Korean J. of Pharmacology
Vol. 31, No. 3, pp. 285-290, 1995

Biphasic Mechanical Responses of Rat Thoracic Aorta to Irradiation with 250~500 nm Light

Hyun Kook

Department of Pharmacology, Chonnam University Medical School, 5 Hak-Dong, Dong-Ku, Kwangju 501-190, Korea

ABSTRACT

This study was undertaken to define the varying responses of vascular smooth muscle to different wavelengths of ultraviolet radiation and to relate them to the changes in cyclic GMP contents. The ring preparations of rat thoracic aorta with intact or removed endothelium were irradiated with the ultraviolet or visible light (UVR) of wavelengths in step of 10 nm between 250 and 500 nm from xenon lamp of a spectrofluorometer, and the changes in vascular tension were recorded. For cyclic GMP assay, the preparations, pretreated with phenylephrine as in the tension experiments, were frozen after irradiation and homogenated in trichloroacetic acid. The supernatant was extracted with ether and the cyclic GMP contents were measured with radioimmunoassay.

In the endothelium-intact preparations, biphasic responses, vasoconstriction (UVR-contraction) followed by vasodilatation (UVR-dilatation), were observed. The maximal UVR-contraction was observed at 320 nm, while the maximal vasodilatation was elicited at 420 nm. In the endothelium-removed rings, however, only vasodilatation was observed, with the maximal vasodilatation taking place at 370 nm. The cyclic GMP contents were not affected by the irradiation with 320 nm for 30 sec or 1 min in the endothelium-intact preparations, while it was significantly increased by 380 and 420 nm. In the endothelium-removed preparations, UVR of 370 nm markedly increased the cyclic GMP contents.

The present study indicates that the increase in cyclic GMP is closely related to vasodilatation induced by UVR of 420 nm in the endothelium-intact or 370 nm in the denuded preparations, whereas it is not involved in the vasoconstriction induced by UVR of 320 nm in the intact rings, and the mechanism leading to UVR-contraction remains to be clarified. These observations suggest that nitric oxide-cyclic GMP system is closely related to the UVR-dilatation in rat aortic preparation, while it is not involved in the UVR-contraction.

Key Words: Ultraviolet light, Vasoconstriction, Vasodilatation, Rat thoracic aorta

INTRODUCTION

It has been reported that UVR produce vasodilatation in various vascular preparations including porcine coronary artery (Baik *et al.*, 1994; Triggle and Bieger, 1990), bovine mesenteric artery (Karlsson *et al.*, 1986), rat thoracic aorta (Mikkelsen *et al.*, 1985) and that the UVR-dilatation is related to the increase in cyclic GMP contents (Furchgott *et al.*, 1984; Matsunaga and Furchgott; 1989). To the contrary, UVR-contraction is reported by Karlsson *et al.* (1986) and Wigilius *et al.* (1990). Baik *et al.* (1992) in this laboratory also observed a vasoconstriction, not a vasodilatation, in the intact preparation of rat thoracic aorta upon

UVR, while the denuded ones responded with relaxation, and they further suggested that the UVR-contraction is related to the tissue cyclic GMP levels, based on the observations that the contraction was potentiated by ACh and reduced by LY83,583, a guanylate cyclase inhibitor (Schmidt *et al.*, 1985). In this study, it was attempted to define the varying responses of vascular preparations to different wavelengths and to relate them to the changes in the cyclic GMP contents.

METHODS

Preparations

The methods of ring preparations and tension experiments were as described by Baik *et al.* (1992). Briefly, Sprague-Dawley rats of either sex weighing 180~250 g were sacrificed and the thoracic aorta was excised immediately. The excised aorta was then cut into rings of 5 mm in length. To prepare the denuded preparations, rings were gently rubbed 2 to 3 times with a metal rod inserted into the lumen of the ring. Ring segments of arteries were mounted in 3 mℓ physiologic salt solution (PSS), and the changes of tension were recorded on a polygraph. The PSS was saturated with 95% O_2 and 5% CO_2 kept at 37℃ (pH 7.4). The completeness of functional removal of the endothelium was ascertained by absence of relaxant response to ACh of the ring precontracted with 10^{-6} M PE.

