High-salt diet impairs vascular relaxation mechanisms in rat middle cerebral arteries

Julian H. Lombard, Francis A. Sylvester, Shane A. Phillips, and Jefferson C. Frisbee

Department of Physiology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

Submitted 18 September 2002; accepted in final form 25 November 2002

Lombard, Julian H., Francis A. Sylvester, Shane A. Phillips, and Jefferson C. Frisbee. High-salt diet impairs vascular relaxation mechanisms in rat middle cerebral arteries. Am J Physiol Heart Circ Physiol 284: H1124–H1133, 2003.—Male Sprague-Dawley rats were maintained on a low-salt (LS) diet (0.4% NaCl) or a high-salt (HS) diet (4% NaCl) for 3 days or 4 wk. PO$_2$ reduction to 40–45 mmHg, the stable prostacyclin analog iloprost (10 pg/ml), and stimulatory G protein activation with cholera toxin (1 ng/ml) caused vascular smooth muscle (VSM) hyperpolarization, increased cAMP production, and dilation in cerebral arteries from rats on an LS diet. Arteries from rats on a HS diet exhibited VSM depolarization and constriction in response to hypoxia and iloprost, failed to dilate or hyperpolarize in response to cholera toxin, and cAMP production did not increase in response to hypoxia, iloprost, or cholera toxin. Low-dose angiotensin II infusion (5 ng·kg$^{-1}$·min$^{-1}$·iv) restored normal responses to reduced PO$_2$ and iloprost in arteries from animals on a HS diet. These observations suggest that angiotensin II suppression with a HS diet leads to impaired relaxation of cerebral arteries in response to vasodilator stimuli acting at the cell membrane.

salt intake; hypertension; angiotensin; hypoxia; vascular smooth muscle; endothelium

Previous studies (8, 11, 19) have demonstrated that both chronic (4–8 wk) volume expanded hypertension caused by reduced renal mass (RRM) with exposure to a high-salt diet and chronic exposure of normotensive rats to a high-salt diet lead to structural changes in arterioles, reductions in microvessel density, and an impaired relaxation of skeletal muscle resistance vessels in response to a variety of vasodilator stimuli, including reduced PO$_2$, the stable prostacyclin analog iloprost, and acetylcholine. Subsequent studies demonstrated that alterations in microvessel structure, density, and reactivity in normotensive animals and RRM hypertensive rats on a high-salt diet develop quite rapidly. For example, Hansen-Smith et al. (15) demonstrated that microvascular rarefaction and profound ultrastructural alterations occur in arterioles of RRM hypertensive rats and normotensive animals after only 3 days on a high-salt diet. Other studies (10–12, 38) have demonstrated that vasodilator responses to reduced PO$_2$, acetylcholine, and iloprost are also impaired after short-term exposure to high-salt diet. The microvascular rarefaction and the reduced relaxation of resistance arteries to vasodilator stimuli in animals on a high-salt diet may be related to the angiotensin II (ANG II) suppression that occurs in response to elevated dietary salt intake because both the reduction of cremasteric microvessel density (16) and the impaired dilation of skeletal muscle resistance arteries in response to acetylcholine, iloprost, and reduced PO$_2$ in animals on a high-salt diet can be prevented by infusion of a low dose of ANG II (37, 38).

To date, the majority of studies investigating changes in vascular control mechanisms with a high-salt diet have focused on alterations of vascular reactivity in skeletal muscle resistance arteries and in situ arterioles of the skeletal muscle microcirculation. However, a recent study (19) demonstrated that pial arterioles of normotensive animals on a high-salt diet fail to dilate in response to acetylcholine and iloprost, in contrast to the relaxation that occurs in response to these vasodilator agonists in arterioles of animals on a normal salt diet. At the present time, the extent and mechanisms of the altered responses to vasodilator stimuli in the cerebral circulation are unknown. This is an important area of investigation in view of the vital role of the cerebral circulation in regulating blood flow to the brain and the differences in vascular control mechanisms that exist in different circulatory beds.

