IN VITRO IRRADIATION OF SMOOTH MUSCLE TISSUES WITH LED RED AND LASER NEAR-INFRARED LIGHT MODULATES NEUROTRANSMISSION PATHWAYS

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ABSTRACT

Alternative therapeutic approaches to mental, neurological and physiological diseases present cures and treatments based on light-emitting electromagnetic radiation. Low-level laser therapy was introduced half a century ago in various medical fields. Nowadays, in the search for cheaper and less absorbed light doses with which the same therapeutic results can be achieved, light-emitting diodes enter boldly into medical practice. Light-emitting diodes have gained popularity though there is no sufficient evidence on the response of various neuromodulators to electromagnetic radiation at a molecular level.

This study was intended to clarify the effect of light-emitting diode red and laser near-infrared light on smooth muscles' reactivity to 5-hydroxytryptamine. The smooth muscle tissues were in vitro irradiated in a self-designed system named wet organ bath, and the specific mechanograms were depicted. Our results demonstrate that in both irradiation cases (light-emitting diode red or laser near-infrared light), smooth muscle contractility is potentiated, which is mediated by the activation of 5-hydroxytryptamine.

Keywords: low-level laser therapy, smooth muscle tissues, electromagnetic radiation, 5-hydroxytryptamine (5-HT), light-emitting diodes (LEDs).

INTRODUCTION

Methods in which light is a crucial player have proven a reliable modality for treating various diseases due to their beneficial effects on biological structures [1]. One of these methods is low-level laser therapy (LLLT), which has been used for over 50 years. The emitted wavelength varies between the red and near-infrared (NIR) parts of the electromagnetic spectrum. Data confirm that LLLT promotes pain relief, induces analgesia, reduces oedema and inflammation, etc. [2]. Targets in LLLT have also been smooth muscles, known for their participation in constructing hollow organs' walls. For example, Aimbire and colleagues investigated the effect of red light on inflamed rat tracheal smooth muscle samples. They found that the flow of photons with a wavelength of 655 nm can reestablish the relaxation of the muscles [3]. Regarding NIR, a study on irradiated vascular smooth muscle (SM) cells demonstrated prolonged vasodilation increasing blood circulation [4].

The advancement of technologies led to greater use

of electromagnetic radiation (ER) in medical practice. In that sense, light-emitting diodes (LEDs) have been practised for several conditions since they allow the irradiation of more significant areas of tissues [5]. Up to date, there is evidence indicating that LED sources can modulate the activity of significant neurotransmitters that affect vital functions of various systems in the human body, including the enteric system. For example, serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neuromodulator that controls peristalsis and motility of the gastrointestinal tract and falls in the classification of "major neurotransmitters" [6]. Moreover, 5-HT regulates processes like vasodilation, neuroprotection and secretion of monoamine in the gut [7]. Literature data confirm the vital role of 5-HT in the modulation of mood, cognition, mental disorders and neurodegenerative diseases. Interestingly, studies on patients suffering from depression have shown elevated levels of 5-HT after exposure to bright light [8].

In previous research, we have designed a novel system for *in vitro* irradiation and proved that ER potentiates the reactivity of the SM tissues (SMT) isolated from the corpus of rat stomach to 5-HT at maximal effective concentration EC_{100} = 5 μM. However, in the case of UV radiation, the contraction of the SMT was decreased due to their radiation exposure [9]. Another study on rat stomach strips demonstrated modulation of SM contractility after tissues' exposure to LED 470 nm, LED 365 nm and Lamp 350 nm [10].

In the current paper, our focus was on the effect of red and NIR light (LED 660 nm и Laser 808 nm) on the reactivity of SMT to 5-HT at a concentration of 5 μM (EC_{100}). Our findings show the improvement of SM contractility after irradiation, which can be explained by the interaction of ER with intracellular enzymes controlling 5-HT levels. Described experiments will uphold our hypothesis later in the text.

