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Inhibition of contractions and ^{45}Ca influx by adenosine in rabbit aorta

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Abstract—The effects of adenosine on contractility and ^{45}Ca-uptake in rabbit aorta have been examined and compared with those of nifedipine. Both adenosine (10^{-5} M) and nifedipine (10^{-8} M) inhibited contractions caused by $CaCl_2$ (0·1–10 mM) in K^+-depolarized preparations. Nifedipine (10^{-7} M) significantly inhibited the contractions of noradrenaline (NA) at all doses (10^{-8}–10^{-4} M), while adenosine inhibited the contractions induced only by lower doses of NA (10^{-8} and 10^{-7} M) without affecting those at higher doses ($> 10^{-7}$ M). Adenosine (10^{-8} M) and nifedipine (10^{-7} M) inhibited K^+-stimulated ^{45}Ca-uptake by aortic strips by 75 and 100%, respectively. Similarly, NA (10^{-4} M)-stimulated ^{45}Ca influx was inhibited by about 70% by both these agents. The results suggest that adenosine inhibits inward Ca^{2+} movement involving both voltage-operated and receptor-operated calcium channels in rabbit aortic smooth muscle.

Adenosine is a potent vasodilator and is known to regulate blood flow to the heart (Berne 1980), skeletal muscle (Berne et al 1971), and brain (Berne et al 1974). Although the mechanism of adenosine-induced vascular smooth muscle relaxation is not fully understood as yet, several hypotheses have been advanced. Inhibition of Ca^{2+} influx into the smooth muscle cells has been suggested as a mechanism in adenosine-mediated relaxation of coronary arteries (Schnaar & Sparks 1972; Fleckenstein et al 1975). Similarly, an inhibition of cellular Ca^{2+}-influx in the presence of adenosine has been suggested from studies related to inhibition of smooth muscle contraction (Herlihy et al 1976) or a decrease in inward Ca^{2+} current (Harder et al 1979). Although the action of adenosine on cellular Ca^{2+} movements remains equivocal, the direct quantitation of Ca^{2+} uptake in vascular smooth muscle cells as influenced by the purine has been reported. Fenton et al (1982) demonstrated an inhibitory action of adenosine on ^{45}Ca-uptake in rat aortic smooth muscle cells. In contrast, adenosine has been shown to have little effect on Ca^{2+}-influx in canine coronary arteries (Dutta et al 1984) but may enhance extrusion of intracellular Ca^{2+} as has been shown with the vasodilators (Cauvin et al 1983).

Adenosine has been shown to relax rabbit aortic strips previously contracted with noradrenaline (NA) (Ghai & Mustafa 1982) but little is known about its mechanism of action in this tissue. The present study was, therefore, undertaken to examine the effects of adenosine on contractility and ^{45}Ca-uptake in rabbit aortic smooth muscle. The effect of calcium channel blockade of adenosine is also presented.

Materials and methods

Material. The chemicals used were: dioxane, POPOP, PPO, naphthalene, ethylene glycol (all from Sisco Research Laboratory, India: scintillation grade), adenosine (Sigma), nifedipine (a gift from Bayer, Germany), noradrenaline (NA) (Sigma) and EGTA (Sigma). All inorganic salts used were of analytical grade from BDH. Nifedipine was dissolved in ethanol and further diluted in deionized and distilled water. All other drug solutions were directly made up in deionized-distilled water. Experiments with nifedipine were performed in the dark.

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Animals. New-Zealand white rabbits, 1·5–2·5 kg, of either sex, obtained from the Laboratory Animal Resource Section of this Institute, were stunned by a blow to the head and subsequently exsanguinated.

Preparation of aortic strips. Thoracic aorta was removed rapidly and placed in physiological saline solution at room temperature (30°C). After removing fat and connective tissue, the aorta was cut spirally and muscle strips of approximately 3 mm × 3 cm were prepared. One end of the strip was attached to the tissue holder and placed into an organ bath containing modified Krebs solution. The other end of the tissue was connected to a force displacement transducer and contractions were recorded isometrically on a polygraph (Medicare, India). The aortic strips were equilibrated in modified Krebs solution (composition in mM: $CaCl_2 \cdot 2H_2O$ 2·51, glucose 11·1, KCl 4·7, $MgSO_4 \cdot 7H_2O$ 1·18, NaCl 118·05, $NaHCO_3$ 11·9, $NaH_2PO_4 \cdot 2H_2O$ 1·04, adjusted to pH 7·4) for 120 min under a resting tension of 2 g. The bathing solution was maintained at 37 ± 0·5°C and continuously bubbled with 95% O_2/5% CO_2. During this equilibration period, the bath fluid was changed every 15 min.

