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Effect of different potassium channel blockers on kaempferol-induced relaxation in the isolated coronary artery of goat

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Abstract

Current study was designed to investigate the effect of different potassium channel blockers on the kaempferol-induced relaxation in the isolated coronary artery of goat. Experiments were performed in the organ bath system by mounting the isolated coronary artery of the goat heart. Small rings of the coronary artery were made and mounted in the tissue bath with the help of stainless steel hooks and tension was recorded using the power lab system. Relaxation response to kaempferol was elicited in the absence and presence of the various potassium channels blockers such as TEA (BK_{Ca} channel blocker), glibenclamide (K_{ATP} channel blocker), 4-aminopyridine (K_v channel blocker) and BaCl₂ (K_{IR} channel blocker). Maximal relaxation induced by kaempferol in KCl precontracted coronary artery was 116.88±10.15%, n=6 in control. However, TEA (1 mM) did not influence the relaxation (113.18±11.55%, n=6) response of kaempferol in the coronary arterial rings of the goat. Further, glibenclamide and BaCl₂ were not able to reduce the relaxation response induced by the kaempferol in this vessel. Nevertheless, 4-aminopyridine (K_v channel blocker) significantly (*p*<0.001) decreased the relaxation response in the goat isolated coronary arterial rings in comparison with control. In conclusion, present investigation suggests that relaxation-induced by the kaempferol in goat coronary artery had shown involvement of K_v channels.

Keywords: Kaempferol, coronary artery, goat, relaxation

1. Introduction

Myocardial ischemia occurs due to an imbalance between myocardial oxygen demand and supply to the heart tissue and leads to angina pectoris which is determined by its pathogenesis and is explained in the terms by increasing myocardial oxygen demand in the presence of stenosis of the epicardial coronary arteries and it is considered as the important cause for the death of the patient [1]. It is very well established fact that endothelial impairment of the coronary artery has a central role in the initiation and progression of coronary atherosclerosis in a patient [1, 2]. Blood pressure control in hypertensive patients is required for their entire life and the drugs used for this treatment often lead to adverse effects, therefore, use of natural plant-derived compounds which are considered less toxic and have more beneficial potential and may be considered as a good approach [3].

Various reports have shown that a diet rich in plant-derived foods has a beneficial and protective effect on the health of human as well as animals [4]. A flavonoid known as kaempferol has present in many plants which are suitable for food purposes such as cabbage, broccoli, kale, beans, tea, tomato [4]. Previously, kaempferol has shown cardioprotective effect in aorta banding, ischemia-reperfusion, angiotensin-II, isoprenaline and hyperglycemia-induced cardiac injury models [5-9]. Further, kaempferol has shown vasodilator and vasorelaxant potential in various vascular beds such as in isolated rat carotid artery [10], porcine coronary artery [11,12], human umbilical vein endothelial cells [13] and rat pulmonary artery [14]. However, with the best of our knowledge, there is no report available on the effect of different potassium channels blockers on the kaempferol-induced vasorelaxation in the isolated coronary artery of goat. Therefore, the current study was designed to assess the effect of kaempferol on a precontracted goat coronary artery in absence and presence of various potassium channels blockers using organ bath system.

2. Materials and Methods

Circumflex coronary arteries were obtained from the hearts of male goats collected immediately after slaughter from slaughter house of the local area. 2–3 mm long coronary arterial rings were prepared under the dissecting microscope. Two stainless steel hooks were

used to mount the goat coronary arterial rings in 10 ml organ bath containing MKHS with composition (in mM): NaCl 118, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, NaHCO₃ 11.9, KH₂PO₄ 1.2 and D-glucose 11.1 (pH 7.4). A temperature of 37±1°C was maintained and solution was continuously aerated with carbogen. Tissues were equilibrated for 90 min with an initial tension of 1.5 g. Force generated by the goat coronary arterial rings was recorded using force transducer and stored in a computer using LabChart 6 Pro version 6.1.3 software (Power Lab, AD Instruments, Australia). High K⁺ (80 mM)-depolarizing solution was used to check the viability of the tissue. Cumulative concentrations–response curves to kaempferol (10⁻⁷-10⁻⁴ M) in goat coronary arterial rings pre-contracted with 30 mM KCl were elicited in presence and absence of various antagonists such as tetraethylammonium chloride (TEA; BK_{Ca} channel blocker) 4-aminopyridine (K_V channel blocker), BaCl₂ (K_{IR} channel antagonist) and glibenclamide (K_{ATP} channel blocker).

