Lithium Upregulates Vascular Endothelial Growth Factor in Brain Endothelial Cells and Astrocytes

Shuzhen Guo, PhD; Ken Arai, PhD; Monique F. Stins, PhD; De-Maw Chuang, PhD; Eng H. Lo, PhD

**Background and Purpose**—We recently reported that delayed lithium therapy can improve stroke recovery in rats by augmenting neurovascular remodeling. We tested the hypothesis that lithium can promote the expression of growth factors in brain endothelial cells and astrocytes.

**Methods**—Human brain microvascular endothelial cells and primary rat cortical astrocytes were exposed to lithium chloride in serum-free medium. We examined 2 representative growth factors: brain-derived neurotrophic factor and vascular endothelial growth factor (VEGF). Cell lysates were collected for Western blot analysis. Conditioned media was analyzed with enzyme-linked immunosorbent assay. SB-216763 and LY294002 were used to assess the roles of the glycogen synthase kinase-3β (GSK-3β) and PI3-K signaling in the lithium-induced responses.

**Results**—No consistent responses were observed for brain-derived neurotrophic factor. However, lithium (0.2 to 20 mmol/L) increased the phosphorylation of GSK-3β and promoted VEGF secretion in a concentration-dependent manner in both endothelial and astrocyte cells. For endothelial cells, the potent GSK-3β inhibitor SB-216763 upregulated VEGF, whereas inhibition of PI3-K with LY294002 suppressed lithium-induced responses in both phospho-GSK-3β and VEGF. In contrast, neither inhibition of GSK-3β nor inhibition of PI3-K had any detectable effects on VEGF levels in astrocytes.

**Conclusions**—Lithium promotes VEGF expression through PI3-K/GSK-3β-dependent and -independent pathways in brain endothelium and astrocytes, respectively. This growth factor signaling mechanism may contribute to lithium’s reported ability to promote neurovascular remodeling after stroke.

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**Key Words:** growth factor ■ neuroprotection ■ neurovascular unit ■ stroke recovery

The mood stabilizer lithium has been reported as a potential neuroprotectant against many central nervous system disorders, including stroke and Alzheimer disease. Although the neuroprotective mechanisms of lithium are still not clearly defined, known molecular targets for lithium include inositol monophosphatase, proteasome, and glycogen synthase kinase-3 (GSK-3). We recently showed that delayed treatment with lithium improved functional MRI outcomes in a rat model of stroke recovery. Within peri-infarct cortex, lithium-treated rats demonstrated increased brain activation after forepaw stimulation, and these areas corresponded with changes in vascular density. Others have shown that brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) contribute to neurovascular remodeling after stroke, and these responses involve both recovering endothelium and reactive astrocytes. Therefore, we now ask whether lithium can upregulate BDNF and VEGF in brain endothelial and astrocyte cells.

**Materials and Methods**

A previously characterized human brain microvascular endothelial cell line was seeded in fibronectin-coated plates and exposed to lithium chloride (LiCl; Sigma) in serum-free medium after 6-hour serum starvation; NaCl (Sigma) was used as a control. Primary cultures of rat cortical astrocytes were prepared following standard techniques with cells from newborn (<2 days) Sprague-Dawley rats seeded in collagen I-coated plates for serum starvation and exposure to LiCl. After 30 minutes incubation, endothelial or astrocyte lysates were collected for Western blot with antibodies against phospho-GSK-3β (Ser9) or total GSK-3β (Cell Signaling). After 20 hours, enzyme-linked immunosorbent assays were used to measure BDNF (Promega) and VEGF (R&D Systems) in endothelial- or astrocyte-conditioned media. SB-216763 (Sigma) and LY294002 (Sigma) were used to inhibit GSK-3β and PI3-K, respectively. Standard lactate dehydrogenase assays confirmed that the treatments were not cytotoxic. Data were analyzed with analysis of variance followed by Tukey-Kramer tests.

**Results**

Levels of 2 representative growth factors, BDNF and VEGF, were assessed in conditioned media. Treatment...
with LiCl (0.2 to 20 mmol/L) for 20 hours did not produce a consistent change in BDNF levels in either endothelial cells or astrocytes (data not shown). Levels of VEGF were easily measured in conditioned media from brain endothelial cells (487.6 ± 33.2 pg/mL) and in astrocytes (46.8 ± 5.3 pg/mL). Exposure to LiCl for 20 hours increased VEGF in a concentration-dependent manner by 2- to 4-fold in both endothelial cells (Figure 1A) and astrocytes (Figure 1B). Treatment with NaCl had no detectable effects.

