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# EFFECT OF DOPAMINE ON TYROSINE HYDROXYLASE IN CULTURED RAT ADRENAL MEDULLA

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**Abstract**—Explants of rat adrenal medulla were cultured in defined medium for up to 22 hr. Addition of dopamine to the medium led to a diminution in the activity of tyrosine hydroxylase (tyrosine 3-monooxygenase; EC 1.14.16.2) in the tissue. The enzyme activity was inversely proportional to the concentration of dopamine in the culture medium. The extent of loss of tyrosine hydroxylase, as measured by immunochemical titration, corresponded to the degree of loss in enzyme activity under the same conditions. The decreased amount of enzyme protein was due to a decrease in the rate of synthesis of tyrosine hydroxylase. However, this effect was not specific in that the relative rate of tyrosine hydroxylase synthesis was not decreased. Metabolites of dopamine when added to the medium did not affect tyrosine hydroxylase activity. Two other adrenal medullary enzymes, monoamine oxidase (EC 1.4.3.4) and acid phosphatase (EC 3.1.3.2), were not affected by addition of dopamine to the medium. The results indicate that elevated cytoplasmic levels of dopamine decrease the concentration of tyrosine hydroxylase by inhibiting protein synthesis.

The activity and amount of tyrosine hydroxylase (TH‡; tyrosine 3-monooxygenase, EC 1.14.16.2), the enzyme catalyzing the rate-limiting step in the biosynthesis of catecholamines, can be induced *in vivo* in central catecholaminergic neurons [1, 2] and in the adrenal medulla [3] by agents that cause release of catecholamines from these tissues. The increased accumulation of TH in these tissues is thought to be due to an increase in the rate of synthesis of the enzyme [4–6]. Conversely, chronic administration of L-dihydroxyphenylalanine (L-dopa), a precursor of catecholamines, *in vivo* reduces TH activity in both brain [7] and adrenal medulla [8]. It has been suggested that elevated cytoplasmic levels of catecholamines may repress the formation of their own biosynthetic enzymes [9–12] and thus may play a role in the regulation of tissue levels of these enzymes. Because of the difficulties in controlling the cellular environment and the potentially toxic effects of large amounts of catecholamines *in vivo*, studies on molecular mechanisms regulating TH have been difficult.

An explant culture of rat adrenal medulla [10] capable of synthesizing catecholamines [13], as well as their biosynthetic enzymes [11, 12, 14], has been developed. In the present study, we examined the biochemical mechanisms that regulate the activity and amounts of TH in adrenal medullary explants. The effects of L-dopa, dopamine and dopamine metabolites on the activity and amounts of TH were examined. We demonstrated that dopamine decreased the activity and amount of TH by decreasing the rate of protein synthesis.

## MATERIALS AND METHODS

Goat antiserum to rabbit IgG, 3,4-dihydroxyphenylacetic acid, dopamine, L-dopa, homovanillic acid, 3-methoxytyramine, *m*-hydroxybenzylhydrazine and fusaric acid were obtained from the Sigma Chemical Co., St. Louis, MO. L-[$^{14}$C]Leucine (0.34 Ci/mmol) and L-[ring-3,5-$^{3}$H]tyrosine (52 Ci/mmol) were purchased from the New England Nuclear Corp., Boston, MA. NCS tissue solubilizer and OCS ligand scintillation fluid were from Amersham, Arlington Heights, IL. Medium 199 with glutamine with Earle's Salts was obtained from Flow Laboratories, Rockville, MD, and medium 199 without leucine was prepared by GIBCO, Grand Island, NY. Haloperidol (haldol) was a gift from McNeil Pharmaceutical, Springhouse, PA.

*Explant culture.* Male Holtzman rats weighing 90–100 g were used in all experiments. Before sacrifice, animals were housed five or six to a cage under controlled conditions of lighting in a room maintained at 21.0 ± 0.5°. Purina rat chow and water were supplied *ad lib.*

After animals were killed by decapitation, adrenal glands were removed rapidly, and the medullae were dissected free of cortex under a dissecting microscope. The tissue was then cultured by the method of Burke *et al.* [10] which is a modification of the method of MacDougall and Coupland [15]. Compounds to be tested were added to the medium at the concentrations noted for each experiment. All compounds in each experiment were added to the medium at the same time except *m*-hydroxy-

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‡ Abbreviations: TH, tyrosine hydroxylase; L-dopa, L-dihydroxyphenylalanine; MAO, monoamine oxidase; and PNMT, phenylethanolamine *N*-methyltransferase.



