The Effect of 5-hydroxytryptamine on Smooth Muscles is Impacted by Broadband UV and LED UV and Blue Light

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Abstract: *Due to their effects, similar to low-intensity therapy light sources such as light-emitting diodes (LED) and broadband spectrum lamps have recently become commonly used in the diagnosis and treatment of neurodegenerative pathologies, cancer, as well as ageing. Despite the proven positive effects of such therapies, deeper understanding of the light therapies' biological effects remains unclear. Even more, the molecular mechanisms through which different neurotransmitters, namely serotonin (5-hydroxytryptamine, 5-HT), mediate the organism's response to radiation are yet indistinct. In this paper, we present the design and development of a specialized system for irradiation of biological objects, which is composed of LED 365 nm and LED 470 nm and a broadband lamp source of UVA/B (350 nm) with intensity, power density and direction, which can be optimized experimentally. The system, named a "water organ bath (wob)", is used in the current work to irradiate smooth muscle stomach strips of rats. The obtained results prove that the modulation of the spontaneous contractile smooth muscle activity and the potentiation of the effects of major neurotransmitters are executed by the emitted light. The probable explanation for the neurotransmitters photoactivation is that it is the resultant effect of electromagnetic radiation on intracellular enzymes signaling systems.*

Keywords: *Water organ bath, Electromagnetic radiation, Serotonin, 5-hydroxytryptamine, Light-emitting diodes.*

Introduction

The beneficial effects of light have made phototherapy an effective approach against neurodegenerative diseases, mental disorders, cutaneous conditions, neurological damages and ageing [1, 7, 16]. Findings have shown that light therapy has been effective in patients diagnosed with seasonal affective disorder (SAD) [21]. Interestingly, one of the factors contributing to SAD is increased reuptake of the extracellular serotonin (5-hydroxytriptamine or 5-HT) via the 5-HT transporter [29]. Patients who suffer from non-seasonal depression after phototherapy had augmented levels of serotonin in their bloodstream [23]. The reason for that, according to Tyrer and colleagues, were decreased levels of 5-HT transporter due to irradiation of patients [29].

The success of light-emitting diodes (LED) as light sources in phototherapy in various fields of medicine has made it widely applicable for treating numerous diseases. Examples of the effects of LEDs can be observed in patients with acute melatonin suppression, in rapid wound healing, for treating *acne vulgaris*, prevention of aging, etc. [32]. The emitted light from LED is monochromatic with different wavelengths, which define the depth of light penetration in biological tissues. Scientific data indicate a different degree of influence on biological tissues depending on whether the light is delivered in a pulsed manner or continuously [19]. LEDs have a significant impact on biological systems through two mechanisms. The generation of heat is the outcome of the first mechanism, due to the conversion of molecules' energy to kinetic such as rotation, translation and vibration. The second mechanism is known as "photochemical" based on the interaction between the light beam and the biological structures, which absorb the incident energy. Thus, the excited molecules move to higher energy levels [4, 20]. UV-LEDs are one of the LED sources often used in laboratory and clinical practice as they emit light with great intensity, independent on temperature. Blue (470 nm) and UV (365 nm) LED lights are known for their antimicrobial capabilities. Though UV and blue LEDs are used in clinical practice, they possess different physicochemical characteristics. For example, UV-light has the ability to break chemical bonds and thus make biomolecules reactive. Studies have shown that free radicals induced by UV-radiation disrupt the integrity of the tissues and the genome [28]. According to Halliwell and Gutteridge the oxidative stress, resulting from free radicals is one of the possible agents causing DNA damages [11]. The most dangerous DNA damages during irradiation are single and/or double-stranded breaks. Data reveal that the generation of DNA photoproducts, which affect processes such as replication and transcription, lead to mutations and ultimately to cell death or malignant transformation [5]. On the other hand, the effectiveness of UV-radiation depends on the wavelength chosen for a specific purpose. For instance, UVA (400-315 nm) has greater wavelength than UVB (315-280 nm) and UVC (280-200 nm) that leads to lower penetration ability of the light. Stimulation of immune system and promotion of wound healing are some of the UV-light's effects [10]. Conversely, blue light is less harmful than the UV radiation and induces lower levels of photodegradation than UV light [14]. LED blue (470 nm) light treats superficial conditions due to its smaller penetration ability – 1mm the most [3]. Authors declare the improvement of thicker lesions such as psoriasis after irradiation with blue light [31]. Clinical studies have shown the positive effect of blue light on *Acne vulgaris*. Moreover, patients suffering from peptic ulcers caused by *Helicobacter pylori* were treated with blue light [18]. In disorders such as the irritable bowel syndrome, diarrhea, or functional dyspepsia, the detected profound abnormalities in 5-HT concentrations were modulated by phototherapy [25]. Data show that phototherapy with LED sources is a good alternative for combatting gastrointestinal pathologies [26]. The contractility of the gastrointestinal tract (GIT) organs is occurring involuntarily as the process initiates after the increase in intracellular concentration of calcium ions. The augmentation of cytosolic Ca^{+2} is due to Ca^{+2} – influx through L-type Ca^{+2}

