Low-dose ouabain constricts small arteries from ouabain-hypertensive rats: implications for sustained elevation of vascular resistance

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Moreover, high-dietary salt increases plasma EO levels in normal men (32).

Elevated EO levels are also observed in rats with DOCA-salt hypertension (20), reduced renal mass hypertension (24), and Milan strain hypertension (14). Additionally, the chronic treatment of normal rats and mice with ouabain elevates BP, i.e., ouabain-induced hypertension (OH) (12, 33, 50). Although digoxin, like ouabain, inhibits Na+ pumps (17), chronic digoxin administration to rats not only fails to elevate BP but even blocks the effect of ouabain on BP (23, 33). This might suggest that the effect of ouabain on BP is not the result of a reduced Na+ pump activity. However, the genetically reduced expression and activity of the ouabain-sensitive Na+ pump α2-catalytic subunit also causes hypertension (4, 51). Furthermore, the mutation of the α2-Na+ pump ouabain binding site abolishes the hypertensinogenic effect of ouabain (12).

In addition to its influence on ion transport, ouabain is a growth factor that activates signaling cascades via a Src kinase pathway linked to the Na+,K+-ATPase but independent of the influence of ouabain on ion distribution (48). Consequently, ouabain may have multiple and complex effects in vivo, possibly including vascular growth and remodeling. Indeed, a number of studies indicate that vascular growth and remodeling may play a central role in the pathogenesis of hypertension (15, 22, 36). In at least several forms of hypertension, however, the remodeling appears to be an adaptive response rather than the cause of the elevated BP (7, 22, 34).

Many investigators have compared the acute effects of ouabain on arteries isolated from normotensive and hypertensive rats, but most have studied large arteries (e.g., aorta) and high concentrations (>10 μM) of ouabain (9, 40). A few studies on arteries from OH rats may provide information about the pathogenesis of the hypertension, but the data are inconsistent. Contractile responses to high K+-, but not phenylephrine (PE), were augmented in the main renal arteries from OH rats, whereas acetylcholine (ACh)-induced relaxation was normal (25). In the mesenteric arteries from OH rats, however, nitric oxide (NO) and prostanoi production were increased, but endothelin-derived hyperpolarizing factor was decreased (41, 49). On the other hand, endothelial NO production was impaired in renal-descending vasa recta from OH rats (8). In isolated, pressurized (80 mmHg) third-order mesenteric arteries from OH rats, “internal diameters were significantly reduced…compared with controls” (~320 μm); nevertheless, norepinephrine-induced vasoconstriction and ACh-induced vaso-dilation were normal (6). These authors also observed an increased collagen deposition in the media (containing 3 to 4

OUABAIN, A MAMMALIAN adrenocortical hormone (4, 20, 21, 28), apparently plays an important role in the pathogenesis of hypertension in humans and rodents. About 50% of patients with essential hypertension and a majority of patients with adenocortical adenomas and hypertension have elevated plasma endogenous ouabain (EO) (42, 45). In normal humans, ouabain infusion increases peripheral vascular resistance, reduces blood flow, and elevates blood pressure (BP) (35, 47).
layers of myocytes), as well as a narrowing of the OH rat artery lumen, but no change in media or adventitia thickness. They suggested that the lumen narrowing might be involved in the genesis of the elevated BP in OH rats, but they ignored the possibility that the circulating ouabain itself might play a dynamic role in the sustained BP elevation.

To explore further the question of how the elevated BP in OH rats is sustained, we focused on fourth-order mesenteric arteries (~165-μm internal diameter, with only a single layer of myocytes) from control and OH rats. Specifically, we asked whether structural changes or the intrinsic contractile properties of the pressurized small arteries or the influence of low-dose ouabain could account for the elevated BP in OH rats. The data indicate that, in contrast to third-order arteries (Ref. 6 and this report), the fourth-order arteries apparently are not structurally altered or narrowed. The normal contractile properties of fourth-order arteries are, however, consistent with similar findings in third-order arteries (6). An analysis of the distribution of resistances indicates that the smaller arteries account for much more of the peripheral resistance than do the third-order arteries. This, plus data on myogenic reactivity and the effect of low-dose ouabain, leads to the conclusion that the vasoconstrictor effect of the circulating ouabain in small arteries, and not structural changes, makes a major contribution to the elevated BP in OH rats.

