J. Pharm. Pharmacol. 2000, 52: 695–707
Received November 25, 1999
Accepted February 11, 2000

© 2000 J. Pharm. Pharmacol.

A Mechanism of Vasodilatory Action of Polyamines and Acetylpolyamines: Possible Involvement of their Ca^{2+} Antagonistic Properties

CHANG-SEON MYUNG, JAMES W. BLANKENSHIP* AND DENIS J. MEERDINK*

*Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, VA 22908 and *Department of Physiology and Pharmacology, University of the Pacific, School of Pharmacy and Health Sciences, Stockton, CA 95211, USA*

Abstract

Polyamines, a class of low-molecular weight organic polycations, have been shown to produce relaxing effects in vascular smooth muscles, although the mechanism has not been carefully examined. In this study, the mechanism of vascular action of polyamines and their metabolites, acetylpolyamines, was pharmacologically examined in the rabbit isolated thoracic aorta focusing on an endothelium-dependent component of vasodilatation and Ca^{2+} influx through plasma membrane channels.

Both polyamines and acetylpolyamines (except N^1-acetylputrescine, which produced no response or very slight contraction) caused concentration-dependent relaxation in preconstricted aortic rings containing an intact endothelium. Aortic rings denuded of endothelium were also responsive to both polyamines and acetylpolyamines. Inhibitors of nitric oxide (reduced haemoglobin and N^{ω}-nitro-L-arginine methyl ester), vasodilator prostaglandins (indomethacin) and guanylyl cyclase (methylene blue) did not affect the relaxation induced by both polyamines and acetylpolyamines in either endothelium-intact or -denuded aortic rings. Both polyamines and acetylpolyamines inhibited the concentration-dependent contraction for phenylephrine and K^+. The Ca^{2+} agonist Bay K 8644 induced concentration-dependent contraction in segments of rabbit aorta partially depolarized with 15 mM KCl, and both polyamines and acetylpolyamines relaxed the Bay K 8644-induced contraction in a concentration-dependent manner. Interestingly, both polyamines and acetylpolyamines also decreased contractions evoked by the Ca^{2+} ionophore A23187. The concentration-response curve to exogenous Ca^{2+} in K^+-depolarization medium ($K^+ = 120$ mM) was shifted to the right by both polyamines and acetylpolyamines. The response elicited by Ca^{2+} was increased by Bay K 8644 (10^{-6} M), and this potentiation was also inhibited by both polyamines and acetylpolyamines.

The results indicate that both polyamines and acetylpolyamines can induce vasorelaxation of rabbit thoracic aorta by an endothelium-independent mechanism in-vitro and relax vascular smooth muscle by acting at the plasma membrane level, decreasing the influx of Ca^{2+}. Therefore, polyamines and acetylpolyamines may have Ca^{2+} antagonistic properties which may, in part, be involved in the mechanism of rabbit aortic vascular smooth muscle relaxation.

Putrescine, spermidine and spermine are ubiquitous polycationic aliphatic polyamines present in all living cells and essential for cell growth and differentiation, presumably through ionic interaction with nucleic acids and proteins (Tabor & Tabor 1984; Pegg 1988). Polyamine concentrations, which vary with the cell cycle and in response to external stimuli such as growth factors and hormones, are controlled by a closely regulated synthetic and catabolic pathway (Morgan et al 1986).

In addition to the well-documented role of polyamines in gene expression and cellular differentiation, polyamines are known to be involved as

Correspondence: C.-S. Myung, Department of Pharmacology, P.O. Box 800735, 1300 Jefferson Park Ave, University of Virginia School of Medicine, Charlottesville, Virginia 22908-0735, USA.
E-Mail: cm8r@virginia.edu

second messengers in Ca^{2+} homoeostasis and phosphoinositide metabolism (Schuber 1989), perhaps by stimulating GTPase activity in heterotrimeric G proteins (Bueb et al 1992). Polyamines enhance Ca^{2+} flux across rat kidney cortex cells and heart myocytes (Koenig et al 1989), stimulate mitochondrial Ca^{2+} uptake and attenuate inositol 1,4,5-triphosphate (IP_3)-induced Ca^{2+} release from endoplasmic reticulum (Lenzen & Rustenbeck 1991) and increase in intracellular Ca^{2+} levels (Moncada et al 1991; Groblewski et al 1992). Moreover, in cultured human umbilical vein endothelium, polyamines in micromolar concentrations stimulate the influx of extracellular Ca^{2+} (Morgan et al 1990). Since polyamines and their metabolites, acetylpolyamines, relax vascular smooth muscle (Chideckel et al 1985a; Wing et al 1993; Johnson et al 1996), these observations suggest that the Ca^{2+}-associated effect of polyamines may involve their vasoactivity.

One possibility is that polyamine-induced increases in intracellular Ca^{2+} may be a mechanism for the release of nitric oxide (NO). NO is a potent vasodilator produced from L-arginine in vascular endothelium, catalysed by NO synthase (NOS) (Moncada et al 1991). Since vascular endothelial cells contain an arginase (Morgan & Baydoun 1994), which catalyses the formation of L-ornithine from L-arginine, and the NO-donating ability of polyamine–NO adducts has been demonstrated under physiological conditions (Hrabie et al 1993), it has been suggested that polyamines represent a novel class of NO donor (Morgan 1994).

Since Ca^{2+} mediates a wide variety of physiological processes such as endocytosis, exocytosis, membrane transport and muscle contraction (Rasmussen & Barrett 1984), the effect of polyamines and acetylpolyamines on Ca^{2+} homoeostasis could be another possible direct mechanism of smooth muscle relaxation. Spermine has been shown to relax rat uterine smooth muscle, an effect which is counteracted by the addition of extracellular Ca^{2+}, suggesting that spermine may prevent Ca^{2+} entry across the membrane in the smooth muscle cells (Hashimoto et al 1973). Acetylspermine and spermine have been shown to decrease the intracellular Ca^{2+} concentration of vascular smooth muscle and to decrease arterial blood pressure (Wing et al 1993). Recently, it has been also suggested that polyamines inhibit rat uterine smooth muscle contraction by acting at the plasma membrane level, decreasing the influx of Ca^{2+} (Fernandez et al 1995). These observations provide a compelling reason to examine whether the vasoactivity of polyamines and their metabolites, acetylpolyamines, are endothelium-dependent or whether the relative direct vasodilatory actions are Ca^{2+}-influx associated.

Ca^{2+} influx essentially occurs through plasma membrane Ca^{2+} channels; receptor-operated channels which are activated by agonists such as noradrenaline and phenylephrine and voltage-operated channels which are activated by K^+ (Cauvin et al 1983; Godfraind et al 1986). Since voltage-operated channels appear to be more sensitive to Ca^{2+} agonists and antagonists than receptor-operated channels, these classes of drugs can be used for the pharmacological investigation of Ca^{2+} modulation across the cell membrane through Ca^{2+} channels in smooth muscle cells (Godfraind et al 1986; Marin 1988). In this study, the pharmacological features of polyamine- and acetylpolyamine-induced vasorelaxation were functionally examined in the rabbit isolated thoracic aorta, focusing on an endothelium-dependent process and on transmembrane extracellular Ca^{2+} influx.

Materials and Methods

Tissue preparation

This investigation conforms to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985). Male New Zealand White rabbits (2·0–2·7 kg) were killed by an overdose of sodium pentobarbital ($50\,mg\,kg^{-1}$, i.v.). The descending thoracic aorta was excised immediately and carefully, and immersed in low-bicarbonate modified Krebs–Henseleit buffer pH 7·4 (composition in mM: NaCl, 120; KCl 4·8; $CaCl_2$, 2·5; KH_2PO_4, 1·2; $MgSO_4$, 1·2; $NaHCO_3$, 6; glucose, 10). Low bicarbonate concentrations were used to prevent the precipitation of calcium carbonate with the polycationic study compounds; Na_2EDTA (0·03 mM) was added to inhibit metal-catalysed oxidation. The aorta was cleared of periadventitial adipose and connective tissues and cut into 3–5 rings, 3–5 mm wide. Care was taken during preparation of the aorta and rings to avoid unnecessary stretching or contact with luminal surfaces to prevent damage to the endothelium.

