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Acetate relaxation of isolated vascular smooth muscle

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Acetate relaxation of isolated vascular smooth muscle. The vasorelaxant effects of acetate in arginine vasopressin (AVP)-contracted rat tail artery strips were examined in order to study mechanism of action. Dose–dependent relaxation by acetate was found in the clinically important range of 4 to 16 mM. Relaxation was not due to complexing of ionized calcium, persisted after mechanical removal of the endothelium, and was not altered by pretreatment with indomethacin. Although acetate also inhibited contraction by alpha-1 and alpha-2 agonists, the relaxant effect was not altered by destruction of sympathetic nerve terminals using 6-hydroxydopamine. The degree of relaxation in this model by various anions correlated with their lyotropic properties; however, the vasorelaxant effect of acetate exceeded that which would be expected on the basis of its position in the lyotropic series. The vasorelaxant effect of acetate was shared by other short–chain fatty acids that can be conjugated with coenzyme A (CoA), such as propionate and malonate. In contrast, a much lesser or absent relaxant effect was found with nonfatty–acid precursors of acetyl CoA, such as pyruvate, lactate, and alanine. The vasorelaxant effect of acetate was abolished by pretreatment with DIDS, an inhibitor of organic anion uptake, suggesting that cellular uptake of acetate is essential to its vasorelaxant action. The results suggest that the relaxant effect of acetate in vascular smooth muscle is non-specific, is not mediated by prostaglandins, does not depend upon the presence of either endothelium or the sympathetic nervous system, and may be due to metabolism of acetate to acetyl CoA with attendant conversion of ATP to AMP.

Acetate is commonly used in hemodialysis as a bicarbonate–generating base. However, patients sometimes become hypotensive when dialyzed against an acetate buffered dialysate [1]. Although acetate has long been known to be a vasodilator [2–6] little is known about the mechanism of its action. In preliminary studies [7, 8], we established that helical strips of the rat caudal artery contracted with potassium chloride (K), phenylephrine, or arginine vasopressin (AVP) could all be relaxed by acetate, with the greatest amount of relaxation being evident in strips contracted with AVP. The objective of the present experiments was to examine the vasorelaxant abilities of acetate under various conditions in an attempt to further delineate its mechanism of action.

Methods

Preparation of tissues

Studies were performed in caudal arteries taken from male Sprague–Dawley rats weighing between 300 and 500 g. Each rat was killed by cervical dislocation. The tail of the rat was then submerged in modified Krebs–Henseleit (KH) solution containing, in mM: Na 140; K 5.0; HCO_3 25; Cl 124; Mg 0.8; Ca 1.35; and dextrose 11. The KH solution was gassed with 95% oxygen and 5% carbon dioxide. The tail artery was carefully removed and cut into helical strips of 1.0 to 1.5 cm in length. Dissection and cleaning of the artery and cutting of the strips were performed at room temperature. The strips were then suspended in warmed (37°C) KH solution and equilibrated under a resting tension of 0.7 g for two hours. The KH solution in the bath was changed every 15 minutes. After the two–hour equilibration period, at 30 minute intervals, the strip was repeatedly contracted by three minute exposure to 100 mM KCl (added from a 4.0 M stock solution). Once the peak phasic contraction to KCl had stabilized, the strip was considered ready for study.

Experimental design

Acetate inhibition of AVP-induced contraction. Dose–response curves to AVP were performed in the presence and absence of acetate. Six strips from each rat ($N = 11$) were randomized into six groups: control, 4 mM acetate, 16 mM acetate, 16 mM acetate in which the concentration of $CaCl_2$ was increased by 5%, 16 mM acetate in which the concentrations of both $CaCl_2$ and $MgCl_2$ were increased by 5%, and 64 mM acetate. The solutions with additional calcium were prepared to counter the fact that, in a 16 mM acetate solution, approximately 5% of the ionized calcium will be complexed [8]. The amount of magnesium complexing by acetate is unknown, but was presumed to be of similar magnitude. In previous studies, we showed that acute reductions in either bath calcium or bath magnesium concentration will cause relaxation in AVP-contracted rat tail artery strips [7].

For each of the acetate groups, a modified KH solution was prepared in which the desired amount of sodium acetate was substituted for an identical amount of sodium chloride. Hence, the sodium level of all the solutions used was identical. Similarly, the bicarbonate concentration in all baths used remained at 25 mM.

Strips assigned to the acetate groups were exposed to the acetate containing solution for 15 minutes prior to performing the AVP dose–response curve. AVP was added in a cumulative fashion from appropriately–diluted stock solutions. Only one dose–response curve was performed per strip. For each strip, the contractions induced by each dose of AVP were normalized

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to the peak phasic contraction to 100 mM K, the latter having been measured during the preconditioning procedure.