channels and calcium release from the sarcoplasmic reticulum of the smooth muscle cells (SMC) [30]. The enzyme calmodulin when activated by intracellular Ca^{+2} phosphorylates the 20-kDa light chain of myosin leading to cross-bridges between thick and thin myofilaments. Neurotransmitters such as acetylcholine (ACh), serotonin (5-HT) and others participate actively in the control of smooth muscle contractility. The involvement of enzymes such as protein kinase C, diacylglycerol, and inositol 1,4,5-trisphosphate in mediation of smooth muscle contraction occurs when neuromodulators bind to receptors located on the cell membrane of SMC. Serotonin also known as 5-hydroxytryptamine or 5-HT is found primarily in the enteric system. It is synthesized by the enterochromaffin cells and acting as a mediator it regulates secretion, peristalsis and vasodilation of the GIT [17]. Scientific evidence reveals that the stimulation of cholinergic neurons by 5-HT leads to release of ACh, which results in smooth muscle contraction [8]. 5-HT being a signaling molecule provokes peristaltic reflexes after stimulation of both vagal and intrinsic afferent nerve fibers [6]. Data demonstrate direct association between gastrointestinal motility and fast and slow excitatory neurotransmission mediated by 5-HT [8].

Here, we present the development and experimental design of a wet organ bath (wob) for photoactivation of smooth muscle tissues under controlled conditions. The specialized system for irradiation of biological objects is composed of LED sources (LED 365 nm and LED 470 nm) and a broadband lamp source, namely UVA/B (350 nm) with intensity, power density and direction, which are optimized experimentally.

Materials and methods

Chemicals and reagents

Acetylcholine and 5-hydroxytryptamine were purchased from Sigma.

The substances were dissolved in distilled water. For the preparation of Krebs solution (KS) were used (Na^+ – 143 mmol/L; K^+ – 5.84 mmol/L; Ca^{2+} – 2.5 mmol/L; Mg^{2+} – 1.19 mmol/L; Cl^- – 133 mmol/L; HCO_3^- – 16.7 mmol/L; $H_2PO_4^-$ – 1.2 mmol/L and $C_6H_{12}O_6$ – 11.5 mmol/L).

Animals

Male Wistar rats were used weighting approximately 250 g. They were bred under standard laboratory conditions (temperature 22 °C ± 1 °C, humidity 45%, 12 h dark/light cycle, food and water *ad libitum*). All the experiments were conducted 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. Along with these requirements additional approvals from the Bulgarian Food Safety Agency (permission number: 229/09.04.2019) and the Ethics Committee of the Medical University – Plovdiv, Bulgaria (Protocol number: 145/09.04.2019) were issued too.