Isolated muscle chamber preparation and tension recording

Thoracic aortic rings with or without endothelium were mounted horizontally between two stainless steel parallel stirrups in individual 10-mL water-jacketed muscle chambers. One stirrup was

anchored by a tissue holder in the muscle chamber, and the other was connected to a force transducer (Grass FT-03, Grass Instruments Co.). The buffer solution in each chamber was continuously oxygenated with 95% O_2–5% CO_2 and maintained at 37 °C. Each ring was allowed to equilibrate for 60–90 min before experimentation by changing the bathing solution every 15 min and continually adjusting preload to maintain a resting tension of 5 g. Pharmacological responses were measured as changes in isometric force, which was recorded on a multichannel polygraph (Grass Model 7B Polygraph) that was calibrated before each experiment. For experiments using rings without endothelium, the endothelium was removed by gently rubbing the intimal surface with a wooden probe on filter paper wetted with bathing solution. The absence of endothelium was confirmed by the inability of acetylcholine (10^{-6} M) to induce relaxation of rings contracted with vasoconstrictors (Martin et al 1985).

Relaxation studies
After stabilization, a concentration–response curve to phenylephrine was obtained for each aortic ring; the half-maximal level of contraction ranged between 4 and 6 g of tension. The rings were subsequently preconstricted to their half-maximal tension with phenylephrine (3×10^{-7} M), and after a stable contractile plateau was obtained, the test compounds were studied using the experimental protocols as described below. In most studies, concentration–response curves in the absence (i.e., control) and in the presence of inhibitory agents were determined on the same rings. Concentration–response studies were performed by cumulatively adding small amounts of concentrated solutions of the study compound (polyamines and acetylpolyamines) into the aerated bathing solution to attain the desired final concentration. Aortic rings with or without functional endothelium were exposed at the beginning of the experiment to 60 mM KCl to check their functional state. Afterwards, the bath medium was changed several times until the resting tone was recovered. Then, cumulative concentration–response curves to phenylephrine, KCl, Bay K 8644, or Ca^{2+} ionophore A23187 were carried out. Only one curve was determined in segments exposed to Bay K 8644 or A23187, because the effects of these drugs did not completely disappear after repeated washing periods. The study compounds were added 15 min before the second concentration–response curve to phenylephrine, K^+, Bay K 8644 or A23187; verapamil (5×10^{-6} M) and nifedipine (10^{-7} M) were also applied 15 min before addition of phenylephrine, K^+, Bay K 8644 or A23187. In most studies, concentration–response curves of control groups were accompanied with test groups using relaxing agents at the beginning and at the end of experiments to rule out changes over time.

Inhibitors studies
N^{ω}-Nitro-L-arginine methyl ester (L-NAME, 10^{-4} M), an inhibitor of NO synthesis from L-arginine in vascular endothelial cells (Rees et al 1990), was added 15 min before phenylephrine precontraction in thoracic aortic rings with endothelium, and cumulative responses to the study compound were then observed. In addition, an equimolar concentration of L-NAME was also added to precontracted aortic rings with endothelium, and cumulative responses to the study compound were obtained. Reduced haemoglobin (10^{-5} M) was added to precontracted thoracic aortic rings with endothelium. The study compound was added cumulatively after reaching the plateau of half-maximal contraction evoked by phenylephrine. Reduced haemoglobin was prepared as described by Martin et al (1985). Indomethacin (10^{-5} M), an inhibitor of prostaglandin synthesis, was added to the incubation media for 20 min before endothelium-intact rings were precontracted with phenylephrine. The study compound was added cumulatively after reaching the plateau of half-maximal contraction evoked by phenylephrine. Effects of indomethacin on relaxation induced by arachidonic acid (3×10^{-5} M) were also assessed in endothelium-intact rings. To determine the involvement of guanosine 3′,5′-cyclic monophosphate (cyclic GMP) in the relaxation induced by polyamines and acetylpolyamines, aortic rings without endothelium were incubated with methylene blue (10^{-5} M), a guanylyl cyclase inhibitor, for 20 min before the addition of phenylephrine, and polyamines or acetylpolyamines (10^{-3}–10^{-2} M) were subsequently added.

Ca^{2+} study
To analyse the effect of polyamines and acetylpolyamines on the extracellular Ca^{2+} concentration-induced contraction, the aortic rings were exposed for 15 min to a Ca^{2+}-free medium before obtaining a concentration–response curve to exogenous Ca^{2+}. In nominally Ca^{2+}-free solution used for tissue preparation, $CaCl_2$ was omitted, and in Ca^{2+}-free solution for washout of extracellular Ca^{2+}, 1 mM EGTA was added. In high-K^+ solutions, NaCl was exchanged for KCl. Thoracic aortic

rings without endothelium were incubated in Ca^{2+}-free solution containing 1 mM EGTA for 10 min. Concentration–response curves to Ca^{2+} (5×10^{-5}–10^{-2} M) were then performed in K^+-depolarization medium ($K^+ = 120$ mM). The rings were readjusted in buffer solution for 30 min before being incubated in Ca^{2+}-free solution containing 1 mM EGTA for another 10 min. These rings were preincubated with the study compound 15 min before the second concentration–response curve was obtained. To assess the effect of Bay K 8644 (10^{-6} M) or nifedipine (10^{-7} M) on contractions caused by Ca^{2+} addition, they were added 15 min before determination of the concentration–response curve to Ca^{2+}.

Drugs

L-Phenylephrine hydrochloride, putrescine dihydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride, N^1-acetylputrescine hydrochloride, N^1-acetylspermidine dihydrochloride, N^8-acetylspermidine dihydrochloride, N^1-acetylspermine trihydrochloride, acetylcholine chloride, L-NAME, haemoglobin (bovine haemoglobin type 1), methylene blue, indomethacin, arachidonic acid, potassium chloride, nifedipine, (±)-verapamil hydrochloride, calcium chloride dihydrate, EGTA (ethylene glycol-bis[β-aminoethyl ether]-*N*,*N*,*N'*,*N'*-tetraacetic acid), EDTA (ethylene-diamine tetra-acetic acid) disodium salt dihydrate and Ca^{2+} ionophore A23187 were purchased from Sigma Chemical Co. (St Louis, MO). *S*(−)-Bay K 8644 [*S*(−)-1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoro-methyl) phenyl]-3 pyridinecarboxylic acid methyl ester] was purchased from Research Biochemicals Incorporated (Natick, MA). All drugs were prepared as aqueous solutions except for L-NAME, Bay K 8644 and A23187, which were dissolved in dimethylsulphoxide (DMSO), and indomethacin which was dissolved in an equimolar Na_2CO_3 solution by sonication, as stock solution and diluted before use. The DMSO concentration in the bath did not exceed 0·001% and neither DMSO nor Na_2CO_3 alone in the applied concentrations was found to have an effect on contractile or relaxation responses (data not shown). All solutions were prepared fresh daily, and concentrations are expressed as the final molar concentrations in bathing solution.

Statistics

Contractile responses induced by drugs were expressed as percentages of the response induced by previous administration of 60 mM KCl. Relaxation was measured as the decrease in tension below the half-maximal tension evoked by phenylephrine, and was expressed as the percentage relaxation of contraction induced by agonists. Results are expressed as mean ± s.e.m. Data expressed as concentration-response curves were fit to sigmoid curves using the fitting routines in the GraphPad Prism software. Statistical differences between the curves were determined using all the individual data points from multiple experiments to calculate the F statistic as described (Motulsky & Ransnas 1987). Statistical significance for paired and unpaired observations was also evaluated by Student's *t*-test. $P < 0{\cdot}05$ was considered significant; n indicates the number of rings.

Results

Relaxant effect of polyamines and acetylpolyamines on precontracted intact aortic rings

Figure 1 shows concentration-response curves to polyamines (A) and acetylpolyamines (B) on vasorelaxation of rabbit isolated aortic rings with intact endothelium. Polyamines relaxed isolated vascular smooth muscle precontracted with phenylephrine in a concentration-dependent manner. Spermine was the most potent vasorelaxant (EC50 = 1·7 mM), and the potency of spermidine (EC50 = 2·8 mM) was greater than that of putrescine (EC50 = 4·6 mM). The maximal relaxation induced by spermine ($V_{max} = 100$) was greater than those induced by both spermidine ($V_{max} = 94{\cdot}9$) and putrescine ($V_{max} = 81{\cdot}5$). Acetylpolyamines also relaxed isolated vascular smooth muscle in a concentration-dependent manner. N^1-Acetylspermine was the most potent vasorelaxant (EC_{50} = 1·6 mM), and the potency of N^1-acetylspermidine (EC_{50} = 2·3 mM) was greater than that of N^8-acetylspermidine (EC_{50} = 2·8 mM). Likewise, the maximal relaxation induced by N^1-acetylspermine ($V_{max} = 91{\cdot}3$) was greater than that induced by either N^1-acetylspermidine ($V_{max} = 86{\cdot}6$) or N^8-acetylspermidine ($V_{max} = 77{\cdot}0$). N^1-Acetylputrescine was found to have no relaxing effect on vascular smooth muscle (data not shown).

