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The relaxant effect of dobutamine on porcine coronary arterial ring segments

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The purpose of this study was to assess the direct effects of dobutamine on porcine coronary arteries and to investigate the mechanism of its action. Rings of coronary arteries from pigs were suspended in baths containing Krebs solution, and isometric tension was measured. The response to dobutamine (10^{-8}–10^{-3} M) was investigated in porcine coronary arterial rings contracted by KCl. The roles of endothelium, nitric oxide (NO), cyclic GMP (cGMP), prostaglandins and the adrenergic β_1, β_2-receptor on dobutamine-induced relaxation were also studied. Dobutamine inhibited the vasocontractivity to KCl and $CaCl_2$, and relaxed porcine coronary artery. The relaxing response to dobutamine in coronary artery was significantly reduced by blockage of the adrenergic β_1-receptor, but not by removal of endothelium, blockage of the adrenergic β_2-receptor, inhibitors of nitric oxide synthase, guanylate cyclase and prostaglandin synthase. Our results suggest that dobutamine induces relaxation of isolated porcine coronary arteries via the adrenergic β_1-receptor.

1. Introduction

Dobutamine is a sympatheticomimetic amine, with peripheral vascular actions (Robie and Goldberg 1975; Sutton 1975; Holloway and Frederickson 1974). Studies have shown that dobutamine increases the systolic pressure but does not alter the mean aortic pressure. It increases cardiac output, but has negligible effects on the renal or mesenteric blood flow. It produces dose-related increases in femoral blood flow and cardiac contractility (Holloway and Frederickson 1974; Driscoll et al. 1979; Ferrara et al. 1995; Chen et al. 2003; Kinoshita et al. 1995) but does not produce selective increases in total liver blood flow (Driscoll et al. 1979). In recent years, studies on dobutamine have focused on chronic heart failure (Eichhorn et al. 2003; Bollano et al. 2003), but, to date, there has been no evidence linked to the direct effects of dobutamine on porcine coronary artery.

Activation of β-adrenoceptors on vascular smooth muscle results in vasorelaxation through activation of adenylyl cyclase (Kuriyama et al. 1982). Vascular endothelial cells may also express β-adrenoceptors (Howell et al. 1988; Molenaar et al. 1988), although their physiological function, if any, remains unclear. Evidence is accumulating, however, that the vascular endothelium may facilitate or mediate β-adrenergic relaxation. Studies *in vitro* showed that β-adrenoceptor mediated relaxation is endothelium dependent in human umbilical vein (Xu et al. 1998), and removal of endothelium or inhibition of nitric-oxide synthases (NOS) impairs relaxation caused by β-adrenoceptor agonists. On the other hand, studies of isolated canine coronary arteries have proved that β-adrenoceptor mediated vasorelaxation was endothelium independent. It seems that different mechanisms mediate vasorelaxation caused by β-adrenoceptor agonists in coronary and peripheral arteries (Macdonald et al. 1987; Bea et al. 1994).

In the present study, we examined the effects of dobutamine on isolated porcine coronary arteries *in vitros* and whether stimulation of β-adrenoceptors in porcine coronary arteries caused endothelium dependent vasorelaxation, whether this occurred through activation of the L-arginine/NO pathway, and what receptor subtype was involved in this event. The possible roles of prostaglandins and calcium influx on porcine coronary arteries were also investigated.

2. Investigations, results and discussion

KCl (40 mM) induced contractile responses in coronary arterial rings either with endothelium or without endothelium (10.06 ± 0.42 g), being the tension induced by K^+. Dobutamine (10^{-8}–10^{-3} M) can relax porcine coronary arterial rings precontracted by KCl in a concentration-related manner $IC50 = (4.43 \pm 1.12) \times 10^{-4}$ M, while in contrast, the normal saline (NS) control had no effect (Fig. 1).

