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Biochemical Pharmacology, Vol. 20, pp. 2087–2089. Pergamon Press, 1971. Printed in Great Britain

Pyrazole-induced inhibition of yeast alcohol dehydrogenase

(*Received* 20 *July* 1970; *accepted* 9 *October* 1970)

Pyrazole, a five-membered cyclic imino compound, has been reported to be a specific inhibitor of liver alcohol dehydrogenase. The activity of yeast alcohol dehydrogenase (YADH) (EC 1.1.1.1) did not seem to be affected.[1]

More recently, Goldberg and Rydberg[2] determined blood alcohol levels in animals treated with pyrazole and found that values obtained with the enzymatic method were consistently lower than the blood alcohol levels obtained with a gas chromatographic method. Since the enzymatic method for alcohol determinations uses YADH, these results suggested that pyrazole might also inhibit the yeast alcohol dehydrogenase. Indeed, addition *in vitro* of 1 mM pyrazole to the ethanol assay system employing YADH decreased the final specific absorption readings by over 80 per cent, indicating an interference with the assay. Since the YADH method for determination of ethanol is widely employed, and pyrazole derivatives are increasingly being used to study the nature of alcohol dehydrogenase action,[3–6] it was decided to investigate the nature of the YADH inhibition by pyrazole and to determine the inhibition constants.

Yeast alcohol dehydrogenase with final activity 40 times that of starting material was prepared according to the method of Racker[7] and kept in 0·01 M potassium phosphate buffer at a pH of 7·5 and −20°. The activity was measured in an assay system consisting of 10 mM sodium pyrophosphate, 50 μM NAD^+ (General Biochemicals, Chagrin Falls, Ohio), 0·1 M ethanol and an aliquot of the enzyme preparation at 23·5° and at a pH of 8·5.[7]

A Beckman DU spectrophotometer was employed in all experiments, using cuvettes having a path length of 1·0 cm. The production of 1·0 μmole NADH as measured by an increase in the optical density at 340 mμ was equal to one standard unit of enzyme. The activity of the enzyme preparation was found to be 75·0 units/mg of protein. Velocity measurements were plotted as the change in the optical density at 340 mμ/min/unit. A 95% (v/v) ethanol solution was used to prepare all concentrations of ethanol employed. Pyrazole (J. T. Baker Chemical Company, Philadelphia, Pa.) was of the highest purity available, with a melting point range of 68–70°.

Under the conditions of our assay, the K_m value of the YADH preparation was found to be $0{\cdot}25 \times 10^{-2}$ M. The data further indicate (Fig. 1) that pyrazole inhibited the YADH activity. This inhibition was competitive in nature and could be overcome by increasing amounts of ethanol. The K_i value was calculated to be $4{\cdot}5 \times 10^{-5}$ M.

In comparison with liver alcohol dehydrogenase, as reported by Theorell and Yonetani[8] (Table 1), the K_m value for YADH is five times greater than that for liver alcohol dehydrogenase under the conditions employed, indicating that the liver enzyme has a greater affinity for ethanol than does yeast alcohol dehydrogenase. The K_i value for yeast alcohol dehydrogenase with pyrazole as the inhibitor is approximately 200 times greater than the K_i value for liver alcohol dehydrogenase under the conditions reported. These results show that pyrazole has a markedly greater affinity for liver alcohol dehydrogenase than for the yeast enzyme.

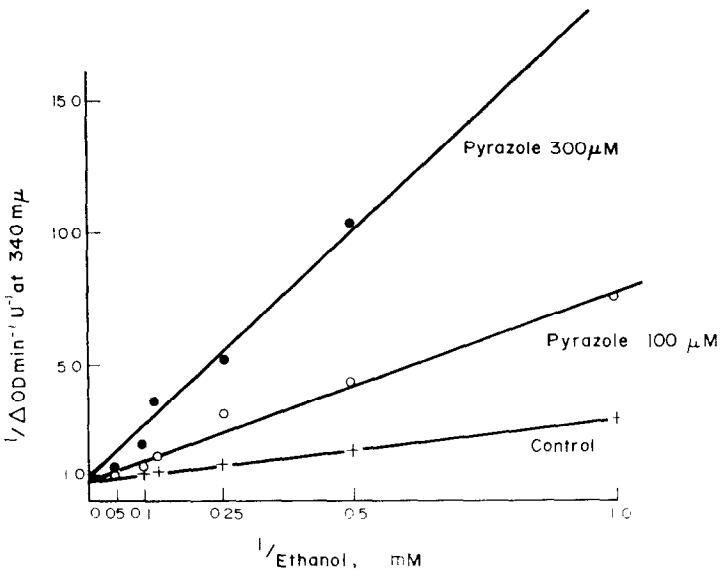

FIG. 1. Lineweaver–Burk plot indicating the competitive nature of the inhibition of yeast alcohol dehydrogenase by pyrazole.