Acetate relaxation of AVP-contracted strips. Six strips from each rat ($N = 7$) were randomized into six groups as above. The strips, while in KH solution, were contracted by addition of 2×10^{-9} M AVP to the bath. After approximately five minutes, when the contraction had peaked, the KH bath containing AVP was isothermically replaced with a second bath containing the same concentration of AVP but modified in composition to include 0, 4, 16, or 64 mM acetate. Again, three 16 mM acetate solutions with varying amounts of calcium and magnesium were used, to give a total of six groups as described above. The percent relaxation of each strip (in terms of the peak AVP contraction) after five minutes of exposure to acetate was the dependent variable.

Effect of position along the tail artery. All strips used in the present studies were taken from the mid-portion of the tail. Because the effect of hydralazine, for example, has been shown to vary with position along the tail artery [9], experiments were done comparing the relaxant effect of 16 mM acetate (substituted for chloride) on AVP-contracted (2×10^{-9} M) strips taken from different positions along the mid-tail. Six 1.5 cm strips were cut from the mid-tail of each rate ($N = 7$). Each strip was contracted with AVP in KH solution and relaxed with acetate, as above. Relaxation was compared among the strips as a function of their position along the tail.

Effect of endothelium removal. Six strips from each rat ($N = 15$) were randomly assigned to two groups, a control group and an endothelium removed group. In the latter, the endothelium was removed by gentle rubbing with a cotton tipped applicator at time of initial preparation. In each group, strips were contracted with AVP (2×10^{-9} M), and then exposed to 0, 4, or 16 mM acetate. The dependent variable was percent relaxation after five minutes exposure to acetate in control versus endothelium–denuded tissue. After each experiment was completed, the completeness of endothelium removal was assessed by contracting the strip with AVP a second time, and assessing the relaxant effect of acetylcholine (10^{-7} through 10^{-5} M). Because ACh is an endothelium dependent vasodilator, the peak relaxation to ACh was used as an indicator of the presence of functioning endothelium.

Effect of indomethacin. Six strips from each rat ($N = 7$) were randomized into two groups, a control, and an indomethacin treated, and then into three subgroups (0, 4, or 16 mM acetate). In those strips randomized into the indomethacin group, indomethacin 10^{-5} M was introduced into the bath after equilibration. Fifteen minutes later, in the continued presence of indomethacin, an AVP contraction was initiated. At the peak of the contraction, the substitution solution containing indomethacin, AVP and 0, 4, or 16 mM acetate was instilled. The percent relaxation at each level of acetate was determined and compared to control experiments performed in identical fashion but in the absence of indomethacin.

Effect of chemical sympathectomy. Six strips from each rat ($N = 10$) were randomized into two groups: a control and a sympathectomized. Sympathectomized strips were exposed thrice (7 minutes each time) to a solution containing 6-hydroxydopamine, phentolamine, and glutathione (0.065 mM), according to a method modified from that described by Aprigliano and Hermsmeyer [10]. The latter solution was not oxygenated to minimize oxidative tissue damage. The control strips were exposed for an identical length of time to a modified solution from which 6-hydroxydopamine, the active agent, had been omitted, Each strip was contracted with AVP (2×10^{-9} M), and then exposed to a relaxation solution containing AVP and 0, 4, or 16 mM acetate, depending upon subgroup assignment. The dependent variable was percent relaxation five minutes after exposure to acetate in control versus sympathectomized tissue. After each study, adequacy of sympathectomy was assessed by performing a dose–response curve to tyramine.

Effect of DIDS. The design was identical to that for indomethacin, except that 1 mM DIDS (4,4′-disothiocyanatostilbene-2,2′-disulfonic acid), a compound that has been shown to block cell uptake of anions [11, 12], was the additive. DIDS addition was begun simultaneously with the addition of AVP. Each group was made up of strips from eight rats.

Acetate effect on dose–response curves to methoxamine and BHT-920. This was the only group of studies in which AVP was not the contracting agent. The purpose was to determine the ability of acetate to inhibit contractions induced by alpha-1 and alpha-2 agonists. Pilot studies showed that several sequential dose–response curves to either of these compounds could be obtained in the same strip without tachyphylaxis. Two strips were taken from each rat ($N = 9$) and randomized into either a methoxamine or a BHT-920 group. Two complete dose–response curves to either methoxamine or BHT-920 (depending upon group assignment) were performed in each strip. However, either the first or the second of the dose–response curves was performed in the presence of 16 mM acetate substituted for chloride (the order of the dose–response curves with and without acetate was balanced and randomized). When acetate was used, exposure to acetate was begun 15 minutes prior to starting the dose–response curve.

The data were analyzed using a curve fitting program [13] which estimated values for agonist ED50 and peak contraction to the agonist. The effect of acetate was expressed as the ratio of agonist ED50 in the presence of acetate to agonist ED50 in the absence of acetate (ED50 dose ratio). The relative potency of acetate against BHT-920 and methoxamine contractions was assessed by comparing the ED50 dose ratios using 16 mM acetate for each of the two agonists.

