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Effect of the Ionophore, A23187, on Contraction and Relaxation of Rat Arteries and Veins (40347)

MARLENE L. COHEN, KATHRYN S. WILEY, AND RALMOND H. TUST

The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46206

Calcium ion plays a crucial role in contraction, relaxation and receptor binding in vascular smooth muscle (1, 2). Many approaches have been used to evaluate the role of calcium in vascular function. The discovery of antibiotic ionophores capable of transporting bivalent ions such as calcium across membranes provides a novel approach to improve our understanding of the importance of calcium in the contractile process. The action of the calcium ionophore, A23187, on contraction of certain blood vessels coupled with data on the role of extracellular calcium in this process may disclose important differences in calcium utilization among blood vessels.

Furthermore, use of A23187 may aid in our understanding of the mechanism by which norepinephrine relaxes the rat jugular vein (3). We have proposed that beta adrenergic receptor stimulation by norepinephrine may be more pronounced in veins than in arteries (4) with the rat jugular vein providing an extreme example of this phenomenon. Changes in calcium availability in this tissue must be examined as a potential explanation for the inability of norepinephrine to contract the rat jugular vein. Therefore, in the present study, we compared ionophore-induced changes in responses of the rat jugular vein to those of the femoral vein, a vein that contracts maximally to norepinephrine (5). The effect of A23187 on the responses of both these veins was then compared with the effect in two rat arteries, the aorta and carotid artery.

Methods. Isolation of vascular tissue. Male Wistar rats (150–300 g) (Harlan Industries, Inc., Cumberland, IN) were killed by a blow to the head. External jugular veins, femoral veins, aortas or carotid arteries were dissected free of connective tissue, cannulated *in situ* with polyethylene tubing (PE #50, OD = 0.97 mm) and placed in Petri dishes containing Krebs' bicarbonate buffer (see below). The tips of two 30 gauge stainless steel hypodermic needles bent into an L-shape were slipped into the polyethylene tubing. Vessels were gently pushed from the cannula onto the needles. The needles were then separated so that the lower one was attached with thread to a stationary glass rod and the upper one was tied with thread to the transducer. This is the procedure for ring preparations (circular smooth muscle) of blood vessels described by Hooker *et al.* (6).

Veins were placed in organ baths containing 10 ml of modified Krebs' solution of the following composition (m*M* concentrations) except when calcium concentration was varied: NaCl, 118.2; KCl, 4.6; $CaCl_2 \cdot 2H_2O$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; dextrose, 10.0 and $NaHCO_3$, 24.8. This solution was maintained at 37° and aerated with 95% O_2 and 5% CO_2. Initial optimum resting force was 4 g for the arteries and 1 g for the veins (3, 5). Isometric contractions were recorded as changes in grams of force on a Beckman Dynograph with Statham UC-3 transducers and microscale accessory attachments. Tissues were allowed to equilibrate 1–2 hr before exposure to drugs.

Effect of A23187 on contractile responses to norepinephrine, serotonin and potassium chloride. Cumulative concentration-response curves were obtained from baseline tension by a stepwise increase in concentration after a steady response occurred to the preceding dose. Tissues were then exposed to either A23187 or a solvent control for one hour and then rechallenged with the contractile agonist. Contractile responses were calculated as the change in grams of force for each concentration of agonist. To minimize variability among preparations, maximum response to each agonist before A23187 was considered 100% and contractile responses after A23187 or a solvent control were calculated as a percent of the initial maximum concentration

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in each tissue. In each experiment, the effect of A23187 was compared with a solvent control.

Effect of A23187 on relaxation responses to norepinephrine, papaverine and nitroglycerin. Jugular veins were contracted to a moderate degree of tone with serotonin (1.78×10^{-7} *M*) or potassium chloride (17–50 m*M*). Once the contraction reached a plateau, relaxant agonists were added and maximum tissue relaxation for each dose was measured. Relaxation of the contracted tissue back to baseline tension represented 100% relaxation. These studies were then repeated after one hour exposure to A23187 or a solvent control.

