5. U. RYDBERG, *Biochem. Pharmac.* **18**, 2425 (1969).
6. G. O. BUSTOS, H. KALANT, J. M. KHANNA and J. LOTH, *Science, N.Y.* **168**, 1598 (1970).
7. E. RACKER, *J. biol. Chem.* **184**, 313 (1950).
8. H. THEORELL and T. YONETANI, *Acta chem. scand.* **23**, 255 (1969).

Biochemical Pharmacology, Vol. 20, pp. 2089–2091. Pergamon Press, 1971. Printed in Great Britain

Chlorpromazine adsorption to brain regions

(*Received* 6 *October* 1970; *accepted* 9 *November* 1970)

CHLORPROMAZINE (CPZ) is a potent local anesthetic[1–3] and immediately suppresses the electrical activity of neurones when applied directly.[4–7] Since the drug nonselectively blocks neurones and stabilizes membranes in a nonspecific fashion identical to other local anesthetics,[8,9] the hypothesis has developed that the apparent specificity of phenothiazines for antinausea and antipsychotic actions results from a particular drug distribution into various brain regions.[8,10,11] It is known that different regions of the brain take up different amounts of chlorpromazine and other phenothiazines after intravenous injection;[11,12–14] frontal cortex and white matter generally do not accumulate much phenothiazine, while thalamus and hippocampus consistently take up moderate to high amounts of the drug. Cassano *et al.*[14] could not observe any relation between regional blood flow and chlorpromazine uptake into brain regions.

It is not known, however, whether the higher uptake by thalamus and hippocampus results from an intrinsically higher affinity (or specific affinity) for chlorpromazine by nerve cells in these regions, or whether a different blood–brain barrier in these regions happens to allow easier transfer into the tissue. In the present study, it was investigated whether differences in tissue affinity for chlorpromazine could cause any of the observed distribution patterns for this drug. Four areas of the cat brain were dissected (frontal cortex, white matter, thalamus and hippocampus) and their affinity for chlorpromazine was determined.

Cats were anesthetized with ether or chloralose and the brains removed. The selected areas were dissected and used immediately or frozen in liquid nitrogen for later use. For adsorption experiments, approximately 0·8 g tissue was homogenized in a Virtis homogenizer with 0·6 ml of a solution containing 0·9% NaCl in 10 mM phosphate buffer. The dry weight of each homogenate was measured by drying at 90° to a minimum weight. The CPZ adsorption of the homogenates was determined by mixing in centrifuge tubes 0·2-ml aliquots of homogenate with 2 ml of a 0·9% NaCl solution containing ^{35}S-labeled chlorpromazine (Amersham, Great Britain). After a 30-min incubation, the tissue was spun down at 36,900 *g* for 20 min. The radioactivity in 0·2-ml samples of the supernatant was determined in a liquid scintillation counter with the liquid scintillator described by Bray.[15] The amount of drug bound per kilogram of dry weight was calculated as described previously.[11] Since previous workers[12,16] had shown that 75–98 per cent of chlorpromazine, which had been added to or incubated with brain tissue, could be recovered as chlorpromazine in short-term experiments, it was assumed in the present experiments that the amount of biotransformation of ^{35}S-chlorpromazine could be neglected.

The results, using five different cat brains, are summarized in Table 1. In the small C_{free} range used in these experiments, the partition coefficients were constant. The free concentration range chosen is known to be associated with local anesthesia[5] and stabilization of the red cell membrane.[9,11,17,18] No significant differences could be detected when the CPZ adsorptions to frontal cortex, thalamus and white matter were compared. The hippocampus showed a small but significant ($P < 0·02$) decrease in drug adsorption.

As it was impossible to explain any of the reported patterns of chlorpromazine distribution by a selective affinity of certain areas, it was investigated whether a selective retention could be the basis for the distribution *in vivo*. Chlorpromazine-^{35}S was adsorbed to tissue homogenates as described for the previous set of experiments and subsequently desorbed by four dilutions with 0·9% NaCl solution.