The present study tested three hypotheses. First, the impaired relaxations to reduced PO$_2$, iloprost, and acetylcholine that were previously demonstrated in skeletal muscle resistance arteries of RRM hypertensive rats on a high-salt diet also occur in middle cerebral arteries (MCA) after both chronic (4 wk) and short-term (3 days) exposure to elevated salt intake. Second, alterations in the electrophysiological response of the vascular smooth muscle (VSM) cells to vasodilator stimuli contribute to the impaired relaxation of cerebral resistance arteries in response to these stimuli. Third, vascular relaxation and the normal electrophysiological responses of resistance arteries to reduced PO$_2$ and iloprost can be restored by preventing the ANG II suppression that occurs in rats on a high-salt diet. An additional goal of the study was to gain insight into...
potential mechanisms of any impaired vasodilator responses that may occur in the MCA of animals on a high-salt diet.

MATERIALS AND METHODS

Experimental animals. Male Sprague-Dawley rats (Harlan; Madison, WI) weighing 250–400 g at the time of the experiment were used for these studies. Rats were fed either a high-salt (4% NaCl) or low-salt (0.4% NaCl) diet (Dyets; Bethlehem, PA) with tap water to drink ad libitum. Rats were maintained on the diet for either 3 days (short term) or 4 wk (chronic) before studies of the isolated vessels. An additional group of instrumented rats on a short-term high-salt diet received an intravenous infusion of a low dose of ANG II to prevent the ANG II suppression that occurs in response to elevated dietary salt intake (16, 38). In those animals, chronic catheters were installed for intravenous infusion of ANG II, as previously described (37, 38). After recovering for a minimum of 5 days, the rats were placed on a high-salt diet for 1 wk and ANG II was infused intravenously at a dose of 5 ng·kg⁻¹·min⁻¹ for the final 3 days before the experiment.

General procedures. On the day of the isolated vessel experiment, the rat was anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg; Abbott Laboratories; North Chicago, IL). The brain was removed, and the proximal portion of the middle cerebral artery (100–250 μm inner diameter) was carefully isolated and placed in warmed physiological salt solution (PSS). The PSS was bubbled with a gas mixture containing 21% O₂-5% CO₂-74% N₂ and was composed of the following (in mM): 119 NaCl, 4.7 KCl, 1.17 MgSO₄, 1.6 CaCl₂, 1.18 NaH₂PO₄, 24 NaHCO₃, 0.026 EDTA, and 5.5 glucose.

After being isolated, the arteries were placed in a heated (37°C) chamber that allowed the lumen of the vessel to be perfused with PSS and the outside of the vessel to be superperfused with PSS from separate reservoirs. This dual perfusion/superfusion system allows extravascular gas concentrations, luminal gas concentrations, and intravascular pressure to be controlled simultaneously (6, 7). Vessel diameters were measured via television microscopy and a video micrometer system. After the artery was mounted on the micropipettes, it was stretched to approximate its in situ length and was equilibrated at an intraluminal pressure of 80 mmHg (7). After the vessel was mounted, the viability of the artery was assessed by measuring the response of the vessel to 100 nM serotonin in the vessel chamber. Any vessel with a control value of 100 μm for a minimum of 5 s and an abrupt return to baseline on exit of the electrode from the cell. Five measurements were made under each condition, and the results were averaged to obtain the final value of Eₘ for that vessel under each experimental condition.

Effects of reduced PO2 on resting diameter and VSM Eₘ. After the control equilibration at 21% O₂, vessel diameter and VSM Eₘ were measured before and during a simultaneous reduction of the O₂ concentration of the PSS in the tissue bath (superfusate) and infow reservoir (luminal perfusate). To test the response of the vessels to 100 μm for a minimum of 5 s and an abrupt return to baseline on exit of the electrode from the cell. Five measurements were made under each condition, and the results were averaged to obtain the final value of Eₘ for that vessel under each experimental condition.

