EFFECT OF ELTROXINE AND NEOMERCAZOL ON MONOAMINE OXIDASE FROM RAT TISSUES

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Summary: The paper describes the effect of two drugs, (Eltroxine and Neomercazol) on the nature and activity of monoamine oxidase (MAO) in albino rats. Four organs namely liver, kidney, brain and heart were examined from this point of view. Biological activity measurements were done using tyramine hydrochloride as the substrate. The liver MAO from normal as well as the drug treated rats were subjected to DEAE gel filtration studies.. These investigations show that there is a significant difference in the biological activity as well as in the actual nature of the enzyme as a result of treatment with the drugs mentioned above.

Key words: monoamine oxidase Eltroxin Neomercazol

INTRODUCTION

It is more or less established now that stresses of various kinds could lead to thyroid disorders (1,2). The biochemical implications of this in thyroxine metabolism is also well known (3). The consequence of this is the effect that these changes have on the amount and nature of MAO. Literature contains enough data on rat liver MAO, (4,5,6). One interesting finding has been that there is more than one form of MAO in rat liver, (7,8). Studies by Axelrod and Kopin (9) have indicated that some of the effects observed depend on the changes in amount or activity of metabolizing enzymes. Thus, for example, thyroid hormone can influence both MAO and catechol-o-methyltransferase (COMT). A few minutes after a dose of epinepherine, the amount found in the heart of the hypothyroid rat is considerably smaller than in the control rat. A comprehensive review of the thyroid in health and disease has recently been published (10). Extensive as the information might be in the literature, there is very little information relating the effect of the drugs on the general metabolism of MAO, and more important, the effect that these drugs have on the nature of MAO itself.

The present studies were undertaken with two objeetives in mind. Firstly, to examine the activity changes if any, of MAO in various organs as a consequence of the administration of these drugs. Secondly, to examine changes in the nature of MAO in liver of rat using the method of DEAE-cellulose gel chromatography. As already stated the two drugs were (i) Eltroxine and (ii) Neomercazol. Eltroxine is known to cause stimulation of the thyroid and produces hyperthyroidism. Consequently, it is administered for hypothyroid conditions. Neomercazol, on the other hand, is known to cause a depression in the production of thyroxine, and therefore produces hypothyroidism, hence this drug is administered for hyperthyroid conditions.

MATERIAL AND METHODS

DEAE- cellulose and Tyramine hydrochloride and 2-4, Dinitrophenyll ydraz i.e were obtained from Sigma Chemical Company (U.S.A.). Water used in all the experiments was deionized and triple distilled. The salts used for the preparation of buffer solution were of analytical reagent grade.

Male albino rats were used for these studies. The rats were 120-150 *gms* in weight. Eltroxine and Neomercazol tablets were from British Drug House and Indian Schering respectively. The rats were divided into three groups. In the first group, the rats were given Eltroxine tablets orally, (dose: 0.02 *mg*/rat/day). The drug was continued for 30 days. The rats were sacrificed by decapitation after every 6 days following the treatment. The tissues were immediately used for the experiments without storing them for any period of time. The enzyme assay was done under conditions of linear assay.

In the second group, the rats were given Neomercazol tablets, (dose: 2.5 *mg*/rat/day). Here also the treatment was continued for 30 days and the rats were decapitated after every 6 days following the treatment. The third group was treated as control. These rats were not subjected to any drug administration.

Preparation of the enzyme:

Liver was quickly removed from the rats and the tissue was immediately put in 0.25M chilled sucrose. The volume of the sucrose solution used was about 10 *ml*/*gm* of tissue. The tissue was homogenized at about 4°C in a motor-driven homogenizer. The homogenate was centrifuged at 1,200 x g in a refrigerated centrifuge (K-70 model) at -10°C for 10 mts. The supernatent fluid obtained by this was centrifuged at the same temperature for 20 mts. at 28,500 x *g*. The crude mitochondrial fraction after a few washings was suspended in 0.1M phosphate buffer, pH 7.4. The volume of the buffer taken was 3 times the original weight of the tissue. The suspension was sonicated in an ultrasonic processor at 20kcs/sec. for 20 mts. The sonicated suspension was centrifuged at 34,00 x *g* for 30 mts. at 0°C. The supernatent fluid obtained by this procedure is the source of the enzyme MAO.

Heart, brain and kidney were not subjected to ultrasonic processing because of the small quantity of the material to start with. In the case of these organs the source of the enzyme was the mitochondrial suspension in the phosphate buffer.

