Front. Optoelectron. China 2009, 2(1): 1–8
DOI 10.1007/s12200-009-0010-3

REVIEW ARTICLE

Homeostatic photobiomodulation

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Abstract Photobiomodulation (PBM) is a modulation of laser irradiation or monochromatic light (LI) on biosystems, which stimulates or inhibits biological functions but does not result in irreducible damage. LI might be of low intensity LI (LIL) (about 10 mW/cm^2), or moderate intensity LI (MIL) (10^2–10^3 mW/cm^2). PBM of LIL or MIL (LPBM or MPBM) is studied from the homeostatic viewpoint in this paper. Homeostasis is redefined as the function-specific homeostasis (FSH), a negative-feedback response of a biosystem which maintains the function-specific conditions inside it. PBM is classified into two kinds, the FSH-specific PBM (fPBM) and developmental PBM (dPBM). For fPBM, there is no PBM of LI on the function in FSH, but there is PBM of LI on the function far from FSH. dPBM can disrupt FSH. It can be found that LPBM is an fPBM, and whether MPBM is fPBM or dPBM depends on MIL dose and cell sensitivity. Low level LI therapy is just clinical applications of fPBM, so that it is a cellular rehabilitation.

Keywords laser, homeostasis, rehabilitation, photobiomodulation (PBM), phototherapy

1 Introduction

Photobiomodulation (PBM) is a modulation of laser irradiation or monochromatic light (LI) on biosystems, which stimulates or inhibits biological functions but does not result in irreducible damage. The LI used in PBM is always low intensity LI (LIL), about 10 mW/cm^2. However, moderate intensity LI (MIL), 10^2–10^3 mW/cm^2, is of PBM if the radiation time is not so long that it damages organelles or cells. The PBM of LIL and MIL are denoted as LPBM and MPBM, respectively. There are almost equal numbers of positive and negative reports on PBM. For example, Campbell et al. found that 3 h of bright light exposure to the area behind the knee caused phase shifts of the circadian rhythms of both body temperature and saliva melatonin in humans [1], while Wright et al. found that the absence of circadian phase resetting in response to bright light behind the knees [2]. Imagine getting over jet lag can be simply attained by strapping a light to your leg and leaving it on while you sleep or read in your airline seat. Campbell et al.'s discovery captured the imagination of scientists and entrepreneurs as well as the public, but Wright et al. have cast doubt on those findings and prospects for commercializing this patented form of light therapy. These confused phenomena will be reviewed from the homeostatic viewpoint.

Received November 13, 2008; accepted November 27, 2008

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2 Function-specific homeostasis

As a classical concept in physiology, homeostasis is a negative-feedback response of a biosystem to maintain the conditions inside the biosystem. A biosystem in homeostasis reacts to every change in the environment, or to every random disturbance, through a series of modifications of equal size and opposite direction to those that created the disturbance.

Homeostasis is one of the most remarkable and most typical properties of a highly complex open biosystem. However, it is too obscure to be studied. In this paper, it is exactly defined as function-specific homeostasis (FSH). FSH is a negative-feedback response of a biosystem to maintain the function-specific conditions inside the biosystem so that the function is perfectly performed. A biosystem in an FSH means the function is in its FSH and it is perfectly performed. A biosystem far from an FSH means the function is far from its FSH and it is dysfunctional. There are two kinds of regulation factors of a function in a biosystem: the homeostatic

regulation factors which modulate the function so that there is no modulation on the function in its FSH and there is modulation on the function far from its FSH, and the developmental regulation factors which disrupt the FSH. Each FSH maintains its function. A developmental regulation factor can disrupt an FSH so that it can change the functions of a biosystem from one to another and can be also called as a non-homeostatic regulation factor.

There are many conditions to maintain an FSH, but FSH-essential conditions may be very sparse. This is supported by the neurobiological studies such as sparse coding and the brainless worker. Several theoretical, computational, and experimental studies suggest that neurons encode sensory information using a small number of active neurons at any given point in time [3]. Recent physiological recordings from sensory neurons have indicated that the sparse coding could be a ubiquitous strategy employed in several different modalities across organisms [3]. For example, Vinje et al.'s experiments have provided direct experimental evidence that primary visual cortex uses a sparse code matched to the underlying sparse structure of natural scenes [4]. Recently, Huber et al. have studied sparse optical microstimulation of barrel cortex in freely moving mice [5]. Their data indicated that mechanisms exist to read out extremely sparse codes from primary sensory areas. Houweling et al. have studied behavioral reports of single neuron stimulation in the somatosensory cortex [6]. Their results demonstrate that single neuron activity can cause a change in the animal's detection behaviour, suggesting a much sparser cortical code for sensations than previously anticipated.

