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STUDIES ON THE METABOLISM OF CATECHOLAMINES IN THE CENTRAL NERVOUS SYSTEM OF THE MOUSE

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1 The distribution of the metabolites of noradrenaline, 1-(3,4-dihydroxyphenyl)ethane-1,2-diol (DOPEG) and 1-(4-hydroxy-3-methoxyphenyl)ethane-1,2-diol (MOPEG), in the brain of the mouse has been investigated.

2 The rate of disappearance of the metabolites after inhibition of the enzyme monoamine oxidase has been used to estimate their turnover rates in the mouse hypothalamus. It was concluded that the turnover of DOPEG was much faster than that of MOPEG.

3 When mice were treated with reserpine dissolved in 5% ascorbic acid solution there was an increase in the hypothalamic concentration of both MOPEG and DOPEG. However, similar increases in the concentrations of the two metabolites were seen when the animals were treated with 5% ascorbic acid solution alone.

4 The administration of tropolone, an inhibitor of the enzyme catechol-*O*-methyl transferase, resulted in an increase in the concentration of DOPEG.

5 Mice, exposed to a temperature of -15°C showed increased hypothalamic concentrations of both DOPEG and MOPEG.

6 The rates of formation in the mouse striatum of 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylacetic acid (HVA), acidic metabolites of dopamine, were compared with the turnover rate of dopamine, estimated from the rate at which this catecholamine disappears after treatment with α-methyl-*p*-tyrosine. It was concluded that the estimate of dopamine turnover obtained by this method is likely to be too large because of the compensatory feedback mechanism which is thought to play a role in the metabolism of dopamine in the brain.

Introduction

Of the possible metabolites of 3,4-dihydroxyphenylethylamine (dopamine), only 3,4-dihydroxyphenylacetic acid (DOPAC), 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid; HVA) and 4-hydroxy-3-methoxyphenylethylamine (3-methoxytyramine) have been clearly demonstrated to occur in the mouse striatum (Carlsson & Lindqvist, 1963; Sharman, 1966; Murphy, Robinson & Sharman, 1969). It has not been possible to identify the corresponding acid metabolites of noradrenaline as normal products of the metabolism of endogenous noradrenaline in the mouse brain, but 2-(4-hydroxy-3-methoxyphenyl)ethan-2-olamine (normetanephrine) is a known metabolite (Carlsson & Lindqvist, 1963). However, two glycol metabolites of noradrenaline, 1-(3,4-dihydroxyphenyl)ethane-1,2-diol (DOPEG) and 1-(4-hydroxy-3-methoxyphenyl)ethane-1,2-diol (MOPEG) have been shown to be present in mouse brain and may represent the major cerebral metabolic transformations of noradrenaline in the mouse (Sharman, 1969). Murphy *et al.* (1969) and Roffler-Tarlov, Sharman & Tegerdine (1971) have demonstrated that the formation of DOPAC is not a simple alternative to the formation of HVA from dopamine in the mouse striatum and that little DOPAC appears to be metabolized to HVA in this tissue. This paper describes an attempt to measure the relative amounts of dopamine and noradrenaline that are metabolized to the deaminated catechol derivatives and to the methoxylated-deaminated compounds.

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Since the publication of a gas-liquid chromatography method for the detection and estimation of small quantities of alcohol metabolites of catecholamines in brain tissue (Sharman, 1969), the experimental procedure has been modified and the details of the method as it is currently employed are given here and include the purification of the reagents used.

Methods

Chemicals and reagents

Acetic anhydride, analytical reagent grade (Hopkin & Williams); distilled three times and stored at 4° C.

Calcium hydride (Hopkin & Williams); dichloromethane (May & Baker) distilled twice; heptafluorobutyric acid (Koch-Light).