Effect of extracellular calcium on contraction in the rat jugular and femoral veins. Initial contractile responses were determined as detailed above in 2.5 m*M* $CaCl_2$ in all tissues. Buffer was then changed to Krebs' solution containing 0.825 m*M* calcium, 0.250 m*M* calcium and finally no added calcium in the presence of 0.1 m*M* Na_2EDTA. The calcium concentration of this solution was estimated to be less than 10^{-6} *M* calcium. In other experiments, buffer was changed to contain 3.75 and 5.0 m*M* calcium. Contractile responses were repeated after approximately 20 min exposure to each calcium concentration. Maximum contraction at each calcium concentration was expressed as a percent of the response in 2.5 m*M* calcium.

Calcium determination. Total tissue calcium was determined in HNO_2-H_2O_2 digests (7) with a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer.

Drugs used. All drugs were prepared daily in saline except A23187 and kept on ice during the course of the experiments. A23187 was prepared as an opalescent aqueous solution (8) by dissolving A23187 in 0.5 ml dimethylsulfoxide (DMSO) and diluting with deionized distilled water. The solvent control was prepared in the same way by omitting the A23187. By this technique, the maximum volume of DMSO added to the 10 ml bath was 5 μl. The source of the drugs used was as follows: *l*-arterenol bitartrate (norepinephrine), *l*-isoproterenol-*d*-bitartrate dihydrate, Sterling Chemical Co.; 5-hydroxytryptamine creatinine sulfate complex (serotonin), Sigma Chemical Co.; potassium chloride, Baker Chemical Co.; nitroglycerin U.S.P., papaverine hydrochloride, A23187, Eli Lilly and Co.

Results. Effect in arteries. The ionophore, A23187 (1.5×10^{-6} *M*) did not have a marked effect on the baseline force of either the aorta or carotid artery. In six out of eight aortas, A23187 (1.5×10^{-6} *M*) produced a small, slow contraction over 1 hr that was 14.8 ± 5.8% of the maximum force generated by the tissue. Three out of seven carotid arteries developed a similar slow contraction over one hour that was 21.9 ± 2.0% of the maximum force. The force developed in the presence of the ionophore was dose-dependent. No increase in force was observed in any solvent treated tissues (aorta $n = 8$; carotid artery $n = 6$). Except for this increase in baseline force, responses of aortas and carotid arteries to serotonin, norepinephrine or potassium chloride (Figs. 1 and 2) did not change after A23187 (1.5×10^{-6} *M*).

Effect in veins. In some jugular veins, A23187 (1.5×10^{-6} *M*) produced a slow small contraction over 1 hr but this was not observed in any of the femoral veins examined ($n = 8$). In the femoral vein, A23187 (1.5×10^{-6} *M*) exposure for 1 hr decreased the contractile response to norepinephrine and serotonin with a marked reduction in maximum force (Fig. 3). Contraction to potassium chloride, however, was not altered.

Similarly, in the jugular vein, there was a reduction in the maximum force produced by serotonin after A23187 (1.5×10^{-6} *M*) (Fig. 4). A23187 did not inhibit contraction to potassium chloride and if anything, produced an enhanced sensitivity to potassium chloride.

Because the rat jugular vein relaxes to many agonists including norepinephrine (3), we examined the effect of the ionophore on vascular relaxation in this issue. After one hour exposure to the ionophore, tissues contracted with low doses of serotonin did not relax completely back to baseline after washing. This was most obvious with 3×10^{-6} *M* but was observed with concentrations as low as 0.75×10^{-6} *M*. Even addition of isoproterenol (10^{-7} *M*) did not reduce force in such tissues although 10^{-8} *M* isoproterenol produced a 55% reduction in serotonin-induced force prior to A23187.

After the ionophore, serotonin-contracted

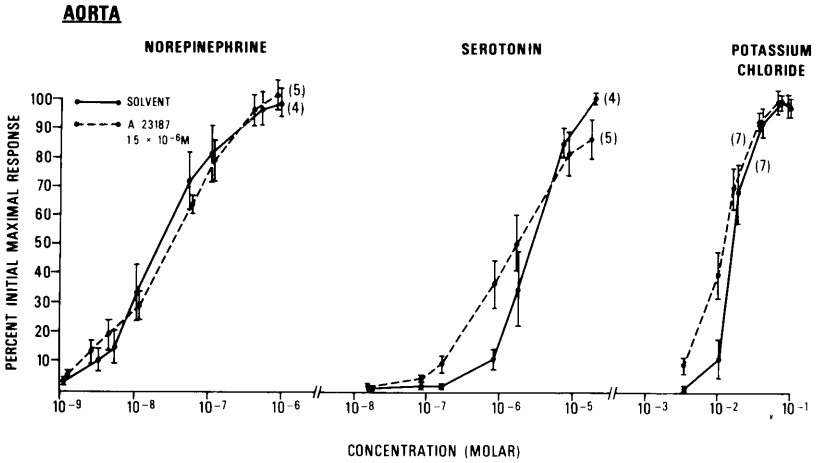

FIG. 1. Effect of A23187 (1.5×10^{-6} M) and solvent treatment (see Methods) on rat aortic contraction to serotonin, norepinephrine and potassium chloride. Points are means ± SE for the number of tissues in parentheses.

