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RESERPINE AS AN UNCOUPLER OF OXIDATIVE PHOSPHORYLATION AND THE RELEVANCE TO ITS PSYCHOACTIVE PROPERTIES

EUGENE C. WEINBACH,*† JONATHAN L. COSTA,‡ C. ELWOOD CLAGGETT,† DENISE D. FAY‡ and TORILL HUNDAL§

†Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20205, U.S.A.; ‡Clinical Neuropharmacology Branch, National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20205, U.S.A.; and §Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

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Abstract—Many drugs differing widely in chemical structure uncouple mitochondrial oxidative phosphorylation *in vitro*. This observation has led to the hypothesis that *in vivo* uncoupling is the basis of their pharmacological activity. Serpasil, a parenteral preparation of reserpine, recently has been shown to uncouple oxidative phosphorylation in vervet monkey kidney mitochondria. Although the drug exhibits some properties of a "classical" uncoupler, our studies show that it has a dual effect on energy conservation. Reserpine released respiratory control in rat liver mitochondria only when dissolved in organic solvents (as in Serpasil) or when deprotonated. Reserpine also released the oligomycin-induced respiratory control in beef heart submitochondrial particles, and inhibited energized uptake of Ca^{2+} by rat liver mitochondria. Reserpine had a dual effect on mitochondrial ATPase: It (a) enhanced ATP hydrolysis by intact liver mitochondria, and (b) inhibited ATP hydrolysis by submitochondrial particles of beef heart. On a molar basis, reserpine was less effective than carbonyl cyanide 3-chlorophenylhydrazone in all bioenergetic reactions examined. Homogenates and mitochondria isolated from brain and liver of rats stuporous from intraperitoneally injections of Serpasil exhibited no detectable abnormalities in respiratory states and responded to known uncouplers in the expected manner. There was no evidence of *in vivo* uncoupling of oxidative phosphorylation as a basis of the pharmacological activity of reserpine, although interference with energy transfer may be involved in toxic manifestations of the drug. The results indicate the need for caution in interpreting the action of drugs formulated in complex pharmaceutical preparations and based solely on *in vitro* experiments.

One of the most striking features of uncoupling agents is the wide diversity in their chemical structure [1, 2]. As many chemotherapeutic compounds are structurally similar to known uncoupling agents, it is not surprising that *in vitro* uncoupling of mitochondrial oxidative phosphorylation has been observed with numerous drugs. Such observations have led to the hypothesis that *in vivo* interference with energy metabolism is the basis of their pharmacological activity [3, 4]. Numerous psychoactive drugs are in this category [5]. Recently, Maina [6] demonstrated that Serpasil acts as an uncoupler of oxidative phosphorylation in vervet monkey liver mitochondria.

In the present study we have examined the effects of Serpasil and its active ingredient reserpine on mitochondrial energy metabolism in rat and bovine tissues. Although the drug exhibits some properties of a "classical"‖ uncoupler, it has a dual effect on energy metabolism. We have also examined, by *in vitro* and *in vivo* methods, a possible relationship between the uncoupling action of reserpine and its psychoactive properties.

MATERIALS AND METHODS

Chemicals. Reserpine, as the crystalline compound, was purchased from the Aldrich Chemical Co., Milwaukee, WI, and Serpasil from the Ciba–Geigy Corp., Summit, NJ. We are indebted to Dr. R. M. Diener, Ciba–Geigy Corp., for providing the formulation and a generous amount of Serpasil vehicle used in the commercial preparation of Serpasil for parenteral injection. Calcium-45 (sp. act. 144 mCi/mg) was obtained from the New England Nuclear Corp., Boston, MA. Generous amounts of reserpine analogs were supplied by Dr. J. W. Daly, NIADDK, National Institutes of Health. All other reagents were commercial products of the highest purity available.

* Address all correspondence to: E. C. Weinbach, Ph.D., Bldg. 5, Rm. 124, NIH, Bethesda, MD 20205, U.S.A.

