Contrasting Degradation Rates of Synthetic Angiotensin Amide and Natural Angiotensin in Human Plasma (34748)

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The rate at which angiotensin loses its biological activity when incubated in plama has been used as an *in vitro* measure of the angiotensinase activity of plasma. Studies of the rate of degradation of synthetic angiotensin II amide (asparaginyl 1-valyl 5-angiotensin) in plasma from patients with hypertension, pregnancy, liver disease, and renal disease have given conflicting results regarding alterations in angiotensinase activity in these diseases (1–5).

Recent studies by Nagatsu *et al.* (6) have suggested that degradation of angiotensin amide requires a different proteolytic enzyme than the one that hydrolyzes the naturally occurring aspartyl 1-valyl 5-angiotensin. Thus, previous studies which have employed the synthetic material may not have afforded a meaningful measure of the rate of loss of biological activity of natural angiotensin.

The purpose of the present study was to compare the rates of *in vitro* destruction of natural and synthetic angiotensin added to human plasma.

Methods. Two forms of angiotensin were studied: Synthetic angiotensin amide (asparaginyl 1-valyl 5-angiotensin II)[1] and natural angiotensin (aspartyl 1-valyl 5-angiotensin)[2]. The natural angiotensin was supplied as the decapeptide angiotensin I which was converted to the octapeptide by exposure to converting enzyme. One ml of renin-free human plasma was added to 10 ml of a solution containing 1300 ng of angiotensin I and incubated at 37° for 15 min. The plasma was obtained from a nephrectomized patient who had repeatedly been demonstrated to have no renin activity in his peripheral blood. After incubation the angiotensin solution was heated in a water bath for 15 min at 75° to destroy enzymatic activity. After centrifugation the supernatant converted angiotensin II solution was collected and tested for activity using a rat ascending colon bioassay system (7). The bioactivity of the aspartyl-antiotensin II was standardized against known amounts of angiotensin amide. This stock solution of aspartyl-angiotensin II remained stable for long periods at temperatures from 4 to 37°.

Aliquots of the two angiotensin solutions were diluted in distilled water and added to human plasma samples so that each 1 ml of the resultant mixture contained 9.3 ng of angiotensin II and 0.3 ml of plasma at a pH of 7.5. Plasma was obtained from normal subjects and from patients with hypertension. The biological activity of the angiotensin was assayed on the rat ascending colon preparation by adding 1 ml of the solution to a 30-ml organ bath immediately after the mixture was made and, at frequent intervals thereafter, during incubation of the mixture at 37°. Test assays were bracketed by standard injections of 5 or 10 ng of angiotensin amide. If the colon preparation was not stable in its response to the standards it was discarded.

Results. The biological activity of the natural aspartyl form of angiotensin II decayed very slowly during incubation with diluted human plasma, whereas the asparaginyl form of angiotensin II lost its biological activity more quickly with the half-life averag-

[1] Kindly supplied by Dr. William Wagner, Ciba Pharmaceutical Company, Summit, New Jersey.

[2] Kindly supplied by Dr. Leonard Skeggs, Cleveland, Ohio.

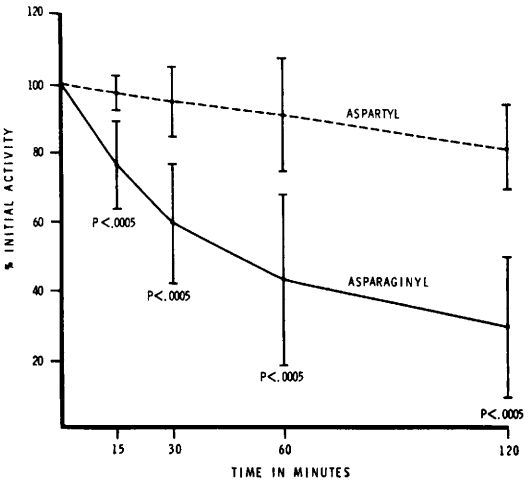

FIG. 1. Mean (± 1 standard deviation) of the decrease with time of biological activity of aspartyl (natural) and asparaginyl (synthetic) angiotensin incubated with human plasma. The data represent the results of 14 separate studies using the asparaginyl form and 9 studies using the aspartyl form.

ing 46.5 min. The differences in decay rates were highly significant ($p<0.0005$) (Fig. 1).

