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Effects of chlorpromazine on the metabolism of catecholamines in dog brain

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1. The effect of chlorpromazine (CPZ) on the metabolism of dopamine and 5-hydroxytryptamine in dog brain was investigated by following the concentrations of the acid metabolites of these amines, homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid, in the ventricular cerebrospinal fluid (C.S.F.) of dogs over a period of 5 hr after intravenous administration of CPZ (2·5, 5, 10 and 15 mg/kg), using the technique of serial sampling of lateral ventricular C.S.F. "Low" doses (2·5–10 mg/kg) produced a rise in the concentration of homovanillic acid and smaller increases in the concentrations of 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid. "High" doses (10–15 mg/kg) had a lesser effect on the concentration of homovanillic acid and had no effect on, or decreased, the concentrations of 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid. The concentration of 3,4-dihydroxyphenylacetic acid was maximal in the ventricular C.S.F. 2 hr after CPZ 5 mg/kg and was unaltered from the control level 2 hr after 15 mg/kg.

2. The effects on the metabolism of brain amines of CPZ (5 mg/kg), doses which the serial sampling of C.S.F. experiments had indicated as producing maximal and minimal effects on dopamine metabolism in brain tissue, were studied by estimating the concentrations of adrenaline, noradrenaline, dopamine, metanephrine, methoxydopamine, homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid in the hypothalamus, midbrain, thalamus, hindbrain, cortex, globus pallidus and caudate nucleus of control dogs and of dogs treated with CPZ intravenously 2 hr before killing. The concentrations of homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid were estimated in samples of ventricular C.S.F. withdrawn from these dogs 2 hr after the injection of CPZ (i.e., immediately before death).

3. The following changes in concentrations were observed. Dopamine: CPZ 5 mg/kg produced no change in the concentration in the caudate nucleus, globus pallidus and midbrain and increased the concentration in the thalamus; CPZ 15 mg/kg appeared to cause a reduction in the concentration of this amine in the caudate nucleus and globus pallidus. Homovanillic acid and 3,4-dihydroxyphenylacetic acid: CPZ 5 mg/kg increased the concentrations of both acids in the caudate nucleus and had no effect on the concentrations

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of the acids in the globus pallidus, hypothalamus and thalamus; CPZ 15 mg/kg produced no change in the concentrations of the acids in any area of the brain. Methoxydopamine: CPZ 5 mg/kg and 15 mg/kg reduced the concentration in the caudate nucleus. Noradrenaline: The concentrations in the hypothalamus, midbrain, thalamus and hindbrain were slightly increased by CPZ 5 mg/kg and 15 mg/kg. Only in the thalamus was a statistically significant increase in noradrenaline observed.

4. It was concluded that the actions of chlorpromazine on catecholamine synthesis and metabolism in the brain of the dog are dose dependent. A dose of CPZ 5 mg/kg was postulated to have the following actions: (i) to increase dopamine synthesis; (ii) to activate mitochondrial monoamine oxidase. A dose of CPZ 15 mg/kg was postulated to act as follows: (i) to decrease dopamine synthesis; or (ii) to release dopamine from its storage sites.

5. The ratios of the concentrations of homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid in the caudate nucleus to the concentrations of these acids in the ventricular C.S.F. were the same in the control dogs as in the dogs treated with CPZ (5 mg/kg and 15 mg/kg). It was concluded that the levels of the acid metabolites of dopamine in lateral ventricular C.S.F. reflect the levels of these acids in the caudate nucleus.

It has been postulated that chlorpromazine affects the synthesis and/or breakdown of catecholamines in the brain (Carlsson & Lindqvist, 1963). Malhotra & Prasad (1962) found that an intravenous dose of 5 mg/kg of this drug increased, while one of 25 mg/kg decreased, the noradrenaline concentrates in the hypothalamus and midbrain of the dog. Gey & Pletscher (1961) found that chlorpromazine in a dose of 20 mg/kg, given intraperitoneally, had no effect on the concentrations of dopamine and noradrenaline in whole rat brain, and Laverty & Sharman (1965b) reported that a dose of 10 mg/kg, given intravenously, caused a fall in the concentration of dopamine in the caudate nucleus of the cat. Possible reasons for these differing results are that the response to chlorpromazine is dependent on the species, the dose and the length of time between administration of the drug and the killing of the animal.

The influence of the latter two factors on chlorpromazine-induced alterations in the levels of some phenolic acids in cerebrospinal fluid (C.S.F.) is demonstrated in this paper, using a method (Ashcroft, Crawford, Dow & Guldberg, 1968) of repeated sampling and analyses of lateral ventricular C.S.F. in dogs.

The use of estimates of the concentration of phenolic acids in ventricular C.S.F. as an indication of the concentrations of these acids in the brain tissue assumes that there is a direct relationship between the concentrations of an acid metabolite in the C.S.F. and in the brain. This hypothesis was tested by comparing the 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid), 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid in the lateral ventricular C.S.F. and in the caudate nucleus from control dogs and from dogs which had received chlorpromazine in a dose (5 mg/kg), which increases the concentrations of these acids in the C.S.F.