Vasorelaxant effect of lyotropic anions. The lyotropic effects of anions are related to their ability to salt out albumin and other proteins from solution [14]. Twelve anions were studied, each in the form of its sodium salt: fluoride, iodate, tartrate, sulfate, (disodium) phosphate, bicarbonate, acetate, bromate, chloride, nitrate, bromide, and thiocyanate. Nine pairs of rats were studied. Twelve strips from each pair were randomly assigned to one of these twelve anion groups. A given strip was first contracted with AVP and then exposed to the anion. In these experiments, the anion in question was added to the bath from a concentrated (1 M) stock solution, and not substituted for chloride as described above. The amount added was such that the final calculated bath concentration of the anion would be 16 mM.

Lyotropic numbers for each anion were obtained from the literature [14, 15], and the percent relaxation five minutes after exposure to a given anion was plotted as a function of the lyotropic number of the anion.

Vasorelaxant effect of compounds of intermediary metabo-

lism. The design was similar to that for lyotropic anions. Eight compounds were studied: L-alanine, pyruvate, DL-lactate, chloride (control), acetate, propionate, malonate, and acetoacetate. Nine pairs of rats were studied. Eight strips from each pair were randomly assigned to the eight compound groups. Each strip was contracted with AVP and then exposed to the compound in question. The compounds were added from 1 M stock solutions, in an amount to result in a final bath concentration of 16 mM. In these studies, as in the lyotropic experiments, the bath osmolality was allowed to increase. All compounds were added in the form of sodium salts, with the exceptions of acetoacetate, which was obtainable only as the lithium salt, and alanine.

Vasorelaxant effects of adenosine and AMP in this model. Because the effects of acetate could conceivably be secondary to metabolic generation of AMP and possibly adenosine, the vascular effects of the latter two compounds were also explored in this model. Strips were randomized into one of three groups: control, adenosine (10^{-3} M), or AMP (10^{-3} M). Each strip was contracted with AVP (2×10^{-9} M), and then exposed to a relaxation solution containing AVP in KH and also containing nothing (control), adenosine, or AMP. The dependent variable was the percent relaxation after five minutes of exposure to the adenine compound.

Studies of pH and ionized calcium

Solutions containing 25 mM bicarbonate and one of each of the lyotropic anions studied (16 mM) were prepared and gassed with 95% oxygen and 5% carbon dioxide to maintain the PCO_2 close to 40 mm Hg. A sample of each solution was then drawn up into a sealed syringe and its pH measured using a blood gas analyzer. The ionized calcium level was also measured using a Nova™ calcium–selective electrode.

Preparation of agonists

All agonists were freshly prepared on the day of use. Potassium chloride was dissolved in 0.9% NaCl to make a 4 M stock solution. Synthetic arginine vasopressin (Bachem, Torrance, California, USA) was first dissolved in 0.9% NaCl (100 mg/liter) also containing 0.1% radioimmunoassay grade, bovine serum albumin (Sigma). Small (0.1 ml) aliquots were frozen at −80°C until the day of use. On the day of the experiment, an aliquot was further diluted to the desired concentration in 0.9% NaCl containing 0.1% radioimmunoassay grade, bovine serum albumin. Methoxamine powder (obtained as a courtesy of Burroughs Wellcome Co., Research Triangle Park, North Carolina, USA) and BHT-920 (Boehringer Ingelheim Ltd., Ridgefield, Connecticut, USA) were dissolved in 0.9% NaCl containing 0.1% radioimmunoassay grade, bovine serum albumin. Indomethacin was dissolved in an aqueous stock solution after first alkalinizing it with sodium carbonate as described by Spokas et al [16]. DIDS and all other compounds were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). All solutions were otherwise made up from reagent grade chemicals dissolved in distilled water.

Statistical analysis

All values are reported as mean ± 1 SEM. In each study, the significance of an overall treatment effect was first assessed using ANOVA. If a significant ($P < 0.05$) F-ratio was obtained, the significance of differences among group means was further explored using the Tukey B procedure [17]. In the BHT-920 and methoxamine studies, when ED50 values were obtained from the curve fitting program used [13], a log transformation was done on the ED50 values. The means and standard deviations of ED50 values were calculated from the log transformed numbers, and then reconverted to their anti-logarithms.

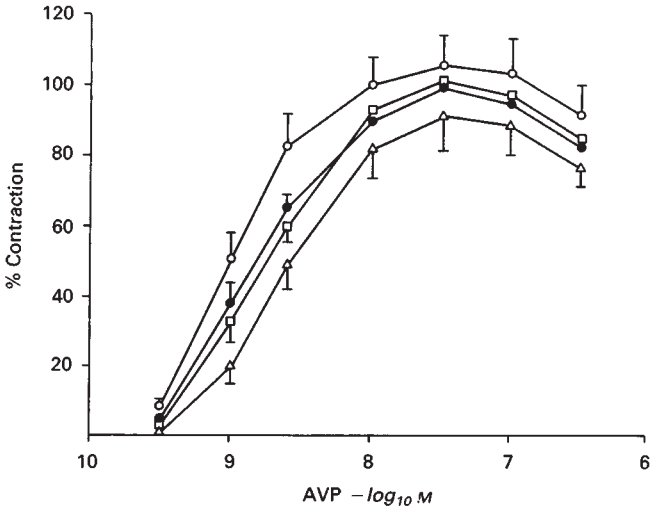

Fig. 1. *Inhibition of contraction induced by cumulative addition of AVP by exposure to 4, 16, or 64 mM acetate.* Symbols are: (○) control; (●) 4 mM acetate; (□) 16 mM acetate (data from 3 groups with different calcium and magnesium levels have been pooled); and (△) 64 mM acetate.

