PHOTOBIOMODULATION AND GLUTAMATE RELEASE FROM CEREBROCORTICAL NERVE TERMINALS

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Introduction: The photons have been shown to affect the functioning of the nervous system. Promising effects of the photobiomodulation (PBM) has been reported in patients with peripheral nerve injury and ischemic stroke. Moreover, the PBM seems to promote axon growth and nerve regeneration, to rescue rodent neurons from neurotoxic injury, to reduce long-term deficits after stroke or traumatic brain injury in animal models, and to enhance memory. In cultured neurons and in ancestral pre-nervous cell-organisms, photons can modify mitochondrial membrane potential, ATP levels, intracellular Ca^{2+} levels, reactive oxygen species, and NO production. However, the mechanisms by which laser light may affect the functioning of the nervous system are not yet understood and their effects on neurotransmitter release have never been investigated.

Materials and methods: To investigate in vitro the effects of photons on glutamatergic transmission, we used cerebrocortical slices, nerve terminals (synaptosomes) and astrocyte processes (gliosomes) obtained from adult mouse cerebrocortex and transferred to superfusion apparatus. During superfusion with standard medium, the preparations were exposed to laser light (808nm, 60 J/cm^2, 1W, 1W/cm^2, 60 s, in continuous wave, CW). The energy parameter used is in line with published data that showed as an 808nm 1W, 1W/cm^2, 60 sec is able to induce PBM in eukaryotic cells, from protozoa and invertebrates up to mammal mitochondria and stem cells. In addition, the wavelength used is in the range of wavelength that is supposed to have a role in cell-to-cell communication by biophotons. Dependence of stimulus-evoked glutamate release on external Ca^{2+} was assessed in synaptosomes superfused with Ca^{2+}-free medium. In each experiment, at least one chamber was used as a control and was not exposed to light. The glutamate released into the superfusate fractions collected from slices, synaptosomes or gliosomes was measured by HPLC.

Results: Laser light evoked glutamate release from slices that retained the complexity of neuron-astrocyte network interaction. Membrane leakage or cell damage was unlikely responsible for glutamate release, as the release raised during exposure to light, and returned to baseline levels after the stimulus had ceased. On investigating this effect separately on the two main sites possibly responsible for releasing glutamate, we found that the nerve terminals, but not the astrocyte processes, were sensitive to light.

Discussion and conclusion: Our finding indicates that photons are mainly involved in activating transmission in neuron networks and might preferentially activate "private" transmission in a neuronal circuit. Notably, the photon-evoked release of glutamate appeared to be dependent on the availability of Ca^{2+}, a finding that is consistent with activation of the vesicular exocytotic release. Regarding the mechanisms that might be involved in the ability of photons to evoke glutamate exocytosis, it should be noted that mitochondria and ATP production are widely recognized targets for PBM and that presynaptic mitochondria and their capacity for ATP production are known to be related to the efficiency of synaptic vesicle exocytosis. Notably, the ultrastructural analysis showed that synaptosomes are equipped with presynaptic mitochondria, while gliosomes are not. Although activation of mitochondrial ATP production might be related to the glutamate-releasing effect of photons, further investigation is required for a better understanding of the ability of photons to activate the vesicular glutamate release from nerve endings. Indeed, we cannot exclude the participation of other mechanisms; for example, NO production—which is reported to be increased by laser light—could also function as a signal to activate vesicular glutamate release from nerve terminals and slices.