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Hypertensive encephalopathy and the blood-brain barrier: is δPKC a gatekeeper?

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Commentary

Hypertensive encephalopathy is a life-threatening condition due to elevation of cerebral perfusion pressure beyond the limits of autoregulation. Breakdown of the blood-brain barrier (BBB) leads to cerebral edema and reduced blood flow. In this issue of the *JCI,* Mochly-Rosen and colleagues demonstrate a novel molecular strategy for preserving the BBB in a model of hypertension-induced encephalopathy (see the related article beginning on page 173). Using a rationally designed peptide inhibitor of δPKC, they stabilized the BBB and improved mortality in hypertensive rats. This study highlights the therapeutic potential of δPKC inhibitors in hypertensive encephalopathy and provides incentive to elucidate δPKC signaling pathways that mediate BBB dysfunction in other disease states.

ularly since the results of the study by Geser et al. suggest that malarial prophylaxis with chloroquine diminished the incidence of Burkitt lymphomas in Tanzania (2). In this regard, antioxidants, which have also been implicated in blocking autophagy, also prevent tumor formation in transgenic mice and xenograft models (16, 20). With chloroquine, this antimicrobial agent was initially thought to diminish lymphomagenesis by inhibiting malaria, but in fact its ability to inhibit autophagy through blocking lysosomal-mediated degradation as demonstrated by the current study by Maclean et al. appears to underpin its antitumorigenic activity in the clinical epidemiological setting (4). These instructive, insightful observations suggest that the use of chloroquine or its improved versions may prove to have a major impact in cancer prevention. It is notable, however, that the long-term effects of prolonged use of a potent autophagy inhibitor may have unexpected side effects, as our understanding of the homeostatic role of autophagy in normal tissues is rudimentary.

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Hypertensive encephalopathy and the blood-brain barrier: is δPKC a gatekeeper?

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Hypertensive encephalopathy is a life-threatening condition due to elevation of cerebral perfusion pressure beyond the limits of autoregulation. Breakdown of the blood-brain barrier (BBB) leads to cerebral edema and reduced blood flow. In this issue of the *JCI*, Mochly-Rosen and colleagues demonstrate a novel molecular strategy for preserving the BBB in a model of hypertension-induced encephalopathy (see the related article beginning on page 173). Using a rationally designed peptide inhibitor of δPKC, they stabilized the BBB and improved mortality in hypertensive rats. This study highlights the therapeutic potential of δPKC inhibitors in hypertensive encephalopathy and provides incentive to elucidate δPKC signaling pathways that mediate BBB dysfunction in other disease states.

Nonstandard abbreviations used: as-, analog sensitive; BBB, blood-brain barrier; RACK, receptor for activated c-kinase; TAT, transactivator of transcription; ZO-1, zonula occludens 1.

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The blood-brain barrier

The blood-brain barrier (BBB) is a gliovascular unit composed of capillary endothelial cells and pericytes surrounded by basal lamina, astrocytic end-feet, and perivascular interneurons (Figure 1A) (1). The tight junctions formed between endothelial cells act as a highly effective physical barrier to selectively permit the entry of required nutrients yet protect the central nervous system from pathogens and potentially harmful small molecules circulating in the blood. The tight junction consists of transmembrane proteins (claudin, occludin, and junction adhesion molecule) and cytoplasmic accessory proteins (Figure 1B) (2). Claudins form dimers and bind to claudins on adjacent endothelial cells to establish the primary gate of the tight junction. The main functions of occludin appear to be to regulate the electrical resistance across the barrier and decrease paracellular permeability. Several accessory cytoplasmic proteins associate with these transmembrane components. Zonula occludens proteins (ZO-1, ZO-2, and ZO-3) serve as recognition proteins for