2.1 Drugs and chemicals

Glibenclamide was procured from Sigma Aldrich, USA. 4-aminopyridine was procured from TCI, USA. Barium chloride was purchased from Glaxo laboratory, India. Kaempferol was purchased from Cayman chemicals, USA. Kaempferol and glibenclamide were dissolved in dimethyl sulphoxide (DMSO). All other drugs were dissolved in distilled water.

2.2 Statistics

Data obtained from the experiments were presented in mean plus standard error. Numbers of experiments were expressed with “n”. E_{max} was used to present the maximal relaxation induced by the kaempferol. pD₂ value denotes the -logEC₅₀. Data were analyzed by two-way ANOVA followed by Bonferroni *post-hoc* test using Graph Pad Prism version 4 (San Diego, California, USA). *p*<0.05 was considered statistically significant in different experiments.

3. Results

Fig. 1 shows the effect of kaempferol (0.1-100 μM) in concentrations-dependent manner on 30 mM KCl precontracted goat isolated coronary artery in absence and presence of tetraethylammonium chloride (TEA, 1 mM; a BK_{Ca} channel blocker). Maximal relaxation induced by kaempferol in KCl precontracted coronary artery was 116.88±10.15%, n=6 in control. However, relaxation response of kaempferol was not significantly different with TEA (1 mM) pretreated coronary arterial rings (113.18±11.55%, n=6). Further, pD₂ value of relaxation response of kaempferol in presence of TEA was not different with control value.

Line diagram in Fig. 2 represents effect of 4-aminopyridine (4-AP; 3 mM; voltage activated potassium channel blocker) on kaempferol (0.1-100 μM)-induced vasodilation in goat isolated coronary artery precontracted with 30 mM potassium chloride. Kaempferol-induced vasodilation was significantly (*p*<0.01; *p*<0.001) reduced (78.06±11.94%, n=5) in presence of 4-AP (a voltage gated potassium channel (K_V) blocker) pretreated coronary arterial rings in comparison with control (109.12±3.33%, n=5).

Effect of ATP activated potassium (K_{ATP}) channel blocker, glibenclamide (10 μM), on kaempferol (0.1-100 μM)-induced vasorelaxation in KCl precontracted isolated coronary artery is shown in Fig. 3. Relaxation response of kaempferol (0.1-100 μM) was 103.98±3.89%, n=3 in presence of glibenclamide (10 μM) pre-incubated coronary arterial rings which was not significantly different with control value 98.33±6.73%, n=3. Further, pD₂ value of relaxation response of kaempferol in presence of K_{ATP} channel blocker, glibenclamide (10 μM), was not different with control value.

Line diagram in Fig. 4 shows the effect of barium chloride (BaCl₂; 30 μM; inward rectifier potassium channel blocker) on kaempferol (0.1-100 μM)-induced relaxation in isolated coronary artery of goat precontracted with 30 mM potassium chloride. Relaxation-induced by the kaempferol was not affected with the presence of BaCl₂ (89.24±3.69%, n=4) in comparison with control (89.51±1.76%, n=4).

