Increase in peripheral blood flow due to extracocular direct irradiation of visible light in rats

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Although there have been reliable experimental efforts to prove the existence of extracocular photosensitivity (23), it has not been believed for a long time (6). Thus the therapeutic effect of the visible light has been at issue (11) despite the fact that the low-power laser irradiation was shown to be successful in treating atrophic ulcers and indolent wounds (9, 12, 16). We hypothesized that visible light irradiation directly applied to the peripheral site increases the peripheral blood flow with the open-close sequence such as fluctuation by heart pulses, respiration, vasomotion (21), and other noises. To avoid the retinal illumination, the plastic box was covered with thick black cloths. The duration of the measurement was 10–20 min. The shutter time (one-half of the open-close period) was 3 s, with an open-to-close time ratio of 1:1. To clarify the implication of nitric oxide (NO) from sixfold coordinated NO-Hb causes vasodilation is postulated for the light-induced blood flow increase.

MATERIALS AND METHODS

Animals. Male Wistar strain rats weighing ~250 g were used. They were housed in a room in which temperature was maintained at 24 ± 1°C, and the room was illuminated for 12 h (0700–1900) by fluorescent tubes (80 lx). Food (type MF; Oriental Yeast) and water were freely available. These rats were adapted to the environmental conditions for at least 1 wk before the experiment.

Experiments. On the experimental day between 1100 and 1600, the rat was fixed in an acrylic resin box with absorbent cotton as a filling up plug. The tail of the rat, which was located outside the plastic box, was fixed by adhesive tape. The monochromatic light was irradiated at the center part of the tail. For the measurement of blood flow, a needle-type Laser Doppler blood flowmeter (ALF21; Advance) was set at the center of the irradiation area. This type of blood flowmeter is capable of continuously monitoring blood flow in the capillaries in the dermis from outside the skin (2). A narrow band-pass filter was inserted in the return path of the probe light (780 nm) to eliminate the mixing of the irradiated light. The blood flow data were sampled at 200 Hz and were averaged synchronously with an open-close signal of the shutter. The processing reduced incoherent variation of the blood flow with the open-close sequence such as fluctuation by heart pulses, respiration, vasomotion (21), and other noises. To avoid the retinal illumination, the plastic box was covered with thick black cloths. The duration of the measurement was 10–20 min. The shutter time (one-half of the open-close period) was 3 s, with an open-to-close time ratio of 1:1. To clarify the implication of nitric oxide (NO), which dilates blood vessels, the effects of administration of 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; Wako Chemicals), an inhibitor of guanylate cyclase, and L-arginine methyl ester, which are inhibitors of nitric oxide synthase, were examined. In the
experiments, changes in blood flow because of the irradiation of 575-nm wavelength lights were compared in groups of rats injected with these agents vs. rats injected with buffers only. Rats with the body weight range of 260–330 g were used in each group. ODQ was dissolved in DMSO and diluted with PBS (pH 7.4), and 2 mg/kg of ODQ were injected intraperitoneally. L-NMMA and L-NAME were dissolved in PBS, and 10 and 30 mg/kg of L-NMMA and L-NAME, respectively, were administered in the peritoneal cavity of rats (15). The same volume of each solvent (vehicle) for ODQ, L-NMMA, and L-NAME was given to control animals. These agents were injected 20 min before the start of the irradiation and nonirradiation cycle. The effects of the vasodilating agents sodium nitroprusside (SNP), S-nitroso-N-acetylpenicillamine (SNAP; both are NO donors), and papaverine (a cAMP synthesis inhibitor) on peripheral blood flow were examined for comparison. Dosages were 0.2, 0.5, and 0.1 mg/kg for SNP, SNAP, and papaverine, respectively. PBS was used as their solvent. The procedure was as follows. First, the basal blood flow was measured at 10 min (baseline). Next, the agent was injected intraperitoneally. After a 5-min resting period, blood flow was measured again for 5–10 min (administration). Average values of blood flow during the above two periods were calculated for each rat, and statistical significance between the baseline value and administration was tested. All measurements were performed in the dark.

RESULTS

Figure 1 shows examples of changes in the blood flow due to visible light (575 nm) irradiation of the rat’s tail during irradiation and nonirradiation periods with various irradiation power densities. The ordinate shows the percentages of the blood flow increment (dB/B₀) per power density (W). Here, B₀ is baseline blood flow, which is the average of blood flow over 0.1–0.5 s of duration before the shutter opens in each on-off sequence. The dB/B₀ per W rises rapidly just after the shutter status changes in both irradiation and nonirradiation periods, as shown in Fig. 1. The percentage of blood flow increase in the irradiated period [dB(L)/B₀W] shows slower decay than that in the nonirradiated period [dB(D)/B₀W]. The most prominent feature of the phenomena is that both dB(L)/B₀W and dB(D)/B₀W are strongly dependent on the power density. They decrease with increasing power density of the irradiation.

