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Differential remodeling responses of cerebral and skeletal muscle arterioles in a novel organ culture system

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Abstract

Evidence suggests that maladaptive changes in the cerebral microcirculation may contribute to ischemia in numerous diseases. We sought, therefore, to develop an ex vivo organ culture system to study early changes in cerebral arteriolar structure and function and to compare associated findings to those for non-cerebral arterioles. Pilot studies revealed that rabbit cerebral arterioles maintained contractility longer when cultured in media containing rabbit-specific plasma rather than fetal bovine serum. Cerebral and skeletal muscle arterioles were cultured in a pressure myograph for 5 days; maximum dilatory and contractile responses were measured at 0, 1, 3, and 5 days. Passive properties were preserved in cerebral arterioles over the entire culture period, although skeletal muscle arterioles underwent constrictive remodeling. Cerebral arterioles also maintained a myogenic capability over the entire culture period, albeit at progressively larger diameters, whereas the skeletal muscle arterioles did so only over 3 days. Culture in rabbit serum, which contains numerous growth factors and clotting factors, did not induce or increase inward remodeling in cerebral and skeletal arterioles, respectively. These results suggest inherent, organ-specific differences in arteriolar remodeling and that extensive results in the literature on non-cerebral arterioles should not be extrapolated to predict responses in the cerebral microcirculation.

Keywords

organ culture; myogenic response; vascular mechanics

1. Introduction

Alterations in vascular structure and function in response to disease or injury vary greatly depending on the perturbation, location of the vascular bed, and size of the vessels involved [15, 31]. These alterations may be induced chemically, as, for example, by a sustained increase in vasoactive factors such as endothelin-1, or mechanically, by sustained changes in blood pressure, flow, or axial loads. Prolonged arteriolar constriction, whether induced chemically or mechanically, is sometimes associated with constrictive remodeling [25], and the resulting semi-permanent reduction in caliber could lead to organ hypoperfusion or ischemia.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The brain is particularly sensitive to decreased perfusion and *in vivo* experiments show that both small cerebral arteries and arterioles exhibit inward remodeling in several animal models of disease [8, 11, 29]. For example, perturbations from the homeostatic mechanical environment due to systemic hypertension in rats decreases the compliance of cerebral arteries [17], which reduces their maximum dilatory capacity. Similarly, subarachnoid hemorrhage (SAH) following rupture of a cerebral aneurysm disrupts the normal biochemical environment of the cerebral vasculature and is thought to cause constrictive remodeling of arteries and perhaps arterioles [35]. Yet, existing studies provide little insight into the initiation of such remodeling responses by either mechanical or biochemical stimuli and they do not reveal time-courses, both of which are critical for developing interventional clinical strategies. Although *in vivo* experiments provide vital insight, they also suffer from numerous shortcomings, including lack of control over physiological variables and the pleiotropy and redundancy of many of the vasoactive hormones and cytokines involved in vascular remodeling. Cell culture experiments, on the other hand, lack the complexity needed to accurately model changes in vascular structure.

We sought, therefore, to develop a cerebral arteriolar culture system and protocol suitable for studying mechanically- or chemically-induced remodeling over short periods. We define remodeling as any process that changes the microstructure and thus mechanical behavior of the vascular wall; inward remodeling, for example, can manifest as a decreased circumferential stretch at a fixed pressure. Delayed cerebral vasospasm, a notable example of chemically-induced cerebrovascular remodeling, develops approximately 3 – 7 days following SAH. Our first goal was thus to maintain cerebral (i.e., pial) arterioles in culture for five days without inducing an inward remodeling response, noting that maintaining maximum dilatory capacity is perhaps most important for preventing cerebral ischemia. For comparison, we also cultured arterioles from skeletal (i.e., the gastrocnemius) muscle under the same conditions, for these arterioles have been cultured successfully by others [7]. We then used this culture system to examine possible chemically-induced remodeling in response to 10% serum in both pial and gastrocnemius arterioles. We hypothesized that culture with high levels of serum, which contains elevated concentrations of vasoactive molecules, growth factors, and transglutaminases, could contribute to constrictive remodeling (cf. [26]). Consistent with prior findings, we found that basal culture conditions caused inward remodeling of gastrocnemius arterioles; in contrast, we found that basal culture conditions did not cause inward remodeling of cerebral arterioles. Culture with serum did not lead to increased inward remodeling in either group of arterioles, however.