Feuillet et al. have reported the brainless white-collar worker [7]. The 44-year-old worker presented with a 2-week history of mild left leg weakness. At the age of 6 months, he had undergone a ventriculoatrial shunt, because of postnatal hydrocephalus of unknown cause. When he was 14 years old, he developed ataxia and paresis of the left leg, which resolved entirely after shunt revision. His neurological development and medical history were otherwise normal. He was a married father of two children, and worked as a civil servant. On neuropsychological testing, he proved to have an intelligence quotient (IQ) of 75: his verbal IQ was 84, and performance IQ was 70. Computer tomography (CT) showed severe dilatation of the lateral ventricles. Magnetic resonance imaging (MRI) revealed massive enlargement of the lateral, third, and fourth ventricles, a very thin cortical mantle and a posterior fossa cyst. Feuillet et al. diagnosed a non-communicating hydrocephalus, with probable stenosis of Magendie's foramen [7]. The leg weakness improved partly after neuroendoscopic ventriculocisternostomy, but soon recurred, however, after a ventriculoperitoneal shunt was inserted. The findings on neurological examination became normal within a few weeks. The findings on neuropsychological testing and CT did not change.

3 Function modulation

Western medicine studies bodies mainly from the anatomical viewpoint, while traditional Chinese medicine (TCM) mainly from the functional viewpoint. The function modulation was discussed from the TCM viewpoint.

Drug therapy is one of the main therapeutic approaches of TCM. FSH-essential conditions may be very sparse, but they are needed. Moreover, FSH-non-essential conditions are also needed. TCM follows the tenet that a formula should have four major ingredients, each playing its unique role while working together synergistically, to achieve the optimum therapy. The four major ingredients have been described in ancient texts as emperor, minister, assistant and delivering servant. Wang et al. have taken one well-known and clinically tested TCM formula for leukaemia therapy as a model and unveiled the biochemical roles of each ingredient [8]. The formula, known as Realgar-Indigo naturalis formula, contains realgar and indigo minerals, as well as the herb red sage root. Through molecular analyses, it showed that arsenic in realgar works as emperor by directly attacking the receptor oncoprotein in leukaemia cells. Indirubin, the active ingredient in indigo, works as assistant by antagonizing the toxicity of arsenic and slowing leukaemia cell growth. Tanshinone, the active ingredient in red sage root, acts as minister by partially restoring those pathways that stop leukaemia spreading. Lastly, indurbin and tanshinone work as delivering servants; these ingredients can enhance the cellular uptake of arsenic by increasing the gene-expression level and therefore the synthesis of carrier pore proteins in the cell membrane.

Generally, the TCM formulae consist of several types of medicinal herbs or minerals, in which one represents the principal component, and others serve as adjuvant ones to assist the effects or facilitate the delivery of the principal component so that the multiple components could hit multiple targets and exert synergistic therapeutic efficacies. PBM might be the principal one or the adjuvant ones. PBM can be then classified into two kinds, FSH-specific PBM (fPBM) in which LI is just a homeostatic regulation, and developmental PBM (dPBM) in which LI is just a developmental regulation.

4 Photobiomodulation of low intensity laser irradiation or monochromatic light

LPBM is mediated by the non-resonant interaction of LIL with the molecules in the membrane of cells or cellular organelles [9,10]. It is so weak that it cannot disrupt the FSH and there is no LPBM on the function in its FSH. Therefore, LPBM is an fPBM. As Karu has pointed out, there are no effects of LIL on the cell on which redox potential is so that the cell normally functions, and the lower the redox potential of a cell is compared with the

normal redox potential, the stronger the LPBM is [11]. The cell which normally functions is in an FSH. As Tunér et al. have summarized, the light energy is thought to reap the greatest benefit where it is most needed [12].

