Biochemical Pharmacology, Vol. 18, pp. 2667–2672. Pergamon Press. 1969. Printed in Great Britain

INDUCTION OF TYROSINE AMINOTRANSFERASE BY PHENTOLAMINE

WILLIAM C. GOVIER and WALTER LOVENBERG

Experimental Therapeutics Branch, National Heart Institute, Bethesda, Md., U.S.A.

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

Abstract—The characteristics of tyrosine aminotransferase induction by phentolamine in rat liver are described. After an initial lag period of about 60 min, phentolamine produced a marked increase in enzyme activity which reached a maximum in about 3 hr. This effect was eliminated by either actinomycin D or cycloheximide. The relation of the induction by phentolamine to adrenal steroids was studied. Phentolamine was found to increase plasma corticosterone levels in normal intact animals. The induction by phentolamine was almost completely abolished by either adrenalectomy or hypophysectomy. However, if a small dose of hydrocortisone was given to adrenalectomized rats, phentolamine then produced a significant increase in enzyme activity. The administration of amino-glutethimide completely eliminated any increase in plasma corticosterone but did not completely eliminate the enzyme induction produced by phentolamine. It is concluded that at least two factors are operative in the induction of tyrosine aminotransferase by phentolamine—(1) a response to an increased plasma corticosterone concentration, and (2) an additional effect which may be a direct substrate type of induction.

TYROSINE aminotransferase (L-tyrosine: 2-oxoglutarate aminotransferase, EC 2.6.1.5) has been a very popular model sustem for the study of induction of a soluble enzyme. This interest stems from the importance of transamination as the initial step in tyrosine catabolism to fumaric and acetoacetic acids, plus the ability to produce relatively large and rapid changes in the activity of the enzyme with a variety of substances.

During a recent study of the possible role of catecholamines in the normal control mechanism for this enzyme, it was found that one adrenergic blocking compound, the *alpha* receptor blocking agent phentolamine, produced a marked increase in tyrosine aminotransferase (TAT) activity.[1] The characteristics of this effect of phentolamine are described in this report. The information derived from these experiments leads to the conclusion that phentolamine induces TAT through at least two separate mechanisms.

METHODS

Adult, female Sprague–Dawley rats were used in these experiments. The rats were fed laboratory chow *ad libitum*, except during the period of an experiment. All rats were killed at 12 noon to eliminate the effect of the normal diurnal variation in the level of TAT. All drugs were given intraperitoneally (i.p.) in 0·9% NaCl. Dosage schedules are described with the individual experiments.

Tyrosine aminotransferase was determined by the method of Diamondstone.[2]

The rat liver was homogenized in 4 vol. 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 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 of protein per hour. Protein was estimated by the method of Lowry *et al.*[3]

Adrenalectomized and hypophysectomized rats were obtained from Hormone Assay Laboratories, Chicago, Ill. These animals were used 5 days after the operative procedure.

The following drugs were used in these experiments: phentolamine HCl (Regitine, CIBA), hydrocortisone (Solucortef, Upjohn), cycloheximide (obtained from Dr. H. Wood, National Cancer Institute), actinomycin D and amino-glutethimide phosphate (Elipten, CIBA).

RESULTS

Administration of phentolamine to rats resulted in a marked increase in hepatic TAT. The effect of drug dosage on enzyme level is presented in Fig. 1. The effect was first noticeable at a dose of 5 mg/kg. The largest dose used, 35 mg/kg, produced a 5-fold increase. The response was linear over the dose range tested.

The time course for development of the response to a single dose of 25 mg/kg of phentolamine is presented in Fig. 2. There was an initial lag period of about 60 min, followed by a rapid increase in enzyme activity over the next 2 hr. The phentolamine effect began to plateau about 3 hr after administration of the drug.