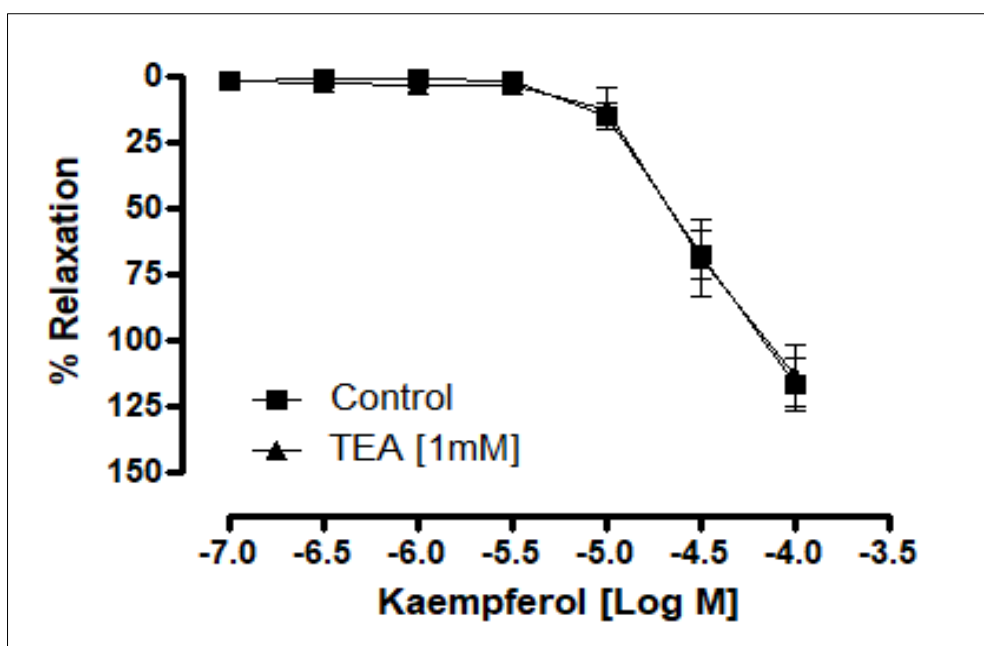


Fig 1: Line diagram shows the effect of tetraethyl ammonium chloride (TEA, BK_{Ca} channel blocker, 1 mM) on kaempferol-induced relaxation in 30 mM KCl pre-contracted coronary arterial rings of goat. The vertical bars represent SEM. The data were analyzed by two-way ANOVA followed by Bonferroni *post-hoc* test.

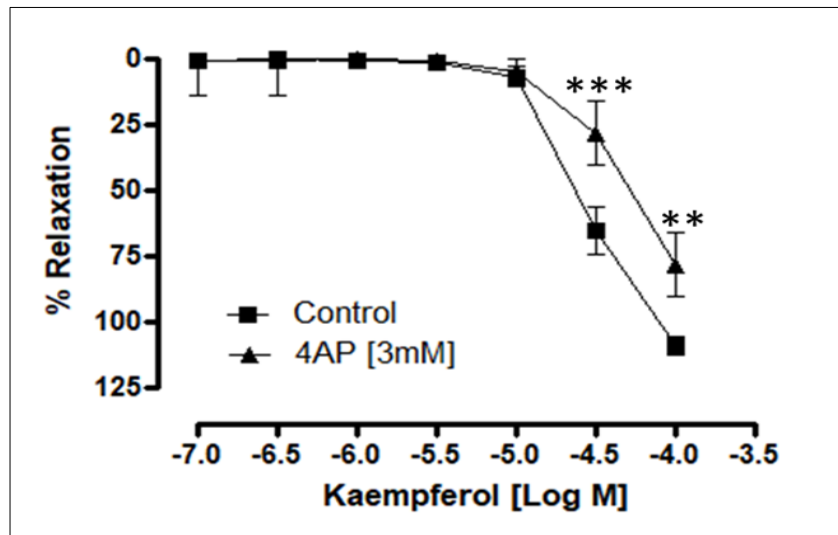


Fig 2: Line diagram illustrates the effect of 4-aminopyridine (K_v channel blocker, 3 mM) on kaempferol-induced relaxation in 30 mM KCl pre-contracted coronary arterial rings of goat. The vertical bars represent SEM. The data were analyzed by two-way ANOVA followed by Bonferroni post-hoc test. *** $p < 0.001$ in comparison with control.

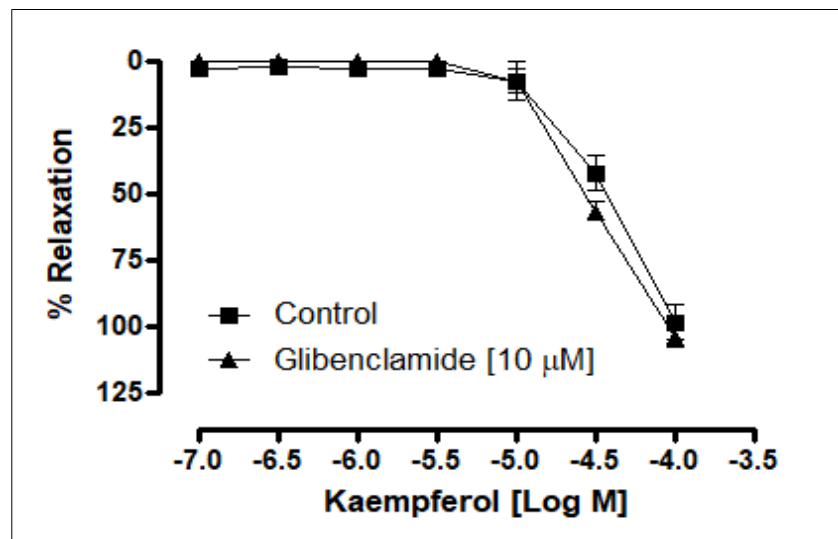


Fig 3: Line diagram depicts the effect of glibenclamide (K_{ATP} channel blocker, 10 μ M) on kaempferol-induced relaxation in pre-contracted coronary arterial rings of goat. The vertical bars represent SEM. The data were analyzed by two-way ANOVA followed by Bonferroni post-hoc test.

