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Analysis of the effects of adenosine in skinned bovine coronary artery

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Abstract—We have investigated the mechanism of adenosine-induced relaxation in relation to its effects on intracellular organelles in Triton X-100- and saponin-skinned bovine coronary arteries. In intact coronary arteries, high K^+ and prostaglandin $F_{2\alpha}$ caused sustained contractions, whereas caffeine produced transient contractions. Triton X-100 treatment abolished these contractions. However, Triton X-100-skinned coronary arteries were responsive to added free calcium. There was no significant difference between calcium concentration-response curves obtained in the absence and presence of adenosine (50 μM). Unlike Triton X-100, in saponin-skinned arteries, caffeine produced transient contractions but high K^+ and prostaglandin $F_{2\alpha}$ did not. Adenosine had no effect on caffeine-induced contractions in saponin-skinned coronary arteries. These data suggest that adenosine had no direct inhibitory effect on either the contractile apparatus or calcium release from sarcoplasmic reticulum in coronary arteries.

Activation of the contractile apparatus in vascular smooth muscle is dependent on increased levels of free cytosolic calcium. Such calcium originates from extracellular sources as well as from the intracellular storage site, sarcoplasmic reticulum (SR). Adenosine dilates the coronary arteries of various species including bovine (Mustafa & Askar 1985) and man (Ramagopal et al 1988) through activation of A_2 adenosine receptors present on the plasma membrane. The mechanisms by which adenosine induces the relaxation of the coronary arteries are not well understood. In this laboratory, it has been shown that adenosine-induced relaxation in bovine coronary arteries involves an extracellular calcium-dependent component (Ramagopal & Mustafa 1988) as well as an extracellular calcium-independent component (Ramagopal et al 1989; Ramagopal & Mustafa 1990). It has also been reported that adenosine interferes with intracellular calcium sequestration (Dutta et al 1984; Frischknecht & Ferrero 1985). Moreover, Bradley & Morgan (1985) reported that in ferret portal vein, adenosine at lower concentrations decreased cytosolic ionized calcium and at higher concentrations desensitizes the smooth muscle myofilaments to cytosolic ionized calcium.

Triton X-100-skinned vascular smooth muscle is devoid of both the surface cell membrane and SR, and is thus useful for studying the action of agents which directly affect the contractile apparatus (Ruegg et al 1983). Saponin-skinned vascular smooth muscle (devoid of the surface cell membrane only) has been useful in studying the intracellular messengers for the release of calcium from SR (Saida et al 1988).

Thus, the purpose of the present study was to test for a direct effect of adenosine on the contractile apparatus or calcium release from its intracellular storage sites (i.e. SR) in bovine coronary arteries functionally skinned either by Triton X-100 or by saponin.

Materials and methods

Bovine coronary artery rings were prepared essentially as described previously (Ramagopal & Mustafa 1990) in 5 mL organ baths containing physiological salt solution (PSS; 37°C; aerated with 95% O_2–5% CO_2). The changes in tension were recorded on a Polygraph. After obtaining reproducible contractions with high K^+ (50 mM), the coronary artery rings were contracted with prostaglandin $F_{2\alpha}$ (10 μM) and caffeine (5 mM).

Triton X-100 skinning. The coronary artery rings were chemically skinned with Triton X-100 by a modification of the procedure described by McMahon & Paul (1985); the protocol is illustrated in Fig. 1. After recording the contractions induced by high K^+, prostaglandin $F_{2\alpha}$ and caffeine, the bath temperature was lowered to 25°C for the remainder of the experiment to prevent the rapid deterioration of the skinned muscles (Iino 1981). The skinning procedure was initiated by exposing the coronary artery rings to a presoak solution for 10 min, followed by 30 min exposure to skinning solution containing 1% Triton X-100. After a rinse to wash out the Triton X-100, the coronary artery rings were incubated in relaxing solution. The tissues were then exposed to high K^+, prostaglandin $F_{2\alpha}$ or caffeine to test the effectiveness of the skinning process. After washing with relaxing solution, a cumulative concentration-response curve to calcium in the absence and presence of adenosine (50 μM) was obtained by incubating the tissues in different contracting solutions containing increased concentrations of free calcium.

