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PREFERENTIAL INHIBITION OF RESPIRATION IN *SACCHAROMYCES CEREVISIAE* BY CHLORIMIPRAMINE

CORRELATION WITH CHLORPROMAZINE

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Abstract—Growth of most yeast strains was inhibited in the presence of 10 μg/ml chlorimipramine and chlorpromazine respectively when the carbon source was glycerol or pyruvate. Concentrations of about 100 μg/ml of the drugs were required to inhibit growth when other non-fermentable substrates (succinate, citrate and α-ketoglutarate) or the fermentable substrate glucose, were used; that is, at this higher concentration there was general toxicity. Inhibition by the drugs of oxygen uptake by cells respiring glycerol or pyruvate was seen in the oxygen electrode. A concentration of about 50 μg/ml inhibited uptake by 50 per cent. There was not detectable inhibition of oxygen uptake at this concentration when Krebs cycle intermediates were being respired. Spontaneous mutants resistant to one or other of the drugs were isolated from glycerol cultures and in all cases there was cross-resistance indicating close similarity in mode of action of the drugs. Mutants were classified as (1) resistant to general toxic effects and (2) resistant to inhibition of respiration. It was concluded that the drugs were most active in blocking entry of pyruvate into the Krebs cycle with chlorpromazine marginally more effective than chlorimipramine. An interpretation of the results in terms of binding of drugs to cellular membranes is discussed.

IT IS generally believed that the central effects of psychotropic drugs are mediated through actions on brain amines and in this respect the anti-depressant action of chlorimipramine (CIMP) apparently results from an effect on the re-uptake of noradrenaline. It is not known how the drug achieves this effect but in view of the finding that it inhibits respiratory processes both in isolated mitochondria and in tissue slices, it is proposed that CIMP and other tricyclic drugs act primarily in restricting energy supply to the nervous system.[1, 2]

Our preliminary finding that both chlorpromazine (CPZ) and CIMP selectively inhibit the yeast mitochondrial system in intact cells[3] opened the way for a more detailed analysis of these effects at the cell level making use of a microbial system.

MATERIALS AND METHODS

Strains of *Saccharomyces cerevisiae* from the collection in this laboratory were used. This species of yeast is a facultative anaerobe and as such can grow and divide without a functional respiratory system provided there is a supply of fermentable substrate. Specific inhibition of the respiratory system by drugs is demonstrated by

inability of cells to grow in the presence of the drug in non-fermentable media while growth proceeds if fermentable substrate is available.

Solid and liquid media containing 1% Difco yeast-extract and 2% bacto-peptone (YEP) were used with glucose (2%) as fermentable substrate (YEP-S), and glycerol, (YEP-G), pyruvate, citrate, α-ketoglutarate and succinate as alternative non-fermentable substrates each at a concentration of 4%. YEP alone is unable to support growth to any extent.

In initial testing for inhibitory effects on growth, the drop-out technique on solid medium was used.[4] This consists of making suspensions of cells of the different yeast strains in water and placing individual drops of suspension (each containing approximately 10^4 cells) at assigned spots on the medium in petri dish series. A system for testing up to 27 strains simultaneously in this way is shown in Fig. 1.

Growth was also followed in YEP-G liquid media. Cells were grown for 24 hr in YEP-G and then inoculated into flasks containing the drug and the optical densities plotted against time.

In testing inhibition of respiration by the drugs, oxygen uptake of whole cells was measured by means of the Rank Oxygen Electrode system. Yeast cells which had been pre-cultured for 24 hr with appropriate substrate were washed and introduced into the container (4 ml capacity) to give a suspension of about 10^7 cells/ml in phosphate buffer (pH 7) together with the same substrate.

Cytochromes spectra of intact cells were recorded in the Unicam SP 800 Spectrophotometer.[5]

Chlorpromazine (May & Baker) and chlorimipramine (Geigy Ltd.) are water soluble and were introduced directly into YEP medium from freshly made stock solutions. In the case of solid medium, this was done just before pouring into petri dishes. The drugs were inoculated in progressive amounts into the oxygen electrode in aqueous solution.

