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Calponin control of cerebrovascular reactivity: therapeutic implications in brain trauma

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Abstract

Calponin (Cp) is an actin-binding protein first characterized in chicken gizzard smooth muscle (SM). This review discusses the role of Cp in mediating SM contraction, the biochemical process by which Cp facilitates SM contraction and the function of Cp in the brain. Recent work on the role of Cp in pathological states with emphasis on traumatic brain injury is also discussed. Based on past and present data, the case is presented for targeting Cp for novel genetic and pharmacological therapies aimed at improving outcome following traumatic brain injury (TBI).

Keywords: vascular reactivity • calponin • brain trauma

A role for calponin (Cp) in mediating smooth muscle (SM) contraction

The basic isoform of Calponin (henceforth denoted as Cp) is an actin-binding protein present in both the cytoskeleton and contractile machinery of SM cells [1]. It has been shown also to be synthesized in endothelial cells (EC) [2–4]. Originally purified from chicken gizzard SM by Takahashi *et al.* [5], Cp was subsequently identified as a 34-kD protein, which bound to tropomyosin [6]. Because of its selective localization, Cp has been deemed a critical protein in the regulation of SM contraction. Mezgueldi *et al.* [7, 8] identified an actin-binding site. This site also contains the part of Cp that, by binding to actin, inhibits myosin ATPase activity.

The mechanism by which Cp regulates SM contraction is somewhat controversial and may involve either direct inhibition of acto-myosin cross-bridging, Cp-specific signalling, or a combination of the two. *In vitro* work revealed that during SM's relaxed state, Cp is bound to acto-myosin, this binding inhibiting acto-myosin cross-bridge formation [8, 9], thus ultimately preventing SM contraction. Worth *et al.* [10] demonstrated increased Cp protein expression in SM's contractile state. Two other *in vitro* works showed that application of Cp in a dose-dependent fashion increase SM contraction [11, 12]. In contrast in whole muscle preparations, Cp appeared to have somewhat different effects. Jaworowski *et al.* [13], using intact skinned SM from guinea pig, reported that while Cp inhibited maximal shortening velocity, it had only a minor influence on the force of contraction (~10% reduction). They further suggested that Cp may modulate the rate of acto-myosin cross-bridging. Similarly, Obara *et al.* [14] presented data supporting the concept that rather than directly inhibiting acto-myosin, Cp modulated the velocity of cross-bridging.

Alternate lines of evidence suggested that Cp involvement in SM contraction could be related to signalling rather than to a direct acto-myosin interaction. In a set of compelling studies, Morgan and colleagues showed that basic Cp not only interacts with PKC alpha and epsilon, but also with ERK [15]. They further showed that Cp is bound directly to the regulatory domain of PKC. Their data, combined with further work showing that during SM contraction ERK redistributed from cytoskeleton to contractile

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domains [16], support a Cp signalling role in PKC and ERK-mediated SM contraction.

It has been suggested that any conflicting data regarding a putative Cp role in regulating SM contraction may be due to either differences in Cp expression, the use of isolated SM cells *versus* intact muscle [17], or both. For example, components of SM cytoskeleton, including Cp, were shown to rapidly down-regulate when SM cells are placed in culture [18–20]. Given that Cp levels in SM may be critical for contractility, such decreased expression may confound interpretation of the *in vitro* data. Nonetheless, the consensus of the literature supports a regulatory role of Cp in mediating contraction of vascular SM.

Cp phosphorylation and vascular contractility

PKC-dependent Cp phosphorylation has been linked to SM contraction. PKC application to cultured ferret aortic SM cells results in enhanced vascular contractility [21, 22]. In contrast, application of staurosporine, a PKC inhibitor, prevents vascular SM contraction [23]. It should be pointed out, however, that results may be difficult to interpret, here, since staurosporine has relatively poor selectivity. Nonetheless, these results provide further evidence that PKC is critical for vasoconstriction. Cp is known to contain five potential phosphorylation sites, serine (SER)-175 and threonine (THR)-170, -180, -184 and -259 [24]. Ser-175 and Thr-184 are PKC-phosphorylation sites. Early on Naka *et al.* [25] showed that Cp is phosphorylated *via* protein kinase C (PKC). Subsequent *in vitro* studies demonstrated that upon phosphorylation, Cp becomes disassociated with acto-myosin, allowing for cross-bridging [26, 27]. Cp dephosphorylation *via* a Cp-specific phosphatase is thought to restore Cp-inhibition of acto-myosin cross bridging, thus returning SM to its relaxed state [28]. In addition only THR-184 was shown to significantly affect (*i.e.* enhance) vasoconstriction [29]. Based on these findings, it can be concluded that PKC phosphorylates at the Cp THR-184 site, which results in vasoconstriction. However, two other groups have provided evidence to the contrary by showing that Cp phosphorylation does not affect SM contraction [17, 30, 31]. Again, this discrepancy could be the result of studying isolated SM cells *versus* intact muscle [17].

Cp localization and function in the brain

Much of the characterization of Cp has been carried out in peripheral tissue (*i.e.* non-CNS) preparations with little work done in brain. In fact, the role of Cp in controlling brain vascular tone has only been recently studied [51]. Furthermore, more fundamentally, whether Cp is found in brain has been the source of controversy. When first purified, Takahashi's laboratory reported that Cp did not exist in either chicken or bovine brain [32, 33]. In 1994, Applegate and colleagues identified an mRNA analogue to Cp in rat brain. However, this pertained to a novel acidic isoform, which did not interact with calcium-calmodulin, suggesting a differential role from that of the basic isoform. Subsequent studies have shown both the acidic and basic isoforms of Cp in various brain regions [34–37]. Using immunofluorescent techniques, we have localized the basic isoform of Cp in SM of reacting microvessels (*i.e.* terminal and precapillary arterioles) from brain regions such as the sensorimotor cortex (smCx) and dorsal hippocampus (hipp) [50–52]. In addition to being located in SM of cerebral blood vessels, Cp has been localized to neurons [35, 36, 41–43]. Ferhat *et al.* [38] demonstrated that Cp is most prevalent in hippocampal neurons during development. Plantier *et al.* [41] further showed that Cp is highly expressed in growth cones in the developing brain. Bannai *et al.* [42] demonstrated a decrease in the number of neurons located in the paraventricular nucleus of Cp gene-deficient mice. These studies, combined with evidence that Cp is up-regulated during dendritic spine plasticity [39], suggest that in addition to its vascular function, Cp may also modulate neuronal growth and development. Therefore, in general, the role of Cp in the brain appears to be heterogeneous; supporting not only vascular contractility but also a broad spectrum of neuronal functions in both the developing and adult brains.