There is no LPBM on the proliferation in proliferation-specific homeostasis (PSH). The chondrocytes in 0%, 2.5%, 5%, and 10% fetal calf serum (FCS) have been irradiated by low intensity He-Ne laser irradiation (LHNL) at 5.74 mW/cm^2 for 2, 8, 16, 30 and 45 min [13]. There was significant PBM on chondrocyte proliferation in 0%, 2.5% and 5% FCS, but there was no PBM on the proliferation in 10% FCS. The chondrocyte proliferation in 10% FCS has been in PSH so that there was no PBM on the proliferation. Dexamethasone (DEX) might induce G1 cell cycle arrest [14] which is in G1 cell cycle arrest-specific homeostasis so that no proliferation differences of the myoblasts in combination with 100 nmol/L DEX were observed between red light at 640 ± 15 nm of light emitting diode array (LED) (RLED 640), simvastatin treatment and the control [15]. After analyzing many experiments, Karu pointed out that only the proliferation of slowly growing subpopulations can be stimulated by LIL, and it is not possible to activate a process which is activated already or occurring at a speed which is near maximal, so that the cells are in PSH [11].

There is no LPBM on the protein production in protein-production-specific homeostasis. Bouma et al. have investigated the effects of LIL on cytokine release by human peripheral blood monocytes *in vitro* [16]. There is no significant difference for the interleukin 6 (IL-6) productions of the cells stimulated by two concentrations of lipopolysaccharides (LPS). In other words, the IL-6 production has been fully stimulated by LPS so that the cells were in IL-6 production-specific homeostasis and LIL fails to modulate the IL-6 production. The chondrocytes used in Lin et al.'s experiment were cultured in 10% FCS, but the cells have been separated from arthritic cartilage and the studied function was stress protein production [17]. Lin et al. have found LHNL promotion on the stress protein production of the chondrocytes, which indicates that the stress protein production of the arthritic chondrocytes in 10% FCS might not be in stress protein production-specific homeostasis although the proliferation might be in PSH [17].

There is no LPBM on the deformability in deformability-specific homeostasis. Iijima et al. have investigated the effect of LHNL on the deformability of human red blood cell (RBC) [18]. RBC solution samples obtained from hematologically normal adult donors by venipuncture were assigned to three groups. Within 2 h after sampling, Group 1 was divided into 7 small groups, and they were irradiated for 0 (control), 1, 3, 5, 10, 15, and 30 min, respectively, but Groups 2 and 3 were stored at 5°C for 24 and 36 h, respectively, and received similar irradiations after 12 h (in both groups), 24 h (in Group 2), and 36 h (in Group 3). The deformability was unchanged in Group 1 (fresh cell group) from the control value, but improved significantly in Groups 2 and 3 (damaged cell groups) after the irradiation. In this case, RBC in Group 1 is in deformability-specific homeostasis.

Although there is no LPBM on the function in its FSH, LIL can inhibit the disruption of a developmental factor. The effects of statins on proliferation of C2C12 myoblast and its PBM [15] have been studied in our laboratory. C2C12 myoblasts in 10% FCS is in PSH, so that simvastatin at 2.0×10^{-6}, 2.0×10^{-7} and 2.0×10^{-8} mol/L had no effects on the myoblast proliferation, and no PBM on the proliferation has been found. However, simvastatin at 2×10^{-5} mol/L inhibited the myoblast proliferation so that only 37.2% of the myoblasts remained to survive, and the inhibited proliferation was promoted with RLED 640 at 0.848 mW/cm^2 and 15 min [15]. Our laboratory has also studied LPBM on the amyloid β protein 25–35 induced apoptosis of PC12 cell *in vitro*, and found RLED 640 at 0.09 mW/cm^2 and 60 min inhibited the apoptosis [19]. Eells et al. have studied red light at 670 nm from LED (RLED 670) on methanol induced mitochondrial dysfunction of cone cells and rod cells *in vivo*, and found RLED 670 at 28 mW/cm^2 and 144 min inhibited the retinal toxicity [20]. LIL is of anti-inflammation. Aimbire et al. found low intensity GaInP/AlGaInP diode laser irradiation at 650 nm (LGAL) might reduce LPS-induced contractile force dysfunction and tumor necrosis factor α (TNF-α) levels in rat diaphragm muscle [21]. After irradiating the rats or mice on the skin over the upper bronchus at the site of tracheotomy after LPS, Aimbire et al. found LIL reduced the rat lung permeability by a mechanism in which the interleukin 1β seems to have an important role and reduced the levels of anti-apoptotic factors in mice lung polymorphonuclear neutrophils (PMNs) by an action mechanism in which the nuclear factor-κB (NF-κB) seems to be involved [22,23].

