

CHANGES IN THE METABOLISM OF 3,4-DIHYDROXYPHENYLETHYLAMINE (DOPAMINE) IN THE STRIATUM OF THE MOUSE INDUCED BY DRUGS

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Several drugs have been shown to increase the concentration of 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid; HVA), a major metabolite of 3,4-dihydroxyphenylethylamine (dopamine), in the striatal tissues of several mammalian species (Andén, Roos & Werdinius, 1963, 1964; Sharman, 1963; Lavery & Sharman, 1965a; Juorio, Sharman & Trajkov, 1966). This report is concerned with the application of a screening test for drugs which increase the concentration of homovanillic acid in the striatum using the albino mouse as the test animal. Possible mechanisms by which such an increase can be produced are suggested.

METHODS

Administration of drugs

The drugs were administered intraperitoneally or, in a few cases, intravenously, dissolved in 0.9% sodium chloride solution with the following exceptions.

Reserpine was injected as the manufacturers' solution.

Tetrabenazine, spiroperidol, haloperidol and triperidol were dissolved in the minimum amount of glacial acetic acid. Dilutions were then made with 0.9% sodium chloride solution.

Probenecid was dissolved in the minimum volume of 1 N-sodium hydroxide and 0.9% sodium chloride solution was then added. If necessary, the pH was adjusted to 7-8 with 0.1 N-hydrochloric acid.

α -Methyl-p-tyrosine was dissolved in the minimum volume of 1 N-sodium hydroxide and the solution diluted with 0.5 M-disodium hydrogen phosphate solution; 0.4 N-hydrochloric acid was then added carefully until a slight precipitate of the amino acid was formed. The final concentration of α -methyl-p-tyrosine was usually 8 mg/ml.

Dissection of tissues

The mice were stunned and decapitated and the brains rapidly dissected out and placed on a glass plate on ice. The part of the brain used for the estimations of homovanillic acid and of dopamine consisted of the striatum together with some orbital cortex. This piece of tissue was selected for ease of reproducibility in the dissection and was obtained by dividing the brain along the midline and exposing the caudate nucleus through the lateral ventricle. The cortical tissue anterior and dorsal to the caudate nucleus was removed and the striatum together with some orbital cortex obtained with a single cut posterior to the caudate nucleus. For the estimation of 5-hydroxyindol-3-ylacetic acid the whole brain anterior to the pons was used. Pooled tissues from two to three mice were used for the estimation of homovanillic acid and from two mice for the estimation of

5-hydroxyindol-3-ylacetic acid. The dopamine estimations were carried out with the tissue from a single animal. Albino mice of both sexes were used.

The estimation of homovanillic acid

The method used to extract and estimate homovanillic acid has been described in detail by Juorio *et al.* (1966). Briefly, the homovanillic acid was adsorbed from the deproteinized tissue extract on to a small column of Dowex 1X2 anion exchange resin. The homovanillic acid was then eluted with 0.1 N-hydrochloric acid and estimated fluorimetrically by a modification of the method of Andén *et al.* (1963) using an Aminco-Bowman Spectrophotofluorometer.

Dopamine was extracted with a column of Dowex 50X8 cation exchange resin as described by Bertler, Carlsson & Rosengren (1958). The dopamine in the eluate from the column was acetylated and estimated fluorimetrically in a Locarte filter fluorimeter after condensation of the acetyl derivative with ethylene diamine (Laverty & Sharman, 1965b). 5-Hydroxyindol-3-ylacetic acid was estimated fluorimetrically as described by Ashcroft & Sharman (1962). All estimations are uncorrected for recoveries.

RESULTS

Effect of drugs on the concentration of homovanillic acid in the striatum

The effect of a number of drugs on the concentration of homovanillic acid in the striatum of the mouse is shown in Table 1. No difference was found between untreated control animals and those treated with the vehicles in which the drugs were dissolved. All of these values have been combined to give the control value in Table 1.