Heptafluorobutyric anhydride. This was prepared by heating equimolar amounts of heptafluorobutyric acid and phosphorus pentoxide together under reflux with the exclusion of moisture, for at least 3 hours. The heptafluorobutyric anhydride was then distilled off, the fraction distilling at 108-109° C being collected. With some batches, the anhydride could be used after one further distillation. More often, peaks, due to contaminating substances, which particularly interfered with the estimation of DOPEG, were observed on the gas chromatograph record when blank determinations were made. These peaks were not present or were greatly reduced in size if the heptafluorobutyric anhydride was first passed through a column (1.5 x 20 cm) of silica gel and then distilled twice.

1,2,3,4,5,6-(γ)-hexachlorocyclohexane; γ-benzene hexachloride; γ-BHC, (gift from Shell Woodstock Agricultural Research Centre); hexane (Hopkin & Williams), dried with calcium hydride and distilled twice; hydrochloric acid (Hopkin & Williams) micro-analytical reagent grade; phosphorus pentoxide, phosphoric oxide (Hopkin & Williams); perchloric acid 72% w/v (May & Baker); potassium chloride (Hopkin & Williams), analytical reagent grade; quinol, hydroquinone (May & Baker); sodium hydrogen carbonate (Hopkin & Williams), analytical reagent grade.

Sodium sulphate, anhydrous granular (Hopkin & Williams), analytical reagent grade, washed by swirling 250 g sodium sulphate in 1-1.5 litres of twice-distilled dichloromethane for several hours. After decanting off the dichloromethane, the sodium sulphate was dried in air in an oven at 110° C.

Silica gel (May & Baker) for chromatography, washed successively with methanol, acetone and benzene and then dried at 110° C.

Tetrahydrofuran (Hopkin & Williams; May & Baker). This was dried over calcium hydride and then distilled. It was stored with the addition of quinol (0.1%). Immediately before use it was redistilled and 1.5% water was added. This concentration of water was found to be optimal for the formation of the heptafluorobutyryl derivative of acetyl-MOPEG. Other organic solvents were tested in this reaction, but only tetrahydrofuran containing 1.5% water allowed the complete formation of the heptafluorobutyryl derivative.

1-(4-Hydroxy-3-methoxyphenyl)-1,2-ethanediol bis-piperazine salt (Calbiochem; Sigma); 1-(3,4-dihydroxyphenyl)-1,2-ethanediol (Calbiochem; Sigma); 4-hydroxy-3-methoxyphenylacetic acid, homovanillic acid (Calbiochem); 3,4-dihydroxyphenylacetic acid (Sigma, recrystallized from benzene).

Double glass distilled water was used throughout.

The drugs used were: ascorbic acid; 2-aminotetralin hydrochloride; cocaine hydrochloride; desmethylimipramine hydrochloride; α-methyl-*p*-tyrosine methyl ester; pargyline hydrochloride; pentobarbitone sodium; probenecid; propranolol hydrochloride; reserpine base; tranylcypromine sulphate; tropolone. Except for α-methyl-*p*-tyrosine methyl ester (water), probenecid (dissolved in 1 M NaOH, neutralized with HCl and diluted with water) and reserpine (5% ascorbic acid solution) the drugs were dissolved in 0.9% w/v NaCl solution (saline).

Unless otherwise stated, the doses refer to the free base or acid.

Homogenization, protein precipitation, acetylation and extraction procedures for brain tissue

Mice were killed by cervical dislocation and the brains were rapidly removed and placed on an ice-cooled glass plate. The brains were dissected, and up to 100 mg of tissue was transferred to a thick-walled, borosilicate glass test tube (75 mm x 7 mm internal diameter) fitted with a ball ended glass pestle. The tissue was homogenized in 0.5 ml ice-cold 0.1 N hydrochloric acid; the homogenate was diluted with 0.5 ml ice-cold water and the mixture rehomogenized. The test tube was then cooled in liquid nitrogen and placed in ice. The procedure was carried out rapidly up to this point in order to prevent losses. The homogenate can be left for 30 min in ice without apparent loss of the glycols.

