Biochemical Pharmacology, Vol. 18, pp. 2661–2666. Pergamon Press. 1969. Printed in Great Britain

STUDIES ON THE ROLE OF CATECHOLAMINES AS REGULATORS OF TYROSINE AMINOTRANSFERASE

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(*Received* 14 *March* 1969; *accepted* 2 *May* 1969)

Abstract—It has been suggested that catecholamines are involved in the control of tyrosine aminotransferase activity. We have studied the effects of drugs which produce adrenergic blockade and catecholamine depletion on the activity of tyrosine aminotransferase (TAT) in rat liver. Phentolamine was found to increase TAT activity while phenoxybenzamine and propranolol had no effect. Of the depleting agents tested reserpine, guanethidine and α-methyl-para-tyrosine increased TAT activity while α-methyl-meta-tyrosine had no effect. All compounds which increased TAT activity also increase the plasma corticosterone concentration; α-methyl-para-tyrosine also increased slightly the enzyme activity in adrenalectomized rats. Thus, TAT activity is influenced by the changes in plasma corticosterone levels which these compounds produced, rather than their adrenergic blocking or catecholamine depleting effects. The response to α-methyl-para-tyrosine in adrenalectomized animals suggests that some additional mechanism is also involved.

TRANSAMINATION is the initial step in the major catabolic pathway of tyrosine.[1,2] This reaction is catalyzed by tyrosine aminotransferase (L-tyrosine: 2-oxoglutarate aminotransferase, EC 2.6.1.5.). The mechanism by which the activity of this enzyme is controlled has been the subject of numerous investigations. It is known from the work of Lin and Knox[3] that elevation of circulating adrenal corticosteroids increases the activity of this enzyme. Recently, Wicks[4] reported that epinephrine increased tyrosine aminotransferase (TAT) activity. On the other hand, Black and Axelrod[5] have suggested that, on the basis of studies with norepinephrine depleting and repleting drugs, norepinephrine may suppress TAT activity directly or indirectly. Thus, although it would seem to be established that adrenal corticosteroids influence TAT activity, there is some disagreement concerning the role of catecholamines. If catecholamines are involved in the regulation of TAT, one would expect that compounds known to affect responses to catecholamines in other systems would affect the enzyme activity. For this reason, we have examined the effects of adrenergic blockade and catecholamine depletion on TAT activity. The results are presented in this report.

METHODS

Adult, female Sprague–Dawley rats weighing 180–220 g were used in these experiments. The animals received laboratory chow and water *ad libitum*, except during the period of an experiment. Food was withheld for the period of each study. All rats were killed at 12 noon to eliminate the effect of the normal diurnal variation on the

level of tyrosine aminotransferase. All drugs were given intraperitoneally (i.p.) in 0·9% NaCl. Dosage schedules are described with the individual experiments.

Tyrosine aminotransferase activity was determined by the method of Diamondstone.[6] Liver was homogenized in 4 vols. of 0·15 M KCl and centrifuged for 20 min at 30,000 *g*. The supernatant fraction was used as the enzyme solution. The reaction was routinely allowed to proceed for 10 min at 37° using 15 μl of enzyme solution in a total volume of 3 ml. Preliminary experiments indicated that the reaction was proportional to the amount of enzyme used under these conditions. Enzyme activity is expressed as μmoles of *p*-hydroxyphenylpyruvate formed per mg protein per hr. Protein was estimated by the method of Lowry *et al.*[7]

Plasma corticosterone levels were determined by the method of Zenker and Bernstein.[8] Adrenalectomized rats were obtained from Hormone Assay Laboratories, Chicago, Ill. Experiments were performed on the fifth day following operation. Norepinephrine was determined by the method of de Champlain *et al.*[9]

The following drugs were used in these studies: phentolamine HCl (Regitine, CIBA), phenoxybenzamine HCl (Dibenzyline, Smith, Kline & French), propranolol HCl (Inderal, Ayerst), reserpine (Serpasil, CIBA), α-methyl-para-tyrosine ethyl ester (Merck, Sharp & Dohme), α-methyl-meta-tyrosine (Merck, Sharp & Dohme), guanethidine (Ismelin, CIBA), and 3,4-dihydroxyphenylalanine (L-DOPA, Calbiochem Inc.).

