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A STUDY OF THE LOCALIZATION OF PHENOTHIAZINES IN DOG BRAIN*

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Abstract—Evidence is presented to show that chlorpromazine and related phenothiazine derivatives concentrate in areas thought to be their loci of action.

Chlorpromazine and prochlorperazine exhibit similar spectra of localization in the fourteen areas of dog brain surveyed.

Thiethylperazine, which is a weak tranquilizer, but potent antinauseant, antiemetic, antivertigo agent concentrates differently from chlorpromazine and prochlorperazine, exhibiting highest concentrations in the cerebellar areas.

The implications of these tissue distribution studies for the mode of action of therapeutic phenothiazines are presented and discussed.

THE brain seems to be the major organ acted upon by chlorpromazine. Within the brain are specific areas judged from electropharmacologic evidence to be the major sites of action.[1] The ability of phenothiazine derivatives to affect many types of membranes, both cellular and subcellular, has been demonstrated.[2–15] Two hypotheses within the framework of a general membrane action present themselves to explain why central nervous system areas should be major sites of action. First, it is possible that within these areas there exist membranes especially sensitive to the drug's presence. The second possibility is that these "site of action" areas concentrate the phenothiazines to a greater degree than other regions do. The present study was instituted to investigate this latter possibility.

The drugs studied were chlorpromazine, prochlorperazine and thiethylperazine (Fig. 1). Chlorpromazine and prochlorperazine were chosen as the prototypes of the promazine and piperazine series of phenothiazine tranquilizers. Thiethylperazine is a piperazine-substituted phenothiazine with weak tranquilizer actions but with very potent anti-nauseant, antivertigo, and antimetic properties. It was felt that the somewhat different spectrum of activity of thiethylperazine might reveal itself in a different localization pattern.

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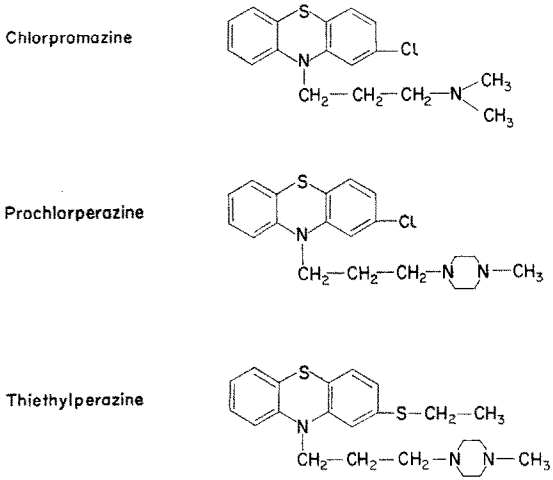

FIG. 1. Formulas of phenothiazines employed.

METHODS

Young mongrel male dogs, 10 to 14 kg, were used in the main experiment; 15, 30, 45, 60, and 90 min after injection of one of the three phenothiazines, the dogs were sacrificed by intravenous air injection, followed immediately by decapitation. The head was perfused through the carotid artery with 500 ml of 0·1 M phosphate buffer solution (pH 7·4). In preliminary experiments the blood was simply allowed to drain. By this method, variable volumes of blood were retained and the fluorescent values varied considerably. The perfusion removed the blood, producing more consistent results with less spread reasonably close to, and with the same rank as, the results in the preliminary studies. The brain was then removed intact. Any brains displaying evidence of disease were discarded. The brains were sectioned as described below. The weights of each zone were recorded and 1 g of each zone was taken for extraction when available. The areas were homogenized in 2·0 ml of 0·1 M phosphate buffer (pH 7·4) in glass hand homogenizers, additional phosphate buffer up to a total of 4·0 ml was added to rinse the homogenizer. The homogenates and rinsings were placed in a test tube and 1 ml of 12 N HCl was added; the test tube was then placed in a boiling water bath for 8 min. After cooling at room temperature and centrifuging at 1,400 $\times$ g for 20 min, 0·2 ml of the supernatant was transferred to a cuvet, 2·8 ml distilled water added, and its fluorescence determined in a Turner Fluorometer.

