BMC Pharmacology

Poster presentation

Imaging of nitric oxide signalling in thalamic neurons

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from 2nd International Conference of cGMP Generators, Effectors and Therapeutic Implications
Potsdam, Germany, 10–12 June, 2005

Published: 16 June 2005

BMC Pharmacology 2005, **5**(Suppl 1):P23 doi:10.1186/1471-2210-5-S1-P23

Although the nucleotide guanosine 3',5'-cyclic monophosphate (cGMP) has been demonstrated to be involved in the regulation of many cellular functions in the brain, little is known about its spatiotemporal dynamics in neurons. The ventrobasal complex (VB) of the thalamus fullfills essential integrative roles in the brain and is innervated by NO/cholinergic fibers arising from the pons contributing to the modulation of attention states.

To investigate the role of NO/cGMP signalling in the VB, we developped a ratiometric imaging method using a genetically encoded fluorescent cGMP sensor (37[1]) expressed in these relay neurons. Direct binding of cGMP to the PKG domain of the sensor induces a conformational change that can be recorded as a decrease of intramolecular fluorescence resonance energy transfer (FRET) between two variants of GFP (ECFP and Citrine) present in the sensor. Neurons in mice brain slices were selectively transfected with the probe construct using a recombinant Sindbis pseudovirus. Ratiometric imaging of the cGMP dynamic was performed 12 h to 24 h after infection.

Application of the non specific phosphodiesterase (PDE) blocker IBMX (200 μM) to the neurons induced a slow and modest increase of the emission ratio (F480/F535: ECFP emission/citrine emission), while the soluble guanylate cyclases (sGC) inhibitor ODQ (10 μM) lead to a decrease indicating that in unstimulated brain slices, a basal state of activation of the sGC and PDE exists. In contrast, activation of the sGC using the NO donor SNAP (100 μM) lead to a very rapid (within 1 to 2 min.) and 4 to 6 times stronger increase in the emission ratio compared to the IBMX response. This SNAP effect was completely abolished when cells were pretreated with ODQ.

Taken together, these results show that a large variety of cGMP fluctuations can be monitored in time and space in living neurons from brain slices. This method will now be used to dissect in further details cGMP signalling in the thalamus and its physiological consequences.

References

1. Honda A, Adams SR, Sawyer CL, Lev-Ram V, Tsien RY, Dostmann WRG: **Spatiotemporal dynamics of guanosine 3',5'-cyclic monophosphate revealed by a genetically encoded, fluorescent indicator.** *PNAS* 2001, **98:**2437-2442.