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DOPAMINERGIC NEURONS—ALTERATION IN THE KINETIC PROPERTIES OF TYROSINE HYDROXYLASE AFTER CESSATION OF IMPULSE FLOW*

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Abstract—Studies *in vitro* conducted on striatal tyrosine hydroxylase have demonstrated that addition of calcium chelators, such as EGTA, results in an alteration in the kinetic properties of tyrosine hydroxylase which can be completely reversed by addition of calcium to the incubation medium. Inhibition of impulse flow in the nigro-neostriatal pathway by either pharmacological or mechanical techniques causes the isolated striatal tyrosine hydroxylase to behave kinetically like the enzyme treated *in vitro* with EGTA. Tyrosine hydroxylase, isolated from the striatum of rats in which impulse flow has been interrupted in the dopamine pathways, has an increased affinity for both substrate and pterin cofactor and a reduced affinity for the end-product inhibitor, dopamine. The latter change is most dramatic; the K_i of the enzyme for dopamine increases more than 700-fold. These alterations in the kinetic properties of tyrosine hydroxylase can also be completely reversed by addition of calcium to the incubation medium. In rats with a pharmacological blockade of impulse flow induced by administration of γ-hydroxybutyrate, subsequent administration of dopamine receptor stimulants causes the isolated tyrosine hydroxylase to have properties similar to those observed for the enzyme prepared from untreated rats. Administration of dopamine receptor blockers prevents the dopamine receptor stimulants from altering the kinetic changes in tyrosine hydroxylase induced by a cessation of impulse flow. These data are discussed in terms of the possible role presynaptic receptors and calcium fluxes may play in the short-term regulation of tyrosine hydroxylase.

When impulse flow is interrupted in dopamine neurons a paradoxical response is observed. Cessation of impulse flow in the nigro-neostriatal pathway induced either pharmacologically or by placement of a lesion in the median forebrain bundle results in a decrease in the release and catabolism of dopamine and a simultaneous increase in dopamine biosynthesis [1–6]. These changes are associated with a dramatic increase in the steady-state level of dopamine in the neuronal terminal network [3]. Once this new steady state level of dopamine is established, the rate of dopamine biosynthesis returns to normal but is not inhibited as it is when the dopamine levels are elevated by treatment with monoamine oxidase inhibitors [4, 5, 7]. The fact that dopamine synthesis is increased while dopamine levels are rising suggests that tyrosine hydroxylase activity in dopaminergic neurons is regulated by mechanisms other than that of end-product inhibition.

Dopamine receptor stimulating agents alter the increase in tyrosine hydroxylase activity observed after acute inhibition of impulse flow [4, 5, 7–9]. This has led to the suggestion that dopamine receptors may play a direct role in the control of dopamine biosynthesis [4, 5, 8]. As demonstrated in the preceding paper [7], drugs which are capable of stimulating dopamine receptors directly or indirectly, such as apomorphine, ET-495 or *d*-amphetamine, prevent the increase in dopamine and tyrosine hydroxylase activity observed after inhibition of impulse flow by administration of γ-butyrolactone. These effects can be nullified by treatment with dopamine receptor blocking agents [7]. Thus drugs which are known to affect dopamine receptors have been shown capable of changing dopamine synthesis via some mechanism which is not dependent upon alterations in impulse flow. The possibility that these drugs may be interacting with a presynaptic dopamine receptor, which in some way alters the activity of tyrosine hydroxylase, has been suggested [4, 5, 8, 9]. If such were the case, decreased levels of dopamine in the synaptic cleft during inhibition of impulse flow might be responsible for triggering the increase in the activity of tyrosine hydroxylase. The reversal of this effect, produced by administration of dopamine receptor stimulating agents, might be due to the ability of these drugs to interact with a presynaptic dopamine receptor. The actual mechanism by which changes in dopaminergic receptor activity can influence tyrosine hydroxylase activity is unknown. However, it is conceivable that these receptors could modulate ion fluxes into the nerve terminal, which in turn might influence tyrosine hydroxylase activity.

Recent studies *in vitro* on striatal tyrosine hydroxylase have indicated that the enzyme isolated from this

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source is allosterically activated by removal of Ca^{2+} ions [9, 10]. Calcium removal by addition of EGTA increases the affinity of tyrosine hydroxylase for both substrate tyrosine and artificial cofactor 6,7-dimethyl tetrahydropterin ($DMPH_4$), while markedly decreasing the affinity of the enzyme for the natural feedback inhibitor, dopamine [9–11]. These results suggest that during a cessation of impulse flow when calcium influx and transmitter release are blocked, the alteration in availability of intracellular calcium might result in an allosteric change in tyrosine hydroxylase ultimately resulting in an increase in tyrosine hydroxylase activity. The following experiments were designed to test this hypothesis.