Fourteen regions of the brain were studied. These were medulla, pons, midbrain, vermis, paraflocculus, hypothalamus, the region just caudal to the thalamus (post-thalamic zone), thalamus, basal ganglia, hippocampus, amygdaloid nucleus, occipital cortex, temporal cortex, and frontal cortex.

The cerebellum was divided into the paraflocculus and anterior central lobe or vermis. The medulla, pons, and midbrain were then dissected away. The cerebrum was cut coronally, starting from the frontal lobe, into four sections. From the first section only frontal cortex was obtained; from the second, basal ganglia and amygdala; from the third, the temporal cortex, hippocampus, hypothalamus, and thalamus; from the fourth, the post-thalamic zone, more hippocampus, and the occipital cortex.

The drugs were always injected into the cephalic vein. The doses were 10 mg (as the salt)/kg and the total volume injected never more than 5·5 to 6·0 ml. Chlorpromazine and prochlorperazine were injected in aqueous solution. Thiethylperazine was dissolved in a sodium bicarbonate:propylene glycol:water vehicle.

The fluorescence of blank extracts of each of the regions of the brain studied was determined in eight control dogs, and the average of these blanks was subtracted from the values obtained in the treated dogs. Results were expressed in concentrations of the salt form of the drug. The filters (activation, Corning 7–60, peak 350 mμ, range 290 to 400 mμ, and emission, Kodak 8, passes wave lengths above 485 mμ) were the same for the three drugs. Chlorpromazine sulfoxide produces less than a third of the fluorescence of chlorpromazine under these conditions. All three drugs produced linear fluorescence-concentration curves between 1 and 100 μg/ml of solution in the cuvet. Recovery experiments were performed by adding known amounts of the drugs to homogenates of the fourteen areas studied. The mixture was incubated for 10 min at 37° to allow for penetration and binding and then subjected to the extraction procedures described above. Three such experiments were performed with each drug for each brain region.

The possible interference by metabolic products of the injected drugs was in part ruled out by the use of filters relatively opaque to the sulfoxide emission. This procedure did not preclude the presence of metabolites such as desmethyl derivatives which seem to have fluorescent properties similar to the parent compound. Therefore, paper chromatography of the extracts was attempted in order to separate and identify the extracted compounds.

Twenty-five to fifty of the extracts were spotted on Whatman No. 1 paper in parallel and subjected to ascending chromatography. The solvent used was the upper phase of a two-phase system resulting from the mixture of n-butanol:water:glacial acetic acid (50:50:12 vol/vol). The spots were visualized with 50% sulfuric acid, 5% ferric nitrate, 0·08% ceric sulfate, ninhydrin dip for primary or secondary amines, nitroprusside–acetaldehyde dip for secondary amines,[16] or ultraviolet light. The standard drugs used were chlorpromazine, chlorpromazine sulfoxide, desmethyl chlorpromazine, desdimethyl chlorpromazine, prochlorperazine, and thiethylperazine. An additional amount of the same drug as injected was added to selected extracts just before chromatography. Development of chromatograms of these mixtures (called co-chromatography) revealed only one spot.

A study employing rabbits and determining thiethylperazine concentrations in cerebrum, cerebellum, and brain stem was also performed.

RESULTS

Recovery Values

The average recovery values of phenothiazine added *in vitro* were similar for all of the fourteen areas (Table 1). Amounts of drug added *in vitro* were calculated to approximate the concentrations extracted from the brains of injected dogs. Recovery values with prochlorperazine and thiethylperazine were of the order of 95 per cent. Recovery of chlorpromazine was somewhat poorer, exhibiting a mean of 88 (range ±4·5%) recovery. This may indicate a greater tissue binding of chlorpromazine than of the other drugs studied.

