Biochemical Pharmacology, Vol. 25, pp. 1157–1166. Pergamon Press, 1976. Printed in Great Britain.

# REGULATION OF ADRENAL TYROSINE HYDROXYLASE ACTIVITY: NEURONAL VERSUS LOCAL CONTROL STUDIED WITH APOMORPHINE*

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(*Received* 7 *June* 1975; *accepted* 10 *October* 1975)

**Abstract** Administration of apomorphine to rats leads to a temporary decrease in adrenal catecholamines and a long-term increase in adrenal tyrosine hydroxylase activity. The kinetic characteristics of tyrosine hydroxylase of the apomorphine-treated and control rats were determined. A significant increase in $V_{max}$ was observed in the treated group as compared to controls; however, there was no change in the $K_m$ for the cofactor $DMPH_4$ or tyrosine between the two groups in undialyzed or dialyzed preparations, indicating that the increase in tyrosine hydroxylase activity was probably due to an increase in enzyme protein. The temporary decrease in adrenal catecholamines was found to be due to increased secretion after apomorphine treatment, even though the concentration of injected apomorphine appeared to be sufficient to inhibit adrenal tyrosine hydroxylase. This was further substantiated by the fact that the decrease in adrenal catecholamines was prevented by adrenal denervation. The delayed increase in tyrosine hydroxylase activity after apomorphine treatment was observed in hypophysectomized rats; however, it was abolished after splanchnic nerve transsection. A relationship was sought between the decrease in adrenal catecholamines and increase in adrenal tyrosine hydroxylase activity. When apomorphine and L-3,4-dihydroxyphenylalanine (L-DOPA) were administered simultaneously, there was no short-term decrease in adrenal catecholamine content but the increased tyrosine hydroxylase activity was still observed. Also, the administration of α-methyl-*p*-tyrosine to rats decreased the concentration of adrenal catecholamines and yet did not affect adrenal tyrosine hydroxylase activity. The results suggest that increased nerve activity, and not adrenal catecholamine concentrations, regulates the induction of adrenal tyrosine hydroxylase. Furthermore, regulation would be by way of some central mechanism involving dopamine-sensitive receptors.

Tyrosine hydroxylase (TH, tyrosine 3-monooxygenase, EC 1.14.16.2) is regarded as the rate-limiting enzyme in the conversion of tyrosine to the catecholamines (CA) [1]. Regulation of adrenal TH is believed to involve two main types of mechanism. The first requires a short-term, rapid alteration in enzyme activity which can be mediated through feedback inhibition [2, 3], allosteric modulators [4, 5, 6], or changes in substrate concentrations [7, 8]. The second mechanism involves delayed, long term changes in enzyme protein and can be neuronally or hormonally mediated. Immobilization, cold exposure, reserpine and 6-hydroxydopamine increase preganglionic nerve acactivity and lead to an increase in adrenal TH in 24–48 hr, which can be prevented by splanchnic transsection [9–11]. ACTH can restore adrenal TH activity in hypophysectomized rats to normal levels [12].

Recently it has been suggested that the concentration of adrenal CA may play a role in the induction of adrenal TH. Dairman and Udenfriend [13] observed a decrease in adrenal TH after rats were given L-DOPA; the effect appeared to be due to a decrease in the amount of enzyme protein [14]. Both neuronal and hormonal factors were excluded and experimental evidence indicated that it was the increased formation of CA from L-DOPA which caused the reduction in adrenal TH. Bhagat [15] showed that the reserpine-induced increase in adrenal TH activity can be reduced by the simultaneous administration of pargyline; this monoamine oxidase inhibitor would be expected to increase CA concentrations. It was also shown [15] that phenoxybenzamine, an α-adrenergic blocker which decreases adrenal CA, causes increased TH activity in innervated adrenals, whereas the α-adrenergic blocking drug, phentolamine, which does not affect adrenal CA concentration, has no effect. Molinoff *et al.* [16] have suggested that the reserpine-induced increase in TH may be controlled by the CA concentrations in the sympathetic postganglionic nerve ending, even though this increase is abolished upon denervation. Work with cultured rat adrenal gland has also shown there is an inverse relationship between TH and CA after exposure to a prolonged depolarizing stimulus in the form of raised extracellular potassium concentration [17]. Experimental evidence contrary to this view has been presented by Mackay and Iversen [18] and Mackay [19]. They found no consistent relationship between TH activity and noradrenaline concentrations of superior cervical ganglia cultured in the presence of ouabain, reserpine or α-methyl-*p*-tyrosine.

In this paper we have used apomorphine, an inhibitor of TH *in vivo* and *in vitro* [20] and a dopamine agonist influencing brain monoamine concentrations [21], as well as the drugs L-DOPA, a CA precursor, and α-methyl-*p*-tyrosine, a TH inhibitor [22], to explore the possible relationship between CA levels and adrenal TH activity. The results presented suggest that it is not adrenal CA concentrations, but pre-

* This work was supported by a grant of the Medical Research Council (Canada).

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ganglionic nerve activity, which regulates the induction of adrenal TH. Furthermore, evidence indicates that the increased neuronal firing which initiates the increase in adrenal TH is due to stimulation of central dopamine receptors.

## MATERIALS AND METHODS

*Drugs.* The following drugs were purchased: 6,7-dimethyltetrahydropterin ($DMPH_4$) from Calbiochem (Los Angeles, CA.); α-methyl-*p*-tyrosine methyl ester (αMPT) and L-DOPA from Sigma (St. Louis, MO.); and L-tyrosine-3,5-[$^3$H] (58 Ci/m-mole) from New England Nuclear Corp. (Boston, MA.). The radioactive tyrosine was purified before use by passage through a Dowex 50W-X8 column, 0.5 × 3.0 cm. Brocresine (*m*-hydroxy-*p*-bromobenzyloxyamine was a gift of Lederle Laboratories (Pearl River, N.Y.); apomorphine hydrochloride and mecamylamine hydrochloride were donated by Merck Sharp and Dohme Research Laboratories (West Point, PA.). All other chemicals were obtained from standard commercial sources.