2. Methods

2.1 Animals and Tissues

All animal procedures were approved by the Institutional Animal Care and Use Committee of Texas A&M University. Tissue was harvested from specific pathogen-free male New Zealand White rabbits (n = 41) between 10 and 13 weeks of age. Animals were sedated with acepromazine maleate (2 mg/kg), then brought to a surgical plane of anaesthesia using sodium pentobarbital (30 mg/kg). During this procedure, whole blood (~20 ml) was collected prior to and following systemic heparinization of the animal (1750 U). The animal was then exsanguinated and the brain and gastrocnemius muscle were harvested and transported in cold physiologic buffered saline (PBS) to the laboratory for dissection of arterioles. The blood was centrifuged (1400 x g, 10 min) and the resulting rabbit plasma or serum (denoted RP and RS, respectively) was collected under sterile conditions and either used immediately or stored at -20°C.

2.2 Arteriole Cannulation, Culture, and Testing

Pial arterioles (~ 150 μm diameter and 1–2 mm long) were harvested from the ventral occipital lobe of the brain. Similarly sized gastrocnemius arterioles were harvested from second and third order branches of the feed artery. Once isolated, they were cannulated in a pressure myograph chamber (Living Systems, Burlington VT) under sterile conditions and secured with 11-0 suture. They were then pressurized to either 30 (cerebral arterioles) or 40 (gastrocnemius arterioles) mmHg using a hydrostatic fluid column and stretched axially until just straight ($\lambda_z = 1.10$ in cerebral arterioles and $\lambda_z = 1.27$ in gastrocnemius arterioles; Fig. 1). These pressures represented the “myogenic threshold” for the two types of vessel, that is, the pressure at which myogenic constriction was first observed in pilot experiments. Leak-free arterioles, as determined by maintenance of luminal pressure, were cultured for up to 5 days in a 5% CO_2 incubator at 37°C while maintained under these mechanical conditions; the time of cannulation was designated as “day 0”, 24 hours post-cannulation as “day 1”, 48 hours post-cannulation as “day 2”, and so on. Throughout the study, the arterioles were bathed both lumenally and adventitially in cell culture media (MegaCell DMEM; Sigma, St. Louis MO) supplemented with penicillin/streptomycin (100 U/100 μg per ml; Invitrogen, Carlsbad CA), L-glutamine (8 mM; Invitrogen, Carlsbad CA), and either 10% RP or 10% RS. Adventitial culture media was changed daily and testing was performed according to the below protocol at 0, 1, 3, and 5 days post-cannulation. Every effort was made to minimize deviation from these time points, and most arterioles were tested within ± 1 h of the prescribed testing time.

During mechanical and functional tests, each of which followed a 15 min equilibration period within the experimental system, myogenic responsiveness was assessed first by varying the pressure from 10 to 60 mmHg. Arterioles were then returned to either 30 mmHg (cerebral) or 40 mmHg (gastrocnemius) and allowed to stabilize; dilation to acetylcholine (ACh, 10 μM ; Sigma, St. Louis MO) was assessed without using a preconstrictor and then constriction to endothelin-1 (ET-1, 1 nM; Sigma, St. Louis MO) was assessed. Finally, the passive pressure-diameter relationship (i.e., maximum dilatory capacity) was determined after multiple rinses with a calcium-free PBS containing 2 mM EGTA. Temperature was maintained at 37°C during testing using feed-back control (Living Systems, Inc.) and pressures were measured via in-line pressure transducers (Living Systems, Inc.) proximal and distal to the vessel. Arterioles were visualized with an inverted microscope and a CCD camera with input to LabView software (National Instruments, Austin TX) that allowed interactive measurements using digital video calipers (resolution 0.3 μm). The software recorded inner diameter, outer diameter, wall thickness at both the top and bottom of the vessel (cf. Fig. 1), and pressure from both transducers. Of note, the testing protocol required the arterioles to be out of the incubator for approximately 1.5 h, with ~0.75 h in DMEM. Although we did not supply CO_2 to the myograph chamber during that period, preliminary experiments showed that the pH of DMEM changed minimally during arteriolar testing. Details of preliminary testing may be found in the Supplementary Information.

