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EFFECTS OF SODIUM NITRITE ON ULTRAVIOLET LIGHT-INDUCED RELAXATION AND ULTRAVIOLET LIGHT-DEPENDENT ACTIVATION OF GUANYLATE CYCLASE IN BOVINE MESENTERIC ARTERIES

I.M. Wigilius,* K.L. Axelsson,*,# R.G.G. Andersson,* J.O.G. Karlsson* and S. Ödman **,+

*Departments of Pharmacology, **Biomedical Engineering and #Biology, Linköping University, Linköping, Sweden

+Swedish Defence Research Establishment, Linköping, Sweden

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It was demonstrated that precontracted strips from different bovine mesenteric arteries showed variation in sensitivity to ultraviolet radiation (366 nm). Some strips relaxed when they were exposed to ultraviolet light, others showed no sensitivity at all and, finally, some showed contraction. However, all arteries relaxed when they were irradiated with UV-light in the presence of 10 μM $NaNO_2$.

Ultraviolet radiation (366 nm) increased the activity of guanylate cyclase in crude homogenate from bovine mesenteric arteries by about 20-fold in the presence of $NaNO_2$, while UV-light in the absence of sodium nitrite had no effect on the guanylate cyclase activation. These results support the notion that nitrite may be essential for vascular smooth muscle relaxation by UV-light, possibly through the release of nitric oxide.

In 1955, Furchgott and coworkers (1) reported that precontracted strips of rabbit aorta relaxed upon ultraviolet (UV) radiation (340-450 nm). Photorelaxation of vascular smooth muscle has been found to be reversible (1,2), independent of oxygen (1,2) and of endothelial cells (5). The relaxation of aortic strips by ultraviolet radiation resembles the relaxation produced by nitrocompounds and endothelium derived relaxing factor [EDRF] in many ways (2, 3, 5, 18, 19). For example, both photoinduced relaxation and relaxation induced by nitrocompounds and EDRF is associated with an elevated cGMP level in smooth muscle tissues (5-11, 20-22). Further, photoinduced relaxation of bovine mesenteric arteries may be due to an activation of soluble guanylate cyclase, since UV-light increased the activity of soluble guanylate cyclase of bovine mesenteric arteries (5, 12), and it is well known that nitrovasodilators and EDRF increase guanylate cyclase activity, and cGMP production, in

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several smooth muscle tissues (13, 14, 25). It has been proposed that relaxation of vascular smooth muscle both by light exposure and by nitrocompounds and EDRF is due to the release or production of some common substance (4, 5). This common substance might be nitric oxide (NO), since relaxation of smooth muscle by several nitrocompounds seems to depend on the generation of NO (15, 16). NO probably interacts with the ferrous heme of guanylate cyclase to cause enzyme activation (15).

In the present report it is demonstrated that sodium nitrite potentiates the UV-induced relaxation of bovine mesenteric arteries and also potentiates the UV-induced activation of guanylate cyclase in crude homogenate from bovine mesenteric arteries.

Materials and Methods

Tension studies: Bovine mesenteric arteries were obtained from a local slaugter house within 30 min. after slaugthering. The organs were transported to the laboratory in warm (37°C) Krebs solution, gassed with 95% O_2 + 5% CO_2. The arteries were carefully dissected free from adjacent fat and adventitia, opened longitudinally, cut into approx. 5 mm long pieces. The arteries were mounted in disposable polyethylene organ baths of 5 ml capacity and immersed in Krebs solution of the following composition (in mM): Na^+ 137, K^+ 6.0, Ca^{2+} 2.2, Mg^{2+} 1.3, Cl^- 134, $H_2PO_4^-$ 1.2, HCO_3^- 15.4 and glucose 5.6. The solution was equilibrated with 95% O_2 + 5% CO_2. The tension of the circular muscle layer was recorded by a Grass FT. 0.3 isometric strain gauge transducer and a Grass polygraph. Before start of the experiment the preparations were allowed to equilibrate for about 90 min. in Krebs solution at 37°C. To allow studies on relaxation each preparation was precontracted by addition of 3 µM phenylephrine to the bathing solution. Radiation of the preparations were performed with an ultraviolet lamp (UV-lamp, Desaga 366 nm, 10 $\mu W/cm^2/m$). The distance from the face of the lamp to the preparations during irradation was about 15 cm and the radiation time was 1 min.

Guanylate cyclase (GC) activity: BMA (from four animals) were homogenized in ice-cold (0.25 M) sucrose containing 5 mM Tris/HCl (pH=7.5). The homogenate was filtrated through gauze. The filtrate was stored under N_2 at -80 °C until used in the GC-assay. The effect of ultraviolet radiation (366 nm) or shielded radiation was studied in the GC-assay in the presence or absence of 0.1 mM $NaNO_2$. The distance from the lamp to the samples during radiation was about 10 cm. The GC assay medium had the following composition: 50 mM Tris/HCl (pH=7.5), 1 mM methylisobutylxanthine, 56 U/ml creatine kinase, 2 mM creatine phosphate, 2 mM Mg^{2+} and GTP in the indicated concentrations. The assay was carried out in a final volume of 100 µl at 37°C and the reaction was started by addition of 10 µl homogenate (about 62 µg protein). After 15 min the reaction was stopped by the addition of 300 µl of 50 mM Tris/HCl (pH=7.5) containing 50 mM EDTA, and by heating for 3 min. at 85°C. Thereafter the samples were batch treated with 50 mg of alumina. The cGMP content were measured by radioimmunoassay according to Steiner et al. (26) using reagents prepared in our laboratory. The results presented are based on the means calculated from duplicate samples in the assay.