Methods

Effect of different doses of chlorpromazine on the concentrations of homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid in lateral ventricular C.S.F. of dogs, using the technique of serial sampling of the C.S.F.

Five beagle dogs, both males and females, weighing about 10 kg and aged 1–3 yr, with chronically implanted guide-tubes to allow sampling of lateral ventricular C.S.F. were anaesthetized intravenously with thiopentone (Pentothal, Abbott Labs.) (Ashcroft *et al.*, 1968). Anaesthesia was maintained throughout the experiments. On different occasions, each dog received intravenously each of the following doses of chlorpromazine (Largactil Inj. B.P., May and Baker)—2·5, 5, 10 and 15 mg/kg, the order of the injections being randomized for each animal with an interval of at least 1 week between doses. In each experiment a 0·5 ml. sample of C.S.F. was withdrawn before the administration of the drug and at intervals of 1 hr afterwards for a period of 5 hr. Body temperature was maintained within 1° C (rectal temperature) by keeping the animals in a thermostatically controlled environmental temperature of 27° C. The samples of C.S.F. were stored at −20° C for not longer than 1 week until the estimations of the concentrations of homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid were carried out (Ashcroft *et al.*, 1968).

Effect of chlorpromazine on the concentrations of the catecholamines and their metabolites in discrete areas of dog brain and on the concentrations of homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid in the lateral ventricular C.S.F. of the same dogs

Beagles, which had been prepared for serial sampling of C.S.F., were also used in these experiments. At least 1 week elapsed between the last sample of C.S.F. taken and these acute experiments. Five to ten minutes after induction of anaesthesia by intravenous sodium thiopentone, 0·5 ml. of lateral ventricular C.S.F. was withdrawn (Ashcroft *et al.*, 1968). Chlorpromazine was then administered, intravenously, to four dogs in a dose of 5 mg/kg and to four dogs in a dose of 15 mg/kg. Another eight dogs, for use as controls, were maintained under thiopentone anaesthesia for 2 hr but did not receive chlorpromazine. Experiments on control and treated dogs were interspersed in time. Two hours after induction of anaesthesia, 0·5 ml. of lateral ventricular C.S.F. was withdrawn (Ashcroft *et al.*, 1968) and the animal killed by exanguination from a cannulated femoral artery.

The vault of the skull was opened and the whole brain removed and halved sagittally. The following areas were dissected and immediately wrapped in aluminium foil and frozen with solid carbon dioxide: (i) hypothalamus—from the optic chiasma to and including the mammillary bodies and demarcated above by the thalamus; (ii) midbrain—that part of the brain stem extending from the posterior border of the hypothalamus to, but excluding, the pons; (iii) thalamus; (iv) hindbrain—the remainder of the brain stem extending from and including the pons, to the beginning of the spinal cord; (v) cortex—the grey matter of the occipital cortex with as little white matter as possible; (vi) globus pallidus—dissection was based on coronal section (Lim, Liu & Moffitt, 1960, Fig. 20, R20) and included globus pallidus and putamen which were found to lie between the

cortex and the internal capsule, after removal of the thalamus ; (vii) caudate nucleus —both nuclei were dissected. The length of time between killing the animal and freezing the samples of tissue varied from 20 to 60 min.

Analyses of C.S.F.

The concentrations of homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid in the samples of C.S.F. were determined fluorimetrically on the day following, overnight storage at −20° C (Ashcroft *et al.*, 1968).

Analyses of brain tissues

The whole of each area removed was homogenized in perchloric acid and the protein precipitate removed by centrifugation (Crawford & Yates, unpublished). Two portions were taken of the perchloric acid extracts of the caudate nucleus and midbrain. Known amounts of the catecholamines under investigation, some of their methoxylated amine metabolites and homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid were added to one portion from each of these extracts to determine the recoveries of the amines and acids through the whole procedure. The following amounts were added to a duplicate portion of the caudate nucleus extract: 2 μg homovanillic acid, 0·5 μg 3,4-dihydroxyphenylacetic acid, 0·5 μg 5-hydroxyindolylacetic acid, 0·2 μg noradrenaline, 0·1 μg or 0·2 μg adrenaline and metanephrine, 1·0 μg dopamine and 0·4 μg methoxydopamine (3-methoxytyramine). To a duplicate portion of the midbrain extract were added: 0·4 μg homovanillic acid, 0·5 μg 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid, 1·0 μg noradrenaline, 0·4 μg adrenaline and metanephrine and 0·2 μg dopamine and methoxydopamine.