‖ "Classical" uncouplers have the following properties in common: They prevent net phosphorylation of ADP to ATP by mitochondria in the presence of oxidizable substrates and maximal respiration, they are moderately weak acids with lipophilic electron-withdrawing groups, they behave as proton ionophores in phospholipid bilayer membranes, and they bind to proteins [1, 2, 7]. Other properties of these compounds are described throughout the text.

Mitochondria. Intact mitochondria were isolated from rat liver [8] and brain [9] by published procedures. Beef heart was the source of submitochondrial particles isolated with EDTA [10].

Assays. Oxygen uptake was determined polarographically as described previously [11]. Respiratory ratios and states were calculated by the method of Chance and Williams [12].* Release of respiratory particles was done as described by Lee and Ernster [10]. ATPase of rat tissue mitochondria was assayed by determining the amount of P_i released from ATP [13]. ATPase activity of beef heart submitochondrial particles was assayed spectrophotometrically with an ATP-regenerating system [14]. ATP-dependent uptake of Ca^{2+} by rat liver mitochondria was estimated by the use of $^{45}Ca^{2+}$ and Millipore filtration [15]. Details of these assays are given in the legends of the figures and tables.

Reproducibility. All polarographic tracings presented in this report are representative of at least three separate experiments. Data on mitochondrial bioenergetics are typically shown in this manner [11]. All assays presented in tabular form were replicated independently at least three times, and the data were calculated as the averages of each set of measurements.

RESULTS

Oxidative phosphorylation. Carefully isolated and washed rat liver mitochondria are "tightly coupled"; that is, they exhibit the phenomenon of respiratory control. Oxidation of exogenous substrates is obligatorily dependent on the phosphorylation of ADP to ATP. Classical uncouplers such as 2,4-dinitrophenol (DNP)† and carbonyl cyanide 3-chlorophenylhydrazone (CCCP) remove this control, and oxidation proceeds without concomitant phosphorylation. These transitions in respiratory states [13] are illustrated in Fig. 1, tracing A, where it is also seen that Serpasil induced the uncoupled State 3_u of respiration, and that CCCP is a much more potent uncoupling reagent.

This effect was observed with succinate, as shown, and with NAD-linked substrates such as glutamate or DL-β-hydroxybutyrate. In our initial experiments, addition of reserpine in aqueous media at the same concentration as in Serpasil had little effect. At first, we considered that some other component of the complex pharmaceutical formulation might be responsible for the apparent release of respiratory control observed with Serpasil. For example, the

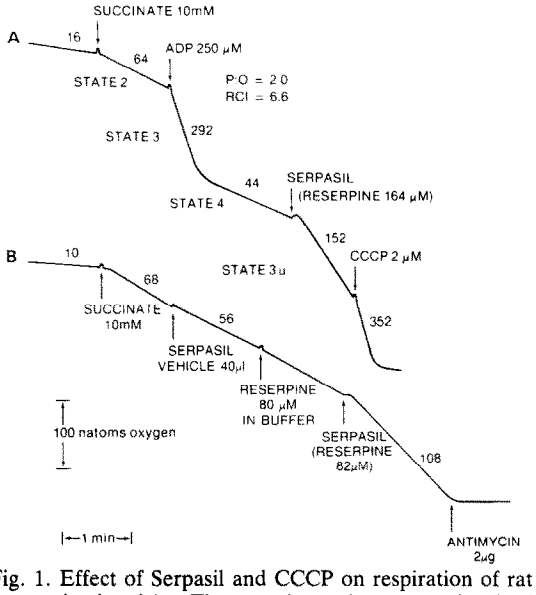

Fig. 1. Effect of Serpasil and CCCP on respiration of rat liver mitochondria. The reaction mixture consisted of 20 mM HEPES (pH, 7.4), 15 mM P_i (pH, 7.4), 5 mM $MgCl_2$ and 0.2 ml of mitochondria (4 mg protein), plus other additions as indicated in a final volume of 1.0 ml. Tracing A: Serpasil contains 4.1 mM reserpine; 40 μl was added. Tracing B: Reserpine was dissolved in 50 mM Tris–Cl buffer, pH 7.4. The numbers on the tracings express the oxygen consumption as nanoatoms per min at 24°. RCI = respiratory control index, i.e. the ratio of the rate of respiration in State 3 to that in State 4.