RESULTS

Growth inhibition. The results of testing a number of genetically different strains for inhibition of growth on drug plates by the drop-out method are given in Table 1 and a typical series of test plates is shown in Fig. 1. In all cases there is greater sensitivity on the non-fermentable medium (YEP-G) up to a 20-fold difference in the case of strain 188. In other words, the respiratory system is much more sensitive to the action of these drugs than other cell processes since comparatively high concentrations are required to inhibit growth on YEP-S medium. A good correlation is seen in the degree of sensitivity of the various strains to the two drugs indicating a similarity in mode of action. Further evidence of a correlation was seen when 22 spontaneous resistant mutants from five different sensitive strains were found to show cross-resistance to the two drugs. These mutants were isolated as individual colonies that came up against a background of inhibited parental cells on YEP-G plates containing 10 μg/ml of one or other of the drugs (Fig. 1) and therefore were showing ostensibly, relative resistance to inhibitory effects on the respiratory system. Mutants had increased tolerance ranging from 4-fold to 7-fold (40–70 μg/ml) in the case of CIMP with corresponding increases of 3-fold to 6-fold (30–60 μg/ml) in tolerance to CPZ. However, all the resistant mutants isolated had simultaneously acquired increased tolerance to the drugs on sugar-containing medium compared to the respective parental strains,

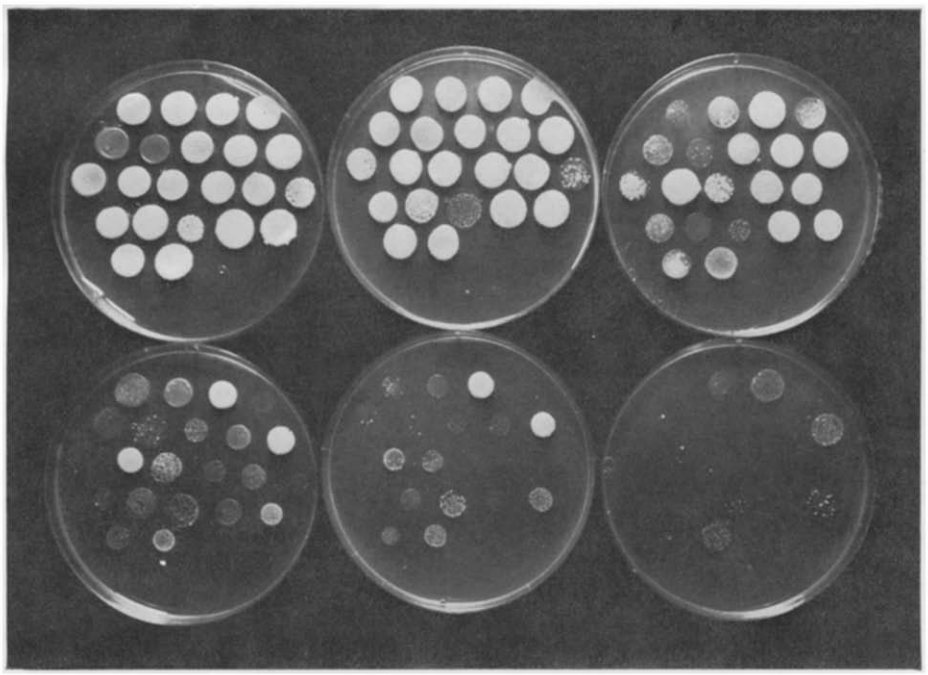

FIG. 1. Screening of yeast strains for sensitivity to chlorimipramine on yeast extract, glycerol medium containing drug in the amounts 0, 5, 10, 20, 40, 70 μg/ml reading from top left to bottom right. Each spot inoculum of each strain contains about 10^4 cells in the replica plates. Spontaneous resistant mutants can be seen as small colonies against a background of inhibited parental cells.

although this was only slight and not proportionate in some cases. Presumably in the latter the mutation has affected mainly or only the inhibitory effect of the drugs on the respiratory system and the extra amount of growth at maximum tolerance level on YEP-S is achieved through respiratory activity. Where a concomitant increase in resistance is seen on the fermentable medium, the most likely mechanism of resistance is an alteration in cell permeability to the drugs. These responses to the drugs were stable features of the parental strains and their mutants.