TABLE 1. PYRAZOLE INHIBITION: COMPARISON OF YEAST ALCOHOL DEHYDROGENASE WITH LIVER ALCOHOL DEHYDROGENASE

Enzyme	K_m (M)	K_i (M)	Conditions
Yeast ADH	$0{\cdot}25 \times 10^{-2}$	$4{\cdot}5 \times 10^{-5}$	pH 8·5; PP_i, 23·5°
Liver ADH	$5{\cdot}0 \times 10^{-4}$	$2{\cdot}0 \times 10^{-7}$	pH 7·14; P_i, 23·5°

The findings of the present study indicate that yeast alcohol dehydrogenase is inhibited by pyrazole though not as effectively as the liver alcohol dehydrogenase. Therefore, the YADH method for the determination of ethanol levels used in experiments with pyrazole will produce erroneous values and should be avoided.

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Addendum—After our experiments were concluded, a publication by Reynier dealing with the inhibition of rat liver alcohol dehydrogenase by pyrazole and retinal oxidation was brought to our attention [M. REYNIER, *Acta chem. scand.* **23**, 1119 (1969)]. In his report, he also mentioned that yeast alcohol dehydrogenase was competitively inhibited by pyrazole.

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Biochemical Pharmacology, Vol. 20, pp. 2089–2091. Pergamon Press, 1971. Printed in Great Britain

Chlorpromazine adsorption to brain regions

(*Received* 6 *October* 1970; *accepted* 9 *November* 1970)

CHLORPROMAZINE (CPZ) is a potent local anesthetic[1–3] and immediately suppresses the electrical activity of neurones when applied directly.[4–7] Since the drug nonselectively blocks neurones and stabilizes membranes in a nonspecific fashion identical to other local anesthetics,[8,9] the hypothesis has developed that the apparent specificity of phenothiazines for antinausea and antipsychotic actions results from a particular drug distribution into various brain regions.[8,10,11] It is known that different regions of the brain take up different amounts of chlorpromazine and other phenothiazines after intravenous injection;[11,12–14] frontal cortex and white matter generally do not accumulate much phenothiazine, while thalamus and hippocampus consistently take up moderate to high amounts of the drug. Cassano *et al.*[14] could not observe any relation between regional blood flow and chlorpromazine uptake into brain regions.

It is not known, however, whether the higher uptake by thalamus and hippocampus results from an intrinsically higher affinity (or specific affinity) for chlorpromazine by nerve cells in these regions, or whether a different blood–brain barrier in these regions happens to allow easier transfer into the tissue. In the present study, it was investigated whether differences in tissue affinity for chlorpromazine could cause any of the observed distribution patterns for this drug. Four areas of the cat brain were dissected (frontal cortex, white matter, thalamus and hippocampus) and their affinity for chlorpromazine was determined.

Cats were anesthetized with ether or chloralose and the brains removed. The selected areas were dissected and used immediately or frozen in liquid nitrogen for later use. For adsorption experiments, approximately 0·8 g tissue was homogenized in a Virtis homogenizer with 0·6 ml of a solution containing 0·9% NaCl in 10 mM phosphate buffer. The dry weight of each homogenate was measured by drying at 90° to a minimum weight. The CPZ adsorption of the homogenates was determined by mixing in centrifuge tubes 0·2-ml aliquots of homogenate with 2 ml of a 0·9% NaCl solution containing ^{35}S-labeled chlorpromazine (Amersham, Great Britain). After a 30-min incubation, the tissue was spun down at 36,900 *g* for 20 min. The radioactivity in 0·2-ml samples of the supernatant was determined in a liquid scintillation counter with the liquid scintillator described by Bray.[15] The amount of drug bound per kilogram of dry weight was calculated as described previously.[11] Since previous workers[12,16] had shown that 75–98 per cent of chlorpromazine, which had been added to or incubated with brain tissue, could be recovered as chlorpromazine in short-term experiments, it was assumed in the present experiments that the amount of biotransformation of ^{35}S-chlorpromazine could be neglected.

The results, using five different cat brains, are summarized in Table 1. In the small C_{free} range used in these experiments, the partition coefficients were constant. The free concentration range chosen is known to be associated with local anesthesia[5] and stabilization of the red cell membrane.[9,11,17,18] No significant differences could be detected when the CPZ adsorptions to frontal cortex, thalamus and white matter were compared. The hippocampus showed a small but significant ($P < 0·02$) decrease in drug adsorption.

As it was impossible to explain any of the reported patterns of chlorpromazine distribution by a selective affinity of certain areas, it was investigated whether a selective retention could be the basis for the distribution *in vivo*. Chlorpromazine-^{35}S was adsorbed to tissue homogenates as described for the previous set of experiments and subsequently desorbed by four dilutions with 0·9% NaCl solution.