0022-3565/83/2272-0322$02.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 227, No. 2
Printed in U.S.A.

Effect of Hydralazine on Tension and Membrane Potential in the Rat Caudal Artery[1]

KENT HERMSMEYER, ANGELO TRAPANI, PETER W. ABEL[2] and MANUEL WORCEL[3]
Department of Pharmacology, University of Iowa, Iowa City, Iowa
Accepted for publication August 1, 1983

ABSTRACT

To determine whether the vasodilator, hydralazine (HYD), produces hyperpolarization of vascular muscle cells, we measured the effect of HYD on membrane potential and contractile responses to phenylephrine and K^+. HYD (1 μM) caused a 4 mV hyperpolarization of phenylephrine-depolarized arteries (compared with controls without HYD), which could possibly account for up to three-fourths of the 39% decrease in tension measured. K^+-contracted vessels were also 34% relaxed by 1 μM HYD without an effect on membrane potential. In addition, HYD further relaxed phenylephrine-stimulated vessels previously relaxed by D-600, suggesting that Ca^{++} channel blockade may not be an important mechanism of vasodilation for HYD. The evidence suggests that a nonmembrane action of HYD on arterial muscle probably multiplies the relaxant effect of membrane potential hyperpolarization. Both mechanisms would attenuate the effects of adrenergic stimulation.

HYD has been used successfully as an antihypertensive drug for more than 25 years. Many pharmacological properties of this compound have been described (see review by Gross, 1978); however, the cellular mechanism by which it reduces blood pressure remains unclear. A number of investigators (Kirpekar and Lewis, 1957; Uchida and Bohr, 1969; Andersson, 1973) have demonstrated the ability of the HYDs to inhibit the contractile effects of many agonists in isolated vascular muscle. The reports can be separated into low, presumably therapeutic concentration (micromolar) and high (millimolar) concentration effects. Studies by McLean *et al.* (1978) showed that high doses of HYD can prevent $^{45}Ca^{++}$ accumulation in K^+-stimulated aortic strips, without affecting $^{45}Ca^{++}$ efflux. However, data which argue against a cell membrane mechanism of action for HYD have also been reported (Diamond and Janis, 1980; Diamond and Shaikh, 1980). These investigators suggest that at least high concentrations of HYD may act at a step in the E-C coupling sequence beyond the regulation of cytoplasmic Ca^{++} levels. Low concentrations have been shown to relax arterial muscle cells by possible membrane (Worcel *et al.*, 1980) and intracellular (Khayyal *et al.*, 1981) actions.

The present study was undertaken to determine whether low concentration HYD produces vasodilation at least partly through an effect on E_m. All of the experiments were conducted on the rat caudal artery which has previously been shown to be extremely sensitive to HYD (Worcel, 1978; Worcel and Chevillard, 1981). HYD was tested at concentrations of 0.1 to 1 μM concentrations which are similar to plasma levels reported in hypertensive patients receiving the drug (Schulert, 1961).

Methods

Biolab or WKY rats (175–250 g) were anesthetized with sodium pentobarbital (30 mg/kg i.p.). Caudal arteries were exposed, freed of surrounding connective tissues and rapidly removed from the animals. All of the vessels used in these experiments were denervated *in vitro* with 6-OHDA to prevent the release or uptake of catecholamines by adrenergic nerve endings from obscuring the direct effect of an intervention on myovascular cells. The technique consisted of 6-OHDA dissolved in a glutathione buffered solution (pH 4.9) and applied to the arteries in a concentration of 300 μg/ml for 10 min with 1 μM phentolamine added to limit stimulation by released norepinephrine (Aprigliano and Hermsmeyer, 1976). After this procedure, the vessels were prepared to record mechanical activity or E_m and allowed to equilibrate for 1 hr before any measurements were made. The isolated arteries were continuously suffused without recirculation, with ISM gassed with 95% O_2-5% CO_2 and of the following composition (millimolar): NaCl, 130; $NaHCO_3$, 16; NaH_2PO_4, 0.5; KCl, 4.7; $CaCl_2$, 1.8; $MgCl_2$, 0.4; $MgSO_4$, 0.4; 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 13; and glucose, 5.5. The solution was maintained at 37°C and had a pH of 7.3. Solutions with an increased K^+ concentration were prepared by replacing NaCl with KCl.