Figure 2 shows the wavelength dependence of the average of dB(L)/B₀W over the shutter time (one-half of the open-close period), ⟨dB(L)/B₀W⟩. These data were obtained by the measurements with irradiation power densities, where the saturation effect was not severe (less than ca. 0.5 mW/cm² for 540- to 580-nm bands and ca. 0.05 mW/cm² for 410- to 420-nm bands). The action spectrum shows the peaks in the regions at 410–420, 540–550, and 570–580 nm. The extent of the increase was about one order of magnitude higher with the light of 410–420 nm than with 540–550 nm and 570–580 nm. The peak positions and intensity ratios among the peaks are very similar to those in the optical absorption spectrum of low-spin ferrous heme complexes such as NO-Hb and NO-myoglobin (17, 18). Data in Fig. 2 indicate the absorption peak positions and the extinction coefficient (in relative scale) of NO-myoglobin (17). The same type of photosensitive reaction of forearm skin blood flow is observed in our preliminary experiment with the two human subjects (data not shown).
dB/B_0W in both the irradiated and nonirradiated periods showed a rapid increase after changing of the shutter status. There was a slower decrease in human subjects after the peak levels were reached than in rats. The action spectrum for dB(L)/B_0W in humans also exhibited peaks at 410–420, 540–550, and 570–580 nm (data not shown) with relative intensities similar to those seen in the case of rats (Fig. 2).

Next, we examined the effect of intraperitoneal injection of ODQ, an inhibitor for guanylate cyclase, and L-NMMA and L-NAME, inhibitors for NOS. ODQ, L-NMMA, and L-NAME were given 20 min before the start of the irradiation and nonirradiation cycle. Figure 3 shows averages of the blood flow increase transients of ODQ (n = 4), L-NMMA (n = 6), and L-NAME (n = 5)-treated groups compared with control vehicle groups of the same sample numbers at a wavelength of 575 nm. The blood flow rates of increase in the control DMSO- and PBS-treated group (Fig. 3A) and the control PBS-treated groups (Fig. 3, B and C) are not much different from those for both irradiated and nonirradiated periods with the similar range of power densities (≤0.4 mW/cm²; Fig. 1, A and B). The blood flow increase of the inhibitor-treated groups in the irradiation period is almost completely suppressed. Contrary, the pulsatile increase at the beginning of the nonirradiated periods remains, even with inhibitor treatment. It becomes steeper and higher in the L-NMMA- and L-NAME-treated groups. The averages of blood flow increases over the irradiated periods were calculated for each rat in each group, and the resultant data were analyzed statistically. The mean (dB(L)/B_0W) values of rats treated with the DMSO-PBS mixture and ODQ, with PBS and L-NMMA, and with PBS and L-NAME were 7.96 ± 1.9 and −0.27 ± 0.86, 6.24 ± 0.33 and −1.41 ± 0.9, and 10.93 ± 1.85 and −0.26 ± 0.56, respectively (in %·mW^−1·cm^−2). The difference between (dB(L)/B_0W) values in each inhibitor-treated and control group were statistically significant: for ODQ vs. vehicle, P < 0.01 by unpaired t-test and P < 0.05 by Mann-Whitney U-test; for L-NMMA vs. vehicle, P < 0.0001 by unpaired t-test and P < 0.005 by Mann-Whitney U-test; for L-NAME vs. vehicle, P < 0.001 by unpaired t-test and P < 0.01 by Mann-Whitney U-test.

Mean values in the dark periods for control PBS- and L-NMMA-treated rats, control PBS- and L-NAME-treated rats, and PBS-DMSO mixture- and ODQ-treated rats were 3.28 ± 1.02 and 3.04 ± 0.89, 7.8 ± 2.87 and 2.6 ± 1.4, and 4.87 ± 1.21 and 2.92 ± 1.44, respectively. These mean values were not different by unpaired t-test and Mann-Whitney U-test.

