Effect of Cross-Transplantation on Normotensive and Spontaneously Hypertensive Rat Arterial Muscle Membrane

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SUMMARY Transplantation of arteries into the anterior eye chamber of rats for 8 weeks was used to test the hypothesis that the neurohumoral environment is important in establishing the altered membrane potential (observable during electrogeneric ion transport inhibition) of vascular muscle in hypertension. When caudal arteries from 12- to 16-week-old spontaneously hypertensive rats (SHR) or genetically matched Kyoto-Wistar normotensive rats (KNR) were transplanted into the opposite strain, there was no change in the transport-inhibited membrane potential ($E_m$) of the arterial muscle cells from that found in freshly excised donor arteries. However, when caudal arteries from 2-week-old animals were transplanted into the anterior eye chamber, the arteries always developed the appropriate $E_m$ for the host animal. In other words, a genetically KNR artery developed the $E_m$ of an SHR artery in an SHR host; conversely, a genetically SHR artery developed the $E_m$ of a KNR artery in the KNR host. These results provide evidence that: 1) the differences between the $E_m$ of caudal arteries from SHR and KNR are not inherent in those muscle cells; 2) the change in $E_m$ is triggered in young animals preceding development of hypertension, but not after hypertension is established; and 3) the $E_m$ alteration of the caudal artery is independent of structural changes that occur in the artery as a result of increased blood pressure (because KNR transplants were not connected in series with the host anterior eye chamber vasculature and subject to the elevated blood pressures). We conclude that the arterial muscle cells up to a certain age respond to an external factor that regulates their $E_m$ and presumably their sensitivity to vasopressor agents. (Hypertension 3: 534-543, 1981)

KEY WORDS • membrane potential • vascular muscle • electrogeneric ion transport • $K^+$ gradient

The role of the inherent properties of the arterial muscle cells vs the neurohumoral environment in the pathogenesis of essential hypertension is not clear, although much attention is directed toward the observed increase in peripheral vascular resistance that appears to be a primary etiologic factor. At present, much of the antihypertensive therapy is directed toward decreasing the vaso-motor drive provided by the sympathetic nervous system. Considering the possibility that there is an inherent change in the muscle, we in this laboratory have provided evidence that an alteration in membrane potential electrogenesis in the spontaneously hypertensive rat (SHR) form of hypertension explains a major part of the increased vasopressor sensitivity. The alteration consists of increased dependence of membrane potential on voltage produced by ion transport and a smaller $K^+$ electrochemical gradient. It is important to understand the origin of the altered membrane properties, in particular, whether the alteration is inherent in the muscle cells or the result of altered environmental factors such as innervation, humoral factors, or pressure. The altered membrane electrogensis has been shown to be specific for arterial muscle in SHR, not occurring in isolated cardiac or venous muscle. Mesenteric veins in situ also appear not to show altered membrane electrogensis when neural function is blocked. Although many studies have focused on the instan-
taneous regulation of contraction of arteries by their adrenergic innervation, it is also possible that the sympathetic nervous system could influence the arterial muscle through a trophic factor. In fact, the trophic factor or factors could include norepinephrine. Furthermore, alteration of muscle sensitivity by a trophic influence of innervation is a well-known phenomenon in autonomically innervated structures. Humoral factors have recently been implicated in the development of increased peripheral resistance in hypertension. Increased systolic blood pressure could also contribute to changes in vascular muscle, as proposed by Folkow et al. The present experiments were intended to determine whether the long term interactions of the neural and humoral components of the whole animal environment might contribute to the altered membrane properties found in hypertension.

Methods

Caudal arteries were from males of the Okamoto-Aoki strain of Kyoto-Wistar spontaneously hypertensive rats (SHR) and genetically matched Kyoto-Wistar normotensive rats (KNR) of The University of Iowa colony maintained by brother-sister mating exclusively. Systolic blood pressure (BP) determinations were made in triplicate on host and adult donor animals before the transplantation procedure and on the host before sacrifice. Until the age of 3 weeks, systolic BPs of SHR and KNR were identical. At later ages, the BP of SHR at 12 weeks was 153 ± 3 mm Hg (17 animals); KNR at 12 weeks was 126 ± 3 mm Hg (12 rats); SHR at 20 weeks was 158 ± 2 mm Hg (17 rats); and KNR at 20 weeks was 129 ± 2 mm Hg (12 rats), all presented as mean ± standard error for animals from the same colony, but not those used for membrane potential measurements.