Recordings of membrane potential. Caudal artery segments 8 to 10 mm in length were pinned in silicone rubber chambers (volume, 2

Received for publication March 14, 1983.
[1] This work was supported by Grants HL 16328 and HL 14388 from the National Institutes of Health and Research Career Development Award HL 00073 from the National Institutes of Health.
[2] Present address: Department of Pharmacology, Emory University, Atlanta, GA 30322.
[3] Present address: Centre de Recherches Roussel Uclaf, BP9, F-93230 Romainville, France.

ABBREVIATIONS: HYD, hydralazine; E_m, membrane potential; 6-OHDA, 6-hydroxydopamine; ISM, ionic solution for mammals; PE, phenylephrine.

ml), without being cut open, and were suffused with ISM at a rate of 3 ml/min. E_m was recorded from the adventitial side of each preparation using glass microelectrodes with resistances of 30 to 100 megohms, filled with 3 M KCl and suspended from a 25-μm Ag wire. The recording microelectrode and superfusing solution were connected to a W-P Instruments M701 high input impedance amplifier by Ag:AgCl half cells. The preamplifier output was displayed on a Tektronix R5031 storage oscilloscope, from which the data were taken. Cell impalements were considered acceptable only if they were consistent with all of the following criteria: 1) cell impalement was indicated by a sharp voltage drop; 2) cell input resistance was between 4 and 40 megohms; and 3) reference potential (±2 mV) and electrode resistance (±1 megohm), compared before and after impalement, were constant.

Experiments using PE and K^+ were conducted using a protocol similar to that of Worcel (1978). Adjacent segments of the same artery were placed in paired vessel chambers. Using this procedure, one segment served as the test (HYD exposed), whereas the other served as control (no HYD). E_m was recorded from either segment during 12-min exposures to 10 μM PE or 60 mM K^+. Depolarization occurred within 1 min after exposure of an artery to PE or K^+ and the E_m remained constant throughout the sampling period. After each sampling period, the chamber was washed free of stimulant and the vessel was suffused for an additional 15 min with ISM, before reexposing it to PE or K^+. During an experiment one of the paired segments was constantly suffused with 1 μM HYD, beginning 30 min before the first challenge with PE or K^+. Whenever possible, an equal number of impalements were obtained from test (HYD) and control segments using the same microelectrode to minimize any variability of E_m measurements caused by different microelectrodes. Both HYD and PE are susceptible to degradation in the presence of oxygen at pH 7.3. Therefore, fresh dilutions of these drugs were prepared every 15 min from refrigerated stock solutions throughout the course of an experiment.

Recordings of mechanical activity. For recording tension, spiral strips were prepared and placed in a muscle chamber of 1.5 ml in volume, with suffusion at a rate of 5 ml/min. The vessels were attached to Grass FT03 or Akers 802 force transducers and the records displayed on a Grass polygraph. A force of 800 dynes was applied to each arterial strip. The resulting initial tension for a vessel was within the range, 3800 to 8900 dynes/mm^2.

The relaxant effect of 1 μM HYD and/or 10 μM D-600 or arterial contractions was also tested. After 1 hr of equilibration in ISM, tissues were exposed to either 10 μM PE for 2.5 min or 60 mM K^+ for 4.5 min. After each stimulation, the arteries were suffused for 15 min with ISM before re-exposing them to 10 μM PE or 60 mM K^+. Exposure to K^+ or PE was continued until three consecutive identical responses were obtained. Once a stable response was established, constant perfusion with HYD (1 μM) and/or D-600 (10 μM) was begun and continued for the duration of the experiment. PE (10 μM) and K^+ (60 mM) contractions were continued, separated by 15-min relaxations, during the 1-hr period of HYD (1 μM) and/or D-600 (10 μM) suffusion. Control helical strips from the same arteries were run in parallel with test (HYD exposed) strips to ensure that responses to PE or K^+ stimulation did not change with time. These vessel segments were treated identically to the test vessels, but were never exposed to HYD.