EXPERIMENTAL

Chemicals and reagents

Acetylcholine (ACh) and 5-HT were purchased from Sigma and dissolved in distilled water. The Krebs solution (KS) contained NaCl - 120 mmol L^{-1}; KCl - 5.9 mmol L^{-1}; $CaCl_2$ - 2.5 mmol L^{-1}; $MgCl_2$ - 1.2 mmol L^{-1}; NaH_2PO_4 - 1.2 mmol L^{-1}; $NaHCO_3$ - 15.4 mmol L^{-1}; glucose 11.5 mmol L^{-1} (pH 7.4).

Animals

All the experiments were conducted with male Wistar rats weighing approximately 250 g and under the requirements by the International Council for Ethical Guidelines for Animal Breeding Labs for Researchers, ARRIVE, and the EU Directive 2010/62/EU for animal experiments. The animals were bred under standard laboratory conditions (temperature 22°C ± 1°C, humidity 45 %, 12 h dark/light cycle, food and water *ad libitum*).

Smooth muscle strips

SM strips were isolated from the corpus of the stomachs of euthanized male Wistar rats and were divided into three groups. The first group was composed of non-irradiated tissues named the "control group". The second and third consisted of SMT exposed to LED 660 nm and Laser 808 nm ER, respectively.

Wet organ bath

For the conduction of the experiments, the SM strips were put in an organ bath free of KS for 1 - 2 min, and during that period, they were exposed to ER. This way, the ER's specific absorption in a liquid medium was avoided. After the SMT irradiation, we investigated the parameters of their spontaneous contractile activity (SCA).

Light sources

For the irradiation of SMT, we have used LED 660 nm and Laser 808 nm. The power of the two sources was constant at 3 W, while the power density of both was 4 mW cm^{-2} as the intensity meter measured the energy per sec. The irradiation of the gastric tissues lasted 60 s, while the SM response was reported at the 15th min after irradiation.

Registration of the parameters of the smooth muscle contractility

For experimental purposes, SM strips isolated from the stomach corpus of 7 male Wistar rats with a length of 20.0 mm ± 1.5 mm without violating the mucus layer. They were put randomly in organ baths, refilled with 15 mL modified KS, and oxygenated with 95 % O_2 and 5 % CO_2 at a standard temperature of 35.5°C ± 0.3°C, defined as standard following other authors' results [11]. Analysis and registration of the SM strips' contractility were done by a three-channel interface

system where the contractile activity record started after the equilibration period. This period was the same for each of the conducted experiments. According to the experimental protocol, each experiment began by treating tissues with ACh 10^{-6} M. Their reaction to the neurotransmitter was recorded for 5 min, after which the baths were washed 3 times. Then the tissues were left in the KS for 20 min so that their contractile activity was normalized, and only then were they irradiated with ER or treated with 5-HT. The registered parameters of the SCA were baseline period (BT), the area under the curve (AUC), mean amplitude (A_{mean}) and frequency.

Exogenous treatment of 5-HT and its effect on smooth muscle contractility

To study the effect of 5-HT on the SCA of SM strips, we used exogenous 5-HT at a concentration of 5 μM. This concentration allowed the measurement of the maximum SM contractility and is designated as EC_{100}. The changes in smooth muscle contractile activity are represented in the 10^{-6} M ACh reaction percentage. The treatments of the SMT in the organ bath initiated with this neurotransmitter and at this concentration to test their reactions. In detecting contractions, the experiment proceeded according to the experimental protocol. After that, all the recorded changes in the SCA as a response to the applied 5-HT at EC_{100} in the organ bath resulted from tissues' stimulation with ACh (10^{-6} M).

Data evaluation and statistical analysis

Statistical analysis was performed using SPSS 17.0. One-sample Kolmogorov-Smirnov test was used to evaluate the normal distribution, and in that case, one-way ANOVA and the Bonferroni posthoc test were employed for multiple comparison analyses. The results were reported as MEAN ± SD (Standard Deviation). The number of tested preparations is given as N. At $p < 0.05$, the results were considered significant.