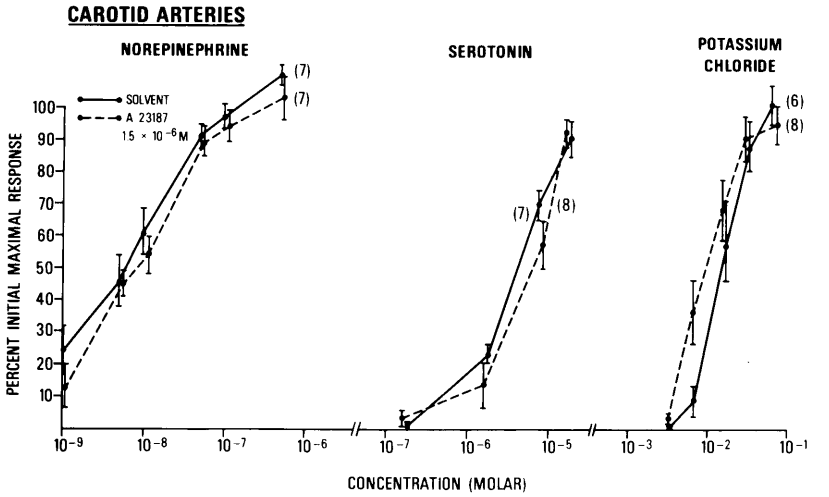

FIG. 2. Effect of A23187 (1.5×10^{-6} M) and solvent treatment (see Methods) on contraction of rat carotid arteries to serotonin, norepinephrine and potassium chloride. Points are means ± SE for the number of tissues in parentheses.

veins relaxed significantly less to all the relaxant agonists examined; i.e., norepinephrine, papaverine and nitroglycerin (Table I). When jugular veins were contracted with potassium chloride, no difference occurred in relaxation to norepinephrine (10^{-5} M) (n = 6) or papaverine (5×10^{-5} M) (n = 7) after A23187 (1.5×10^{-6} M). Thus, in the jugular vein, the defective relaxation demonstrated with norepinephrine, papaverine and nitroglycerin may be related to the inhibitory effect of A23187 on serotonin-induced contractions. It is of interest that norepinephrine in concentrations up to 2×10^{-4} M even after A23187 did not contract the rat jugular vein.

Role of extracellular calcium in venous responses to serotonin and potassium chloride. Since A23187 differentially affected serotonin and potassium chloride-induced contractions in the jugular and femoral veins, we investigated the role of extracellular calcium in the contraction to these agonists. For comparison, a similar analysis has previously been reported for both serotonin and potassium chloride in the rat aorta (9).

As extracellular calcium concentration was reduced, maximum force developed to both serotonin and potassium chloride declined in jugular and femoral veins (Table II). Decline in maximum response was similar for sero-

