ATP Activation of the Neutral Proteolytic Activity. A Widespread Phenomenon[1] (35825)

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Recent work in our laboratory has shown that the addition of exogenous ATP to liver homogenates increases the rate of neutral proteolysis (1). Since this activation could be linked to the presence of aspartic acid and also to the presence of "amino acid activating enzymes," it was suggested that the activating species might be the aspartyl adenylate.

The level of the neutral proteolytic activity in liver has been found to vary with stress (2) and conceivably with other types of physiological situations. The decrease observed during acute stress was found to be reversible by the addition of ATP suggesting that the observed change in proteolytic activity might be part of the adaptation process.

The study of the type of synthetic substrates hydrolyzed by neutral homogenates showed the presence of a great variety of aminopeptidases, a carboxypeptidase system and an endopeptidase system susceptible to the activation by ATP (3). These findings suggested a possible mechanism for the degradation of proteins at neutral pH in which the rate determining step is thought to be the endopeptic attack of the proteins followed by the concerted attack of the newly formed peptides by the aminopeptidase and carboxypeptidase system.

The fact that the endopeptidase system seemed to be inhibited by its own products and that this inhibition could be released by the addition of ATP or "amino acid activating enzymes" suggested that one possible regulatory mechanism for the process of neutral protein degradation might be the level of aspartyl adenylate. The level of this compound would depend not only on its rate of synthesis but also on the rate of utilization for incorporation into new protein molecules. Under these conditions a close relationship between the rate of protein degradation and the rate of protein synthesis could be expected, the net result being the phenomenon of regulated protein turnover.

If, as proposed in our article (3), the neutral proteolytic activity were to be intimately involved in the process of protein turnover, it could be expected that the proposed regulatory mechanism, activation by an ATP derivative, should be present in all the tissues known to exhibit the phenomenon of turnover. To investigate this possibility, homogenates made with the main organs of the rat were studied with respect to their ability to increase the rate of neutral autolysis after the addition of exogenous ATP. The finding that all the tissues examined showed the ATP activation effect further supports our contention that the neutral proteolytic activity may be an intimate part of the process of protein turnover.

Materials and Methods. This study was carried out with the organs of male albino rats, weighing between 200 and 250 g. The organs were heart, muscle, brain, spleen, and kidney. Studies with liver homogenates have been presented extensively elsewhere (1–3).

The organs were homogenized as follows. Heart and muscle (dissected from the hind legs) were initially passed through a sieve with 1 mm perforations using a manual tissue press (Harvard Apparatus Co., Dover, Massachusetts). After repeating this procedure 2 to 4 times, the heart pulp was homogenized in 16 ml of 0.6 *M* KCl/g of tissue while the muscle pulp was homogenized in 12.5 ml of the same medium with a

[1] This work was accomplished with the support of the School of Dentistry of the University of Minnesota.