TABLE 1. PER CENT RECOVERY OF ADDED DRUG*

	Chlorpromazine	Prochlorperazine	Thiethylperazine
Medulla	91 ± 3	97 ± 3	96 ± 2
Pons	89 ± 5	95 ± 2	98 ± 1
Midbrain	93 ± 3	94 ± 4	95 ± 3
Vermis	87 ± 5	93 ± 5	92 ± 4
Paraflocculus	92 ± 3	96 ± 2	94 ± 6
Hypothalamus	82 ± 3	89 ± 6	87 ± 8
Amygdala	86 ± 9	93 ± 5	91 ± 4
Post-thalamus	83 ± 6	92 ± 3	92 ± 3
Thalamus	83 ± 8	93 ± 6	90 ± 6
Basal ganglia	89 ± 6	94 ± 3	96 ± 2
Hippocampus	93 ± 3	94 ± 2	97 ± 1
Occipital cortex	87 ± 6	93 ± 5	92 ± 5
Temporal cortex	93 ± 1	88 ± 5	92 ± 6
Frontal cortex	92 ± 3	94 ± 4	92 ± 1

* Values are means of three determinations for each drug (± standard error of the mean).

Chromatography

Examination of the chromatograms of tissue extracts never revealed more than one spot. This spot had substantially the same Rf as the authentic injected drug. Rf's were: chlorpromazine, 0·55; prochlorperazine, 0·57; thiethylperazine, 0·57; desmethyl chlorpromazine, 0·59; desdimethyl chlorpromazine, 0·57; chlorpromazine sulfoxide, 0·60 to 0·61. The average running front was 32 cm. Co-chromatography again revealed only one spot. No colors were revealed by the various visualizing reagents other than that due to the original parent drug. Finally, no reaction was ever obtained in chromatograms of extracts to the primary and secondary amine dips.

TABLE 2. CONCENTRATION OF THIETHYLPERAZINE*

	Tissue (μg/g)		
	Cerebellum	Cerebrum	Brain stem
Mean value	166	24	34
Range of values	98–315	16–38	24–46

* Preliminary results of investigation into thiethylperazine distribution in rabbit brain 1 hr after intraperitoneal injection of 10 mg/kg; mean values are averages of determinations in six rabbits.

Rabbits

A preliminary experiment using 26 rabbits to determine the spectrum of concentration of thiethylperazine in cerebrum, cerebellum, and brain stem was conducted. The extraction and assay methods were identical with the dog studies. Table 2 shows the concentrations of thiethylperazine in these areas 1 h after injection of 10 mg/kg, i.p. Values compare well with those obtained in the dogs.

TABLE 3. DRUG CONCENTRATIONS IN BRAIN AREAS*

	Chlorpromazine					Prochlorperazine					Thiethylperazine				
	Minutes after intravenous injection:					Minutes after intravenous injection					Minutes after intravenous injection				
	15	30	45	60	90	15	30	45	60	90	15	30	45	60	90
Medulla	und.	165 ± 34	196 ± 41	187 ± 46	162 ± 39	und.	83 ± 29	132 ± 42	79 ± 28	81 ± 20	11 ± 3	80 ± 31	29 ± 8	27 ± 5	11 ± 3
Pons	und.	15 ± 3	49 ± 9	91 ± 21	110 ± 31	und.	21 ± 10	59 ± 22	82 ± 19	43 ± 8	und.	und.	und.	und.	und.
Midbrain	und.	110 ± 29	70 ± 14	56 ± 10	118 ± 28	und.	161 ± 43	131 ± 34	92 ± 24	103 ± 42	und.	und.	11 ± 3	14 ± 4	16 ± 4
Vermis	und.	18 ± 4	und.	20 ± 4	und.	und.	11 ± 3	16 ± 7	28 ± 6	11 ± 3	18 ± 10	148 ± 28	396 ± 52	240 ± 22	180 ± 26
Paraflocculus	und.	und.	32 ± 8	16 ± 8	21 ± 3	und.	11 ± 3	34 ± 9	39 ± 9	29 ± 9	14 ± 8	210 ± 43	320 ± 46	226 ± 33	150 ± 12
Hypothalamus	und.	191 ± 31	210 ± 42	157 ± 38	147 ± 46	und.	123 ± 38	142 ± 43	108 ± 41	97 ± 19	und.	11 ± 3	und.	und.	und.
Amygdala	und.	53 ± 11	81 ± 15	77 ± 19	93 ± 29	und.	62 ± 21	70 ± 12	91 ± 23	89 ± 13	11 ± 0	59 ± 16	11 ± 3	11 ± 3	und.
Post-thalamus	und.	11 ± 3	31 ± 14	und.	16 ± 8	und.	13 ± 2	13 ± 4	und.	11 ± 3	und.	52 ± 23	75 ± 21	73 ± 21	64 ± 20
Thalamus	und.	87 ± 19	113 ± 37	129 ± 23	160 ± 37	und.	62 ± 11	99 ± 31	106 ± 39	94 ± 27	und.	40 ± 16	60 ± 16	40 ± 9	19 ± 5
Basal ganglia	und.	104 ± 37	121 ± 23	142 ± 31	73 ± 15	und.	115 ± 19	109 ± 27	77 ± 12	87 ± 19	und.	40 ± 13	40 ± 11	43 ± 8	und.
Hippocampus	und.	139 ± 39	87 ± 11	90 ± 15	106 ± 18	und.	157 ± 32	146 ± 46	68 ± 21	68 ± 29	10 ± 0	60 ± 21	70 ± 27	90 ± 19	31 ± 9
Occipital cortex	und.	32 ± 11	27 ± 4	41 ± 11	16 ± 8	und.	und.	und.	20 ± 7	und.	und.	und.	und.	und.	und.
Temporal cortex	und.	und.	11 ± 3	9 ± 0	17 ± 4	und.	und.	11 ± 3	13 ± 4	11 ± 3	und.	34 ± 10	32 ± 7	28 ± 4	18 ± 4
Frontal cortex	und.	und.	und.	9 ± 0	13 ± 1	und.	und.	11 ± 3	11 ± 3	und.	und.	35 ± 9	30 ± 11	28 ± 4	17 ± 4

