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Review article

Calcium signal communication between glial and vascular brain cells

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Abstract

The brain is composed of neurons that communicate electrical signals over neurites and chemical signals across synapses, and non-neuronal cells like glial and vascular cells that communicate calcium signals among each other. Calcium ions have an important signaling function in the cytoplasm that depends on their amplitude, time course of change and subcellular localisation. Work over the last decade has added an additional dimension to this rich repertoire by including the possibility that calcium signals can be communicated between cells. In astrocytes and endothelial cells, connexins appear to be at the crossroad of calcium signal communication pathways, because they are the building blocks of gap junction channels that functionally connect cells, and because they can arrange as hemichannels that act as a conduit for cellular ATP release, thus initiating paracrine purinergic signaling. The two pathways appear to be operational in astrocytes and endothelial cells and we review in this paper possible functions of astrocyte-to-blood vessel calcium signaling at the level of arterioles where blood flow is controlled, at the level of capillaries where the blood-brain barrier is located and at the level of blood immune cells.

Key words : Intercellular calcium signaling ; glia ; brain vessels ; neurovascular ; gliovascular ; neurobarrier coupling ; gap junctions ; connexin hemichannels.

Introduction

The proper functioning of a complex multicellular network like the brain depends on an elaborate cell-to-cell communication network. Recently, considerable progress has been made to unravel this network. Neuronal electrochemical impulses are no longer seen as the only mechanism underlying information processing in the nervous system and there is now growing evidence that electrically non-excitable cell types such as glial cells and brain vessel cells communicate with each other and interact with the neurons through changes in intracellular calcium concentration (Dani *et al.*, 1992 ; Charles, 1994 ; Nedergaard, 1994 ; Kang *et al.*, 1998 ; Braet *et al.*, 2001). Calcium ions play a pivotal role in non-excitable brain cells as an important *intra*cellular messenger but also as an *inter*cellular signal because the calcium message can be further communicated to surrounding cells. Because there is a certain degree of homology between action potentials and calcium transients these signals provide non-excitable cells with a form of excitability that is called 'calcium excitability' (Verkhratsky *et al.*, 2002). Intercellular calcium signals are transient changes in cytoplasmic free calcium that are, analogous to action potentials in neurons, characterized by an initiating trigger followed by a mechanism that propagates the calcium signal to neighboring cells (Berridge *et al.*, 2000). Just like electrical signals, calcium signals can be communicated between cells by gap junctions, i.e. channels that connect the intracellular space of two adjacent cells, and by paracrine communication involving the release of an extracellular messenger. Calcium signals are thus organized at multiple levels : they are characterized by their amplitude, their temporal behaviour (transient, steady or oscillatory changes), their subcellular localization (e.g. subplasmalemmal versus global cytoplasmic changes) and, in addition, their pattern of spread towards neighboring cells. This provides the signal with the necessary complexity to encode a large range of information converging onto this single ion, to be further decoded and rediverged to specific cellular responses through the action of various intracellular calcium-binding proteins.

Communicated calcium signals in cell cultures and brain slices

The communication of calcium signals in the brain is a feature of almost all cell types resident in the brain, including neurons, glial cells and vascular cells (Dani *et al.*, 1992 ; Charles, 1994 ; Charles *et al.*, 1996 ; Leybaert *et al.*, 1998 ; Braet *et al.*, 2001). Most evidence is however available for astrocytes, which are stellate glial cells that are in contact with both neurons and vascular cells. There are indications that neuronal synaptic activity is able to trigger calcium signals in surrounding astrocytes and various neurotransmitters such as glutamate, acetylcholine, GABA, ATP or NO have been

implicated as the triggering transmitter substance. Astrocytes are, on their turn, able to release certain neurotransmitters themselves such as glutamate and ATP, which trigger calcium responses in surrounding neurons or modulate signal transmission across synapses. The topic of glial-neuronal signaling has been the subject of numerous recent reviews and will not be dealt with in the present paper (Araque *et al.*, 1999 ; Bezzi and Volterra, 2001 ; Haydon, 2001 ; Fields and Stevens-Graham, 2002 ; Perea and Araque, 2002 ; Zonta and Carmignoto, 2002).

