PHYSIOLOGY

Effect of Laser Irradiation on Adrenoreactivity of Pial Arterial Vessels in Rats

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Experiments on WKY and SHR rats showed that low-intensity laser irradiation reduced the tone of pial arterial vessels thereby potentiating the subsequent constrictor effect of norepinephrine. Irradiation in the red region of the spectrum produced a more pronounced effect in the blue region. The observed effects were less pronounced in SHR rats compared to normotensive WKY rats.

Key Words: *laser radiation; pial arterial vessels; vascular tone; adrenoreactivity*

Laser therapy based on stimulating action of low-intensity laser radiation (LILR) on microcirculation is widely used in the therapy of various diseases. The mechanisms of LILR stimulation are still not clearly understood, though many hypotheses were put forward [2,3-5,11]. New data are accumulating supporting current views on the dependence of light-induced activation at wavelengths affecting some primary chromophores and on absorption of radiation in some spectrum regions by biological tissues [1,5,6,12].

Our aim was to examine the effects exerted by blue (λ=473 nm) and red (λ=650 nm) LILR on the arterial tone in *pia mater* (PM) of normotensive and spontaneously hypertensive rats. In addition, we tried to assess the effects of LILR on adrenoreactivity of the pial arteries.

MATERIALS AND METHODS

The experiments were carried out on normotensive WKY rats weighing 230-300 g (n=16) and spontaneously hypertensive SHR rats weighing 210-345 g (n=16) under intraperitoneal urethane narcosis (150 mg/100 g body weight). In narcotized WKY and SHR rats, the mean blood pressure (BP) was 86 and 125 mm Hg, respectively. Irrigation of PM with norepinephrine solution (2×10^{-5} g/ml) produced no effect on BP. The irrigation system could rapidly exchange the superfusing physiological solution for the norepinephrine solution and *vice versa*.

Two series of experiments were carried out with laser radiation in blue (λ=473 nm) and red (λ=650 nm) regions. In both series, the lasers with output power of 20 mW were used (irradiation intensity of 20 mW/cm^2).

The experimental protocol was as follows: *1*) the baseline diameters of pial vessels were recorded during irrigation of the brain surface with physiological solution; *2*) the brain surface was irrigated with norepinephrine solution, and the vascular reactions were recorded on minute 1 after the onset of irrigation (in superfusion system, the physiological saline was temporarily changed for the norepinephrine solution); *3*) PM was irradiated for 5 min during irrigation of PM with a temperature-stabilized physiological solution (to prevent the heating action of laser radiation); *4*) the vascular reactions were recorded immediately after

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irradiation; *5*) the brain surface was irrigated with norepinephrine solution, and the vascular reactions were recorded on post-irrigation minute 1.

The responses of PM arteries were examined by intravital microphotography (×470) [8]. These responses were recorded in the bed of medial cerebral artery, where the diameter of pial arteries ranged 34-171 μ and 23-178 μ in WKY and SHR rats, respectively. The number of measured sites in pial arteries was 456. The changes of the lumen of pial arteries and the number of constricted or dilated sites were determined using Matrox Inspector software [8], thereafter the total section area (TSA) of the examined sites was calculated. To assess the changes in vascular reactivity, the number of examined arterial sites and their lumens were normalized to 100 and 1, respectively. Thus, 100 arterial sites each with a diameter of 1 rel. unit (RU) corresponded to a standard section $100\times\pi(d/2)^2=100\times3.14(0.5)^2=314\times0.25=78.5$ square rel. units. This standard section area (SSA) was taken as 100%. The changes of TSA of the examined arteries in response to a stimulus (measured relatively SSA) characterized vascular reactivity. In such tests, it was possible to evaluate the individual contributions of dilated, constricted, or unchanged arterial sites to TSA. The data were processed statistically using Microsoft Excel 2002 statistical software.

RESULTS

In normotensive rats, irradiation of PM with 473-nm laser increased TSA of pial arteries to 113.40±3.26% ($p\leq0.05$), but produced no effect in hypertensive rats (101.22±6.48%). Irradiation of PM with laser light at 650 nm increased TSA to 132.78±7.66% ($p\leq0.05$) and 117.24±5.97% ($p\leq0.05$) in normo- and hypertensive rats, respectively (Fig. 1, *a*). Analysis of contributions of various reactions into changes in TSA showed that irradiation at 473 nm induced dilation of the majority of examined arteries in normotensive rats and their contribution to TSA was 59% (Table 1). After irradiation of PM at 650 nm, TSA of the dilated arteries was 78% (Fig. 1, *b*). In hypertensive rats with PM irradiated at 473 nm, the shares of constricted and dilated sites were virtually identical (25 and 27%, respectively), while about half TSA (48%) was contributed by nonresponsive arteries. After irradiation of PM at 650 nm, the contribution of dilated area into TSA was 50% (Fig. 1, *c*). Thus, the increase in TSA of the examined arteries in response to irradiation resulted from dilation, and this effect was more pronounced in the red region of the spectrum (at 650 nm) in comparison with blue light (473 nm); in addition, it was more pronounced in normotensive rats.

