Br. J. Pharmac. (1971), **42**, 505–511.

Some observations on the estimation of 3-methoxytyramine in brain tissue

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Summary

1. A new method for the estimation of 4-hydroxy-3-methoxyphenylethylamine (3-methoxytyramine) in brain tissue is described. This is based on the formation of a fluorescent derivative by oxidation with potassium ferricyanide in ammonium hydroxide solution.
2. The effects of some drugs on the concentration of 3-methoxytyramine in the brain are reported.
3. The significance of changes in the striatal concentration of 3-methoxytyramine is discussed.

Introduction

3-Methoxytyramine (4-hydroxy-3-methoxyphenylethylamine) is an intermediate in the metabolism of dopamine (3,4-dihydroxyphenylethylamine) in the brain. The presence of this substance in the mammalian brain was reported by Carlsson & Waldeck (1964), who described a fluorimetric method for its estimation based on the formation of a hydroxyindole compound when 3-methoxytyramine is oxidized with iodine and the resulting compound subjected to a molecular rearrangement. Methods for estimating 3-methoxytyramine based on this procedure have been used by Carlsson & Lindqvist (1963), Laverty & Taylor (1968), Lisch, Aigner & Hornykiewicz (1968), Jonas & Scheel-Krüger (1969) and Tagliamonte, Tagliamonte & Gessa (1970). This procedure also gives rise to a fluorescent derivative if applied to dopamine. Crawford & Yates (1970) have estimated 3-methoxytyramine in brain tissue using paper chromatographic separation of its acetyl derivative (Hagopian, Dorfman & Gut, 1961) followed by demethylation and condensation with 1,2-diaminoethane. Corrodi & Werdinius (1965) mentioned that 3-methoxytyramine could give rise to a fluorescent derivative if subjected to the reaction used for the estimation of 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA) (Andén, Roos & Werdinius, 1963) in which HVA is oxidized by potassium ferricyanide in an ammonium hydroxide solution. We have found that if the reaction is carried out in a more concentrated solution of ammonium hydroxide then the intensity of the fluorescence is similar to that which can be obtained from HVA, and have observed that a more intense fluorescence was obtained if the reaction was carried out using a solution of 3-methoxytyramine prepared with perchloric acid which had been neutralized with potassium carbonate.

Since both dopamine and 3-methoxytyramine can be eluted together from a column of cation exchange resin by 2 N perchloric acid, the application of this

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method of estimating 3-methoxytyramine, which does not produce fluorescence with dopamine, was investigated.

Methods

The animals were killed by cervical dislocation and the brains were removed and placed on an ice-cooled glass plate. The corpora striata were dissected out and homogenized immediately or stored at −17° C until analysed. The tissues were homogenized in four volumes of ice-cold 0·1 N hydrochloric acid containing 5 mg/ml ascorbic acid. The homogenate was then diluted with an equal volume of ice-cold distilled water. Concentrated (72%) perchloric acid was added to give a final concentration of 0·6 N and the homogenate mixed thoroughly. After removal of the precipitated protein by centrifuging at 14,000 *g* for 5 min at 0° C, the pH of the clear supernatant was adjusted to 4·0 with 20% and then 2% potassium carbonate solutions. The mixture was centrifuged at 1,000 *g* for 5 min at 0° C to remove the insoluble potassium perchlorate. The clear supernatant was allowed to come to room temperature and was decanted into a reservoir above a 2·2 × 0·4 cm column of carefully cleaned Dowex 50 × 8 (sodium form) cation exchange resin. After the solution had passed through, the reservoir and the column of resin were carefully washed with 8 ml 0·4 N hydrochloric acid. The sides of the reservoir were washed down with 5 ml distilled water which was also allowed to pass through the resin. This ensured that all of the ascorbic acid was washed from the reservoir and the column.

Dopamine and 3-methoxytyramine were eluted together from the column with 10 ml of 2 N perchloric acid. The pH of the eluate was adjusted to 7 by the addition of saturated and 2% potassium carbonate solutions, with vigorous stirring to avoid local alkalinity in the solution. The solution was cooled and the precipitated potassium perchlorate removed by centrifugation. The resulting clear supernatant was used for the estimation of 3-methoxytyramine. Dopamine was estimated in a portion of the same solution after acetylation and condensation with 1,2-diaminoethane (Laverty & Sharman, 1965).