MATERIALS AND METHODS

Lesion. Male Sprague–Dawley rats (250–300 g) obtained from Charles River Breeding Labs were used in all experiments. Unilateral electrothermic lesions were made in the nigro-neostriatal pathway under halothane anesthesia at the level of the rostral end of the supramamillary decussation essentially as described by Hökfelt and Ungerstedt [12] and modified by Walters *et al.* [3]. The location and extent of the lesion was verified histologically. The lesion was spherical, approximately 1·5 mm in diameter and included the medial forebrain bundle, part of the Fields of Forel and the medial edge of the crus cerebri.

Drug treatments. Drugs were administered intraperitoneally (i.p.). Gamma-butyrolactone (GBL; Matheson, Coleman & Bell) was used in preference to the sodium salt of gamma-hydroxybutyric acid (GHB), since it is more rapidly and uniformly absorbed after intraperitoneal injection. Other drugs used were apomorphine HCl and chlorpromazine (Smith, Kline & French), ET-495 [1-(2″-pyrimidyl)-piperonyl-piperazine, trivastal, Laboratories Servier] and haloperidol (McNeil Laboratories). Thirty min after electrothermic lesion or at 90 min after GBL administration, animals were killed by decapitation. The striata were rapidly dissected over ice as described by Bunney *et al.* [13], frozen on dry ice, weighed and kept at −70° until the time of assay. Striata were either assayed singly or pooled for determination of tyrosine hydroxylase kinetics.

At the time of assay, striata were homogenized (ten strokes) in 10 vol. of ice-cold 0·05 M Tris–acetate buffer, pH 6·0 in an all-glass homogenizer (Duall Grinder, Kontes Glass Co.) with a clearance of 0·010 cm. The homogenate was then centrifuged at 105,000 *g* for 90 min and the clear supernatant decanted. The supernatant served as the source of the soluble tyrosine hydroxylase.

Assay. Tyrosine hydroxylase activity was assayed according to the method of Shiman *et al.* [14] with minor modifications as described in detail elsewhere [10, 11]. The reaction was carried out in a total volume of 1 ml containing 100 μl supernatant. Dihydropteridine reductase, purified through the first ammonium sulfate fractionation according to the method of Kaufman [15], was added in 4- to 10-fold excess (100 μl containing 2·6 mg protein). Each reaction mixture contained 3300 units catalase in 200 μl of glass-distilled water and 1·0 mM NADPH. Catalase, NADPH and ultrapure Tris base were purchased from Schwarz Mann. $DMPH_4$ was obtained from Calbiochem. 3,4-Dihydroxyphenylethylamine and ethyleneglycol-bis-(β-amino-ethyl ether)*N*,*N*′-tetra-acetic acid (EGTA) were purchased from Sigma Chemical Co. L-[3,5-^{3}H]tyrosine (30 Ci/m-mole) was obtained from New England Nuclear and purified according to the method of Coyle [16]. All other reagents were of the maximal obtainable purity. The activity of tyrosine hydroxylase was expressed as pmoles DOPA/mg of protein/min. Protein was determined according to the method of Lowry *et al.* [17] using bovine serum albumin as a standard. Data were analyzed using a paired *t*-test and kinetic data were calculated according to the method of Wilkinson [18].

RESULTS

The effect of a lesion in the nigro-neostriatal pathway on the activity of striatal tyrosine hydroxylase is shown in Table 1. Thirty min after the lesion was made, a marked (4-fold) increase in the activity of tyrosine hydroxylase was observed on the side of the lesion, as compared to the control side. This increase in activity was completely reversed by the addition of 50 μM calcium chloride to the reaction mixture prior to incubation. The addition of 10 μM EGTA to the homogenate obtained from the non-lesioned control side caused an increase in tyrosine hydroxylase activity similar to that seen in the tyrosine hydroxylase isolated from the striatum on the side with the lesion. EGTA was without further effect on the enzyme isolated from the striatum on the lesioned side (Table 1).

The kinetics of the tyrosine hydroxylation in homogenates of striatum were determined after the placement of the lesion in the nigro-neostriatal pathway (Table 2). The K_m for tyrosine was decreased 7-fold from 55·3 to 7·8 μM. The K_m for synthetic cofactor $DMPH_4$ was decreased 8-fold from 0·91 to 0·11 mM, and the K_i for DA was increased nearly 700-fold, from 0·11 to 75·6 mM.

