RESEARCH HIGHLIGHT

Adrenomedullin and Glucocorticoids interaction at the glial/endothelial interface: two sides of the same regulatory coin?

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Adrenomedullin is a vasodilatatory peptide, important during the inflammation process and also able to regulate blood-brain barrier function. Adrenomedullin and its receptors have been shown to be glucocorticoid-dependent in many cell types, including primary T cells and the major cellular components of the blood-brain barrier. Considering that the immunosuppressant, glucocorticoids are also well-known to regulate inflammation and blood-brain barrier properties, in this research highlight we review the evidence for glucocorticoid modulation of adrenomedullin secretion and adrenomedullin receptor expression at the glial/endothelial interface during physiological and inflammatory conditions. This view would offer a platform for consideration of new therapeutic options aiming to restore or maintain the blood brain barrier.

Keywords: adrenomedullin, glucocorticoids, blood-brain barrier, RAMPs

Abbreviations: Adrenomedullin, AM; blood-brain barrier, BBB; cerebral endothelial cells, CEC; calcitonin receptor-like receptor, CLR; glucocorticoids, GC; receptor-activity modifying protein, RAMP.

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Adrenomedullin (AM), a recently-discovered vasodilatory peptide, circulates in the plasma and is produced by a variety of cells [1]. AM effects are mediated through a G-protein coupled receptor, calcitonin receptor-like receptor (CLR) associated with receptor-activity modifying protein (RAMP) 2 or 3 [2]. AM-AM receptor binding can increase intracellular cAMP levels [3, 4] and/or cause calcium mobilization [4, 5]. Importantly, considering that AM is highly produced by cerebral endothelial cells (CEC), this peptide concentration is greater in the brain than in the peripheral vasculature [6]. Interestingly, astrocytes seem to have a key role in blood-brain barrier (BBB) AM production, as when endothelial cells are co-cultured with astroglia or astrocyte-conditioned media, they increase their AM production [6] suggesting that AM up-regulation may depend on astrocyte soluble

factors. Indeed, AM has also been shown to be important in the regulation of BBB properties such as increased trans-endothelial electrical resistance and decreased endothelial permeability [7]. Exogenous application of AM does activate adenylate cyclase, therefore increase cAMP levels in CEC [7]. Since cAMP is a key second messenger in maintaining BBB functions [8], this suggests a direct role for AM as an autocrine and/or paracrine regulator of BBB. Furthermore, a tightening of intercellular junctions and changes in tight junction principal protein expression (i.e. occludin, claudin and ZO) were observed in response to treatment with AM [7], and is supported by further studies demonstrating an increase in claudin-5 after AM exposure in a dose-dependent manner [9]. Finally, AM can activate P-glycoprotein indicating a role for this peptide in the regulation of specific BBB efflux transporter systems [7].

AM and its receptors have been shown to be glucocorticoid (GC)-dependent in cultured rat ventricular myocytes [10], human vascular endothelial cells [11] and T98G human glioblastoma cells [12]. Both AM secretion and mRNA levels were elevated in a dose-dependent and time-dependent manner [10, 11] following dexamethasone treatment. Furthermore, previous investigations have reported that GCs could augment AM concentration and expression dose-dependently also *in vivo* [13].Interestingly, GCs are not alone in that, other hormone group could regulate AM secretion and receptors expression [14,15].

In opposition to its role in maintaining BBB function, AM is also an important mediator of inflammation and its concentration seems to increase under inflammatory conditions [16-18]. Indeed, endothelial cells and vascular smooth muscle cells display augmented AM production following exposure to IL-1, TNF-α and LPS [19]. Similar data were obtained with astrocytes: when cells were treated with TNF-α, IL-1 and INF-γ, their production of AM was significantly increased [20]. *In vivo* experiments reported that the peptide could reduce inflammation levels in experimental arthritis where it was able to ameliorate not only the incidence but also severity of the disease [21] . These anti-inflammatory properties of AM were also observed in two different models of sepsis where treatments with AM could significantly diminish the levels of immuno-inflammatory mediators [22]. Interestingly, AM was observed concomitant with the development of neuroinflammatory lesions in a rat paradigm of multiple sclerosis [23]. Considering that GCs are the best-known immunosuppressant, exerting an important role during the inflammatory process [24], our group investigated GC-dependency for AM and its receptor in primary T cells before and after stimulation [25]. In these cells AM receptor presentation is GC-sensitive, which was highly dependent on the stimulation state. As a result, the interaction of these two molecules seems important during inflammation, as well as under physiological conditions.

It is well-known that the BBB is hormonally regulated as its features are also influenced by circulating peripheral hormones [26]. Indeed, GCs were able to restore BBB properties when disrupted as well as maintain its physiological conditions [24, 27, 28]. Since both endothelial cells and astrocytes are fundamental components of the functional BBB [29], and as both GCs and AM have been shown to play a relevant role in regulating BBB characteristics [7, 30], our further studies aimed to identify whether these two molecules could interact in these cell types [31]. GCs were able to alter RAMP2 expression in endothelial-like cells, hence regulate AM-sensitivity, while they mainly influenced AM secretion in astrocytes. Our results have implications not only for the BBB characteristics under physiological conditions but also but also during inflammatory responses. More specifically, both AM [7] and GCs [30] have been closely related with acute pathological states, beside their ability to restore the BBB characteristics during neuro-inflammation. Moreover, AM was able to protect the BBB against oxidative stress, as during hypoxia addition of AM attenuated CEC permeability and preserved cell viability in an *in vitro* BBB model [32].

In conclusion, our studies have shown that the ability of GCs to influence AM secretion and AM sensitivity intimately depended on the cell type. This is in line with our previous observations in T cells, where GCs could alter AM receptor expression depending on the stimulation state. Taken together, these data strongly suggest that GC's modulation of AM secretion and AM sensitivity is a key feature for steroids during inflammation as well as under physiological conditions. As a result, AM has the potential for consideration as a novel therapeutic target in pathologies where there is a neuroinflammatory component.

Conflicting interests

The authors have declared that no competing interests exist.

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