RESULTS AND DISCUSSION

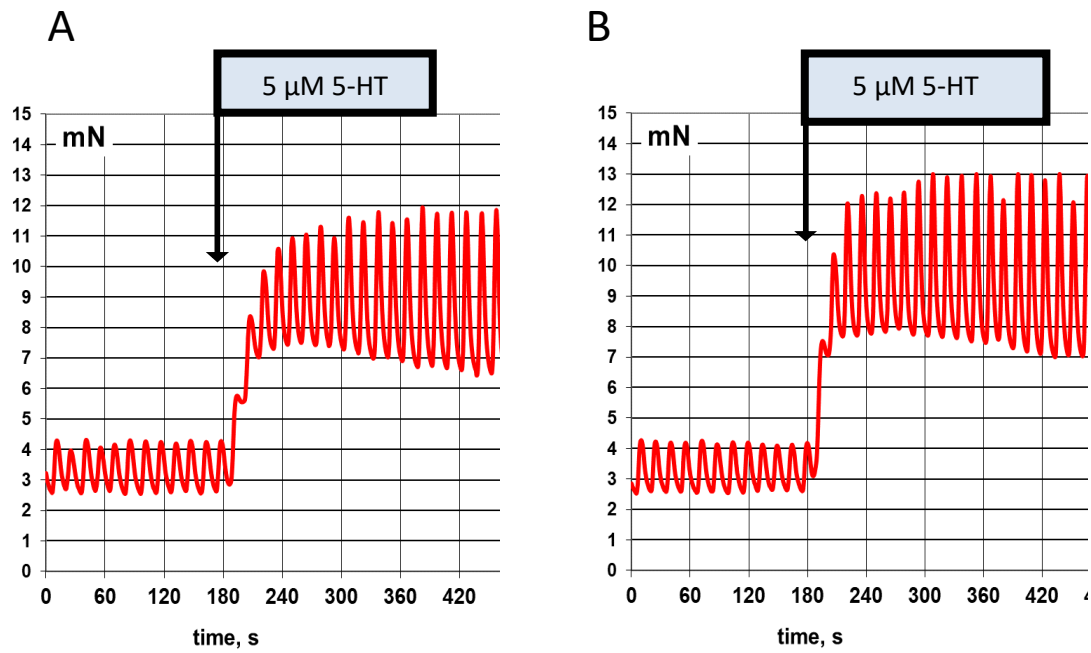
Our work aimed to investigate the influence of ER, namely LED 660 nm and Laser 808 nm, on SMT'contractility in the presence of 5-HT. All tissues isolated from the corpus of rat stomachs were positioned in KS and were exposed to the abovementioned radiations in a wet medium (without a buffer) named wet organ bath, abreviated WOB, for 1-2 min. Thus, any interference from the specific absorption of the LED and laser beam in a liquid medium was avoided. Furthermore, 5-HT to a concentration of 5 μM was applied to the SMT in the organ bath, where registration of the 5-HT-induced SM contractions of the tissues was further recorded.

As has already been described previously, the SM strips have been classified into non-irradiated (control - group 1), irradiated with LED 660 nm (group 2), and those with Laser 808 nm (group 3). The results obtained from group 1 were considered the basic level for all comparisons.

Our first task was to evaluate the changes in the parameters of SCA of group 1 against the irradiated SMT with LED 660 nm. Fig. 1 gives a representative diagram comparing the effect of 5-HT at EC_{100} in control (Fig. 1A) and group 2 (Fig. 1B) tissues. Our findings showed that the ER provoked significant alterations in SM contractility for non-irradiated biological structures. In the case of irradiation with LED 660 nm, the strips' SCA increased significantly by 1 mN ($n = 7$, $p < 0.05$) as a response to the appllication of exogenous 5-HT. Statistical analysis is presented in Figure 1C. Precisely, we observed an increase in the tissues' SCA of about 6 % after exposure to LED 660 nm and treatment with exogenous 5-HT (5 μM).

Keeping up with the experimental protocol, we proceeded with the laser source emitting NIR light at a wavelength of 808 nm to investigate its efficacy on the reactivity of SMT to 5-HT at a concentration of 5 μM. Fig. 2 illustrates the mechanograms of both groups of tissues (groups 1 and 3) after light irradiation and treatment of 5 μM 5-HT. The mechanograms demonstrate the changes in the parameters of SCA of group 3 (Fig. 2B) against group 1 (Fig. 2A) after the treatment with 5-HT. Surprisingly, the most remarkable alterations were in the phasic contractions of the irradiated tissues, which have been augmented by approximately 4 mN compared to group 1. In addition, statistical data analysis showed significant potentiation of SM contractility after exposure to NIR light, around 43 % ($n = 7$, $p = 0.001$) (Fig. 2C).