2.3 Histology

Freshly isolated and cultured arterioles were fixed overnight in neutral buffered formalin, embedded in paraffin, and sectioned to a thickness of 5 μm . Sections were mounted on glass slides and stained with hematoxylin and eosin (H&E) or Masson’s trichrome.

2.4 Calculations and Statistics

Axial stretch of cannulated vessels was calculated as $\lambda_z = l/L$ where L is the unstretched length at a nearly unloaded pressure (2–3 mmHg) and l is the stretched length (e.g., length at which the arteriole does not bend or buckle when at a physiological pressure or 30 or 40 mmHg). Circumferential stretch was calculated as $\lambda_\theta = a/A$, where a is the inner radius

under loaded conditions and A is the inner radius at the nearly unloaded state (2–3 mmHg). Mean circumferential stress was calculated via the Laplace equation as $\sigma_\theta = Pa/h$, where P is the transmural pressure, a the current inner radius, and h the average of two measured current wall thicknesses (i.e., as seen simultaneously in a side view): $h = (h_1 + h_2)/2$ at any given P . Wall cross-sectional area was calculated as $(\pi/4)(d_o^2 - d_i^2)$, where d_o is the current outer diameter and d_i the current inner diameter. CSA denotes the mean cross-sectional area calculated across all pressures, and fixed axial length, for a given group of arterioles.

Data were compared using either ANOVA for repeated measures or a paired Student's t -test, as appropriate. Statistical analyses were performed using R software, a modification of the S programming language (The R Project for Statistical Computing, <http://r-project.org>). A p value less than 0.05 was considered sufficient to reject the null hypothesis. All data are presented as a mean $\pm$ standard error.

3. Results

3.1 Culture with 10% rabbit plasma

Based on preliminary results, we cultured both types of arterioles in DMEM with 10% RP. Cerebral arterioles maintained acute levels of myogenic contractility for 1 day, showed a slight reduction in contractile capacity at 3 days, and a more marked decrease on day 5, but nevertheless retained throughout culture the ability to resist passive distension to increasing pressure (Fig. 2). Endothelial-dependent dilation to ACh was virtually absent by day 3 and constriction to ET-1 was minimal on day 5, however (Table 1). The lack of response to receptor-mediated vasoactive agents, despite a continued ability to constrict myogenically (Fig. 2), might have resulted from a number of different factors, including the loss of the endothelial layer during culture. To address this possibility, we performed histological staining of cross-sections of cultured arterioles. However, because the cerebral arteriolar wall only contains a single smooth muscle layer plus the endothelium, which is not bound by an internal elastic lamina, we were unable to conclusively determine whether or not the endothelial layer was lost during culture. Passive responses did not change during the 5 day culture period (Figs. 3 and 4) indicating that the control culture protocol did not alter the maximal dilatory capacity as desired. A transient increase in wall CSA was seen on day 3 ($p < 0.05$; data not shown), and circumferential stress was slightly higher on day 5 than on day 1 ($p < 0.05$; Fig. 4). Adherence to the protocol resulted in an 85% success rate, with almost all failed cultures due to contamination. Interestingly, daily performance of the testing procedure or a simulated test was necessary to maintain arteriolar contractility; it appeared as though the change in pressure (as occurred during tests of myogenic reactivity) helped preserve the contractile phenotype. Alternatively, omission of the passive pressure-diameter test (in calcium-free PBS) on day 0 resulted in a total loss of contractility and assumed death at 1 day ($n = 4$; data not shown).