The various solutions used in this protocol have the following composition (mM): presoak solution—imidazole 20, EGTA 5, KCl 50, sucrose 150 (pH 7·4); skinning solution—as above with 1% Triton X-100, dithioerythritol 0·5, phenylmethyl sulphonyl fluoride 1; relaxing solution—K^+ 21, Na^+ 36, Mg^{2+} (total) 10, EGTA 4, ATP 7·5, imidazole 20, Cl^- 35, azide 1, and ATP-regenerating system consisting of phosphocreatinine 10 and creatine phosphokinase 10 units mL^{-1} (pH 6·7); contracting solution—relaxing solution in which EGTA was partly replaced with calcium—EGTA to increase the free calcium. The concentration of free calcium was calculated by means of the computer program developed by Fabiato (1988). Calmodulin (4 μM) was added to both the relaxing and contracting solutions to prevent the gradual depletion of calcium from the cells.

Percent contraction with free calcium (pCa) was calculated by taking the magnitude of contraction induced at pCa = 4·5 as 100%.

Saponin skinning. The bovine coronary arteries were skinned with saponin by the method of Itoh et al (1981). Fig. 3 illustrates the protocol used in skinning the coronary arteries with saponin. After recording the contractions induced by high K^+, prostaglandin $F_{2\alpha}$ or caffeine in calcium-containing PSS, the tissues were incubated with skinning solution containing 50 μg mL^{-1} saponin for 20 min at 25°C. Later, the preparation was washed with relaxing solution. The tissues were then exposed to high K^+, prostaglandin $F_{2\alpha}$ and caffeine to test the effectiveness of the saponin skinning process. After rinsing, the saponin-treated tissues were exposed to contracting solution to load the SR with calcium. After calcium loading, the saponin-treated tissues were exposed to caffeine in the absence and presence of 50 μM adenosine.

The composition of the solutions used in this protocol were (mM): relaxing solution—KCl 130, Tris maleate 20, $MgCl_2$ 5,

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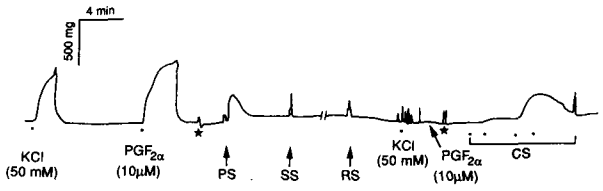

FIG. 1. Protocol for measuring calcium sensitivity in Triton X-100-skinned rings of bovine coronary arteries. See methods for details. Addition of caffeine (5 mM) is denoted by *.

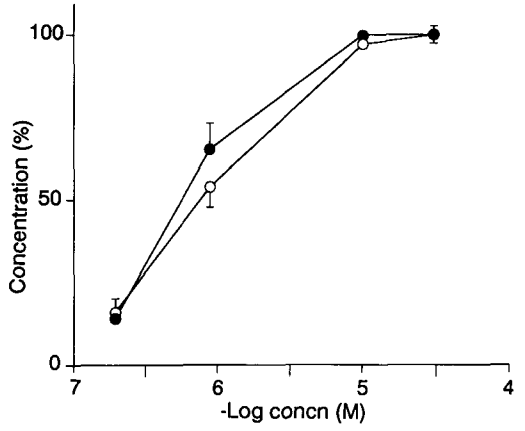

FIG. 2. Concentration-response curves for free calcium (pCa) in the absence (○) and presence (●) of adenosine (50 μM) in Triton X-100 skinned bovine coronary arteries. Isometric tension responses have been normalized to the force obtained at pCa = 4·5 in each vessel. Each point represents the mean ± s.e. of at least 6 artery rings obtained from 4 to 5 bovine hearts.