Astrocyte-blood vessel calcium signals and coupling of neuronal activity to vessel function

Astrocytes are intermediately positioned between the neurons and the brain vessels and therefore occupy a key signaling position between these two important players (Attwell, 1994). Astrocytes are in contact with smooth muscle cells of arteriolar brain vessels, which determine the vessel diameter and thus blood flow, and with endothelial cells of capillary vessels, which form the blood-brain barrier that controls the composition of the brain interstitium. Recent work has indicated that astrocytic calcium signals play a pivotal role as an intermediate signaling step in the cascade that brings about increased blood flow following neuronal activation (Zonta *et al.*, 2003). The coupling of neuronal activity to blood flow is called *neurovascular coupling* or functional hyperemia (Lou *et al.*, 1987 ; Iadecola, 1993 ; Wahl and Schilling, 1993 ; Villringer and Dirnagl, 1995). In addition to neurovascular coupling, there is also *neurometabolic coupling* which denotes adaptations of the glucose metabolism in response to neuronal activation (Magistretti and Pellerin, 1999 ; Chih and Roberts, 2003). We have investigated the possibility that astrocytic calcium signals triggered by neuronal activity, are further communicated towards the blood vessels, more specifically towards blood-brain barrier endothelial cells. At this final step of the neuron-to-astrocyte-to-endothelium signaling cascade, calcium signals can be expected to influence the important molecular transports taking place over this barrier, thereby functioning as a signal involved in what we propose to call *neurobarrier coupling* (Braet *et al.*, 2004a), i.e. the coupling of neuronal activity to the activity of carrier-mediated transport across the blood-brain barrier.

Intercellular calcium signals and connexins

Neuronal activity can trigger calcium signals in astrocytes and work of our group has demonstrated in a co-culture model that astrocytes can communicate these calcium signals further towards endothelial cells (Leybaert *et al.*, 1998 ; Paemeleire and Leybaert, 2000 ; Braet *et al.*, 2001). We identified

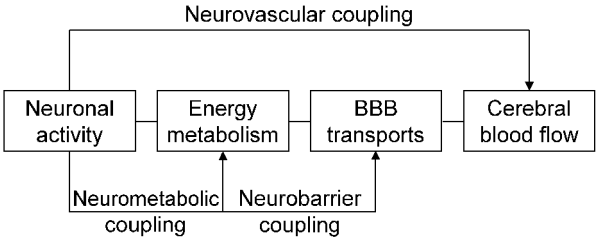

FIG. 1. — Neuronal activation triggers various responses that include local dilation of blood vessels causing an increased blood flow, called neurovascular coupling, and stimulation of the energy metabolism of glucose, dubbed neurometabolic coupling. The framework of our investigations is the hypothesis that neuronal activation brings about changes in the intrinsic activity of carrier-mediated transport across the blood-brain barrier, a phenomenon we propose to call *neurobarrier coupling*.

two mechanisms that support astrocyte-endothelial calcium signal communication : the first mechanism involves the diffusion of the calcium mobilizing intracellular messenger $InsP_3$ through gap junction channels and the second one relies on paracrine purinergic signaling involving the release of ATP, its binding to receptors on neighboring cells and activation of downstream signaling cascades that lead to an increase of cytoplasmic free calcium in the target cell (Braet *et al.*, 2001). Further investigations of endothelial ATP release indicated that the release was triggered by an increase of intracellular calcium and that it was mediated by a connexin-related mechanism (Braet *et al.*, 2003a ; Braet *et al.*, 2003b). Connexins are the subunits that, as a dodecamer (12 subunits), form gap junction channels. Work with peptides that mimic a short extracellular sequence of the connexin 43 subunit revealed drastic inhibitory effects on cellular ATP release. These effects can be explained by interaction of the mimetic peptides with connexin subunits that, as a hexamer, form so called connexin hemichannels. Connexin hemichannels are half gap junction channels that, in contrast to gap junction channels, are not involved in cell coupling but form a large conductance conduit between the cells' interior and the extracellular space (Goodenough and Paul, 2003 ; Leybaert *et al.*, 2003). Further work is directed towards the role of intracellular calcium as a trigger signal to activate this new connexin-related release pathway and to determine its involvement relative to the other release pathways such as vesicular release.