TABLE 1. DRUG SENSITIVITY OF STRAINS OF *SACCHAROMYCES CEREVISIAE* WITH GLUCOSE (YEP-S) AND GLYCEROL (YEP-G) AS CARBON SOURCES RESPECTIVELY

Strain	Concentrations* inhibiting cell growth (μg/ml)			
	Chlorimipramine		Chlorpromazine	
	YEP-S	YEP-G	YEP-S	YEP-G
13-2D	100	100	100	100
22-5A	100	100	100	100
188	100	5	70	5
S878	70	10	70	5
A18	70	10	50	10
A74	70	10	50	10
A7F	70	10	50	10
A15	70	10	50	10
A30	70	10	70	10
38-3A	70	10	50	10
1-9C	70	10	50	10
1323-1	70	10	50	10
A26	50	10	40	10
26-482	50	10	50	10
A433	50	10	40	10
A33	50	10	50	10
A43	50	10	50	10
11-1	50	10	50	10
121-4B	50	10	50	10

* Range of drug concentrations used: 5, 10, 20, 40, 50, 70 and 100 μg/ml.

Growth curves obtained in liquid YEP-G showed that strain A26 is totally inhibited in the presence of 15 μg/ml CIMP (Fig. 2a) while growth is only partially affected in the case of the resistant mutant 262 (derived from A26) at 40 μg/ml (Fig. 2b).

Strains A18, A26 and A74 were grown to stationary phase on YEP-S medium containing 25 μg/ml CIMP and CPZ respectively. Cells were then harvested and their absorption spectra recorded. Peaks indicating the presence of cytochromes *a*, *b* and *c* were obtained. From these results it appeared that the inhibition of growth on non-fermentable medium by the drugs at this concentration was not due to an effect on the biosynthesis of the mitochondrial system but presumably on its function.

Inhibition of oxygen uptake. Oxygen uptake was studied in the presence and absence of drug in strains A18, A26 and A74 and their respective resistant mutants 279, 262 and 275. Glycerol-grown cells were used with glycerol as added substrate in the oxygen electrode. The results are recorded in Fig. 3 for parental strains and from which it can be seen there is inhibition of oxygen uptake by CIMP although considerably higher concentrations of drug are required to inhibit this process than to

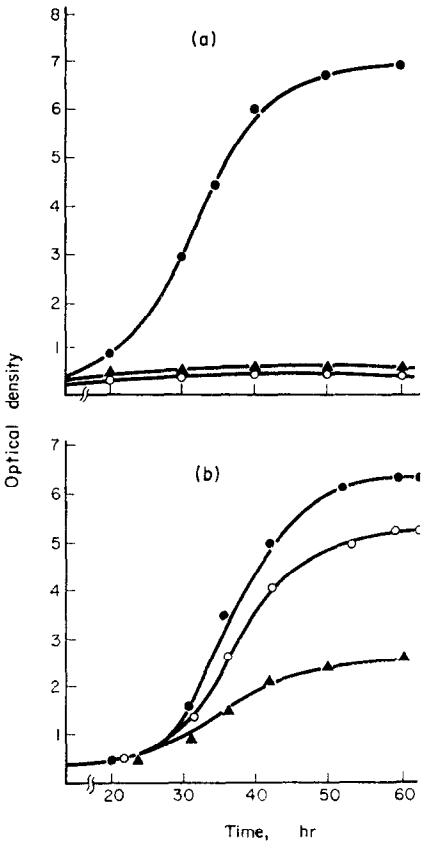

FIG. 2. Growth of strain A26 (a) and its resistant mutant 262 (b) in the presence and absence of chlorimipramine. ●——● without drug in a and b; ▲——▲ 10 μg ml in a, 40 μg ml in b; ○——○ 15 μg ml in a, 25 μg/ml in b.

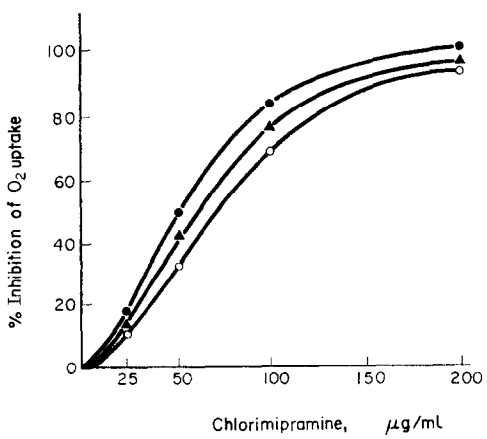

FIG. 3. Inhibition of oxygen uptake by chlorimipramine in yeast strains. ●——● strain A26, ▲——▲ strain A18, ○——○ strain A74.

