INHIBITORY ACTION OF L-CYSTEINE ON FORMATION OF AMYLOID FIBRILS IN VITRO AND IN VIVO

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Addition of L-cysteine to a solution of amyloid reduces the amount of precipitate formed when the pH is changed from 11 to 7 by 1.54 times compared with the control. This is evidence of the inhibitory action of L-cysteine on the formation of amyloid fibrils in vitro. In experiments on C57B1 mice, a delay in the development of amyloidosis of the spleen was found in animals receiving L-cysteine and casein by comparison with a control group, which received injections of casein alone.

A distinguishing feature of the amyloid which is deposited in various organs in amyloid disease is its fibrillary structure [1, 2, 3, 5, 8].

While it differs considerably in many of its properties from the other fibrillary proteins of connective tissue, amyloid possesses some features which are common to all fibrillary protein and which determine their ultrastructure.

According to the general theory of protein structure, an essential condition for aggregation of protein molecules into fibrils is the presence of free SH-groups in the tertiary structure of the protein. The results of most investigations [3, 6, 7] have shown that amyloid contains a large proportion of cysteine (1.5-2.9 moles %), the SH-groups of which facilitate aggregation of the amyloid substance into fibrillary structures.

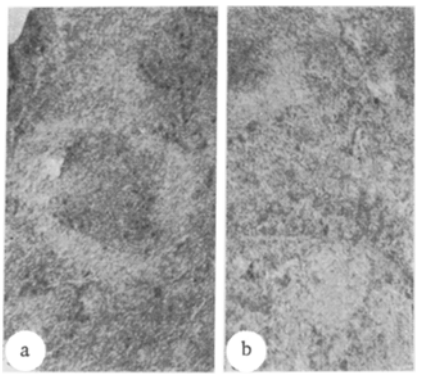

Fig. 1. Massive deposition of amyloid in reticular tissue of the spleen in control mice (a) and slight deposition of animals around follicles in experimental mice (b).

Under certain conditions protein molecules form sulfhydryl bridges between each other through their free SH-groups; in addition, individual subunits are held by interaction between different polar groups. Since the dominant factor in the process of fibril formation is the creation of sulfhydryl bridges, it is possible to stop aggregation artificially by the administration of agents binding free mercapto-groups.

The most effective inhibitors of the process of formation of protein fibrils are amino acids and the simplest peptides containing free SH-groups, because of their close relationship with the protein molecule. The simplest compound of this type is cysteine, which contains a mercapto-group and has a very small molecular volume.

On the basis of these theoretical considerations an investigation was carried out to study the inhibitory action of L-cysteine on the formation of amyloid fibrils.

EXPERIMENTAL METHOD AND RESULTS

Amyloid, isolated from mouse spleen [4], was dissolved at pH 11, and an equal volume of an aqueous solution of L-cysteine was

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added to the solution (an equal volume of water was added to the control sample), after which the pH of the mixture was adjusted to 7 by the addition of 1% HCl.

The degree of aggregation was estimated from the height of the column of precipitate, which was separated by centrifugation at 3000 rpm for 15 min, in sealed capillary tubes.

The degree of aggregation differed considerably in the control and experimental tests. The height of the columns of precipitates in the samples with cysteine was less than in the control on the average by 1.54 times. Consequently, L-cysteine has an inhibitory action on the formation of amyloid fibrils in vitro.

In view of the inhibitory action of L-cysteine on the aggregation of animals into fibrils in vitro, it was assumed that L-cysteine would have the same action in vivo.

To test this hypothesis, two series of observations were made on 20 C57B1 mice. In the experiments of series I (control), the mice received an intramuscular injection of 1.5 ml of 5% casein solution. In the experiments of series II, simultaneously with casein, the mice received 0.15 ml of 1% cysteine hydrochloride by intraperitoneal injection.

The animals were sacrificed by decapitation after 5, 10, 15, 20, and 25 injections.

No amyloid was found in the organs of the control or experimental groups of mice sacrificed after 5, 10, 15, and 20 injections. In the mice of the control group sacrificed after 25 injections, considerable deposition of amyloid was found in the spleen (Fig. 1a). No deposition of amyloid was found in the other organs. In all mice of the experimental group receiving 25 injections, the deposition of amyloid was much less marked (Fig. 1b).

The results suggest that administration of L-cysteine, by preventing the formation of fibrillary structures, may retard the development of experimental amyloidosis.

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