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MODIFICATION BY DRUGS OF THE METABOLISM OF 3,4-DIHYDROXYPHENYLETHYLAMINE, NORADRENALINE AND 5-HYDROXYTRYPTAMINE IN THE BRAIN

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There have been a number of studies of the effects of drugs on the content of various amines found in brain tissue. In particular, the concentrations in the brain of noradrenaline (Vogt, 1954) and 5-hydroxytryptamine (Paasonen & Vogt, 1956; Brodie, Shore & Pletscher, 1956) have been determined before and after treatment with drugs, and many attempts have been made to correlate the change in brain amine concentration with an observable change in animal behaviour. Later, brain dopamine (3,4-dihydroxyphenylethylamine) has been included in such studies (Bertler, 1961).

In the present experiments, the content of dopamine, noradrenaline and 5-hydroxytryptamine in different parts of cat and dog brain was measured, and so was the content of homovanillic acid (4-hydroxy-3-methoxyphenylacetic acid) and 5-hydroxyindol-3-ylacetic acid, the major acid metabolites of dopamine and 5-hydroxytryptamine. The corresponding acid metabolite of noradrenaline, vanillylmandelic acid (4-hydroxy-3-methoxymandelic acid), has not been detected in brain tissue (Andén, Roos & Werdinius, 1964; Sharman, unpublished) and so could not be determined. The simultaneous determination of an amine and its metabolite permitted a study of the effect of the drug not only on the content of the amine but also on a possible indicator of the rate of metabolism of the amine.

The concentrations of both dopamine and homovanillic acid are highest in the brain in the caudate nucleus and putamen; since the caudate nucleus is easy to dissect out reproducibly it was used for the estimation of these two compounds. The caudate nucleus is thought to form part, with other basal ganglia which also contain dopamine, of the " extrapyramidal motor system", though recent evidence suggests that it has a more generalized function, acting as an integrative centre for the whole of the cortex (Laursen, 1963; Carman, Cowan & Powell, 1963). In the brains from patients with Parkinson's syndrome, Bernheimer, Birkmayer & Hornykiewicz (1963) found a much-reduced concentration of dopamine in the caudate nucleus, as well as smaller reductions in the concentrations in the brain of noradrenaline and 5-hydroxytryptamine. This suggests a relationship between brain dopamine metabolism and Parkinson's syndrome, as do the results of Barbeau &

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Sourkes (1961) who found a reduced urinary excretion of dopamine in patients with Parkinsonism. For this reason we have studied a number of drugs which are reported to influence or induce a Parkinson-like syndrome in animals or man. In addition, some drugs were studied which cause or mimic central sympathetic excitation. Preliminary reports of some of the results have been published (Laverty, 1963; Sharman, 1963b).

METHODS

Adult cats, dogs and rabbits were used. Whenever possible litter mates were used as controls for the treated animals. If litter mates were not available, the experimental and control groups were usually matched for sex and size. The brains from control animals were analysed simultaneously with those from treated animals; in most experiments, control and treated animals were housed under identical conditions before and during treatment.

Drugs were administered subcutaneously, except for some cats where intraperitoneal or oral administration was used and a few rabbits to which the drugs were given either intraperitoneally or intravenously into a marginal ear vein.

The animals were killed by bleeding during chloroform anaesthesia. The brain was removed and the caudate nuclei and the hypothalamus were removed and deep-frozen; in cats the thalamus (excluding the medial geniculate bodies) was also removed and frozen. All tissues were weighed frozen. Within 2 hr of removal of the tissue from the animal, the hypothalamus and caudate nuclei were each homogenized with at least twice their weight of 0.1 N-hydrochloric acid, with the addition of a few mg of ascorbic acid. The protein in the hypothalamic homogenate and in a portion of the caudate nucleus homogenate was precipitated with an equal volume of 0.8 N-perchloric acid and centrifuged off at 0° C. The *p*H of the supernatant fluid was adjusted to *p*H 4 with 3 N-potassium carbonate; the precipitated potassium perchlorate was removed by further centrifugation at 0° C. The supernatant fluid was then applied to a Dowex 50 X-8 ion-exchange resin column (25 × 4 mm) prepared as described by Bertler, Carlsson & Rosengren (1958). The column was fitted with a capillary tube which maintained a flow rate of 8 to 10 ml./hr without applied pressure. When the supernatant fluid had run through, the column was washed with 4 ml. of water; then the noradrenaline was eluted with 8 ml. of 0.4 N-hydrochloric acid. A further fraction which contained dopamine could be obtained by eluting with 8 ml. of 2 N-hydrochloric acid. The columns used for caudate nucleus extracts were washed with 6 ml. of 0.4 N-hydrochloric acid instead of water to remove any noradrenaline or dihydroxyphenylalanine, and the dopamine was eluted with 8 ml. of 2 N-hydrochloric acid. Edetic acid disodium salt (20 μg) was added to the dopamine eluates which were then brought to *p*H 4 with solid sodium bicarbonate.