*Animals.* Male Sprague–Dawley rats weighing 130–160 g were obtained from Canadian Breeding Farms and Laboratories, Ltd., St. Constant, Quebec. Hypophysectomized and sham-operated rats were purchased from the same supplier. Hemi-splanchnicectomies were performed under sodium pentobarbital anesthesia in this laboratory. Because the weight of denervated and innervated adrenals were identical (8.5 ± 0.2 mg and 8.4 ± 0.2 mg, respectively; 18 organs in each mean), i.e. no glands had atrophied, there is assurance that the vascular supply had remained intact. Experiments were begun 3–4 days after denervation.

Apomorphine·HCl, α-methyl-*p*-tyrosine methyl ester and mecamylamine·HCl were administered to rats i.p. The drugs were dissolved in distilled water and injected in a volume of 0.2 ml at the indicated doses (expressed in terms of apomorphine, α-methyl-*p*-tyrosine and mecamylamine). When apomorphine·HCl and α-methyl-*p*-tyrosine methyl ester were both administered, they were injected simultaneously in a volume of 0.2 ml. The controls were injected with 0.2 ml of distilled water at the same times as the experimental group. L-DOPA was injected s.c. as a fine suspension in 0.9% NaCl in a volume of 0.5 ml. The controls received 0.5 ml saline. The dosages and timing of the experiments are indicated in the legends to the figures and tables.

In the cold exposure experiments, rats were kept at 4° singly in wire cages. For the immobilization experiments, the animals' limbs were placed through gaps in a specially prepared metal grid. The two fore limbs and the hind limbs were then bound using adhesive tape.

*Preparation of the tissue.* Animals were killed by decapitation. The adrenals were immediately removed and placed in ice-cold 0.3 M sucrose. The fat and connective tissue were removed, the adrenals weighed and each pair homogenized in 0.9 ml of 0.3 M sucrose for 10 sec with an Ultraturrax homogenizer. An aliquot (0.2 ml) was used for assay of TH activity. In some of the experiments the adrenal homogenates were dialyzed for 5 hr against 300 volumes 5 mM Tris acetate buffer pH 7.0 at 0°; the final volume of dialysate was adjusted to 2.0 ml with the buffer and a 0.3-ml aliquot was used for the enzyme assay, which was done immediately afterwards.

*Tyrosine hydroxylase assay.* TH was assayed according to the method of Nagatsu *et al.* [23] with certain modifications. The incubation mixture, in a total volume of 0.5 ml, contained: 0.05 ml 0.3 ml enzyme preparation; 0.1 ml of a mixture of 100 μmoles sodium acetate, 5 μmoles potassium phosphate, 1000 U catalase and 50 nmoles brocresine, pH 6.1; 0.05 ml solution of 50 nmoles L-tyrosine-3,5-[$^3$H] containing approximately 200,000 cpm. This was preincubated for 5 min at 30° in a shaking water bath. The reaction was begun by the addition of 0.5 μmoles $DMPH_4$ and 1.25 μmoles ascorbate in 0.05 ml. After 20 min the reaction was stopped by the addition of 0.1 ml 25% trichloroacetic acid (TCA) and the precipitated proteins sedimented by centrifugation. The supernatant was passed through a Dowex 50W-X8 column (0.5 cm × 2.0 cm), previously equilibrated with 1 ml TCA pH 1.8; the columns were then washed with 0.8 ml TCA pH 1.8. Both effluents were collected and assayed for radioactivity in a liquid scintillation counter.

*Catecholamine determinations.* Adrenal CA were determined in aliquots (0.05 ml) of the sucrose homogenates, which were acidified with 8 ml 0.4 N perchloric acid. The mixture was centrifuged and the protein-free supernatant brought to pH 7.5–8.0 using 4 N NaOH containing 250 mM EDTA. The CA were then adsorbed on an alumina column (0.5 cm × 2 cm), previously equilibrated with a solution of 10 mM $Na_2EDTA$ 10 mM β-mercaptoethanol. The CA were eluted with 5 ml 0.1 N perchloric acid [24]. Noradrenaline and adrenaline were then assayed by the hydroxyindole technique of Laverty and Taylor [25] with slight modifications: 0.5 ml of a 250 mM citrate solution was added to each 5-ml aliquot, which was titrated to pH 4.0 using 1 N NaOH containing 500 mM $NaH_2PO_4$. A 2-ml aliquot was taken for the adrenaline estimation which was done in the standard way. The remaining solution was titrated to pH 6.7 using 1 N NaOH containing 500 mM $NaH_2PO_4$. The noradrenaline measurements were done on a 2-ml aliquot, with development of fluorescence at 100° for 3 min. For blanks the oxidant and reductant were added in reverse order. All samples were read immediately after the heating period.

*Apomorphine determinations.* Apomorphine was measured in the following way. The tissue (4 pairs of rat adrenals) was homogenized with a Teflon homogenizer in a mixture containing 1.5 ml 0.1 N HCl, 0.5 ml ethyl acetate, 0.025 ml 10% $Na_2SO_4$ and 0.025 ml 10% $Na_2EDTA$ [26]. Approximately 0.5 g NaCl was added after which the apomorphine was extracted into 2.5 ml ethyl acetate. The procedure of Van Tyle and Burkman [27] was then followed.

*Purification of bovine adrenal TH.* The purification process was carried out essentially as described by Nagatsu *et al.* [28] with the following modification. After ammonium sulfate precipitation, the resuspended enzyme preparation was passed through a Sephadex G-25 column, previously equilibrated with 10 mM Tris acetate buffer pH 7.6 containing 0.3 M sucrose, for the removal of CA [29]. The column was

