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Cerebrovascular dysfunction and blood-brain barrier permeability induced by oxidized LDL are prevented by apocynin and magnesium sulfate in female rats

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Abstract

Oxidized low-density lipoprotein (oxLDL) is elevated during several neurologic conditions that involve cerebral edema formation, including severe preeclampsia and eclampsia; however, our understanding of its effect on the cerebral vasculature is limited. We hypothesized that oxLDL induced blood-brain barrier (BBB) disruption and changes in cerebrovascular reactivity occurs through NADPH oxidase-derived superoxide. We also investigated the effect of MgSO₄ on oxLDL-induced changes in the cerebral vasculature as this is commonly used in preventing cerebral edema formation. Posterior cerebral arteries (PCA) from female rats were perfused with 5μg/mL oxLDL in rat serum with or without 50μM apocynin or 16mM MgSO₄ and BBB permeability and vascular reactivity were compared. oxLDL increased BBB permeability and decreased myogenic tone that were prevented by apocynin. oxLDL increased constriction to the nitric oxide synthase inhibitor L-NNA that was unaffected by apocynin. oxLDL enhanced dilation to the NO donor sodium nitroprusside that was prevented by apocynin. MgSO₄ prevented oxLDL-induced BBB permeability without affecting oxLDL-induced changes in myogenic tone. Thus, oxLDL appears to cause BBB disruption and vascular tone dysregulation through NADPH oxidase-derived superoxide. These results highlight oxLDL and NADPH oxidase as potentially important therapeutic targets in neurologic conditions that involve elevated oxLDL.

Keywords

blood-brain barrier permeability; oxidized LDL; magnesium sulfate; cerebral arteries; myogenic responses

Introduction

It has become recognized that oxidized low-density lipoprotein (oxLDL) is one of the key factors in the pathogenesis of many cardiovascular diseases.¹ Oxidative modification of physiological native LDL (nLDL) into oxLDL occurs in numerous disease states as a result of oxidative stress and the presence of reactive oxygen species.² The formation of oxLDL

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initiates multiple pathways in both endothelial and vascular smooth muscle cells, mostly through binding to its receptor lectin-like oxLDL receptor (LOX-1).³ oxLDL binding to LOX-1 generates complex signaling cascades leading to induction of the inflammatory pathway and increased production of superoxide that can further promote vascular dysfunction.¹⁻³ Although less understood than in peripheral or cardiovascular disease, oxLDL also appears to contribute to cerebrovascular disease and stroke. Previous studies showed a significant association between increased circulating levels of oxLDL and cerebral ischemic lesions in stroke patients that may be related to the presence of oxidative stress.^{4, 5} In addition, high doses of oxLDL increases BBB permeability in cultured cerebral endothelial cells⁶ and in isolated cerebral arteries that was prevented by LOX-1 inhibition and scavenging peroxynitrite.⁷ Thus, oxLDL may be an important therapeutic target for cerebrovascular disease and stroke, especially under conditions of oxidative stress and cerebral edema formation.

Oxidative and nitrosative stress are known to have detrimental effects on cerebrovascular reactivity and BBB permeability, however, the role of oxLDL in these events is largely unknown. oxLDL activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase to produce superoxide and may have an important role in cerebrovascular dysregulation.^{8, 9} Superoxide and peroxynitrite are known to affect vascular tone and myogenic reactivity that are important for control of cerebral blood flow (CBF) and cerebrovascular resistance (CVR).¹⁰⁻¹² In addition, increased BBB permeability caused by oxLDL may also promote vasogenic edema formation and contribute to life-threatening neurologic symptoms associated with conditions such as ischemic stroke, seizure, and severe preeclampsia. In fact, our previous study showed that increased circulating levels of oxLDL in severe preeclamptic women induced BBB disruption that was prevented by scavenging peroxynitrite.¹³ However, the mechanism by which oxLDL causes peroxynitrite generation to induce BBB disruption is not known but may be important to understand since cerebral edema is thought to be a primary mechanism by which seizure can occur during preeclampsia.¹⁰ In addition, data concerning the effect of oxLDL on cerebrovascular reactivity and myogenic responses are limited, however, potentially important in neurologic symptoms in preeclampsia where impaired cerebral autoregulation is an important contributor to formation of cerebral edema and neurologic complications.¹⁰ In the present study, we hypothesize that oxLDL to the level of women with severe preeclampsia would decrease myogenic tone and reactivity of cerebral arteries that could contribute to vascular dysfunction and the formation of cerebral edema. We further hypothesized that NADPH oxidase-derived superoxide is involved in oxLDL-induced changes in the cerebral vasculature.