Noradrenaline in the eluates was determined fluorimetrically by a ferricyanide oxidation method (Sharman, Vanov & Vogt, 1962). The fluorescence was measured using a Locarte filter fluorimeter with a Chance 0X1 primary filter, and an Ilford 625 secondary filter. This filter combination gave equivalent fluorescence with equal amounts of noradrenaline and adrenaline; the results given for noradrenaline include any adrenaline present. In many experiments the adrenaline content of the noradrenaline eluate was also measured by a differential method using a second filter set; the primary was a combination of Corning 3389 and Corning 5113 and the secondary, Chance OY4. With this set, adrenaline gave approximately three times the fluorescence given by an equal amount of noradrenaline.

Dopamine was measured fluorimetrically by a modification of the method of Weil-Malherbe & Bone (1952). To 4 ml. of neutralized column eluate was added 0.3 ml. of ethylenediamine and 0.2 ml. of 2 N-hydrochloric acid (instead of 0.2 ml. of 2 N-ethylenediamine dihydrochloride) and the mixture was heated in the dark for 20 min at 65° C. The reaction mixture was saturated with sodium chloride and extracted with 3 ml. of isobutanol. The fluorescence of the isobutanol extract was read in an Aminco-Bowman spectrophotofluorimeter at 415/520 mμ (uncorrected instrumental wavelengths). In some later experiments the dopamine in the eluate was acetylated before condensation (Laverty & Sharman, 1965); this increased the sensitivity of the method.

5-Hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid were measured fluorimetrically as described by Ashcroft & Sharman (1962); in dogs a portion of the caudate nucleus homogenate and in cats the

thalamus was used. Homovanillic acid was measured in portions of the homogenates from cat and dog caudate nuclei by the method described by Sharman (1963a). In rabbits only catechol amines were estimated.

In experiments with tritiated dopamine, the radioactivity was measured using a Packard Tri-Carb liquid scintillation counter. Samples of aqueous solution, usually 1 ml., were added to 15 ml. of scintillator solution in dioxane (Bray, 1960) and counted directly with an efficiency of 10 to 13% against an internal standard of tritiated dopamine. The 1-[^{3}H]-dopamine was obtained from New England Nuclear Corp. (150 μC/mg).

The following drugs were used: atropine sulphate (B.D.H.), atropine methylbromide, caramiphen hydrochloride (Geigy), chlorpromazine hydrochloride (May & Baker), dexamphetamine sulphate, 3,4-dihydroxyphenylalanine (dopa, L. Light), diethazine hydrochloride (May & Baker), ephedrine hydrochloride, guanethidine (Ciba), morphine hydrochloride (B.D.H.), oxotremorine (May & Baker), pentobarbitone sodium (Abbott), reserpine (Ciba), β-tetrahydronaphthylamine hydrochloride (Theo. Schuchardt, Munich), trifluoperazine dihydrochloride (Smith, Kline & French), thioproperazine dimethanesulphonate (May & Baker) and thioridazine hydrochloride (Sandoz). Doses are given in terms of the salts.

Standard solutions of amines and metabolites were prepared, in terms of free base or acid, from adrenaline base (Burroughs Wellcome), dopamine hydrochloride (California Corp. for Biochemical Research), homovanillic acid (California Corp. for Biochemical Research), 5-hydroxytryptamine hydrogen oxalate (Regis), 5-hydroxyindol-3-ylacetic acid (L. Light) and noradrenaline bitartrate (L. Light).

RESULTS

Recovery experiments. A number of experiments were carried out on the recovery of dopamine and noradrenaline added to various tissue samples. The recovery from 0.2 μg of noradrenaline added to cat, dog and rabbit brain tissue samples was 78$\pm$2.6% (mean and standard error) in twenty-eight determinations and was reasonably consistent and reproducible. Dopamine recoveries tended to be more variable; the mean recovery from 0.5 μg of dopamine added to cat, dog and rabbit tissue in fifty-five estimations was 64$\pm$3.3%.

The recovery figures were calculated from the difference measured between two samples of tissue homogenate, one of which contained added amine; each was run through the entire column extraction procedure. In an attempt to reduce the variability arising from the use of two separate columns, radioactive dopamine was added to a tissue homogenate and the recovered radioactivity measured. This should enable the estimation of the

TABLE 1

COMPARISON OF THE RECOVERIES OF ADDED DOPAMINE (0·5 μG) ESTIMATED BY FLUORIMETRIC AND RADIOACTIVE TRACER TECHNIQUES

Values are means and standard errors

Tissue	No. of experiments	Recovery (%)	
		Fluorimetric	Radioactive
Cat caudate nucleus	6	74$\pm$ 7·9	83$\pm$1·1
Cat cortex	5	67$\pm$ 1·3	82$\pm$2·1
Rabbit caudate nucleus	6	79$\pm$10·2	72$\pm$6·2
Rabbit cortex	10	53$\pm$ 7·5	62$\pm$1·7

unknown dopamine content and of the recovery in the same sample. A number of control experiments (Table 1) were done in which the recovery estimated by fluorimetric means was compared with the recovery estimated by radioactivity. It will be seen that the two recoveries were similar but that the recovery estimated by fluorimetric means was much more variable. This may reflect losses in some experiments of the dopamine in the final

eluate without a corresponding loss of radioactivity, or may reflect a larger experimental error involved in fluorimetric estimations.