TABLE 1
THE EFFECT OF DRUGS ON THE CONCENTRATION OF HOMOVANILIC ACID IN THE STRIATUM OF THE MOUSE

Except where otherwise stated drugs were injected intraperitoneally

* Significantly different ($P < 0.01$) from control value. A single observation showing an increase of 0.24 $\mu\text{g/g}$ above the control value can be taken to indicate a significant increase

Drug	Dose (mg/kg)	Duration of treatment (hr)	Concn. of homovanillic acid ($\mu\text{g/g}$ tissue \pm s.e.m.) No. of observations in parentheses
Control			0.31 \pm 0.01 (76)
<i>Tranquillizing drugs</i>			
Body temperature maintained where necessary			
Chlorpromazine HCl	2.5	3	0.45 ; 0.31
	5.0	3	0.70 \pm 0.16 (10)*
	10.0	3	0.91 \pm 0.15 (3)*
Thioridazine HCl	10.0	3	0.49 ; 0.49
Prothipendyl HCl	10.0	3	0.30 ; 0.28
	50	3	0.50
	100	3	0.69 ; 0.81
Chlorprothixene	10	3	1.28 \pm 0.08 (3)*
Reserpine	2.5	3	0.54 ; 0.36
	5	3	0.48 \pm 0.01 (3)
Tetrabenazine	50	1.5	0.73 \pm 0.02 (3)*
Spiroperidol	0.1	2	0.72 ; 0.86
		3	0.66 \pm 0.04 (9)*
	0.25	3	0.48 ; 0.72
	0.5	3	0.91 \pm 0.07 (4)*
Haloperidol	0.5	2	0.50 ; 0.92
Triperidol	0.5	2	1.01 ; 0.82
Chlordiazepoxide	50	1	0.43 ; 0.41
	100	1	0.49 \pm 0.06 (4)*
	200	1	0.54 ; 0.66

TABLE 1—continued

Drug	Dose (mg/kg)	Duration of treatment (hr)	Concn. of homovanillic acid ($\mu\text{g/g}$ tissue \pm s.e.m.) No. of observations in parentheses
<i>Excitant drugs</i>			
Methylphenidate	20	3	0.18 ; 0.10
	50	2	0.31 \pm 0.03 (3)
β -Tetrahydronaphthylamine	50	2	0.91 \pm 0.18 (3)*
<i>Morphine and associated compounds</i>			
Morphine HCl	45 mg/kg in two doses at 2-hr interval		0.42 \pm 0.07 (3)
	Total duration 4 hr		
M.99	0.1	1	0.53 ; 0.58
		2	0.88 \pm 0.06 (3)*
		4	0.79 \pm 0.12 (3)*
		8	0.27 \pm 0.01 (3)
Body temperature maintained	0.1	2	0.58 \pm 0.07 (3)*
	0.2	2	0.84 ; 0.76
M.285	0.5	2	0.32 \pm 0.02 (4)
Phencyclidine HCl	5	1	0.36 ; 0.32
		4	0.25 ; 0.30
	10	1	0.34 ; 0.42
<i>Central cholinomimetic drugs</i>			
These mice were pretreated with atropine methyl bromide 2 mg/kg s.c.			
Arecoline HBr	2 I.V.	2	0.31 ; 0.30
	6 I.V.	2	0.28
	10 I.V.	2	0.30
Pilocarpine HCl	2 I.V.	2	0.27
	4 I.V.	2	0.34 ; 0.34
<i>Anti-depressant drugs</i>			
Pargyline HCl	400	0.5	0.20 ; 0.18
		1	0.20 ; 0.26
		4	0.08 ; 0.06
Imipramine	20	4	0.40 \pm 0.09 (4)
Desmethyylimipramine	20	4	0.23 ; 0.25
<i>Other drugs</i>			
Probenecid	200	3.5	0.75 ; 0.78
	270	3.5	1.31 \pm 0.10 (4)*
Zoxazolamine	90 mg/kg divided into 3 doses at 1-hr intervals		0.25 \pm 0.02 (3)
	Total duration 2.5 hr		
Tolazoline	35 mg/kg divided into 4 doses at 30-min intervals		0.28 \pm 0.02 (5)
	Total duration 2 hr		
Bulbocapnine HCl	40	0.5	0.51 \pm 0.05 (4)*
Benzhexol HCl	50 I.V.	2	0.43 ; 0.40

The large increases caused by most of the tranquillizing drugs are in agreement with the observations of Andén *et al.* (1964) and Roose (1965) on the rabbit, Lavery & Sharman (1965a) on the cat and Juorio *et al.* (1966) on four rodents. The small increase seen after chlordiazepoxide was seen only after doses of this drug which produce a loss of the righting reflexes.