Magnesium sulfate (MgSO₄) has been shown to be protective of the BBB and prevent cerebral edema during numerous conditions including traumatic brain injury, septic encephalopathy, hypoglycemia, and preeclampsia.¹⁴⁻¹⁶ In fact, MgSO₄ is one of the primary treatments for prevention of vasogenic brain edema in severe preeclampsia.^{15, 16} However, if MgSO₄ is also specifically protective of oxLDL-induced BBB permeability or vascular tone dysregulation remains unknown. Thus, our last aim was to investigate the effect of MgSO₄ on oxLDL-induced BBB permeability and cerebral vascular dysfunction.

Methods

Animals

All animal procedures were approved by the University of Vermont Institutional Animal Care and Use Committee and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Female virgin nonpregnant Sprague Dawley rats (12–14 weeks; 250–300 grams) were used for all experiments. The animals were purchased from Charles River (Saint-Constant, QB, Canada) and housed until experimentation at the

University of Vermont Animal Care Facility, an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility. Animals had access to food and water ad libitum and maintained a 12-hour light/dark cycle.

Rat serum samples

Serum samples were obtained from trunk blood from female Sprague Dawley rats and collected in serum separator tubes. After a waiting period of 30 minutes, the tubes were centrifuged for 10 minutes at 2500 revolutions per minute, and the serum pooled from ~10 animals and aliquoted. The pooled serum was then stored at -80 °C until experimentation. For each experiment, one aliquot of pooled serum was used.

BBB permeability studies

To investigate the effect of oxLDL on BBB permeability and determine the mechanism by which oxLDL increases permeability, hydraulic conductivity (L_p), the critical transport parameter that relates water flux to hydrostatic pressure, was measured in isolated PCAs from female nonpregnant rats, as described previously.¹⁷ This method of measuring BBB permeability has been successfully used in several previous studies.^{17–19} Briefly, a third-order branch of the PCA was carefully dissected out of the brain and the proximal end mounted on a glass microcannula in an arteriograph chamber. PCAs were perfused intraluminally with 20% v/v serum from female rats with (n=8) or without (n=8) the presence of 5 µg/ml oxLDL or 5 µg/ml native LDL (nLDL; n=4) in HEPES buffer for 1 hour at 75.0 ± 0.3 mmHg and $37.0 \text{ °C} \pm 0.2 \text{ °C}$. The concentration of oxLDL used for these experiments was based on a previous study that determined the concentration of oxLDL in plasma of severe preeclamptic women to be approximately 5 µg/ml.¹³ To determine the involvement of NADPH oxidase activity in oxLDL-induced BBB permeability, a separate set of PCAs were perfused with 50 µM of the NADPH oxidase inhibitor apocynin (n=8) in addition to the oxLDL-serum mixture. To determine the effect of MgSO₄ on oxLDL-induced BBB permeability, another set of PCAs was perfused with 16 mM MgSO₄ in addition to the oxLDL-serum mixture. This dose of MgSO₄ was chosen because it is within the therapeutic range for MgSO₄ for seizure prophylaxis.^{16, 20} After the equilibration period, intravascular pressure was increased to $80.0 \text{ mmHg} \pm 0.1 \text{ mmHg}$ and the drop in pressure due to transvascular filtration across the vascular wall in response to hydrostatic pressure was measured for 40 minutes. The decrease in intravascular pressure per minute (mmHg/min) was converted to volume flux across the vessel wall (3m³) using a conversion curve, as previously described.¹⁷ L_p was then determined by normalizing flux to surface area and oncotic pressure of the serum perfusate.