Ca^{2+} induced contractions. The method for recording calcium-induced contractions in rabbit aorta was adapted from Godfraind (1983). In brief, the tissues were pre-incubated for 10 min in Ca-free modified Krebs solution containing 0·1 mM EGTA then washed three times with this solution. Subsequently, the tissues were exposed to K^+-depolarizing solution (K^+, 40 mM replaced an equimolar concentration of NaCl in modified Krebs solution and $CaCl_2$ was omitted). Concentration-dependent contractions were elicited by adding $CaCl_2$ (0·03, 0·1, 0·3, 1·0, 3·0 and 10 mM) cumulatively. After a 60 min recovery period, a second reproducible concentration-response relationship was elicited. The tissues were then incubated with either adenosine (for 5 min) or nifedipine (for 15 min) before initiating contractions with Ca^{2+}.

NA induced contractions. The following experimental protocol was followed to initiate contractions with NA. After the equilibration period, concentration-related contractions were elicited with different concentrations of NA (10^{-8}–10^{-4}M), added cumulatively. When the concentration-response relationship was consistent, the effects of adenosine and nifedipine were examined by pre-incubation of the tissues for a period of 5 min and 15 min, respectively, before contractions with NA were elicited. The change in NA response in the presence of either adenosine or nifedipine was expressed as a percentage of the control response.

Uptake of ^{45}Ca. The net uptake of Ca^{2+} into smooth muscle cells of rabbit aortic strips was determined by measuring the increase in ^{45}Ca content, adapted from the procedures described by Godfraind (1983) and Chiu et al (1986). Taking nifedipine as a specific blocker of calcium channels, its own effect as well as that of adenosine was examined on K^+ (10^{-1} M) and NA (10^{-4} M)-stimulated ^{45}Ca-uptake. La^{3+} was used to remove ^{45}Ca from the extra-cellular space (Van Breemen et al 1972).

The thoracic aorta was cut spirally and strips (approximately 3 × 3 mm) weighing about 4–8 mg were prepared and equili-

brated for 60 min in Tris-buffered solution (composition in mM: $CaCl_2 \cdot 2H_2O$ 2·51, glucose 11·1, KCl 4·7, NaCl 118·05, Tris 12, pH adjusted to 7·4) at 37 ± 0·5°C with continous O_2 bubbling. After this equilibration period, tissues were preincubated with either adenosine (10^{-5} M) or nifedipine (10^{-7} M) for a period of 3 min and 15 min, respectively. The aortic strips were incubated for 10 min in ^{45}Ca-containing Tris-buffered solution (^{45}Ca 1–1·5 $\mu Ci\ mL^{-1}$) containing the inhibitors. To stimulate ^{45}Ca uptake, the tissues were then challenged by 2 min exposure to either K^+ or NA. Basal (unstimulated) ^{45}Ca movement was determined by avoiding administration of both stimulants and inhibitors. The tissues were then soaked for 20 min in 5 mL chilled (2°C) La^{3+} Tris-buffered solution (composition in mM: $CaCl_2 \cdot 2H_2O$ 2·5, glucose 11·1, KCl 4·7, $LaCl_3$ 50, NaCl 118·05, Tris 12, pH adjusted to 7·4) with continuous O_2 bubbling. The strips were then lightly blotted on a filter paper, placed in scintillation vials and weighed. Subsequently, the samples were digested in 0·1 mL of colourless concentrated nitric acid for 5–10 min at 70°C. The vials were cooled and 0·9 mL of Tris (0·75 M) was added. Ten mL of Bray's scintillation fluid was added to the vial and the radioactivity of the samples was measured in a liquid scintillation spectrometer (Packard Tricarb Model 3320). The radioactivity was converted to the apparent tissue content of ^{45}Ca (mmol kg^{-1} wet weight of tissues). The stimulated influx is designated as the difference between the uptake measured in the presence of the stimulant and the corresponding control (unstimulated uptake).

