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Effect of drugs used in psychoses on cerebral dopamine metabolism

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Summary

1. Chlorpromazine 15 mg/kg, given daily to cats for 2 weeks, produced a rise in homovanillic acid (HVA) content of the caudate nucleus, whereas the same dose of thioridazine lacked this effect. Of these two drugs, only chlorpromazine causes a high incidence of drug-induced Parkinsonism in man.

2. In the mouse, chlorpromazine, thioridazine and haloperidol increased striatal concentrations of HVA and accelerated the disappearance of dopamine (DA) after inhibition of catecholamine synthesis with α-methyltyrosine. Low doses of the three compounds increased, whereas high doses reduced, the concentration of DA in the striatum. In their effects on the DA metabolism of the mouse, chlorpromazine and thioridazine had the same potency, but haloperidol was between 10 and 100 times more active than the other two drugs. In producing hypothermia and sedation, the three compounds were equiactive.

3. Oxypertine, another drug apt to produce Parkinsonism in man, caused a severe reduction in striatal DA and hypothalamic noradrenaline (NA). Though the clinical signs produced in the mouse were indistinguishable from those seen after the same dose of chlorpromazine, the biochemical changes in the brain were thus quite different.

4. Though all the drugs used caused temporary motor disabilities in animals, these bore no resemblance to human Parkinsonism, even when treatment was continued for 7 weeks or more as it was in cats and monkeys. The latter were treated with chlorpromazine 7·5 mg/kg daily, a dose chosen to avoid loss of weight and which may have been too small to produce toxic side-effects. It caused no changes in striatal DA turnover.

5. Even at the high dose of 50 mg/kg, phenoxybenzamine did not increase DA turnover in mouse brain, but it sedated the mice as did the tranquillizers.

6. Atropine sulphate, 25 mg/kg, reduced the HVA content of mouse striatum and partially antagonized the rise in HVA produced by phenothiazines. The effect was surmountable. Possible modes of action of atropine are discussed.

7. At present we know of two types of biochemical changes which may occur in the brain of animals after treatment with drugs apt to cause Parkinsonism in man: a loss of cerebral catecholamines, as seen after

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reserpine or oxypertine, or an increase in turnover of DA as after phenothiazines and butyrophenones.

Introduction

Andén, Roos & Werdinius (1964) have shown that chlorpromazine and haloperidol increase the concentration of the acid metabolites of dopamine (DA) in the rabbit striatum, particularly that of homovanillic acid (HVA). The same phenomenon was seen in the cat and the mouse (Laverty & Sharman, 1965b; Sharman, 1966). The effect is interpreted as an increase in turnover of dopamine. These drugs do not appreciably increase the turnover of cerebral noradenaline and 5-hydroxytryptamine, so they offer an opportunity to study correlations between abnormal DA metabolism and any changes in motor function which they may cause. In man, the chief side-effect of these two drugs is the production of Parkinsonism in a proportion of patients. They share this property with most phenothiazines and all butyrophenones which have proved successful in the treatment of schizophrenia. Among the phenothiazines used in schizophrenia, however, one, thioridazine, stands out clinically because of the low incidence of Parkinsonian side-effects when doses which are effective against psychoses are used (Cole & Clyde, 1961). Its action was therefore compared (Laverty & Sharman, 1965b) with that of three other related compounds, for which the incidence of Parkinsonism was high. After prolonged treatment of cats with the four drugs, only thioridazine did not produce a rise in striatal HVA. The present paper is a study of the quantitative aspects of changes in DA metabolism following the use of drugs liable to produce Parkinsonism, and of the relation these changes may bear to motor abnormalities produced either in man or animals.

Methods

The effect of drugs was investigated on a few adult female monkeys (*Macaca mulatta*), adult cats and rabbits of both sexes, litters of dogs aged a few months, some adult dogs and female albino mice weighing 15–30 g.

Drugs were injected subcutaneously into monkeys and some dogs, and intraperitoneally into mice and rabbits; they were given in gelatin capsules (size No. 3) to cats and most dogs. Except for trifluoperazine-2HCl (Stelazine, Smith, Kline and French), which was only administered in capsules, solutions or suspensions of the following drugs were prepared for injection: chlorpromazine HCl (Largactil, May and Baker), thioridazine HCl (Melleril, Sandoz), methixene hydrochloride (Wander) and atropine sulphate were dissolved in 0·9% sodium chloride solution; haloperidol (R1625, Janssen Pharmaceutica) and oxypertine (5,6-dimethoxy-2-methyl-3-[2-(4-phenyl-1-piperazinyl)ethyl] indole, Win 18501-2, Winthrop Products Co.) were made up in 0·1% ascorbic acid, except when stated otherwise. Spiperone (R5147, Janssen Pharmaceutica) was dissolved in a minimum of glacial acetic acid, diluted with water and then diluted further with 0·9% NaCl solution. L-α-methyl-*p*-tyrosine (Merck Sharp and Dohme, Inc.) was dissolved in a minimum of concentrated HCl, diluted with water and the pH adjusted to 4 with NaOH, which caused the formation of a fine suspension; phenoxybenzamine HCl (Dibenyline, Smith, Kline and French) was dissolved in a small volume of

acidified ethanol and diluted with water; L-3,4-dihydroxyphenylalanine (Koch-Light) was suspended in 0·9% sodium chloride solution. Control animals were injected with the vehicles.

Dissections

Monkeys, cats and dogs were killed by bleeding from the neck under chloroform anaesthesia. Rabbits were bled after the neck had been broken. Mice were stunned and then decapitated. The brain was removed from the skull and dissected on ice.

The " substantia nigra " was obtained by cutting a slice of tissue dorsal to the peduncles thick enough to include this structure. The hypothalamus of the rabbit included the median eminence, but the optic chiasma and the mammillary bodies were discarded. The striatum of the mouse was dissected by opening the lateral ventricles to expose the caudate nuclei, removing the cortex dorsally and anteriorly to this nucleus and making a vertical cut posterior to it to cut away midbrain and hindbrain. The remaining piece of brain included with the striatum some orbital cortex and diencephalon. It weighed approximately 0·1 g. Estimations of dopamine were made on the two striata of one mouse; the striata of two mice were pooled for measuring homovanillic acid.

The tissues were weighed immediately after dissection. Monkey, cat, dog and rabbit tissue was frozen and kept at $-18°$ C for not more than an hour before being homogenized. Mouse tissue was not frozen but was kept on ice for not more than 10 min until homogenization.

Chemical estimations

Dopamine in the striatum was estimated according to Laverty & Sharman (1965a) by homogenizing the tissue in 0·1 N HCl, deproteinizing, and adsorbing the amine on to the cation exchange resin Dowex 50WX – 8. The dopamine in the eluate was acetylated, condensed with ethylene diamine, the fluorophor extracted into isobutanol and the fluorescence read in a filter fluorimeter. Readings were carried out at two wavelengths so as to be able to correct for fluorescence due to the blank or to any noradrenaline present. Recoveries were (mean $\pm$ S.E.M.) $76·0 \pm 1·8\%$ (fifty-three experiments).