Discussion. These studies demonstrate that aspartyl angiotensin persists during *in vitro* incubation in human plasma considerably longer than asparaginyl angiotensin. Previous studies provide a biochemical explanation for this finding. It has been demonstrated that hydrolysis of the N-terminal amino acid (aspartic acid or asparagine) from angiotensin II reduces the pressor effect by one-half, while further cleavage of the arginine amino acid to form the hexapeptide leaves only minimal pressor activity. We have confirmed these findings on the rat colon preparation (Fig. 2). Nagatsu *et al.* (6) observed that the cleavage of arginine from aspartyl angiotensin was inhibited until the terminal aspartic acid residue had been removed from the octapeptide. In contrast, arginine cleavage from asparaginyl angiotensin progressed without regard to the presence of asparagine. Thus, the asparaginyl form would be reduced to the biologically inactive hexapeptide more quickly. Furthermore, differences in the characteristics of the aminopeptidase which hydrolyze asparaginyl and aspartic acid suggest that the degradation of aspartyl and asparaginyl angiotensin depend upon different aminopeptidases (6).

It is now apparent that angiotensin is much more rapidly destroyed in transit through tissue vascular beds than in blood alone (8). Despite this evidence minimizing the importance of circulating angiotensinases in the *in vivo* destruction of angiotensin, the *in vitro* rate of destruction of angiotensin II in plasma is still being used as a guide to angiotensinase activity in various disease states. Furthermore, tissue destruction may depend on an enzymatic system similar to that demonstrated in plasma. Recent studies assessing angiotensinase activity have utilized the β-naphthylamide derivative of aspartic

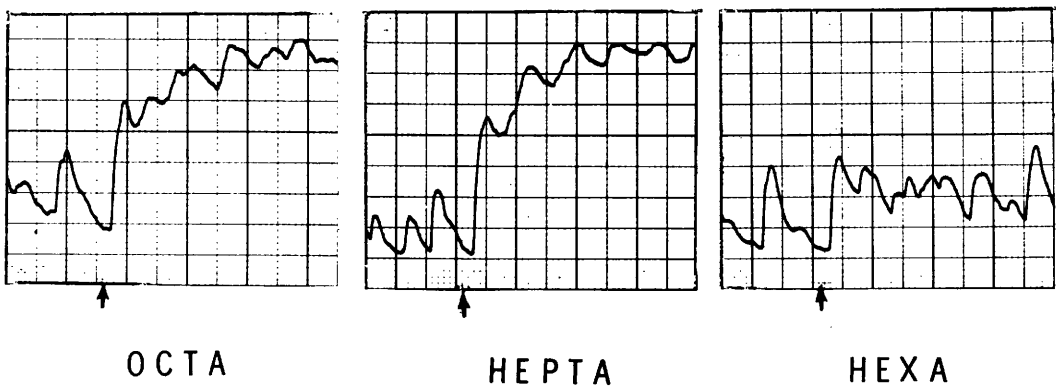

FIG. 2. Comparison of the biological activity on the rat ascending colon of equimolar solutions of the octapeptide (0.5 ml injected), the heptapeptide (1 ml injected) and the hexapeptide (1 ml). The heptapeptide produces approximately one-half the smooth muscle shortening effect of the octapeptide while the hexapeptide is biologically practically inert.

acid as a substrate, since hydrolysis of this aspartic acid apparently is mediated by an enzyme identical to that acting on aspartyl angiotensin (9, 10). However, assay of this reaction assesses degradation only to the heptapeptide, which retains considerable pressor activity. It would seem that meaningful studies of angiotensinase activity should therefore be performed using natural angiotensin.

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