

Role of Various Potassium Channels in Caffeine-induced Aortic Relaxation in Rats

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ABSTRACT

Background: Studies done on caffeine-induced changes in aortic rings have demonstrated inconclusive results. Moreover, the role of various potassium channels in caffeine-induced effects has not been explored so far. The present *in vitro* study was designed to explore the direct effects of caffeine on rat aortic rings and the role of various potassium channels in those changes/effects.

Materials and Methods: This study was carried out in College of Medicine, University of Dammam. Aortic rings obtained from Sprague Dawley rats were mounted in the organ bath. Tension in the aortic rings was measured with an isometric force transducer and recorded with a PowerLab data-acquisition system. Aortic rings in relaxed and contractile state were exposed to caffeine and various potassium channel blockers (glyburide, 4-aminopyridine, or tetraethylammonium).

Results: Caffeine produced significant relaxation of isolated aortic rings (baseline tension: 1.26 ± 0.30 g, tension after adding cumulative concentrations of caffeine: 1.12 ± 0.31 g, $P < 0.05$) in the absence or presence of norepinephrine (NE) (tension induced by NE: 1.06 ± 0.37 g, tension after adding cumulative concentrations of caffeine: 1.01 ± 0.36 g, $P < 0.05$). Caffeine's vasodilatory effects were, however, blocked in aortic rings pretreated with different types of potassium channel blockers such as 4-aminopyridine (tension induced by NE: 1.52 ± 0.41 g, tension after adding cumulative concentrations of caffeine: 1.50 ± 0.37 g, $P > 0.05$), glyburide (tension induced by NE: 0.82 ± 0.35 g, tension after adding cumulative concentrations of caffeine: 0.79 ± 0.42 g, $P > 0.05$), and tetraethylammonium (tension induced by NE: 0.68 ± 0.34 g, tension after adding cumulative concentrations of caffeine: 0.67 ± 0.33 g, $P > 0.05$).

Conclusion: Caffeine causes significant dilation of aortic rings, and this vasodilatory effect may involve ATP-dependent, calcium-mediated, or voltage-dependent potassium channels.

Key words: Aorta, caffeine, potassium channels

ملخص البحث:

أجريت هذه الدراسة في المختبر لاكتشاف الآثار المباشرة للكافيين على الفئران في حلقات الشريان الأبهري ودور قنوات البوتاسيوم على تلك التغيرات. أجريت هذه الدراسة على حلقات الشريان الأبهري في صمام الفئران العضوي المائي وقد تم قياس التوتر في هذه الحلقات مع قوة محلول متساوي القياس وسجلت مع نظام الباور لاب. تعرضت حلقات الشريان الأبهري وقنوات البوتاسيوم لتقلص واسترخاء ناتجا عن مادة الكافيين. وسبب الكافيين استرخاء كبيراً لحلقات الشريان الأبهري المعزولة وكان التوتر الناتج عن ذلك يختلف باختلاف تركيز

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الكافيين التراكمية والتغيرات الحاصلة من قنوات البوتاسيوم مثل الامينوباييرين والغليبيريد ورباعي ايثيل الامونيوم. خلصت الدراسة إلى أن الكافيين يسبب تمداً كبيراً لحلقات الشريان الابهرى ويمكن أن تؤدي هذه التأثيرات إلى توسع الأوعية الدموية المعتمدة على الكالسيوم وقنوات البوتاسيوم المعتمدة على الجهد.