For the estimation of dopamine and noradrenaline in the hypothalamus, the compounds were acetylated and the acetates isolated by paper chromatography (Laverty & Sharman, 1965a). Recoveries from brain tissue (twelve estimations) were dopamine $68·1 \pm 3·5\%$ and noradrenaline $51·7 \pm 8·4\%$. Estimations of HVA were first carried out following the specification of Juorio, Sharman & Trajkov (1966), and later by the simpler and more sensitive procedure suggested by Portig, Sharman & Vogt (1968). Recoveries were $63·1 \pm 2·0\%$ (forty experiments) with the first, and $67·4 \pm 1·3\%$ (thirty-six experiments) with the second method. Corrections for losses have not been made in the tables.

Dopamine and homovanillic acid were obtained as A grade from Calbiochem. The Dowex resins were 50W hydrogen form, 8% cross-linked, and -1 chloride form, 2% cross-linked, both 200–400 mesh. Several reagents were purified before use: acetic anhydride, ethylene diamine, isobutanol and dichloromethane by redistilling 3 times, methanol by distilling first over sodium hydroxide and re-

distilling, *n*-butylacetate, ethylacetate and toluene by redistilling once, and l-cysteine hydrochloride by recrystallization from ethanol.

Results

Cats

In the experiments of Laverty & Sharman (1965b), cats treated for 2 weeks with thioridazine by mouth had a normal HVA content of the caudate nucleus, whereas the concentration was raised after 2 weeks on chlorpromazine. The doses had been chosen to cause about the same degree of sedation, and were somewhat higher for chlorpromazine than for thioridazine. To ascertain that the difference in the effects obtained had not been related to dosage, the experiments were now repeated with equal doses of the two compounds (15 mg/kg daily orally for 2 weeks), with the last dose, as before, 24 hr before death. The same result was obtained (Fig. 1): the HVA content of the caudate nucleus was significantly elevated after chlorpromazine, but not after thioridazine. It was, however, not obvious from these results whether the action of thioridazine was of a different nature, or only more short-lived than that of chlorpromazine. Whereas it had been shown (Laverty & Sharman, 1965b) that 4 hr after a single subcutaneous injection of either drug the HVA content of the brain was raised, there was no information as to whether this rise also followed oral administration and persisted for 24 hr, the time which had been allowed to elapse after the last oral dose of the chronic treatment. In the present experiments (Fig. 1) no significant rise in HVA content was found after thioridazine at any time after a single or after a series of oral doses, whereas, with chlorpromazine, there was always a rise,

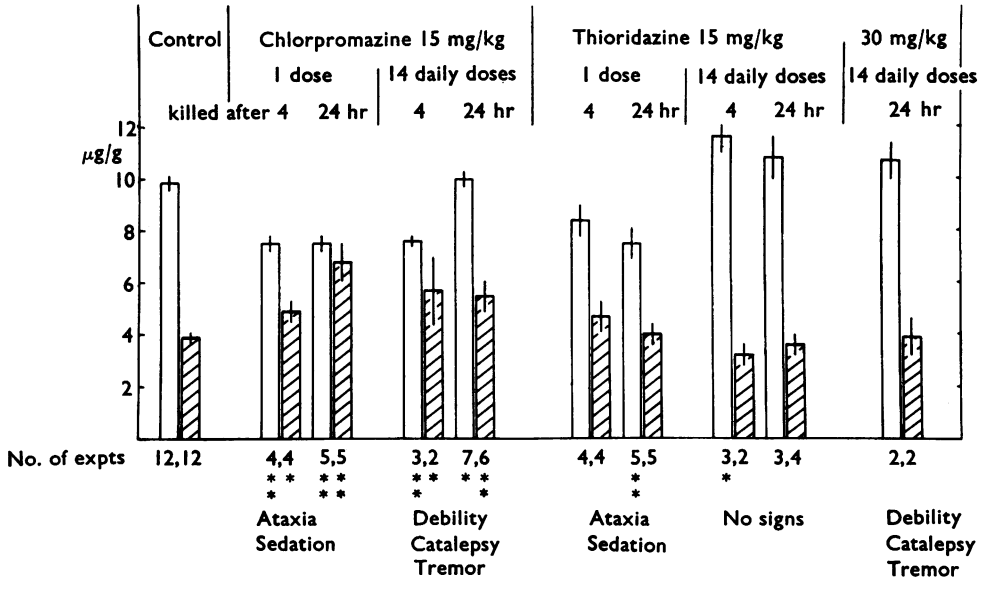

FIG. 1. Mean concentration of dopamine (clear columns) and homovanillic acid (shaded columns) in the caudate nucleus of the cat after single and repeated oral doses of chlorpromazine and thioridazine. Vertical bars indicate S.E. of the mean. Asterisks denote significant differences from controls (*$P<0.05$, **$P<0.01$). Values in µg/g fresh tissue, uncorrected for losses.

and it was highest 24 hr after a single dose. On the other hand, a difference between the effect of the two drugs on dopamine concentration was evident in the chronic experiments 4 hr, but not 24 hr, after the last dose, and consisted of a fall occurring only after chlorpromazine. Since single doses of thioridazine lowered dopamine concentrations, the disappearance of this effect after 14 days shows that some form of adaptation had developed to the action of thioridazine.

Clinical signs

A search was made for correlations between clinical signs and biochemical findings. Both drugs (at 15 mg/kg) caused ataxia and sedation for about 9 hr during the first 1 or 2 days of treatment. During the second week, six out of ten cats given chlorpromazine (and none given thioridazine) lost weight, stopped cleaning themselves, moved little, and showed loss of muscular tone. When walking, they showed a fine tremor of the whole body, and some of them showed catalepsy. These six cats had the highest concentrations of HVA in the caudate nucleus of the eight animals whose brains were analysed. Yet there seems to be no causal relation between these toxic signs and the change in DA metabolism, for two cats given thioridazine 30 mg/kg orally for 2 weeks developed the same type of debility, tremor and catalepsy and yet had normal concentrations of DA and HVA in the caudate nucleus.

A few experiments were carried out in which, in order to simulate more closely human therapeutic procedure, chlorpromazine was administered for up to 50 days and the animals were carefully watched for signs which might resemble human Parkinsonism. Table 1 summarizes the results. Three cats tolerated 15 mg/kg of the drug for 4 weeks and showed no motor disturbances of any kind, whereas a fourth cat died after 32 days with signs of debility. In the surviving cats, the concentration of HVA in the caudate nucleus 24 hr after the last dose was normal, so that in that respect tolerance had obviously been established. Three further cats were given smaller doses, two of them 8 mg/kg for 50 days; except for ataxia on the first day, they showed no ill effects and no abnormality in striatal dopamine metabolism (Table 1). The same was true of a single cat given trifluoperazine 8 mg/kg for 50 days.

Monkeys

A small number of monkeys were used for the next experiment, because it has been shown (Poirier, Sourkes, Bouvier, Boucher & Carabini, 1966) that midbrain

TABLE 1. *Effect of prolonged oral administration of chlorpromazine on concentration (μg/g) of DA and HVA in the caudate nucleus of the cat*

Dose (mg/kg per day)	Duration of treatment (days)	Dopamine (μg/g)	Homovanillic acid (μg/g)
15	27	9·4	4·3
15	28	9·7	2·9
15	28	10·0	2·6
5	28	13·1	3·8
8	50	10·2	3·7
8	50	10·7	3·0
0	—	9·6±0·3	3·9±0·2

The brain was examined 24 hr after the last dose. There were no neurological signs. Control figures obtained on twelve cats.

lesions which resembled those occurring in patients suffering from Parkinsonism, produced at least some signs analogous to those of the human disease. The dose chosen was the highest amount tolerated without serious loss of weight; this required that the aminals were not sedated throughout the whole day and thus took their food. A dose of 7·5 mg/kg injected subcutaneously once a day proved suitable, except for a period of 9 days in one monkey, when this dose had to be reduced to 5 mg/kg; treatment lasted between 51 and 58 days. Sedation was more pronounced than in cats, and, though it diminished in length and intensity, recurred daily after the injection throughout the experiment. There was neither tremor nor other motor disability. The analysis of the striatum (Table 2) showed no difference between controls and treated monkeys in mean values for DA and HVA, but the standard errors indicate a fair amount of individual variation. The dopamine content of the substantia nigra was significantly higher in the treated monkeys; this region is not visible to the naked eye and the dissection is difficult to reproduce, so the finding requires confirmation on a larger number of animals. The suspicion that a dissection artefact might be involved is supported by the finding that the absolute amounts of DA in the pieces of tissue containing the substantia nigra did not differ significantly in controls and treated monkeys.