It has been shown that GBL, a drug which blocks impulse flow in the nigro-neostriatal pathway, and acute lesion of the nigro-neostriatal cause similar increases in the levels of dopamine in the neostriatum

Table 1. Effect of acute lesion of the nigro-neostriatal pathway on the tyrosine hydroxylase activity of the neostriatum*

Additions	Tyrosine hydroxylase activity	
	Control side	Lesioned side
None	22·9 ± 3·2	87·2 ± 6·1
Calcium (50 μM)	23·3 ± 2·6	21·5 ± 1·9
EGTA (10 μM)	79·4 ± 8·1	87·9 ± 4·2

* Results are expressed as pmoles of DOPA/mg of protein/min + S. E. M. of triplicate determinations of individual striata obtained from six animals. The animals were sacrificed 30 min after placement of a lesion in the median forebrain bundle. Tyrosine hydroxylase activity was determined *in vitro* as described in Methods. Calcium chloride and EGTA were added to the incubation medium 5 min before initiation of the reaction by addition of labeled tyrosine.

Table 2. Effect of acute lesion of the nigro-neostriatal pathway on the kinetics of striatal tyrosine hydroxylase*

Kinetic parameter	Control side	Lesioned side
K_m Tyrosine (μM)	55·3 ± 4·2	7·8 ± 1·1
K_m $DMPH_4$ (mM)	0·91 ± 0·04	0·12 ± 0·01
K_i DA (mM)	0·11 ± 0·01	75·6 ± 2·3

* K_m values for tyrosine and $DMPH_4$ were determined by the method of Lineweaver and Burk [19] at six substrate concentrations with six separate determinations at each substrate concentration. The K_i for dopamine was determined by the method of Dixon [20] at six dopamine concentrations and three $DMPH_4$ concentrations. Tyrosine hydroxylase was obtained from the 105,000 *g* supernatant of six pooled striata. Statistics were determined by the method of Wilkinson [18].

[3]. These increases are accompanied by an increase in the accumulation of DOPA following inhibition of DOPA decarboxylase, suggesting an apparent increase in the activity of tyrosine hydroxylase [2, 4, 5]. In order to determine whether GBL has the same effect as mechanical inhibition of impulse flow on soluble tyrosine hydroxylase activity, rats were pretreated with GBL (750 mg/kg) 90 min prior to sacrifice. The effect of GBL on tyrosine hydroxylase activity is shown in Table 3. GBL caused a 4-fold increase in the activity of striatal tyrosine hydroxylase. This increase was indistinguishable from that caused by electrothermic lesion of the median forebrain bundle in that both increases were readily reversible by the addition of calcium to the incubation medium (cf. Tables 1 and 3).

The K_m for tyrosine following the administration of GBL is shifted 6-fold from 53·9 to 8·9 μM (Fig. 1). This decrease in K_m caused by GBL pretreatment is also readily reversed by the addition of calcium to the incubation medium. The effect of GBL on $DMPH_4$ kinetics is shown in Table 4. GBL caused a 6-fold decrease in the K_m from 0·88 to 0·13 mM.

Table 3. Effect of GBL on striatal tyrosine hydroxylase activity*

Treatment	N	Tyrosine hydroxylase activity (pmoles DOPA/mg protein/min)
Saline	8	21·6 ± 2·7
GBL (750 mg/kg)	8	83·3 ± 5·1
GBL (750 mg/kg) + 50μM calcium†	8	21·1 ± 3·6

* Eight male Sprague Dawley rats (275–300 g) were pretreated with GBL (750 mg/kg) or saline 90 min prior to sacrifice. Striata were removed, frozen on dry ice and assayed for tyrosine hydroxylase activity. Tyrosine hydroxylase activity is expressed as pmoles DOPA/mg of protein/min ± S. E. M. of triplicates of each sample.

† Calcium chloride was added to the supernatant obtained from the GBL-treated rats after a 5-minute preincubation period at 37°.

This activation could also be readily reversed by addition of calcium to the incubation mixture.

Since electrothermic lesion caused a marked change in the affinity of striatal tyrosine hydroxylase for the feedback inhibitor, dopamine, we investigated the effects of GBL administration on the kinetics of dopamine inhibition of tyrosine hydroxylase. GBL causes a 730-fold increase in the K_i for dopamine, making the striatal tyrosine hydroxylase isolated from the GBL-pretreated animals almost completely insensitive to inhibition by dopamine (Fig. 2). This alteration in K_i is also reflected by the failure of other catechol-type inhibitors to inhibit tyrosine hydroxylase isolated from striata of GBL-pretreated animals. α-Propyldopacetamide (H-22/54) at a concentration of 20 μM caused a 55 ± 6 per cent (mean ± S. E. M., N = 12) inhibition of striatal tyrosine hydroxylase from untreated control animals, whereas the same concentration of inhibitor caused only an 18 ± 4 per cent (N = 12) inhibition of striatal tyrosine hydroxylase isolated from GBL-pretreated rats.