Role of endothelium in polyamine- and acetylpolyamine-induced vasorelaxation

Figure 2 shows the effect of endothelium on vasorelaxation induced by polyamines. The vasorelaxant effect of putrescine, spermidine and spermine did not significantly differ between endothelium-intact and endothelium-denuded

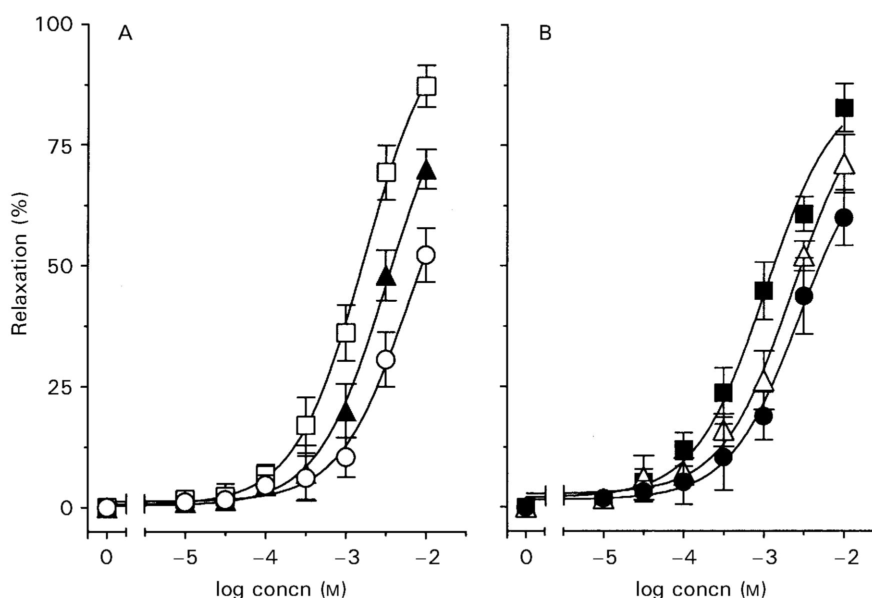

Figure 1. Concentration–response curves of (A) polyamines (○, putrescine; ▲, spermidine; □, spermine) and (B) acetylpolyamines (●, N^8-acetylspermidine; △, N^1-acetylspermidine; ■, N^1-acetylspermine) on endothelium-intact rabbit aortic rings. Responses were expressed as percentage of precontraction induced by 3×10^{-7} M phenylephrine ($4{\cdot}9 \pm 0{\cdot}3$ g). Values are means ± s.e.m. (n = 28 for polyamines; n = 15 for acetylpolyamines).

Table 1. Effect of the presence of endothelium on acetylpolyamine-induced vasorelaxation in endothelium-intact and endothelium-denuded rabbit aortic rings.

Concn (mM)	Relaxation (%)					
	N^8-Acetylspermidine (n = 15)		N^1-Acetylspermidine (n = 13)		N^1-Acetylspermine (n = 15)	
	Endo (+)	Endo (−)	Endo (+)	Endo (−)	Endo (+)	Endo (−)
0·01	1·4 ± 0·3	2·6 ± 0·1	1·7 ± 0·4	1·9 ± 0·3	2·4 ± 0·5	3·9 ± 0·5
0·03	3·2 ± 0·9	5·1 ± 0·1	6·1 ± 2·1	7·5 ± 3·2	5·2 ± 1·0	8·2 ± 1·4
0·1	7·2 ± 1·9	8·6 ± 1·0	10·3 ± 3·5	11·6 ± 3·2	11·9 ± 2·8	12·1 ± 1·8
0·3	14·3 ± 2·8	16·3 ± 2·6	19·9 ± 6·0	17·6 ± 3·1	23·7 ± 5·7	17·1 ± 2·7
1·0	22·9 ± 4·5	23·2 ± 4·3	30·2 ± 8·1	27·0 ± 1·9	34·9 ± 7·5	37·5 ± 9·8
3·0	47·7 ± 3·2	48·1 ± 5·5	56·1 ± 5·9	51·9 ± 2·5	60·8 ± 6·6	57·9 ± 5·5
10·0	64·0 ± 2·4	64·3 ± 5·1	75·2 ± 2·7	72·8 ± 1·1	80·9 ± 3·0	82·8 ± 3·3

Endo (+), endothelium-intact aortic rings; Endo (−), endothelium-denuded aortic rings. Responses are expressed as a percentage of the precontraction induced by 3×10^{-7} M phenylephrine ($4{\cdot}9 \pm 0{\cdot}3$ g tension). Values are means ± s.e.m. The differences of vasorelaxation between endothelium-intact and endothelium-denuded aortic rings at any given concentration of the test compound were not statistically significant.

preparations. Table 1 summarizes the observed vasorelaxation induced by acetylpolyamines in endothelium-intact and -denuded aortic rings. These results suggest that the vasorelaxation produced by both polyamines and acetylpolyamines is not dependent upon intact endothelium.

Effect of L-NAME, reduced haemoglobin, indomethacin and methylene blue on polyamine- and acetylpolyamine-induced vasorelaxation

The data shown in Table 2 demonstrate that neither reduced haemoglobin (10^{-5} M) nor L-NAME (10^{-4} M) inhibited the vasorelaxation induced by polyamines or acetylpolyamines over the concentration range 10^{-3}–10^{-2} M in rings with intact endothelium. Indomethacin (10^{-5} M) significantly reduced the transient relaxation induced by arachidonic acid (3×10^{-5} M) by $93{\cdot}0 \pm 2{\cdot}1$% in endothelium-intact aortic rings precontracted with phenylephrine (data not shown); an equal dose of indomethacin, however, did not affect the relaxation induced by both polyamines and acetylpolyamines in rings with intact endothelium. Incubation with the guanylyl cyclase inhibitor, methylene blue (10^{-5} M), for 20 min before contraction with phenylephrine had no effect on

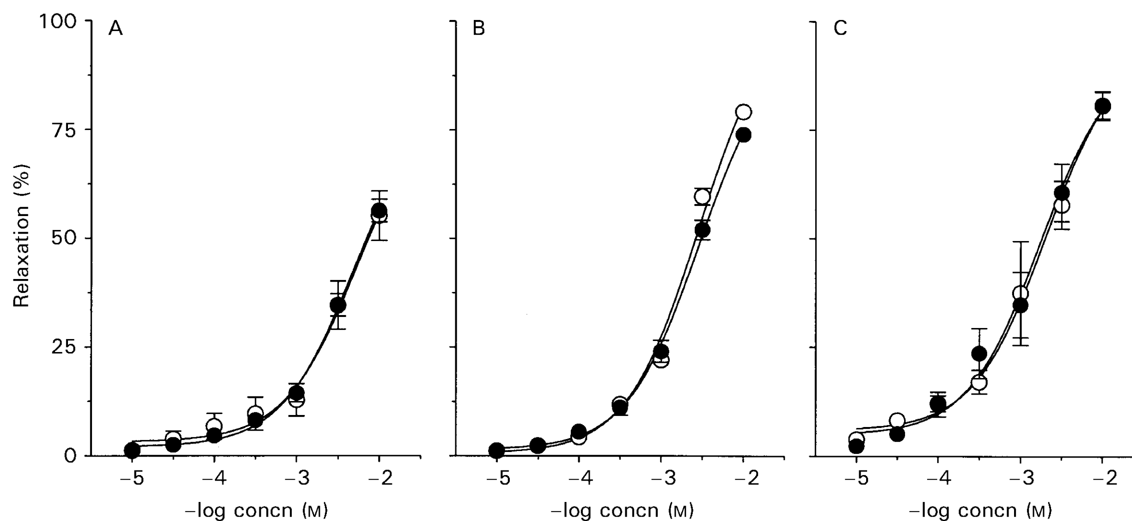

Figure 2. Effect of the presence of endothelium on the vasorelaxation produced by putrescine (A; n = 24), spermidine (B; n = 27) and spermine (C; n = 28) on rabbit aortic rings. Responses were expressed as a percentage of the precontraction induced by 3×10^{-7} M phenylephrine (4·9 ± 0·3 g). Values are means ± s.e.m. The differences in vasorelaxation between endothelium-intact (●) and endothelium-denuded (○) aortic rings at any given concentration of the test compound were not statistically significant.

relaxation induced by both polyamines and acetylpolyamines in rings without endothelium (i.e., relaxation induced by spermine at 10^{-3} M, 5×10^{-3} M and 10^{-2} M was 29·3 ± 3·3%, 66·8 ± 3·9% and 83·8 ± 3·4%, respectively, in the presence of methylene blue, and 31·7 ± 1·8%, 67·6 ± 2·3% and 87·1 ± 1·2% in controls). These results suggest that both polyamines and acetylpolyamines dilate the vascular smooth muscle independently of endothelium-derived NO, vasodilator prostaglandins and the activation of soluble guanylyl cyclase.

