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Short communication

The hemoregulatory peptide *N*-acetyl-ser-asp-lys-pro impairs angiotensin I-induced contractions in rat aorta

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Abstract

The hemoregulatory peptide *N*-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP) is degraded by ACE. This study was designed to examine the effect of Ac-SDKP on the contractions to angiotensin I. Experiments were performed on rat aortic rings with endothelium exposed to nitro-L-arginine. Ac-SDKP (10 and 100 μM) significantly augmented angiotensin I ED_{20} (from 2.0 ± 0.4 to 4.2 ± 1.0 and 5.0 ± 0.9 nM) and ED_{50} (from 4.3 ± 0.7 to 8.6 ± 1.0 and 10.7 ± 1.3 nM, respectively), but did not alter its maximal response. The contractions to angiotensin II were not affected by Ac-SDKP. No degradation of exogenous Ac-SDKP nor detectable release of endogenous Ac-SDKP were observed in the incubation medium. These results suggest that Ac-SDKP impairs angiotensin I response by inhibiting ACE and subsequent angiotensin II formation.

Keywords: Angiotensin II; Angiotensin I; Angiotensin converting enzyme; Vascular tone; Aorta; (Rat); Ac-SDKP (*N*-acetyl-seryl-aspartyl-lysyl-proline)

1. Introduction

N-Acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP) is a regulatory factor of hematopoiesis secreted by bone marrow and ubiquitously found in vivo, including in plasma (Pradelles et al., 1990, 1991). The tetrapeptide is degraded preferentially by ACE in vitro and in vivo (Rieger et al., 1993; Azizi et al., 1996, 1997). Angiotensin converting enzyme also contributes to the formation of the potent vasoconstrictor angiotensin II from inactive angiotensin I. Although angiotensin I and Ac-SDKP are ACE substrates with respective K_m of 16 and 41 μM (Wei et al., 1991; Jaspard et al., 1993; Rousseau et al., 1995), Ac-SDKP is present in plasma and tissues at much higher concentrations than angiotensin I (Pradelles et al., 1991, Campbell et al., 1994). These observations suggest that Ac-SDKP may interfere with the formation of angiotensin II. As a consequence, Ac-SDKP could alter the functional effect of angiotensin I, mediated by its conversion in angiotensin II. In addition, the structure of the natural ACE substrate Ac-SDKP is very close to that of the angiotensin converting enzyme synthetic inhibitor lisinopril (*N*-[(*S*)-1-carboxy-3-phenyl-propyl]-L-lys-L-pro), which was discovered earlier than the endogenous tetrapeptide (Brunner et al., 1981).

In order to determine whether or not Ac-SDKP affects the conversion of angiotensin I in angiotensin II, we examined in vitro the effect of increasing concentrations of Ac-SDKP on the contractions to exogenous angiotensin I in the rat aorta.

2. Material and methods

Experiments were performed in vitro using the thoracic aorta of male Wistar rats (15 week old; Iffa-Credo, Domaine des Oncins, St Germain sur l'Abresle). The rats were anesthetized with pentobarbital (50 mg/kg i.p.) following the standards of French Ministère de l'Agriculture for animal care and euthanasia. The thoracic aorta was dissected and placed in an ice-cold control solution (composition in mM: 118.3, NaCl; 4.7, KCl; 2.5, $CaCl_2$; 1.2, KH_2PO_4; 1.2, $MgSO_4$; 25, $NaHCO_3$; 11, glucose; 0.026,

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EDTA). For measurement of endogenous level of Ac-SDKP, the rat aortas were then immediately frozen in liquid nitrogen and kept at −80°C until assay.

2.1. Organ chamber experiments

The preparations, cut into rings (4–5 mm long), were suspended in conventional organ chambers for the recording of isometric tension. The preparations were brought to the maximum of their length–tension relationship and were exposed to KCl (60 mM). After removing KCl, presence of the endothelium was confirmed by complete relaxation to acetylcholine (10 μM) during phenylephrine contraction (10–300 nM). After washing the agonists, all preparations were exposed to nitro-L-arginine (an inhibitor of nitric oxide synthase; 30 μM; for 30 min) to prevent both release of nitric oxide (NO) and down-regulation of angiotensin II receptors (Ito et al., 1991; Boulanger et al., 1995). All experiments were performed in parallel rings, either in the absence (control) or the presence of increasing concentrations of Ac-SDKP.

2.2. Measurement of Ac-SDKP in the incubation medium and in rat aorta

At the end of the dose–response curves to angiotensin I, aliquots of incubation medium were frozen at −20°C until assay. Aortas were homogenized in the presence of acetic acid (2 N, 2 ml) followed by centrifugation at 10,000 × *g* (10 min; 4°C). The supernatant was evaporated and the resulting dry extract dissolved in enzyme immunoassay buffer. The concentration of Ac-SDKP was determined by competitive enzyme immunoassay as described earlier (Ezan et al., 1994). The limit of detection was 0.5 nmol/l.