The contractility of SMT from all groups (groups 1, 2 and 3) is compared in Fig. 3. The changes in the SCA are given in percentages due to response to 5-HT addition (5 μM). We observed significant differences in



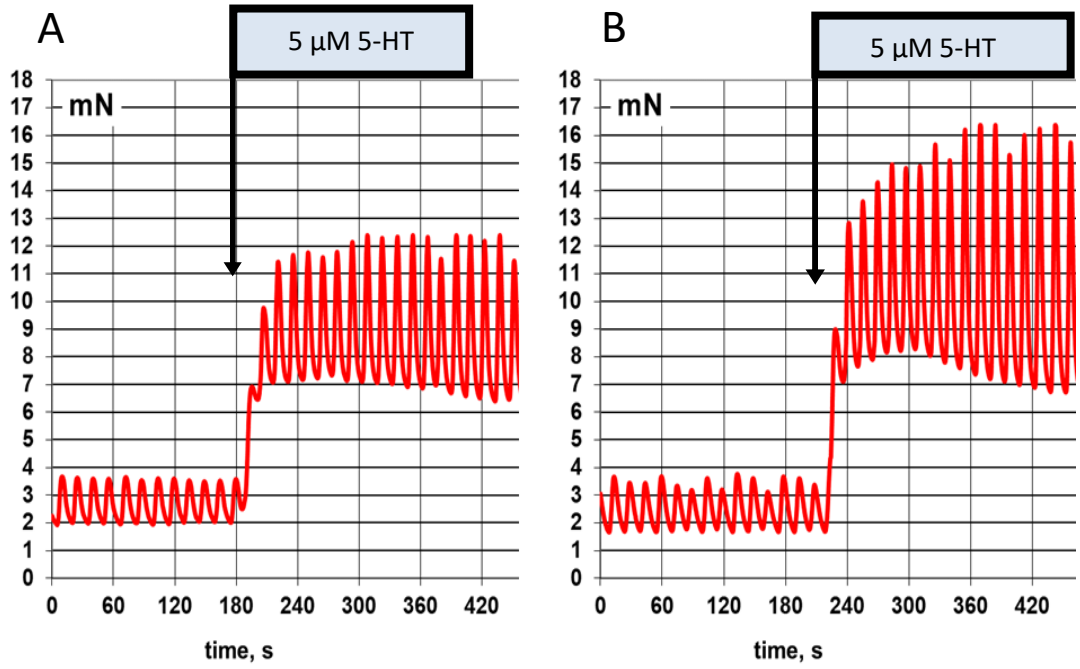
C

Changes in the SCA of the WOB group after treatment with 5-HT at 5 µM				*Changes in the SCA of the group 2 after treatment with 5-HT at 5 µM and irradiation with LED 660 nm*			
parameters	*Relative increase*			*parameters*	*Relative increase*		
	Average value	*Percentiles 25%*	*Percentiles 75%*		*Average value*	*Percentiles 25%*	*Percentiles 75%*
BT	*2.78*	*2.7*	*2.85*	*BT*	*2.99*	*2.95*	*3.04*
BT + A_{mean}	*2.85*	*2.79*	*2.88*	*BT + A_{mean}*	*3.15*	*3.12*	*3.24*
AUC	*2.68*	*2.6*	*2.68*	*AUC*	*3.01*	*3.00*	*3.14*
Frequency	*1.00*	*1.00*	*1.00*	*Frequency*	*1.00*	*1.00*	*1.00*
P=0.03							

Fig. 1. Representative mechanograms of smooth muscle contraction: A) A mechanogram demonstrating the effect of 5-HT at concentration EC100 on the SM contractility of group 1 tissues; B) A mechanogram illustrating the effect of 5-HT at concentration EC100 on the SM contractility of group 2 tissues (LED 660 nm); C) Statistical analysis of the recorded SCA parameters.

both comparisons (group 2 to group 1 and group 3 to group 1). The obtained data indicate that distinct light sources modulated the effect of neurotransmitter 5-HT biological structures differently.

