INTERACTION OF LIVER ALCOHOL DEHYDROGENASE WITH NEUROLEPTICS CHLOROPROTHIXENE AND CHLOROPROMAZINE*

L. SKURSKÝ, J. KOVÁŘ
Department of Biochemistry, Faculty of Sciences, University J. E. Purkyně, 611 37, Brno, Czechoslovakia

and

J. MICHALSKÝ
Pharmaceutical Factory Farmakon, Olomouc, Czechoslovakia

Received 17 January 1975

1. Introduction

Basic textbooks of pharmacology warn against simultaneous alcohol consumption during the treatment of patients by modern psychopharmaca and declare acute alcohol intoxication a strict contraindication for the administration of most of these drugs. Liver injuries are reported to occur sometimes as a side effect in the treated patients. For the thiaxanthene derivative Chloroprothixene, it has been experimentally established that it significantly prolongs the ethanol anesthesia [1].

Strong inhibitory activity of Chloroprothixene and Chloropromazine on horse liver alcohol dehydrogenase (EC.1.1.1.1.) (LADH) reported in this paper, might be related to the pharmacologically undesirable effects since, in preliminary experiments, the human enzyme was also strongly inhibited by the tested drugs.

2. Materials and methods

Horse LADH was isolated according to the method of Theorell et al. [2]. Enzyme concentration was determined spectroscopically [3] and its purity on

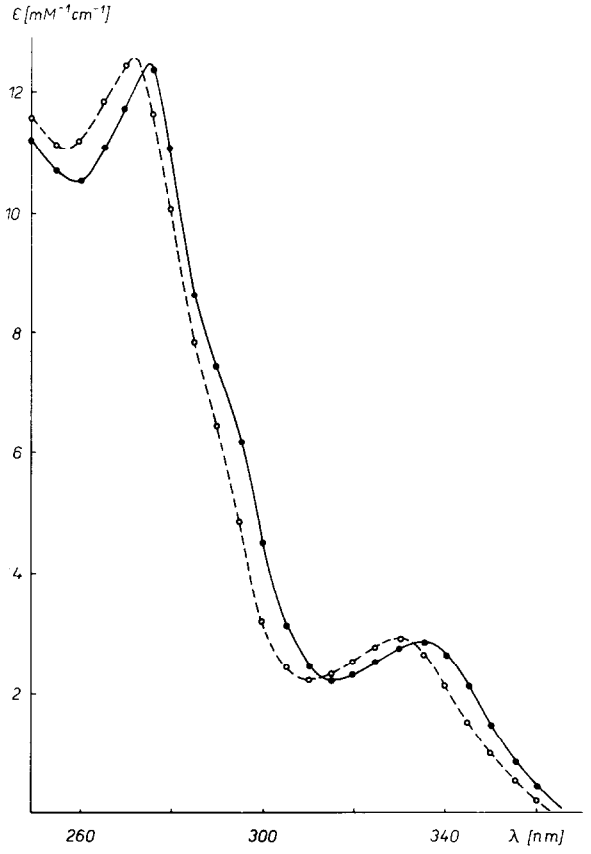


Fig. 1. Absorption spectra of free Chloroprothixene (-○-○-) and of the drug bound to horse LADH (—●—●—).

*This paper is dedicated to the 60th Birthday of Professor František Šantavý.

the basis of $\epsilon_{280} = 0.455$ mg^{-1} cm^2 [4] was 80–90%. Human LADH preparations were roughly purified by fractionation of the crude homogenate by ammonium sulphate according to [5]. The human enzyme was found to be of the 'normal' type in terms of von Wartburg's [6] classification.

NAD was Boehringer's product (grade II). Chloroprothixene, Chloropromazine and Prothiadene were hydrochlorides of the purity required for the manufacture of commercial remedies. The content of the pharmacologically inactive *cis* forms of Prothiadene and Chloroprothixene was less than 3%. Other common compounds were of A.R. grade.

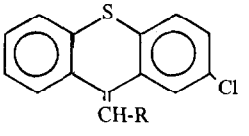
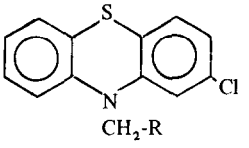
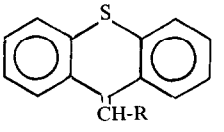
For kinetic measurements a Beckman Model DU Spectrophotometer was used either with the standard equipment (spectrophotometry) or with its commercial fluorometric attachement. Absorbance or fluorescence intensity increase in time were scanned by means of the Energy Recording Attachement ERA of Beckman and the Honeywell recording millivoltmeter.

All optical measurements were carried out at 23.5°C. Difference spectrum of the bound and free Chloroprothixene was measured on a Cary 118 spectrophotometer in a mixture of 51 μN horse LADH and 10 μM Chloroprothixene which ensures at least 96% inhibitor in the bound form. (Reference beam passed through two cuvettes with the same compounds separated.) Spectrum of the bound drug shown in fig.1 represents the superposition of the difference spectrum of the free drug.