When cultured in 10% RP, arterioles from the gastrocnemius muscle maintained myogenic reactivity similar to cerebral arterioles, although with more variability and a near loss of any contractility by day 5 (Fig. 2). These arterioles constricted in response to ET-1 on day 1, but not thereafter, and only 2 of 5 arterioles dilated to ACh on day 1 (Table 1). No significant change in diameter in response to these drugs was observed for the remainder of the culture period. The poor response of these arterioles to any of the three stimuli tested (pressure, ACh, and ET-1) suggested a generalized loss of contractility or perhaps cell death. Indeed, histology revealed a clear loss of the endothelium by day 3 of culture. Yet, passive behavior changed consistent with an inward remodeling: unloaded diameter increased from 94.09 ± 5.36 to 125.66 ± 4.46 ($p < 0.05$), while maximally loaded diameter (60 mmHg) decreased from 168.01 ± 8.01 to 155 ± 2.44 ($p < 0.05$). As a result, circumferential stretch gradually and progressively decreased from day 0 to day 5 (Fig. 3), indicating a stiffening of the wall.

This change was accompanied by a parallel decrease in circumferential stress (Fig. 4). Average wall CSA tended to be elevated on day 3 ($7958 \pm 369 \mu\text{m}^2$ on day 0 vs. $9553 \pm 227 \mu\text{m}^2$ on day 3; $p = 0.07$) and day 5 ($9517 \pm 103 \mu\text{m}^2$; $p = 0.08$).

3.2 Culture with 10% rabbit serum

Cerebral arterioles cultured with 10% RS did not maintain a myogenic ability as long as those cultured with 10% RP, as evidenced by their inability to resist pressure-induced dilation on day 5 (Fig. 5). Contrary to expectations, these arterioles did not exhibit inward remodeling, as evidenced by a lack of change in passive behavior (Figs. 6 and 7) and average wall CSA ($5259 \pm 241 \mu\text{m}^2$ on day 0 vs. $5339 \pm 158 \mu\text{m}^2$ on day 5). Similar results were seen in gastrocnemius arterioles. When cultured in RS, they displayed a highly variable myogenic response (Fig. 5) and, like those cultured in RP, did not exhibit the period of myogenic responsiveness seen in cerebral arterioles. No changes were observed in passive diameter (Figs. 6 and 7) or average wall CSA (data not shown), indicating a lack of inward remodeling.

4. Discussion

During the process of developing and improving a system and protocol for the culture of cerebral arterioles, we discovered that cerebral arterioles cannot be maintained under the same culture conditions as skeletal muscle arterioles; rather, they require an enhanced media formulation and species-specific plasma or serum. These specific control conditions, while adequate to support cerebral arteriolar structure and passive behavior for up to 5 days, induced progressive, constrictive remodeling in skeletal muscle arterioles. Furthermore, in apparent contradiction of the prevailing paradigm of vascular remodeling, serum did not cause inward remodeling of arterioles from either vascular bed.

4.1 Culture Techniques

One goal was to develop an organ culture system and protocol for studying potential mechanisms of cerebral microvascular remodeling for up to 5 days, a period corresponding roughly to the time to onset of cerebral vasospasm in humans following SAH. Of primary importance in preventing cerebral ischemia, of course, is preservation of the maximum dilatory capacity, which derives from passive properties. We are unaware of any other organ culture system capable of maintaining the passive behavior and sustaining even partial myogenic responsiveness of such arterioles for 5 days. Note, however, that Bakker et al. [7] reported the first arteriolar organ culture system in 2000. Using non-cerebral arterioles and small arteries from vascular beds such as the heart, cremaster, and mesentery from the rat and pig, they have since amassed an impressive collection of data describing remodeling responses of these vessels to a variety of stimuli, including ET-1, hypertension, low flow conditions, and transglutaminases [3, 4, 6, 30]. Yet, their culture system/protocol induces significant inward remodeling under no-flow conditions (i.e., a decrease in passive inner diameter; [7]), they have not reported cultures past 4 days, and they have not evaluated the ability of their system to culture cerebral arterioles. Unlike Bakker et al. [7], we did not see inward remodeling of skeletal muscle arterioles when cultured with serum, even when we omitted mechanical property testing on day 0 to render our culture conditions as similar to theirs as possible. This discrepancy might be due to the source of the serum, however; they used commercially available FBS and we used non heat-inactivated rabbit serum. In addition, Bakker et al. also used serum only in the luminal media, whereas our experiments included serum or plasma in both luminal and extraluminal compartments. In our preliminary investigations, however, we were not able to maintain viability of cerebral arterioles without extraluminal plasma.