Specific data show that abnormal quantities of 5-HT are among the significant reasons for the emergence of neurological, mental or physiological disorders. 5-HT receptors are located mainly on postsynaptic cell



C

Changes in the SCA of the WOB group after treatment with 5-HT at 5 µM				*Changes in the SCA of the group 3 after treatment with 5-HT at 5 µM and irradiation with Laser 808 nm*			
parameters	*Relative increase*			*parameters*	*Relative increase*		
	Average value	*Percentiles 25%*	*Percentiles 75%*		*Average value*	*Percentiles 25%*	*Percentiles 75%*
BT	*3.48*	*3.45*	*3.53*	*BT*	*4.56*	*4.5*	*4.63*
BT + A_{mean}	*3.23*	*3.2*	*3.3*	*BT + A_{mean}*	*4.21*	*4.2*	*4.28*
AUC	*3.11*	*3.15*	*3.14*	*AUC*	*3.89*	*3.86*	*3.99*
Frequency	*1.00*	*1.00*	*1.00*	*Frequency*	*1.00*	*1.00*	*1.00*
P=0.001							

Fig. 2. Representative mechanograms of smooth muscle contraction: A) A mechanogram demonstrating the effect of 5-HT at concentration EC100 on the SM contractility of the control tissues; B) A mechanogram illustrating the effect of 5-HT at concentration EC100 on the SM contractility of the irradiated tissues with NIR light (Laser 808 nm); C) Statistical analysis of the recorded SCA parameters.

membranes mediating the effect of 5-HT [12]. Hyper or hypofunction of enzymes that degrade biogenic amines (like 5-HT) can affect their effectiveness. Monoamine oxidase, known as MAO, is an enzyme that catalyzes the oxidative deamination of amine neurotransmitters, which regulates their quantities [13]. Data reveals that ER modulates the activity of MAO. In the early '70s, authors reported alternations of MAO activity to ionizing

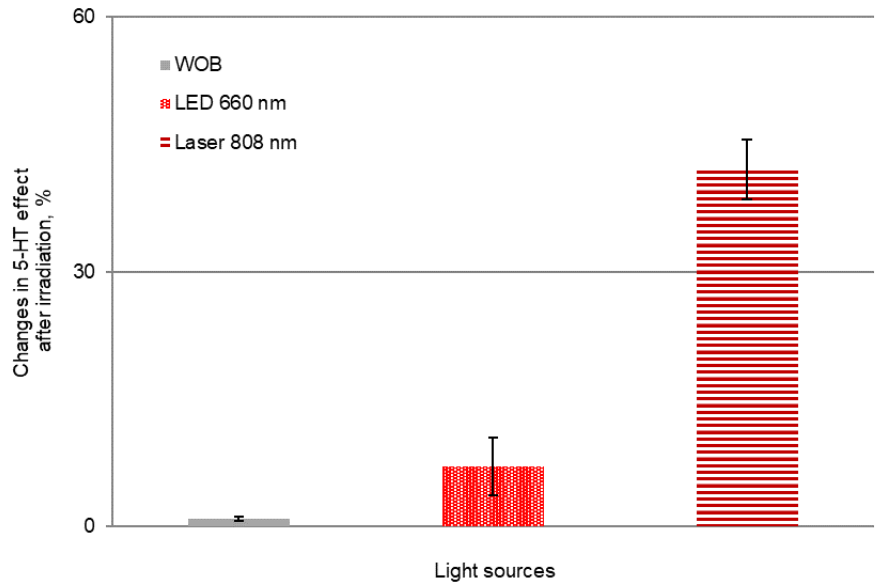


Fig. 3. Comparative diagram illustrates changes in the SM SCA in response to light sources emitting electromagnetic radiation at different wavelengths and exogenous treatment of 5-HT at a concentration of 5 μM. SM SCA parameters are presented as MEAN % ± STDV of three repetitions.