Radioactive dopamine was used to detect where the losses occurred in the extraction procedure. When radioactive dopamine was added to a homogenate of mouse lung and the dopamine losses followed at various stages it was found, in four experiments, that approximately 5% was trapped in the precipitated protein and 2% in the potassium perchlorate precipitate; a further 3% was not adsorbed on the resin and the main loss (12%) occurred when the resin was washed with 0.4 N-hydrochloric acid. The remaining radioactivity (78%) was recovered in the 2 N-hydrochloric acid eluate.

Radioactive tracer methods also gave information about the efficiency of the ethylenediamine reaction and isobutanol extraction. It was found that 39% of the radioactivity in the eluate was recovered in the isobutanol extract used to measure the fluorescence developed. The exact reaction products are unknown (Harley-Mason & Laird, 1959) but it is apparent that the reaction between dopamine and ethylenediamine to form an isobutanol-soluble fluorescent product was being operated at less than maximum yield.

Recoveries of 5-hydroxytryptamine (0.2 μg) and 5-hydroxyindol-3-ylacetic acid (0.2 μg) added to tissue samples averaged $69 \pm 1.4\%$ (eleven experiments) and $51 \pm 2.0\%$ (eleven experiments) respectively. The mean recovery of homovanillic acid (2 to 5 μg) added to dog tissue was $72 \pm 6.2\%$ (eleven experiments).

The results presented in this paper are uncorrected for losses.

The amounts of adrenaline and dopamine in the hypothalamus of the animals used in this study were measured, but the results have not been given in detail, as the amounts present were too close to the lower limits of sensitivity of the methods for great reliance to be placed upon them. Single doses of morphine, β-tetrahydronaphthylamine, dexamphetamine, ephedrine, pentobarbitone or atropine, and chronic treatment with phenothiazines did not change the ratio of adrenaline to noradrenaline in the cat hypothalamus. The adrenaline found in the hypothalamus as a percentage of the total adrenaline plus noradrenaline was 4% in cats and 11% in dogs. This agreed with previous results (Vogt, 1954).

The determination by the ethylenediamine reaction of small amounts of dopamine found in the hypothalamus yielded values of about 0.5 μg/g but cannot be relied upon as tissue blank figures were high relative to the amount of dopamine present. By using a more sensitive method involving acetylation, separation of the acetylated amine and measurement at two different fluorescence wavelengths (Laverty & Sharman, 1965) it is possible to show that the dopamine content of cat and dog hypothalamus is approximately 0.2 μg/g.

A striking feature of the present experiments is the extreme variability of the dopamine content of the caudate nucleus (range 2.1 to 12.3 μg/g in the cat). This is not due to excessive variability in the extraction methods, since in fifteen experiments in which duplicate estimations were made on each sample there was agreement between the duplicate samples and the variation between animals was still present. The variability of the dopamine content appeared to be as great or greater between different groups of animals as within groups, particularly with the cats, and was probably due to poor control over their previous environment. In dogs and rabbits the use of litter mates reduced but did not eliminate the variability. A wide range (0.12 to 0.49 μg/g) was also observed for the concentration of 5-hydroxytryptamine in the thalamus of the cat.

Excitant drugs

Biochemical effects. The effect on the concentration of amines and metabolites in the brain of a group of drugs which cause forms of excitation involving sympathetic mechanisms in cats was studied in the cat, dog and rabbit. The results of these experiments are summarized in Table 2.

Cats. In confirmation of previous work (Vogt, 1954), it was found that the noradrenaline content of the cat hypothalamus was low 4 hr after injection of morphine and β-tetrahydronaphthylamine, but normal after ephedrine. In the caudate nucleus, the homovanillic acid content was raised 4 hr after the first two, but not after the third drug. Dexamphetamine only slightly lowered the hypothalamic noradrenaline and slightly raised the homovanillic acid level. Yet dexamphetamine was the only drug of these four to lower significantly the dopamine content of the caudate nucleus. In whole rat brain dexamphetamine was seen to lower the noradrenaline content without affecting that of dopamine (Baird & Lewis, 1963).

The peculiar " rage-like " effect seen in cats after the injection of tremorine or its metabolite, oxotremorine (Toman, 1963), prompted a study of the effects of oxotremorine. The drug was given in two doses (0.25 and 0.125 mg/kg, intravenously) spaced at an interval of 2 hr in cats protected from the peripheral effects of the drug by an injection of atropine methylbromide (1.5 mg/kg, subcutaneously) 15 min before the first injection. The only biochemical change found after treatment with oxotremorine was a rise in the homovanillic acid content of the caudate nucleus. Small rises in the 5-hydroxytryptamine concentration were obtained with morphine and β-tetrahydronaphthylamine and a small fall with dexamphetamine; in addition, morphine produced a small increase in the concentration of 5-hydroxyindol-3-ylacetic acid.