Of the two excitant drugs, only β -tetrahydronaphthylamine produced an increase in the level of homovanillic acid, an effect also seen in the cat (Lavery & Sharman, 1965a). These authors also observed that morphine elevated the homovanillic acid in the cat. When tested in the mouse morphine did not show this effect even after 45 mg/kg. However, the very active morphine-like compound M.99 (6,14-endoetheno-7-(2-hydroxy-

2-pentyl)-tetrahydro-oripavine hydrochloride) produced a clear increase after a dose of only 0.1 mg/kg. M.285 (N-cyclopropylmethyl-6,4-endoetheno-7-(2-hydroxy-2-propyl)-tetrahydro-nororipavine hydrochloride), which has strong nalorphine-like properties, did not produce an increase in homovanillic acid when 0.5 mg/kg were given. With a larger dose (1 mg/kg) a possible small increase in the homovanillic acid was seen (Table 2).

TABLE 2

THE EFFECT OF PRETREATMENT ON DRUG-INDUCED INCREASES IN THE HOMOVANILLIC ACID CONCENTRATION IN THE STRIATUM OF THE MOUSE

Except where stated otherwise all drugs were injected intraperitoneally

* Significant difference ($P < 0.01$) from M.99 alone

† Significant difference ($P < 0.01$) from all controls (Table 1); not significantly different from parallel control values

Drug	Dose (mg/kg)	Duration of treatment (hr)	Pretreatment 15-60 min before (dose in mg/kg)	Concn. of homovanillic acid ($\mu\text{g/g}$ tissue \pm s.e.m.) No. of observations in parentheses
Chlorpromazine HCl	5	3		0.70 ± 0.05 (10)
			Diethazine 10	0.66 ; 0.77
			Caramiphen 10	0.76 ; 0.61
			Desmethylinipramine 20	0.78 ± 0.06 (3)
			Imipramine 20	0.93 ± 0.01 (4)
			Methylphenidate 20	0.80 ; 0.72
			Methylphenidate 50	0.60 ; 0.65
Spiroperidol	0.1	2	M.285 1	0.70 ± 0.03 (11) 0.63 ± 0.04 (8)
Spiroperidol	0.1	3		0.66 ± 0.04 (9)
			Phencyclidine 5 Benzhexol 10 I.V.	0.59 ; 0.67 0.51 ; 0.81
M.99	0.2	2		0.75 ± 0.06 (7)
M.285	1	2	M.285 1	0.53 ± 0.04 (8)*
Control animals	(Estimations made in parallel with above three sets of results)			0.45 ± 0.04 (7)† 0.37 ± 0.05 (6)

Inhibition of monoamine oxidase by pargyline resulted in a fall in the homovanillic acid level, an effect seen in the rabbit after treatment with the monoamine oxidase inhibiting drug nialamide (Andén *et al.*, 1963). The two other anti-depressant drugs tested, which are not inhibitory to monoamine oxidase, had no significant effect on the concentration of homovanillic acid. The drugs causing central cholinergic stimulation were also ineffective in this respect. Of the other drugs tested, probenecid is of interest as an inhibitor of organic acid transport in the renal tubules; it produced a large elevation of the level of homovanillic acid in the striatum. An increase in the concentration of 5-hydroxyindol-3-ylacetic acid in the brain of the rat after treatment with probenecid has been reported by Neff, Tozer & Brodie (1964). Zoxazolamine has uricosuric properties which can be additive with those of probenecid, and is a centrally acting muscle relaxant, but it did not affect the concentration of homovanillic acid in striatal tissues.

Antagonism of drug induced increases in the concentration of homovanillic acid

Because the major tranquillizing drugs are known to produce side effects which resemble Parkinson's disease, some drugs which are used to treat this disease were tested to see if they would prevent the increase in homovanillic acid produced by chlorpromazine and spiroperidol. The drugs were administered intraperitoneally 15–60 min before the tranquillizing drug.

