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HISTAMINE RELEASE FROM RAT PERITONEAL MAST CELLS EXPOSED TO ULTRAVIOLET LIGHT

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Abstract. After irradiation with ultraviolet light, rat peritoneal mast cells incubated with the histamine liberator compound 48/80 were found to have reduced capacity to release histamine. The action spectrum for histamine release inhibition seemed to be in the wavelength region of UVB. UV-light *per se* did not induce histamine liberation other than for dosages ≥ 1.45 J/cm^2. No such release was observed with the doses of UVA studied (3.4–20.5 J/cm^2). The metabolic inhibitor 2.4-dinitrophenol (DNP), which almost completely inhibited compound 48/80-elicited histamine release, did not influence the UV-induced histamine release, indicating that the latter was not a secretory process but due to cytotoxic leakage of histamine from the cells. These results suggest that inhibition of histamine-releasing capacity or reduction of skin histamine due to photolysis of mast cells may explain the beneficial effect of UV in pruritic disorders where histamine release from mast cells is involved.

In a previous paper we reported that exposure of human subjects to ultraviolet light, alone or in combination with oral psoralen (UVB, UVA or PUVA), was followed by a decrease in itch and flare responses induced by intradermal injection of the histamine-liberating agent compound 48/80 (3). This was as a rule not observed with intradermally injected histamine. Thus, UV-light probably reduced compound 48/80-induced histamine release from dermal mast cells. Conceivably this could occur by inhibition of cellular release mechanisms, or due to a decrease in cellular histamine content. In the present work these possibilities were studied in rat mast cell suspensions exposed to UV light in vitro. It was found that moderate doses of UVB-light inhibited the histamine releasing effect of compound 48/80, whereas higher doses seemed to cause a cytotoxic histamine leakage from the mast cells. UVA light seemed to be of minor or no influence *per se* but probably augmented the inhibiting effect of UVB.

MATERIAL AND METHODS

Mast cells

Suspensions of mixed peritoneal cells, containing about 3–5% mast cells, were prepared from male Sprague-Dawley rats as described previously (2). In each experiment, cells from one rat were prepared, washed once and resuspended in 25–30 ml buffered saline (NaCl, 154 mM; KCl, 2.7 mM; $CaCl_2$, 0.9 mM; Na_2HPO_4, 4.0 mM; KH_2PO_4, 2.7 mM), pH 7.0, containing 0.1% serum albumin. Four-ml portions of this suspension were irradiated for different time periods in quartz cuvettes at a distance of 15 cm from the light sources under continuous cooling by an electric fan. In inhibitor experiments the uncoupling agent 2,4-dinitrophenol (DNP) was added before the irradiations. The cell suspensions were never exposed to temperatures exceeding +36°C.

In the experiments with light cabins, irradiation started simultaneously for all cuvettes which were then removed at appropriate time points and placed in ice-water until further handling. In the experiments with the xenon arc lamp only one cuvette could be irradiated at a time. Meanwhile, the remaining cell portions were kept in darkness in room temperature (+25°C) before irradiation and subsequent transfer of the cuvettes to ice-water.

After the irradiation procedures which lasted for at most about 65 min, 0.9 ml samples of irradiated and control cells were transferred to centrifuge tubes each containing 0.1 ml of compound 48/80 or buffered saline. After incubation for 10 min at +37°C the cells were centrifuged at 350 *g* at 0°C for 10 min. The supernatants were carefully decanted. The sediments were resuspended in 2 ml of saline and boiled for 3 min to extract histamine. The histamine was determined in the supernatant and sediment by the fluorometric method described by Shore et al. (15), omitting the purification steps (4). Histamine relase was calculated and expressed as a percentage of the total amount in each tube.

To evaluate the effect of heat, 4-ml aliquots of light-protected cells were treated for varying time periods in water baths of varying temperature before incubation with compound 48/80 or buffered saline and further handling as described.