To check whether vasodilation causes an increase in peripheral blood flow observed by laser blood flowmeter, the effects of vasodilator administrations were examined under the dark condition. Papaverine (100 µg/kg ip) caused an increase of 66.7 ± 14% compared with baseline (n = 6), whereas the increase in vehicle (PBS) was −3.4 ± 2.68% (n = 5). The 10 times higher dosage of papaverine caused a decrease in blood flow (−18.5 ± 16.6% for n = 3). SNP (0.2 mg/kg ip) and SNAP (0.5 mg/kg ip), which are typical NO donors, caused no significant change [−2.93 ± 10.27% (n = 6) and −6.38 ± 11.13% (n = 5), respectively]. Results of the NO donor administrations might be explained by the findings that NO donated usually forms NO-Hb in the systemic circulation (13). Finally, we examined effects of NO donor administration on the light-induced blood flow increase. Figure 4A shows the average of the blood flow increase in (dB(L)/B_0W) over the irradiated duration as a function of irradiation power density with and without SNAP administration (n = 2 for each case). These data were obtained by the on-off modulation technique. The (dB(L)/B_0W) for the vehicle-treated rats saturates and slightly decreases as irradiation density exceeds 0.5 mW/cm², as seen in Fig. 1. SNAP treatment apparently enhances (dB(L)/B_0W) until power density reaches −1.5 mW/cm². (dB(L)/B_0W) rapidly decreases in the higher power density region. Figure 4B shows the blood flow increase transient of vehicle-treated and SNAP-treated rats for a single-step irradiation at 2.1 mW/cm² (n = 2 for each case). The vehicle-treated rats showed a transient increase in which dB(L)/B_0W rapidly increased at the onset of the irradiation step and diminished within 0.7–0.8 s. Although the transient shows a shape very similar to that obtained with the on-off modulation technique (Fig. 1), it is about two orders of magnitude more intense (peak increment was −1% at 2.1 mW/cm² for nontreated rats with on-off modulation). The SNAP-treated rats exhibited a rapid increase at the onset to a level about 10 times higher than expected from the on-off modulation.
data (~3% at 2.1 mW/cm²) for SNAP-treated rats in Fig. 4A) and sustained the level at least 3–4 s.

DISCUSSION

The present results reveal that tail skin blood flow increases with visible light irradiation. One of the prominent features of the phenomenon is the very fast response of the reaction. It should be noticed that the apparent rise times of the observed waveforms are mostly determined by the time constant of the measurement apparatus. The response time of the blood flow increase is then estimated to be less than ~100 ms. The characteristic time constant for the thermal response is given as \((c/\kappa)\rho S\), where \(c\), \(\kappa\), \(\rho\), and \(S\) are heat capacity, thermal conductivity, density, and interface area between irradiated volume and surrounding nonirradiated tissues, respectively. Assuming that thermophysical properties of tissues of interest are determined by water, we obtained a characteristic thermal time constant of \(\sim 7 \times 10^2\) s. This value is too long for the explanation of the present results. Also, the irradiation power density is so low that the temperature rise at the irradiated region is estimated to be trivial. These strongly suggest that the phenomenon is caused by a series of chemical reactions in which a photochemical process plays a main role.

The results in Fig. 3 clearly present the inhibitory effects of ODQ, L-NMMA, and L-NAME to the light-induced blood flow increase. The baselines tended to decrease by the inhibitor administrations (control-to-inhibitor ratios were 1.195 ± 0.06 vs. 0.854 ± 0.160, 1.824 ± 0.203 vs. 1.087 ± 0.189, and 1.033 ± 0.245 vs. 1.026 ± 0.192 for ODQ, L-NMMA, and L-NAME groups, respectively). We have done recursive analysis for the dB/B₀W vs. B₀ relation in vehicle-treated rats and found that the relation was expressed as dB/B₀W = −0.278B₀ + 8.48 (n = 31) for B₀ in the range of 0.21–2.24 with irradiation density of 0.2–0.6 mW/cm². This result ensures that dB/B₀W is almost independent of the baseline change due to the inhibitor administrations mentioned above. The dB/B₀W is apparently attenuated by the administration of ODQ (Fig. 3A). These data suggest that NO is involved in the mechanism of the light-induced increase in the blood flow. The attenuations by L-NMMA (Fig. 3B) and L-NAME (Fig. 3C) reveal that the light-induced NO is synthesized in endothelium of the blood vessel. The dB(D)/B₀W was not attenuated by the inhibitor administrations (Fig. 3). This indicates that mechanisms of the two kinds of blood flow increase (at lights on and lights off) are quite different.

Characteristic peaks of the action spectrum of the blood flow increase coincide well with those of the B (Soret band 410–420 nm) and Q (\(\alpha\) 540–550 nm and \(\beta\) 570–580 nm) bands in the optical absorption of the low-spin sixfold coordinated ferrous heme (17, 18). All of these features of the phenomenon led us to postulate a simple model in which the optical absorption at sixfold coordinated heme centers gives rise to the increase in the free NO concentration, which then causes dilatation of the vasculatures and increases the capillary blood flow.