Smooth muscle strips

Smooth muscle (SM) strips were dissected from the corpus of the stomachs of euthanized male Wistar rats and were divided into four groups. The first group was considered the "control group" and composed of SM preparations that were not irradiated. Muscle strips irradiated with the broadband UVA/B light source constituted the second group. SM preparations exposed to LED UV and LED blue radiation formed the third and fourth groups, respectively (Table 1).

Table 1. Description of the rat SM sample groups used in the study

Samples	Sample groups
Control – non-irradiated SM strips	Group 1
SM, irradiated with broadband lamp emitting UVA/B (350 nm)	Group 2
SM, irradiated with LED UV light (365 nm)	Group 3
SM, irradiated with LED blue light (470 nm)	Group 4

Tissue irradiation with electromagnetic light in the wet organ bath

Wet organ bath

To avoid specific absorption of the electromagnetic irradiation in the KS in which the preparations were submerged, wet organ bath was applied [15]. The studies of spontaneous contractile activity (SCA) of the SM strips were conducted under conditions without buffer for a period of 2 min. No alterations in the parameters of SCA occurred. In a period of 5, 10, 20 and 30 min after the exposure of the sample to 2 min in the wob, the reactivity of SM tissues to 5-HT (5 μM) after the application of ACh (10 μM) has been assessed. The ACh allowed the measurement of SM preparations' maximal response to 5-HT. The mechanograms with ACh are not shown, as these experiments are control experimentations for the vitality of the tested smooth muscles.

Parameters of irradiation with the broadband UV, and blue and UV LED light sources

The light sources in the current work were light-emitting diodes (LEDs) and a broadband UV lamp. The LEDs functioned with constant power of 3 W while emitting light at a wavelength of 470 nm and 365 nm. Alternatively, the broadband lamp's power was 6 W and it emitted UV radiation at a wavelength of 350 nm. SM strips from the corpus of rat stomachs were irradiated with UVA/B, LED UV and LED blue light for 60 sec while the effect was reported at the 15^{th} min after the act. The power density of each light source was 4 mW/cm^2 as the intensity meter device measured the energy per sec. The LED sources and the lamp were equipped with an autonomous power supply and an optical system enabling the collimation of the electromagnetic energy flow and ensuring the desired power density. A programmable unit regulated the exposure of the light beam to comply with the experimental protocol.

Registering the smooth muscle contractility: parameters

SM strips were prepared from the stomach corpus of 18 male Wistar rats having a length of 20.0 mm ± 1.5 mm without violating the mucus layer, randomly put in organ baths, prefilled with 15 mL modified Krebs' solution, oxygenated with 95% O_2 and 5% CO_2 at 35.5 ± 0.3 °C. A three-channel interface system performed analysis and registration of the contractility of the SM samples as the record of the normal contractile activity started after the equilibration period. The baseline tone and relative change in the muscle contraction were analyzed for a 5 min period and were used for further comparative analysis. To investigate the effect of ACh (10 μM), the substance was added to the organ baths and the alterations of the SM strips' spontaneous activity were recorded for 5 min. After each trial, the solution in the organ baths was flushed and replaced with a new one in which ACh was added to 10 μM final concentration to test the SM preparations' contractile response by activation of cholinergic receptors.

Exogenous application of serotonin and effect on smooth muscle contractility

With the usage of exogenous application of 5-HT at a concentration of 0.005 mM corresponding to EC_{100}, the studies were conducted to determine the effect of 5-HT on the SCA of SM strips. The reason for using EC_{100} is that at this concentration the SM preparations demonstrated

maximum contractility. All the above-mentioned parameters were analyzed at the same time and were represented in percentage of 10^{-5} M ACh reaction.

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 post-hoc test were employed for multiple comparison analysis. The results were reported as MEAN ± SEM. The number of tested preparations is given as *N*. At $p < 0.05$, the results were considered significant.