INTRODUCTION

Caffeine is the most widely consumed psychoactive substance in the world. It is found in coffee, tea, soft drinks, chocolate, analgesics, appetite suppressants, decongestants, and diuretics. The estimated daily consumption of caffeine is 2.4 and 0.7 mg/kg in adults over the age of 25 and children under the age of 12, respectively.^[1]

Studies conducted on the effects of caffeine on blood vessels have demonstrated that both vasoconstriction and vasodilation are induced by caffeine.^[2-4] Caffeine constricts the vessels by activating ryanodine receptors and releasing calcium from intracellular stores.^[2] Caffeine dilates the vessels by producing nitric oxide (NO) or inhibiting phosphodiesterase in the smooth muscle cell.^[4,5]

Caffeine is a xanthine derivative, which acts in the body's cells by several mechanisms of action and on a variety of molecular targets. Despite investigating a number of mechanisms for caffeine-induced vascular effects, research on one of the probable mechanisms, namely the role of potassium channels is far from conclusive. There is a lack of concrete and continuous information regarding the effects of caffeine on vascular potassium channels. A human study has shown that caffeine has no effect on vascular potassium channels,^[6] whereas, animal studies have revealed activation of potassium channels by caffeine.^[7-9]

Insufficient data and inconclusive results on the effect of caffeine on vascular potassium channels urged us to investigate the direct effect of caffeine on potassium channels in isolated aortic rings from normal healthy rats. To the best of our knowledge, this is the first study to explore the effects of caffeine on various types of potassium channels in rat aorta. This study will improve our understanding of the vascular effects of caffeine.

The objectives of this study were to test the reproducibility of aortic relaxation induced by caffeine and to determine the role of different potassium (K⁺) channels in caffeine-induced vascular effects by using various K⁺ channel blockers.

MATERIALS AND METHODS

The study was conducted at the Department of Physiology, College of Medicine, University of Dammam and was approved by the Institutional Review Board. The Animal welfare guidelines and research protocols were strictly adhered to.

Six adult male Sprague Dawley rats weighing 200–250 g were anaesthetized with ketamine (2–10 mg/kg body weight intraperitoneum). The thoracic aorta was removed carefully and cleaned of fat and adherent connective tissues. The vessel was then immersed in ice-cold Krebs-Henseleit (KH) solution (pH 7.4) containing (mMol/L): NaCl 118.4, KCl 4.7, CaCl₂ 2.52, MgSO₄ 1.18, KH₂PO₄ 1.18, NaHCO₃ 25.0, and D-glucose 11.1 along with adequate oxygenation.

The dissected aorta was cut into 1.8 mm long ring segments and mounted onto two stainless-steel stirrups. The lower stirrup was anchored to the tissue mounting hook, and the upper stirrup was connected with a thread to the isometric force transducer for tension measurement and recording with a PowerLab data-acquisition system. The aortic rings were then immersed in a 20 ml organ bath containing KH buffer continuously bubbled with 95% O₂ and 5% CO₂ and maintained at 36.5°C.

The organ bath used was ML0146 (AD Instruments) equipped with four tissue chambers, preheating reservoir coils, gas diffusers, tissue holders, micro positioners, a water pump, and thermostat controller. Tissue responses were recorded using force transducer MLT0201, Quad bridge amplifier FE224, and PowerLab® PL3508 data-acquisition system with LabChart Pro®.

The rings were stretched to a resting tension of 1g and allowed to equilibrate for at least 1 h, with the bath fluid (KH) being changed every 15 min.^[10] Baseline tension was recorded after calibration. Aortic rings in both relaxed and contractile state (contraction induced by norepinephrine [NE]) were exposed to varying strengths of caffeine starting from low concentration to high concentration, i.e., 10⁻⁷-10⁻³. The relaxation response was defined as a decrease in tension from the resting value after equilibration or maximal contraction induced by NE.

Since the maximum effect of NE was produced by concentration 10^{-4} , in subsequent set of experiments, we used 10^{-4} NE to produce contraction. To elucidate the role of potassium channels in caffeine-induced vasorelaxation, and aortic rings preincubated for 30 min with glyburide (ATP-dependent potassium channel blocker) or 4-aminopyridine (voltage-dependent potassium channel blocker) or tetraethylammonium (calcium-activated potassium channel blocker) were contracted with NE and then exposed to varying strengths of caffeine (10^{-7} - 10^{-3}).

