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Abstract

The applications of ultraviolet (UV) light (336 nm) on the upper oesophageal strips of frog elicited relaxant responses in the presence of NaNO2 (50 microM). The tissues were mounted under the tension 0.5 g in an organ bath containing Ringer solution, maintained at 25 degrees C and gassed with 100% O2. The responses were recorded on a kymograph via an isotonic lever. Antimegaloblastic agents, including hydroxocobalamin (1, 10, and 100 microM), cyanocobalamin (1, 10, 25, and 100 microM), and folic acid (1, 10, 50, 100, and 200 microM), significantly attenuated the relaxation response to UV light. Folinic acid (1, 10, 25, and 100 microM), however, enhanced the relaxation. Pyrogallol (50 microM), hydroquinone (50 microM), and diethyldithiocarbamic acid (8 mM) were found ineffective for attenuation, though FeSO4 (200, 400, and 500 microM) and hemoglobin (50 microM), respectively, exerted significant inhibition. L-arginine methylester (500 microM) did not impair UV-induced relaxation. Based on these results, we concluded that a mechanism involving undefined action(s) of antimegaloblastic drugs may cause alterations in the UV light-induced relaxation of the tissue used.

KEYWORDS: ultraviolet light, frog oesophageal strip, hydroxocobalamin, cyanocobalamin, folic acid

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Inhibitory Actions of Hydroxocobalamin, Cyanocobalamin, and Folic Acid on the Ultraviolet Light-Induced Relaxation of the Frog Upper Oesophageal Strip

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The applications of ultraviolet (UV) light (336 nm) on the upper oesophageal strips of frog elicited relaxant responses in the presence of $NaNO_2$ (50 μM). The tissues were mounted under the tension 0.5 g in an organ bath containing Ringer solution, maintained at 25 °C and gassed with 100% O_2. The responses were recorded on a kymograph via an isotonic lever. Antimegaloblastic agents, including hydroxocobalamin (1, 10, and 100 μM), cyanocobalamin (1, 10, 25, and 100 μM), and folic acid (1, 10, 50, 100, and 200 μM), significantly attenuated the relaxation response to UV light. Folinic acid (1, 10, 25, and 100 μM), however, enhanced the relaxation. Pyrogallol (50 μM), hydroquinone (50 μM), and diethyldithiocarbamic acid (8 mM) were found ineffective for attenuation, though $FeSO_4$ (200, 400, and 500 μM) and hemoglobin (50 μM), respectively, exerted significant inhibition. L-arginine methylester (500 μM) did not impair UV-induced relaxation. Based on these results, we concluded that a mechanism involving undefined action(s) of antimegaloblastic drugs may cause alterations in the UV light-induced relaxation of the tissue used.

Key words: ultraviolet light, frog oesophageal strip, hydroxocobalamin, cyanocobalamin, folic acid

Previous experimental data (1-4) obtained from vascular smooth muscle suggest that ultraviolet (UV) light-evoked relaxations is due to the release of nitric oxide (NO). A response of this sort is independent of the existence of endothelial cells and progressively declines after repeated exposure of the preparation to UV light. A reasonable explanation for this independence and decline is still lacking, though one hypothesis (5) has put forward the presence of a photoactivable and depletable NO pool in vascular smooth muscle. Notably, nonvascular smooth muscles are insensitive or less sensitive to UV light, though addition of $NaNO_2$ or N^{ω}-L-nitro-arginine into the bathing medium makes these tissues sensitive to UV treatment (6-8). Similar enhancement in sensitivity to UV light also occurs in the mouse gastric fundus (9). On the other hand, hydroxocobalamin, an antimegaloblastic agent, inhibits NO-mediated relaxation in the aorta with intact endothelium and anococcygeal muscle of rat and in the mouse corpus cavernosum (10-13). Additionally, the antimegaloblastic agent can inhibit UV-light induced relaxation of the mouse gastric fundus in the presence of $NaNO_2$ (9). An interesting pharmacological preparation, upper oesophageal strip of frog, relaxes with electrical field stimulation, and a nitrergic mechanism seems to be responsible (14, 15). These results prompted us to determine whether UV light evokes relaxation of the tissue in the presence of $NaNO_2$. Further studies were also carried out to elucidate the effect of antimegaloblastic agents on this relaxation.