Results

Dose–dependent inhibitory effect of acetate on AVP contraction

The inhibitory effect of acetate on AVP contraction is depicted in Figure 1. As shown, the AVP contraction curve was shifted to the right in a dose–dependent manner from 4 to 64 mM. ANOVA revealed a significant overall acetate effect at only the three lowest AVP dose levels: 0.3×10^{-9} M, $F = 2.83$, $P = 0.05$; 1×10^{-9} M, $F = 5.01$, $P = 0.003$; 3×10^{-9} M, $F = 3.13$, $P = 0.03$. A trend in the data did suggest that acetate also diminished the peak AVP response.

In these studies, the maximum phasic contraction to 100 mM KCl (administered during the conditioning phase) was 1240 ± 43 (SEM) mg. There was no difference in the phasic contraction to KCl among the six study groups ($F = 0.25$).

Dose–dependent relaxation of AVP constricted strips

In Figure 2, the relaxant effect of acetate against AVP contraction is shown. Relaxation with 4 mM acetate was significantly different from control ($P < 0.05$), and relaxation with 16 mM acetate was significantly greater than with 4 mM ($P < 0.05$). Relaxation was not different among the subgroups exposed to the three 16 mM acetate solutions, two of which contained supplemental calcium and/or magnesium. The amount of relaxation with 64 mM acetate was not significantly greater than that with 16 mM acetate.

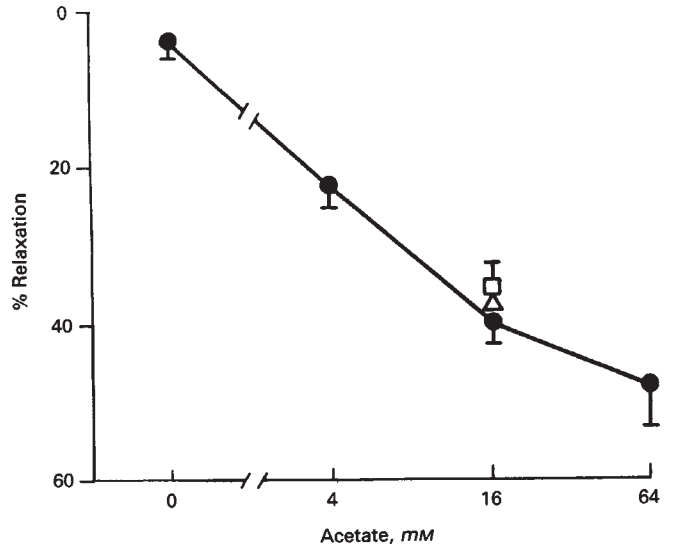

Fig. 2. *Relaxation of AVP-contracted strips induced by 4, 16, or 64 mM acetate.* Symbols are: (●) baths unadjusted for calcium complexing; (△) bath with 5.0% added calcium; and (□) bath with 5.0% added calcium and 5.0% added magnesium.

In these studies the peak phasic contraction to KCl averaged 1098 ± 40 mg, and was similar among the six groups (F = 0.40, *P* NS).

Effect of position along the tail artery on acetate relaxation. The percent relaxation five minutes after exposure to 16 mM acetate averaged 31% ± 1.6 (SEM) for the six groups of strips combined. As one proceeded along the mid-tail from the most proximal to the most distal group, the relaxation percentages were 30, 33, 37, 31, 30, and 28, respectively. These vaues were not significantly different (F = 0.45, *P* NS). The peak phasic contractions to KCl in these studies averaged 1176 ± 4.9 mg, and were not different among the segments.

Effect of endothelial rubbing on acetate relaxation. Endothelial rubbing had no effect on the magnitude of acetate induced relaxation. When endothelium was present, the spontaneous (control) relaxation of 10% ± 4.5 (SEM) was increased to 37% ± 5.6 or to 64% ± 5.0 in the presence of 4 or 16 mM acetate, respectively. When endothelium was absent, the spontaneous relaxation of 0% ± 1.6 was similarly increased to 30% ± 4.5 or 53% ± 3.8 in the presence of acetate. The values obtained with rubbed strips and unrubbed strips were not significantly different.

The peak phasic contraction to KCl in these studies averaged 950 ± 23 (SEM) mg, and was similar in the rubbed and unrubbed strips. The peak relaxation to ACh in the rubbed strips (2.0% ± 1.6) was significantly less than in the unrubbed tissue (44% ± 4.5, $P < 0.001$).