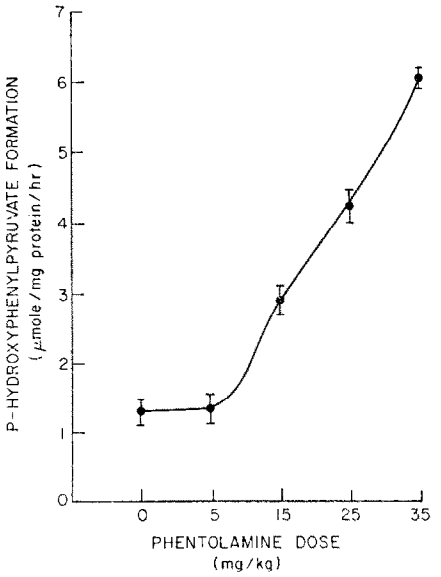

FIG. 1. Dose–response curve for phentolamine induction of tyrosine aminotransferase. Rats received various doses of phentolamine i.p. They were killed and the enzyme assayed 3 hr after injection. Each point represents the mean of five experiments. Vertical bars indicate standard error of the mean.

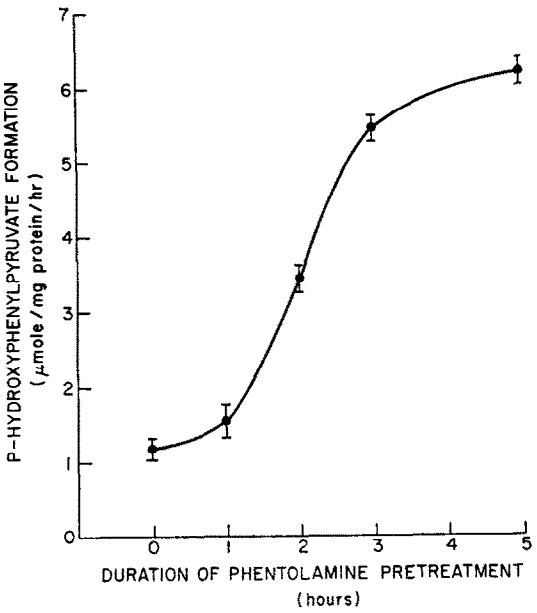

FIG. 2. Time course of the induction of rat liver tyrosine aminotransferase by phentolamine. Rats received 25 mg/kg phentolamine i.p. and were killed at various intervals. Each point represents the mean of five experiments. Vertical bars indicate standard error of the mean.

Phentolamine had no effect on TAT activity when added in concentrations up to 10^{-4} M to systems *in vitro* of either liver homogenate or liver slices.

The effects of cycloheximide and actinomycin D on the response to phentolamine were studied. Cycloheximide prevents the transfer of amino acids from RNA to polypeptides,[4] whereas actinomycin D prevents the synthesis of new RNA.[5] The results are shown in Table 1. Either of these compounds completely prevented the effect of phentolamine on TAT activity. Neither compound affected the enzyme activity when given alone.

In earlier experiments, it was found that phentolamine increased the concentration of plasma corticosterone 4- to 5-fold above values found in control animals.[1]

TABLE 1. EFFECTS OF CYCLOHEXIMIDE AND ACTINOMYCIN D ON RAT LIVER TYROSINE AMINOTRANSFERASE INDUCTION BY PHENTOLAMINE*

Treatment	Enzyme activity (μmoles product/mg protein/hr)
Control (saline)	1·45 ± 0·25
Phentolamine (25 mg/kg)	4·30 ± 0·30
Cycloheximide (50 mg/kg)	1·25 ± 0·22
Phentolamine (25 mg/kg) + cycloheximide (50 mg/kg)	1·45 ± 0·20
Actinomycin D (0·5 mg/kg)	1·30 ± 0·22
Phentolamine (25 mg/kg) + actinomycin D (0·5 mg/kg)	1·40 ± 0·24

* All drugs were given i.p. in a volume of 0·5 ml 3 hr before sacrifice. Each group contained six animals. Enzyme activity values represent the mean ± standard error.

