Effects of a Crystalline Proteinase from *Bacillus subtilis* (Nagarse) on Angiotensins I and II

The vasopressor polypeptide substance A is formed when fraction IV-4 of human plasma is incubated with crude preparations of α-amylase obtained from the growth medium of *Bacillus subtilis*[1,2]. According to WALASZEK et al.[3], this substance is very similar to the octapeptide angiotensin II, and in fact, CARLINI and HUGGINS[4] have shown that crude preparations of α-amylase form angiotensin II from its precursor angiotensin I. However, the latter authors were unable to demonstrate any converting activity in a crystalline sample of the enzyme. This suggested that the peptidic activity might rather be due to impurities contaminating the crude preparations of the carbohydrate-splitting enzyme. In accordance with this are the findings of HUGGINS et al.[5] who, using the crystalline proteinase Nagarse, also secreted by *B. subtilis*, obtained a vasopressor polypeptide identical to substance A and also similar to angiotensin II; these authors suggested further that Nagarse probably is, as an impurity in α-amylase preparations, responsible for the formation of substance A.

All these data seem to indicate that Nagarse might have the property of converting angiotensin I into angiotensin II, and was responsible for the converting activity found in α-amylase by CARLINI and HUGGINS[4]. The experiments described here were performed in an attempt to verify this possibility.

Material and methods. The eventual conversion of angiotensin I into angiotensin II was measured on isolated rat uteri. The measure of oxytocic activity can be considered as a reliable index of conversion because according to CARLINI et al.[6] angiotensin I has no significant activity on isolated uteri, whereas angiotensin II and the heptapeptide, arginyl-valyl-tyrosyl-isoleucyl-histidyl-prolyl-phenylalanine, are the only peptides formed from angiotensin I that show significant oxytocic activity (BUMPUS et al.[7]).

Virgin rats weighing about 150 g were injected with 10 μg of diethylstilbestrol per 100 g of body weight. 24 h later the animals were killed by a blow on the head, and one uterine horn was suspended in a 10 ml chamber containing aerated de Jalon's solution at 30 °C. The pH of the de Jalon's solution was 7.4. Contractions were recorded on a smoked drum with a frontal lever giving fourfold magnification. Valine[5]-angiotensin I-amide, valine[5]-angiotensin II-amide and crystalline Nagarse (1650 proteolytic U of Nagarse per mg) were diluted in de Jalon's solution. Incubations of enzyme with the angiotensins were carried out at 37 °C. 0.2 and 0.4 ml aliquots of the incubation mixtures were assayed on the rat uteri every 6 min, from 20 sec up to 60 min of incubation. The angiotensin solutions were also heated at 37 °C for identical periods of time.

Results. Effects of Nagarse on angiotensin II. It was first decided to investigate the effects of Nagarse on the octapeptide. If Nagarse was very active, this would make the measurement of any eventual conversion of angiotensin I into angiotensin II difficult. In fact, it was verified in 9 experiments that Nagarse, at concentrations of 0.0125–0.05 μg/ml, inactivated angiotensin II at the end of 40 min. In some experiments, with concentrations of 0.5 and 5.0 μg/ml, the oxytocic activity of angiotensin II was lost after only 20 sec of incubation; on the other hand, 0.002 μg/ml of enzyme, in 3 experiments, did not destroy the angiotensin II at the end of 46 min of incubation.

Effects of Nagarse on angiotensin I. It was verified in 3 experiments that 0.002 μg/ml of Nagarse, the dose which did not inactivate angiotensin II, had not the property to convert angiotensin I into an oxytocic principle. Among 13 incubations with concentrations of the enzyme ranging from 0.0125 to 0.5 μg/ml, only 0.0125 μg/ml in 2 out of 4 experiments demonstrated a discrete converting activity.

Discussion. The data of the present study indicate that Nagarse inactivates angiotensin II rather efficiently and either does not convert angiotensin I into II or inactivates the oxytocic principle once formed. The former results are not surprising in view of the wide proteolytic activity of Nagarse[8]. It appears also that angiotensin II is much more susceptible to inactivation by Nagarse than bradykinin, which is not destroyed completely even after 75 min of incubation with 50 μg of enzyme per ml of incubate (PRADO et al.[9]).

On the other hand, the present results are in disagreement with the work of WALASZEK et al.[3] and HUGGINS et al.[5], who claim that substance A and angiotensin II are probably one and the same substance. Thus, our findings that angiotensin II is destroyed within a few minutes by 0.5 μg/ml of Nagarse, whereas substance A remains undestroyed even when incubated with 10 μg/ml of the enzyme for much longer periods of time[5], rather suggest that angiotensin II and substance A are different chemical compounds. The recent findings of HUGGINS (personal communication) who obtained evidence from amino acid analysis that substance A is not identical to angiotensin II[10], agree with this.

Zusammenfassung. Nagarse, eine vom *Bacillus subtilis* gewonnene kristalline Proteinase, bewirkte eine intensive Inaktivation von Angiotensin II, wobei keine Umwandlung von Angiotensin I in Angiotensin II festzustellen war. Die mögliche Identität zwischen einem aus einer menschlichen Plasmafraktion gewonnenen Polypeptid nach Nagarse-Impfung mit Angiotensin II wird geprüft.

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[1] C. G. HUGGINS and E. J. WALASZEK, Proc. Soc. exp. Biol. Med. *100*, 100 (1959).

[2] E. J. WALASZEK and C. G. HUGGINS, J. Pharmac. exp. Ther. *126*, 258 (1959).

[3] E. J. WALASZEK, R. D. BUNAG, and C. G. HUGGINS, J. Pharmac. exp. Ther. *138*, 139 (1962).

[4] E. A. CARLINI and C. G. HUGGINS, Biochem. Pharmac. *11*, 171 (1962).

[5] C. G. HUGGINS, L. PAVESI, and F. ARIAS, Biochem. Pharmac. *13*, 697 (1964).

[6] E. A. CARLINI, Z. P. PICARELLI, and J. L. PRADO, Bull. Soc. chim. biol. *40*, 1825 (1958).

[7] F. M. BUMPUS, P. A. KHAIRALLAH, K. ARAKAWA, I. H. PAGE, and R. R. SMEBY, Biochim. biophys. Acta *46*, 38 (1961).

[8] K. OKUNUKI, H. MATSUBARA, S. NISHIMURA, and B. HAGIHARA, J. Biochem., Japan *43*, 857 (1956).

[9] J. L. PRADO, E. S. PRADO, and A. JURKIEWICZ, Archs int. Pharmacodyn. Thér. *147*, 53 (1964).

[10] Acknowledgment: This work was supported by a grant from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). – Our thanks to Prof. J. L. PRADO for his advice and generous gift of Nagarse, and to Produtos Quimicos CIBA for the supply of angiotensins.