The pH of the perchloric acid extracts was adjusted to 4·0 with potassium hydroxide to precipitate potassium perchlorate. The extract was then acidified to pH 1–2, saturated with sodium chloride and the acid metabolites extracted into ethyl acetate (Crawford & Yates, unpublished). Mechanical carry-over of traces of dopamine from the aqueous into the ethyl acetate phase could give rise to overestimates of 3,4-dihydroxyphenylacetic acid, because both react similarly in the fluorimetric method of estimation. In order to reduce the amounts of dopamine and other amines which might be present in the ethyl acetate, the ethyl acetate fraction was shaken for 5 min with 2 ml. 0·01 N hydrochloric acid, which had been saturated with sodium chloride. This acid wash was discarded in the first group of experiments, consisting of four control dogs and four dogs which had received chlorpromazine 5 mg/kg, since preliminary experiments had shown that, in pure solution, there was no detectable loss by carry-over of the catecholamines into ethyl acetate from an acid aqueous solution. It was subsequently found that a significant amount of noradrenaline was removed into ethyl acetate from acid extracts of hypothalamus, and therefore the acid wash was added to the aqueous phase left after extraction with ethyl acetate in the second group of experiments of four control dogs and four dogs treated with chlorpromazine 15 mg/kg. The ethyl acetate fraction containing the acid metabolites was stored overnight at −20° C. On the following day the acid metabolites were extracted into alkaline Tris buffer and the concentrations of homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid determined fluorimetrically in separate portions of this

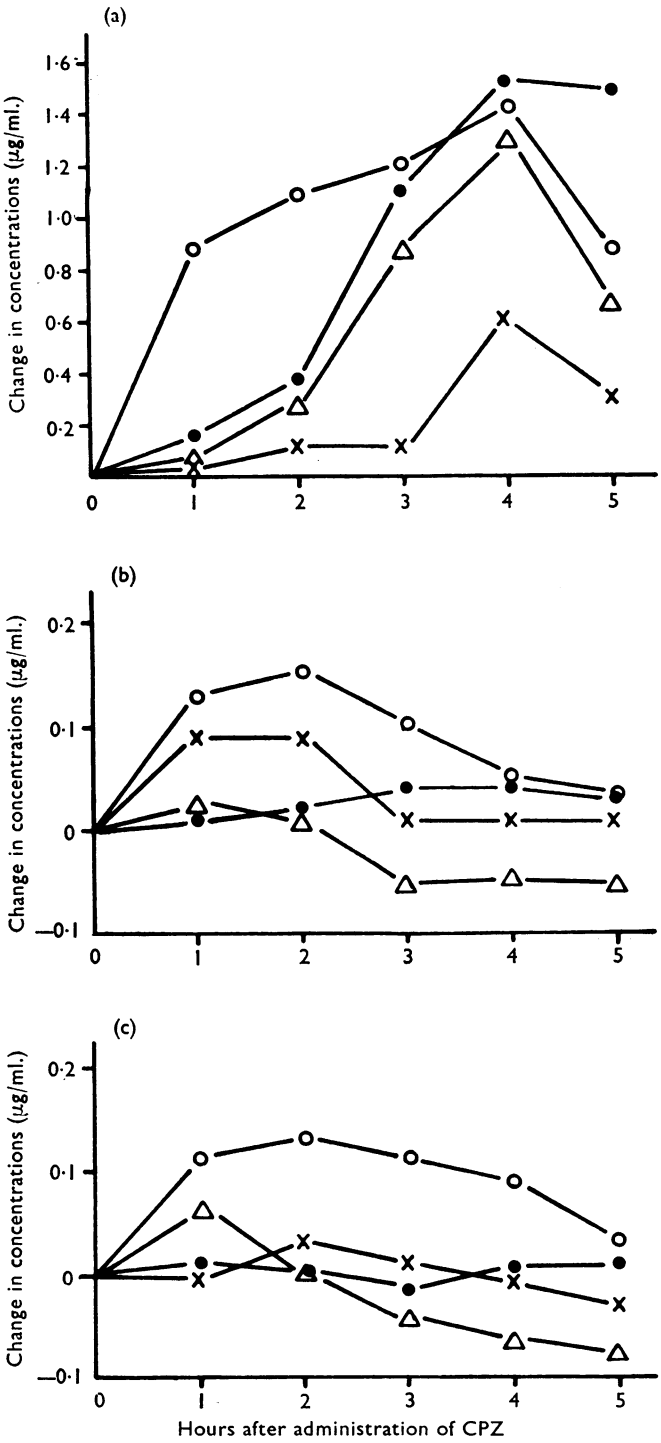

FIG. 1. Effect of chlorpromazine (CPZ) administered intravenously in doses of 2·5 (×——×), 5 (○——○, 10 (●——●) and 15 (△——△) mg/kg on the concentrations of homovanillic acid (a), 3,4-dihydroxyphenylacetic acid (b) and 5-hydroxyindolylacetic acid (c) in lateral ventricular C.S.F. of a dog. Concentrations (μg/ml. C.S.F.) of each acid expressed as change from the "control concentration" at zero time. The results are from experiments on the same dog.

buffer extract (Ashcroft *et al.*, 1968). The cortical extract was used as a tissue blank in the estimation of the phenolic acids. In control experiments, there was no detectable quenching of the fluorescence from 200 mμg of homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid by extracts from control and chlorpromazine treated (15 mg/kg) dogs. Internal standards were therefore not included routinely in the estimations.

