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DISTRIBUTION OF SOME STRUCTURALLY RELATED PHARMACOLOGICAL AGENTS IN RAT BRAIN

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Abstract—Single doses of the four structurally related pharmacological agents chlorpromazine, chlorprothixene, chlorperphenthixene and perphenazine were injected intravenously into the rat. At four different times after injection the distribution of the drugs in ten parts of the brain were measured. A striking similarity between the patterns of the three first drugs was observed, while that of perphenazine deviated. An explanation for this phenomenon based on chemical structure is presented and the distribution in the brain is discussed in view of the pharmacological activity.

ALTHOUGH during the last two decades the use of psycho–pharmacological drugs has improved the condition of many patients, little is known concerning their mechanism of action. Of a number of compounds, there is evidence of an inhibitory effect on enzymes (e.g. monoamino oxidase inhibitors), and of others, like the phenothiazines and iminodibenzyl-derivatives, the surface-active action on membranes has been generally accepted as one of the ways in which they exert their therapeutic influence; but besides that we can only assume that the transfer of nerve-stimuli in brain is being influenced.

Therefore we thought that knowledge concerning the difference in regional distribution of psychoactive agents in brain might give an indication towards the question why chemically related compounds give different therapeutic results. For our study we used four major tranquillizers (neuroleptics) which either contain a common ring system (phenothiazine or thioxanthene) or have a similar side chain (dimethylaminopropylic or propylpiperazine-ethanolic) as can be seen in Fig. 1. The compounds chlorpromazine (CPZ), perphenazine (PPh), chlorprothixene (CPX) and chlorperphenthixene (CPPhX) can be classified two and two, and it would be interesting to see if their localization in rat brain is influenced by the ring system or their side chain.

MATERIALS AND METHODS

The four compounds used were analyzed for their nitrogen, chloride and sulphur content which corresponded well with the calculated percentages. The u.v.-spectra and melting points agreed with values cited in the literature.

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† The author wishes to dedicate this article to Prof. Dr. H. Veldstra, on the occasion of his retirement from the Chair of Biochemistry at the University of Leiden.

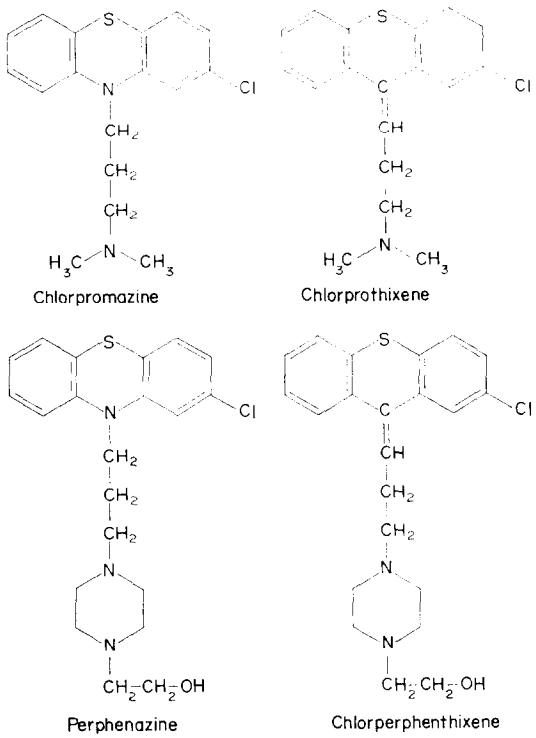

FIG. 1. The neuroleptics which have been used.

Male Wistar rats (Strain: Pharmacological laboratory, State University, Leiden) 190–210 g were injected intravenously with 0·5 ml of an isotonical solution containing 22×10^{-3} M of one pharmacon. At different times after injection the animals were decapitated; the brains were dissected at 4° into ten different parts, viz. medulla spinalis (1), medulla oblongata (2); cerebellum (3); midbrain (4); olfactory bulbs (5); hypothalamus (6); hippocampus (7); basal ganglia (8); cortex cerebri (9); and white matter such as the pons, corpus callosum and chiasma opticum (10). The numbers correspond to those used in the Figs. 2–4.

Ten per cent homogenates (w/v) in 0·32 M sucrose were made, using a Potter homogenizer. The assay of the phenothiazine derivatives was based on the procedure of Udenfriend *et al.*[1] and was as follows.

Chlorpromazine determination. 0·3 ml homogenate, 0·7 ml 0·32 M sucrose, 1 ml 8 N NaOH and 1 ml 4×10^{-3} M sodium deoxycholate were heated for 5 min at 100°.

