Hyperpolarization and Relaxation of Resistance Arteries in Response to Adenosine Diphosphate Distribution and Mechanism of Action

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Membrane hyperpolarization appears to be an important mechanism of vasodilation induced by many pharmacological and endogenous vasodilators. The objective of the present study was to determine the mechanisms of vasodilation induced by ADP, an endogenous vasodilator, in various resistance arteries isolated from the rabbit. ADP hyperpolarized (12–15 mV) and relaxed mesenteric and skeletal muscle resistance arteries. The hyperpolarization was abolished by glibenclamide, an inhibitor of ATP-sensitive potassium channels. Glibenclamide inhibited part of the ADP-induced relaxations of these arteries; thus, a portion of the relaxation appears to result directly from the change in membrane potential. Hyperpolarizations and relaxations to low concentrations of ADP (<0.3 μM) were abolished by removal of the endothelium, but responses to higher concentrations of ADP were partially independent of the endothelium. ADP did not hyperpolarize but did relax small-diameter middle cerebral arteries, and glibenclamide had no effect on these ADP-induced relaxations. Relaxations of small cerebral arteries to all concentrations of ADP were endothelium dependent. These studies support the hypothesis that activation of ATP-sensitive potassium channels is an important single mechanism of vasodilation, including responses of resistance arteries. However, this generalization may not apply to small pial arteries of the rabbit. (Circulation Research 1991;69:1415–1420)

Evidence is accumulating that membrane hyperpolarization is an important general mechanism of vasodilation.1 Vascular responses to pharmacological dilators such as cromakalim,2–3 minoxidil,4 and pinacidil5 as well as endogenous substances such ascalcitonin gene-related peptide6 and vasoactive intestinal polypeptide2 are mediated wholly or in part via this mechanism. The hyperpolarization induced by cromakalim is probably due to direct activation of ATP-sensitive potassium (KATP) channels located in the vascular smooth muscle cell membrane.2 Responses to calcitonin gene-related peptide appear to involve activation of peptide receptors on vascular smooth muscle cells and subsequent second-messenger–induced increases in the activity of KATP channels.6 An inhibitory factor, endothelium-derived hyperpolarizing factor, released from the vascular endothelium in response to activation of cholinergic receptors also hyperpolarizes vascular smooth muscle,7,8 and this response is blocked by inhibitors of KATP channels.9 Studies to date in this area have focused on the behavior of large arteries. Still unresolved is the role of membrane hyperpolarization in dilation of resistance arteries and the possible involvement of KATP channels in this response. The objective of the present study was to determine the relation between membrane potential and ADP-induced decreases in vascular tone in small arteries isolated from rabbits and to evaluate the role of KATP channels in this response.

Materials and Methods

Preparation of Arteries

Adult male New Zealand White rabbits were anesthetized with sodium pentobarbital (35 mg/kg) and exsanguinated. The brain was removed and placed in cold physiological saline solution (PSS) of the following composition (mM): NaCl 118.5, KCl 4.7, NaHCO3 24.0, KH2PO4 1.2, EDTA 0.023, CaCl2 1.6,
MgSO₄ 1.2, and glucose 11.0. A small segment of pial artery, ~2.5 cm from the origin of the middle cerebral artery, was gently dissected away from the surface of the brain and placed in a dissection dish containing cold PSS. Adhering pial membrane was removed, and a segment of artery 1.8 mm in length was mounted in a myograph for simultaneous measurement of force and membrane potential as previously described.⁹ Distal segments of superior mesenteric artery taken from an area that supplies the proximal duodenum were isolated as described by Mulvany and Halpern.¹⁰ Skeletal muscle resistance arteries were isolated from the vastus medialis muscle as described by Nilsson and Mulvany.¹¹ Connective tissue and adhering lipids were removed from the arteries, and they were mounted in the myograph. The arteries were then continuously superfused with warmed PSS (37°C) that was gassed with 95% O₂–5% CO₂ to maintain pH at 7.4. Mounted arteries were stretched until passive force was just detectable, and the luminal diameter at this point was determined using a video dimension analyzer.¹² Average luminal diameters for mesenteric, skeletal muscle, and cerebral arteries were 120±6 μm (n=7), 131±11 μm (n=5), and 138±18 μm (n=7), respectively. Ten minutes after the initiation of superfusion, the arteries were stretched to a resting tension that was optimal for force development as determined in preliminary experiments (mesenteric and femoral arteries, 100 mg; cerebral arteries, 75 mg).

Removal of Endothelium

In some experiments, before mounting the arteries in the myograph, the endothelium was removed by passing a human hair through the lumen as described by Osol et al.¹³ Disruption of endothelial function was assessed later in the experiment, as indicated by the absence of a dilator response to acetylcholine (1 μM).

