Involvement of the kallikrein-kinin system in the antihypertensive effect of the angiotensin converting enzyme inhibitors

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1 Studies were performed in normal subjects and in rats to assess the effect of angiotensin converting enzyme (ACE) inhibition on the kallikrein-kinin system. As ACE is identical to kininase II, one of the enzymes physiologically involved in bradykinin degradation, bradykinin may be expected to accumulate during ACE inhibition.

2 A competitive antagonist of bradykinin was used to explore in unanaesthetized rats the contribution of circulating bradykinin to blood pressure control under ACE inhibition.

3 No evidence was found for a role of this vasodilating peptide in the blood pressure lowering effect of acute ACE inhibition.

4 The plasma activity of carboxypeptidase N (= kininase I), another pathway of bradykinin degradation, remained intact during a 1 week course of treatment with an ACE inhibitor in normal subjects. This therefore indicates that bradykinin formed during ACE inhibition can still be metabolized.

Keywords bradykinin kallikrein-kinin captopril ACE-inhibition renin-angiotensin

Introduction

The regulation of blood pressure is permanently under the control of pressor and depressor systems and hormones. The renin-angiotensin and the kallikrein-kinin systems are opposing forces with regard to the control of vascular tone. The activation of the renin-angiotensin system leads to the generation of angiotensin II, an octapeptide with potent vasoconstrictor properties (Oparil & Haber, 1974). On the other hand, the activation of the kallikrein-kinin system results in the formation of bradykinin, a potent vasodilating nonapeptide (Levinsky, 1979; Carretero & Scicli, 1980; Margolius, 1984; Fuller & Funder, 1986). There exists a major link between the two systems: the angiotensin I-processing carboxy-dipeptidase, angiotensin converting enzyme (ACE), is identical to kininase II, one of the

enzymes physiologically involved in the degradation of bradykinin (Erdo, 1975). Conceivably therefore, ACE inhibition may produce an accumulation of bradykinin.

One of the aims of the present work was to review what is known about the impact of ACE inhibition on the kallikrein-kinin system. In addition, we summarise our experience with a competitive antagonist of bradykinin which we utilized recently in unanaesthetized rats to explore the contribution of circulating bradykinin to cardiovascular homeostasis. The effect of prolonged ACE inhibition on kininase I activity is also reported. This enzyme represents one possible pathway of bradykinin metabolism during kininase II inhibition (Sheikh & Kaplan, 1986).

The kallikrein-kinin system

The basic elements of the kallikrein-kinin system are illustrated in Figure 1. They consist of proteases (kallikreins) which release kinins from precursor proteins (kininogen). There exist two kinds of kallikreins, plasma and glandular kallikrein. The active plasma kallikrein is derived from the inactive prekallikrein when it is exposed, for instance, to the active fragment of factor XII (Alving *et al.*, 1978). Plasma kallikrein produces bradykinin from a high molecular weight substrate. Kallikrein present in the renal tissue and in the urine is of the glandular subtype. Renal prekallikrein may be activated by phospholipase A₂ and arachidonic acid, suggesting a role for prostaglandins in the regulation of the renal kallikrein-kinin system (Nishimura *et al.*, 1980). Renal kallikrein cleaves both low and high molecular kininogen to produce kallidin, a decapeptide. Kallidin is then converted to bradykinin by an aminopeptidase.

Relationships between the renin-angiotensin and the kallikrein system

There are important interrelations between the renin-angiotensin-aldosterone and the kallikrein-kinin systems. As already pointed out, the enzyme which is necessary for the production of angiotensin II at the same time plays a role in bradykinin inactivation (Erdos, 1975). Mineralocorticoids represent another link between the two hormonal systems. These salt-retaining steroids are indeed known to increase the release of renal kallikrein (Margolius *et al.*, 1974). Also

to be considered is the fact that the vasoconstrictor effect of angiotensin II may be counteracted by bradykinin (Aubert *et al.*, 1988) and that both angiotensin II and bradykinin stimulate prostaglandin synthesis (McGiff *et al.*, 1972; Blumberg *et al.*, 1977). Finally, it is possible that kallikrein is involved in the activation of prorenin (Sealy *et al.*, 1978; Derkx *et al.*, 1979).