Irrigation of PM with norepinephrine solution constricted the pial arteries in the rats of both lines so that TSA decreased by 5-10% (Table 2). At this, the contribution of the constricted arteries in TSA was 34% and 26% in normotensive and spontaneously hypertensive rats, respectively.

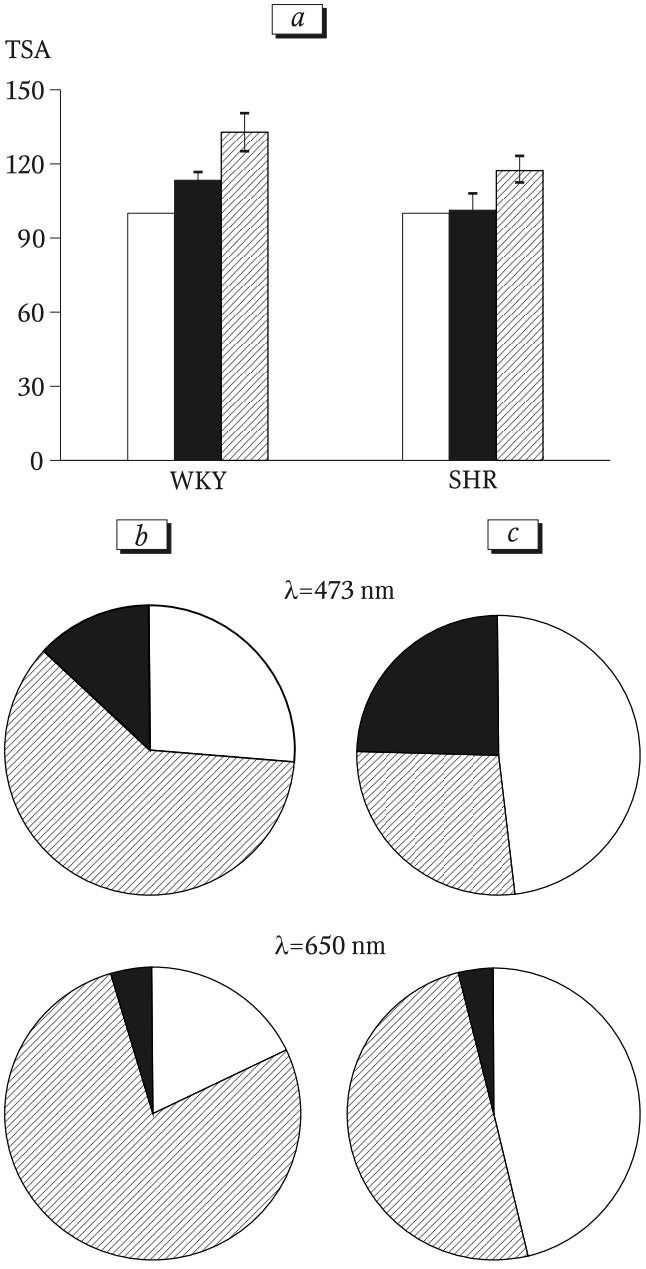

Fig. 1. Responses of pial arteries to irradiation of PM at 473 and 650 nm (*a*) and contribution of different radiation-induced reactions into total section area of examined vascular segments in WKY (*b*) and SHR (c) rats. *a*) Open bars: the standard section area; closed and hatched bars: total section area of examined arterial sites after irradiation at 473 nm and 650 nm, respectively. *b*) and *c*) open area shows contribution of nonresponsive sites; the closed and hatched areas show contribution of the constricted and dilated sites, respectively, in percentage to total section area of the examined arterial sites.

After preliminary irradiation of PM in rats of both strains with blue laser (473 nm), application of norepinephrine decreased TSA by 6-7% demonstrating approximately the same constrictor effect as in control. However, after preliminary irradiation at 650 nm, the constrictor effect was significant: TSA in normotensive and hypertensive rats decreased by 22 and 11%, respectively (Table 2). In normotensive rats, preliminary irradiation of PM at 473 nm increased contribution of the constricted arteries to TSA from 34% (control value) to 39% (after norepinephrine application) and halved the contribution of dilated arteries from 23% (control value) to 10% (norepinephrine). Similar values for the normotensive rats irradiated at 650 nm were 34 to 73% (constricted arteries) and 23 to 10% (dilated arteries), respectively (Table 1). In spontaneously hypertensive rats, blue light produced no significant effect on the constrictor action of norepinephrine, but the share of the constricted arteries in TSA increased from 26 to 35% (in comparison to the control value) due to a decrease in the contribution of the nonresponsive arteries in TSA. However, the preliminary irradiation of PM at 650 nm exerted greater effect on the action of norepinephrine. Now contribution of the constricted arteries into TSA increased from 26 to 37% (in comparison with control) while the contribution of dilated arteries decreased from 14 to 9%.

