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3,4-Dihydroxyphenylacetic acid and 4-hydroxy-3-methoxyphenylacetic acid in the mouse striatum: a reflection of intra- and extra-neuronal metabolism of dopamine?

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Summary

1. The administration of probenecid to mice increased the concentration of 4-hydroxy-3-methoxyphenylacetic acid (HVA) in the striatum, but did not raise the concentration of 3,4-dihydroxyphenylacetic acid (DOPAC).

2. After drug treatments which normally increase the concentration of HVA several-fold, inhibition of catechol-*O*-methyltransferase (COMT) by tropolone greatly reduced the concentration of HVA but resulted in only a small increase in the concentration of DOPAC in the striatum of the mouse.

3. These results indicate that HVA and DOPAC do not occur at the same location in the tissue of the striatum and that DOPAC is not normally metabolized to HVA to any great extent in this tissue.

4. When mice were treated with reserpine, which is thought to prevent the intraneuronal storage of dopamine, there was an increase in the striatal concentration of DOPAC which preceded an increase in the concentration of HVA. Since non-cholinergic nerve endings of rat brain contain mitochondria and show monoamine oxidase activity, this result suggests that DOPAC is formed intraneuronally.

5. It is concluded that the DOPAC in the striatum represents intraneuronal metabolism of dopamine and that only the HVA which is sensitive to the action of probenecid represents entirely extraneuronal metabolism of this amine. Some of the HVA is not sensitive to the action of probenecid. This suggests that part of the metabolism of dopamine involves both locations.

6. A group of drugs which are chemically related to amphetamine were tested for their effects on the concentrations of DOPAC and HVA in the striatum. It is suggested that D-amphetamine, 2-aminotetralin and 1,2,3,4-tetrahydroisoquinoline reduced the intraneuronal metabolism of dopamine whereas adamantanamine did not.

Introduction

There are thought to be two locations in the vicinity of the peripheral sympathetic adrenergic nerve ending where noradrenaline can be metabolized. One of these is intraneuronal where oxidative deamination by monoamine oxidase (MAO) takes

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place, and the other is extraneuronal where, in addition to oxidative deamination, 3-*O*-methylation by catechol-*O*-methyl transferase (COMT) is involved (Kopin & Gordon, 1962; Kopin & Axelrod, 1963; Jonason, 1969). If dopamine is a transmitter substance in the central nervous system a similar situation might hold for the metabolism of this amine since both *O*-methylated and deaminated products of dopamine metabolism can be found in brain tissue. The contributions of the two locations to the metabolism of endogenously formed dopamine in the central nervous system are difficult to assess, but the observation by Murphy, Robinson & Sharman (1969) that in the mouse brain in which COMT activity had been inhibited, the formation of 3,4-dihydroxyphenylacetic acid (DOPAC) was not a simple alternative to the formation of 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA), suggests that metabolism at the two locations need not be sequential. This report examines the hypothesis that the concentration of DOPAC is an index of intraneuronal metabolism, and the concentration of HVA is an index of extraneuronal metabolism of dopamine in the striatum of the mouse.

Methods

Albino mice weighing 20–40 g were killed by cervical dislocation, decapitated and the brains were rapidly removed and cooled on a glass plate kept at 0° C. The striatal tissue was dissected as described by Sharman (1966). DOPAC and HVA were estimated fluorimetrically in the same sample of tissue, pooled from at least four animals, as described by Murphy, Robinson & Sharman (1969). Recovery of authentic HVA and DOPAC was, respectively, $62{\cdot}7 \pm 1{\cdot}4\%$ (S.E.M.) and $66{\cdot}4 \pm 1{\cdot}7\%$ (S.E.M.); $n=87$. Dopamine was estimated fluorimetrically (Laverty & Sharman, 1965) after adsorption from tissue extracts on to a column of Dowex 50 X-8 cation exchange resin and elution with 2 M hydrochloric acid. Recovery of dopamine added to tissue homogenates was $82 \pm 4\%$ (S.E.M.); $n=29$. The results are given uncorrected for recoveries. All drugs were injected intraperitoneally. Reserpine was dissolved in 10% w/v ascorbic acid. Probenecid was dissolved in the minimum volume of 1 M NaOH, diluted with water and if necessary, the pH of the solution adjusted to 7·0 with 0·1 M HCl. Spiperone and haloperidol were dissolved in the minimum volume of glacial acetic acid, the solution was diluted with water and final dilutions were made in 0·9% w/v NaCl solution. The pH of the solution was adjusted to 7·0, if necessary, with sodium hydrogen carbonate. Drug doses are given in terms of the free base.