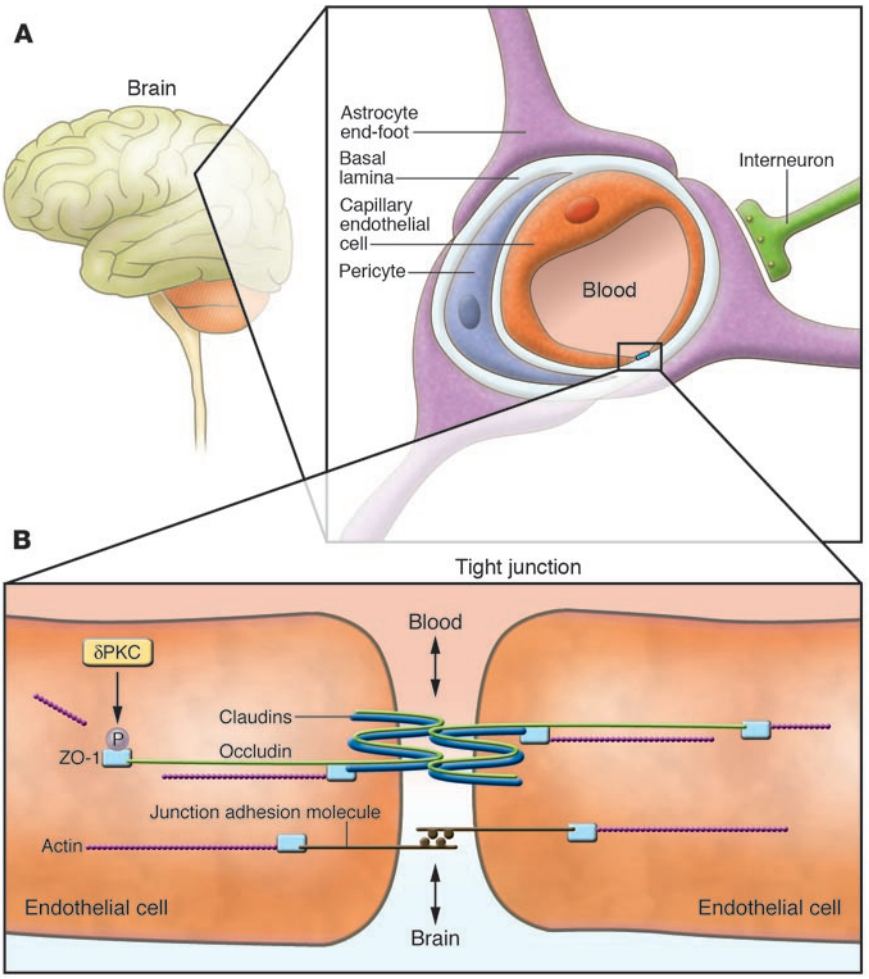

Figure 1
Structure of the BBB and tight junction. (**A**) The BBB is formed in the central nervous system by capillary endothelial cells and surrounding perivascular elements (basal lamina, pericyte, astrocyte end-foot, and interneurons). (**B**) The tight junction is established by the interaction between the transmembrane proteins (claudins, occludin, and junction adhesion molecule) on adjacent endothelial cells. The C terminal of these transmembrane proteins is linked to cytoskeletal actin through ZO-1. In response to pathological stimuli, δPKC may directly or indirectly increase phosphorylation of ZO-1, thus disrupting the association between ZO-1 and the actin cytoskeleton. The disorganization of proteins at the tight junction may result in the aberrant permeability of the BBB.

tight junction placement and as support for signal transduction proteins. ZO-1 connects transmembrane proteins of the tight junction to the actin cytoskeleton. This interaction is critical for the stability and function of the tight junction because dissociation of ZO-1 from the cytoskeletal complex is frequently associated with increased barrier permeability (3). ZO-1 is phosphorylated by both serine/threonine and tyrosine kinases, which can, in turn, regulate ZO-1 localization and BBB function. Thus, activation of PKC by phorbol esters promotes the migration of ZO-1 to the plasma membrane and increases the permeability of the BBB (4).

Impairment of tight junctions leads to increased barrier permeability and occurs in a number of neurological disorders, including stroke, trauma, brain tumor, epilepsy, multiple sclerosis, Alzheimer disease, and hypertension (5). In hypertensive encephalopathy, BBB dysfunction is widespread and severe and produces cerebral edema. The main goal of therapy is to reduce systolic blood pressure with drugs such as nitroprusside, adrenergic receptor blockers, and angiotensin-converting enzyme inhibitors or angiotensin receptor blockers to reduce levels of rennin and angiotensin II produced in response to renal ischemia (6). However, there are no treatments that specifically target the disrupted BBB.

δPKC peptide inhibitor: TAT–δV1-1

The PKC family contains nine structurally related but functionally distinct serine/threonine kinases that transduce signals from lipid second messengers. The δPKC isozyme is expressed in several tissues and has been implicated in growth inhibition, cell death, and ischemic injury (7). Over a decade ago, D. Mochly-Rosen proposed a mechanism of PKC activation that involves the binding of an activated PKC isozyme to an isozyme-specific anchoring protein called a receptor for activated c-kinase (RACK) (8). Based on this model, her laboratory has designed selective peptides to inhibit the binding of PKCs to their anchors and thereby inhibit PKC isozyme function. To allow these peptides to be delivered into cells, they can be conjugated with positively charged sequences, such as the HIV transactivator of transcription (TAT) protein-transduction domain (9).