Response to iloprost, aprikalim, cholera toxin, forskolin, and Ca2⁺-free solution. In addition to determining the response of the vessels to reduced PO2, we assessed the response of arteries from each group of rats to the stable prostanoid analog iloprost (10 pg/ml); the stimulatory G (G)ₐ protein activator cholera toxin (1 ng/ml); and forskolin (10 μM), a direct activator of adenyl cyclase. The concentrations of the vasoactive agents used in these experiments were selected on the basis of prior experiments conducted in our laboratory (8–10, 12, 13, 18, 36, 37). In an additional series of experiments to evaluate the effect of high-salt diet on ATP-sensitive K⁺ (KATP) channel function, the response of vessels to the KATP channel opener aprikalim (0.1 μM-10 μM) was evaluated by measuring vessel diameter and VSM Eₘ in MCA isolated from animals on short-term high-salt and low-salt diets. Vessel responses to aprikalim were compared in the presence and absence of the KATP channel inhibitor glibenclamide (1 μM). To administer the drugs, superfusion of the vessel was briefly interrupted and an appropriate amount of drug was added to the PSS to achieve the final desired concentration in the vessel chamber. All measurements were made with the vessel fully pressurized by clamping the outflow pipette.

During exposure to the vasodilator agonists, vessel diameter was monitored continuously, and the final measurement corresponded to the maximum change in steady-state diameter attained during exposure to the drug. After a steady-state diameter was achieved, the agonist was washed out of the chamber and diameter was allowed to return to the control value that existed before application of the agonist. After the response of the vessels to the different vasodilator stimuli had been determined, active tone and maximum diameter of the arteries were determined by measuring the diameter increase that occurred during maximal dilation with a Ca²⁺-free relaxing solution containing the following (in mM): 119 NaCl, 4.7 KCl, 1.17 MgSO₄, 1.6 CaCl₂, 1.18 NaH₂PO₄, 24 NaHCO₃, 0.026 EDTA, and 5.5 glucose. Any
vessel that did not exhibit a large dilation in response to Ca2+ -free solution was not included in the analysis of the data.

Cyclooxygenase and thromboxane synthase inhibition. Previous studies by our group (7, 20) have indicated that endothelium-derived cyclooxygenase (COX) metabolites, most likely prostacyclin, mediate hypoxic dilation in response to reduced PO2 in rat MCA. Several other studies (1, 28, 34) have suggested that a COX product, most likely prostaglandin E2 (PGE2) or thromboxane A2 (TXA2), acts as a constrictor factor in several forms of hypertension. To evaluate the role of COX metabolites in mediating the response of the vessels to reduced PO2 in animals on high- and low-salt diets, vessel diameters were measured before and during inhibition of cyclooxygenase with 1 μM indomethacin. The role of TXA2 in contributing to the altered response of vessels to reduced PO2 was tested by measuring changes in vessel diameter during exposure to hypoxia in the presence and absence of the thromboxane synthase inhibitor dazoxiben (10 μM) in the tissue bath.

Determination of prostacyclin and TXA2 production. In an additional series of experiments, we assessed the production of prostacyclin I2 (PGI2) and TXA2 by cerebral arteries during exposure to reduced PO2, as described previously (20). In those studies, brains were removed from anesthetized rats that were on either low-salt or high-salt diet for 1 wk. MCA and other arteries from the circle of Willis, or branching from the circle of Willis, were isolated, pooled, and incubated in 1 ml of PSS for 30 min under control conditions (21% O2) and during equilibration of the PSS with reduced PO2 (5% O2), to lower bath PO2 to 35–40 mmHg for an additional 30 min. After each 30-min equilibration period, the PSS was removed from the incubation chamber, frozen in liquid N2, and stored at −80°C. PGI2 and TXA2 release by vessels under the different O2 levels was assessed in the Physiology Department Biochemical Assay Core Facility at the Medical College of Wisconsin, with the use of commercially available enzyme immunoassay kits purchased from Cayman Chemical (Ann Arbor, MI). Prostacyclin release was evaluated by measuring the level of the stable PGI2 metabolite 6-keto-prostaglandin F1α (PGF1α) in the incubation medium, and TXA2 release was assessed by measuring the levels of TXB2, the stable metabolite of TXA2, in the incubation medium.