For the estimation of 3-methoxytyramine 1 ml of the neutralized perchloric acid solution was placed in a glass test tube. One millilitre of a solution of potassium ferricyanide (20 μg/ml) in concentrated (S.G.=0·880) ammonium hydroxide was added. After 2 min 0·1 ml of a solution of cysteine (1 mg/ml) was added and mixed. The fluorescence of the resulting solution was measured in an Aminco-Bowman Spectrophotofluorometer (activation wavelength 315 nm; fluorescence wavelength 430 nm; uncorrected instrumental values) fitted with an interference filter (maximum transmission wavelength 429 nm) in the fluorescence light path.

A blank determination was made by reversing the order of the addition of the ferricyanide-ammonia solution and the solution of cysteine. Authentic 3-methoxytyramine was also added to a portion of the neutralized eluate and the fluorescence developed to check for quenching. Recoveries (mean ± S.E.M.) of authentic 3-methoxytyramine and dopamine added to tissue homogenates were 73 ± 3% (n=16) and 62 ± 8% (n=8) respectively. The results are uncorrected for recovery.

Drugs were injected intraperitoneally into mice and intravenously into an ear vein of rabbits.

The following drugs were used: D-amphetamine sulphate, Smith, Kline & French Laboratories Ltd; chlorpromazine hydrochloride, May & Baker Ltd.; benzhexol

hydrochloride, Lederle Laboratories Division; 1-adamantanamine hydrochloride, Ralph N. Emanuel Ltd.; cocaine hydrochloride B.P., B.D.M. Ltd.; pheniprazine (JB-516), Lakeside Laboratories Inc.; tranylcypromine sulphate, Smith, Kline & French Laboratories Ltd.; pargyline hydrochloride, Abbott Laboratories Ltd.; α-ethyltryptamine acetate, The Upjohn Co. Ltd.; DL-α-methyl-*p*-tyrosine methyl ester hydrochloride, Kistner A.B. Drugs were dissolved in 0·9% sodium chloride solution for injection, except for benzhexol which was dissolved in 20% ethanol. Doses are given in terms of the free base except for α-methyl-*p*-tyrosine which was used as the ester salt. Other chemicals and reagents were of analytical reagent grade or of known purity. Cysteine was recrystallized from ethanol and stored *in vacuo*. Control animals were injected with 0·9% sodium chloride solution.

Results

Observations on the method of estimating 3-methoxytyramine

The fluorescence obtained with 3-methoxytyramine has similar characteristics to that obtained with HVA (Andén *et al.*, 1963) and other vanyl compounds (Gjessing, Vellan, Werdinius & Corrodi, 1967), and is presumably due to the formation of a dimer as reported for HVA by Corrodi & Werdinius (1965). Oxidation times of 0·5–2 min did not affect the intensity of the fluorescence developed, but if the oxidation was allowed to proceed for 5 min the fluorescence yield was reduced. The intensity of the fluorescence developed was in a linear relation to the amount of 3-methoxytyramine present in the sample up to at least 1 μg.

Several acids were tested as possible eluting solvents, but only perchloric acid neutralized with potassium carbonate allowed the development of an intense fluorescence from 3-methoxytyramine. The method appears to be of a similar sensitivity to that of the hydroxyindole method as reported by Laverty & Taylor (1968). In pure solutions the blank was equivalent to 15 ng of 3-methoxytyramine and the blank obtained with tissue extracts and representing approximately 0·05 g of tissue was equivalent to 20 ng of the amine. The method was suitable for the estimation of 3-methoxytyramine in the striatal tissue from one rabbit and in the pooled striatal tissues from five mice, four rats or two guinea-pigs, and appears to have a reasonable specificity. Several amines which might be present in eluates from cation exchange resin columns were tested. Of these only 3-methoxytyramine gave rise to a fluorescence. Tyramine, noradrenaline, α-methyl noradrenaline, adrenaline, normetanephrine, metanephrine, dopamine and α-methyl dopamine did not produce a significant fluorescence. The acetyl derivative of 3-methoxytyramine also yields an intense fluorescence and it is probable that α-methyl-3-methoxytyramine would be converted to a fluorescent compound.