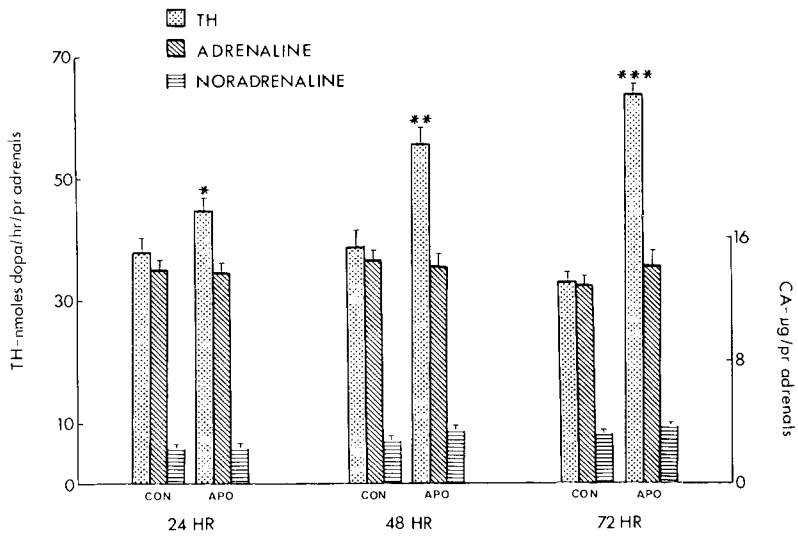


Fig. 1. The effect of apomorphine on tyrosine hydroxylase (TH) activity and catecholamine (CA) concentrations in rat adrenal glands. The first group of rats received 7 injections of 10 mg/kg apomorphine (APO) at 1.5-hr intervals and were killed 24 hr after the first injection. The second and third groups received the same treatment for 2 and 3 successive days, respectively, and were killed 48 and 72 hr after the initial injections. In all experiments controls received equivalent volumes of water at the same time as the experimentals. Significance of differences from control (CON) are indicated as follows: $*P < 0.05$ $**P < 0.01$ $***P < 0.001$. Each bar represents the mean ± S.E.M. of 9–10 animals.

eluted with this buffer and the enzyme preparation diluted with it to adjust the protein concentration to about 2 mg/ml. The preparation yielded about 0.15 μmoles DOPA/hr/mg protein. The enzyme was stored at −20 : it remained stable for 4 weeks.

## RESULTS

*The effect of apomorphine on rat adrenal TH activity and CA concentration.* The effect of 1, 2 or 3 days of injection with apomorphine on adrenal TH activity and CA concentrations is shown in Fig. 1. Twenty-four hr after the first of a series of injections, providing 70 mg/kg over a 10.5-hr period (see legend to Fig. 1), a small (18%) but significant increase in TH activity was observed in the apomorphine-treated rats; there was no change in the CA concentrations. After 2 days of the injection schedule a 44% increase in TH activity occurred. There was a small increase in the noradrenaline concentration ($P < 0.05$), but no change in adrenaline. An increase of 91% in the TH activity of the adrenals was observed after 3 days of injection with apomorphine with no alteration in the CA concentration.

An experiment was also done to determine whether 1 or 2 days of injections would be sufficient to elicit a significant increase in TH activity measured 72 hr after the initial injection. When treatment was discontinued 1 or 2 days prior to killing the animals, no increase in enzyme activity or changes in CA concentrations were observed.

*The effect of injected apomorphine on the kinetic characteristics of TH.* The increase in TH activity noted in Fig. 1 could stem from actual increases in the amount of enzyme protein or from changes in the kinetic characteristics of the enzyme that were induced by the drug treatment. For this reason kinetic studies were done with adrenal preparations obtained from apomorphine-treated and control rats. There was no change in the $K_m$ for $DMPH_4$ or tyrosine between the two groups, whether the enzymic preparations were dialyzed or not; however, there was a significant increase in $V_{max}$ ($P < 0.001$) in the treated group as compared to controls. Because changes in enzyme activity could be due to the formation of activators or loss of inhibitors, adrenal homogenates from control and apomorphine-treated rats were combined. In all cases the enzyme activities were additive. These results indicate that the increase in TH activity was due to an increase in the amount of enzyme protein.

*The effect of apomorphine on adrenal CA concentrations immediately after a series of injections.* Several investigators [13–17] have suggested that there is a relationship between alterations in the adrenal CA content and delayed changes in adrenal TH activity. We therefore considered the possibility that apomorphine had actually caused some significant alteration in the CA levels but that these had been restored to normal 24 hr after the initial injection. Rats were injected with apomorphine according to the 1-day schedule (10 mg/kg, every 1.5 hr on 7 occasions) and were then killed 1.5 hr after the seventh injection. The results are shown in Table 1, experiment A. It is clear that the treatment with apomorphine brought about large decreases in the concentration of the CA; 20% in the case of noradrenaline and 30% in the case of adrenaline.

*Concentration of apomorphine in adrenal glands after a single i.p. injection of 10 mg/kg.* The decrease obtained in adrenal CA after a series of injections could occur in several ways. The first mechanism we considered was the inhibition of TH activity by apomorphine. Goldstein *et al.* [20] have shown that apomorphine inhibits rat brain TH *in vivo* and *in vitro* at concentrations as low as $10^{-6}$ M. Kinetic studies

Table 1. Effect of α-MPT and L-DOPA on the apomorphine-induced decrease in adrenal CA

| Experiment | Treatment | Adrenaline μg pr adrenals | Adrenaline Control % | Noradrenaline μg pr adrenals | Noradrenaline Control % | Total catecholamines μg pr adrenals | Total catecholamines Control % |
|---|---|---|---|---|---|---|---|
| A | Control | 13.2 ± 0.5 | 100 | 2.9 ± 0.1 | 100 | 16.1 ± 0.5 | 100 |
| | Apomorphine | 9.2 ± 0.5** | 72 | 2.3 ± 0.2* | 80 | 11.5 ± 0.6** | 73 |
| B | Control | 12.1 ± 0.4 | 100 | 3.0 ± 0.2 | 100 | 15.1 ± 0.3 | 100 |
| | Apomorphine | 9.0 ± 0.1**,‡ | 74 | 3.7 ± 0.5 | 123 | 12.7 ± 0.6* | 83 |
| | αMPT | 11.5 ± 0.5 | 95 | 3.0 ± 0.5 | 100 | 14.5 ± 0.7 | 96 |
| | Apomorphine + αMPT | 6.0 ± 1.0**,‡,§ | 50 | 1.8 ± 0.1**,‡,§ | 60 | 7.8 ± 1.0**,‡,§ | 52 |
| C | Control | 10.5 ± 0.6 | 100 | 3.1 ± 0.2 | 100 | 13.6 ± 0.8 | 100 |
| | Apomorphine | 8.5 ± 0.3*, | 81 | 2.6 ± 0.2 ¶ | 84 | 11.2 ± 0.3 §§ | 82 |
| | L-DOPA | 9.7 ± 0.6 | 92 | 3.4 ± 0.2 | 110 | 13.1 ± 0.8 | 96 |
| | Apomorphine + L-DOPA | 10.5 ± 0.6 | 100 | 3.6 ± 0.2 | 116 | 14.1 ± 0.5 | 104 |

