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EVIDENCE FOR THE INVOLVEMENT OF Ca^{2+}-CALMODULIN AND CYCLIC AMP IN THE REGULATION OF THE TYROSINE HYDROXYLASE SYSTEM IN RAT STRIATAL TISSUE SLICES

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Abstract—To determine if both the Ca^{2+}-calmodulin system and the cyclic AMP system may regulate tyrosine hydroxylase (TH) activity *in situ*, rat striatal tissue slices that contain all of the components of the TH, cyclic AMP and Ca^{2+}-calmodulin systems were subjected to experimental manipulations. Incubation of striatal tissue slices in a medium containing W-7 [*N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide], a specific inhibitor of calmodulin, resulted in a dose-dependent decrease of DOPA formation. The concentration of W-7 producing 50% inhibition (3×10^{-5} M) of DOPA formation was in good agreement with the binding affinity of W-7 to calmodulin. W-7 did not affect TH activity *in vitro* or cellular cyclic AMP level. A structurally unrelated calmodulin antagonist, trifluoroperazine, also inhibited DOPA formation. On the other hand, incubation of striatal tissue slices in a medium containing dibutyryl cyclic AMP (DBcAMP) increased DOPA formation dose dependently. Kinetic analysis revealed that the enzyme in homogenates of control tissue slices had two different K_m values for a cofactor, 6-methyl-5,6,7,8-tetrahydropterin ($6MPH_4$), indicating the presence of two forms in striatal tissue slices: a less active form with a relatively low affinity for the pterin cofactor and a more active form with a relatively high affinity. W-7 produced an increase of the high K_m form and a decrease of the low K_m form respectively. In contrast, incubation of tissue slices in the presence of DBcAMP resulted in almost complete activation of the enzyme to the low K_m form. These results suggest that both the Ca^{2+}-calmodulin-dependent system and the cyclic AMP-dependent system may regulate TH activity in the rat striatum *in vivo*. The different types of kinetic change produced by DBcAMP and W-7 indicate that the two processes may act in different fashions and that the basal level of catechol formation in striatal tissue slices may be dependent upon an activated form of TH.

There is considerable evidence that the synthesis of catecholamines in neural tissue is subjected to multiple modes of acute regulation. In particular, tyrosine hydroxylase (TH‡, tyrosine 3-monooxygenase, EC 1.14.16.2) [1], which catalyzes the rate-limiting step of catecholamine biosynthesis [2], can be modulated by several distinct mechanisms [3]. One mode of regulation may involve a Ca^{2+}-dependent event [4]. Some studies have demonstrated that the Ca^{2+}-calmodulin system may regulate the activity of TH. Yamauchi and Fujisawa [5] found that rat brainstem TH is activated *in vitro* by incubation with Ca^{2+} in the presence of Mg^{2+} and ATP and that the activation requires the presence of calmodulin. El Mestikawy *et al.* [6] also demonstrated that TH from the rat striatum could be activated by a Ca^{2+}-dependent process, probably by means of calmodulin, and that this activation is not observed when the striatal slices are incubated in K^+-enriched medium. Another mode of regulation may be phosphorylation by cyclic AMP-dependent protein kinase [7–9]. Dibutyryl cyclic AMP (DBcAMP) was demonstrated to activate rat striatal TH system [10–13].

W-7 [*N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide] was first reported to be a vasodilating compound that interacts directly with the actomyosin system from bovine aorta smooth muscle [14]. This compound also inhibits stimulation of cyclic nucleotide phosphodiesterase by calmodulin [15]. It was suggested that this selective inhibitor of calmodulin, W-7, can serve as an effective pharmacological tool for elucidating the regulatory mechanism in muscle contraction. This led us to investigate whether W-7 also inhibits TH activity by interaction with a Ca^{2+}-calmodulin dependent process in striatal slices. Recently, we reported a method for the measurement of TH activity in tissue slices, using high-performance liquid chromatography with electrochemical detection, by estimating DOPA accumulation from endogenous tyrosine in the presence of an inhibitor of aromatic L-amino acid decarboxylase [16]. In this study, we used this slice system to elucidate the regulation of TH in intact cells. Because all of the components of the enzyme system (TH, tetrahydrobiopterin, dihydropteridine reductase, NADH, and NADH-generating system) and the possible regulatory compounds may be present at physiological levels, this tissue slice system is suitable

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‡ Abbreviations: TH, tyrosine hydroxylase; DBcAMP, *N*-6,2′-*O*-dibutyryl cyclic AMP; and $6MPH_4$, 6-methyl-5,6,7,8-tetrahydropterin.

to study the regulation of TH under conditions like those *in vivo*. In addition, we have attempted to characterize the nature of the inactivation produced by W-7 in comparison to the activation produced by DBcAMP and to examine the combined effects of W-7 and DBcAMP. The results suggest that the basal level of catechol formation in striatal tissue slices is dependent upon an activated form of TH.