* In micrograms per gram; mean values of six determinations in six dogs (± standard error of the mean); und. means the concentration was below the level of detection of the method.

Dogs

Table 3 and Fig. 2 depict numerically and in histogram form the distribution of the three drugs in the various areas of dog brain studied over 15 to 90 min of the period.

The concentrations of prochlorperazine in the different areas of the brain are reasonably similar to those of chlorpromazine.

The thiethylperazine spectrum of concentration is quite different. The high concentration of the drug found in the vermis and paraflocculus at all the times considered, confirms the results found for the cerebellum of the rabbit.

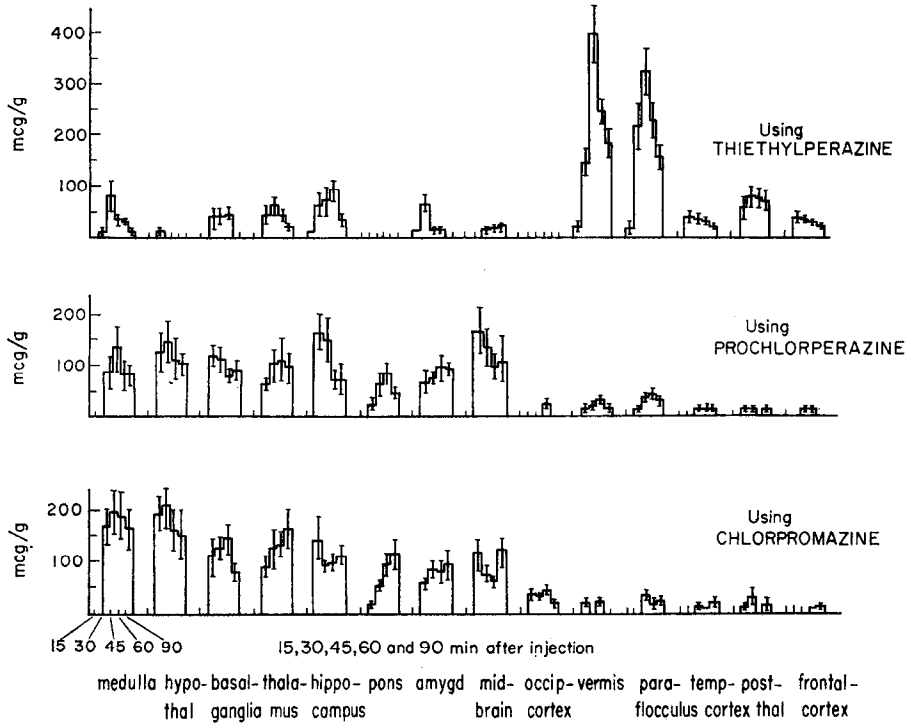

FIG. 2. Histogram of localization of three phenothiazines in fourteen areas of dog brain. Each vertical bar represents the mean of six determinations in six dogs at a particular interval after injection. I———I indicates standard error. The various brain areas are arranged so that from left to right the concentration of chlorpromazine is highest to lowest. The absence of a vertical bar (I) indicates that the method used was unable to detect any drug.