In experiment A rats were given 7 injections of 10 mg/kg apomorphine (or distilled water for controls) i.p. at 1.5-hr intervals. In experiment B rats were injected with 10 mg/kg apomorphine every 1.5 hr, 7 times; with one injection of 125 mg/kg αMPT and 6 subsequent injections of 50 mg/kg every 1.5 hr; or a combination of 10 mg/kg apomorphine plus 125 mg/kg αMPT for the first injection and 10 mg/kg apomorphine plus 50 mg/kg αMPT for 6 subsequent injections given every 1.5 hr. Controls were injected with carrier solution at the same time as experimentals. In experiment C rats were injected s.c. with 1 g/kg L-DOPA or saline 15 min prior to 7 i.p. injections of 10 mg/kg apomorphine or distilled water given at 1.5-hr intervals. In all experiments, rats were killed 1.5 hr after the last injection of apomorphine or αMPT or distilled water. Each value represents the mean ± standard error of 10 rats in experiment A and 4–6 rats in experiments B and C.

* Significantly different from controls ($P < 0.05$).
† Significantly different from controls ($P < 0.01$).
** Significantly different from controls ($P < 0.001$).
‡ Significantly different from αMPT ($P < 0.001$).
§ Significantly different from apomorphine ($P < 0.01$).
Significantly different from L-DOPA-treated rats ($P < 0.05$).
¶ Significantly different from apomorphine-treated plus L-DOPA group ($P < 0.05$).
§§ Significantly different from apomorphine-treated plus L-DOPA group ($P < 0.01$).

with partially purified bovine adrenal TH confirmed these findings. Double reciprocal plots of activity against substrate concentration showed that apomorphine inhibits the enzyme cooperatively with respect to the pteridine and uncompetitively with respect to tyrosine.*

For this reason we measured the amount of apomorphine in rat adrenals immediately after a single injection of 10 mg/kg to determine whether sufficient apomorphine reached the adrenals to be inhibitory. The adrenal glands of 4 rats, killed at given time intervals, were pooled for each determination. A peak concentration of 188 ng/pr adrenals occurred after 3 min; this decreased to 28 ng/pr adrenals at 75 min and 6 ng at 90 min (Fig. 2). A second experiment of this kind gave similar results. A content of 188 ng/pr adrenals weighing 20 mg would be equivalent to $3 \times 10^{-5}$ moles/kg. At 75 min the content would be $4.4 \times 10^{-6}$ moles/kg and at 90 min, $10^{-6}$ moles/kg. If the apomorphine were distributed evenly throughout the adrenal this would correspond to a concentration of $3 \times 10^{-5}$ M, declining to $10^{-6}$ in 1.5 hr.

*The effect of apomorphine on the adrenal CA concentrations in rats treated with αMPT.* If apomorphine is decreasing adrenal CA concentrations by inhibition of TH activity, administration of a known inhibitor of TH, αMPT, or a combination of αMPT and apomorphine, should lead to the result obtained with apomorphine alone. αMPT was therefore given to

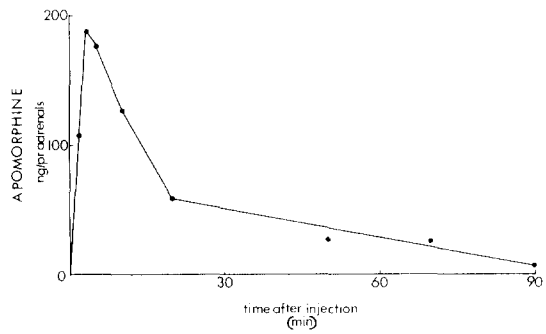


Fig. 2. Concentration of apomorphine in adrenal glands after a single injection. Rats were sacrificed at various time intervals after receiving 10 mg/kg apomorphine i.p. The adrenals from 4 rats were pooled and the amount of apomorphine determined as described in Materials and Methods.

rats receiving the schedule of apomorphine injections and to suitable controls. In these experiments the total amount of αMPT injected was 400 mg/kg over a 10.5-hr period. This dose is known to inhibit TH activity about 100 per cent [22]. The results in Table 1, experiment B, show that αMPT alone had no effect on adrenal CA presumably due to the slow turnover rate [30]. Apomorphine again caused a very highly significant decrease (26%) in the adrenaline concentrations; however, there was no change in the noradrenaline concentration in this experiment, in contrast

* M. Quik and T. L. Sourkes, unpublished data.

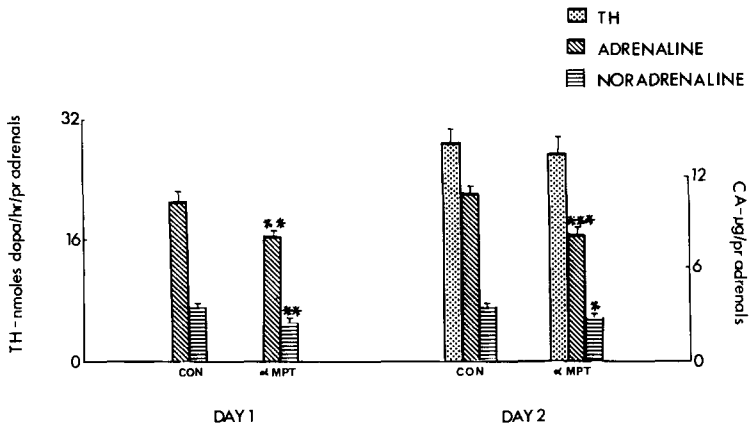


Fig. 3. The effect of αMPT on rat adrenal TH and CA concentrations. Rats were given an initial injection of 200 mg/kg αMPT and 2 subsequent injections of 100 mg/kg at 4.5-hr intervals for 1 or 2 days and were sacrificed 24 and 48 hr, respectively, after the initial injection. An aliquot of the adrenal homogenate was removed for CA determinations and the remainder dialyzed as described in Materials and Methods. Each bar represents the mean ± S.E. of 4–8 rats. Significance of differences from control (CON) are indicated as follows: **P < 0.01 ***P < 0.001.

to results in Table 1, experiments A and C. Administration of both drugs lead to an even greater decrease than with apomorphine alone: a 45% decrease in adrenaline and a 40% decrease in noradrenaline. These results suggest that apomorphine increases secretion of adrenal CA.