MATERIALS AND METHODS

Materials. NSD-1055 (brocresine, 4-bromo-3-hydroxybenzyloxyamine dihydrogen phosphate) was obtained from Nakarai Chemicals (Kyoto, Japan); Amberlite CG-50 was from Rohm & Hass (Philadelphia, PA, U.S.A.); aluminium oxide was from Merck (Darmstadt, G.F.R.) and α-methyldopa, 6-methyl-5,6,7,8-tetrahydropterin ($6MPH_4$) and L-tyrosine were from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). DBcAMP and cyclic AMP assay kits were obtained from Yamasa Biochemicals (Tokyo, Japan).

Amberlite CG-50 (type I, 100–200 mesh) was washed by cycling through acid and sodium forms with 2 M HCl and 2 M NaOH and finally with water, equilibrated with 0.4 M potassium phosphate buffer, pH 6.1, and stored as suspension in the same buffer. Aluminium oxide was heated at 200° for 2 hr before use [17] W-7, W-5 [*N*-(6-aminohexyl)-1-naphthalenesulfonamide)] and trifluoroperazine were supplied by Dr. H. Hidaka (Mie University, School of Medicine, Tsu, Japan).

Tolbutamide was supplied by Dr. T. Kanamori (Aichi-Gakuin University, School of Dentistry, Nagoya, Japan).

All other chemicals used were of analytical grade.

The molar concentration of 6-methyl-5,6,7,8-tetrahydropterin ($6MPH_4$) was estimated from the molar extinction coefficient of 14,700 $M^{-1}cm^{-1}$ at 265 nm in 0.1 M HCl.

Preparation of tissue slices and measurement of DOPA formation. Rat striatal tissue slices were prepared as previously described [16]. Briefly, male Wistar rats (200–300 g) were decapitated, and striata were immediately dissected and cut into slices (0.22 mm in thickness) on a McIlwain tissue chopper. The slices were washed extensively before use in Krebs–Ringer bicarbonate medium composed of 118 mM NaCl; 4.7 mM KCl; 2.5 mM $CaCl_2$; 25 mM $NaHCO_3$; 0.1 mM $MgSO_4$; 1.4 mM KH_2PO_4; and 8 mM glucose. The medium was saturated with an atmosphere of 95% O_2/5% CO_2. Incubation was carried out at 37° for 1 hr in 1 ml of Krebs–Ringer medium containing NSD-1055 and the drugs examined. DOPA formation proceeded linearly for 1 hr at 37°. Amounts of DOPA formed from endogenous tyrosine were determined by high-performance liquid chromatography with electrochemical detection (HPLC-ED), as described previously [16].

Kinetic studies of tyrosine hydroxylase. The striatal slices, incubated at 37° for 1 hr in various conditions, were separated from the medium and homogenized with 0.5 ml of 0.32 M sucrose; the homogenates were quickly used for kinetic studies of TH. The enzyme activity was assayed according to the method of Nagatsu *et al.* [18] with slight modifications. The standard incubation mixture consisted of the following components in a total volume of 0.5 ml (final concentrations in parentheses): potassium phosphate buffer, pH 6.3 (0.2 M), 2-mercaptoethanol (0.1 M), catalase (50 μg), $6MPH_4$ (1 mM or at various concentrations for kinetic studies), L-tyrosine (0.1 mM or at various concentrations for kinetic studies), sucrose (32 mM) containing enzyme and water. Incubation was at 37° for 10 min. The reaction proceeded linearly during incubation. For the blank, incubation was carried out without enzyme, and 100 pmoles of DOPA was added to another blank as an internal standard for DOPA.

Measurement of cyclic AMP. The striatal tissue slices, incubated at 37° for 30 min in the conditions indicated, were homogenized by sonication with 1.0 ml of ice-cold 6% trichloroacetic acid (TCA). The extract was centrifuged at 1500 *g* for 10 min at 4°. The resulting supernatant fraction was shaken with 4 ml of water-saturated ether for 1 min, three times. The water phase was lyophilized, dissolved in 0.5 ml of water, and a 0.1-ml aliquot was used for the radioimmunoassay of cyclic AMP [19].

Protein was measured according to the method of Hartree [20], using bovine serum albumin as the standard.