ATP 5, Na (as Na_2ATP) 10, EGTA 4 (pH 6·8); skinning solution—relaxing solution with 50 μg mL^{-1} saponin; contracting solution—relaxing solution in which EGTA was partly replaced with calcium-EGTA to increase the free calcium.

The data were analysed by Student's *t*-test for paired and unpaired observations, as appropriate. $P < 0.05$ was considered to be statistically significant.

All chemicals were obtained from Sigma Chemicals (St Louis, MO). Calmodulin was a gift from Dr R. K. Sharma, University of Calgary, Canada. Drug solutions were prepared daily and all concentrations are expressed as final molar (M) concentrations.

Results

In normal PSS, high K^+ (50 mM) and prostaglandin $F_{2\alpha}$ (10 μM) caused contractions which reached a maximum (1·15 ± 0·17 and 1·40 ± 0·19 g, respectively) in 6–8 min (Figs 1, 3). In normal PSS, caffeine induced a transient contraction (0·175 ± 0·02 g; Figs 1, 3). Triton X-100 treatment abolished the contractions due to high K^+, prostaglandin $F_{2\alpha}$ and caffeine indicating that the detergent has functionally skinned the plasma membrane and intracellular stores (Fig. 1). However, as shown in Figs 1 and 2, Triton X-100-skinned coronary artery rings contracted in response to an increase in intracellular calcium concentration. Adenosine (50 μM) did not affect the calcium concentration-response curve (Fig. 2). The EC50 for calcium contraction in control skinned coronary artery rings was 0·76 ± 0·22 μM. In the presence of 50 μM adenosine, the EC50 for calcium was 0·57 ± 0·11 μM, which was not significantly different from the control value ($P > 0.05$). Similarly, the maximum tension attained at pCa 4·5 in the presence of adenosine (0·275 ± 0·08 g) was not significantly different from the control value (0·236 ± 0·07 g; $P > 0.05$).

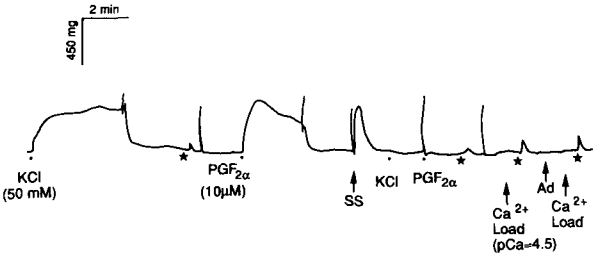

FIG. 3. Protocol for measuring caffeine (*, 5 mM)-induced contractions in saponin skinned rings of bovine coronary arteries. See methods for details.

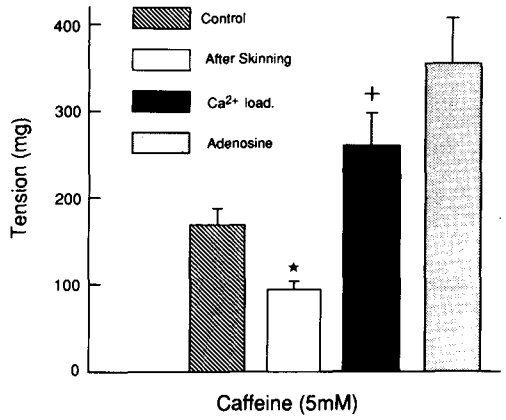

FIG. 4. The transient contractions induced by caffeine (5 mM) in normal and saponin skinned bovine coronary arteries and the effect of adenosine (50 μM) on caffeine-induced contractions after calcium (Ca^{2+}) loading (pCa = 4·5) in saponin-skinned coronary arteries. *$P < 0.05$ between control and after skinning, †$P < 0.05$ between before and after calcium loading. Values are mean ± s.e. of at least 6 artery rings obtained from 4 to 5 bovine hearts.