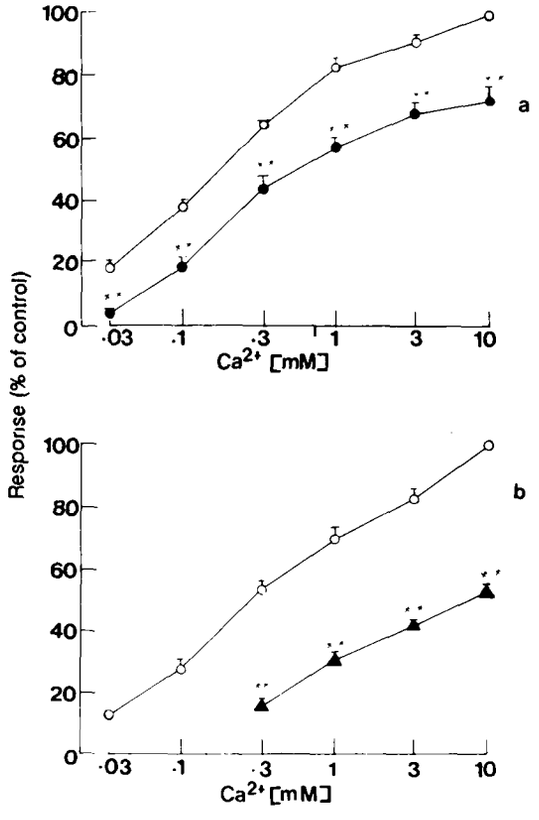

FIG. 1. Effects of adenosine and nifedipine on contractions caused by cumulative addition of $CaCl_2$ in rabbit thoracic aorta previously depolarized with K^+ (40 mM). (a) Inhibition of control concentration response curve (○) in the presence of adenosine (10^{-5} M; ●) n = 7 and (b) a downward shift of the control concentration-response curve (○) in the presence of nifedipine (10^{-8} M; ▲) n = 4. Vertical bars indicate s.e.m. ** $P < 0.01$.

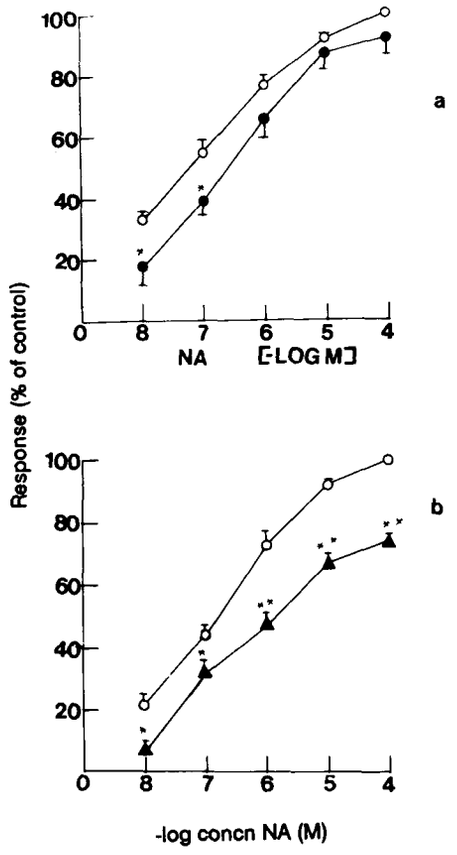

FIG. 2. Effects of adenosine and nifedipine on concentration-related contractions obtained with NA (10^{-8}–10^{-4} M) added cumulatively. (a) Concentration-response curves were developed in the absence (○) and presence of adenosine (10^{-5} M; ●) n = 5 and (b) downward displacement of control concentration-response curve (○) in the presence of nifedipine (10^{-7} M; ▲) n = 4. Vertical bars indicate s.e.m. * $P < 0.05$; ** $P < 0.01$.

Results are given as mean ± s.e.m. Student's *t*-test was used to test for significance with a probability level of 0·05.