TABLE 1. *Concentrations of 3-methoxytyramine in the striatal tissue of different species*

Species	3-Methoxytyramine in striatal tissue: Corpus striatum	Caudate nucleus
Rabbit	0·22±0·01 (8)	0·70±0·11 (3)
Rat	0·32±0·01 (3)	—
Mouse	0·25±0·02 (5)	—
Guinea-pig	0·19±0·01 (3)	—
Dog	—	0·48±0·04 (4)
Pig	0·52 ; 0·44	0·30

Concentration (μg/g wet tissue) ±S.E.M. (No. of estimates).

TABLE 2. *Effects of drugs on the concentrations of 3-methoxytyramine and dopamine in the striatum of the rabbit*

Treatment	Dose (mg/kg i.v.) 1st drug	Dose (mg/kg i.v.) 2nd drug	Duration of action (h) 1st drug	Duration of action (h) 2nd drug	3-Methoxytyramine (μg/g tissue)	Dopamine (μg/g tissue)
None	–	–	–	–	0·22±0·01 (8)	2·13±0·16 (8)
Pheniprazine	10	–	3	–	0·53±0·06 (5)	2·82±0·35 (5)
Chlorpromazine (CPZ)	10	–	2	–	0·19 ; 0·29	0·81 ; 1·93
CPZ+pheniprazine	5	10	2	3	1·94±0·41 (3)*	1·88±0·51 (3)
Pheniprazine+CPZ	10	5	2	3	1·48±0·15 (3)*	1·54±0·19 (3)
Amphetamine+pheniprazine	2·5	10	2	3	0·66±0·02 (3)	3·52±0·62 (3)
Pheniprazine+amphetamine	10	10	3	1	0·97±0·08 (3)*	3·20±0·15 (3)
Pheniprazine+adamantanamine	10	25	3	1	0·38±0·04 (5)	3·24±0·32 (5)
Pheniprazine+benzhexol	10	1	3	1	0·39±0·06 (5)	3·03±0·28 (5)
Pheniprazine+cocaine	10	2	3	1	0·37±0·04 (5)	2·90±0·18 (5)

Means±S.E.M. (No. of estimations). *Difference from treatment with pheniprazine alone $P<0·01$ (Student's t test).

The formation of a fluorescent dimer from HVA and related compounds is known to be easily inhibited by the presence of other substances or ions in the reaction mixture. When the method, as described, was applied to quantities of striatal tissue greater than 1 g, or when a high concentration of dopamine was present in the reaction mixture, there was some reduction in the fluorescence derived from 3-methoxytyramine. Quenching due to the presence of dopamine was overcome by passing the eluate (pH 7) through a column of acid washed alumina before carrying out the fluorescence reaction. With extracts from 0·5 g of brain tissue there was usually no quenching of the fluorescence.

Concentration of 3-methoxytyramine in the striatal tissue of different species

Some estimates of the concentration of 3-methoxytyramine in striatal tissues of six mammalian species are shown in Table 1. The concentrations given here are not obviously different from those reported by other authors (Carlsson & Waldeck, 1964 ; Lisch *et al.*, 1968 ; Guldberg & Yates, 1969, and Tagliamonte *et al.*, 1970).

The effects of some drugs which modify dopamine metabolism in the central nervous system, and also of some drugs used in the treatment of Parkinson's disease, on the concentrations of 3-methoxytyramine and dopamine in the striatum of the rabbit are given in Table 2.

The possibility that there might be pathways for the removal of 3-methoxytyramine from the brain alternative to its metabolism to HVA by monoamine oxidase was examined in mice pretreated with a monoamine oxidase inhibiting drug. Table 3 shows the effect of three different monoamine oxidase inhibiting drugs on the concentration of 3-methoxytyramine in the mouse striatum. Of these drugs, tranylcypromine caused the largest increase in the concentration of 3-methoxytyramine and was used in the following experiment. After giving tranylcypromine (10 mg/kg i.v.) the synthesis of dopamine was inhibited by giving α-methyl-*p*-tyrosine methyl ester hydrochloride (200 mg/kg i.p.) and the concentrations of 3-methoxytyramine and dopamine in the striatum were estimated after increasing

TABLE 3. *Effects of different MAO inhibitors on 3-methoxytyramine in mouse striatum*

Drugs	Dose (mg/kg i.p.)	Duration of action (h)	3-Methoxytyramine (μg/g tissue)
None	–	–	0·22±0·01
Pargyline	100	3	0·44±0·02
α-Ethyltryptamine	10	1	0·35±0·03
Tranylcypromine	10	3	0·70±0·05

Means±S.E.M. of three estimations.