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benzylhydrazine which was added 3 hr prior to the addition of L-dopa. Ascorbic acid was added directly to the medium in a final concentration of 5.7 mM. We have shown previously that this stabilized catecholamines in this medium [10]. Tissue was dispensed so that one half of the same medulla served as a matched control for the experimental piece. Incubations were at 37° in an atmosphere of 95% oxygen–5% carbon dioxide for a maximum of 22 hr. To determine viability of the tissue, medullae cultured in medium 199 containing 1.0 mM dopamine were fixed in 10% formalin. Sections were cut, stained with hematoxylin and eosin, and examined by light microscopy. In other experiments viability of the explants was determined using 0.1% trypan blue by the method of Sawicki *et al.* [16].

*Preparation of homogenates for enzyme assays.* At the end of the culture period, tissue was homogenized in 0.5 ml of 0.5 M potassium-phosphate buffer, pH 6.0, containing 0.2% Triton X-100. After centrifugation at 12,000 *g* for 10 min, the supernatant fraction was taken for TH assay. In experiments in which dialysis was used prior to TH assay, the homogenates were dialyzed overnight at 4° against 3.5 liters of 0.1 M potassium-phosphate buffer, pH 7.4, containing 10% sucrose and 127 μM ferrous ammonium sulfate [10]. There was no loss of TH activity with dialysis against this medium [17]. For measurement of monoamine oxidase (MAO) and acid phosphatase activities, the tissue was homogenized in 0.5 ml of 5 mM potassium-phosphate buffer, pH 7.4, containing 0.2% Triton X-100 and dialyzed overnight at 4° against 5 mM potassium-phosphate buffer, pH 7.4, containing 10% sucrose prior to assay.

*Enzyme assays.* TH activity was assayed by a modification of the methods of Reis *et al.* [1] and Lerner *et al.* [18]. In summary, the assay mixture contained: 50 μl of cytosol (or 50 μl of homogenizing buffer for blanks), 10 μl of 0.5 M potassium-phosphate, pH 6.64 (or pH 5.9 if the dialysate was used as the source of enzyme); 10 μl of 0.2 M ascorbic acid; 10 μl of 1 mM ferrous ammonium sulfate; 2400 units of catalase; 15 μl of 10 mM 7-methyl-5,6,7,8-tetrahydropterine (reduced with $PtO_2$ and hydrogen gas in 0.01 N HCl immediately prior to assay); 10 μl of 10 mM *m*-hydroxybenzylhydrazine; 2 μCi of L-[3,5-$^3$H]tyrosine (52 Ci/mmol) (dried with a stream of $N_2$ prior to assay); and water to make the total volume of the assay mixture 125 μl. The reaction was initiated by the addition of 50 μl of supernatant fraction and was stopped after 15 min with 5 ml of 0.5 M potassium-phosphate buffer, pH 8.5, containing 30 mg $Na_2S_2O_5$, 100 mg disodium ethylenediaminetetracetic acid and 1 μg of carrier L-dopa. The product was absorbed immediately onto 200 mg of alumina [19] by shaking for 5 min. The alumina was then washed three times with 10-ml aliquots of water. The [$^3$H]dopa was then eluted by shaking the alumina with 3 ml of 0.4 N perchloric acid for 5 min. The recovery of L-dopa with this method [20] was 72 ± 2.3%. Two milliliters of the eluate was then counted in PCS counting fluid in a Beckman scintillation spectrometer. The values were not corrected for recovery. Enzyme activity was linear for the time used in the assay.