Iterative improvement of both our culture system and protocols required careful assessment of the homeostatic biochemomechanical environment of cerebral arterioles. We previously showed that, *in situ*, these vessels are under an axial pre-stretch $\lambda_z \approx 1.24$, whereas the common method of stretching pressurized, cannulated arterioles until they no longer are bent produces a $\lambda_z = 1.10$ (Steelman et al., 2010). In contrast, stretching cannulated, pressurized gastrocnemius arterioles until just straight produced an axial stretch of $\lambda_z \approx 1.27$. Because axial stretch within this range did not affect myogenic constriction of cerebral arterioles, we cultured at the lower value of λ_z to prevent possible damage by overstretching. The luminal pressure applied during culture of cerebral arterioles was determined from the literature: we subtracted normal rabbit cerebrospinal fluid pressure (5–10 mmHg; [33]) from normal rabbit cerebral mean arteriolar pressure (~40 mmHg; [34]) and cultured at 30 mmHg. In pilot experiments, this value was also found to be the “myogenic threshold”, that is, the pressure at which myogenic constriction initiated. Lacking hemodynamic data for rabbit gastrocnemius arterioles, we cultured these vessels at their observed myogenic threshold pressure (~40 mmHg) in an attempt to maintain consistency between culture conditions for cerebral and skeletal muscle arterioles. Thus, both groups of arterioles were cultured at a pressure that would elicit myogenic tone, although 40 mmHg is significantly lower than the expected *in vivo* pressure for similarly sized skeletal muscle arterioles. We are thus unable to rule out the possibility that these arterioles were cultured below their *in vivo* transmural pressure. In addition to the biomechanical environment, we attempted to preserve the *in vivo* biochemical environment as much as possible. Although the incubator temperature was slightly lower than the body temperature of a normal rabbit (37°C vs. 38–39°C) to maintain comparability with previously published work from our laboratory, we cannot rule out a possible effect of this discrepancy. The CO₂ level (5%), however, was roughly equivalent to the partial pressure of CO₂ in the brain [18]. The discovery herein of species-specific plasma as the key to successful culture was fortuitous, but we suspect that the heparin present in the blood may have aided cell survival by enhancing interactions of growth factors with their receptors [22]. The reason for the failure of cultures with FBS is unknown, although there may be species differences (bovine vs. rabbit) in the protein sequences of required growth factors.

Careful evaluation of any technique can reveal both strengths and weaknesses. We used our culture system primarily to study possible changes in vascular structure and mechanics over a modest period, but it could also be used to study alterations in vascular permeability, cell-cell contacts, matrix production and degradation, gene expression, and so forth. Yet, the gradual loss of myogenic constriction renders the current protocol unsuitable for studying contractility or smooth muscle cell phenotype. Changes in ET-1 receptor subtypes have been seen in cultured arteries [1, 2], but it is not known whether other molecular changes may be caused by the culture system itself. Our protocol did not preserve endothelial function and resulted in the loss of the endothelial layer of gastrocnemius arterioles by day 3 and potentially caused endothelial cell loss in cerebral arterioles as well. This limitation may be improved by the addition of luminal flow during culture [21] just as smooth muscle contractility may be improved by subjecting the vessel to pulsatile rather than mean pressure. We initially attempted to supply flow using a syringe pump, but found that a large backpressure developed after only 1 day. A carefully controlled pressure drop, applied through resistance-matched cannulae, is most likely the solution to this problem, although the accurate replication of *in vivo* shear stress in an *in vitro* environment will be a significant challenge [32]. Another potential problem is the repeated exposure of arterioles to a calcium-free solution during testing. To rule out the possibility that passive testing on day 0 prevented subsequent cerebral arteriolar remodeling, we omitted testing on day 0 in one group of vessels. These vessels demonstrated a dramatic loss of compliance and were non-contractile, however. It is also possible that trauma to the vessels during isolation contributed to the remodeling response; however, we noted throughout the duration of the

study that any damage to the arterioles whatsoever during isolation or cannulation caused loss of viability within 24 h. Nevertheless, our success rate was >80%, with most losses due to contamination, although this must be established for each laboratory and taken into account when planning experiments.