Thus, in both rat strains, potentiation of norepinephrine-induced vascular constriction by red light resulted from recruitment of previously dilated or nonresponsive arteries into the constrictor reaction.

When comparing the responses of pial arteries to norepinephrine expressed by TSA relatively not to SSA (as in the above estimates), but to TSA obtained immediately after irradiation and before application of norepinephrine (Table 3), the constrictor effect of this agent would be expressed even stronger.

Potentiation of norepinephrine-induced constrictor effect by irradiation of PM is probably related to weakening in the vascular tone caused by irradiation. Red light weakened vascular tone more pronouncedly than blue light, which probably explains the fact that the constrictor effect of norepinephrine was greater after irradiation of PM with red light than with the blue one. According to literature, red irradiation affects the endogenous porphyrins of the cell membranes, endotheliocytes included [3,4]. The porphyrins are known as photosensitizers. By absorbing LILR, they induce LPO in cell membranes resulting in increase in calcium permeability. Elevation of intracellular calcium concentration augments functional activity of the cell and up-regulates synthesis of biologically active substances (NO included) which finally lead to vasodilation. The acceptors of blue radiation are the hemoprotein nitrosyl complexes such as hemoglobin [4].

TABLE 1. Contribution of Different Responses of Pial Arteries to Norepinephrine into TSA Prior (Control) and After Irradiation of PM in WKY and SHR Rats

Index		WKY	SHR
After irradiation (λ=473 nm)	Dilated sited	58.88^{+}	27.26
	Constricted sites	12.60^{+}	24.67
	Nonresponsive sites	28.52	48.07^{+}
After irradiation (λ=650 nm)	Dilated sited	77.59^{+}	50.10
	Constricted sites	4.34	3.88^{+}
	Nonresponsive sites	18.07^{+}	46.02
Response to norepinephrine prior to irradiation	Dilated sited	22.90	14.20
	Constricted sites	33.70	26.40
	Nonresponsive sites	43.40	59.40
Response to norepinephrine after irradiation (λ=473 nm)	Dilated sited	10.37*	14.63
	Constricted sites	39.26	34.78*
	Nonresponsive sites	50.37	50.59
Response to norepinephrine after irradiation (λ=650 nm)	Dilated sited	10.40*	8.50
	Constricted sites	72.84*	36.51*
	Nonresponsive sites	16.76*	54.99

Note. $p \leq 0.05$: $^{+}$in groups of arteries with various responses; *in comparison with the reaction to norepinephrine prior to irradiation (in control).

TABLE 2. Reaction (TSA Relatively SSA) of Pial Arteries to Norepinephrine Prior to (Control) and After Irradiation at λ=473 nm and λ=650 nm in WKY and SHR Rats

Stage of experiment		WKY	SHR
After irrigation of PM with norepinephrine		90.00±3.19	95.00±2.24
After preliminary irradiation followed by irrigation of PM with norepinephrine	473 nm	92.56±2.65	94.38±3.57
	650 nm	77.55±3.37	88.54±1.67

TABLE 3. Reaction (TSA) of Pial Arteries (in Percentage to TSA Measures Immediately After Irradiation) to Norepinephrine After Irradiation at λ=473 nm and λ=650 nm in WKY and SHR Rats

Stage of experiment		WKY	SHR
After preliminary irradiation followed by irrigation of PM with norepinephrine	473 nm	81.61±2.34	93.24±8.47
	650 nm	58.41±2.54	75.52±1.42

Blue radiation induces decay of the nitrosyl complexes of erythrocytic hemoglobin HbNO with the release of free NO and activation of soluble guanylate cyclase, which means releasing NO in erythrocytes. Probably by this reason, blue radiation produced no significant weakening in pial arterial tone, so the following changes in adrenoreactivity were rather moderate.

Thus, LILR in both red and blue spectrum regions weakened the tone of pial arteries thereby augmenting the following constrictor effect of norepinephrine. Comparison of LILR effects in blue and red spectrum regions showed that red light produced more pronounced effect on the pial arteries and modulated the adrenergic reactions in these vessels to a stronger degree.

Comparison of the vascular reactions in normo- and spontaneously hypertensive rats showed that in hypertensive animals the effect of laser irradiation was smaller than that in the normal ones. This observation can be explained by the appearance of genetically determined defects in the cell membranes during chronic hypertension that are manifested by insufficient membrane control over cytoplasmic calcium concentration [7,9,10] required for activation of NO synthesis. According to some researchers [3,4], namely NO released due to interaction between laser radiation with the primary chromophores such as endogenous porphyrins and hemoprotein nitrosyl complexes results in vasodilation and improvement of microcirculation, which does not occur deterministically in spontaneously hypertensive animals.

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