4. J. F. FOSTER in *The Plasma Proteins*, F. W. PUTNAM, Ed., vol. 1, pp. 194–202. Academic Press, New York (1960).
5. B. B. BRODIE and C. A. M. HOGBEN, *J. Pharm. Pharmacol.* **9**, 345 (1957).
6. J. F. DOUGLAS, B. J. LUDWIG and A. SCHLOSSER, *J. Pharmacol. exp. Ther.* **138**, 21 (1962).
7. J. F. DOUGLAS, B. J. LUDWIG, T. GINSBERG and F. M. BERGER, *J. Pharmacol. exp. Ther.* **136**, 5 (1962).
8. S. S. WALKENSTEIN, C. M. KNEBEL, J. A. MACMULLEN and J. SEIFTER, *J. Pharmacol. exp. Ther.* **123**, 254 (1958).
9. F. M. BERGER and B. J. LUDWIG, U.S. Patent 2,937,119 (May 17, 1960).
10. I. M. KLOTZ, F. M. WALKER and R. B. PIVAN, *J. Amer. chem. Soc.* **68**, 1486 (1946).
11. A. H. ANTON, *J. Pharmacol. exp. Ther.* **129**, 282 (1960).
12. A. J. HOFFMAN and B. J. LUDWIG, *J. Amer. pharm. Ass., sci. Ed.* **48**, 740 (1959).
13. J. D. TERESI and J. M. LUCK, *J. biol. Chem.* **194**, 823 (1952).

A comparative study of chlorpromazine and its demethylated derivatives: Potency and tissue distribution

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A PREVIOUS STUDY from our laboratory[1] showed the N-demethylated derivatives of chlorpromazine*—namely Nor_1CP and Nor_2CP—less potent than CP in inducing sedation in rats and rabbits, as seen both in behavioral and electroencephalographic experiments. Nor_1CP and CP appeared to be equally potent as based upon the electroencephalographic parameter of elevating recruitment threshold (rabbits). Data from rat experiments included only behavioral evaluations at a 90-min postinjection period and rat brain phenothiazine quantitation again for this 90-min period. Drug doses used in this former study ranged from 0·042–0·126 mmoles/kg.[1]

In the present study we have extended our work, using rats. Behavioral evaluations and brain phenothiazine determinations were made at 90 min and at 20 min after i.p. injection of a single equimolar drug dose level (0·084 mmole/kg). This was done in order to confirm whether or not the comparative potencies of Nor_1CP and CP differed on a temporal basis in rats, as had been the case with rabbits.[1] We have refined our evaluation of pharmacological effectiveness further by quantifying the pharmacological potency of these drugs in terms of an index. In addition, the drug tissue distributions were determined in liver, fat, and brain and compared with the respective chloroform-buffer and benzene-buffer partition coefficients.

METHODS AND MATERIALS

The methods used in behavioral evaluation of rat as well as quantitation of phenothiazine and phenothiazine sulfoxide have been presented in the previous publication.[1] Depressions of reactivity are denoted by a negative sign, and statistical evaluations were not attempted on the behavioral judgments. A general discussion of methods used in animal behavioral evaluation are available.[2,3]

The depression of reactivity was divided by the drug level found in the brain. This quotient is referred to as an index of pharmacological effectiveness and represents the depression of reactivity which theoretically results from a brain drug level of 1 μmole drug/g brain. The higher this index of pharmacological effectiveness, the greater the potency of a compound in producing sedation, as judged by our parameters.

Statistical analysis of mean drug values per gram of tissue included the F test for homogeneity of variance and Student's 't' test. All P values have been calculated from Student's 't' table based on size of samples.[4,5]

Chloroform-buffer and benzene-buffer partition coefficients were established by shaking the drug derivative in a solution of 0·1 M phosphate buffer (pH 7·4) at room temperature with an equal volume of chloroform or benzene for 1 hr. Fat tissue was taken from perirenal and testicular depots.

* The following abbreviations are used: chlorpromazine (tertiary amine) CP; desmonomethyl chlorpromazine (secondary amine) Nor_1CP; desdimethyl chlorpromazine (primary amine) Nor_2CP; chlorpromazine sulfoxide, CPSO.

RESULTS AND DISCUSSION

Table 1 shows the demethylated derivatives of CP to be pharmacologically weaker than CP in exerting depression of behavioral reactivity in rats, as based upon the listed indexes of pharmacological effectiveness. Nor_1CP exhibited intensity of behavioral depression equal to that of CP in the initial 20-min postinjection period as previously described,[1] but this equality of action is achieved

TABLE 1. RAT BRAIN LEVELS AND BEHAVIORIAL RESPONSES 20 AND 90 MIN AFTER INJECTION OF DRUG.*

	20-min data			90-min data‡			
Injected drug	CP	Nor_1CP	Nor_2CP	CP	Nor_1CP	Nor_2CP	CPSO
No. of rats	6	6	4	12	12	12	6
Mean drug value (μmoles/g brain ± standard error of mean)	0·048 ± 0·005†	0·103 ± 0·013†	0·058 ± 0·009	0·070 ± 0·005	0·135 ± 0·014	0·095 ± 0·009	0·030 ± 0·004
Mean depression or reactivity	−1·7	−1·6		−2·3	−1·6	−0·9	0
Index of pharmacological effectiveness§	−34·6	−15·6		−33·4	−12·0	−9·5	0

* 0·084 mmole/kg, i.p.
† These means were significantly different from one another ($P<0·01$) at 20 min.
‡ The means of CP, Nor_1CP, and CPSO at 90 min after i.p. injection were significantly different ($P<0·01$) from one another. However, CP and Nor_2CP were not significantly different from each other ($P>0·01$). This was also true of Nor_1CP and Nor_2CP ($P>0·01$).
§ $\frac{\text{Drug brain conc. (μmole/g brain).}}{\text{Mean depression of reactivity}}$

when Nor_1CP has accumulated to twice the brain level of CP. We have already shown that a linear type of relationship exists between brain drug levels of CP, Nor_1CP, or Nor_2CP and the depression of reactivity induced by the particular compound.[1] CPSO has little or no effect in depressing rat reactivity.

