Biochemical Pharmacology, Vol. 22, pp. 1677–1680. Pergamon Press, 1973. Printed in Great Britain.

Effect of chloropromazine on ^{3}H-phenazine methosulfate uptake into various regions of rat brain and into subcellular fractions of brain and liver (*in vitro*)

(*Received* 11 *September* 1972; *accepted* 12 *January* 1973)

THIS PAPER reports results obtained on uptake of ^{3}H-phenazine methosulfate (^{3}H-PMS) *in vivo* into whole rat brain, cerebral cortex, cerebellum, mid-brain, hypothalamus and brain stem under the influence of chlorpromazine (CPZ). In addition, it examines uptake of ^{3}H-PMS into mitochondrial and microsomal-cytosol fractions of rat brain and liver. The effects of CPZ on neurochemistry,[1–7] and CPZ on mitochondrial permeability and physiology[8–12] suggested that CPZ might alter the uptake of PMS into both tissue and subcellular fractions from brain and liver.

In vitro, CPZ decreases respiration and Ca^{2+} transport in rat brain mitochondria.[13] *In vitro*, CPZ also inhibits PMS transport in rat liver mitochondria.* In liver mitochondria, PMS transport is both respiration and Ca^{2+} dependent.[14] In the present study, we were particularly interested in whether CPZ would inhibit PMS transport *in vivo*. In addition, we were interested in whether the effect of CPZ on the brain was regional or general in view of the selective blood–brain barrier to CPZ.[15]

In six experiments, male rats of the Wistar strain (400–500 g) were used. The rats were fed a basal diet previously described.[16] Rats were anesthetized with ether, injected intraperitoneally (i.p.) with CPZ, 50 μmoles/kg body wt (Smith, Kline & French Labs) 90 min before ^{3}H-PMS was given i.p. (325 μCi/335 g; New England Nuclear). Controls were given water in lieu of CPZ.

Determination of ^{3}H-PMS uptake into tissue. After 5 min, blood was taken from the heart of an ether-anesthetized rat and the brain was excised. The brain was washed in 20 ml of ice-cold buffer consisting of 0·32 M sucrose, 0·1 mM EDTA, and 5 mM Tris (pH 7·4). The brain was sliced longitudinally with a razor blade and one-half was dissected into aliquots from the cerebral cortex, cerebellum, mid-brain, hypothalamus and brain stem.[17] The brain tissue was disrupted in a small Tri-R glass-Teflon homogenizer, using a motor-driven pestle, in a small buffer volume. The homogenates and blood samples were digested for 2 hr in Protosol (New England Nuclear) at 55°, a fraction of the blood and tissue digests were transferred to a glass scintillation vial containing 15 ml of Aquasol (New England Nuclear), and the samples were counted on a Beckman LS-233 liquid scintillation counter. Chemiluminescence was reduced with glacial aldin acid and samples were counted after 12 hr. Corrections for quench and background activity were made and the specific activity was calculated (dis/min of ^{3}H-PMS/mg of protein). Proteins were determined by the method of Lowry *et al.*[18] with bovine serum albumin as a standard. Data were calculated as the ratio of the specific activity in the tissue relative to blood or as the specific activity of the mitochondrial and microsomal-cytosol fractions compared to blood.

The rationale for computing tissue/blood ratios depends on the fact that tissue uptake of ^{3}H-PMS is directly dependent upon the amount of ^{3}H-PMS in the blood. Since it is practically impossible to accurately reproduce ^{3}H-PMS blood levels on a day-to-day basis with different animals, knowledge of the blood ^{3}H-PMS specific activity (dis/min/mg protein) provides a baseline upon which tissue uptake can be interpreted.

Determination of ^{3}H-PMS in mitochondrial and microsomal fractions. The methods of isolation of brain and liver mitochondria have been described previously.[19,20] Homogenates were prepared in a 50-ml Tri-R homogenizer with a motor-driven Teflon pestle. Homogenates and isolated fractions were treated as described for tissue preparations. Brain[19] and liver[20] microsomal-cytosol fractions were obtained from the supernatants of the first mitochondrial purification steps for each procedure. For the liver microsomal-cytosol fraction, the method was modified from 9000 *g*/10 min to 8 min at 2000 *g* followed consecutively by a 2-min spin at 9000 *g*.

Brain tissue uptake of ^{3}H-PMS. The specific activity in all areas of the brain from the control group was higher than in the blood (Table 1). In contrast, brain ^{3}H-PMS specific activity in the CPZ-treated rats never exceeded that of blood. Analysis of the data, using the F-test, revealed that none of the separate regions of the brain had an uptake significantly different from that of the whole brain. Hence, PMS was uniformly distributed in the brain regions examined.

The blood specific activity between both the treated and untreated rats was not different. Hence, any decrease in the tissue/blood ratio represents inhibition of ^{3}H-PMS uptake by CPZ. The per cent of inhibition of ^{3}H-PMS uptake by CPZ was not significantly different in any one brain region compared with CPZ inhibition of ^{3}H-PMS by whole brain. This uniform effect by CPZ was not expected, in view of the fact that accumulation of CPZ in rat brain follows widely varying degrees of

* S. W. French and D. S. Palmer, unpublished data.