Mice

The preceding experiments have shown great individual variability in cerebral concentrations of DA and HVA; in order to compare the quantitative aspects of the effect of different doses and drugs, work was carried out on mice which could be used in greater numbers.

Chlorpromazine and thioridazine

A first experiment compared the effects of single injections of two phenothiazines, chlorpromazine and thioridazine, using 10 mg/kg. In mice, both drugs increased the cerebral content of HVA to the same extent and with an identical time course, which had a maximum of 3 times normal 2–4 hr after the intraperitoneal injection. Also indistinguishable were the time course of sedation, of hypothermia, and of loss of muscle tone. With this dose, neither of the two drugs modified the concentration of cerebral dopamine. Groups 3 and 5 (Table 3) show the value for DA and HVA 2 hr after 10 mg/kg of the phenothiazines, and groups 7 and 9 after 100 mg/kg. The higher dose of both drugs caused a significant fall in DA concentration.

TABLE 2. *Effect of repeated subcutaneous injections of chlorpromazine on concentration (μg/g fresh tissue, mean ±S.E.M.) of DA and HVA in different regions of monkey brain, analysed 24 hr after the last injection*

	Dopamine (μg/g)			Homovanillic acid (μg/g)		
Treatment	Caudate nucleus	Putamen	Substantia nigra	Caudate nucleus	Putamen	Substantia nigra
Chlorpromazine	11·8±1·0	14·0±3·3	2·0*±0·2	5·7±0·6	7·2±0·4	2·4±0·3
Saline	10·3±0·9	12·4±2·2	0·8 ±0·1	5·7±0·9	7·9±2·8	2·2±0·7

Sedation for part of the day was the only sign observed. Injections were given once daily for 51–58 days, the dose being 7·5 mg/kg, except in one monkey which had 5 mg/kg during 9 of the 57 days. Control monkeys were injected with saline. Three monkeys in each group.
* Difference from controls significant ($P < 0.005$).

Mice have an active transport mechanism for the removal of organic acids from the brain (Sharman, 1966). Before a rise in HVA can be interpreted as an increase in turnover of DA, the possibility has to be ruled out that the drug acts by inhibiting transport of the acid. This can be done by combining the drug with an inhibitor of dopamine synthesis, such as α-methyltyrosine, and estimating the rate of disappearance of DA. If only the transport of HVA is affected, the combination will not result in an accelerated fall in DA content of the tissue following the arrest of synthesis; if, however, the drug accelerates turnover, the fall in DA concentration caused by the inhibitor should be augmented by the drug. Table 3, groups 2, 4 and 6, shows that chlorpromazine and thioridazine, 10 mg/kg, accelerated the disappearance of DA after the administration of α-methyltyrosine. The effect of 100 mg/kg of either drug cannot be readily interpreted, because the phenothiazines on their own reduced the DA content of the tissue. Hence, the system is not in a steady state and turnover cannot be calculated using steady state kinetics.

If the mice given phenothiazines were kept at room temperature (about 21° C), hypothermia ensued, and experiments were therefore designed to check how far this affected the results. An environmental temperature of 30° C kept rectal temperatures normal after any dose of the phenothiazines, and experiments were done in parallel at ambient temperatures of 21° and 30° C.

"Sedation" was much more severe when body temperature was allowed to fall, but the changes in brain content of DA or HVA were not significantly different at the temperatures of 21° and 30° C after 10 mg/kg of either chlorpromazine or thioridazine. Yet temperature was not without effect on the changes in DA metabolism brought about by the drugs; thus a greater fall in DA and a greater rise in HVA concentration was found at 30° than at 21° C when the doses of the phenothiazines were increased to 100 mg/kg; furthermore, the effect of the lower doses of phenothiazines on striatal DA turnover were not identical at 21° and 30° C, because injection of these substances 5 min after the administration of α-methyltyrosine caused a greater fall in DA concentration than α-methyltyrosine

TABLE 3. *Comparison of the effects of chlorpromazine and thioridazine on DA metabolism in mouse striatum*

Group	Drugs used	Dose of phenothiazine (mg/kg)	Dopamine (μg/g) (mean±S.E.M.)	Homovanillic acid (μg/g) (mean±S.E.M.)
1	None	0	2·99±0·15 (7)	0·30±0·04 (6)
2	α-Methyltyrosine	0	1·92±0·13 (7)‡	0·22±0·02 (7)
3	Chlorpromazine	10	3·47±0·16 (6)	1·15±0·10 (6)‡
4	Chlorpromazine with α-methyltyrosine	10	1·18±0·14 (6)**	0·23±0·03 (5)
5	Thioridazine	10	2·99±0·30 (4)	1·40±0·11 (6)‡
6	Thioridazine with α-methyltyrosine	10	1·33±0·18 (6)*	
7	Chlorpromazine	100	2·07±0·10 (4)†	1·17±0·09 (6)‡
8	Chlorpromazine with α-methyltyrosine	100	0·79±0·12 (5)**	
9	Thioridazine	100	1·33±0·26 (5)‡	
10	Thioridazine with α-methyltyrosine	100	0·87±0·07 (5)**	

* ($P<0·05$), ** ($P<0·01$) significantly different from group 2.
† ($P<0·01$), ‡ ($P<0·001$) significantly different from group 1.
All mice injected with drugs liable to cause hypothermia were kept at 30° C. DA and HVA estimated 2 hr after the intraperitoneal injections of one of the phenothiazines or of 0·9% sodium chloride solution. In the even-numbered groups, the injections were given 5 min after an intraperitoneal injection of α-methyltyrosine 80 mg/kg. Number of estimations in brackets.

alone at 30° C, but failed to do so at 21° C (Table 4). This led to an experiment designed to test whether hypothermia as such, by slowing down metabolic processes, would retard the fall in tissue DA after α-methyltyrosine alone. Mice were cooled by placing them into small cages at a temperature of 4° C for 2 hr, by which time their body temperature was as low as that of mice kept at 21° C and given phenothiazines. Control mice were restrained in a similar way. The cerebral DA content was not changed by cooling (group 10, Table 4), but the DA concentration after α-methyltyrosine was 34% higher at 4° than at 21° C (groups 12 and 11, Table 4). The difference was only at the verge of significance at the 5% level, but suggests that the retardation of metabolic processes is likely to have contributed to, or even to have been responsible for, the apparent lack of effect on DA turnover of the

TABLE 4. *Effect of body temperature on turnover of DA in the striatum of mice injected with two phenothiazines* (10 *mg/kg intraperitoneally*)