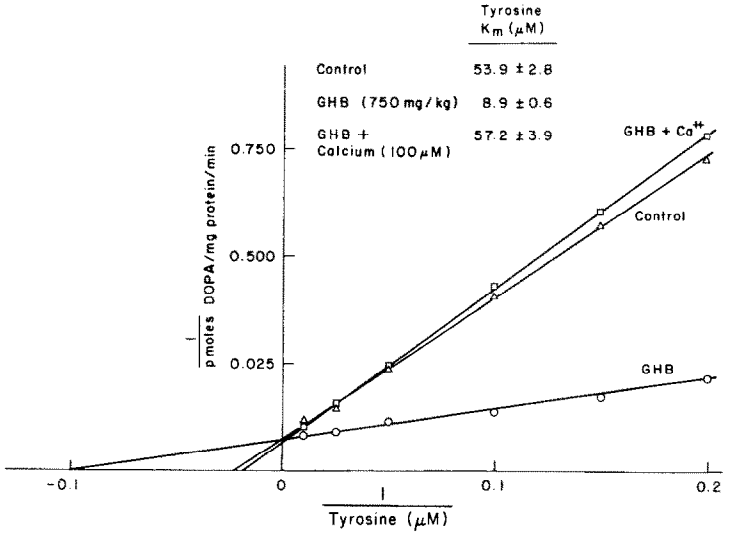

Fig. 1. Effect of GBL administration on the K_m for tyrosine of tyrosine hydroxylase isolated from the rat striatum. The K_m for tyrosine was determined according to the method of Lineweaver and Burk [19] at a $DMPH_4$ concentration of 1·0 mM and six tyrosine concentrations ranging from 5 to 100 μM. Each K_m value is the mean ± S. E. M. of the intercepts generated from six separate lines.

Table 4. Effects of GBL on the kinetic properties of striatal tyrosine hydroxylase for pterin cofactor*

Treatment	K_m $DMPH_4$	V_{max}
Control	0·88 ± 0·12	107·5
GBL (750 mg/kg)	0·13 ± 0·04	117·3
GBL (750 mg/kg) + calcium† (100 μM)	0·83 ± 0·09	123·3

* The K_m and V_{max} for $DMPH_4$ were determined by the method of Lineweaver and Burk [19] using six concentrations of $DMPH_4$ ranging from 1 to 0·01 mM and a tyrosine concentration of 0·1 mM. Each value is the mean of the intercepts generated from six separate lines. V_{max} results are expressed in terms of pmoles of DOPA/mg of protein/min.

† Calcium chloride was added to the supernatant obtained from GBL-treated rats after a 5-minute preincubation period at 37°.

Similar kinetic activations of tyrosine hydroxylase were observed in the enzyme isolated from olfactory tubercles of rats pretreated with GBL (Table 5). However, no significant alteration in tyrosine hydroxylase activity was observed in the median eminence following GBL pretreatment.

Studies *in vivo* have shown that dopamine agonists are able to block and reverse the apparent insensitivity of tyrosine hydroxylase to inhibition by endogenous dopamine observed after cessation of impulse flow [5, 7]. To determine whether these drugs also reversed the kinetic changes in tyrosine hydroxylase caused by decreased impulse flow, animals were injected with GBL 90 min prior to sacrifice. Forty min prior to sacrifice, they were given either saline, ET-495 (10 mg/kg) or apomorphine (2 mg/kg). The striata were removed and tyrosine hydroxylase activity and kinetics were determined on the high-speed supernatant. The results are summarized in Table 6. Both DA-receptor agonists, ET-495 and apomorphine, reversed the activation of striatal tyrosine hydroxylase resulting from inhibition of impulse flow. The reversal of this activation by the dopamine agonist pretreatment was indistinguishable from the reversal obtained by addition of calcium to the incubation medium *in vitro* (Fig. 1). The dopamine agonists had no effect on tyrosine hydroxylase activity when added directly to the incubation medium even in relatively high concentrations. The ability of apomorphine to reverse the GBL-induced activation of tyrosine hydroxylase was blocked by the administration of chlorpromazine (10 mg/kg) or haloperidol (1 mg/kg) (Table 7). The enzyme prepared from animals treated with GBL, apomorphine and an antipsychotic drug such as haloperidol was still susceptible to the calcium reversal *in vitro* (data not shown).