Table 2. Effect of L-NAME, reduced haemoglobin, indomethacin and methylene blue on the vasorelaxation produced by polyamines and acetylpolyamines in rabbit aortic rings.

		Relaxation (%)				
		Control	L-NAME	Haemoglobin	Indomethacin	Methylene blue
Polyamines						
Putrescine (28)	1·0 mM	12·7 ± 1·8	9·4 ± 2·0	15·6 ± 3·9	13·0 ± 1·7	10·3 ± 1·9
	3·0 mM	34·3 ± 3·4	34·0 ± 4·9	38·2 ± 6·7	35·5 ± 2·8	36·8 ± 3·4
	10·0 mM	53·5 ± 4·2	52·1 ± 5·9	57·2 ± 7·1	55·5 ± 4·1	55·4 ± 3·8
Spermidine (32)	1·0 mM	23·1 ± 1·8	23·1 ± 1·7	22·1 ± 3·1	20·0 ± 3·4	22·1 ± 2·3
	3·0 mM	52·3 ± 3·1	59·2 ± 3·4	52·9 ± 2·9	49·5 ± 3·1	54·8 ± 3·8
	10·0 mM	73·8 ± 1·8	77·2 ± 2·4	72·3 ± 0·7	70·4 ± 3·4	74·0 ± 3·2
Spermine (29)	1·0 mM	31·7 ± 1·8	31·3 ± 1·5	37·9 ± 4·9	28·4 ± 4·0	29·3 ± 3·3
	3·0 mM	67·6 ± 2·3	72·2 ± 1·8	76·0 ± 3·0	66·6 ± 5·5	66·8 ± 3·9
	10·0 mM	87·1 ± 1·2	87·9 ± 0·9	88·8 ± 2·0	83·5 ± 4·2	83·8 ± 3·4
Acetylpolyamines						
N^8-Acetylspermidine (15)	1·0 mM	22·1 ± 2·0	22·1 ± 4·8	19·3 ± 3·6	15·9 ± 2·9	21·7 ± 5·0
	3·0 mM	48·0 ± 2·0	48·7 ± 4·0	45·9 ± 3·3	43·8 ± 2·9	46·2 ± 3·6
	10·0 mM	64·7 ± 1·7	63·6 ± 3·3	63·9 ± 3·1	61·2 ± 3·9	60·5 ± 1·6
N^1-Acetylspermidine (15)	1·0 mM	28·8 ± 3·0	29·4 ± 6·9	26·1 ± 4·2	23·6 ± 8·6	30·9 ± 6·4
	3·0 mM	54·8 ± 2·3	56·2 ± 5·1	51·4 ± 4·3	49·1 ± 7·5	55·5 ± 5·2
	10·0 mM	74·4 ± 1·4	74·1 ± 1·9	73·6 ± 2·7	67·1 ± 5·1	74·2 ± 1·9
N^1-Acetylspermine (13)	1·0 mM	34·6 ± 3·5	32·7 ± 6·4	28·4 ± 5·0	26·6 ± 5·9	32·7 ± 5·4
	3·0 mM	60·5 ± 2·7	59·8 ± 4·8	53·8 ± 6·3	55·2 ± 6·1	59·8 ± 4·8
	10·0 mM	79·9 ± 2·2	80·0 ± 4·7	77·6 ± 2·0	77·2 ± 3·0	80·0 ± 4·6

Responses are expressed as a percentage of the precontraction induced by 3×10^{-7} M phenylephrine (4·9 ± 0·3 g tension). Whereas the effect of L-NAME, haemoglobin and indomethacin on the vasorelaxation induced by test compound was measured in endothelium-intact aortic rings, the effect of methylene blue was measured in endothelium-denuded rings. Values are means ± s.e.m. and n values are in parentheses. There is no significant difference from control at any given concentration of the test compound.

Effect of polyamines and acetylpolyamines on the concentration–response curve to phenylephrine and K^+

Phenylephrine induced concentration-dependent contractions in segments of rabbit aorta without endothelium. Figure 3A shows that concentration-dependent contractions induced by phenylephrine were significantly reduced by the Ca^{2+}-channel blockers nifedipine (10^{-7} M) and verapamil (5×10^{-6} M), but were not increased by the Ca^{2+} agonist Bay K 8644 (10^{-6} M, 15-min preincubation). Both spermine (Figure 3C) and N^1-acetylspermine (Figure 3E) shifted the concentration-response curve to phenylephrine to the right. The data shown in Figure 3B demonstrate that K^+ (7·5–120 mM) produced concentration-dependent contractions in isolated vascular smooth muscle from rabbit thoracic aorta without functional endothelium. The responses induced by K^+ were reduced by both nifedipine (10^{-7} M) and verapamil (5×10^{-6} M) and significantly increased by Bay K 8644 (10^{-6} M, 15-min preincubation). The concentration–response curve to K^+ was shifted to the right by both spermine (Figure 3D) and N^1-acetylspermine (Figure 3F). Spermidine and putrescine were less potent in relaxing phenylephrine- or K^+-induced contractions than spermine, and the potency of N^1-acetylspermine was greater than that of either N^1- or N^8-acetylspermidine (data not shown). Both polyamines and acetylpolyamines relaxed the phenylephrine- and K^+-induced contraction as did Ca^{2+}-channel blockers (nifedipine and verapamil). The potentiation of K^+ responses elicited by Bay K 8644 was also reduced by both polyamines and acetylpolyamines (data not shown). Thus, these results suggest that the relaxing effect of polyamines and acetylpolyamines may be through Ca^{2+} channels at the plasma membrane level.

Effect of polyamines and acetylpolyamines on the concentration–response curves to Bay K 8644 and A23187

Bay K 8644 did not alter the basal tone, but when the segments were partially depolarized with 15 mM K^+ it produced concentration-dependent contraction (control in Figure 4A). The estimated EC50 value for Bay K 8644 was $2{\cdot}24 \times 10^{-7}$ M. Figure 4A shows that spermine (15-min preincubation) shifts the concentration–response curve to Bay K 8644 to the right in endothelium-denuded aortic rings. Likewise, N^1-acetylspermine concentration-dependently inhibited the response elicited by Bay K 8644 (Figure 4C). The presence of nifedipine (10^{-7} M) also reduced the response elicited by Bay K 8644 (data not shown). Therefore, both polyamines and acetylpolyamines inhibited the vascular smooth muscle contraction caused by Ca^{2+} agonist Bay K 8644, as did Ca^{2+}-channel blockers. Cumulative addition of the Ca^{2+} ionophore, A23187 (10^{-7}–10^{-4} M), induced slowly-developing concentration-dependent contractions in the rabbit thoracic aorta without functional endothelium (control in Figure 4B). The maximal response was $2{\cdot}68 \pm 0{\cdot}83$ g tension, which corresponded to $44{\cdot}7 \pm 9{\cdot}4\%$ of contractions elicited by 60 mM K^+. Nifedipine (10^{-7} M; $n = 6$) or verapamil (10^{-5} M; $n = 6$) did not significantly alter the A23187 curve (data not shown). However, both spermine (Figure 4B) and N^1-acetylspermine (Figure 4D) modified the vascular smooth muscle contraction evoked by A23187 in a concentration-dependent manner, suggesting that the mechanism of increase in intracellular Ca^{2+} level by A23187 is different from the mechanisms involving the dihydropyridine-sensitive Ca^{2+} channel (voltage-operated)-activators such as Bay K 8644, and the vasorelaxing effect of polyamines and acetylpolyamines may be through their Ca^{2+} antagonistic property.