Drugs. The following drugs were used in these experiments: HYD HCl (Sigma Chemical Company, St. Louis, MO), 6-OHDA HBr (Sigma), PE HCl (Winthrop Laboratories, Inc., New York, NY), D-600 HCL (Knoll, Ludwigshafen am Rhein, Germany).

Statistics. For all contraction and E_m experiments, control (no HYD) and test (HYD exposed) segments from the same artery were

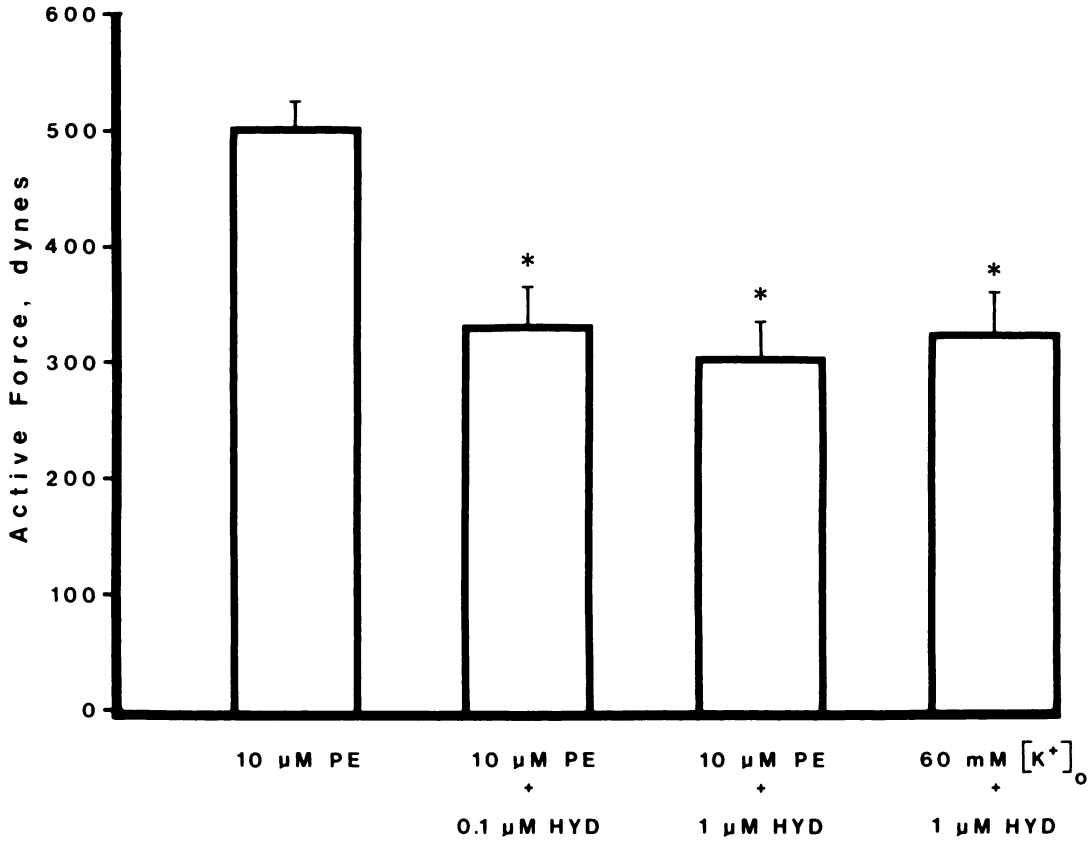

Fig. 1. Effect of HYD on PE or K^+ tonic phase contractions in caudal artery strips. The responses are expressed as contraction in dynes (1 dyne is the weight of 1.02 mg at sea level). Note that both K^+ and PE contractions were significantly relaxed by HYD. Active tension induced by PE was decreased significantly by both 0.1 and 1 μM HYD. Vertical brackets represent S.E.s for 20 animals. Asterisks indicate statistically significant difference from 10 μM PE (second and third bars) or from 60 mM K^+ (fourth bar), respectively ($P < .05$), by paired t test.

analyzed. Tension experiments were compared as the difference between control and test segments from the same animal using Student's paired t test. E_m was compared as the difference based on total number of impalements (group t test). The $P < .05$ level of probability was accepted as significant for all comparisons.

