Stress and the State of Activation of the Neutral Proteinases of Rat Liver[1] (35583)

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Recent work in our laboratory (1) has confirmed the finding that adenosine-5′-triphosphate (ATP) can activate the rate of protein degradation at neutral pH. Our experiments also showed that this activating effect is not due to a direct reaction of ATP with the proteinases but most probably is due to the formation of an amino acid derivative which subsequently acts as a proteinase activator. Indirect evidence suggested that the activating species might be the aspartyl adenylate.

The possibility that an adenyl derivative of an amino acid might be involved in the control of the neutral proteolytic activity has lent further support to the concept that protein metabolism can be regulated not only by changes in the rate of protein synthesis but also by changes in the rate of protein catabolism. Our work has suggested that changes in the latter might be secondarily accomplished by changes in the rate of amino acid utilization for protein synthesis. The faster their utilization for this purpose, the less available the activating species would be for activation of the proteinases and the slower the rate of protein catabolism.

If this were to be the case, it could be expected that physiologic conditions which affect the rate of protein synthesis should also affect the state of activation of the neutral proteinases. To test this possibility, it was decided to study the effect of an acute stress situation on the state of activation of the neutral proteinases of rat liver. This situation was selected because of its ability to induce the release of glucocorticoid hormones from the adrenal cortex, which in turn have been shown to increase the rate of protein synthesis in the liver (2–4).

Materials and Methods. These studies were carried with the liver of male albino rats, weighing between 180 to 200 g. One group of rats was decapitated with a minimum of manipulation to reduce the possibility of any stress reaction. This group is referred to as the nonstressed group.

The second group was decapitated after each animal had been kept in a restraining cage for 30 min. The restraining conditions were such that once the animal had been introduced in the cage it could not turn around or even change its initial position. This group is referred to as the stressed group.

After bleeding the decapitated animals for 1 min, 1 g of tissue was excised from the liver and was subsequently homogenized with a Dounce-type homogenizer in 50 ml of 0.22 *M* phosphate buffer (pH 7.5) containing 0.2% Triton X-100. These procedures were carried out as close to 0° as possible. The amount of total protein in the homogenate was determined according to the method of Lowry *et al.* (5) using bovine serum albumin as standard.

The total neutral proteolytic activity of the homogenate was assayed in the presence and in the absence of exogenous ATP as previously described (1). Basically, the incubation mixture contained 2.0 ml of a 2% solution of pepsin degraded albumin, 1 ml of liver homogenate, and 6 ml of 0.22 *M* phosphate buffer (pH 7.5). Two mixtures were prepared for each homogenate, one was used as the control and the other one received 1 mg of the sodium salt of ATP. After 5 min of equilibration at 37° 5-ml aliquots were taken and pipetted into 2 ml of 10% trichloroacetic

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TABLE I. Effect of ATP on the Neutral Proteolytic Activity of the Liver of Stressed and Nonstressed Rats.[a]

Group	(μmoles of L-tyrosine equivalents/g of liver proteins) Control	Plus ATP	% Change
Nonstressed	(10) 292.6 ± 12.2	(6) 378.3 ± 37.8 $p = 0.05$[b]	30
Stressed	(10) 214.4 ± 14.8 $p = 0.001$[c]	(6) 343.5 ± 10.4 $p = 0.001$[b]	60

[a] Number of rats used in each experiment is given in parentheses. The results are given as the mean value ± 1 SE.

[b] The significance level (p) was calculated with respect to the corresponding control values.

[c] p value calculated with respect to control of the nonstressed group.

acid (TCA). The incubation was continued for another 15 min when a second aliquot was similarly pipetted into TCA. After complete removal of the precipitated protein by centrifugation and filtration, the amount of Ninhydrin-positive material was determined in the clear TCA supernatants according to the method of Spies (6) using L-tyrosine as standard. The amount of color in the 5-min aliquot was subtracted from the amount present in the 15-min aliquot. The results were expressed as the number of micromoles of L-tyrosine equivalents liberated per gram of liver protein under the described conditions. The statistical significance of the difference between means was obtained with the t test.

Results and Discussion. The results are presented in Table I. As in the case of our previous studies (1), the proteolytic activity of the liver of the nonstressed rats was significantly increased after the addition of exogenous ATP. This phenomenon can be interpreted as an indication that, under normal physiological conditions, the neutral proteolytic activity exists in the liver in an active form and in an inactive form which can be reactivated by the addition of exogenous ATP. In a previous article (1), evidence has been presented indicating that this activating effect of ATP is not due to any direct reaction of this compound with the proteinases but most probably to the formation of an intermediate with aspartic acid which then acts as the activator.

The effect of the acute stress produced by complete body restraint was to decrease the level of activity of the neutral proteinases. The addition of exogenous ATP increased the activity of the neutral proteinases. The addition of exogenous ATP increased the activity to levels similar to those determined in the liver of the nonstressed group after the addition of ATP. These results show that the lower proteolytic activity found in the stressed rats is due to a decrease in the active form of the neutral proteinases which could conceivably result in a lower rate of protein degradation. That this lower rate of catabolism can be restored to values found in nonstressed animals by the addition of ATP strongly suggests that the observed changes might be of physiological significance and to be intimately related to the mechanism that controls the rate of protein catabolism in the cell.

The extreme complexity of the endocrine reaction elicited by acute stress precludes the assignment of the changes in proteolytic activity to the effect of a particular type of hormone. However, the fact that stress situations result in the liberation of large quantities of glucocorticoid steroids from the adrenal cortex, together with the fact that this type of steroids has been shown to increase the rate of protein synthesis and the accumulation of protein in the liver (2, 3), make it tempting to speculate that the faster rate of utilization of the activated form of the amino acids might reduce the amount of activator of the neutral proteases, and therefore, the rate of protein catabolism. These conditions would certainly favor the accumulation of protein observed by several investigators af-

ter the repeated injection of cortisol (2–4).

The possibility that the amount of protein in the cell could be regulated not only by the rate of protein synthesis but also by changes in the rate of protein catabolism, as suggested by our results, makes it probable that some other physiological situations might also influence the rate of protein catabolism at neutral pH through endocrine changes. These hormonal changes could act indirectly by the removal or the increased production of proteinase activator, at this point believed to be the activated form of aspartic acid.

Summary. Neutral proteolytic activity in the liver exists in an active and an inactive form which can be reactivated by the addition of ATP. The acute stress elicited by complete body restraint produced a significant decrease in the active form of the proteinases conceivably reducing the rate of protein catabolism at neutral pH. Full levels of proteolytic activity could be restored by the addition of exogenous ATP to the homogenates. The fact that stress situations produce a large release of steroids able to increase the rate of protein synthesis in the liver has suggested that the decreased proteolytic activity could be due to a decreased availability of the proteinase activator, at this point believe to be the aspartyl adenylate.

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