METHODS

Experimental Animals

Ethical approval. All rat protocols were approved by the Institutional Animal Care and Use Committee of the University of Maryland School of Medicine.

Induction of ouabain hypertension. Normal male Sprague-Dawley rats (Charles River, Wilmington, MA) were used for this study; body weights were 500–600 g at the time of euthanasia. Under halothane anesthesia, hypertensive rats were induced by implanting 1.5-mg slow-release ouabain pellets (Innovative Research of America, Sarasota, FL) subcutaneously between the scapulae; control rats received vehicle pellet implants. BP rose to an elevated plateau in 2 to 3 wk in the ouabain pellet-implanted rats [e.g., see Fig. 6A in Cao et al. (8)]. Arteries from four groups of 10–12 rats were studied; in each group, half were ouabain-treated and half were controls.

BP Measurements

Systolic arterial BP (SBP) was measured in conscious, restrained rats using tail-cuff plethysmography (25). Three to five reproducible (within 15%) determinations were averaged to obtain a single BP value. The SBP data collection was performed “double blind,” i.e., the individual measuring the SBP was unaware of the status of the rats (OH vs. control) at the time of the measurements.

Arterial Structure

Arterial diameter and wall thickness. The rats were euthanized by CO2 overdose followed by decapitation. Small (4th order) arteries from the superior mesenteric artery arcade were isolated. The arteries were cannulated at both ends, pressurized to 70 mmHg (with no internal flow), and superfused with physiological salt solution (PSS) at 35–37°C to permit myogenic tone (MT) to develop (51, 52). In most experiments, the artery external diameters were continuously monitored with a Nikon TMS microscope (Melville, NY) with a ×10 objective and with a monochrome CCD camera operated by LabView software (National Instruments, Austin, TX). Unless specifically noted, the reported diameters are external diameters because the electronic edge detector was often unable to discriminate clearly the inner edge of the artery wall in the low-magnification images required for the diameter measurements. Some studies were therefore performed on an Olympus IX81 inverted microscope equipped with a ×40 water immersion objective (Olympus America, Center Valley, PA); images were processed with Slidebook 4.2 software (Intelligent Imaging Innovations, Denver, CO). This enabled us to measure directly the wall thickness in pressurized arteries in various states of dilation and constriction to calculate the internal diameters.

Visualization of myocytes and endothelial cells. To visualize individual myocytes or endothelial cells within the arteries were visualized in a Zeiss LSM 5 Live inverted microscope equipped with a ×40 water immersion objective (Carl Zeiss, Stuttgart, Germany). This enabled us to obtain z-axis stacks of x-y plane images, 1 μm apart, at a rapid rate (up to two 30-plane z-stacks/s with 512 × 100 pixels/plane). Zeiss Efficient Navigation (Carl Zeiss) software was then used to construct three-dimensional (3-D) images of small artery segments as a function of time. Approximately 1% of the fluorochrome was bleached during the accumulation of each z-stack. This and numerous spontaneous, local fluctuations of fluo-4 fluorescence, and thus Ca2+ concentration, precluded the detection of the anticipated small ouabain-induced increase in myocyte Ca2+ concentration (51). Also, the diameters of the arteries employed in this study were too large to obtain high-resolution x-y images or z-stacks through the entire artery cross section. Nevertheless, we were able to obtain high-resolution images of individual myocytes within small, crescent-shaped segments of the rat artery wall during vasoconstriction and dilation. Histology. Small segments of the mesentery, including the fourth-order mesenteric artery arcade and the two third-order feeder arteries that feed the ends of the arcade (10), were isolated from control and OH rats. These mesentery segments were incubated in Ca2+-free PSS (2 × 10 min) and Ca2+-free PSS with 10 μM Na+-nitroprusside (1 × 10 min), fixed in 10% formalin (48 h), dehydrated in ethanol, and embedded in paraffin. Thin sections of the third- and fourth-order arteries were stained with Masson’s trichrome.