Results

Effect of HYD on PE and K^+ contractions. When caudal artery strips were exposed to 10 μM PE, they reached a plateau level of tension which was maintained during the 2.5-min exposure period. Vessels responded to 60 mM K^+ with a phasic contraction lasting from 1 to 2 min, followed by a tonic component of tension which was maintained as long as 60 mM K^+ was present. Only the tonic component of the K^+ contraction was measured, as this corresponded to the time period in which E_m was recorded in parallel experiments. The tonic contractile response to 60 mM K^+ was measured as the difference between base line and the tension level at 4 min of 60 mM K^+ exposure and averaged 80% of the 10 μM PE maximum.

Pretreating arteries for 30 min with 1 μM HYD significantly decreased both 10 μM PE and 60 mM K^+ contractions when compared with respective controls without HYD (fig. 1). HYD relaxed the PE contractions by 39%, whereas the tonic phase of the K^+ contractions were decreased by 34% ($n = 20$). Using a protocol of challenging the vessels with PE or K^+ after 15-min relaxations (see "Methods"), we observed a partial decrease in contraction even after 15 min of suffusion with 1 μM HYD. The maximum relaxant effect of HYD (shown in fig. 1) was measured at 30 min, with no further decrease in contraction seen with longer exposures to the vasodilator. Pretreating arteries for 30 min with 0.1 μM HYD significantly reduced PE contractions by 33% and 60 mM K^+ contractions by 28% compared with respective controls ($n = 20$). Neither 0.1 nor 1 μM HYD had any relaxant effect on the baseline tension of unstimulated arteries.

Effect of 1 μM HYD on PE and K^+ depolarization. The results of experiments to evaluate the effect of HYD on PE depolarization in the caudal artery are presented in figure 2. Arteries exposed to 10 μM PE were depolarized by 23 mV ($E_m = -34 \pm 0.7$ mV) when compared with the predrug E_m of -57 ± 1.0 mV. Continuous suffusion with 1 μM HYD prevented 4 mV ($E_m = -38 \pm 0.7$) of the PE depolarization. HYD (0.1 μM) prevented 2 mV ($E_m = -36 \pm 0.6$) of the PE depolarization. E_m recordings from arteries exposed to 1 μM HYD alone measured -57 ± 1.0 mV (data not shown), which was not significantly different from control.

To test whether HYD hyperpolarization could occur even in high K^+ solution, the effect of 1 μM HYD on 60 mM K^+ depolarization was measured (fig. 3). The vascular muscle cells were depolarized 33 mV ($E_m = -23 \pm 0.5$ mV) by 60 mM K^+ from the control value of -56 ± 0.4 mV. In HYD the E_m of -24 ± 0.6 mV was not significantly different from E_m in 60 mM K^+.

Effect of HYD and D-600 on PE contractions. We also tested the possible Ca^{++} channel blocking action of HYD by combination with D-600. The Ca^{++} antagonist D-600 (10 μM) completely blocked 60 mM K^+ contractions ($n = 4$, data not shown). However, when arteries were suffused with 10 μM D-600 they generated 37% of the control tension obtained with 10 μM PE (fig. 4). HYD (1 μM) significantly reduced the tension remaining (after 10 μM D-600 blockade) to 24% of control tension.