TABLE 1. EFFECT OF CPZ ON ^{3}H-PMS UPTAKE INTO VARIOUS REGIONS OF RAT BRAIN

Tissue	Specific activity (dis/min/mg protein)		Tissue/blood ratio of dis/min/mg protein		% Inhibition of ^{3}H-PMS uptake of CPZ*
	Control	CPZ	Control	CPZ	
Blood	10,283·9± 518·0	11,915·2±1010·0			
Brain	12,984·2± 663·0	11,878·0± 915·0	1·24±0·036†‡	0·99±0·028	19·4
Cerebral cortex	12,865·5± 471·0	11,111·0± 978·0	1·21±0·021‡	0·93±0·010	23·1
Cerebellum	12,352·2±1438·0	10,750·9±1232·0	1·26±0·104§	0·90±0·027	28·7
Mid-brain	11,902·6± 734·0	11,326·7±1106·0	1·18±0·011‡	0·95±0·037	19·6
Hypothalamus	13,127·5± 377·0	10,402·2±1180·0	1·18±0·044‖	0·87±0·044	26·4
Brain stem	13,514·2±1075·0	12,299·0±2826·0	1·39±0·024§	1·01±0·110	27·3

* Calculated from tissue/blood ratios.
† Three animals per group; mean±S. E.
‡ $P < 0·005$ compared with the CPZ-treated rats.
§ $P < 0·05$ compared with the CPZ-treated rats.
‖ $P < 0·01$ compared with the CPZ-treated rats.

TABLE 2. EFFECT OF CPZ ON ^{3}H-PMS UPTAKE INTO FRACTIONS OF RAT BRAIN AND LIVER

Tissue	Specific activity (dis/min/mg protein)		Tissue/blood ratio of dis/min/mg protein		% Inhibition of ^{3}H-PMS uptake of CPZ*
	Control	CPZ	Control	CPZ	
Blood	10,999·1± 1525·0	11,343·5± 1290·0			
Brain homogenate	14,531·7± 1756·0	11,471·7± 1007·0	1·29 ±0·003†‡	1·02±0·026	22·5
Brain mitochondria	5177·4± 840·0	2917·9± 308·0	0·467±0·011†	0·256±0·017	45·2
Brain microsome-cytosol	16,640·6± 3253·0	19,418·7± 2159·0	1·74 ±0·027	1·72 ±0·120	1·2
Liver homogenate	71,400·7± 9536·0	57,647·4±12,671·0	6·49 ±0·047§	4·98 ±0·547	24·5
Liver mitochondria	21,611·3± 3088·0	13,329·5± 2027·0	1·96 ±0·077‖	1·20 ±0·265	38·6
Liver microsome-cytosol	103,498·7±11,760·0	68,683·1±10,574·0	9·51 ±0·494‡	6·00 ±0·242	36·9

* Calculated from tissue/blood ratio.
† Three animals in each group; mean ± S. E.
‡ $P < 0.005$ compared with the CPZ-treated group.
§ $P < 0.05$ compared with the CPZ-treated group.
‖ $P < 0.01$ compared with the CPZ-treated group.

uptake according to the region studied.[21] Despite this, CPZ inhibited to the same degree the uptake of ^{3}H-PMS *in vivo* in different regions of the brain.

^{3}H-PMS uptake into brain and liver fractions. Table 2 gives an indication of the effects of CPZ on ^{3}H-PMS uptake *in vivo* at the subcellular level. Uptake was greater in the liver compared to the brain suggesting that a blood–brain barrier to PMS exists. CPZ inhibited ^{3}H-PMS accumulation by 22·5 per cent in the brain and by 24·5 per cent in the liver. At the mitochondrial level, CPZ caused 45·2 per cent inhibition of uptake into the brain and 38·6 per cent inhibition in the liver. The uniformity of effect in these different tissues is not followed at the microsomal-cytosol level, however. Only the liver microsome-cytosol fraction appears to be sensitive to CPZ.

The present data suggested that CPZ might decrease the blood–brain barrier permeability to ^{3}H-PMS. CPZ has previously been shown to decrease vascular permeability to a toxic chemical mixture[22] and to delay the passage of protein through capillary walls.[23] In addition, CPZ administered subcutaneously together with Coramine and strychnine delays the onset of convulsions.[24] However, in the present study it was shown that CPZ inhibited the uptake of ^{3}H-PMS by the brain and liver homogenates to the same extent. Since there is no anatomical blood–liver barrier, it is more likely that CPZ inhibited active transport of ^{3}H-PMS to the same extent in both liver and brain.