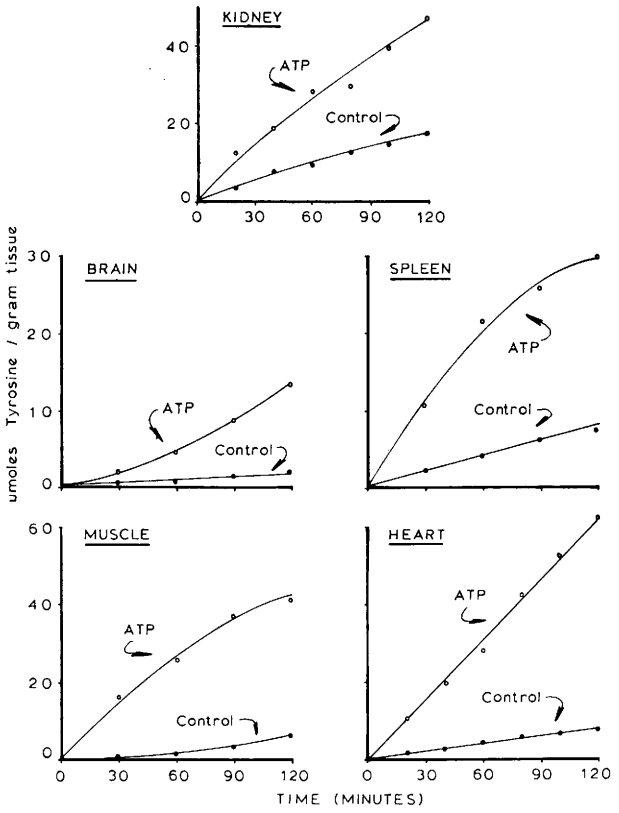

FIG. 1. Response of the neutral proteolytic activity of different organs of the rat to the periodical addition of exogenous ATP during the process of neutral autolysis: Each curve represents the average of two different experiments in which the organs of two or more rats were pooled together for homogenization.

Sorvall Omni-Mixer homogenizer run 3 min at a high speed. The heart and muscle homogenates were each filtered through cheesecloth and homogenized again for 1 and 3 min, respectively.

Kidney and spleen were homogenized in 12.5 ml of 0.22 *M* phosphate buffer (pH 7.5)/g of tissue using the Sorvall homogenizer at full speed for 3 min. The homogenate was then filtered through cheesecloth.

Brain was homogenized in 0.22 *M* phosphate buffer (pH 7.5)/g of tissue, with a Dounce homogenizer. This homogenate was also filtered through cheesecloth.

Before incubation all the homogenates were diluted by adding 5 ml of 0.22 *M* phosphate buffer (pH 7.5) to 10 ml of the original homogenate; the final organ concentration was 4% for heart and 5.3% for the other organs.

The diluted homogenates were then divided into two parts of 7.5 ml each. One part was used as the control and the other received 1 mg of ATP[2] before the initiation of the assay and every 20 or 30 min immediately after taking the aliquots. This large, unphysiological amount of ATP had to be added to provide high levels of ATP for the activation process in the presence of conceivably high rates of ATP hydrolysis and ATP binding to the homogenate constituents.

The control and the ATP containing flasks were placed in a constant temperature water bath at 37°. After 5-min equilibration, 1 ml of homogenate was pipetted into 1 ml of 10% trichlororoacetic acid (TCA) (zero-time aliquot). Similar samples were taken every 20 min for heart and kidney and every 30 min

[2] ATP was obtained as the disodium salt from Sigma Chemical Co., St. Louis, Missouri.

for the other organs for the next 2 hr; the autolytic process was stopped, as for the zero-time aliquot, by pipetting the samples into TCA. After 30-min standing, the precipitated proteins were removed by centrifugation and subsequent filtration; and the amount of Ninhydrin-positive material was determined in the clear TCA supernatant according to the method of Spies (4) using L-tyrosine as standard.

The amount of proteolytic degradation was calculated by subtracting the amount of Ninhydrin-positive material in the zero-time aliquot from the values obtained at each time interval. The results were expressed as micromoles of L-tyrosine released per gram of tissue under the described conditions. Each point in Fig. 1 represents the average of two different experiments in which the organs of two or more rats were pooled.

Results and Discussion. Figure 1 represents the response of the neutral proteolytic activity of all the organs examined to the addition of exogenous ATP. As in the case of liver (1, 3), all the organs responded to ATP with a large increase in the rates of proteolysis, lending support to the hypothesis that the rate of protein degradation can possibly be regulated through the levels of an ATP derivative, and that this regulatory mechanism may be an intimate part of the process of turnover (3).

Ahn and Rosemberg (5) have shown that the rate of proteolysis in thyroid slices can be decreased by anoxia, 2,4-dinitrophenol, antimycin A, and cyanide, suggesting that the neutral proteolytic activity of this gland is also susceptible to regulation by the availability of ATP.

At this point, it is not possible to decide whether or not the mechanism of activation in all the organs examined is similar to the one proposed for liver (3). However, the widespread presence of this activation phenomenon certainly points to its physiological importance.

Summary. The experiments presented clearly showed that the ATP activation of the neutral proteolytic activity is a widespread phenomenon and, therefore, a possible regulatory system for the catabolic phase of protein turnover.

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Received March 31, 1971. P.S.E.B.M., 1971, Vol. 138.