*The effect of prolonged treatment with αMPT on rat adrenal TH and CA.* In order to determine whether the increase in TH activity after apomorphine treatment could be due to the preliminary decrease in adrenal CA, we made further use of αMPT. Figure 3 shows that both 24 and 48 hr after the initial injection statistically significant decreases in the adrenaline and noradrenaline concentrations are observed in αMPT-treated rats; however, injection of the drug for 2 days did not result in an increase in TH activity over controls. Failure to obtain an increase in enzyme activity in αMPT-treated rats was not due to the presence of any residual αMPT which could inhibit the enzyme *in vitro*. The adrenal preparations of the treated and control rats had been dialyzed, and mixing experiments showed the enzyme activities to be additive.

*The effect of apomorphine on TH activity and CA concentrations of hypophysectomized rats.* Table 2 gives the results obtained with apomorphine treatment of hypophysectomized rats. The values for sham-operated animals correspond to those for intact rats (Fig. 1): there was a 76% increase in the TH activity of the apomorphine-injected rats, while the CA concentrations remained unchanged. In the apomorphine-treated hypophysectomized rats there was a highly significant increase in TH activity; however, there was also an 88% decrease in the adrenaline concentration and a 40% decrease in the noradrenaline concentration.

Because TH is inhibited by catechols [3, 28] the possibility exists that the increase in TH activity in the apomorphine-injected hypophysectomized rat may be due to the low concentrations of adrenaline and noradrenaline *in vitro*. For this reason, adrenal homogenates of hypophysectomized rats injected with apomorphine and controls were dialyzed. The results (Table 2) show that after dialysis the same type of increase in TH activity occurs.

*Effect of apomorphine treatment on adrenal TH activity and CA concentrations in hemi-splanchnicectomized rats.* To determine whether the increase in TH activity was neuronally mediated the left splanchnic nerve was cut. Rats were then injected with apomor-

Table 2. Effect of apomorphine on adrenal TH activity and CA concentrations in hypophysectomized rats

| Group | Apomorphine | No. rats | Tyrosine hydroxylase (nmoles dopa/hr/pr adrenals) | Adrenaline μg/pr adrenals | Noradrenaline μg/pr adrenals |
|---|---|---|---|---|---|
| Sham-operated | – | 4 | 38.75 ± 5.33 | 16.5 ± 1.4 | 3.0 ± 0.3 |
| | + | 4 | 68.29 ± 3.67[b] | 15.8 ± 0.7 | 4.5 ± 0.3[b] |
| Hypophysectomized | – | 9 | 31.47 ± 2.20 | 12.8 ± 0.8 | 2.7 ± 0.1 |
| | + | 7 | 47.23 ± 2.74[c] | 1.6 ± 0.2[c] | 1.6 ± 0.2[c] |
| Hypophysectomized[a] | – | 8 | 21.85 ± 1.24 | 9.90 ± 0.3 | 2.9 ± 0.3 |
| | + | 8 | 37.99 ± 3.00[c] | 2.83 ± 0.3[c] | 2.1 ± 0.2[b] |

Animals were given 10 mg/kg apomorphine i.p. at 1.5-hr intervals 7 times daily for 3 days. Seventy-two hr after the first injection the rats were killed and the TH activity and CA concentrations determined.

[a] The adrenal homogenates were dialyzed prior to the TH assay as described in Materials and Methods.

[b] Significantly different from control (P < 0.01).

[c] Significantly different from control (P < 0.001).

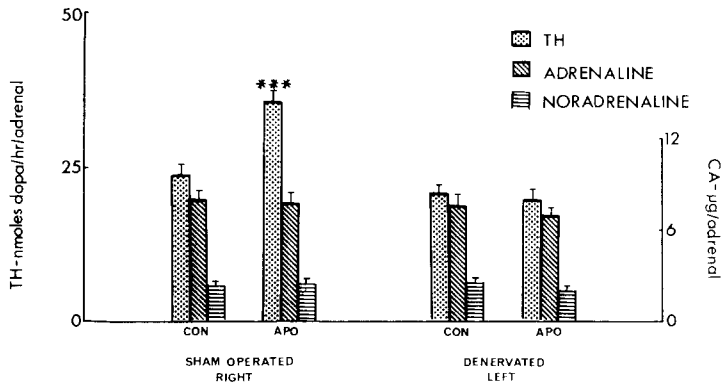


Fig. 4. Effect of adrenal denervation on the apomorphine-induced increase in TH activity and CA concentrations of rat adrenals. Five days after left splanchnic transsection, rats were given injections of apomorphine (APO) according to the 3-day schedule described in the legend to Fig. 1. They were killed 72 hr after the initial injection. Each bar represents the mean ± S.E.M. of 8–11 animals. Significance of differences from control (CON) are indicated as follows: ***$P < 0.001$.

phine for 3 days. The results (Fig. 4) show there were slight decreases in the TH activity and CA concentrations in the denervated control adrenal (left) as compared to the sham-operated control (right); however, these were not significantly different ($P > 0.05$). After apomorphine treatment, the expected increase in TH activity was no longer observed in the denervated adrenal. Moreover, the adrenaline and noradrenaline concentrations were identical to those in the denervated adrenal glands of rats receiving no apomorphine.

The effects of adrenal denervation and of apomorphine treatment on the short term adrenal CA concentrations were also determined. Five days after denervation of the left adrenal rats were injected with apomorphine, 10 mg/kg i.p., 7 times at 1.5-hr intervals. They were killed 1.5 hr after the last injection. The decrease which occurred in the CA concentration after apomorphine treatment was no longer observed when the splanchnic nerve was cut.