4.2 Cerebral versus Skeletal Muscle Arterioles

Whereas much is known regarding skeletal muscle arterioles under both physiological and pathological conditions, there is a dearth of information about cerebral arterioles. Limited numbers of studies have investigated the myogenic response [13, 14], hypertensive remodeling [8–11], shear stress [12], and potassium and TRPC channels [13, 24] in these vessels, yet a comprehensive view of cerebrovascular responses to insult or injury remains wanting. In addition, we are just now beginning to understand the process of vascular remodeling; we know that certain integrins, cytoskeletal proteins, contractile elements, and matrix components are necessary [16, 27], but we do not know how all these players come together to effect a permanent change in vascular structure and behavior. It is thus difficult to propose a mechanism that would cause inward remodeling in skeletal muscle arterioles but not in cerebral arterioles under identical conditions. Such remodeling likely begins with active contraction of vascular smooth muscle cells that enables a rearrangement and elongation of these cells around a smaller lumen [5, 15, 20, 26, 28]. This new geometry is then entrenched by changes in the extracellular matrix, which could include production of new matrix or crosslinking of existing matrix proteins by transglutaminases. Interestingly, cerebral (pial) arterioles exhibited a stronger myogenic response as well as a persistently reduced caliber for the first 1 – 2 days of culture, consistent with the hypothesis of smooth muscle cell rearrangement, but entrenchment did not occur at this smaller diameter. Skeletal muscle (gastrocnemius) arterioles became entrenched, presumably via crosslinking of adventitial collagen, without undergoing a period of enhanced contractility. Further exploration of the effects of transglutaminases on cerebral arterioles may prove enlightening.

4.3 Delayed Cerebral Vasospasm

Subarachnoid hemorrhage subsequent to rupture of a cerebral aneurysm is thought to cause delayed cerebral vasospasm, the angiographically visible narrowing of a cerebral artery. Attention has recently turned to the potential role of the cerebral microcirculation in this disease [23]; we thus sought to study this phenomenon *in vitro*. Consistent with the prevailing paradigm that clotted blood promotes constriction and inward remodeling of cerebral blood vessels [19, 35], we hypothesized that culture with a high concentration of serum (noting that serum is the supernatant of a blood clot) would mimic this situation *in vitro*. Contrary to our hypothesis, serum reduced contractility and did not induce remodeling, although we did not specifically investigate the effects of intravascular versus extravascular presentation of serum. The possibility exists that the factor(s) responsible for vasospasm are not present in serum, but are either released from the clot itself as it degrades or are produced by activated astrocytes, apoptotic neurons, or infiltrating inflammatory cells. Further investigation into effects of factors released from clotted blood and glial cells seems warranted.

4.4 Conclusion

We described herein a unique organ culture system suitable for limited studies of arterioles for up to five days. Using this system, we found fundamental differences in both the requisite culture media for and subsequent remodeling responses of cerebral and skeletal muscle arterioles. These results suggest that extensive results in the literature on non-cerebral arterioles should not be extrapolated to predict responses in the cerebral microcirculation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.
Representative image of a cerebral arteriole, after cannulation, on day 0 of culture. Note that some of the resolution available via the computer was lost in reproduction.

Fig. 2.

Cannulated rabbit cerebral (top panel) and gastrocnemius (bottom panel) arterioles were cultured for 5 days in media containing 10% rabbit plasma. All arterioles showed a progressive decrease in the degree of myogenic constriction after 1 day of culture, although the cerebral arterioles retained an ability to resist pressure-induced distension. Passive pressure-diameter relationships obtained on day 0 of culture are shown for comparison (solid line). Data are presented as mean $\pm$ SEM, $n = 4$ to 7 arterioles.

Fig. 3.

Cannulated rabbit cerebral (top panel) and gastrocnemius (bottom panel) arterioles were cultured for 5 days in media containing 10% rabbit plasma. When tested under passive conditions, inner diameter of cerebral arterioles did not change during the culture period, whereas gastrocnemius arterioles showed a progressive loss of compliance. Data are presented as mean $\pm$ SEM, $n = 4$ to 7 arterioles.