TABLE 1. ADSORPTION OF CHLORPROMAZINE TO BRAIN REGIONS

Brain region	Range of free concn. of CPZ (mM)	Bound CPZ range (m-moles/kg dry wt.)	Partition coefficient (mean ± S.D.)	No. of determinations
Frontal cortex	0·016–0·028	16·7–25·9	916 ± 98	9
Hippocampus	0·019–0·026	15·0–18·9	753 ± 39	4
Thalamus	0·013–0·024	13·7–24·1	935 ± 141	6
White matter	0·017–0·021	16·0–18·0	899	2

For the first dilution, 1 ml of the CPZ–homogenate mixture was added to 20 ml of 0·9% NaCl. Further desorption was carried out in three steps at 30-min intervals by adding 3 ml of diluted drug–homogenate mixtures to 10 ml of 0·9% NaCl. At each step, duplicate samples were taken, spun down and analyzed for radioactivity. In preliminary experiments, it was shown that the desorption of CPZ from brain homogenates occurs within 5 min and does not change during a 7-hr incubation.

The results of the desorption experiments are presented in Table 2. It can be seen that cortex, thalamus and hippocampus do not differ significantly in respect to the release of CPZ bound to these areas.

TABLE 2. DESORPTION OF CHLORPROMAZINE FROM BRAIN REGIONS

Brain region		CPZ remaining after one to four washes (%)			
		1	2	3	4
Frontal cortex	100	37	15	7	7
	100	36	9	3	3
	100	31	9	1	0
	100	38	14	6	4
Hippocampus	100	34	10	3	3
	100	39	12	1	0
Thalamus	100	39	11	2	2
	100	46	16	9	6
Average	100	37·5	12·0	4	3

Finally, the importance of tissue integrity and cell organization was tested. Small pieces (approximately 300 mg) of cortex, thalamus and hippocampus were incubated in 10 ml of a solution containing ^{35}S-labeled chlorpromazine. The disappearance of CPZ from the supernatant was measured as a function of time. Duplicate experiments showed that the drug was slowly adsorbed by all the tissues and that at 29 hr no equilibrium had been reached. The adsorption at that time amounted to only half the adsorption by homogenates under similar conditions. Again no differences could be detected between the rate of penetration into the three areas tested.

In summary, these results show that chlorpromazine readily adsorbs or desorbs to homogenates and slices of four regions of the brain with equal affinity. These similarities *in vitro* indicate that the observed differences in chlorpromazine uptake by different brain regions *in vivo* must result from different permeability properties of the blood–brain barrier to chlorpromazine. The present finding of nonspecific adsorption together with the fact that chlorpromazine is a nonspecific anesthetic *in vitro*[1–11] suggests the simple conclusion that chlorpromazine action may be directly related to the amount present in any one brain region. As explained previously,[8] such a view is compatible with the well established finding that chlorpromazine inhibits the uptake of noradrenaline into sympathetic nerve fibers.[19]

Acknowledgements—This work was supported by grants (No. 274 and E.G. 46) from the Ontario Mental Health Foundation and Grant MA-2951 of the Medical Research Council of Canada.