Dogs. Only β-tetrahydronaphthylamine and dexamphetamine were tested. Both lowered hypothalamic noradrenaline content but, in contrast to the observation on cats, the homovanillic acid level did not rise, nor did that of dopamine fall after amphetamine, indicating a greater susceptibility of the dopamine metabolism in cats than in dogs.

Rabbits. Morphine, β-tetrahydronaphthylamine and dexamphetamine had no effect on the dopamine content of the caudate nucleus of rabbits. Though, with the exception of morphine, these drugs had pronounced excitatory effects, only a slight fall in hypothalamic noradrenaline level was observed after amphetamine.

Behavioural effects. Morphine in cats caused an obvious central excitation which ranged from "hallucinations " to an increase in motor activity, hissing and occasional convulsions. There was also increased salivation, mydriasis and panting. Rabbits were, if anything, sedated by morphine and respiration was depressed.

β-Tetrahydronaphthylamine caused in cats, dogs and rabbits pupillary dilatation, salivation and intense excitement characterized by increased alertness, apprehension and bouts of violent activity. From time to time, some cats and rabbits showed muscular rigidity and tremor. There was usually panting and an increase in body temperature.

Dexamphetamine caused alertness in all species, but differed from β-tetrahydronaphthylamine in causing specific patterns of motor activity. Cats showed rhythmic head movements and paced about the cage, dogs continually circled the pen and rabbits chewed the bars of the cage. These activities were accompanied by a lack of response to external stimuli but

TABLE 2

THE EFFECT OF SOME EXCITANT DRUGS ON THE MEAN CONTENTS OF SOME AMINES AND THEIR ACID METABOLITES IN BRAIN TISSUE

The noradrenaline content was measured in the hypothalamus, dopamine and homovanillic acid in the caudate nucleus and the indole derivatives in the thalamus of cats and the caudate nucleus of dogs. Values are means and standard errors with numbers of observations in parentheses; one determination of each compound was made in each animal. With groups of less than three animals, actual values are given. * $P<0.05$ and ** $P<0.01$, significant difference from own control animals; † $P<0.05$ and ‡ $P<0.01$, significant difference from all control animals. I.p., Intraperitoneal; s.c., subcutaneous; i.v., intravenous

Species	Drug	Dose (mg/kg) and duration of treatment	Mean tissue content (μg/g fresh tissue) of: Noradrenaline	Dopamine	Homovanillic acid	5-Hydroxy-tryptamine	5-Hydroxyindol-3-yl-acetic acid
Cat	Morphine	50 i.p., 1 hr	1·6±0·25 (3)	8·3±0·6 (3)	—	—	—
	Morphine	30 i.p., 4 hr	0·9±0·17 (4)‡	10·0±1·8 (4)	—	—	—
	Control	—	1·4±0·19 (4)	10·0±1·5 (4)	—	—	—
	Morphine	30 s.c., 4 hr	1·0±0·12 (8)‡**	9·2±0·5 (9)	4·1±0·8 (9)‡	0·38±0·03 (9)‡	0·34±0·03 (9)‡*
	Control	—	2·0±0·25 (4)	8·0±1·1 (4)	2·1±0·3 (4)	0·29±0·04 (4)	0·23±0·02 (4)
	β-Tetrahydro-naphthylamine	30 s.c., 4 hr	0·9±0·08 (7)‡**	10·2±1·2 (7)	6·8±0·9 (7)‡*	0·47±0·03 (5)‡*	0·23±0·04 (5)
	Control	—	1·7±0·15 (3)	11·3±0·9 (3)	2·5±1·0 (3)	0·36±0·02 (3)	0·24±0·02 (3)
	Dexamphetamine	10 s.c., 4 hr	1·6±0·15 (12)‡**	4·0±0·4 (13)‡**	2·7±0·3 (6)†*	0·22±0·02 (13)‡*	0·22±0·02 (6)
	Control	—	2·0±0·10 (14)	7·1±0·7 (14)	2·1±0·1 (9)	0·29±0·02 (14)	0·21±0·01 (9)
	Ephedrine	50 s.c., 4 hr	2·5, 2·2	5·4, 6·9	1·6, 1·9	0·35, 0·39	0·22, 0·23
	Control	—	2·2	6·2	1·6	0·37	0·23
	Oxotremorine	0·375 i.v., 3 hr	1·7±0·27 (4)	7·0±0·3 (4)	4·1±0·2 (4)‡**	0·23±0·03 (4)	0·25±0·01 (3)
	Control	—	2·0±0·22 (4)	7·2±0·4 (4)	1·7±0·2 (4)	0·20±0·02 (4)†	0·23±0·01 (4)
	Total controls	—	1·9±0·09 (30)	8·0±0·5 (30)	2·0±0·2 (21)	0·29±0·02 (26)	0·22±0·01 (21)

TABLE 2—(*continued*)

