J. Pharm. Pharmacol. 1983, 35: 668–670
Communicated March 2, 1983

Effect of miconazole on *Saccharomyces cerevisiae*

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Miconazole nitrate [1-(2,4-dichloro-8-(2,4-dichlorobenzyloxy)phenyl) imidazole nitrate] is a potent antimycotic agent (Godefroi et al 1969), which has a wide spectrum of antimicrobial activity. It effects especially the growth of dermatophytes, yeasts and Gram positive bacteria (Van Cutsem & Thienpont 1972; Holt 1974; Swamy et al 1974; Sawer et al 1975). Several workers have shown that the ultrastructure of cell membranes was altered by imidazole derivates (De Nollin & Borgers 1974; Preusser & Rostek 1979; Borgers et al 1981), and biochemical investigations by Swamy et al (1974) have indicated that miconazole affects the plasma membrane of *Candida albicans*. At low concentrations miconazole (Van den Bossche et al 1978) affects ergosterol synthesis in yeast cells resulting in an accumulation of 14 α-methylsterols known to impair membrane functions, whereas higher concentrations of the agent lead to a shift from unsaturated to saturated fatty acids (Van den Bossche et al 1981). Furthermore, miconazole seems to change the lipid organization in the membrane without binding to the lipids (Van den Bossche et al 1982). Recently, Arndt et al (1982) have shown that miconazole affects the electrical conductivity of artificial bilayer membranes.

In the present publication we describe the effect of miconazole on the proton-gradient of the plasmamembrane of *Saccharomyces cerevisiae* and the uptake of purine bases, glucose and amino acids. We also show that the effects caused by miconazole are not due to an inhibition of RNA or protein synthesis.

Materials and methods

Cells of the diploid strain R XII of *Saccharomyces cerevisiae* (a kind gift of Dr A. Kotyk, Prague) were grown in GYNP-medium containing 2% glucose, 1% Difco Yeast Nitrogene Base and 0·5% peptone at 30 °C, and harvested in the early stationary growth phase (about 10^8 cells ml^{-1}). For the isolation of nuclei and the preparation of the cell-free translational system cells were harvested in the logarithmic growth phase.

The manometric assay of respiration and fermentation was carried out with a Warburg-apparatus (Braun-Melsungen, SL 85) at 30 °C as described earlier (Hempel & Laskowski 1964). For the isolation of yeast nuclei a modification of the low temperature procedure described by Schibler and Weber (1974) was used. The cells were suspended in a buffer consisting of 30 mM Tris-HCl pH 7·9, 20 mM NaCl, 2 mM $MgCl_2$, 1 mM sperimidine, 6 mM 2-mercaptoethanol, 400 mM sucrose and 40% glycerol. The cells were kept on ice for 20 min and then broken in a French-Pressure Cell (American Instrument Co.) (Schulz-Harder et al 1979). Following the disruption of the cells the homogenate was centrifuged at 4000*g* (HB 4 rotor, Sorvall) for 15 min at −20 °C. The pellet was resuspended in buffer and centrifuged under the same conditions. Both supernatants were pooled and centrifuged for 15 min at 16 000*g* (−20 °C). The pellet was resuspended in 20 ml buffer and centrifuged again for 15 min at 4000*g*. Finally the supernatant was centrifuged for 15 min at 16 000*g*, and then the pelleted nuclei were resuspended in the homogenzation buffer and stored at −25 °C. In-vitro RNA synthesis was performed as described earlier (Schulz-Harder & Tata 1982). The cell-free protein synthesis system of yeast was prepared and used according to the method of Kreutzfeldt (1983).

For the measurement of the initial rate of uptake of purine bases, cells of a 50 ml culture (stationary cells) were suspended in 250 ml glucose citrate buffer (50 mM sodium citrate, pH 5·4, 2% glucose), and incubated at 30 °C under aeration for 60 min. Then the initial uptake velocities were measured according to the method of Forêt et al (1978). In order to measure the effect of miconazole on the proton-gradient of the plasma membrane of yeast, stationary cells were preincubated