Astrocyte-endothelium calcium signals and their possible functions

Recent work by Simard *et al.* (2003) has demonstrated that endothelial cells in brain slices do not express P_2Y_2 and P_2Y_4 metabotropic receptors, pre-

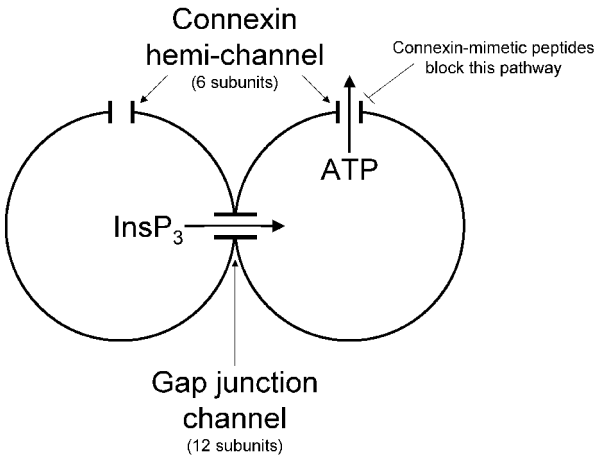

FIG. 2. — Connexin subunits are the building blocks of both gap junction channels and connexin hemichannels. The latter are composed of six subunits that together form a channel between the cytoplasm and the extracellular space. Two hemichannels on neighboring cells dock together to form a gap junction channel that connects the cytoplasm of the two cells. Gap junction channels allow the passage of intracellular messengers like $InsP_3$ to communicate calcium signals between cells. Brain cells like astrocytes and vascular endothelial cells release ATP as a paracrine transmitter substance and work with connexin mimetic peptides (peptides that mimic a sequence on the connexin subunit) demonstrated that these are very effective blockers of cellular ATP release. Based on this evidence we have proposed that ATP release occurs through connexin hemichannels, but because this is still a hypothesis we prefer to denote this kind of ATP release as 'connexin-related'. Taken together, connexins are involved in the two pathways of cell-to-cell communication of calcium signals, one pathway using the gap junction channels and the other making use of connexin-related ATP release that acts on purinergic receptors on neighboring cells.

cluding the purinergic calcium signaling pathway in the astrocyte-to-endothelial direction. Further work will thus be needed to identify possible other messenger systems acting between the two cell types. Several possibilities should be considered concerning the possible role of astrocyte-to-endothelium calcium signal communication. Changes of endothelial calcium are considered a key step in disrupting the tight junctions between endothelial cells, thereby opening the blood-brain barrier (Abbott, 1998 ; Mayhan, 2001 ; Brown and Davis, 2002 ; Wolburg and Lippoldt, 2002). Astrocyte-endothelial calcium signals might thus be involved in the process of barrier opening under pathological conditions. A second possibility is that, according to our neurobarrier coupling hypothesis, endothelial calcium signals have effects on the transports occurring over the blood-brain barrier. An important example of these transports is the GLUT-1 (glucose transporter type-1) mediated shuttle of glucose over the barrier. Preliminary work from our group suggests that certain neurotransmitters acting on endothelial calcium are able to stimulate glucose uptake in these cells (Braet and Leybaert, 2000) and recent work by Loaiza *et al.* (2003) has explored this possibility at the level of astrocytes where glutamate was shown to stimulate astrocytic glucose uptake (the role of intracellular calcium changes was however not investigated in this paper). A third possibility is that astrocyte-endothelium calcium signals control the release of vasodilating agents such as nitric oxide (NO) and the prostaglandins PGE_2 or PGI_2, thereby becoming an essential part of the signaling cascade involved in neurovascular coupling. Possibly, endothelial cells communicate calcium signals between each other from the capillary level