Group	Material injected	Ambient temperature (°C)	Fall in rectal temperature (°C)	Dopamine (μg/g) (Mean ± S.E.M.)
1	0·9% NaCl solution	21	1	2·99 ± 0·13 (13)
2		30	0	2·87 ± 0·12 (11)
3	α-Methyltyrosine	21	1	1·85 ± 0·10 (12)
4	and 0·9% NaCl solution	30	0	1·93 ± 0·11 (9)
5	α-Methyltyrosine	21	11	2·08 ± 0·10 (8)
6	and chlorpromazine	30	0	1·18 ± 0·14 (6)**
7	α-Methyltyrosine	21	11	2·06 ± 0·25 (6)
8	and thioridazine	30	0	1·33 ± 0·18 (6)*
9	0·9% NaCl solution	21	1	3·42 ± 0·15 (11)
10	(mice restrained)	4	12	3·59 ± 0·26 (10)
11	α-Methyltyrosine	21	1	2·25 ± 0·14 (12)
12	(mice restrained)	4	13	2·74 ± 0·21 (10)

* $P < 0·05$, ** $P < 0·01$ for significance of difference from group 4.
Turnover assessed by fall in DA concentration after inhibition of synthesis with α-methyltyrosine (80 mg/kg injected intraperitoneally 5 min before the phenothiazine). Number of mice in brackets.

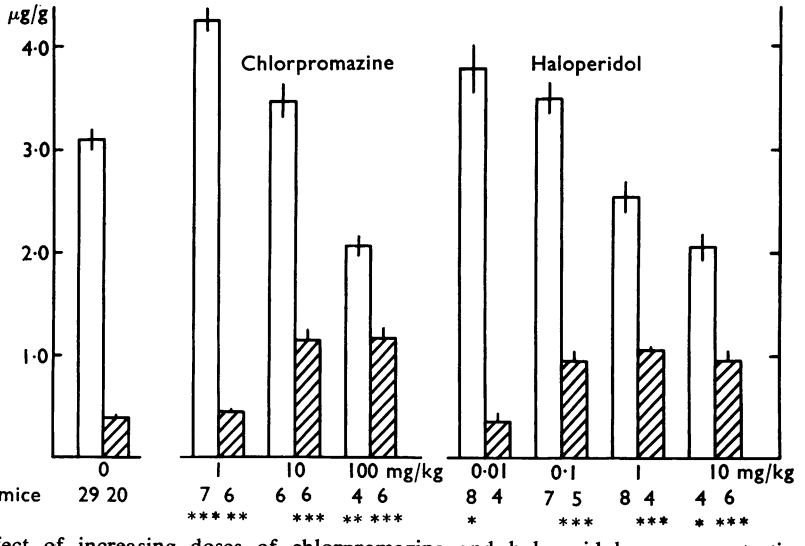

FIG. 2. Effect of increasing doses of chlorpromazine and haloperidol on concentration of DA (clear columns) and HVA (shaded columns) in the striatum of the mouse. Mice killed 2 hr after intraperitoneal injection of the drugs. Significant differences from controls are indicated as $*P<0·05$, $**P<0·01$, $***P<0·001$. Vertical bars represent S.E.M. Mice were kept at 30° C whenever the drug would have caused hypothermia.

phenothiazines at the ambient temperature of 21° C. Table 4 further shows that the DA concentrations in the mice which were restrained at 21° C were higher than in the corresponding groups (1 and 3) of unrestrained mice. Whether this was a result of the stress of restraint or had other causes was not investigated.

Dose-response curves of chlorpromazine and haloperidol

In patients, the effective dose of haloperidol, one of the butyrophenones useful in the treatment of schizophrenia, is approximately one-fiftieth of that of chlorpromazine (Haase & Janssen, 1965), and the same ratio is valid for the production of Parkinsonian side-effects. The dose response relations of chlorpromazine and haloperidol were compared in mice and the doses determined which produced changes in DA metabolism, body temperature and behaviour. Figure 2 illustrates some of the changes in DA metabolism, and is based entirely on experiments in which hypothermia was prevented by raising the ambient temperature when necessary. Small doses of either drug caused a rise in striatal content of DA and of HVA, large doses a fall in DA and a further rise in HVA till a plateau had been reached. The rise in DA was observed with chlorpromazine 1 mg/kg and haloperidol 0·01 mg/kg, with 10 times that dose of either drug DA content was normal, and significant falls were seen with chlorpromazine 100 mg/kg and haloperidol 1 and 10 mg/kg. Combination of haloperidol 0·1 or 1 mg/kg, doses which do not change the DA concentration, with α-methyltyrosine, showed a greater loss of tissue DA than after α-methyltyrosine alone. The figure with 0·1 mg/kg (group 3, Table 5) and that obtained for chlorpromazine 10 mg/kg (group 4, Table 3) were the same, and so were the figures with haloperidol 1 mg/kg (group 4, Table 5) and chlorpromazine 100 mg/kg (group 8, Table 3). From these data it appears that chlorpromazine and haloperidol affect the turnover of DA in precisely the same way, haloperidol being more active by a factor of 100 or a little less. In contradistinction, the effect on body temperature of the two drugs was about the same for equal doses. In mice kept at 21° C, hypothermia did not exceed about 1° C with 1 mg/kg of either substance, and amounted to approximately 10° C with 10 mg/kg. For comparable effects on spontaneous activity, equal doses of the two drugs were also required. After haloperidol 0·001, 0·01 and 0·1 mg/kg, motility of the mice was normal, and only after 1 mg/kg did they move less and rather slowly. This degree of sedation occurred with chlorpromazine after the same dose. With 10 mg/kg of either drug there was occasional catalepsy, and there was neither rigidity nor loss of righting reflex. Haloperidol 100 mg/kg killed the three mice to which it was given ; the same dose of chlorpromazine caused convulsions.

TABLE 5. *Effect of haloperidol on turnover of DA in mouse striatum*

Group	Intraperitoneal injection of	DA μg/g (Mean±S.E.M.)
1	0·1% ascorbic acid*	3·21±0·17 (12)
2	α-Methyltyrosine and 0·1% ascorbic acid	1·81±0·08 (6)
3	α-Methyltyrosine and haloperidol 0·1 mg/kg	1·18±0·05 (5)†
4	α-Methyltyrosine and haloperidol 1 mg/kg	0·82±0·09 (3)†

* Solvent for haloperidol. † Difference from group 2 significant ($P<0·001$).
Number of mice in brackets.
Turnover is assessed by the fall in DA after arrest of synthesis by α-methyltyrosine 80 mg/kg given 5 min before the haloperidol.

Atropine and haloperidol

The beneficial effects of atropine in Parkinsonism aroused interest in the possibility that it might have an effect on the metabolism of DA. It was therefore injected into mice on its own and in combination with haloperidol, and the concentration of DA and HVA in the striatum determined. A dose of atropine sulphate 25 mg/kg was without effect on brain DA, nor did it modify the fall in DA concentration or in body temperature produced by haloperidol 10 mg/kg. There was, however, a reduction in the striatal HVA concentration, and this was true of mice given atropine alone and atropine in combination with moderate doses of haloperidol or chlorpromazine. Figure 3 shows how this effect persisted up to a dose of 1 mg/kg of haloperidol and 10 mg/kg of chlorpromazine. This action of atropine was produced without attenuation of the effects of the two tranquillizers on motor activity.

