NONLINEAR PHOTOBIOSTIMULATION: THE MECHANISM OF VISIBLE AND INFRARED LASER-INDUCED STIMULATION AND REDUCTION OF NEURAL EXCITABILITY AND GROWTH

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Non-linearity in photobiostimulation is described as a process where linear optical absorption produces active chemicals such as cytoplasmic H^+ and Ca^{2+} which participate in chemical reactions whose reaction rates depend non-linearly on the concentration of these photoproducts active chemicals, thus allowing very sensitive light control of non-linear biological reactions. Important contributions to neural excitability and growth include photostimulation of ATP production which fuels the action potential and fills the synaptic ATP vesicles. As was recently shown, the synaptic ATP plays the important role of an extracellular neurotransmitter. The importance of this effect on the possibility of systemic laser therapy of nervous disorders by photostimulation of ATP release by migrating macrophages is mentioned. The connection between light-enhanced electrical activity of the nerve and neurite growth is also analysed.

KEY WORDS Monoliner photobiostimulation Neural excitability ATP Neurotransmitters

Primary Photoacceptors and Possible Ways by which Visible and Infrared Laser Energy is Converted to Proton-Motive Force by the Photoacceptors

We assume that the laser light is absorbed in the membranes of the mitochondria by endogenous protoporphyrins¹ or pigments of the respiratory chain denoted collectively by P, leading to the corresponding excited species, P^* . In one possible mechanism we consider photosensitization in which energy of P^* is transferred to molecular oxygen in the ground state, denoted by O_2 .^{2,3}

Replacing O_2 by singlet oxygen, 1O_2 , a potent oxidizer,² stimulates the redox activity of the respiratory chain. Electronic transport of the redox reactions pumps H^+ from the mitochondrial matrix to the exterior according to Mitchell's chemiosmotic hypothesis.⁴ Cell photostimulation via 1O_2 production, as proposed here, is similar to the stimu-

lation in the dark, obtained by adding methylene blue, another potent oxidizer, to cell cultures.⁵

It is well known, however, that when porphyrins are introduced artificially into the tissues, as in photodynamic therapy, destruction of the (malignant tumour) cells by 1O_2 is obtained.⁶ This would seem to indicate, as proposed by Karu,⁵ that laser stimulation and damage result from qualitatively different causes: Cell damage is caused by photosensitized 1O_2 and stimulation arises from a different type of photoactivation of the redox reactions of the respiratory chain, discussed below. We propose to regard photobiostimulation as a non-linear process: The action of light at low intensity and fluence is assumed to cause stimulation of the cells, and damage results at higher light intensity and fluence where this stimulation becomes too strong, exhausting the energy reserves of the cell.⁷

In addition to the production of 1O_2 , laser light also activates the redox activity of the respiratory chain since excitation of the component chromophores of the chain will increase their reactivity.⁵ We assume that electronic excitation is converted to vibrational energy in the electronic ground state of the chromophores, via radiative or radiationless transitions. The vibrationally 'hot' molecules react faster than the 'cold' vibrationally unexcited molecules. It is also possible that the weak absorption in the far-red infrared of one of the chromophores, cytochrome aa_3 , corresponds not to electronic tran-

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sition, but to excitation of vibrational overtones in the electronic ground state, due to 'intensity borrowing'.⁷

Moreover, instead of photoactivation, via vibrationally hot molecules, of the redox reactions of the respiratory chain, the absorption of a laser photon can itself produce a photochemical reaction capable of producing proton transmembrane transport. This is similar to the proton gradient produced by the irradiation of bacteriorhodopsin of the halophilic bacterium *Halobacterium halobium*.⁸ Here the cytochrome oxidase plays the role of bacteriorhodopsin and acts as a proton pump, as has been stated in the literature.⁹ The absorbed photon excites the enzyme electronically and the excited electronic state decays in part to a chemically different configuration of the enzyme. Overtone excitation by far-red infrared irradiation of cytochrome oxidase, facilitated by intensity borrowing, may produce a similar effect when the vibrationally hot molecule has sufficient energy to cross the potential barrier toward chemical transformation. Suppose that the weak overtone excitation by far-red infrared light has a relatively large probability of reaching the vibrational states that lead to chemical transformation. Then the weak infrared excitation may become (at least) as efficient as the relatively strong electronic excitation by visible light since the electronically excited state will only in part decay to the reactive vibrational states. This explains the stimulation of Na^+/K^+ -ATP activity in rat saphenous nerve tissue obtained using irradiation by the 830 nm, 60 mW continuous beam gallium aluminium arsenide diode laser.¹⁰ Less than linearly increasing stimulation was obtained in the range of fluences between 0.360 J/cm² and 0.9 J/cm². With higher fluences, the stimulation steadily decreased from the maximum value. No measurement with unmyelinated peripheral saphenous nerve or with visible light was carried out. It is interesting to note, however, that direct irradiation of the rat sciatic nerve with a 632.8 nm, 0.3 mW continuous wave helium neon laser yields stimulation of the action potential at 0.2 J/cm².¹¹ This indicates that the strongly absorbed visible light and the weakly absorbed infrared light produce stimulation at fluences which are of the same order of magnitude. In order to obtain reduction of the action potential in rat sciatic nerve much higher fluences, of the order of 100 J/cm² obtained by using 5 min (3000 pulses) irradiation with pulses of 8 ms duration and pulse energy from 10–100 J/cm² and peak power from 181–180 × 10⁶ W/cm² of the 1064 nm Nd: yag laser had to be used.¹² Reduction of excitability of rat cerebellum nerve cells was obtained with visible dye-laser pulses of 1 μs duration, again with fluences of the order of 100 J/cm² and peak powers of the order of 100 × 10⁶ W/cm². In these measurements the reduction of excitability with fluence was mark-