Determination of vascular cAMP levels. In a separate series of experiments, we assessed vascular cAMP production during exposure to different challenges in cerebral arteries of rats on low-salt or high-salt diet. In these experiments, MCA and other arteries from the circle of Willis or branching from the circle of Willia were isolated from rats on low-salt or high-salt diet for 1 wk. The vessels were immediately placed in PSS containing 1 mM 3-isobutyl-1-methylxanthine, a non-specific inhibitor of cellular phosphodiesterases, to minimize the degradation of cAMP produced by the vessel (29). The PSS for these experiments was equilibrated with a 21% O2-5% CO2-74% N2 gas mixture. After a 60-min equilibration period, vessels from rats on low-salt or high-salt diet (pooled vessels from 6 rats for all challenges) were then either exposed to hypoxia (5% O2) for 30 min; challenged with 1 ng/ml iloprost, 1 ng/ml cholera toxin, or 0.1 μM forskolin; or maintained under the 21% control condition (normoxia). After imposition of the different challenges, the vessels were immediately frozen in liquid N2. The frozen vessels were subsequently homogenized to a powder and resuspended in 0.1 M hydrochloric acid. After centrifugation, the supernatant was removed and cAMP concentration in the supernatant was determined with a commercially available competitive binding immunoassay kit (Assay Designs; Ann Arbor, MI). The resulting values of cAMP concentration in the supernatant were corrected for dilution effects and normalized to protein content using the Bradford method for protein determination (30).

Statistical analysis. In all experiments, data were summarized as means ± SE. Differences between multiple means were assessed using analysis of variance with a subsequent Newman-Keuls test. A paired Student’s t-test was used to assess the response of the vessels to a single reduction of perfusate/superfusate oxygen concentration, or to a single concentration of agonist. A probability of P < 0.05 was considered to be statistically significant.

RESULTS

Effect of vasodilator stimuli on diameter of MCA of animals on high- and low-salt diets. Figure 1 summarizes the effect of chronic (A) and short-term (B) high-

![Graph A](http://ajpheart.physiology.org/DownloadedFrom/http://ajpheart.physiology.org/)

![Graph B](http://ajpheart.physiology.org/DownloadedFrom/http://ajpheart.physiology.org/)
salt diets on the response of MCA to reduced PO2 and the stable prostaacyclin analog iloprost. Arteries from rats maintained on either a short-term or a chronic low-salt diet dilated in response to reduced PO2 and iloprost, as previously reported for MCA of animals on a normal salt diet (7, 20). However, arteries of rats maintained on the high-salt diet for either 3 days or for 4–8 wk exhibited a paradoxical constriction in response to both reduced PO2 and iloprost.

Maintenance of plasma ANG II levels by intravenous infusion of a low-dose of ANG II for 3 days restored the dilator response to reduced PO2 and iloprost in MCA of rats fed a short-term high-salt diet (Fig. 1B). Dilation of the vessels in response to direct activation of adenylyl cyclase with forskolin was unaffected by dietary salt content or ANG II infusion. In animals on chronic low- and high-salt diets, the mean diameter increase in response to forskolin was 96 ± 6 μm (n = 12) and 92 ± 6 μm (n = 11), respectively. In animals on short-term diet, mean diameter increase in response to forskolin was 77 ± 10 μm (n = 12) in animals on the low-salt diet, 94 ± 3 μm (n = 8) in animals on the high-salt diet, and 106 ± 7 μm (n = 8) in animals on the short-term high-salt diet with low-dose ANG II infusion.

Effect of reduced PO2, iloprost, and forskolin on VSM Em in MCA of rats on low- and high-salt diets. Figure 2 summarizes the effect of reduced PO2, iloprost, and forskolin on VSM Em in pressurized MCA of rats on low- and high-salt diets. In these studies, resting Em of the VSM cells before exposure to reduced PO2 or vasodilator agonists was similar in arteries of animals on low- and high-salt diets. In arteries from rats on short- and chronic low-salt diets, exposure to reduced PO2 and iloprost caused a significant hyperpolarization of the VSM cells, and an increase in vessel diameter similar to that reported in our previous studies of animals on a normal salt diet (20). In arteries from animals on short-term and chronic high-salt diets, reduced PO2 and iloprost caused depolarization of the arterial smooth muscle cells, which is consistent with the paradoxical vasoconstrictor response to these dilator stimuli in arteries from animals on a high-salt diet (Fig. 1). Forskolin caused a large hyperpolarization of the VSM cells that was similar in magnitude in MCA of animals on high- and low-salt diets.