Measurements of Arterial Function

Myogenic tone. The magnitude of the myogenic constriction is expressed as a percentage of the passive external diameter (PD), measured in Ca2+-free PSS (at the end of each experiment; i.e., MT = (myogenic constriction/PD) × 100. PE- and K+ -evoked vasoconstriction. Vasocostrictions evoked by PE or an elevated external K+ concentration are expressed as the evoked vasoconstriction relative to PD: Vasoconstriction = [(PD – constrained diameter)/PD] × 100. Note that in this method of expressing the constriction (43), the vasocostrictor-induced constriction is superimposed on MT.

ACh-induced vasodilation. Arteries were constricted with 5 μM PE and then treated with 10-9–10-4 M ACh in the absence and presence of 1 μM Nω-nitro-L-arginine methyl ester (L-NAME), a NO synthase inhibitor. Relaxation is expressed in terms of the decline in the PE-evoked constriction (=100% in the absence of ACh).
Estimation of sarcoplasmic reticulum Ca$^{2+}$ store content. The relative size of the inositol trisphosphate-releasable and caffeine-releasable (ryanodine sensitive) sarcoplasmic reticulum (SR) Ca$^{2+}$ stores were estimated from, respectively, the 5 μM PE- or 10 mM caffeine-evoked vasosconstriction in the absence of external Ca$^{2+}$. The Ca$^{2+}$-free medium was introduced 2 min before either the PE or caffeine was added; thus, the arteries were diluted to PD at the time the PE or caffeine was added. The PE was washed out after 2 min and the caffeine after 30 s, and normal Ca$^{2+}$-replete PSS was restored 2 min later.

Reagents and Solutions

Artery dissection solution contained (in mM) 145 NaCl, 4.7 KCl, 1.2 MgSO$_4$·7H$_2$O, 2.0 MOPS, 0.02 EDTA, 1.2 NaH$_2$PO$_4$, 2.0 CaCl$_2$·2H$_2$O, 5.0 glucose, and 2.0 pyruvate and 1% albumin (pH 7.4 at 5°C). The PSS perfusion solution contained (in mM) 112 NaCl, 25.7 NaHCO$_3$, 4.9 KCl, 2.5 CaCl$_2$, 1.2 MgSO$_4$·7H$_2$O, 1.2 KH$_2$PO$_4$, 11.5 glucose, and 10 HEPES (adjusted pH to 7.3–7.4 with NaOH). High (10–75 mM) K$^+$ PSS was made by replacing NaCl with equimolar KCl. Ca$^{2+}$-free PSS was made by omitting Ca$^{2+}$ and adding 0.5 mM EGTA. Solutions were gassed with 5% O$_2$-5% CO$_2$-90% N$_2$ at 35–37°C; the measured O$_2$ level in the open artery was ~12%.

The reagents and sources were as follows: ouabain, caffeine, PE, ACh, Na$^+$-nitroprusside, and t-NAME were from Sigma-Aldrich, St. Louis, MO, and fluo-4 was from Molecular Probes, Eugene, OR. Other reagents were reagent grade or the highest grade available.

Data Analysis and Statistics

The data are expressed as means ± SE; n denotes the number of animals or the number of arteries studied (1 artery per animal). Comparisons of data were made using Student’s paired or unpaired t-test, as appropriate; two-way ANOVA was used where indicated (see figure legends). Differences were considered significant at P < 0.05. Images were analyzed with customized Interactive Data Language software (IDL, Research Systems, Boulder, CO).

RESULTS

Blood Pressure

On average, the rats in the four control groups used here had a mean SBP of 124 ± 4 mmHg (n = 20). The mean SBP of the OH rats was 138 ± 3 mmHg (n = 23) at 4 to 5 wk after implanting the ouabain pellets (P < 0.01 vs. controls). The time course of the changes in SBP in one of the four groups of rats used in this study is shown in Fig. 6A of Cao and colleagues (8).