Anterior Eye Chamber Transplantation

The host animals were males, 12 weeks of age. Donors were 2-week-old males and eight 12- to 16-week-old males. The causal artery was carefully and aseptically dissected free and placed in sterile M199 cell culture medium with 50 μg/ml gentamicin at room temperature. The adventitia of the caudal artery was carefully dissected free and matched with the host caudal artery, pinned side-by-side. Eight weeks after transplantation the animals were exsanguinated while under ether anesthesia, and the eyeball containing the transplant was dissected out. Two of the eyeballs were placed on special electrodes that allowed stimulation of the nerves. Upon stimulation, contraction of the iris sphincter could be easily observed, and in the two cases tested, responses of the transplant were also observed, indicating functional innervation.

In three animals, pieces of aorta from an adult donor were transplanted into the same anterior eye chamber as a piece of caudal artery.

Electron Microscopy

Eyeballs were split in half, the lens displaced, and the anterior half dropped into phosphate-buffered 5% glutaraldehyde (pH 7.4) for 15 minutes. The transplant was then dissected free from the iris, cut into smaller pieces, and placed in fresh fixative for a further 45 minutes. It was then washed at 2°C overnight in phosphate-buffered sucrose and further fixing for 1 hour in phosphate-buffered OsO₄. After further fixation for 8 hours in a saturated solution of uranyl acetate, the tissue was dehydrated and embedded in Epon or Araldite. Some tissue was fixed in KMnO₄.

Thin sections were stained with lead citrate and uranyl acetate and examined with a Siemens Elmiskop III electron microscope.

Fluorescence Histochemistry

The transplant was frozen in liquid propane at liquid nitrogen temperature, freeze-dried, then incubated in paraformaldehyde vapor at +80°C for 1 hour. The tissue was then vacuum-embedded in Paraplast Plus, and 10 μm sections taken for fluorescence microscopy.

Electrophysiology

Experiments were carried out on caudal arteries transplanted from 2-week-old and 12- to 16-week-old SHR and KNR donors, and on host animal caudal arteries. With 2-week-old animals, the whole tail was removed, the caudal artery carefully dissected free from the connective tissue to avoid stretching, and the tail pinned in a silicone rubber chamber. Experimental pairs consisted of an anterior eye chamber transplant matched with the host caudal artery, pinned side-by-side. The effect of release of endogenous norepinephrine was eliminated by superfusing for 10 minutes at 37°C temperature, with a solution of 6-hydroxydopamine (200 μg/ml) in a glutathione antioxidant buffer with protection of the artery against released norepinephrine by 1 μM phentolamine.

The solutions used for superfusion were gassed with 95% O₂ and 5% CO₂ and had the following composition (mM): 150.0 Na⁺, 2.7 K⁺, 1.0 Mg⁺⁺, 145.3 Cl⁻, 11.9 HCO₃⁻, 1.06 H₃PO₄, 1.8 Ca⁺⁺, 7.8 dextrose, and 0.029 CaNa₂ ethylene-diaminetetraacetate acid (EDTA). The superfusion rate was 3 ml/min, and
chamber volume was 1 ml. All measurements were made at 16°C to suppress electrogenic ion transport and reveal the $K^+$ gradient. Further details of the methods used are described elsewhere.²

The muscle cells were impaled from the adventitial side by KCl-filled glass microelectrodes. All data that met the criteria of stable electrode tip potential and resistance compared before, during, and after impale-

FIGURE 1. Catecholamine fluorescence histochemical demonstration of the density and distribution of innervation of 12 week SHR caudal artery (in situ). (× 190)

FIGURE 2. Fluorescence histochemical demonstration of the pattern and density of innervation of a 2-week-old SHR caudal artery (in situ). (× 250)

FIGURE 3. Transverse section through 12-week-old SHR caudal artery (in situ) demonstrating the density of innervation. Note most axons (A) are outside the external elastic lamina (EEL), although one axon has penetrated the outer muscle cells of the media (A*). (× 7,600)
ment were included. To overcome the problem of microelectrode penetration of the dense connective tissue surrounding the transplant, all arteries were soaked in 3 mg collagenase/ml suffusion at 37°C for 1-3 minutes. At least 60 minutes were allowed after collagenase treatment for establishment of a steady state before data were collected. Previous experiments have shown that, although collagenase exposure depolarizes cells, recovery is complete in 90% of the cells by 30 minutes and 100% by 60 minutes using non-treated caudal arteries for comparison. An equal number of cell impalements with the same microelectrode were performed alternatively on the transplant and host caudal artery to eliminate variability introduced by different microelectrodes.