Effect of indomethacin on acetate relaxation. Incubation in indomethacin had no effect on acetate induced relaxation. In control strips, relaxation at five minutes in the presence of 0, 4, or 16 mM acetate averaged 19, 25, or 47%, respectively. In the indomethacin incubated strips, the respective relaxation percentages were 16, 36, and 38. There was no significant indomethacin effect at any of these acetate levels.

The peak phasic contraction to KCl in these studies averaged 850 ± 85 mg. The response averaged 790 mg in the control strips, and 934 in the indomethacin treated strips (*P* NS). The magnitude of the AVP contraction tended (*P* NS) to be higher in the indomethacin treated strips, averaging 81% ± 7.5 of the peak phasic contraction to K, versus 69% ± 6.5 in controls.

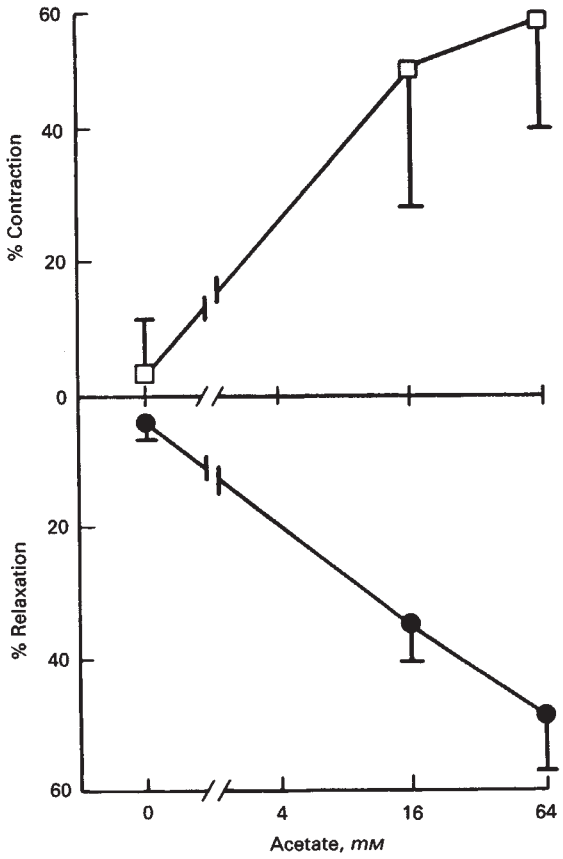

Fig. 3. *Effect of DIDS on the relaxant effect of acetate.* Symbols are: (●) control studies; and (□) studies in the presence of 10^{-3} M DIDS.

Effect of chemical sympathectomy on acetate relaxation. Chemical sympathectomy had no significant effect on the magnitude of acetate induced relaxation. In control strips, the percent relaxation at five minutes in the presence of 0, 4, or 16 mM acetate averaged 9.3% ± 2.4, 34% ± 3.0, and 49% ± 3.1, respectively. In denervated strips, the respective relaxation percentages were: 8.1% ± 2.1, 37% ± 2.9, and 54% ± 1.4.

The peak phasic contraction to KCl in the control strips was 1029 ± 60 mg; significantly higher than in the denervated strips (775 ± 37 mg, $P < 0.001$). In control strips, the peak contraction to tyramine averaged 819 ± 72 mg; significantly greater than in denervated strips (82 ± 16 mg, $P < 0.001$).

Modulating effect of DIDS on acetate relaxation. Results are shown in Figure 3. In the DIDS group, in the absence of acetate, there was a slight, transient contraction just after changing the bath at the peak of the AVP contraction. However, by five minutes, the relaxation (relative to the tension at time of bath change) was similar in the control (4.0%) and DIDS (−3.4%) groups (*P* NS). In the control group, relaxation in the presence of 16 or 64 mM acetate averaged 35% or 47%, respectively. In the DIDS group, however, a large contraction was obtained upon exposure of the strips to acetate (Fig. 3).

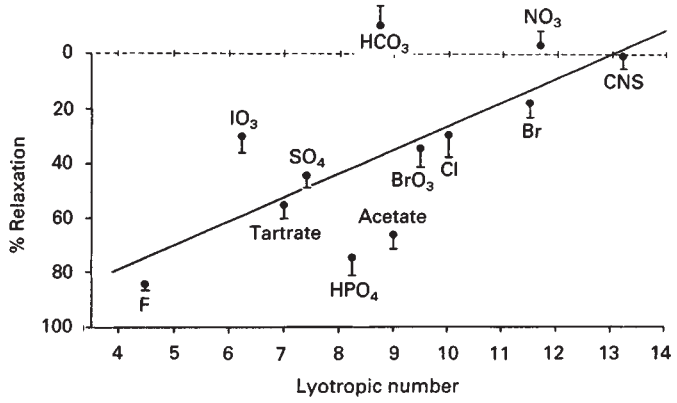

Fig. 4. *Vasorelaxant effect of different baths containing 16 mM concentrations of various anions as a function of their lyotropic numbers.* The regression line calculated by least squares is shown ($r = -0.65$, $P < 0.001$). Abbreviations are: F, fluoride; IO_3, iodate; SO_4, sulfate; HPO_4, disodium phosphate; HCO_3, bicarbonate; BrO_3 bromate; Cl, chloride; Br, bromide; NO_3, nitrate; CNS, thiocyanate.