Ca^{2+} study

In Ca^{2+}-free solution, subsequent Ca^{2+} addition (5×10^{-5}–10^{-2} M) produced concentration-dependent contractions in rabbit thoracic aorta without functional endothelium (control in Figure 5). The concentration–response curve to Ca^{2+} in high K^+-depolarization medium ($K^+ = 120$ mM) was shifted to the right after incubation with spermine (Figure 5A) and N^1-acetylspermine (Figure 5B). Maximal contraction was reduced by $12{\cdot}8 \pm 0{\cdot}9\%$ (spermine) and $8{\cdot}6 \pm 0{\cdot}9\%$ (N^1-acetylspermine). The estimated EC50 values of Ca^{2+} in control and after incubation (10^{-2} M) with spermine and N^1-acetylspermine were $3{\cdot}9 \pm 0{\cdot}1$ M (control), $2{\cdot}9 \pm 0{\cdot}1$ M (spermine) and $3{\cdot}2 \pm 0{\cdot}2$ M (N^1-acetylspermine), respectively. The potency of spermine was greater than that of either spermidine or putrescine, and N^1- or N^8-acetylspermidine was less potent in relaxing Ca^{2+}-induced contraction than N^1-acetylspermine (data not shown). These observations suggest that both polyamines and acetylpolyamines have Ca^{2+} antagonistic property.

Effect of polyamines and acetylpolyamines on the potentiation of Ca^{2+} response elicited by Bay K 8644

Figure 6A shows that the Ca^{2+}-induced contraction was increased by the Ca^{2+} agonist Bay K 8644 (10^{-6} M), and reduced by the Ca^{2+} antagonist nifedipine (10^{-7} M). The potentiation of Ca^{2+}-induced contraction was reduced by nifedipine (10^{-7} M). Both spermine and N^1-acetylspermine

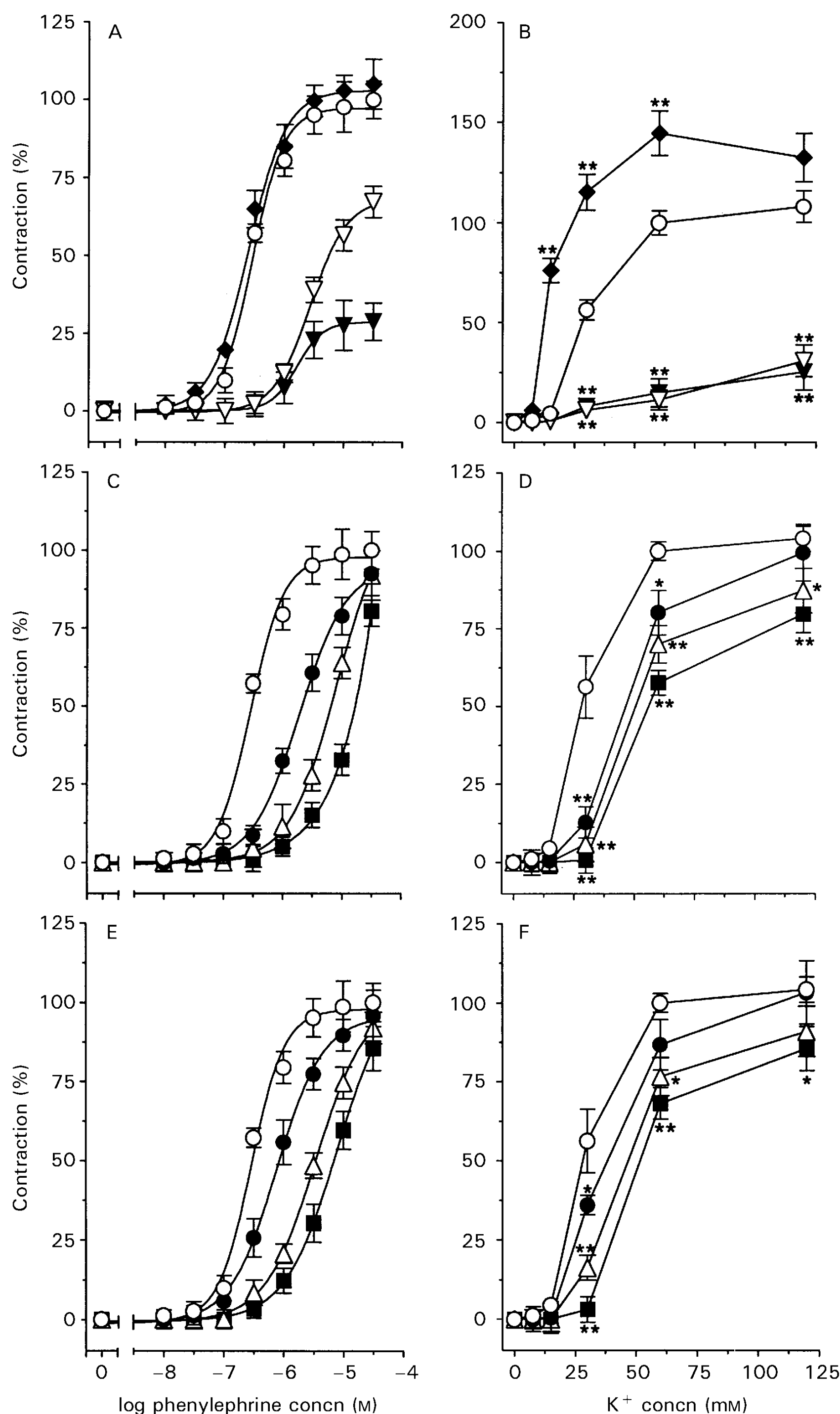

Figure 3. A. Effect of Ca^{2+} agonist, Bay K 8644 (◆, 1 μM) and antagonists, verapamil (▽, 5 μM) and nifedipine (▼, 0.1 μM) on the concentration-response curve to phenylephrine (○, control) in rabbit aortic rings. The difference between curves to control (phenylephrine only) and verapamil or nifedipine was statistically significant ($P < 0{\cdot}001$), but not statistically significant between control and Bay K 8644. B. Effect of Bay K 8644 (◆, 1 μM), verapamil (▽, 5 μM) and nifedipine (▼, 0.1 μM) on the concentration-response curves to K^+. $^{**}P < 0{\cdot}001$, $^{*}P < 0{\cdot}05$, significantly different response to K^+ compared with corresponding controls.

C and D. Effect of spermine (○, control; ●, 1 mM; △, 5 mM; ■, 10 mM) on the concentration-response curve to phenylephrine and K^+, respectively. The difference between concentration-response curves to phenylephrine in control and experimental group (pretreatment with spermine) at every concentration was statistically significant ($P < 0{\cdot}001$).

E and F. Effect of N^1-acetylspermine (○, control; ●, 1 mM; Δ, 5 mM; ■, 10 mM) on the concentration-response curve to phenylephrine and K^+, respectively. Responses were expressed as percentage of the previous contraction induced by 5×10^{-5} M phenylephrine ($8{\cdot}4 \pm 0{\cdot}7$ g) and 60 mM K^+ ($5{\cdot}0 \pm 0{\cdot}4$ g) in endothelium-denuded aortic rings. The difference between concentration–response curves to phenylephrine in control and experimental group (pretreatment with N^1-acetylspermine) at every concentration was statistically significant ($^{*}P < 0.05$; $^{**}P < 0{\cdot}001$). Values are means ± s.e.m., n = 12.