Small Artery Structure

Live arteries. The fluorescent images in Fig. 1 are views of small portions of the wall from control rat mesenteric small (4th order) arteries pressurized to 70 mmHg. In Fig. 1A, all the myocytes, but no endothelial cells, are stained because the fluo-4 was loaded from the superfusion fluid and not the lumen. Artery wall longitudinal cross sections reveal that there is only a single layer of myocytes in the wall (Fig. 1A, ii and iii). The individual myocytes are best seen, however, in rotated views of the 3-D reconstructions (Fig. 1A,iv and supplemental video clip VC1; note: all supplemental material may be found posted with the online version of this article). The structure of the control rat artery segments was indistinguishable from that of OH rat arteries (not shown).

The stained endothelial cell layer is shown en face in Fig. 1B,i and in the cross section in Fig. 1B,ii. In this artery, fluo-4 was loaded via the lumen and was omitted from the superfusion fluid. The endothelial cells form a single, thin, virtually confluent, cobble stone-like layer lining the lumen. Rotated views of this figure are presented in supplemental video clip VC2. Note that in the pressurized artery, the endothelial cell layer does not exhibit the folding that would be expected in a markedly constricted artery (Fig. 2). This flat surface is mirrored in the adjacent smooth internal elastic lamina surface in the 3-D image of the artery wall intrinsic tissue fluorescence (Fig. 1C,i). The intrinsic tissue fluorescence, viewed from the external surface of the artery, reveals numerous neuronal processes coursing through the adventitia (Fig. 1C,ii). The endothelial cells and the myocytes situated between the internal elastic lamina and the adventitia are unstained and have very little intrinsic fluorescence; they are, therefore, invisible in these images.

Fixed arteries. The structure of fixed, stained mesenteric small arteries from control and OH rats was also examined; the arteries were stained with Masson’s trichrome to distinguish between myocytes (red) and collagen (blue) (Fig. 2A). As illustrated, the third-order feeder arteries (10) used by Briones and colleagues (6) were considerably larger than the fourth-order arcade arteries (10) whose physiology is described in this report. The fixed arteries were markedly constricted despite the incubation in Ca$^{2+}$-free PSS and exposure to Na$^+$-nitroprusside before fixation. The internal diameters of the fixed fourth-order arteries (~75–85 μm, Fig. 2B,ii) were much narrower than those of comparable fresh arteries with MT (~165 μm at 70 mmHg; Fig. 3). The obvious endothelial folding in both third- and fourth-order arteries (arrows in the higher magnification images in Fig. 2A; and see Fig. 4A in Ref. 6) contrasts with the smooth endothelial cell layer and internal elastic lamina in isolated, pressurized arteries (Fig. 1, B and C). In the fixed, constricted fourth-order arteries, the myocytes overlap so that the arterial media appears to be, on average, about 2 myocytes thick (Fig. 2A). This clearly differs from the situation in the arteries with MT in which the myocytes are arrayed in only a single layer (Fig. 1A).

The summarized data in Fig. 2B show that the external diameter, internal diameter, and wall thickness are all significantly smaller in OH third-order arteries than in controls. The narrower diameter of these OH arteries is consistent with the results of Briones and colleagues (6). In striking contrast, the diameter and wall thickness of OH fourth-order arteries do not differ significantly from the respective measurements in control rat arteries (Fig. 2B).

Myogenic Responses

When pressurized to 70 mmHg and warmed to 35–37°C, rat small arteries spontaneously constrict; i.e., they develop MT (52). The mean MT in arteries from control rats in this study corresponded to a constriction of 19 ± 1% of PD (n = 16). Mean MT in arteries from OH rats was 21 ± 2% of PD (n = 17), slightly, but not significantly, greater than that in controls.

The transmitted light images in Fig. 3A illustrate the measurement of wall thickness in isolated, cannulated arteries. Mean wall thickness in control arteries with MT at 70 mmHg was 19.8 μm and declined to 11.3 μm at PD (Fig. 3B) in part
because the myocytes presumably elongated without changing cell volume. Very similar results were obtained in OH rat arteries: wall thickness was 20.4 μm in arteries with MT and declined to 12.3 μm at PD (Fig. 3B). Supplemental video clip VC3 shows the dynamic change in wall thickness as a representative artery dilates from a constricted state (MT) to PD during the washout of Ca²⁺. The calculated internal diameters shown in Fig. 3C are based on the wall thickness data in Fig. 3B and the external diameters in Fig. 4B.