Caffeine, norepinephrine, 4-aminopyridine, tetraethylammonium, glyburide (diluted in absolute ethanol), and chemicals were needed to prepare the KH solution. All drugs were purchased from the Sigma pharmaceutical company. Strengths prepared were 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , and 10^{-7} . A stock solution of norepinephrine (10^{-2}) was made with deionized water (90%), HCl (10% to ensure solubility), and ethylenediaminetetraacetic acid (ethylenediaminetetraacetic acid; 100 $\mu\text{g}/1$ ml to prevent oxidation of NE). All the drugs were serially diluted in distilled water before each experiment. The concentration of a drug is expressed as the final concentration in the bath solution.

Maximum response was calculated for all doses through LabChart[®]. Microsoft Excel Program was used for data analysis. Cumulative concentration-response curves were obtained. The change in tension was reported as mean \pm standard deviation paired *t*-test was used to compare the results between different drugs and the change in tension. $P < 0.05$ was considered significant.

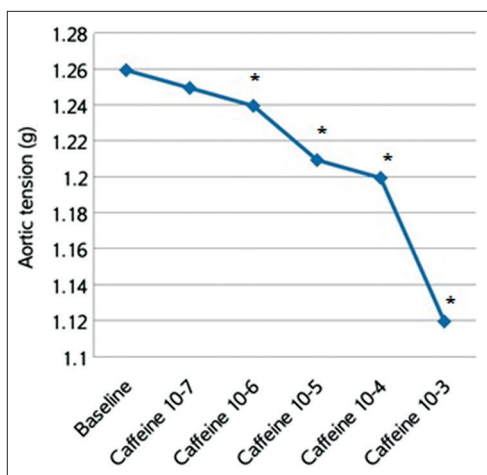


Figure 1: Effects of varying concentrations of caffeine on aortic rings ($n = 6$). * $P < 0.05$

RESULTS

The mean values for different doses of caffeine from 10^{-7} - 10^{-3} on the relaxed and precontracted aorta were compared with baseline readings. In relaxed aorta, there was a significant dose-dependent decrease in the aortic tension and maximum decline was observed after adding caffeine 10^{-3} [Figure 1]. Exposure of the aortic rings to norepinephrine caused them to contract; increasing the tension from baseline (0.82 ± 0.25 g) to a new level of 1.06 ± 0.37 g [Figure 2]. The addition of varying strengths of caffeine to these precontracted aortic rings produced a dose-dependent relaxation which was statistically significant with caffeine 10^{-3} only [1.01 ± 0.36 g, $P < 0.05$; Figure 2]. Caffeine failed to produce any significant relaxation in the aortic rings preincubated for 30 min with any of the three different potassium channel blockers (glyburide, 4-aminopyridine, and tetraethylammonium) [Figure 3].

DISCUSSION

Caffeine induced a dose-dependent significant relaxation of the aorta with and without NE. However, caffeine's vasodilatory effects disappeared in the presence of different types of potassium channel blockers. Our results are in agreement with other studies that reported the vasodilatory effects of caffeine on rat aorta.^[3] The mechanism behind this caffeine-induced relaxation could be due to the production of NO.^[5] The NO diffuses into vascular smooth muscles and causes them to relax.^[4] This effect has been proven by using the NO pathway blockers such as NAME, oxyhemoglobin, and methylene blue.^[11] Caffeine-induced vasodilation