*The effect of mecamylamine on the increase in TH activity induced by various treatments.* Costa *et al.* [31] have shown that the ganglionic blocker mecamylamine can prevent the increase in adrenal TH activity induced by cold exposure if the blocker is injected prior to the treatment. A series of experiments was carried out in which rats received 10 mg/kg mecamylamine i.p. The drug treatment was divided into 4 doses and given over the same period the animals were receiving apomorphine (see legend to Fig. 1). The first injection of mecamylamine was given 15 min prior to the apomorphine treatment. There was no effect of the blocker on TH activity in control or apomorphine-treated rats.

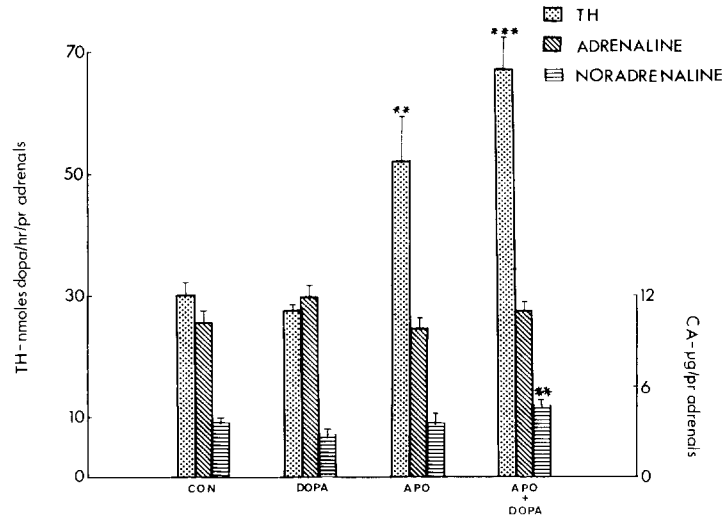


Fig. 5. The effect of L-DOPA on the apomorphine-induced increase in adrenal TH. Rats were injected with 10 mg/kg apomorphine (APO) i.p. every 1.5 hr, 7 times daily for 4 days; with 1 g/kg L-DOPA s.c. given once daily for 4 days; or with one injection of 1 g/kg L-DOPA given 15 min prior to the first of a series of 7 injections of apomorphine. The rats were sacrificed 96 hr after the initial injection. An aliquot of the adrenal homogenate was removed for CA determinations and the remainder dialyzed prior to assay as described in Materials and Methods. Each bar represents the mean ± S.E.M. of 6 animals. Significance of differences from control (CON) are indicated as follows: **$P < 0.01$ ***$P < 0.001$.

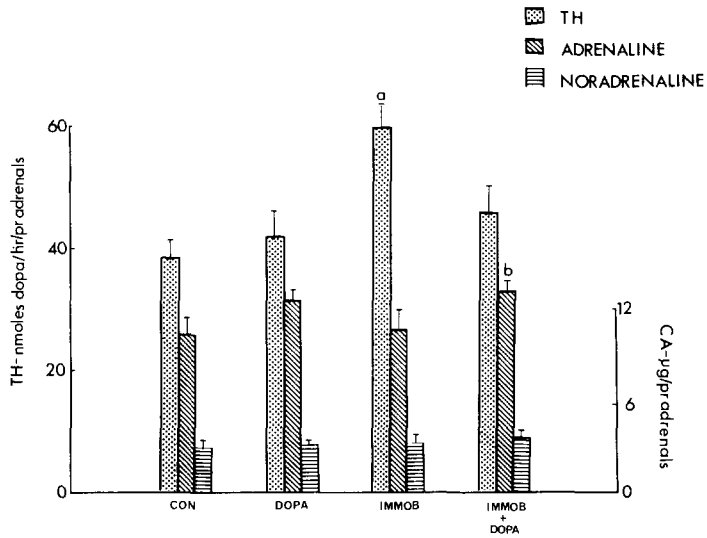


Fig. 6. The effect of L-DOPA on the immobilization-induced increase in adrenal TH. Rats were subjected to a 3-hr immobilization (IMMOB) period daily; injected with 1 g/kg L-DOPA s.c. once daily; or were injected with 1 g/kg L-DOPA s.c. once daily 15 min prior to a 3-hr immobilization period. The results presented here are those pooled from a 3 and 4 day experiment; thus the rats were killed 72 or 96 hr after the initial treatment. An aliquot of the adrenal preparation was removed for CA measurements and then the preparations were dialyzed prior to TH assay as described in Materials and Methods. Each bar for TH represents the mean ± S.E.M. of 10 12 animals; each bar for CA the mean ± S.E.M. of 5 6 rats. [a]Significantly different from controls $P < 0.01$; from L-DOPA treatment $P < 0.01$; from L-DOPA plus immobilization treatment $P < 0.05$. [b]Significantly different from controls $P < 0.05$.

In additional experiments animals were immobilized 3 hr/day for 3 days; mecamylamine, 4.2 mg/kg, was injected 15 min prior to the immobilization, immediately after it and 3 and 6 hr later. Rats subjected to 10.5-hr cold exposure received the blocking agent in 4 injections, each 2.5 mg/kg, at 3-hr intervals with the first injection given 15 min prior to treatment. The inclusion of the mecamylamine injection did not prevent the immobilization-induced or cold-induced increase in TH activity.

*The effect of* L-*DOPA on the apomorphine-induced increase in adrenal TH activity.* Both the prevention of the apomorphine-induced increase in TH activity by denervation and the negative results obtained with the 2 day αMPT experiment suggest that the increase in TH activity is regulated neuronally and not locally. Further proof of this is evident from experiments using apomorphine in combination with L-DOPA. If the apomorphine-induced increase in TH activity is mediated through a local decrease in CA then the administration of L-DOPA, which would favour repletion of CA, together with apomorphine should prevent the increase in enzyme activity. If, on the other hand, the increase in TH is neuronally mediated L-DOPA would have no effect. Figure 5 shows that apomorphine given for 4 days increases adrenal TH activity by 70%, with no changes in the CA concentrations. One injection of 1 g/kg L-DOPA, given daily for 4 days, caused no statistically significant changes in adrenal TH or CA. When both apomorphine and L-DOPA were administered, the increase in TH activity was 120% over controls and more than 20% greater than the activity obtained with apomorphine alone (Fig. 5). A significant increase was also observed in the noradrenaline concentration. These results favour the view that the increase in TH is central in origin.