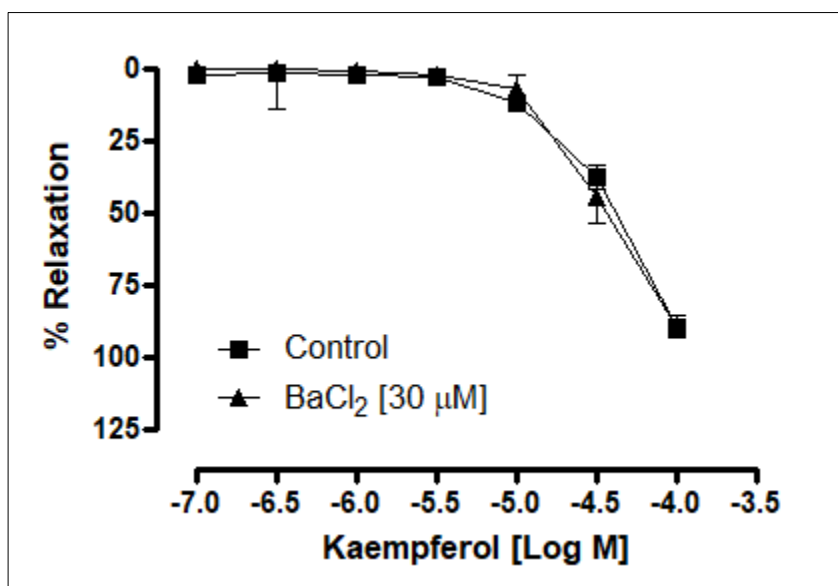


Fig 4: Line diagram shows the effect of BaCl₂ (K_{IR} channel blocker, 30 μ M) on kaempferol-induced relaxation in pre-contracted coronary arterial rings of goat. The vertical bars represent SEM. The data were analyzed by two-way ANOVA followed by Bonferroni post-hoc test.

4. Discussion

Present study was designed to investigate the effect of different potassium channels blockers on the kaempferol-induced vasorelaxation in the isolated coronary artery of goat. The following observations were recorded during the study as kaempferol-induced relaxation was not affected in the presence of large conductance calcium activated potassium channel blocker, tetraethyl ammonium chloride (TEA), in the goat isolated coronary artery. Further, glibenclamide (K_{ATP} channel blocker) and $BaCl_2$ (Kir channel blocker) did not affect the relaxation response induced by the kaempferol in this vasculature. However, 4-aminopyridine (4-AP) significantly reduced the kaempferol-induced relaxation in the coronary artery of goat.

Previous report suggests that involvement of potassium channels activation is important for the in the vascular smooth muscle cells relaxation induced with the flavonoid [14]. Membrane hyperpolarization occurs due to the increase in K^+ ion permeability across the membranes and leads to the vasorelaxation [15]. Maintenance of the contractile and vasodilator tone of the smooth muscle occurs by the presence of different types of potassium channels on the membrane [15,16]. The current investigation showed that TEA (BK_{Ca} channel blocker), glibenclamide (K_{ATP} channel blocker) and $BaCl_2$ (Kir channel blocker) did not influence the relaxation response induced by the kaempferol in the goat isolated coronary artery. In contrary to the present investigation, a recent report showed the involvement of the large-conductance Ca^{2+} -activated K^+ channels (BK_{Ca}) in the kaempferol-induced relaxation in smooth muscle of porcine coronary artery [12] and in human umbilical vein endothelial cells (Xu *et al.*, 2008)[13]. 4-aminopyridine (Kv channel blocker) significantly attenuated the kaempferol-induced relaxation in the goat coronary artery in the current investigation. Nevertheless, voltage gated potassium channels and small conductance calcium activated potassium channels were not involved in the kaempferol-induced relaxation in the human umbilical vein endothelial cells as reported previously [13]. However, present study suggests partial involvement of voltage gated potassium channels in kaempferol-induced vasorelaxation in the goat coronary artery which may be due to species variation.

In conclusion, present study shows that relaxation-induced by the kaempferol in goat coronary artery had shown partial involvement of Kv channels.

5. Acknowledgement

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6. Conflict of Interest

None

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