Himwich & Glisson (1967) gave haloperidol 7·5 mg/kg orally to dogs for 3 days. They reported a tremor and a high degree of debility after that treatment. They also showed that the DA in the caudate nucleus was reduced to 60% of normal. In view of the interest in tremor as one of the signs of Parkinsonism, these experiments were repeated and extended. In a first litter, three doses of 7·5 mg/kg were given orally to two dogs as had been done by Himwich & Glisson, and the animals were killed 1 hr after the last dose. Ataxia, loss of muscle tone and a fine tremor were seen for a number of hours after the first two doses. Tremor was also produced in two dogs of a second litter given a single dose of drug only. After both these treatments the DA concentration in the caudate nucleus was reduced to about 60% of control values, and the HVA content was elevated 2·6 fold (Table 6). Tremor, however, was not a consistent effect of single doses, whereas loss of muscle tone was invariably seen. A small dose (0·1 mg/kg) was given to three dogs ; in two of them the caudate nucleus showed a reduction in DA concentration, and in all of them there was a rise in HVA which averaged 61% ($P<0{\cdot}002$). No tremor or

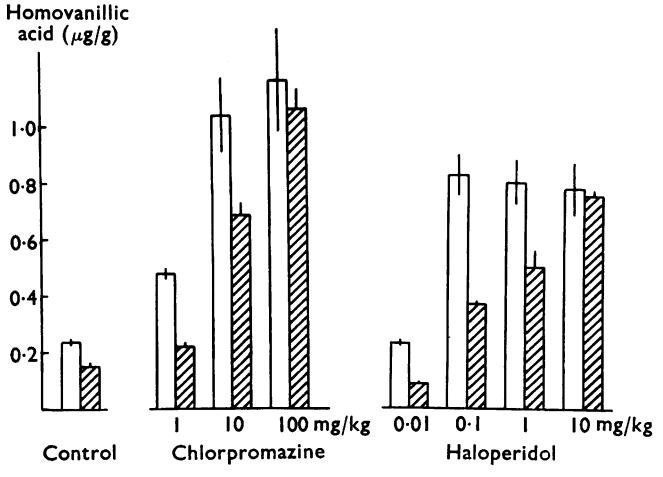

FIG. 3. Effect of atropine, injected on its own or before a tranquillizer, on HVA content of mouse striatum. Shaded columns, atropine sulphate 25 mg/kg injected subcutaneously 5 min before control solvent, chlorpromazine or haloperidol. Clear columns, controls. The mice were kept at 30° C whenever the drugs were liable to cause hypothermia. Vertical bars: S.E.M.

other motor disturbance was seen. A single cat given haloperidol 7·5 mg/kg and killed 4 hr later showed a large fall in DA concentration and a rise in HVA content of the caudate nucleus ; it too lost its muscle tone and showed a fine tremor ; this was obviously of a nature different from that seen after chronic treatment with other phenothiazines, for it occurred before chronic treatment had caused debility. In an experiment in which a very large dose of haloperidol (15 mg/kg) was given subcutaneously to a dog, convulsions ensued, and this suggests that the tremor after haloperidol is a precursor of the convulsions ; the latter can also be produced by large doses of chlorpromazine, 13 mg/kg in the dog, 32 mg/kg in the cat (Moran & Butler, 1956), and were seen in this work with 100 mg/kg in the mouse.

If the tremor elicited by haloperidol bore any relation to Parkinsonian tremor, one would have expected that atropine or methixene would reduce the tremor. A dog was given haloperidol 7·5 mg/kg by mouth ; when a fine tremor had developed, atropine sulphate 2·5 mg/kg was injected subcutaneously and followed an hour later by 5 mg/kg. The tremor was unaffected, but a control dog, given atropine only, developed some tremor after the second dose. A similar experiment was then carried out with methixene hydrochloride, reputed to be particularly effective against human Parkinsonian tremor. In contrast to atropine, methixene alone, up to 8 mg/kg, had no visible effects on the dogs. Tremor which had developed in a dog after haloperidol 8 mg/kg given subcutaneously was not cured, though perhaps reduced, after two consecutive doses of methixene 1 mg/kg ; two older dogs given haloperidol 11 mg/kg showed no tremor, and thus another animal was injected with 15 mg/kg. It showed tremor, jerks and slight clonic convulsions ; none of these movements were arrested by methixene 2 mg/kg or by a further dose of 6 mg/kg. The dog recovered uneventfully from the drugs.

Oxypertine in mice

The claim (Matsuoka, 1964 ; Matsuoka, Ishii, Shimizu & Imaizumi, 1965) that oxypertine lowers the NA but not the DA content of rabbit brain was of interest because this substance, too, is used for treating psychoses and causes Parkinsonism in patients. The question was whether its mode of action was different from that of the other drugs known to cause Parkinsonism ; that is, different from that of the phenothiazines, which increase DA turnover without lowering NA content, and different from that of reserpine, which lowers the concentration of all monoamines.

In a first series of experiments carried out on mice, a difficulty arose because propylene glycol, the solvent for oxypertine used by other workers, had effects of its own when injected intraperitoneally. Thus 10 ml/kg of 50% propylene glycol

TABLE 6. *Effect of haloperidol given orally on the concentration of DA and HVA in the caudate nucleus*

Animal	Haloperidol (mg/kg per day)	Duration of treatment (days)	Dopamine (μg/g)	Homovanillic acid (μg/g)
Dog	0		7·4±0·04 (4)‡	12·1±0·7 (4)‡
Dog	7·5	3*	3·2; 5·5	32·3; 30·3
Dog	7·5	1†	5·0; 4·9	30·5; 33·1
Dog	0·1	1†	5·8±0·51 (3)‡	19·5±0·39‡
Cat	0		8·3	2·9
Cat	7·5	1†	4·3	6·6

* Killed 1 hr after last dose. † Killed 4 hr after drug. ‡ Mean±S.E.M. Number of dogs in brackets.

doubled the concentration of HVA in the brain; furthermore, its injection was resented as was that of 30% propylene glycol. However, 0·1% ascorbic acid proved a suitable solvent which was devoid of any noticeable effects on behaviour or brain catecholamines.

At the dose of 10 mg/kg, oxypertine, like chlorpromazine, caused sedation, ptosis, loss of muscle tone and hypothermia for a period of a few hours, but the changes in DA metabolism were quite different. There was, after oxypertine (Table 7), a fall in DA concentration to about 50% of normal not seen after the same dose of chlorpromazine. Sixteen hours after the injection the values were back to normal. Concentrations of HVA were about twice normal at 2 hr, and were no longer significantly raised at 4 hr. With chlorpromazine, the rise in HVA concentration was larger and lasted longer. On the other hand, a combination of oxypertine with α-methyltyrosine caused a greater loss of DA from the tissue than the combination of chlorpromazine with α-methyltyrosine (Table 4, row 8). This, of course, does not reflect a greater DA turnover with oxypertine, but is due to the DA releasing action of the drug. This is probably also the reason why the effect was almost the same, whether or not hypothermia had been prevented by raising the ambient temperature (rows 7 and 8, Table 7).

Oxypertine in rabbits

The next experiments were done on rabbits, the animals used by Matsuoka *et al.* (1965). Their brains are large enough to estimate the NA and DA content of the hypothalamus. For the estimation of the small concentrations present in the hypothalamus, it was necessary to acetylate the DA and separate it from NA by paper chromatography (see **Methods**).