Ultraviolet or visible light irradiation

To minimize the glass interference on the incident light, quartz cuvette was employed instead of glass organ bath (Fig. 1). As the light source, a 150W xenon lamp of spectrofluorometer was used and the distance between the tissue and the lamp was 30 cm. And a convex lens made of quartz (30 D.) was placed inbetween to intensify the light. The ultraviolet light was irradiated sequentially from 250 to 500 or reversely in steps of 10 nm for 3 min each. The UVR did not affect the temperature of the bath fluid. All UVR experiments were performed in the dark room lit with a 5-Watt red lamp. In assaying tissue cyclic GMP levels, the tissue was irradiated the same way as described above.

Radioimmunoassay of cyclic GMP

The cyclic GMP contents were assayed according to the method described by Ryu *et al.* (1992). Briefly, the rings prepared as described above were incubated in 3 ml PSS in cuvette. After 2-hour equilibration 10^{-6} M PE was added to the bath fluid. Immediately upon terminating UVR, the preparations were frozen and were stored at −70℃ until use. Frozen tissues were homogenized in 1 ml of 10% trichloroacetic acid at 1~4℃ and the homogenate was centrifuged at 2500×g for 30 min at 4℃. The pellet was used for protein assay and the supernatant was extracted 5 times with 4 ml of water-saturated ether. The [^{125}I] radioimmunoassay kits (DuPont) were used to determine cyclic GMP concentrations.

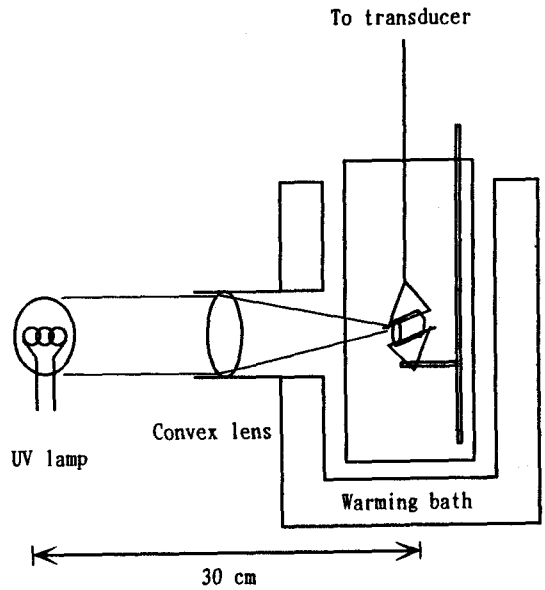

Fig. 1. The diagram of ultraviolet or visible light irradiation (UVR) system imployed to observe the mechanical responses of the vascular preparations to UVR. A ring preparations was mounted in the cuvette. See text in details.

Drugs and statistics

Composition of PSS in mM was 122 NaCl, 4.7 KCl, 1.6 $CaCl_2$, 1.2 KH_2PO_4, 15 $NaHCO_3$, 11.5 dextrose, 0.026 EDTA and 0.12 ascorbic acid. Phenylephrine (PE) HCl was obtained from Sigma, trichloroacetic acid from Fisher Scientific, and diethylether from J.T. Baker Inc. All drugs were dissolved in and diluted with distilled water. Statistical significance was examined by unpaired Student's t-test.

RESULTS

Changes in vascular tension induced by ultraviolet or visible light

The UVR did not affect the resting tension of the preparations. In the intact prerations, 10^{-6} M PE elicited vasoconstriction producing tension of 0.70±0.12 g (n=7), and UVR with 250~320 nm produced further vasoconstriction in an irradiation-time dependent manner and the maximal tension produced was 0.21±0.02 g at 320 nm. The UVR-contraction was reduced with further increase of the wavelengths, and the vasoconstriction was reversed to a vasodilatation at 380 nm. Then, the longer wavelengths, the greater vasodilatation became. And finally, the ring reached the maximal dilatation of 0.10 ±0.02 g at 420 nm. And then, the tension slowly recovered to the basal level as the wavelength further increased up to 500 nm (Fig. 2). The range of wavelength to produce contractile responses was 270~370 nm and the range to cause relaxtion was 410~450 nm. The same biphasic pattern of the tension was obtained when the UVR was started from 500 down to 250 nm.