parenteral preparation contains 68 mM adipic acid (1,4-dibutanedicarboxylic acid), and it is known that fatty acids uncouple oxidative phosphorylation [16]. Adipic acid was tested and found to be devoid of uncoupling properties. Serpasil is formulated with sodium sulfite, and liver mitochondria readily oxidize sulfite [17]. This possibility also was examined, but sulfite oxidation was unaffected by antimycin, whereas release of respiratory control by Serpasil was inhibited completely by antimycin, indicating that the stimulated oxidation proceeded via the respiratory chain (Fig. 1, tracing B). Serpasil contains ascorbic acid but, under conditions of these experiments, ascorbate was not oxidized by rat liver mitochondria. When the Serpasil vehicle became available, it was tested and found to have no effect on coupled respiration (Fig. 1, tracing B).

Uncoupling and solubility of reserpine. Crystalline reserpine is relatively insoluble in aqueous media. When reserpine was dissolved in a minimal amount of glacial acetic acid and then added to the aqueous incubation medium buffered at pH 7.4, precipitation occurred when the final reserpine concentration exceeded 88 μM. However, when reserpine was dissolved in *N,N*-dimethylacetamide (as is done in the Serpasil formulation) and then added to the incubation medium, final reserpine concentrations as high as 328 μM could be maintained without apparent precipitation. Addition of large volumes of reserpine dissolved in glacial acetic acid lowered the pH of the incubation medium and diminished the rate of release of respiratory control. Addition of

* In their original formulation, State 2 designated conditions when the concentrations of ADP and O_2 were high and the level of exogenous substrate was low. For convenience, we have used State 2 to designate conditions where the concentrations of substrate and O_2 are high and that of ADP low.

† Abbreviations: DNP, 2,4-dinitrophenol; CCCP, carbonyl cyanide 3-chlorophenylhydrazone; FCCP, carbonyl cyanide 4-trifluoromethoxyphenylhydrazone; HEPES, *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonate; MES, 2-(*N*-morpholino) ethanesulfonate; DMA, *N,N*-dimethylacetamide; SUCC, succinate; PYR, pyruvate; MAL, malate; OLIG, oligomycin; and SMP, submitochondrial particles.

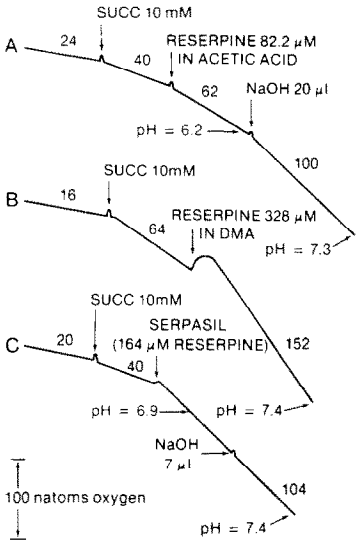

Fig. 2. Effect of pH. The composition of the reaction mixture was the same as indicated in the legend of Fig. 1. Duplicate reaction vessels were prepared and, where indicated, the pH values were determined. Crystalline reserpine was dissolved in a minimal amount of glacial acetic acid to effect solution and diluted with water to the desired concentration. Microliter amounts of 1 N NaOH were added where indicated. Other conditions were as shown in Fig. 1. The initial pH of Serpasil was 5.5.