It is known from the work of Lin and Knox[6] that the administration of glucocorticoids to rats results in an increase in tyrosine aminotransferase activity. Therefore, the relationship between the increase in plasma corticosterone and the increase in tyrosine aminotransferase activity produced by phentolamine was studied. The results are presented in Table 2. If rats were either adrenalectomized or hypophysectomized,

TABLE 2. RELATION OF RAT LIVER TYROSINE AMINOTRANSFERASE INDUCTION BY PHENTOLAMINE TO ADRENAL CORTICOSTEROIDS*

Treatment	Enzyme activity (μmoles product/mg protein/hr)		
	Condition of animal		
	Normal	Adrenalectomized	Hypophysectomized
Control (saline)	1·30 ± 0·15 (8)	1·45 ± 0·18 (8)	1·65 ± 0·20 (6)
Phentolamine (25 mg/kg)	4·50 ± 0·20 (8)	1·70 ± 0·15 (8)	1·80 ± 0·24 (6)
Amino-glutethimide (25 mg/kg)	1·28 ± 0·15 (8)		
Phentolamine (25 mg/kg) + amino-glutethimide (25 mg/kg)	1·85 ± 0·20 (8)		
Hydrocortisone (12 mg/kg)	4·30 ± 0·20 (6)	4·24 ± 0·30 (6)	
Phentolamine (25 mg/kg) + hydrocortisone (12 mg/kg)		6·75 ± 0·35 (6)	

* All drugs were given i.p. in a volume of 0·5 ml. Phentolamine and hydrocortisone were administered 3 hr before sacrifice. Amino-glutethimide was administered 17 and again 3 hr before sacrifice. Operative procedures were performed 5 days before the experiment. Enzyme activity values represent the mean ± standard error. The number of animals in each group is indicated in parenthesis.

the response to phentolamine was almost completely abolished. If rats were pretreated with amino-glutethimide, a compound which blocks the conversion of cholesterol to pregnenolone and thereby inhibits the production of corticosterone in the rat,[7] the response to phentolamine was similarly reduced. However, a small effect was still obtained in these animals. This residual response in amino-glutethimide pretreated rats was statistically significant ($P < 0·05$). After pretreatment with amino-glutethimide, the administration of phentolamine did not increase the plasma corticosterone concentration (Table 3). Nevertheless, phentolamine still produced

TABLE 3. EFFECT OF PHENTOLAMINE AND AMINO-GLUTETHIMIDE ON RAT PLASMA CORTICOSTERONE CONCENTRATION*

Treatment	Plasma corticosterone (μg/100 ml)
Control (saline)	40 ± 5
Phentolamine (25 mg/kg)	160 ± 15
Amino-glutethimide (25 mg/kg)	38 ± 5
Phentolamine (25 mg/kg) + amino-glutethimide (25 mg/kg)	45 ± 6

* Each group contained eight animals. All drugs were given i.p. in a volume of 0·5 ml. Phentolamine was injected 3 hr before sacrifice. Amino-glutethimide was administered 17 and again 3 hr before sacrifice. Values represent the mean ± standard error.

a slight increase in enzyme activity. Hydrocortisone produced a marked increase in enzyme activity, as has been reported previously by others.[6] In adrenalectomized rats, phentolamine did not produce a significant change. However, if a small priming dose of hydrocortisone was given to adrenalectomized rats, the administration of phentolamine then resulted in a significant additional rise in TAT activity.

DISCUSSION

The activity of tyrosine aminotransferase fluctuates widely in response to a wide variety of substances.[8] Induction of this enzyme has been considered to be entirely dependent upon a supply of glucocorticoids.[9] Recently, however, an additional mechanism for this induction has been proposed which involves insulin and glucagon and appears to be steroid independent.[10] Our attention was drawn to phentolamine during a study of the possible role of catecholamines in the normal control of TAT activity.[1] Phentolamine produced a marked increase in enzyme activity which was unrelated to the *alpha* adrenergic blocking action of this compound. It was felt that a study of the mechanism of this increase in TAT activity could be of interest in regard to the overall control of this enzyme.

On the basis of the experiments with actinomycin D and cycloheximide, one can say that the increase in enzyme activity after phentolamine administration involves the formation of new RNA and the synthesis of new protein. This increase, therefore, appears to be a true enzyme induction.