Assays of MAO and acid phosphatase activity in 50-μl aliquots of dialyzed homogenates of medullary tissue were performed by the methods of Wurtman and Axelrod [21] and of Andersch and Szczypinski [22] respectively. The unit of enzyme activity reported for TH and MAO corresponds to the formation of 1 nmol of radioactive produce/hr. For acid phosphatase, the unit of activity is the hydrolysis of 1 mmol *p*-nitrophenylphosphate/hr.

*Catecholaminase analysis.* Catecholamines were assayed in tissue homogenates and in dialysates using high pressure liquid chromatography with electrochemical detection according to a method previously described [23].

*Immunochemical titration of TH.* Explants of adrenal medullae were homogenized and centrifuged at 12,000 *g*. An aliquot of the supernatant fraction was taken for measurement of protein content by the method of Lowry *et al.* [24]; the remainder was used to measure the relative concentrations of TH. To stabilize enzyme activity during incubation with the antibody, 38,000 units of catalase were added to 1 ml of this supernatant. Antibody to rat adrenal TH was prepared and judged to be specific according to a method previously described [3, 25]. This antibody was used to titrate [3, 26] the amount of enzyme in soluble extracts from rat medullae that had been incubated previously for 20 hr in either the presence or absence of 1.0 mM dopamine. Portions of the soluble extract measuring 5–50 μl were added to tubes containing 15 μl of antibody. The volume of each tube was adjusted to 65 μl with 0.15 M NaCl. This mixture was allowed to stand at 4° with occasional swirling, for 60 min, after which the tubes were centrifuged at 6000 *g* for 10 min.

A 50-μl aliquot of the supernatant fraction was removed from each tube, and the activity of TH was determined. From a graph of enzyme activity in the supernatant fraction versus addition of medullary extract, the equivalence point between enzyme and antibody was determined in duplicate as previously described [1, 3].

*Measurement of rates of synthesis and degradation of TH.* Opposite halves from 32 adrenal medullae (16 pairs in each medium) were cultured for 10 hr at 37° in medium 199 in either the presence or absence of 1.0 and 2.0 mM dopamine. The medullae were transferred to fresh leucine-free medium to which 40 μCi of [$^{14}$C]leucine was added and cultured for an additional 4 hr. For determination of the relative rate of TH synthesis, half of the medullae were removed from each culture dish and each pair of medullae were homogenized in 310 μl of 5 mM potassium-phosphate, pH 7.0, containing 0.2% Triton X-100. Aliquots were precipitated with 0.4 N perchloric acid for determination of [$^{14}$C]leucine incorporation into total protein and protein content of the tissue. The rest of the homogenate was centrifuged at 15,000 *g* for 15 min. To 200 μl of the supernatant fraction, 20 μl of antibody to TH was added and the mixture was incubated for 60 min at room temperature and overnight at 4°. To precipitate the total TH antibody complex, 150 μl of secondary antibody goat antirabbit IgG was added to the mixture. The mixture was incubated for 90 min with occasional shaking, and the TH antibody secondary antibody

Table 1. Effect of L-dopa in the presence and absence of a decarboxylase inhibitor on TH activity in cultured explants of adrenal medulla*

| Compound added | TH activity (units/mg protein) |
|---|---|
| None | 0.86 ± 0.15 |
| 1.0 mM L-Dopa | 0.28 ± 0.12† |
| 0.5 mM *m*-Hydroxybenzylhydrazine | 0.71 ± 0.17 |
| 1.0 mM L-Dopa + 0.5 mM *m*-hydroxybenzylhydrazine | 0.80 ± 0.22 |

* Adrenal medullary explants were cultured for 20 hr in medium 199 or medium 199 containing the compound listed at the concentration indicated. Homogenates of these explants were assayed for TH as outlined under Materials and Methods. Each mean ± SE was derived from five replicate cultures.
† $P < 0.02$.

complex was collected by centrifugation. The [$^{14}$C] protein complex was washed twice with 500 μl of NCS tissue solubilizer and counted in 20 ml of OCS liquid scintillation fluid with 10 μl acetic acid to prevent photon interference with counting.

To determine the rate of degradation of TH, the remaining pairs of medullae were transferred to fresh medium of the same composition without [$^{14}$C]leucine and maintained for an additional 8 hr. The radioactive leucine remaining in TH was measured by the method described above.