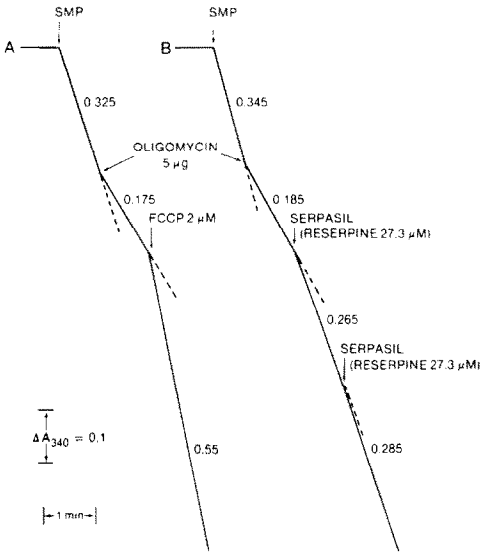

Fig. 3. Effect of reserpine on oligomycin-induced respiratory control of beef heart submitochondrial particles. The reaction mixture consisted of 50 mM Tris–Cl, pH 7.6, 0.2 mM NADH, and 250 mM sucrose, plus additions as indicated, in a final volume of 3.0 ml. Submitochondrial particles (SMP), 100 μg protein, were added to initiate the reaction which was determined at 30° by the decrease of absorbance at 340 nm in a recording spectrophotometer. The numbers on the tracings refer to the ΔA per min.

alkali raised the pH, and State 3_u respiration was increased (Fig. 2, tracing A). In contrast, addition of Serpasil (initial pH, 5.5) released respiratory control at a rate that was unaffected by subsequent addition of alkali (Fig. 2, tracing C). As shown in Fig. 2, tracing B, addition of reserpine dissolved in DMA released respiratory control to the extent observed with Serpasil. DMA alone had no effect on mitochondrial respiration. Further studies (not shown) revealed that other organic solvents (*N*,*N*-dimethylformamide, dimethyl sulfoxide) served equally well as vehicles for reserpine.

Reserpine analogs. Data summarized in Table 1 show that uncoupling of oxidative phosphorylation, as evidenced by release of respiratory control, is property shared by analogs of reserpine. It is also evident that the ability of a particular analog to release respiratory control is not correlated with its ability to release norepinephrine from mouse heart [18].

Submitochondrial particles. In addition to its uncoupling action on intact rat liver mitochondria, Serpasil released the oligomycin-induced respiratory control of beef heart submitochondrial particles, although to a lesser extent than did FCCP (Fig. 3, tracings A and B).

Rat brain mitochondria. The preparations used here [9] did not exhibit the sharp transitions of the

Table 1. Comparison of the effects of reserpine and reserpine analogs on the respiratory states of mitochondria isolated from rat liver*

Compound added after succinate	Release of [^{3}H]norephinephrine from mouse heart† (%)	Ratio of respiratory states ($3_u/2$)	Ratio of respiratory states ($3_u/2$) after oligomycin (5 μg/ml)
Reserpine	88	1.61	1.56
Methyl reserpate	75	1.25	1.00
Yohimbine	40	1.50	1.39
Coryanthine	31	1.56	1.26
Methyl triepireserpine	15	1.50	1.20
N-Methyl methyl reserpate	9	1.36	1.14
Sarpagine	2	1.32	1.32
Raunitidine	0	1.57	1.24

* Experimental conditions are given in the legend to Fig. 1. The final concentration of each compound was 200 μM.

† As reported by Creveling *et al.* [18].

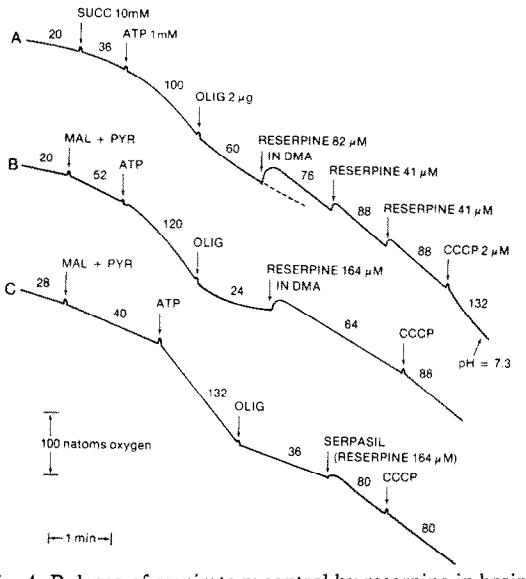

Fig. 4. Release of respiratory control by reserpine in brain mitochondria. Experimental conditions are as indicated in the legend of Fig. 1, except that the reaction medium also contained 15 units units of hexokinase and 10 mM glucose. Malate (0.5 mM) and pyruvate (10 mM) were added as indicated in tracings B and C.

