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Role of sensory C fibers in hypoxia/ reoxygenation-impaired myogenic constriction of cerebral arteries

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Abstract

Objective—Hypoxia/reoxygenation (H/R) associated with extracorporeal membrane oxygenation disrupts cerebral autoregulation. However, the underlying mechanisms remain poorly understood. The present study was designed to investigate the role of sensory C-fibers in myogenic responsiveness of cerebral arteries.

Methods—Arterial diameter and intraluminal pressure were simultaneously measured *in vitro* on rat posterior cerebral arteries.

Results—Cerebral arteries constricted in response to graded increase in intraluminal pressure (20–100 mmHg, in 20 mmHg increments). *In vitro* C-fiber desensitization with capsaicin (1 μ mol/l, 20 minutes) significantly suppressed myogenic constriction by over 50%, but did not affect 5-hydroxytryptamine (0.01–10 μ mol/l) and KCl (120 mmol/l)-induced constriction. Capsazepine (5 μ mol/l, 30 minutes), a selective blocker of neuronal vanilloid receptor TRPV1, had similar inhibitory effect on cerebral myogenic constriction to elevated pressure. Cerebral myogenic constriction was significantly attenuated by H/R; the impairment by H/R was further enhanced after C-fiber desensitization (except at a pressure level of 100 mmHg).

Discussion—These findings indicate that C-fiber activity contributes to myogenic constriction of cerebral arteries under normal and H/R conditions. H/R-impaired myogenic responsiveness is exaggerated by C-fiber dysfunction. These results raise the possibility that therapeutic strategies directed toward preserving C-fiber nerve endings or supplying its constituent neuropeptides could be developed.

Keywords

Cerebral arteries; C-fibers; hypoxia/reoxygenation; myogenic constriction; vascular response

INTRODUCTION

Cerebral autoregulation effectively maintains its normal blood flow over a wide range of perfusion pressure. This autoregulatory mechanism is impaired under pathological conditions such as uremia, hypertension and stroke. Disrupted cerebral autoregulation is also seen in infants with respiratory failure subjected to extra-corporeal membrane oxygenation (ECMO) ^{1–3}. However, the underlying mechanisms remain poorly understood.

Autoregulation is one of the critical determinants of cerebral vascular tone. Cerebral vascular tone is regulated by various factors, one of which derives from myogenic origin. Myogenic reactivity is an intrinsic property of smooth muscle cells where, in response to stretch, the cells constrict. In arteries, this property results in a decrease in vessel diameter in response to increased intraluminal pressure and is an important regulator of peripheral vascular resistance. Myogenic constriction is endothelium independent and Ca^{2+} dependent, resulting from pressure-induced depolarization leading to the subsequent opening of voltage-gated Ca^{2+} channels and/or mechanosensitive cation channels and from cytoskeletal tension^{4–6}. Furthermore, sensory C-fibers and neuronal vanilloid receptor TRPV1 have been found to be involved in myogenic constriction of mesenteric arteries⁷. Although myogenic response plays an essential role in the regulation of vascular tone and blood flow, differences between species and between vascular beds could make the underlying mechanisms highly complex.

Like other resistance arteries, the presence of nerve fibers accompanying cerebral vessels has been proven. A subset of unmyelinated and finely myelinated sensory nerves comprises C- and θ -fibers. Evidence implicates that some cardiovascular diseases are associated with abnormal sensory perception or progressive damage and loss of unmyelinated and myelinated nerve fibers⁸. Loss of innervation seems to be connected with some vascular diseases. However, very little is known about the role of sensory nerves in the regulation of myogenic responsiveness of cerebral vessels under hypoxia/ reoxygenation (H/R) conditions.

In addition to the known endothelial and direct smooth muscle effects of these factors, an effect through local peripheral nerve fibers also can be supposed. Hypoxia/ischemia leads to metabolic changes and the production of a number of factors that potentially could affect these sensory nerve endings⁹. These factors include lactic acid¹⁰, cyclooxygenase metabolites¹¹ and hydroxy free radicals¹². Reactive oxygen species plays a key role in tissue injury after cerebral ischemia/ reperfusion^{13–15}. In particular, H/R-induced cerebral endothelial dysfunction is mediated by superoxide overproduction coupled to Rho kinase and NF- κ B-dependent pathway^{16,17}.

The present study was designed to investigate the role of capsaicin sensory C-fibers in cerebral myogenic constriction, with a specific interest in the interaction of sensory nerve and vascular reactivity during H/R. We hypothesized that C-fibers activity contributed to myogenic constriction of rat cerebral arteries under normal and H/R conditions. Because cerebral artery tone increases in response to a rise in intraluminal pressure (i.e. myogenic constriction) and changes in this physiological process may conceivably reflect changes in cerebral autoregulation under pathological conditions, we chose myogenic constriction of cerebral arteries as a criterion to study.