Results

Effect of probenecid on the concentration of DOPAC and HVA in the striatum of the mouse

The concentrations of DOPAC and HVA in the striatum of the mouse after a dose of probenecid (200 mg/kg i.p.), which produces a maximal change in the concentration of HVA, are illustrated in Fig. 1. This shows that there is a linear increase in the concentration of HVA for at least 2 h after the injection of probenecid whereas there is no significant increase in the concentration of DOPAC.

The administration of major tranquillizing drugs such as chlorpromazine or haloperidol causes an increase in the concentration of both DOPAC and HVA in

the brain (Andén, Roos & Werdinius, 1964). In the mouse, treatment with haloperidol (0·1 mg/kg i.p.) or spiperone (0·1 mg/kg i.p.) increased the concentration of HVA in the striatum from $0·15 \pm 0·01$ μg/g (mean $\pm$ S.E.M.) to $0·61 \pm 0·05$ μg/g and $0·50 \pm 0·03$ μg/g, respectively, after 2 hours. When probenecid (100 mg/kg i.p.) was given to mice in combination with these major tranquillizing drugs there were increases in the concentration of HVA after 2 h to $1·46 \pm 0·08$ μg/g with haloperidol and to $0·95 \pm 0·04$ μg/g with spiperone. The treatments with haloperidol or spiperone alone resulted in increases in the striatal concentration of DOPAC from $0·18 \pm 0·01$ μg/g to $0·37 \pm 0·05$ μg/g and $0·49 \pm 0·03$ μg/g, respectively. However, when probenecid was given in addition to the tranquillizing drugs there was no further increase in the concentration of DOPAC in the striatum.

The effects of inhibition of COMT by tropolone on the concentrations of HVA and DOPAC are illustrated by Table 1, showing that tropolone only causes a small fall in the concentration of HVA in normal animals. However, in animals treated with probenecid, where an increase in HVA reflects the rate at which this acid is being formed, it can be seen that tropolone (25 mg/kg) appears to prevent formation of HVA completely. Table 1 also shows that tropolone reduces or prevents the increase in the concentration of HVA caused by reserpine alone or combined with probenecid or by spiperone combined with probenecid, whereas it has little effect on the concentration of DOPAC.

Andén, Roos & Werdinius (1964) reported that when rabbits were treated with reserpine, the concentrations of DOPAC and HVA in the corpus striatum were increased and the concentration of DOPAC appeared to rise somewhat earlier than

