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The role of nitric oxide in neurovascular coupling

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HIGHLIGHTS

- A mathematical model of nitric oxide in a neurovascular unit is presented.
- Results show that nitric oxide leads to an increase of resting regional blood flow.
- Arteriolar dilation during neuronal activation is enhanced.
- The model helps investigate underlying mechanisms.

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ABSTRACT

Nitric oxide (NO) is a neurotransmitter known to act as a potent cerebral vasodilator. Its role in neurovascular coupling (NVC) is discussed controversially and one of the main unanswered questions is which cell type provides the governing source of NO for the regulation of vasodynamics.

Mathematical modelling can be an appropriate tool to investigate the contribution of NO towards the key components of NVC and analyse underlying mechanisms. The lumped parameter model of a neurovascular unit, including neurons (NE), astrocytes (AC), smooth muscle cells (SMC) and endothelial cells (EC), was extended to model the NO signalling pathway.

Results show that NO leads to a general shift of the resting regional blood flow by dilating the arteriolar radius. Furthermore, dilation during neuronal activation is enhanced. Simulations show that potassium release is responsible for the fast onset of vascular response, whereas NO-modulated mechanisms maintain dilation. Wall shear stress-activated NO release from the EC leads to a delayed return to the basal state of the arteriolar radius. The governing source of vasodilating NO that diffuses into the SMC, which determine the arteriolar radius, depends on neuronal activation. In the resting state the EC provides the major contribution towards vasorelaxation, whereas during neuronal stimulation NO produced by the NE dominates.

1. Introduction

Our understanding of the human brain is constantly deepening, however, crucial coherences and signalling pathways remain undiscovered. There exist still many reasons for the onset of cerebral diseases, and neurodegenerative conditions need to be fully investigated in order to provide possible preventions and medical treatment. Research findings within the last decades have suggested that there exists a stronger link between cerebral function and vascular perfusion of the brain than previously thought (Girouard and Iadecola, 2006; Zlokovic, 2011; Aoi et al., 2012).

Changes in neuronal activity adjust local perfusion, and hence glucose and oxygen supply, via direct and indirect pathways that include signalling at a molecular level (Lok et al., 2007). This interaction between neurons and arteriolar vessels is commonly called neurovascular coupling (NVC) or functional hyperaemia, and involves multiple cell types dynamically performing together. Neurons, together with astrocytes, smooth muscle cells, and endothelial cells, form a functioning unit, commonly known as neurovascular unit (NVU), building the major contributor for maintenance of homeostasis (Drewes, 2012).

Impaired NVC is associated with several cerebral diseases such as hypertension, Alzheimer's Disease, cortical spreading depression, atherosclerosis and stroke (Girouard and Iadecola, 2006; Iadecola, 2004). Therefore, it is crucial to understand the underlying mechanisms – both in the healthy as well as in the pathological brain.

Cellular signalling involves numerous pathways and transmitter molecules. In Furchgott and Zawadzki (1980) discovered an

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endothelium-derived relaxing factor (EDRF) in the cardiovascular system. They found that this unknown signal molecule was only produced by an intact endothelium. Ignarro et al. (1987) later proved that EDRF was nitric oxide (NO) and that cyclic guanosine monophosphate (cGMP) is its second messenger. For these discoveries Robert F. Furchgott, Louis J. Ignarro and Ferid Murad were awarded the Nobel Prize in Medicine or Physiology in 1998.

The identification of NO as a vasodilating signalling molecule in cardiovascular, and especially also in neurovascular systems was a surprise to the scientific community, because it did not fit into the traditional definition of neurotransmitters since it is not produced in advance and stored. Furthermore, due to its small size NO diffuses widely and readily in all three dimensions and is not limited to local effects, which sets it apart from other central nervous system signalling molecules. It is extremely diffusible in both aqueous and lipid environments which allows rapid spreading even through membranes. Nevertheless, NO is an unstable gaseous free radical and therefore has a very short life-time, which limits its activity temporally (Garthwaite and Boulton, 1995; Dobutovi et al., 2011).

NO is known to be produced in a large number of different tissues playing a wide variety of physiological roles, including relaxation of blood vessels (Dawson et al., 1991; Alderton et al., 2001). However, it still is not fully understood as to how exactly NO contributes to NVC and particularly which source of NO plays the major role (Butler and Life, 2003). The biochemical reaction that synthesises NO is catalysed by the enzyme family of nitric oxide synthases (NOS) (Förstermann et al., 1998; Alderton et al., 2001; Rafikov et al., 2011).

