SUPERACTIVE INHIBITORS OF ANGIOTENSIN CONVERTING ENZYME

Analogs of BPP_{9a} containing dehydroproline

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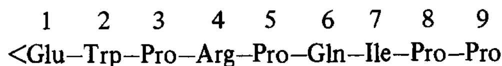
1. Introduction

As part of a program to examine the pharmacokinetics of the angiotensin converting enzyme inhibitor BPP_{9a} (also known as SQ 20,881):

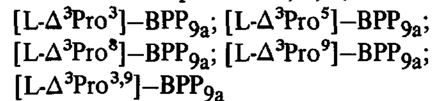

1 2 3 4 5 6 7 8 9
<Glu–Trp–Pro–Arg–Pro–Gln–Ile–Pro–Pro

we have synthesized a series of its analogs containing L-3,4-dehydroproline [1] (Δ^3Pro) in place of the proline residues at position 3, 5, 8 or 9. These analogs were synthesized to facilitate preparation of tritium-labelled BPP_{9a} by catalytic hydrogenation in tritium gas of the unsaturated carbon–carbon bond of Δ^3Pro. However, during the course of our studies, the Δ^3Pro–BPP_{9a} analogs were assayed in vitro and in vivo for their abilities to inhibit angiotensin converting enzyme (ACE). All of the analogs were found to be more potent than BPP_{9a} itself.

Several bradykinin potentiating peptides (BPP) have been isolated from the venom of *Bothrops jararaca*, a South American pit viper [2,3]. The most potent is the nonapeptide, BPP_{9a}. Part, if not all, of the effects of BPP_{9a} as a bradykinin potentiating agent are attributable to its ability to inhibit angiotensin converting enzyme [4,5]. Gavras and colleagues [6,7] have shown that BPP_{9a}, injected intravenously at 1–4 mg/kg body wt, acts as a potent anti-hypertensive agent in patients with renin-related hypertension. Of equal or greater importance is that some patients with essential hypertension can also be made normotensive by treatment with BPP_{9a} in combination with a diuretic such as furosemide [8,9].

The means by which BPP_{9a} exerts its anti-hypertensive effects are not fully understood, but one of its major mechanisms of action is that of inhibition of angiotensin converting enzyme. In addition to its ability to inhibit the conversion of angiotensin I (AI) into the potent vasoconstrictor octapeptide angiotensin II (AII) [10,11], BPP_{9a} can also prevent the inactivation of the hypotensive hormone bradykinin by ACE [5]. It is still uncertain whether the blood pressure lowering effect of BPP_{9a} is attributable to inhibition of the formation of AII, to the preservation of bradykinin, or to actions as yet undefined.

In efforts to understand further the mechanisms of action of BPP_{9a} and to determine its metabolic fate in vivo, we have synthesized 5 analogs of BPP_{9a} containing L-3,4-dehydroproline (Δ^3Pro) in place of the proline residues at position 3, 5, 8, or 9 of BPP_{9a}:


[L-Δ^3Pro^3]–BPP_{9a}; [L-Δ^3Pro^5]–BPP_{9a};
[L-Δ^3Pro^8]–BPP_{9a}; [L-Δ^3Pro^9]–BPP_{9a};
[L-Δ^3Pro^{3,9}]–BPP_{9a}

Upon catalytic reduction in tritium gas, the double bond of the Δ^3Pro residue can be reduced to yield BPP_{9a} labelled with two tritium atoms per original dehydroproline residue [12].

2. Materials and methods

The Δ^3Pro–BPP_{9a} analogs were synthesized by the solid-phase technique using a Schwarz-Mann automatic peptide synthesizer, as described for Δ^3Pro-bradykinin

analogs [13]. The α-amino nitrogen or arginine was protected with the amyloxycarbonyl (Aoc) group and the guanidino nitrogen with the tosyl protecting group. β-Mercaptoethanol (1%) was added to the deprotection reagent after incorporation of tryptophan in order to prevent oxidation of its indole ring [14]. To avoid diketopiperazine formation from the Pro–Pro dipeptide resin, inverse addition of dicyclohexylcarbodiimide (DCC) and Boc–Ile for the third coupling was carried out according to the recommendation in [15]. Cleavage of a given peptide from the resin, with simultaneous removal of protecting groups and formation of the carboxyl terminal acid, was effected with anhydrous, liquid hydrogen fluoride (HF) in the presence of 10% anisole. The crude deprotected peptides were purified by gel filtration and partition chromatography on Bio-Gel P-2, Sephadex G-25, and Sephadex LH-20, and were analyzed for purity as in [13].