**Statistics**

Comparisons of difference for statistical significance at $p < 0.05$ were made by group $t$ test, except where host and transplant comparisons with data gen-

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**Figure 4.** Transverse section through a 2-week-old SHR caudal artery (in situ). The lumenal side of the artery is at the top, with endothelium and internal elastic lamina (IEL) at its edge. Muscle cells (M) separated by collagen-filled narrow extracellular space (ecs) form the media. One cell contains numerous organelles (org) in the cytoplasm. The adventitia contains axon terminals (A), collagen (col), and fibroblasts (Fi). Scale bar is 1 μ. (× 12,000)
erated by equal numbers of impalements were made, for which paired t comparisons were made.

Results
In Situ Caudal Artery and Aorta Structure

The caudal artery of the adult rat was muscular, densely innervated, and 300 to 400 μm in diameter, with a media usually 7 to 8 muscle cells thick. The most striking feature was the density of innervation observed both with fluorescence histochemistry (figs. 1 and 2) and electron microscopy (fig. 3). These nerves were localized to the medial-adventitial border, although a few penetrated a short distance into the media (figs. 1 and 3).

In rats 2 weeks of age, the caudal artery was well developed, composed of 5-7 layers of 1-3 μm diameter cells, and about 150 μm in diameter in the proximal region of the tail. Muscle cells were separated by a narrow extracellular space and contained thick and thin myofilaments and greater numbers of other organelles (e.g., Golgi and mitochondria) in the perinuclear region than in the adult rats (fig. 4). Sympathetic nerves were also present at the medial-adventitial border in this region (figs. 2 and 4).

The media of the thoracic aorta consisted of circularly oriented muscle cells arranged in lamellae separated by elastin fibers. No nerves were observed in the adventitia or media of aorta.

Anterior Eye Chamber Transplant Structure

At 8 weeks after transplantation, transverse sections through the caudal arteries indicated a similar pattern and arrangement of cells as in the in situ artery, with no differences between SHR and KNR. The muscle cells contained the normal complement of myofilaments and other organelles, but were smaller, more irregular in shape, and separated by a wider extracellular space than in the in situ artery (fig. 5). Myofilaments and plasmalemmal vesicles were found in many of the muscle cells (fig. 6). These did not penetrate far into the wall, as is also the case in the in situ artery, and appeared predominantly norepineph-
rine-containing, as determined by KMnO₄ fixation. Formaldehyde-induced fluorescence indicated that transplants of adult caudal artery were reinnervated in a pattern similar to that in the in situ artery (fig. 7). The 2-week-old caudal artery transplants were also rich in catecholamine-containing nerves (fig. 8).

Transplants of adult aorta maintained the structure of muscle cells separated by elastic lamellae (fig. 9), with no differences between SHR and KNR. Very few nerves were observed with fluorescence histology or electron microscopy on the outside of the aorta transplants, and none within, indicating specificity of reinnervation.

Membrane Electrical Properties of Caudal Arteries

Adult donor caudal artery transplanted into the anterior eye chamber of the same strain of rat did not change the membrane property that identifies the strain (fig. 10). The difference between the membrane potential (Eₘ) of the muscle cells of the host arteries.
FIGURE 7. Catecholamine fluorescence histochemical demonstration of degree and pattern of reinnervation of 12 week SHR caudal artery transplanted to the anterior eye chamber of an SHR for 8 weeks. (× 225)

FIGURE 8. Pattern and density of reinnervation of 2-week-old KNR caudal artery transplanted to the anterior eye chamber of an SHR for 8 weeks. (× 250)

FIGURE 9. Transverse section through muscle cells of 12-week-old SHR aorta transplanted to the anterior eye chamber for 8 weeks. The cells are still separated by extracellular space and elastic lamellae (EL) as in situ. (× 8,500)
of KNR and SHR (with electrogenic ion transport inhibited) is evident. In both cases, the transplants tended to have slightly lower (absolute) values than the host artery from the same strain (fig. 10 A and B), but the SHR-KNR difference in $E_m$ due to the lower $K^+$ gradient in SHR persisted. In all cases, variability reflects differences in cells rather than between animals.