CPZ has been shown to inhibit the active uptake of ^{32}P into brain tissue[25] and the uptake of ^{14}C-acetate for incorporation into brain ATP.[26] CPZ also inhibits the uptake and incorporation of ^{14}C-leucine, glycine and lysine into brain proteins.[27,28] Active uptake of ^{24}Na and ^{42}K into the brain is also CPZ inhibited.[29] *In vitro*, PMS requires Ca^{2+}-supported respiration and electron-flow for active uptake to occur.[30,31] CPZ has previously been demonstrated to inhibit both Ca^{2+} uptake and electron transport.[9,13,32] Data on CPZ inhibition of respiration-supported PMS uptake *in vitro** and CPZ inhibition of brain mitochondrial respiration[13] suggest that, in the present work, CPZ inhibits ^{3}H-PMS uptake *in vivo* by decreasing the active uptake of this phenazine. This conclusion is supported by the fact that CPZ inhibited ^{3}H-PMS uptake by the brain and liver to the same extent.

Department of Pathology,
University of British Columbia,
Vancouver 8, British Columbia, Canada

DOUGLAS S. PALMER
SAMUEL W. FRENCH

REFERENCES

1. J. W. PHILLIS and K. JHAMANDAS, *Comp. gen. Pharmac.* **2,** 306 (1971).
2. M. HUANG, H. SHIMIZU and J. W. DALY, *J. med. chem.* **15,** 462 (1972).
3. M. HUANG and J. W. DALY, *J. med. chem.* **15,** 458 (1972).
4. H. NYBÄCK and G. SEDVALL, *J. Pharmac. exp. Ther.* **162,** 294 (1968).
5. H. NYBÄCK, *Acta physiol. scand.* **84,** 54 (1972).
6. H. NYBÄCK, *Acta pharmac. tox.* **30,** 372 (1971).
7. P. UZUNOV and B. WEISS, *Neuropharmacology* **10,** 697 (1971).
8. S. W. FRENCH, T. TODOROFF and M. L. NORUM, *Expl molec. Path.* **16,** 270 (1972).
9. S. TJIOE, N. HAUGAARD and C. P. BIANCHI, *J. Neurochem.* **18,** 2171 (1971).
10. T. MATSUBARA and B. HAGIHARA, *J. Biochem. Tokyo* **63,** 156 (1968).
11. N. S. SHAH, *Brain Res.* **38,** 391 (1972).
12. S. W. FRENCH and T. TODOROFF, *Res. Commun. Chem. Path. Pharmac.* **2,** 206 (1971).
13. S. A. TJIOE, A. A. MANIAN and J. O'NEILL, *Biochem. biophys. Res. Commun.* **48,** 212 (1972).
14. S. W. FRENCH, D. S. PALMER and W. A. SIM, *Can. J. Biochem. Physiol.*, in press.
15. W. O. KWANT and P. SEEMAN, *Biochem. Pharmac.* **20,** 2089 (1971).
16. S. W. FRENCH, *J. Nutr.* **91,** 292 (1967).
17. J. GLOWINSKI and L. L. IVERSEN, *J. Neurochem.* **13,** 655 (1966).
18. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. biol. Chem.* **193,** 265 (1951).
19. K. IZAWA, K. SETA, H. TAKEDA, K. ANDO, H. HONDA and C. ASAKI, *J. Biochem., Tokyo* **59,** 501 (1966).
20. D. F. PARSONS and G. R. WILLIAMS, in *Methods in Enzymology* (Eds. S. W. COLOWICK and N. O. KAPLAN), p. 443. Academic Press, New York (1955.)
21. A. W. WASE, J. CHRISTENSEN and E. POLLEY, *A.M.A. Archs Neurol. Psychiatry* **75,** 54 (1956).
22. A. T. HASEGAWA and R. I. H. WANG, *Radiat. Res.* **45,** 364 (1971).
23. R. GUILLEMIN and C. FORTIER, *Endocrinology* **48,** 617 (1951).
24. E. N. GARBATOVA, *Pharmac. Toxic.* **22,** 74 (1959).
25. T. M. SHTUTMAN, *Ukr. biokhem. Zh.* **31,** 405 (1959).
26. H. G. ALBAUM and L. J. MILCH, *Ann. N.Y. Acad. Sci.* **96,** 190 (1962).
27. A. J. GLASKY, *Fedn Proc.* **22,** 272 (1963).
28. E. ZÖLLER, K. SCHREIER and P. R. YANG, *Arzneimittel-Forsch.* **8,** 238 (1958).
29. J. CHRISTENSEN, Y. S. I. FENG, E. POLLEY and A. W. WASE, *Fedn Proc.* **17,** 358 (1958).
30. S. W. FRENCH, D. S. PALMER and B. A. FRENCH, *Fedn Proc.* **31,** 289 (1972).
31. S. W. FRENCH, D. S. PALMER and W. A. SIM, *Proc. Can. Fedn Biol. Soc.* **15,** 676 (1972).
32. C. H. GALLAGHER, J. H. KOCH and D. M. MANN, *Biochem. Pharmac.* **14,** 789 (1965).