Species	Drug	Dose (mg/kg) and duration of treatment	Mean tissue content (μg/g fresh tissue) of				
			Noradrenaline	Dopamine	Homovanillic acid	5-Hydroxy-tryptamine	5-Hydroxyindol-3-yl-acetic acid
Dog	β-Tetrahydro-naphthylamine	30 s.c., 4 hr	0·4±0·12 (3)‡*	7·7±0·3 (3)	10·0±1·1 (3)	0·23±0·05 (3)	0·14±0·04 (3)
	Control	—	1·3, 1·1	7·1, 7·1	13·3, 13·5	0·23, 0·24	0·08, 0·19
	Dexamphetamine	10 s.c., 4 hr	0·9±0·12 (4)‡*	5·7±0·4 (4)	9·9±0·2 (4)†	0·15±0·02 (4)‡*	0·17±0·03 (4)
	Control	—	1·6, 1·7	5·8, 6·0	10·8, 17·4	0·26, 0·31	0·19, 0·20
	Total controls	—	1·6±0·09 (4)	6·5±0·3 (4)	13·8±1·4 (4)	0·26±0·02 (4)	0·17±0·03 (4)
Rabbit	Morphine	15–80 i.v., 1 hr	1·1±0·20 (5)†	9·1±1·1 (5)	—	—	—
	Control	—	1·6±0·25 (5)	9·9±0·8 (5)	—	—	—
	Morphine	50 i.p., 4 hr	1·7±0·16 (5)	8·9±0·8 (4)	—	—	—
	Control	—	1·5±0·14 (6)	6·9±1·4 (6)	—	—	—
	β-Tetrahydro-naphthylamine	15 i.v., 4 hr	1·5±0·17 (4)	10·3±0·7 (4)	—	—	—
	Control	—	1·7±0·12 (4)	8·3±0·7 (4)	—	—	—
	Dexamphetamine	15 s.c., 4 hr	1·0±0·06 (3)†*	5·9±1·1 (3)	—	—	—
	Control	—	1·5±0·15 (4)	4·5±0·2 (4)†	—	—	—
	Total controls	—	1·5±0·09 (17)	7·8±0·6 (16)	—	—	—

occasionally also by aggressiveness. There was salivation, mydriasis and panting, and an increased body temperature. Ephedrine was used in two cats as a control for amphetamine; they became alert and irritable but did not show the specific patterns of motor activity seen after amphetamine.

Oxotremorine caused an immediate response consisting of extreme " rage " and aggression, which was, however, quite different from that produced by other drugs. The animal's actions were much more co-ordinated, closely resembling the actions of a normal cat playing with its captured prey; there was purposeful response to external stimuli such as an immediate attack upon another cat placed in the same cage. The effect lasted approximately 1.5 hr; a subsequent dose (0.125 mg/kg, intravenously) given 2 hr after the first caused a lesser and briefer response of a similar pattern.

Tranquillizing phenothiazines

Biochemical effects. Four phenothiazine derivatives used as tranquillizers in patients were examined in cats for their effect on the content of amines and their metabolites in brain tissue. Table 3 summarizes the results. Chlorpromazine and thioridazine were compared 4 hr after a single administration. Chlorpromazine (10 mg/kg) lowered the dopamine content and increased the homovanillic acid content of the caudate nucleus but did not lower the hypothalamic noradrenaline level. In contrast, single doses of thioridazine (10 and 50 mg/kg) decreased the concentration of noradrenaline but did not lower the dopamine level. Like chlorpromazine, they increased the homovanillic acid content of the caudate nucleus. The 5-hydroxytryptamine content was lowered by the larger dose. After chronic administration of chlorpromazine, trifluoperazine and thioproperazine the only change observed was an increase in the content of homovanillic acid in the caudate nucleus, whereas thioridazine was without effect on any of the compounds measured.

Behavioural effects. After a single administration of chlorpromazine the cats became quiet, withdrawn and ataxic. The nictitating membranes were relaxed; the animals were not hypothermic when killed. A single injection of thioridazine (10 mg/kg) had little apparent effect initially, but 4 hr after injection the animals were quiet, walked clumsily and had relaxed nictitating membranes. The high dose of thioridazine (50 mg/kg) caused relaxation of the nictitating membranes and anal sphincter and diarrhoea; the cats were not hypothermic when killed. Cats treated with thioridazine were less quiet than after chlorpromazine, showing periods of calling and walking about the cage, and one cat treated with the large dose had pronounced rigidity of all limbs and tremor.

Treatment with phenothiazine drugs was prolonged in an attempt to produce conditions similar to those of patients showing Parkinsonism-like side-effects on chronic phenothiazine treatment. Trifluoperazine was given subcutaneously; this caused ulceration at the site of injection in some animals. For this reason, the other drugs were given orally, in capsules. All drugs were given in doses sufficient to cause an observable degree of tranquillization, without causing loss of weight or other noticeable deterioration in condition. No signs of tremor, rigidity, plasticity or anything that could be described as a Parkinsonian syndrome were observed. The animals moved little of their own volition, but were quite capable of normal movement. Any aggression or fear in response to handling or administration of drugs remained unchanged throughout treatment. Thioridazine produced side-effects, the animals suffering from diarrhoea, which prevented a higher dose from being used; some of the treated animals had relaxed nictitating membranes.