LIL can also promote the disruption of a developmental factor. Spleen cells at rest might be in rest-specific homeostasis (RSH). An object of phagocytosis Candida albicans can disrupt the RSH and activate their respiration burst which might be evaluated by the luminol-amplified chemiluminescence (LDC). Karu et al. have studied the respiration burst in murine spleen cells after treatment with an object of phagocytosis Candida albicans and LHNL, and found the irradiation effect was detectable only in these cases when the cells were treated first with a low concentration of Candida albicans (5×10^7 particle/mL), and no additional activation by LHNL was possible at the concentration of 1×10^8 particle/mL at which the chemiluminescence was maximally activated so that the cells were in respiration burst-specific homeostasis (RBSH) [24]. Aimbire et al. have studied PBM on trachea muscle relaxation response in rats with TNF-α-mediated smooth airway muscle dysfunction, and found LIL promoted TNF-α induced 3'-5'-cyclic adenosine monophosphate (cAMP) accumulation [25]. Karu et al. have studied donors of nitric

oxide (NO) and LPBM on cell attachment to extracellular matrices, and found LIL promoted the inhibition of sodium nitroprusside on the cell attachment [26].

The individual difference of LPBM had been studied in our laboratory [27]. The PMNs were isolated from peripheral blood of 13 volunteers (10 ordinary persons, 3 athletes) and treated with RLED 640 at 50, 100, 300, 500 and 1000 J/m^2 and a fixed 100 s duration. Blood samples of athletes were extracted at different times in the 10 km non-interrupted long-distance running, before running, 1 h after the start of running, just finishing the running, resting for 1 and 2 h after running. We found that there were three types of modulation of RLED 640 on the respiratory burst of three types of PMNs respectively, promotion for the one of sub-activated PMNs, inhibition for the one of over-activated PMNs and none for the PMNs in RBSH.

5 Photobiomodulation of moderate intensity laser irradiation or monochromatic light

The concentration of endogenous photosensitizers is so low that LPBM may be mainly mediated by the non-specific pathways, but MPBM may be mainly mediated by the specific pathways. Wu et al. have studied the apoptotic effect of moderate intensity He-Ne laser irradiation (MHNL) (200 mW/cm^2) on ASTC-a-1 cells, and found immediate generation of mitochondrial reactive oxygen species (ROS) following MHNL, reaching a maximum level 60 min after irradiation [28]. Zhang et al. used fluorescence resonance energy transfer (FRET) to visualize the dynamic Src activation (pathway 2) in HeLa cells immediately after irradiated with MHNL (64.4 mW/cm^2), and found that it was ROS that mediated Src activation by MHNL [29].

The PBM of MIL of higher intensity is dPBM. The PMNs from 20 healthy male volunteers is normal in RBSH, but can be attenuated by the infrared diode laser (GaAlAs), continuous wave at 830 nm and 150 mW/cm^2 [30]. For the NCTC 2544 keratinocytes in 10% FCS, the proliferation is in PSH so that mitogen-activated protein kinase (MAPK)/extracellular signaling regulated kinase (ERK) (MAPK/ERK) was strongly activated, but can be further activated by ultraviolet A (320–400 nm) (UVA) (4 mW/cm^2, 75 min) [31]. DEX might induce G1 cell cycle arrest (function 1) [14], but MIL (GaAlAs diode 780 nm laser, 250 mW/cm^2, 12 s) acts as a proliferative stimulus (function 2) on osteoblast-like cells, even under the influence of DEX [32]. Agkistrodon contortrix laticinctus myotoxin might induce muscle injury, in which regeneration cannot be promoted by fPBM of LIL (GaAs diode 904 nm laser, 7.5 mW/cm^2, 2.2 or 8 min) [33], but can be promoted by dPBM of MHNL (371 mW/cm^2, 7 s) [34]. The chondrocytes have been cultured in 10% normal goat serum so that they were in PSH, but the ROS level induced by pulsed GaAlAs diode 780 nm laser irradiation (300 J, 1 W, 10 min) (MIL) is high enough to destroy the PSH and further promote chondrocyte proliferation in an Italian group [35]. As the lack of vascularity in cartilage *in vivo* leads to the relative hypocellularity, the Italian group's cellular model might not be the best cellular model of cartilage regeneration. Apoptosis and proliferation are two kinds of cellular functions, and their transformation into each other can only be realized by dPBM. MPBM induced anti-apoptosis has been observed for serum-free induced apoptosis of myofibers and their adjacent cells, as well as cultured myogenic cells with MHNL (177 mW/cm^2, 3 s) [36], and nutritional deficiency induced Cho K-1 cell apoptosis with a GaAlAs semiconductor laser irradiation (810 nm, 1990 mW/cm^2, 1 s) [37]. Both Shefer et al. [36] and Carnevalli et al. [37] have found MIL induced proliferation (function 1) of the cells in serum-deprivation-induced apoptosis (function 2), but it was mediated by MIL induced ROS and its MAPK activation [36]. In these cases, MPBM can change functions from one into another so that it is not a homeostatic regulation.