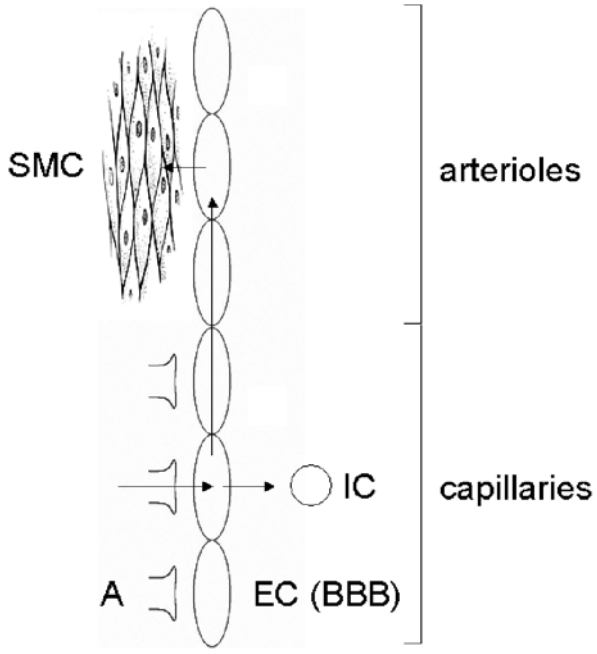

FIG. 3. — Our work, performed in co-culture systems and in brain slices, has shown that astrocytic calcium signals can be communicated towards endothelial cells (EC, A = astrocytes) through the pathways described in the previous figure. Increases in endothelial calcium are known to disrupt the blood-brain barrier (BBB) that is formed by tightly connected endothelial cells, and astrocyte-endothelial calcium signals might thus be involved in the opening of the barrier under pathological conditions like brain trauma or stroke. Endothelial calcium changes are furthermore hypothesized to affect the activity of transporters acting to shuttle substances like glucose over the barrier and might thus be involved in neurobarrier coupling. Alternatively, calcium signals can be communicated between endothelial cells in an upstream direction (towards the arterioles), where endothelial relaxing factors are released to cause relaxation of smooth muscle cells (SMC) and increased blood flow, a pathway of neurovascular coupling that is still hypothetical. Endothelial cells release ATP in response to various triggers, and the released ATP is known to have proinflammatory effects on immune cells (IC) in the blood. This communication pathway possibly modulates the interactions of leukocytes and lymphocytes with the blood-brain barrier upon neuroinflammation.

to the level of the arterioles, to cause dilation of upstream located resistance vessels (Iadecola, 1993). Evidence from vascular systems outside the brain indeed implicate capillary-to-arteriole endothelial calcium signals as a short-range signaling system (Sarelius *et al.*, 2000 ; Dora *et al.*, 2003).

Astrocyte-endothelium calcium signals in brain slices

In recent work we have investigated astrocyte-endothelium calcium signal communication in brain slices. Acutely isolated brain slices can typically be maintained functional during several hours after isolation and form an excellent model to investigate calcium signal communication. We have found that electrical stimulation of cortical brain slices with a microelectrode approximately 100 μm away from a small vessel (± 12 μm in diameter), triggers calcium signals in the glial cells that are communicated towards endothelial cells and smooth muscle cells of the vessel (unpublished observation). In accordance to these observations, the vessels reacted with either dilation or constriction, which is likely to be explained by communication of the calcium signal either to the endothelial cells, resulting in smooth muscle relaxation, or directly to the smooth muscle cells causing them to contract. These findings furthermore illustrate that astrocyte-endothelium calcium signals can also be observed in a preparation that largely resembles the *in vivo* organisation of the neural tissue.