*Measurement of the absolute rate of protein synthesis.* Opposite halves from 20 medullae were cultured for 10 hr at 37° in medium 199 in either the presence or absence of 1.0 mM dopamine. The medullae were transferred to fresh leucine-free medium to which 20 μCi of [$^{14}$C]leucine was added and cultured for an additional 4 hr. The tissue was homogenized in 100-μl aliquots of methanol to precipitate protein* and centrifuged at 20,000 *g* for 30 min. The amount of [$^{14}$C]leucine incorporated into protein and the protein content of the tissue were determined. The concentration of methanol in the supernatant fraction was decreased to 50% with the addition of $H_2O$. Aliquots of the supernatant fraction were then counted in 10 ml of PCS to determine the amount of free [$^{14}$C]leucine in the soluble fraction. Aliquots of 100 μl of the supernatant fraction were then added to 100 μl of ophthaldialdehyde reagent [27] concentrated five times to reduce the volume needed for the reaction. After derivatization, the amount of leucine in the soluble fraction was determined using high pressure liquid chromatography with electrochemical detection according to a method previously described [27,*]. The mobile phase was 40% methanol in a 0.1 M sodium-phosphate buffer, pH 7.0.

## RESULTS

Exposure of adrenal medullary explants to 1.0 mM L-dopa for 20 hr resulted in a 67% decrease in TH activity in tissue homogenate (Table 1). This decreased TH activity was not due to L-dopa itself because the effect was blocked completely by the addition of an aromatic amino acid decarboxylase inhibitor, *m*-hydroxybenzylhydrazine, to the culture medium (Table 1). To determine whether the effect of L-dopa was mediated by a metabolite, we tested the effects of a variety of metabolites in the culture medium on medullary TH activity. At a concentration of 1.0 mM, dopamine, the decarboxylated metabolite of L-dopa, caused a 49% decrease in the medullary TH activity after a 20-hr incubation (Table 2, Expt. 1). As the concentration of dopamine in the medium was increased 5-fold, there was a corresponding decrease in TH activity in homogenates of cultured explants (Table 2, Expt. 1). The dopamine content of the tissue was similarly increased 6-fold (control: 7 ± 2 ng/μg protein vs 1 mM dopamine treated: 45 ± 8). However, its metabolite norepinephrine was not increased (control: 153 ± 27 ng/μg protein vs 1 mM dopamine treated: 115 ± 20). This effect of dopamine was not due to a direct inhibitory effect of dopamine or other catecholamine inhibitors in the tissue because the decreased TH activity was present in medullary homogenates that had been dialyzed to remove catecholamines prior to assay (Table 2, Expt. 2). Assay of these dialyzed homogenates for dopamine showed that it was undetectable to electrochemical detection measured at 2 nA/V which allows detection of no less than 500 pg of dopamine, indicating a concentration of less than 40 nM. This is well below the 100 μM dopamine reported to produce a 38% inhibition of adrenal TH [28].

To ascertain the viability of the tissue being cultured in the presence of 1.0 mM dopamine, several variables were measured. Although 5–10% of the tissue took up trypan blue, this was due to tissue damage during dissection. There was no difference in uptake of this vital stain between control and dopamine-treated tissue. The activities of two other medullary enzymes, MAO and acid phosphatase, were not affected by addition of dopamine to the culture medium (Table 2, Expt. 2). The degradation rate of neither medullary total protein (control: 0.60 ± 0.09 vs 2.0 mM dopamine treated: 0.55 ± 0.13) nor TH (see Table 6) was increased by dopamine. Tissue exposed for 20 hr to 2.0 mM dopamine was structurally intact as verified by examination of stained sections under light microscopy.

To determine whether this effect on TH activity in cultured explants was due to dopamine or to one of its metabolites, we added 1.0 mM dopamine to

* M. H. Joseph and P. Davies, *Current Separations Bioanalytical Systems* **4**, 62 (1982).