inhibit growth on YEP-G medium. Results obtained with CPZ were similar although in general this drug was about 20 per cent more active than CIMP, a fact also apparent from growth inhibition findings. It was found that the resistant mutant strains, classified as resistant on the basis of ability to grow in drug concentrations that inhibited parental strains, were inhibited to about the same extent as parental strains regarding oxygen uptake. This apparent lack of correlation between inhibition of growth and inhibition of respiration can be explained if the former is a long-term effect and oxygen uptake inhibition an immediate manifestation of drug action requiring high concentrations. Evidence of a cumulative effect on oxygen uptake with time at low drug concentration was seen when cells of A18 were grown in sugar medium containing 20 μg/ml for 12 hr. These cells showed no detectable oxygen uptake in the oxygen electrode. On the other hand, cells of the resistant mutant 279 treated in the same way were respiratory active. These results permit the conclusion that the inhibition of cell growth by the drugs on YEP-G medium is due to the inhibition of respiration of glycerol.

In attempts to establish at which point in the respiratory process inhibition was occuring, the same six strains were grown using one or other of the non-fermentable substrates pyruvate, citrate, α-ketoglutarate and succinate. Growth on citrate and succinate was generally poor possibly because of difficulty of entry of these substrates into the cells and it was necessary to select within the strains for better ability to use these compounds as carbon sources. Pyruvate and α-ketoglutarate were similar to glycerol in supporting growth. Cell samples of each culture were introduced into the oxygen electrode and the same substrate as for the culture was added to the system. There was inhibition of oxygen uptake by the two drugs once again and similar to that described for glycerol in the case of pyruvate respiration. On the other hand, when citrate, α-ketoglutarate and succinate were used as substrates there was little or no inhibition of oxygen uptake at concentrations of drug as high as 300 μg/ml. Furthermore, in the glycerol/pyruvate series when maximum inhibition had been brought about, addition to the electrode of succinate restored oxygen uptake by as much as 30 per cent of the original rate while in some cases α-ketoglutarate restored about 20 per cent generally.

Growth inhibition of cells using these substrates in the presence of the drugs paralleled the oxygen uptake findings: there was inhibition of growth on the pyruvate medium at drug concentrations that inhibited on the glycerol medium but inhibition on Krebs cycle intermediates required drug amounts similar to those for inhibition on glucose. These results indicate that the main activity of the drugs is to block the utilization of pyruvate (glycerol of course, is converted to pyruvate before entry into the Krebs cycle) while allowing the Krebs cycle and respiratory chain to remain unaffected, apparently.

DISCUSSION

Løvtrup[6] carried out an extensive biochemical and biophysical study of the effects of CPZ and IMP on isolated mitochondria of liver and brain. He concluded that the drugs have no specific chemical activity but rather their inhibition of various aspects of respiration is probably due to adsorption of the drugs to membrane, a view also advanced by other workers in the field as he points out. Kwant and Seeman[7] for example have demonstrated that CPZ binds to the erythrocyte membrane. The drug

apparently competes for attachment sites in the membrane with Ca^{2+} which it displaces. An interpretation of the results with yeast cells based on reactions of the drugs with cell membranes is feasible. Since activity of this kind would clearly affect membrane-associated enzymes, it may be concluded that the functional aspects of mitochondrial membranes concerned with pyruvate utilization are much more sensitive to these effects of CPZ and CIMP than other aspects of organellar membrane function or to the impairment by the drugs of cell membranes generally. Provided that drug concentrations are high (of the order of 100 μg/ml) most, if not all membrane functions appear to be affected in bringing about general toxicity. The hypothesis favoured here then, is one of indiscriminate binding of these drugs to cell membranes in achieving their results. The greater sensitivity of the pyruvate system may reflect the overall complexity of the reaction involved in pyruvate metabolism, requiring as it does the activity of several mitochondrial enzymes. This aspect of drug activity is being investigated in more detail at the biochemical level in conjunction with the analysis of resistant mutants.

Although the connection between clinical potency and activity against the yeast mitochondrion is at present tenuous, preliminary results from the screening of a number of derivatives of CIMP and related drugs are encouraging and indicate a correlation.

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