METHODS OF TREATMENT AND PHARMACEUTICAL COMPOSITION

This application is a continuation application of U.S. patent application Ser. No. 10/341,868 filed on Jan. 14, 2003 and claims benefit of U.S. Provisional Pat. Appl. No. 60/386,792, filed Jun. 7, 2002 and U.S. Provisional Pat. Appl. No. 60/349,660, filed Jan. 17, 2002, the entire disclosures of which are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

The renin angiotensin system is a complex hormonal system comprised of a large molecular weight precursor, angiotensinogen, two processing enzymes, renin and angiotensin converting enzyme (ACE), and the vasoactive mediator angiotensin II (Ang II). See *J. Cardiovasc. Pharmacol.*, Vol. 15, Suppl. B, pp. S1-S5 (1990). The enzyme renin catalyzes the cleavage of angiotensinogen into the decapeptide angiotensin I, which has minimal biological activity on its own and is converted into the active octapeptide Ang II by ACE. Ang II has multiple biological actions on the cardiovascular system, including vasoconstriction, activation of the sympathetic nervous system, stimulation of aldosterone production, anti-natriuresis, stimulation of vascular growth and stimulation of cardiac growth. Ang II functions as a pressor hormone and is involved the pathophysiology of several forms of hypertension.

The vasoconstrictive effects of angiotensin II are produced by its action on the non-striated smooth muscle cells, the stimulation of the formation of the adrenergenic hormones epinephrine and norepinephrine, as well as the increase of the activity of the sympathetic nervous system as a result of the formation of norepinephrine. Ang II also has an influence on electrolyte balance, produces, e.g., anti-natriuretic and anti-diuretic effects in the kidney and thereby promotes the release of, on the one hand, the vasopressin peptide from the pituitary gland and, on the other hand, of aldosterone from the adrenal glomerulosa. All these influences play an important part in the regulation of blood pressure, in increasing both circulating volume and peripheral resistance. Ang II is also involved in cell growth and migration and in extracellular matrix formation.

Ang II interacts with specific receptors on the surface of the target cell. It has been possible to identify receptor subtypes that are termed, e.g., AT 1- and AT 2-receptors. In recent times great efforts have been made to identify substances that bind to the AT 1-receptor. Such active ingredients are often termed Ang II antagonists. Because of the inhibition of the AT 1-receptor such antagonists can be used, e.g., as anti-hypertensive's or for the treatment of congestive heart failure, among other indications. Ang II antagonists are therefore understood to be those active ingredients which bind to the AT 1-receptor subtype.

Inhibitors of the renin angiotensin system are well-known drugs that lower blood pressure and exert beneficial actions in hypertension and in congestive heart failure as described. See, e.g, *N. Eng. J. Med., Vol.* 316, No. 23, pp. 1429-1435 (1987). A large number of peptide and non-peptide inhibitors of the renin angiotensin system are known, the most widely studied being the ACE inhibitors, which includes the drugs captopril, enalapril, lisinopril, benazepril and spirapril. Although a major mode of action of ACE inhibitors involves prevention of formation of the vasoconstrictor peptide Ang II, it has been reported in *Hypertension*, Vol. 16, No. 4, pp. 363-370 (1990), that ACE cleaves a variety of peptide substrates, including the vasoactive peptides bradykinin and substance P. Prevention of the degradation of bradykinin by ACE inhibitors has been demonstrated, and the activity of the ACE inhibitors in some conditions has been reported in *Circ. Res.*, Vol. 66, No. 1, pp. 242-248 (1990), to be mediated by elevation of bradykinin levels rather than inhibition of Ang II formation. Consequently, it cannot be presumed that the effect of an ACE inhibitor is due solely to prevention of angiotensin formation and subsequent inhibition of the renin angiotensin system.

Neutral endopeptidase (EC 3.4.24.11; enkephalinase; atriopeptidase; NEP) is a zinc-containing metalloprotease that cleaves a variety of peptide substrates on the amino terminal side of aromatic amino acids. See *Biochem. J.*, Vol. 241, pp. 237-247 (1987). Substrates for this enzyme include, but are not limited to, atrial natriuretic factors (ANFs), also known as ANPs, brain natriuretic peptide (BNP), met and leu enkephalin, bradykinin, neurokinin A and substance P.

ANPs are a family of vasodilator, diuretic and anti-hypertensive peptides which have been the subject of many recent reports in the literature. See, e.g., *Annu. Rev. Pharm. Tox.*, Vol. 29, pp. 23-54 (1989). One form, ANF 99-126, is a circulating peptide hormone which is released from the heart during conditions of cardiac distension. The function of ANF is to maintain salt and water homeostasis, as well as to regulate blood pressure. ANF is rapidly inactivated in the circulation by at least two processes: a receptor-mediated clearance reported in *Am. J. Physiol.*, Vol. 256, pp. R469-R475 (1989), and an enzymatic inactivation via NEP reported in *Biochem. J.*, Vol. 243, pp. 183-187 (1987). It has been previously demonstrated that inhibitors of NEP potentiate the hypotensive, diuretic, natriuretic and plasma ANF responses to pharmacological injection of ANF in experimental animals. The potentiation of ANF by two specific NEP inhibitors is reported by Sybertz et al., *J. Pharmacol. Exp. Ther.*, Vol. 250, No. 2, pp. 624-631 (1989), and in *Hypertension*, Vol. 15, No. 2, pp. 152-161 (1990), while the potentiation of ANF by NEP in general was disclosed in U.S. Pat. No. 4,749,688. In U.S. Pat. No. 4,740,499, Olins disclosed the use of thiorphan and kelatorphan to potentiate atrial peptides. Moreover, NEP inhibitors lower blood pressure and exert ANF-like effects, such as diuresis and increased cyclic guanosine 3',5'-monophosphate (cGMP) excretion in some forms of experimental hypertension. The anti-hypertensive action of NEP inhibitors is mediated through ANF because antibodies to ANF will neutralize the reduction in blood pressure.

Darrow et al. in European Patent Application No. 498361 disclose treating hypertension or congestive heart failure with a combination of certain Ang II antagonists or certain renin inhibitors with certain NEP inhibitors.

Powell et al. in European Patent Application No. 726072 disclose treating hypertension or congestive heart failure with a combination of the Ang II antagonist 2-butyl-6,7,8,9-tetrahydro-3-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1,3-diazaspiro[4.4]nonan-4-one with a NEP inhibitor or a dual acting vasopeptidase inhibitor (single molecular entity with both ACE and NEP inhibitory activities). Prolonged and uncontrolled hypertensive vascular disease ultimately leads to a variety of pathological changes in target organs, such as the heart and kidney. Sustained hypertension can lead as well to an increased occurrence of stroke. Therefore, there is a strong need to evaluate the efficacy of anti-hypertensive therapy, an examination of additional cardiovascular endpoints, beyond those of blood pressure lowering, to get further insight into the benefits of combined treatment.

The nature of hypertensive vascular diseases is multifactorial. Under certain circumstances, drugs with different mechanisms of action have been combined. However, just