The most potent stimulus to new enzyme formation after phentolamine administration appears to be an increase in circulating glucocorticoids. Induction of TAT has been considered to occur solely in response to glucocorticoids.[9] Consistent with this view are our results showing that phentolamine does markedly increase plasma corticosterone levels. Also, the time course of the enzyme response to phentolamine is very similar to that reported for hydrocortisone.[11] It is possible, however, that the induction which occurs in the presence of adrenal steroids is the result of some action of these compounds on cell membranes which affects the distribution or availability of endogenous tyrosine. The ultimate effect would then be a substrate type of induction rather than a response to the steroid itself.

Although an increase in plasma corticosterone concentration is clearly an important factor in the induction of TAT by phentolamine, the response to phentolamine was not completely eliminated under circumstances in which plasma glucocorticoid level could not increase. The response to phentolamine was markedly reduced in animals which had been either adrenalectomized or hypophysectomized. Although the changes produced by phentolamine in these animals were not statistically significant, there was a tendency for phentolamine to increase the enzyme activity. Phentolamine did produce a significant increase in enzyme activity in adrenalectomized rats if a small amount of exogenous glucocorticoid was supplied. A similar effect has been noted with several compounds other than phentolamine.[6,9] Under these circumstances, steroid is present in a relatively fixed, but certainly not increasing, concentration. The fact that the enzyme still responds to phentolamine suggests that the compound has some intrinsic effect on TAT activity. The administration of amino-glutethimide to a normal rat produces a model similar to the adrenalectomized rat which has received exogenous steroid. The animal can no longer increase its plasma corticosterone concentration upon demand, but a certain amount of corticosterone is still present

in the plasma. In such rats treated with amino-glutethimide, it was possible to demonstrate that phentolamine could still produce a significant increase in TAT activity.

Thus, our results indicate that at least two mechanisms are involved in the induction of TAT by phentolamine. The major portion of the induction is related in some manner to the increase in plasma corticosterone concentration which is produced by phentolamine. It is not clear whether this effect represents a direct induction by the steroid or an indirect response due to some effect on endogenous substrates. In addition, there appears to be an induction of TAT by phentolamine which occurs under circumstances in which plasma corticosterone concentration cannot increase. The magnitude of this response is consistent with the response to insulin or glucagon as described by Holton and Kenney.[10] The work of Porte[12] on adrenergic receptors and insulin release suggests that phentolamine may have increased the circulating insulin concentration in our animals. One may also postulate that the supra-steroid effect of phentolamine represents a direct action of this compound on the enzyme synthesizing machinery, producing an effect similar to a substrate induction.

REFERENCES

1. W. C. GOVIER, W. LOVENBERG and A. SJOERDSMA, *Biochem. Pharmac.* **18**, 2661, (1969).
2. T. I. DIAMONDSTONE, *Analyt. Biochem.* **16**, 395 (1966).
3. O. N. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. biol. Chem.* **193**, 265 (1951).
4. H. L. ENNIS and M. LUBIN, *Science, N.Y.* **146**, 1474 (1964).
5. E. REICH, R. M. FRANKLIN, A. J. SHATKIN and E. L. TATUM, *Science, N.Y.* **134**, 556 (1961).
6. E. C. C. LIN and W. E. KNOX, *Biochim. biophys. Acta* **26**, 85 (1957).
7. A. M. CAMACHO, R. CASH, A. J. BROUGH and R. S. WILROY, *J. Am. med. Ass.* **202**, 114 (1967).
8. G. LITWACK and T. I. DIAMONDSTONE, *J. biol. Chem.* **237**, 469 (1962).
9. F. T. KENNEY and R. M. FLORA, *J. biol. Chem.* **236**, 2699 (1961).
10. D. HOLTON and F. T. KENNEY, *J. biol. Chem.* **242**, 4372 (1967).
11. E. C. C. LIN and W. E. KNOX, *J. biol. Chem.* **233**, 1186 (1958).
12. D. PORTE, *J. clin. Invest.* **46**, 86 (1967).