A small volume (0.1 ml) of 72% perchloric acid

was then added to, and mixed thoroughly with, the homogenate using a small glass rod, in order to precipitate the proteins. The perchlorate was then removed by adding solid potassium chloride in a slight excess of saturation, with thorough mixing. After the mixture had been centrifuged for 2 min at 2000 *g* in the test tube, the clear supernatant was transferred to a 15 ml glass-stoppered test tube. Great care was taken not to transfer any of the fatty layer which floated on the supernatant, otherwise the final extract contaminated the electron capture detector. By tipping the tube carefully this material was made to adhere to the side of the tube. Acetic anhydride, 0.1 ml, was added to the extract and mixed. This was followed by sodium hydrogen carbonate added in small quantities, each approximately 15 mg, with mixing until there was a slight excess at the bottom of the tube and there was very little evolution of carbon dioxide on adding more solid sodium hydrogen carbonate. The reaction usually takes 15 to 20 min to complete and is thought to acetylate only the phenolic hydroxyl groups of DOPEG and MOPEG. Dichloromethane (5 ml) was then added and the tube cooled in liquid nitrogen. The acetylated glycol metabolites of noradrenaline were extracted into the dichloromethane by shaking on a vortex mixer. The tubes were then centrifuged for 1 min at 2000 *g* to separate the two phases.

The lower, organic phase was taken up into a pipette and the dissolved water removed by passing through a column of anhydrous sodium sulphate (0.6 x 2.5 cm). Immediately before use the column was washed with 5 ml dichloromethane. The column of anhydrous sodium sulphate was conveniently prepared in a commercially available, long form pasteur pipette fitted with a small glass bead to retain the crystals.

The dried dichloromethane extract was collected into a borosilicate glass test tube (75 x 8 mm internal diameter) and evaporated to dryness under a stream of nitrogen in a heating block thermostatically controlled at 50° C.

The formation of heptafluorobutyryl esters

This reaction was carried out in a fume cupboard. Tetrahydrofuran, 100 µl, was added to the tube in which the extract had been dried; 50 µl heptafluorobutyric anhydride was added and mixed by rotation to dissolve any material on the side of the tube and the mixture left for 10 min at room temperature. It was then evaporated under a stream of nitrogen at 50° C. The nitrogen was passed into the tube for a further 25 min to remove all of the volatile material present.

Preparation of the solution for injection into the gas-liquid chromatograph

A carefully measured volume of a solution of γ-benzene hexachloride (50 ng/ml) in dry hexane was added to the tube containing the heptafluorobutyryl esters. The volume was usually 100 µl. If a smaller volume of the γ-benzene hexachloride was used, the volume was made up to 100 µl with dry hexane. Volumes (0.3-3 µl) of this solution were injected into the gas-liquid chromatograph.

Gas-liquid chromatography

A Pye Model 104 gas-liquid chromatograph fitted with a ^{63}Ni electron capture detector was used. The glass column (150 cm x 4 mm) was packed with 3.8% SE 30 on Diatomite C 'Q', 100-120 mesh. The derivatives of MOPEG and DOPEG appeared to be destroyed by the glass fibre plug at the inlet end of the column. The column was therefore used without this plug. The carrier gas was Argon/5% methane and was used at a pressure of 20 kg/cm^2. Two sets of operating conditions were used: oven temperature 135° C, detector oven temperature 170° C and gas flow 50 ml/min; or oven temperature 180° C, detector oven temperature 195° C and gas flow 60 ml/minute. The higher operating temperature allowed a more rapid estimation of MOPEG but interference with the estimation of DOPEG by contaminants was more frequently encountered than at the lower operating temperature which required a much longer time (1.5-2 h) for the completion of each assay.

The amounts of MOPEG and DOPEG in the tissue samples were calculated by the peak height ratio method used by Sharman (1969). The lower limit of detection of the method was approximately 0.05 ng for both MOPEG and DOPEG in a tissue sample.

Recovery of authentic MOPEG and DOPEG (10-20 ng) added to tissue samples was 33 ± 3% (mean ± s.e.m.), $n = 16$ and 50 ± 8%, $n = 7$ respectively.