Does Cp play a role in pathologic states?

While the mechanism by which Cp normally regulates SM contraction may not yet be entirely elucidated, Cp's role in vascular contractility (or vasoreactivity) has been recently implicated in several pathologic states, including renal glomerular nephritis [46], ischaemia/reperfusion [47], hypertension [48], subarachnoid haemorrhage [49, 50], vasospasm [51, 52], and more recently by us in traumatic brain injury (TBI) [38–40]. While there are several experimental models of TBI documenting alterations in vasoreactivity and the cerebral microcirculation, very little work with these models has focused on the pathophysiologic mechanism underlying these alterations. Because of the seminal role of Cp in SM contraction and vasoreactivity as explained above, we focus here on the role of Cp in vasoreactivity in a rodent model of diffuse TBI where one of this model's pathologic events is a persistent state of enhanced vasoreactivity and decreased cerebral blood flow (CBF) [53].

Cp and TBI

TBI results in a chronic state of enhanced vasoreactivity of reacting microvessels, which leads to hypoperfusion (decreased CBF) and hypoxia of the brain parenchyma [53–56]. Because these resulting pathophysiologies from brain primary injury are thought

to contribute to the development of secondary injury (*i.e.* nerve cell injury/death), our laboratory has dedicated the last decade to understanding the molecular mechanisms that underlie enhanced vasoreactivity following TBI. To date, research relating to disruption of the brain microcirculation following TBI has focused primarily on receptors [57–59], growth factors [60, 61] and neurochemicals [54, 62–64]. However, none of these investigations have studied the signal transduction mechanism underlying either control of normal vascular tone or the dysfunctional sustained SM contraction that follows after TBI. Related to these issues and at a more fundamental level, little work has been done to demonstrate the role of contractile proteins in mediating vasoconstriction in the normal and injured brain. Because of the above Cp work supporting a role in SM contraction, we undertook to study the spatial and temporal patterns of Cp expression in brain reacting microvessels that may underlie enhanced vascular reactivity and decreased CBF as observed after TBI.

We first sought to determine whether TBI had an effect on Cp brain cellular expression and found significant increases in Cp immunoreactivity (IR) in SM of reacting microvessels from smCx and hipp as early as 4 hrs after injury. These increases were sustained up to 48 hrs after TBI [38] and correlated temporally with the previously observed enhanced vascular reactivity and decreased CBF [53]. Only trace amounts of Cp IR were detected in endothelial cells at all time points of the study which is consistent with the findings by Birukov *et al.* [65]. Analysis of single SM cells revealed a Cp shift from the cytosol towards plasma membrane of SM during sustained vasoreactivity after TBI (Fig. 1) [38, 39]. This finding is in concert with the *in vitro* work by Morgan and colleagues who also showed Cp migration during SM contraction [15]. The significance of the Cp migration is not fully understood at this point. One explanation is that Cp migration facilitates ERK or PKC translocation prior to various phosphorylation events [66–68]. Alternatively, Cp migration may be important in maintaining cytoskeletal integrity of SM during its contraction. Cp has been shown to interact with caldesmon (Cd) [69], another cytoskeletal protein known to be located in endothelium and at the SM plasma membrane [38]. As such, Cp's migration and its molecular linkage with Cd could provide overall SM structural integrity during its contraction.

Because Cp phosphorylation could modulate SM contracion, we undertook a study of such process and its association with sustained vasoreactivity and CBF alteration after TBI. Cp was phosphorylated from 4 to 48 hrs after TBI [40], this phosphorylation temporally coinciding with enhanced vasoreactivity. This provided *in vivo* evidence that Cp phosphorylation was temporally associated both with the SM sustained contraction and hypoperfusion of the cerebral microcirculation after TBI. In order to further test these last conclusions, we designed an experiment in which a peptide that bound to the THR184 phosphorylation site on Cp (177FASQQGM**T**A185, Invitrogen, Carlsbad, CA, USA) was injected intracerebroventricularly (ICV) prior to TBI [70]. Subsequently assessment of Cp phosphorylation and CBF were carried out by Western immunobloting and arterial spin labelling magnetic resonance imaging (ASL-MRI), respectively. A single bolus injection of 20 nmol of the peptide effectively blocked Cp phosphorylation (Western) and ameliorated the TBI-induced hypoperfusion in smCx and hipp as detected by ASL-MRI (Fig. 2). We then asked the question whether the observed molecular and functional changes correlated with alterations in cognitive behaviours, which are also known to occur after TBI. As such, our rodent model of diffuse TBI causes significant food acquisition and retention memory loses performed in an automated radial arm maze, with such deficits persisting up to 27 days after impact (Fig. 3). Using the same behaviour paradigm, we found that ICV peptide administration improved performance in the radial arm maze compared to that of animals that only received vehicle injections (Fig. 3). More recently, we are exploring the feasibility of using Cp gene therapy as a therapy for improving CBF and cognitive outcome following TBI.