The dose of oxypertine was very large, 70 mg/kg, but was chosen because it was the one used by Matsuoka (1964). The results (Table 8) show that 3 or 4 hr after the injection of the drug the DA content of the caudate nucleus had fallen to 28% of normal and that of hypothalamic NA to 25% of normal. Dopamine concentration in the hypothalamus was not significantly changed (74% of control figure).

TABLE 7. *Effect of oxypertine, 10 mg/kg, given intraperitoneally in 0·1% ascorbic acid solution, on DA metabolism of female mice*

Group	Substances injected	Time elapsed since injection (hr)	Ambient temperature (°C)	Dopamine (μg/g) (Mean±S.E.M.)	Homovanillic acid (μg/g) (Mean±S.E.M.)
1	0·1% ascorbic acid (10 ml/kg)	2	21 or 30	3·29±0·19 (8)	0·32±0·02 (4)
2	Oxypertine	2	21	1·72±0·12 (8)	0·75±0·03 (4)
3	Oxypertine	2	30	1·98±0·18 (8)	0·80±0·06 (4)
4	Oxypertine	4	21	1·68±0·12 (3)	0·49±0·02 (4)
5	Oxypertine	16	21	2·95±0·36 (8)	
6	α-Methyltyrosine and ascorbic acid	2	21 or 30	2·05±0·10 (7)	
7	α-Methyltyrosine and oxypertine	2	21	0·82±0·11 (8)	
8	α-Methyltyrosine and oxypertine	2	30	0·60±0·10 (8)	

Groups 6, 7 and 8 had α-methyltyrosine, 80 mg/kg, intraperitoneally 5 min before the injection of either ascorbic acid or oxypertine. Number of mice in brackets.

The HVA in the caudate nucleus was 2·3 times normal. Thus after oxypertine, hypothalamic NA and striatal DA content were both low, whereas hypothalamic DA, considered to be " precursor " DA, was slightly but not significantly reduced, and certainly not raised as found by Matsuoka (1964). With this dose, effects on behaviour were profound. There was sedation from which the rabbit was not aroused by touch or noise. There were autonomic effects, such as deep and rapid respiration, salivation, lachrymation and vasodilatation. There was no catalepsy. The signs were most pronounced from 30 to 120 min after the injection.

Butyrophenones in rabbits

The object of the experiment was to test whether the effect of butyrophenones on turnover of DA was restricted to the striatum and whether even high doses spared hypothalamic NA. A small, but not significant, rise in DA had been found in the hypothalamus of the dog after repeated doses of haloperidol (Himwich & Glisson, 1967).

Two rabbits were injected with haloperidol 7·5 mg/kg: whereas striatal DA concentration was reduced to half its control value, hypothalamic DA remained unchanged; so did the NA concentration of the hypothalamus. Behaviour was affected in a very peculiar way: no abnormality could be detected for most of the time, but during repeated periods of about a minute's duration the rabbits remained immobile with eyes half-closed and showed no resistance or other response to touch or passive movements of limbs and ears.

Homovanillic acid was not estimated in the brain of the rabbits given haloperidol, but was measured in a group of rabbits injected with the even more potent tranquillizer, the butyrophenone spiperone. The concentration of HVA was examined in the striatum and two other parts of the brain. Table 9 shows that although the concentration of HVA in the caudate nucleus and putamen was greatly elevated, no increase was observed in either the hypothalamus or the substantial

TABLE 8. *Effect of oxypertine, 70 mg/kg, injected intraperitoneally into rabbits, on DA, NA and HVA concentrations in different parts of the brain*

	Caudate nucleus		Hypothalamus	
	Homovanillic acid	Dopamine	Dopamine	Noradrenaline
Controls	1·8; 2·9	8·8±0·5 (5)	0·19±0·02 (3)	1·27; 0·94
Oxypertine	4·4; 6·5	2·5±0·7 (5)	0·14±0·01 (3)	0·35; 0·21

The rabbits were killed 3 hr or, sometimes, 4 hr after the injection. Values are either single estimations (μg/g fresh tissue) or means ±S.E.M. Number of rabbits in brackets.

TABLE 9. *Effect of spiperone on the concentration of homovanillic acid (HVA) in the rabbit brain*

Dose of spiperone (mg/kg)	Duration of experiment (hr)	Concentration of HVA (μg/g)			
		Caudate nucleus	Putamen	Substantia nigra	Hypothalamus
0*		3·2±0·3†	4·2±0·2	1·0±0·1	0·6±0·2
0·5 mg/kg i.p.	4·5	8·6	8·4	1·0	1·1
0·5 mg/kg i.p., 3 hr later 0·5 mg/kg i.p.	5	4·1; 5·4	8·5; 6·7	0·8; 1·1	0·9; 1·0
0·5 mg/kg i.v.	3	6·1; 8·1	7·1; 7·5	1·0; 1·0	0·3; 1·1
0·5 mg/kg i.v.	4·5	12·6; 6·8	16·5; 7·4	1·3; 0·8	0·8; 0·6

* Four untreated animals. † S.E. of mean.

nigra. The dose of spiperone used was large and caused the animals to remain still and unresponsive. In one animal (not included in Table 9) an intraperitoneal injection seemed to have no effect on behaviour and there was no increase in the concentration of HVA in the striatum.

Phenoxybenzamine in mice

The increase in turnover of DA observed after chlorpromazine has been interpreted as a compensatory phenomenon due to the block of " monoaminergic receptors " (Carlsson & Lindqvist, 1963). Yet α-adrenoceptors (Ahlquist, 1948) appeared not to be involved, because the same authors showed that there was no increase in the formation of 3-methoxytyramine, as there was with chlorpromazine, after injection of the α-blocking agent phenoxybenzamine (20 mg/kg) to mice pretreated with a monoamine oxidase inhibitor. Nor was there a rise in the concentration of HVA in the rabbit striatum after this dose of phenoxybenzamine (Andén *et al.*, 1964), but larger intravenous doses given to rabbits had an effect on motoneurone activity similar to that of chlorpromazine (Arvidsson, Roos & Steg, 1966). Dopamine turnover was therefore examined by combining a large dose (50 mg/kg) of phenoxybenzamine with α-methyltyrosine. This dose of phenoxybenzamine was found to sedate the mice to about the same degree as chlorpromazine 10 mg/kg and also to cause an equal degree of hypothermia if the animals were left at room temperature. The experiments were therefore carried out at 30° C. Table 10 shows that, by itself, phenoxybenzamine did not alter the DA concentration in the brain, nor did it accelerate the fall produced by arresting synthesis with α-methyltyrosine. There was thus no evidence that DA turnover was increased.

Discussion

One aim of the present paper was to test whether there is a close correlation between the tendency of a drug to produce Parkinsonian side-effects in man and its ability to increase DA turnover in the brain of animals. The work confirmed the finding of Laverty & Sharman (1965b) that thioridazine, which rarely induces Parkinsonism in man, does not elevate the HVA concentration in the caudate nucleus of cats given the drug for 2 weeks ; this contrasted with the rise in HVA seen in cats treated for the same time and with the same dose of chlorpromazine, a substance which frequently causes Parkinsonian side-effects. The same difference between the actions of the two drugs was observed 24 hr after a single oral dose, only chlorpromazine causing a rise in striatal HVA. Yet thioridazine is not altogether devoid of actions on the metabolism of DA in cat brain: 24 hr after

TABLE 10. *Effect of phenoxybenzamine, 50 mg/kg intraperitoneally, on DA turnover in mouse striatum*

Drugs given	Dopamine (μg/g) (mean $\pm$S.E.M.)
Solvent*	3·09±0·18
Phenoxybenzamine	2·76±0·18
Solvent* 5 min after α-methyltyrosine	1·69±0·09
Phenoxybenzamine 5 min after α-methyltyrosine	1·89+0·21

* Solvent for phenoxybenzamine, consisting of dilute acidified ethanol.
Arrest of DA synthesis by α-methyltyrosine 80 mg/kg injected intraperitoneally. Mice were kept at 30° C and were killed 2 hr after the injections. The results are means of three estimations.

a single oral dose, DA concentration was somewhat lowered, as it was after one dose of chlorpromazine. Four hours after repeated doses, however, DA concentration was reduced after chlorpromazine but not after thioridazine.