Purinergic communication at the blood-brain barrier

ATP release by astrocytes and endothelial cells is not only involved in astrocyte-endothelial interactions but is also an essential element of the paracrine communication pathway between the bloodvessels and the blood cells. Endothelial ATP has indeed been demonstrated to act as a proinflammatory signal on blood immune cells such as leukocytes and lymphocytes (Di Virgilio *et al.*, 2001). We have put forward the hypothesis that ATP release through connexin hemichannels forms a high-capacity mechanism that might act to overcome dilution and washout of the endothelial ATP signal by the bloodflow, based on the fact that a single stimulus with ten micromolar concentrations $InsP_3$ triggers the release of quite a substantial fraction (1-2%) of the cellular ATP pool (Braet *et al.*, 2004a ; Braet *et al.*, 2004b). Interactions of immune cells with capillary endothelial cells are important in the disruption of the blood-brain barrier associated with neuroinflammatory diseases such as mutliple sclerosis (Poser, 1993). The breaching of the barrier involves the action of cytokines like TNF-α, IL1-β and IFN-γ and an increase of endothelial cytoplasmic calcium (de Vries *et al.*, 1996 ; Etienne-Manneville *et al.*, 2000). Our working hypothesis is that calcium signals communicated between endothelial cells may act to spatially spread and thus amplify the calcium-induced opening of the blood-brain barrier. We investigated in this context whether TNF-α has a modulatory influence on the communication of calcium signals between capillary endothelial cells (Vandamme *et al.*, 2004). We found that this cytokine inhibits two connexin-related communication pathways, i.e. the gap junctions and the connexin hemichannels. TNF-α appeared to be a blocker of ATP release through connexin hemichannels and because this is the first step of the paracrine signaling pathway, purinergic cell-cell communication will be expected to be silenced by this cytokine (Vandamme *et al.*, 2004). This might have a profound influence on the complex interactions of blood-brain barrier endothelial cells with blood immune cells. Further investigations will be needed to determine the role of these TNF-α effects at the blood-brain barrier upon inflammation.

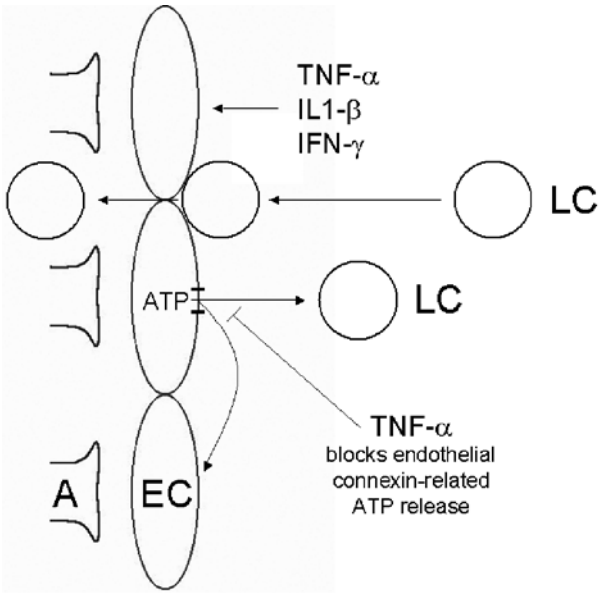

FIG. 4. — Cytokines like TNF-α, IL1-β and IFN-γ are known to be involved in the adherence of lymphocytes to endothelial cells and in the transmigration of these cells towards the brain under neuroinflammatory conditions. In addition to this, transmigration and blood-brain barrier opening also involves changes in endothelial calcium concentration. Endothelial cells (EC) release ATP in response to calcium changes and the released ATP can act on neighboring endothelial cells to increase their cytoplasmic calcium and thus to propagate blood-brain barrier opening. Such amplification effect can also be mediated by gap junctional communication (not shown). Endothelially released ATP has proinflammatory effects on lymphocytes (LC), as mentioned in the previous figure. We found that TNF-α supresses endothelial connexin-related ATP release, an effect that can be hypothesized to limit blood-brain barrier opening to the very place of lymphocyte transmigration. Further work will be needed to validate this hypothesis.

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