Biochemical Pharmacology, 1965, Vol. 14, pp. 1073–1076. Pergamon Press Ltd., Printed in Great Britain.

INHIBITION OF TRANSAMINASES BY CINCHOPHEN AND ITS DERIVATIVES

OSMO HÄNNINEN and KAARLO HARTIALA

Department of Physiology, University of Turku, Turku, Finland

(*Received* 25 *January* 1965; *accepted* 11 *March* 1965)

Abstract—It has been shown that cinchophen and its derivatives 3-, 6-, 7- and 8-hydroxycinchophens inhibit rat liver glutamate oxaloacetate and glutamate pyruvate transaminases.

Glutamate pyruvate transaminase was considerably more sensitive to these inhibitors than glutamate oxaloacetate transaminase. Cinchophen and its derivatives were more powerful inhibitors than salicylic acid in both cases. Usually the hydroxylation of cinchophen increased its effect. 3-hydroxycinchophen was especially powerful inhibitor of glutamate pyruvate transaminase.

Addition of pyridoxal phosphate to reaction mixture had no effect on the inhibition of glutamate pyruvate transaminase by 3-hydroxycinchophen. 3-hydroxycinchophen seems to compete with amino group donator for the enzyme.

CINCHOPHEN stops effectively the biosynthesis of duodenal and gastric mucus[1] and readily causes ulcerations in the pylorus and duodenum and changes in the macromolecules of the gastric wall.[2]

It has been earlier demonstrated that cinchophen inhibits succinic, lactic, malic and α-glycerophosphate dehydrogenases[3] and also alchohol and uridinediphosphate-glucose dehydrogenases in these cases at least the mechanism being mainly the competition with nicotinamideadenine dinucleotide.[4] Whitehouse and Haslam[5] have shown that cinchophen and 3-hydroxycinchophen uncouple oxidative phosphorylation.

The main component of the gastric and duodenal mucus is protein.[6]

The aim of the present study was to explore, if the drug and its derivatives has any primary effects on the preliminary steps of protein biosynthesis, on transamination reactions.

METHODS

Glutamate-oxaloacetate (GOT) and glutamate-pyruvate (GPT) transaminases were prepared from rat liver. The animal was bled, the liver cut in pieces in crushed ice and homogenized in nine parts of 0·15 M KCl in a Potter-Elvehjem type all glass homogenizer. Homogenate was centrifuged at 2° for 10 min at 1000 *g*. For GOT studies the obtained supernatant was used. For GPT the procedure of Otto[3] was followed to calcium phosphate gel step (including). The activity determinations based on 2·4-dinitrophenylhydrazine method.[1] Incubation time in both studies was 30 min.

Cinchophen was obtained from Schering Ag, (Berlin), 3-hydroxy-cinchophen from Karl Roth and other cinchophen derivatives were synthetized and recrystallized at our own laboratory (6- and 8-hydroxycinchophens according to Döbner and Fettbach[7] and 7-hydroxycinchophen according to Borsche).[8]

RESULTS

Cinchophen and its derivatives have so high an absorption in the u.v. region that it was not possible to use direct and continuous spectrophotometric methods to determine the reaction velocities. Since the ability of the drugs to inhibit dehydrogenases also disturbed in this respect, the 2·4-dinitrophenylhydrazine method had to be employed. Reactions proceded linearly with time and the concentration of the enzyme preparation under the conditions used. The determination of pyruvate as hydrazone was affected by the variation of the concentration of α-ketoglutarate and by the addition of pyridoxal phosphate to the reaction mixture.

The concentrations which were needed for 50 per cent inhibition of GOT and GPT under the reaction conditions used have been collected to Table 1. For comparison the same concentrations of salicylic acid are also included in the study. The purification of GPT did not affect significantly the concentrations needed for 50 per cent inhibition.

TABLE 1. INHIBITION OF RAT LIVER GLUTAMATE OXALOACETATE AND GLUTAMATE PYRUVATE TRANSAMINASES BY CINCHOPHEN, ITS DERIVATIVES AND SALICYLIC ACID.