Estimation of homovanillic acid, 3,4-dihydroxyphenylacetic acid and dopamine

HVA and DOPAC were estimated by the fluorimetric method described by Murphy *et al.* (1969), except that each acid was determined in a separate tissue sample. Recovery of authentic HVA and DOPAC added to tissue samples was 66 ± 7%, $n = 6$ and 48 ± 4%, $n = 17$ respectively. Dopamine was estimated by the fluorimetric

method of Laverty & Sharman (1965) after adsorption onto and elution from a column of Dowex 50 x 8 cation exchange resin. Recovery of dopamine added to tissue samples was 71 ± 3%, $n = 24$.

Where direct comparisons of concentrations of substances estimated in tissues are made, the figures given are uncorrected for recovery. Values used in estimates of turnover are corrected for corresponding recoveries.

Results

Metabolites of noradrenaline

The distribution of the glycol metabolites of noradrenaline in the brain of the mouse is shown in Table 1. The highest concentrations of both MOPEG and DOPEG were observed in the hypothalamus. With the present method which can also be used to estimate alcohol metabolites of dopamine we have been unable to detect any free 1-(4-hydroxy-3-methoxyphenyl)ethan-2-ol in the striatum of the mouse although the small amount of MOPEG in this tissue is easily measured.

Effect of drugs

Because the hypothalamus showed the highest concentrations, this region of the brain was used to study the effect of some drugs on the relative concentrations of the two metabolites. Table 2 shows that cocaine (30 mg/kg, i.p.; 1 h) and desmethylimipramine (20 mg/kg, i.p.; 1 h) had no significant effect on the concentrations of MOPEG and DOPEG in the hypothalamus of the mouse. However, there was a significant reduction in the

Table 1 The distribution of 1-(4-hydroxy-3-methoxyphenyl)ethane-1,2-diol (MOPEG) and 1-(3,4-dihydroxyphenyl)ethane-1,2-diol (DOPEG) in the mouse brain

Brain region	*No. of observations*	*Concentration (ng/g tissue ± s.e.m.)* MOPEG	DOPEG
Hypothalamus	51	43 ± 2	25 ± 2
Midbrain	8	26 ± 3	18 ± 2
Massa intermedia of thalamus	8	25 ± 2	22 ± 3
Olfactory lobes	6	15 ± 2	11 ± 3
Cerebral cortex	4	14 ± 1	11 ± 3
Corpus striatum	5	11 ± 1	9 ± 2
Cerebellar cortex	5	9 ± 2	7 ± 2

Table 2 The effect of some drugs on the concentrations of 1-(4-hydroxy-3-methoxyphenyl)ethane-1,2-diol (MOPEG) and 1-(3,4-dihydroxyphenyl)ethane-1,2-diol (DOPEG) in the hypothalamus of the mouse

Treatment	*No. of observations*	*Time (h)*	*MOPEG (ng/g ± s.e.m.)*	*DOPEG (ng/g ± s.e.m.)*
Saline	8	1.0	39 ± 4	24 ± 3
Cocaine 30 mg/kg i.p.	8	1.0	33 ± 2	24 ± 4
Saline	8	1.0	39 ± 4	24 ± 3
Desmethylimipramine 20 mg/kg i.p.	8	1.0	35 ± 2	20 ± 2
Saline	6	3.0	38 ± 1	20 ± 2
Desmethylimipramine 20 mg/kg i.p.	6	3.0	26 ± 1*	17 ± 3
Saline	8	1.5	36 ± 3	25 ± 4
Tropolone 50 mg/kg i.p.	9	1.5	37 ± 2	55 ± 8*

* $P < 0.01$ when compared with own controls, Student's t test.

concentration of MOPEG 3 h after the administration of desmethylimipramine (20 mg/kg i.p.). Tropolone (50 mg/kg i.p.), a drug which inhibits the enzyme catechol-*O*-methyl transferase (COMT) (Belleau & Burba, 1963), caused an increase in the concentration of DOPEG but left the concentration of MOPEG unchanged after 1.5 hours.