The reported effects of NO and the endothelial cell on vasorelaxation induced by β-adrenoceptor agonists are not consistent. The difference between arteries such as the coronary and peripheral arteries under investigation may contribute to these differences (Bea et al. 1994; Graves and Poston 1993; Blankesteijn and Thien 1993). We have proved, in the present work, that dobutamine induced considerable relaxation of porcine coronary arteries precon-

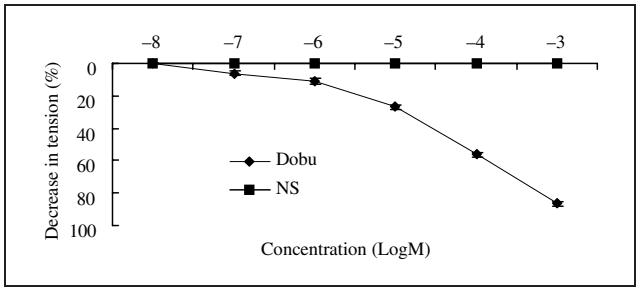

Fig. 1: Line plot shows the effect of dobutamine (Dobu) (10^{-8}–10^{-3} M) on porcine coronary arteries precontracted with KCl (40 mM). ◆, Dobu; ■, NS. Data are expressed as percentage relaxation of the tension induced by KCl. (Mean SEM, n = 8)

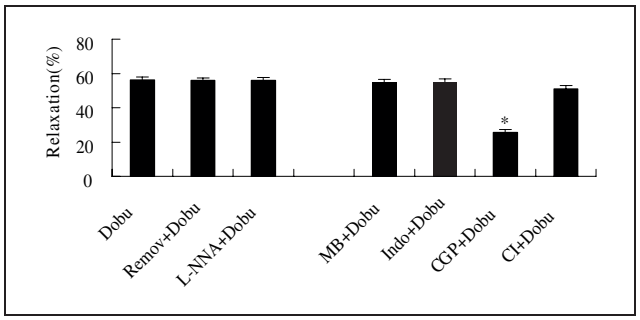

Fig. 2: Bar graph shows the effects of removal of endothelium (Remov), L-NNA, methylene blue (MB), indomethacin (Indo), CGP20712 (CGP), ICI118551 (ICI) on relaxation induced by dobutamine (Dobu) (10^{-4} M) in porcine coronary arteries precontracted with KCl (40 mM). Data are expressed as percentage relaxation of the tension induced by KCl (Mean ± SEM, n = 8). Vertical bars represent the SEM. * $p < 0.05$ vs Dobu data

tracted by KCl, while removed of endothelium, N^{ω}-nitro-L-arginine (L-NNA), an inhibitor of EDRF (particularly NO) synthesis (Rees et al. 1990) and methylene blue, an inhibitor of cGMP synthesis (Martin et al. 1985) did not reduce the relaxation induced by dobutamine (10^{-4} M). These results suggest that *in vitro* acute relaxation of porcine coronary arteries caused by dobutamine is not dependent on NO and cGMP. Fig. 2 illustrates the relaxation directly caused by dobutamine (10^{-4} M) and the effects of removal of endothelium, L-NNA (10^{-4} M), methylene blue (10^{-5} M), indomethacin (10^{-5} M), CGP20712A (300 nmol $\cdot L^{-1}$) (β_1-adrenoceptor antagonist), and ICI118551 (100 nmol $\cdot L^{-1}$) (β_2-adrenoceptor antagonist) on the relaxation.

The endothelium might release a number of prostaglandins, either vasodilators or constrictors (Otter and Austin 1990). Indomethacin inhibits the synthesis of prostaglandins (Ferreira et al. 1973) and markedly inhibits the transient relaxation induced by arachidonic acid in rabbit coronary arteries (Jiang et al. 1991). However, indomethacin did not affect dobutamine induced relaxation in endothelium-intact porcine coronary artery (Fig. 2). This result indicates that the release of vasodilator prostanoids is not involved in dobutamine-induced coronary artery relaxation *in vitro*.

The results of the present study also show that β_1-adrenoceptor blockade (CGP20712A) can abolish the vasorelaxation caused by dobutamine in coronary arteries, but β_2-adrenoceptor antagonization (ICI118551) cannot (Fig. 2). It suggests that the vasorelaxation induced by dobutamine occurs through direct stimulation of β_1-adrenoceptors on smooth muscle cells.