Adult donor caudal arteries cross-transplanted into the anterior eye chamber of a rat of the opposite strain had insignificantly different $E_m$ values (Fig. 10 C and D). Therefore, caudal arteries from SHR transplanted into KNR had $E_m$ typical for the SHR donor rather than the KNR host; and the caudal arteries of KNR transplanted into SHR maintained $E_m$ characteristics of KNR rather than of the SHR host.

Arteries transplanted from rats old enough to have developed hypertension (fig. 10) showed a different $E_m$, electrogenesis before the transplant. Arteries from young animals were thus studied. At 2 weeks of age,
there was no significant difference in $E_m$ between KNR and SHR caudal artery at 16°C (or 36°C). Figure 11 compares freshly isolated arteries of 12- to 16-week-old KNR and SHR with those of 2-week old KNR and SHR. There was no significant difference in $E_m$ between the KNR and SHR at 2 weeks, although both values were less negative than the 12- to 16-week-old KNR.

Cross-transplantation experiments were then performed to see if membrane properties of young donor arteries would develop host characteristics. Figure 12A shows that caudal arteries from 2-week-old SHR donors transplanted into KNR hosts (for 8 weeks) developed the more negative $E_m$ characteristic of the KNR strain into which they had been transplanted, rather than the $E_m$ electrogensis associated with the SHR strain. In addition, arteries from 2-week-old KNR donors transplanted into SHR hosts developed the less negative $E_m$ characteristic of the SHR hosts (fig. 12B). The cross-transplantation thus appeared to result in a crossover of membrane potential electrogensis.

Discussion

The most striking finding in these experiments was that transplanted 2-week-old rat arterial muscle membrane potentials ($E_m$) developed as a result of the neurohumoral environment, rather than being inherent properties of the muscle cells. Despite slightly less negative $E_m$ measured in transplanted arteries, vascular muscle from young animals always followed properties found in the host animal. The changes are unlikely to be a coincidence because subsequent experiments showed that 2-week-old caudal artery transplantation within the same strain resulted in a $E_m$ no different from that of the host animals (unpublished observations). These data suggest that the $E_m$ alteration associated with hypertension is not an inherent property of the muscle cells but is a response to some extrinsic influence. The likelihood that the factor that determines the $E_m$ is a trigger event is suggested by the failure of arteries from adult animals to follow the properties of host animals in equivalent transplantations. It would appear that after the degree of dependence of $E_m$ on the $K^+$ gradient is specified, placing the artery in the opposite type of environment cannot alter the $K^+$ gradient. The time at which such properties are specified is probably shortly after the 2-week age we chose because the differences between the BPs of normotensives and hypertensives appear to become significant after 3 weeks of age. Additional experiments to define the ontogenic changes occurring at this age in SHR are needed.

These experiments also present evidence that high arterial BP is not the causative factor in the membrane conversion since the KNR artery in the anterior eye chamber is not stretched by the high BP of the SHR host, and yet develops the SHR membrane properties. The caudal artery in the anterior eye chamber does not form connections with the host circulatory system that would provide perfusion through its lumen. Rather, the ≤100 µm thick media wall appears to be nourished by diffusion from vessels that reach the outside of the transplanted caudal artery. The changes in membrane properties thus appear not to be a result of muscle hypertrophy, which Folkow et al. have shown can contribute to the elevated peripheral resistance seen in hypertension.

The type of artery transplanted into the anterior eye chamber determines the degree of reinnervation, consistent with the hypothesis that target organs play an important role in determining the type and pattern of innervation. Rat caudal artery transplanted to the anterior eye chamber became attached to the iris and was served by the iris circulation in a few days. Nerves
These experiments are in situ. Innervation, demonstrated both by electron microscopy and histochemical fluorescence, was dense and oriented much like that of the adult caudal artery in situ. Transplants of the aorta, however, demonstrated little reinnervation, consistent with the innervation of adult aorta in situ. These experiments are similar to the differences found between sympathetic innervation of rabbit aortic muscle cells (sparse) or rabbit ear artery cells (dense) that forms in joint tissue culture.16

In summary, these experiments have demonstrated that there is some neural or humoral trigger that occurs at an early age to determine membrane properties of the caudal artery. These properties appear to determine normal or exaggerated responsiveness to vasoconstrictor agents in SHR. The experiments allow the elimination of high systolic BP as a cause of the changed membrane properties. The dense innervation of the caudal artery provides a close association of nerve and muscle cells, which might be important in the trophic influence apparently controlling the type of membrane properties that develop.

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