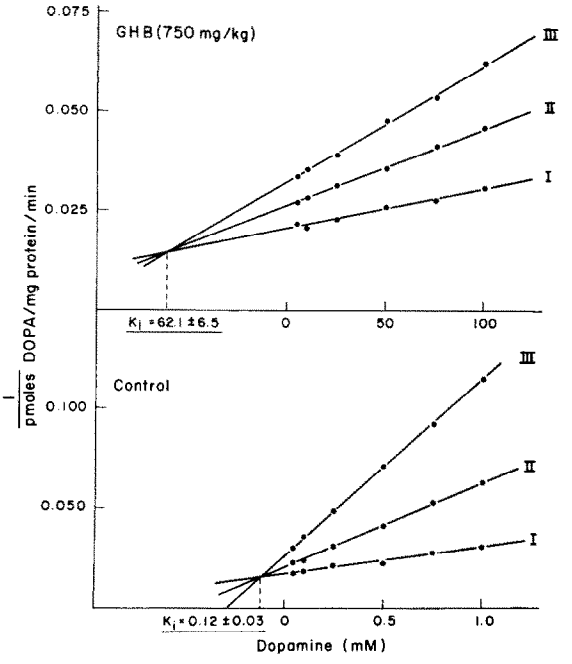

Fig. 2. Effect of GBL administration on the K_i for dopamine of tyrosine hydroxylase isolated from the rat striatum. The K_i for dopamine was determined by the method of Dixon [20] at three $DMPH_4$ concentrations: 100 μM (I); 10 μM (II) and 1 μM (III) and six concentrations of dopamine. The K_i is expressed as the mean ± S. E. M. of six determinations.

DISCUSSION

Catechols have been shown to depress the activity of tyrosine hydroxylase *in vitro* by competing with the pterin cofactor for the same form of the enzyme [21]. It has been proposed that endogenous dopamine, localized in some 'strategic' pool, may regulate the activity of dopaminergic tyrosine hydroxylase *in vivo* by a similar mechanism [22–25]. In fact, an increase in the endogenous levels of striatal dopamine does result in an apparent decrease in striatal tyrosine hydroxylase activity under some conditions [25]. For example, as the previous paper [7] indicates, the increase in dopamine caused by administration of a monoamine oxidase inhibitor (MAOI) is associated

Table 5. Effect of GBL administration on the tyrosine hydroxylase isolated from the olfactory tubercles and median eminence*

Tissue	Treatment	K_m tyrosine (μM)	K_m $DMPH_4$ (mM)	K_i DA (mM)
Olfactory tubercles	None	51·6 ± 6·6	0·96 ± 0·13	0·19 ± 0·02
Olfactory tubercles	GBL (750 mg/kg)	1·91 ± 0·24	0·15 ± 0·03	85·9 ± 9·3
Median eminence	None	50·6 ± 3·7	0·80 ± 0·11	0·13 ± 0·03
Median eminence	GBL (750 mg/kg)	54·1 ± 4·9	0·83 ± 0·13	0·17 ± 0·02

* K_m values for tyrosine and $DMPH_4$ were determined by the method of Lineweaver and Burk [19] at six substrate concentrations with three separate determinations at each substrate concentration. The K_i for dopamine was determined by the method of Dixon [20] at six dopamine concentrations and three $DMPH_4$ concentrations. Tyrosine hydroxylase was obtained from the 105,000 *g* supernatant of tissue pooled from three to six rats.

Table 6. Reversal of the GBL-induced activation of tyrosine hydroxylase by dopamine agonists*

Treatment	N	Tyrosine hydroxylase activity (pmoles/DOPA/mg protein/min)	K_m tyrosine (μM)	K_m $DMPH_4$ (mM)	K_i DA (mM)
Control	6	23·2 ± 2·6	53·9 ± 5·1	0·89 ± 0·03	0·11 ± 0·01
ET-495 (10 mg/kg; 40 min)	6	24·4 ± 3·1	55·5 ± 3·7	0·85 ± 0·02	0·13 ± 0·01
APO (2 mg/kg; 40 min)	6	21·5 ± 6·6	49·8 ± 4·5	0·90 ± 0·03	0·11 ± 0·01
GBL (750 mg/kg; 90 min)	6	85·2 ± 4·6	8·9 ± 0·6	0·13 ± 0·01	73·9 ± 8·3
GBL, 90 min; ET-495 40 min	6	26·7 ± 4·1	52·6 ± 3·8	0·86 ± 0·06	0·10 ± 0·01
GBL, 90 min; APO, 40 min	6	20·1 ± 3·0	50·4 ± 4·3	0·90 ± 0·05	0·12 ± 0·01

* Male Sprague–Dawley rats (250–300 g) were injected i.p. with 750 mg/kg of GBL or saline 90 min prior to sacrifice. ET-495 (10 mg/kg), apomorphine (APO, 2 mg/kg) or saline was administered 40 min prior to sacrifice (50 min after GBL). Striata were removed, frozen and assayed for tyrosine hydroxylase activity and kinetics determined as described in Methods. Kinetics were determined by pooling tissue from six animals and the assay was carried out in triplicate. GHB (10^{-3}), APO (10^{-4}) and ET-495 (10^{-4} M) added directly to the incubation media had no significant effect on tyrosine hydroxylase activity.

with a reduced accumulation of dihydroxyphenylalanine (DOPA) in the neostriatum following DOPA decarboxylase inhibition [7].