After cooling, the mixture was extracted by shaking for 15 min with 6 ml *n*-heptane containing 1·7% (v/v) isoamylalcohol. The two layers were separated by centrifugation, 5 ml of the heptane layer were extracted with 2·5 ml glycin–HCl buffer pH 3, 0·5 M. To 2 ml of the acid extract 0·1 ml 30% H_2O_2 was added, the mixture was heated for 10 min at 100° to oxidize the chlorpromazine and after cooling the fluorescence was measured on an Aminco–Bowman spectrophotofluorometer. Excitation 345 nm, emission 385 nm (uncorrected).

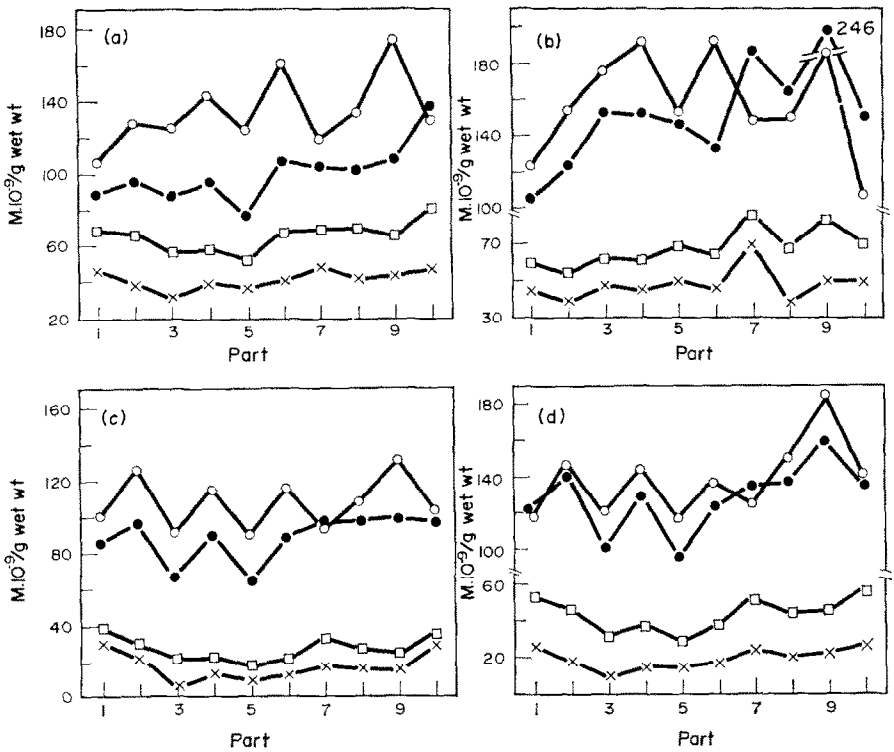

FIG. 2. Distribution pattern of the drugs over the different parts at different times after injection (a) = Chlorpromazine; (b) = perhpenazine; (c) = chlorprothixene; (d) = chlorperphenthixene ○—○ 5 min; ●—● 1 hr; □—□ 4 hr; ×—× 6 hr.

Perphenazine determination. Essentially the same as for chlorpromazine, but in the first extraction 4 N NaOH and 0·2 ml 4 × 10^{-3} M deoxycholate were used and no isoamylalcohol was added to the heptane. The recovery was for chlorpromazine 93–95 per cent and for perphenazine 78–80 per cent.

The thioxanthenes were measured by mixing 0·5 ml of brain homogenate with 2 ml orthophosphoric acid (85 per cent), according to Mellinger *et al.*[2] After exactly 1 hr the fluorescence was measured (exc. 385 nm/emiss. 555 nm, uncorrected). Because the tissue showed fluorescence also, homogenates of dissected brains of non-treated animals were used as blanks. Lipids were extracted according to Folch *et al.*[3] After dialysis of the upper layer for 48 hr against distilled water NANA was determined.[4] By multiplicating with 4·5 the amount of gangliosides was calculated.

In the lower layer, the total amount of lipids was determined gravimetrically, and phospholipid-phosphorus was measured according to Hooghwinkel and Van Niekerk.[5] Protein was determined by the method of Lowry *et al.*[6] Thin layer chromatography was done on heptane or dichloromethane extracts of brain tissue for the determination of metabolites of the phenothiazines and thioxanthenes, respectively. Plates were prepared with Silica gel DO (Fluka). Chloroform–ethanol–ammonia (80:20:1) and benzene–ethylacetate–diethylamine (7:2:1) were used as solvent systems. Spots were revealed by spraying the plate with a mixture of sulphuric acid (d = 1·40) and ethanol (96%) (1:1), after it had been dried.