various respiratory states seen with liver mitochondria possibly because of contamination with ATPase-containing structures such as myelin fragments and nerve-ending particles [19]. Addition of ADP or ATP (in the presence of hexokinase and glucose) elicited State 3 respiration that was sensitive to oligomycin (Fig. 4, tracings A, B and C). Reserpine or Serpasil released the oligomycin block. Figure 4 also shows that reserpine was equally effective with NAD-linked substrates (malate/pyruvate) as it was with succinate. Similar effects were observed with brain mitochondria isolated by two other methods [19, 20]. The rate of respiration with brain mitochondria was acutely sensitive to small changes in pH. Addition of CCCP after addition of 164 μM reserpine in DMA was less effective in releasing respiratory control in brain mitochondria than in liver mitochondria (compare Fig. 4, tracings A, B, and C with Fig. 1, tracing A). It is of interest that in brain, but not in liver mitochondria, addition of CCCP after Serpasil elicited little or no increase in State 3_u respiration, whereas CCCP added alone was effective in releasing respiratory control. This anomaly remains unexplained.

Parenterally administered reserpine. Rats injected intraperitoneally with Serpasil appeared highly sedated. Liver and brain mitochondria isolated from these animals exhibited normal respiratory transitions with succinate or glutamate as substrates, with no evidence of uncoupling (Table 2). Addition of reserpine, Serpasil, CCCP, or DNP released respiratory control in a fashion similar to that observed with control rats injected with Serpasil vehicle. To examine the possibility that the drug was removed from the organelles during the preparation-related washings, assays were made of the homogenates. These showed little evidence of uncoupled respira-

Table 2. Effect of reserpine *in vivo**

Fraction	Additions	Respiration [natoms O · min^{-1} · (mg protein)$^{-1}$]			
		Liver		Brain	
		Control	Serpasil	Control	Serpasil
Homogenate	None	5.9	6.4	6.6	8.0
	ADP	20.1	11.4	16.1	12.0
Mitochondria	None	4.6	5.0	8.0	7.7
	Succinate	20.0	15.0	16.8	21.7
	ADP	74.0	53.7	38.4	49.0
Respiratory control index		5.6	4.0	†	†

* Rats were injected intraperitoneally with Serpasil (reserpine, 0.75 mg/kg) once daily for 3 days. Liver and brain homogenates and mitochondria were isolated, as cited in Materials and Methods, and assayed immediately with the polarograph. The composition of the reaction mixture and other experimental conditions were as given in the legend of Fig. 1.

† Because of the variable amounts of ATPase activity present in the brain preparations, it was not possible to determine consistent RCI values.

Table 3. Effects of reserpine and CCCP on calcium uptake by isolated rat liver mitochondria*

Additions to medium	Amount of Ca^{2+} uptake† [nmoles · min^{-1} · (mg protein)$^{-1}$]	Inhibition (%)
None (control)	174 ± 24	
CCCP (2 μM)	17.7 ± 2.9	90
DNP (400 μM)	17.9 ± 1.9	90
DMA (80 μl)	157 ± 35	10
Reserpine (164 μM) in DMA	81 ± 12	53
Serpasil	38 ± 2.5	78

* Each reaction vessel contained 10 mM Tris-maleate buffer, pH 7.0, 10 mM succinate, 4 mM P_i, 8 mM NaCl, 10 mM $MgCl_2$, 3 mM ATP, 2.0 mM $CaCl_2$ (containing 0.1 μCi $^{45}Ca^{2+}$), 2 mg of mitochondrial protein, other additions as indicated, and sufficient 0.25 M sucrose to make a final volume of 2.0 ml. Before addition of mitochondria to initiate the reaction, the pH of those vessels containing reserpine or Serpasil was adjusted to 7.4. After incubation at 24° for 5 min, the reaction mixture was rapidly filtered by vacuum through Millipore filters (0.45 μm pore size) and the filters were transferred to liquid scintillation mixtures and counted in a Beckmann LS9000 spectrometer (Palo Alto, CA). Under these conditions, the uptake of Ca^{2+} was linear with time.