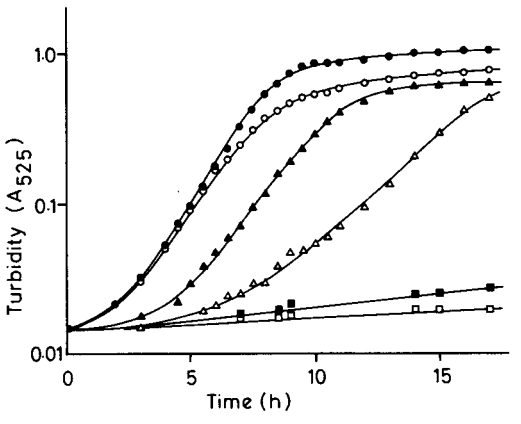

FIG. 1. Effect of miconazole on the growth of *Saccharomyces cerevisiae*. The cells were cultivated in GYNP-medium at 30 °C ●——● without miconazole and in the presence of various concentrations of miconazole, ○——○ 0·3 μM, ▲——▲ 5 μM, △——△ 10 μM, ■——■ 15 μM, □——□ 20 μM.

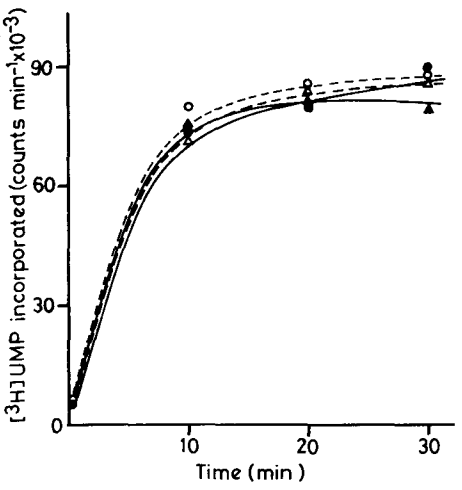

FIG. 2. Effect of miconazole on the incorporation of [^{3}H]UTP into RNA in the isolated yeast nuclei. Nuclei were incubated for the times indicated at 30 °C ●——● without miconazole and in the presence of various miconazole concentrations, ○ - - - ○ 50 μM, ▲——▲ 100 μM, △ - - - △ 200 μM.

in glucose citrate buffer as described above and resuspended in 2% glucose solution at 30 °C under aeration for 60 min at a concentration of 2×10^7 cells ml^{-1}. Thereafter the cell-suspension was stirred at room temperature (20 °C), and the pH was monitored with a pH meter connected with a recorder.

Results and discussion

Miconazole impairs growth of yeast at a concentration of 3×10^{-7} M, but a complete inhibition of growth is reached at a high concentration of 2×10^{-5} M (Fig. 1). Because yeast can change from glycolytic to aerobic metabolism when the glucose concentration decreases (Beck & Meyenburg 1968), it was necessary to investigate the effect of miconazole on fermentation and respiration. We have found 50% inhibition of the CO_2-production and the O_2-uptake at a miconazole concentration of $1{\cdot}25 \times 10^{-5}$ M (results not shown). Another target for the drug could be the RNA or protein synthesis because both systems are integrated into membrane structures. Therefore, we incubated isolated yeast nuclei in the presence of miconazole and determined the incorporation of [^{3}H]UMP into acid insoluble material. As seen in Fig. 2, miconazole at concentrations up to 2×10^{-4} M has no effect on transcription in-vitro. Also, the cell-free translational system was not affected by the drug (results not shown).

Since miconazole has been found to interact with membrane lipids it is likely that the agent affects the transport systems of various molecules. Van den Bossche (1974) described an inhibition of the uptake of purine bases in *Candida albicans* at miconazole concentrations lower than those affecting growth. In contrast to the results of Van den Bossche, we have found an inhibition of the hypoxanthine transport in *Saccharomyces cerevisiae* at much higher miconazole concentrations (Fig. 3A, B). Furthermore, the uptake of other purine bases, amino acids and glucose was markedly reduced (results not shown). These results could be due to the different yeast strains used or to different experimental conditions, or both. Van den Bossche measured the effect of miconazole on the purine uptake with growing cells, while we investigated the inhibition of the transport system with stationary cells, which were preincubated in glucose citrate buffer. Under our conditions miconazole affects the purine uptake within a few minutes. However, miconazole concentrations which inhibit the transport system of *Saccharomyces*