Myogenic reactivity to progressive increases in intraluminal pressure was measured in arteries isolated from control and OH rats. The intraluminal pressure was lowered from 70 to 10 mmHg and then raised to 130 mmHg in 20-mmHg increments; Fig. 4A depicts a set of original data from a control rat artery. The diameter versus pressure curves in Fig. 4B show that PD was slightly, but not significantly, larger in the OH rat arteries than the control arteries at all pressures. The respective myogenic reactivity curves from the OH and control arteries incubated in normal Ca²⁺-containing PSS were, however, nearly superimposable; thus internal diameters at 70 mmHg were nearly identical in control and OH arteries (~165 μm; Fig. 3C). Nevertheless, normalization of the data in Fig. 2B to PD at 70 mmHg [i.e., classic myogenic reactivity curves (48)] reveals that the OH rat arteries exhibited significantly augmented myogenic constriction (Fig. 4C).

Effect of Ouabain

The circulating ouabain level is elevated in OH rats, but ouabain is washed out when the arteries are isolated. To understand better the in vivo situation, we tested the effect of an acute ouabain application on pressurized arteries from control and OH rats. Figure 5A shows that the ouabain dose-response curve in normal rat arteries is biphasic, with a plateau between ~10 and ~300 nM, as in mouse arteries (51). Ouabain concentrations between 0.01 and 100 nM, which selectively inhibit α₂-Na⁺ pumps in rodents (39), augment MT with a half-maximal effect at 0.66 nM (EC₅₀). This is close to the EC₅₀ in mouse arteries (1.3 nM) (51). In most experiments, 100 nM ouabain was used because this dose rapidly and reversibly inhibits all Na⁺ pumps with an α₂-subunit but has a negligible effect on rodent Na⁺ pumps with an α₁ (low ouabain
Mobilization of Sarcoplasmic Reticulum Ca\(^{2+}\) increase the resistance to flow by about 90%. According to Poiseuille’s law (29), this constriction should narrow the diameter by
\[
\frac{H_{11011}}{D_{165}}\text{m}\cdot \text{H}_{140}\text{m}.\]
Effect on OH and control arteries: on average, it narrowed the effect on OH and control arteries: on average, it narrowed the artery radius (assuming concentric constriction); the downward displacement from “i” to “ii” illustrates the recovery.

Summarized data on the 100 nM ouabain-induced vasoconstriction, reported as changes in MT in control and OH rat arteries, are shown in Fig. 5D. This constriction was accompanied by a 2.8 ± 0.3 μm (n = 6) increase in wall thickness (see Fig. 3A for method); thus ouabain decreased the internal diameter by ~26 μm. Note that ouabain had a comparable effect on OH and control arteries: on average, it narrowed the lumen from 165 μm (Fig. 3C) to ~140 μm at 70 mmHg. According to Poiseuille’s law (29), this constriction should increase the resistance to flow by about 90%.

**Mobilization of Sarcoplasmic Reticulum Ca\(^{2+}\) Stores**

To estimate whether the inositol trisphosphate-releasable and ryanodine-sensitive SR Ca\(^{2+}\) stores were altered in the OH rats, we measured the maximal vasoconstrictions evoked by 5 μM PE and by 10 mM caffeine, respectively, in Ca\(^{2+}\)-free media (Figs. 6 and 7). Figure 6A shows the protocol for the PE experiments. The results, summarized in Fig. 6B, reveal that the evoked vasoconstriction was marginally, but not significantly, greater in isolated OH rat arteries than in controls. The caffeine-induced vasoconstriction, too (see protocol in Fig. 7A), was slightly, but not significantly, greater in OH rat arteries than in controls (Fig. 7B).

**PE and High-K\(^{+}\) Constriction Dose-Response Curves**

To determine whether vasoconstrictor responses were altered in the isolated OH rat arteries, we compared the K\(^{+}\) and PE cumulative dose-response curves in control and OH rat arteries. High K\(^{+}\)-evoked vasoconstrictions were virtually identical in the arteries from the two groups of rats (Fig. 8). Likewise, except at very high nonphysiological PE concentrations, the two PE dose-response curves were indistinguishable (Fig. 9). In control arteries, 100 nM ouabain did not significantly augment the contractile responses to 0.1–1 μM PE (not shown).

