concentration of dopamine in the tyrosine hydroxyl-

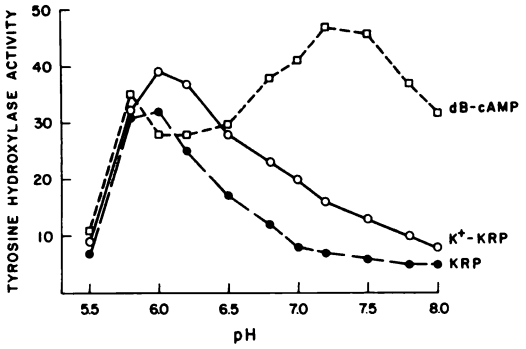

FIG. 4. *pH dependency of tyrosine hydroxylase isolated from striatal slices exposed to potassium or dB-cAMP*

Striatal slices were incubated for 15 min in KRP, K^+-KRP or KRP containing 1 mM dB-cAMP. Slices were homogenized in 10 mM MES at pH 6.0. Tyrosine hydroxylase was assayed in the 20,000 × *g* supernate at the illustrated pH using 50 mM MES buffer in the pH range 5.5–7.0 and 50 mM MOPS buffer in the pH range of 6.8–8.0. Tyrosine hydroxylase activity is expressed as picomoles dopa per min per mg protein. Values represent the mean ± S.E.M. of 3–5 determinations.

ase assay. Since all the assays in the current experiments were carried out on crude supernate fractions, it was of interest to determine if the observed activation might occur as a result of alterations in endogenous levels of dopamine. A substantial depletion of endogenous dopamine in treated striatal slices could theoretically result in an increase in tyrosine hydroxylase activity in the *in vitro* assay system, provided that under the assay conditions employed endogenous dopamine concentrations are high enough to exert a significant inhibitory effect on enzyme activity. Experiments summarized in Table 3 indicate that when slices are incubated in KRP prior to homogenization and analysis of tyrosine hydroxylase activity, the concentration of endogenous dopamine in the tyrosine hydroxylase assay is 0.33 μM. Incubation of slices in K-KRP (60 mM) results in about a 29% decrease in endogenous dopamine levels and the concentration of dopamine in the final assay medium is 0.22 μM. Under these conditions greater than a 100% increase in tyrosine hydroxylase activity is observed. However, if slices are incubated in K-KRP (20 mM), or in the presence of dB-cAMP, no significant change in endogenous dopamine is observed and the concentration of dopamine in the assay mixture is not significantly different from control conditions. Yet under identical assay conditions K-KRP (20 mM) and dB-cAMP resulted in a 100% and 120% increase, respectively, in tyrosine hydroxylase activity. These experiments argue strongly against the likelihood that changes in endogenous dopamine levels are responsible for the activation of tyrosine hydroxylase observed in homogenate prepared from slices exposed to high potassium or dB-cAMP.

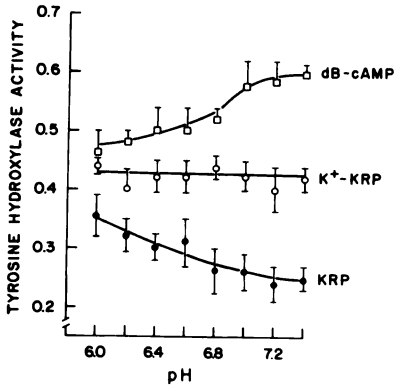

FIG. 5. *Effect of pH on tyrosine hydroxylase isolated from striatal slices exposed to potassium or dB-cAMP*

Striatal slices were incubated for 15 min in KRP, K^+-KRP or KRP containing 1 mM dB-cAMP. Tyrosine hydroxylase was assayed in the 20,000 × *g* supernatant at the indicated pH using Tris-acetate buffer (50 mM). Tyrosine hydroxylase activity is expressed as picomoles dopa per min per mg tissue. Values represent the mean ± S.E.M. of 3-5 determinations.

DISCUSSION

Previous *in vivo* and *in vitro* studies in our laboratory have demonstrated similar-

TABLE 3

Dopamine levels in striatal slices after incubation under different conditions

Slices from each striatum were incubated in the KRP media under indicated conditions for 15 min and then homogenized in 1.0 ml MES buffer, 10 μM, pH 6.0 with 0.2% Triton. After centrifugation at 20,000 × *g* to 0.4 ml of supernatant were added 3.0 ml of TCA 15% and centrifuged again. The supernatant was used for dopamine determination.