The tension levels at five minutes in the control versus DIDS groups were highly significantly different at both levels of acetate ($P < 0.001$).

The peak KCl contractions in these studies averaged 1400 mg ± 89, and were not significantly different between the DIDS and control groups.

Dose-response curves to methoxamine and BHT-920

In the control KH solution, the peak contraction to methoxamine averaged 1478 ± 128 (SEM) mg, whereas the peak contraction to BHT-920 (in other strips) was less (735 ± 56 mg, $P < 0.001$). In the presence of acetate, the peak contraction to either of these agonists was not significantly reduced. In the control bath, the ED50 of methoxamine was $8.0 + 1.5/-1.0 \times 10^{-7}$ M), a value that was significantly increased in the presence of 16 mM acetate ($1.5 + 1.5/-1.0 \times 10^{-6}$M, $P < 0.01$). The ED50 of BHT-920 in the control bath was $4.4 + 1.8/-1.2 \times 10^{-7}$ M). Acetate also shifted the ED50 of BHT-920 to the right ($P < 0.01$), to $2.24 + 1.23/-0.66 \times 10^{-6}$ M). The ED50 dose ratio (ED50 with acetate/ED50 without acetate) was greater for BHT-920 (6.7 ± 1.5) than for methoxamine (2.1 ± 0.29, $P < 0.05$).

Vasorelaxant effect of the lyotropic anions

Results are shown in Figure 4. As can be seen, the lyotropic anions, added to give a final bath concentration of 16 mM, had a vasorelaxant effect that was in proportion to their lyotropic number. The overall correlation coefficient for this relationship was −0.68, $P < 0.01$. The regression line calculated by least squares is shown in the figure.

The percent relaxation with 16 mM acetate in these studies, in which acetate was added from a concentrated stock solution, averaged 66 ± 6.1, a value considerably greater than that observed in the experiments in which acetate was substituted for chloride (Fig. 2). The peak KCl contractions in these studies averaged 1300 mg, and were not significantly different among the 12 groups by ANOVA (F = 0.39).

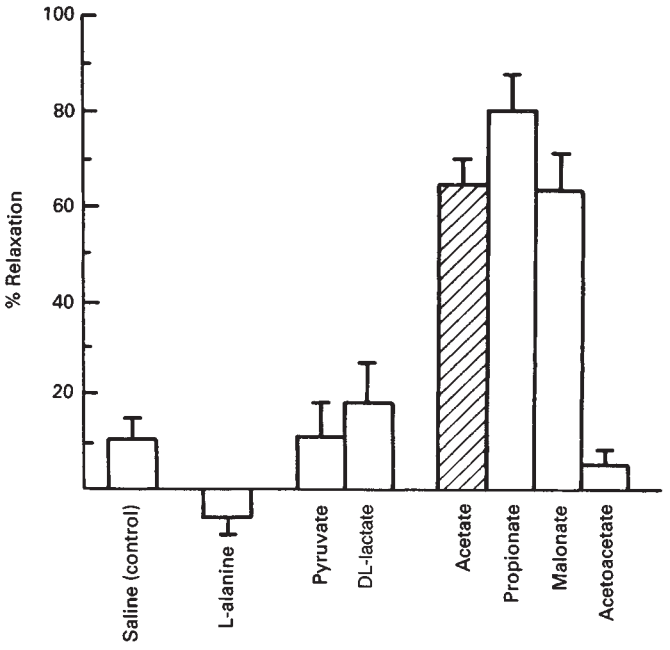

Fig. 5. *Effect of various compounds of intermediary metabolism on AVP-induced contraction.*

Vasorelaxant effect of fatty acid fragments and Krebs intermediates

Results are depicted in Figure 5. As shown, the short-chain fatty acids malonate and propionate were also quite efficacious vasorelaxant agents, whereas acetoacetate was not. Relaxations obtained with acetate, propionate, and malonate were not significantly different, but were significantly greater ($P <$ 0.05 or less) than those obtained with all other agents. Pyruvate and lactate appeared to be weak vasodilators, although their relaxant ability compared to sodium chloride (controls) did not quite attain statistical significance. Relaxations with sodium chloride, alanine, and acetoacetate were not significantly different, although there was a trend for alanine to be associated with a slight vasoconstriction.

Because acetoacetate could only be obtained commercially as the lithium salt, several further experiments were done in which the control bath contained 16 mM lithium chloride, and in which lithium acetoacetate was isosmotically substituted for lithium chloride during the contraction. These studies confirmed the lack of vasorelaxant effect of acetoacetate in this model.