DISCUSSION

Initially, extraction and assay determinations were carried out by the methods of Salzman and Brodie[17] and Berger and Forrest.[18] The recoveries from brain reported by these authors in their publications were 40 per cent and 95 per cent respectively. With the method of Berger and Forrest, which depends upon the oxidation of the phenothiazine nucleus to the more easily extractable sulfoxide, our recoveries were poor (50%). This was probably due to inconstant oxidation produced by their method in our laboratory. Chromatograms of the Berger-Forrest extraction mixture revealed

a series of spots that represented a mixture of oxidation levels of chlorpromazine (chlorpromazine sulfoxide and chlorpromazine sulfone).

It still cannot be said definitely from the results whether the compounds that are recovered from brain are the same as the drugs injected, or some metabolites, or both. Chlorpromazine sulfoxide, when read fluorometrically at concentrations of the same strength as chlorpromazine, produced only about 30% of the emission of chlorpromazine. Unfortunately, in the solvent system used, the Rf's were rather similar. However, only single spots were obtained and these had the same Rf values as the authentic drugs. The amine dips yielded no positive tests for primary or secondary amines. It is possible that the metabolites may have been present in less than detectable concentrations. For these reasons it seems likely that only parent compound was being measured.

The sites of action of chlorpromazine have been recently reviewed by Domino.[1] The areas cited as being centers of action of chlorpromazine and presumably prochlorperazine are precisely those showing the greatest concentration of these drugs in this study. Those not shown to be affected by reasonably small doses of chlorpromazine such as cerebral cortex, show low concentrations of the drug.

Thiethylperazine manifests antiemetic, antinauseant, and antivertigo activities[19] in various experimental procedures and clinical situations which surpass those of chlorpromazine or prochlorperazine. In an extended series of experiments reviewed by Tyler and Bard[20] the integrity of the flocculonodular lobe of the cerebellum of the dog was found to be necessary for the development of motion sickness. Studies by Wang and Chinn[21] further indicate that stimulation of labyrinthine receptors may result in vestibular impulses activating the vomiting center via the cerebellum (through the juxtarestiform body) and chemoreceptor trigger zone. Thus the ability of the cerebellar areas to take up this phenothiazine, thiethylperazine, may directly result in the desired therapeutic effect.

Although pysiological factors such as blood supply undoubtedly exert an influence on drug distribution to various areas of the brain, it seems reasonably clear from the data that chemical factors may be more important. Thus the hypothalamus is known to be one of the most highly vascularized areas in the brain, yet this area showed no detectable concentration of thiethylperazine.

It is impossible to make a general statement correlating drug concentration and sites of action. Many, or perhaps most, drugs do not seem to concentrate at their sites of action, at least when whole tissue extracts are made. Thus, digitalis concentrates in small intestine, and most drugs show high concentrations in liver.[22] The important point is whether a drug requires a very special receptor found only in its "site of action" tissue or whether it is capable of affecting receptors found in many different organs for the expression of its activity. If the latter case applies to the phenothiazines—and it would seem to—then the site of highest concentration becomes the site of greatest action.

A second important consideration is the level at which measurements are made. Thus, a study comparing whole-organ concentrations of phenothiazines would find the brain showing considerably lower concentrations than liver, kidney, or lung.[23] A study of the concentration among areas of the same organ, such as the present investigation, demonstrates important differences of distribution among these areas. A study of the distribution among subcellular particles of areas exhibiting high

concentrations might well show that hypothalamic mitochondria have concentrations exceeding those of any other mitochondria. Berger,[24] as well as Guth,[25] showed that mitochondria isolated from brain seemed to have a greater affinity for chlorpromazine than have liver mitochondria.