	Dopamine	% change	Dopamine in TH assay[a]	Tyrosine hydroxylase activity
	(ng/mg protein)			
nKRP	137 ± 9.0 (6)	0	0.33 μM	8.0 ± 0.9
KRP + K^+ (20 mM)	154 ± 8.0 (4)	12	0.30 μM	15.9 ± 0.3
KRP + K^+ (60 mM)	98 ± 11.0* (4)	−28.5	0.22 μM	17.4 ± 2.5
KRP + dB-cAMP (3 mM)	149 ± 16.0 (4)	8.8	0.34 μM	17.7 ± 2.9

[a] Calculation based on the mg of protein actually measured in the tyrosine hydroxylase assays shown in this table since a fixed volume (50 μl) of striatal supernatant rather than a final amount of protein was added to each assay.

* $p < 0.01$ when compared to slices incubated in normal KRP.

ities between the properties of striatal tyrosine hydroxylase activated by increased impulse flow *in vivo* and exposure of the isolated enzyme to phosphorylation conditions (2, 24). In both cases the isolated enzyme had an increased affinity for substrate and cofactor and a decreased affinity for dopamine. In both *in vivo* and *in vitro* studies there appears to be a short latency period before the enzyme becomes maximally activated. Also, once activated the enzyme remains in this state for a finite period of time. The enzyme activated by either condition (stimulation *in vivo* or addition of cAMP *in vitro*) remains in the activation state following Sephadex treatment (24). However, this manipulation is complicated by the fact that Sephadex treatment also causes an increase in enzyme activity (18, 19). In one study we observed that the enzyme activated by stimulation of the nigrostriatal pathway could not be further activated by addition of optimal concentration of cAMP (2). However, in other studies in which phosphorylation conditions were optimum (addition of ATP, optimal concentrations of Mg^{2+} and in some cases protein kinase), it was observed that the activity of tyrosine hydroxylase activated by neuronal stimulation could be further enhanced by exposure of the isolated enzyme to phosphorylating conditions (Roth, unpublished). Studies in synaptosomes have also demonstrated that the increase in tyrosine hydroxylase activity induced by depolarizaton with veratridine can be enhanced by incubation with dB-cAMP (10). The experiments described in this paper also disclose some similarities, as well as subtle differences between the activation of tyrosine hydroxylase induced by depolarization and those produced by incubation of tissue slices with dB-cAMP. Both treatments require a period of time before tyrosine hydroxylase is maximally activated and once activated, the activated enzyme has a similar time course of inactivation. Differences are also apparent in the two treatments. (a) The activation produced by depolarization appears to be calcium dependent while the activation produced by addition of cAMP to the medium bathing the slices is not dependent upon the presence of calcium in the medium. (b) The enhanced activation produced by depolarization with optimal concentrations of potassium is additive with the activation produced by supra-optimal concentrations of dB-cAMP. (c) the pH optima for the tyrosine hydroxylase isolated from the tissue exposed to high K^+ and to dB-cAMP differs substantially. It is important to note that this difference in pH optima is most apparent when enzyme activity is measured over a broad pH range and in the appropriate buffer system. This latter observation of a different pH optimum of the two activated forms of tyrosine hydroxylase and the fact that treatment with potassium and dB-cAMP results in more than an additive increase in tyrosine hydroxylase activity is difficult to reconcile with the hypothesis that the depolarization induced activation of tyrosine hydroxylase and the activation produced by cAMP occur by a similar process. Since experiments by Lovenberg's group (18) have stressed the importance of the presence of endogenous catecholamines in tissue extracts for demonstration of the activation of tyrosine hydroxylase by phosphorylating conditions we tested to see if changes in endogenous dopamine could be responsible for any of the changes observed in the present experiments. Treatment of slices with dB-cAMP did not result in any significant change in the levels of endogenous dopamine in the slices and thus in the assay medium. Depolarization of slices with 60 mM potassium resulted in a small (about 30%) but significant depletion of endogenous dopamine in slices and a reduction in the concentration of dopamine in the final assay medium from 0.33 μM to 0.22 μM. This small reduction in endogenous dopamine, however, is probably not sufficient to influence tyrosine hydroxylase activity. The K_i for dopamine under the conditions of the assay employed in these experiments is 6 μM (14). However treatment of slices with 20 mM potassium, a condition that resulted in about a 100% increase in tyrosine hydroxylase activity, did not cause any depletion of dopamine. In addition, if striatal dopamine levels were depleted by pretreatment of rats with reserpine, the activation effects induced by

potassium and dB-cAMP were unchanged (Bustos and Roth, to be published). These experiments all support our conclusion that changes in the levels of endogenous dopamine are not responsible for the activation of tyrosine hydroxylase produced by incubation of striatal slices with dB-cAMP or by depolarization of slices in an enriched potassium medium. Experiments are currently in progress in an attempt to elucidate the mechanisms involved in the depolarization-induced activation of tyrosine hydroxylase.

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