Endothelin (ET)-1, its receptors (ETrA, ETrB) and Cp-mediated vasoreactivity: therapeutic implications

Because our work and that of others support a pivotal role of ET-1 and its receptors ETrA and ETrB in the control of vascular tone in normal and injured brains, we have included here, a brief discussion on these substances. Likewise, the causal association between ET-1 receptor-mediated vasoreactivity and Cp-mediated SM contractility is also explained.

ET-1 is a powerful vasoconstrictor, which exerts vasoactive actions through its two G-protein-coupled receptors, A and B [71]. Activation of either receptor is coupled with activation of PKC. However activation of ETrA results in vasoconstriction, whereas that of ETrB causes vasodilation [72, 73]. Beginning in the early 1990s, clinical trials using ETrA/B antagonists were introduced for its potential role in ameliorating vasospasm in several pathologic states [74, 75]. Since then, ET-1 has been a target for improving blood flow to several organs in a number of pathologic states including hypertension [76] hepatorenal syndrome [77], heart failure [78] and decreased cerebral blood flow and hypoxia [79]. In 1995, Luscher and Wenzel published one of the first reviews where ET-1 receptor antagonists were identified as therapeutic agents to treat vascular disorders in clinical settings [80]. In their 1999 review, Benigni and Remuzzi [81] summarized data from pre-clinical and clinical studies, which showed promise for the use of specific ETrA antagonists in controlling hypertension.

While clinical trials using pharmacological blockade of ET-1 receptors for a variety of peripheral tissue pathologies have increased in recent years, until recently few experimental studies have addressed the *mechanism* by which these therapies may be working. Therefore our laboratory has concentrated on elucidating

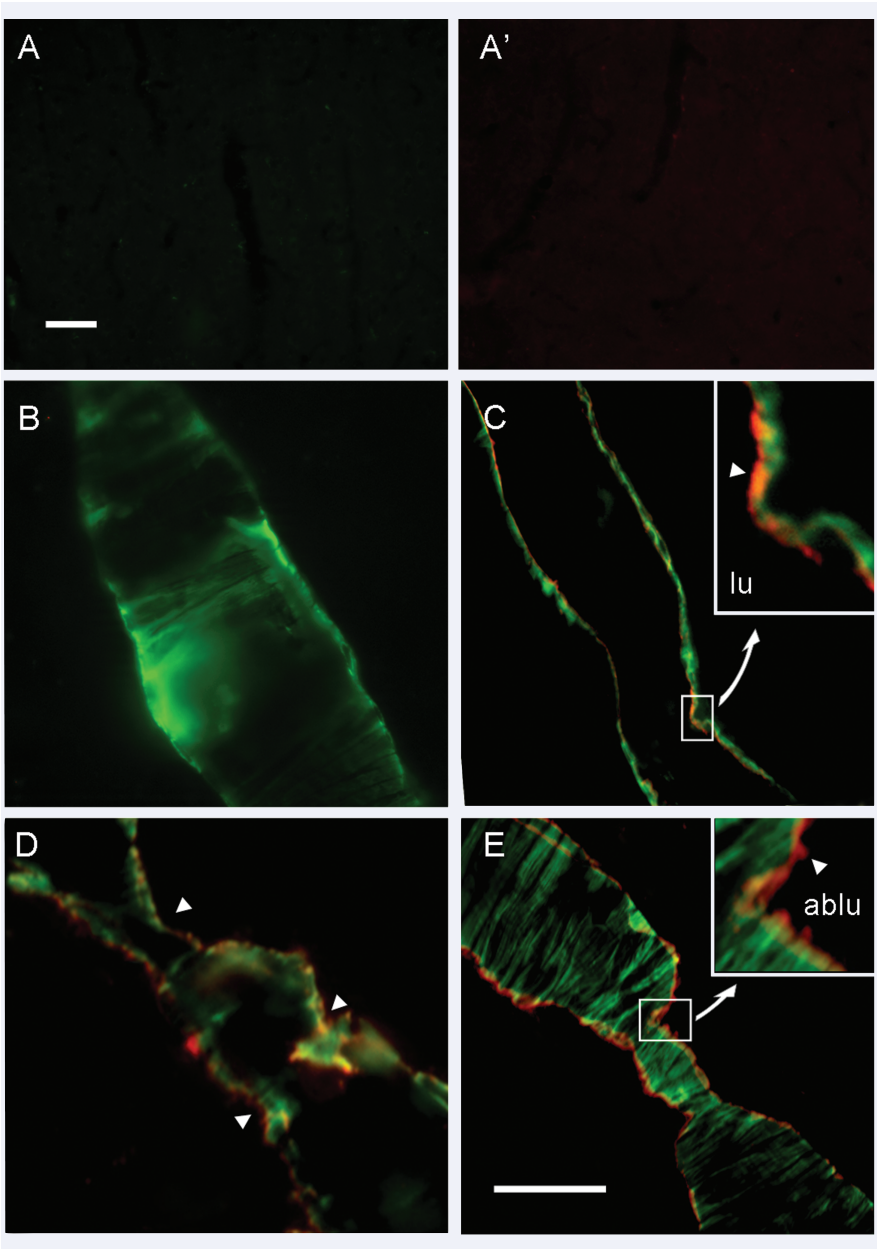

Fig. 1 Calponin redistribution following TBI is causally associated with sustained vascular reactivity (reprinted with permission from Kreipke *et al.*, 2007). Photomicrographs depicting double immunofluorescence-labelled α smooth muscle actin (SMA; green) + Cp (red) positive smooth muscle cells in reacting vessels from control (sham), 4, 24 and 48 hrs after TBI. (**A**) control immunofluorescence for FITC minus primary antibody (SMA); (**A'**) control immunofluorescence for Texas red (TR) minus primary antibody (Cp); (**B**) control (sham lesioned) vessel in the relaxed state labelled for SMA and Cp. Note that only trace amounts of Cp are expressed within the control; (**C**) microvessel 4 hrs after TBI showing a significant increase in Cp within SM. Inset depicts a ×300 enlargement of a portion of the vessel showing the SM location of Cp primarily beside the membrane of the SM closest to the vessel's lumen (lu) (arrowhead); (**D**) vessel 24 hrs after TBI. Notice increase in the yellow channel (overlap between SMA and Cp). Arrowheads point to segment of the reacting arteriole significantly contracted; (**E**) microvessel 48 hrs after TBI. A significant increase in Cp beside the abluminal (ablu) membrane of SM was observed. Inset shows ×300 enlargement of a contracted portion of the vessel. Arrowhead highlights intense Cp signal along the ablu face.