Compound	50% inhibition ($\times 10^{-3}$ M)	
	GOT	GPT
Cinchophen	12	1·4
3-hydroxycinchophen	4·8	0·047
6-hydroxycinchophen	9·5	0·65
7-hydroxycinchophen	3·9	0·50
8-hydroxycinchophen	4·0	1·5
Salicylic acid	>30	5·5

In order to study the mechanism of the inhibition, the effect of 3-hydroxycinchophen upon GPT-preparation was selected. Addition of pyridoxal phosphate (up to 0·06 mM concentration) had no effect on the degree of inhibition. The effects of different amino group donator (alanine) and acceptor (α-ketoglutarate) concentrations are shown in Figs. 1 and 2 respectively. Results have been plotted according to Lineweaver and Burk.[10]

The transaminase preparations from rat duodenum were similarly inhibited by the drugs.

DISCUSSION

Cinchophen and its derivatives were found to be powerful inhibitors of GOT and GPT. The former was, however, less sensitive to the action of the drugs. The comparison with salicylic acid revealed that it was considerably less effective.

Hydroxylation of cinchophen increased its effectivity as an inhibitor with the exceptions of 6-hydroxycinchophen in the GOT series and 8-hydroxycinchophen in GPT series. By far the most powerful inhibition was demonstrated with 3-hydroxycinchophen on GPT.

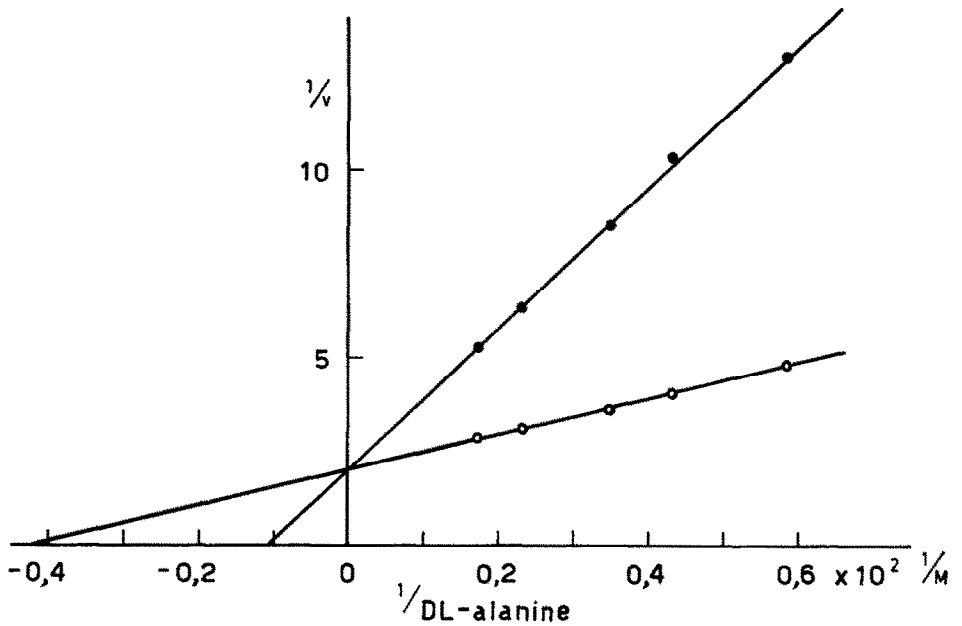

FIG. 1. Reciprocal plots for GPT with DL-alanine as varied substrate and 3-hydroxycinchophen (●) as inhibitor. α-Ketoglutarate concentration 1·43 mM, 3-hydroxycinchophen concentration 0·071 mM. K_i $2{\cdot}5 \times 10^{-5}$.