MATERIALS AND METHODS

General preparations

Our study conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). All procedures were approved by the Institutional Animal Care and Use Committee at Children's National Medical Center. Male Sprague–Dawley rats (weight, 250–300 g; from Taconic, Germantown, NY, USA) were anesthetized with sodium pentobarbital (60 mg/kg i.p.) along with heparin (1000 U/kg, i.p.) before exsanguination. The brain was rapidly removed and placed in ice-cold physiological salt solution (PSS) with the following composition (in mmol/l): NaCl 119, KCl 4.7, CaCl_2 1.6, MgSO_4 1.17, KH_2PO_4 1.18, NaHCO_3 24.9, ethylenediaminetetraacetic acid 0.026 and dextrose 5.5, pH 7.4. The posterior cerebral artery (PCA) was carefully isolated.

Measurement of vascular diameter

As previously described¹⁷, segments of the PCA (1–2 mm in length, $120 \pm 10 \mu\text{m}$ in internal diameter) were placed in a microvascular chamber containing PSS aerated with 16% O₂/5% CO₂/79% N₂ (pH = 7.4) at room temperature. Vessels were cannulated with glass pipettes and pressurized to 60 mmHg after making sure that there were no potential leaks. The cannulated vessel was warmed to 37°C and allowed to equilibrate for 30–60 minutes under no flow condition, with a longitudinal stretch to approximate its *in situ* length, until stable myogenic tone developed. The blood vessel was imaged using a video camera and a dimension analyser (Living Systems Instrumentation) linked to a chart recorder (Model 23, Perkin-Elmer). Inner diameter and intravascular pressure were measured continuously throughout the experiment. All pharmacological reagents were added to the superfusion solution. At the end of each experiment, the diameter of maximal dilation was determined in the presence of Ca²⁺-free medium with ethyleneglycoltetraacetic acid (2 mmol/l).

EXPERIMENTAL PROTOCOLS

Effects of *in vitro* H/R

Hypoxia/reoxygenation was accomplished by changing the superfusate pO₂ in an airtight arteriograph reservoir system¹⁷. After vascular reactivity was stabilized, the vessels were exposed to hypoxia (95% N₂/5% CO₂, superfusate pO₂ 10–15 mmHg) for 30 minutes and then to reoxygenation (16% O₂/5% CO₂/79% N₂, superfusate pO₂ 100–160 mmHg) for 30 minutes.

Cerebral contractile response to graded increase in intraluminal pressure from 20 to 100 mmHg (in 20 mmHg increments) was examined in the absence and presence of H/R. In our preliminary studies, time control experiments showed that myogenic response to pressure was reproducible on the same preparation under the same superfusate condition during the time course of our study (4–4.5 hours). Constrictive responses to 5-hydroxytryptamine (5-HT) (0.01–10 $\mu\text{mol/l}$) and KCl (120 mmol/l) at a pressure level of 60 mmHg were compared before and after H/R.

Effect of C-fiber desensitization

In vitro C-fiber desensitization was induced using the selective neurotoxin capsaicin (1 $\mu\text{mol/l}$) for 20 minutes, followed by a washout for 40 minutes. A 40 minute washout period ensures removal of any residual neuropeptide that may have been released. It is noted that acute exposure to capsaicin activates the C-fiber; however, prolonged exposure to capsaicin, as applied in the current protocol, will result in desensitization of the nerve ending to further activation^{7,18}.

After C-fiber desensitization, myogenic constriction to a graded increase in transmural pressure from 20 to 100 mmHg was examined under either normal or H/R conditions. In another separated experimental series, constriction to 5-HT (0.01–10 $\mu\text{mol/l}$) and KCl (120 mmol/l) were compared before and after C-fiber desensitization.

Effect of capsazepine

Because TRPV1 channels have been identified as a major activation site on C-fiber nerve endings⁷, we examined the effect of a selective TRPV1 receptor antagonist capsazepine. Myogenic constriction to increased intraluminal pressure from 20 to 100 mmHg was assessed in the presence or absence of pre-treatment with capsazepine (5 $\mu\text{mol/l}$) for 30 minutes. The effect of gadolinium (Gd³⁺, 10 $\mu\text{mol/l}$), a stretch-activated non-selective cation channel blocker, on myogenic constriction was also evaluated. The concentrations were chosen based on other studies^{7,18}.

DATA ANALYSIS

All data are presented as mean $\pm$ SE, and n indicates the number of animals for the experiments performed.

Myogenic constriction (%) at each pressure = $100 \times [(D_{\text{Ca-free}} - D_{\text{PSS}}) / D_{\text{Ca-free}}]$, where D is the arterial diameter in Ca^{2+} -free buffer or PSS. Under Ca^{2+} -free conditions, a negative value is used to state a decrease in tone level, opposing to an increase in myogenic tone.