RESULTS

Effects of adrenergic blocking agents. The *alpha* adrenergic blocking agents phentolamine and phenoxybenzamine and the *beta* adrenergic blocking agent propranolol were given to intact and adrenalectomized rats in dosage schedules known to result in maximal adrenergic blockade and the effects on liver TAT activity were determined. The results are summarized in Table 1. In normal, intact rats the administration of phentolamine resulted in a 3-fold increase in enzyme activity. Phenoxybenzamine and propranolol were without effect. In adrenalectomized animals none of the adrenergic blocking agents induced the enzyme activity.

Effects of catecholamine-depleting agents. Black and Axelrod[5] have reported that the catecholamine-depleting drugs, reserpine and α-methyl-para-tyrosine, increase liver TAT in adrenalectomized rats. They found that the administration of L-DOPA

TABLE 1. EFFECTS OF ADRENERGIC BLOCKING AGENTS ON RAT LIVER TYROSINE AMINOTRANSFERASE*

Treatment	Enzyme activity (μmoles product/mg protein/hr)	
	Normal	Adrenalectomized
Control (saline)	1·30 ± 0·20	1·35 ± 0·22
Phentolamine (25 mg/kg)	3·85 ± 0·25	1·40 ± 0·18
Phenoxybenzamine (25 mg/kg)	1·65 ± 0·22	1·70 ± 0·20
Propranolol (25 mg/kg)	1·60 ± 0·30	1·68 ± 0·25

* Each group contained six animals. Phentolamine was injected i.p. 3 hr before sacrifice. Phenoxybenzamine was injected 17 and again 3 hr before sacrifice. Propranolol was injected 3 and again 1 hr before sacrifice. All drugs were given in a volume of 0·5 ml. Enzyme activity values represent the mean ± standard error.

to replenish norepinephrine stores effectively blocked the production of an increased enzyme activity by α-methyl-para-tyrosine. They suggest, therefore, that the increased enzyme activity produced by reserpine or α-methyl-para-tyrosine is a result of norepinephrine depletion. We have performed similar experiments in both normal and adrenalectomized rats. The results are presented in Table 2. In normal rats, α-methyl-para-tyrosine produced a 6-fold increase in enzyme activity. The administration of

TABLE 2. EFFECTS OF α-METHYL-*p*-TYROSINE AND 3,4-DIHYDROXYPHENYLALANINE ON RAT LIVER TYROSINE AMINOTRANSFERASE*

Treatment	Enzyme activity (μmoles product/mg protein/hr)	
	Normal	Adrenalectomized
Control (saline)	1·35 ± 0·20 (6)	1·59 ± 0·18 (11)
α-Methyl-*p*-tyrosine	7·88 ± 0·80 (6)†	2·62 ± 0·29 (7)‡
L-DOPA	1·19 ± 0·18 (6)	1·17 ± 0·13 (8)
α-Methyl-*p*-tyrosine + L-DOPA	7·10 ± 0·90 (6)†	1·85 ± 0·26 (8)

* Rats receiving α-methyl-para-tyrosine (ethyl ester) were injected with 200 mg/kg at 10 p.m. and 50 mg/kg at 8 a.m. Those receiving dihydroxyphenylalanine (L-DOPA) were injected with 100 mg/kg at 8 a.m. Rats treated with both drugs received α-methyl-*p*-tyrosine 200 mg/kg at 10 p.m. and 50 mg/kg at 8 a.m. and L-DOPA 100 mg/kg at 8 a.m. All injections were i.p.. Rats were killed at 12 noon. Enzyme activity values represent the mean ± standard error. The number of animals in each group is indicated in parenthesis.

† Significantly different from control ($P < 0·001$).