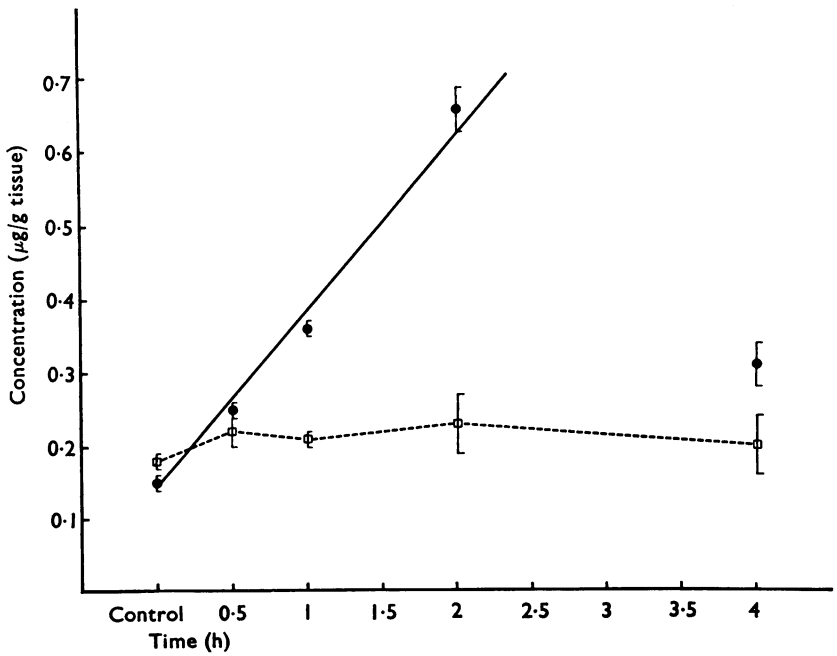

FIG. 1. Time course of the changes in the concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylacetic acid (HVA) in the striatum of the mouse in response to a maximally effective dose of probenecid (200 mg/kg, i.p.). Vertical bars indicate the S.E.M. No. of observations: for controls $n=55$ and for treated mice $n=5$. (●——●), HVA; (□- - -□), DOPAC.

TABLE 1. *Effect of inhibition of catechol-O-methyltransferase on the concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylacetic acid (HVA) in the striatum of the mouse*

Drug dose and duration of treatment	No. of observations	Concentration of phenolic acid in the striatum (μg/g±S.E.M.) HVA	Change in concentration	DOPAC	Change in concentration
Control	6	0·19±0·01		0·19±0·01	
Tropolone 50 mg/kg; 2 h	6	0·14±0·01	−0·05	0·36±0·01	+0·17
Spiperone 2 mg/kg +Probenecid 200 mg/kg } 1·5 h	3	1·55±0·04		0·68±0·02	
Spiperone 2 mg/kg +Probenecid 200 mg/kg +Tropolone 25 mg/kg } 1·5 h	3	0·30±0·04	--1·25	0·86±0·02	+0·18
Spiperone 2 mg/kg; 2 h +Probenecid 1·5 h	2	2·67 ; 2·87		1·36 ; 1·16	
Spiperone 2 mg/kg; 2 h +Probenecid 200 mg/kg +Tropolone 100 mg/kg } 1·5 h	2	0·09 ; 0·07	−2·69	1·34 ; 1·50	+0·18
Probenecid 200 mg/kg; 1·5 h	2	0·80 ; 0·99		0·33 ; 0·28	
Probenecid 200 mg/kg +Tropolone 100 mg/kg } 1·5 h	2	0·09 ; 0·07	−0·81	0·54 ; 0·55	+0·24
Reserpine 5 mg/kg; 2 h	6	0·60±0·03		0·50±0·03	
Reserpine 5 mg/kg+tropolone 25 mg/kg; 2 h	3	0·34±0·01	−0·26	0·76±0·01	+0·26
Reserpine 5 mg/kg; 2 h+probenecid 200 mg/kg; 0·5 h	3	1·09±0·02		0·50±0·01	
Reserpine 5 mg/kg+tropolone 25 mg/kg; 2 h+probenecid 200 mg/kg; 0·5 h	3	0·60±0·02	−0·49	0·81±0·01	+0·31

All drugs were given intraperitoneally. Tropolone, 50 mg/kg, will almost prevent the formation of HVA from administered 3,4-dihydroxyphenylalanine (DOPA) in the brain of the mouse (Murphy, Robinson & Sharman, 1969).