The analogs were tested in vitro for their abilities to inhibit the activity of angiotensin converting enzyme. Inhibitory activity was measured in terms of the ability of a given analog to inhibit hydrolysis of the substrate [^{3}H]benzoyl–Gly–His–Leu ([^{3}H]Hip–His–Leu) by ACE partially purified from human urine. Enzyme was used in a quantity sufficient to hydrolyze 8% of substrate ([^{3}H]Hip–His–Leu plus unlabelled Hip–His–Leu at 100 μM) on incubation at 37°C for 15 min in Hepes buffer containing 0.1 M NaCl and 0.75 M Na_2SO_4 at pH 8 [16]. Inhibitor concentrations varied from 10^{-6}–10^{-11} M.

Each of the Δ^3Pro–BPP_{9a} analogs was assayed in vivo for the ability to inhibit the vasopressor response to angiotensin I. Normotensive Sprague-Dawley rats were anesthetized with pentobarbital, 50 mg/kg intraperitoneally. Cannulas were inserted for intravenous injection and for direct measurement of mean arterial blood pressure. Angiotensin I, 200 ng/kg, was injected intravenously at timed intervals before and after injection of an inhibitor. Systemic arterial blood pressure was recorded via the femoral artery with a Statham pressure transducer connected to a polygraph recorder.

3. Results

Each of the synthetic Δ^3Pro–BPP_{9a} analogs was homogeneous, as judged by thin-layer chromatography, paper electrophoresis, and high-performance liquid chromatography. By these techniques, the analogs were essentially indistinguishable from BPP_{9a}. Each analog on amino acid analysis showed the requisite residues in the theoretical molar ratios.

Table 1
Inhibition of angiotensin converting enzyme by Δ^3Pro-BPP_{9a} analogs

Peptide	I_{50} (nM)
BPP_{9a}	25
[L-Δ^3Pro^3]–BPP_{9a}	0.6
[L-Δ^3Pro^5]–BPP_{9a}	0.6
[L-Δ^3Pro^8]–BPP_{9a}	0.3
[L-Δ^3Pro^9]–BPP_{9a}	0.2
[L-Δ^3Pro^{3,9}]–BPP_{9a}	0.5

Assay protocol is as in section 2 and also in [16]

In vitro, the Δ^3Pro–BPP_{9a} analogs inhibit the activity of angiotensin converting enzyme. That concentration of inhibitor which reduces enzyme activity by half is defined as the inhibitory potency and is expressed as the I_{50} value. The I_{50} values for BPP_{9a} and its Δ^3Pro analogs are listed in table 1. Two of the analogs, [L-Δ^3Pro^3]–BPP_{9a} and [L-Δ^3Pro^5]–BPP_{9a}, proved to be ~40-times more potent than BPP_{9a} itself (respective I_{50} values, 0.6 nM, 0.6 nM, and 25 nM). [L-Δ^3Pro^8]–BPP_{9a} (I_{50}, 0.3 nM) and [L-Δ^3Pro^9]–BPP_{9a} (I_{50}, 0.2 nM) are ~100-times more potent than BPP_{9a}. The doubly substituted analog [L-Δ^3Pro^{3,9}]–BPP_{9a} (I_{50}, 0.5 nM) is ~50-times more potent than BPP_{9a}.

Each of the Δ^3Pro-BPP_{9a} analogs is also active in vivo in inhibiting the vasopressor response to angiotensin I in anesthetized normotensive rats (fig.1). [L-Δ^3Pro^9]–BPP_{9a} is not only as potent as Squibb's new ACE inhibitor, Captopril (SQ 14,225) [17,18], on a molar basis but also has a longer duration of action. The doubly substituted analog [L-Δ^3Pro^{3,9}]–BPP_{9a} is also as active as the Δ^3Pro^3 analog but has a slightly shorter duration of action. As expected, the durations of action of the analogs were increased with increasing dosage (fig.1b).