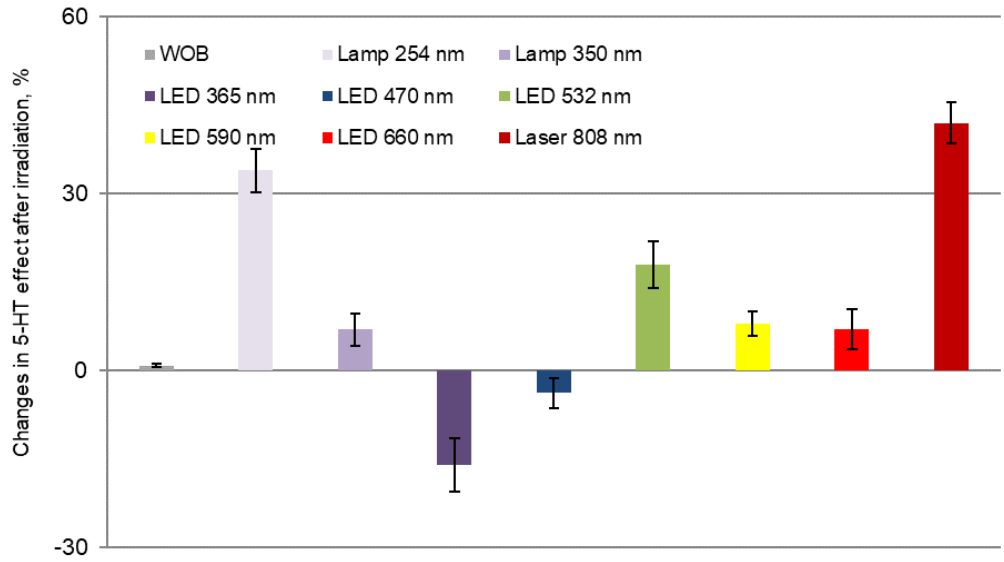


Fig. 4. Comparative diagram illustrating the change in the SM SCA in response to light sources emitting ER emitted at different wavelengths and exogenous ER of 5-HT at a concentration of 5 μM.

radiation [14]. Dolgaceva and colleagues investigated the effect of ER on the enzymatic activity of model animals and saw a clear correlation [15].

As a summary and for a better presentation of our results, we have provided a general graphic (Fig. 4), which presents a summarization of all investigated light sources and wavelengths on the reactivity of SMT to the treatment of 5 μM 5-HT. The same graphic contains

results from previously published papers [9, 10]. The light sources were the following: Lamp 254 nm, Lamp 350 nm, LED 365 nm, LED 470 nm, Laser 532 nm, LED 590 nm, LED 660 nm and a Laser 808 nm. The differences in the SCA of the SMT after irradiation with these light sources were apparent. Interestingly, sources such as LED 365 nm and 470 nm decreased the 5-HT effect on the SMT SCA, which meant that we still observed the SM strips' contraction after treatment of 5-HT, but it was less than this of the control ones.

Conversely emitters like Lamp 254 nm, Lamp 350 nm, Laser 532 nm, LED 590 nm, LED 660 nm and Laser 808 nm augmented the contractility of the SM strips. Our allegations were based upon the retrieved mechanograms, similar to Figs. 1A, 1B and 2A, 2B. A possible explanation of these findings could be the diverse sensitivity of the tissues to the different wavelengths and hence, the inversely influenced molecular pathways in the activity and metabolism of 5-HT. However, these possibilities to date are only hypotheses and need further molecular studies.

CONCLUSIONS

This study showcases changes in the contractility of SMT isolated from the corpus of rat stomachs when irradiated with LED 660 nm and Laser 808 nm in a wet medium. Our findings demonstrate that the designed WOB system regulates SM contractility induced by 5-HT and gives a deeper insight into the impact of different radiation wavelengths on the reactivity of SMT to 5-HT. Furthermore, we hypothesize that the light sources influence the activity of enzymes involved in the 5-HT metabolism disparately though further experiments are needed to validate this statement.

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