In the paper by Mochly-Rosen et al. in this issue of the *JCI*, the authors report on their induction of hypertensive encephalopathy in Dahl salt-sensitive rats by feeding them a high-salt diet for 10 weeks beginning at 6 weeks of age (10). The hypertension disrupted BBB integrity, as demonstrated by increased BBB permeability and swelling of astrocytes and endothelial cells, and increased mortality, with 60% of salt-fed rats dying by the age of 15 weeks. The disorganization of tight junctions in these animals correlated with several biochemical changes, including reduced levels of

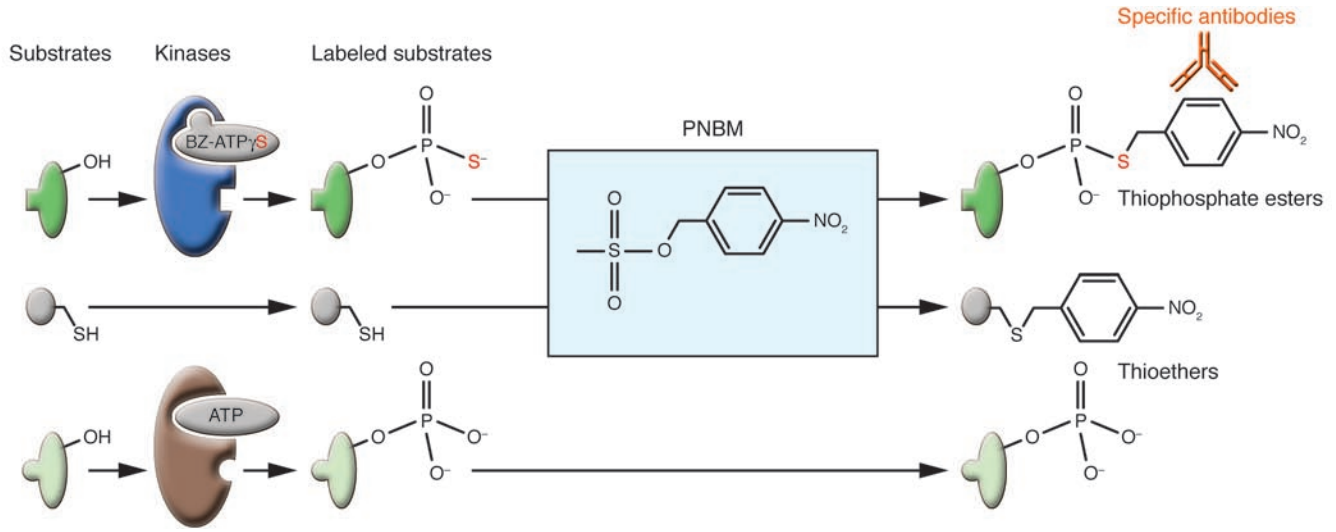

Figure 2
The chemical genetics approach. An as-kinase with a hole (in blue) recognizes the *N*6-(benzyl)-ATP-γS (BZ-ATPγS) with a bump and phosphorylates its unique substrates. Only the as-kinase, not wild-type kinases (in brown), can use N6-substituted ATP analogs (BZ-ATPγS) as phosphate donors. In the second step, alkylation with *p*-nitrobenzylmesylate (PNBM) forms thiophosphate esters and thioethers. Only esterified as-kinase substrates are recognized by specific antibodies discriminating thiophosphate esters from thioethers.

ZO-1 and occludin associated with the cytoskeleton, enhanced ZO-1 phosphorylation, and importantly, increased membrane translocation of δPKC (Figure 8 in ref. 9). Sustained administration of the selective δPKC inhibitor peptide TAT–δV1-1 (11) beginning at week 11 attenuated phosphorylation of ZO-1, increased the association of ZO-1 and occludin with the actin cytoskeleton, reduced leakage at tight junctions, and improved the survival rate to greater than 90% at week 15. The beneficial effect of TAT–δV1-1 was likely due to prevention of BBB breakdown, since treatment did not reduce the blood pressure or alter cardiac function. These results are quite striking, though a limitation to this study is that treatment was begun before the onset of encephalopathy. For practical therapeutic use, it will also be important in the future to determine whether the δPKC peptide inhibitor can reverse BBB breakdown after signs of encephalopathy appear.

The TAT–δV1-1 peptide has been used successfully in several other experimental disease models, including cardiac ischemia and reperfusion (11–14), cerebral ischemia and reperfusion (15, 16), and chronic hypertension (10, 16). The peptide reduces infarct size in the heart (11–14) and brain (15, 16), possibly by attenuating apoptosis. The TAT–δV1-1 peptide also increases blood flow and reduces microvascular pathology after cerebral ischemia and reperfusion (16).