5-Hydroxytryptamine-induced constriction (%) = $100 \times [(d_{\text{KPSS}} - b) / (d_{\text{KPSS}} - a)] - 100$, where d_{KPSS} is the diameter obtained in PSS containing 120 mmol/l KCl, b is the diameter reached by the vessel at a given concentration of the agonist and a is the diameter before administration of the agonist.

Data were analysed by the paired Student's t -test for single comparisons and by one-way analysis of variance, for multiple comparisons, followed by Bonferroni's test when appropriate. Differences were considered significant when $p < 0.05$.

RESULTS

Hypoxia/reoxygenation impaired cerebral myogenic constriction

Under control conditions, ~98% of the rat PCA segments developed myogenic tone with PSS (37°C) within 1 hour at 60 mmHg intramural pressure. The cerebral arteries constricted in response to graded increase in intraluminal pressure from 20 to 100 mmHg (in 20 mmHg increments), with maximal tone level of $38.7 \pm 2.6\%$ at 100 mmHg pressure (Figure 1). Hypoxia/reoxygenation significantly attenuated myogenic constriction (Figure 1). Hypoxia/reoxygenation had little influence on constrictor responses to 5-HT (0.01–10 $\mu\text{mol/l}$, data not shown) and to high K^+ 120 mmol/l (tone increase $68.76 \pm 6.33\%$ and $65.95 \pm 4.10\%$, control versus H/R, $n = 5$, $p > 0.05$). The myogenic constriction was sensitive to a stretch-activated non-selective cation channel blocker gadolinium (Gd^{3+} , 10 $\mu\text{mol/l}$) and abolished by removal of extracellular Ca^{2+} (Figure 2). These results support the property of myogenic constriction and the susceptibility of cerebral myogenic response to H/R insults.

Effect of C-fiber desensitization

In vitro C-fiber desensitization with capsaicin (1 $\mu\text{mol/l}$) remarkably inhibited >50% of myogenic constriction of PCA at each pressure (Figure 3A). Because neuronal vanilloid receptor TRPV1 has been identified as a major activation site on C-fiber nerve endings, the effect of a selective TRPV1 receptor antagonist capsazepine on myogenic response of PCA was also examined. Like capsaicin, capsazepine (5 $\mu\text{mol/l}$) had a significant inhibitory effect on cerebral artery myogenic constriction to elevated pressure (Figure 3A). These results indicated that C-fibers activity contributed to myogenic constriction of cerebral arteries.

Furthermore, H/R-impaired myogenic constriction at pressure levels of 20, 40, 60 and 80 mmHg, but not at 100 mmHg, was further attenuated after C-fiber desensitization with capsaicin (1 $\mu\text{mol/l}$; Figure 3B). However, C-fiber desensitization did not alter vasoconstriction to 5-HT (0.01–10 $\mu\text{mol/l}$; Figure 4) and KCl (120 mmol/l) (Figure 5).

DISCUSSION

The major findings of this study include the following: (1) desensitization of C-fibers remarkably inhibits myogenic constriction of cerebral arteries *in vitro* to graded increase in intraluminal pressure under normal or H/R conditions and (2) H/R significantly attenuates cerebral myogenic constriction, and C-fiber desensitization further reduced this response.

Adequate cerebral circulation depends on various mechanisms, one of which is the myogenic autoregulatory response (Bayliss effect). Sensory C-fibers play an important role in the regulation of this process. Our results have demonstrated that desensitization of C-fibers with capsaicin profoundly blunts normal myogenic constriction to increased intraluminal pressure. Because TRPV1 channels have been identified as a major activation site on C-fiber nerve endings, we explored the effect of the selective TRPV1 receptor antagonist capsazepine. We found that capsazepine (5 $\mu\text{mol/l}$) exhibits a similar inhibitory effect on myogenic constriction of cerebral arteries. On the other hand, vasoconstrictor responses to 5-HT and high K^+ are not modified by C-fiber dysfunction. Collectively, these data support a regulatory role of C-fiber activity in cerebral myogenic constriction. Although acute exposure to capsaicin activates sensory nerve C-fibers via TRPV1 to release neuropeptides, like calcitonin gene-related peptide and substance P, prolonged exposure to capsaicin, as the protocol applied in studies of our own and others, results in desensitization of the nerve ending to further activation^{17,18}. Noticeably, capsaicin-sensitive nerves are widely distributed in the vasculature of humans and animals^{19,20}. Our findings from cerebral arteries are consistent with the studies of Scotland *et al.*⁷ regarding the role of C-fibers in mediating myogenic constriction in rat mesenteric resistance arteries. A critical role for other transient receptor potential channels (e.g. TRPM4 and TRPC6) in pressure-induced smooth muscle depolarization and myogenic constriction of cerebral arteries has also been established²¹.