There is an apparent contradiction, however, between this product inhibition hypothesis and the changes in striatal tyrosine hydroxylase activity observed after impulse flow is inhibited in dopaminergic neurons. Sixty to ninety min following the administration of GBL or the placement of a lesion in the median forebrain bundle (conditions which result in a cessation of impulse flow in the nigro-neostriatal pathway and a rapid increase in dopamine levels), the activity of striatal tyrosine hydroxylase does not appear to be depressed below control rates [5]. A combination of MAO inhibition and GBL treatment produces an even greater increase in dopamine than does GBL treatment alone, yet still does not result in a decrease in dopamine synthesis below control [7].

A possible explanation for this phenomenon is provided by the studies *in vitro* presented here which suggest that the physical properties of tyrosine hydroxylase are altered when impulse flow is blocked in the dopamine neurons. The result is a drastic reduction in the enzyme's sensitivity to inhibition by endogenous dopamine. When striatal tyrosine hydroxylase is isolated from the neostriatum of rats treated with GBL or from rats which have acute median forebrain bundle lesion, the kinetic parameters of the enzyme are considerably different from those observed when the enzyme is isolated from control untreated animals; the K_m for tyrosine and pterin cofactor is decreased and the K_i for dopamine is markedly increased. This increase in the K_m for tyrosine may not affect the rate of dopamine synthesis *in vivo*, as the concentration of tyrosine in the dopamine terminal is probably saturating. However, the enzyme's increased affinity for pterin cofactor and decreased sensitivity to inhibition by dopamine may well account for the elevation in the rate of dopamine synthesis *in vivo* observed after impulse flow is blocked in the dopamine neurons. The decreased affinity of tyrosine hydroxylase for dopamine may also explain the fact that dopamine synthesis is not depressed below control levels by the relatively high levels of dopamine accumulating in the dopamine terminals after cessation of impulse flow in these neurons [5].

The functional significance of the increase in dopamine synthesis which occurs with inhibition of dopaminergic impulse flow is unclear. Single-unit recording studies in anesthetized and paralyzed rats have shown that the dopamine neurons in the substantia nigra and the ventral tegmental areas are tonically active [13]. However, perhaps under some circumstances such as anoxia or during some specific behaviors, these neurons cease firing and some operational advantage is gained by having an extra supply of transmitter present in the terminals when firing

Table 7. Reversal of apomorphine inhibitory effect on the activation of tyrosine hydroxylase produced by GHB*

Treatment	Tyrosine hydroxylase activity (pmoles DOPA/mg protein/min)	K_m tyrosine (μM)	K_i dopamine (mM)
Saline	23·2 ± 2·6	53·9 ± 5·1	0·11 ± 0·01
GBL	84·9 ± 3·6	8·9 ± 0·6	73·9 ± 8·3
GBL + APO	20·1 ± 3·0	50·4 ± 4·3	0·12 ± 0·01
GBL + HAL + APO	86·0 ± 3·7	8·9 ± 1·3	76·0 ± 3·6
GBL + CPZ + APO	76·2 ± 2·6	11·5 ± 1·6	67·5 ± 10·6

* Rats received GBL (750 mg/kg) 90 min prior to sacrifice. Haloperidol (HAL, 1 mg/kg) or chlorpromazine (CPZ, 10 mg/kg) was administered 50 min prior to sacrifice, followed in 10 min by either saline or apomorphine (APO, 2 mg/kg). Striata were removed, frozen and assayed for tyrosine hydroxylase. There were six animals in each group. Kinetics were determined by pooling tissue from six rats; the assays were carried out in triplicate.

resumes. It is interesting that a similar increase in acetylcholine levels occurs when impulse flow is blocked in cholinergic neurons [26]. In contrast, there is no significant increase in the concentration of norepinephrine in the areas of the brain containing noradrenergic terminals when impulse flow is blocked in noradrenergic neurons [27, 28].