Statistical differences between controls and treated groups were subjected to analysis by Student's *t*-test or the Welch procedure whenever variances were unequal [21].

Enzyme kinetic constants were determined by the weighed linear-regression analysis technique of Wilkinson [22].

RESULTS

The effect of W-7, a specific inhibitor of calmodulin, on the formation of DOPA from endogenous tyrosine was examined. Incubation of striatal slices for 1 hr in the presence of W-7 led to a dose-dependent decrease in DOPA formation (Fig. 1). W-7 had no effect on TH activity *in vitro* when added to crude enzyme at a concentration of 10^{-4} M, whereas DOPA formation was inhibited by 75% at 10^{-4} M in tissue slices. The effect of W-7 on the cyclic AMP level was also examined. When the striatal slices were incubated at 37° for 30 min with 10^{-5} M or 10^{-4} M W-7, there was no change in the cyclic AMP content of the slices. Results (cyclic AMP, pmoles/mg protein) were: control, 4.88 ± 0.47; W-7 at 10^{-5} M, 5.22 ± 0.24; and W-7 at 10^{-4} M, 4.36 ± 0.21.

W-5, an analogue of W-7, which inhibits calmodulin-activated protein kinase at concentrations about ten times higher than W-7 [23], also reduced the DOPA formation at concentrations higher than W-7 (Fig. 1). W-5 had no effect on TH activity *in vitro* at a concentration of 10^{-4} M.

Trifluoroperazine, a structurally unrelated calmodulin antagonist, also reduced the formation of DOPA (70 ± 3% at 10^{-5} M and 41 ± 3% at 10^{-4} M as compared to controls, N = 4).

The effect of W-7 on the activation by depolarization was examined in the slices incubated with 20 mM K^+ at 37° for 30 min. DOPA accumulation in the presence of 20 mM K^+ was 200% of the

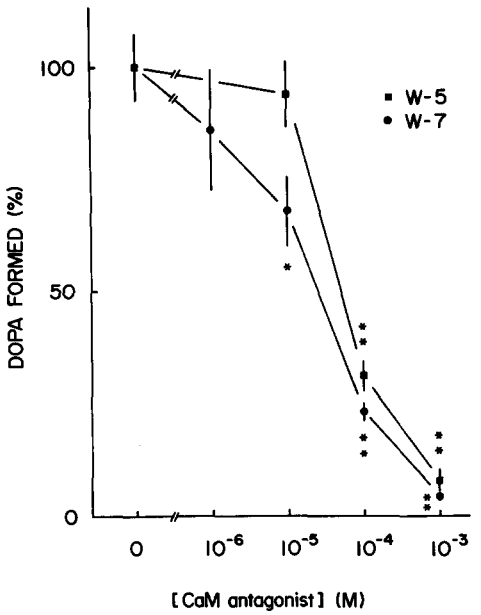

Fig. 1. Effect of W-7 or W-5 on the formation of DOPA in striatal tissue slices of the rat. Each value is the mean (± S.E.M. of four experiments) as a percentage of the mean control level: control (100%) value for W-7, 294.6 ± 21.2 pmoles DOPA formed/hr/mg protein; control value for W-5, 213 ± 25 pmoles DOPA formed/hr/mg protein. Key: (*) $P < 0.05$ and (**) $P < 0.01$ for the difference from control

control, and W-7 at 10^{-4} M inhibited the stimulation by K^+ to 150%.

W-7 might act as a dopamine autoreceptor agonist to decrease DOPA formation as a consequence of stimulating dopamine receptor. Therefore, we also tested the effect of sulpiride, a dopamine receptor antagonist, on W-7-induced inhibition of DOPA formation in the striatal slices. Sulpiride (5×10^{-5} M) did not affect the inhibition of DOPA formation caused by W-7 (5×10^{-5} M).

To determine whether or not DBcAMP stimulates DOPA formation inhibited by W-7, we investigated the effect of DBcAMP in the presence or absence of 10^{-4} M W-7. DBcAMP alone stimulated the formation of DOPA at a concentration of 10^{-3} M, but not of 10^{-4} M. DBcAMP at 10^{-4} stimulated DOPA formation in the presence of 10^{-4} M W-7, and the formation of DOPA under inhibition by 10^{-4} M W-7 was completely recovered to the control level by 10^{-2} M DBcAMP (Fig. 2A).

When the slices were incubated with 10^{-2} M DBcAMP and various concentrations of W-7, the formation of DOPA under activation by DBcAMP was inhibited by 10^{-4} M W-7 but not by 10^{-5} M W-7. DBcAMP at 10^{-2} M recovered DOPA formation to the control level even in the presence of 10^{-3} M W-7, in which condition TH had almost no activity in the tissue slices (Fig. 2B).