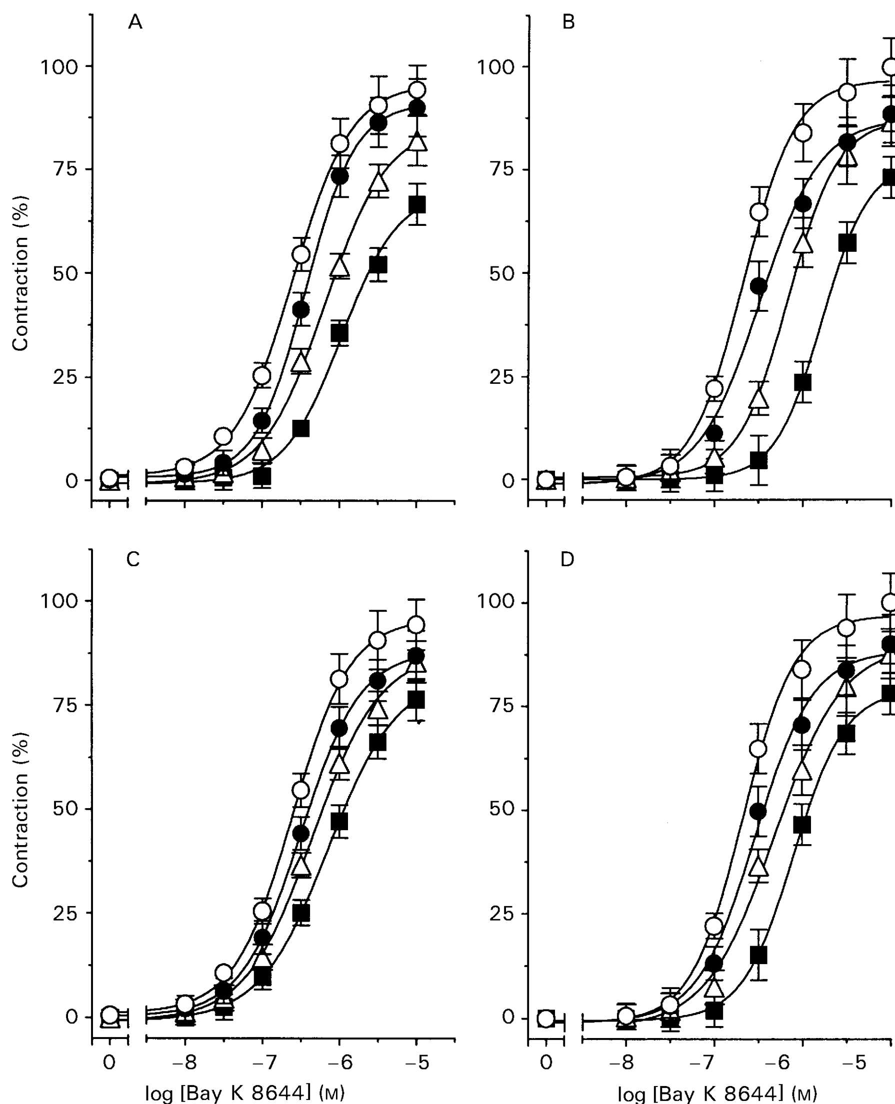

Figure 4. A and B. Effect of spermine (○, control; ●, 1 mM; △, 5 mM; ■, 10 mM) on the concentration–response curve to Bay K 8644 and A23187 in endothelium-denuded aortic rings, respectively. Responses were expressed as percentage of the previous contraction induced by 60 mM K^+ (5·0 ± 0·4 g) or 10^{-4} M A23187 (2·7 ± 0·3 g). The difference between curves to control (Bay K 8644 only or A23187 only) and experimental group (pretreatment with spermine) at every concentration (1 mM, $P < 0·01$, and 5 and 10 mM, $P < 0·001$) was statistically significant. C and D. Effect of N^1-acetylspermine (○, control; ●, 1 mM; △, 5 mM; ■, 10 mM) on the concentration–response curve to Bay K 8644 and A23187 in endothelium-denuded aortic rings, respectively. Responses were expressed as percentage of the previous contraction induced by 60 mM K^+ (5·0 ± 0·4 g) or 10^{-4} M A23187 (2·7 ± 0·3 g). The difference between curves to control (Bay K 8644 only or A23187 only) and experimental group (pretreatment of N^1-acetylspermine) at every concentration (1 mM, $P < 0·01$, and 5 and 10 mM, $P < 0·001$) was statistically significant. Values are means ± s.e.m., n = 13.

also inhibited the potentiation of Ca^{2+} concentration-dependent contraction by Bay K 8644 (Figure 6B). Thus, these results suggest that both polyamines and acetylpolyamines inhibit Ca^{2+} concentration-dependent contraction in rabbit thoracic aortic rings without functioning endothelium.

Discussion

This study demonstrates two major findings. Firstly, both polyamines and acetylpolyamines induce relaxation independent of the presence of endothelium in phenylephrine-precontracted rabbit thoracic aorta. Furthermore, reduced haemoglobin, L-NAME, indomethacin and methylene blue did not affect this relaxation. Secondly, both polyamines and acetylpolyamines relax the phenylephrine-, K^+- and Bay K 8644-induced contraction in a concentration-dependent manner. They also modified contractions evoked by the Ca^{2+} ionophore A23187 and shifted the concentration-dependent contraction curve to Ca^{2+} in high K^+

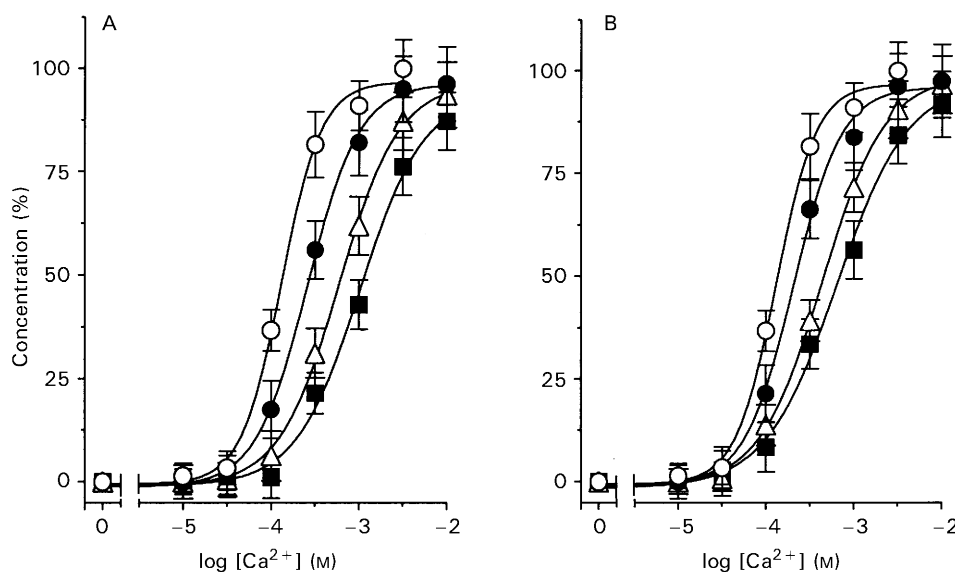

Figure 5. Effect of (A) spermine (○, control; ●, 1 mM; △, 5 mM; ■, 10 mM) and (B) N^1-acetylspermine (○, control; ●, 1 mM; △, 5 mM; ■, 10 mM) on Ca^{2+} concentration-dependent contraction curve in endothelium-denuded rabbit aortic rings. Responses were expressed as percentage of the previous contraction induced by 5 mM Ca^{2+} (6·3 ± 0·4 g). The difference between curves to control (Ca^{2+} only) and experimental group (pretreatment with either spermine or N^1-acetylspermine) at every concentration (1 mM, $P < 0{\cdot}01$ and 5 and 10 mM, $P < 0{\cdot}001$) was statistically significant. Values are means ± s.e.m., n = 12.