δPKC peptide inhibitors are attractive as therapeutics since they are rationally designed to be specific for the δPKC isozyme. Also, the peptides are water soluble, stable in solution, and easy to produce in large quantity. However, in general, the pharmacological properties of peptides are limited by short life due to proteolytic cleavage, uneven tissue distribution, and inadequate subcellular targeting. Although the effects of δPKC peptide inhibitors may provide useful information about the normal function of δPKC, the exact mechanism of action for TAT–δV1-1 could be more complicated than originally anticipated (7). For example, activation of δPKC requires phosphorylation by Src tyrosine kinases and serine/threonine kinases. Binding of the activated kinase to its RACK is thought to target the kinase to its specific substrates. It is theoretically possible that TAT–δV1-1 interference with δPKC-RACK binding may still allow δPKC to be active but at an unusual subcellular location, leading to phosphorylation of atypical substrates. Furthermore, in response to apoptotic stimuli, a constitutively active C terminal catalytic fragment of δPKC is cleaved from the N terminal domain responsible for binding to RACK and TAT–δV1-1. The cleaved δPKC fragment appears to be essential for mediating apoptosis and is increased after transient cerebral ischemia (17). How TAT–δV1-1 could interfere with the production or function of the δPKC catalytic fragment is not clear. Therefore, although the beneficial effects of TAT–δV1-1 suggest that δPKC serves a deleterious role under certain pathological conditions, it will be important to confirm such findings using additional methods such as genetic approaches to gain a clear understanding of the role of δPKC in disease.

Molecular mechanisms of disease progression: identification of δPKC substrates

The beneficial effects of TAT–δV1-1 in models of ischemia and hypertension have increased interest in understanding the molecular and cellular actions of δPKC in disease progression. A detailed understanding of δPKC signaling pathways will ultimately require identification of δPKC substrates. Traditionally, δPKC substrates have been identified using pharmacological strategies with kinase activators or inhibitors, but lack of specificity of these modulators limits interpretation of these studies. However, recent work by Shokat and colleagues (18) has led to a novel chemical genetics approach to identifying immediate substrates of kinases. The approach targets the structurally conserved ATP-binding pocket within all kinases to generate mutant alleles that can utilize specific ATP analogs in addition to ATP. The mutation creates a "hole" by replacing a conserved bulky amino acid (e.g., valine or methionine) with a small residue

(alanine or glycine) in the ATP-binding pocket (Figure 2). A series of ATP analogs (benzyl-ATP) possessing a side group to fit into the engineered hole have been generated. Only the analog-sensitive (as-) mutant, not the wild-type kinase, can efficiently use these ATP analogs as phosphate donors. Therefore, only unique substrates of the as-kinase are labeled by the ATP analogs. A modification of this approach that generates a thiophosphate ester at the phosphorylation site for detection with an antibody has been successfully used to identify direct substrates of as-kinases (18). In our view, an as-δPKC mutant will be an important tool for identifying specific δPKC substrates phosphorylated in response to ischemia and reperfusion, hypertension, and microvascular dysfunction. The δPKC signaling pathways revealed during disease progression should facilitate the development of additional δPKC-based therapeutic strategies.

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A skewed view of X chromosome inactivation

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X chromosome inactivation involves a random choice to silence either X chromosome early in mammalian female development. Once silenced the inactive X is stably inherited through subsequent somatic cell divisions, and thus, females are generally mosaics, having a mixture of cells with one or the other parental X active. While in most females the number of cells with either X being active is roughly equal, skewing of X chromosome inactivation is observed in a percentage of women. In this issue of the *JCI*, Bolduc and colleagues address whether skewing of X chromosome inactivation in humans is influenced by an X-linked locus that can alter this initial random inactivation (see the related article beginning on page 333). Their data indicate that most of the skewing observed in humans results from secondary events rather than being due to an inherited tendency to inactivate a particular X chromosome.

Nonstandard abbreviations used: *Xce*, X chromosome controlling element; XCI, X chromosome inactivation; *Xist*, X (inactive)–specific transcript.

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What is skewed X chromosome inactivation?

X chromosome inactivation (XCI) — a process originally hypothesized by Lyon in 1961 (1) and by which one of the two copies of the X chromosome present in females is inactivated — achieves dosage equivalency for X-linked genes between XY males and XX females. A critical tenet of this hypothesis was that the initial choice of which X (maternal or paternal) to inactivate was random but then this choice was stably inherited. An individual cell's decision to inactivate either the paternal or maternal X is made early in development, at approximately the time of implantation (2). A deviation from equal (50%) inactivation of each parental allele is known as skewing, with common criteria for "skewed" inactivation being arbitrarily defined as the observation of inactivation of the same allele in 75% or 80% of cells (and very skewed inactivation resulting in 90% or 95% of cells with the same allele inactive).

A solid understanding of the causes of skewed XCI is needed because skewing is often used as a tool in the clinical setting