Results and discussion

Some authors verified the fundamental role of 5-hydroxytryptamine in the proper functionality of the gut by demonstrating profound abnormalities in 5-HT concentrations in many gastrointestinal disorders such as irritable bowel syndrome, diarrhoea or functional dyspepsia [25]. Therefore, we wanted to compare the role of 5-HT in gastric contractile activity under irradiation of rat SM strips with different light sources. For the purpose, we have developed an original system for irradiation of biological tissues allowing precise irradiation of SM strips in *in vitro* conditions. The advantages of the developed system were optimization of the exposure time of SM tissues and the dose of the electromagnetic energy that they absorbed [33]. The difference between the classical organ bath and wob was that the irradiation in the wob was through air.

The contractile activity of Group 1 and 2 SM strips (exposed to broadband UVA/B light source with a wavelength 350 nm) was studied and results are shown as mechanograms in Fig. 1.

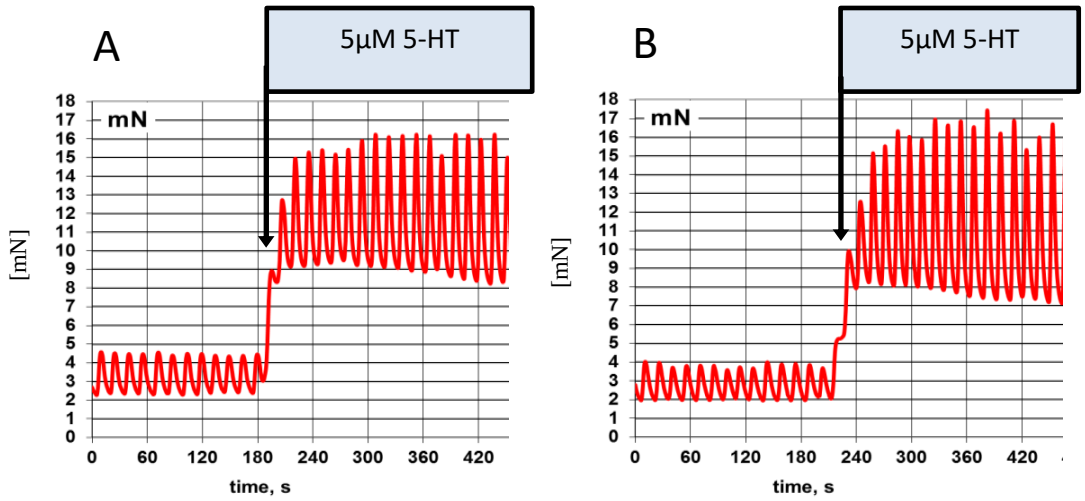

Fig. 1 Mechanograms, illustrating SM contractile activity in response to broadband UVA/B (350 nm) light source and exogenous application of 5-HT at a concentration 5 μM (5×10^{-6} M): A) A representative mechanogram, showing the effect of 5-HT on the contractility of control SM strips – Group 1; B) A mechanogram, demonstrating the 5-HT impact on contractility of irradiated SM preparations with broadband UVA/B light source – Group 2. The axis *x* in all mechanograms represents the time measured in seconds, while the ordinate y – the force of contraction measured in mN.

Fig. 1A represents the spontaneous contractile activity (SCA) of the tissues before irradiation – Group 1, while Fig. 1B demonstrates the way the SCA of the SM changed after irradiation with the broadband lamp light source (Group 2 SM strips). In both cases exogenous application of

5-HT to a final concentration of 5 μM (5×10^{-6} M) was applied. The comparison between the two mechanograms on Fig. 1 demonstrates that the SCA after application of 5-HT differed significantly between Groups 1 and 2. The irradiated SM strips reacted with 1mN stronger than the control ones. The statistical analysis demonstrated increased levels of the detected contractile activity in all SCA parameters, with statistically significant differences between Group 1 and Group 2 ($p < 0.001$) (Table 2). The results were presented in percentages and for Group 2 the detected increase in the SM SCA after UVA/B irradiation was approximately 8% higher than in Group 1.