Table 2. Effect of dopamine on activities of adrenal medullary enzymes in cultured explants*

| Expt. | Dopamine added (mM) | Enzyme activity (units/mg protein) TH | MAO | Acid phosphatase |
|---|---|---|---|---|
| 1 | None | 0.82 ± 0.16 | | |
| | 1.0 | 0.42 ± 0.11† | | |
| | 5.0 | 0.08 ± 0.01‡ | | |
| 2 | None | 0.82 ± 0.15 | 35.21 ± 5.56 | 35.55 ± 5.18 |
| | 1.0 | 0.41 ± 0.05† | 39.24 ± 6.67 | 36.04 ± 4.65 |

* Rat adrenal medullary tissue was cultured for 20 hr in median 199 or medium 199 containing dopamine at the concentrations indicated. Homogenates of the tissue were assayed for TH, MAO or acid phosphatase as outlined under Materials and Methods. Homogenates in Expt. 1 were not dialyzed prior to assay. Homogenates in Expt. 2 were dialyzed overnight, as described in the text, prior to assay. Each mean ± SE was derived from five replicate cultures.

† $P < 0.05$.

‡ $P < 0.01$.

the culture medium in the presence or absence of 0.1 mM fusaric acid, a dopamine β-hydroxylase inhibitor. After culturing tissue for 20 hr with fusaric acid, which inhibits the conversion of dopamine to norepinephrine, the effect of dopamine on TH activity in medullary homogenates was not prevented (Table 3). Norepinephrine at a concentration of 2.0 mM had no effect on medullary TH after tissue was cultured for 20 hr (control: 0.81 ± 0.1 units/mg protein vs norepinephrine treated: 0.74 ± 0.06). In addition, the other metabolites of dopamine, produced by oxidative decarboxylation and methylation, had no effect on TH activity when added to the explant medium at a concentration of 1.0 mM (Table 4).

To discover whether this effect of dopamine on TH was mediated by dopamine receptors, medullae were cultured for 20 hr in the presence of 1.0 mM dopamine with or without $10^{-4}$ M haloperidol. Haloperidol in the medium had no effect on TH activity (control: 1.21 ± 0.18 units/mg protein vs haloperidol treated: 1.15 ± 0.27), nor did haloperidol block the effect of dopamine on TH activity in cultured medullae (dopamine treated: 0.42 ± 0.11 units/mg protein vs dopamine + haloperidol treated: 0.59 ± 0.16).

To determine the naturally occurring dopamine concentration, medullae were dissected free of cortex, weighed, homogenized, and analyzed for total dopamine content. From four adrenal medullae analyzed separately, the mean concentration of dopamine was 1.14 ± 0.03 mM. This value may be compared to earlier data of Udenfriend [29] who found that the entire adrenal gland of sheep contained 28 μg of dopamine (0.183 μmol/g tissue). If the volume of the medulla relative to the entire gland is 20% [30], the concentration of dopamine in the medulla may be estimated to be not less than 0.92 mM.

To ascertain whether the change in TH activity was due to a change in the amount of TH or to a shift to a less active form, immunochemical titration was carried out in duplicate on tissue cultured in the presence or absence of 1.0 mM dopamine. The equivalence point of extracts of medullae cultured in the presence of 1.0 mM dopamine for 20 hr was shifted significantly ($P < 0.025$) to 0.65 ± 0.04 μg protein from 0.35 ± 0.02 μg observed for the control explants (Fig. 1). This 46% diminution in relative amount of TH agreed with the 53% loss in measurable TH activity in the same extract of tissue incubated with dopamine (1299 dpm/μg protein) compared to tissue incubated in basal medium (2775 dpm/μg protein).

To determine whether the decrease in amount

Table 3. Effect of dopamine in the presence or absence of a dopamine β-hydroxylase inhibitor on TH activity in cultured explants of adrenal medulla*

| Compound added | TH activity (units/mg protein) |
|---|---|
| None | 1.23 ± 0.11 |
| 1.0 mM Dopamine | 0.63 ± 0.12† |
| 0.1 mM Fusaric acid | 1.34 ± 0.26 |
| 1.0 mM Dopamine + 0.1 mM fusaric acid | 0.51 ± 0.12‡ |

* Adrenal medullary explants were cultured for 20 hr in medium 199 or medium 199 containing the compound listed at the concentration indicated. Homogenates of the explants were assayed for TH as outlined under Materials and Methods. Each mean ± SE was derived from five replicate cultures.