Light sources

Light sources used were Waldmann radiation cabins UVA 1000 and UVB 1000 (H. Waldmann GmbH + Co, Werk

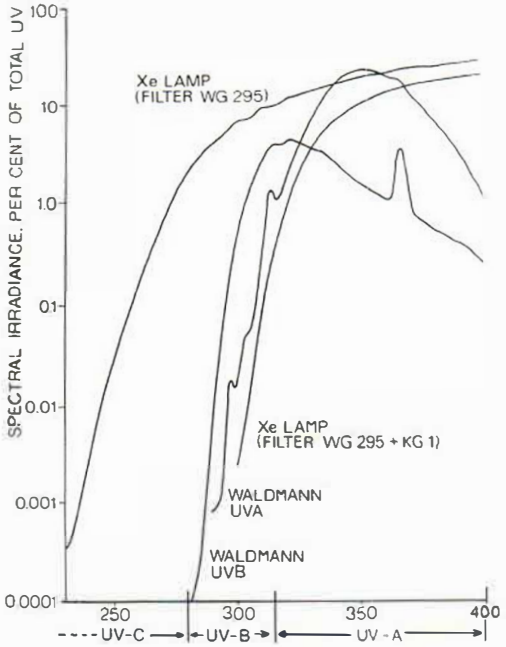

Fig. 1. Relative spectral irradiance of the following light sources; an Osram high pressure xenon (Xe) arc lamp (XBO 150W) equipped with filter WG295 or WG295 + KG 1 and Waldmann radiation cabins either UVB 1000 equipped with Sylvania lamps F75 85W/UV6 or UVA 1000 equipped with Sylvania lamps F75 85W PUVA.

für Lichttechnik, Villingen-Schwenningen, W. Germany) used in clinical practice and an Osram high pressure xenon arc lamp (XBO 150 W).

Technical data for the Waldmann cabins have been presented in a previous paper (3). The xenon arc lamp, equipped with a 2 mm Schott 295 filter to eliminate UVC gave a sun-spectrum-like radiation including UVB and UVA. A 2-mm KG 1 filter was used to filter out UVB. A 2-mm RG 715 filter was used to eliminate wavelengths below the infrared region. The lamps were allowed to stabilize during a warm-up period of 5 min before use.

Computer-plotted relative spectral irradiance expressed as percentage of total UV emission from the lamps was determined for each nanometer in the spectral interval from 230 to 400 nm. Spectral irradiance was measured in a plane of reference with a laboratory based narrow bandwidth spectroradiometer system from EG & G Inc., Salem, Massachusetts, type 580/585. The slit width was 3.5 nm. The EG & G spectroradiometer was calibrated against a 1 000 W halogen lamp, type G. E. DXW 1 000 from Statens Provningsanstalt, Borås, Sweden, with data traceable to the National Bureau of Standards (NBS), USA. The spectroradiometer was in close contact with the lamps from the Waldmann devices, whereas the irradiation was measured at a distance of 15 cm from the xenon arc lamp aperture. The reference irradiation was compared with corresponding readings from a battery-operated portable digital wide bandwidth radiometer J-260 with sensors for 297 and 365 nm from Ultraviolet Product Inc. (San Gabriel, California). Thus calibrated, the instrument was used to measure the ultraviolet irradiation at a distance of 15 cm from the lamps in the Waldmann light cabins. For further information concerning technical details, see Wester (19). Relative spectral irradiance from the different lamps are presented in Fig. 1. Light intensitites of the radiation sources are presented in Table 1.

Table 1. *Light intensities of the radiation sources at a distance of 15 cm from lamp apertures*

Radiation source	Light intensity (mW/cm²) UVC	UVB	UVA
Waldmann UVB 1000	–	0.27	1.30
Waldmann UVA 1000	–	0.06	9.60
Xenon arc lamp			
with filter WG 295	0.18	2.41	20.76
with filter WG 295 + KG 1	–	0.02	11.38

The irradiance level of the infrared radiation at a distance of 15 cm from the xenon arc lamp was determined by a battery-operated portable opto-meter from United Detector Technology (UDT) Inc. (Santa Monica, California), model 40X, calibrated at 632.8 nm against a standard (UDT) traceable to NBS. The opto-meter equipped with a radiometric filter assured a flat response from 450–950 nm. Light intensity from the xenon arc lamp with filters WG 295, WG 295+KG1 or WG 295+RG 715, exceeded the maximum value measurable with the device, i.e. 10 mW/cm². For comparison, light intensities at the same distance from lamps in the Waldmann cabins were as follows: for UVA cabin 2.4 mW/cm² and for UVB cabin 0.8 mW/cm².