Fig. 4.

Cannulated rabbit cerebral (top panel) and gastrocnemius (bottom panel) arterioles were cultured for 5 days in media containing 10% rabbit plasma. When tested under passive conditions, the slope of the circumferential stress-stretch relationship of cerebral arterioles did not change during the culture period, although circumferential stress was slightly elevated at day 5 compared to day 1 ($p < 0.05$). Gastrocnemius arterioles, however, showed a progressive increase in stiffness with no change in circumferential stress as evidenced by the leftward shift in the stress-stretch relationship. Data are presented as mean $\pm$ SEM, $n = 4$ to 7 arterioles.

Fig. 5.

Cannulated rabbit cerebral (top panel) and gastrocnemius (bottom panel) arterioles were cultured in media containing 10% rabbit serum for 5 days. The arterioles showed large variability in myogenic responsiveness and a pronounced loss of tone. The passive pressure-diameter relationship obtained on day 0 of culture is shown for comparison (solid line). Data are presented as mean $\pm$ SEM, $n = 4$ to 7 arterioles.

Fig. 6. Cannulated rabbit cerebral (top panel) and gastrocnemius (bottom panel) arterioles were cultured for 5 days in media containing 10% rabbit serum. When tested under passive conditions, inner diameter of cerebral and gastrocnemius arterioles did not change during the culture period. Data are presented as mean $\pm$ SEM, $n = 4$ to 7 arterioles.

Fig. 7.

Cannulated rabbit cerebral (top panel) and gastrocnemius (bottom panel) arterioles were cultured for 5 days in media containing 10% rabbit serum. When tested under passive conditions, the slope of the circumferential stress-stretch relationship of cerebral and gastrocnemius arterioles did not change during the culture period. Data are presented as mean $\pm$ SEM, $n = 4$ to 7 arterioles.

Table 1

Change in diameter of pial or gastrocnemius arterioles during short-term culture in DMEM. Change in diameter is expressed as percentage change from resting diameter. The number of arterioles in each group is noted in parentheses. Constriction to endothelin-1 (ET-1, 1 nM) and dilation to acetylcholine (ACh, 10 μ M) were measured after incubation for 0.5 or 1 h, or after culture for 1, 3, or 5 days. Gastrocnemius arterioles cultured in RS were not tested on days 1 or 5. Dilation to ACh was not tested if myogenic tone was absent.

		% Change in Diameter (n)	
Day	Treatment	ET-1	ACh
Pial Arterioles			
0	DMEM + RS (10%)	-15.9 ± 2.1 (6)	93.94 ± 4.6 (6)
	DMEM + RP (10%)	-46.5 ± 9.1 (4)	33.57 ± 26.3 (5)
1	DMEM + RS (10%)	-	-
	DMEM + RP (10%)	-37.1 ± 5.7 (6)	16.68 ± 7.4 (6)
3	DMEM + RS (10%)	-0.6 ± 5.1 (5)	23.20 ± 22.8 (4)
	DMEM + RP (10%)	-14.6 ± 6.5 (6)	-0.13 ± 3.4 (5)
5	DMEM + RS (10%)	-0.9 ± 0.4 (4)	-
	DMEM + RP (10%)	-0.8 ± 0.5 (7)	0.96 ± 0.4 (4)
Gastrocnemius Arterioles			
0	DMEM + RS (10%)	-51.2 ± 15.4 (5)	83.04 ± 54.1 (5)
	DMEM + RP (10%)	-7.0 ± 5.3 (4)	5.00 ± 3.4 (4)
1	DMEM + RS (10%)	-	-
	DMEM + RP (10%)	-48.5 ± 20.0 (5)	32.35 ± 29.5 (5)
3	DMEM + RS (10%)	0.2 ± 0.5 (3)	-
	DMEM + RP (10%)	-1.4 ± 1.0 (7)	-2.11 ± 2.2 (6)
5	DMEM + RS (10%)	-	-
	DMEM + RP (10%)	0.5 ± 1.2 (5)	0.32 ± 0.5 (4)

Data are presented as mean $\pm$ SEM, n = 3 to 7. RS – rabbit serum, RP – rabbit plasma.