The results are shown in Table 2 and they indicate that diethazine and caramiphen do not prevent the increase in homovanillic acid produced by chlorpromazine and that benzhexol is ineffective against spiroperidol. Imipramine, desmethylimipramine and methylphenidate were also inactive. Table 2 also shows that M.285, in a dose that blocks the behavioural effects of M.99 administered 15 min later, can partially antagonize the increase in homovanillic acid produced by the latter drug. The same dose of M.285 did not change the homovanillic acid increase after spiroperidol (0.1 mg/kg).

Investigation of the mechanisms by which drugs can elevate the concentration of homovanillic acid in the striatum

Five of the drugs found to increase the homovanillic acid concentration in the striatum of the mouse were selected for further investigation into the mechanisms by which they produce this effect. These were spiroperidol, M.99, β -tetrahydronaphthylamine, probenecid and bulbocapnine.

(a) Behavioural effects

These five drugs produce very different behavioural effects in the mouse.

Spiroperidol in a dose of 0.1 mg/kg had little effect on the gross behaviour of the mice. They remained fairly active but frequently showed ptosis. With a larger dose (0.5 mg/kg) the animals became inactive but did not collapse.

The response to M.99 (0.2 mg/kg) was extremely variable. A pronounced Straub effect on the tail was the first sign of the action of the drug. The animals then became excited and moved erratically about the cage. A rigid catalepsy then developed and in many but not all of the experiments the animals lost their righting reflexes. There was a pronounced fall in temperature.

β -Tetrahydronaphthylamine caused piloerection on the back of the neck and excitement.

Probenecid (200 mg/kg), had no obvious effect on the behaviour of the mice.

Bulbocapnine (40 mg/kg) caused a flaccid catalepsy which wore off after one hour. None of these behavioural effects was prevented by the administration of α -methyl-p-tyrosine.

(b) Effects on the rate of utilization of dopamine

The effects of these five drugs on the concentrations of dopamine and homovanillic acid in the striatum of the mouse were studied after treatment with the drugs alone and after pretreatment with α -methyl-p-tyrosine (80 mg/kg). This amino acid competitively inhibits the enzyme tyrosine hydroxylase (Nagatsu, Levitt & Udenfriend, 1964; Udenfriend, Zaltzman-Nirenberg & Nagatsu, 1965), which catalyses the rate-limiting step

in the synthesis of dopamine *in vivo*. Spector, Sjoerdsma & Udenfriend (1965) have shown that the administration of this amino acid results in a fall of the dopamine concentration in the caudate nucleus and suggested that the rate of the decrease in dopamine concentration in a tissue, after treatment with α -methyl-p-tyrosine, would reflect the turnover rate of the dopamine in that tissue.

The results obtained in this series of experiments are given in Tables 3 and 4. A maximum inhibition of the tyrosine hydroxylase was effected in these experiments because a larger dose of α -methyl-p-tyrosine (160 mg/kg) did not produce a larger fall in the concentration of dopamine than that following a dose of 80 mg/kg. Table 3 shows that of the five drugs only β -tetrahydronaphthylamine changes the dopamine concentration when given alone. In a dose of 30 mg/kg this drug caused a significant increase in the concentration of dopamine in the striatum of the mouse. Table 3 also shows that after α -methyl-p-tyrosine, the administration of spiroperidol in doses of 0.1 mg/kg and 0.5

TABLE 3

THE EFFECT OF DRUGS ON THE DECREASE IN THE CONCENTRATION OF THE DOPAMINE IN THE STRIATUM OF THE MOUSE PRODUCED BY THE ADMINISTRATION OF α -METHYL-p-TYROSINE

α -Methyl-p-tyrosine (80 mg/kg) administered 15 min before drug

* Significant difference ($P < 0.01$) from corresponding control value

† Significant difference ($P < 0.01$) from control animals not treated with α -methyl-p-tyrosine