**ACh-Induced Vasodilation**

The effect of ACh on the constriction evoked by 10 μM PE (a near-maximal dose; Fig. 9) was also tested on isolated, pressurized small arteries from control and OH rats. The representative cumulative ACh concentration experiment from a control artery (Fig. 10A) shows that ACh antagonizes PE-induced vasoconstriction and dilates the artery. To determine how much of this vasodilation was due to NO, the dose-
response to ACh was repeated after treatment with L-NAME. L-NAME concentrations of 1–5 μM block NO synthase (18, 26), whereas 100 μM L-NAME also constricts small pressurized arteries by an endothelium- and NO synthase-independent mechanism (37). We tested the effects of pretreatment with 0.1–100 μM L-NAME on the vasorelaxation induced by 10 μM ACh, a maximally effective concentration (Fig. 10A), in PE-constricted arteries. We, too, observed the vasoconstriction with 10–100 μM L-NAME; moreover, 1 μM was almost as effective as 100 μM L-NAME in inhibiting the response to ACh (not shown). Therefore, a 10-min exposure to 1 μM L-NAME was employed subsequently, as illustrated by the representative experiment in Fig. 10B. Indeed, the summary

Fig. 3. Wall thickness in Ctrl and OH rat 4th-order mesenteric arteries. A: transmitted light longitudinal cross-sectional images of the walls of a Ctrl artery (left) and an OH artery (right); the artery lumen is on the right in each panel. The arteries were pressurized to 70 mmHg (35°C). A, top: walls of arteries with myogenic tone (MT). A, bottom: walls of the same arteries, respectively, dilated to passive diameter (PD) in Ca²⁺-free physiological salt solution (PSS). Scale bar = 50 μm. Supplemental video clip VC3 shows the wall thickness decreasing as a Ctrl rat artery dilates to PD. B and C: summary data of wall thickness (B) and internal diameter (C). The data in B are from 6 Ctrl and 5 OH rat arteries. The data in C were calculated by subtracting twice the wall thickness (from B) from the external diameters of, respectively, Ctrl and OH arteries with MT at 70 mmHg (data from Fig. 4B).

Fig. 4. Myogenic reactivity (MR) in Ctrl and OH rat mesenteric small arteries. A: representative original vasoconstriction record from a Ctrl artery illustrates the diameter changes (top thick line) in response to the step pressure changes (in mmHg) indicated by the bottom line. B: mean external diameter of the Ctrl and OH rat arteries is graphed as a function of translumenal pressure in response to 20-mmHg step increases in pressure starting at 10 mmHg (see A). The MR curves were obtained in normal PSS. The arteries were then superfused with Ca²⁺-free PSS, and the sequence of pressure changes was repeated to obtain the PD curves. The PD curve for OH arteries is shifted slightly, but not significantly, upward relative to the Ctrl artery PD curve (ANOVA, P = 0.152). C: the data from B are normalized to PD of Ctrl arteries at 70 mmHg (48). The normalized MR curve for the OH arteries is shifted downward, relative to the Ctrl curve (ANOVA, P < 0.01); n = 12 and 11 for Ctrl and OH groups, respectively.
hypothesis is, What initiates and what sustains the elevation of hypertension is still vigorously debated. Structural changes such as increased collagen deposition, media thickening, lumen narrowing, and myocyte hypertrophy or hyperplasia are documented in small resistance arteries, (22) as well as large arteries, in most forms of hypertension (2, 22, 36), including ouabain-induced hypertension (6). Nevertheless, small artery diameters appear to be normal, however, in some models of salt-sensitive hypertension (2).

At least two problems arise, however, in assessing the cause and effect of the increased TPR. One is that sustained function of hypertension is unimpaired in fourth-order mesenteric small arteries from OH rats (vs. controls). About half of the ACh-induced vasodilation is unimpaired by this l-NAME pretreatment in both the control and the OH arteries.