‡ Significantly different from control ($P < 0·01$).

L-DOPA did not significantly alter the response to α-methyl-para-tyrosine. In adrenalectomized animals, the results (Table 2) followed the same general pattern reported by Black and Axelrod,[5] although the differences between groups were considerably smaller. The TAT activity of animals treated with α-methyl-para-tyrosine was significantly higher than that of controls. The administration of L-DOPA tended to counteract the effect of α-methyl-para-tyrosine, but the change produced was not statistically significant.

In another series of experiments, we compared the induction of TAT and the depletion of myocardial norepinephrine produced by reserpine, guanethidine, α-methyl-para-tyrosine and α-methyl-meta-tyrosine. The results are presented in Table 3. All of these compounds produced a significant reduction in myocardial norepinephrine content. However, only reserpine, guanethidine and α-methyl-para-tyrosine increased the enzyme activity. α-Methyl-meta-tyrosine, an excellent norepinephrine depleting agent, had no effect on enzyme activity in either intact or adrenalectomized rats. Among those compounds which did increase enzyme activity, no correlation could be demonstrated between the degree of norepinephrine depletion and the increase in enzyme activity. The compound which produced the greatest increase in TAT activity, α-methyl-para-tyrosine, produced the least depletion of norepinephrine.

Relation of tyrosine aminotransferase induction to plasma corticosterone concentration. Lin and Knox[3] have demonstrated that glucocorticoids induce TAT activity. Both reserpine and α-methyl-para-tyrosine have been reported by Carr and Moore[10] to increase rat plasma corticosterone concentration. We have examined the effects

TABLE 3. EFFECTS OF CATECHOLAMINE-DEPLETING AGENTS ON RAT LIVER TYROSINE AMINOTRANSFERASE ACTIVITY AND MYOCARDIAL NOREPINEPHRINE CONTENT*

Treatment	Enzyme activity (μmoles product/mg protein/hr)	Myocardial norepinephrine (μg/g)
Control (saline)	1·30 ± 0·10	0·85 ± 0·02
Reserpine (5 mg/kg)	5·74 ± 0·40	< 0·01
Guanethidine (25 mg/kg)	3·08 ± 0·15	0·21 ± 0·01
α-Methyl-*p*-tyrosine (200 mg/kg)	8·83 ± 0·60	0·38 ± 0·02
α-Methyl-*m*-tyrosine (400 mg/kg)	1·38 ± 0·15	0·17 ± 0·02

* Each group contained ten animals. Reserpine and α-methyl-*p*-tyrosine (ethyl ester) were given i.p. 17 and again 3 hr before sacrifice. Guanethidine and α-methyl-*m*-tyrosine were given i.p. 3 hr before sacrifice. Values represent the mean ± standard error.

TABLE 4. EFFECT OF VARIOUS COMPOUNDS ON RAT PLASMA CORTICOSTERONE CONCENTRATION*

Treatment	Plasma corticosterone (μg/100 ml)
Control (saline)	45 ± 5
Phentolamine (25 mg/kg)	150 ± 10
Reserpine (5 mg/kg)	175 ± 12
Guanethidine (25 mg/kg)	225 ± 20
α-Methyl-*p*-tyrosine (200 mg/kg)	150 ± 15
α-Methyl-*m*-tyrosine (400 mg/kg)	40 ± 8

* Each group contained ten animals. Reserpine and α-methyl-*p*-tyrosine (ethyl ester) were given i.p. 17 and again 3 hr before sacrifice. Phentolamine, α-methyl-*m*-tyrosine and guanethidine were given i.p. 3 hr before sacrifice. All values represent the mean ± standard error.

of several compounds on the plasma corticosterone concentration. The results are presented in Table 4. Compounds which had been shown to increase TAT activity, i.e. phentolamine, reserpine, guanethidine, and α-methyl-para-tyrosine, all increased the plasma corticosterone level 4 to 5-fold. α-Methyl-meta-tyrosine did not increase the enzyme activity and did not increase the corticosterone concentration.