Drug	Dose (mg/kg)	Duration of treatment (hr)	Dopamine concn. ($\mu\text{g/g}$ tissue \pm s.e.m.) No. of observations in parentheses	
			Drug alone	After α -methyl-p-tyrosine
Control			3.61 \pm 0.14 (20)	1.86 \pm 0.05 (25)†
Spiroperidol	0.1	2	2.91 \pm 0.15 (6)	1.49 \pm 0.11 (12)*
	0.5	2	2.67 \pm 0.09 (6)	1.16 \pm 0.06 (6)*
β -Tetrahydro-naphthylamine	30	2	4.35 \pm 0.13 (17)*	2.01 \pm 0.11 (6)
M.99	0.2	2	3.17 \pm 0.08 (8)	1.98 \pm 0.10 (6)
Bulbocapnine HCl	40	1.25-1.5	2.90 \pm 0.22 (6)	1.83 \pm 0.13 (9)
Probencid	200	2	2.85 \pm 0.20 (6)	2.07 \pm 0.21 (9)

TABLE 4

THE EFFECT OF α -METHYL-p-TYROSINE ON THE DRUG-INDUCED INCREASE IN THE LEVEL OF HOMOVANILLIC ACID IN THE STRIATUM OF THE MOUSE

α -Methyl-p-tyrosine (80 mg/kg) administered 15 min before drug

* Significant difference ($P < 0.01$) from corresponding control value

† Significant difference ($P < 0.01$) from control animals not treated with α -methyl-p-tyrosine

Drug	Dose (mg/kg)	Duration of treatment (hr)	Concn. of homovanillic acid ($\mu\text{g/g}$ tissue \pm s.e.m.) No. of observations in parentheses	
			Drug alone	After α -methyl-p-tyrosine
Control			0.31 \pm 0.01 (76)	0.18 \pm 0.01 (10)†
Spiroperidol	0.1	2	0.70 \pm 0.03 (11)*	0.22 \pm 0.01 (6)
	0.5	2	1.04 \pm 0.05 (5)*	0.34 \pm 0.03 (7)*
β -Tetrahydro-naphthylamine	30	2	0.55 \pm 0.05 (12)*	0.19 \pm 0.01 (4)
M.99	0.2	2	0.75 \pm 0.06 (7)*	0.26 \pm 0.02 (10)
Bulbocapnine HCl	40	1.25-1.5	0.70 \pm 0.06 (12)*	0.21 \pm 0.01 (4)
Probencid	200	2	0.90 \pm 0.02 (6)*	0.47 \pm 0.03 (8)*

mg/kg results in a significantly larger fall in the concentration of dopamine than occurs after the amino acid alone. The other four drugs did not change the fall in dopamine concentration caused by the α -methyl-p-tyrosine.

The specificity of the dopamine estimations. To confirm that the apparent increase in the dopamine concentration seen after β -tetrahydronaphthylamine was due to dopamine and not to a metabolite of the drug, striatal tissues from treated mice were extracted as for the estimation of dopamine. After the acetylation step in the procedure the solution was extracted with dichloromethane and the material in this extract subjected to paper chromatographic separation as described by Lavery & Sharman (1965b). Fluorescence was only found on the chromatogram in the region of triacetyldopamine, and this was present in an intensity which corresponded with the increased amount found by direct estimation in the tissue. Furthermore, no increase in dopamine was detected in the hypothalamic region of the mouse brain after treatment with β -tetrahydronaphthylamine, where metabolites of β -tetrahydronaphthylamine, if such were formed, should also be detected.

Udenfriend *et al.* (1965) have shown that a small amount of α -methyl-3,4-dihydroxyphenylalanine can be formed from α -methyl-p-tyrosine *in vitro* by the enzyme tyrosine hydroxylase. If this transformation were to occur *in vivo* then it is possible that the dopamine estimations given in Table 3 would include any α -methyl dopamine formed by decarboxylation of the α -methyl-3,4-dihydroxyphenylalanine. However, no α -methyl dopamine could be detected in extracts of striatal tissue from mice treated with α -methyl-p-tyrosine when these were examined by the methods described above for the identification of dopamine since these allow the separation of dopamine and its methylated derivative α -methyl dopamine. Using these methods it was possible to demonstrate that α -methyl dopamine was formed in striatal tissue 2 hr after the administration of α -methyl-3,4-dihydroxyphenylalanine (80 mg/kg).

