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Research Report

Acute blood–brain barrier permeabilization in rats after systemic *Phoneutria nigriventer* venom

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ABSTRACT

A highly controlled transport of substances at the interface between blood and brain characterizes the blood–brain barrier (BBB), fundamental for maintenance of the homeostasis of the cerebral milieu. In this study, we investigated the time course (15 min, 1, 2, and 5 h) of BBB opening induced by intravenous (i.v.) injection of *Phoneutria nigriventer* spider venom (PNV) using quantitative and morphological approaches on cerebellum and hippocampus vessels for assessment of BBB permeability. The results showed vasogenic edema and tracer extravasation faster and severalfold higher in hippocampus than in cerebellum. Reactive astrocytes with swollen perivascular end-feet processes were found only in cerebellum. An immediate and total degradation of laminin in capillaries occurred resulting in the disappearance of the basement membrane. In medium-sized vessels, this effect was less prominent. The changes were transient, with cerebellum in general presenting a faster recovery. However, at 5 h laminin was overexpressed, principally in hippocampus. The rapid and abrupt shift of laminin expression in capillaries (at 15 min) coincided with the immediate and severe signs of intoxication shown by the animals, but not with the peak of leakage of vessels and vasogenic edema, which occurred later (1–2 h). The findings suggest a complex regulatory mechanism, since the extension of BBB impairment caused by PNV depends on the region of the SNC, and on the vessels types. It is suggested that the components of the BBB (gliovascular unit) have a critical role in these differences. *P. nigriventer* venom can be a useful tool to explore the mechanisms of BBB.

1. Introduction

Blood–brain barrier (BBB) is a diffusion barrier essential for the normal functioning of the CNS. Endothelial walls from brain blood vessels differ from peripheral ones by being continuous, fenestration-free and possessing an extensive highly resistant tight junction (TJ) occluding the intercellular pathway as a route for transit of molecules. In addition, a very selective transcellular transport largely controlled by membrane receptors, carriers and metabolic barriers acting-enzymes expressed in the

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endothelial plasma membrane limits the entrance of substances into brain. Nutrients such as glucose and amino acids enter the brain via cell transporters. Membrane receptor-mediated endocytosis controls the uptake of larger molecules such as insulin, leptin, and iron transferring (Zhang and Pardridge, 2001). In contrast, small lipophilic substances, such as O_2 and CO_2, diffuse freely across plasma membranes to tissue according concentration gradient (Grieb et al., 1985). In addition, outer coverings represented by capillary basement membrane (BM), perivascular astroglial end-feet, pericytes, perivascular microglia and neurons are also important part of the BBB complex (Ramsauer et al., 2002). These cells contribute to the synthesis of proteins of the extracellular matrix which in turn influences behavior and differentiation of the cells. The BBB complex has been referred to as neurovascular unit (Abbott, 2002).

The BBB can be altered in several brain diseases. A variety of pathological conditions may either weaken the barrier efficiency or contribute to the development of the disease processes (Neuwelt, 2004). Examples of BBB dysfunction were demonstrated in Alzheimer's disease (Zlokovic, 2004), neuroinflammatory diseases, such as multiple sclerosis (Plumb et al., 2002; Werring et al., 2000), HIV encephalitis (Toborek et al., 2003), and brain tumors (Schlageter et al., 1999). Natural toxins contained in the venom of caterpillars of the saturniid moth *Lonomia obliqua* (Silva et al., 2004), and in the venom of the armed spider *Phoneutria nigriventer* (Le Sueur et al., 2003, 2004) are among a large list of xenobiotics which can disturb the tight-controlled bi-directional transport blood–nervous tissue.

P. nigriventer venom has long been investigated by Brazilian researchers because the majority of accidents caused by venomous spiders, in São Paulo State, Brazil, were due to this species (Bucaretchi et al., 2000). The victims of bite complain of intense local pain (92.1%) and edema (33.1%). The accidents are classified as mild (89.8%), moderate (8.5%) and severe (0.5%) (Bucaretchi et al., 2000). Severe accidents occur mainly in children and elderly people who may develop acute pulmonary edema and eventually death. Experimental animals injected systemically with the whole venom show excitatory signs including salivation, flaccid followed by spastic paralysis of hindlimbs, tremors, spasms and tonic convulsions, which are indicate that venom toxins affect autonomic (Antunes et al., 1993; Gomez et al., 1995), peripheral (Fontana and Vital-Brazil, 1985; Cruz-Höfling et al., 1985; Love and Cruz-Höfling, 1986; Love et al., 1986) and central nervous system (Le Sueur et al., 2003, 2004; Zanchet et al., 2004).