The procedure from this stage onwards was as described by Crawford & Yates (unpublished). After removal of the phenolic acids into ethyl acetate, the amines in the acid aqueous extract were acetylated. The acetylated amines were extracted into dichloromethane, separated by paper chromatography and estimated fluorimetrically. Internal standards were not determined routinely, because it was found that the fluorescence intensities from 100 mμg amounts of the acetylated amines to eluates of chromatograms from extracts of caudate nucleus and midbrain from both control and chlorpromazine (15 mg/kg) treated dogs were not significantly different from aqueous standards.

The mean recoveries ($\pm$S.D.) of homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid added to tissue homogenates were $84 \pm 12\%$, $84 \pm 20\%$ and $79 \pm 12\%$, respectively. Recoveries of the amines added to homogenates varied but did not appear to reflect the recoveries of the corresponding endogenous amines (Crawford & Yates, unpublished). No correction for recovery of the acid or amine estimates was made.

The significance of difference in concentrations of amines and of acid in brain regions of control and drug-treated animals was determined by Student's *t* test.

TABLE 1. *Mean increase in the concentrations of homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid in lateral ventricular C.S.F. of dogs treated with chlorpromazine 5 mg/kg and 15 mg/kg, given intravenously*

Time after administration of chlorpromazine (hr)	Mean increase in acid concentrations after intravenous chlorpromazine in doses of: 5 mg/kg	15 mg/kg
Homovanillic acid		
0	0(2·36±0·73	0(2·29±0·46)
1	0·40±0·30	0·09±0·07
2	0·54±0·30	0·34±0·24
3	0·81±0·29	0·86±0·60
4	0·92±0·30	0·61±0·56
5	0·62±0·22	0·56±0·61
3,4-Dihydroxyphenylacetic acid		
0	0(0·24±0·07)	0(0·21±0·06)
1	0·05±0·05	−0·01±0·05
2	0·07±0·05	0·01±0·03
3	0·06±0·02	0·01±0·05
4	0·03±0·01	−0·05±0·05
5	0·02±0·01	−0·01±0·02
5-Hydroxyindolylacetic acid		
0	0(0·28±0·04)	0(0·32±0·04)
1	0·04±0·07	0·04±0·03
2	0·07±0·03	0·02±0·05
3	0·05±0·04	0·00±0·03
4	0·04±0·05	−0·02±0·03
5	0·00±0·02	−0·01±0·00

The mean increase (μg/ml. C.S.F.) with the standard deviation, quoted for any one time interval is the average rise observed in experiments on five different animals. Control values for the concentrations (μg/ml. C.S.F.) of the acids in C.S.F. ($\pm$S.D.) are given in parenthesis at zero time.

Results

Effect of different doses of chlorpromazine on the concentrations of homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid in the lateral ventricular C.S.F. of dogs, using the technique of serial sampling of C.S.F.

The changes in the concentrations of homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid in the C.S.F. induced by chlorpromazine were found to be dependent on the dose and the time interval between the administration of the drug and the sampling of the C.S.F. In Fig. 1 the changes in the concentrations of these acids in the C.S.F. are plotted against the time after administration of four different doses of chlorpromazine to the same dog. The results shown in this figure are typical of the observations made in the four other dogs although there was considerable variation between dogs in the dose giving a maximum response and in the magnitude of the responses (Guldberg, 1967). These variations are illustrated by the wide scatter of the average changes in the concentrations of homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid in the ventricular C.S.F. of five dogs at the same time intervals after intravenous administration of 5 and 15 mg/kg chlorpromazine (Table 1). An increase in the homovanillic acid concentration in the C.S.F. was usually observed with all doses of chlorpromazine, but the largest rise, as well as an early sharp increase in the concentration of homovanillic acid, followed the administration of the lower dose (5 mg/kg in Fig. 1) of chlorpromazine. A depression of the initial rate of increase and of the peak concentration of homovanillic acid was observed at higher dose levels (Fig. 1). Small increases, maximal at 2 hr, in the concentrations of 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid were found after the lower doses (2·5 and 5 mg/kg in Fig. 1, 5 mg/kg in Table 1) of chlorpromazine while the higher doses (10 and 15 mg/kg in Fig. 1, 15 mg/kg in Table 1) either had no effect on the concentrations of these two acid metabolites or produced a fall below the control concentrations.