RESULTS

Xenon arc lamp

After exposing mast cells to sun spectrum-like irradiation, histamine release induced by compound 48/80 (0.3 and 1.0 μg/ml) was inhibited (Fig. 2*a*). A dose–response relationship was observed between moderate light doses (0.07–0.72 J/cm² of UVB and 0.6–6.2 J/cm² of UVA) and inhibition of histamine release. Further increase of the dosages (UVB≥1.45 J/cm² and UVA≥12.5 J/cm², i.e. ≥10 min exposure) resulted in increasing 'spontaneous' release of histamine. This spontaneous release was not significantly augmented by subsequent incubation of the cells with compound 48/80.

In order to study the effect of UVA a KG 1 filter was used to eliminate most of the UVB radiation from the xenon arc lamp. The cells were exposed to 3.4–20.5 J/cm², i.e. 5–30 min exposure time. However, no inhibition of the 48/80-induced histamine release occurred and no 'spontaneous' histamine

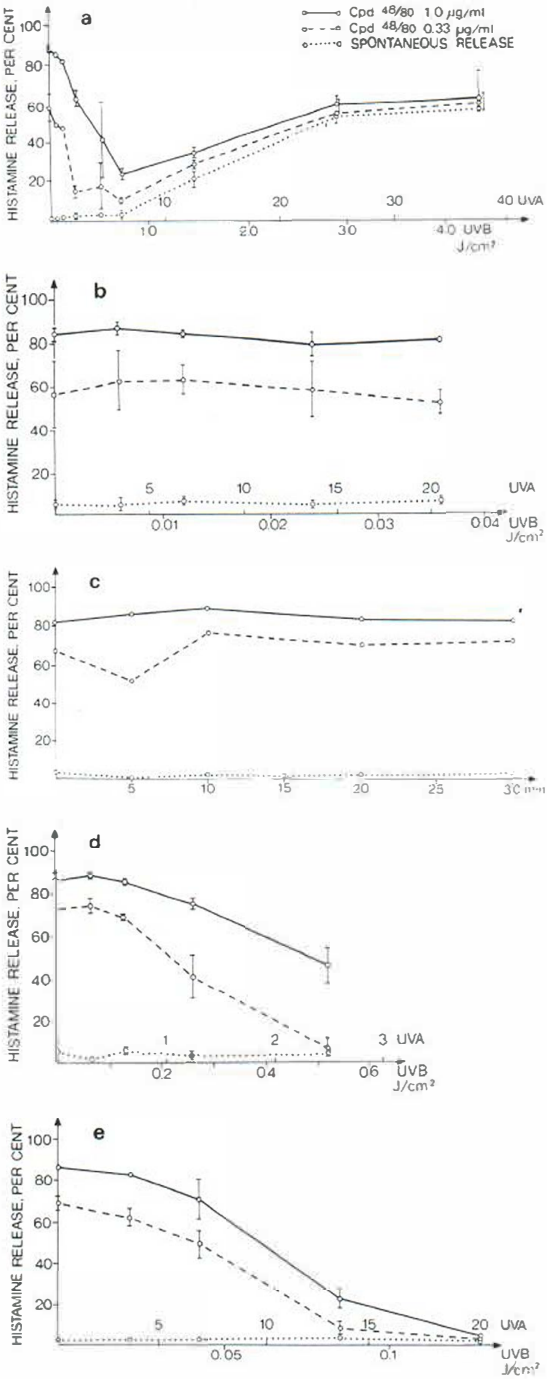

Fig. 2 a–e. Histamine release (mean and range based on two experiments; 2c based on one experiment) from rat mast cells induced by compound 48/80 following UV preexposure. Light sources utilized were an Osram high pressure xenon arc lamp equipped with (*a*) filter, WG 295, (*b*) filter WG 295 + KG 1 or (*c*) filter WG 295 + RG 715 and Waldmann radiation cabins, (*d*) UVB 1000 or (*e*) UVA 1000.