4. Discussion

BPP_{9a} and related peptides exert their effects on

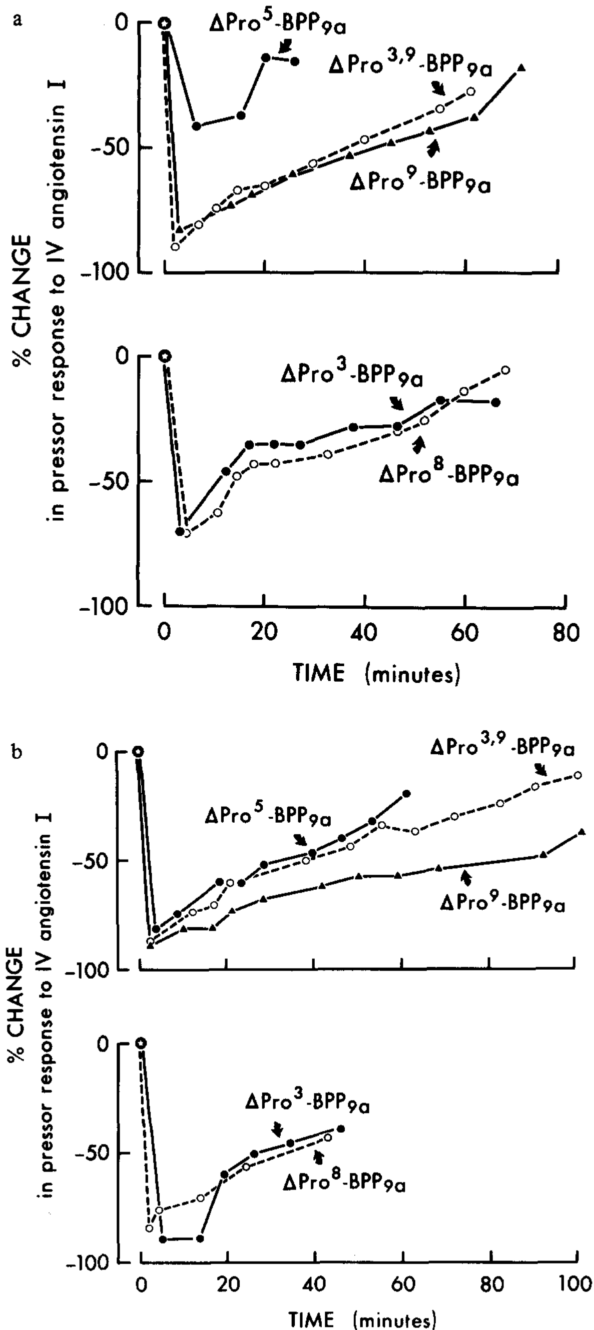


Fig.1. Effects of intravenous ΔPro–BPP_{9a} analogs on the vasopressor response to angiotensin I (200 ng/kg) in normotensive rats. (a) Inhibitor at 0.45 μmol/kg; (b) Inhibitor at 1.37 μmol/kg. Blood pressure effects of angiotensin I given after inhibitor are expressed in terms of % of the control response.

ACE by binding at or near the active site of the enzyme. The high potencies of the Δ^3Pro–BPP_{9a} analogs may be due to preferential π–π interaction of the deformed electron cloud of the double bond of the dehydroproline residue with binding sites on the enzyme. The high potency of [L-Δ^3Pro^9]–BPP_{9a} emphasizes the importance of the C-terminal proline residue for binding to the active site of the enzyme [19,20]. It has been postulated that introduction of unsaturation into a binding element of a peptide can enhance its affinity for some of its receptors [21]. For example, [L-Δ^3Pro^7]–oxytocin has increased uterotonic effects compared to oxytocin itself [21]. By analogy, it would appear that inhibitor–enzyme binding is similarly influenced.

In addition, the double bond of dehydroproline, allylic to the asymmetric center, tends to restrict the conformational flexibility of the proline ring. Therefore, it is reasonable to consider that substitution of dehydroproline into the BPP_{9a} sequence may restrict further the number of possible conformations which the peptide can assume and thereby alter its inhibitory potency [13,22].

In experiments using our assay technique, results obtained with Squibb's new synthetic ACE inhibitor, Captopril (SQ 14,225) [17,18], are highly variable (I_{50}, 1–20 nM), possibly owing to the lability of the drug. To our knowledge, [L-Δ^3Pro^9]–BPP_{9a} is the most potent ACE inhibitor known. Since the costs of synthesis of BPP_{9a} and its Δ^3Pro analogs are essentially the same, there may be distinct economic and manufacturing advantages in the use of [L-Δ^3Pro^9]–BPP_{9a} for the treatment of renovascular and essential hypertension.

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