Reactivity studies

Another set of experiments was performed to determine the effect of oxLDL on reactivity of PCAs. A third-order branch of the PCA was carefully dissected from the brain of female rats and the proximal end mounted on one glass cannula in an arteriograph chamber. PCAs were perfused intraluminally with 20% v/v serum from female rats with (n=8) or without (n=8) the presence of 5 µg/ml oxLDL or 5 µg/ml nLDL (n=4) in a HEPES buffer for 2 hours at 80.0 ± 0.3 mmHg and $37.0 \text{ °C} \pm 0.2 \text{ °C}$. The distal end of the vessel was tied off to avoid flow-mediated responses. The proximal cannula of the arteriograph was connected to a pressure transducer and servo controller that maintained intravascular pressure at a set pressure or changed manually. Lumen diameter of the PCA was measured via video microscopy during the experiment. After the equilibration period, intravascular pressure was increased in steps of 20 mmHg to 140 mmHg and lumen diameter was recorded at each pressure once stable. Pressure was then returned to 80 mmHg for the remainder of the experiment. A single concentration (0.1 mmol/L) of the nitric oxide synthase (NOS) inhibitor nitro-L-arginine (L-NNA) was added to the bath and constriction in response to

NOS inhibition was measured after approximately 20 minutes. In the presence of L-NNA, the NO donor sodium nitroprusside (SNP) was cumulatively added to the bath (10^{-8} M- 10^{-5} M) and the dilation to SNP was measured at each concentration. Finally, zero- Ca^{2+} HEPES was added to the bath to obtain fully relaxed diameters. Passive diameters were then recorded at pressures from 140 to 5 mmHg.

Data Calculations

Percent tone was calculated as the percent decrease in diameter from the fully relaxed diameter in zero- Ca^{2+} HEPES by the equation: $((\phi_{\text{passive}} - \phi_{\text{active}}) / \phi_{\text{passive}}) \times 100\%$, where ϕ_{passive} is the diameter in zero- Ca^{2+} HEPES and ϕ_{active} is the diameter when the vessels had tone. Percent constriction to L-NNA was calculated with the equation: $((\phi_{\text{baseline}} - \phi_{\text{drug}}) / \phi_{\text{baseline}}) \times 100\%$ where ϕ_{baseline} is diameter before adding the L-NNA and ϕ_{drug} is diameter with presence of L-NNA. Percent sensitivity to the NO donor SNP using a concentration-response curve was calculated with the equation: $((\phi_{\text{drug}} - \phi_{\text{minimum}}) / (\phi_{\text{maximum}} - \phi_{\text{minimum}})) \times 100\%$ where ϕ_{drug} is diameter at a specific concentration of SNP, ϕ_{minimum} is diameter at the lowest concentration of SNP and ϕ_{maximum} is the diameter at the highest concentration of SNP. The effective concentration that produced half maximal dilation to SNP (EC_{50}) was calculated from individual plots of each curve and averaged.

Drugs and solutions

HEPES physiological salt solution was made fresh daily and consisted of (mmol/L): 142.0 NaCl, 4.7 KCl, 1.71 MgSO_4 , 0.50 EDTA, 2.8 CaCl_2 , 10.0 HEPES, 1.2 KH_2PO_4 , and 5.0 dextrose. HEPES without Ca^{2+} was also made daily but Ca^{2+} was left out of the solution. Apocynin, L-NNA and SNP were purchased from Sigma (St Louis, MO, USA). The concentrated apocynin was diluted in double distilled H_2O to reach the concentration of 50 μM and was stored in -20°C until used. L-NNA and SNP were made as stock solutions and diluted. Highly oxLDL was purchased from Kalen Biomedical (LLC, Montgomery Village, Mn, USA; 770252-7) and stored at 4°C until experimentation.