Results

$CaCl_2$ (0·03–10 mM), added cumulatively to rabbit aortic strips previously depolarized with K^+ (40 mM), initiated concentration-dependent contractions. Both adenosine (10^{-5} M) and nifedipine (10^{-7} M) significantly ($P < 0.01$) inhibited the contractile responses induced by $CaCl_2$ and shifted the dose-response curves downwards (Fig. 1). In the presence of adenosine and nifedipine, the maxima obtained with Ca^{2+} (10 mM) were inhibited by 29·0 ± 4·9 and 50·25 ± 1·9%, respectively.

The effects of adenosine (10^{-5} M) and nifedipine (10^{-7} M) on the contractile response induced by cumulative addition of NA (10^{-8}–10^{-4} M) in rabbit aorta are shown in Fig. 2. Adenosine significantly ($P < 0.05$) inhibited the contractions caused by lower concentrations of NA (10^{-8} and 10^{-7} M). On the other hand, it had little effect on the contractile response induced by higher concentrations of NA (> 10^{-7} M). Nifedipine, however, caused a significant downward shift of the dose-response curve of NA (10^{-8}–10^{-4} M). The maximal isometric tension (1·95 ± 0·2 g) developed by NA (10^{-4} M) was inhibited by 27 ± 1·9% (n = 4) in the presence of nifedipine (10^{-7} M).

The effects of adenosine (10^{-5} M) and nifedipine (10^{-7} M) on K^+ (100 mM)- and NA (10^{-4} M)-stimulated ^{45}Ca-uptake into

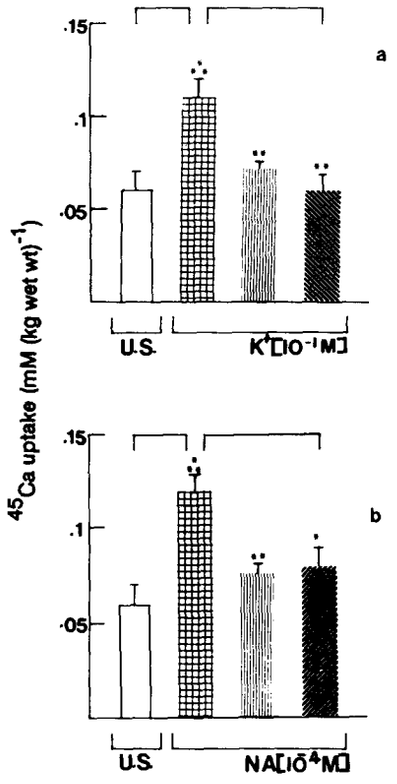

FIG. 3. Effects of adenosine (10^{-5} M) and nifedipine (10^{-7} M) on ^{45}Ca uptake into rabbit aortic strips stimulated with (a) K^+ (10^{-1} M) and (b) NA (10^{-4} M); U.S. represents unstimulated uptake (n = 10); crossed column represents control K^+/NA-stimulated uptake (n = 5). Vertical striped column represents K^+/NA-stimulated uptake in the presence of adenosine (n = 7); hatched column represents K^+/NA-stimulated uptake in the presence of nifedipine (n = 7), vertical bars indicate s.e.m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

rabbit aortic strips are shown in Fig. 3. Both K^+ and NA caused almost two-fold increase in the ^{45}Ca-uptake into smooth muscle strips. Adenosine (10^{-5} M) significantly ($P < 0.01$) reduced ^{45}Ca-influx in aortic strips stimulated either with K^+ or NA, the percentage of inhibition being about 75% in either case. Similarly, nifedipine caused significant ($P < 0.01$, 0.05) inhibition in ^{45}Ca-uptake stimulated by K^+ and NA; the per cent inhibition was 100 and 68, respectively.

Discussion

It is generally believed that the content of the intracellular Ca^{2+} must be elevated to activate the contractile proteins in muscle and this is achieved primarily through influx of extracellular Ca^{2+} during the activation process. The results from several earlier studies suggest that membrane depolarization by high K^+ and receptor activation by the action of NA facilitate the influx of extracellular Ca^{2+} into aortic smooth muscle through voltage-operated (VOC) and receptor-operated (ROC) calcium channels, respectively (Bolton 1979; Meisheri et al 1981; Chiu et al 1986; Godfraind et al 1986). Keeping in view the influx of extracellular Ca^{2+} as a vital link in signal transduction during vascular smooth muscle contractions, the effects of adenosine on contractility and ^{45}Ca uptake were studied in rabbit aorta and were compared with those of nifedipine, a known calcium channel blocker.

