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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† Crystalline D-aldosterone kindly supplied by Dr. J. J. Chart, Ciba Inc., Summit, N.J.

concentrations. The contents of the flask and manometers were gassed with 100% O_2 for 10 min while shaking in a 37·5° constant-temperature tank. At the end of this equilibration period the flasks were sealed and O_2 uptake continuously recorded for 2 to 4 hr. At the end of the experiment the tissues were removed, blotted lightly on filter paper, and dried in an oven (95 to 110°) overnight for the determination of dry weights.

The results obtained in these experiments are shown in Table 1. Ouabain ($2{\cdot}8 \times 10^{-6}$ M) produced by a significant increase (93·7%) in the O_2 uptake of the atria relative to the untreated controls.

TABLE 1. EFFECT OF D-ALDOSTERONE, OUABAIN, AND ALDOSTERONE + OUABAIN ON THE OXYGEN UPTAKE (QO_2) OF RABBIT LEFT ATRIAL TISSUE

Treatment	No. of experiments	QO_2*
Controls	7	5·52 ± 0·35
D-Aldosterone† ($1{\cdot}1 \cdot 10^{-4}$ M)	6	5·08 ± 0·27‡
Ouabain ($2{\cdot}8 \cdot 10^{-6}$ M)	9	10·69 ± 0·73§
D-Aldosterone + ouabain	9	12·09 ± 0·59¶

* QO_2 = microliters O_2 per milligram dry weight per hour; mean, ± S.E.M.
† Crystalline D-aldosterone (acetate).
‡ $P > 0{\cdot}05$ compared to controls.
§ $P < 0{\cdot}05$ compared to controls.
¶ $P > 0{\cdot}05$ compared to ouabain alone.

D-Aldosterone alone ($1{\cdot}1 \cdot 10^{-4}$ M) failed to affect the O_2 uptake of the tissues. When ouabain and D-aldosterone were present together in the medium, the effect was similar to the response seen with ouabain alone.

The data presented here, coupled with previous measurements of other myocardial parameters,[1, 2] fail to support the view that aldosterone may antagonize or modify the actions of ouabain on isolated cardiac muscle. Since D-aldosterone alone is without significant action on the QO_2 of atrial tissue, including that from rat and man,[7] we were unable to test the alternative possibility that ouabain may antagonize the actions of this hormone on cardiac respiration.[4] In other tissues such as the frog skin[8] and toad bladder,[3] where these steroids may be mutually antagonistic insofar as their effects on SCC are concerned, no data are available to indicate whether O_2 consumption changes might parallel the electrical changes.

Thus, while the results presented here and elsewhere are not compatible with the view that aldosterone may antagonize the actions of ouabain on isolated atrial muscle, the possibility is not excluded that, under certain conditions and in certain tissues, these two steroids may be mutually antagonistic.

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REFERENCES

1. J. V. LEVY and V. RICHARDS, *Arch. int. Pharmacodyn.* **146**, 363 (1963).
2. J. V. LEVY and V. RICHARDS, *Proc. Soc. exp. Biol. (N.Y.)* **114**, 280 (1963).
3. G. SHARP and A. LEAF, *J. clin. Invest.* **42**, 978 (1963).
4. C. MATARAZZO and R. CHIUMMO, *Boll. Soc. ital. Biol. sper.* **39**, 95 (1963).
5. I. GLYNN, *J. Physiol. (Lond.)* **136**, 148 (1957).
6. F. GROSS, *Germ. med. Mth.* **7**, 21 (1962).
7. J. V. LEVY and V. RICHARDS, *Fed. Proc.* **22**, 185 (1963).
8. W. WILBRANDT and E. WEISS, *Arzneimittel-Forsch.* **10**, 409 (1960).