The effect of low-dose ANG II infusion on the electrophysiological responses to reduced PO2, iloprost, and forskolin in MCA of animals subjected to a short-term elevation in dietary salt intake is also summarized in Fig. 2B. Consistent with the protective effect of ANG II to restore vascular relaxation in response to reduced PO2 and iloprost, these vasodilator stimuli caused a significant hyperpolarization of the smooth muscle cells in arteries of animals on a high-salt diet and receiving low-dose ANG II infusion. This response to reduced PO2 and iloprost was in direct contrast to the VSM depolarization that occurred in response to these stimuli in vessels of animals that were on a high-salt diet but not receiving ANG II infusion. In angiotensin-infused rats, forskolin caused a large hyperpolarization of the arterial smooth muscle cells that was similar in magnitude to that occurring in noninfused rats on a high-salt diet.

Effect of high-salt diet on response of MCA to aprikalim and cholera toxin. Figure 3 shows the effect of the KATP channel opener aprikalim on diameter (Fig. 3A) and VSM Em (Fig. 3B) in the MCA of rats on short-term low-salt and high-salt diets. In these experiments, aprikalim caused a glibenclamide-sensitive dilation and VSM hyperpolarization that was similar in magnitude in arteries of animals on short-term high- and low-salt diets.

Figure 4 shows the effect of the Gs protein activator cholera toxin on diameter (Fig. 4A) and VSM Em (Fig. 4B) in the MCA of rats on short-term low-salt and high-salt diets.
B) in MCA of animals on short-term low-salt and high-salt diets. In these studies, cholera toxin caused vasodilation and VSM hyperpolarization in arteries of animals on a low-salt diet. MCA of animals on high-salt diet failed to dilate in response to cholera toxin, and VSM $E_m$ was unaffected by cholera toxin.

**Vascular cAMP levels.** Figure 5 compares cAMP formation in response to different vasodilator stimuli in cerebral vessels from rats on a low-salt or high-salt diet for 1 wk. In animals on a low-salt diet, cAMP levels rose significantly in response to reduced $P_{O_2}$, iloprost, cholera toxin, and direct activation of adenylyl cyclase with forskolin. In contrast, animals on a high-salt diet did not show an increase in cAMP levels during exposure to reduced $P_{O_2}$, iloprost, or cholera toxin. However, vessels of animals on the high-salt diet exhibited an increase in cAMP content in response to forskolin that was similar to the increase in cAMP content occurring in vessels from animals on a low-salt diet.

**Effect of endothelium removal on response to reduced $P_{O_2}$ and iloprost in arteries of animals on high- and low-salt diet.** The effect of endothelium removal on the response to hypoxia and iloprost in MCA isolated from animals on short-term low- and high-salt diets is shown in Fig. 6. Removal of the endothelium eliminated the dilation in response to hypoxia in MCA of animals on a low-salt diet but did not affect vessel responses to iloprost. In MCA of animals on a high-salt diet, endothelial removal significantly attenuated the vasoconstrictor response to hypoxia, while the constric- tor response to iloprost before and after endothelial removal was not significantly different.

**Effect of indomethacin and thromboxane synthase inhibition on response to reduced $P_{O_2}$ in arteries of animals on high- and low-salt diet.** Figure 7 compares the effect of the cyclooxygenase inhibitor indomethacin and thromboxane synthase inhibitor on response to hypoxia in MCA isolated from animals on short-term low- and high-salt diets.
on the responses to reduced PO₂ in MCA of animals on short-term low-salt and high-salt diets. In these experiments, indomethacin eliminated the hypoxic dilation in MCA of animals on a low-salt diet and abolished the paradoxical vasoconstriction in response to reduced PO₂ in MCA of animals on a high-salt diet. Treatment with the solvent vehicle for indomethacin had no effect on hypoxic dilation of MCA from rats on a low-salt diet or hypoxic vasoconstriction of MCA from rats on a high-salt diet.