† Values are expressed as means ± S.E.M.; N = 5.

tion (Table 2); the responses to ADP were inhibited by oligomycin, and subsequently released by CCCP (data not shown).

Calcium uptake. Classical uncouplers of oxidative phosphorylation impede the energized (ATP-dependent) uptake of Ca^{2+} by isolated rat liver mitochondria [21]. Results summarized in Table 3 show that CCCP, DNP, reserpine, or Serpasil had a similar inhibitory effect. It is clear that, on a molar basis, CCCP was much more potent than reserpine or Serpasil, but that the latter was about as effective as DNP. DMA alone was weakly inhibitory.

Effects on ATPase. In view of the known action of classical uncouplers to promote the hydrolysis of ATP in freshly isolated rat liver mitochondria [13], it was of interest to determine whether or not reserpine had a similar effect. As detailed in Table 4, addition of reserpine or Serpasil weakly enhanced the low endogenous ATPase activity of liver mitochondria, whereas CCCP produced the expected strong stimulation. None of these compounds had any significant effect on the high endogenous level of ATPase activity of brain mitochondria. Unexpectedly, studies with the ATPase from beef heart particles disclosed that Serpasil had an *inhibitory*, oligomycin-like effect on the catalytic activity of these preparations (Fig. 5).

Reserpine and partially-uncoupled oxidative phosphorylation. Mitochondria isolated from the livers

Table 4. ATP hydrolysis*

Conditions	P_i released from ATP [nmoles · min^{-1} · (mg protein)$^{-1}$]	
	Liver	Brain
Control	9	26
CCCP (5 μM)	61	27
Reserpine in DMA (164 μM)	24	18
DMA (40 μl)	8	26
Serpasil (40 μl) (reserpine, 164 μM)	18	29
Serpasil vehicle (40 μl)	9	27

* The reaction mixture contained 60 mM Tris–Cl buffer, pH 7.4, 6 mM ATP, 2 mg protein of either rat liver or brain mitochondria, other additions as indicated, and sufficient 0.25 M sucrose to make a final volume of 1.0 ml. Before addition of mitochondria to initiate the reaction, the pH of those vessels containing reserpine or Serpasil was adjusted to 7.4. Incubation time, 10 min; temperature, 30°.

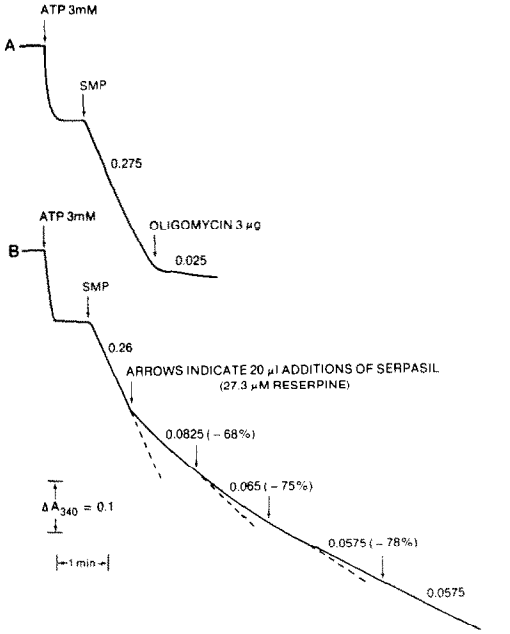

Fig. 5. Reserpine inhibition of ATPase from beef heart. The assay medium consisted of 25 mM Tris-acetate, pH 7.6, 30 mM K acetate, 3 mM Mg acetate, 1 mM phosphoenolpyruvate, 1.67 μM rotenone, 0.2 mM NADH, 25 units of lactate dehydrogenase and 55 units of pyruvate kinase plus other additions as indicated in a final volume of 3.0 ml. Decrease in absorbance at 340 nm was followed at 30° after the addition of the oligomycin-sensitive ATPase of beef heart submitochondrial particles (SMP; 46 μg protein). Numbers on the tracings refer to the ΔA per min.