Department of Pharmacology,
University of Toronto,
Toronto 5, Canada

W. O. KWANT
P. SEEMAN

REFERENCES

1. J. SIGWALD, D. BOVET and G. DUMONT, *Revue Neurol.* **11–12**, 581 (1946).
2. B. N. HALPERN, G. PERRIN and P. B. DEWS, *J. Physiol., Paris* **40**, 210A (1948).
3. S. COURVOISIER, J. FOURNEL, R. DUCROT, M. KOLSKY and P. KOETSCHET, *Archs int. Pharmacodyn. Thér.* **92**, 305 (1952).
4. P. ROSENBERG and S. EHRENPREIS, *Biochem. Pharmac.* **8**, 192 (1961).
5. J. M. RITCHIE and P. GREENGARD, *J. Pharmac. exp. Ther.* **133**, 241 (1961).
6. H. C. SABELLI, R. MARKS and J. E. P. TOMAN, *Fedn Proc.* **22**, Abstract No. 1374 (1963).
7. P. B. BRADLEY, J. H. WOLSTENCROFT, L. HÖSLI and G. L. AVANZINO, *Nature, Lond.* **212**, 1425 (1966).
8. P. SEEMAN, *Int. Rev. Neurobiol.* **9**, 145 (1966).
9. P. SEEMAN and J. WEINSTEIN, *Biochem. Pharmac.* **15**, 1737 (1966).
10. G. A. V. DE JAMARILLO and P. S. GUTH, *Biochem. Pharmac.* **12**, 525 (1963).
11. W. O. KWANT and P. SEEMAN, *Biochim. biophys. Acta* **183**, 530 (1969).
12. B. GOTHELF and A. G. KARCZMAR, *Int. J. Neuropharmac.* **2**, 39 (1963).
13. A. W. WASE, J. CHRISTENSEN and E. POLLEY, A.M.A., *Archs Neurol. Psychiatry* **75**, 54 (1956).
14. G. B. CASSANO, S. E. SJOSTRAND and E. HANSSON, *Archs int. Pharmacodyn. Thér.* **156**, 48 (1965).
15. G. A. BRAY, *Analyt. Biochem.* **1**, 279 (1960).
16. I. S. FORREST, A. G. BOLT and M. T. SERRA, *Biochem. Pharmac.* **17**, 2061 (1968).
17. W. O. KWANT and J. VAN STEVENINCK, *Biochem. Pharmac.* **17**, 2215 (1968).
18. P. SEEMAN, W. O. KWANT and T. SAUKS, *Biochim. biophys. Acta* **183**, 499 (1969).
19. L. L. IVERSEN, *J. Pharm. Pharmac.* **17**, 62 (1965).

Biochemical Pharmacology, Vol. 20, pp. 2091–2094. Pergamon Press, 1971. Printed in Great Britain

Relative redistribution of [^{3}H]histamine and [^{14}C]spermidine in homogenates of dog brain*†

(*Received* 5 *June* 1970; *accepted* 9 *November* 1970)

MICHAELSON and Dowe[1] employed *n*-butanol extraction of alkalinized acid extracts of tissue for the *o*-phthaldialdehyde (OPT)[2] fluorometric analysis of brain histamine. Their study suggested that histamine is associated with synaptosomes. Organic solvent extracts[2] contain histamine as well as spermidine, which reacts with OPT to produce a fluorophore with spectral characteristics very nearly like those of histamine.[3,4] A reinvestigation of our earliest findings[1] and chromatographic separation of histamine from spermidine[3,4] in the crude mitochondrial fraction demonstrated that 65 per cent of the recovered spermidine is associated with synaptosomes.[5] Other workers[6] have pointed out that the amount of polyamine found in subcellular fractions does not necessarily reflect the distribution within the intact cell because of the high affinity of these basic substances for cellular polyanions. Redistribution of polyamines in a variety of animal tissues after homogenization has been reviewed by Tabor and Tabor.[7] This communication examines the question of secondary redistribution of histamine and spermidine after mechanical disruption of dog hypothalamus tissue. We have tested this possibility by studying the distribution of radioactive histamine and spermidine added: (1) to the homogenized hypothalamus of the dog before it is fractionated by differential centrifugation, and (2) to the "crude mitochondrial" fraction from the hypothalamus before it is further fractionated on a discontinuous sucrose density gradient.

* Supported by U.S.P.H.S., National Institute of Neurological Diseases and Stroke, Grant NB-06053 from the National Institutes of Health.

† A portion of this work was presented before the Fourth International Pharmacology Congress, Basel, Switzerland (July 1969).