the mechanism by which ET-1 and its receptors may, in part, underlie enhanced vasoreactivity and hypoperfusion of the brain parenchyma after TBI. Using experimental animal models, we and others have shown that ET-1 and ET-1 receptor signalling may underlie vascular dysfunction following TBI [82, 83]. Specifically, we have reported that ET-1, ETrA and ETrB are up-regulated following TBI [84, 85]. Further, we have demonstrated that by blocking the ETrA receptor, we can block the hypoperfusion that follows brain injury [86].

How Cp may be implicated in the signal transduction of ET-1 and its receptors in normal and injured brain was further demonstrated by us. As such, ETrA antagonism also blocked Cp phosphorylation and improved hypoperfusion and CBF after TBI [85]. In order to further establish a cause-effect relationship between Cp phosphorylation and ET-1 receptor signalling, we blocked PKC-induced phosphorylation of Cp by application of chelerythine. It is known that ETrA is coupled to PKC signalling [72, 73]. Therefore, when we injected chelerythrine to block PKC prior to TBI, this intervention

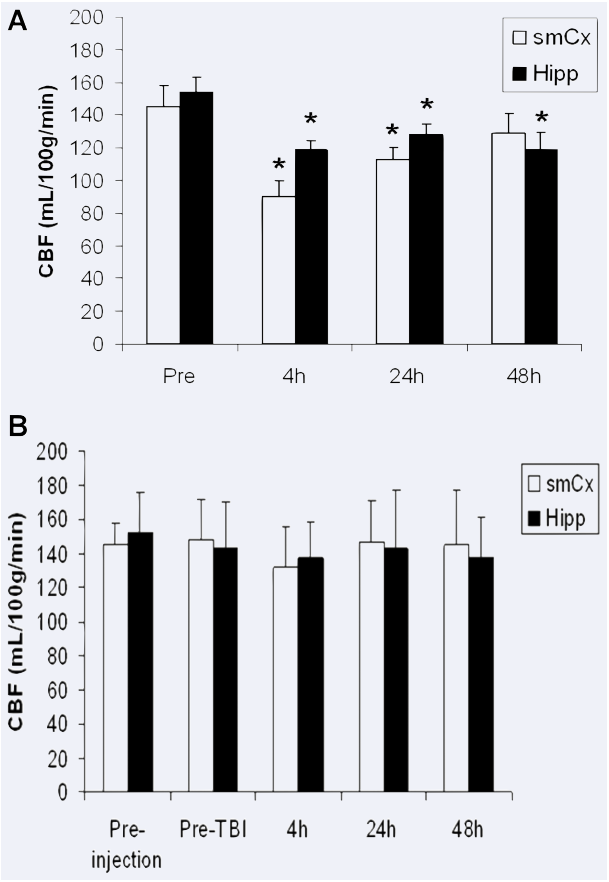

Fig. 2 Effect of blocking Cp phosphorylation on CBF following TBI. A peptide designed to block the THR184 phosphorylation site on Cp was injected into the ventricles of rats (50 ng per side, 100 ng total) 1 hr prior to inducing TBI. Results show that TBI, alone, causes reduced CBF (*$P < 0.05$) (**A**). However, while blocking Cp phosphorylation had no significant effect in control animals, it improved CBF following injury as indicated by the lack of a significant reduction in CBF at any time point (**B**).

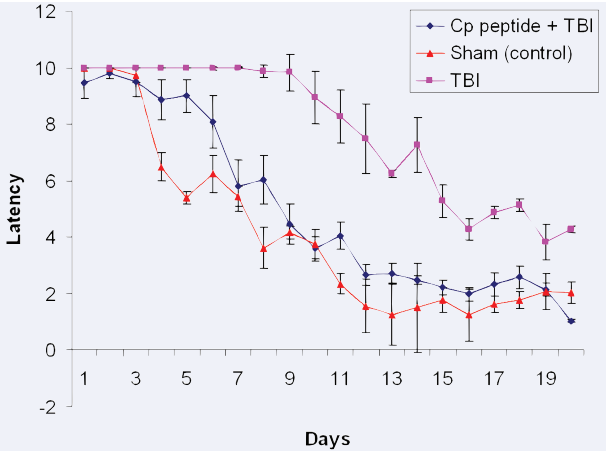

Fig. 3 Effect of blocking Cp phosphorylation on behavioural outcome following TBI. Cp peptide (20nmol) was administered ICV 1 hr prior to injury in male Sprague–Dawley rats ($n = 8$, 6 receiving TBI, 2 receiving sham operation). Injury was induced using a weight-drop model of TBI. One day following TBI, animals were tested for 20 consecutive days on the radial arm maze. The results were compared with animals that received sham operation (red) or TBI alone (no Cp-peptide injection) (pink). Cp peptide (dark blue) improved performance on the radial arm maze compared to TBI suggesting that blocking Cp phosphorylation improves behavioural outcome following injury.