(c) *Effects on the concentration of homovanillic acid*

Table 4, in agreement with Table 1, shows that all five drugs can produce a significant increase in the homovanillic acid concentration in the striatum of the mouse. Inhibition of tyrosine hydroxylase by α -methyl-p-tyrosine causes a reduction in the level of the homovanillic acid in this tissue. Furthermore, α -methyl-p-tyrosine given 15 min before the drug reduces the increase in homovanillic acid produced by probenecid and the higher dose of spiroperidol and abolishes that caused by the lower dose of spiroperidol, β -tetrahydronaphthylamine, M.99 and bulbocapnine.

(d) *Effects on 5-hydroxyindol-3-ylacetic acid*

The five drugs were also examined for their effect on the concentration of 5-hydroxyindol-3-ylacetic acid in the brain. The results are given in Table 5. Probenecid was found to cause an increase in the concentration of 5-hydroxyindol-3-ylacetic acid in the brain whereas β -tetrahydronaphthylamine produced a reduction in the concentration of this acid. No effect on the 5-hydroxyindol-3-ylacetic acid level was observed after spiroperidol, M.99 and bulbocapnine.

TABLE 5

THE EFFECT OF DRUGS ON THE CONCENTRATION OF 5-HYDROXYINDOL-3-YLACETIC ACID IN THE BRAIN OF THE MOUSE

* Significantly different ($P < 0.01$) from control values

Drug	Dose (mg/kg)	Duration of treatment (hr)	Concn. of 5-hydroxyindol-3-ylacetic acid ($\mu\text{g/g}$ tissue \pm s.e.m.) No. of observations in parentheses
<i>First series of experiments</i>			
Control			0.21 \pm 0.01 (4)
Spiroperidol	0.5	3.5	0.24 \pm 0.03 (4)
Probenecid	200	3.5	0.49 \pm 0.06 (4)*
<i>Second series of experiments</i>			
Control			0.18 \pm 0.01 (24)
Spiroperidol	0.5	2	0.22 \pm 0.02 (6)
β -Tetrahydronaphthylamine	30	2	0.09 \pm 0.02 (6)*
M.99	0.2	2	0.22 \pm 0.03 (6)
Bulbocapnine HCl	40	1.25-1.5	0.18 \pm 0.02 (6)
Probenecid	200	2	0.35 \pm 0.02 (6)*

DISCUSSION

The method for estimating homovanillic acid described by Juorio *et al.* (1966) has proved to be sensitive enough to be used in a screening test for drugs which affect the homovanillic acid in the striatum, using the albino mouse as the test animal. This enables several observations to be made when only a limited amount of a drug is available.

The dopamine in the striatum is thought to have a physiological role of its own and the major final metabolite of dopamine in this region of the brain appears to be homovanillic acid. An increased formation of homovanillic acid could follow an increased synthesis of the amine. If the dopamine storage mechanism is not saturated under normal conditions, this increased synthesis would be accompanied by an increase in the amount of dopamine present in the tissue. An inhibitor of dopamine synthesis would abolish drug-induced increases in the concentrations of both dopamine and homovanillic acid. Secondly, an increased requirement for dopamine could result in a compensatory increase in the synthesis of dopamine through a feedback mechanism which maintains the level of the stored amine. Inhibition of the synthesis of dopamine would reduce the increase in homovanillic acid, but the increased requirement for the amine would be reflected in a depletion of the stored dopamine greater than that produced by inhibition of the synthesis of dopamine under normal conditions. When the formation of dopamine is inhibited a small increase in homovanillic acid might be produced by a drug acting in this way but this will depend on the magnitude of the increase in the rate of depletion of the stored amine.

Finally, a drug might also reduce the rate of outflow of homovanillic acid from the brain. After inhibition of the synthesis of dopamine an increase in homovanillic acid would still be seen. If such a drug is not acting specifically on the mechanism which removes homovanillic acid from the brain then a concomitant increase in other acidic substances such as 5-hydroxyindol-3-ylacetic acid should be seen.

These mechanisms are illustrated diagrammatically in Fig. 1.

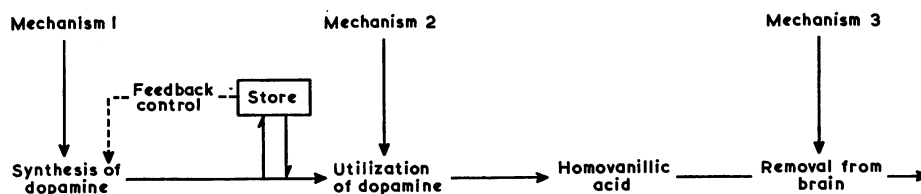


Fig. 1. The sites in the major metabolic pathway of dopamine in the striatum at which a drug might act to produce an increase in the concentration of homovanillic acid.