Intravascular low energy laser therapy (ILELT) is an intravascular application of MIL. MPBM might promote ROS generation, but the concentration of endogenous photosensitizers is so low and the radiation time is so short that the generated ROS cannot disrupt the FSH in ILELT and ILELT is just a clinical application of fPBM. Mi et al. [38] have studied the effects of 632.8 nm (150 mW/cm^2, 540 J/cm^2) and 532 nm (150 mW/cm^2, 90 or 180 J/cm^2) laser irradiation on some rheological factors in human blood *in vitro*, and found no PBM on the rheological factors in blood from healthy persons. Wang et al. [39] have studied MPBM of extracorporeally circulatory blood on ATP phosphohydrolase (ATPase) activities of erythrocyte membrane in 13 cases of patients with insulin dependent diabetes mellitus. The results showed that ATPase was significantly lower in insulin dependent diabetes mellitus than that in control healthy subjects in FSH ($P < 0.01$), ILELT could markedly activate the Na^+/K^+-ATPase, Ca^{2+}, Ma^{2+}-ATPase of the patients with insulin dependent diabetes mellitus ($P < 0.05$ or $P < 0.01$), but could not significantly affect the ones of the control ($P > 0.05$). ROS generation in whole blood can be registered with LDC. Acute pneumonia and asthmatics or bronchial asthma [40] patients with intensive LDC exposed to ILELT retained free radical oxidation defects and the disease symptoms because of the reduced enzymic and non-enzymic antioxidant activities in acute pneumonia [41] and the reduced activities of antioxidant enzymes in asthmatics [42], but ILELT activated ROS generation and raised treatment effectiveness in low intensity of blood LDC.

Xiao et al. [43] have treated 21 and 18 patients of cerebral infarction by intranasal low intensity laser therapy (ILILT) with LGAL at 3.5–4.0 mW for 30 min and ILELT with LGAL at 2.5–3.0 mW for 30 min, respectively, and

then used single photon emission computed tomography (SPECT) of brain perfusion imaging to study the changes of regional cerebral blood flow (rCBF) and brain blood flow function change rate. They found that the ratio of local rCBF vs whole brain rCBF and brain blood flow function change rate increased in the focus side of the brain after the treatment of either ILILT or ILELT, but there was no change in the mirror regions.

6 Cellular rehabilitation

Phenomenology of PBM indicated that PBM is cell-specific. Kipshidze et al. have determined the effect of LIL on the growth of rabbit and human aortic endothelial cells (ECs) and smooth muscle cells (SMCs) *in vitro* [44]. All cell cultures were irradiated with single dose LIL using LHNL with different energy densities. Both human and rabbit ECs revealed enhanced growth rate and reached confluence faster following laser irradiation with 0.54 J/cm^2 than control non-irradiated cells. Higher doses of LHNL, however, decreased cell growth. In contrast, the experiments on SMCs revealed that nontoxic doses of the LHNL did not enhance growth rate and there was no difference in comparison with control cultures. Higher doses of LI were cytotoxic for both ECs and SMCs and decreased their growth. In this case, the cell-specific LHNL at 0.54 J/cm^2 promoted EC proliferation, but it has no PBM on SMC proliferation.