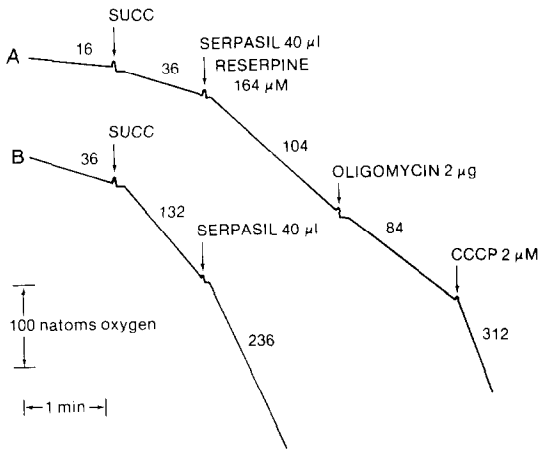

Fig. 6. Effect of reserpine on partially-uncoupled oxidative phosphorylation. Liver mitochondria were isolated from rats injected intraperitoneally twice daily with either saline (tracing A) or with pargyline, 20 mg/kg (tracing B), and assayed as described in the legend of Fig. 1.

of rats injected intraperitoneally with pargyline, an irreversible monoamine oxidase inhibitor [22], exhibited partially uncoupled oxidative phosphorylation (Fig. 6), and greater than 80% inhibition of monoamine oxidase (data not shown). Addition of Serpasil (tracing B) elicited a much higher rate of respiration than that observed with mitochondria from control (saline-injected) rats (tracing A). CCCP elicited the same degree of uncoupling in the mitochondria from either animal (data not shown).

DISCUSSION

The results of the present study support the conclusion of Maina [6] that Serpasil exhibits properties of an uncoupler of oxidative phosphorylation in that it releases respiratory control, impedes energized uptake of Ca^{2+}, and increases proton permeability in isolated vervet monkey kidney mitochondria. On the other hand, our studies have shown that the drug has a dual effect on mitochondrial energy conservation. Contrary to the action of classical uncouplers, reserpine only weakly enhanced ATPase activity in intact rat liver mitochondria, and had an oligomycin-like effect on the ATPase from beef heart submitochondrial particles in that it *inhibited* enzymatic activity (Fig. 5). Also in contrast to classical uncouplers [11], the action of reserpine was neither prevented nor reversed by a 3-fold molar excess of defatted bovine serum albumin (data not shown). It is of interest that reserpine was more effective as an uncoupler in liver mitochondria from pargyline-injected rats than in control rats; the State 3_u rate approached that of CCCP. Oxidative phosphorylation in these mitochondria was partially uncoupled as a result of the pargyline injections (Fig. 6). Pargyline has been shown to act as an uncoupler when added *in vitro* to isolated rat hepatocytes [23]. Our work thus suggests that reserpine has multiple sites of action on aerobic energy metabolism.

It is evident from our study that the solubility of reserpine markedly influences its biochemical activity. The limited solubility of reserpine in neutral aqueous media requires acidification or the use of organic solvents to achieve adequate concentrations. Because reserpine is more soluble in aqueous media under acidic conditions, it is more likely to enter lipophilic biological compartments (i.e. cell membranes) under conditions where it is deprotonated.

Other work has suggested that, for reserpine to elicit amine release, it must be capable of acting as a protonophore, i.e. the tertiary nitrogen in ring C must *not* be complexed so that it always carries a positive charge [18]. Similarly, the uncoupling action of reserpine and the analogs examined here, all of which have a free tertiary nitrogen in ring C, may be the result of protonophoric action at this locus. Other factors (e.g. steric configurations) may also be necessary for amine release to occur, since releasing potency and uncoupling activity are not well correlated (Table 1).

