THE EFFECT OF STEREOISOMERS OF CYCLOSERINE ON THE FORMATION AND TRANSFORMATION OF FREE γ-AMINOBUTYRIC ACID IN BRAIN TISSUE

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The use of the natural stereoisomer of cycloserine (D-cycloserine) in the treatment of tuberculosis has been restricted to some extent by its neurotoxicity. Experience of the clinical use of the racemate of cycloserine is limited, and no complete assessment of the character and degree of its side effects can be given.

In connection with reports in the literature that cycloserine acts as an antagonist in relation to the toxic action of isoniazid, N. I. Smol'nikova investigated the effect of D-cycloserine and the racemate of cycloserine on convulsions in mice caused by isoniazid [5]. She found that D-, L-cycloserine, as opposed to D-cycloserine, has the property of restraining the convulsions and prolonging the period of survival of the mice.

According to Sklenovsky [7], convulsions after administration of large doses of isoniazid are accompanied by changes in the concentration of free amino acids in the brain, and especially by a decrease in the concentration of γ-aminobutyric acid which, according to present ideas, inhibits the activity of the neurons and delays synaptic transmission.

The above effect of D-, L-cycloserine may be due to the influence of this antibiotic on the concentration of γ-aminobutyric acid in the brain. This hypothesis is supported by the fact that the concentration of free γ-aminobutyric acid in the brain is controlled by processes catalyzed by phosphopyridoxal enzymes [2], and some phosphopyridoxal enzymes are depressed by cycloserine [1, 3, 4, 8].

We accordingly investigated the effect of the stereoisomers of cycloserine on the formation of γ-aminobutyric acid in the process of the decarboxylation of glutamic acid, and on the disappearance of γ-aminobutyric acid in the process of its transamination with α-ketoglutaric acid in the brain tissue. In order to make the experimental conditions as close as possible to those in vivo, we studied the effect of the cycloserine stereoisomers on these processes at pH 7.5, and not at the optimal pH values for these processes.

EXPERIMENTAL METHOD

Albino rats weighing 200-300 g were decapitated, and the brain rapidly extracted and homogenized during cooling in a glass homogenizer with 0.1 M phosphate buffer at pH 7.5. A 20% brain homogenate was used in the experiments.

The samples were placed in the small containers of a Warburg's apparatus. The composition of the samples during investigation of the effect of cycloserine on the decarboxylation of glutamic acid was: I) 0.5 ml of the cycloserine preparation at pH 7.0, 2 ml of homogenate, and 0.5 ml of a 0.05 M solution of sodium glutamate (in the side tube); II) the cycloserine solution was replaced by a buffer solution; III) 2 ml of homogenate and 1 ml of buffer.

During the investigation of the effect of cycloserine on the transamination of γ-aminobutyric acid with α-ketoglutaric acid, the composition of the samples was as follows: I) 0.5 ml of cycloserine solution, 2 ml of homogenate and, in the side tube, 0.25 ml each of a 0.02 M solution of α-ketoglutaric and γ-aminobutyric acids; II—without cycloserine; and III—as described above.

The experiments were carried out in an atmosphere of N_2, purified by passage through pyrogallol solution. Incubation was for 45 min at 37°. The reaction was stopped by the addition of 0.31 ml of 80% trichloroacetic acid.

The decarboxylation of glutamic acid was judged by the CO_2 given off in the Warburg's apparatus and by the formation of γ-aminobutyric acid, estimated by paper chromatography; the progress of transamination was judged by the formation of glutamic acid, also estimated chromatographically. For the chromatographic estimation of the amino acids, the trichloroacetic acid was removed from the samples by ether extraction, repeated five times. Chromatography was carried out in a butanol : acetic acid : water mixture in proportions of 4 : 4 : 1.

After the solvent had been passed through three times, the chromatogram was treated by Giri's method [6]. The indices of sample III were subtracted from the indices of samples I and II. The inhibition of the process in the presence of the cycloserine preparations was calculated as a percentage. The intensity of the process in the absence of the cycloserine preparation was taken as 100%.

EXPERIMENTAL RESULTS

The mean results of five experiments showed that D-, L-cycloserine in a concentration of 10^{-3} M acted much more weakly than the D-isomer in depressing both the process of formation of γ-aminobutyric acid and the process leading to its disappearance: 40% inhibition of decarboxylation of glutamic acid compared with 9%, and 45% inhibition of transamination compared with 0 (or even activation by 20%).

In normal conditions the concentration of γ-aminobutyric acid in adult animals is maintained at a uniform level [2], so that the amount of γ-aminobutyric acid disappearing in unit time evidently equals the amount formed. Since the formation and disappearance of γ-aminobutyric acid were almost equally depressed by D-, L-cycloserine, its administration to the animals evidently could not change the concentration of free γ-aminobutyric acid significantly. However, the relationship between these processes could be modified by administration of D-, L-cycloserine after previous administration of isoniazid. The latter is also known to have the property of depressing the activity of certain phosphopyridoxal enzymes.

If the convulsions during administration of large doses of isoniazid were associated with a change in the concentration of free γ-aminobutyric acid in the brain, in this case isoniazid must depress the formation of this acid more strongly than its disappearance. The additional administration of D-, L-cycloserine, which depresses not only the formation, but also the disappearance of the acid, must in fact lead to the slowing of the latter process.

According to our observations, D-cycloserine in the same concentration depresses the formation of γ-aminobutyric acid only weakly, but may actually cause some degree of activation of its disappearance; consequently, if used in this particular concentration, it may be expected to produce a slight decrease in the concentration of this acid.

It is possible that the effect of D-cycloserine on the concentration of γ-aminobutyric acid in the brain may have a direct bearing on the mechanism of its neurotoxic action.

SUMMARY

A study was made of the effect produced by the stereoisomers of cycloserine on decarboxylization of glutamic acid and transamination of γ-aminobutyric acid with α-ketoglutaric acid in the homogenates of the rat brain at pH 7.5. As revealed, D,L-cycloserine in a concentration of 10^{-3} M depressed the first process by 40% and the second one by 45%. In the same conditions D-cycloserine depressed glutamic acid decarboxylation by 9%; it somewhat activated γ-aminobutyric acid transamination. A discussion is presented on the possible effect of cycloserine isomers on the content of free γ-aminobutyric acid in the brain (in vivo) following the administration of isoniazid in large doses.

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