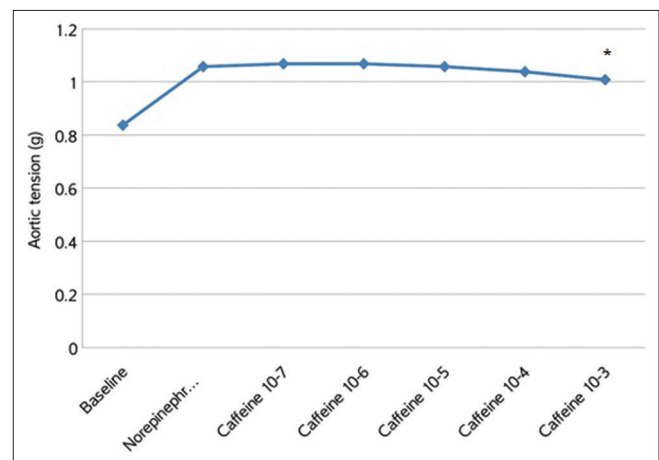


Figure 2: Effects of varying concentrations of caffeine on aortic rings precontracted with norepinephrine 10^{-4} ($n = 10$). * $P < 0.05$

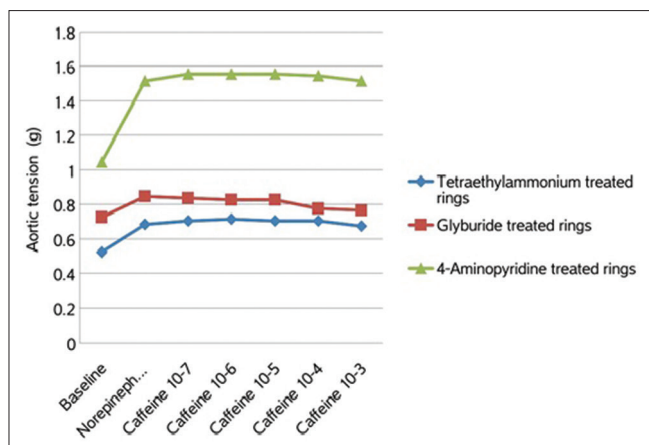


Figure 3: Effects of varying concentrations of caffeine on aortic rings preincubated with various potassium channel blockers ($n = 4$)

might also involve inhibition of phosphodiesterase in the smooth muscle cell leading to increased (cyclic adenosine monophosphate) concentration, which then leads to relaxation by inhibiting myosin light chain kinase enzyme.^[4,12] Another postulated mechanism is that caffeine could have inhibited the inositol triphosphate receptor and the contractile apparatus directly leading to vasodilation.^[13] Some more suggested mechanisms involve the inhibition of voltage gated calcium channels or blockade of adenosine receptors.^[14,15]

Literature supports the presence of three types of potassium channels in the aorta; voltage-dependent potassium channels, calcium-activated potassium channels and ATP-dependent potassium channels.^[16-18] The present study which evaluated the role of these potassium channels in caffeine-induced relaxation found that caffeine failed to produce any significant relaxation in the presence of any of the potassium channel blockers, suggesting that stimulation of these channels was involved in the vasodilatory effects of caffeine. Whether, this stimulation is a direct effect of caffeine on potassium channels or secondary to caffeine's effect on intracellular regulatory enzymes is yet to be explored.

Previous studies have reported mediation of ATP-dependent potassium channels by caffeine through its antiphosphodiesterase activity.^[7-9] Caffeine has also been reported to activate calcium-dependent potassium channels in aortic cells by causing release of calcium from internal stores.^[19] Montes *et al.* explored the effects of caffeine on internal mammary artery (IMA) and described a potent vasodilation.^[20] However, in contrast to our study, they discovered that ring relaxation was not influenced by preincubation with different potassium

channel blockers. The discrepancy between our results and Montes can be explained on the basis of different experimental design and/or cell type. The present study used rat aorta, whereas Montes used IMA obtained from patients undergoing cardiac bypass.

CONCLUSION

Our results suggest that caffeine causes significant vasodilation of rat aortic rings. The mechanism of caffeine-induced relaxation may be secondary to the activation of ATP-dependent, calcium activated, or voltage-dependent potassium channels.

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Conflicts of interest

There are no conflicts of interest.

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