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Mechanism of action of antipsychotic drugs on biological electron transport

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SEVERAL pharmacological agents are known to be potent inhibitors of the respiratory chain in bacteria, yeast and mammalian mitochondria. Chlorpromazine, other phenothiazine derivatives, and several of the barbiturates[1] have been shown to cause inhibition of mitochondrial electron transport and coupled phosphorylation.[2] Interestingly enough, it has been shown that there is some correlation between the tranquilizing activity of chlorpromazine and its inhibition of mitochondrial electron transfer reactions; it acts as an uncoupler of oxidative phosphorylation at low concentrations (0–80 μM) and as an inhibitor of electron transport at high concentrations (80–265 μM).[3] We have recently observed an interesting interaction between an antipsychotic drug, Clomacran phosphate (CLO), SK & F 14,336, (2-chloro-9-[3-dimethylaminopropyl] acridan, phosphate salt) and the monosodium salt of riboflavin-5-phosphate (FMN);[4] the latter was shown to catalyze the oxidation of CLO to its acridine derivative (2-chloro-9-[3-dimethylaminopropyl] acridine) in the presence of visible light under anaerobic conditions. As the FMN was not reduced during this reaction, the action of the flavin was attributed to a triplet–triplet energy transfer between light-excited FMN and CLO. These observations plus the fact that phenothiazines and dihydroacridines exhibit structural similarities prompted us to investigate the mechanism of action of CLO on the biological electron transport chain. The preliminary data indicate that CLO interferes with electron transfer in the NADH-dehydrogenase segment of the respiratory chain in *Pseudomonas saccharophila*, a facultative autotrophic bacterium.

The organism was grown aerobically in a liquid medium described by Doudoroff[5] with succinate as the carbon and energy source and NH_4Cl as the sole nitrogen source. After 16 hr of incubation at 30°, the cells were harvested, washed twice with 0·05 M tris-HCl buffer (pH 7·2). The cell paste containing 10 g of wet-packed cells were suspended in 10 ml of the sonication medium employed by Aleem[6] for the preparation of actively phosphorylating particles from *Nitrobacter agilis*. The medium contained: 0·3 M sucrose, 1·0 mM $MgCl_2$, 0·5 mM EDTA-Na_2, 0·5 mM reduced glutathione and 0·05 M tris-HCl (pH 7·2). The cells were disrupted by passing twice through an AMINCO-French

TABLE 1. EFFECT OF CLOMACRAN AND 9-AMINOMETHYLACRIDAN ON OXIDATIVE AND PHOSPHORYLATIVE ACTIVITY IN *P. saccharophila**

Substrate	Inhibitor	Inhibitor concn (mM)	O_2 consumed (μatoms)	ATP formed (μmoles)	P/O
NADH	Complete system		0·379	0·206	0·54
	Clomacran	0·01	0·386	0·081	0·21
	Clomacran	0·02	0·373	0·050	0·00
	9-AMA	0·1	0·304	0·049	0·16
	9-AMA	0·2	0·276	0·016	0·06
Succinate	Complete system		0·248	0·132	0·53
	Clomacran	0·1	0·248	0·141	0·57
	9-AMA	1·0	0·221	0·116	0·53

* The experimental conditions were described in the text, except Clomacran and 9-AMA were added where indicated. The substrates used were 3·3 mM NADH and 7 mM succinate; the reaction mixture contained 1·0 mg enzyme protein per 2·0 ml when NADH was the substrate and 2·26 mg enzyme protein per 2·0 ml when succinate was the substrate.

pressure cell at about 18,000 psi. The resulting extract was centrifuged at 15,000 *g* for 30 min and the cell-free supernatant was used as the enzyme source. Protein content of the cell-free preparations was determined by the biuret method of Gornall *et al.*[7]

The oxidation of NADH and succinate was measured polarographically at 30°. The test system for the measurement of oxidation and coupled phosphorylation consisted of 10 mM sucrose, 5 mM

