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Research Report

Progesterone attenuates thrombin-induced endothelial barrier disruption in the brain endothelial cell line bEnd.3: The role of tight junction proteins and the endothelial protein C receptor

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ABSTRACT

This study examines the effects of progesterone on blood-brain barrier (BBB) integrity following thrombin administration. Thrombin is expressed in many diseases which affect neural tissue and is associated with breakdown of the BBB. Progesterone has shown protective effects on the BBB in stroke and traumatic brain injury.

Methods: Mouse brain endothelial (bEnd.3) cells were treated with progesterone (20 μmol/l) for 24 h before thrombin administration (60 U/ml). BBB permeability was measured by transendothelial electrical resistance (TEER), because TEER decrease is associated with BBB compromise. Tight junction (TJ) proteins (occludin, claudin-5, and zonula occludens-1) and endothelial protein C receptor (EPCR) were analyzed.

Results: Thrombin decreased TEER and progesterone prevented that decrease. TJ proteins and EPCR were also decreased after thrombin treatment and progesterone treatment blocked that effect. Conclusion: Progesterone can attenuate thrombin-induced BBB disruption by blocking the degradation of TJ proteins and EPCR in bEnd.3 cells.

1. Introduction

Loss of blood–brain barrier (BBB) integrity is associated with the pathophysiology of many brain diseases, including acute and chronic cerebral ischemia, brain trauma, multiple sclerosis, Alzheimer's disease, brain tumors, and brain infections (Rosenberg, 2012). Although the cellular and molecular mechanisms leading to BBB damage are not fully understood, serum components such as thrombin might exert a direct toxic effect on the BBB and in the brain parenchyma. Accumulating data suggest

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that thrombin is a pleiotropic molecule in brain tissue that can cause vascular disruption (Chen et al., 2010), inflammatory response (Xue and Del Bigio, 2001), oxidative stress (Kameda et al., 2012), and direct cellular toxicity (Gingrich et al., 2000). Taken together, these reports suggest that thrombin contributes to brain injury through multiple mechanisms (Choi et al., 2003; Ishida et al., 2006).

The BBB is maintained by cell–cell contacts formed by various kinds of tight- (TJ) and adherence-junction proteins. Recent evidence shows that thrombin can exert deleterious effects on BBB integrity by directly disassembling the TJ protein claudin-5 through its actions on protease-activated receptor 1 (PAR-1) (Kondo et al., 2009). Activation of thrombin can also act indirectly to disrupt the integrity of the neurovascular signaling system because it is cytotoxic to neurons and glia (Donovan et al., 1997), and because it can induce an inflammatory response in brain tissue that then mediates further apoptosis in hippocampal neurons (Park et al., 2009). The deleterious effects of thrombin on BBB integrity can then accelerate the progression of stroke and other CNS injuries (Chapman, 2006; Sokolova and Reiser, 2008; Liu et al., 2010; Alabanza and Bynoe, 2012). Administration of thrombin inhibitors after stroke or the intraventricular injection of thrombin in the rat brain can help to reveal the effect of thrombin on vasculature and neuronal injury (Liu et al., 2010; Chen et al., 2012).

Progesterone is known to have neuroprotective effects on inflammation, BBB permeability, and edema in a variety of animal injury models (Ishrat et al., 2010; Wei and Xiao, 2013). Progesterone is converted to metabolites such as allopregnanolone and 5α-dihydroprogestrone (5α-DHP) in the brain, and these can have beneficial effects equal to or better than those of progesterone. The inhibition of progesterone conversion to 5α-DHP and allopregnanolone has been shown to eliminate progesterone's neuroprotective effects (Ciriza et al., 2006).

We recently showed that treatment with progesterone or allopregnanolone alleviated BBB disruption in rodent models of permanent ischemic stroke or tPA-induced stroke (Ishrat et al., 2010; Won et al., 2014). In vitro, progesterone also decreased tPA-induced vascular permeability and the dissociation of TJ proteins in brain endothelial cell line b.End.3. This cell line is often used for experiments on BBB integrity after injury and for examining the effects of drugs (Fernandes et al., 2014; Shin et al., 2014; Wan et al., 2014; Won et al., 2014).

The mechanisms of BBB protection by progesterone are not yet fully understood, but the modulation of matrix metalloproteinases (MMPs) and inflammation is believed to be a factor associated with progesterone's beneficial effects (Ishrat et al., 2010). Progesterone's protective effect on the endothelial protein C receptor (EPCR) has also remains to be studied. This is relevant because the EPCR plays a role in determining the permeability of the BBB in response to stroke and its treatment. Here, we tested the hypotheses that: (1) ischemic brain injury would induce an increase of endogenous thrombin expression; (2) treatment with progesterone would reverse that increase; (3) increased thrombin expression would lead to endothelial damage *via* the dissociation of TJ proteins; (4) this effect would be prevented by progesterone treatment in a mouse brain endothelial cell line (bEnd.3); and (5) these beneficial effects would be associated with the modulation of EPCR expression.

2. Results

2.1. Effect of progesterone on increased thrombin expression after stroke

We observed a significant increase in thrombin expression in ischemic brain tissue, and a significant decrease in thrombin expression following ischemia and progesterone treatment in the brain tissue extracted from the rats ($p<0.001$, Fig. 1A).

2.2. Effect of progesterone on BBB permeability after thrombin injury

To test the direct effect of thrombin or progesterone+thrombin on BBB permeability, we then measured the TEER after thrombin or progesterone+thrombin treatment in bEnd.3 cells. There were significant differences in TEER values between control, thrombin, and progesterone+thrombin cells at 60 and 90 min ($p<0.01$, Fig. 1B). Thrombin treatment resulted in a significant decrease in TEER at both 60 and 90 min compared to the control cells ($p<0.05$, Fig. 1B) but there was a significant increase of TEER in the progesterone-treated cells after 60 and 90 min compared to the thrombin-alone cells ($p<0.05$, Fig. 1B).

2.3. Effect of progesterone on TJ expression in bEnd.3 cells after thrombin injury

Because pathologic BBB permeability is a consequence of the disassembly of junction proteins in endothelial cells, we examined the TJ proteins occludin, claudin-5 and ZO-1 with Western blot assays as biomarkers of this process. There was a significant reduction in the 65 kDa occludin band in the thrombin-treated cells compared to controls (Fig. 2). Progesterone treatment significantly prevented the loss of occludin expression compared to thrombin-treated cells ($p<0.05$, Fig. 2). Thrombin induced a marked reduction of claudin-5 expression (23 kDa) compared to controls which was significantly prevented by progesterone treatment compared to treatment with thrombin alone ($p<0.05$, Fig. 2). A reduction in the 210 kDa ZO-1 band was observed in the thrombin-treated cells compared to controls. Progesterone significantly increased ZO-1 expression compared to the thrombin-treated cells ($p<0.05$, Fig. 2).

2.4. Progesterone's effect on EPCR expression in bEnd.3 cells after thrombin injury

We used Western blots to investigate whether progesterone can affect EPCR after thrombin injury. Compared to controls, the thrombin-treated cells displayed a significantly reduced level of EPCR ($p<0.05$, Fig. 3). EPRC expression in the progesterone-treated cells was significantly higher than that seen in cells treated with thrombin alone and was similar to controls ($p<0.05$, Fig. 3). We interpret this to suggest that progesterone prevents thrombin-induced EPCR down-regulation.