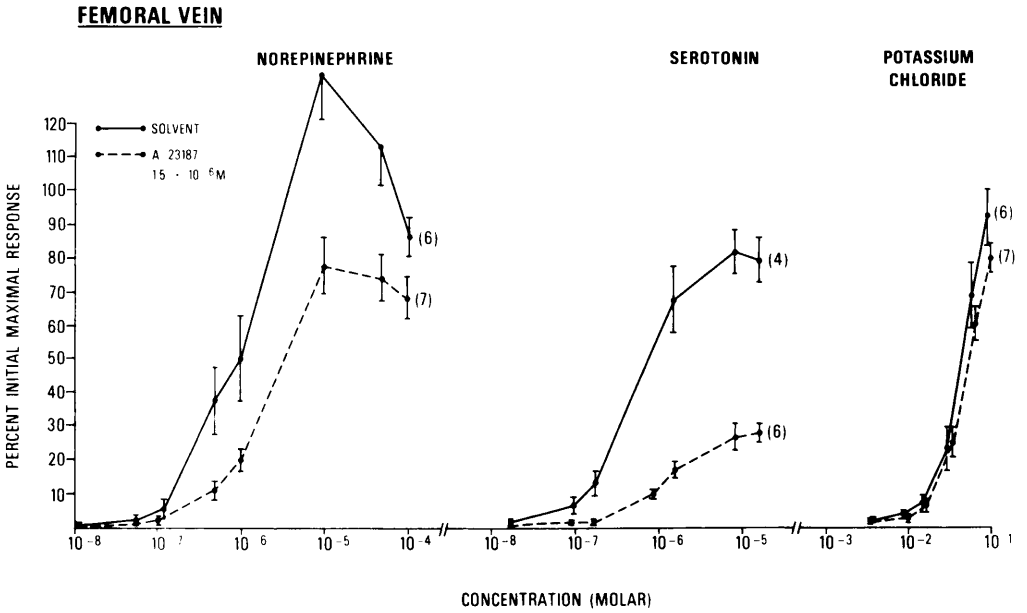

FIG. 3. Effect of A23187 (1.5×10^{-6} *M*) and solvent treatment (see Methods) on contraction of rat femoral veins to serotonin, norepinephrine and potassium chloride. Points are means ± SE for the number of tissues in parentheses.

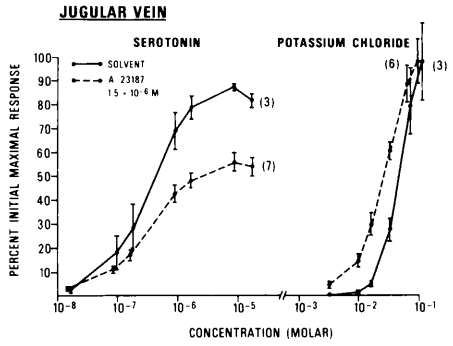

FIG. 4. Effect of A23187 (1.5×10^{-6} *M*) and solvent treatment (see Methods) on contraction of rat jugular veins to serotonin and potassium chloride. Points are means ± SE for the number of tissues in parentheses.

tonin and potassium chloride. In the femoral vein, lowering extracellular calcium also reduced responses to norepinephrine, but reduction in norepinephrine contraction was less than for serotonin and potassium chloride at 0.825 m*M* and 0.250 m*M* calcium. In jugular veins, higher calcium concentrations did not significantly affect the maximum response to either serotonin or potassium chloride (Table II).

Tissue calcium. Total calcium did not differ between jugular veins (.055 ± .004 μeq Ca^{2+}/mg dry tissue; $n = 11$) and femoral veins (.058 ± .007 μeq Ca^{2+}/mg dry tissue; $n = 7$).

Discussion. The effect of the calcium ionophore, A23187, on vascular smooth muscle has not been widely studied. The present investigation in both rat arteries and veins confirms the slow and minimal direct contractile effect of A23187 shown on aortic tissue (10). The lack of a marked contraction of rat blood vessels to A23187 is in contrast to its reported contractile effectiveness in guinea pig fundus, taenia coli (11), ileum (12), bronchi (13), atrium (8) and the stomach muscularis from *Bufo marinus* (14). Other smooth muscle preparations such as the vas deferens (12) have been reported not to contract to A23187. Differences in the contractile effectiveness of A23187 in various smooth muscles is consistent with the concept of differences in the calcium availability or utilization among such tissues.

Another way to evaluate an action of A23187 in vascular tissue is to determine its effect on contractile responses to other agonists. No enhancement of maximum contraction to serotonin, norepinephrine or potassium chloride occurred in any vessel examined. The use of A23187 in vascular tissue revealed two major findings: (1) A23187 rather than enhancing contractile responses,

TABLE I. EFFECT OF A23187 ON RELAXANT RESPONSES IN RAT JUGULAR VEIN.

	Control	After solvent[a]	After A23187 (1.5×10^{-6} M)[a]
		Percent relaxation[b]	
Norepinephrine (10^{-6} M)	51.9 ± 6.8 (6)	64.5 ± 7.3 (6)	21.2 ± 6.4 (6)[c]
Papaverine (10^{-5} M)	46.1 ± 6.8 (6)	65.8 ± 8.8 (5)	−4.0 ± 7.7 (3)[c]
Nitroglycerin (10^{-7} M)	44.2 ± 5.0 (6)	39.6 ± 3.2 (5)	18.9 ± 5.2 (3)[c]

[a] Solvent (see Methods) or A23187 were in contact with the tissue for 1 hr.