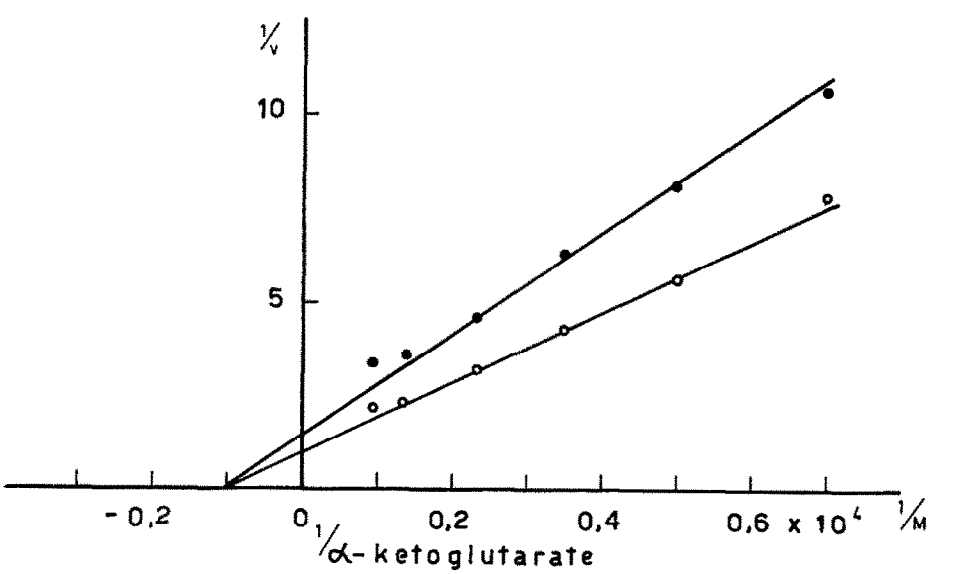

FIG. 2. Reciprocal plots for GPT with α-ketoglutarate as varied substrate and 3-hydroxycinchophen (●) as inhibitor. DL-alanine concentration 0·143 M, 3-hydroxycinchophen concentration 0·071 mM. K_i $1{\cdot}4 \times 10^{-4}$.

Smith and his co-workers[11] have shown that the inhibition of glutamate pyruvate transaminase is enhanced if the *ortho*-position of benzoic acid derivatives is hydrozylated. In the present study the 3-hydroxyderivative was the most potent inhibitor of the same enzyme confirming the importance of the immediate neighbourhood of carboxy and hydroxy groups in the biochemical activity of aromatic compounds. It has been also demonstrated that *ortho*-hydroxylation in benzoic acid derivatives enhances their binding to albumin.[12]

The mechanism of the inhibition was studied only with GPT and 3-hydroxycinchophen and may differ in other cases. 3-hydroxycinchophen competed effectively with the amino group donator. Possibly the hydroxy group in the immediate neighbourhood of the carboxy group strives to exclude alanine from the active centre of the transaminase. Excessive α-ketoglutrate concentrations seemed to cause substrate inhibition with GPT as it has been shown to inhibit GOT.[13]

The gastroduodenal ulceration produced by cinchophen, which is probably due to a defect in the protective mucus layer, may thus be caused by the summation of the effects of the drug on oxidative phosphorylation, uridinediphosphoglucuronic acid (mucopolysaccharide) biosynthesis and transamination reactions (protein biosynthesis). As a result of this, changes in the production of macromolecules by the gastrointestinal wall can be provoked as we have also been able to demonstrate in this laboratory.[2]

Acknowledgements—A preliminary report of the study has been presented.[4] The synthesis of the cinchophen derivatives was carried out by T. Terho M.Sc. at our laboratory. This work has been supported by a grant from the Sigrid Jusélius Foundation.

REFERENCES

1. K. HARTIALA, A. C. IVY and M. J. GROSSMAN, *Am. J. Physiol.* **162**, 110 (1950).
2. K. HARTIALA and I. HÄKKINEN, in press (Gastroenterology).
3. C. LUTWAK-MANN, *Biochem. J.* **36**, 706 (1942).
4. O. HÄNNINEN and K. HARTIALA, *Biochem. J.* **92**, 15P (1964).
5. M. W. WHITEHOUSE and J. M. HASLAM, *Nature, Lond.* **196**, 1323 (1962).
6. I. WERNER, *Acta Soc. Med. Upsaliensis* **58**, 1 (1953).
7. H-U. BERGMEYER and E. BERNT, in *Methoden der enzymatischen Analyse* (edited by H-U. BERGMEYER) pp. 842 and 851. Berlag Chemie GMBH, Weinheim (1962).
8. O. DÖBNER and H. FETTBACH, *Ann. Chem. Justus Liebigs* **281**, 7 and 11 (1894).
9. W. BORSCHE, *Ber. deut. chem. Ges.* 3889 (1908).
10. H. LINEWEAVER and D. BURK, ref. in Enzymes (M. DIXON and E. C. WEBB) p. 22. Longmans, London (1960).
11. R. A. STEGGLE, A. K. HUGGINS and M. J. H. SMITH, *Biochem. Pharmac.* **7**, 151 (1961).
12. C. DAVIDSON and P. K. SMITH, *J. Pharmac.* **133**, 161 (1961).
13. C. P. HENSON and W. W. CLELAND, *Biochemistry* **3**, 338 (1964).
14. O. HÄNNINEN and K. HARTIALA, to be published (1964).
15. K. OTTO, *Hoppe-Seylers Z. physiol. Chem.* **336**, 69 (1964).