Statistical analysis

All data are expressed as mean $\pm$ standard error of the mean. Data were analyzed using one-way ANOVA and post hoc Student-Newman Keuls test for multiple comparisons. Differences were considered statistically significant when $P < 0.05$.

Results

oxLDL increased BBB permeability that was prevented by apocynin

Figure 1 shows BBB permeability of PCAs in response to oxLDL. oxLDL significantly increased BBB permeability in PCAs compared to controls without oxLDL ($P < 0.05$). Importantly, the presence of apocynin abolished the oxLDL-induced BBB permeability ($P < 0.05$), suggesting that increased NADPH oxidase activity was involved in oxLDL-induced BBB disruption. To confirm that BBB permeability was increased due to the oxidative state of LDL, the effect of nLDL on BBB permeability was compared to oxLDL-induced BBB permeability. nLDL did not increase BBB permeability compared to controls, demonstrating that the increased BBB permeability was a result of the oxidative modification of LDL.

oxLDL decreased myogenic tone of PCAs that was prevented by apocynin

Figure 2A shows active diameter changes in response to increased intravascular pressure. All groups had modest constriction in response to increased pressure, demonstrating myogenic reactivity was present in all vessels. PCAs perfused with oxLDL had larger diameters at all pressures measured, but this was not statistically significant ($P = 0.17$).

However, Figure 2B shows that oxLDL significantly decreased myogenic tone compared to the control group ($P < 0.05$), suggesting that the larger diameters were due to diminished basal tone. Importantly, apocynin prevented the oxLDL-induced decrease in myogenic tone in PCAs ($P < 0.05$). Also for these experiments, measurements were repeated with the presence of nLDL instead of oxLDL. nLDL did not cause any changes in myogenic responses or myogenic tone compared to the control group without oxLDL (data not shown), suggesting that the effects were due to the oxidative state of LDL.

oxLDL increased constriction to NOS inhibition and enhanced NO-mediated vasodilation

To determine the effect of oxLDL on basal endothelium-derived NO, a single high concentration of L-NNA was added to PCA from all 3 groups and changes in diameter measured (Figure 3A). All vessels constricted in response to L-NNA, suggesting basal NO was present and inhibited tone. The presence of oxLDL caused a significant increase in constriction to L-NNA compared to the controls ($P < 0.01$). However, there was no effect of apocynin on the increased constriction to NOS inhibition induced by oxLDL. Figure 3B shows the effect of circulating oxLDL on dilation to SNP in PCA. oxLDL caused PCAs to have increased sensitivity to SNP compared to the control group that was abolished by the presence of apocynin ($P < 0.05$). The EC_{50} values for SNP were $1.9 \pm 0.2 \times 10^{-7}$ M in the oxLDL group compared to $5.0 \pm 0.7 \times 10^{-7}$ M in the control group and $4.0 \pm 0.6 \times 10^{-7}$ M in the apocynin group ($P < 0.05$).

MgSO₄ prevented oxLDL-induced BBB permeability

Figure 4 shows the effect of MgSO₄ on BBB permeability induced by oxLDL. MgSO₄ prevented the oxLDL-induced BBB permeability ($P < 0.05$) that was similar to controls, suggesting a direct protective effect on oxLDL-induced BBB permeability.

MgSO₄ did not affect oxLDL-induced decrease in myogenic tone

The effect of MgSO₄ on oxLDL-induced changes in myogenic reactivity and tone are shown in Figures 5A and 5B. MgSO₄ did not affect the active myogenic response in the PCAs or prevented the oxLDL-induced decrease in myogenic tone, suggesting the protective effect of MgSO₄ was selective for BBB permeability.