All of low level/intensity/energy LI therapy, soft LI or cold LI therapy and LI therapy based on the biostimulation or PBM used the same principles. The same principle should be fPBM from the above discussion, and all the therapeutic approaches should be the clinic applications of fPBM so that they are unified to be denoted as low level LI therapy (LLLT). Of course, LLLT included ILILT and ILELT.

Cell rehabilitation rate has once been used to assay the cytotoxicity of radiation [45] or drugs [46]. It represents the recover function of the cells cultured in fresh media for 72 h after they are irradiated at the given dose or cultured in the media with a drug for 24 h. LLLT is a clinic application of cell-specific fPBM. It may promote the cellular function recovery and elevate the cell rehabilitation rate so that it is of cellular rehabilitation. LLLT might be the first therapeutic approach which is found to be of cellular rehabilitation.

There are almost equal numbers of positive and negative results on fPBM or LLLT. In our opinion, there was something wrong with the negative results. For example, the negative results might be due to its trying to modulate the function in its FSH or its inattentive research on dose relationship [10]. At this point, only positive results are discussed.

7 Photobiomodulation of diagnostic laser irradiation or monochromatic light

LI has always been used to diagnose biosystems especially after green fluorescent protein pioneers shared the 2008 Nobel Prize in chemistry. We have witnessed remarkable advances over the past decade in the application of optical techniques to visualize the genetically encoded fluorescent proteins (FPs) in living systems. The imaging of the FPs inside living cells has become an essential tool for studies of cell biology and physiology. FPs are now available that span the visible spectrum from deep blue to deep red, providing a wide choice of genetically encoded fluorescent markers. Furthermore, some FPs have been identified that have unusual characteristics that make them useful reporters of the dynamic behaviors of proteins inside cells. These additions to the FP toolbox are now being used for some very innovative live-cell imaging applications [47]. At this point, the effects of LI on living cells should be paid attention to. The effect of the damage effects of high intensity laser irradiation or the photodynamic effects of MIL on the diagnostic biosystem has been widely discussed. However, the effect of LPBM on the diagnostic biosystems, which might be called diagnostic PBM (DPBM), has been little discussed and will be discussed in this section.

Gao et al. have studied LIL induced protein kinase C (PKC) activation by using FRET of yellow fluorescence protein (YFP) and cyan fluorescence protein (CFP) on laser scanning confocal microscopy (LCSM) [48]. In order to monitor PKC activity in living cells in real time, Gao et al. transfected and screened ASTC-a-1 cells stably expressing C kinase activity reporter (CKAR) constructed based on FRET technique [48]. The increasing dynamics of PKCs activity is monitored by using FRET imaging. Based on Gao et al.'s paper [48], Figs. 1 and 2 are marked with a horizontal long line above which the up arrow shows how much the DPBM is. In Fig. 1, Go 6983 was used to inhibit PKC activation. There is no DPBM for the cells in Fig. 1(b) which were grown in DMEM supplemented with 15% FCS because the cellular PKC activation were in cellular PKC activation-specific homeostasis (PKCSH), but there is DPBM for the cells in Fig. 1(a) which were starved for 24 h because the cellular PKC activation of the staved cells were far from PKCSH. Duan et al. have found that Aβ25-35 induced PC12 apoptosis can be inhibited by LIL [19]. In Fig. 2, PKC activation was induced by LHNL (Fig. 2(a)) and phorbol 12-myristate 13-acetate (PMA) (Fig. 2(b)) respectively. There should be DPBM because the starved cells were far from PKCSH so that LHNL and then the diagnostic LIL can promote PKC activation, but there is no DPBM for the PMA-treated cells because PMA induced PKC activation was in PKCSH. For Gao et al.'s experiment [48], DPBM