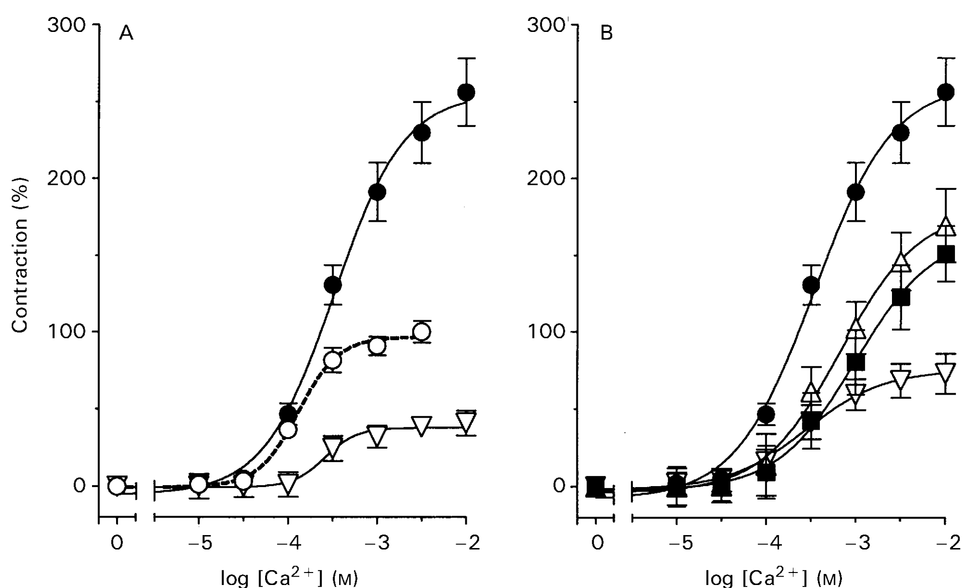

Figure 6. A. Effect of Ca^{2+} agonist, Bay K 8644 (○, 0 μM; ●, 1 μM) and antagonist, nifedipine (▽, 0·1 μM) on Ca^{2+} concentration-dependent contraction curve in endothelium-denuded rabbit aortic rings. The difference between curves to control and experimental group (pretreatment with either Bay K 8644 or nifedipine) was statistically significant ($P < 0{\cdot}001$). Bay K 8644, n = 8; nifedipine, n = 7. B. Effect of nifedipine (▽, 0·1 μM), spermine (■, 10 mM) and N^1-acetylspermine (△, 10 mM) on the potentiation of Ca^{2+} concentration-dependent contraction curve by Bay K 8644 (1 μM; ●, alone) in endothelium-denuded aortic rings. The difference between curves to control (pretreatment with Bay K 8644) and experimental group (pretreatment with either nifedipine, spermine or N^1-acetylspermine before pretreating with Bay K8644) was statistically significant ($P < 0{\cdot}001$). Responses were expressed as a percentage of the previous contraction induced by 5 mM Ca^{2+} (6·3 ± 0·4 g). Values are means ± s.e.m., n = 12 for spermine; n = 8 for N^1-acetylspermine.

medium to the right. These results suggest that both polyamines and acetylpolyamines dilate vascular smooth muscle by an endothelium-independent mechanism in-vitro and independent of endothelium-derived NO, vasodilator prostaglandins or activation of soluble guanylyl cyclase, and that they relax vascular smooth muscle by acting at the plasma membrane level, decreasing the influx of Ca^{2+}. Therefore, both polyamines and acetylpolyamines may have Ca^{2+} antagonistic properties

and a Ca^{2+} antagonistic property may be involved in the mechanism of rabbit aortic vascular smooth muscle relaxation by polyamines and acetylpolyamines.

Since the relaxing effect of polyamines was first demonstrated in gastrointestinal smooth muscle by De Meis (1967), spermine and spermidine have shown a relaxant effect in the uterus (Hashimoto et al 1973), respiratory tract (Chideckel et al 1985b) and vasculature (Chideckel et al 1985a; Wing et al 1993; Johnson et al 1996). Because polycationic polyamines and their metabolites can interact with negatively charged molecules, the number of positive charges on the molecule might play an important role in the function of both polyamines and acetylpolyamines. As seen in Figure 1, the relaxant effect of polyamines is positively correlated to the number of positive charges on the molecule (i.e., the vasodilatory effect of polyamines is in the order of spermine $(+4) > $ spermidine $(+3) > $ putrescine $(+2)$). Likewise, acetylspermine $(+3)$ is more effective than acetylspermidine $(+2)$ in decreasing vascular smooth muscle contractility. N^1-Acetylputrescine $(+1)$ had no effect on vascular smooth muscle, suggesting that a $+2$ charge may be the minimum to exert their vasorelaxant action. In these results, acetylspermine $(+3)$ is less effective than spermine $(+4)$ in relaxing vascular smooth muscle. Since in general, acetylation reduces the number of positive charges on the polyamine molecule, the metabolic process of polyamines may lead to a decrement in their vasorelaxant effect. However, the potency of spermidine $(+3)$ in relaxing vascular smooth muscle is less than that of acetylspermine $(+3)$, suggesting that there may be factors other than cationic effects in the action of polyamines and acetylpolyamines.

Polyamines bind to phospholipids (Schuber et al 1983), and this investigation supports the concept that polyamines may modulate membrane-related activities, such as Ca^{2+} movement. Since transmembrane Ca^{2+} influx via specific Ca^{2+} channels plays a crucial role in the excitation–contraction coupling of smooth muscle (Bolton 1979), a Ca^{2+} antagonistic property of polyamines and acetylpolyamines is likely to modulate muscular contractility, suggesting that they may act as naturally occurring Ca^{2+} antagonists. However, no direct evidence exists concerning changes in Ca^{2+} influx and contractile forces in vascular smooth muscle in response to polyamines.

Our results show that Bay K 8644 induces small, but not significant, contractile responses in segments of rabbit aortic rings without endothelium in basal condition. However, the moderate depolarization with 7·5 mM or 15 mM K^+ produced a marked potentiation of these responses, which were inhibited by both polyamines and acetylpolyamines, and by Ca^{2+} antagonists such as nifedipine and verapamil. Phenylephrine-induced contraction was not significantly increased by Bay K 8644, but K^+-induced contraction was increased by Bay K 8644. Nevertheless, Ca^{2+} antagonists block the potentiation of both phenylephrine- and K^+-induced contractions by Bay K 8644 (Godfraind et al 1986; Marin 1988). Bay K 8644 is known to facilitate Ca^{2+} influx (Freedman & Miller 1984; Salaices et al 1985; Gil-Longo et al 1992). Phenylephrine-induced contraction is dependent on the intra- and extracellular Ca^{2+} concentration (Weiss 1977; Bolton 1979; Cauvin et al 1983). Thus, these observations indicate that Bay K 8644 essentially facilitates Ca^{2+} entry through voltage-operated channels in this vascular preparation and is unable to produce an important Ca^{2+} influx through voltage-operated channels in resting conditions. In keeping with other observations (Rico et al 1990; Barrus et al 1995), these findings suggest that Bay K 8644 is a useful pharmacological tool to discriminate between voltage-operated and receptor-operated channels in this vascular preparation.

K^+ (60 mM)-induced constrictions were abolished in Ca^{2+}-free medium. Addition of Ca^{2+} produced concentration-dependent responses, consistent with other observations that the responses produced by K^+ are dependent upon extracellular Ca^{2+} (Godfraind et al 1986), which were reduced by nifedipine and increased by Bay K 8644. Both polyamines and acetylpolyamines relax the vascular smooth muscle contraction induced by phenylephrine and K^+ in this vessel. They also inhibit a sustained contraction by Bay K 8644 and the potentiation of phenylephrine- and K^+-induced contractions by Bay K 8644. These results suggest that both polyamines and acetylpolyamines may act on Ca^{2+} channels in the cell membrane regardless of receptor-operated or voltage-operated channels.

However, the possibility of another mechanism to transport Ca^{2+} across the cell membrane cannot be excluded however. The Ca^{2+} ionophore A23187 is known to form complexes with Ca^{2+} and transports this ion across membranes by a mechanism in which Ca^{2+} channels appear not to be involved (Reed & Lardy 1972). A23187 produces contractions in different smooth muscle preparations (Pressman 1973; Watson 1978) by its ability to increase the intracellular Ca^{2+} concentration. High concentrations of nifedipine and verapamil, which markedly reduced the responses elicited by phenylephrine and, particularly, by K^+, did not affect A23187-induced contractions, as reported (Watson

1978). However, both polyamines and acetylpolyamines reduced the response elicited by A23187. These results indicate that polyamines and acetylpolyamines are unable to discriminate between Ca^{2+} influx through Ca^{2+} channels (receptor-operated or voltage-operated channels) and that produced by another mechanism independent of these channels, as in the case of A23187. This suggests that polyamines and acetylpolyamines may act on the cell membrane to interfere with Ca^{2+} influx regardless of whether this influx is via Ca^{2+} channels or by a mechanism independent of these channels.