TABLE 3

THE EFFECT OF SOME PHENOTHIAZINE TRANQUILLIZERS ON THE MEAN CONTENTS OF SOME AMINES AND THEIR ACID METABOLITES IN BRAIN TISSUE

See Table 2 for detailed caption. All experiments were on cats

Drug	Dose (mg/kg) and duration of treatment	Mean tissue content (μg/g fresh tissue) of				
		Noradrenaline	Dopamine	Homovanillic acid	5-Hydroxy-tryptamine	5-Hydroxyindol-3-yl-acetic acid
Acute experiments						
Chlorpromazine	10 s.c., 4 hr	2·0±0·25 (5)	4·6±0·4 (9)‡**	3·6±0·4 (5)‡*	0·27±0·04 (5)	0·18±0·01 (5)
Control	—	1·6±0·19 (5)	6·7±0·5 (8)	2·0±0·4 (5)	0·24±0·03 (5)	0·28±0·07 (5)
Thioridazine	10 s.c., 4 hr	2·0±0·15 (4)**	6·2±0·3 (4)	3·7±0·3 (4)‡**	0·23±0·07 (4)	0·28±0·09 (4)
Thioridazine	50 i.p., 4 hr	1·3±0·13 (4)‡**	7·0±0·8 (4)	6·0±1·2 (4)‡*	0·12±0·003 (3)†*	0·33±0·08 (3)
Control	—	2·7±0·08 (4)	7·4±0·9 (4)	2·1±0·1 (4)	0·21±0·03 (4)	0·19±0·03 (4)
Total controls	—	2·1±0·21 (9)	6·9±0·4 (12)	2·1±0·2 (9)	0·22±0·02 (9)	0·24±0·04 (9)
Chronic experiments						
Chlorpromazine	20 oral, 14 days	1·4±0·08 (4)	5·3±0·3 (4)	3·1±0·2 (4)‡**	0·32±0·05 (4)	0·15±0·06 (4)
Control	—	1·8±0·10 (4)	6·6±0·9 (4)	2·1±0·2 (4)	0·37±0·03 (4)	0·26±0·06 (3)
Trifluoperazine	8 s.c., 12 days	1·8±0·12 (4)	5·5±0·9 (4)	3·6±0·2 (4)‡**	0·23±0·03 (4)	0·30±0·05 (4)
Control	—	1·8±0·14 (4)	6·8±0·1 (4)	2·2±0·4 (4)	0·19±0·05 (4)	0·34±0·07 (4)
Thioproperazine	100 oral, 14 days	1·6, 1·7	5·7±0·4 (3)	3·5±0·4 (3)‡*	0·28±0·04 (3)	0·16±0·03 (3)
Control	—	1·4±0·09 (3)	3·5±0·9 (3)†	1·6±0·5 (3)	0·29±0·03 (3)	0·25±0·03 (3)
Thioridazine	15 oral, 14 days	1·6±0·04 (4)	8·3±0·3 (4)	2·4±0·3 (4)	0·25±0·02 (4)	0·17±0·006 (4)
Control	—	1·4±0·05 (3)	8·7±1·2 (3)	2·6±0·2 (3)	0·34±0·06 (3)	0·15±0·004 (3)
Total controls	—	1·6±0·07 (14)	6·5±0·6 (14)	2·1±0·2 (14)	0·29±0·03 (14)	0·24±0·03 (14)

Other drugs

A number of other drugs were examined before and during the course of the above experiments. The first four were used to test the methods; the remaining three in order to investigate drugs used clinically to alleviate Parkinsonism. The results are given in Table 4.

Dopa, the amino acid precursor of dopamine, was used to increase dopamine metabolism. It was given intraperitoneally to cats; in dogs anaesthetized with pentobarbitone it was infused slowly into one carotid artery, the other having been tied off. In both species, the dopamine content of the caudate nucleus increased, and so did the homovanillic acid content in dogs. In cats the homovanillic acid content did not rise, due probably to the short duration of the experiment.

Reserpine (2 mg/kg) reduced the content of all amines in the cat brain and caused a corresponding increase in the two acid metabolites. Guanethidine given to cats had no appreciable effect on catechol amine or homovanillic acid contents. Pentobarbitone anaesthesia had no effect on the brain amines or the acids.

Atropine given in a large dose (25 mg/kg, intraperitoneally) to cats had no effect on amines or their metabolites. It produced pronounced peripheral and mild behavioural effects, the animals remaining quiet when undisturbed but later becoming excitable when handled.

Diethazine (50 mg/kg) and caramiphen (1 mg/kg) had no effect on brain amines and metabolites when given to dogs as a single dose. No behavioural effects were observed.