The myogenic constriction was sensitive to a stretch-activated non-selective cation channel blocker gadolinium (Gd^{3+} , 10 $\mu\text{mol/l}$) and abolished by the removal of extracellular Ca^{2+} . We are aware of that gadolinium at the concentration used in our study has been described as a potent blocker of voltage-dependent Ca^{2+} channels²². Since these channels play a leading role in the myogenic constriction, the effect of gadolinium may result from the action of this inhibitor on cation channels as well as on calcium channels. With these action modes, these results still validate the property of myogenic constriction.

It is apparent that C-fiber dysfunction may worsen impaired myogenic constriction of cerebral arteries during H/R. Our results have shown that H/R significantly impairs cerebral myogenic constriction, indicating the susceptibility of cerebral myogenic response to H/R insults. These changes are enhanced by desensitization of capsaicin-sensitive C-fibers. It seems that H/R has a specific effect on cerebral myogenic constriction, since constrictive responses to 5-HT and high K^+ are unaffected by H/R. Our previous *in vivo* and *in vitro* studies have shown that short-term exposure of the newborn lambs to venoarterial ECMO alters the response of cerebral arteries to increased intravascular pressure, resulting in disrupted cerebral autoregulation^{3,23}. However, how ECMO-related autoregulatory disruption occurs and what regulates myogenic response under H/R conditions remain virtually unknown. The present study has expanded our previous observations and described a novel mechanism fundamental to the regulation of cerebral myogenic constriction during H/R.

Since our data are based on *in vitro* experiments with isolated cerebral arteries, it is difficult to extend our findings directly to *in vivo* conditions, where multiple endogenous factors (e.g. hormonal, metabolic and neuronal factors) contribute simultaneously to vascular tone and blood flow. These factors may also influence C-fiber function. However, because the specific antagonists used in this study preferentially block the activity of C-fibers and changes in the vessel diameter correspond well to the changes in either C-fiber activity or oxygenation status, our findings are directly correlated to the function of C-fibers in these arteries under normal and H/R conditions. Nevertheless, a better understanding of the relationship between sensory C-fibers and vasculature may provide the foundation to develop new therapies for various vascular and neurological disorders. This study provides novel insight into the pathogenesis of cerebral diseases in which both sensory nerves and cerebrovascular dysfunction are involved.

In summary, our findings indicate that C-fiber activity contributes to myogenic constriction of cerebral arteries under both normal and H/R conditions. Cerebral myogenic responsiveness is significantly impaired during H/R, and the defect is exaggerated by C-fiber dysfunction. These results also suggest the importance of therapeutic strategies based on preserving C-fiber nerve endings or providing its constituent neuropeptides.

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Figure 1. Effect of hypoxia/reoxygenation (H/R) on myogenic constriction of isolated rat PCAs to graded increase in intraluminal pressure (20–100 mmHg). Values are expressed as mean $\pm$ SE, $n = 8$ each group, * $p < 0.05$ versus control

Figure 2.

Influence of gadolinium (10 $\mu\text{mol/l}$), a stretch-activated non-selective cation channel blocker and 0- Ca^{2+} plus EGTA (2 mmol/l) on myogenic constriction of rat PCA. Under Ca^{2+} -free conditions, a negative value is used to express a decrease in tone level, opposing to an increase in myogenic tone. Values are expressed as mean $\pm$ SE; $n = 5-10$, ** $p < 0.01$ compared with control

Figure 3.

Effect of *in vitro* desensitization of C-fibers with capsaicin. **(A)** Normal myogenic constriction of rat PCA to elevated intra-luminal pressure in the presence or absence of capsaicin (1 $\mu\text{mol/l}$ for 20 minutes) and the TRPV1 receptor antagonist capsazepine (5 $\mu\text{mol/l}$ for 30 minutes). Values are expressed as mean $\pm$ SE, $n = 8$ each group, * $p < 0.05$, ** $p < 0.01$ versus control. **(B)** Myogenic constriction of rat PCA to elevated intraluminal pressure in the presence or absence of C-fiber desensitization with capsaicin under H/R conditions. Values are expressed as mean $\pm$ SE, $n = 5$ each group. * $p < 0.05$, compared with H/R alone

Figure 4.

Effect of capsaicin on 5-HT-induced vasoconstriction. Concentration-dependent constriction of rat PCA to 5-HT before and after *in vitro* treatment with capsaicin (1 $\mu\text{mol/l}$, 20 minutes). Values are mean $\pm$ SE, $n = 6$; $p > 0.05$ compared with control

Figure 5.

Effect of capsaicin on high K⁺-induced vasoconstriction. Constriction of rat PCA to high K⁺ (120 mmol/l) before and after *in vitro* treatment with capsaicin (1 μmol/l, 20 minutes). Values are mean ± SE, *n* = 5; *p* > 0.05 compared with control