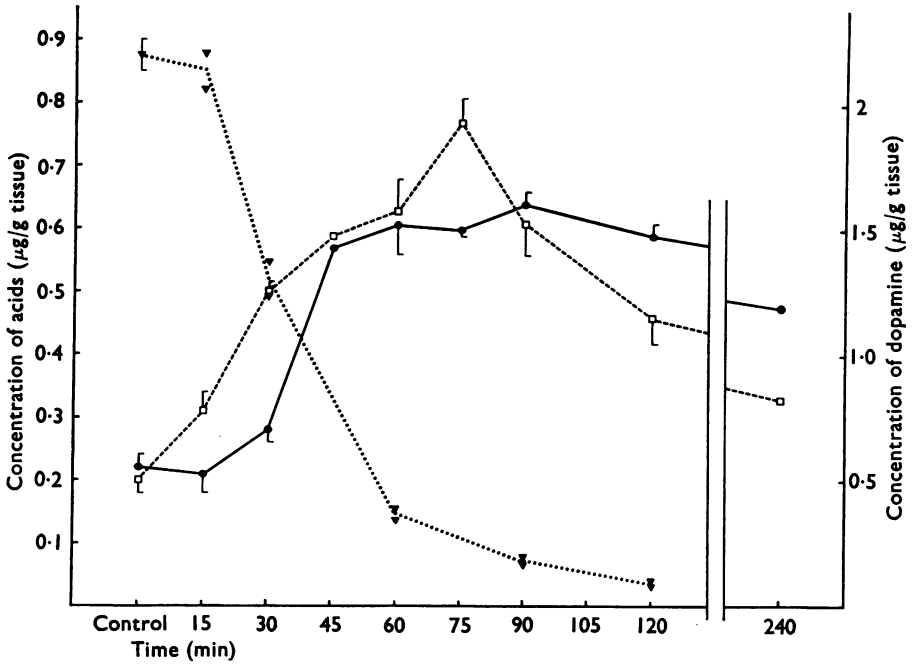

FIG. 2. Effect of reserpine (5 mg/kg i.p.) on the concentrations of dopamine and its acid metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylacetic acid (HVA) in the striatum of the mouse. (▼ · · · · ▼), Dopamine; (●——●), HVA; (□– – –□), DOPAC. Vertical bars are S.E.M. obtained from at least four observations. Values without S.E.M. are single observations or means from two observations.

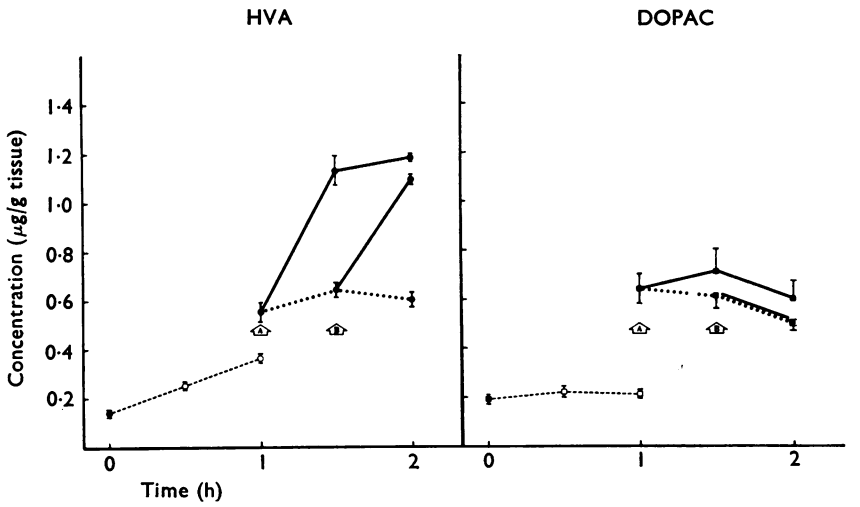

FIG. 3. Effect of probenecid on the concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) [right] and 4-hydroxy-3-methoxyphenylacetic acid (HVA) [left] in the striatal tissue of mice treated with reserpine. (○– –○; □– –□), probenecid (200 mg/kg, i.p.); (●· · ·●; ■· · ·■), reserpine (5 mg/kg, i.p.), injected at time 0; (●——●; ■——■), reserpine (5 mg/kg, i.p.) injected at time 0 then probenecid (200 mg/kg, i.p.) injected at time A or B. Vertical bars are S.E.M. obtained from at least three observations.

that of HVA. Figure 2 shows that a similar result is obtained with the mouse. A clear increase in the concentration of DOPAC in the striatum was seen 15 min after the administration of reserpine (5 mg/kg i.p.) at which time the concentration of dopamine was still close to its normal value. A significant increase in the concentration of HVA did not occur until 45 min after the reserpine treatment. The concentrations of HVA and DOPAC remained high for at least 4 h after reserpine but the concentration of DOPAC appeared to be changing towards its normal value faster than that of HVA. In Fig. 3, the effect of probenecid, in a maximally effective dose of 200 mg/kg i.p., on the concentration of HVA and DOPAC in the striatal tissue of mice treated with reserpine (5 mg/kg i.p.) is illustrated. Again, the concentration of HVA is increased by probenecid whereas that of DOPAC is not. Furthermore, the increase in HVA is not linear with respect to time as in normal animals. The concentration of HVA in the striatal tissues of reserpine treated mice was greatly increased 30 min after the administration of probenecid but there was no further increase after 1 hour. However, if probenecid was injected at a point on the time course of the reserpine treatment 30 min later than in the above experiment, then there was as large an increase in the concentration of striatal HVA after 30 min as that seen in the first experiment. These results indicate that after reserpine the rate of formation of HVA in the striatum of the mouse is at least 5 times the normal rate.