In saponin-skinned coronary artery rings, the contractions evoked by high K^+ and prostaglandin $F_{2\alpha}$ were abolished (Fig. 3) and caffeine-induced transient contractions were significantly reduced ($P < 0.05$; Figs 3, 4). As can be seen in Fig. 4, in saponin-skinned coronary arteries, calcium loading increased the caffeine-induced transient contraction. This contraction was significantly greater than the response obtained before calcium loading ($P < 0.05$; Fig. 4). Adenosine (50 μM) pretreatment before calcium loading, did not change significantly the caffeine-evoked transient contraction ($P > 0.05$; Fig. 4).

Discussion

The present study shows that in skinned bovine coronary arteries, adenosine had no direct inhibitory effect either on the contractile apparatus or on caffeine-induced calcium release from SR, suggesting that adenosine-induced relaxation is probably mediated through its receptors present on the surface membrane.

In calcium buffer, bovine coronary artery rings were able to contract in response to either high K^+ or prostaglandin $F_{2\alpha}$. It has been proposed that, in vascular smooth muscle, K^+ depolarization was due mainly to the stimulation of calcium influx through potential-operated calcium channels from superficial and extracellular calcium sources, whereas, prostaglandin $F_{2\alpha}$-induced contractions were, in large part, due to an increase in calcium influx possibly through distinct receptor-operated calcium channels and to a lesser extent, a result of calcium

release from plasma membrane-bound sites and intracellular storage sites (Bolton 1986). Furthermore, in the present study, caffeine induced a slight transient contraction in normal PSS, which could be due to calcium release into the cytosol by caffeine from intracellular storage sites (Saida et al 1988).

A lack of contractile response to high K^+, prostaglandin $F_{2\alpha}$ and caffeine in Triton X-100-treated coronary artery rings indicated that Triton X-100 functionally destroyed the plasma membrane and intracellular calcium storage sites, i.e. SR. Furthermore, an increase in free calcium, concentration-dependently contracted the Triton X-100-treated coronary artery rings suggesting that the contractile apparatus was not affected by Triton X-100 treatment and that the resulting contraction was probably due to a direct effect of calcium on the contractile apparatus. The similar EC50 values and magnitude of free calcium-induced contraction in the absence and presence of adenosine in Triton X-100-skinned coronary artery rings indicated that adenosine had no direct inhibitory effect on the contractile apparatus to calcium in the coronary arteries. Long & Stone (1986) reported similar results in Triton X-100-skinned rat aortic fibres and suggested that adenosine may not have any direct inhibitory effect on calcium-induced contractions.

Although caffeine-induced transient contractions in saponin-skinned coronary arteries were reduced, the contractions induced by high K^+ and prostaglandin $F_{2\alpha}$ were abolished indicating that saponin functionally damaged the plasma membrane without destroying the SR and the contractile apparatus. Van Breemen et al (1986) reported that upon saponin skinning of arterial smooth muscle cells, it is possible to maintain SR in a functional state. In the present study, calcium loading significantly increased the ability of caffeine to evoke contractions indicating that calcium-depleted intracellular stores may take up the added calcium to be subsequently released by caffeine. It has been reported that SR takes up calcium that has just entered the cells as a superficial buffer barrier and thereby controls the calcium that is added to the cytoplasm (Van Breemen et al 1986). Caffeine-induced contractions after calcium loading suggests that SR takes up calcium. The lack of effect of adenosine on caffeine-induced contractions indicates that adenosine had no apparent inhibitory effect on calcium release from SR. The slight increase in caffeine-evoked contractions by adenosine suggests that adenosine might be increasing the internal store of calcium (Frischknecht & Ferrero 1985) probably by directly stimulating the SR membrane.

In conclusion, the results of the present study suggests that adenosine-induced relaxation of bovine coronary artery is mediated probably through its extracellular receptors present on the surface membrane and that adenosine probably had no direct inhibitory effect on the contractile apparatus and on intracellular calcium release from SR. The extracellular calcium-independent component (Ramagopal et al 1989; Ramagopal & Mustafa 1990) of adenosine-induced coronary vasodilation in the presence of contracting agents might be due to the modulation of intracellular calcium levels by adenosine.

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