In the mouse, β -tetrahydronaphthylamine seems to be acting by the first mechanism proposed, increasing the synthesis of dopamine, since both the concentration of the amine and its acid metabolite are increased. Both of these effects are abolished when the synthesis of dopamine is inhibited by α -methyl-p-tyrosine. Because β -tetrahydronaphthylamine does not accelerate the fall in the dopamine concentration caused by α -methyl-p-tyrosine, there is no increase in the requirement for dopamine. The enzyme tyrosine hydroxylase catalyses the rate-limiting step in the synthesis of dopamine and it is tempting to ascribe the action of β -tetrahydronaphthylamine to an effect on this enzyme.

Spiroperidol might be acting on the second mechanism. It appears to act by increasing the turnover of dopamine, since, after inhibition of the synthesis of dopamine there is a faster rate of depletion of the amine in mice treated with this drug than in untreated animals. Only the larger dose of spiroperidol increased the concentration of homovanillic acid after treatment with α -methyl-p-tyrosine. It would further appear that part of the action of this drug is dependent upon an intact synthetic pathway for dopamine.

Probenecid is a typical example of a drug which acts by generally preventing the outflow of acidic substances from the brain. This drug has no effect on the concentration of dopamine when given alone or after α -methyl-p-tyrosine. After the latter treatment, probenecid still produces a large increase in homovanillic acid. This increase is not as large as in the normal animal and reflects a lowering of the turnover rate of dopamine as the "stored" amine is reduced. The general action of probenecid is illustrated by the fact that it was the only drug of the five examined which elevated the concentration of 5-hydroxyindol-3-ylacetic acid in the brain of the mouse. Neff *et al.* (1964) have demonstrated an active system for transporting 5-hydroxyindol-3-ylacetic acid out of the brain of the rat, which can be inhibited by probenecid. The observations presented in this paper confirm the presence of a similar system in the mouse.

On the evidence presented here, the actions of M.99 and bulbocapnine in elevating homovanillic acid do not appear to fit any of the proposed schemes. These drugs did not increase the concentration of dopamine like β -tetrahydronaphthylamine and in the presence of an inhibitor of dopamine synthesis there was no increase in the rate of disappearance of dopamine, as had been seen with spiroperidol. Their action requires an intact synthetic pathway for dopamine, and they do not act by preventing the outflow of homovanillic acid from the brain. A possible explanation of the action of M.99 and bulbocapnine is that they produce a diversion of the dopamine after the amine is formed by the decarboxylation of 3,4-dihydroxyphenylalanine and that it is metabolized before it comes under the influence of the "storage" mechanisms. Such a diversion would tend to reduce the amount of dopamine in the "store" and this, in turn, might lead to an

increased synthesis of dopamine to maintain the "store." This diversion of the metabolism of dopamine could also play a part in the action of spiroperidol.

Although it is suggested that spiroperidol, β -tetrahydronaphthylamine, M.99, probenecid and bulbo-carpine produce an increase in homovanillic acid by different mechanisms, the possible correlation between these different mechanisms and the behavioural effects of these drugs cannot be attempted until more detailed behavioural studies have been made.

SUMMARY

1. A rapid method is described for the detection of drugs which affect the concentration of 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid) in the striatum, using the albino mouse as the test animal.

2. Among the drugs which were found to increase the concentration of homovanillic acid in the striatum of the mouse, spiroperidol, β -tetrahydronaphthylamine, M.99, bulbo-carpine and probenecid were selected for a fuller analysis. This included the effect of these drugs on the fall in brain concentration of 3,4-dihydroxyphenylethylamine (dopamine) elicited by α -methyl-p-tyrosine.

3. These observations, and the study of the effects of these drugs on the concentration of 5-hydroxyindol-3-ylacetic acid in the brain, have suggested a number of mechanisms by which drugs can produce an increased concentration of homovanillic acid in the striatum.

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