Assay of activity:

The enzyme activity was assayed spectrophotometrically by the method of Green (11) in 0.2M phosphate buffer, pH 7.6, in the presence of 0.1M tyramine hydrochloride neutralized by NaOH to pH 7.4 at room temperature. The method is based on the measurement of the aldehyde formed during the enzymic oxidation of tyramine. The unstable aldehyde is prevented from decomposition by the addition of semicarbazide 0.05M, in the assay mixture. The

semicarbazone thus formed is converted to the corresponding 2-4, dinitrophenyl-hydrazone. The intensity of the color of this material in alkaline solution at 470 *nm* provides a sensitive measure of the enzyme activity.

Chromatography:

DEAE- cellulose chromatography was performed using a column (15 x 1.5 *cm*), at 0°C. The column was jacketted through which it was possible to pass ice cold water. The DEAE-cellulose was obtained from Sigma Chemical Company. The preparation of the gel before loading the column was according to the instructions of the suppliers. The column was equilibrated with 0.1M phosphate buffer, pH 7.6 containing 0.01M NaCl. Ice cold water was passed through the Jacket of the column so that the entire DEAE-cellulose chromatography was done in the cold. 2.0 *ml* sample was loaded and 3.0 *ml* fractions were collected. The O.D. at 280 *nm* of the fractions were measured with a spectrophotometer and these O.D. values were plotted against elution volume.

RESULTS

(I) Activity measurements of MAO from different organs:

As already explained in the introduction, since the amount of material was small in the case of kidney, heart and brain it was not possible to extend the purification procedure to include ultra sonication. This step was therefore omitted in the case of heart, brain and kidney. In the case of liver however as already mentioned purification of the sonicated mitochondrial suspension was also done. The mitochondrial suspension of these organs was tested for biological activity of MAO, using tyramine hydrochloride as the substrate by the procedure explained above. The O.D. at 470 *nm* was measured from the mitochondrial suspension from rats which were sacrificed at 6th, 12th, 18th, 24th and 30th day of the treatment.

Fig. 1 contains data relating to O.D. at 470 *nm*, which is proportional to MAO activity in the four organs as a function of number of days of administration of Eltroxine. Fig. 2 contains similar data for the four organs in the case of Neomercazol treated rats. The corresponding activity of MAO in the tissues of heart, brain, kidney and liver of normal rats are: 0.24, 0.28, 0.12 and 0.25 respectively. It is necessary to point out here that the aim and scope of the present studies is only to see the effect of any individual drug on the four organs and not to compare the absolute activity values of the enzyme from among the individual organs. The activity values plotted as O.D. at 470 *nm* in the ordinate of Figs. 1 and 2 are therefore not representative of the specific activity of the enzyme but are indicative only of the total activity in the organ.

(II) DEAE-cellulose chromatographic studies:

As explained above, for want of adequate material, DEAE-cellulose gel chromatographic studies were done for MAO only from liver. The experiments were repeated in atleast 5-6 different batches in the case of normal rats and twice in the case of Eltroxine and Neomercazol treated rats respectively. The DEAE elution profile for normal rats is shown by elution profile marked 1 in Fig. 3. This pattern was found to be quite reproducible from experiment to experiment. Protein peaks were obtained at elution volumes of 21 *ml*, 30 *ml* and 47 *ml*.

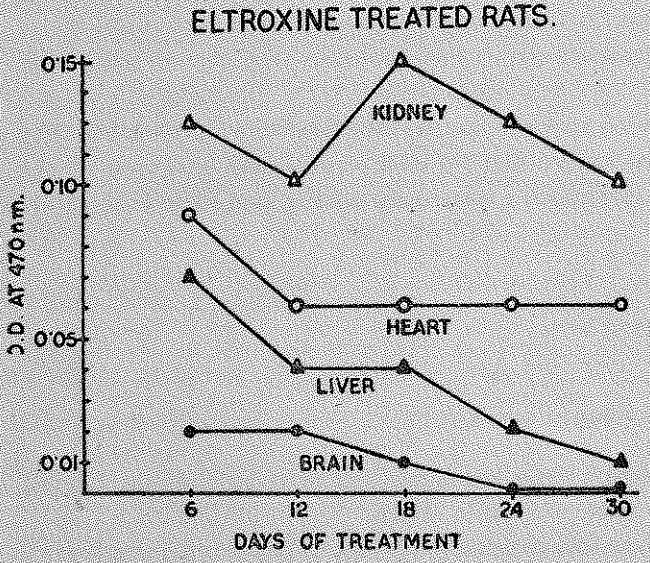

Fig. 1: MAO activity in four organs as a function of number of days after administration of Eltroxine.