DISCUSSION

We have examined the possibility that catecholamines are involved in the normal control *in vivo* of tyrosine aminotransferase activity. Conflicting reports concerning the effects of catecholamines on the activity of this enzyme have appeared. Wicks[4] has reported that epinephrine increases the activity of TAT in tissue cultures of fetal rat liver. Using a different experimental system, the adrenalectomized rat *in vivo*, Black and Axelrod[5] have reported that a reduction in endogenous norepinephrine content results in an increase in TAT activity. If catecholamines were affecting the normal activity of this enzyme, one would expect that this effect would be mediated by one of the known adrenergic receptor mechanisms. However, no systematic relationship between adrenergic blockage and alteration in enzyme activity could be demonstrated in our experiments. Although phentolamine was found to be an effective inducing agent for TAT, a second *alpha* blocking agent, phenoxybenzamine, was

found to be without effect on the enzyme activity. These findings indicate that the response of the enzyme to phentolamine is unrelated to *alpha* receptor blockade. Similarly, no effect of *beta* receptor blockade could be demonstrated.

Our results agree with the findings of Black and Axelrod[5] that the catecholamine-depleting agents reserpine and α-methyl-para-tyrosine increase TAT activity. These authors proposed that the presence of norepinephrine suppresses the synthesis of TAT. However, we have found that α-methyl-meta-tyrosine, a compound which effectively depletes catecholamines, did not increase the activity of the enzyme. It is known that α-methyl-meta-tyrosine is converted *in vivo* to metaraminol, which may then act as a false transmitter. One could postulate that a false transmitter may assume the role of norepinephrine and prevent an increase in TAT activity when the endogenous norepinephrine level has been reduced. However, the false transmitter in this case, metaraminol, is considerably less potent as a sympathomimetic agent than norepinephrine. It is unlikely, therefore, that metaraminol could completely counteract the effect of a marked norepinephrine depletion. The effects on TAT activity observed after administration of α-methyl-meta-tyrosine may, therefore, be considered to represent the effects of uncomplicated norepinephrine depletion. Our findings indicate that norepinephrine depletion *per se* does not affect tyrosine aminotransferase activity.

Although reserpine and α-methyl-para-tyrosine are catecholamine-depleting agents, this action is not their only common factor. Carr and Moore[10] have demonstrated that both compounds also increase the plasma corticosterone concentration. We have confirmed these findings and have also shown that phentolamine and guanethidine increase the plasma corticosterone level. Adrenal corticosteroids are known to induce TAT activity.[3] Therefore, it is probable that the induction of TAT by these compounds in intact rats is primarily a result of the increased plasma corticosterone concentration. However, the entire response of the enzyme to α-methyl para-tyrosine cannot be due to the increased corticosterone concentration since some response was obtained in adrenalectomized rats. The basis for this residual response is not immediately apparent. It has been reported by Lin and Knox[3] and by Kenney and Flora[11] that the primary substrate of this enzyme, tyrosine, does not increase enzyme activity in adrenalectomized rats. Thus, the close structural similarity between tyrosine and α-methyl-para-tyrosine does not appear to account for the residual effect of α-methyl-para-tyrosine in adrenalectomized rats. Nor can this response be a result of catecholamine depletion since α-methyl-meta-tyrosine was without effect in adrenalectomized rats.

In summary, several compounds which affect either the response to catecholamines or tissue catecholamine content have been found to increase TAT activity. These compounds include phentolamine, reserpine, guanethidine, and α-methyl-para-tyrosine. However, other similar compounds, including phenoxybenzamine, propranolol, L-DOPA and α-methyl-meta-tyrosine did not alter the enzyme activity. Thus, although several compounds which affect catecholamine metabolism also increase TAT activity, it is not clear that this action is related to their catecholamine effects. It would appear that the increase in TAT activity which these compounds produce is primarily related to an increase in circulating plasma corticosterone. However, the response to α-methyl-para-tyrosine in adrenalectomized animals suggests that a second mechanism is also involved.

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