Whereas there was a clear difference between the actions of chlorpromazine and thioridazine on cat brain, this difference was absent in mice. Equal doses of both drugs accelerated turnover of DA, whether this was measured by a rise in striatal HVA concentration or by an accelerated fall in striatal DA after arrest of catecholamine synthesis. All phenothiazines undergo rapid metabolism in the body, and so differences in drug metabolism between cat and mouse may account for the differences in responses. Among rodents, species differences in susceptibility to the two drugs are very obvious in behavioural responses: in the mouse sedation, hypothermia, loss in muscle tone were the same for equal doses of the two phenothiazines, whereas the rat needs much higher doses of thioridazine than of chlorpromazine for immobilization and other behavioural changes to become evident (Shillito, 1967).

Comparison, in the mouse, of the actions of chlorpromazine with those of haloperidol, another tranquillizer, gave a very different picture. In man, the dose of haloperidol required for the treatment of psychoses, or apt to cause Parkinsonian side-effects, is about one-fiftieth of that of chlorpromazine. The same potency ratio of approximately 50 (somewhere between 10 and 100) was observed in respect of DA metabolism in mouse striatum; this was true of the biphasic effect on DA concentration (a rise with small and a fall with large doses), of the rise in HVA content and of the increase in DA turnover as measured by arresting catecholamine synthesis with α-methyltyrosine. Whereas haloperidol was thus much more potent than chlorpromazine in modifying DA metabolism in mouse brain, the two drugs showed the same potency when compared on motor behaviour of the mouse. Therapeutic and toxic effects in man thus run parallel with biochemical changes in mouse brain, but not with "sedation" of the mouse; behavioural effects in other species, however, such as inhibition of conditioned avoidance responses or of apomorphine-induced emesis in the dog, have been known for some time to be good indices of clinical potency (Janssen, 1961).

It was not only in the mouse but also in other animal species that motor disabilities failed to run parallel to changes in dopamine turnover. In the cat, single oral doses of thioridazine or chlorpromazine, 15 mg/kg, caused ataxia and "sedation", but only chlorpromazine increased concentrations of HVA in the caudate nucleus. Furthermore, tremor and catalepsy developed when cats received appropriate doses of thioridazine and chlorpromazine for 2 weeks, but only chlorpromazine increased the turnover of DA (Fig. 1). When single doses of haloperidol were given to dogs, 7·5 mg/kg produced loss of muscle tone and tremor, whereas 0·1 mg/kg had no visible effect. Yet the latter caused a very appreciable rise in DA turnover (the content of HVA in the caudate nucleus increased by 61%), so that it is difficult to believe that the motor effects were a consequence of the change in DA turnover.

When an increase in HVA was found to result from the injection of drugs into mice, it was interpreted as a sign of accelerated turnover of DA. The justification of this view has been demonstrated for chlorpromazine by Sharman (1967). It was now confirmed for thioridazine and haloperidol by showing that these drugs caused an accelerated loss of DA from the tissue if DA synthesis had been arrested

by administration of α-methyltyrosine. This effect can be obscured if the drugs produce a serious fall in body temperature, and the experiments should therefore be conducted at an environmental temperature which prevents such a fall.

In all species given phenothiazines and related drugs, particular attention was paid to signs which might resemble those of Parkinsonism. A fine tremor of high frequency was, in fact, seen in cats and dogs after phenothiazines or haloperidol; in cats given phenothiazines, it was associated with muscular weakness and debility, and in dogs, which showed it after haloperidol, it appeared to be the precursor of convulsions. It was never accompanied by rigidity; in fact, loss of muscle tone was the change most frequently induced in animals. Kaelber & Joynt (1956) reported tremor in cats injected with chlorpromazine; it occurred in 46% of their animals, often after a single injection, and showed little relation to dose; it was different from the tremor seen in the present work, for it occurred in the most active cats and only in certain relaxed postures.

In monkeys, in which destruction of the substantia nigra may produce signs resembling those of human Parkinsonism (Poirier *et al.*, 1966), prolonged treatment with chlorpromazine 7·5 mg/kg, the highest dose tolerated without impairment to health, produced neither changes in motility nor in cerebral DA metabolism, though "sedation" was evident for a while after each injection. We do not know what changes in cerebral metabolism accompany drug-induced Parkinsonism in man, or why only some subjects are susceptible, so it is not possible to tell whether the lack of acceleration of DA metabolism in the monkey is due to greater resistance of this species, the treatment of too few animals, or the fact that in primate brain the chemical changes produced by chlorpromazine are not the same as in cats, dogs or mice. We completely failed to produce in any species a motor response which could be considered analogous to drug-induced Parkinsonism. It may be that catalepsy is the best guide available so far; this is the view of Cole & Edwards (1964), who found a good correlation between the incidence of catalepsy in rats and reports of Parkinsonian side-effects in clinical trials of tranquillizers.

Oxypertine was of interest because, in its therapeutic actions and in its production of Parkinsonian signs, it is considered to resemble the phenothiazines (Hollister, Overall, Kimbell, Bennett, Meyer & Caffey, 1963). Yet, biochemically, it was reputed greatly to lower brain noradrenaline but not striatal dopamine. At present there are only two known patterns of biochemical changes associated with tranquillizers which produce Parkinsonism: a large fall in concentrations of all catecholamines after a drug like reserpine, or changes which take place chiefly in the metabolism of DA as seen after phenothiazines and butyrophenones. In the mouse, oxypertine was indeed found to mimic the action of phenothiazines on behaviour: at the same dose of 10 mg/kg, sedation, hypothermia, ptosis and loss of muscle tone was similar after the two types of compound. The biochemical changes caused by oxypertine, however, resembled those of a short-acting reserpine-like drug: a 50% fall in striatal dopamine and, at the time when DA had reached its lowest concentration, a rise in HVA which was far smaller than that occurring after chlorpromazine. The fall in striatal dopamine caused by oxypertine was confirmed in experiments on rabbits to which the dose of 70 mg/kg used by Matsuoka (1964) was administered. In the course of 3 hr, the concentration of DA in the caudate nucleus and of NA in the hypothalamus fell to about one quarter of normal. In order to rule out the possibility that oxypertine interfered

with the estimation of dopamine in tissue, the recovery of DA was tested with tissue from oxypertine-treated mice and found not to differ from that obtained with normal mice. The difference between our results and those of the Japanese workers who reported normal concentrations of striatal DA might be due to the use of the solvent propylene glycol, which can by itself modify metabolism of DA, and to the necessity for elaborate methods of isolation and purification when the concentrations of catecholamines to be estimated in tissue are small (see also Sharman & Vogt, 1965).