Several other studies of phenothiazine distribution in mammalian nervous system revealed no, or small, differences between brain areas. Federov and Schnol,[26] using ^{35}S-chlorpromazine, found no differences in accumulation, while Wechsler and Roizin[23] found lower concentrations in spinal cord and only traces in cerebellum and pons of monkeys. On the other hand, Wase *et al*[27] found strikingly greater accumulations of ^{35}S-chlorpromazine in hypothalamus and other areas of rats. Hayashi[28] likewise, found differences between radioactivity of midbrain, pons, and cerebellum after ^{35}S-chlorpromazine injection. The differences in results among these studies are probably explicable on three grounds: sampling times, presence of metabolites (which could contribute to radioactivity assays), and species differences. Further investigation of central nervous system localization of phenothiazines, particularly thiethylperazine, among cerebellar areas is under way.

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REFERENCES

1. E. F. DOMINO, *Ann. Rev. Pharmacol.* **2**, 215 (1962).
2. E. T. ECKHARDT and W. M. GOVIER, *Proc. Soc. exp. Biol., N.Y.* **17**, 124 (1958).
3. J. R. LEDUC, *Rev. canad. Biol.* **8**, 439 (1949).
4. H. MAISIN, *C.R. Soc. Biol., Paris* **145**, 456 (1951).
5. O. SCHALES, *Proc. Soc. exp. Biol., N.Y.* **83**, 593 (1953).
6. H. HALPERN, B. DREYFUS and G. BOURDON, *Presse méd.* **58**, 1151 (1950).
7. A. R. FREEMAN and M. A. SPIRTES, *Biochem. Pharmacol.* **11**, 161 (1962).
8. M. E. GREIG and A. J. GIBBONS, *Amer. J. Physiol.* **181**, 313 (1955).
9. K. F. BAUER and H. LEONHARDT, *J. comp. Neurol.* **109**, 363 (1956).
10. J. CHRISTENSEN, Y. S. L. FENG, E. POLLEY and A. W. WASE, *Fed. Proc.* **17**, 000, (1958).
11. M. A. SPIRTES and P. S. GUTH *Nature, Lond.* **190**, 274 (1961).
12. P. S. GUTH, *Fed. Proc.* **21**, 1100 (1962).
13. J. LOISELEUR and L. CATINOT *C.R. Soc. Biol., Paris* **349**, 2403 (1959).
14. O. J. RAFAELSEN, *Acta psychiat. scand.* **34**, suppl. **136**, 73 (1959).
15. T. GATI, G. HARMOS and G. LUDANY, *Arch. int. Pharmacodyn.* **114**, 251 (1958).
16. F. FEIGL, *Spot Tests in Organic Chemistry*. Elsevier, Amsterdam (1956).
17. N. P. SALZMAN and B. B. BRODIE, *J. Pharmacol. exp. Ther.* **118**, 46 (1956).
18. M. BERGER and I. S. FORREST, *J. Neurochem.* **4**, 366 (1959).
19. D. C. BROWNE and R. SPARKS, *Sth. med. J., Bgham, Ala.* **54**, 953 (1961).
20. D. B. TYLER and P. BARD, *Physiol. Rev.* **29**, 311 (1949).
21. S. C. WANG and H. I. CHINN *Amer. J. Physiol.* **185**, 617 (1956).
22. V. DRILL, *Pharmacology in Medicine*, 2nd ed. McGraw-Hill, New York (1958).
23. M. B. WECHSLER and L. ROIZIN, *J. ment. Sci.* **106**, 1501 (1960).
24. M. BERGER, *J. Neurochem.* **2**, 30 (1957).
25. P. GUTH, Ph.d. Thesis. Hahnemann Medical College, Philadelphia (1958).
26. N. A. FEDEROV and E. C. SCHNOL, *Zh. Nevropat. Psikhiat.* **2**, 139 (1956).
27. A. W. WASE, J. CHRISTENSEN and E. POLLEY, *Arch. Neurol. Psychiat., Chic.* **75**, 54 (1956).
28. N. HAYASHI, *Tokyo Jikeika Ikadaigaku Zasshi*, **75**, 23 (1960).