2.3. Drugs

Acetylcholine, angiotensin I, angiotensin II, nitro-L-arginine, phenylephrine were obtained from Sigma (Saint-Quentin Fallavier, France). Ac-SDKP was graciously provided by Dr. E. Cohen, Beaufour-Ipsen Industrie (Dreux, France). Perindoprilat was a kind gift from Dr. N. Thibaud, Institut de Recherches International Servier (Courbevoie, France). All compounds were prepared daily and dissolved in distilled water (stock solutions 0.1 to 10 mM), except for nitro-L-arginine which was dissolved in water after sonication.

2.4. Statistical analysis

The data are given as mean ± S.E.M. ED_{20} and ED_{50} represent the concentration of angiotensin I or II which induces 20 or 50% of the maximal contraction induced by the peptides, respectively. They were measured from individual preparations and pooled to obtain mean values (±S.E.M.). For each experiment, linear correlations between the logarithm of Ac-SDKP concentrations on one hand, and angiotensin I ED_{20} and ED_{50} values on the other hand, were obtained using Instat 200 (GraphPad Software). Statistical analysis (on data given in mg increase in tension) was performed using analysis of variance for repeated measures (ANOVA) followed by Bonferonni's test;

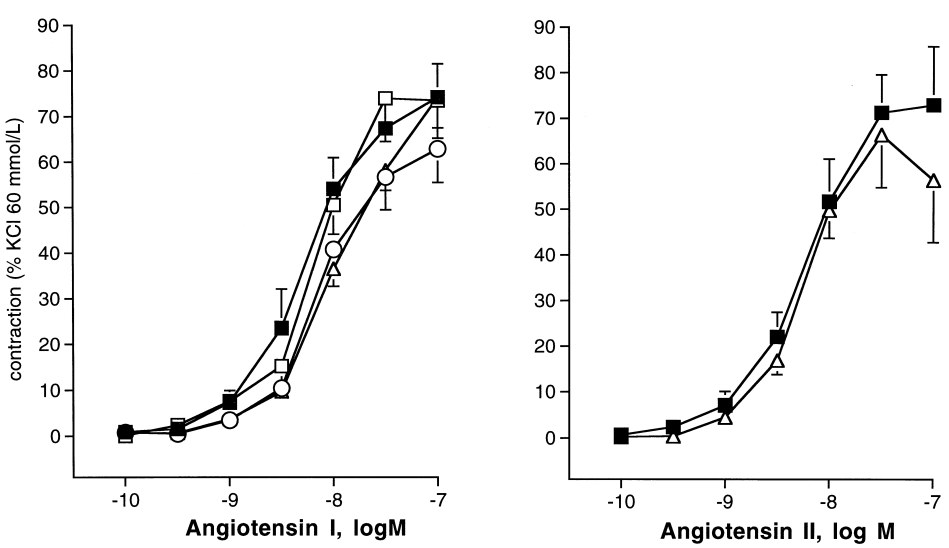

Fig. 1. Effect of Ac-SDKP on the response to angiotensin I (left) and angiotensin II (right) in rings with endothelium of the rat aorta. Experiments were performed in parallel, under control conditions (■) or in the presence of Ac-SDKP (1 μM: □; 10 μM: ○;100 μM: Δ). For the sake of clarity, the effect of Ac-SDKP (0.1 μM) is not shown. Data are expressed as percent of the response of each preparation to potassium chloride (60 mM) which were not different between all the groups. For experiments dealing with angiotensin I (left), the response to KCl 60 mM was: control, 2.4 ± 0.1 g; Ac-SDKP 1 μM: 2.1 ± 0.2 g; Ac-SDKP 10 μM: 2.2 ± 0.3 g; Ac-SDKP 100 μM: 2.2 ± 0.2 g; $n = 6$). For experiments dealing with angiotensin II (right), the response to KCl 60 mM was: control, 2.1 ± 0.2 g; Ac-SDKP 100 μM: 2.1 ± 0.1 g ($n = 5$).

Table 1
Effect of Ac-SDKP on the maximal responses to angiotensin I ($n = 6$) and angiotensin II ($n = 5$)

Ac-SDKP	Angiotensin I			Angiotensin II:		
	ED_{20} (nM)	ED_{50} (nM)	Maximum (g)	ED_{20} (nM)	ED_{50} (nM)	Maximum (g)
0 (control)	2.0 ± 0.4	4.3 ± 0.7	1.6 ± 0.2	2.1 ± 0.8	5.4 ± 1.2	1.5 ± 0.3
0.1	2.9 ± 0.5	5.4 ± 1.0	1.4 ± 0.2	n.d.	n.d.	n.d.
1	3.1 ± 0.7	7.5 ± 1.3	1.7 ± 0.3	n.d	n.d.	n.d.
10	4.2 ± 1.0[a]	8.6 ± 1.0[a]	1.3 ± 0.2	n.d	n.d.	n.d.
100	5.0 ± 0.9[a]	10.7 ± 1.3[a]	1.5 ± 0.1	2.5 ± 0.3	5.0 ± 0.8	1.3 ± 0.2

n.d.: Not determined.
[a] Indicates a significant effect of Ac-SDKP as compared with control.

n indicates the number of animals in each group. Differences were considered significant when P was less than 0.05.