The data presented in Table 2 show that the drug-tissue distributions of these compounds differ markedly from one congener to the next. The chloroform-buffer and benzene-buffer partition

TABLE 2. DRUG TISSUE DISTIBUTION 90 MIN AFTER INJECTION OF ALL DRUGS*

Compound	Benzene buffer	Chloroform buffer partition coefficient†	Brain, μmole/g	Fat, μmole/g	Liver, μmole/g
CP	18·7	31·3	0·070 (12)‡	0·806 (6)	0·227 (12)
Nor_1CP	8·9	19·2	0·135 (12)	0·181 (6)	0·321 (13)
Nor_2CP	8·3	14·1	0·095 (14)	0·147 (6)	0·231 (13)
CPSO	4·5	45·5	0·030 (6)	0·001 (6)	0·437 (3)

* 0·084 mmole/kg.
† All partition coefficients represent the mean of four determinations.
‡ (Number of animals.)

coefficients agree in their relative comparative levels of these drugs in fat, except for CPSO. It may well be that the very high chloroform-buffer partition coefficient for CPSO is due to the fact that this is a much more polar molecule than CP, Nor_1CP, or Nor_2CP. Chloroform, being a polar organic solvent, should solubilize more CPSO than any of the other drugs. CPSO is shown least soluble in benzene. Little agreement between the drug partition coefficients and their relative comparative brain levels is, however, seen. CP exerted a longer-lasting depression than the other drugs. This more enduring effect may be due to enhanced storage in fat, which thus fosters protection from enzymatic

destruction and aids the prevention of rapid excretion. The liver drug values (means of 12 rats) show the Nor_1CP level to be significantly higher than CP or Nor_2CP at the 0·005 level of confidence. The greater accumulation of Nor_1CP than CP in brain cannot be attributed solely to the action of a selective blood-brain barrier phenomenon because similar differential accumulations are seen in liver as well.

This study shows that Nor_1CP accumulates in the brain to a greater extent than CP, Nor_2CP, of CPSO. This faster and greater accumulation may account for the more rapid onset of depression of reactivity with Nor_1CP and also the equal depression of reactivity between CP and Nor_1CP in the initial half-hour postinjection period. When, however, the brain level is taken into account, as in our indexes of pharmacological effectiveness, it is clear that the demethylated congeners are pharmacologically weaker than CP through the entire postinjection interval. CPSO is shown to be devoid of potency in depressing rat behavioral reactivity. The enhanced accumulation of Nor_1CP in brain cannot be completely accounted for in terms of a blood-brain barrier phenomenon.

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REFERENCES

1. G. G. BRUNE, H. H. KOHL, W. G. STEINER and H. E. HIMWICH, *Biochem. Pharmacol.* **12**, 679 (1963).
2. S. IRWIN, *Science* **136**, 123 (1962).
3. S. IRWIN, *Program Notes*, p. 133. Gordon Research Conference on Medicinal Chemistry, Colby Junior College, New London, N.H., Aug. 3–7 (1959).
4. A. L. EDWARDS, *Experimental Design in Psychological Research*, p. 163. Rinehart, New York (1950).
5. N. M. DOWNIE and K. W. HEATH, *Basic Statistical Methods*, H. H. REMMERS, Ed., p. 123. Harper, New York. (1959).

Failure of D-aldosterone to affect ouabain-augmented oxygen uptake of isolated rabbit atria*

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RECENT work has indicated that the changes in contractile, electrical, and ionic parameters of isolated heart muscle produced by ouabain cannot be significantly modified by the potent mineralosteroid D-aldosterone.[1, 2] However, other evidence has appeared indicating that changes in certain tissue functions such as the short-circuit current (SCC) of toad bladder[3] and the oxygen uptake by slices of rat ventricle[4] induced by aldosterone may be reversed by the addition of a cardiac glycoside. Moreover, other evidence has shown that while ouabain inhibits active Na^+ transport across cell membranes,[5] aldosterone may enhance active Na^+ transport.[6] These observations led us to test the working hypothesis that if these steroids are mutually antagonistic with regard to membrane ion transport effects, the antagonism might apply to a modification by aldosterone of the stimulatory effect of ouabain on cardiac O_2 consumption.

The oxygen uptake (QO_2) of isolated rabbit left atria was measured by the direct method of Warburg, utilizing an automated device which permitted continuous direct recording of O_2 uptake in microliters. Female albino rabbits (1·5 to 2·5 kg) were sacrificed, exsanguinated, and their hearts quickly removed. The left atrial appendage was dissected from the rest of the heart and placed in Warburg flasks containing 3·0 ml of a Krebs-Ringer phosphate medium with 5·5 mM glucose as substrate. The center well contained 0·2 ml of 20% KOH. Drug effects were studied by placing appropriate amounts of ouabain or D-aldosterone† in the medium to yield the indicated final molar

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