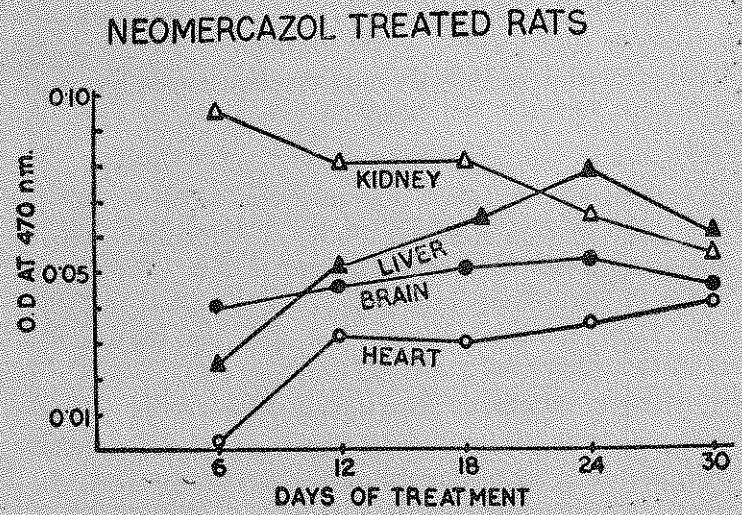

Fig. 2: MAO activity in four organs as a function of number of days after administration of Neomercazol.

The elution profile makred 2 in Fig. 3 shows the behaviour of MAO from the liver of Eltroxine treated rats. Here again, the position of the peaks were reproducible with different batches of rats. Two protein peaks were observed: One at elution volume of around 7-8; and another around 24-25 *ml*. The elution profile marked 3 in Fig. 3 shows the data for MAO obtained from Neomercazol treated rats. Two protein peaks were observed: One at around 21 *ml* and the another one at around 35 *ml*.

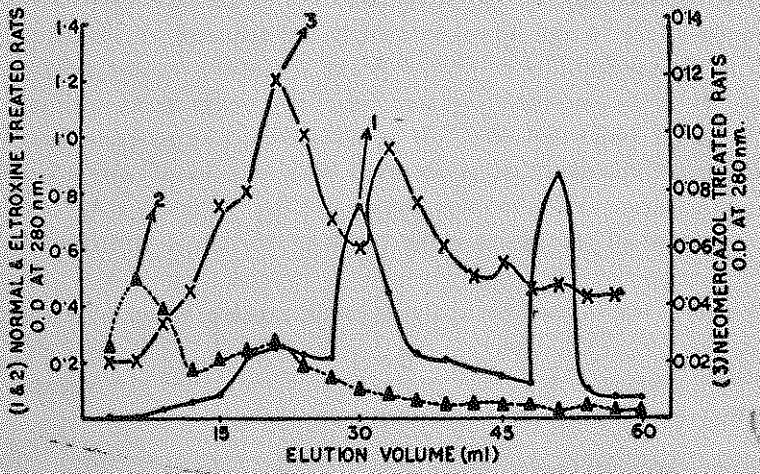

Fig. 3: Elution profiles of rat liver MAO:
(1) Control rats
(2) Eltroxine treated rats
(3) Neomercazol treated rats

DISCUSSION

The role of MAO in thyroid metabolism and its interrelationship with Noradrenaline is well known (12). Furthermore, the effect of stress on neurohumors is also well known (13). Even more important, the nature and form of MAO seem to play an important role in its physiological functions (14). Two drugs namely Eltroxine and Neomercazol are generally clinically used in thyroid disorders. Their function seems to directly or indirectly control the thyroid hormone which in turn either controls the amount and/or the nature of MAO. It would therefore be very essential to study the nature and amount of MAO as a consequence of the administration of these drugs.

Examination of Fig. 1 has to be done with a certain amount of caution. It would appear from the figure that in general for all the four organs studied there is a decrease in the amount of MAO and therefore its activity as the number of days of treatment of the drugs is increased. However, the actual decrease in activity as a result of increasing the number of days of treatment cannot be ascertained with a great accuracy because such a data would necessitate a large number of experiments from which the average values will have to be taken. This was not done in our recent studies. Nevertheless, it is correct to say that Fig. 1 indicates that the amount of MAO decreases as one increases the number of days of administration of the drug.