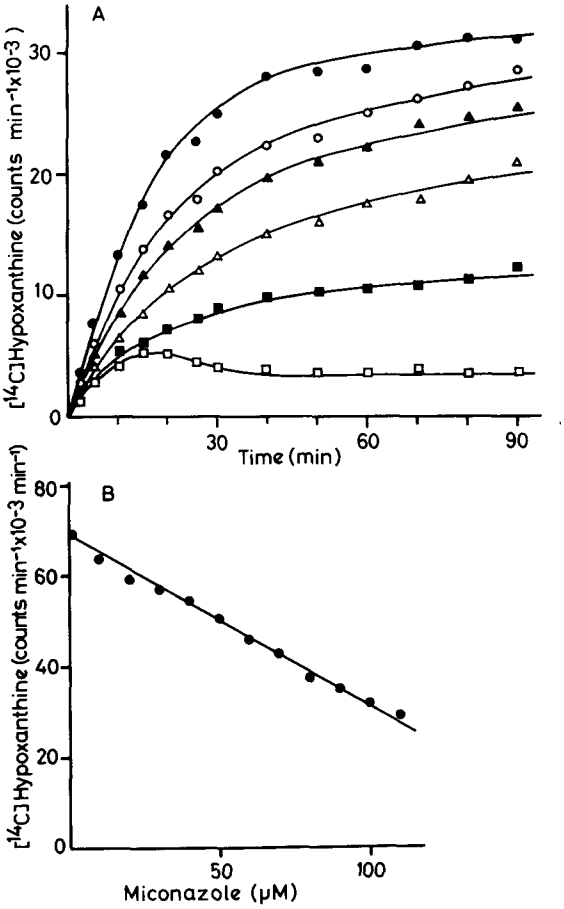

FIG. 3. Effect of miconazole on the hypoxanthine uptake of *Saccharomyces cerevisiae*. Stationary cells were preincubated in glucose citrate buffer for 60 min at 30 °C, then the cells were incubated in the presence of [^{14}C]hypoxanthine (100 μM; 0·1 μCi ml^{-1}) and miconazole at a concentration of 1×10^7 cells ml^{-1}. (A) Capacity of the [^{14}C]hypoxanthine uptake ●——● without miconazole and at different miconazole concentrations, ○——○ 12·5 μM, ▲——▲ 25 μM, △——△ 50 μM, ■——■ 75 μM, □——□ 125 μM. (B) Initial uptake velocities of [^{14}C]hypoxanthine at various miconazole concentrations.

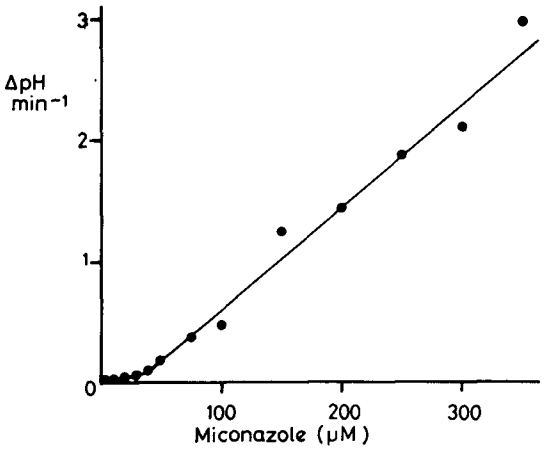

FIG. 4. Effect of miconazole on the proton-gradient of *Saccharomyces cerevisiae*. Increase of the rate of the pH-change induced by miconazole.

cerevisiae also lead to a vitiation of the proton-gradient at the plasma membrane which is known to be necessary for purine uptake. As seen in Fig. 4, the addition of miconazole to yeast cells preincubated in 2% glucose results in an increase of the external pH. The velocity of the pH-change in the medium is dependent on the concentration of the agent. Although the increase of the external pH is very slow at low miconazole concentrations, we cannot exclude a secondary effect of the drug, caused by alterations of intracellular ion-concentrations, which could lead to an impairment of many cell-functions.

Foury et al (1976) presented data demonstrating an almost immediate effect of miconazole on the exchange of K^+ for extracellular H^+. Those findings and the fact that miconazole increases the electrical conductivity of artificial bilayer membranes (Arndt et al 1982) could be explained by the suggestion of Van den Bossche et al (1982) that miconazole changes the lipid organization of the membrane.

We wish to thank Janssen Pharmaceuticals for their gift of miconazole, Dr U. Reichert for helpful discussions, and Mrs Forêt for excellent technical assistance.

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