When mice were injected with reserpine (5 mg/kg i.p.) dissolved in 5% w/v ascorbic acid solution, the concentrations of both DOPEG and MOPEG in the hypothalamus increased, reaching maxima 45 min after the injection. However, Table 3 shows that when 5% w/v ascorbic acid solution was injected alone, then, after 30 min, the increases in the concentrations of DOPEG and MOPEG were as large as those seen with reserpine dissolved in 5% w/v ascorbic acid solution.

Figure 1 shows the rates of disappearance of MOPEG from the hypothalamus of the mouse consequent upon the administration of two drugs, pargyline and tranylcypromine, which inhibit the enzyme monoamine oxidase (MAO). The calculated rate constant for the disappearance of MOPEG, assuming first order kinetics, was, after tranylcypromine (10 mg/kg i.p.), 2.8 times as large as that found after pargyline (100 mg/kg i.p.). No DOPEG (<1 ng/g) was detected in the hypothalamus 30 min after the administration of pargyline (100 mg/kg i.p.). After tranylcypromine (10 mg/kg i.p.) the concentration of DOPEG also declined rapidly. Five minutes after the administration of the drug there was still a detectable amount of DOPEG present in the tissue but none (<1 ng/g) could be measured after 10 minutes.

Pentobarbitone sodium (60 mg/kg i.p.; 1.5 h) and propranolol (0.5 mg/kg i.p.; 1 h) did not change the hypothalamic concentration of MOPEG. 2-Aminotetralin (30 mg/kg i.p.; 1.5 h) caused a 41% increase in the concentration of MOPEG.

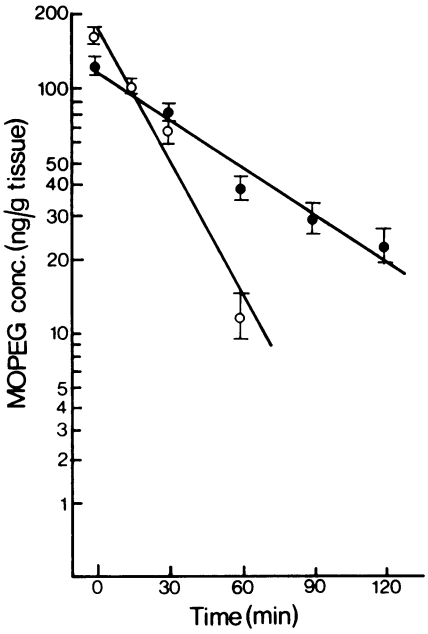

Fig. 1 The decline in the concentration of 1-(4-hydroxy-3-methoxyphenyl) ethane-1,2-diol (MOPEG) in the hypothalamus of the mouse after treatment with monoamine oxidase inhibiting drugs. (●) After pargyline (100 mg/kg, i.p.); (○) after tranylcypromine (10 mg/kg, i.p.).

Effect of low temperature

Mice, in groups of 5-8, were exposed to a temperature of −15°C for 1 h in cages which allowed the animals to huddle together on a

Table 3 The concentration of free 1-(4-hydroxy-3-methoxyphenyl)ethane-1,2-diol (MOPEG) and 1-(3,4-dihydroxyphenyl)ethane-1,2-diol (DOPEG) in mouse hypothalamus after the administration of reserpine in 5% w/v ascorbic acid solution and after ascorbic acid solution alone

Experiment	*No. of observations*	*Duration of treatment (min)*	*MOPEG (ng/g ±s.e.m.)*	*DOPEG (ng/g ±s.e.m.)*
Untreated controls	5	30	46 ± 3	28 ± 3
Saline (0.3 ml)	11	30	42 ± 2	29 ± 2
5% w/v ascorbic acid solution (0.3 ml)	6	30	86 ± 6*	40 ± 3*
Reserpine (5 mg/kg) in %5 w/v ascorbic acid solution	6	30	93 ± 7*	46 ± 4*

* Comparison with NaCl treated animals; Student's *t* test, $P < 0.01$.

dividing wire mesh wall to enable them to keep their heat loss at a minimum. The concentration of MOPEG in the hypothalamus rose from 27 ± 3 ng/g (n = 9) (estimated in corresponding control animals kept at room temperature) to 69 ± 6 ng/g (n = 9); and the concentration of DOPEG increased from 31 ± 2 ng/g (n = 9) to 58 ± 9 ng/g (n = 9).