3. Results and discussion

The inhibitor concentrations $I_{0.5}$ which cause a 50% inhibition of ethanol oxidation by the enzyme are shown in table 1. $I_{0.5}$ strictly equals to the inhibition

Table 1

Compound	Structure[a]	$I_{0.5}$ at pH 7 (μM)[b]	$I_{0.5}$ at pH 10 (μM)[c]
Chloroprothixene		1.3	3.5
Chloropromazine		17.5	25.0
Prothiadene		190	> 300

[a] R: $-(CH_2)_2\ N(CH_3)_2$

[b] 4.0 ml of 7 mM ethanol and 0.35 mM NAD in a 0.1 μ Na-phosphate buffer pH 7 (measured fluorometrically)

[c] 3.0 ml of 11 mM ethanol and 0.5 mM NAD in a 0.67 M NaOH–glycine buffer pH 10 (measured spectrophotometrically).

constant K_i only under specific prerequisits in case of a noncompetitive behaviour of the inhibitor. Nevertheless it can serve for a comparison of the inhibitory power of related compounds.

The strongest inhibitor among the tranquilisers tested up to now is the Chloroprothixene. Though it is not possible to set up structural requirements for the inhibition from this small series it seems clear that even a minor change of the heteroaromatic system brings about great change in the inhibition power. (Low affinity of the dibenzothiepine thymoleptic drug Prothiadene to the enzyme might also depend on the absence of the chlorine atom.) The dependence of the inhibition intensity on pH suggests the possibility that the side chain nitrogen atom with its positive charge may also be involved.

Kinetic behaviour of the Chloroprothixene shown in table 2 indicates that the 'tranquilizer binding site' of horse LADH is different from the coenzyme binding site. Possible overlapping of it with the substrate binding site remains to be examined. (It is not proven by the found mixed-competitive pattern vs. ethanol due to the validity of the Theorell-Change mechanism and due to the experimental conditions used; cf. J. Kovář, in preparation.)

The non-competitiveness of Chloroprothixene vs. NAD also excludes the possibility to explain the inhibition being caused by direct binding of the drug with the free coenzyme.

Existence of a binary enzyme–Chloroprothixene complex supported by kinetic pattern has been proven by the UV absorption spectra (fig.1). Both absorption maxima of Chloroprothixene at 269 and 325 nm get red-shifted upon binding and the shoulder at 290 nm becomes more pronounced.

In order to find out whether the observed tranquilizer–enzyme interaction could be of any importance in man the influence of Chloroprothixene on a crude LADH preparation from human liver has been tested. The activity of this impure enzyme dropped under 70% in the presence of 10 μM Chloroprothixene. This favours the possibility of a direct influence of tranquilizers on alcohol metabolism in man via their binding with the LADH.

Recently a very strong bond between the rat LADH and the carcinogenic aminoaz dye, the 3′-methyl-4-dimethylaminoazobenzene has been reported [7]. In the cited case the enzyme seems to serve as some sort of target (or storage) protein for the noxious drug (although this one does not inhibit the enzymic activity).

The speculative hypothesis on liver alcohol dehydrogenase as a possible target protein for certain tranquilizers seems to deserve further attention.

Table 2
Kinetic parameters of the inhibition of horse LADH by Chloroprothixene

Substrate	Pattern	(K_i) slope (μM)	(K_i) intercept (μM)
NAD	Noncompetitive	1.4	1.4
Ethanol	Mixed-competitive	0.8	1.6

Measured fluorometrically on 0.1 μ Na-phosphate buffer pH 7.0 with the final enzyme concentration of 0.1 μN. Variable ethanol, NAD and inhibitor concentrations varied between 200–5000 μM, 34–340 μM and 0–5 μM respectively. Concentrations of saturating components were 5800 μM and 680 μM for ethanol and NAD respectively.

Acknowledgements

We thank Dr J. Kamarýt, Research Institute for Pediatrics, Brno, for valuable discussions, help in material supply and for the human enzyme type testing.

Professor Hugo Theorell, Stockholm, is cordially acknowledged for his encouragements and manifold assistance.

References

[1] Scheckel, C. L. (1969) in: The Thioxanthenes. Mod. Probl. Pharmacopsychiat. (Lehmann, H. E. and Ban, T. A. eds.) Vol. 2, pp. 1–14, Karger, Basel/New York.
[2] Theorell, H., Taniguchi, S., Åkeson, Å. and Skurský, L. (1966) Biochem. Biophys. Res. Commun. 24, 603–610.
[3] Dalziel, K. (1957) Acta Chem. Scand. 11, 397–398.
[4] Bonnichsen, R. (1950) Acta Chem. Scand. 4, 715–718.
[5] Mourad, N. and Woronick, C. L. (1967) Arch. Biochem. Biophys. 121, 431–439.
[6] Von Wartburg, J. P., Papenberg, J., Aebi, H. (1965) Can. J. Bioch. 43, 889–899.
[7] Tokuma, Y. and Tarayama, H. (1973) Biochem. Biophys. Res. Commun. 54, 341–349.