Tolbutamide has been reported to inhibit cyclic AMP-dependent protein kinase in rat parotid glands [24]. Tolbutamide at 9.5 mM did not affect the con-

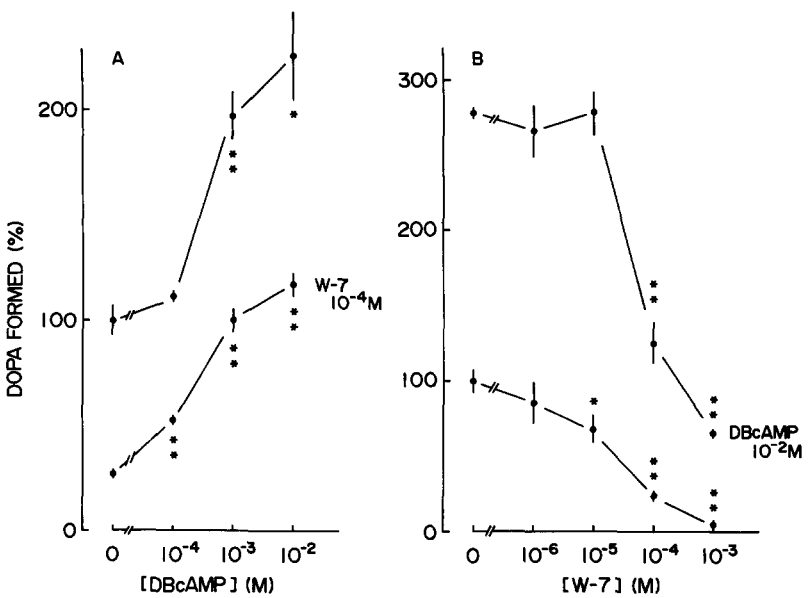

Fig. 2. (A) Effect of dibutyryl cyclic AMP (DBcAMP) on the formation of DOPA in striatal tissue slices of the rat in the absence or presence of W-7. Each value is the mean (± S.E.M. of four experiments) as a percentage of the mean control level: control (100%) values were 295.1 ± 19.4 (for the experiment with W-7) and 219.4 ± 4.0 (for the experiment without W-7) pmoles DOPA formed/hr/mg protein. Key: (*)$P < 0.05$ and (**)$P < 0.01$ for the difference from each control. (B) Effect of W-7 on the formation of DOPA in striatal tissue slices of the rat in the absence or presence of DBcAMP. Each value is the mean (± S.E.M. of four experiments) as a percentage of the mean control level: control (100%) values were 294.6 ± 21.2 (in the absence of DBcAMP) and 245.2 ± 16.4 (in the presence of DBcAMP) pmoles DOPA formed/hr/mg protein. Key: (*)$P < 0.05$ and (**)$P < 0.01$ for the difference from each control.

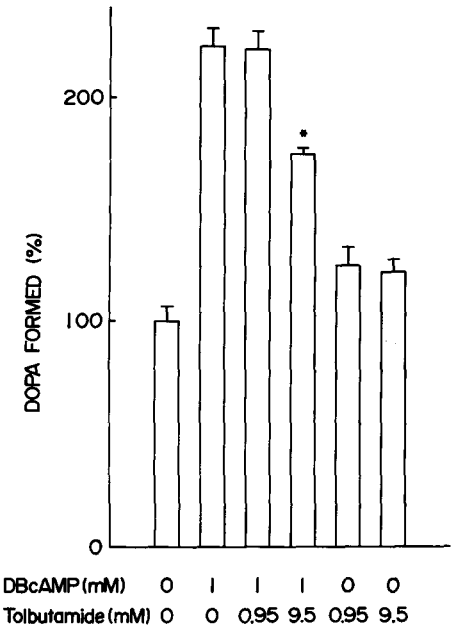

Fig. 3. Effect of tolbutamide on DBcAMP-stimulated DOPA formation in striatal tissue slices of the rat. Each value is the mean ± S.E.M. of four experiments; control value was 205.1 ± 13.1 pmoles DOPA formed/hr/mg protein. Key: (*)$P < 0.05$, compared to slices incubated with DBcAMP.

trol level of DOPA formation, but partially inhibited DBcAMP (10^{-3} M) induced increase of DOPA formation (Fig. 3).