Effect of chlorpromazine on the concentrations of catecholamines and their metabolites in discrete areas of dog brain

The effect of chlorpromazine (5 mg/kg and 15 mg/kg) on the concentrations of homovanillic acid, 3,4-dihydroxyphenylacetic acid, 5-hydroxyindolylacetic acid, noradrenaline, dopamine and methoxydopamine in different areas of dog brain and on the three phenolic acids in the lateral ventricular C.S.F. is shown in Tables 2 and 3. The concentrations of adrenaline and metanephrine were also estimated in these experiments. In the first and second control groups of animals adrenaline was found in the hypothalamus in a concentration of $0{\cdot}05 \pm 0{\cdot}02$ (S.D.) μg/g and $0{\cdot}08 \pm 0{\cdot}03$ μg/g, respectively. Neither dose of chlorpromazine altered the hypothalamic adrenaline concentrations. In all other areas of brain, both in control and chlorpromazine treated animals, adrenaline was below the level of detection, $<0{\cdot}03$ μg/g. Metanephrine was just detectable ($0{\cdot}03 \pm 0{\cdot}01$ μg/g) in the hypothalami from the four dogs which had received 5 mg/kg chlorpromazine, but these concentrations were not significantly different from the control values of $<0{\cdot}05$ μg/g. No metanephrine ($<0{\cdot}03$ μg/g) was found in any other area.

TABLE 2. *Effect of chlorpromazine (CPZ), 5 mg/kg given intravenously 2 hr before killing the animals, on the concentrations of catecholamines and their metabolites and of 5-hydroxyindolylacetic acid in various areas of the brains of beagle dogs and on the concentrations of homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid in lateral ventricular C.S.F. of these same dogs immediately before killing*

	Homovanillic acid		3,4-Dihydroxyphenyl-acetic acid		5-Hydroxyindolyl-acetic acid		Noradrenaline		Dopamine		Methoxydopamine	
	Control	CPZ	Control	CPZ	Control	CPZ	Control	CPZ	Control	CPZ	Control	CPZ
Hypothalamus	2·5(3) ±0·1	2·4 ±0·16	0·09(3) ±0·03	0·33(3) ±0·25	0·67 ±0·11	0·79 ±0·11	0·40 ±0·12	0·60 ±0·19	0·15 ±0·05	0·22 ±0·04	<0·06	0·04 0·03
Midbrain	1·8 ±0·3	1·4 ±0·17	0·05(3) ±0·03	0·15 0·09	0·82 ±0·14	1·1 ±0·28	0·22 ±0·06	0·31 ±0·12	0·12 ±0·05	0·12 ±0·04	0·02(3) ±0·02	0·01 0·02
Thalamus	1·6 ±0·4	2·0 ±0·26	0·04 0·08	0·1 0·03	0·48 ±0·16	0·65 ±0·17	0·13 ±0·02	0·24† ±0·06	0·03 ±0·01	0·13* ±0·06	<0·03	0·02(3) ±0·01
Cortex	Not assayed—used as blank						0·04 ±0·03	0·06 0·05	0·06 ±0·03	0·03 0·03	<0·04	0·02 0·02
Globus pallidus	3·9 ±0·8	2·8 ±2·0	0·17(3) ±0·03	0·14 0·44	0·36 ±0·17	0·34 ±0·09	0·11 ±0·08	0·08(3) ±0·05	0·54 ±0·5	0·46 ±0·19	0·10 ±0·04	0·07(3) ±0·05
Caudate nucleus	13·4 ±1·1	17·0† ±1·1	1·3 ±0·27	1·9 ±0·42	0·35 ±0·06	0·46* ±0·04	<0·20(3)	0·13(3) ±0·02	5·5 ±1·4	5·6 ±1·1	0·38(3) ±0·24	0·07(3) 0·16
Lateral ventricular C.S.F.	1·7 ±0·39	2·4 ±0·67	0·21 ±0·04	0·33* ±0·05	0·25 ±0·05	0·35 ±0·10						

Both control and CPZ-treated animals were maintained under thiopentone anaesthesia for the 2 hr period. Concentrations are given in μg/ml. C.S.F. (mean± standard deviation from four experiments unless otherwise indicated by number in parenthesis. Uncorrected for recoveries). The "less than" estimated were calculated on the basis that the smallest measurable amount of a compound was that amount giving rise to a fluorescence reading which was half that of the blank. Differences between mean values of "drug" and control groups when statistically significant are denoted: * $P<0.05$; † $P<0.01$.
The lowest detectable concentration of noradrenaline in the caudate nucleus is about 3–5 times higher than in any other area because the weight of caudate nucleus processed was only 1/3–1/5 the weight of the other areas processed.