Discussion

The results presented here demonstrate that a hyperpolarization underlies the HYD relaxation of PE contractions in

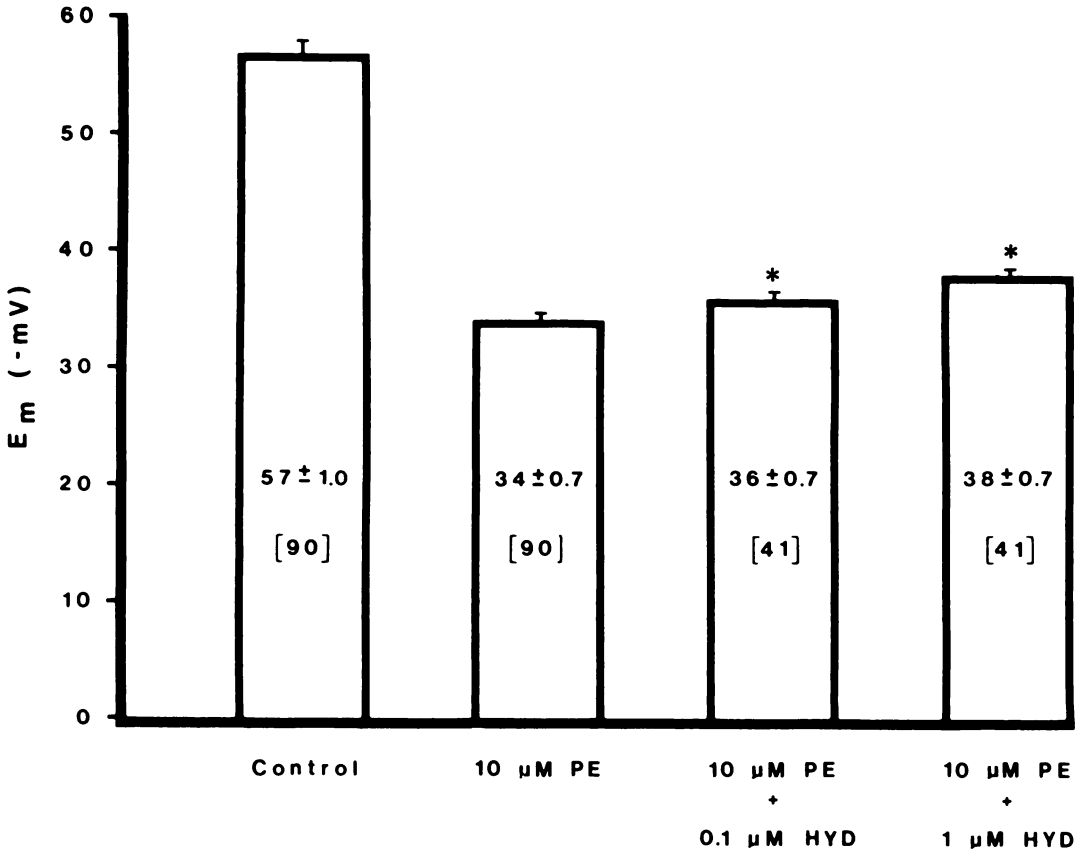

Fig. 2. E_m measurements of PE-stimulated vascular muscle cells showed partial prevention of depolarization by HYD. Exposure to 10 μM PE caused the myovascular cells to depolarize to −34 mV, which was significantly different ($P < .05$ by group t test) from the control E_m. Pretreatment with 1 μM HYD caused a 4 mV less depolarization by PE, whereas 0.1 μM HYD caused 2 mV less depolarization (asterisks indicate differences from −34 mV at $P < .05$ confidence level by group t comparison). The mean E_m and S.E.s are listed for 12 animals (control and PE) or 6 animals (HYD bars), followed by the total number of impalements in parentheses.

