# Human placental leucine aminopeptidase (P-LAP) as a hypotensive agent

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*Summary.* The administration of leucine aminopeptidase purified from human placenta was found to be effective in lowering the blood pressure in rats with experimental hypertension induced by the infusion of angiotensin II or renin.

Systemic hypertension is one of the most common chronic diseases; its diagnosis and treatment constitutes one of our greatest medical challenges today. The renin-angiotensin system is an important regulator of blood pressure in normal and hypertensive individuals. The renal enzyme renin, reacting with a substrate present in blood, produces first an inactive decapeptide, angiotensin I; angiotensin I is then converted to the active octapeptide angiotensin II which is the most potent vasoconstrictor hormone.

Since the demonstration by Brunner and Gavaras[3] that receptor antagonists of angiotensin II, such as saralasin, and inhibitors of the enzyme responsible for the conversion of angiotensin I to angiotensin II, such as teprotide[4] and captopril[5], reduce blood pressure in severely hypertensive patients, treatment of hypertension by regulating the angiotensin concentration has been the focus of interest. However, these agents have not yet received unequivocal acceptance; it has been reported that these agents cause serious side-effects, such as agranulocytosis and renal failure[6,7].

We present here, using animal models, a different method for the treatment of hypertension due to overactivity of the renin-angiotensin system, using leucine aminopeptidase (arylamidase, LAP), which directly destroys angiotensin in the circulation.

*Materials and methods.* The membrane-bound LAP from human placenta (EC 3.4.11.2) was purified approximately 137-fold with a yield of about 16%, essentially according to the method of Hiwada and Kokubu[8]. The purified enzyme was shown to be homogeneous by polyacrylamide gel electrophoresis in sodium dodecyl sulphate and had a mol. of about 260,000. Its optimal pH was around 7.0 and the enzyme was stable between pH 6.0 and pH 10.0. LAP activity was estimated by the method of Takenaka[9]; the sp.act. was 8.09 units/mg protein. 1 unit of enzyme activity was defined as the amount of enzyme catalyzing the formation of 1 µmole of $\beta$-naphthylamine per min at 37 °C. Angiotensinase activity was estimated according to the biological method described previously[10,11]. 8 male Wistar albino rats weighing 150–200 g received a continuous infusion of angiotensin II (Protein Research Foundation, Osaka, Japan) into the femoral vein, or a single injection of renin prepared from pig kindney (Milles Laboratories Inc. Indiana) (figs 1 and 2). After the rats had been made hypertensive by these procedures, each rat was given an i.v. injection of the purified enzyme. The changes in blood pressure before and after administration of enzyme were recorded. Protein concentration was measured by the method of Lowry et al.[12].

*Results and discussion.* As figures 1 and 2 demonstrate, continuous infusion of angiotensin II (70 ng/min), or a single injection of renin (0.1 mg) caused a marked rise in

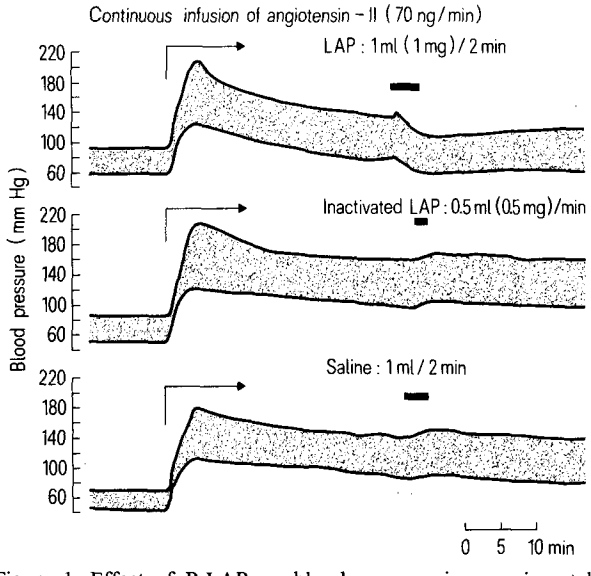


Figure 1. Effect of P-LAP on blood pressure in experimentally hypertensive rats prepared by injection of angiotensin II. Male Wistar rats, anesthetized by injections of urthane (1.4 g/kg, i.m.) and pentolinium tartrate (5 mg/kg, i.v.), were made hypertensive with continuous infusion of angiotensin II (70 ng/min). The figure shows typical examples out of 8 animals giving essentially the same results.

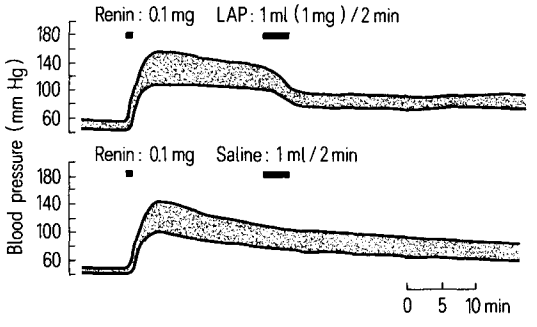


Figure 2. Effect of P-LAP on blood pressure in experimentally hypertensive rats prepared by injection of renin. Experimental conditions were the same as specified in figure 1, except that the rats were made hypertensive by a single injection of renin (0.1 mg).