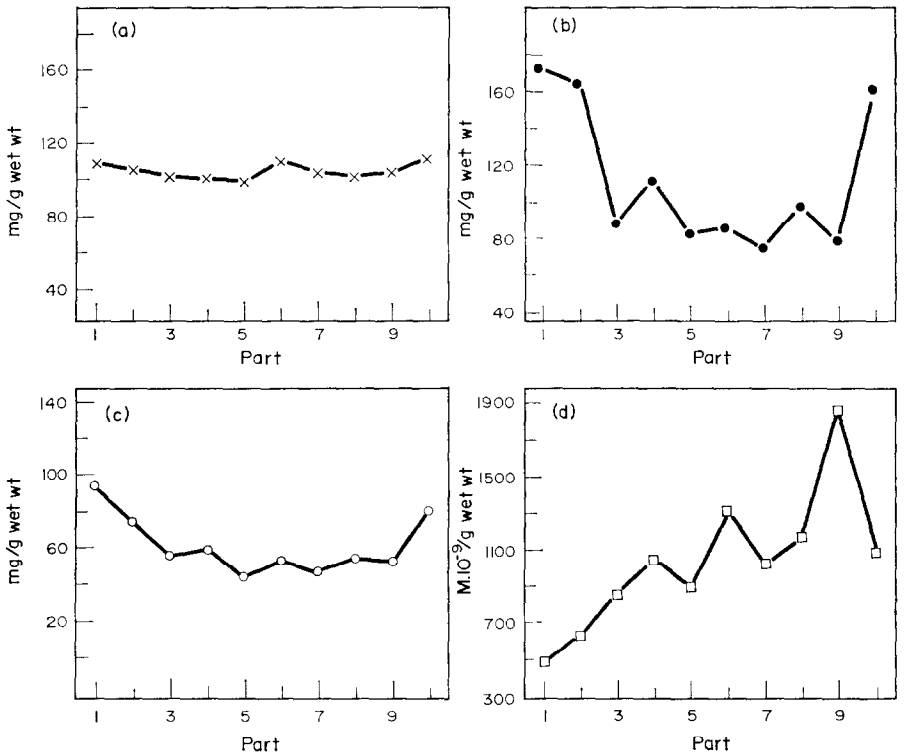

FIG. 3. Amounts of protein and lipids in the parts studied. (a) = Protein; (b) = total lipids; (c) = phospholipids; (d) = gangliosides.

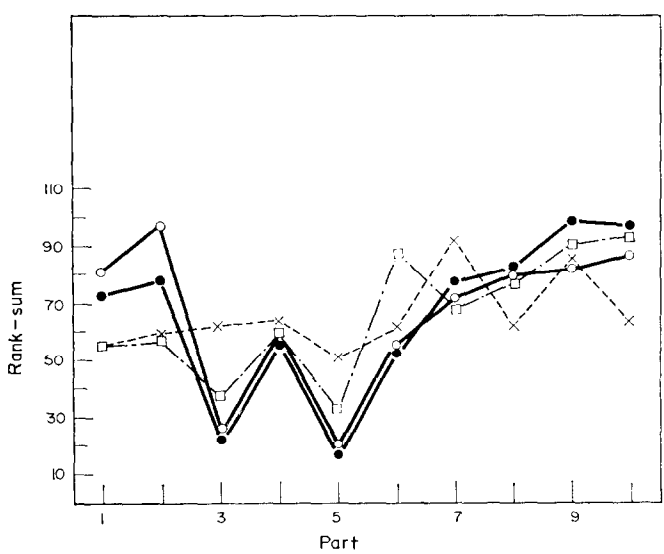

FIG. 4. Rank-sums per part.

RESULTS

The distribution of the pharmacological agents in the ten different regions is shown in Fig. 2. The points indicated in this figure are the means of six values obtained in three experiments. The standard error does not exceed 8 per cent of these means. Distribution patterns of CPZ, CPX and CPPhX appear to agree with each other at all four times at which determinations were made, viz. 5 min, 1, 4 and 6 hr after injection. The amounts found in the cerebellum (3) and olfactory (5) are always low.