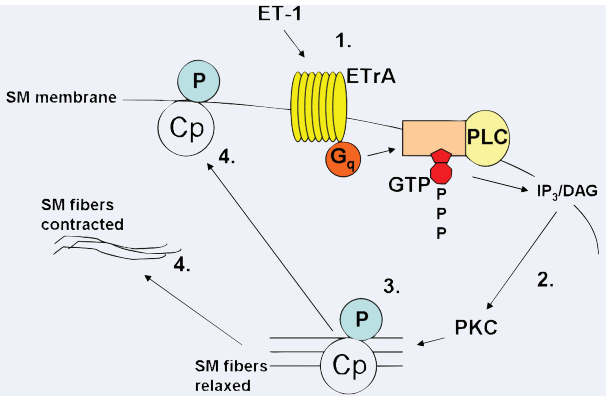

Fig. 4 Signal transduction leading to smooth muscle (SM) contraction (adapted from Kreipke *et al.*, 2007). 1. ET-1 binds to its receptor, ETrA, a G-protein coupled receptor. 2. PKC is activated and translocates to. 3. phosphorylate Cp. 4. Cp phosphorylation causes it to disassociate from the acto-myosin complex, which allows for contraction where it then translocates to the SM membrane.

resulted in a reduction both of Cp phosphorylation, as detected by 2-dimensional gel electrophoresis, and cerebral hypoperfusion (*i.e.* enhanced CBF) as detected by ASL-MRI after TBI [70]. Taken together, we propose the following mechanism leading to vasoconstriction (Fig 4): ET-1 binds to its receptor, ETrA, which activates PKC, leading to phosphorylation of Cp and ultimately leading to vasoconstriction of reacting microvessels. Following TBI, we posit that this signal transduction cascade may be enhanced, leading to an increase in vasoconstriction. Future studies will aim to control the persistent vasoreactivity and decreased CBF by pharmacologic intervention of the same signal transduction cascade, that is, by blocking either at the ET-1 receptor level using selective and non-selective inhibitors, Cp phosphorylation level, or both.

Conclusion

Cp is a critical component of SM contractile machinery leading to vasoconstriction. While the precise molecular mechanism by which Cp regulates SM contraction is somewhat controversial,

past and current work support the notion that Cp phosphorylation is essential to mediate this contraction. While Cp has been implicated in several pathologic states, our work supports a role for Cp in mediating both enhanced brain vasoreactivity and hypoperfusion (*i.e.* decreased CBF) resulting from TBI. Because these pathophysiologic effects of primary brain injury in turn are likely to lead to the development of secondary injury (*i.e.* nerve cell injury/death), understanding the signal transduction cascade *via* ET-1 and its receptors, as well as their modulation of Cp action in SM contraction are paramount to design rational therapies that can be implemented effectively to improve TBI-induced neurological deficits in the clinical setting.