In the endothelium-removed rings precontracted by PE, however, no vasoconstriction was observed. The UVR starting from 250 nm gradually reduced the 10^{-6} M PE-induced tension, reaching the nadir at 370 nm with the magnitude of 0.16±0.04 g (n=6) as shown in

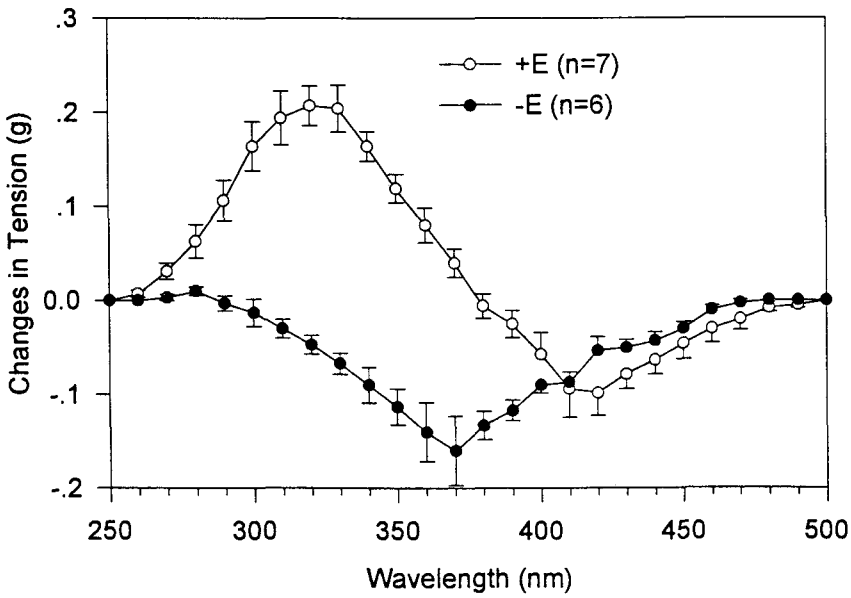

Fig. 2. Responses of rat thoracic aorta preparations to irrdiation of wide spectrum light. The endothelium-intact (+E) preparations show biphasic responses, whereas the endothelium-removed ones elicited only dilatation. The preparations were preconstricted by 10^{-6} M PE. Each dot represents mean±SEM. Numbers of preparations were given in parentheses. See text in details.

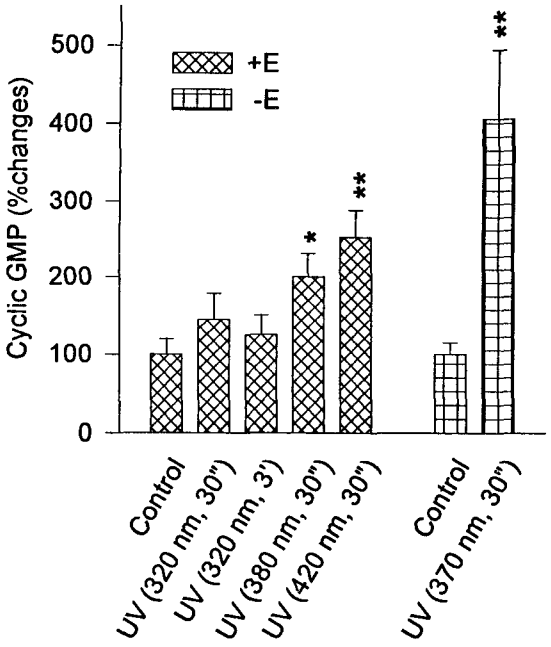

Fig. 3. Changes in cyclic GMP contents induced by UVR in the preparations of rat thoracic aorta with intact (+E) or removed (-E) endothelium. All rings were pretreated with 10^{-6} M PE. Irradiation was done for either 30 sec or 3 min at wavelengths indicated. Each bar represents mean±SEM. Asterisks indicate significant differences from their controls (*$p<0.05$, **$p<0.01$).