In mammals, three isoforms of NOS have been identified: neuronal (nNOS), mainly expressed in neurons, endothelial (eNOS), mainly expressed in EC, and inducible (iNOS), a transcriptionally regulated enzyme, which is present in multiple cell types (Förstermann et al., 1998). Literature suggests different NO production rates ranging from $0.035\,\mu M\,s^{-1}$ up to $68\,\mu M\,s^{-1}$ (Chen and Popel, 2006). All isoforms have important biological functions, strongly linked with their location and output, and it has been suggested that changes in their expression may have physiological and pathophysiological consequences (Förstermann et al., 1998). Their layout of catalytic domains is similar to a C-terminal reductase and an N-terminal oxygenase section, including binding sites each for calmodulin (CaM), L-arginine (L − Arg) and for several cofactors (Mayer and Hemmens, 1997). The catalytic activity of the constitutively expressed isoenzymes, nNOS and eNOS, is regulated by the intracellular calcium (Ca^{2+}) that builds a complex with CaM, but evidence indicates that these enzymes are also regulated by other factors such as physical stimuli (Forsythe et al., 2001).

The physiological role of NO is still a matter of debate (Attwell et al., 2010) and the molecule has been described to have a "Janus-faced" character (Förstermann, 2006; Calabrese et al., 2007): NO can act either neuroprotectively by controlling the vascular smooth muscle tone and blood flow, therefore preventing ischaemic cell injury, or neurotoxically leading to severe cerebral degeneration. Literature suggests that the production source and quantity determines the character of its functioning (Aurelia Zorrilla-Zubilete et al., 2010). This emphasises the necessity of a functional model that predicts NO release from different cell types and its biophysical influence, ideally including vessel control and interaction with feedback from wall shear stress (wss), intracellular Ca^{2+} levels, and oxygen delivery and consumption (Buerk, 2001).

Because of its high diffusivity, it is difficult to measure the exact NO production and concentration experimentally. Mathematical modelling can hence be a useful tool to elucidate physiological processes and guiding directions for further experiments.

2. Model development

The potential of NO as an important vasodilating messenger molecule is assessed using a holistic mathematical model that includes the dynamics of NO in the NVU. With the help of this model the most crucial signalling pathways are analysed and the influence of NO in NVC investigated, including the localisation of the main contributing source.

Therefore, our previous foundation NVU model (Farr and David, 2011; Dormanns et al., 2015) is extended by mathematical equations that represent production, diffusion and consumption of NO in different cell types, as well as the interaction of NO with other biochemical species and ion channel open probabilities.

As in the previous models of an NVU (Farr and David, 2011; Dormanns et al., 2015) we divide the full model into seven compartments: the neuron (NE), the synaptic cleft (SC), the astrocyte (AC), the perivascular space (PVS), the smooth muscle (SMC) and endothelial (EC) compartments, and the arteriolar lumen (LU). The compartments are coupled predominantly by ion fluxes through ion channels, and membrane potential differences.

Our NO model focusses on NO production by the two constitutive isoforms of nitric oxide synthase, nNOS and eNOS (Fleming and Busse, 1999). Both enzymes' activation is mediated by intracellular Ca^{2+} in the NE and EC, respectively. In addition, eNOS gets activated by blood flow induced wall shear stress in cerebral arterioles (Joannides et al., 1995).

Due to its high diffusion coefficient NO diffuses rapidly into other compartments, shown in experiments (Malinski et al., 1993) and in kinetic simulations (Lancaster, 1994). When NO reaches the SMC it interacts with intracellular enzyme activation and regulates SMC relaxation (Yang et al., 2005).

A schematic representation of the compartments and the NO signalling pathway in the NVU is given in Fig. 1 spanning NO synthesis in the NE and the EC through the relaxation of the SMC.

The dynamics of NO in the involved compartments are described mathematically using mass balance formulations. The concentration of NO in each domain is determined by the balance between production $p_{NO,m}$, consumption $c_{NO,m}$ within the cell, i.e. the reaction with oxygen or other molecules, and the diffusion $d_{NO,m}$ from and into other compartments.

The NO concentration $[NO]_m$ is given by the solution of the following generic first-order non-linear differential equation:

$$\frac{d[NO]_m}{dt} = p_{NO,m} - c_{NO,m} + d_{NO,m}, \tag{1}$$

where $m \in \{n, k, i, j\}$ denotes the cell indices for NE, AC, SMC and EC, respectively.

2.1. NO production

The production rate of NO is dependent on the concentration of activated nitric oxide synthase. L-Arginine (L − Arg), oxygen (O_2) and nicotinamide adenine dinucleotide phosphate (NADPH) are the biochemical substrates needed for the NO production (Chen and Popel, 2006), where L-Arg is the requisite and sole nitrogen donor (Forsythe et al., 2001). L − Arg is oxidated to L-Citrulline and the biochemical reaction leads to the production of $NADP^+$, water