A major conclusion that emerges from this study is that uncoupling of oxidative phosphorylation *in vivo* is unlikely to be the basis of the psychoactive effect of the drug. Mitochondria isolated from rats stuporous from intraperitoneally injections of Serpasil exhibited normal parameters of coupled respiration and responded to known classical uncouplers (CCCP, DNP) in the usual way. If uncoupling were involved in the pharmacological activity of reserpine, the brain homogenates would be expected to show it. Previous studies have shown that appreciable levels of reserpine accumulate in both brain and liver following intraperitoneally injection of tritriated reserpine [24]. Washed mitochondria may have contained the drug *in vivo* which was lost during the washings. Unwashed mitochondria (homogenates), however, exhibited little evidence of drug-induced abnormalities of energy conservation (Table 2). Furthermore, *in vivo* uncoupling would be expected in our experiments, although other actions of reserpine may have obscured its effect on temperature. Therefore, it appears unlikely that reserpine, in concentrations that cause unmistakable psychoactive effects, interferes with mitochondrial energy production *in vivo*. On the other hand, the action of reserpine as an uncoupler of oxidative phosphorylation *in vitro* with both intact and submitochondrial particles, as well as its interference with the energy transfer reaction in the membrane-bound ATPase, may indicate a biochemical basis for its toxic effects in concentrations greater than normal pharmacological doses. It should be pointed out that, even in the homogenates, dilution of the mitochondria may have lowered the drug to a concentration below the minimal amounts needed to uncouple respiration *in vitro*.

Finally, these observations indicate the need for caution in interpreting the action of drugs which are dissolved in complex pharmaceutical preparations, and based solely on *in vitro* experiments.

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REFERENCES

1. E. C. Weinbach and J. Garbus, *Nature, Lond.* **221**, 1016 (1969).
2. P. G. Heytler, *Pharmac. Ther.* **10**, 461 (1980).
3. T. M. Brody, *Pharmac. Rev.* **7**, 335 (1955).
4. E. Noack, *Trends pharmac. Sci.* **2**, 225 (1981).
5. E. Bachmann and G. Zbinden, *Biochem. Pharmac.* **28**, 3519 (1979).
6. G. Maina, *Biochim. biophys. Acta* **333**, 481 (1974).
7. R. J. Kessler, C. A. Tyson and D. E. Green, *Proc. natn. Acad. Sci. U.S.A.* **73**, 3141 (1976).
8. E. C. Weinbach, *Analyt. Biochem.* **2**, 335 (1961).
9. E. C. Weinbach and J. Garbus, *J. biol. Chem.* **234**, 412 (1959).
10. C. P. Lee and L. Ernster, in *Methods in Enzymology* (Eds. R. W. Estabrook and M. E. Pullman), Vol. X, p. 543. Academic Press, New York (1967).
11. E. C. Weinbach and J. Garbus, *J. biol. Chem.* **241**, 3708 (1966).
12. B. Chance and G. R. Williams, *Adv. Enzymol.* **17**, 65 (1956).
13. E. C. Weinbach, *J. biol. Chem.* **221**, 609 (1956).
14. M. E. Pullman, H. S. Penefsky, A. Datta and E. Racker, *J. biol. Chem.* **235**, 3322 (1960).
15. P. Caroni and E. Carafoli, *Nature, Lond.* **283**, 765 (1980).
16. B. C. Pressman and H. A. Lardy, *Biochim. biophys. Acta* **21**, 458 (1956).
17. H. J. Cohen, S. Betcher-Lange, D. L. Kessler and K. V. Rajagopalan, *J. biol. Chem.* **247**, 7759 (1972).
18. C. P. Creveling, J. W. Daly, R. T. Parfitt and B. Witkop, *J. med. Chem.* **11**, 596 (1968).
19. S. Lovtrup and T. Zelander, *Expl Cell Res.* **27**, 468 (1962).
20. J. B. Clark and W. J. Nicklas, *J. biol. Chem.* **245**, 4724 (1970).
21. F. D. Vasington and J. V. Murphy, *J. biol. Chem.* **237**, 2670 (1962).
22. R. R. Rando, *Science* **185**, 320 (1974).
23. F. P. A. Carr, S. A. Smith and C. I. Pogson, *Biochem. Pharmac.* **29**, 1103 (1980).
24. R. E. Stitzel, *Pharmac. Rev.* **28**, 179 (1977).