The effects of some drugs with chemical structures related to amphetamine are given in Table 2. Of these, 2-aminotetralin, 1,2,3,4-tetrahydroisoquinoline and D-amphetamine reduced the concentration of DOPAC and increased the concentration of HVA. Adamantanamine (Amantadine) was devoid of any effect on the concentration of the two acids.

Discussion

In the mouse brain two acidic metabolites of dopamine are 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylacetic acid (HVA). Our results show that normally and when the concentrations of these acids are changed by drug treatment only the concentration of HVA and not that of DOPAC is increased by the administration of probenecid, a drug which inhibits acid transport

TABLE 2. *Effects of drugs which are structurally related to amphetamine on the concentration of* 3,4-*dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylacetic acid (HVA) in the striatum of the mouse*

Drug dose and duration of treatment	No. of observations	Concentration of phenolic acid in the striatum (μg/g±S.E.M.) HVA	DOPAC
D-Amphetamine 20 mg/kg; 1 h	8	0·21±0·04	0·08±0·01†
Control	8	0·16±0·03	0·18±0·01
D-Amphetamine 20 mg/kg; 2 h	8	0·31±0·02†	0·09±0·01†
Control	7	0·14±0·02	0·20±0·03
Tetrahydroisoquinoline 100 mg/kg; 1·5 h	8	0·21±0·01*	0·11±0·01*
Control	4	0·15±0·01	0·15±0·01
2-Amino-tetralin 30 mg/kg; 1·5 h	10	0·28±0·01†	0·10±0·01†
Control	8	0·20±0·01	0·17±0·01
Adamantanamine 100 mg/kg; 1·5 h	12	0·26±0·03	0·20±0·01
Control	6	0·20±0·01	0·19±0·01

Student's *t* test probability when compared with own controls. *$P<0.05$, †$P<0.01$; all drugs were given intraperitoneally.

in the renal tubules and the active transport of acid substances out of the brains of rodents (Neff, Tozer & Brodie, 1964 ; Sharman, 1966, 1967) and birds (Ahtee, Sharman & Vogt, 1970). This indicates that the two acids do not occur at the same site in the striatum of the mouse. Since the cerebral, probenecid sensitive, active transport process is also concerned with the removal of 5-hydroxyindolylacetic acid from the rodent brain (Neff *et al.*, 1964 ; Sharman, 1966) it is unlikely that the observed effect is due to a specific action of probenecid on the transport system for HVA.

The conclusion of Murphy *et al.* (1969) that the formation of DOPAC was not a simple alternative to the formation of HVA has been confirmed for even after drug treatments which accelerate the synthesis of HVA from endogenous dopamine, inhibition of COMT by tropolone only caused a small increase in the concentration of DOPAC. This result suggests that most, if not all, of the HVA is formed, not from DOPAC, but from the amine intermediate, 4-hydroxy-3-methoxyphenylethylamine (methoxytyramine).