**DISCUSSION**

A central, but still unresolved, issue pertaining to most forms of hypertension is, What initiates and what sustains the elevated BP? In particular, in salt- and plasma volume-dependent hypertension, the initial rise in BP has usually been attributed to increased cardiac output (19, 38). The sustained elevation of BP was explained as a blood flow-induced compensatory shift (whole body autoregulation) to increased vasoconstriction and total peripheral vascular resistance (TPR) (11, 19). We have suggested that this shift to increased TPR is mediated by elevated circulating EO (3, 4). The ability of exogenously administered ouabain to induce hypertension in normal rodents (8, 12, 33, 50) follows from this idea. Ouabain-induced hypertension depends on the (normal) high-ouabain affinity binding site on Na\(^+\) pumps with an \(\alpha_2\)-catalytic subunit (12). Moreover, mice with a null mutation in one \(\alpha_2\)-allele have elevated BP, and their arteries exhibit increased MT and augmented myogenic reactivity (51).

**Mechanism of Increased TPR in Hypertension: the Structure-Function Debate**

Elevated TPR is the hallmark of chronic hypertension (11), but whether this has a structural or functional basis is still vigorously debated. Structural changes such as increased collagen deposition, media thickening, lumen narrowing, and myocyte hypertrophy or hyperplasia are documented in small resistance arteries, (22) as well as large arteries, in most forms of hypertension (2, 22, 36), including ouabain-induced hypertension (6). Nevertheless, small artery diameters appear to be normal, however, in some models of salt-sensitive hypertension (2).

At least two problems arise, however, in assessing the cause and effect of the increased TPR. One is that sustained func-
tional increases in artery constriction and tone usually induce compensatory structural changes or remodeling (34). Secondly, when arteries are removed from hypertensive animals, the structural changes remain and usually can be readily detected (6, 22), whereas the in vivo function cannot necessarily be ascertained by studying the isolated arteries in vitro. This is especially important if the functional changes depend on circulating factors that are washed out in isolated artery studies, but such a possibility has generally been overlooked. Indeed, it has been noted that the evidence for altered arterial function is much weaker than the evidence that the structural changes are responsible for the increased TPR in hypertension (36). For this reason, in vivo augmentation of vasoconstrictor-evoked responses in hypertensive humans (13) and animals (5) might be attributed to the altered arterial structure.

Most of the peripheral vascular resistance to blood flow, and the regulation of flow and BP, occurs at the level of small resistance arteries (16). In the rat mesenteric vasculature, these are believed to be the arteries with in vivo internal diameters of 100–300 μm, but the locus of the main resistance to flow is uncertain (10).

Recently, Briones and colleagues (6) studied isolated resistance arteries from control and OH rats. These third-order mesenteric feeder arteries (10), with internal diameters of ~320 μm, had limited myogenic reactivity, especially when compared with the fourth-order arteries used here (Fig. 11).
Chronic in vivo ouabain treatment increased collagen deposition and wall stiffness and narrowed the artery lumen from ~320 to ~280 μm (at 80 mmHg) without altering the adventitia or media thickness (6). Nevertheless, the physiological responses of the isolated OH rat third-order arteries to norepinephrine and ACh were normal. Briones and colleagues (6) therefore concluded that vessel narrowing “could play a role in the pathogenesis of [the] hypertension” in OH rats. The authors did not, however, address the question, Does circulating ouabain have a dynamic effect on the arteries?

**Arterial Diameter and Resistance to Flow**

In this report, we described mesenteric arteries with internal diameters of ~165 μm at 70 mmHg, and with only a single layer of smooth muscle cells. The isolated, pressurized fourth-order arteries from OH rats had diameters and wall thicknesses similar to control arteries. Even fixed, constricted fourth-order control and OH arteries had comparable diameters and comparable wall thicknesses (Fig. 2). In contrast, fixed, third-order OH rat arteries, like the isolated, pressurized OH arteries (6), had significantly smaller diameters than did the controls (Fig. 2).