† $P < 0.01$.

‡ $P < 0.005$.

Table 4. Effects of dopamine metabolites on TH activity in cultured explants of adrenal medulla*

| Compound added | TH activity (units/mg protein) |
|---|---|
| None | 0.73 ± 0.08 |
| Homovanillic acid | 0.81 ± 0.24 |
| 3-Methoxytyramine | 0.79 ± 0.10 |
| 3,4-Dihydroxyphenylacetic acid | 0.74 ± 0.11 |

* Adrenal medullary explants were cultured for 20 hr in medium 199 or medium 199 containing one of the compounds listed at a concentration of 1.0 mM. Homogenates of the explants were assayed for TH as outlined under Materials and Methods. Each mean ± SE was derived from five replicate cultures.

Table 5. Effect of dopamine on synthesis of TH in cultured adrenal medulla*

| Expt. | Dopamine (mM) | [$^{14}$C]TH (dpm/μg protein) |
|---|---|---|
| 1 | 0 | 1150 ± 119 |
| | 1.0 | 572 ± 85† |
| 2 | 0 | 1160 ± 100 |
| | 2.0 | 119 ± 37‡ |

* Explants of adrenal medullae were cultured for 10 hr at 37° in medium 199 in either the presence or absence of dopamine. Tissue was transferred to fresh medium of the same composition but without leucine. [$^{14}$C]Leucine (40 μCi) was added to each culture dish. After an additional 4 hr of incubation, the tissue was removed for determination of [$^{14}$C]leucine incorporation into TH (see Materials and Methods). Each mean ± SE was derived from five (Expt. 1) or four (Expt. 2) replicate cultures.

† $P < 0.02$.

‡ $P < 0.01$.

of TH in dopamine-treated medullae was due to a decrease in the rate of TH synthesis, the incorporation of [$^{14}$C]leucine into TH at 14 hr and the [$^{14}$C]leucine remaining in the TH at 22 hr were determined. When dopamine was present in the culture medium, there was a dose-dependent decrease, of 50% with 1.0 mM and 90% with 2.0 mM dopamine, in the incorporation of [$^{14}$C]leucine into TH at 14 hr compared to the control culture (Table 5). However, there was no change in the relative rate of TH synthesis, i.e. [$^{14}$C]leucine into TH/[$^{14}$C]leucine into total protein × 100 (control: 4.60 ± 0.18 vs 1.0 mM dopamine: 4.94 ± 0.16; control: 4.78 ± 0.57 vs 2.0 mM dopamine: 4.80 ± 0.27). To investigate whether the decreased incorporation of [$^{14}$C]leucine into TH and protein was due to inhibition of transport of this amino acid across the cell membrane or to alterations in the specific activity of the intracellular leucine pool by dopamine, we measured the uptake of [$^{14}$C] and the specific activity of the leucine pool. Medullae were cultured for 10 min in the presence or absence of 2.0 mM dopamine with 20 μCi of [$^{14}$C]leucine. Neither the total uptake of [$^{14}$C]leucine (control: 109.8 ± 8.0 dpm/μg protein vs 2.0 mM dopamine: 109.8 ± 5.2) nor uptake of [$^{14}$C]leucine into the acid-soluble fraction, after removal of protein with 0.4 N PCA (control: 61 ± 0.58 dpm/mg protein vs 2.0 mM dopamine 65.8 ± 0.77) was affected by dopamine in the medium. This decreased incorporation of [$^{14}$C]leucine into TH and protein in dopamine-treated tissue was also not due to an alteration in specific activity of the intracellular leucine pool since the specific activity of this pool after a 14-hr culture with 4-hr exposure to [$^{14}$C]leucine was not changed by dopamine (control: 140 ± 9.3 nCi/nmol vs 1.0 mM dopamine 153 ± 1.8). The rate of total protein synthesis was decreased significantly by 65% in dopamine-treated tissue (control: 6.5 ± 1.4 pmol leucine/μg protein/hr vs 1.0 mM dopamine: 2.3 ± 0.9; $P < 0.005$).