The effect of the thromboxane synthase inhibitor dazoxiben on the response of MCA to reduced PO₂ in animals on short-term low-salt or high-salt diet is summarized in Fig. 8. In these experiments, dazoxiben eliminated hypoxic vasoconstriction of MCA from animals on a high-salt diet but did not affect vasodilation in response to reduced PO₂ in MCA of animals on a low-salt diet. In a separate series of experiments to verify the effectiveness of dazoxiben, thromboxane release by MCA of Sprague-Dawley rats on standard chow fell from 92.8 ± 7.4 pg/mg before treatment with...
AJP-Heart Circ Physiol • VOL 284 • APRIL 2003 • www.ajpheart.org

Fig. 9. Effect of reduced PO2 on prostacyclin (PGF1α) (A) and thromboxane (TxB2) (B) release from cerebral arteries of rats on short-term low-salt and high-salt diet for 1 wk. Data are expressed as mean percentage of 21% O2 low-salt control (± SE) and n = 4 rats per treatment. *Significant difference from the control value determined in vessels of animals on a low-salt diet during equilibration of the PSS with 21% O2; †significant difference from 21% O2 in the high-salt group.

Effect of high-salt diet on prostacyclin and thromboxane release. Figure 9 shows the effect of reduced PO2 on prostacyclin and TxA2 release from cerebral arteries of rats maintained on a low-salt diet or a high-salt diet for 1 wk, as evaluated by the release of their stable metabolites, PGF1α and TxB2, respectively. In our previous study (20), MCA from rats on short term and chronic high-salt diet exhibited a paradoxical vasoconstriction and depolarization of the VSM cells during exposure to reduced PO2.

The paradoxical constriction and smooth muscle depolarization occurring in response to hypoxia in vessels of animals on a high-salt diet could be due to a variety of factors, including the liberation of vasoconstrictor factors from the endothelium, an intrinsic alteration in the response of the VSM cells themselves to reduced PO2, or an altered response of the smooth muscle cells to the prostacyclin that is released from the endothelium in the rat middle cerebral artery (20). As discussed in detail below, it appears that enhanced production of TxA2, a vasoconstrictor metabolite of arachidonic acid, is a crucial contributor to the impaired response of the vessel to reduced PO2 in animals on a high-salt diet.

In addition to enhanced TxA2 release, MCA of animals on a high-salt diet also exhibit an intrinsic alteration in their response to PGI2 because vessels from animals on a high-salt diet constricted in response to...
direct application of the stable prostacyclin analog iloprost, rather than diluting like MCA from rats on a low-salt diet (Fig. 1) or normal salt diet (20). The hypothesis that the paradoxical vasoconstrictor response to PGL2 is due to intrinsic alterations in the response of the VSM cells to PGL2 is supported by the observation that endothelium denuded vessels of animals on a high-salt diet also constricted in response to iloprost, whereas endothelium denuded vessels from animals on a low-salt diet dilated in response to iloprost. However, the role of altered PGL2 responses in contributing to an impaired relaxation of the vessels during exposure to reduced PO2 in animals on high-salt diet is not clear because PGL2 production by vessels from animals on high-salt diet appears to decrease during hypoxia (see below).

Role of TxA2 and altered PGL2 release in contributing to impaired dilation of cerebral arteries of animals on high-salt diet. Previous studies have suggested that overproduction of vasoconstrictor factors, such as PGH2 and TxA2 via cyclooxygenases, can contribute to an impaired relaxation in response to endothelium-dependent vasodilator stimuli in spontaneously hypertensive rats (1, 28) and spontaneously hypertensive hamsters (34). The results of the present study suggest that TxA2 plays a role in mediating the paradoxical constriction in response to reduced PO2 in MCA of animals on a high-salt diet because TxA2 production was significantly elevated during resting conditions in vessels from rats on high-salt diet and TxA2 release tended to increase during hypoxia in vessels from animals on high-salt diet. Hypoxic constriction of MCA from rats on a high-salt diet was also blocked by the thromboxane synthase inhibitor dazoxiben. In contrast, cerebral arteries from rats on a low-salt diet showed a reduced release of TxA2 in response to hypoxia, and hypoxic dilation was unaffected by dazoxiben.

In the present study, PGI2 release increased in response to reduced PO2 in cerebral arteries of animals on a low-salt diet, as previously reported for arteries of animals on a normal salt diet (20). However, in contrast to our earlier findings in skeletal muscle resistance arteries (18), PGI2 release during resting conditions was significantly higher in arteries of animals on high-salt diet and decreased in response to reduced PO2. In conjunction with the lack of a dilator response to iloprost in arteries of animals on a high-salt diet, these observations suggest that enhanced PGI2 release plays an important role in mediating hypoxic dilation in MCA of animals on a low-salt diet, but factors other than altered PGI2 release are responsible for the impaired dilation in response to reduced PO2 in arterial vessels of animals on a high-salt diet.