*The effect of* L-*DOPA on the apomorphine induced decrease in adrenal CA.* To ensure that L-DOPA was indeed repleting adrenal CA in apomorphine treated rats, the adrenaline and noradrenaline concentrations were measured 1.5 hr after a series of 7 injections of apomorphine. L-DOPA had been injected 15 min prior to the first apomorphine injection. As is evident from Table 1, experiment C the decrease in CA observed after apomorphine treatment is restored by L-DOPA administration.

*The effect of* L-*DOPA on the increase induced by immobilization in adrenal TH activity.* L-DOPA was unable to counteract the effect of apomorphine on TH activity (Fig. 5); however, Dairman *et al.* [32] have shown that the reserpine-induced increase in adrenal TH, which is neuronally mediated, can be completely blocked by the simultaneous administration of L-DOPA. To gain insight into this problem we looked at the effect of this amino acid on the immobilization-induced increase in TH activity. Immobilization 3 hr/day for 3 days increased rat adrenal TH activity by 58% (Fig. 6). Injection of L-DOPA 1 g/kg, given once daily, again caused no significant change in adrenal TH activity or CA concentrations. When L-DOPA was administered prior to a 3-hr immobilization period the increase in TH activity was no longer observed. The implications of this experiment are dealt with in the discussion.

## DISCUSSION

These results show that apomorphine can increase TH activity in rat adrenal glands. A schedule of 7

injections of apomorphine per day for 1, 2 or 3 days significantly increases adrenal TH *in vitro* when it is measured 15 hr after the last injection. The CA concentrations measured at each time period are the same for apomorphine-treated and control animals. The only exception to this is at 48 hr when there is a small but significant increase in the noradrenaline concentration; however, total CA concentrations are the same in the two groups. The gradual increase obtained in TH activity over the 3-day period is in accord with the concept that this increase is the result of a slow process in which the transcription phase takes approximately 18–24 hr to be completed, and translation another 24–36 hr [33].

A single injection of reserpine [34], a 2-hr swim stress [35] or a 2-hr cold stress [10] is sufficient to initiate an increase in rat adrenal TH detectable 24–48 hr after the initial stimulus. However, when rats are injected with apomorphine for 1 or 2 days and sacrificed 72 hr after the first injection, no significant increase in TH is observed. Interruption of the stimulus appears to allow adaptive mechanisms to restore enzyme activity to control levels.

Activation of TH in the adrenals of apomorphine-treated rats by an effector molecule was ruled out through kinetic studies as well as mixing and dialysis experiments. Kinetic studies of adrenal TH preparations from apomorphine-injected and control animals showed there was no change in the $K_m$ of the enzyme for the pteridine cofactor, $DMPH_4$, or tyrosine. However, an approximately 2-fold increase in $V_{max}$ was observed. When preparations made from adrenals of apomorphine-treated and control rats were incubated together and then assayed, the enzyme activities were additive; this indicates that the increase in activity was probably due to an increase in enzyme protein and not to the presence of an activator or loss of an inhibitor. To substantiate this point further, the adrenal preparations were dialyzed and the kinetic parameters were again determined. The increase in enzymic activity was still observed after dialysis and the $K_m$ values for control and apomorphine-treated rats were identical. Thus apomorphine treatment increases TH activity and this appears to occur through an increase in the amount of enzyme protein.

It has been suggested [13, 17, 36] that a relationship may exist between adrenal CA and adrenal TH activity. Although we found CA levels to be identical in apomorphine-treated and control rats when measured 24, 48 and 72 hr after the initial injection, that is, 15 hr after the last of a series of 7 injections (Fig. 1), a decrease was noted when the CA concentrations were determined only 1.5 hr after the daily schedule of injections was completed (Table 1).

Goldstein *et al.* [20] have shown that apomorphine inhibits rat brain TH at concentrations as low as $10^{-6}$ M and our *in vitro* studies confirmed these findings. To determine whether apomorphine could be inhibiting TH *in vivo*, the concentration was measured in rat adrenals after a single injection of the drug. After 3 min, a peak concentration corresponding to $3 \times 10^{-5}$ M was obtained which decreased to $10^{-6}$ M at 90 min. *In vivo* inhibition of adrenal TH is therefore a possibility. In a further effort to determine this, apomorphine was given simultaneously with the TH inhibitor αMPT over a 1-day period; the adrenaline and noradrenaline concentrations were measured 1.5 hr after the last injection of apomorphine (Table 1). The αMPT was injected in amounts sufficient to produce nearly complete inhibition of adrenal TH [22]. If the action of apomorphine is through enzyme inhibition, the apomorphine-induced decrease in adrenal CA should be similar to that with αMPT; also injection of both drugs should result in a decrease in CA similar to or slightly greater than with αMPT (since αMPT may not be inhibiting TH 100%). The results show, however, that administration of αMPT in the conditions used in these experiments did not cause a significant decrease in adrenal CA; this can be explained by the long half-life of adrenal CA [30]. More surprisingly, the simultaneous administration of αMPT with apomorphine potentiated the decrease caused by the latter drug alone. That joint inhibition of TH by the two drugs is responsible for this effect does not seem likely, because the decrease in CA is observed only as long as adrenal innervation is intact. This favours the interpretation that apomorphine is causing increased secretion of CA by means of increased nerve stimulation.

To determine whether the adrenal CA may play a regulatory role in the induction of adrenal TH further use was made of αMPT. Administration of this drug caused highly significant decreases in the adrenal CA concentrations after 1 and 2 days; however, the TH activity 48 hr after the beginning of treatment was identical to that of controls (Fig. 3). This result is, of course, not consistent with the concept that adrenal CA are important in regulating the induction of adrenal TH.

