THE EFFECT OF D,L-CYCLOSERINE ON THE PROCESS OF TRANSAMINATION

E. D. Vyshepan, K. I. Ivanova and A. M. Chernukh

From the Institute of Pharmacology and Chemotherapy of the AMN SSSR (Director — Active Member AMN SSSR V.V. Zakusov), Moscow

(Received April 3, 1959. Presented by Active Member AMN SSSR V. V. Zakusov)

According to the findings of Yamade, Sawaki and Hayami [5], the new antituberculous drug D-cycloserine suppresses the decarboxylation and decomposition of tryptophan in cell-free preparations of bacterial enzymes in lower concentrations than does isoniazid. The addition of pyridoxal or phosphopyridoxal restores the suppressed processes.

These authors also showed that cycloserine forms complexes with vitamin B_6.

N. K. Kochetkov and his co-workers suggest that the antibacterial action of cycloserine is based on disturbance of nitrogen metabolism as the result of the formation of azomethine compounds of cycloserine with the pyridoxal coenzyme. In this connection it appeared to be of interest to study the influence of both cycloserine itself and of other compounds with analogous functional groups on the process of transamination.

According to A. E. Braunshtein and M. M. Shemyakin [1], the process of transamination begins by the formation of an azomethine complex of phosphopyridoxal-protein with an amino acid. It is, therefore, quite possible that the distinctive amino acid cycloserine plays the role of an antimetabolite in the transamination reaction.

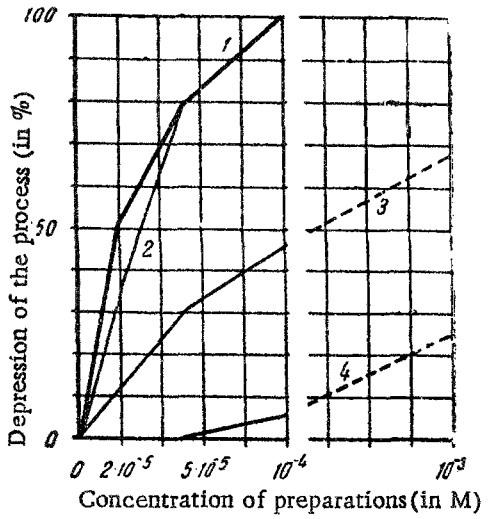
In the present research we studied the effect on transamination of D,L-cycloserine and of three other compounds obtained by N. K. Kochetkov and his co-workers: cyclothreonine (preparation KKh-11), the dihydrochloride of the ethyl ester of β-aminooxyalanine (preparation KKh-9) and the semihydrochloride of β-aminooxyalanine (preparation KKh-14). The effect of these compounds on the process of transamination was investigated in rat liver homogenates, incubated with glutaminic and pyruvic acids.

EXPERIMENTAL METHOD

White rats, weighing 100-150 g, were decapitated and the liver was rapidly extracted and homogenized in the cold, in a glass homogenizer, with a phosphate buffer at pH = 7.4. Composition of the samples: 0.7 ml phosphate buffer, 0.1 ml of a 0.2 M solution of glutaminic acid, 0.1 ml of a 0.2 M solution of pyruvic acid and 0.1 ml of 20 % homogenate.

In a control sample, the pyruvic acid was replaced by buffer. Inhibitor solutions of various concentrations were added in volumes of 0.1 ml instead of buffer. The solutions of glutaminic acid and pyruvic acid, and also the inhibitor solutions, were preliminarily neutralized with NaOH.

The samples were incubated at 37° for 1 hour and then fixed by the addition of 1 ml of 20% trichloroacetic acid. The latter was removed from the filtrates by extraction five times with ether; after removal of all traces of ether, the amino acids were separated by the method of ascending chromatography on paper, in a mixture of butanol, acetic acid and water (in proportions of 4: 4: 1). Alanine and glutaminic acid were estimated



The effect of D,L-cycloserine and preparations KKh-9, KKh-14 and KKh-11 on transamination in rat liver homogenates.

quantitatively by the method of Giri and Rao [3]. The intensity of the process was estimated by the decrease in the glutaminic acid content and the increase in that of alanine.