TABLE 3. *Effect of chlorpromazine (CPZ), 15 mg/kg given intravenously 2 hr before killing the animals, on the concentrations of catecholamines and their metabolites and of 5-hydroxyindolylacetic acid in various areas of the brains of beagle dogs and on the concentrations of homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid in lateral ventricular C.S.F. of these same dogs immediately before killing.*

	Homovanillic acid		3,4-Dihydroxyphenyl acetic acid		5-Hydroxyindolyl-acetic acid		Noradrenaline		Dopamine		Methoxydopamine	
	Control	CPZ	Control	CPZ	Control	CPZ	Control	CPZ	Control	CPZ	Control	CPZ
Hypothalamus	1·1 ±0·4	1·1 ±0·22	0·18(1)	0·21 0·21	0·35 ±0·1	0·40 ±0·2	0·59 ±0·22	0·86 ±0·34	0·21 ±0·03	0·16 ±0·05	<0·04	<0·04(3)
Midbrain	1·04 ±0·4	1·2 ±0·5	0·16(1)	0·17 0·17	0·51 ±0·17	0·52 ±0·4	0·44 ±0·21	0·44 ±0·15	0·14 ±0·04	0·12 ±0·07	0·02 0·02	<0·03(3)
Thalamus	1·1 ±0·3	1·3 ±0·4	0·18(1)	0·10 (1)	0·31 ±0·09	0·31 0·1	0·20 ±0·07	0·41(3)† ±0·06	0·04 ±0·01	0·04 ±0·02	<0·02	<0·02(3)
Hindbrain	0·17(3) ±0·12	0·24 ±0·05	<0·05(3)	<0·05(3)	0·33(3) ±0·04	0·31 ±0·06	0·28(3) ±0·07	0·43(3) ±0·11	0·02(3) ±0·01	0·02(3) ±0·01	<0·02	<0·02(3)
Cortex	Not assayed—used as blank						0·08(3) ±0·01	<0·12	<0·04	<0·04	<0·02	<0·03(3)
Globus pallidus	2·2 ±1·2	2·2(3) ±0·7	<0·2(2)	0·2 0·2	0·29 ±0·13	0·15 ±0·08	<0·19(3)	0·18(3) ±0·06	0·81 ±0·42	0·27(3) ±0·21	0·09 ±0·04	<0·08(3)
Caudate nucleus	12·5 ±1·1	12·5 ±0·9	1·0 ±0·12	0·94(3) ±0·3	0·24 ±0·05	0·24 ±0·07	0·28(1)	0·29(3) ±0·18	5·5 ±1·6	3·8 ±0·4	0·61 ±0·30	0·17(3)* ±0·05
Lateral ventricular C.S.F.	1·7 ±0·57	1·6(3) ±0·39	0·22 ±0·06	0·20(3) ±0·02	0·19 ±0·08	0·24(3) ±0·05						

Both control and CPZ-treated animals were maintained under thiopentone anaesthesia for the 2 hr period. Concentrations are given in μg/g tissue or μg/ml. C.S.F. (mean±standard deviation from four experiments unless otherwise indicated by number in parenthesis). Uncorrected for recoveries. The "less than" estimates were calculated on the basis that the smallest measurable amount of a compound was that amount giving rise to a fluorescence reading which was half that of the blank. Differences between mean values of "drug" and control groups when statistically significant are denoted: * $P<0.05$, † $P<0.01$.

TABLE 4. *Relation between the concentrations of the phenolic acids in the caudate nucleus and in the lateral ventricular C.S.F. of the dog before and after treatment with chlorpromazine (5 mg/kg and 15 mg/kg intravenously, 2 hr before killing).*

	Chlorpromazine (5 mg/kg intravenously)						Chlorpromazine (15 mg/kg intravenously)					
	Homovanillic acid		3,4-Dihydroxyphenyl-acetic acid		5-Hydroxyindolyl-acetic acid		Homovanillic acid		3,4-Dihydroxyphenyl-acetic acid		5-Hydroxyindolyl-acetic acid	
	Control	CPZ	Control	CPZ	Control	CPZ	Control	CPZ	Control	CPZ	Control	CPZ
Mean of the ratios of concentrations (caudate nucleus μg/g: C.S.F. μg/ml.)	8·1	7·3	6·0	5·9	1·4	1·4	7·7	8·0	5·0	4·8	1·4	1·0
(No. of estimations)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(2)	(4)	(3)	(4)	(3)