MgSO₄ decreased oxLDL-induced dilation to SNP without affecting oxLDL-induced constriction to NOS inhibition

Figure 6A and 6B show the constriction to NOS inhibition and dilation to the NO-donor SNP of PCAs due to oxLDL with or without the presence of MgSO₄. MgSO₄ treatment did not significantly affect the constriction to L-NNA, however, it did prevent the oxLDL-induced enhanced dilation to SNP in PCAs ($P < 0.05$). The EC_{50} values for SNP were $4.9 \pm 0.8 \times 10^{-7}$ M in the MgSO₄ group that was not different from the control group ($5.0 \pm 0.7 \times 10^{-7}$ M) but significantly higher compared to the oxLDL group ($1.9 \pm 0.2 \times 10^{-7}$ M; $P < 0.05$).

Discussion

oxLDL has become a known key factor in the pathogenesis of many cardiovascular diseases such as hypertension, atherosclerosis and coronary heart disease. However, information regarding the effect or mechanism of oxLDL in the cerebral vasculature remain limited. In the present study, we determined the effect of oxLDL on BBB permeability and cerebral vascular function and investigated the mechanism by which it acts to increase permeability and diminish myogenic tone. In addition, we examined the possible beneficial effects of MgSO₄ on oxLDL-induced changes in BBB permeability and cerebral vascular reactivity, as

it has been shown to be beneficial in treating several neurologic conditions that are associated with elevated oxLDL, including severe preeclampsia.^{7,13} The results of the present study demonstrate that oxLDL, but not native LDL, significantly increased BBB permeability and decreased myogenic tone in PCAs that was associated with increased constriction to NOS inhibition and dilation to SNP. Inhibition of NADPH-derived superoxide with apocynin prevented much of the detrimental effects of oxLDL, including increased BBB permeability and sensitivity to SNP, but not constriction to NOS inhibition. Treatment with MgSO₄ also protected the BBB and prevented oxLDL-induced BBB disruption as well, but without affecting the oxLDL-induced changes in myogenic tone. Taken together, these results suggest that oxLDL causes BBB disruption and cerebrovascular tone dysregulation through activation of NADPH oxidase whereas the effect of MgSO₄ treatment on oxLDL-induced vascular dysfunction appears more limited to preventing BBB disruption.

In the present study, we demonstrate for the first time that increased levels of oxLDL significantly increased BBB permeability that was prevented by apocynin, suggesting the effect was mediated by NADPH oxidase-derived superoxide. We also show that this effect was specifically due to the oxidative modification of LDL, as nLDL did not affect BBB permeability. It is generally accepted that increased levels of superoxide rapidly bind NO to generate peroxynitrite, a toxic radical that causes endothelial dysfunction.²¹ Thus, we speculate that oxLDL increases superoxide production through NADPH oxidase that leads to subsequent generation of peroxynitrite, resulting in BBB disruption. Our proposed mechanism is supported by several previous reports using cell-culture models showing that increased levels of oxLDL generate superoxide production through NADPH oxidase, leading to increased production of peroxynitrite.^{8, 22} Importantly, because NADPH oxidase activity is greater in the cerebral vasculature compared to the systemic vasculature,²³ oxLDL-induced increased activity of NADPH oxidase may result in excessive production of superoxide in the cerebral vasculature leading to extensive damage of the BBB and vasogenic brain edema. Thus, oxLDL and NADPH oxidase may be important therapeutic targets for treatment and/or prevention of neurologic symptoms in preeclampsia and possibly other neurologic conditions where BBB disruption and edema formation is involved.

In the present study, we found that acute intraluminal exposure of oxLDL caused PCAs to have larger diameters and significantly decreased myogenic tone, an effect that could lead to diminished CVR, hyperperfusion, and BBB disruption due to high hydrostatic pressure.¹⁰ To our knowledge, this is the first report to show the detrimental effects of 5 µg/ml oxLDL on PCA function. We used this concentration because this is what was measured in women with severe, early-onset preeclampsia.¹³ A previous study investigated the effect of 10 µg/ml oxLDL on PCAs from rabbits and found that oxLDL increased myogenic tone, which is in contrast to the results of the present study.²⁴ However, the concentration used in the previous study was higher than in the present study. Previous reports have described differential effects of oxLDL on the systemic vasculature depending on the concentration such that oxLDL > 10 µg/ml increased myogenic tone due to decreased NO production, while concentrations of oxLDL < 10 µg/ml increased NO production and decreased myogenic tone.^{25, 26} The difference between these studies may also be due to species or gender differences as the previous study used PCAs from male rabbits.