References

1. **North AJ, Gimona M, Cross RA, Small JV**. Calponin is localized in both the contractile apparatus and the cytoskeleton of smooth muscle cells. *J. Cell Sci.* 1994; 107: 437–44.
2. **Birukov KG, Stepanova OV, Nanaev AK, Shirinsky VP**. Expression of calponin in rabbit and human aortic smooth muscle cells. *Cell Tissue Res.* 1991; 266: 579–84.
3. **Sakihara C, Nishimura J, Kobayashi, Takahashi S, Kanaide H**. Expression of calponin mRNA in porcine aortic endothelial cells. *Biochem Biophys Res Commun.* 1996; 222: 195–200.
4. **Bandopadhyay R, Orte C, Lawrenson JG, Reid AR, De Silva S, Allt G**. Contractile proteins in pericytes at the blood-brain and blood-retinal barriers. *J Neurocytol.* 2001; 30: 35–44.
5. **Takahashi K, Hiwada K, Kokubu T**. Isolation and characterization of a 34,000-dalton calmodulin- and F-actin-binding protein from chicken gizzard smooth muscle. *Biochem Biophys Res Commun.* 1986; 141: 20–6.
6. **Takahashi K, Abe M, Hiwada K, Kokubu T**. A novel troponin T-like protein (calponin) in vascular smooth muscle: interaction with tropomyosin paracrystals. *J Hypertens Suppl.* 1988; 6: S40–3.
7. **Mezgueldi M, Fattoum A, Derancourt J, Kassab R**. Mapping of the functional domains in the amino-terminal region of calponin. *J Biol Chem.* 1992; 267: 15943–51.
8. **Mezgueldi M**. Calponin. *Int J Biochem Cell Biol.* 1996; 28: 1185–9.
9. **Winder S, Walsh M**. Inhibition of the actomyosin MgATPase by chicken gizzard calponin. *Prog Clin Biol Res.* 1990a; 327: 141–8.
10. **Worth NF, Rolfe BE, Song J, Campbell GR**. Vascular smooth muscle cell phenotypic modulation in culture is associated with reorganisation of contractile and cytoskeletal proteins. *Cell Motil Cytoskeleton.* 2001; 49: 130–45.
11. **Lin Y, Ye LH, Ishikawa R, Fujita K, Kohama K**. Stimulatory effect of calponin on myosin ATPase activity. *J Biochem.* 1993; 113: 643–5.
12. **Yang JX, Feng XH, Zhang Y, Tang ZY, Lin Y**. The influence of trace amount of calponin on the smooth muscle myosins in different states. *Biochem Biophys Res Commun.* 2004; 318: 904–10.
13. **Jaworowski A, Anderson KI, Arner A, Engstr^m M, Gimona M, Strasser P, Small JV**. Calponin reduces shortening velocity in skinned taenia coli smooth muscle fibres. *FEBS Lett.* 1995; 365: 167–71.
14. **Obara K, Szymanski PT, Tao T, Paul RJ.** Effects of calponin on isometric force and shortening velocity in permeabilized taenia coli smooth muscle. *Am J Physiol.* 1996; 270: C481–7.
15. **Gangopadhyay SS, Takizawa N, Gallant C, Barber AL, Je HD, Smith TC, Luna EJ, Morgan KG**. Smooth muscle archvillin: a novel regulator of signaling and contractility in vascular smooth muscle. *J Cell Sci.* 2004; 117: 5043–57.
16. **Khalil RA, Menice CB, Wang CL, Morgan KG**. Phosphotyrosine-dependent targeting of mitogen-activated protein kinase in differentiated contractile vascular cells. *Circ Res.* 1995; 76: 1101–8.
17. **Bárány M, Rokolya A, Bárány K**. Absence of calponin phosphorylation in contracting or resting arterial smooth muscle. *FEBS Lett.* 1991; 279: 65–8.
18. **Gimona M, Herzog M, Vandekerckhove J, Small JV**. Smooth muscle specific expression of calponin. *FEBS Lett.* 1990; 274: 159–62.
19. **Bochaton-Piallat ML, Gabbiani F, Ropraz P, Gabbiani G**. Cultured aortic smooth muscle cells from newborn and adult rats show distinct cytoskeletal features. *Differentiation.* 1992; 49: 175–85.
20. **van Eys GJ, Völler MC, Timmer ED, Wehrens XH, Small JV, Schalken JA, Ramaekers FC, van der Loop FT**. Smoothelin expression characteristics: development of a smooth muscle cell *in vitro* system and identification of a vascular variant. *Cell Struct Funct.* 1997; 22: 65–72.
21. **Walsh MP, Horowitz A, Clement-Chomienne O, Andrea JE, Allen BG, Morgan KG**. Protein kinase C mediation of Ca (2+)-independent contradictions of vascular smooth muscle. *Biochem Cell Biol.* 1996; 74: 485–502.
22. **Horowitz A, Clément-Chomienne O, Walsh MP, Morgan KG**. Epsilon-isoenzyme of protein kinase C induces a Ca(2+)-independent contraction in vascular smooth muscle. *Am J Physiol.* 1996; 271: C589–94.
23. **Moreland RS, Cilea J, Moreland S**. Staurosporine decreases stiffness but not stress in endothelin-1-stimulated arterial muscle. *Am J Physiol.* 1992; 262: C862–9.
24. **Kaneko T, Amano M, Maeda A, Goto H, Takahashi K, Ito M, Kaibuchi K**. Identification of calponin as a novel substrate of Rho-kinase. *Biochem Biophys Res Commun.* 2000; 273: 110–6.
25. **Naka M, Kureishi Y, Muroga Y, Takahashi K, Ito M, Tanaka T**. Modulation of smooth muscle calponin by protein kinase C and calmodulin. *Biochem Biophys Res Commun.* 1990; 171: 933–7.
26. **Winder SJ, Walsh MP**. Smooth muscle calponin. Inhibition of actomysin MgATPase and regulation by phosphorylation. *J Biol Chem.* 1990b; 265: 10148–55.
27. **Tani E**. Molecular mechanisms involved in development of cerebral vasospasm. *Neurosurg Focus.* 2002; 12: ECP1.
28. **Winder SJ, Allen BG, Fraser ED, Kang HM, Kargacin GJ, Walsh MP**. Calponin phosphorylation *in vitro* and in intact muscle. *Biochem J.* 1993; 296: 827–36.
29. **Nakamura K, Toda H, Kakuyama M, Nishiwada M, Yamamoto M, Hatano Y, Mori K**. Identification of the regulatory site in smooth muscle calponin that is phosphorylated by protein kinase C. *J Biol Chem.* 1993; 9: 6194–201.

