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Short communication

Vascular effects of a tripeptide fragment of novokinine in hypertensive rats: Mechanism of the hypotensive action

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ABSTRACT

Background: Activation of angiotensin AT_2 receptors (AT_2R) counteracts vasoconstrictor effects of AT_1R stimulation and contributes to blood pressure control. We examined effects on mean arterial pressure (MAP) and renal hemodynamics of LKP, a tripeptide fragment of novokinine, an established AT_2R agonist.
Methods: Effects of intravenous LKP infusion and then superimposed losartan (AT_1R antagonist) on MAP, total renal (RBF, Transonic probe) and renal medullary blood flows (laser-Doppler), and on renal excretion, were examined in anesthetized (1) Wistar rats with acute norepinephrine-induced hypertension, untreated or pretreated with AT_2R antagonist (PD 123319) and (2) spontaneously hypertensive rats (SHR) maintained on standard or high-sodium (HS) diet.
Results: In Wistar rats LKP decreased MAP (−4%, $p < 0.01$) and increased renal medullary perfusion, these effects were abolished in rats pretreated with PD 123319 in which a post-LKP increase in MAP (+6%, $p < 0.02$) occurred. LKP did not alter MAP in SHR; in those on HS diet RBF decreased (−14%, $p < 0.02$), the response that was reverted by losartan. Addition of losartan always decreased or tended to decrease MAP.
Conclusions: LKP lowered MAP in norepinephrine-induced hypertension, probably *via* activation of AT_2R. At reduced availability of AT_2R, as in SHR, LKP appeared to bind to different receptors, possibly AT_1, and induced systemic or renal vasoconstriction. Compared to the parent novokinine, a smaller LKP molecule might be easier absorbed after oral application and more useful in therapy.

Introduction

The renin-angiotensin system (RAS), especially its main active agent angiotensin II (AngII), is critically important for cardiovascular control in physiology and disease. AngII exerts its action *via* two receptors types, AT_1R and AT_2R, expressed in many tissues including the kidney, heart and blood vessels. RAS activity affects the vascular tone as well as renal excretion and fluid balance. Activation of AT_1R can induce hypertension, *via* vasoconstrictor action and, indirectly, *via* salt and water retention [1,2]. Therefore, AT_1R blockade is a crucial component of antihypertensive therapy, beside blockade of AngII synthesis using angiotensin converting enzyme (ACE) inhibitors and application of diuretics.

More recent studies indicate that activation of AT_2R counteracts pro-hypertensive effects of AT_1R stimulation, e.g. through a release of nitric oxide and eicosanoids [2,3]. A precise functional characterization of AT_2R has still not been achieved, largely because of its relatively low expression and dominating influence of AT_1R. The palette of selective AT_2R agonists is limited and attempts to lower blood pressure by AT_2R stimulation were not always successful. There is evidence that hypotensive and vasorelaxant actions of the hexapeptide novokinine (Arg-Pro-Leu-Lys-Pro-Trp) are mediated by activation of AT_2 and also of prostaglandin IP receptors [4,5]. In this study we examined if similar antihypertensive and vasorelaxant actions, possibly also mediated by AT_2R, can be demonstrated for LKP, a tripeptide (Leu-Lys-Pro) fragment of novokinine. We reasoned that LKP, possibly an active metabolite of novokinine, could be better absorbed after oral application than the larger parent molecule and prove more

Abbreviations: CBF, cortical perfusion (laser-Doppler flux); IMBF, inner medullary perfusion (laser-Doppler flux); LKP, a tripeptide (Leu-Lys-Pro) fragment of novokinine; LOS, losartan; MAP, mean arterial pressure; OMBF, outer medullary perfusion (laser-Doppler flux); RBF, renal blood flow.

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efficacious as an antihypertensive or vasorelaxant drug. Before, LKP was reported to have ACE-inhibitory properties [6] while here we focus for the first time on its possible AT_2R stimulatory action, similar as that found for novokinine.

Since subtle AT_2-mediated blood pressure lowering effects can easier be demonstrated at elevated baseline pressure level [7], we tested the drug in rats with norepinehrine-induced acute mild hypertension and in spontaneously hypertensive rats (SHR). It is known that hypotensive action of AT_2R stimulation can easier be demonstrated under inhibition of AT_1R [8–10]. Here, we thought it of interest to examine effects of AT_1R blockade with losartan when superimposed on sustained AT_2R stimulation. High sodium (HS) intake can elevate blood pressure ("salt-dependent hypertension"), possibly also by modulating the expression of AngII receptors [11]. Therefore we also examined effects of LKP and losartan in SHR maintained on standard or HS diet.

Methods

The experiments were approved by the extramural IV Local Ethical Committee, Warszawa. Wistar rats or spontaneously hypertensive rats (SHR, aged 10 weeks, with established hypertension) were used. Male Wistar rats used were bred at the Animal House, Mossakowski Medical Research Centre, PAN, Warszawa, Poland. Male SHR were purchased at the Polish Mother's Memorial Hospital Research Institute, Łódź, Poland.