Vasorelaxant effect of adenosine and AMP

Both adenosine and AMP, when applied at the peak of the AVP induced contraction, caused transient increases in tension, peaking at one minute (adenosine: +37% ± 4.2, AMP: +34% ± 3.7). However, after five minutes, tension in the continued presence of adenosine (−4.8% ± 4.5 relative to tension at time of solution substitution) was no different from that in controls (−6.0% ± 3.4, *P* NS). With AMP, however, the tension after five minutes (−28% ± 6.2) was less than in controls (−2.0% ± 4.5, $P < 0.05$), indicating a relaxant property of AMP in this model.

Studies of pH and ionized calcium values

Solutions containing acetate. Isotonic or hyperosmotic solutions containing 0, 4, 16, or 64 mM acetate and also containing 25 mM bicarbonate did not differ with respect to pH. When the PCO_2 was kept at 40 mm Hg, the pH of all these solutions was very close to 7.38. The ionized calcium values, as measured by the Nova calcium-selective electrode, did decrease slightly, by 2.2, 5.1, and 24%, respectively, for solutions containing 4, 16, and 64 mM acetate, as demonstrated by us earlier [8].

Solutions containing other lyotropic anions. In solutions containing 25 mM bicarbonate and 16 mM of each of the other lyotropic anions, the pH was also very close to 7.38 when the PCO_2 was kept at 40 mm Hg. The only exception was when an additional 16 mM of sodium bicarbonate was added, in which case the pH shifted to 7.45. The electrode-determined ionized calcium values for the various ion solutions (as percent of control) were: fluoride—out of range, iodate—89%, tartrate—62%, sulfate—77%, phosphate—out of range, bicarbonate—83%, acetate—94%, bromate—97%, chloride—100%, nitrate—98%, bromide—99%, and thiocyanate—69%. There was no correlation between complexing of calcium as measured by the electrode and the degree of vasorelaxation.

Discussion

Our results suggest that acetate has a marked relaxant effect on isolated vascular smooth-muscle. The vasorelaxant effect is dose-related and is demonstrable at levels which are commonly achieved in the plasma during dialysis [18]. Our results thus extend and confirm previous reports of the vasodilating effect of acetate in a number of animal models [2–8].

In previous studies we showed that acetate relaxes rat tail artery strips constricted with a variety of different agonists, including potassium chloride (KCl), phenylephrine, and arginine vasopressin (AVP) [7, 8]. In the present study, we also found that acetate inhibits contraction by methoxamine (an alpha-1 agonist), and by BHT-920 (an alpha-2 agonist). Taken together, the findings suggest that acetate relaxes smooth muscle by a non-specific mechanism. The effect of acetate against alpha-2 induced contraction in our studies was greater than against alpha-1 mediated contraction. Van Zwieten, van Meel and Timmermans have suggested that preferential inhibition of alpha-2 versus alpha-1 generated tension is a property shared by the calcium entry blockers [19]. Accordingly, preferential inhibition of alpha-2 contraction by acetate might be interpreted as evidence that acetate may be acting as a calcium uptake blocker. However, acetate is a very weak inhibitor of contractions induced by KCl (Daugirdas, unpublished information), suggesting that calcium entry blockade is not a primary mechanism of action. Furthermore, the preferential inhibition of alpha-2 contractions by acetate can also be explained on the basis of receptor occupancy theory: in addition to calcium channel blockers, many other vasodilators, including hydralazine and sodium nitroprusside, have been shown to inhibit alpha-2 contractions more than alpha-1 contractions [20]. The postulated mechanism relates to the fact that the weaker alpha-2 agonist requires a greater proportion of cell surface receptors to be occupied to obtain an equivalent degree of contraction, and hence is especially susceptible to the action of vasodilators.

The rat tail artery is a richly innervated blood vessel, and it has been shown that K-generated tension in this tissue is partially dependent upon norepinephrine release [21]. In a conscious dog model, we have shown that chemically sympathectomized animals develop severe (albeit transient) hypotension when exposed to an acetate buffered dialysate [6]. Thus, some of the effects of acetate in our model might conceivably have been due to acetate induced suppression of norepinephrine release in the wall of the vessel strip. However, in the present experiments, acetate was equally potent in relaxing control and chemically sympathectomized tissue, suggesting that the relaxant effect of acetate is not mediated by inhibition of the sympathetic nervous system. Of note, the effect of hydralazine, another "non-specific" vasodilator, can be enhanced in the proximal tail artery by prior chemical sympathectomy [9]. Our results do not rule out a separate effect of acetate on sympathetic nerve transmission, however.

Having established that acetate relaxes the tail artery by a direct, non-specific action, the possibility remained that the action of acetate might be mediated by the endothelium or by prostaglandin synthesis. Our findings do not support this possibility, as the amount of relaxation in vessel strips from which the endothelium had been removed and in vessel strips treated with indomethacin was similar to that in control tissues.