Kosaka et al. (13) demonstrated that a certain part of Hb in the circulating arterial blood binds NO as the sixth ligand to form the low-spin ferrous-heme complex by in vivo electron spin resonance (ESR) measurements of cytokine-treated rats. The unpaired electron of NO transfers and delocalizes through the \(d_z^2\) orbital of Fe²⁺ (here, \(d_z^2\) denotes Fe3d atomic orbital with symmetry axis along direction perpendicular to porphyrin plane). The B and Q absorption bands of this type of complex come from the optical transitions between \(\pi\) and \(\pi^*\) orbitals of the porphyrin ring with configuration interaction. Thus it may be expected that energy transfer to Fe²⁺-NO bonding through assumed \(d_z^2(Fe^{2+})-\pi^*\) (porphyrin) interaction breaks the bonding.

Figure 4 provides further support to this mode. In the on-off modulation mode (Fig. 4A), the irradiation-induced blood flow increase saturates already in the very low power density and diminishes in a very short time in the single-step irradiation mode (Fig. 4B).
These facts are reasonably explained to be due to the exhaustion of NO-Hb in the peripheral vasculature of the irradiated region. SNAP administration, which increases NO-Hb concentration, might enhance the saturation level (Fig. 4A) and prolong the increase in transient (Fig. 4B). The available amount of NO, which is linear to the blood flow increment dB in the small signal limit, is proportional to B0 in the steady-state condition of the on-off modulation. Thus the insensitivity of the dB/B0Wt to B0 is reasonably interpreted in the same frame work.

The question arises as to why the NO release from NO-Hb occurs in such a very low irradiation density, whereas flash photolysis results reveal that recombination of released NO to heme is very fast (10, 19). At this issue, we speculate that NO binding to the R state heme in the sixfold coordination may have a tendency to release NO. Kosaka et al. (13) showed that NO is bound to both α- and β-hemes in the R state with sixfold coordination in the high oxygen saturation; however, they are converted to more stable fivefold coordinate αNO-heme as oxygen saturation decreases, accompanying T-to-R transition by ex vivo ESR experiments.

Lia et al. (14) indicated that S-nitrosohemoglobin plays important roles in the allosteric control of NO-Hb. They also pointed out that highly reactive SH groups of the 93rd cystein in the β-subunits are significant. They discussed a mechanism of NO transfer from intraerythrocyte Hb to the endothelial surface through direct contact between erythrocyte membrane-endothelial surfaces. This model, which includes no diffusion process, is favorable to explain the short blood flow increase rise time in the present experiments.

Abu-Soud et al. (1) reported that NOS self inactivates by forming a sixfold coordinated ferrous-nitrosyl complex during aerobic catalysis. A majority of the NOS quickly converted to this catalytically inactive complex, causing the enzyme to operate at only a fraction of its maximum possible activity. If the optical absorption causes release of NO from this nitrosyl complex, then the NOS is to be activated again, and, consequently, free NO production increases. It is noticed, however, that the ferrous NO-NOS complex exhibits absorption peaks at 436 nm (1). This seems not to coincide with our observation, i.e., a peak at 410–420 nm. The third possibility is the release of NO from the assumed photoreleasable storage in the smooth muscle of the vascular system, as suggested by Furchgott and co-workers (5, 7, 8) using rabbit endothelium-denuded aorta. This phenomenon was shown to fade out if the periodical irradiation is repeated and was shown to recover with NO donor treatment (22). The response time of the phenomenon is much longer (by minutes) than that in the presently observed phenomenon (22). Involvement of a certain thermal process is doubtful. It should be noticed that the irradiation power densities are estimated to be about two orders of magnitude greater than those in the present experiments, and infrared light was not eliminated in these experiments. Whether these are the causes may be clarified in the future.

Recently, Campbell and Murphy (3) reported that phases of the human circadian rhythm are shifted by the irradiation of the extraretinal (popliteal) region with visible light. This phenomenon might be explained by the release of NO (3), because it is suggested that NO is implicated in the mechanism of synchronization or generation of the circadian rhythm (4, 20).

Finally, we would like point out that the present discoveries might have therapeutic value. The saturation value of the light-induced blood flow increment is only a few percent (Fig. 4A). This value is one order of magnitude less than the effect of papaverine. This limitation is caused by the shortage of NO-Hb in the peripheral blood vessels where the blood flow is very low (typically 1 ml·min⁻¹·100 g tissue⁻¹). If we have enough supply of blood flow or NO to the irradiated region, we could have a blood flow increase comparable to vasodilating agents, such as papaverine, with moderate irradiation densities of 1–10 mW/cm².

REFERENCES