The kinetic properties of the enzyme were studied using homogenates of striatal tissue slices incubated in the control medium and in the medium containing DBcAMP or W-7. The striatal TH incubated in the control medium did not obey normal Michaelis–Menten kinetics toward $6MPH_4$. As shown in Fig. 4, two different apparent K_m values were obtained: one relatively lower apparent K_m (0.24 mM) for $6MPH_4$ and the other relatively higher apparent K_m (1.8 mM) for the cofactor. In TH from the striatal slices incubated with W-7, the same, two different, apparent K_m values as in controls were observed, but the fraction with high K_m values was increased, and the fraction with low K_m values was decreased. The results suggest the conversion of the low K_m form to the high K_m form. Pretreatment of the tissue slices in the medium with DBcAMP showed normal Michaelis–Menten kinetics, and the enzyme exhibited a single, low apparant K_m value (0.13 mM) (Fig. 4).

On the other hand, the Lineweaver–Burk plots for tyrosine gave a straight line as shown in Fig. 5. Incubation of striatal tissue slices in a medium with W-7 resulted in a significant decrease in the apparent V_{max} without a significant change in the apparent K_m for tyrosine. In the enzyme isolated from striatal tissue slices incubated in a DBcAMP medium, the apparent V_{max} increased without significant change in the apparent K_m for tyrosine.

DISCUSSION

TH has been demonstrated to be activated *in vitro* under phosphorylating conditions by cyclic AMP-dependent protein kinase [7–9] and by Ca^{2+}-calmodulin-dependent protein kinase [5]. However, it is not yet clear whether the activation of TH by the cyclic AMP system and/or by the Ca^{2+}-calmodulin system operates *in vivo*.

The present results demonstrate that the calmodulin antagonists W-7, W-5, or trifluoroperazine decreased the rate of DOPA formation in striatal tissue slices. The inhibition by a calmodulin antagonist seemed to be mediated by the Ca^{2+}-calmodulin system for the following reasons. (1) The concentration of W-7 or W-5 producing 50% inhibition of basal TH activity in tissue slices was in good agreement with the binding affinity of W-7 or W-5

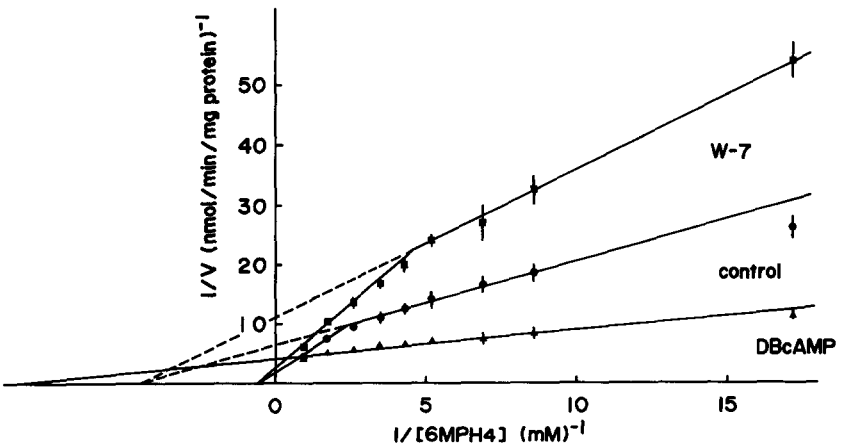

Fig. 4. Lineweaver–Burk plots of enzyme activity vs 6-methyl-5,6,7,8-tetrahydropterin ($6MPH_4$) concentration for soluble TH prepared from rat striatal tissue slices that had been incubated for 1 hr in the presence of 10 mM DBcAMP or 1 mM W-7. Results are means ± S.E.M. of four experiments.

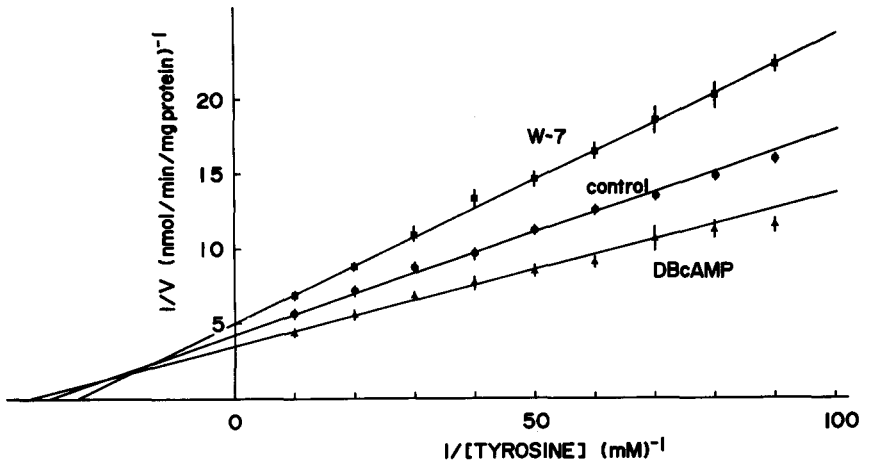

Fig. 5. Lineweaver–Burk plots of enzyme activity vs tyrosine concentration for soluble TH prepared from rat striatal tissue slices that had been incubated for 1 hr in the presence of 10 mM DBcAMP or 1 mM W-7. Results are means ± S.E.M. of four experiments.