Since spermine has been shown to inhibit calmodulin or protein kinase C, leading to relaxation of smooth muscle (Mezzetti et al 1988; Walters & Johnson 1988), as well as inhibiting Ca^{2+} entry, the intracellular actions of both polyamines and acetylpolyamines could also be involved in their vascular effects. Therefore, the effect of polyamines and acetylpolyamines of inducing smooth muscle relaxation by mechanisms unrelated to extracellular Ca^{2+} remains to be determined.

In conclusion, the present investigation indicates that polyamines and acetylpolyamines induce endothelium-independent relaxation by an action at the plasma membrane level, decreasing the influx of Ca^{2+} in rabbit isolated thoracic aortic rings. Therefore, polyamine- and acetylpolyamine-induced relaxation may play an important role in the regulation of vascular tone as endogenous paracrine factors, independent of NO or NO-related mechanisms. Polyamines and acetylpolyamines may have Ca^{2+} antagonistic activity which may, in part, be the mechanism by which they produce their vasodilatory action in rabbit aortic vascular smooth muscle.

References

Barrus, M. T., Reviriego, J., Marin, J. (1995) Effect of Ca^{2+} agonist Bay K 8644 in human placental arteries. Gen. Pharmacol. 26: 989–996

Bolton, T. B. (1979) Mechanisms of action of transmitters and other substances on smooth muscle. Physiol. Rev. 59: 606–718

Bueb, J. L., Da Silva, A., Mousli, M., Landry, Y. (1992) Natural polyamines stimulate G-proteins. Biochem. J. 282: 545–550

Cauvin, C., Loutzenhiser, R., Van Breemen, C. (1983) Mechanisms of calcium antagonist-induced vasodilation. Annu. Rev. Pharmacol. Toxicol. 23: 373–396

Chideckel, E. W., Dedhia, H. V., Fedan, J. S., Teba, L., Jain, A. (1985a) Polyamines relax vascular and respiratory tract smooth muscle. Clin. Res. 44: 175A

Chideckel, E. W., Fedan, J. S., Mike, P. (1985b) Polyamines and putreanine relax respiratory tract smooth muscle in the guinea-pig. Eur. J. Pharmacol. 116: 187–190

De Meis, L. (1967) Relaxing effect of spermine and spermidine on intact and glycerol-treated muscle. Am. J. Physiol. 212: 92–96

Fernandez, A. I., Cantabrana, B., Sanchez, M., Hidalgo, A. (1995) Extracellular and intracellular effects of polyamines on smooth muscle contractions. Life Sci. 57: 855–861

Freedman, S. B., Miller, R. J. (1984) Calcium channel activation: a different type of drug action. Proc. Natl Acad. Sci. USA 81: 5580–5583

Gil-Longo, J., Orallo, F., Verde, I., Campos, M., Calleja, J. M. (1992) Role of the endothelial system in Bay K 8644 enantiomer and nifedipine vasomodulator action in rat aorta. Eur. J. Pharmacol. 221: 1–8

Godfraind, T., Miller, R., Wibo, M. (1986) Calcium antagonism and calcium entry blockade. Pharmacol. Rev. 38: 321–416

Groblewski, G. E., Hargittai, P. T., Seidel, E. R. (1992) Ca^{2+}/calmodulin regulation of putrescine uptake in cultured gastrointestinal epithelial cells. Am. J. Physiol. 262: C1356–C1363

Hashimoto, H., Unemoto, T., Hayashi, M. (1973) Inhibitory action of spermine on the contractions of rat uterus. Am. J. Physiol. 225: 743–746

Hrabie, J. A., Klose, J. R., Wink, D. A., Keefer, L. K. (1993) New nitric oxide-releasing zwitterions derived from polyamines. J. Org. Chem. 58: 1472–1476

Johnson, T. D., Childres, W. F., Bryan, R. J. (1996) The *in vitro* action of polyamines on rat basilar and femoral artery contractile activity. Life Sci. 59: 529–536

Koenig, H., Fan, C. C., Goldstone, A. D., Lu, C. Y., Trout, J. J. (1989) Polyamines mediate androgenic stimulation of calcium fluxes and membrane transport in rat heart myocytes. Circ. Res. 64: 415–426

Lenzen, S., Rustenbeck, I. (1991) Effects of IP_3, spermine, and Mg^{2+} on regulation of Ca^{2+} transport by endoplasmic reticulum and mitochondria in permeabilized pancreatic islets. Diabetes 40: 323–326

Marin, J. (1988) Vascular effects of calcium antagonists. Uses in some cerebrovascular disorders. Gen. Pharmacol. 19: 295–306

Martin, W., Villani, G. M., Jothianandan, D., Furchgott, R. F. (1985) Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. J. Pharmacol. Exp. Ther. 232: 708–716

Mezzetti, G., Monti, M. G., Moruzzi, M. S. (1988) Polyamines and the catalytic domain of protein kinase C. Life Sci. 42: 2293–2298

Moncada, S., Palmer, R. M., Higgs, E. A. (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol. Rev. 43: 109–142

Morgan, D. M. (1994) Polyamines, arginine and nitric oxide. Biochem. Soc. Trans. 22: 879–883

Morgan, D. M., Baydoun, A. R. (1994) Polyamine transport and arginine pool size in vascular endothelial cells. Biochem. Soc. Trans. 22: 387S

Morgan, J. E., Blankenship, J. W., Matthews, H. R. (1986) Association constants for the interaction of double-stranded and single-stranded DNA with spermine, spermidine, putrescine, diaminopropane, N^1- and N^8-acetylspermidine, and magnesium: determination from analysis of the broadening of thermal denaturation curves. Arch. Biochem. Biophys. 246: 225–232

Morgan, D. M., Coade, S. B., Pearson, J. D. (1990) Polyamines stimulate calcium uptake by human vascular endothelial cells. Biochem. Soc. Trans. 18: 1223–1224

Motulsky, H. J., Ransnas, L. A. (1987) Fitting curves to data using nonlinear regression: a practical and nonmathematical review. FASEB J. 1: 365–374

Pegg, A. E. (1988) Polyamine metabolism and its importance in neoplastic growth and a target for chemotherapy. Cancer Res. 48: 759–774

Pressman, B. C. (1973) Properties of ionophores with broad range cation selectivity. Federation Proc. 32: 1698–1703

Rasmussen, H., Barrett, P. Q. (1984) Calcium messenger system: an integrated view. Physiol. Rev. 64: 938–984

Reed, P. W., Lardy, H. A. (1972) A23187: a divalent cation ionophore. J. Biol. Chem. 247: 6970–6977

Rees, D. D., Palmer, R. M., Schulz, R., Hodson, H. F., Moncada, S. (1990) Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. Br. J. Pharmacol. 101: 746–752

Rico, I., Alonso, M. J., Salaices, M., Marin, J. (1990) Pharmacological dissection of Ca^{2+} channels in the rat aorta by Ca^{2+} entry modulators. Pharmacology 40: 330–342

Salaices, M., Marin, J., Sanchez-Ferrer, C. F., Reviriego, J. (1985) The effects of BAY-K-8644 on the contraction of cat middle cerebral and femoral arteries. Biochem. Pharmacol. 34: 3131–3135

Schuber, F. (1989) Influence of polyamines on membrane functions. Biochem. J. 260: 1–10

Schuber, F., Hong, K., Duzugunes, N., Papahadjopoulos, D. (1983) Polyamines as modulators of membrane fusion, aggregation and fusion of liposomes. Biochemistry 22: 6134–6140

Tabor, C. W., Tabor, H. (1984) Polyamines. Annu. Rev. Biochem. 53: 749–790

Walters, J. D., Johnson, J. D. (1988) Inhibition of cyclic nucleotide phosphodiesterase and calcineurin by spermine, a calcium-independent calmodulin antagonist. Biochim. Biophys. Acta 957: 138–142

Watson, E. L. (1978) Effects of ionophores A23187 and X537A on vascular smooth muscle activity. Eur. J. Pharmacol. 52: 171–178

Weiss, G. B. (1977) Calcium and contractility in vascular smooth muscle. In: Takahashi, T., Bianchi, C. P. (eds) Advances in General and Cellular Pharmacology. Vol. II, Plenum Press, New York, pp 71–152

Wing, L. Y., Chan, C. F., Jiang, M. J. (1993) Acetylpolyamines decrease blood pressure, $[Ca^{++}]_i$ and isometric force of vascular smooth muscle. J. Pharmacol. Exp. Ther. 266: 179–183