The rate of degradation of TH was determined by measurement of [$^{14}$C]leucine remaining in TH at 22 hr after culturing medullae in the presence of radiolabeled leucine from 10 to 14 hr. In contrast to the rate of TH synthesis, its rate of degradation was not changed from control values when medullae were cultured in the presence of 2.0 dopamine (Table 6).

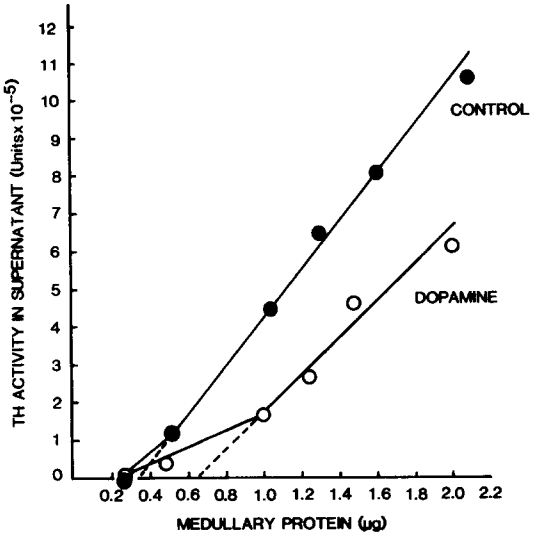


Fig. 1. Amount of immunotitratable TH in medullary explants cultured in the presence or absence of dopamine. Explants of adrenal medullae were cultured for 20 hr at 37° in medium 199 in either the presence or absence of 1.0 mM dopamine. At the end of the incubation period, tissue was homogenized and centrifuged at 12,000 *g*. The resulting soluble portion of the tissue homogenate was added to specific antibody to TH, and the activity of the remaining unprecipitated enzyme was determined as outlined under Materials and Methods. Duplicate determinations were made for each point.

## DISCUSSION

In intact animals, chronic administration of drugs such as reserpine [1, 3, 31] or prolonged stress [2, 30] causes release of catecholamines, including dopamine [32], from storage sites and leads to elevated TH activity in both brain and adrenal medulla. Inhibitors of protein biosynthesis [4] and immunological methods [1, 3] have been used to show that the elevations in TH in response to prolonged transsynaptic activity are due to biosynthesis of new protein and not to activation of pre-existing molecules. The induction of TH is selective but not specific

Table 6. Effect of dopamine on degradation of TH in cultured adrenal medulla*

| Dopamine (mM) | [$^{14}$C]TH (dpm/$\mu$g protein) At 14 hr | At 22 hr | [$^{14}$C]TH (At 22 hr/at 14 hr) |
|---|---|---|---|
| 0 | 1160 ± 100 | 679 ± 116 | 0.58 ± 0.07 |
| 2.0 | 119 ± 37 | 62 ± 24 | 0.52 ± 0.19 |

* Explants of adrenal medullae were cultured for 10 hr at 37° in medium 199 in either the presence or absence of dopamine. Tissue was transferred to fresh medium of the same composition but without leucine. [$^{14}$C]Leucine (40 $\mu$Ci) was added to each culture dish. After an additional 4 hr of incubation, half the tissue from each dish was removed for determination of [$^{14}$C]leucine incorporation into TH (see Materials and Methods). The remaining tissue was placed in fresh nonradioactive medium of the same composition and maintained for an additional 8 hr after which the [$^{14}$C]leucine remaining in TH was determined. Each mean ± SE was derived from four replicate cultures.

because it is accompanied by increases in some enzyme proteins, i.e. dopamine $\beta$-hydroxylase and phenylethanolamine *N*-methyltransferase (PNMT) but not others, e.g. MAO [33]. Conversely, chronic administration of L-dopa, a precursor of catecholamines, leads to a decrease in TH activity in both adrenal medulla [8] and brain [7]. These findings have led to the hypothesis that catecholamines may repress the synthesis of their own biosynthetic enzymes [9].