The R_f was determined for all compounds capable of being developed by ninhydrin. Cycloserine and preparation KKh-9, in the same concentrations as glutaminic acid, produced lightly stained, small spots with and R_f equal to that of glutaminic acid. Since cycloserine and preparation KKh-9 were used in the experiments in considerably lower concentrations than glutaminic acid, we considered it possible to disregard superimposition of these spots. The spots of preparations KKh-14 and KKh-11, also lightly stained, were almost completely merged with the alanine spot.

In some cases these preparations were used in concentrations only 10 times lower than the concentration of the substrate, but in these cases, too, the results obtained in respect to decrease in the glutaminic acid content did not differ appreciably from those obtained in respect to the increase in alanine.

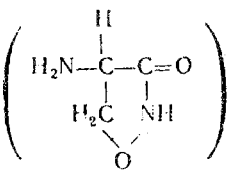
EXPERIMENTAL RESULTS

The experimental results (see figure) show that, starting at concentrations of $2 \cdot 10^{-5}$ M, cycloserine caused considerable depression of the process of transamination A similar action was shown by the preparation KKh-9. Differences in the actions of this compound and cycloserine could be observed only at very low concentrations. The preparation KKh-14 depressed transamination much more weakly. The least effect on the transamination process was shown by preparation KKh-11 .

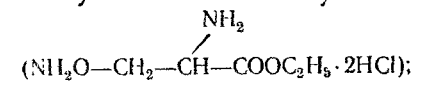
The high activity of preparation KKh-9 was probably explained by the fact that this compound very readily underwent a cyclic change, and at a pH of 7.4 was converted into cycloserine [2] . The possibility of the direct action of this preparation itself cannot , however, be excluded.

The direct conversion of preparation KKh-14 into cycloserine in mild conditions is impossible [2], and at the same time it inhibited transamination more weakly , but, nevertheless perceptibly.

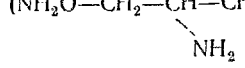
It may be suggested that the cyclic structure and the unsubstituted hydrogen in position 5 of the cycloserine molecule had some connection with the stabilization of its azomethine compound with pyridoxal or with phosphopyridoxal, since the presence of a free amino group in the preparations KKh-14 and KKh-11 enabled the formation of azomethine compounds, and the effect of these

1—D,L-cycloserine  ;

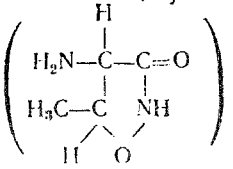
2- preparation KKh-9, the dihydrochloride of the ethyl ester of β-aminooxyalanine



3- preparation KKh-14, the semihydrochloride of β-aminooxyalanine



4- preparation KKh-11, cyclothreonine



* It was shown by special experiments that on replacing glutaminic acid by ammonium carbonate, only negligible amounts of alanine were formed, and this was unaffected by cycloserine. We consider, therefore, that when liver homogenates were incubated with glutaminic and pyruvic acids, cycloserine depressed the formation of alanine by the transamination reaction but not by direct amination.

The Effect of Pyridoxal and Phosphopyridoxal on the Restoration of the Process of Transamination when Suppressed by D,L-Cycloserine

Test No.	Added to the complete test	Decrease in glutaminic acid content, in mM / g tissue	Increase in alanine content per g tissue
1	—	0,65	0,50
2	$5 \cdot 10^{-5}$ M D,L-cycloserine	0,13	0,10
3	$5 \cdot 10^{-5}$ M D,L-cycloserine + $5 \cdot 10^{-4}$ M pyridoxal	0,10	0,01
4	$5 \cdot 10^{-5}$ M D,L-cycloserine + $5 \cdot 10^{-4}$ M pyridoxal + 10^{-4} M ATP	0,15	0,01
1	—	0,70	0,60
2	$5 \cdot 10^{-5}$ M D,L-cycloserine	0,20	0,12
3	$5 \cdot 10^{-5}$ M D,L-cycloserine + $2,5 \cdot 10^{-4}$ M phosphopyridoxal	0,20	0,10
1	—	0,70	0,54
2	$2 \cdot 10^{-5}$ M D,L-cycloserine	0,34	0,20
3	$5 \cdot 10^{-5}$ M D,L-cycloserine + 10^{-4} M phosphopyridoxal	0,25	0,20