Chlorpromazine (5 mg/kg) significantly increased the concentrations of the acid metabolites, had no effect on the dopamine concentration and decreased the methoxydopamine concentration in the caudate nucleus. As was demonstrated in Fig. 1, the concentrations of homovanillic acid and 3,4-dihydroxyphenylacetic acid in the C.S.F. were increased by 5 mg/kg chlorpromazine, although in the present experiments the increase was statistically significant only in the case of 3,4-dihydroxyphenylacetic acid. The means of the estimates of the acid metabolites in the C.S.F. samples from the control dogs after 5–10 min anaesthesia did not differ significantly from the means of the concentrations of these acids in samples of C.S.F. taken from the same dogs after 2 hr anaesthesia. These results are in agreement with those of Ashcroft *et al.* (1968), who showed that the withdrawal of 0·5 ml. ventricular C.S.F. from an anaesthetized dog, if repeated at intervals of longer than 30 min, did not alter the concentrations of homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindolylacetic acid in the successive samples. Chlorpromazine (15 mg/kg) appeared to decrease the concentrations of dopamine and methoxydopamine in the caudate nucleus but had no effect on the concentrations of the acid metabolites of dopamine in the caudate nucleus or, as was also shown in the serial sampling experiments (Fig. 1), in the C.S.F. The reduction in dopamine concentration was only significant at the 10% level. The only results to show significant changes in the other regions of the brain investigated were the concentrations of noradrenaline and dopamine in the thalamus. In this region, chlorpromazine at a dose of 5 mg/kg increased the concentrations of noradrenaline and of dopamine and at a dose of 15 mg/kg increased the concentration of noradrenaline but had no effect on the concentration of dopamine.

Effect of chlorpromazine on the relation between the concentrations of the phenolic acids in the caudate nucleus and in the lateral ventricular C.S.F. in dogs

The concentrations of homovanillic acid in the caudate nucleus and in the lateral ventricular C.S.F. of each dog were expressed as a ratio (Table 4). The mean of these ratios obtained from control dogs was the same as those from dogs which had received chlorpromazine, 5 mg/kg or 15 mg/kg intravenously, 2 hr before killing. The ratios of the concentration of 3,4-dihydroxyphenylacetic acid and of 5-hydroxyindolylacetic acid in the caudate nucleus to the concentration of these acids in the lateral ventricular C.S.F. were likewise unaltered by treatment of the animals with chlorpromazine.

Discussion

Distribution of catecholamines and their metabolites in dog brain

The distributions and concentrations of noradrenaline and dopamine in different regions of dog brain do not differ greatly from the results given by other workers (Vogt, 1954; Bertler & Rosengren, 1959; Malhotra & Prasad, 1962; Laverty & Sharman, 1965a, b). However, the concentration of noradrenaline in the hypothalamus found in the present experiments is some 40% lower than the values given by Vogt (1954), Bertler & Rosengren (1959), Laverty & Sharman (1965a) and Malhotra & Prasad (1962). It is difficult to explain this discrepancy in view of the close agreement of our estimates of the noradrenaline concentrations in other

areas with the estimates reported in the literature. It may be that 1–2 yr old beagles have less noradrenaline in the hypothalamus than the dogs (breed unspecified) used by other workers.

The wide scatter in our estimates of the concentrations of the amines and acids in the globus pallidus can be attributed to difficulties encountered in dissecting this area reproducibly. Nevertheless, the proportions of 3,4-dihydroxyphenylacetic acid to dopamine and methoxydopamine to dopamine were found to be approximately the same in the globus pallidus as in the caudate nucleus. Carlsson & Waldeck (1964) analysed tissues pooled from several animals using a method of estimation about three times less sensitive than the methods of Crawford & Yates (unpublished), and found concentrations of methoxydopamine in pig and sheep caudate nucleus of 0·83 and 1·2 μg/g respectively. These estimates are of the same order as the estimates of methoxydopamine in the caudate nucleus observed in the two control groups of dogs in the present experiments. Homovanillic acid, unlike methoxydopamine and 3,4-dihydroxyphenylacetic acid, was detected, in every dog, in all areas of brain investigated, but its concentration was greatest in the basal ganglia. Our estimates for homovanillic acid in the caudate nucleus agree closely with the estimate of 13·8 μg/g found by Laverty & Sharman (1965b).

The estimates of 5-hydroxyindolylacetic acid in different areas of brain were similar to those quoted by Eccleston, Ashcroft, Moir, Parker-Rhodes, Lutz & O'Mahony (1968).

There was no significant difference between the estimates of noradrenaline, dopamine and methoxydopamine in the two groups of control animals. The estimates of homovanillic acid in the hypothalamus, midbrain, thalamus and globus pallidus and 5-hydroxyindolylacetic acid in the hypothalamus, midbrain and caudate nucleus were, however, slightly greater in the group 1 control experiments than in the group 2 control experiments. These differences may be due to variation between animals; they could not be attributed to different recoveries of the acids through the two control groups of experiments.

Relation between the phenolic acid concentrations in the caudate nucleus and lateral ventricular C.S.F. of the dog before and after treatment with chlorpromazine

On the basis of the good evidence that the phenolic acids do not pass readily from blood to brain (Carlsson & Hillarp, 1962) or from blood to C.S.F. (Guldberg & Yates, 1968), Ashcroft *et al.* (1968) suggested that the concentrations of homovanillic acid and 3,4-dihydroxyphenylacetic acid which they measured in the lateral ventricular C.S.F. of dogs were proportional to the concentrations of these acids in the caudate nucleus. We have confirmed this relation and shown that it is maintained when the acid concentrations in the caudate nucleus are increased by treatment with chlorpromazine (5 mg/kg). The relation between the phenolic acid concentrations in brain and C.S.F. has been discussed fully by Guldberg (1969).