Materials and Methods

The animal subject, a frog (Rana pipiens) weighing 20-25 g, was decapitated and pitched. The oesophagus was rapidly removed and its upper segment was incised longitudinally to obtain a strip (approximately 10 mm long and 3 mm wide). The preparation thus obtained was mounted under the tension of 0.5 g in an organ bath filled with Ringer solution (in mM; NaCl 111.1, KCl 1.88, $CaCl_2$

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1.08, Na H_2 PO_4 0.083, $NaHCO_3$ 2,38 and glucose 10.1) maintained at 25 °C and gassed with 100% O_2. Throughout the experiment, the bathing medium containing $NaNO_2$. Changes in the muscle length were recorded on a kymograph via an isotonic lever (x8–10 magnification). The UV lamp (Vilmar Lournet VL GL) was placed next to the outer wall of the organ bath, at a distance of about 3 cm from the strip. After 60 min at equilibrium, the strip was precontracted with 1 μM carbochol. When contraction reached a plateau (at approximately 10 min), the tissue was exposed to the UV-light (360 nm) for 10 min. The same procedure was repeated after the resting period of 30 min interval. At the end of each UV light application, the preparation was washed with fresh solution. Thus, control responses were obtained.

In the first series of experiments, following the first application of UV light, the tissue was incubated with hydroxocobalamin (1, 10, or 100 μM), cyanocobalamin (1, 10, 50, or 100 μM), folic acid (1, 10, 50, 100, or 200 μM), or folinic acid (1, 10, or 100 μM) for 30 min. The tissue after those pretreatments were again precontracted with 1 μM carbochol and exposed again to UV light. In the second series of experiments, the effects of hydroquinone (50 μM), pyrogallol (50 μM), diethyldithiocarbamic acid (DETCA; 8 mM), $FeSO_4$ (100, 200, 400, or 500 μM), human hemoglobin (50 μM), or L-arginine-methylester (L-AME; 500 μM) on the UV light-induced responses were investigated by adding these agents into the bathing medium. Similar protocol to that used in the first series was applied. All of the chemicals except DETCA were included the bath throughout the second phase of the experiment, DETCA was washed out before the addition of carbachol. The experiments for each concentration of chemicals were performed on a separate group of strips.

Drugs and solutions. $NaNO_2$ was purchased from Merck Co. (Darmstadt, F.R., Germany). All other chemicals and drugs were purchased from Sigma (St. Louis, MO, USA). Each was dissolved in distilled water before use.

Statistical considerations. Responses of the strip to UV light were expressed as percent of the first response (% control), and comparisons were made by use of Student's *t*-test. $P < 0.05$ was considered significant

Results

In the presence of $NaNO_2$, UV light (366 nm) evoked reproducible relaxations of the upper oesophageal strips of frog precontracted with 1 μM carbochol (Fig. 1). The effect of UV light was reversible; length of the tissue was consistently restored back to base line value. No significant difference was observed between the responses to the first and the second UV applications.

Hydroxocobalamin (1, 10 and 100 μM) cyanocobalamin (1, 10, 25 and 100 μM) and folic acid (1, 10, 50, 100 or 200 μM) significantly inhibited UV light-evoked relaxations (Figs. 1, 2 and 3). The responses to UV light almost disappeared at the highest concentration tested. In contrast, folinic acid (1, 10, 25 or 100 μM) enhanced the relaxations (Fig. 3). All of the substances affected tissue response in a dose-dependent manner.

Pyrogallol (50 μM) and hydroquinone (50 μM) did not significantly alter the relaxant responses (Table 1), nor were these responses affected by DETCA (8 mM). However, $FeSO_4$ (200, 400 or 500 μM) exerted a significant inhibitory effect on UV-induced responses in a concentration-dependent manner. Hemoglobin (50 μM) completely abolished the relaxant responses. No observable change could be demonstrated after addition of (500 μM) L-NAME.

Discussion

The present study clearly shows that hydroxocobalamin, cyanocobalamin, and folic acid, but not folinic acid, significantly inhibit the UV-evoked relaxation of frog oesophageal strips in the presence of $NaNO_2$. The inhibitory action of hydroxocobalamin has been demonstrated on NO-induced relaxation in previous studies on the endothelium-intact aortic rings and the anococcygeus muscle of rat (10–12). Hydroxocobalamin has also been shown to reduce the relaxant responses induced by electrical field stimulation and acidified $NaNO_2$ in the mouse corpus cavernosum (13, 16). Further, it was reported that UV light-evoked relaxations of the strips of mouse gastric fundus can be inhibited by the same chemical (9). In the present study, we demonstrated that hydroxocobalamin has similar inhibitory effect on the relaxant responses of frog oesophageal strips to UV light. Likewise, cyanocobalamin exerted an inhibitory effect identical to that of hydroxocobalamin. Folic acid, also an antimegaloblastic drug, might be expected to inhibit UV

