Treating seizures and epilepsy with anticoagulants?

Nicola Maggio1,2*, Ian Blatt2,3, Andreas Vlachos4, David Tanne2,3, Joab Chapman2,3 and Menahem Segal5

1 Tel Aviv Medical Leadership Program; The Chaim Sheba Medical Center; Tel Hashomer, Israel
2 Department of Neurology, The J. Sagol Neuroscience Center, The Chaim Sheba Medical Center; Tel Hashomer, Israel
3 Department of Neurology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
4 Institute of Clinical Neuroanatomy, Neuroscience Center, Goethe-University Frankfurt, Frankfurt/Main, Germany
5 Department of Neurobiology, The Weizmann Institute of Science, Rehovot, Israel

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Thrombin is a serine protease playing an essential role in the blood coagulation cascade. Recent work, however, has identified a novel role for thrombin-mediated signaling pathways in the central nervous system. Binding of thrombin to protease-activated receptors (PARs) in the brain appears to have multiple actions affecting both health and disease. Specifically, thrombin has been shown to lead to the onset of seizures via PAR-1 activation. In this perspective article, we review the putative mechanisms by which thrombin causes seizures and epilepsy. We propose a potential role of PAR-1 antagonists and novel thrombin inhibitors as new, possible antiepileptic drugs.

THROMBIN SIGNALING IN THE BRAIN

Thrombin is a serine protease, which plays an essential role in the blood coagulation cascade (Siller-Matula et al., 2011). Upon its formation following the enzymatic cleavage of prothrombin by activated Factor X, thrombin regulates a cascade of proteolytic events ultimately leading to the formation of blood clots (Lippi et al., 2012). Lately, however, novel signaling cascades mediated by thrombin have been discovered (Siller-Matula et al., 2011). Specifically, through the activation of the protease-activated receptors (PARs), thrombin seems to directly affect the activity of multiple cell types and regulate a variety of biological functions, such as inflammation, leukocyte migration, cellular proliferation, vascular permeability and tone, edema formation, and other processes related to tissue repair (Coughlin, 2000, 2001; Sambrano et al., 2001; Chen and Duling, 2009; Schuepbach et al., 2009; Spiel et al., 2011).

Protease-activated receptors belong to a unique family of G protein-coupled receptors (Luo et al., 2007). Their activation is initiated by an irreversible site-specific proteolytic cleavage in the N-terminal extracellular region. The uncovering N-terminal region then acts as a tethered ligand which activates the receptor (Gingrich and Traynelis, 2000). PARs are expressed in the brain and while PAR-2 represents a class of trypsin/tryptase-activated receptors, PAR-1, PAR-3, and PAR-4 are most effectively activated by thrombin (Gingrich and Traynelis, 2000). In the brain, PAR-1 has been detected in both neurons and astrocytes, with the latter demonstrating stronger immunoreactivity in human brain tissue (Junge et al., 2004). High levels of PAR-1 are detected in the hippocampus, cortex, and striatum of humans (Junge et al., 2004).

While the molecular pathways activated by PAR-1 in neurons are yet under investigation, in the brain PAR-1 activation has been shown to modulate synaptic transmission and plasticity through the enhancement of N-methyl-D-aspartate (NMDAR) receptor (NMDAR) currents (Gingrich et al., 2000; Lee et al., 2007; Maggio et al., 2008). In addition, PAR-1 knockout animals present profound deficits in hippocampal–dependent learning and memory processes (Almonte et al., 2007, 2013). Altogether, it seems that PAR-1 plays a critical role in memory formation and synaptic plasticity.

Interestingly, a variety of pathological conditions have been associated with changes in the expression of PAR-1 in the brain. In Parkinson’s disease, a significant increase in the number of astrocytes expressing PAR-1 has been reported in the substantia nigra pars compacta (Shih et al., 2006). In addition, upregulation of PAR-1 in astrocytes has been observed in HIV encephalitis, (Bovens et al., 2003) indicating that this receptor might be implicated in the pathogenesis of neuroinflammation. This idea is supported by the evidence of elevated levels of thrombin in an experimental model of multiple sclerosis (Belin et al., 2005) as well as in other inflammatory brain diseases (Chapman, 2006). Stimulation of PAR-1 by thrombin causes proliferation of glia and potentially produces reactive gliosis, infiltration of inflammatory cells, and angiogenesis (Striggow et al., 2001). Finally, expression of PAR-1 is increased in experimental models of Alzheimer’s disease (Pompili et al., 2004) and brain ischemia (Striggow et al., 2001).

