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EXPERIMENTAL MODEL FOR IN VITRO HYDROXYLATION ; MICROMYCETES MUCOR HIEMALIS COMPARED TO LIVER MICROSOMAL FRACTION

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Microorganisms, among them fungi, effect a variety of biotransformations of foreign chemical compounds (1). Microorganisms perform nearly every conceivable type of oxidative reactions with suitable substrates and such reactions have been practically exploited (2, 3). In the present work, the ability of a micromycetes strain, Mucor hiemalis, to oxidize model substrates, like biphenyl or N-methyldiphenylamine, was studied and compared to mammalian systems.

MATERIALS AND METHODS

The cultures of Mucor hiemalis were maintained at 27°C in a flask or an incubator with synthetic medium (glucose 140 mM, KH_2PO_4 11 mM, $Mg\ SO_4\ 7\ H_2O$ 2 mM, NH_4NO_3 19 mM, $(NH_4)_2\ SO_4$ 11 mM, L-leucine 4 mM, L-asparagine 4 mM, Vit. B_1 0.7 µM. Sterilised air was continuously bubbled through the medium. The substrates at various concentrations (0.20-0.45 mM) were added to each culture after 24 h of growth. Biphenyl and aminopyrine and N-methyldiphenylamine were purchased from Merck. Per-hexiline maleate was a gift from Merrell Toraude. After 5 days, the mycelium was filtered and the culture filtrate was extracted with 3 volumes of diethylether and 2 volumes of ethyl acetate. The solvents were removed in vacuo at 30°C. The liver microsomal fractions from male Sprague-Dawley rat liver (200 g body weight, Iffa Credo, L'Arbresle, F) or from Abattoir pig liver were prepared by ultracentrifugation. The microsomes were incubated at 37°C for 30 minutes with the substrate and a NADPH generating system. The metabolic reaction was stopped by adding 0.5 ml HCl 0.6 N. The incubation medium was extracted with 2 volumes of diethylether. The solvent was removed. Metabolic products were determined by gas chromatography and analysed by GC/MS (LKB 2091 or Ribermag R 10/10 Sidar).

RESULTS AND DISCUSSION

Metabolism of biphenyl. The monohydroxylation of biphenyl occured at three positions to produce 2-hydroxy, 3-hydroxy and 4-hydroxy derivatives, the latter being the main metabolite. 4-Hydroxybiphenyl was also the main metabolite formed by pig liver microsomes. 2-Hydroxybiphenyl used as substrate was further metabolized by Mucor hiemalis, into 2,5-dihydroxybiphenyl, which is also present in rat urine after in vivo administration of 2-hydroxybiphenyl (4). Moreover the fungal cultures were also able to produce a phase II metabolite : biphenyl-O-glucoside. There is ample evidence to indicate that a wide variety of fungi oxidize biphenyl in a similar pathway to that observed in mammals and by similar mechanisms (5-8). Mucor hiemalis was one more example among fungal strains able to hydroxylate foreign compounds.

Metabolism of perhexiline maleate. Perhexiline maleate, an antianginal agent, hydroxylated in vitro by microsomal preparation from pig liver, remained unmetabolized after incubation with Mucor hiemalis.

O and N-demethylation reactions. 4-methoxybiphenyl O-demethylation was performed by Mucor hiemalis but 2-methoxybiphenyl was not demethylated by this fungus. Both 4-methoxy- and 2-methoxybiphenyl were hydroxylated. Rat liver microsomes transformed these chemicals into 2-hydroxybiphenyl, 2-methoxy-hydroxybiphenyl and 4-hydroxybiphenyl. This example pointed out the importance of the position of the methyl substituent for the metabolic fate of the compound.

N-demethylation was studied with N-methyldiphenylamine. Nine metabolites were produced by Mucor hiemalis. Some of these metabolites also appeared in mammalian systems. N-methyldiphenylamine was not only demethylated but also hydroxylated and again O-glucosides were observed.

The negative examples consisted of aminopyrine and antipyrine. None of the usual metabolites, observed with mammalian liver microsomes, were observed with the present fungal cultures.

Presence of cytochrome P-450. Cytochrome P-450 was present (0.1 nmol.mg^{-1} protein) in Mucor hiemalis and was detected by the reduced-carboxy cytochrome spectrum. Metyrapone in the culture medium inhibited the hydroxylation of 2-hydroxybiphenyl into 2,5 hydroxy derivative. The metabolite production was reduced by 90 % of the routine production.

In conclusion the present microorganism Mucor hiemalis was able to produce some of the metabolites observed with pig or rat liver microsomes (biphenyl, N-methyldiphenylamine). Some drugs resisted the metabolism by Mucor hiemalis. When the metabolic pattern is close enough to the mammalian species, Mucor hiemalis offer an interesting model to produce metabolites in quantity for analytical or pharmaco-toxicological purposes as already demonstrated with microbial models (9).

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