[b] Tissues were contracted to a moderate tone with serotonin (1.8×10^{-7} M) and when contraction reached a plateau, relaxant agonist was added. Relaxation was measured three min later. Values are means ± SE for the number of tissues in parentheses.

[c] Relaxation was significantly less ($P < .05$) than control relaxation as determined with Student's t test.

TABLE II. EFFECT OF EXTRACELLULAR CALCIUM CONCENTRATION ON THE CONTRACTILE RESPONSES OF RAT JUGULAR AND FEMORAL VEINS TO POTASSIUM CHLORIDE, SEROTONIN AND NOREPINEPHRINE.

	Extracellular calcium concentration (mM)					
	0[a]	0.25	0.825	2.5	3.75	5.0
	Percent contraction in 2.5 mM calcium[b]					
Jugular vein						
Potassium chloride (4) (130 mM)	27.6 ± 6.5	49.3 ± 2.7	58.8 ± 3.6	100	93.3 ± 6.2	99.4 ± 6.8
Serotonin (4) (9×10^{-6} M)	0.6 ± 0.2	23.7 ± 3.1	54.8 ± 8.1	100	81.7 ± 4.8	81.8 ± 2.4
Femoral vein						
Potassium chloride (7) (130 mM)	15.2 ± 1.6	19.1 ± 1.4	45.7 ± 3.9	100	—	—
Serotonin (7) (9×10^{-6} M)	1.6 ± 0.9	33.0 ± 3.2	49.9 ± 3.6	100	—	—
Norepinephrine (5) (10^{-5} M)	5.3 ± 2.7	51.0 ± 11.8	72.1 ± 10.2	100	—	—

[a] Buffer contains no added calcium in the presence of 0.1 mM Na_2 EDTA.

[b] Values are means ± SE for the number of tissues in parentheses.

selectively inhibited the maximum force developed to serotonin and norepinephrine but not to potassium chloride, and (2) this effect only occurred in the two rat veins examined and not in the aorta or carotid artery.

We considered the possibility that in veins, contractile responses to serotonin and norepinephrine might utilize tissue calcium stores that differ from those utilized or mobilized during contractile responses to potassium chloride and that dependence on extracellular calcium in veins differed from arteries. However, our data indicate that serotonin and potassium chloride both rely on extracellular sources of calcium in veins, yet only the response to serotonin was reduced after A23187. Additionally, norepinephrine dependence on extracellular calcium in the femoral vein was similar to the aorta (9, 15–17), yet A23187 did not affect aortic responses. Thus, there was no correlation between dependence on extracellular calcium and the inhibitory effect of A23187 in veins. The possibility that in veins, A23187 produced a large increase in intracellular calcium, that actually inhibited the response to serotonin or norepinephrine is also unlikely. When extracellular calcium was raised in the jugular vein, response to serotonin was not markedly inhibited as occurred with A23187.

Differences in calcium utilization between jugular and femoral veins have been proposed to explain the opposite effects of norepinephrine in these tissues, i.e., norepinephrine relaxed the rat jugular vein (3) and contracted the femoral vein (5). Since both veins responded similarly to A23187 and to manipulation of extracellular calcium, calcium uti-

lization does not appear to differ between the jugular and femoral veins. Furthermore, we considered the possibility that total calcium levels may be lower in the jugular vein than in the femoral vein. However, there was no difference in calcium levels between these veins. Thus, although based on indirect evidence, we propose that differences in calcium handling do not provide a satisfactory explanation for the unusual responsiveness of the rat jugular vein.

Summary. The present study describes differences in the effect of the ionophore, A23187, on contraction and relaxation in certain rat arteries and veins. A23187 selectively inhibited maximal contraction to receptor agonists such as serotonin and norepinephrine in veins but not arteries. Furthermore, based on the role of extracellular calcium, the action of A23187 and measurement of total calcium levels, no difference in calcium handling was apparent between the rat jugular and femoral veins. Therefore, relaxation of the rat jugular vein to norepinephrine is probably unrelated to any uniqueness in calcium utilization.

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