The present investigation has utilized cultured explants of adrenal medullary tissue bathed in a medium of known composition to study under controlled conditions the effects of L-dopa and its metabolite, dopamine, on TH. The initial experiment using a decarboxylase inhibitor, which blocked the effect of L-dopa on TH activity in the explants, indicated that L-dopa itself did not reduce TH activity in the cultured explants but rather that a metabolite of L-dopa produced this effect. Artificially elevating the concentration of dopamine in the cytoplasm of adrenal medullary explants by addition of 1 mM dopamine to the culture medium diminished TH activity. That this decrease was present after catecholamines were removed by dialysis indicates that the decrease in TH was not due to direct inhibition of the enzyme by dopamine. The concentration of dopamine in the medium which produced this effect was comparable to that normally present in the medullary cells (1.14 mM). That fusaric acid, which inhibits the conversion of dopamine to norepinephrine, did not block the effect of dopamine and that norepinephrine concentration in the tissue did not rise in the presence of dopamine indicate that dopamine itself and not another catecholamine produced this effect. The identification of dopamine as the regulator of TH levels was further established by showing that neither norepinephrine nor the oxidative or methylated metabolites of dopamine reduced TH when added to the medium.

Immunochemical titration showed that the decrease in TH activity with addition of dopamine to the culture medium was due to a decrease in the amount of enzyme protein rather than to a shift to a deactivated form of TH [34]. The decrease in the amount of TH was entirely due to a decrease in the rate of synthesis of the enzyme with no change in the rate of degradation. The relative rate of TH synthesis was also correspondingly decreased, indicating that this effect was not specific. Assuming a mass fraction of leucine in a medullary protein of 13% [35], only 2.6% of the medullary proteins are being synthesized at 10–14 hr. Our results indicate that dopamine affects this small family of rapidly turning over proteins. Although the effect was not specific, it was selective in that two other medullary enzymes, MAO and acid phosphatase, were not affected by dopamine. This selectivity may be due to slower turnover rates for these proteins compared to TH. Distinct families of proteins in the same tissue, with different turnover rates, have been reported previously by Lajtha [36]. Our results indicate that the approximate half-life of TH is 8.3 to 9.5 hr estimated from our *in vitro* data. This is remarkably similar to the half-life of TH that can be calculated from the data reported by Chuang and Costa [37] for the *in vivo* medulla. These results suggest that the turnover of TH may be more rapid than previously thought [38].

The molecular mechanisms which mediate the effect of dopamine on TH and protein synthesis are not known. However, that the effect was not blocked by haloperidol indicates that the effect was not a cell membrane receptor-mediated effect as has been demonstrated for dopamine on brain protein synthesis [39].

The effects of dopamine on total protein and catecholamine enzyme synthesis differ from those of epinephrine on these variables. Medullary tissue cultured for 20 hr in the presence of 1 mM epinephrine shows only a 10% decrease in incorporation of [$^{14}$C]leucine into protein [12]. Dopamine at the same concentration in the medium produced a 65% decrease in protein synthesis after only 10 hr. The effect of epinephrine on PNMT synthesis in cultural medullae is also more specific than that of dopamine since fewer proteins are affected and the relative rate of PNMT synthesis is decreased by epinephrine [12]. However, because of the decrease in total protein synthesis elicited by dopamine, no specific effect on TH synthesis could be demonstrated. The cause of the different effects of these two catecholamines on protein synthesis is not known. Further studies are required to determine the molecular mechanisms by

which dopamine affects protein synthesis. We are currently investigating how catecholamines affect synthesis of specific proteins.

It is unclear whether this effect of dopamine on TH is physiological or mainly pharmacological. The concentrations of dopamine we used in these experiments at first seem high for physiological effects; however, the amount of dopamine in the cytosol which is free to mediate this effect is unknown. Our studies indicate that at least 76% of the dopamine was bound to the storage vesicles (our unpublished observation). However, this value may be low due to leakage from the vesicle during the isolation of vesicles from cytosol. In addition, dopamine in the cytosol may be bound to catabolic enzymes as well as to other proteins and chemicals. Further studies are needed to determine the precise levels of free dopamine in the cytosol and how these are regulated.

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