Western blot of cell lysates demonstrated that Ser-9 phosphorylation of GSK-3β was increased by LiCl in a concentration-dependent manner in endothelial cells (Figure 2A). GSK-3β activity is decreased by Ser-9 phosphorylation. Consistent with this phenomenon, the GSK-3 inhibitor SB-216763 similarly elevated VEGF levels in this brain endothelial cell model (Figure 2B). Next, we examined the closely related PI-3K pathway. The potent PI3-K inhibitor LY294002 prevented the phosphorylation of GSK-3β by LiCl (Figure 2C). Concomitantly, inhibition of PI3-K also prevented the lithium-induced upregulation of VEGF (Figure 2D).

In astrocyte cells, the phosphorylation of GSK-3β was also increased by LiCl (Figure 3A). However, surprisingly, inhibition with SB-216763 did not elevate VEGF levels (Figure 3B). The PI3-K inhibitor LY294002 prevented the lithium-induced phosphorylation of GSK-3β (Figure 3C), but it had no effect on lithium-induced VEGF levels (Figure 3D).

Discussion

Recently, we showed that delayed treatment with lithium may augment neurovascular remodeling and improve vasculature in brain endothelial cells. **Figure 1.** A, LiCl increased VEGF production by brain endothelial cells. B, LiCl increased VEGF production by brain astrocyte cells. *P<0.05 versus CON. N=3 independent experiments performed in triplicate.

**Figure 2.** A, Exposure to LiCl triggered GSK-3β signaling as indicated by elevated levels of phospho-GSK-3β in cell lysates. B, The GSK-3 inhibitor SB-216763 also significantly upregulated VEGF production. C, LiCl-induced GSK-3β signaling requires PI3-K, because the PI3-K inhibitor LY294002 (LY) suppressed phospho-GSK-3β levels. A total of 20 mmol/L LiCl and 5 μmol/L LY294002 was used. D, LY294002 prevented the LiCl-induced upregulation of VEGF in brain endothelial cells. *P<0.05 between controls (CON) versus LiCl-treated cells. #P<0.05 between LiCl alone versus LiCl plus LY294002. N=3 independent experiments performed in triplicate. DMSO was used as a vehicle control.
stroke recovery. The present study suggests that some of lithium’s effects might involve its ability to upregulate VEGF in brain cells.

Although lithium can trigger a wide range of biological actions in cells, our data here suggest that the GSK-3β/H9252 and PI3-K signaling pathways may play a central role. GSK-3β can modulate VEGF expression and help regulate angiogenesis. It has been shown that lithium increases VEGF mRNA in myocardium after cardiac ischemia. Here, the ability of lithium to upregulate VEGF in brain endothelial cells also appeared to function through the GSK-3β/H9252 and PI3-K pathway. It has also been reported that lithium can induce GSK-3-independent mechanisms in neural cells. Surprisingly, however, lithium-induced VEGF in astrocytes appears to be independent of GSK-3β and PI3-K signaling, at least in our system. Besides the glial and vascular compartment, neurons may also be beneficially affected. Lithium prevents VEGF reduction in immature neurons of hippocampus after chronic mild stress in rats. Hence, the ability of lithium to upregulate VEGF in brain endothelium and astrocytes might allow both autocrine and paracrine protection to the entire neurovascular unit after stroke.

Nevertheless, several questions remain. Lithium increases BDNF secretion in cultured rat cortical neurons and protects them from glutamate toxicity. Recently, lithium was found to selectively promote the BDNF promoter IV activity, causing an increase in exon IV-containing BDNF mRNA and total BDNF protein levels in rat neurons. However, in our brain endothelial cell and astrocyte models, BDNF was not affected. Why? Besides VEGF and BDNF, what other growth factors might be altered by lithium signaling? Besides GSK-3β and PI3-K signals, what other molecular pathways may mediate VEGF, especially in astrocytes?

Finally, besides stroke, might neurovascular actions of lithium also apply in other central nervous system disorders? A small clinical trial recently suggested that lithium may improve survival in ALS patients. Others have proposed that VEGF may protect neurons in experimental models of ALS, whereas early neurovascular alterations may contribute to ALS pathophysiology. Hence it is possible that the ability of lithium to upregulate endothelial or astrocytic VEGF and other growth factors may contribute to its beneficial effects in ALS and other neurodegenerative diseases. These broader neurovascular signaling actions of lithium warrant further investigation.

In conclusion, lithium or other GSK-3β-modulating drugs may provide a therapeutic approach for augmenting VEGF and perhaps promoting neurovascular remodeling during stroke recovery. The initial cell data presented here require further confirmation and analysis with in vivo model systems.

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Disclosures
None.

References


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