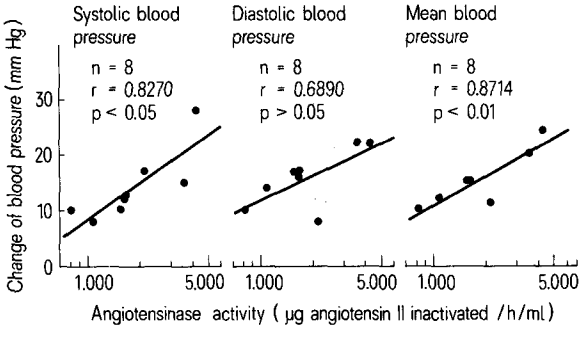


Figure 3. Correlation between the decrease in blood pressure and blood angiotensinase activity in experimental hypertensive rats. 8 rats were used for these experiments; each point represents the result obtained from 1 animal.

blood pressure. I.v. infusion of 1 mg of the enzyme during 2 min resulted in a rapid and significant decrease in blood pressure, whereas no changes were observed with the inactivated enzyme or saline (figs 1 and 2). These results were confirmed in all rats used (total 8 rats). The activity of the enzyme (100 µg) administered to rats lasted for 90 min. The changes in blood pressure of the 8 rats were also plotted against those of angiotensinase activity in circulation in the individual rats; significant correlations between pressure drop and enzyme activity were observed for the systolic and for the mean blood pressures (fig. 3).

In the present communication, we have presented a new possibility for treating hypertension, using LAP. This method depends on the direct enzymatic destruction of angiotensin II; the hypotensive effect is very rapid (figs 1 and 2). Therefore, this method seems suitable for the emergency therapy of a hypertensive crisis caused by overactivation of the renin-angiotensin system.

Pregnancy is characterized by a marked activation of the renin-angiotensin system[13,14]; however, the blood pressure generally remains stable. Page[15] suggested that there is an elevation in the angiotensinase activity in pregnancy serum, probably due to a direct contribution of the placenta. It seems likely that angiotensinase newly appearing in pregnancy sera is involved in the regulation of plasma and tissue concentrations of angiotensin II, and thus protects women from the elevation of blood pressure. Since we previously showed that angiotensinase activity newly present in pregnancy serum could not be distinguished from placental LAP[11], it seems reasonable to suppose that placental LAP, from the point of view of the physiology of pregnancy, counteracts the hypertensive effects of renin-angiotensin release.

Although aminopeptidase M prepared from pig kidney, which is commercially available (Sigma) was also effective in lowering blood pressure as estimated by the same procedure (unpublished data), we recommend the human placental enzyme for possible human use, for immunological reasons. Furtheremore, human placenta is easily obtainable as an enzyme source.

The potential of this enzyme for treating hypertensin caused by overactivity of the renin-angiotensin system seems promising, although extensive work is required before using this method on man.

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## Temperature dependence of the deformability of Carp *(Cyprinus carpio)* red blood cells

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*Summary.* Measurements were made of the deformability of the red cells of carp at different temperatures and compared with previous results obtained with another teleost fish (yellowtail) and human red cells. Changes with temperature are similar to those obtained with yellowtail, but interpretation of differences in terms of mean corpuscular volume alone are insufficient to account for the variations, which seem to be mainly due to differences in cellular deformability of the cells themselves.

Blood viscosity increases with decreasing temperature, causing a reduced fluidity of blood in vessels at low temperatures. Red blood cell deformability, which is a critical factor for blood passage through capillary vessels, might be severely affected by temperature. Recently Hughes et al.[4] studied the temperature dependence of the passage of whole blood through micropores (5 µm diameter) in a Nuclepore filter membrane using fish (yellowtail, *Seriola quinqueradiata*) and human blood. Good fluidity of fish blood through the micropores was observed throughout the temperature range studied (10–37 °C); blood passage time decreased considerably as temperature was increased from the normal 15 to 37 °C. However, when human blood was cooled, a small change observed between 37 and 20 °C was followed by a marked reduction in fluidity through the filter below 18 °C. Blood flow through the micropores was almost impossible at 10 °C but good fluidity was regained when the temperature was raised above 18 °C[5].

This difference in the effect of temperature change on fish and human blood fluidity through micropores may reflect differences in the structure of fish and human red blood cells. Further, the temperature dependence of blood fluidity through capillary vessels may be relevant to the adaptability of animals to environmental temperature fluctuations. In order to throw light on this problem, it is necessary to study blood from a wide variety of animals. This report is the beginning of such a comparative study and describes