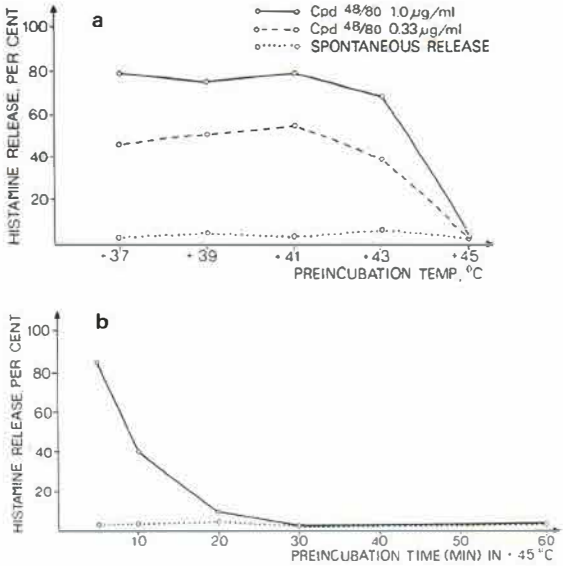

Fig. 3 a, b. (*a*) Temperature influence on histamine release from rat mast cells. The mast cells were preincubated at different temperatures for 30 min and exposed to compound 48/80 for 10 min at +37°C. (*b*) Time course of temperature influence on histamine release. The mast cells were preincubated at +45°C for varying time periods and exposed to compound 48/48 for 10 min at +37°C.

release was observed, even after the highest light doses (Fig. 2*b*). The concomitant emission in the UVB region did not exceed 0.04 J/cm².

Infrared light studied by use of a RG 715 filter neither induced histamine liberation *per se* nor influenced mast cells subsequently incubated with compound 48/80 (Fig. 2*c*).

Waldmann light cabins

Histamine release from mast cells, induced by compound 48/80, was inhibited following irradiation for 4–32 min in the UVB cabin (0.07–0.52 J/cm²) or for 6–35 min in the UVA cabin (3.4–20.5 J/cm²). In both cases the inhibition was related to the dose of UV light (Fig. 2*d, e*). No spontaneous release of histamine was observed. A low dose of UVB was emitted in the UVA cabin, e.g. together with 20.5 J/cm² of UVA there was an emission of 0.13 J/cm² of UVB, i.e. more than 3 times that from the xenon arc lamp equipped with filter WG 295+KG 1. This might be of importance to explain the inhibition observed after irradiation in the UVA cabin. The concomitant UVA emission in the UVB cabin did not exceed 2.5 J/cm².

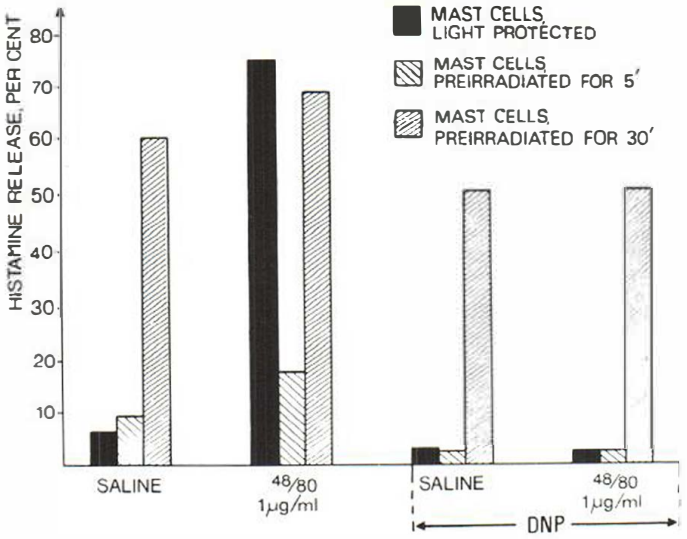

Fig. 4. Influence of 2,4-dinitrophenol (DNP), 10^{-4} M, on histamine release from rat mast cells induced by (i) UV irradiation *per se*, and by (ii) compound 48/80 following UV pre-exposure to an Osram high pressure xenon arc lamp equipped with filter WG 295.

Temperature influence

The mast cell suspensions were continuously cooled by an electric fan during the UV exposures. The cell suspensions were never exposed to temperatures exceeding +36°C. We confirmed that higher temperatures were required to induce histamine release or influence mast cell reactivity. Thus, incubation of mast cells in water baths of the following temperatures: +37°C, +39°C, +41°C and +43°C for 30 min did not induce spontaneous histamine release or influence the histamine release stimulated by compound 48/80. Only when the mast cells had been incubated in +45°C for 10 min, was a significantly decreased response to compound 48/80 observed (Fig. 3*a*, *b*).

Metabolic inhibition

The metabolic uncoupler of oxidative phosphorylation, 2,4-dinitrophenol (DNP) 10^{-4} M virtually abolished compund 48/80-induced histamine release from UV-pre-irradiated as well as light protected mast cells. However, it had no influence on UV-induced spontaneous release (Fig. 4).