Fig. 2. As the wavelengths were further increased, the vasodilatation decreased, and the PE-induced basal tension was fully regained at 470 nm. The maximal relaxation in the denuded preparations was not significantly different from that in the intact ones. The range of wavelength to elicit vasodilatation was 310~450 nm.

Effects of UVR on tissue cyclic GMP contents

Cyclic GMP level in the endothelium-intact preparations without any pretreatment was 81.4 ±15.7 pmole/mg protein and that with PE-pretreatment was 98.4±13.8 pmole/mg protein. In Fig. 3, the cyclic GMP contents in the irradiated tissue was presented in percentage of the PE-pretreated control. UVR of 320 nm, which produces vasoconstriction in the endothelium-intact rings, did not produce any significant change in cyclic GMP contents (145±33% on 30 sec UVR and 126±25% on 1 min UVR). However, UVR of 380 and 420 nm for 30 sec markedly increased the cyclic GMP contents to 200±30% ($p<0.05$, $n=4$) and 252±36% ($p<0.01$, $n=4$), respectively. In endothelium-removed preparations, UVR of 370 nm for 30 sec markedly increased the cyclic GMP contents up to 407±88% ($n=6$, $p<0.01$).

DISCUSSION

In this study using UVR of varying wavelengths ranging from 250 to 500 nm, it was found that the rat thoracic aorta with intact endothelium elicited a biphasic response: vasoconstriction, with range of 270~370 followed by vasodilatation in the range from 410 to 450, whereas the denuded preparations elicited only vasodilatation with range of 310~450, whose maximal relaxation occurred at 370 nm. In the intact endothelial preparation the peak contraction was elicited at 320 nm wavelength and the peak dilatation at 420 nm. Baik *et al.* (1992) observed, applying UVR fixed at 365 nm, that the isolated rat thoracic aorta with intact endothelium responded with vasoconstriction, while the endothelium-removed preparations elicited vasodilatation, and on the bases of further observations that it is potentiated by pretreatment with acetylcholine or nitroprusside, but attenuated by LY83,583, they suggested that the vasoconstriction is related to tissue cyclic GMP and to the inhibition of EDRF and/or the release of EDCF.

In disagreement with these reports that UVR produced vasodilatation and that increase in cyclic GMP is closely related to the vasodilatation in various preparations (Baik *et al.*, 1994; Triggle and Bieger, 1990; Karlsson *et al.* 1986; Mikkelsen *et al.*, 1985), some researchers reported to have observed UVR-contraction. Karlsson *et al.* (1986) reported that contraction may occurs with 300 nm or shorter wavelengths, and

Wilgilius et al. (1990) also suggested that the differences among the UVR (365 nm)-induced responses might have resulted from the difference in the amount of endogenous substrate for nitric oxide in bovine mesenteric artery.

The wavelengths which elicited maximal contraction (320 nm) and maximal dilatation (420 nm) as observed in this study are clearly different from those of other investigators. One of the possibilities accounting for the discrepancy to be considered is the interference and distortion of the incident light by the glass organ bath which is commonly made of double-lumened glasses with circulating water for warming. The facts that both vasoconstriction and vasodilatation occur in the same preparations according to the wavelengths of UVR and that the conversion of the responses takes place within such a narrow distance as 30~40 nm implicate the possibility that the differences might also be caused by subtle discrepancies or inaccuracies of the apparatus employed in UVR. Of course, the species and tissue differences might be of the utmost importance because porcine coronary artery was dilated by irradiation with the same ultraviolet source which constricted the rat thoracic aorta as reported by Baik *et al.* (1992; 1994).