Table 2. Statistical analysis of our results verifies the significant differences between all the groups after application of 5-HT at 5×10^{-6} M and irradiation with the three light sources

Bonferroni's multiple comparison test	**Mean difference**	***p*-values**
Group 1 vs Group 2	-7.338	$p < 0.001$
Group 1 vs Group 3	15.54	$p < 0.001$
Group 1 vs Group 4	5.575	$p < 0.001$
Group 2 vs Group 3	22.88	$p < 0.001$
Group 2 vs Group 4	12.91	$p < 0.001$
Group 3 vs Group 4	-9.963	$p < 0.001$

The next step was to investigate the reactivity of Group 3 SM strips using the same concentration of exogenous 5-HT after exposure to LED UV light source (365 nm). The obtained results are shown on Fig. 2, where two representative mechanograms of the SCA of SM strips from Group 1 and 3 are presented.

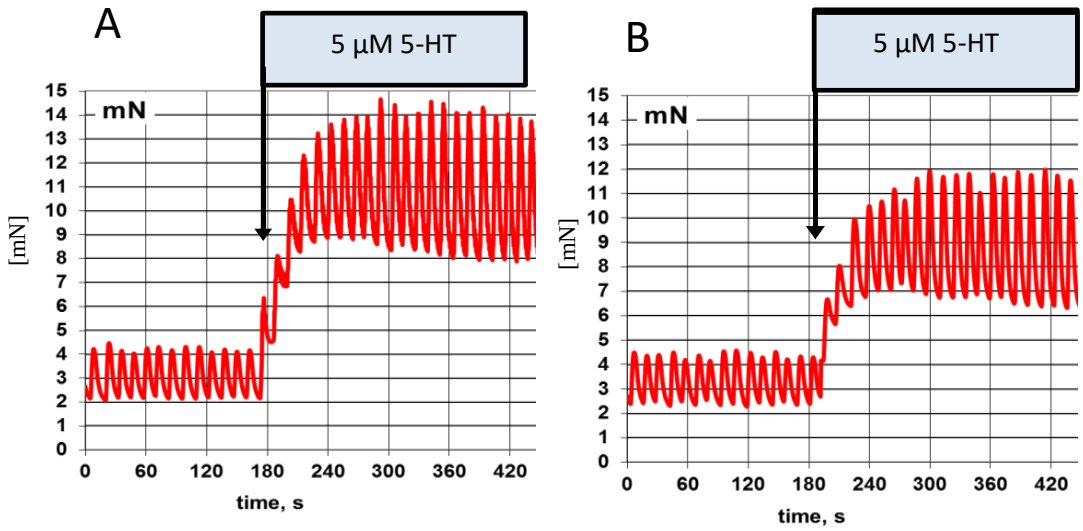

Fig. 2 Mechanograms, illustrating SM contractile activity as response to LED UV (365 nm) radiation and exogenous application of 5-HT at concentration 5 μM (5×10^{-6} M):
A) A mechanogram, showing the effect of 5-HT on the contractility of control SM strips from Group 1; B) A mechanogram demonstrating the 5-HT impact on contractility of irradiated SM preparations with LED UV light – Group 3.

The performed comparative SCA data analysis showed that with the LED UV irradiation the SM strips of Group 3 decrease by 15% in the contractile activity in response to the exogenous application of 5-HT. The mechanograms in Fig. 2 show a decrease in the SCA of about 2 mN

in the SCA of SM strips of Group 3 in comparison to Group 1. This comparison demonstrates a statistically significant reduction in the reactivity of SM strips of Group 3 to 5-HT after irradiation with LED UV ($p < 0.001$, Table 2). Apparently, the LED UV light induced a decrease in the contractions of Group 3 SM strips.