Metabolites of dopamine

Mice were injected with α-methyl-*p*-tyrosine methyl ester (200 mg/kg, i.p.) and the concentration of dopamine in the striatum was measured 30 min and 60 min later. Figure 2a shows the fall in the concentration of dopamine that was observed, and also the fall in the concentration of DOPAC in the mouse striatum which was observed 30 min and 60 min after the administration of the monoamine oxidase inhibiting drug pargyline (100 mg/kg i.p.). From the rate constants calculated from these observations, assuming first order kinetics, the turnover of dopamine was 189 pmol g^{-1} min^{-1} and the turnover of DOPAC was 38 pmol g^{-1} min^{-1}. The rate of formation of HVA in the mouse striatum was estimated by measuring the straight line increase in the concentration of this acid during the first hour after the administration of probenecid (200 mg/kg i.p.). This is shown in Figure 2b. The rate of formation of HVA was calculated to be 56 pmol g^{-1} min^{-1}.

Discussion

The distribution of the glycol metabolites of noradrenaline in the mouse brain approximates to that of noradrenaline reported by Saelens, Schoen & Kovacsics (1967). A similar distribution for the sulphate conjugate of MOPEG in the rat brain has been observed by Meek & Neff (1972). Using the fluorimetric method developed by the latter authors we have been unable to detect any MOPEG-sulphate (<6 ng/g) in the whole mouse brain. There have been reports that 4-hydroxy-3-methoxyphenylethanol (MOPET) is present in the rat brain as a conjugate (Karoum, Ruthven & Sandler, 1971; Braestrup, 1972; Wendel, Waterbury & Pearce, 1972). We have been unable to find free MOPET in the striatum of the mouse. When mice were treated with cocaine or desmethylimipramine, drugs which inhibit the reuptake of noradrenaline into the terminals of peripheral noradrenergic nerves (Iversen, 1967) no effect was observed on the concentration of the two glycols in the hypothalamus after 1 hour. However, 3 h after desmethylimipramine the concentration of