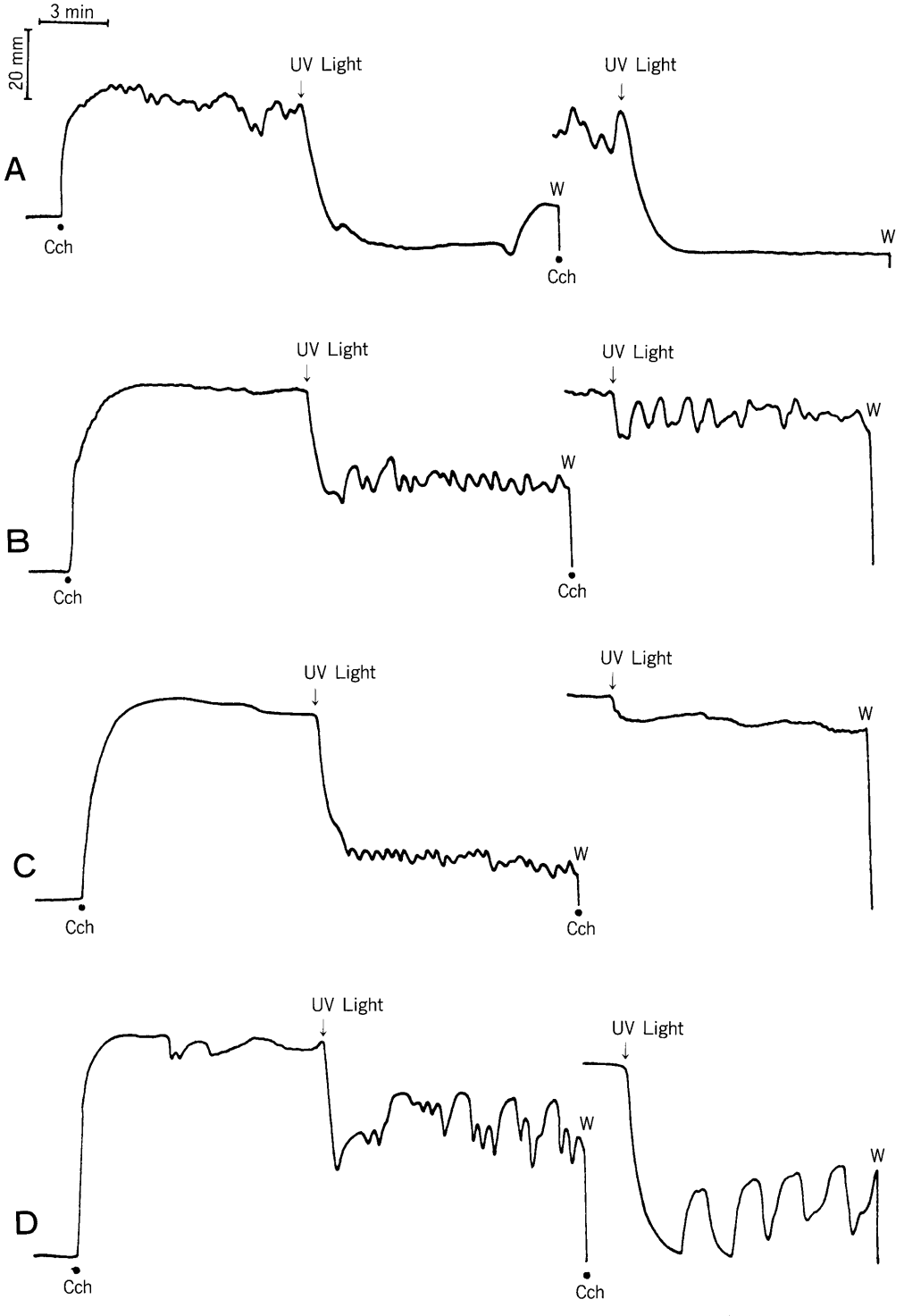

Fig. 1 Typical tracings showing photorelaxations of the frog upper oesophageal tissue precontracted with 1 μM carbachol (Cch). Second responses were obtained in the absence (A) and presence of 10 μM hydroxocobalamin (B), 100 μM folic acid (C), or 100 μM folinic acid (D). Following application of ultraviolet (UV) light, recording was stopped and the tissue was washed (w); after the resting period of 30 min, carbachol was added into the bathing medium and recording recommenced when the tissue tone had approximately reached that of the previous level.