Effect of chlorpromazine on the metabolism of the catecholamines in dog brain

It was observed that the rise in the concentration of 3,4-dihydroxyphenylacetic acid in the lateral ventricular C.S.F. produced by chlorpromazine was maximal 2 hr after intravenous administration of a 5 mg/kg dose. However, although at this

time the concentrations of homovanillic acid and 3,4-dihydroxyphenylacetic acid in the caudate nucleus, were also increased, the dopamine concentration in this tissue was unaltered while the concentration of methoxydopamine was significantly decreased.

Since the distribution of the acids between the caudate nucleus and the C.S.F. showed no significant change following treatment at either dose level, it may be deduced that the drug did not affect the passage of the acids from the brain tissue to the C.S.F.

Recent experiments, in which the concentrations of homovanillic acid in the lateral ventricular and cisternal C.S.F. of dogs were estimated before and after administration of chlorpromazine (5 mg/kg and 15 mg/kg) have not indicated any alteration in the ratio of the concentrations of this acid in the lateral ventricular and cisternal C.S.F. Such an alteration would be expected if chlorpromazine affected the active transport system, located at some point between the lateral ventricle and the cisterna (Guldberg, Ashcroft & Crawford, 1966), which transfers homovanillic acid from C.S.F. to blood.

Our results could in part be explained if chlorpromazine at the lower dose (5 mg/kg) increased the rate of synthesis of dopamine in the basal ganglia. This might result from an increased requirement for dopamine, induced by chlorpromazine, and operating by a feedback mechanism, possibly from the receptor sites (Carlsson & Lindqvist, 1963) and affecting the regulation of dopamine synthesis by involving the rate-limiting enzyme tyrosine hydroxylase (Burkard, Gey & Pletscher, 1967). Alternatively, chlorpromazine might affect the regulatory processes for dopamine synthesis by a direct action on the mechanisms concerned with this.

However, an increased turnover of dopamine in the caudate nucleus after chlorpromazine 5 mg/kg would be expected to cause a rise, and not a fall as we observed, in the levels of methoxydopamine. A reduction in methoxydopamine could be brought about by the facilitated conversion of methoxydopamine to homovanillic acid, possibly by a mechanism making methoxydopamine readily available to the action of the extraneuronal monoamine oxidase. Similarly, the small rise in the 3,4-dihydroxyphenylacetic acid concentration which was observed after chlorpromazine, rather than being a reflection of an increased turnover of dopamine, may be the result of an increased substrate (dopamine) availability for intraneuronal monoamine oxidase. Chlorpromazine in high concentrations (10^{-3}M) has been shown to inhibit brain mitochondrial monoamine oxidase *in vitro* (Nakajima, 1959), but there are no experimental data to suggest that low doses activate monoamine oxidase. There is considerable evidence that high and low doses of chlorpromazine have opposite effects on the permeability characteristics of membranes (Guth & Spirtis, 1964).

Chlorpromazine, in a dose of 15 mg/kg, appeared to reduce the concentration of dopamine and significantly reduced the concentration of methoxydopamine in the caudate nucleus but did not alter the concentrations of the acid metabolites of dopamine. Two possible explanations for the fall in the dopamine levels are: (i) reduction of dopamine synthesis and (ii) release of dopamine from its storage sites in the brain tissue. It is difficult to reconcile the fall in the dopamine concentration with the unchanged levels of the acid metabolites found after chlorpromazine 15 mg/kg.

In those areas of brain containing more noradrenaline than dopamine—the hypothalamus, thalamus and midbrain—the levels of noradrenaline were increased by chlorpromazine 5 mg/kg although only in the case of the thalamus was this increase statistically significant. The fact that the dopamine concentrations in the hypothalamus and thalamus were also slightly increased by this dose of chlorpromazine suggests that chlorpromazine 5 mg/kg may stimulate catecholamine synthesis in these areas. Chlorpromazine in the higher dose, 15 mg/kg, still appeared to increase the noradrenaline concentrations in the hypothalamus, thalamus and hindbrain (Table 3). However, the concentrations of dopamine in the thalamus and hypothalamus were unaltered by this dose.

The significant rise in the concentration of 5-hydroxyindolylacetic acid in the caudate nucleus together with the increases, although not significant, in the levels of this acid in the thalamus, hypothalamus and midbrain, produced by chlorpromazine 5 mg/kg suggest that this dose of chlorpromazine may also stimulate the turnover of 5-hydroxytryptamine.

It would appear that low and high doses of chlorpromazine have different effects on the metabolism of the catecholamines in brain tissue, possibly due to a dose-dependent action of the drug on subcellular membranes. The main effect of the low dose was considered to be a stimulation of dopamine synthesis, although our results could not be explained entirely on this basis; the main effects of the high dose may be to reduce dopamine synthesis and/or to release amines from storage sites.

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