30. **Bárány M, Bárány K**. Calponin phosphorylation does not accompany contraction of various smooth muscles. *Biochim Biophys Acta.* 1993; 1179: 229–33.
31. **Itoh T, Suzuki S, Suzuki A, Nakamura F, Naka M, Tanaka T**. Effects of exogenously applied calponin on Ca(2+)-regulated force in skinned smooth muscle of the rabbit mesenteric artery. *Pflugers Arch.* 1994; 427: 301–8.
32. **Takahashi K, Hiwada K, Kokubu T**. Occurrence of anti-gizzard P34K antibody cross-reactive components in bovine smooth muscles and non-smooth muscle tissues. *Life Sci.* 1987; 41: 291–6.
33. **Takahashi K, Nadal-Ginard B**. Molecular cloning and sequence analysis of smooth muscle calponin. *J Biol Chem.* 1991; 266: 13284–8.
34. **Trabelsi-Terzidis H, Fattoum A, Represa A, Dessi F, Ben-Ari Y, der Terrossian E**. Expression of an acidic isoform of calponin in rat brain: western blots on one- or two-dimensional gels and immunolocalization in cultured cells. *Biochem J.* 1995; 306: 211–5.
35. **Represa A, Trabelsi-Terzidis H, Plantier M, Fattoum A, Jorquera I, Agassandian C, Ben-Ari Y, der Terrossian E**. Distribution of caldesmon and of the acidic isoform of calponin in cultured cerebellar neurons and in different regions of the rat brain: an immunofluorescence and confocal microscopy study. *Exp Cell Res.* 1995; 221: 333–43.
36. **Agassandian C, Plantier M, Fattoum A, Represa A, der Terrossian E**. Subcellular distribution of calponin and caldesmon in rat hippocampus. *Brain Res.* 2000; 887: 444–9.
37. **Ibanez C, Ito D, Zawadzka M, Jeffery ND, Franklin RJ**. Calponin is expressed by fibroblasts and meningeal cells but not olfactory ensheathing cells in the adult peripheral olfactory system. *Glia.* 2007 15; 55: 144–51.
38. **Ferhat L, Charton G, Represa A, Ben-Ari Y, der Terrossian E, Khrestchatisky M**. Acidic calponin cloned from neural cells is differentially expressed during rat brain development. *Eur J Neurosci.* 1996; 8: 1501–9.
39. **Ferhat L, Esclapez M, Represa A, Fattoum A, Shirao T, Ben-Ari Y**. Increased levels of acidic calponin during dendritic spine plasticity after pilocarpine-induced seizures. *Hippocampus.* 2003; 13: 845–58.
40. **Pape M, Doxakis E, Reiff T, Duong CV, Davies A, Geissen M, Rohrer H**. A function for the calponin family member NP25 in neurite outgrowth. *Dev Biol.* 2008; 321: 434–43.
41. **Plantier M, Fattoum A, Menn B, Ben-Ari Y, Der Terrossian E, Represa A**. Acidic calponin immunoreactivity in postnatal rat brain and cultures: subcellular localization in growth cones, under the plasma membrane and along actin and glial filaments. *Eur J Neurosci.* 1999; 11: 2801–12.
42. **Bannai M, Yoshimoto R, MiTsui-Saito M, Hori M, Nishihara M, Takahashi K, Yamamura H, Taniguchi S, Katsuki M, Ozaki H, Karaki H**. Increased locomotor activity, increased food and water intake and decreased PVN neurons in H1 calponin gene-deficient mice. *J Vet Med Sci.* 2003; 65: 153–5.
43. **Sugenoya Y, Yoshimura A, Yamamura H, Inui K, Morita H, Yamabe H, Ueki N, Ideura T, Takahashi K**. Smooth-muscle calponin in mesangial cells: regulation of expression and a role in suppressing glomerulonephritis. *J Am Soc Nephrol.* 2002; 13: 322–31.
44. **Juan YS, Li S, Levin RM, Kogan BA, Schuler C, Leggett RE, Huang CH, Mannikarottu A**. The effect of ischemia/reperfusion on rabbit bladder-role of Rho-kinase and smooth muscle regulatory proteins. *Urology.* 2008; epub.
45. **Hu JJ, Ambrus A, Fossum TW, Miller MW, Humphrey JD, Wilson E**. Time courses of growth and remodeling of porcine aortic media during hypertension: a quantitative immunohistochemical examination. *J Histochem Cytochem.* 2008; 56: 359–70.
46. **Doi M, Kasuya H, Weir B, Cook DA, Ogawa A.** Reduced expression of calponin in canine basilar artery after subarachnoid haemorrhage. *Acta Neurochir.* 1997; 139: 77–81.
47. **Sun H, Kanamaru K, Ito M, Suzuki H, Kojima T, Waga S, Kureishi Y, Nakano T**. Myosin light chain phosphorylation and contractile proteins in a canine two-hemorrhage model of subarachnoid hemorrhage. *Stroke.* 1998; 29: 2149–54.
48. **Kim I, Leinweber BD, Morgalla M, Butler WE, Seto M, Sasaki Y, Peterson JW, Morgan KG**. Thin and thick filament regulation of contractility in experimental cerebral vasospasm. *Neurosurgery.* 2000; 46: 440–6.
49. **Tani E**. Molecular mechanisms involved in development of cerebral vasospasm. *Neurosurg Focus.* 2002; 12: ECP1.
50. **Kreipke CW, Morgan N, Petrov T, Rafols J**. Calponin and caldesmon cellular domains in reacting microvessels following traumatic brain injury. *Microvasc Res.* 2006; 71: 197–204.
51. **Kreipke CW, Morgan R, Roberts G, Bagchi M, Rafols JA**. Calponin phosphorylation in cerebral cortex microvessels mediates sustained vasoconstriction after brain trauma. *Neurol. Res.* 2007a; 29: 369–74.
52. **Kreipke CW, Morgan RL, Petrov T, Rafols JA**. Subcellular redistribution of calponin underlies sustained bascular contractility following traumatic brain injury. *Neurol Res.* 2007b; 29: 604–9.
53. **Rafols J., Kreipke CW, Petrov T**. Alterations in cerebral cortex microvessels and the microcirculation in a rat model of traumatic brain injury: a correlative EM and laser Doppler flowmetry study. *Neurol Res.* 2007; 29: 339–47.
54. **Petrov T, Rafols JA**. Acute alterations of endothelin-1 and iNOS expression and control of the brain microcirculation after head trauma. *Neurol Res.* 2001; 23: 139–43.
55. **Steiner J, Rafols D, Park HK, Katar MS, Rafols JA, Petrov T**. Attenuation of iNOS mRNA exacerbates hypoperfusion and upregulates endothelin-1 expression in hippocampus and cortex after brain trauma. *Nitric Oxide.* 2004; 10: 162–9.
56. **Stys, PK**. White matter injury mechanisms. *Curr Mol Med.* 2004; 4: 113–30.
57. **Fee DB, Sewell DL, Andresen K, Jacques TJ, Piaskowski S, Barger BA, Hart MN, Fabry Z.** Traumatic brain injury increases TGF beta RII expression on endothelial cells. *Brain Res.* 2004; 1012: 52–9.
58. **Szmydynger-Chodobska J, Chung I, Koźniewska E, Tran B, Harrington FJ, Duncan JA, Chodobski A**. Increased expression of vasopressin v1a receptors after traumatic brain injury. *J Neurotrauma.* 2004; 21: 1090–102.
59. **Sköld MK, von Gertten C, Sandberg-Nordqvist AC, Mathiesen T, Holmin S**. VEGF and VEGF receptor expression after experimental brain contusion in rat. *J Neurotrauma.* 2005; 22: 353–67.
60. **Nag S, Eskandarian MR, Davis J, Eubanks JH**. Differential expression of vascular endothelial growth factor-A (VEGF-A) and VEGF-B after brain injury. *J Neuropathol Exp Neurol.* 2002; 61: 778–88.
61. **Suzuki R, Fukai N, Nagashijima G, Asai JI, Itokawa H, Nagi M, Suzuki T, Fujimoto T**. Very early expression of vascular endothelial growth factor in brain oedema tissue associated with brain contusion. *Acta Neurochir Suppl* 2003; 86: 277–9.
62. **Rafols D, Steiner J, Rafols JA, Petrov T**. Intracellular coexpression of endothelin-1 and inducible nitric oxide synthase underlies hypoperfusion after traumatic brain injury in rat. *Neurosci Lett.* 2004; 362: 154–7.