The mechanism by which oxLDL caused decreased myogenic tone is not clear, however, it may be due to increased endothelium-dependent NO production as we also found oxLDL increased constriction to LNNA. However, the increased constriction to LNNA may be due either increased NO production by the endothelium or increased sensitivity of the smooth muscle to NO. Because we found the oxLDL also increased sensitivity to SNP, it is possible

the decrease in tone is due to increased sensitivity to endothelium-derived NO and not to increased NO production. The effect of oxLDL on dilation to SNP has been examined in several studies. In the abdominal aorta, oxLDL did not affect the dilation to SNP.²⁷ However, another study found that oxLDL decreased dilation to SNP in carotid arteries without affecting dilation to SNP in basilar arteries, suggesting oxLDL may affect smooth muscle function differently in different vascular beds.²⁸ Importantly, all these previous studies used concentrations that were at least 10-fold higher in concentration than used in this study.

The present study also found that the presence of the NADPH oxidase inhibitor apocynin prevented the oxLDL-induced decrease in myogenic tone, suggesting superoxide production may be involved in this response. In a previous study, we demonstrated that high cholesterol-treated rats had PCAs with decreased myogenic tone that was due to generation of peroxynitrite.^{7, 11} It is possible that oxLDL-induced superoxide production combines with NO to generate peroxynitrite that is the vasoactive component. This hypothesis is supported by previous reports that found that peroxynitrite is a powerful vasodilator at high concentrations and decreases myogenic tone in the cerebral vasculature.^{11, 29, 30} However, apocynin did not prevent the increase in constriction to LNNA, suggesting that only superoxide, and not NO, is involved in oxLDL-induced decrease in myogenic tone. Further studies are needed to specifically address the underlying mechanism by which oxLDL decreases tone and the role of NO, superoxide and peroxynitrite.

In addition, we determined the effect of MgSO₄ on oxLDL-induced BBB disruption and vascular tone dysregulation. MgSO₄ is widely used and effective in the management of severe preeclampsia for prevention of neurologic complications such as seizures.³¹ Here, we found that MgSO₄ abolished oxLDL-induced BBB permeability without affecting oxLDL-induced changes in myogenic tone. These results confirm earlier reports that MgSO₄ is protective of the BBB without causing significant dilation in the cerebral vasculature. However, it is not clear from this study if MgSO₄ directly antagonizes the mechanism of oxLDL that leads to BBB disruption or if MgSO₄ had an overall protective effect on the BBB. It is possible that the mechanism by which MgSO₄ acts is through its ability to scavenge free radicals such as superoxide and decrease lipid-peroxidation.³² Although this may explain the protective effect of MgSO₄ on BBB disruption, oxLDL-induced decrease in myogenic tone was not prevented, suggesting MgSO₄ may act primarily to protect the cerebral endothelium.

Conclusion

The present study demonstrates for the first time that oxLDL causes increased BBB permeability and cerebral vascular tone dysregulation that was prevented by apocynin, suggesting involvement of NADPH oxidase derived superoxide in these oxLDL-induced effects. In addition, treatment with MgSO₄ prevented oxLDL-induced BBB disruption without affecting cerebral myogenic tone. These results demonstrate the significance of oxLDL in the occurrence of cerebral vascular dysfunction in conditions such as severe preeclampsia and may be an important marker in the pathogenesis of many neurologic conditions that involve cerebral edema formation.

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Figure 1. Effect of apocynin on oxLDL-induced BBB permeability

Graph showing hydraulic conductivity (L_p) as a measure of BBB permeability in response to perfusion of 20% v/v rat serum alone (CTL) compared to rat serum containing 5 μ g/ml oxLDL without (oxLDL) and with 50 μ M apocynin (oxLDL+apo). oxLDL significantly increased BBB permeability in PCAs from female rats compared to controls. The addition of apocynin significantly abolished the oxLDL-induced BBB permeability. Native LDL (nLDL) did not change BBB permeability compared to controls. (* $P < 0.05$ vs. all other groups by one-way ANOVA).

