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The vasodilatory effect of allopurinol mediates its antihypertensive effect: Effects on calcium movement and cardiac hemodynamics

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ABSTRACT

Despite the reported reduction in blood pressure in hypertensive patients treated with allopurinol, the mechanism of the allopurinol hypotensive effect is still unclear. In the current study, the hypotensive effect of allopurinol has been fully investigated in hypertensive rats. Hypertension was induced in rats by angiotensin II (120 ng/min/kg) infusion for two weeks. Rats were then subjected to real-time recording of blood pressure, left ventricular pressure and volume and surface ECG. After 10 min of basal recording, allopurinol was slowly injected into the femoral vein with a dose of 10 µmole/kg. Then, invasive blood pressure, cardiac hemodynamics and ECG were continuously recorded for an additional 20 min. In addition, the vasodilation effect of allopurinol was studied using the isolated artery technique. Allopurinol injection reduced systolic, diastolic and pulse blood pressure. Allopurinol suppressed both cardiac systolic and diastolic hemodynamics as is apparent from the reduction in the rate of rise and the rate of fall in left ventricular pressure. Allopurinol reduced the general cardiac output quickly. Allopurinol addition to the organ bath (10–1000 µM) produced significant vasodilation of PE pre-constricted aortae that was not affected by endothelium denudation, L-NAME or indomethacin. However, allopurinol ameliorated the calcium induced contraction of aorta pre-constricted with KCl in calcium-free media. Erk or ROCK inhibition did not attenuated allopurinol produced vasodilation. In conclusion, allopurinol has an antihypertensive effect that is mediated, probably, by reducing cardiac output and decreasing vascular resistance. The vasodilator effect of allopurinol is most likely mediated by calcium blocking activities.

1. Introduction

Hypertension is defined as a persistent blood pressure above 140/90 mmHg. It is a condition with a complex etiology and it is usually associated with abnormal endothelial function, abnormal renal function, metabolic disease, diabetes and excessive release of angiotensin II [1]. Recent studies have shown that 25.5% of the Saudi population suffers from some type of hypertension [2]. Although hypertension may seem harmless at first, it leads to the development of various fatal cardiovascular disorders. There is therefore a clear need for the discovery of new drug targets that can be used to treat this debilitating condition.

Angiotensin II is a peptide hormone that is produced from its precursors: angiotensinogen and angiotensin I, by the Angiotensin-Converting Enzyme (ACE) in the lungs and kidney. It is well known for its strong vasoconstricting effect leading to increased blood pressure. Angiotensin II can be used to induce hypertension in rats via infusion through an osmotic pump for a period of 14 days [3,4]. This model may be used to effectively induce hypertension in rats and allows us to study the effects of a variety of compounds on a pre-existing hypertensive state.

Allopurinol is a xanthine oxidase enzyme inhibitor, which commonly used to treat a variety of inflammatory disorders such as gout and hyperuricemia. Studies in various laboratories have shown that in addition to its traditional use in gout, allopurinol may be used to treat cardiovascular disease [1,2]. Studies have shown that the inhibition of the xanthine oxidase enzyme alleviates many of the cardiac complications which are associated with insulin resistance [5]. In this study, allopurinol alleviated the impaired ventricular relaxation associated with insulin resistance, corrected the ECG abnormalities seen due to cardiac ischemia in this model and reduced elevated angiotensin II and its receptor [5].

However, the reduction in blood pressure has been reported in hypertensive patients treated with allopurinol, reviewed in [6], very limited data currently exist regarding the mechanism of allopurinol hypotensive effect. Thus, in the current study, we investigated the effect of allopurinol on the cardiovascular function of angiotensin-induced hypertensive rats using invasive blood pressure and left ventricular

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pressure-volume technique as well as recording cardiac conductivity. The vasodilator effect of allopurinol on isolated rat aortic blood vessels was examined in order to elucidate the mechanism of action of allopurinol.

2. Methods

2.1. Animals

Six-week-old male Wistar rats with a weight of 250 g were obtained from King Abdul-Aziz University (KSA). They were kept in clear cages made of polypropylene and with good ventilation (3–4 rats in each cage), under constant environmental conditions of 22 ± 2 °C temperature, 50–60% relative humidity and 12-h day and night cycle. Unlimited rodent pellet food and purified water were provided to the rats. Approval for every experiment was obtained from the Biomedical and Research Ethical Committee of the Faculty of Medicine at King Abdul-Aziz University in Jeddah, Saudi Arabia. Furthermore, the experiments complied with the Saudi research bioethics regulations.

2.2. Induction of hypertension

Male Wistar rats (250–275 g) were infused with angiotensin II (120 ng/min/kg, two weeks) via osmotic minipump Model #2002 (0.5 ul/hr, Alzet®, Cupertino, CA) for two weeks as previously described [7].