The increase in cyclic GMP is closely related to the vasorelaxation in the intact preparations as well as in the endothelium-removed ones. However, vasoconstriction does not seem to be caused by changes in cyclic GMP, that is, 'decrease' in the vasodilatory intracellular messenger, as expected, because no decrease in the cyclic GMP contents were evident, but rather, a tendency toward an increase was observed with UVR at 320 nm. This suggest that, other than cyclic GMP, a certain contractile mechanism, which is closely related to endothelium, might be activated by UVR of 320 nm. The finding that the maximal relaxation was 'shifted' to 370 nm in the denuded preparations might implicate the existence of certain contractile mechanism in the endothelium, which is activated by UVR of shorter wavelengths. It seems thus clear that the cyclic GMP is not involved in the UVR-contraction. However, this finding contradicts the observations of Baik *et al.* (1992) that cyclic GMP also may be involved in the contraction because the contraction was potentiated by the guanylate cyclase activators such as acetylcholine, nitroprusside, and atrial natriuretic peptide. We have at the present no evidence at hand to reconcile the discrepancy. The changes in vascular response according to varying wavelengths in the guanylate cyclase-activated conditions as employed by them remain to be investigated.

Overall, the present study indicates that the increase in cyclic GMP is closely related to vasodilatation induced by UVR of 420 nm in the endothelium-intact or 370 nm in the denuded preparations, whereas it is not involved in the vasoconstriction induced by UVR of 320 nm in the intact rings, and the mechanism leading to UVR-contraction remains to be clarified.

ACKNOWLEDGEMENT

This work was supported by "The Promotion of Basic Medical Science Fund 1994" of the Korean Medical Association. The author greatly appreciates the suggestions by Prof. Soo Wan Chae and Yong-Geun Kwak in Department of Pharmacology, Chonbuk University.

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=국문초록=

돼지 관상동맥 및 흰쥐 흉부대동맥에서 자외선 및 가시광선 조사시 파장에 따른 기계적 반응과 Cyclic GMP의 농도변화

전남대학교 의과대학 약리학교실

국 현

이 실험은 여러 파장(240~520 nm)의 자외선 또는 가시광선(이후 '광선'이라 표기함)을 흰쥐 흉부대동맥에 조사하여 이때의 혈관장력의 변화 및 조직내 cyclic GMP농도의 변화를 관찰하기 위하여 시행하였다. 돼지관상동맥 또는 흰쥐 흉부대동맥의 환상표본에 spectrofluorometer의 xenon lamp를 이용하여 여러 파장의 광선을 조사하고 이때의 장력변동을 polygraph상에 기록하였다. Cyclic GMP농도변화는 표본에 광선을 조사한 직후 조직을 얼리고 homogenization 및 원침시킨 후 상청액을 ether로 추출하여 RIA kit로 측정하였다.

Phenylephrine으로 수축된 내피존재 흰쥐 흉부 대동맥에서는 광선조사로 수축반응을 보였고 320 nm에서 최대수축반응을 일으켰다. 그 이상의 파장에서는 점차 수축반응이 감소되어 420 nm에서는 최대 이완반응을 일으킨 후 점차 기본장력으로 회복되었다. 그러나 내피제거 표본에서는 전파장에서 이완반응만을 일으켰고 이때 최대 이완반응은 370 nm에서 관찰되었다. 내피존재 표본에서 320, 380 및 420 nm의 광선을 30초간 조사한 결과 380과 420 nm에서 현저한 cyclic GMP의 증가가 관찰되었으나 320 nm에서는 유의한 변동이 없었다. 한편, 내피제거 표본에서는 370 nm의 광선조사로 cyclic GMP 함량이 약 4배 증가하였다.

이상의 성적으로부터 흰쥐 흉부대동맥은 광선조사에 의하여 내피존재표본에서는 수축-이완의 이상성반응을, 제거표본에서는 이완반응만을 일으키고 양 표본의 이완반응은 nitric oxide-cyclic GMP계의 활성화에 기인하나 수축반응은 cyclic GMP계와 직접 관련성이 없는 것으로 추론하였다.