to calmodulin [23]. Both W-7 and W-5 inhibited DOPA formation, but the concentration of W-5 that produced significant inhibition was higher than that of W-7. This finding is consistent with the observation that W-5 is about ten times less effective than W-7 with respect to the concentration needed for 50% inhibition of the binding to calmodulin [23]. (2) Since W-7 at a concentration of 10^{-4} M had no effect on TH activity in cell-free preparations *in vitro* or on the cyclic AMP level in striatal tissue slices, the possibility that the W-7-induced inhibition of TH in tissue slices was due to direct inhibition of TH or to reduction of cellular cyclic AMP level can be ruled out. If W-7 were interfering with a normal effect of calmodulin on the activation of a phosphodiesterase, an increase in cyclic AMP would have been detected, but the cyclic AMP level did not change. The reason is not clear. (3) Sulpiride, a dopamine receptor antagonist, had no effect on the inhibition of DOPA formation caused by W-7. Therefore, it seems unlikely that W-7 acts as a dopamine receptor agonist to decrease DOPA formation as a consequence of stimulating presynaptic receptors. (4) W-7 was not metabolized in tissue slices during short incubation times (37° 1 hr) (H. Hidaka, personal communication). Thus, W-7 might not be changed to metabolites that inhibit TH activity. (5) Okumura-Noji and Tanaka [25] have reported recently that W-7 at 2×10^{-5} M produces only a small increase of [^{3}H] DA release. Thus, the possibility that W-7 might act as tyramine to release dopamine from vesicular storage sites can be ruled out.

These results indicate that W-7, a calmodulin antagonist, inhibits DOPA formation in striatal tissue slices by direct interaction with the Ca^{2+}-calmodulin system and that the Ca^{2+}-calmodulin system may operate to maintain the basal level of catechol formation in striatal tissue slices which is dependent upon an activated form of TH.

In accord with this result, there have been some studies which have shown that Ca^{2+} and calmodulin may regulate TH activity. In the cell-free *in vitro* system, TH is activated under Ca^{2+}-calmodulin phosphorylating conditions in the presence of an activator protein [26]. Bustos *et al.* [27] have reported that incubation of striatal slices in a sodium-free medium results in an increase in TH activity in the high speed supernatant fraction of homogenates prepared from the slices. Since this condition has been shown to increase the intraneuronal concentration of free Ca^{2+} [28], these observations were interpreted as the activation of TH mediated by a rise in the free intraneuronal Ca^{2+} concentration. Moreover, it has been reported that TH prepared from striatal slices depolarized by high K^+ could not be further stimulated by Ca^{2+}-dependent phosphorylating conditions [6].

Although our slice system is useful to assess TH activity under physiological conditions, the basal activity of TH in striatal tissue slices may not exactly reflect the resting state of TH activity in dopaminergic neurons. The work on DOPA accumulation *in vivo* shows that not only is hydroxylation of tyrosine, measured by DOPA accumulation, enhanced in response to electrical stimulation of dopamine neurons, but DOPA accumulation also increases in striatal terminals when impulse-flow is blocked (surgically or with γ-butyrolactone) [29]. Thus, the question arises as to what basal enzyme activity represents in striatal tissue *in vivo* or in tissue slices. There is the possibility that TH activity in control striatal slices is in an activated state and is similar to the *in vivo* condition when impulse-flow is blocked.

We examined the effect of depolarization by high K^+ in the absence and presence of W-7. High K^+ activated DOPA formation, and this increase was blocked by W-7. This result agrees with that by El Mestikawy *et al.* [6]. On the other hand, the present result also indicates that Ca^{2+}-calmodulin dependent phosphorylation may maintain the "basal" enzyme activity.

The relative importance of the Ca^{2+}-calmodulin system in comparison to the cyclic AMP-dependent system for regulating TH activity is not known. As shown in Fig. 2A, W-7 decreased basal TH activity

in the absence of DBcAMP. The inhibitory effect of W-7 in the presence of DBcAMP can therefore be interpreted as being largely due to the decrease in basal DOPA synthesis which may be mediated by the Ca^{2+}-calmodulin-dependent system.