edly non-linear. Nerve regeneration in rats via neurite growth induced by long-term, low fluence (10 J/cm²) transcutaneous 621.8 nm, 16 mW/cm² continuous wave HeNe laser irradiation has also been reported.¹⁴ This fluence is of the same order of magnitude as that which produces transcutaneous stimulation of the action potential of the nerves.

Finally, we suggest that the action of the laser light on the pigments of the respiratory chain may be similar to the photophysical and photochemical processes triggered in photosynthesis. As in the thylakoid membrane there may be one or two photochemical reaction systems.¹⁵ The electron transport product in this way is assumed to produce proton transport creating a transmembrane electrochemical proton gradient.

Laser-Induced ATP Production and Ca^{2+} Release in the Cytoplasm Responsible for Non-Linear Stimulation and Reduction of Neural Excitability and Neurite Growth

In the preceding section we have discussed various processes by which laser energy is converted via chemiosmosis, or rather photochemiosmosis, into a transmembrane proton gradient. The proton-motive force produced thereby is known to generate ATP in the membranes of the mitochondria together with Ca^{2+} release from the mitochondrial matrix to the cytoplasm by an antiport process:

In ordinary eukaryotic cells these effects enhance cell proliferation.⁷ We assume that in neurons the same effects enhance neurite growth. Moreover, the ATP production activates the Na^+/K^+ ATP-ase pumps. This activity charges the battery that powers the action potential. This action potential, together with the Ca^{2+} release by the mechanism described by equation 3 increase the Ca^{2+} concentration in the axon terminal eliciting the exocytic release of acetylcholine responsible for synaptic transmission of the action potential from one nerve cell to another or to a muscle cell. This explains the biostimulating effect of low-intensity laser radiation on the action potential and on its synaptic transmission. Note that the laser-enhanced production of ATP also fuels the Ca^{2+} pumps, expelling more Ca^{2+} into the surrounding medium and increasing the Ca^{2+} concentration gradient of the medium relative to the cytoplasm. This triggers an increased influx of Ca^{2+} from the medium into the cytoplasm via the ion channels. This additional Ca^{2+} enhances the activation of calmodulin:

and the activated calmodulin Ca^{2+} complex participates in protein generation and transformation needed for cell growth. The laser-enhanced electrical activity of the nerve also contributes to its healing by neurite growth and to the success of laser-aided nerve transplantation in animal neurosurgery.¹⁴ This is due to the fact that the increased ATP fuels transient influx of Na^+ ions, changes protein conformation and influences cell growth.¹⁶

Recent observations indicate that the light-induced increase in ATP production will also give a direct contribution to nerve-communication enhancement. This is inferred from recently confirmed results showing that the ATP stored in synaptic vesicles is a neurotransmitter both in the peripheral and in the central nervous system.¹⁷ The ATP acts as an extracellular messenger on ATP receptors. The extracellular transmitter property of ATP will probably stimulate the study of systemic effects in low-level laser therapy of nervous disorders since irradiation of peripheral blood vessels activates circulating macrophages, stimulating ATP release by these cells.¹⁸

The stimulating and regeneration effect is obtained using low laser fluences. At higher fluences, using high-intensity laser pulses, reduction of nerve excitability has been reported.^{12, 13} This can be explained by a large influx of Ca^{2+} into the cytoplasm which causes hyperactivity of Ca^{2+} ATP-ase pumps and exhausts the ATP reserves of the cytoplasm. This paralyzes the Na^+-K^+ ATPase pumps responsible for generating and maintaining the K^+ gradient and decreases the membrane potential.

As can be seen from equations 3 and 4, the processes described depend non-linearly on the cytoplasmic H^+ and Ca^{2+} concentrations. Moreover, the influx of Na^+ and Ca^{2+} through the ion channels are also highly non-linear processes which depend on the available ATP concentration. Now, in the case of photobiostimulation, the cytoplasm concentration of the H^+ , Ca^{2+} and ATP all depend on the laser intensity and flux. Therefore photobiostimulation in general is expected to be a non-linear process both with respect to laser fluence and, at constant fluence, with respect to laser intensity. This is in accordance with recent observations which demonstrate the high sensitivity of non-linear biological reactions with respect to light control.¹⁹

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