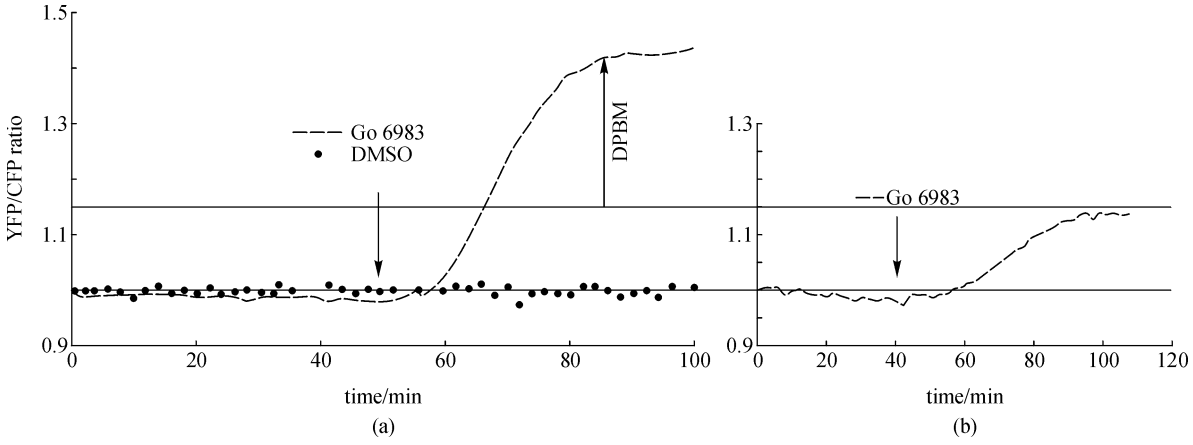

Fig. 1 Go 6983 inhibited PKC activation. (a) 24 h starved cells, DPBM; (b) cells in 15% FCS, no DPBM

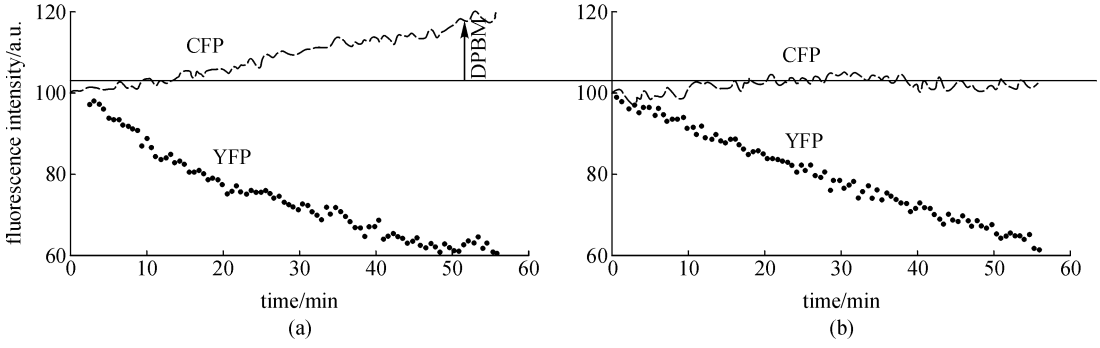

Fig. 2 PKC activation. (a) 24 h starved ASTC-a-1 cells (after LIL), DPBM; (b) 6 h starved ASTC-a-1 cells (after PMA), no DPBM

has not changed the experimental results, but has fine quantitative effect. This should be noted for the future research.

8 Discussion

There are almost equal numbers of positive and negative reports on PBM. Whether the modulated function is in FSH might be one of the causes. There is no fPBM on the function in FSH. The *neiguan* acupoint and then heart vibration of healthy non-smoking males is in the corresponding FSH, respectively, so that there is no effect of laser acupuncture stimulation applied to the *neiguan* acupoint on the heart rate variability in the randomized, double-blinded, placebo-controlled trial [49]. However, the *neiguan* acupoint and then heart vibration of young male night shift workers are far from the corresponding FSH respectively, so that laser acupuncture stimulation applied to the *neiguan* acupoint increased vagal activity and suppressed cardiac sympathetic nerves [50]. The popliteal region, the area directly behind the knee joint, of the participants in ambient light of less than 20 lux, and then the circadian rhythms of both body temperature and saliva melatonin are far from the corresponding FSH respectively, so that 3 h of bright light exposure to the area behind the knee caused phase shifts of the circadian rhythms of both body temperature and saliva melatonin in humans [1]. However, the popliteal region of the participants in ambient light of 0 lux, total darkness, and the circadian rhythms of both body temperature and saliva melatonin are in the corresponding FSH respectively, so that no circadian phase resetting in response to bright light behind the knees has been found [2].

Acknowledgements This work was supported by the National Natural Science Foundation of China (Grant Nos. 60878061, 60478048, 60178003 and 60278012), the National 973 Basic Project of China (No. 2005CB523502) and the National Postdoctoral Foundation of China (No. 20070420143).

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