TABLE 4. *Effect of tranylcypromine and α-methyl-*p*-tyrosine methyl ester hydrochloride on 3-methoxytyramine and dopamine in mouse striatum*

Drugs and durations of action (h)			
Tranylcypromine (10 mg/kg i.p.)	α-MPT (200 mg/kg i.p.)	3-Methoxytyramine Means (μg/g tissue)±S.E.M.	Dopamine of three estimations
3	–	0·75±0·05	2·43±0·34
4	–	0·65±0·04	3·00±0·35
4	1	0·67±0·05	2·33±0·54
5	–	0·73±0·04	2·77±0·54
5	2	0·89±0·04	2·30±0·35
6	–	0·70±0·06	3·00±0·92
6	3	0·75±0·08	1·77±0·17

periods of time. The results of this experiment are given in Table 4. This shows that when the synthesis of dopamine is inhibited in mice treated with tranylcypromine little change in the concentration of 3-methoxytyramine was observed during the subsequent 3 hours. There was a slow reduction in the concentration of dopamine during this time. These results indicate that the major pathway for the removal of 3-methoxytyramine from the mouse striatum involves its conversion to HVA by monoamine oxidase.

Discussion

3-Methoxytyramine is formed from dopamine by the action of the enzyme catechol-*o*-methyl transferase. The measurement of the rate at which this conversion takes place *in vivo* would be useful in studying the function of dopamine in the central nervous system. However, in normal animals 3-methoxytyramine is an intermediate in a metabolic chain, and the conditions in the tissue might be such that a large change in the rate at which dopamine is metabolized by catechol-*o*-methyl transferase need not produce a large change in the concentration of 3-methoxytyramine.

Our method for estimating 3-methoxytyramine is simple, fairly rapid and many samples can be processed at the same time. In addition, dopamine can be estimated in the same eluate used for 3-methoxytyramine. The results obtained using this method have in general confirmed those obtained by other authors (Carlsson & Waldeck, 1964; Lisch *et al.*, 1969; Guldberg & Yates, 1969; Jonas & Scheel-Krüger, 1969, and Tagliamonte *et al.*, 1970), both in the amount of 3-methoxytyramine present in striatal tissues and in the effects of amphetamine and chlorpromazine, drugs which can produce an increase in the concentration of 3-methoxytyramine in the striatal tissues of animals pretreated with drugs which inhibit monoamine oxidase. It was not possible to conclude from these experiments whether or not any of the other drugs tested reduced the rate at which 3-methoxytyramine is formed in the striatum.

The largest changes in the concentration of 3-methoxytyramine previously reported have been observed in animals treated with monoamine oxidase-inhibiting drugs. Our experiments indicate that, at least in the mouse, there is no major pathway for the removal of 3-methoxytyramine other than metabolism by monoamine oxidase, so that the accumulation of 3-methoxytyramine after inhibition of this enzyme can be taken as the rate at which dopamine is methylated by catechol-*o*-methyl transferase.

In using the concentration of 3-methoxytyramine to study the effects of drugs on dopamine metabolism the best approach appears to be an examination of the rate at which 3-methoxytyramine accumulates after inhibition of monoamine oxidase subsequent to the administration of the drug to be studied. However, many such drugs have a short duration of action and the comparison of results so obtained may be difficult. Most monoamine oxidase inhibiting drugs are long acting and it has been more convenient to study the effects of drugs on the concentration of 3-methoxytyramine in the brains of animals which have been pretreated with a long-acting monoamine oxidase inhibiting drug. Yet our results in the mouse, in which both the synthesis of dopamine and monoamine oxidase activity are inhibited, would indicate that this latter approach suffers from a major drawback. Only those drugs which increase the rate at which dopamine is *o*-methylated will be

easily detected; for if a drug reduces this rate its effect will not be seen because of the slow rate of removal of 3-methoxytyramine from the brains of animals treated with a monoamine oxidase inhibiting drug.

During this work H.C.G. was in receipt of scholarships from the British Council and the Norwegian Pharmaceutical Society.

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(*Received May* 6, 1971)