The reactivity of the control and irradiated SM tissues with LED blue light to 5-HT, applied at 5 µM was further examined. Fig. 3 shows the SCA of Group 1 and Group 4. The mechanograms show the magnitude with which electromagnetic radiation affected the reaction of SM preparations to 5-HT. The reactivity of the irradiated samples to 5-HT was significantly reduced than this of the wob samples Group 1 ($p < 0.001$) (Table 2).

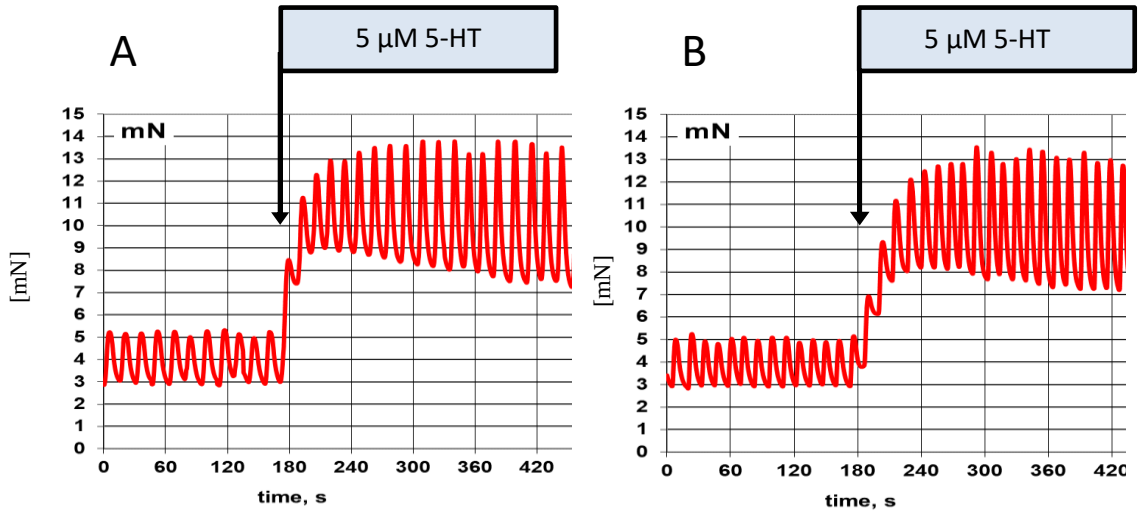

Fig. 3 Mechanograms, illustrating SM contractile activity in response to LED blue (470 nm) radiation and exogenous application of 5-HT at concentration 5 µM (5×10^{-6} M):
A) Representative mechanogram showing the effect of 5-HT on the contractility of control SM strips – Group 1; B) Mechanogram demonstrating the 5-HT impact on contractility of irradiated SM preparations with LED blue light – Group 4.

A possible explanation of these phenomena could be explained by an increased activity of enzymes that regulate 5-HT concentrations upon LED UV and blue light photoactivation. However, this hypothesis needs further studies.

In order to compare the biological effects of the three lights on SM contractility at the exogenous application of 5-HT at a concentration of 5×10^{-6} M, we calculated the percentage of SM contractility for all four groups and compared each group with all others. Fig. 4 illustrates the reactivity of SM samples in percentage. The results show an increase in SM strips' reactivity to 5-HT after exposure to radiation emitted by the broadband lamp at a wavelength of 350 nm by 8.1% ± 2%, while the two LED sources: the UV and the blue LED light emitters decreased the 5-HT-induced contractions of SM tissues by 14.75% ± 4% and 4.78% ± 1.5%, respectively.

The detected differences between the SCA of the tested groups were statistically significant ($p < 0.001$) when compared to the control (Groups 2, 3 and 4 versus Group 1) and between each other as seen in Table 2.