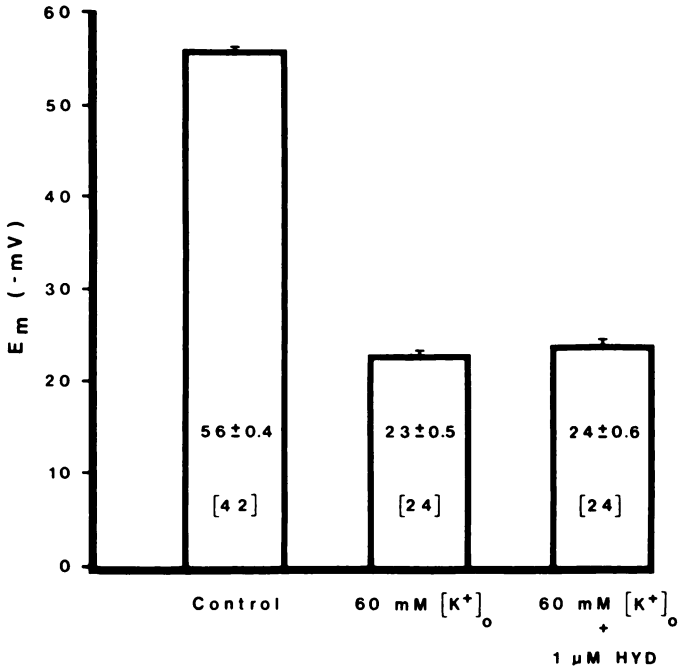

Fig. 3. Mean E_m values obtained from 60 mM K^+-stimulated cells with and without HYD present. K^+ (60 mM) produced a significant depolarization of the vascular muscle cells ($P < .05$). HYD (1 μM) had no significant effect on the K^+ depolarization. Mean $E_m \pm$ S.E.s are listed for four animals, followed by the total number of impalements in parentheses.

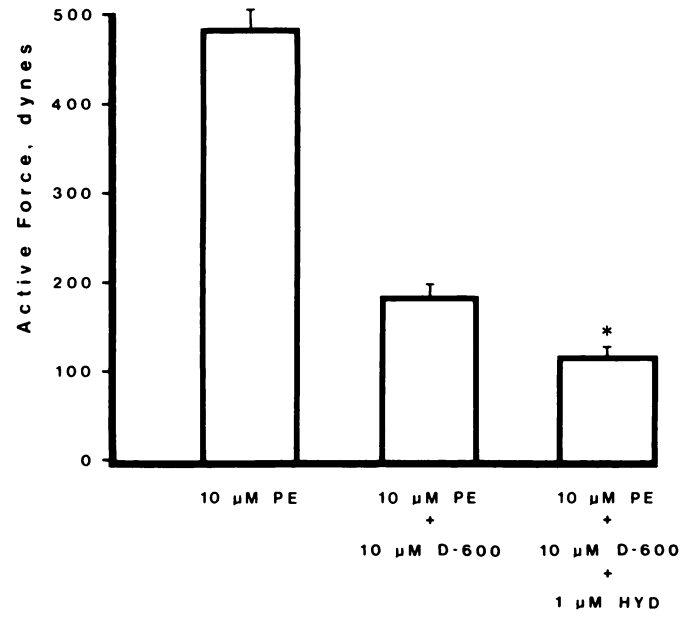

Fig. 4. Relaxation of PE contracted arteries by D-600 with and without HYD. Note that 1 μM HYD caused further relaxation of vessels which were suffused with 10 μM D-600 (asterisks indicate $P < .05$ by paired *t* test). Bars and brackets represent means and S.E.s, respectively, for four animals.

isolated strips of rat caudal artery. The potency of HYD in blocking both PE and K^+ contractions is similar to that previously recorded for rat caudal artery (Chevillard *et al.*, 1981b), occurring at submicromolar concentrations. The 4 mV hyperpolarization might be sufficient to explain three-quarters of the nearly 40% reduction in tension based on the tension-E_m relationship in caudal artery (Hermsmeyer *et al.*, 1981; Hermsmeyer, 1982). In the range of E_m around −35 mV, hyperpolarization by 4 mV would translate into 30% reduction of the maximum contractile force generated by the vessel (Hermsmeyer, 1982). The hyperpolarization could thus contribute to the HYD relaxation in this range of E_m.

However, when E_m is much less negative (−23 mV), as it was in the case of the 60 mM K^+, HYD failed to cause significant hyperpolarization, even though there was nearly as large a fraction of the K^+ contraction blocked. This result suggests that HYD relaxation could occur by other than E_m mechanisms (even though in a normally polarized vessel the hyperpolarization would contribute to the relaxation). HYD did not cause hyperpolarization at rest, only preventing a part of the PE depolarization. Possibly the HYD hyperpolarization occurs only in a certain range of E_m, which might suggest a basis for differential action. In fact, the first 2 mV of prevented depolarization reduced tension 33% (0.1 μM HYD), whereas the second 2 mV reduced tension by only an additional 6% of maximum (1 μM HYD). These experiments suggest that at least in the caudal artery HYD would relax contractions by multiple mechanisms, with E_m possibly most important at lower HYD concentrations.