As shown in Fig. 2B, W-7 at a concentration of 10^{-5} M inhibited the formation of DOPA in the absence of DBcAMP but not in the presence of DBcAMP. This result suggests an interrelation between the cyclic AMP-dependent process and the Ca^{2+}-calmodulin-dependent process. It is possible that DBcAMP may increase the intracellular level of calmodulin and counteract the inhibitory effect of W-7. Indeed, it has been reported that a purified cAMP-dependent protein kinase, in the presence of ATP and cAMP, stimulates the release of the endogenous phosphodiesterase activator, calmodulin, from the particulate fraction of rat brain and adrenal medulla [30].

The observation that tolbutamide at 9.5 mM inhibited the activation induced by DBcAMP without affecting basal activity (Fig. 3) suggests that the effect of DBcAMP may be mediated by cyclic AMP-dependent protein kinase, and that the cyclic AMP-dependent system may play a minor role in a basal state, at least under our control conditions in striatal slices.

Kinetic analysis (Fig. 4) suggests that TH present in rat striatal tissue slices that are incubated in the control medium exists in two forms: a less active form with a relatively low affinity for a pterin cofactor and a more active form with a relatively high affinity. Incubation of slices in a medium containing a calmodulin antagonist, W-7, resulted in an increase in the less active form with a relatively low affinity for a pterin cofactor. This may indicate that inhibition of the Ca^{2+}-calmodulin system decreases the active form and that the Ca^{2+}-calmodulin system is operating to activate TH in the basal state of striatal slices. On the other hand, incubation of slices in the presence of DBcAMP resulted in almost complete activation of the enzyme to the highest affinity form. This activation of TH was also asssociated with an increase in the V_{max} of the enzyme for the pterin cofactor. Hamon's group [6] also demonstrated both a Ca^{2+}-calmodulin activation and a cyclic nucleotide activation and different kinetic effects, as V_{max} and K_m activation respectively. Thus, the present data confirm the earlier papers. The data regarding the K_m and V_{max} values for $6MPH_4$ do not reveal a significant effect of any treatment on the V_{max} and only show a convincing effect of DBcAMP on the K_m of the enzyme. One reason for this may be having chosen to measure the enzyme activity at pH 6.3 instead of at pH 7.0. Several groups have demonstrated that the activation is not adequately expressed when the enzyme is measured at the more acidic pH optimum [13, 31]. Thus, increase in V_{max} by DBcAMP may be even greater when measured at pH 7.0.

The kinetic results also indicate that the activated form by the cyclic AMP system may have a higher affinity for the pterin cofactor than the active form present in control tissue slices. This suggests that the mechanism of activation by the cyclic AMP system and the Ca^{2+}-calmodulin system may be different. If both cyclic AMP and Ca^{2+}-calmodulin activate TH by phosphorylation, the sites of phosphorylation could be different. The existence of interconvertible states of the enzyme is reported by Weiner *et al.* [32], Togari *et al.* [33], and Bustos *et al.* [27]. Weiner *et al.* [32] have demonstrated that TH in the adrenergic neurons of the vas deferens preparation appears to exist in two forms and that both nerve stimulation and incubation of intact vas deferens in the presence of 8-methylthio cyclic AMP result in similar kinetic changes in the soluble enzyme which is prepared from these organs. However, the observations of several laboratories have questioned the physiological significance of DBcAMP-induced activations of the enzymes for mediating depolarization-induced increases in enzyme activity [6]. Thus, the relevance of the finding to activate TH by incubating slices in the presence of DBcAMP to physiological conditions, e.g. neuronal firing, remains uncertain.

As shown in Fig. 5, TH from control tissue slices and from slices incubated with W-7 or DBcAMP had similar K_m values for tyrosine, indicating that neither inhibition by a calmodulin antagonist nor activation by DBcAMP changed the affinity of TH for tyrosine significantly.

Although what is known of the regulation of TH activity in cell-free preparations seems to be similar to regulation in slices, a difference must be considered between enzyme from striatal dopaminergic terminals versus enzyme from noradrenergic neurons (e.g. vas deferens). It remains uncertain whether these enzymes are regulated in the same manner *in vivo* under all circumstances.

In conclusion, TH in striatal tissue slices under conditions similar to physiological state may be regulated by the cyclic AMP-dependent system and by the Ca^{2+}-calmodulin system. The Ca^{2+}-calmodulin system may result in interconversion from a less active form to an active form in a basal state, and the cyclic AMP system may maximally activate TH. The basal level of catechol formation in striatal tissue slices is dependent upon an activated form of TH.