The effect of reserpine on the concentrations of HVA and DOPAC in the mouse striatum confirms the results of Andén *et al.* (1964) on the rabbit. Reserpine is thought to reduce the concentration of dopamine in the brain by preventing the storage of the amine in intraneuronal granules. The non-cholinergic nerve endings of rat brain (de Robertis, Pellegrino de Iraldi, Rodriguez de Lores Arnaiz & Salganicoff, 1962) contain mitochondria and show MAO activity (Rodriguez de Lores Arnaiz & de Robertis, 1962). After reserpine the dopamine inside the neurone would no longer be protected from metabolizing enzymes and the first deaminated metabolite to show an increase in concentration is most likely to be formed inside the neurone. In the mouse striatum after reserpine treatment the concentration of DOPAC increased before that of HVA and thus DOPAC is probably formed intraneuronally. A delayed increase in the concentration of HVA in the striatum of rats after treatment with reserpine was observed by Juorio, Sharman & Trajkov (1966). Guldberg & Broch (1971) have shown that in the rat the increase in the concentration of HVA coincides with the onset of stereotypy and is preceded by an increase in the concentration of striatal DOPAC. These observations allow a simple explanation for the difference in the effect of probenecid on the concentrations of DOPAC and HVA ; the former acid is formed and located inside the neurone and the latter acid is formed and located outside the neurone. However, it appears that not all of the HVA is located extraneuronally. Sharman (1967) observed in mice that after a low dose (0·1 mg/kg) of spiperone, the increase in HVA was not sensitive to the action of probenecid. In our experiments it was observed that the relative increase in the concentration of HVA caused by a dose of probenecid was less in spiperone treated mice than in haloperidol treated mice, although the effects of the two tranquillizing drugs, given alone, on the concentration of HVA were not significantly different from each other. In addition, tropolone, given to normal mice in a dose known to inhibit strongly the formation of HVA from administered 3,4-dihydroxyphenylalanine (Murphy *et al.*, 1969) only slightly reduced the concentration of HVA in the striatum.

These observations might be explained if the HVA is present in the tissue at two sites. At one of these sites the concentration of HVA does not immediately reflect changes in the total rate of formation of this acid and also at this site the HVA does not have access to the probenecid sensitive active transport system. At the other site the concentration of HVA rapidly reflects the rate of formation of the

acid which is then removed by the active transport system. If there is an active uptake mechanism for dopamine at the neuronal membrane of the dopamine containing neurones it is possible that the methylated metabolite of dopamine, 4-hydroxy-3-methoxyphenylethylamine (methoxytyramine) also enters by this mechanism into the neurone where it could be deaminated to HVA. This HVA, like DOPAC, would not be sensitive to the action of probenecid. Such a mechanism would also explain the small constant increase in DOPAC seen after inhibition of COMT when the extraneuronally formed, methoxytyramine would be replaced by dopamine.

It is thus necessary to modify the original hypothesis proposed in this paper. It seems that the concentration of DOPAC represents its intraneuronal formation from dopamine. On the other hand, only that HVA, the cerebral efflux of which is blocked by probenecid, could be taken as an index of completely extraneuronal metabolism. The contribution of the two acid metabolites to the total metabolism of dopamine in the mouse striatum has not been assessed and it remains to be examined whether changes in the concentration of DOPAC can be directly related to changes in the rate of its formation.

An effect of amphetamine on the 'membrane pump' of dopamine and noradrenaline containing neurones in the rat brain was demonstrated by Carlsson, Lindqvist, Dahlström, Fuxe & Masuoka (1965). Glowinski, Axelrod & Iversen (1966) showed that in the rat brain the formation of deaminated catechol metabolites from radioactive noradrenaline injected into the cerebral ventricles was reduced by treatment with amphetamine. The uptake of radioactive dopamine by homogenates of the rat striatum can be inhibited by both D- and L-amphetamine (Coyle & Snyder, 1969) and Rutledge (1970) has concluded that amphetamine inhibits the oxidative deamination of noradrenaline in the brain primarily by inhibiting the uptake of this amine into cerebral neurones.

Our observations suggest that D-amphetamine, 1,2,3,4-tetrahydroisoquinoline and 2-aminotetralin but not adamantanamine, a structurally related compound used in the treatment of Parkinson's disease, share the property of reducing intraneuronal oxidative deamination of dopamine in the striatum of the mouse probably by reducing the uptake of dopamine into neurones.

Our value for the concentration of HVA in the striatum of the normal mouse ($0{\cdot}15 \pm 0{\cdot}01$ μg/g) is lower than the values of $0{\cdot}31 \pm 0{\cdot}01$ μg/g; $0{\cdot}28 \pm 0{\cdot}01$ μg/g reported by Sharman (1966, 1967). This may be due to a strain difference. The different methods used to extract HVA in these reports were compared and found to give the same result when applied to the same tissue sample.

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REFERENCES

AHTEE, L., SHARMAN, D. F. & VOGT, M. (1970). Acid metabolites of monoamines in avian brain; effects of probenecid and reserpine. *Br. J. Pharmac.*, **38**, 72–85.