However, the rat caudal artery differs from the rabbit renal artery in this respect because in rabbit renal artery K^+ contractions (or K^+ + norepinephrine contractions) were not relaxed by HYD whereas norepinephrine contractions were relaxed (Khayyal *et al.*, 1981). The difference in vascular muscle type probably accounts for much of the difference, although depolarization was by 45 mM K^+ in the rabbit renal artery experiments (Khayyal *et al.*, 1981). If rabbit renal artery membrane excitation by 45 mM K^+ was less dependent on intracellular Ca^{++} release than 60 mM K^+ stimulation of rat caudal artery, HYD might be expected to better relax rat caudal artery.

Several different mechanisms of intracellular action of HYD have been suggested by other workers. For example, Seidel *et al.* (1980) have suggested that reduced actomyosin content might be involved, at least in rat aorta. Worcel and Chevillard (1981) and Chevillard *et al.* (1981 a,b) have suggested that cell HYD receptors, with which purines interact, are an important key to the mechanism of action of HYD on rat caudal artery. Khayyal *et al.* (1981) have suggested that an action of HYD on excitation-contraction coupling, perhaps on the movements of intracellular Ca^{++}, might be the most important action of HYD, at least in rabbit renal artery. Other investigators (Limas and Cohn, 1974) have suggested that HYD might act through stimulation of Na^+-K^+/adenosine triphosphatase in rat mesenteric arteries. Diamond and Shaikh (1980) have also argued that HYD might cause vasodilation of rat aorta at a step in the E-C coupling sequence beyond regulation of cytoplasmic Ca^{++} levels. The results of the D-600 experiment (fig. 4) make it unlikely that HYD acts mainly as a Ca^{++} channel blocker.

The cellular mechanism of action of HYD may remain unclear until direct measurements of intracellular Ca^{++} can be made in vascular muscle. Any mechanism that explains the actions of HYD must deal with the following characteristics. The onset of action of HYD is slow (Chevillard *et al.*, 1981b). In some preparations (*e.g.*, rabbit renal artery, rabbit ear artery and bovine mesenteric artery), there is strong selectivity for relaxation of adrenergic contractions over K^+ contractions (Worcel *et al.*, 1980). There is also a selectivity for dilation of arterial muscle as compared with venous muscle (Collier *et al.*, 1978).

If there is a HYD receptor, direct demonstration of receptor-like characteristics (affinity, saturability and stereospecificity) should be documented. If there is an intracellular action of HYD on the coupling between membrane excitation and contraction, identification of the normal contractile mechanism in vascular muscle must be made before the effects of HYD on it can be appreciated. From the data presented herein, there appears to be a significant membrane hyperpolarization produced by HYD on PE-stimulated rat caudal artery. The hyperpolarization appears to be sufficient to explain part of the relaxation and can be taken to be an important component of vasodilation in normally polarized caudal artery. Electrogenic ion transport stimulation might fit the selectivity characteristics because regulating arteries, such as the caudal artery, show greater dependence of E_m on ion transport than do veins. There would also be greater effect of electrogenic ion transport in certain voltage ranges, *e.g.*, more effect on PE contractions than on K^+ contractions because E_m is in the steep part of the voltage-tension curve with PE. Relaxations of contractions by such a mechanism might be only transient, following the time course demonstrated for electrogenic ion transport (Hermsmeyer, 1976, 1982). If the hyperpolarization occurs through stimulation of electrogenic ion transport, further studies of the mechanism, such as by deliberate stimulation of electrogenic ion transport (Hermsmeyer, 1982), will be useful in quantitating the importance of the mechanism.

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Send reprint requests to: Dr. Kent Hermsmeyer, Department of Pharmacology, University of Iowa BSB, Iowa City, IA 52242.