Protein determination: Protein was measured according to Bradford (27) with the use of a commercial protein reagent (Pierce Chemical Company, USA).

Results

Ultraviolet radiation (366 nm) of bovine mesenteric arteries precontracted with 3 μM phenylephrine induced a reversible relaxation in most of the tissue specimens (Fig. 1a). However, some strips appeared to be insensitive to UV-radiation or even responded with a small contraction (Fig. 1b-c). Addition of 10 μM $NaNO_2$ *per se* evoked a relaxation of the arteries, and markedly potentiated the relaxatory response to UV-radiation (Fig. 1a-c). In order to ensure that this potentiated relaxation was not an effect of the reduction in tension elicited by $NaNO_2$, further doses of phenylephrine was added until the original tension level was attained (Fig. 1a-c). This photo-induced relaxation was also reversible.

The guanylate cyclase activity in the crude homogenate from bovine mesenteric arteries was determined with MgGTP as substrate. The effect of ultraviolet

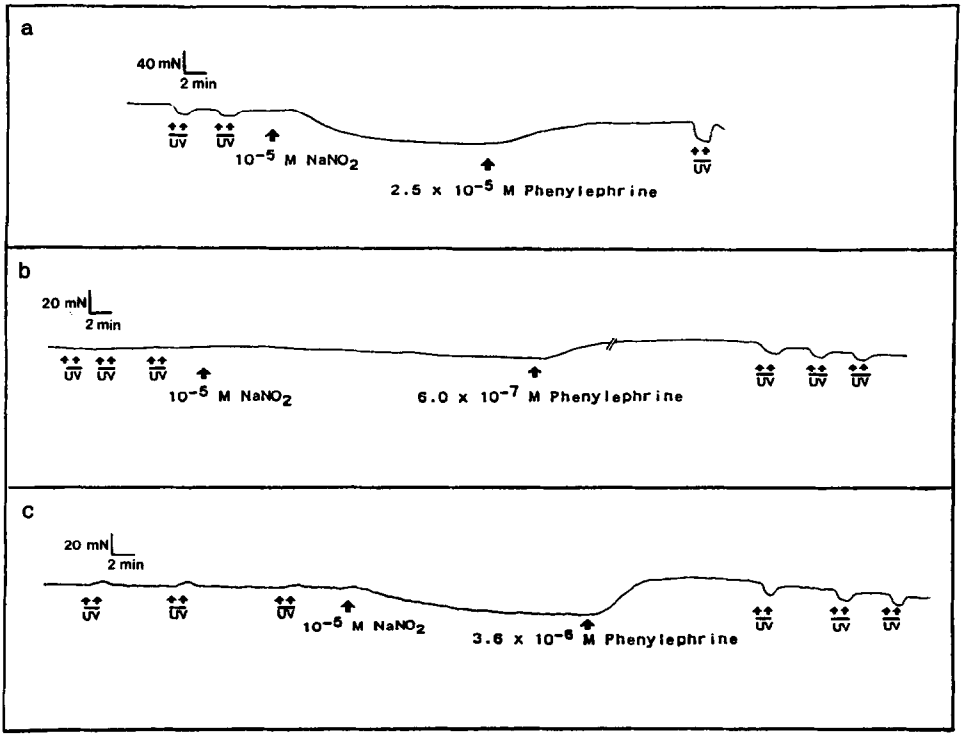

Figure 1.
Effects of ultraviolet radiation (366 nm) on phenylephrine-contracted (3 μM) bovine mesenteric arteries from three animals in the absence and presence of 10^{-5} M $NaNO_2$. Fig. 1 a-c shows variation in sensitivity to UV-light before an addition of $NaNO_2$, while all arteries relaxed upon radiation in the presence of $NaNO_2$. UV in the figures indicate UV irradiation (1 min.)