ANDÉN, N.-E., ROOS, B.-E. & WERDINIUS, B. (1964). Effects of chlorpromazine, haloperidol and reserpine on the levels of phenolic acids in rabbit corpus striatum. *Life Sci.*, **3**, 149–158.

CARLSSON, A., LINDQVIST, M., DAHLSTRÖM, A., FUXE, K. & MASUOKA, D. (1965). Effects of the amphetamine group on intraneuronal brain amines *in vivo* and *in vitro*. *J. Pharm. Pharmac.*, **17**, 521–524.

COYLE, J. T. & SNYDER, S. H. (1969). Catecholamine uptake by synaptosomes in homogenates of rat brain; stereo-specificity in different areas. *J. Pharmac. exp. Ther.*, **170**, 221–231.

DE ROBERTIS, E., PELLEGRINO DE IRALDI, A., RODRIGUEZ DE LORES ARNAIZ, G. & SALGANICOFF, L. (1962). Cholinergic and non-cholinergic nerve endings in rat brain—I. Isolation and subcellular distribution of acetylcholine and acetylcholinesterase. *J. Neurochem.*, **9**, 23–55.

GLOWINSKI, J., AXELROD, J. & IVERSEN, L. L. (1966). Regional studies of catecholamines in the rat brain. IV. Effects of drugs on the disposition and metabolism of H^3-norepinephrine and H^3-dopamine. *J. Pharmac. exp. Ther.*, **153**, 30–41.

GULDBERG, H. C. & BROCH, O. J. Jr. (1971). On the mode of action of reserpine on dopamine metabolism in the rat striatum. *Eur. J. Pharmac.*, **13**, 155–167.

JONASON, J. (1969). Metabolism of catecholamines in the central and peripheral nervous system. A study with special reference to the enzymes involved. *Acta physiol. scand.*, suppl. 320.

JUORIO, A. V., SHARMAN, D. F. & TRAJKOV, T. (1966). The effect of drugs on the homovanillic acid content of the corpus striatum of some rodents. *Br. J. Pharmac. Chemother.*, **26**, 385–392.

KOPIN, I. J. & AXELROD, J. (1963). The role of monoamine oxidase in the release and metabolism of norepinephrine. *Ann. N.Y. Acad. Sci.*, **107**, 848–855.

KOPIN, I. J. & GORDON, E. K. (1962). Metabolism of norepinephrine-H^3 released by tyramine and reserpine. *J. Pharmac. exp. Ther.*, **138**, 351–359.

LAVERTY, R. & SHARMAN, D. F. (1965). The estimation of small quantities of 3,4-dihydroxyphenylethylamine in tissues. *Br. J. Pharmac. Chemother.*, **24**, 538–548.

MURPHY, G. F., ROBINSON, D. & SHARMAN, D. F. (1969). The effect of tropolone on the formation of 3,4-dihydroxyphenylacetic acid and 4-hydroxy-3-methoxyphenylacetic acid in the brain of the mouse. *Br. J. Pharmac.*, **36**, 107–115.

NEFF, N. H., TOZER, T. N. & BRODIE, B. B. (1964). Indole metabolism Part II. A specialised transport system to transfer 5-HIAA directly from brain to blood. *Pharmacologist*, **6**, 194.

RODRIGUEZ DE LORES ARNAIZ, G. & DE ROBERTIS, E. D. P. (1962). Cholinergic and non-cholinergic nerve endings in the rat brain—II. Subcellular localisation of monoamine oxidase and succinate dehydrogenase. *J. Neurochem.*, **9**, 503–508.

RUTLEDGE, C. O. (1970). The mechanisms by which amphetamine inhibits oxidative deamination of norepinephrine in brain. *J. Pharmac. exp. Ther.*, **171**, 188–195.

SHARMAN, D. F. (1966). Changes in the metabolism of 3,4-dihydroxyphenylethylamine (dopamine) in the striatum of the mouse induced by drugs. *Br. J. Pharmac. Chemother.*, **28**, 153–163.

SHARMAN, D. F. (1967). A discussion of the modes of action of drugs which increase the concentration of 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid) in the striatum of the mouse. *Br. J. Pharmac. Chemother.*, **30**, 620–626.

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