Figure 2. Effect of apocynin on oxLDL-induced changes in myogenic reactivity and tone of posterior cerebral arteries

Graphs showing (A) active lumen diameter and (B) percent myogenic tone in response to increased intravascular pressure in posterior cerebral arteries from female rats that were perfused with 20% v/v rat serum alone (CTL) compared to serum containing 5 μ g/ml oxLDL without (oxLDL) or with 50 μ M apocynin (oxLDL + apo). oxLDL caused larger lumen diameters compared to CTL at all pressures that was prevented by apocynin. oxLDL significantly decreased percent myogenic tone of PCA compared to CTL without oxLDL. The effect of oxLDL on basal tone was prevented by apocynin. (* $P < 0.05$ vs. all other groups by one-way ANOVA).

Figure 3. Effect of apocynin on oxLDL-induced changes in constriction to NOS inhibition and dilation to sodium nitroprusside (SNP) in posterior cerebral arteries

Graphs showing (A) constriction to NOS inhibition with L-NNA and (B) sensitivity to the NO donor sodium nitroprusside (SNP) in posterior cerebral arteries from female rats that were perfused with 20% v/v rat serum alone (CTL) compared to rat serum plus 5 μ g/ml oxLDL without (oxLDL) and with 50 μ M apocynin (oxLDL+apo). Perfusion of oxLDL caused a significant increase in constriction to L-NNA in PCA compared to CTL. Apocynin did not affect the oxLDL-induced increase in constriction to L-NNA. oxLDL significantly increased sensitivity to SNP in PCA that was abolished by apocynin. (** $P < 0.01$ vs. all other groups by one-way ANOVA; * $P < 0.05$ vs. all other groups by one-way ANOVA).

Figure 4. Effect of MgSO₄ on oxLDL-induced BBB permeability

Graph showing hydraulic conductivity (L_p) as a measure of BBB permeability in response to perfusion of 20% v/v rat serum alone (CTL) compared to serum plus 5 μ g/ml oxLDL without (oxLDL) and with 16 mM MgSO₄ (oxLDL+ MgSO₄). oxLDL caused an increase in BBB permeability that was prevented by MgSO₄. (* $P < 0.05$ vs. all other groups by one-way ANOVA).

Figure 5. Effect of MgSO_4 on oxLDL-induced changes in myogenic tone of posterior cerebral arteries

Graphs showing (A) active myogenic responses and (B) myogenic tone in posterior cerebral arteries in response to increased intravascular pressure after perfusion with 20% v/v rat serum alone (CTL) compared to serum plus 5 $\mu\text{g}/\text{ml}$ oxLDL without (oxLDL) and with 16 mM MgSO_4 (oxLDL+ MgSO_4). There was no difference in lumen diameters between groups. In addition, MgSO_4 did not prevent the decrease in myogenic tone induced by oxLDL. (* $P < 0.05$ vs. all other groups by one-way ANOVA).

Figure 6. Effect of MgSO₄ on oxLDL-induced constriction to NOS inhibition and dilation to sodium nitroprusside (SNP) in posterior cerebral arteries

Graphs showing (A) constriction to NOS inhibition with L-NNA and (B) sensitivity to the NO donor SNP in posterior cerebral arteries in response to perfusion of 20% v/v rat serum alone (CTL) compared to serum plus 5 μ g/ml oxLDL without (oxLDL) or with 16 mM MgSO₄ (oxLDL+ MgSO₄). The presence of MgSO₄ did not significantly affect the oxLDL-induced increase in constriction to L-NNA. However, MgSO₄ prevented the increased sensitivity to SNP in response to oxLDL. (** $P < 0.01$ vs. all other groups by one-way ANOVA; * $P < 0.05$ vs. all other groups by one-way ANOVA).