The source of the increased cyclooxygenase activity that produces the elevated levels of TxA2 and PGI2 in vessels of animals on high-salt diet is most likely in the endothelium, since the expression of COX-1 and COX-2 is up to 20 times greater in the endothelium than in the smooth muscle cells (5). In addition, thromboxane can be produced by the endothelium, in addition to its primary source of production in the platelets (5). Our observation that hypoxic dilation of MCA of rats on a low-salt diet and hypoxic constriction of arteries from rats on a high-salt diet can both be eliminated by endothelial removal also supports the hypothesis that the endothelium is the source of PGI2 and TxA2 in the present studies.

Role of impaired signal transduction pathways in contributing to loss of vasodilator responses in MCA of rats on a high-salt diet. The present study indicates that the impaired relaxation and the lack of VSM hyperpolarization in response to hypoxia and iloprost in MCA of rats subjected to short-term and chronic elevations in dietary salt intake are most likely due to alterations that occur early in the signal transduction pathway, e.g., changes in receptor function or G protein-coupling mechanisms, rather than alterations in the function of membrane K+ channels or in second messenger function. Of particular importance in this regard is the observation that the electrical and mechanical responses of the vessels to the Gs protein activator choleran toxin are impaired in animals on the high-salt diet, i.e., vessels of animals on the low-salt diet exhibit vasodilation and hyperpolarization in response to choleran toxin, whereas MCA from animals on a high-salt diet do not dilate or hyperpolarize in response to choleran toxin (Fig. 4). In contrast, the glibenclamide-sensitive dilation of the MCA in response to activation of KATP channels with aprikalim (Fig. 3) and the dilation and VSM hyperpolarization occurring in response to direct activation of adenyl cyclase with forskolin (Fig. 2) were unaffected by short-term or chronic exposure to high-salt diet, as previously reported for skeletal muscle resistance arteries (18, 37).

The results of the present study are consistent with those of Stekiel and co-workers (33), who demonstrated that in situ arterioles and venules of reduced renal mass hypertensive rats exhibited an impaired hyperpolarization of their VSM cells in response to β-adrenergic stimulation with isoproterenol and direct activation of the Gs protein with choleran toxin, whereas the electrophysiological response of the vessels to direct activation of adenyl cyclase with forskolin in that study was unaltered in the hypertensive animals. Subsequent studies (4, 14, 31) have indicated that impaired coupling between membrane receptors and downstream signal transduction events also occurs in spontaneously hypertensive rats. The present study is the first to demonstrate that a high-salt diet alone can lead to impaired coupling between membrane receptors and downstream messenger systems.

Because prostacyclin is a hyperpolarizing vasodilator that acts via the adenyl cyclase system (22, 25, 32) and enhanced prostacyclin release is a crucial mediator of hypoxic dilation of MCA of rats on normal salt diet (20), it is likely that a similar impairment of this pathway may contribute to loss of the vasodilator response to iloprost (and to reduced PO2) that we observed in arteries of animals on a high-salt diet. In this respect, the present report provides the initial evidence that high-salt diet can also lead to an impaired coupling between membrane receptors and the ultimate signal transduction events.
electrophysiological response to reduced PO₂ in cerebral resistance arteries. The hypothesis that coupling between membrane receptors and downstream-second messengers is impaired at the level of the G protein is supported by the results of the cAMP studies, which demonstrated that the increase in cAMP production in response to hypoxia, iloprost, and the G protein activator, cholera toxin are impaired in rats on a high-salt diet, whereas the increase in cAMP production in response to direct activation of adenylyl cyclase with forskolin is normal. The latter results are consistent with the results of recent studies from our laboratory (12), which demonstrated that vasodilation, VSM membrane hyperpolarization, and cAMP production are also impaired in skeletal muscle resistance arteries of normotensive rats on a high-salt diet.