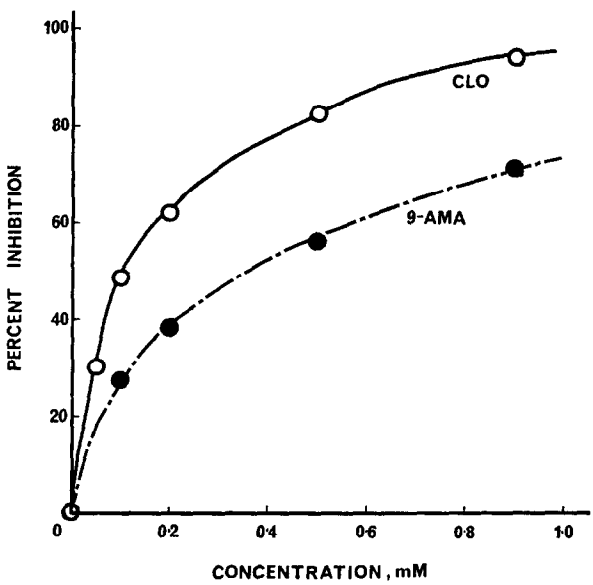

FIG. 1. Effect of Clomacran (CLO) and 9-aminomethylacridan (9-AMA) on the oxidation of NADH. The reaction mixture in a total volume of 3·0 ml contained *P. Saccharophila* cell-free extracts containing 3 mg of enzyme protein, 285 μmoles of tris–HCl (pH 7·0) and inhibitor concentration as shown. The enzyme preparations were pre-incubated for 3 min with the inhibitor before addition of 10 μmoles of NADH to start the reaction.

$MgCl_2$, 10 mM KF, 4 mM inorganic phosphate, 2 mM ADP, 100 mM tris–HCl (pH 7·0) and an appropriate enzyme concentration as indicated in Table 1. The same reaction mixture without substrate was used as a control in all experiments. The reaction was initiated by the addition of substrate; after 3 min of incubation at 30°, the samples for ATP measurements were deproteinized according to

the procedure of Gibson and Morita.[8] The amount of ATP formed was determined as the difference in the amount of ATP between sample and control, by the Luciferin-Luciferase method of Strehler.[9]

The data in Fig. 1 show that about 60 per cent inhibition of the NADH oxidase occurred in the presence of 0·2 mM CLO, whereas the same concentration of chlorpromazine caused about 100 per cent inhibition. It was also observed that a 0·2 mM concentration of an acridine derivative of CLO, i.e. [2-chloro-9-(3-dimethylaminopropyl) acridine dihydrochloride], caused only slight inhibition of the NADH oxidase system. The dihydroacridine compound 9-aminomethylacridan (9-AMA), which is structurally similar to CLO, showed only 37 per cent inhibition of electron transport when used at a concentration of 0·2 mM. The activity as well as the inhibition pattern of the NADH oxidase was virtually unaffected by the type of reaction mixture used either in Fig. 1 or in Table 1. When succinate was used as an electron donor, no inhibition of the electron transport occurred in the presence of 0·5 mM CLO or 0·2 mM 9-AMA.

The oxidative phosphorylation coupled to NADH oxidation was inhibited almost completely in the presence of 0·02 mM CLO or 0·2 mM 9-AMA, while 0·1 mM CLO and 1·0 mM 9-AMA showed no effect on phosphorylation coupled to succinate oxidation (Table 1). The electron transport from NADH or succinate to O_2 was observed to be mediated by cytochrome components of the *b*, *c*, and *o* type, as identified spectrophotometrically. However, in the presence of 1·0 mM CLO the reduction of the cytochrome system was completely blocked when NADH served as the electron donor while the reduction of cytochromes by succinate remained unaffected. We have observed that the ATP-dependent reduction of NAD^+ by succinate catalyzed by the cell-free preparations from *P. saccharophila* was also markedly sensitive to CLO or 9-AMA when used at a concentration of 1·0 mM. Although the respiration, phosphorylation and the reverse electron flow processes were not equally sensitive to CLO or 9-AMA, the results indicate that the suppression by these inhibitors of respiration and coupled phosphorylation, as well as the inhibition of the ATP-linked reversal of electron transfer in *P. saccharophila*, appear to occur at a component not common to the NADH- and succinate-oxidase systems.

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