In accordance with the latest data analyses, depression has become one of the most common diseases worldwide. Studies indicate how the deprivation of blue light ameliorates the mental

state of patients by augmenting the 5-HT levels in brain [13]. A confirmative number of papers reveal the association of 5-HT with various neurological, mental or neurodegenerative conditions. In all these cases, the 5-HT levels are abnormal according to various reports [2]. The most common treatment against these disorders is drug therapy, in particular antidepressants. Importantly, therapies in which light is the key player represent a good substitution in curing mood disorders like SAD [22] or disturbances in circadian rhythms [24]. In gastroenterology, ionizing radiation is used for treating and diagnosing cancers. In cases of chemotherapy, according to Spiller emesis was prevented using 5-HT_3 blockers [27].

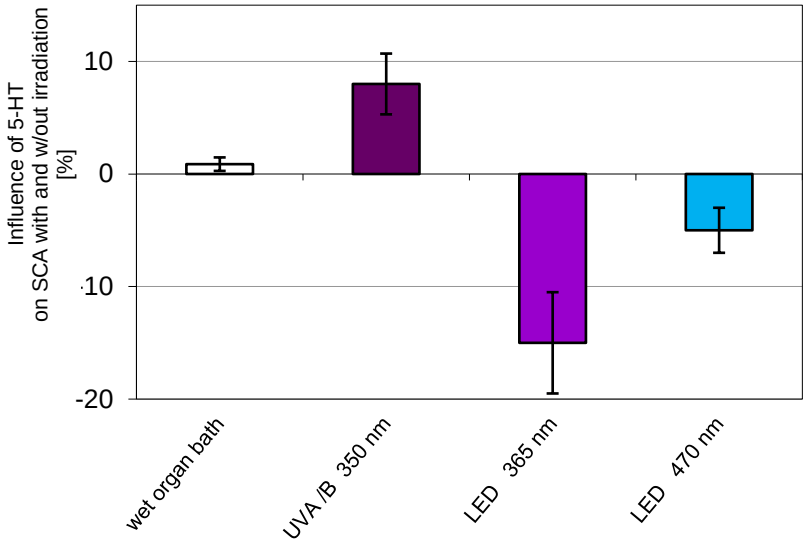

Fig. 4 Comparative diagram, illustrating the change in the SM SCA in response to broadband UVA/B light emitter (350 nm), LED UV (365 nm) and blue (470 nm) radiation and exogenous application of 5-HT at a concentration 5 μM (5×10^{-6} M)

Additionally, other authors showed that non-ionizing radiation was a good tool against *Helicobacter pylori*, which leads to gastric adenocarcinoma [12]. Our *in vitro* studies on reactivity of SM tissues dissected from rat stomach to 5-HT show that the alternations in 5-HT levels resulted from the interaction of different wavelengths of the electromagnetic radiation with the biological structures. In any case, we believe that light can regulate the expression of 5-HT.

Conclusions

In this paper we present the design and development of a specialized system for irradiation of biological tissues, which consists of a wob installation for irradiation of samples and broadband light and LED sources (UVA/B and LED UV and blue) with intensity, power density and direction that can be optimized experimentally. We demonstrate that this system allows modulation of SM contraction via direct interactions of the light with the biological tissues without any waste of energy in the buffer used under conventional conditions. Moreover, the wob allows the potentiation of the biological functions by activating neuronal 5-HT-controlled pathways. Our results show that the broadband lamps emitting light at a wavelength of the UVA/B spectrum increased the effect of 5-HT on the SCA of rat stomach SM samples, while the incoherent radiation with wavelengths LED UV 350 nm and LED 470 nm reduced the 5-HT effect on SM strips. This suggests different biological mechanisms of the modulation of the SM contractility induced by the exogenous application of 5-HT and under different

irradiation conditions. The latter indicates the need for further experiments that aim at the elucidation of these molecular mechanisms for the eventual development of phototherapy.

Abbreviations

LED, light-emitting diodes; 5-HT, 5-hydroxytryptamine; wob, water organ bath; ACh, acetylcholine; SAD, seasonal affective disorder; GIT, gastrointestinal tract; KS, Krebs' solution; SCA, spontaneous contractile activity; SM strips, smooth muscle strips.

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