Membrane Potential

Membrane potential was measured using intracellular microelectrodes as described previously.⁹ Microelectrodes were filled with 0.5 M KCl and had tip resistances of 100–150 megohms. Smooth muscle cells were impaled from the Advential side of the preparation, and standard criteria for acceptance of recordings were applied. These criteria are 1) an abrupt change in potential on impalement of cells, 2) stable membrane potential for at least 3 minutes before experimental manipulations, 3) maintained impalement throughout the experimental protocol, 4) unchanged tip resistance before and after impalements, and 5) tip potentials of <7 mV.

Procedures

One hour after the preload was applied, arteries were maximally contracted by elevating the extracellular potassium to 120 mM. After washout of potassium, the arteries were constricted to ~70% (71±5%) of the maximum potassium-induced tone using norepinephrine (1–3 μM) in mesenteric and femoral arteries or a combination of histamine (1–2 μM) and serotonin (0.5–1 μM) in cerebral arteries. For initial measurements of membrane potential and force, cells were impaled, and ADP, acetylcholine, or glibenclamide was then added directly to the myograph solution or to the superfusion solution, as indicated. For determination of the full dose–response relations for ADP, arteries were first contracted with the appropriate agonist, and then increasing concentrations of ADP were added directly to the myograph bath. After maximal relaxation occurred, the arteries were washed to normal PSS and 10 minutes later were perfused with PSS containing the contractile agonists and glibenclamide. After a steady-state level of contraction was obtained, the full dose–response relation for ADP in the presence of glibenclamide was determined.

Chemicals

All chemicals were obtained from Sigma Chemical Co., St. Louis, Mo.

Statistical Methods

The concentration of ADP causing half-maximal inhibition of tone (IC₅₀) was determined using a desktop computer program (PCs). Data are expressed as mean±SEM or, for IC₅₀ values, as geometric mean and 95% confidence interval (CI). Results were analyzed by analysis of variance and were considered significantly different at p<0.05.

Results

ADP hyperpolarized mesenteric and skeletal muscle resistance arteries (Figures 1A and 1B), and the hyperpolarizations were associated with decreases in vascular tone. Addition of glibenclamide near the peak of the responses reversed the hyperpolarization and partially inhibited the relaxation of mesenteric resistance arteries (Figure 1A). Similarly, pretreatment of skeletal muscle resistance arteries with glibenclamide abolished the ADP-induced hyperpolarization and reduced the magnitude of the relaxation (Figure 1B). Interestingly, ADP did not hyperpolarize but did relax small distal branches of the middle cerebral artery (Figure 1C). The relaxation of the cerebral artery was not affected by glibenclamide. Glibenclamide had no direct depolarizing or contractile effect on vascular smooth muscle in any of these arteries in the absence of ADP.

Mean data from five to seven experiments examining the effects of glibenclamide and of removal of the endothelium on ADP-induced hyperpolarizations in each type of resistance artery are shown in Figure 2. Hyperpolarizations to low (10⁻⁷ M) and high (10⁻⁵ M) concentrations of ADP were blocked by glibenclamide. In mesenteric and skeletal muscle arteries, removal of the endothelium nearly abolished the response to 10⁻⁷ M ADP but only partly inhibited the hyperpolarization to 10⁻⁵ M ADP (Figure 2, top and middle panels). Little or no effect of ADP on membrane potential in distal middle cere-
The effects of glibenclamide and of removal of endothelial cells on the full dose–response curves for ADP in the different arteries are shown in Figure 4. Glibenclamide caused substantial inhibition of the relaxing effects of ADP in the mesenteric and skeletal muscle resistance arteries. Glibenclamide increased the IC50 values for ADP by nearly 18-fold in mesenteric arteries (control IC50, 1.8[0.2–16]×10^-8 M; glibenclamide-treated IC50, 3.2[0.5–19]×10^-7 M; p<0.05 versus control) and by nearly fivefold in skeletal muscle arteries (control IC50, 2.1[1.1–3.6]×10^-7 M; glibenclamide-treated IC50, 9.4[3.2–23]×10^-7 M; p<0.05 versus control). The response to ADP in the distal segment of the middle cerebral artery was unaffected by glibenclamide (control IC50, 1.2 [0.3–5.5]×10^-6 M; glibenclamide-treated IC50, 1.2[0.1–11.2]×10^-6 M. Removal of endothelial cells inhibited the responses to ADP in all three arteries, but substantial endothelium-independent responses
to higher concentrations of ADP were evident in mesenteric and skeletal muscle resistance arteries (Figure 4).

