ABSTRACT

In this Thesis we have been studied in experimental models the antihypertensive potential of two types of bioactive peptides: on the one hand, peptides derived from different zones of the sequence of the bovine lactoferrin (LF), included its antimicrobial domain lactoferricin (LfcinB), on the other hand, heptapeptides obtained by rational design from parental hexapeptides. Three types of assays have been realized: peptides were assayed *in vitro* to determine their capacity to inhibit the angiotensin converting enzyme (ACE) activity using both synthetic and natural substrates; *ex vivo* functional assays in isolated rabbit arteries, to analyze inhibitory effect of peptides on angiotensin I-induced vasoconstriction; and *in vivo* assays to study the antihypertensive effects after peptides administration in spontaneously hypertensive rats (SHR). In some cases, also *in vitro* tests have been realized to discard citotoxic effects on the part of the non natural peptides in cultured cells and simulated gastrointestinal digestion tests to analyse the bioavailability of peptides.

A set of lactoferricin B (LfcinB)-derived peptides were obtained from different elongations both at the C-terminal and N-terminal ends of the representative peptide $LfcinB_{20-25}$ (RRWQWR). These peptides showed different *in vitro* inhibitory effects on ACE activity and, except one, they showed *ex vivo* inhibitory effects on ACE-dependent vasoconstriction. No clear correlation between *in vitro* and *ex vivo* inhibitory effects was found. Only $LfcinB_{20-25}$ and one of its fragments (WQ), generated after a simulated gastrointestinal digestion, showed significant *in vivo* antihypertensive effect. However, fragment WQ did not show any effect on ACE-dependent vasoconstriction in contrast to the inhibitory effect showed by $LfcinB_{20-25}$. On the other side, a bovine lactoferrin pepsin hydrolysate with molecular mass lower than 3KDa (LFH<3KDa) was prepared. This hydrolysate showed antihypertensive effects maintained manner up to 24 h after oral administration. LFH>3KDa was further fractionated by semi-preparative

high performance liquid chromatography (HPLC), 38 peptides, contained in the ACE inhibitory fractions, were identified by using ion trap mass spectrometer and, based on the peptide abundance, a total of 11 peptides were chemically synthesized. Three of them (LIWKL, RPYL and LNNSRAP) exerted different *in vitro* inhibitory effects on ACE and showed *in vivo* antihypertensive effects, though only two of them, LIWKL and RPYL, showed *ex vivo* inhibitory effect on ACE-dependent vasoconstriction. Finally, six heptapeptides obtained by means of rationally design showed different grades of *in vitro* ACE inhibitory activity and *ex vivo* inhibitory effects on ACE-dependent, but not inhibitory effect on ACE-independent angiotensin II-induced vasoconstriction. The heptapeptides PACEI50L (RKWHFLW) and PACEI52L (RKWLFHW), as well as the parental hexapeptide PACEI32L (RKWHFW) showed *in vivo* antihypertensive effects on SHRs, but not modify the systolic blood pressure on normotensive rats. When the D-amino acid enantiomeric peptides was used, the potency against *in vitro* ACE activity was strongly reduced, the inhibitory effect on *ex vivo* ACE-dependent vasoconstriction was missing at the concentration used, and showed antihypertensive effect after intravenously administration but did not show after oral administration. The potencies of these synthetic peptides at reducing cell viability were in the millimolar range, very much higher than the micromolar peptide concentration with ACE inhibitory effect.

In conclusion, we demonstrated antihypertensive potential of LfcinB-derived peptide representative of the antimicrobial motif of LF ($LfcinB_{20-25}$), of pepsin hydrolysate of bovine LF enriched in low molecular weight peptides (LFH<3KDa), of peptides contained in saying hydrolysate proceeding from other lactoferrin regions different from the LfcinB (LIWKL, RPYL and LNNSRAP), and of hexa- and heptapeptides obtained by rational design (PACEI32L, PACEI50L and PACEI52L). In most of them, the antihypertensive effect associates to their vasoactive effect for their aptitude to ACE activity inhibitory capacity.