THROMBIN CAUSES SEIZURES AND EPILEPSY THROUGH PAR-1 ACTIVATION

Serine proteases are normally expressed in the brain at very low level (Luo et al., 2007). Nevertheless, their concentration can increase abnormally following the breakdown of the blood–brain barrier (BBB). Under this scenario, a large, non-selective increase in the permeability of brain capillaries and tight junctions takes place, allowing the entry of high molecular weight proteins (Bullabh et al., 2004) and blood components into the cerebral tissue. This event can occur under several neurological...
facilitated the response to the lower concentration of K
spontaneous seizure-like activity in the slice. Strikingly, thrombin
upon PAR-1 activation induces membrane and synaptic changes
studies clearly indicate a proepileptic effect of thrombin which
15 mM. Similarly, 500
amplitude of mIPSCs (Maggio et al., 2012). T aken together, these
quency and amplitude of mEPSCs while reducing frequency and
transmission in hippocampal CA3 neurons by enhancing both fre-
nalyzing a BBB breakdown in the slice (Chen and Swanson, 2003; Beart
and O’Shea, 2007), we exposed neurons to thrombin in presence
et al., 2008). In hippocampal slices, thrombin at a concentration
induced seizures are mediated by activation of PAR-1 (Maggio
predicts serum and other proteases can freely dif-
ner BBB breakdown due to uncontrolled seizures may lead
ence of elevated [K+]o or low levels of glutamate. In normal slices,
addition of 4 mM K+ did not produce any noticeable sponta-
neous seizures, which were clearly seen when [K+]o were raised by
5 mM. Similarly, 500 μM but not 100 μM glutamate produced
spontaneous seizure-like activity in the slice. Strikingly, thrombin
facilitated the response to the lower concentration of K+ (4 mM) and
and glutamate (100 μM) to produce seizure-like activity. This
activity was mediated by PAR-1 activation, since it was mimicked
by a peptide agonist of the receptor and blocked by its antagonist
INap
nformation is currently available on the possible role of thrombin
hyperexcitability (Heinemann et al., 2002; Heinemann, 2004), no
of hemorrhage, BBB breakdown may activate the coagulation cas-
take place of thrombin and PAR-1 activation in this situation.
PAR-1 ANTAGONISTS AND THROMBIN INHIBITORS AS NEW
ANTIEPILEPTIC DRUGS?
Seizures and epilepsy are commonly observed in conjunction with
stroke, TBI, and central nervous system infections, all conditions
known to result in compromised BBB function (Tomsikins et al.,
2001; Ballabh et al., 2004). Regional patterns of BBB breakdown
have been described during epileptiform seizures induced in ani-
mal models by various convulsive agents (Nitsch and Klatzo, 1983).
Following BBB breakdown, seizures result from the exposure of the
brain to serum components such as thrombin due to the increased
permeability of the BBB (Kelly, 2008). In fact, even in the absence
of hemorrhage, BBB breakdown may activate the coagulation cas-
cade leading to intracerebral generation of thrombin (Stein et al.,
2002; Chodobski et al., 2011; Pissapati et al., 2012). In this setting an
enduring BBB breakdown due to uncontrollable seizures may lead to
a continuous leak of thrombin into the brain, which in turn
sustains the epileptic process (Figure 2).
If thrombin indeed is the major reason for seizures in this condition, it is tempting to speculate that PAR-1 antagonists and/or thrombin inhibitors could act as potential antiepileptic drugs. PAR-1 antagonists are a class of drugs currently tested in the context of cardiovascular diseases (Abin et al., 2003; Landis, 2007; Lee and Hamilton, 2012). They are non-peptide small molecular compounds which differ in their effectiveness to inhibit PAR-1 (Abin et al., 2003). They have both anticoagulant (Wielders et al., 2007) and antiaggregant (Lee and Hamilton, 2012) properties. However, unlike a direct thrombin inhibitor, they are thought to have minimal bleeding side-effects due to the inability of blocking the enzymatic action of thrombin in the coagulation cascade. We (Maggio et al., 2008, 2012) and others (Iusea et al., 2012) have indeed shown that PAR-1 antagonists block the proepileptogenic effects of thrombin in vitro, however, no data currently exist on the role of PAR-1 antagonists as antiepileptic drugs in animal models of epilepsy following BBB breakdown. Furthermore, it is not known whether thrombin and PAR-1 levels are increased in the brains of experimental animals undergoing chronic epilepsy. In this context, our preliminary data based on L127+ pilocarpine treated animals do show that this might indeed be the case. Interpretation of clinical data might provide important insights as well. Cardiac surgery has been associated with a high rate of seizures in the post-operative setting (Goldstone et al., 2011a,b; Hervey-Jumper et al., 2011). Thus, are patients treated with PAR-1 antagonists as prevention to reduce the prothrombotic risk occurring in cardiothoracic surgery (Landis, 2007) going to show less seizures in their post-operative outcomes?

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