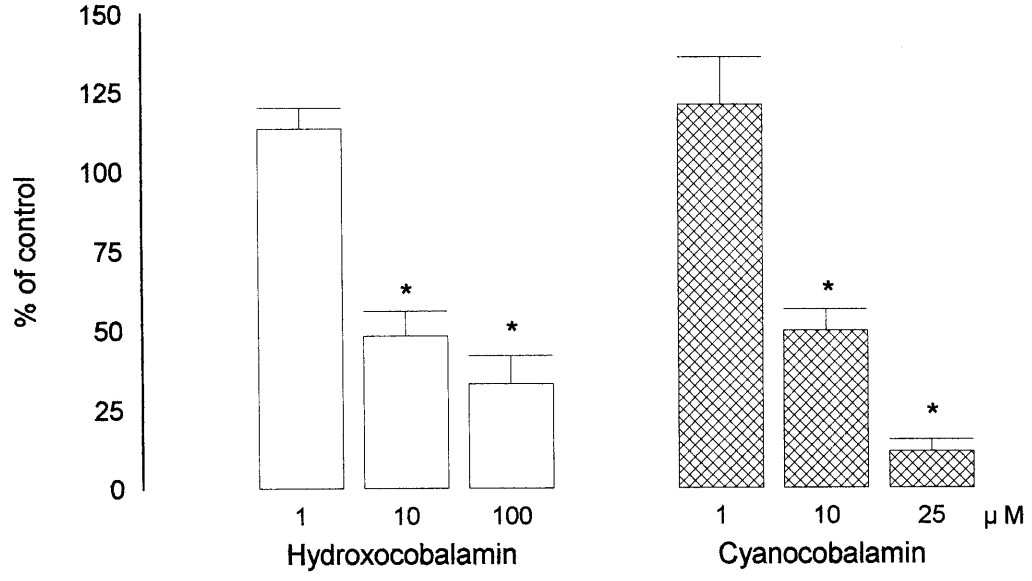

Fig. 2 Concentration-dependent inhibitory effects of cobalamins on photorelaxation in the presence of $NaNO_2$. *indicates statistical significance when compared with control ($P < 0.05$).

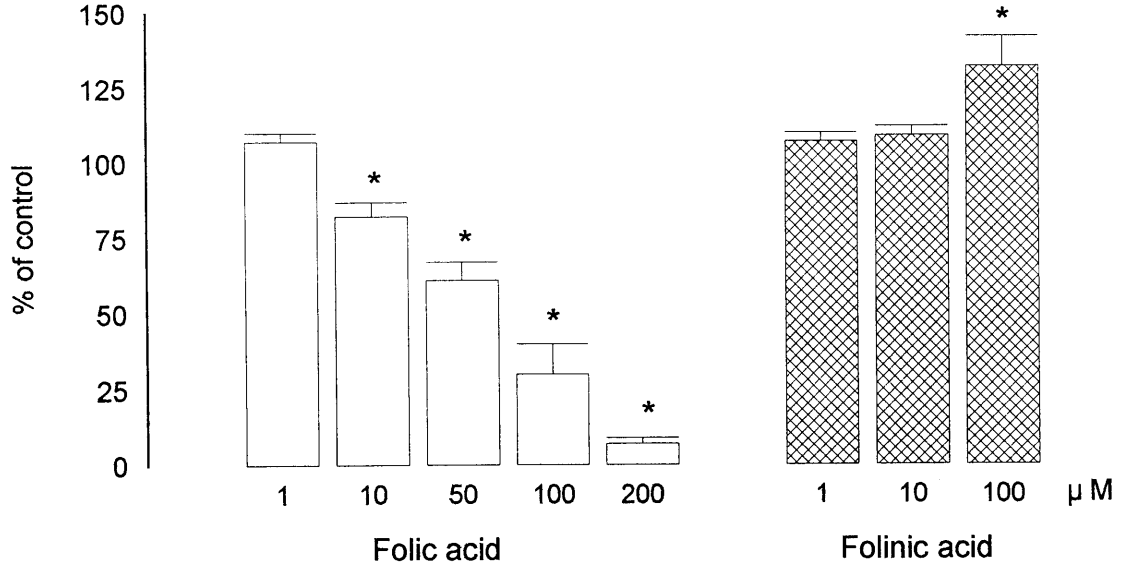

Fig. 3 Effects of folic and folinic acid on photorelaxation in the presence of $NaNO_2$. *indicates statistical significance when compared with control ($P < 0.05$).