Experimental studies have shown that the venom affects the excitability of nerve fibers by producing conformational changes in Na^+ channels that are highly concentrated at nodes of Ranvier, to allow inward current during depolarization to sustain saltatory conduction. Electrophysiological studies in sciatic nerve–soleus preparations of mice and on dorsal and ventral nerve roots of rats indicate that the venom delays inactivation of sodium channels (Baker et al., 1985; Cruz-Höfling et al., 1985). The electrophysiological disturbances were associated with striking changes in the appearance of myelinated fibers, which were prevented if pretreatment with tetrodotoxin, a sodium channel blocker, was applied (Cruz-Höfling et al., 1985; Love et al., 1986; Love and Cruz-Höfling, 1986). More recently, it was shown that the venom of *P. nigriventer* increases the microtubule-mediated transendothelial transport at the brain microvasculature in rats (Le Sueur et al., 2004). The paracellular transport at the periods then observed (1 and 9 days after envenoming) seemed unaffected, although the interendothelial cleft was seen filled with an intravenous (i.v.)-injected extracellular tracer (Le Sueur et al., 2003).

In this work, we investigate the permeation of the BBB of cerebellum and hippocampus to the extracellular tracer lanthanum nitrate in rats provoked by the intravenous injection of PNV at the first stages of the systemic envenoming (15 min, 1, 2 and 5 h). In addition, the integrity of the basement membrane (BM) of the microvessels was evaluated by the expression of its major protein, laminin through immunofluorescence. This study is part of a series aimed at characterizing the time course of the alterations involving impairment of the blood–brain barrier caused by the *P. nigriventer* spider venom.

2. Results

To investigate the time-course of BBB breakdown and the participation of basement membrane on it caused by PNV in hippocampus and cerebellum at acute stages of envenoming, 32 rats were divided into four treated groups and four control groups ($n=4$ rats/period after venom or saline i.v. injection, respectively).

Clinically, the animals presented immediate signs of intoxication after PNV injection, such as hyperemia, tremors, eye paleness, salivation and motionless. Whereas hyperemia and eye paleness disappeared almost as quickly as they appeared, the other signs persisted and some minutes after the animals also showed flaccidity followed by spastic paralysis of hindlimbs. These signs persisted until their sacrifice. Of the four rats used in each period of envenoming, one or two showed temporary tonic convulsion. Some animals that died by cardiac–respiratory arrest were discarded and have to be replaced. Necropsy of these animals revealed lung edema. Saline-injected animals were clinically normal in appearance.

LM histological observations of 1-μm-thick TB-stained sections showed that the neural parenchyma looked normal, and so neuronal and glial cells, both in saline- and PNV-injected rats. However, whereas all segments of the microvasculature of the cerebellum and hippocampus showed no abnormality in control groups, blood vessels with wide spaces around, characterizing perivascular edema were present in both regions of PNV-treated rats (Fig. 1).

Quantification of the affected vessels aimed at evaluating the extension of barrier impairment permitted estimation of the time-course of the alterations from 15 min to 5 h post-injection (p.i.). It also permitted comparison between hippocampus and cerebellum. At the hippocampus, a marked 7.4-fold increase of vessels with vasogenic edema was seen 1 h after PNV injection in comparison to controls ($P<0.01$). In the succeeding periods examined (2 and 5 h), there was reduction in number of hippocampal vessels with perivascular edema (Fig. 2A). In cerebellum, the perivascular edema was not so marked as for hippocampus, nor was so high as the percentage of affected vessels. Probably, as a consequence of this, the highest number of affected vessels in cerebellum was achieved later than in hippocampus, i.e., it occurred at 2 h p.i., after which there was reduction, being all changes not significant in