63. **Armstead WM, Cines DB, Al-Roof Higazi A**. Altered NO function contributes to impairment of uPA and tPA cerebrovasodialation after brain injury. *J Neurotrauma.* 2004; 21: 1204–11.
64. **Ahn MJ, Sherwood ER, Prough DS, Lin CY, DeWitt DS**. The effects of traumatic brain injury on cerebral blood flow and brain tissue nitric oxide levels and cytokine expression. *J Neurotrauma*. 2004; 21: 1431–42.
65. **Birukov KG, Stepanova OV, Nanaev AK, Shirinsky VP**. Expression of calponin in rabbit and human aortic smooth muscle cells. *Cell Tissue Res.* 1991; 266: 579–84.
66. **Menice CB, Hulvershorn J, Adam LP, Wang CA, Morgan KG**. Calponin and mitogen-activated protein kinase signaling in differentiated vascular smooth muscle. *J Biol Chem.* 1997; 272: 25157–61.
67. **Leinweber BD, Leavis PC, Grabarek Z, Wang CL, Morgan KG**. Extracellular regulated kinase (ERK) interaction with actin and the calponin homology (CH) domain of actin-binding proteins. *Biochem J.* 1999; 344: 117–23.
68. **Leinweber B, Parissenti AM, Gallant C, Gangopadhyay SS, Kirwan-Rhude A, Leavis PC, Morgan KG**. Regulation of protein kinase C by the cytoskeletal protein calponin. *J Biol Chem.* 2000; 275: 40329-36.
69. **Graceffa P, Adam LP, Morgan KG**. Strong interaction between caldesmon and calponin. *J Biol Chem.* 1996; 271: 30336–9.
70. **Kreipke CW, Schafer SM, Schafer PC, Rafols JA**. Inhibition of calponin phosphorylation by endothelin receptor-A antagonism ameliorates hypoperfusion and improves behavioral outcome following traumatic brain injury. *J Neurotrauma*. 2008; Abstracts of the 2008 National Neurotrauma Meeting.
71. **Sakurai T, Yanagisawa M, Masaki T**. Molecular characterization of endothelin receptors. *Trends in Pharacol Sci.* 1992; 13: 103–8.
72. **Touzani O, Galabraith S, Siegl P, McCulloch J**. Endothelin-B receptors in cerebral resistance arterioles and their functional significance after focal cerebral ischemia in cats. *J Cereb Blood Flow Metab.* 1997; 17: 1157–65.
73. **Jacobs A, Preston IR, Gomberg-Maitland M**. Endothelin receptor antagonism in pulmonary arterial hypertension—a role for selective ET(A) inhibition? *Curr Med Res Opin.* 2006; 22: 2567–74.
74. **Vierhapper H, Wagner O, Nowotny P, Walhäusl W**. Effect of endothelin-1 in man. *Circulation.* 1990; 81: 1415–8.
75. **Baldys-Waligórska A, Szybiński Z**. Plasma endothelin 1/2 levels in healthy blood donors as measured by RIA–a clinical application. *Endokrynol Pol.* 1992; 43: 7–12.
76. **Baldys-Waligórska A, Szybiński Z.** Plasma endothelin 1/2 levels in healthy blood donors and in hypertensive patients: clinical application. *Endocr Regul.* 1993; 27: 83–7.
77. **Epstein M**. Hepatorenal syndrome: emerging perspectives of pathophysiology and therapy. *J Am Soc Nephrol.* 1994; 4: 1735–53.
78. **Sakai S, Miyauchi T, Sakurai T, Yamaguchi I, Kobayashi M, Goto K, Sugishita Y**. Pulmonary hypertension caused by congestive heart failure is ameliorated by long-term application of an endothelin receptor antagonist. Increased expression of endothelin-1 messenger ribonucleic acid and endothelin-1-like immunoreactivity in the lung in congestive heart failure in rats. *J Am Coll Cardiol.* 1996; 28: 1580–8.
79. **Therkelsen K, Jensen KA, Freundlich M, Thorshauge H, BünemannL, Bøgeskov Nielsen L**. Endothelin-1 and cerebral blood flow: influence of hypoxia, hypercapnia and indomethacin on circulating endothelin levels in healthy volunteers. *Scand J Clin Lab Invest.* 1994; 54: 441–51.
80. **Lüscher TF, Wenzel RR**. Mechanisms of acute coronary syndrome. *Schweiz Rundsch Med Prax.* 1995; 84: 155–64.
81. **Benigni A, Remuzzi G**. Endothelin antagonists. *Lancet.* 1999; 353: 133–8.
82. **Armstead WM**. Superoxide generation links protein kinase C activation to impaired ATP-sensitive K+ channel function after brain injury. *Stroke.* 1999; 30: 153–9.
83. **Rafols, JA,** editor. Neurological research special edition: diffuse traumatic brain injury. Volume 29; Number 4. Cambridge, England: Maney Publishing, 2007.
84. **Petrov T, Steiner J, Braun B, Rafols JA**. Sources of endothelin-1 in hippocampus and cortex following traumatic brain injury. *Neuroscience.* 2002; 115: 275–83.
85. **Kallukuri S, Kreipke CW, Rossi N., Rafols JA, Petrov T**. 2007. Spatial alterations in endothelin receptor expression are temporally associated with the altered microcirculation after brain trauma. *Neurol Res.* 2007; 29: 362–8.
86. **Kreipke CW, Schafer PC, Rossi N, Rafols JA**. Differential effects of endothelin receptor-A and B antagonism on cerebral hypoperfusion following traumatic brain injury. *Neurological Research* 2008; epub.