Since local control does not appear to be a factor in regulating the induction of TH, hormonal and neuronal control was investigated. Mueller *et al.* [12] have shown that hormonal control is important for maintaining the basal level of TH and that in hypophysectomized rats ACTH can restore the decreased TH activity to normal. To determine whether apomorphine was causing the increase in TH activity through pituitary-mediated mechanisms, the drug was administered to hypophysectomized rats. The increase in enzyme activity observed after apomorphine treatment was the same in operated as in sham-operated animals; however, a large decrease was observed in the adrenaline and noradrenaline content of adrenals of hypophysectomized rats given apomorphine. This parallels the effect observed by Kvetnansky *et al.* [37] in hypophysectomized rats after immobilization.

In all previous reports, intact innervation was required if increases in adrenal TH were to be observed: reserpine, 6-hydroxydopamine, phenoxybenzamine [11], cold exposure [10] and immobilization [9] no longer cause an increase in TH activity after the nerve fibers to the adrenal gland are cut. The apomorphine-induced increase in adrenal TH and short term decrease in CA also require intact innervation, indicating that increase in enzyme synthesis required nervous input.

To demonstrate further that the apomorphine-induced increase in adrenal TH required intact innervation, we attempted to use the observation of Costa *et al.* [31] that injection with the ganglionic blocker mecamylamine prevents the increase in adrenal TH

observed after cold exposure. Using the same dose of mecamylamine as described [31], and with the first injection given 15 min prior to the apomorphine treatment, we were unable to prevent the increase in adrenal TH activity. In a further experiment this drug, in a dose nearly twice as large as prescribed, was administered to rats subjected to immobilization and again it did not prove effective in blocking the induced increase in TH. To reproduce the conditions of the original experiment [31] rats were exposed to cold; the increase in TH activity was not blocked. No other ganglionic blockers were tested as Mueller *et al.* [38] have shown them to be ineffective in blocking increases in adrenal TH activity.

Further studies were undertaken to investigate the reason for the increase in TH activity after apomorphine treatment. When L-DOPA was administered prior to the apomorphine treatment the induced increase in TH activity was obtained (Fig. 5), despite the restoration of adrenal CA to control levels (Table 1). The facts that (1) L-DOPA was totally ineffective in blocking the increase in TH after apomorphine; (2) the initial decrease in CA and the long term increase in TH activity were prevented by adrenal denervation; and (3) αMPT decreases adrenal CA and yet does not increase adrenal TH, indicate that increased preganglionic nerve activity is the major controlling factor in the induction of adrenal TH.

Experiments by Dairman *et al.* [32] appear to contradict the results we obtained after the simultaneous administration of apomorphine and L-DOPA. It has been shown that reserpine increases adrenal TH activity and decreases adrenal catecholamines [34]. When L-DOPA and reserpine were given simultaneously the increase in TH was completely abolished and control enzyme levels were obtained. This might lead to the conclusion that adrenal CA are regulatory, despite the fact that the increase after reserpine can only occur with intact innervation [11].

Further work was done using immobilized rats since this treatment induces adrenal TH and bears some resemblances to the action of apomorphine. Kvetnansky and Mikulaj [39] showed that adrenal CA are decreased significantly immediately after a daily 2.5-hr immobilization up till the 14th day of immobilization. There is also a delayed increase in adrenal TH [9]. Both the increase in TH activity and the decrease in CA concentrations can be abolished by splanchnicectomy [40]. In the present work L-DOPA was administered 10 min prior to a 3-hr immobilization period; in this case, in contrast to apomorphine treatment the increase in TH was not observed (Fig. 6).

Thus, L-DOPA was able to overcome the reserpine- and immobilization-induced increase in adrenal TH, but not the apomorphine-induced increase. This apparent contradiction can be interpreted if the actions of the drugs are considered at the central and not the local level. Watanabe *et al.* [41] have shown that increasing central nervous system CA with L-DOPA produces a generalized reduction of efferent sympathetic nerve activity. Reserpine, in contrast, depletes brain monoamines [42] and produces a reflex increase in sympathetic nerve activity [43]. The combination of L-DOPA and reserpine may result in a balance in brain CA and, thus, normal firing of preganglionic sympathetic fibers. A similar situation to that with reserpine may exist with immobilization; Bliss and Zwanziger [44] have shown that immobilization leads to a decrease in brain noradrenaline.

Since the apomorphine-induced increase in adrenal TH activity was still observed with L-DOPA pretreatment the mechanism by which the increase occurs must be different from that caused by immobilization stress and reserpine. This implies that apomorphine is not producing its effect in the present instance through the stress of the induced stereotyped behaviour [21] or by alterations in the caliber of the peripheral vasculature [45].

Apomorphine, however, is known to stimulate dopamine receptors in brain [21, 46, 47], a condition which, from our results, appears to cause increased activity of splanchnic nerve fibers and an increase in adrenal TH. Stimulation of these central dopamine receptors would occur in the presence or absence of L-DOPA with resultant nerve firing in either case. This implies that a dopaminergic system is involved in the induction of adrenal TH.

The claim that alterations in brain activity can regulate adrenal TH has been made by several authors. Reis *et al.* [48] have shown that stimulation of certain brain areas can increase adrenal TH. Smith *et al.* [49] have shown that diazepam, a central nervous system depressant, can increase the induction of adrenal TH by isoproterenol. It has been proposed that this occurs through the ability of the drugs to interfere with the firing of CNS neurons. Specific brain systems have also been implicated in the induction of adrenal TH. Mueller *et al.* [50] and Breese *et al.* [51] have suggested that interference with serotonin nerve function potentiates the induction of adrenal TH by various treatments. That a dopaminergic system may be important is evident from the work of Beuning and Gibb [52]. Methamphetamine, a drug which releases brain dopamine, increases adrenal TH activity; however, when haloperidol, a dopamine receptor blocker, is administered in combination with methamphetamine, the increase in TH is decreased about 50 per cent.

In conclusion, we have shown that increases in adrenal TH are mediated centrally, and not locally through alterations in the CA concentration. Furthermore, the results suggest that stimulation of central dopamine receptors by apomorphine alters the activity of preganglionic neurons which innervate the adrenal medulla and results in an increase in the TH activity.

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