We examined acute effects on mean arterial blood pressure (MAP), total and regional perfusion of the kidney, and on renal excretion of a tripeptide fragment of novokinine (LKP: Leu-Lys-Pro). LKP has been synthesized in house by solution step-by-step method and crude material purified by preparative RP-HPLC; the final tripeptide as hydrochloride salt has been used in our experiments. When effects of LKP have stabilized, we examined the subsequent actions of superimposed administration of losartan (LOS, a gift from Adamed Company, Sp. z. o.o., Pieńków, Poland), an antagonist of AT_1 angiotensin II receptors.

Surgical preparations

The rats were anesthetized with thiopental (Sandoz GmbH, Kundl, Austria), 100 mg/kg i.p., which provided stable anesthesia for at least 4 h. A tracheal cannula was placed to ensure free airways. In Wistar rats only, right-side nephrectomy was first performed from a flank incision; this helped obtain stable elevation of blood pressure with moderate dosage of norepinephrine (NE) [12]. Body temperature was maintained at 37 °C using a servo-controlled heating pad. The femoral vein was cannulated for infusion of fluids and drugs and the femoral artery for measurement of mean arterial blood pressure (MAP). Fluid losses were compensated by infusion of 3% bovine serum albumin in Ringer solution at 8 ml/h/kg BW. After completed surgery, this infusion was replaced by isotonic saline containing the drugs used in the experiments (see below), given at a total volume rate of 10 ml/kg/h. The left kidney was exposed from a flank incision and immobilized in a plastic holder; the ureter was cannulated for timed urine collection. A noncannulating probe connected with a Transonic flowmeter (T106, Transonic Systems Inc., Ithaca, NY, USA) to measure the total renal blood flow (RBF), was placed on the renal artery, with precautions being taken to avoid damage to renal nerves. Blood perfusion of the renal outer and inner medulla (OMBF, IMBF) was measured separately as laser-Doppler fluxes using Periflux 4001 system (Perimed AB, Jarfalla, Sweden) using two needle probes (PF 402) inserted into the kidney to the depths of 3 and 5 mm, respectively.

Twenty to 40-min equilibration was required until stabilization of the variables measured. We have repeatedly shown that with the above procedures stable MAP, and renal hemodynamics and excretion are recorded over at least 150 min.

Urine volume was determined gravimetrically, urine osmolality using a cryoscopic osmometer (Osmomat 030, Gonotec, Berlin, Germany) and urine sodium concentration by flame photometry (Jenway PFP7, Essex, UK).

Protocols

Group 1 ($n = 7$, body weight 301 ± 3 g). In uninephrectomized Wistar rats maintained on standard diet the left kidney hemodynamics and excretion were measured continuously. After completing surgery, to increase MAP, norepinephrine (Sigma–Aldrich, St. Louis, MO) infusion was first started and then was infused intravenously throughout experiments, at 240 μg/kg/h. After stabilization of MAP, renal hemodynamics and urine flow, two 30-min measurement and urine collection periods (control) were made. Subsequently, i.v. infusion of LKP in isotonic saline was added, at 120 μg/kg/h, to be continued till the end of the study, and two 30-min measurement and urine collection periods were made. In preliminary experiments we established that the double dose was not more effective at lowering blood pressure and the fourfold lower dose was without any consistent effect. One hour after starting LKP infusion, losartan, 10 mg/kg, was added i.v. during 5 min (6 ml/kg/h), and measurements were continued during final two 30-min periods. This losartan dose was reported to significantly decrease MAP in anesthetized rats [13].

Group 2 ($n = 5$, BW 299 ± 8 g). The protocol described above was used except that after stabilization of MAP and renal hemodynamics under NE infusion, 10–15 min before LKP administration, an i.v. infusion of PD 123319, an antagonist of angiotensin II AT_2 receptors was started at 50 μg/kg/min and continued till the end of the study. We have shown previously that in a similar experimental set-up this PD123319 dose inverted the usual renal medullary vasodilator response to Ang II. [14].

Because of a too-late-detected technical problem with one of the flowmeter's laser channels, IMBF data for this group were thought unreliable and were discarded.

Group 3 ($n = 6$, BW 253 ± 3 g). In SHR fed standard sodium MAP and renal hemodynamics were measured as usual. Control data were first recorded during 30 min. The subsequent experimental procedures (LKP infusion and short infusion of losartan) were as in group 1.

Group 4 ($n = 7$, BW 253 ± 5 g). The same protocol was used in SHR maintained on high sodium diet (4% Na, w/w SSNIFF GmbH, Soest, Germany) over three weeks preceding an acute experiment.

Since at different time-points the experimental protocols involved infusion of from one to four drugs (NE, PD123319, LKP, losartan), at each stage the total volume infused was adjusted, by adding saline as needed, to the total infusion rate of 10 ml/kg/h.

In all groups the data for the latest 10 min of the continuous recordings in the control, LKP and losartan periods were averaged and presented. The values for renal excretion parameters were those for the second 30-min control, LKP or losartan urine collection period.

After experiments the rats were killed with an overdose of the anesthetic. The position of the laser Doppler probes was verified by cross-sectioning the kidney.

Statistics

In each group the differences between mean values for saline control (or PD123319 pretreatment in group 2) periods *versus* LKP periods, and between LKP *versus* losartan periods, were first evaluated using repeat measurement ANOVA followed by *post hoc* modified *t* test as described by Wallenstein et al. [15]. Statistical inter-group or multiple comparisons were not done. When needed,