light-evoked relaxations. Indeed, we found that folic acid produced significant inhibition of UV light-induced relaxations. The exact mechanisms by which different antimegaloblastic substances inhibit UV light-induced relaxation remains to be identified. These substances may directly protect the tissue against UV light. The hypothesis that they prevent UV-evoked NO liberation from $NaNO_2$ may also be suggested to explain their actions. This hypothesis reasonable, when we take into account the previous observation that UV light causes NO generation from a series of chemicals including $NaNO_2$ (17). Both mechanisms may be involved in mediating the

Table I Effects of various agents on the photorelaxation induced by ultraviolet light in the presence of $NaNO_2$ of frog upper oesophageal strips

	Untreated	Treated
Control	68.4 ± 6.7	76.4 ± 5.0
500 μM L-AME	62.5 ± 4.9	76.2 ± 5.9
50 μM Pyrogallol	46.8 ± 7.0	60.8 ± 9.9
50 μM HQ	47.9 ± 4.4	57.8 ± 4.5
8 mM DETCA	32.2 ± 8.4	43.1 ± 8.8
400 μM $FeSO_4$	38.4 ± 5.3	14.3 ± 6.2*
50 μM Hemoglobin	36.4 ± 7.3	0*

Results (mean ± SE) are presented as % relaxations (n = 4–11). *indicates statistical significancy when compaired with untreated (first) series ($P < 0.001$). DETCA, diethyldithiocarbamic acid; HQ, hydroquinone; L-AME, L-arginine methylester.

inhibition where the net effect will be dependent on the relative contribution of each process. Folic acid-evoked inhibition may be resulted not solely from the action of the parent molecule but also from that of photodegratation product(s), given that folic acid is well known to be decomposed by light into paraaminobenzoic acid, pterine 6-carboxylic acid, para aminobenzoyl L-glutamic acid, and pteroic acid (18). Surprisingly, folinic acid enhanced UV relaxations. This was unexpected given that folic acid, a chemically related substance, was found to inhibit the same phenomenon. It is difficult to explain why folinic acid action qualitatively differs from those of other antimegaloblastics. After devoting some thought to the elucidation of this unusual action, we supposed the following. First, folinic acid may enhance NO generation from $NaNO_2$; this suggestion may be plausible, based on the fact that 5 methyl tetrahidrofolate, an active form of folic acid, has been shown to inhibit xanthine oxidase-induced superoxide generation and to restore NO activity in familial hypercholesterolemia (4). Second, this particular chemical may improve the tissue responses to the UV light by an as yet unidentified mechanism.

Superoxide anion-generating agents such as hydroquinone and pyrogallol did not impair the relaxation. This unexpected finding cannot easily be explained. It is possible that NO is destroyed by superoxide anions. Further studies are needed to account for this discrepancy. DETCA was also found to be ineffective. This can be attributed to the possibility that the DETCA-sensitive enzyme (superoxide dismutase) activity is absent in the frog oesophagus; if this were so, it would follow that UV-released NO exerts its action without being affected by this type of agent. Further, we examined the effects of $FeSO_4$ and hemoglobin on the UV-induced relaxations; both were found to be inhibitory, and mitigated the responses. This finding is consistent with those of an earlier study (9), which was carried out on the mouse gastric fundus by use of UV light. Little is known about the mechanism underlying this interaction. However, it is not surprising that such an action of iron-containing chemicals (inorganic or organic) would be observed; supporting evidence shows that NO suppresses xanthine oxidase activity by directly binding the iron sulphate moiety of the enzyme. It is quite possible that NO synthase does not make any contribution to UV-induced relaxations, based on the finding that no significant impairments on the responses to UV light were observed after the treatment of strips with L-AME.

In conclusion, experimental data presented here clearly showed that antimegaloblastic substances significantly impair UV light-evoked relaxation. Hydroxocobalamin, cyanocobalamin, and folic acid significantly protected the tissue exposed to UV light, and therefore may have potential use in several clinical situations such as extreme sunburn and similar disorders resulting from exposure to excessive amounts of UV light. To our knowledge, data indicating the beneficial effects of these substances on such clinical lesions are currently lacking. Nevertheless, evidence suggesting that the release of NO or peroxynitrite may be responsible for the pathogenesis of sunburn erythema and inflammation is available (19). At present, there is no firm evidence to support the therapeutic usefulness of these substances in such clinical situations. Further studies are necessary to confirm this. Investigation of the extent to which pathological changes are dependent on UV light is also needed.

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