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REFERENCES

1. T. Nagatsu, M. Levitt and S. Udenfriend, *J. biol. Chem.* **239**, 2910 (1964).
2. M. Levitt, S. Spector, A. Sjoerdsma and S. Udenfriend, *J. Pharmac. exp. Ther.* **148**, 1 (1965).
3. T. Nagatsu, in *Advances in Experimental Medicine: A Centenary Tribute to Claude Bernard* (Eds. H. Parvez and S. Parvez), p. 527. Elsevier/North-Holland Biochemical Press, New York (1980).
4. V. H. Morgenroth, III, M. C. Boadle-Biber and R. H. Roth, *Molec. Pharmac.* **11**, 427 (1975).
5. T. Yamauchi and H. Fujisawa, *Biochem. Int.* **1**, 98 (1980).

6. S. El Mestikawy, J. Glowinski and M. Hamon, *Nature, Lond.* **302**, 830 (1983).
7. T. H. Joh, D. H. Park and D. J. Reis, *Proc. natn. Acad. Sci. U.S.A.* **75**, 4744 (1978).
8. T. Yamauchi and H. Fujisawa, *J. biol. Chem.* **254**, 503 (1979).
9. A. M. Edelman, J. D. Raese, M. A. Lazar and J. D. Barchas, *J. Pharmac. exp. Ther.* **216**, 647 (1981).
10. B. Anagnoste, C. Shirron, E. Friedman and M. Goldstein, *J. Pharmac. exp. Ther.* **191**, 370 (1974).
11. M. Goldstein, R. L. Bronaugh, B. Ebstein and C. Roberge, *Brain Res.* **109**, 563 (1976).
12. R. L. Patrick and J. D. Barchas, *J. Pharmac. exp. Ther.* **197**, 97 (1976).
13. J. R. Simon and R. H. Roth, *Molec. Pharmac.* **16**, 224 (1979).
14. H. Hidaka, M. Asano, S. Iwadare, I. Matsumoto, T. Totsuka and N. Aoki, *J. Pharmac. exp. Ther.* **207**, 8 (1978).
15. H. Hidaka, T. Yamaki, T. Totsuka and M. Asano, *Molec. Pharmac.* **15**, 49 (1979).
16. Y. Hirata, A. Togari and T. Nagatsu, *J. Neurochem.* **40**, 1585 (1983).
17. T. Nagatsu, K. Oka, Y. Numata (Sudo) and T. Kato, *Analyt. Biochem.* **93**, 82 (1979).
18. T. Nagatsu, K. Oka and T. Kato, *J. Chromat.* **163**, 247 (1979).
19. M. Honma, T. Satoh, J. Takezawa and M. Ui, *Biochem. Med.* **18**, 257 (1977).
20. E. F. Hartree, *Analyt. Biochem.* **48**, 422 (1972).
21. B. L. Welch, *Biometrika* **34**, 28 (1947).
22. G. N. Wilkinson, *Biochem. J.* **80**, 324 (1961).
23. H. Hidaka, M. Asano and T. Tanaka, *Molec. Pharmac.* **20**, 571 (1981).
24. T. Kanamori, T. Hayakawa and T. Nagatsu, *Biochem. biophys. Res. Commun.* **57**, 394 (1974).
25. K. Okumura-Noji and R. Tanaka, *Neurochem. Int.* **6**, 91 (1984).
26. T. Yamauchi, H. Nakata and H. Fujisawa, *J. biol. Chem.* **256**, 5404 (1981).
27. G. Bustos, J. Simon and R. H. Roth, *J. Neurochem.* **35**, 47 (1980).
28. W. L. Stahl and P. D. Swanson, *J. Neurochem.* **19**, 2335 (1972).
29. R. H. Roth, J. R. Walters and G. K. Aghajanian, in *Frontiers in Catecholamine Research* (Eds. S. H. Snyder and E. Costa), p. 567. Pergamon Press, New York (1973).
30. M. E. Gnegy, E. Costa and P. Uzunov, *Proc. natn. Acad. Sci. U.S.A.* **73**, 354 (1976).
31. M. A. Lazar, A. J. Lockfeld, R. J. W. Truscott and J. D. Barchas, *J. Neurochem.* **39**, 409 (1982).
32. N. Weiner, F-L. Lee, E. Dreyer and E. Barnes, *Life Sci.* **22**, 1197 (1978).
33. A. Togari, T. Kato and T. Nagatsu, *Biochem. Pharmac.* **31**, 1729 (1982).