It is instructive to consider the effects of these artery diameter differences on the resistance to blood flow. According to Poiseuille’s law, the resistance to flow in the control fourth-order arteries will be about 14-fold greater per unit artery length than the resistance of third-order arteries. Moreover, even with the reduced diameter of third-order OH arteries, the resistance to flow will still be about 8.3-fold greater in fourth-order arteries, ignoring the possible dynamic influence of circulating ouabain. Because the third-order arteries are, on average, only about 20–25% longer than fourth-order arteries (15.5 ± 0.8 vs. 12.7 ± 1.4 mm, n = 10 for both), the implication is that these fourth-order arteries contribute much more to the peripheral vascular resistance than do the third-order arteries.

**The Functional Consequences of Circulating Ouabain**

Importantly, the isolated third-order (6), as well as the fourth-order, OH rat mesenteric arteries had normal responses to vasodilators and constrictors. For example, the OH rat fourth-order arteries exhibited normal vasoconstrictor responses to high K⁺ and moderate doses of PE, and normal vasodilator responses to ACh (Figs. 8–10).

Vasoconstriction to PE and to caffeine in Ca²⁺-free media, measures of the mobilization of SR-releasable Ca²⁺ stores, was also not significantly changed by the chronic ouabain treatment (Figs. 6 and 7). The similar MT in the control and OH rat arteries suggests that the baseline cytosolic Ca²⁺ concentration is similar in control and OH artery myocytes. Thus, even though the relationship between cytosolic Ca²⁺ concentration and vasoconstriction is nonlinear, the data in Figs. 6 and 7 imply that the releasable Ca²⁺ stores are not markedly altered in OH rat arterial myocytes.

We did observe augmented myogenic constriction in the smaller arteries from OH rats, however, as well as significantly greater constriction at high-dose (~1 μM) PE. The latter may be the result of enhanced Ca²⁺ entry through receptor-operated channels. The diameters of the control and OH fourth-order arteries (Fig. 4, B and C), but not the larger arteries (Fig. 11) (6), were substantially reduced when intraluminal pressure was increased above ~50 mmHg; this is the classical myogenic response (48). Furthermore, despite the increased MT of the fourth-order OH arteries, the diameters of the myogenically constricted control and OH arteries were virtually identical (Fig. 4B). Because wall thickness did not differ significantly between control and OH rat arteries (Figs. 2B and 3B), these data raise the possibility that the OH arteries may be more constricted to compensate for high pressure-induced stretch, so that the normal lumen diameter is maintained.

Most important is our observation that the myogenically constricted OH arteries responded normally to 100 nM ouabain (Fig. 5); thus the chronic in vivo exposure to ouabain did not downregulate the response. As noted in RESULTS, the 100 nM ouabain-induced constriction should increase resistance to blood flow by about 90%. With the assumption that ouabain also reduces the third-order artery diameter by the same amount (~26 μm), the fourth-order arteries will still be about 11-fold more resistant to flow than the larger arteries. Furthermore, the still smaller branches that emanate from the fourth-order arcade and enter the intestinal wall (10) will have an even greater resistance. Even at a concentration close to EC₅₀ of 0.66 nM (Fig. 5A), which is and within the range of EO concentrations observed in normal humans on a high-salt diet (32), ouabain should increase vascular resistance by ~35%. The implication is that circulating ouabain in OH rats should augment MT and TPR. This finding, plus the absence of small vessel lumen narrowing, suggests that the dynamic effect of ouabain, rather than the remodeling of resistance arteries, may be a key contributor to the sustained elevation of BP in OH rats. Indeed, if most (or all) small and muscular arteries are similarly affected by low-dose ouabain, as seems likely (for example, mouse cremaster small arteries, <100 μm diameter) are also constricted by 100 nM ouabain (H. Raina and M. P. Blaustein, unpublished), this may be the dominant factor in the increased TPR in OH rats. Moreover, these same mechanisms may have a much broader implication, given the evidence summarized in the Introduction that EO is involved in the pathogenesis of hypertension (4). This could explain, for example, why Digibind, digoxin-specific antibody Fab fragments with high affinity for ouabain (44), rapidly (within minutes) lowers BP in several rat hypertension models (24, 27, 30) and in human pregnancy-induced hypertension (1).
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