A somewhat similar situation arises with Neomercazol treated rats. For the same reason explained in Fig. 2 in the previous paragraph, it will not be correct to quantitate the changes

in the activity of MAO as a function of number of days of treatment. Nevertheless, it is again correct to say that MAO in the case of liver, brain and heart seems to increase as a function of number of days of treatment at least in the initial state.

The data contained in Fig. 3 merit discussion. As already reported, these DEAE-cellulose chromatographic studies were done at least more than once for each case, and the elution profiles were found to be reproducible. Furthermore, activity investigations under various peaks showed the solution under these peaks to be active. In the case of normal rats, essentially 3 peaks are seen at elution volumes of 21,30 and 48 *ml*. Similarly, in the case of Eltroxine treated rats, the material seems to elute essentially under two peaks of elution volume around 7 *ml* and 21 *ml*. Again in the case of Neomercazol treated rats, two peaks at the elution volume of 21 and 33 *ml* seem to be the important ones. It is also interesting to note from Fig. 3 that the protein peaks at elution volume 21 *ml* is observed both in normal, and drug treated rats and therefore is a known feature in all the three elution profiles. Furthermore, one can hypothesize, with a certain amount of caution that the forms of MAO are different in different thyroid conditions.

DEAE-cellulose chromatography is not in itself an analytical tool, capable of quantitative macromolecular characterization, but it is well known that the theory behind DEAE-cellulose method of separation involves ion exchanges. It is very probable that the Liver MAO from the drug treated rats and the normal rats are different in their molecular nature.

REFERENCES

1. Lipton, M.A. and A.J. Prange. Increased rate of norepinephrine biosynthesis in hypothyroid rats. *Fed. Proc.*, **27** : 399, 1968.
2. Prange, A.J., Jr., J.L. Meek and M.A. Lipton. Diminished rate of synthesis in rat brain and heart after thyroxine pretreatment. *Life Science*, **9** : Part 1, 901, 1970.
3. Waldstein, M.D. and S. Sheldon. Thyroid-Catecholamine interrelations. *Annu. Rev. Med.*, **17** : 123, 1966.
4. Zile Maija and H.A. Lardy. Monoamine oxidase activity in liver of thyroid fed rats. *Arch. Biochem. & Biophys.*, **82** : 411, 1959.
5. Ho-Van Hap, A. et al. Hormonal action of monoamine oxidase activity in rats. *Canad. J. Biochem.*, **45** : 355, 1967.
6. Diaz Borges, J.M. and A. D'Lorio. Polyacrylamide gel electrophoresis of rat liver mitochondrial monoamine oxidase. *Canad. J. Biochem.*, **51** : No. 7, 1089, 1973.
7. Sierens, L. and A. D'Lorio. Multiple monoamine oxidase in rat liver mitochondria. *Canad. J. Biochem*, **48**: 659, 1970.
8. Diaz Borges, J.M. and A. D'Lorio Multiple forms of mitochondrial Monoamine oxidase. *Advanc. Biochemical Psychopharmacology*, **5** : 79, 1972.
9. Wurtman, R.J., J. Arelrod and I.J. Kopin. Thyroid function and the Cardiac disposition of catecholamines. *Endocrinology*, **73** : 63, 1963.
10. Udupa, A., S. Srinivasan and K.N. Udupa. Monoamine oxidase in Health and Disease. *Ind. J. Physiol. Pharmac.*, **18** : 73, 1974.
11. Green, A.L. and M.H. Thelma. A colorimetric method for the estimation of Monoamine oxidase. *Biochem. J.*, **72** : 1961.
12. Kopin, I.J. and J. Arelord. Annals of New York Academy of Sciences, **107**: 848, 1963. The role of Monoamine oxidase in the release and metabolism of norepinephrine.
13. Weiner, N. and R. Bjur. Role of intraneuronal Monoamine oxidase in the regulation of norepinephrine synthesis. *Advanc. in Biochemical Psychopharmacology*, **5** : 409, 1972.
14. Sandler, M. and M.B.H. Youdim. Multiple forms of Monoamine oxidase: Functional significance. *Pharmacological Review*, **24** : No. 2, 331, 1972.