DISCUSSION

This investigation shows that histamine release, induced by compound 48/80, was inhibited in rat mast cells pre-exposed to ultraviolet light from a high pressure xenon arc lamp or from fluorescent lamps in Waldmann UVA and UVB cabins. When the UVB spectrum had been eliminated from the xenon light there was no inhibition of the subsequent histamine release, although dosages up to 20.5 J/cm^2 of UVA were given. Thus, UVB seemed to be essential for the inhibiting effect. Accordingly, similar doses of UVB from the xenon lamp and the UVB cabin had approximately the same inhibitory effect. The inhibition following irradiation in the Waldmann UVA cabin was therefore unexpected, since corresponding doses of UVA from the xenon lamp were inactive after eliminating most of the UVB by addition of the filter KG 1 to WG295. One explanation for this observation could be the remaining concomitant emission of UVB which in the light cabin was more than three times higher that in the xenon lamp equipped with the filters. Although only subthreshold doses of UVB were given in the UVA cabin, it is conceivable that photo-augmentative interactions between UVB and UVA were responsible for the inhibition induced by irradiation in the UVA cabin.

The light-induced inhibition was dose related up to a critical level where further increase in the UV-doses was accompanied by histamine release. This UV-induced 'spontaneous' release was not significantly increased when the cells were subsequently incubated with compound 48/80. The metabolic inhibitor DNP which markedly inhibited release induced by compound 48/80, had no effect on the UV-induced histamine liberation. Thus, it seems likely that the latter process was a passive leakage of histamine due to UV-induced cytotoxic effects.

The mechanism for the UV-induced inhibition is

unknown. Since membrane events are of importance both for the active release process, e.g. initiated by compound 48/80 (11, 14) and for the storage of histamine in the mast cell granules (1) it might be speculated that the UV effects were membrane related and that moderate doses of UVB caused minor disturbances of the cell membrane leading to decreased capacity to secrete histamine, whereas higher doses produced more pronounced membrane lesions leading to leakage of histamine from the mast cells.

It is known that temperature elevation can induce profound cellular changes, e.g. denaturation of critical proteins and increase in plasma cell membrane permeability (13). We therefore considered the possibility that the present observations could be due to heat effects. However, this seems unlikely, since higher temperatures than observed in the present investigation were required to induce histamine release *per se* or influence the histamine liberation evoked by compound 48/80. This is consistent with previous reports (5, 9, 18).

The inhibited histamine-releasing capacity following exposure to moderate doses of UV and the photolysis following higher doses might provide an explanation for our previously reported findings of reduced itch and flare reponses in UV-irradiated human skin (3). We found that regular exposure of human skin to UVB, UVA or PUVA for 4 weeks was followed by inhibited itch and flare reponses. The inhibition was more pronounced for the responses induced by compound 48/80 than for those evoked by histamine. However, great caution is warranted when comparing the results obtained in vivo in humans with those obtained in vitro in rat mast cells. Different sensitivities in these two systems are known to exist (2). Furthermore, in the present study the cells were exposed to light only once, whereas in the former investigation the human subjects were repeatedly exposed.

That mast cells may be affected and histamine released by UV irradiation has been reported in several studies. After exposing rat peritoneum to UVA and UVB light, the mast cells display microscopical injuries, whereas no affection is seen in other connective tissue elements (7). Release of histamine has been reported in venous blood draining UVB-irradiated healthy skin (17). UVA has been reported to cause degranulation of mast cells in human skin (12) but in clinical work irradiation of patients with uremic pruritus with repeated UVA exposures does not affect the number of dermal mast cells or induce changes in their morphology (8). PUVA therapy in urticaria pigmentosa (6) and psoriasis (16) is accompanied by a gradual reduction in the number of dermal mast cells, but there is no evidence of immediate histamine release in association with the PUVA therapy (6). These observations indicate that inhibition of mast cell proliferation may explain the successful antipruritic effect of PUVA. However, there is only one previous report which in analogy to ours indicates that UV light also may induce an increase in the mast cell degranulation threshold (10).

To summarize: UV light in moderate doses may affect rat peritoneal mast cells by inhibiting the histamine-releasing capacity, while higher doses seem to induce mast cell damage and a cytotoxic leakage of histamine. The action spectrum seems to be in the wavelength region of UVB. UVA does not seem to affect mast cells *per se* but may photoaugment the effect of UVB. These effects may be of clinical significance in treating pruritus in conditions where histamine release is involved.

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