Acetate, as well as other anions, share, to a greater or lesser extent, what have been described as lyotropic properties, defined as the ability to "salt out" proteins, for example, albumin, from solution [14]. The lyotropic properties of anions have been shown to correlate very well with a number of other superficially unrelated attributes, such as the ability to differentially congregate at air–fluid interfaces, and the ability to affect the electrical properties of transmission across the cell membrane [15]. In fact, acetate is known to hyperpolarize certain cell membranes [22, 23]. Thus, it is conceivable that acetate and other lyotropic anions relax vascular smooth muscle via effects on either the resting transmembrane potential, or on ion conductance through the membrane. Our model permitted only a crude test of such a hypothesis. Although the lyotropic ability of the anions studied did correlate with their ability to relax AVP-induced contractions, the effect of acetate appeared to be greater than that which would have been expected based on the position of acetate in the lyotropic series.

DIDS is an agent that may block cell uptake of many anions [11, 12]. DIDS does not affect the action of certain vasodilators believed to act at the cell membrane, such as ouabain [12]. DIDS may thus be a tool for evaluating the importance of cellular uptake of an anion to its mechanism of action. DIDS completely blocked the vasorelaxant effect of acetate in our model. This finding suggests that cellular penetration of acetate is important to its mode of action.

If cellular uptake of acetate is indeed necessary for it to relax vascular smooth muscle, it is conceivable that acetate induces relaxation via formation of intermediary metabolites. Such a "metabolic" hypothesis of acetate action was first advocated by Liang and Lowenstein [24]. During acetate infusion, these investigators found elevated muscle levels of adenosine monophosphate (AMP) and increased cardiac levels of adenosine. They hypothesized that conjugation of acetate with coenzyme A in the mitochondria to form acetyl CoA, a reaction that consumes ATP [25], resulted in increased tissue levels of both

AMP and adenosine. The potential clinical importance of this mechanism was recently highlighted by the demonstration of ATP depletion in circulating platelets during acetate buffered hemodialysis [26]. How acute conversion of ATP to AMP by acetate might cause vasorelaxation is completely unknown. Many of the adenine nucleotides can act as vasodilators in preconstricted vessel strips, possibly by virtue of their effect on purinergic receptors [27]. The presence of the latter on the presynaptic nerve terminal, on the postsynaptic smooth muscle surface, and on the endothelium, is now well established [27]. Adenosine may also act at an intracellular site to cause vasorelaxation [28]. Acute conversion of ATP to AMP might, therefore, cause vasorelaxation by virtue of leakage of AMP out of the cell and activation of a purinergic receptor. Adenosine might also be generated from AMP via salvage pathways, and the adenosine could act either intracellularly or outside of the cell. Alternatively, acute changes in the ATP balance of the cell might cause vasorelaxation by affecting intracellular cyclic nucleotide levels, the intracellular calcium concentration, or the inositol triphosphate and protein kinase C systems, all of which have been implicated in vascular contraction/relaxation events.

In an attempt to indirectly validate the "metabolic" hypothesis of acetate action, we assessed the vasorelaxant abilities of a number of other compounds that can also feed into the Krebs cycle. Propionate can be conjugated to propionyl CoA in a reaction that consumes ATP [25]. The metabolic hypothesis would predict that propionate, as well as the chemically similar short-chain fatty acid derivatives malonate and acetoacetate, should also have vasorelaxant effects. In fact, both propionate and malonate evidenced strong vasorelaxant properties in our model. The lack of vasodilatation with acetoacetate is interesting, and might be explained by the fact that there exists an enzyme which is capable of synthesizing the acetoacetate CoA derivative by transferring CoA from succinate to acetoacetate, in a reaction which does not involve the direct consumption of ATP [29]. The finding that pyruvate, lactate and alanine were not strong vasodilators in our model also supports the metabolic hypothesis of acetate action. Like acetate, pyruvate, lactate, and alanine are eventually metabolized to acetyl CoA. However, the reaction sequence for the last three compounds does not result in direct consumption of ATP [25].

In further exploration of ramifications of the metabolic hypothesis of acetate action, we looked for the ability of AMP and adenosine to relax the AVP-contracted rat tail artery. Previous investigators have reported that adenosine and AMP have little effect on tension in this system [9], or have noted a vasoconstrictive response [30]. We found that AMP, but not adenosine, did relax the AVP-constricted rat tail artery when added to the tissue bath in a high concentration. We also identified a transient vasoconstrictive effect of both AMP and adenosine.

In conclusion, we have established a rat tail-artery model wherein the mechanism of action of acetate can be pursued. All data were consistent with the metabolic hypothesis of action of acetate; namely, that acetate induced vasorelaxation is mediated by cellular uptake of acetate with subsequent metabolism to acetyl CoA and attendant conversion of ATP to AMP. The intracellular links between acetate metabolism and vasorelaxation remain to be defined.

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