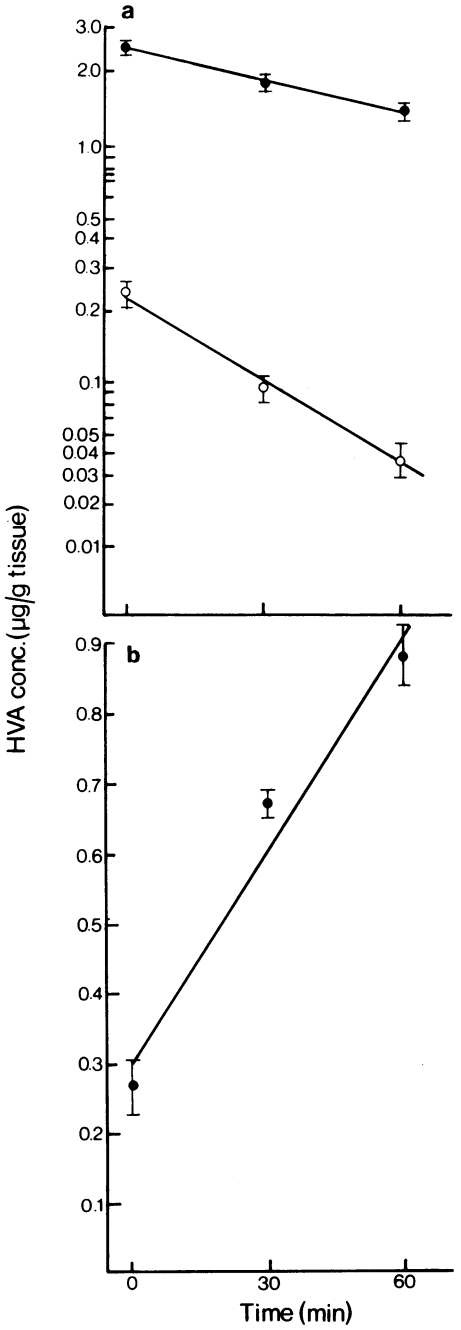

Fig. 2 (a) The decline in the striatal concentration of dopamine after the administration of α-methyl-*p*-tyrosine methyl ester (200 mg/kg i.p.) and the decline in the striatal concentration of 3,4-dihydroxyphenylacetic acid (DOPAC) after the administration of pargyline (100 mg/kg i.p.) to the mouse. (•) Dopamine; (○) DOPAC. (b) The accumulation of 4-hydroxy-3-methoxyphenylacetic acid (HVA) in the striatum of the mouse after the administration of probenecid (200 mg/kg, i.p.).

MOPEG fell. Roffler-Tarlov & Langer (1971) have shown that, *in vitro*, the metabolism of radioactive noradrenaline, released in the guinea-pig atrium by field stimulation, can be inhibited by these two drugs. The fall in the concentration of MOPEG in the mouse hypothalamus 3 h after desmethylimipramine might indicate an inhibition of the metabolism of noradrenaline, but this explanation is difficult to reconcile with the observations of Dingell, Sulser & Gillette (1964) who showed that desmethylimipramine is rapidly metabolized in the mouse, its half life being 50 minutes.

2-Aminotetralin has been shown to increase the concentration of HVA in the striatum of the mouse (Sharman, 1966) and its action on the concentration of MOPEG in the hypothalamus was similar. When COMT was inhibited with tropolone the effects on noradrenaline metabolism again resembled those seen on the metabolism of dopamine (Roffler-Tarlov *et al.*, 1971); in both cases there was a significant increase in the deaminated catechol metabolite of the catecholamine but little change in the concentration of the corresponding methoxy derivative.

The concentrations of hypothalamic MOPEG and DOPEG were reduced after the administration of MAO inhibiting drugs. The rate of disappearance of a deaminated metabolite of a biogenic amine from the brain, after inhibition of MAO, has been used by Tozer, Neff & Brodie (1966) to study the turnover of the major metabolite of 5-hydroxytryptamine, 5-hydroxyindol-3-ylacetic acid (5-HIAA), in the rat brain. Our results show that after inhibition of MAO with pargyline and tranylcypromine, the concentration of MOPEG in the hypothalamus appears to fall exponentially but at different rates, the rate of fall being faster after tranylcypromine. The decline in the concentration of DOPEG was five times faster than that of MOPEG after both drugs, indicating that in the mouse hypothalamus, noradrenaline is mainly metabolized directly to DOPEG rather than to MOPEG. This is in keeping with the conclusions of Kopin & Gordon (1962) who suggested that deamination was the major route of inactivation of noradrenaline retained within the endings of peripheral adrenergic nerves. However, we have not investigated the further metabolism of DOPEG.