As has been discussed in the previous paper [7], dopamine agonists or drugs which increase the release of dopamine block the increase in dopamine synthesis that occurs with inhibition of impulse flow in the nigro-neostriatal pathway. It has been postulated that this effect is mediated by a dopaminergic receptor located on the presynaptic side of the dopamine synapse which is influenced by the levels of dopamine or dopamine agonists in the synaptic cleft [2, 4, 5, 9]. The present experiments demonstrate that when apomorphine or ET-495 is administered to animals after GBL treatment and the kinetic parameters of striatal tyrosine hydroxylase are determined, the activity of the enzyme, the K_m for tyrosine and pterin cofactor, and the K_i for dopamine are all similar to those of control animals. Thus these studies *in vitro* corroborate the observation *in vitro* [7] that the ability of dopamine to inhibit tyrosine hydroxylase after cessation of impulse flow is restored by the administration of dopamine agonists. The finding that dopamine receptor blockers such as chlorpromazine and haloperidol prevent the reversal in kinetic parameters produced by the dopamine agonists supports the idea that the agonists are exerting their effect at a site which is similar to the post-synaptic dopamine receptor but located on the presynaptic side of the synapse.

The enzyme studies described here suggest a possible way in which decreases in impulse flow and presynaptic receptor stimulation may induce changes in tyrosine hydroxylase activity. When calcium chelators are added to the incubation medium containing striatal tyrosine hydroxylase the activity of the enzyme is markedly increased. This activation is very similar in all its kinetic parameters to the activation caused by a blockade of impulse flow in the dopamine neurons *in vivo*. Moreover, when the activated enzyme is prepared from animals with impaired impulse flow, the addition of calcium to the incubation medium returns the activity and the kinetic parameters of the enzyme to normal. Thus the removal of calcium *in vitro* appears to bring about a conformational change in tyrosine hydroxylase which results in an increase in the enzyme's affinity for cofactor and substrate, and a marked decrease in its sensitivity to inhibition by dopamine. Similarly, it seems possible that *in vivo*, a cessation of impulse flow may alter calcium fluxes or the levels of ionized calcium in some critical intraneuronal pool and induce a similar change in tyrosine hydroxylase, rendering it much less sensitive to inhibition by endogenous dopamine. The ability of dopamine agonists to reverse this activation by interacting with what appears to be a presynaptic dopamine receptor suggests that stimulation of this receptor may restore some calcium fluxes and permit the enzyme to return to its normal form in the same way that the addition of calcium reverses the activation *in vitro*.

The activity of tyrosine hydroxylase is increased in the olfactory tubercles as well as in the neostriatum following the administration of GBL. This finding is in agreement with the observation that GBL inhibits the firing of dopamine cells in both the ventral tegmental areas and the substantia nigra (J. H. Walters, R. H. Roth and G. K. Aghajanian, unpublished observations). However, an increase in tyrosine hydroxylase activity was not produced in the median eminence after GBL administration, although the enzyme isolated from this region of the brain does appear to be activated by calcium chelating agents *in vitro* [29]. This might imply that the dopamine cells projecting to the median eminence are not tonically active or that their activity is not inhibited by GBL. It is also possible that with these neurons a blockade of impulse flow does not result in a decrease in intraneuronal calcium at the enzyme site.

Unlike the enzyme isolated from areas of the brain containing predominantly dopamine terminals, the activity of tyrosine hydroxylase prepared from areas with predominantly noradrenergic innervation is not increased *in vitro* by the removal of calcium from the incubation medium [10, 11]. Moreover, as mentioned above, no increase in norepinephrine levels is observed after inhibition of impulse flow in these neurons [23, 24]. These results suggest that there may be different forms of tyrosine hydroxylase present in the noradrenergic and dopaminergic neurons. A similar conclusion has recently been reached by Joh and Reis [30] employing chromatographic and immunochemical techniques.

It also appears that the tyrosine hydroxylase in dopaminergic terminals can be activated by more than one mechanism. Electrical stimulation of the dopamine cell bodies in the substantia nigra also results in activation of the enzyme [31–33]. This increase in activity is associated with an increased affinity for cofactor and substrate and a decreased affinity for dopamine. The activation of tyrosine hydroxylase observed after nigral stimulation and the activation observed after abolition of impulse flow in the nigro-neostriatal pathway appear to be produced by different mechanisms, however, since only the latter is reversed by addition of calcium to the incubation medium. The increase in the K_i of the enzyme for dopamine is also much greater when impulse flow is inhibited than when the cells are electrically stimulated. Since removal of calcium does not closely mimic the activation observed with increased impulse flow and addition of calcium *in vitro* does not reverse it, it would appear that other factors are involved in the mechanism mediating the increase in dopamine synthesis observed with electrical stimulation of the dopamine cells or following the administration of drugs which cause an increase in dopaminergic impulse flow.

Thus, it is becoming increasingly apparent that the control of tyrosine hydroxylase activity in the central catecholamine systems is quite complex. Although the inhibitory effect of intraneuronal dopamine may be an important factor in regulating the activity of tyrosine hydroxylase under normal conditions, the sensitivity of the enzyme to this type of inhibition as well as the affinity of the enzyme for substrate and pterin cofactor may be altered by several factors such as impulse flow, ionic environment and presynaptic receptor stimulation.