Effects of ACE inhibition on the components of the kallikrein-kinin system

Some investigators have reported increases in circulating bradykinin during acute ACE inhibition (Crantz *et al.*, 1980; Swartz *et al.*, 1980). In most studies, however, no consistent changes in circulating bradykinin could be detected, either during short- or long-term inhibition of ACE (Hulthen & Hokfelt, 1978; Johnston *et al.*, 1979, 1982; Ogihara *et al.*, 1981). Interestingly, a decrease in the number of bradykinin tissue receptors has been observed in the experimental animal following prolonged captopril administration (Johnston *et al.*, 1982). The same investigators also demonstrated a down-regulation of these receptors by exogenous bradykinin. Based on these results, it was speculated that bradykinin may have accumulated in tissues rather than in the blood.

The renal kallikrein-kinin system is presumably implicated in renal sodium handling (Levinsky, 1979; Carretero & Scicli, 1980; Margolius, 1984). Urinary kallikrein is reduced by ACE inhibition (Ohman *et al.*, 1983; Quilley *et al.*, 1987), probably as a result of the decreased levels of

Figure 1 Components of the kallikrein-kinin system.

aldosterone. During acute ACE inhibition, urinary kinin excretion was reported to increase in the absence of a concomitant change in circulating bradykinin (Vinci *et al.*, 1979). However, captopril administered by other investigators for several days did not change urinary kinin excretion (Quilley *et al.*, 1987). Whether the renal kallikrein-kinin system facilitates natriuresis while ACE activity is inhibited therefore remains speculative.

Response to endogenous and exogenous bradykinin during ACE inhibition

There is no doubt that ACE inhibition enhances the vascular response to exogenous bradykinin (Green *et al.*, 1972; Textor *et al.*, 1981; Kiowski *et al.*, 1982). In this respect, the recent findings of an increased thickness of weals caused by intradermal injection of bradykinin during ACE inhibition is interesting (Ferner *et al.*, 1987; Fuller *et al.*, 1987). On the other hand, the bronchial reactivity to bradykinin is not enhanced by ACE inhibition (Dixon *et al.*, 1987). This is of interest since bradykinin is known to provoke bronchoconstriction.

It is difficult to ascertain whether activation of the plasma kallikrein-kinin system may lead to the formation of bradykinin in an amount sufficient to exert systemic effects. In an attempt to answer this question, unanaesthetized rats were treated with the active fragment derived from factor XII (factor XIIIf) (Waeber *et al.*, 1984).

This fragment known to activate *in vitro* plasma prekallikrein (Kaplan & Austen, 1970) was found to induce a dose-dependent hypotension (Figure 2). That the blood pressure fall was indeed due to bradykinin generation was suggested by the observed potentiation of the hypotensive reaction to factor XIIIf by captopril while blockade of angiotensin II at the vascular receptor level by saralasin did not accentuate the blood pressure response to the active fragment.

Blood pressure effect of a bradykinin antagonist during ACE inhibition

Recently, competitive antagonists of bradykinin have been synthesised (Vavrek & Stewart, 1985). We used one of them [DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DPhe-Thi-Arg-TFA (Thi = beta-(2-thienyl)-L-alanine; Hyp = L-4-hydroxyproline; TFA = trifluoroacetic acid)] to evaluate in conscious semirestrained rats the contribution of circulating bradykinin to blood pressure control following ACE inhibition. This antagonist was kindly provided by Dr J. Stewart (Denver, CO, USA).

The bradykinin-antagonist was injected intravenously as a bolus of 400 µg. This dose was shown in the normotensive rat to attenuate markedly for at least 5 min the blood pressure lowering effect of exogenous bradykinin (Aubert *et al.*, 1988). The bradykinin-antagonist was found also to prevent to a large extent the factor XIIIf-induced hypotension (Figure 3). These

Figure 2 Maximal mean (s.e. mean) blood pressure response induced by factor XIIIf in unanaesthetized rats pretreated with either captopril (●) or its vehicle (○). * $P < 0.05$.