In its short-lasting effects on monoamine metabolism, oxypertine is closest to substances like tetrabenazine or prenylamine ; with the latter it shares, in the rat, the preferential attack on catecholamines while 5-hydroxytryptamine (5-HT) is spared (Spector, Melmon & Sjoerdsma, 1962 ; Juorio & Vogt, 1965). This poses another problem. Sedation after reserpine and tetrabenazine can be related to an action of these drugs on the binding of 5-HT (Brodie, Comer, Costa & Dlabac, 1966). In keeping with this view, the sedative action of prenylamine is negligible in the rat but very pronounced in the pigeon, in which it lowers the brain concentration of 5-HT as well as of the catecholamines (Juorio & Vogt, 1967), so that in this species the action of prenylamine is almost identical with that of reserpine. The sedation produced by oxypertine must obviously have a cause which is not related to the binding of 5-HT.

It was surprising to find that atropine is not without action on turnover of DA. It may either act directly by slowing DA metabolism, or indirectly by inhibiting the action of acetylcholine at a site where this normally activates a dopaminergic pathway. Such a site could be the substantia nigra, from which the cell bodies of dopaminergic neurones are thought to send axons into the striatum. Smelik & Ernst (1966) have carried out an experiment which they interpret as a demonstration of activation of this dopaminergic pathway by acetylcholine. They elicited gnawing in rats by implanting solid physostigmine salicylate into the region of the substantia nigra. A similar response followed the implantation of DOPA into the striatum of rats ; assuming that the response to implanted DOPA is an exaggeration of the normal activity of the dopaminergic nigro-striatal pathway, gnawing after physostigmine implantation should indicate cholinergic activation of this pathway.

The complexity of the problem is enhanced by the fact that chlorpromazine has some inhibitory action on cholinesterase ; this property was considered to explain the observation that sedation of rats by larger doses (16 mg/kg) of chlorpromazine was not abolished by amphetamine alone but only by a combination of amphetamine with atropine (Maickel, 1968).

In a few experiments on rabbits, drugs which lowered striatal concentration of DA were found to be without effect on hypothalamic DA. This was so for oxypertine, which lowers, and for haloperidol, which does not affect, hypothalamic NA. Thus these drugs affect DA differently at sites where it is acting as precursor and at sites where it is presumed to be released as transmitter. The absence of any effect of spiperone on HVA in the hypothalamus and substantia nigra is in keeping with the possibility that, in these regions, the HVA is formed as a result of wastage of DA.

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REFERENCES

AHLQUIST, R. P. (1948). A study of the adrenotropic receptors. *Am. J. Physiol.*, **153**, 586–600.

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., Oxford*, **3**, 149–158.

ARVIDSSON, J., ROOS, B.-E. & STEG, G. (1966). Reciprocal effects on α- and γ-motoneurones of drugs influencing monoaminergic and cholinergic transmission. *Acta physiol. scand.*, **67**, 398–404.

BRODIE, B. B., COMER, M. S., COSTA, E. & DLABAC, A. (1966). The role of brain serotonin in the mechanism of the central action of reserpine. *J. Pharmac. exp. Ther.*, **152**, 340–349.

CARLSSON, A. & LINDQVIST, M. (1963). Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. *Acta pharmac. tox.*, **20**, 140–144.

COLE, J. O. & CLYDE, D. J. (1961). Extrapyramidal side effects and clinical response to the phenothiazines. *Revue can. Biol.*, **20**, 565–574.

COLE, J. O. & EDWARDS, R. E. (1964). In *Animal Behaviour and Drug Action*, Ciba Foundation Symposium, ed. Steinberg, H., de Reuck, A. V. S. and Knight, J., pp. 286–298. London: Churchill.

HAASE, H. J. & JANSSEN, P. A. J. (1965). *The Action of Neurcleptic Drugs*. Amsterdam: North-Holland Publishing Company.

HIMWICH, W. A. & GLISSON, S. N. (1967). Effect of haloperidol on caudate nucleus. *Int. J. Neuropharmac.*, **6**, 329–332.

HOLLISTER, L. E., OVERALL, J. E., KIMBELL, I., JR., BENNETT, J. L., MEYER, F. & CAFFEY, E., JR. (1963). Oxypertine in newly admitted schizophrenics. *J. new Drugs*, **3**, 26–31.

JANSSEN, P. A. J. (1961). Vergleichende pharmakologische Daten über sechs neue basische 4'-Fluorobutyrophenon-Derivate. *Arzneim. Forsch.*, **11**, 819–824; 932–938.

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.

JUORIO, A. V. & VOGT, M. (1965). The effect of prenylamine in the metabolism of catecholamines and 5-hydroxytryptamine in brain and adrenal medulla. *Br. J. Pharmac. Chemother.*, **24**, 566–573.

JUORIO, A. V. & VOGT, M. (1967). Monoamines and their metabolites in the avian brain. *J. Physiol., Lond.*, **189**, 489–518.

KAELBER, W. W. & JOYNT, R. J. (1956). Tremor production in rats given chlorpromazine. *Proc. Soc. exp. Biol. Med.*, **92**, 399–402.

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

LAVERTY, R. & SHARMAN, D. F. (1965b). Modification by drugs of the metabolism of 3,4-dihydroxyphenylethylamine, noradrenaline and 5-hydroxytryptamine in the brain. *Br. J. Pharmac. Chemother.*, **24**, 759–772.

MAICKEL, R. P. (1968). Diverse central effects of chlorpromazine. *Int. J. Neuropharmac.*, **7**, 23–27.

MATSUOKA, M. (1964). Function and metabolism of catecholamines in the brain. *Jap. J. Pharmac.*, **14**, 181–193.

MATSUOKA, M., ISHII, S., SHIMIZU, N. & IMAIZUMI, R. (1965). Effect of Win 18501-2 on the content of catecholamines and the number of catecholamine-containing granules in the rabbit hypothalamus. *Experientia*, **21**, 121–123.

MORAN, N. C. & BUTLER, W. M., JR. (1956). The pharmacological properties of chlorpromazine sulfoxide, a major metabolite of chlorpromazine. A comparison with chlorpromazine. *J. Pharmac.*, **118**, 328–337.

POIRIER, L. J., SOURKES, T. L., BOUVIER, G., BOUCHER, R. & CARABIN, S. (1966). Striatal amines, experimental tremor and the effect of harmaline in the monkey. *Brain*, **89**, 37–52.

PORTIG, P. J., SHARMAN, D. F. & VOGT, M. (1968). Release by tubocurarine of dopamine and homovanillic acid from the superfused caudate nucleus. *J. Physiol., Lond.*, **194**, 565–572.

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.

SHARMAN, D. F. & VOGT, M. (1965). The noradrenaline content of the caudate nucleus of the rabbit. *J. Neurochem.*, **12**, 62.

SHILLITO, E. E. (1967). The effect of chlorpromazine and thioridazine on the exploration of a Y-maze by rats. *Br. J. Pharmac. Chemother.*, **30**, 258–264.

SMELIK, P. G. & ERNST, A. M. (1966). Role of nigro-neostriatal dopaminergic fibres in compulsive gnawing behaviour in rats. *Life Sci., Oxford*, **5**, 1485–1488.

SPECTOR, S., MELMON, K. & SJOERDSMA, A. (1962). Evidence for rapid turnover of norepinephrine in rat heart and brain. *Proc. Soc. exp. Biol. Med.*, **111**, 79–81.

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