3. Results

Ac-SDKP (from 1 nM to 0.1 mM) did not cause contractions nor relaxations in rat aortic rings exposed to phenylephrine ($n = 3$; data not shown).

Contractions to angiotensin I (1 to 100 nM) were abolished by the angiotensin converting enzyme inhibitor perindoprilat (1 μM) in aortic rings with endothelium ($n = 6$). Ac-SDKP (10 to 100 μM) caused a significant concentration-dependent inhibition of the contractions induced by low concentrations of angiotensin I (1 to 10 nM; $P = 0.001$) (Fig. 1). Ac-SDKP (10 and 100 μM) significantly augmented angiotensin I ED_{20} and ED_{50} ($P < 0.008$; Table 1). The slope of the linear correlations between angiotensin I ED_{20} or ED_{50} on one hand, and Ac-SDKP concentrations on the other hand, averaged 75 ± 23 and 159 ± 41 and they were significantly different from zero, respectively ($P = 0{,}026$ and $P = 0{,}012$, respectively; $n = 6$ each). Ac-SDKP did not decrease significantly the maximal response to angiotensin I ($P = 0.43$; Table 1). Under the same experimental conditions, the response to angiotensin II, even at low concentrations, was not affected by Ac-SDKP (100 μM; $P = 0.24$) (Fig. 1; Table 1).

At the end of the experiment, the concentration of Ac-SDKP was measured in the bathing solution. It averaged 0.095 ± 0.006, 1.04 ± 0.06 and 12.07 ± 0.56 μM for incubation solution of Ac-SDKP 0.1, 1 and 10 μM, respectively ($n = 4$–6). In addition, the bathing medium of preparations not exposed to exogenous Ac-SDKP contained no detectable amounts of the tetrapeptide (below 1 nM; $n = 5$). The endogenous concentration of Ac-SDKP averaged 33 ± 2 pmol/g tissue in the rat aorta with endothelium ($n = 5$).

4. Discussion

The present results demontrate that Ac-SDKP, a physiological substrate of ACE, impairs the contractions evoked by low concentrations of angiotensin I in the rat aorta.

The contractile response to angiotensin I in the rat aorta results preferentially from its conversion to angiotensin II by ACE, as illustrated in the present study by the full inhibition of ACE inhibitor perindoprilat on angiotensin I response and the comparable response to equimolar concentrations of angiotensin I and angiotensin II (Dzau and Pratt, 1994; Vidal et al., 1994). The concentration-dependent inhibitory effect of Ac-SDKP, although more moderate than that caused by synthetic ACE inhibitor, is likely due to a decreased formation of the potent vasoconstrictor angiotensin II resulting from an inhibition of the conversion of angiotensin I.

Several data support the interpretation that the inhibitory effect of Ac-SDKP on the contractions to angiotensin I may occur at ACE level. First, a non-specific effect of Ac-SDKP can be ruled out since the tetrapeptide has no vasoactive effect and does not decrease the response to angiotensin II in rat aorta. In addition, the tetrapeptide has no effect on the contraction to phenylephrine. Interestingly, the inhibitory effect of Ac-SDKP is observed for concentrations consistent with its K_m (41 μmol/L) for the wild type membrane-bound recombinant converting enzyme (Rousseau et al., 1995). Although circulating Ac-SDKP concentrations (in the nanomolar range) are 500 to 1000 time higher than those of angiotensin I in humans (Azizi et al., 1996), the present results are not in favor of an inhibitory role of Ac-SDKP in angiotensin II formation in the plasma compartment. Indeed, both Ac-SDKP and angiotensin I plasma concentrations are much lower than their respective K_m and the substrate to enzyme ratio is very low.

However, Ac-SDKP could play a role in the regulation of local renin angiotensin system in tissues and in the vessel wall (Cassis et al., 1988; Naftilan et al., 1991). Angiotensin I concentrations averaged 38 ± 3 fmol/g and 3.3 ± 0.5 fmol/g in the rat kidneys and lungs, respectively (Campbell et al., 1994). These concentrations are much lower than those of Ac-SDKP measured in male Wistar rat kidneys (220 ± 24 pmol/g) and lungs (220 ± 21 pmol/g, Ezan et al., unpublished data). Thus, in rat tissues, the ratio of Ac-SDKP to angiotensin I concentrations varies between 30,000 and 700,000 (Campbell et al., 1994; Ezan et al. unpublished data). Therefore, Ac-SDKP may permanently, but mildly, compete with angiotensin I on both

active sites of ACE in tissues where Ac-SDKP concentrations are close to its K_m and where the ratio Ac-SDKP vs. angiotensin I is very high.

Since the local renin angiotensin system may generate sufficient amounts of angiotensin II to regulate both local and downstream blood vessel functions (Qiu et al., 1994; Henrion et al., 1997), Ac-SDKP may participate in the physiological regulation of angiotensin II formation in tissues where its endogenous concentration reaches its K_m value for the converting enzyme.

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