Role of ANG II in maintaining vasodilator responses of MCA. Previous studies in our laboratory (37, 38) suggested that suppression of ANG II may play a role in the impaired reactivity of skeletal muscle resistance arteries to vasodilator stimuli in animals on a high-salt diet. In the present study, low-dose ANG II infusion in animals on a short-term high-salt diet restored both the relaxation and the VSM hyperpolarization that normally occurs in response to reduced PO₂ and iloprost. These observations suggest that ANG II mediates its protective effect by preserving the normal electrophysiological responses of the smooth muscle cells to these vasodilator stimuli. Judging from the nature of the impaired dilator responses in vessels of animals on a high-salt diet but not receiving ANG II infusion, it appears that this protective effect of ANG II infusion is mediated by an action of the peptide to maintain normal signal transduction mechanisms at the level of the cell membrane receptor(s) or G proteins.

One question regarding the mechanism of action of ANG II in restoring vasodilator responses in animals on a high-salt diet concerns the possible role of reactive oxygen species in mediating or modulating the protective effect of ANG II to restore vascular relaxation mechanisms. ANG II infusion leads to upregulation of reduced NAD(P)H oxidase subunits and to increased vascular superoxide production (21, 27). This increase in superoxide production has been postulated to lead to an inactivation of nitric oxide and reduced endothelium-dependent dilation. Whereas the relationship of increased superoxide production to the protective effect of ANG II to restore normal reactivity to vasodilator stimuli in vessels of animals on high-salt diet remains to be determined, we believe that it is unlikely that increased superoxide production contributes to the restoration of vascular relaxation mechanisms in animals on a high-salt diet, because studies showing increased superoxide production with ANG II infusion have generally employed fairly large doses of ANG II (0.7–1.0 mg·kg⁻¹·day⁻¹) to produce hypertension (21, 27), whereas NAD(P)H oxidase activity was not increased in animals receiving a lower dose (0.3 mg·kg⁻¹·day⁻¹) of ANG II (27). In addition, previous studies (37) in our laboratory have demonstrated that infusion of ANG II in doses similar to those employed in the present experiments restores acetylcholine-induced dilation of skeletal muscle resistance arteries, which would not be expected if that level of ANG II infusion produced a substantial increase in superoxide production.

Perspectives. The novel aspects of the current study are the demonstration that the relaxation of cerebral arteries and the electrophysiological responses of cerebral arterial smooth muscle cells to reduced PO₂ and the prostacyclin analog iloprost are profoundly impaired by both short-term (3 day) and chronic exposure to a high-salt diet and that ANG II has a protective effect to maintain normal vascular relaxation and VSM hyperpolarization in MCA of animals on a high-salt diet. Endothelial function also appears to be altered by elevated salt intake, because short-term exposure to high-salt diet eliminates acetylcholine-induced dilation of MCA. It also appears that short-term exposure to high-salt diet leads to significant alterations in arachidonic acid metabolism in MCA, because these vessels exhibited significant increases in both PGÎ2 and TxA2 release in response to high-salt diet. In addition, the pattern of PGÎ2 and TxA2 release was also altered by high-salt diet, such that vessels of animals on a low-salt diet exhibited an increase in PGÎ2 production and a decrease in TxA2 production during exposure to hypoxia, whereas PGÎ2 production was reduced and TxA2 production tended to increase when vessels from animals on a high-salt diet were exposed to reduced PO₂.

The observation that a high-salt diet leads to rapid and profound changes in the regulation of active tone in resistance arteries of normotensive animals may be crucial in identifying factors that could predispose an individual to salt-sensitive forms of hypertension. The demonstration of salt-induced decreases in the reactivity of resistance arteries to vasodilator stimuli in normotensive animals may also reveal previously unknown defects in the ability of normotensive individuals on a high-salt diet to regulate blood flow and to respond to circulatory stresses such as hypoxia, hemorrhage, and exercise.

The authors are grateful to Donna Bizub and Tianjian Huang for outstanding technical assistance and to Schering and Pfizer for the generous gifts of iloprost and dazoxiben, respectively.

This study was supported by National Heart, Lung, and Blood Institute Grants HL-29587, HL-37374, and HL-65289.

REFERENCES

HIGH-SALT DIET AND CEREBRAL VASODILATION

28. Rapoport RM and Williams SP. Role of prostaglandins in acetylcholine-induced contraction of aorta from spontaneously hypertensive and Wistar-Kyoto rats. Hypertension 28: 64–75, 1996.