DISCUSSION

The aim of the present study was to investigate the effect of drugs on the concentrations in the brain of certain amines and their major metabolites in an attempt to see whether there was any correlation between the behavioural effect of the drug and changes in the content or rate of turnover of an amine in the brain. An increase in the rate of turnover of dopamine was suspected to have taken place whenever an increase in the content of homovanillic acid was observed, particularly when this occurred without a corresponding increase in the metabolite derived from 5-hydroxytryptamine. However, the possibility that a restriction on the outflow of acidic substances from the brain can be produced by a drug cannot be discounted especially when more than one acidic metabolite is increased.

The effects of the drugs which cause central sympathetic excitation on the concentration of the amines in the brain confirm and extend earlier observations and once again illustrate the different responses that are observed with different species. The changes observed with these drugs in the 5-hydroxytryptamine concentration were increases after morphine and β-tetrahydronaphthylamine and a fall after dexamphetamine. None of these effects were very pronounced. Previous authors (Brodie *et al.*, 1956, and Paasonen & Giarman, 1958, using other species, and Maynert, Klingman & Kaji, 1962, using the dog) found morphine ineffective and dexamphetamine was a depleting agent only in very high doses (Paasonen & Vogt, 1956). Whereas the concentration of dopamine was only exceptionally affected in the cat by drugs which cause central sympathetic excitation, that of homovanillic acid was raised by four of the five drugs tested. The metabolism of dopamine in the caudate nucleus of the cat, like that of the noradrenaline in the hypothalamus of this species, is peculiarly sensitive to drug action. This is illustrated by the fact that the two

TABLE 4

THE EFFECT OF SOME OTHER DRUGS ON THE MEAN CONTENTS OF SOME AMINES AND THEIR ACID METABOLITES IN BRAIN TISSUE

See Table 2 for details. I.c.a., Intracarotid arterial. ø 5-Hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid were measured in the massa intermedia of the thalamus

Species	Drug	Dose (mg/kg) and duration of treatment	Mean tissue content (μg/g fresh tissue) of				
			Noradrenaline	Dopamine	Homovanillic acid	5-Hydroxy-tryptamine	5-Hydroxyindol-3-ylacetic acid
Cat	Dopa	300 i.p., 30 min	—	8·1, 11·7*	1·1, 1·4	—	—
	Reserpine	2 i.p., 2 hr	1·4, 1·8	<1·0 (5)‡**	5·1±0·5 (5)‡**	0·33, 0·38ø	0·47, 0·70ø
	Control	—	3·3	4·9±0·7 (4)	2·0±0·5 (4)	0·98ø	0·16ø
	Guanethidine	50 i.p., 4 hr	2·1, 2·3	7·8, 6·3	2·1, 4·3	—	—
	Control	—	1·4	7·0	4·0	—	—
	Pentobarbitone	50 i.p., 0·5–1 hr	1·4, 1·9	9·5, 11·7	1·5, 3·2	0·18, 0·29	0·10, 0·23
	Control	—	1·9, 2·0	9·4, 11·1	2·7, 3·3	0·33, 0·44	0·36, 0·42
	Atropine	25 i.p., 3 hr	1·5, 2·2	7·6, 9·9	2·1, 2·3	0·23, 0·25	0·23, 0·28
	Control	—	2·0	8·9	2·7	0·25	0·25
	Total controls	—	2·1±0·3 (5)	6·9±1·0 (8)	2·6±0·4 (8)	—	—
Dog	Dopa	15 i.c.a., 1–1·5 hr	—	6·6±0·7 (3)**	17·1±1·5 (3)†*	—	—
	Control	—	—	2·7±0·3 (4)	10·0±1·2 (4)	—	—
	Diethazine	50 s.c., 4·5 hr	1·4, 1·6	5·8, 6·3	12·8, 13·8	0·28, 0·30	0·17, 0·22
	Caramiphen	1 s.c., 4·5 hr	1·4, 1·5	8·0, 10·0	13·2, 13·5	0·27, 0·28	0·14, 0·22
	Control	—	1·1, 1·8	6·0, 7·2	11·6, 14·8	0·30, 0·35	0·18, 0·23
	Total controls	—	—	4·0±0·9 (6)	11·4±1·4 (6)	—	—

drugs (β-tetrahydronaphthylamine and dexamphetamine) which were tested in cats and dogs increased the homovanillic acid in the cat only. If one wished to correlate the behavioural effect of the two related drugs, dexamphetamine and ephedrine, with their different actions on the metabolism of dopamine in the cat, the only clear sign produced by dexamphetamine and not by ephedrine was the stereotyped forced motor activity.

Oxotremorine is the active metabolite formed in the liver from tremorine (1,4-pyrrolidine-2,3-butyne; Cho, Haslett & Jenden, 1961; Welch & Kocsis, 1961), a substance reported to cause tremor in mice, rats and dogs similar to Parkinsonian tremor (Everett, 1961). In cats, an intense, co-ordinated excitation was produced with little or no tremor, and this was accompanied by an increase in the homovanillic acid in the caudate nucleus.