The inhibition of Ca^{2+}-induced contractions in K^+-depolarized preparations by adenosine suggests that the purine might have caused a decrease in inward Ca^{2+}-movement through VOCs. This is substantiated by the direct evidence that adenosine significantly reduced K^+-stimulated ^{45}Ca-influx in aortic strips. Although the calcium channel blocker nifedipine had similar effects, it was more potent than adenosine both in respect of inhibition of contractility as well as ^{45}Ca uptake. There is increasing evidence for a structural relationship between cell membrane nucleoside transporters and calcium channels (Glossmann et al 1985; Striessnig et al 1985). Thus, it is quite possible that adenosine might interact with calcium channels to decrease the inward movement of Ca^{2+}. The results of the present study with respect to adenosine inhibiting Ca^{2+}-influx through VOCs are in conformity with those observed in rat aortic smooth muscle cells (Fenton et al 1982).

NA-induced contractions in aortic smooth muscle have been shown to result from both Ca^{2+}-influx as well as release of intracellular Ca^{2+} (Flaim 1982; Karaki & Weiss 1984). Furthermore, in a recent report, concentration-dependent activation of VOCs, ROCs and intracellular Ca^{2+} release with NA has been shown in rabbit aorta (Hester 1988). Hester's studies demonstrated that while low concentrations of NA activate VOCs, the higher concentrations of the agonist activate ROCs and cause release of intracellular Ca^{2+}. Thus, the differential susceptibility of NA contractions to adenosine in rabbit aorta as observed in the present study could be explained in terms of differential modes of transport of Ca^{2+} into the cell and alternatively the sources of activator Ca^{2+} contributing to the contractions. In contrast to the antagonism of contractions by nifedipine throughout the range of NA concentrations, adenosine only inhibited the contractions elicited at lower concentrations of the agonist. Possibly, this might be related to adenosine-induced inhibition of inward movement of Ca^{2+} through ROCs activated by low concentrations of NA. The involvement of VOCs in the Ca^{2+} transport is ruled out because of the lack of membrane depolarizing action of NA in rabbit aorta (Cauvin & Van Breemen 1985 a,b). On the contrary, the lack of effect of adenosine at higher concentrations of NA could be because there is now a significant contribution of released intracellular Ca^{2+} contributing to the contractile response instead of Ca^{2+} influx through adenosine-sensitive slow Ca^{2+}-channels. This is further supported by the findings that a significant inhibition of NA (10^{-4} M)-stimulated ^{45}Ca uptake by the purine was observed in rabbit aortic strips. The release of intracellular Ca^{2+} by high doses of NA is further supported by the finding that nifedipine (10^{-7} M) inhibited NA (10^{-4} M)-stimulated ^{45}Ca-uptake by 75% but only reduced contractions to NA (10^{-4} M) by about 27%. The present observation is in accordance with an earlier report where nifedipine produced a maximal inhibition (25%) of NA contractions in rabbit aorta (Razzetti et al 1984). Considering the inhibitory effects of adenosine and nifedipine on Ca^{2+}- and NA-stimulated contractions, it appears that adenosine has an apparent selectivity to VOCs compared with ROCs in rabbit aorta. However, these observations are at variance with previous reports involving actions of adenosine on calcium channels in vascular smooth muscles (Karaki 1987).

In conclusion, the present study suggests that (1) inhibition of Ca^{2+}-induced contractions and K^+-stimulated ^{45}Ca uptake in rabbit aorta might be due to antagonism of VOCs by adenosine, (2) the antagonism of contractions to lower concentrations of NA by adenosine and also the significant inhibition of NA (10^{-4} M)-stimulated uptake of ^{45}Ca implicate the blockade of ROCs and (3) the resistance of the contractions elicited by higher concentrations of NA could be due to release of intracellular Ca^{2+}.

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