Perphenazine on the contrary shows a deviating pattern. The differences between PPh and the other agents are chiefly observed in the cerebellum (3) and olfactory (5), in which more, and basal ganglia (8) and hypothalamus (6) in which, especially later than 5 min after injection, less is found. After 5 min there exists a good agreement with the ganglioside distribution (Fig. 3D). Also in another respect PPh distributes exceptionally since the concentration of the three other compounds decreases slowly with time, but the perphenazine content is at 1 hr after injection higher than at 5 min, particularly in the hippocampus, basal ganglia, cortex cerebri and white matter. Symchowicz *et al.*[7] have shown the same phenomenon for PPh in some parts of dog brain.

Determination of some macromolecular components of rat brain (Fig. 3) reveals little difference in protein content between the ten parts (100–115 mg protein/g wet wt). The amount of total lipids shows, as expected, a great difference between those regions considered to be "white" and those entitled as "grey". The amounts range from 90 to 160 mg/g wet wt. The same pattern is found for the phospholipids with amounts from 50–80 mg/g wet wt.

Lipid-bound gangliosides can be found mainly in grey matter and to a lesser extent in white matter. Difficulties in preparing the region named "white matter" (10) totally pure might explain the relatively high amount of gangliosides found in it. The points shown in Fig. 3 are also obtained in three experiments. The standard error for protein, total lipids, phospholipids and gangliosides is 5, 9, 9 and 10 per cent of the means, respectively.

Thin layer chromatography 5 min and 1 hr after injection did not reveal metabolites of the four drugs. Neither u.v.-spectra nor thin layer chromatography shows any influence of the extraction procedure on the compounds under investigation.

DISCUSSION

An answer to our preposed question concerning what part of the drug molecules governs their distribution can best be given on the basis of their distribution patterns, as shown in Fig. 2. By providing the concentrations in the different brain regions with rank numbers, per drug per point of time and per experiment, the zero hypothesis can be tested whether the differences between the regions as to their drug contents are measured accidentally. Combination of the rank numbers results in rank-sums per part (Fig. 4) and from these and Fig. 2 the conclusion is reached that chlorpromazine, chlorprothixene and chlorperphenthixene are distributed rather identically at all times, while perphenazine shows a clear deviation from this pattern. The separate position of perphenazine might be caused by differences in protein binding or biological half-life, but we do not have indications for this.[8] However, one of the discrepancies in sterochemical view is that PPh might be able to assume a cyclic conformation by an interaction between the hydroxy group of the side chain and the sulphur

atom of the ring system, as can be shown from investigations on molecular models. For CPPhX this possibility is blocked by the double bond in the side chain (Fig. 1). In this connection the results of de Jaramillo and Guth[9] are important. They showed that for prochlorpromazine the distribution in dog brain equals that of chlorpromazine. Prochlorpromazine differs from perphenazine only by the side chain on the piperazine-nitrogen atom:

$$-CH_3 \text{ versus } -CH_2-CH_2-OH.$$

It is not clear whether the rather strong affinity of PPh towards some parts of the limbic system, viz. olfactory (5) and hippocampus (7) correlates with the action of this psychopharmacological agent seen in patients, assuming that distribution in rat brain may be compared with human brain. For psychoactive drugs in general Eckert and Hopf[10] attribute a real sense to this observation.

The strong "Parkinson-like" phenomena seen as a side effect of perphenazine medication made us expect a higher concentration of this drug in the basal ganglia, but in view of the fact that only one of the nuclei might be responsible for this, a "dilution" with other nuclei might be introduced by our preparation technique. Valdman[11] described an inhibitory effect on the reticular formation in the brain stem as the primary action of chlorpromazine. The wide branching of this structure in brain would result in a total depression of brain activity. The highest concentration of cells belonging to this reticular formation is found in the mesencephalon (4) and its high content of the drugs studied by us, 5 min and 1 hr after injection, might have a functional meaning.

In the distribution pattern in the brain in general, lipophility plays an important role.[12] Firstly, in the penetration into brain tissue, as demonstrated for phenothiazines by Mahju and Maickel[13] and by Jänchen and Krieglstein.[14] After that, the pharmacological agents we studied follow the distribution pattern of psychotropic drugs as described also by Cassano and Placidi:[15] firstly, a high concentration in the "grey" regions, notably the cortex (9), induced by the vascularization, and second, after a certain course of time, their lipid solubility leads to a predominance in the "white" regions.

A binding to lipids might be, therefore, one of the important factors governing the regional distribution of the four psychopharmacological agents.

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