Potential-dependent calcium channel (PDCs) are activated by depolarization of the plasma membrane when the extracellular k^+ concentration is increased (McDonald et al. 1994; Karaki et al. 1997). In this experiment, dobutamine relaxed KCl-precontracted coronary artery rings, and preincubation with dobutamine not only inhibited the KCl concentration-dependent contractions in normal Krebs solution, but also decreased the calcium concentration-dependent contraction in porcine coronary arteries in high K^+ depolarization medium. These results showed that dobutamine can inhibit KCl and $CaCl_2$ concentration-response curves and this effect may be through exciting β_1 adrenoceptors, or by another mechanism. However, it is reported that K^+ opens the potential-dependent calcium channel (PDCs). When the β_1-adrenoceptor is activated, adenylate cyclase (AC) can be activated, and cAMP increased, and then the L type calcium channel can be opened (Gang 1999). This seems to contradict our results. So it is possible that other mechanisms participated in this mechanism besides the β_1-adrenoceptor, but this needs further experimentation to test it (Fig. 3; Fig. 4). Fig. 3 illustrates the effect of dobutamine (10^{-4} M) on KCl concentration-dependent contractile responses. The EC50 values in KCl and after incubation with dobutamine (10^{-4} M) were (38.18 ± 2.12) mM (n = 8, KCl) and (65 ± 4.12) mM (n = 8, $p < 0.05$ vs KCl) respectively. Fig. 4 illustrate the effect of dobutamine (10^{-4} M) on the calcium concentration-dependent contractile curve. The EC50 values of calcium for control and dobutamine (10^{-4} M) were $(3.76 \pm 0.58) \times 10^{-3}$ M (n = 8, Calcium) and $(6.65 \pm 0.91) \times 10^{-3}$ M (n = 8, $p < 0.05$ vs Calcium).

In summary, we have shown that dobutamine has a relaxant effect on isolated porcine coronary artery. The mechanism of the action involves β_1-adrenoceptors, but does not involve NO, cGMP, indomethacin and β_2-adrenoceptors.

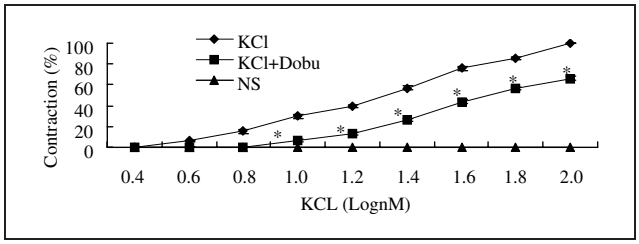

Fig. 3: Line plot shows the effect of dobutamine (Dobu) (10^{-4} M) on KCl concentration-dependent contraction curves in porcine coronary arteries. ◆, KCl; ■, Dobu + KCl; ▲, NS. Data are expressed as percentage of maximal contraction induced by KCl in controls. (Mean SEM, n = 8, in each group). * $p < 0.05$ vs KCl

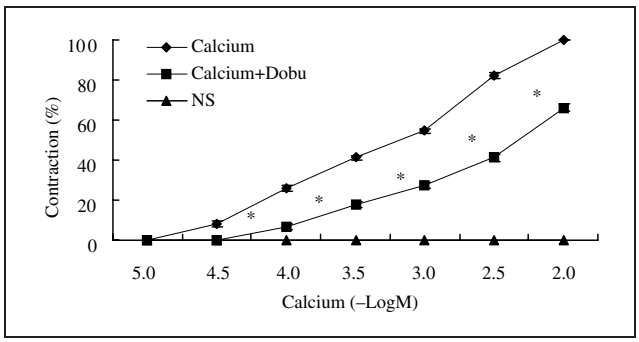

Fig. 4: Line plot shows the effect of dobutamine (Dobu) (10^{-4} M) on calcium concentration-dependent contraction curve in porcine coronary arteries. ◆, Calcium; ■, Dobu + Calcium; ▲, NS. Data are expressed as percentage of maximal contraction induced by calcium in controls (Mean SEM, n = 8, in each group). * $p < 0.05$ vs calcium