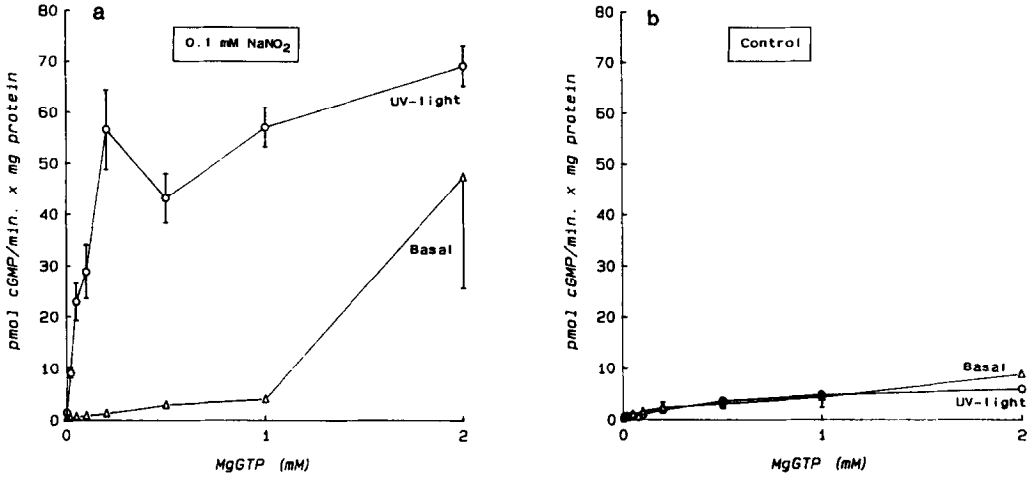

Figure 2.
Effects of ultraviolet radiation (366 nm) on guanylate cyclase activity of bovine mesenteric arteries in a) presence and b) absence (i.e. control) of 0.1 mM $NaNO_2$. The enzyme activity was determined under basal conditions (Δ-Δ) (i.e. shielded radiation) and in the presence of UV-light (O-O). Vertical lines indicate S.E.M.; n= 3.

irradiation (366 nm) was investigated in the presence or absence of 0.1 mM $NaNO_2$ (Fig. 2). The basal guanylate cyclase activity in the absence of $NaNO_2$ was 8.79 ± 0.45 pmol cGMP/mg protein x min and no effect of UV-radiation could be seen (Fig. 2b). 0.1 mM $NaNO_2$ in the absence of UV-radiation did not have any effect on the activity of guanylate cyclase in the GTP concentration range between 2 μM and 1.0 mM (Fig. 2a). However, at 2 mM GTP the guanylate cyclase activity was increased about 10-fold. Ultraviolet radiation in the presence of 0.1 mM $NaNO_2$ gave a potentiation of the guanylate cyclase activity which was most marked at low substrate concentrations. The maximal enzyme activity was 69.4 ± 4.0 pmol cGMP/mg protein x min in the presence of UV-radiation (Fig. 2a).

Discussion

The present study shows that sodium nitrite potentiates the relaxation elicited by UV-light (366 nm) in bovine mesenteric arteries and potentiates the UV-dependent activation of guanylate cyclase in crude homogenate from bovine mesenteric arteries. Relaxation of vascular smooth muscle by UV-radiation has previously been reported (1-5), and potentiation of this relaxation by sodium nitrite was recently shown in rabbit aorta (4). It was

suggested that this potentiation was due to UV-dependent release of some vascular smooth muscle relaxant factor from nitrite, and it was speculated that this factor could be identical to nitric oxide (4). UV-induced vascular smooth muscle relaxation has been shown to be mediated by cGMP (5), and cGMP is also the mediator for vascular smooth relaxation induced by various nitrocompounds (5-11) and EDRF (22). The mechanism responsible for this increase in cGMP is thought to involve production of nitric oxide from the nitrocompounds or the endothelium, and it has been suggested that the nitric oxide subsequently interacts with and activates guanylate cyclase (15-17, 23). The involvement of nitric oxide in UV-mediated guanylate cyclase activation is also in accordance with our previous finding that scavengers of oxygen derived free radicals potentiates this effect (12). Oxygen derived free radicals such as O_2^- is known to rapidly inactivate nitric oxide.

The lack of effect of UV-radiation on guanylate cyclase activity in the absence of sodium nitrite, as opposed to our previous findings (5, 12) is not known. It might be that the use of different guanylate cyclase preparations (ie. crude homogenate in the present study and crude soluble guanylate cyclase in previous investigations) is one explanation. Another explanation might be that the amount of endogenous substrate for generation of the guanylate cyclase activator nitric oxide varies in different artery preparations.

One other finding made in the present study was that arteries from different animals responded differently to UV-radiation. Some arteries showed relaxation, some gave no response while others responded with a contraction. The reason for these differences is not known, although it could be speculated that the amount of endogenous substrate for generation of nitric oxide is one important factor which determines degree of relaxation obtained. The observation that addition of sodium nitrite to the bathing medium in all cases sensitized the arteries to the relaxatory effect of UV-light may support this assumption. Furthermore, it has previously been shown that other smooth muscle tissues which is insensitive to UV-induced relaxation (eg. rat stomach muscle), exhibits photorelaxation after addition of sodium nitrite (1). Another possible explanation could be that different arteries exhibit

slightly different spectral sensitivity. We have previously shown that UV-light of shorter wavelength than 366 nm elicits contractions in bovine mesenteric arteries (24).

Further studies are obviously needed in order to clarify the mechanism by which UV-light activates guanylate cyclase and induces smooth muscle relaxation, and the reason for the varying responsiveness to UV-relaxation among different smooth muscle preparations.

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