2.3. Blood pressure and cardiac hemodynamic recording

The cardiac hemodynamics were recorded invasively in real time following the procedure outlined in our previous publications [5,8]. The rats were subjected to anaesthesia with single intraperitoneal injection of 100 mg/kg ketamine and 10 mg/kg xylazine. Animals' body temperature was held at 37 °C by means of a rectal probe and automated heating pads. A micro-tip pressure volume catheter (PV catheter, SPR-901, Millar Instruments, Houston, TX, USA) was inserted via a small opening into the right carotid artery and extended into the left ventricle. This instrument is able to continuously and concurrently monitor both ventricular pressure and volume of the intact, functioning hearts, while a second pressure sensor concurrently monitors the arterial pressure. The micro-tip catheter was linked via a Power Lab Data Interface to a computer running the Lab Chart professional software (v8.0, AD Instruments, Bella Vista, Australia) incorporating the PV and blood pressure (BP) modules. Following a stabilisation time of 5 min, readings were continuously recorded. The BP module was employed to monitor the systolic and diastolic blood pressure. The PV module analyzes the relationship between the LV pressure and volume signals. Systolic and diastolic BP were monitored via the BP module.

2.4. Electrocardiogram (ECG) recording

A Powerlab® system (AD Instruments, Bella Vista, Australia) linked to a computer running the LabChart professional software with the ECG module was employed to record the standard surface ECG according to the methodology outlined in a previous report by our group [8]. The ECG module quantitatively assesses the various elements of the ECG.

2.5. Injection of allopurinol

After 10 min (stabilization period) of basal recording of invasive blood pressure, cardiac hemodynamics and ECG, allopurinol was slowly (over 2 min) injected into the femoral vein in a dose of 10 μmole/kg (dissolved in 0.3 ml saline). The dose 10 μmole/kg of allopurinol was chosen based upon preliminary experiment testing the antihypertensive effect of dose range from 1 to 1000 μmole/kg allopurinol. Saline (0.3 ml) was injected in time control experiments. Then, invasive blood pressure, cardiac hemodynamics and ECG were continuously recorded for an additional 20 min.

2.6. Studying the vasodilation effect of allopurinol

2.6.1. Isolation of aortae

Animal lives were terminated by rodent guillotine. The thoracic aortae were then excised using ice-cold Krebs–Henseleit buffer. Then the isolated aortae were cleaned of connective tissue and fat before cutting into rings (2–3 mm).

2.6.2. Vasodilatation experiments

The vasodilation effect of allopurinol was studied using the isolated artery techniques previously described in our work [9–11]. In these experiments, allopurinol cumulative concentrations (1–1000 μM) were added to organ baths containing the isolated aortae preconstricted with 10 μM of PE. The decreases in tension were then recorded.

In other sets of experiments, mechanically denudated isolated aortae were used to study the role of endothelium in allopurinol vasodilation. In addition, the nitric oxide synthase inhibitor Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME, 100 μM), the cyclooxygenase inhibitor indomethacin (5 μM), the RhoA/Rho-kinase (ROCK) Fasudil (10 μM) or the Erk inhibitor array 162 (1 μM) were added 30 min before investigating the vasodilation effect of allopurinol as above.

The role of extracellular Ca^{2+} on allopurinol-induced vasodilation was examined as previously described in our work [12]. In brief, aortic rings were allowed to stabilize for 30 min in normal Krebs solution, which was then replaced with Ca^{2+}-free Krebs buffer for 10 min. Then high-KCl (80 mM) Krebs solution was added. The vascular contraction in response to $CaCl_2$ (1.25–5 mM) was recorded after a 20 min incubation with different concentrations of allopurinol (100, 300 and 1000 μM) or the vehicle (0.1% DMSO; control group).

2.7. Drugs and chemicals

The following drugs and chemicals were used: Angiotensin II (LKT Laboratories, Inc.Paul, Minnesota, USA), allopurinol (Sigma-Aldrich, Munich, Germany), ketamine (Tekam®, Hikma Pharmaceutical, Amman, Jordan), xylazine (Seton®, Laboratories Calier, Barcelona, Spain) (Sigma-Aldrich, St. Louis, MO, USA).

2.8. Statistical analysis

Expression of values took the form of mean ± SEM. Statistical analysis consisted of repeated measures Two way analysis of variance (ANOVA) and Newman-Keuls' post-hoc test ($p < 0.05$ significance level) conducted with the Prism 5 software (Graph Pad, USA).

3. Results

3.1. Effects of allopurinol on blood pressure

As shown in Fig. 1A and B, intravenous injection of allopurinol (10 μmole/kg) led to a reduction in systolic blood pressure in angiotensin II-induced hypertensive animals from (177.6 ± 4.708 mmHg) to (155.5 ± 3.625 mmHg) after 10 min of allopurinol injection. The reduction in systolic blood pressure was gradual ultimately reaching a plateau and was statistically significant 8 and 10 min (both at $p < 0.05$) after allopurinol injection.

A similar effect of allopurinol injection on diastolic blood pressure was observed (Fig. 1C). The reduction in diastolic blood pressure by allopurinol reached nearly normotensive values after 10 min of allopurinol injection (109.1 ± 4.411 vs 124.3 ± 1.649 before allopurinol injection). The reduction in diastolic blood pressure was also gradual and reached a plateau with statistical significance observed after 8 and