**Discussion**

Previous experiments using large-diameter arteries have revealed significant endothelium-dependent hyperpolarizations of vascular smooth muscle in response to acetylcholine. An increased potassium conductance has been indicated as a possible mechanism of action underlying this response. In large cerebral and mesenteric arteries from the rabbit, these membrane responses and the associated vasodilation are inhibited by glibenclamide, which selectively inhibits $K_{ATP}$ channels. $K_{ATP}$ channels are present in vascular smooth muscle, and they are activated by pharmacological and endogenous vasodilators. In light of these observations, activation of $K_{ATP}$ has been proposed as an important general mechanism of vasodilation. The present study indicates that this mechanism of vasodilation is also important in mesenteric and skeletal muscle resistance arteries and with respect to dilation mediated by ADP, a compound that may be an important endogenous vasodilator after release from blood platelet cells. Inhibition of the membrane hyperpolarization caused significant inhibition of ADP-induced dilations in mesenteric and skeletal muscle resistance arteries. This effect was most apparent using concentrations of ADP that induced 10–60% of maximal relaxation. In this lower range of ADP concentrations, glibenclamide inhibited 40–80% of the dilator response, indicating a causal relation between the membrane hyperpolarization and part of the vasodilation. The hyperpolarization is apparently of sufficient magnitude to close voltage-dependent calcium channels and thereby decrease calcium influx, which ultimately leads to inhibition of vascular tone.

It is clear from previous studies that the response to endogenous vasodilators such as vasoactive intestinal peptide, calcitonin gene-related peptide, and endothelial factors is mediated by several pathways, only one of which involves membrane hyperpolarization. This is true for mesenteric and skeletal muscle resistance arteries as well. Dilations induced by higher concentrations of ADP ($>3\times10^{-6}$ M) achieved the same amplitude in the absence or
presence of glibenclamide. The primary mechanism of action of endothelium-derived relaxing factor, which is probably nitric oxide or a related substance, is thought to involve activation of guanylate cyclase, which leads to increased intracellular cGMP. Although subsequent steps in this pathway are not entirely clear (possibilities include increased extrusion or sequestration of calcium, altered calcium sensitivity of the contractile proteins, and phosphorylation of myosin light chain kinase), none of these steps would necessarily be linked to or be dependent on membrane hyperpolarization. Thus, changes in cGMP or other second messengers could play a role in the mechanism of dilation that is independent of membrane hyperpolarization.

Responses of mesenteric and skeletal muscle arteries to high concentrations of ADP were partly independent of the endothelium. Mathieson and Burnstock observed that ATP-induced dilations of large mesenteric arteries of the rabbit were independent of the endothelium and concluded that ATP probably directly activates P2-purinoceptors on the vascular muscle of this artery to inhibit tone. Although direct tests of the purinoceptor types involved in the response of resistance arteries were not undertaken in the present study, it appears likely that inhibitory P2-receptors are also present on the vascular muscle membrane of some systemic resistance arteries of the rabbit. Since glibenclamide blocked the hyperpolarizations induced by ADP in arteries with and without endothelium, the responses both to endothelium-derived hyperpolarizing factor and to activation of P2-receptors directly on the vascular cells apparently involve activation of K<sub>ATP</sub> channels.

Membrane hyperpolarization-independent mechanisms of dilation predominate in the distal segments of the middle cerebral artery; ADP relaxed this artery with no effect on membrane potential, and glibenclamide had no effect on the relaxations. This is somewhat unexpected because membrane hyperpolarization represents an important mechanism of dilation in the larger proximal segments of the same artery. The explanation for such differences is unclear, although substantial differences in reactivity of large and small cerebral arteries have been noted previously. A role for perivascular nerves in determining the distribution of calcium channel subtypes in large and small cerebral arteries of the rat has been suggested by Hill et al. A similar phenomenon might also pertain to the distribution of potassium channels in cerebral arteries of the rabbit, but clearly any such neural influence on potassium channels remains to be determined.

In summary, these studies demonstrate that ADP hyperpolarizes and relaxes mesenteric and skeletal muscle resistance arteries, in part via endothelium-dependent pathways, and that a portion of the vasodilator response results directly from the change in membrane potential. These observations support the hypothesis that K<sub>ATP</sub> channels are centrally involved in dilation of both muscular and resistance arteries in response to a variety of agents, including substances released from the endothelium. This does not appear to be the case for small distal segments of the middle cerebral artery, which are not hyperpolarized by ADP or other agents that normally activate K<sub>ATP</sub> channels. Such channels may be present only in very low density in these small cerebral arterial segments, or if present, they are unreactive to agents that activate K<sub>ATP</sub> channels in other tissues. The present studies further demonstrate the unique physiological and pharmacological characteristics of cerebral arteries, both when they are compared with extracerebral arteries and when they are compared with other cerebral arteries from different anatomic sites within this vascular bed.
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References


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