In studying the metabolism of dopamine we have used a different method for estimating the turnover of HVA. Neff, Tozer & Brodie (1967) showed that the rate of accumulation of 5-HIAA in the rat brain after a maximally effective dose of probenecid was equal to the rate at which 5-HIAA was formed. We have used this technique for studying the rate of formation of HVA. The estimated rates of turnover of HVA and DOPAC only account for one half of the turnover rate of dopamine in the striatum estimated from the rate of disappearance of this amine from this tissue after inhibition, with α-methyl-*p*-tyrosine methyl ester, of the enzyme tyrosine hydroxylase (Spector, Sjoerdsma & Udenfriend, 1965). Spano & Neff (1972) have shown that the turnover of radioactive dopamine in the caudate nucleus of the guinea-pig can be accounted for by the formation and elimination of HVA and DOPAC. The higher rate of turnover of dopamine, calculated from the rate of disappearance after α-methyl-*p*-tyrosine, might arise from the activity of the feedback system which is thought to be involved in the normal release of dopamine in the striatum. It has been demonstrated that drugs such as chlorpromazine and haloperidol increase the rate of formation of dopamine metabolites in the brain (Carlsson & Lindqvist, 1963; Andén, Roos & Werdinius, 1964) and that apomorphine reduces the turnover of dopamine and the concentration of homovanillic acid in the striatum of the rat (Andén, Rubenson, Fuxe & Hökfelt, 1967; Roos, 1969). A compensatory increase (Carlsson & Lindqvist, 1963) or decrease in the activity of the dopaminergic neurones in response to receptor blockade or activation has been suggested to explain these responses. As the concentration of dopamine falls after the administration of α-methyl-*p*-tyrosine with a concomitant reduction in the release of dopamine, the compensatory system would tend to increase the neuronal activity in an attempt to counteract this reduced release of transmitter. Thus the rate constant for the fall in the dopamine concentration after inhibition of tyrosine hydroxylase would increase to a maximum value, giving rise to a rate constant that is higher than that which applied before the enzyme was inhibited. If a compensatory mechanism is active after the synthesis of dopamine is inhibited, it would be the time taken to reach the maximum rate constant that would vary with different turnover rates.

Stress has been shown to increase the turnover of dopamine in the brain (Bliss, Ailion & Zwanziger, 1968; Thierry, Blanc & Glowinski, 1971) and it is possible that the stress of the injection of α-methyl-*p*-tyrosine methyl ester results in an increased rate of disappearance of dopamine which is reflected in an increased rate of fall of the concentration of the amine. A similar response may occur after the injection of probenecid, leading to an increased rate of accumulation of HVA; but the estimates of the turnover of DOPAC based on its disappearance after MAO inhibition are theoretically not changed by an increase in the turnover of dopamine, unless there is a concomitant change in the rate of

removal of DOPAC from the tissue. The three different methods give estimates of the rate of accumulation or elimination of dopamine or its metabolites under the conditions of the experiments used in their determination but it might not be correct to equate the results, which they give, with each other. However, the estimated turnover rates of DOPAC and HVA were of a similar magnitude, in agreement with the findings of Spano & Neff (1972) in the caudate nucleus of the guinea-pig.

From the results of the present experiments we are not able to describe the action of reserpine on the concentrations of the two metabolites of noradrenaline in the mouse hypothalamus. It is possible that the effects produced by the administration of the ascorbic acid vehicle are maximal and that any effect of reserpine on the storage of noradrenaline may not increase the rate at which this amine is metabolized. Sjöstrand (1970) has observed that a dose of 100 mg/kg ascorbic acid injected intravenously into mice had no effect on the turnover of noradrenaline in the brain but that a similar dose of dehydroascorbic acid increased the cerebral turnover of this catecholamine. The intraperitoneal injection of ascorbic acid (500 mg/kg) to rats has been shown to reduce the concentration of dopamine and increase the concentration of noradrenaline in the brain (Izquierdo, Jofré & Acevedo, 1968). These authors suggested that ascorbic acid was increasing the rate of formation of noradrenaline in the brain by participating in the action of the enzyme dopamine-β-hydroxylase. The increase in the concentration of the glycol metabolites in the hypothalamus of the mouse after the intraperitoneal injection of ascorbic acid may reflect the metabolism of noradrenaline so formed.

The release of cerebral noradrenaline increases under conditions of stress (Maynert & Levi, 1964). Simmonds (1969) demonstrated an increase in the turnover of noradrenaline in the hypothalamus of the rat exposed to a low temperature. The present experiments indicate that the concentrations of DOPEG and MOPEG in the brain rise when there is an increased turnover of noradrenaline.

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