REFERENCES

1. J. R. Walters and R. H. Roth, *Biochem. Pharmac.* **19,** 3001 (1972).
2. A. Carlsson, W. Kehr, M. Lindqvist, T. Magnusson and C. V. Atack, *Pharmac. Rev.* **24,** 371 (1972).
3. J. R. Walters, R. H. Roth and G. K. Aghajanian, *J. Pharmac. exp. Ther.* **186,** 639 (1973).
4. R. H. Roth, J. R. Walters and G. K. Aghajanian, in *Frontiers in Catecholamine Research* (Eds. E. Usdin and S. H. Snyder), p. 567. Pergamon Press, New York (1973).
5. J. R. Walters and R. H. Roth, *J. Pharmac. exp. Ther.* **191,** 82 (1974).
6. Y. Agid, F. Javoy and J. Glowinski, *Brain Res.* **74,** 41 (1974).
7. J. R. Walters and R. H. Roth, *Biochem. Pharmac.* **25,** 649 (1976).
8. W. Kehr, A. Carlsson, M. Lindqvist, T. Magnusson and C. V. Atack, *J. Pharm. Pharmac.* **24,** 744 (1972).
9. R. H. Roth, J. R. Walters and V. H. Morgenroth, III, in *Neuropsychopharmacology of Monoamines and Their Regulatory Enzymes* (Eds. E. Usdin), p. 369. Raven Press, New York (1974).
10. V. H. Morgenroth, III, M. C. Boadle-Biber and R. H. Roth, *Molec. Pharmac.*, in press.
11. V. H. Morgenroth, III, M. Boadle-Biber and R. H. Roth, *Proc. natn. Acad. Sci. U.S.A.* **71,** 4283 (1974).
12. T. Hökfelt and U. Ungerstedt, *Acta physiol. scand.* **76,** 415 (1969).
13. B. S. Bunney, J. R. Walters, R. H. Roth and G. K. Aghajanian, *J. Pharmac. exp. Ther.* **185,** 560 (1973).
14. R. Shiman, M. Akino and S. Kaufman, *J. biol. Chem.* **246,** 1330 (1971).
15. S. Kaufman, in *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan), Vol. 5, p. 812. Academic Press, New York (1962).
16. J. T. Coyle, *Biochem. Pharmac.* **21,** 1935 (1972).
17. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
18. G. N. Wilkinson, *Biochem. J.* **80,** 324 (1961).
19. H. Lineweaver and D. Burk, *J. Am. chem. Soc.* **56,** 658 (1934).
20. M. Dixon, *Biochem. J.* **55,** 170 (1953).
21. S. Undenfriend, P. Zaltsman-Nirenberg and T. Nagatsu, *Biochem. Pharmac.* **14,** 837 (1965).
22. D. R. Masuoka, H. F. Schott and L. Petriello, *J. Pharmac. exp. Ther.* **139,** 73 (1963).
23. E. Costa and N. H. Neff, in *Biochemistry and Pharmacology of the Basal Ganglia* (Eds. E. Costa, L. Cote and M. D. Yahr), p. 141. Raven Press, New York (1966).
24. J. E. Harris and R. H. Roth, *Molec. Pharmac.* **7,** 593 (1971).
25. F. Javoy, Y. Agid, D. Bouvet and J. Glowinski, *J. Pharmac. exp. Ther.* **182,** 454 (1972).
26. V. H. Sethy, M. J. Kuhar, R. H. Roth and M. H. Van Woert, *Brain Res.* **55,** 481 (1973).
27. J. Korf, R. H. Roth and G. K. Aghajanian, *Eur. J. Pharmac.* **23,** 276 (1973).
28. B. E. Herr and R. H. Roth, *Proc. Fifth Int. Congr. Pharmacology*, p. 100 (1972).
29. V. H. Morgenroth, III, M. C. Boadle-Biber and R. H. Roth, *Molec. Pharmac.*, in press.
30. T. H. Joh and D. J. Reis, *Brain Res.* **85,** 146 (1975).
31. R. H. Roth, J. R. Walters, L. C. Murrin and V. H. Morgenroth, III, *Pre- and Post-synaptic Receptors. Proc. 135th Annual Meeting American College Psychopharmac.* (Eds. E. Usdin and W. E. Bunney), pp. 5–48. Marcel Dekker Press, New York (1975).
32. R. H. Roth, L. C. Murrin and V. H. Morgenroth, III, *Antipsychotic Drugs, Pharmacodynamics and Pharmacokinetics, Wenner-Green Center Symposium Series.* Pergamon Press, New York, in press.
33. L. C. Murrin, V. H. Morgenroth, III and R. H. Roth, *Pharmacologist* **16,** 213 (1974).