3. Experimental

3.1. Arterial tension recording

Tissue preparation: Porcine hearts were obtained from a local abattoir and immediately immersed in cold normal saline (NS) before transfer to the laboratory. The left anterior descending coronary artery was dissected free from the surrounding myocardium. After cleaning adherent fat and connective tissue, the artery was cut into rings of 4–5 mm in length, 4–6 coronary arterial rings being prepared from each heart. The rings were suspended horizontally between two parallel stainless steel hooks for the measurement of isometric tension in individual organ baths containing Krebs solution composed of (mM) NaCl 118, KCl 4.7, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $NaHCO_3$ 14.5, $CaCL_2$ 2.5 and glucose 11.5, and bubbled through with a mixture of 95% O_2 and 5% CO_2, and the temperature was maintained at 37 °C throughout the experiment. Isometric tension generated by the vascular artery rings was measured using a force transducer (JH-2), and recorded with a BL-420E Experiment System of Biological Function (TME, China) through an IBM computer.
In some rings, the endothelium was removed by gently rubbing with a wooden probe with cotton. The absence or presence of endothelial cells was confirmed by the absence or presence of relaxation to the endothelium-dependent vasodilator bradykinin (10^{-6} M).

3.2. Relaxing effects of dobutamine on precontracted porcine coronary arteries

Coronary arterial rings with or without endothelium were stabilized under 2 g of constant tension for 90 min before being contracted with KCl (40 mM). Dobutamine (10^{-8}–10^{-3} M) was dissolved in NS, or the equivalent NS was added 15 min after addition of the KCl.

3.3. Effects of removing endotheliums L-NNA, methylene blue, indomethacin, CGP20712A, ICI118551 on dobutamine-induced relaxation

Removal of endothelium was done before cutting the artery into rings. L-NNA, (10^{-4} M), methylene blue (10^{-5} M), indomethacin (10^{-5} M), CGP20712A (300 nmol · L^{-1}), and ICI118551 (100 nmol · L^{-1}) were added, respectively to the organ bath 15 min before being contracted with KCl (40 mM). Dobutamine (10^{-4} M) was added 15 min after addtion of KCl (40 mM), or the equivalent NS was added 15 min after addition of the KCl (40 mM).

3.4. Effects of dobutamine on KCl concentration-dependent contractile response in porcine coronary arteries

Some rings were stabilized under 2 g constant tension for 90 min in Krebs solution, the concentration-response curves to KCL (4–100 mM) were obtained, and then the arteries were washed repeatedly with Krebs solution until the rings returned to the original constant tension. The tissues were then incubated with dobutamine (10^{-4} M) or the same volume of normal saline for 20 min and then KCl was once again added cumulatively.

3.5. Effects of dobutamine on calcium concentration-dependent contractile response in porcine coronary arteries

Porcine coronary arterial rings were incubated in calcium-free solution containing 0.1 mM EGTA for 60 min. Afterwards, a calcium concentration-dependent contraction curve experiment was performed in K^+ depolarization medium (80 mM KCl). After being washed with calcium-free solution and original tension being restored, the rings were incubated with dobutamine (10^{-4} M) or the same volume of normal saline for 20 min; the calcium concentration-dependent contraction curve experiment was then repeated.

3.6. Drugs

The following drugs were used: dobutamine (ShangHai First Pharmaceutical Factory, China), bradykinin, L-NNA, (Sigma Chemical Co.), methylene blue (Merck, Darmstadt), indomethacin (Fluka), ICI118551 ($C_{23}H_{25}F_3N_4O_5 \cdot CH_3SO_3H$) (Zeneca), CGP20712A ($C_{17}H_{27}NO_2 \cdot HCl$) (Ciba-Geigy). Drugs were dissolved in NS.

3.7. Calculation and data analysis

All results are expressed as mean ± SEM, where n refers to the number of animals from which blood vessels were taken. Relaxation was expressed as percentage relaxation of the tension induced by KCl (40 mM). In experiments on the KCl or $CaCl_2$ concentration-response curves, the results were expressed as the percentage of control maximal contractile response induced by 100 mM KCl and 10^{-2} M $CaCl_2$ respectively. Statistical significance between two groups was evaluated by student's *t*-test for paired and unpaired observations. Comparison between multiple groups was made by analysis of variance (ANOVA). If a significant F value was found, the Student-Neumann-Keuls test was applied to determine significant difference among the data groups using a computer statistical package (SPSS 8.0 for Windows). EC_{50} was determined by the Scott method for each individual vessel. Each group was compared with the time-matched NS. A probability level of less than 0.05 was considered significant.

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