Figure 3 Effect of factor XIIIf on mean (s.e. mean) blood pressure of rats pretreated with a bradykinin-antagonist or its vehicle. ** $P < 0.01$.

results are of particular interest since they confirm that bradykinin may be formed in the circulation in an amount sufficient to exert haemodynamic effects.

The bradykinin-antagonist had no blood pressure effect *per se* in normotensive rats (Aubert *et al.*, 1988). In spontaneously hypertensive rats, however, circulating bradykinin seemed to participate actively in cardiovascular control since blood pressure rose immediately following injection of the antagonist. The magnitude of this blood pressure increase was not influenced by acute ACE inhibition with captopril. Moreover, it was similar when the renin-angiotensin system was blocked with saralasin, a competitive antagonist of angiotensin II, rather than by inhibiting kininase II activity (Figure 4) (Waeber *et al.*, 1986). In normotensive rats also, ACE inhibition did not alter the blood pressure response to the bradykinin-antagonist (Aubert *et al.*, 1987). Other investigators have used in normotensive rats, as well as in rats with renovascular hypertension, the same antagonist to assess the role of bradykinin in the depressor action of ACE inhibition (Benetos *et al.*, 1986a, b). The results of these studies suggested that bradykinin may actually contribute to the decrease in blood pressure produced by ACE in-

Figure 4 Mean (s.e. mean) blood pressure changes induced by a bradykinin-antagonist in spontaneously hypertensive rats pretreated with captopril, 2.5 mg intravenously, or infused with saralasin, 6 $\mu\text{g min}^{-1}$ intravenously. • $P < 0.05$ vs base line.

hibition. It should be noted that the bradykinin inhibitor was either infused intravenously for 10 min at a rate of 40 $\mu\text{g min}^{-1}$ or injected intra-arterially in two 1 mg doses 5 min apart. It is however, unknown whether the mode of administration of the antagonist accounts for the differences in the observations.

Effect of ACE inhibition on kininase I activity

Kininase II is not the only enzyme capable of degrading bradykinin. This vasodilating peptide can also be metabolized in human plasma by carboxypeptidase N (= kininase I) (Sheikh & Kaplan, 1986), an enzyme which, like ACE, is located on the surface of endothelial cells (Ryan *et al.*, 1982). We therefore studied in normal volunteers the effect of repeated doses of an ACE inhibitor on the plasma activity of kininase I. For this purpose, the subjects were treated for 7 days with either a long-acting ACE inhibitor (CGS 14824A) or a placebo (Waeber *et al.*, 1987). Two doses of CGS 14824A were tested (2 and 10 mg orally once daily). They both effectively inhibited ACE activity throughout the day. Figure 5 shows the levels of kininase I activity determined serially using a spectrophotometric assay on the first and the last day of treatment (Plummer & Kimmel, 1980). It appears that ACE inhibition for 1 week has no inhibitory action on plasma kininase I. This means therefore that bradykinin, at least when formed in the circulation, can be inactivated even in the face of a sustained blockade of kininase II.

Conclusions

There exist important connections between the renin-angiotensin and the kallikrein-kinin systems. Much has been learned about the function of the renin axis by blocking the synthesis or by antagonizing the effect of angiotensin II. The same investigational approach is used today to evaluate the contribution of circulating bradykinin to cardiovascular homeostasis. After administering a competitive antagonist of bradykinin to unanaesthetized rats, we did not detect any evidence for a consistent involvement of bradykinin in the blood pressure response to acute ACE inhibition. Bradykinin does not seem to accumulate following inhibition of ACE, probably because of the action of the other bradykinin-processing enzyme, kininase I.

Figure 5 Effect of a 7 day treatment with the ACE inhibitor CGS 14824A on plasma carboxypeptidase N (= kininase I) activity (mean $\pm$ s.e. mean). Normotensive volunteers received daily either a 2 mg (●—●) or a 10 mg (○—○) dose of the inhibitor. Other subjects were given a placebo (●—●). The enzyme activity was measured at different times both on the first and the last day of the study.

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