The increase in homovanillic acid in the caudate nucleus frequently occurs together with a loss of noradrenaline from the hypothalamus. This points to the possibility that under these conditions both dopamine and noradrenaline are being utilized to a greater extent than normal. Since the synthesis of dopamine is faster than that of noradrenaline (Holzer & Hornykiewicz, 1959; Udenfriend & Zaltzman-Nirenberg, 1963), it is not surprising that the dopamine content of the tissue is unchanged whereas that of noradrenaline is reduced. One might expect that under these conditions not only the acid metabolite of dopamine, homovanillic acid, but also the corresponding metabolite of noradrenaline, vanillylmandelic acid, would be raised in those tissues in which the metabolism of noradrenaline is increased. The investigation of this question has not yet been possible because the methods for determining vanillylmandelic acid are not yet sufficiently sensitive.

The phenothiazine derivatives selected for examination were chlorpromazine, trifluoperazine, which causes a high incidence of Parkinsonian side-effects in man (Ayd, 1961), thioridazine, for which a low incidence of these side-effects has been reported (Cole & Clyde, 1961), and thioproperazine, which has a potent catatonic action in rats (Leslie & Maxwell, 1964). Chlorpromazine has been reported to increase the acid metabolites of dopamine in the brain of the rabbit (Andén *et al.*, 1964). Though evidence was obtained in the present work that all four drugs are able to affect the metabolism of dopamine, the effect of thioridazine in the doses used differed from that of the other drugs. After a single dose of thioridazine the caudate nucleus was not depleted of dopamine as was observed with chlorpromazine. After repeated administration any changes in the content of homovanillic acid which were produced initially had disappeared, whereas they persisted with the other three phenothiazine derivatives. In addition, the acute administration of thioridazine caused a depletion of the hypothalamic noradrenaline, an effect which was not seen with chlorpromazine. After thioridazine, the incidence of Parkinsonism is reported to be much lower than after the other phenothiazine drugs used in this work. The observation that it affects amine metabolism in the brain differently from its congeners may have some bearing on this property.

The experiments also show that drugs may have similar behavioural effects but different biochemical actions on amines in the brains of different species; furthermore, that drugs as closely related chemically as the phenothiazine tranquillizers may each have characteristic behavioural and biochemical effects. It is only possible to conclude that there are no simple rules by which the behavioural effect of a drug can be correlated with changes in the metabolism of amines in the brain.

SUMMARY

1. The effects of sixteen drugs on the content of certain amines and their metabolites in brain tissue were studied in an attempt to see whether drugs with central effects caused any change in amine metabolism.

2. The contents of noradrenaline in the hypothalamus and dopamine in the caudate nucleus of cats, dogs and rabbits, of homovanillic acid in the caudate nucleus of cats and dogs, and of 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid in the thalamus of cats and the caudate nucleus of dogs, were measured fluorimetrically following chromatographic separation or solvent extraction.

3. Of those drugs which cause central sympathetic stimulation or " excitement " in cats, morphine (30 to 50 mg/kg), β-tetrahydronaphthylamine (30 mg/kg) and dexamphetamine (10 mg/kg) lowered the noradrenaline content, but only dexamphetamine lowered the dopamine content. All three drugs and also oxotremorine (0.38 mg/kg) increased the homovanillic acid concentration. Morphine and β-tetrahydronaphthylamine slightly increased the 5-hydroxytryptamine content whereas dexamphetamine slightly reduced it; morphine caused a small rise in the 5-hydroxyindol-3-ylacetic acid content. Ephedrine (50 mg/kg) had no effect on any amine or metabolite content.

4. In dogs, β-tetrahydronaphthylamine (30 mg/kg) and dexamphetamine (10 mg/kg) lowered hypothalamic noradrenaline and dexamphetamine lowered the 5-hydroxytryptamine content. No effect on dopamine metabolism was seen. In rabbits, morphine (15 to 80 mg/kg), β-tetrahydronaphthylamine (15 mg/kg) and dexamphetamine (15 mg/kg) produced no change in dopamine level, but dexamphetamine reduced the hypothalamic noradrenaline.

5. Of the phenothiazine derivatives tested in cats, chlorpromazine (10 mg/kg) and thioridazine (10 to 50 mg/kg) given as single injections increased the homovanillic acid but only chlorpromazine lowered the dopamine content. Thioridazine, however, lowered the hypothalamic noradrenaline and, in a large dose, the thalamic 5-hydroxytryptamine content. After treatment for 12 to 14 days with chlorpromazine (20 mg/kg, orally), trifluoperazine (8 mg/kg, subcutaneously) and thioproperazine (100 mg/kg, orally), the homovanillic acid content of the caudate nucleus was increased but the other compounds were not changed; after 14 days' treatment with thioridazine (15 mg/kg, orally) no measurable biochemical change had occurred. Thus thioridazine, which, unlike the other phenothiazine derivatives tested, rarely causes Parkinsonism in man, differs from these compounds in its effects on the metabolism of brain catechol amines.

6. Atropine (25 mg/kg), diethazine (50 mg/kg) and caramiphen (1 mg/kg), drugs associated with the treatment of Parkinsonism, were without effect on brain amine metabolism.

7. No simple correlation between the biochemical and the behavioural effects of the drugs could be found.

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