By analogy with the action of cycloserine on the process of decarboxylation it may be suggested that pyridoxal or phosphopyridoxal will also restore the process of transamination, when suppressed by this antibiotic.

To test this hypothesis we carried out experiments in the same conditions as before*. Pyridoxal or phosphopyridoxal were added to the mixture buffer, substrate, cycloserine, and 10-15 minutes later the homogenate was added.

As will be seen from the experimental results shown in the table, neither pyridoxal, alone or with ATP, nor phosphopyridoxal restored the process. It was evident that the azomethine compound of cycloserine with free pyridoxal and phosphopyridoxal was unstable in the absence of a breakdown enzyme. If this were not so, pyridoxal or phosphopyridoxal, when added to cycloserine before the addition of the hemogenate, would be bound to neutralize it. It may thus be postulated that a stable complex is formed as a result of the combination of phosphopyridoxal-protein with cycloserine, and that the protein is of importance in the stabilization of the azomethine compound of cycloserine with phosphopyridoxal.

Hicks and Cymerman-Craig [4] came to a similar conclusion in respect to other transamination poisons — the hydrazides. They consider that hydrazides are combined, not with free phosphopyridoxal, but with phosphopyridoxal combined with protein. From this point of view, no explanation can be given of the findings of Yamade and his co-authors [5], who not only observed restoration of the processes of decarboxylation and decomposition of tryptophan by free pyridoxal or phosphopyridoxal, but also, by means of the absorption spectrum and by separation by means of paper chromatography, showed that a cycloserine — pyridoxal complex was formed.

This problem, as also that of the influence of cycloserine on the other aminoferases, is at present being investigated by us.

SUMMARY

The authors studied the effect of D,L-cycloserine, the dihydrochloride of the ethyl ether of β-aminooxyalanine (preparation KKh-9), semihydrochloride of β-aminooxyalanine (preparation KKh-14) and of cyclothreonine (preparation KKh-11) on the amino group shift from glutaminic acid to pyruvic acid in rat liver homogenates.

* Phosphopyridoxal was kindly made available to us by Prof. A. E. Braunshtein to whom we extend our deep gratitude.

D,L-cycloserine and preparation KKh-9 in 10^{-5}M to 10^{-4}M concentration, tend to depress this process by 50-100 %. Preparation KKh-14 depresses the process by 45% in 10^{-4}M concentration and by 70% in 10^{-3}M concentration. 10^{-3}M concentration of preparation KKh-11 depresses the process by 25% only.

An admixture of pyridoxal or phosphopyridoxal, in concentration 5 times above that of D,L-cycloserine, did not restore the process depressed by the latter.

It is conjectured that as a result of the combination of phosphopyridoxal-proteid with cycloserine a stable complex compound is formed; for the stabilization of the above complex the cyclic structure and the non-substituted hydrogen of the cycloserine molecule in the 5th position is important.

LITERATURE CITED

[1] A. E. Braunshtain, M. M. Shemyakin, Biokhimiya, 18, 4, 393-411 (1953).

[2] N. K. Kochetkov, R. M. Khomutov, M. Ya. Karpeiskii et al., Zhur. Obshchei Khimii, 28, 11, 3013-3019 (1958).

[3] K. W. Giri, A. N. Rao, J. Indian Inst. Sc. v. 35, p. 343 (1953).

[4] R. M. Hiks and Cymerman-Craig, J. Biochem. J. v. 67, p. 353-357 (1957).

[5] K. Yamade, S. Sawaki and S. Hayami, J. Vitaminol. v. 3, No. 1, p. 68-72 (1957).