INTRAVITAL STUDY OF THE EFFECT OF HISTAMINE AND SEROTONIN ANTAGONISTS ON INFLAMMATION

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A local ultraviolet burn causes inflammation of the mucous membrane of the retrobuccal pouch of the hamster. Substances with D-antiserotonin activity, if applied to the mucous membrane before exposure to UV light, prevent the inflammatory changes. Antihistamine substances do not affect its development.

Key words: ultraviolet burns; serotonin; histamine; microcirculation.

Biologically active amines have recently been shown to play a role in the development of inflammation. In particular, after burns of the human skin caused by a single exposure to UV light, histamine and serotonin are liberated [2].

The object of this investigation was to study the action of histamine and serotonin antagonists on the inflammatory focus.

EXPERIMENTAL METHOD

Experiments were carried out on 142 male hamsters weighing 100-120 g. A UV spot burn was obtained by irradiating the mucous membrane of the retrobuccal pouch with long-wave (410 nm) UV light through a 20× objective on an ML-2 luminescence microscope for an exposure of 20 min [6]. To investigate the direct effect of UV rays on the microcirculation the method of direct observation of the mucous membrane of the hamster retrobuccal pouch was used [10]. The development of inflammation as a result of UV-irradiation and the effect of histamine and serotonin antagonists on it were studied by the writers' modification [4] of the implanted chamber [9] method. The period of observation was 10 days, corresponding according to earlier data [5] to the period of function of the blood vessels of the mucous membrane in the control. The diameter of the vessels was measured with an ocular micrometer and by the split image method [1]. Antagonists of histamine (diphenhydramine hydrochloride) and serotonin (morphine hydrochloride, D-LSD-25) and tipindole [3] were used in concentrations of 1×10^{-4}-1×10^{-8} g/ml. In different variants of the experiments the antagonists were applied to the intact mucous membrane before UV-irradiation, the antagonists were introduced daily into the chamber after its implantation, they were introduced into the chamber once before the UV-burn of the mucous membrane and then again daily for the next 10 days, or they were introduced daily into the chamber after the UV-burn only.

EXPERIMENTAL RESULTS AND DISCUSSION

After 5 min of irradiation constriction of the arterioles and venules of the mucous membrane developed and reached a maximum after 15-18 min. A reduction in the constriction of these microvessels was observed 15 min after the UV-burn. However, they did not regain their original diameter.

The mucous membrane in the region of the burn 24 h after exposure to UV light was cloudy in 92.1% of cases and the microvessels were dilated. The number of functioning capillaries per unit area fell from 1.2 to 0.9 (in conventional units). Later considerable slowing of the blood flow, reversible and irreversible

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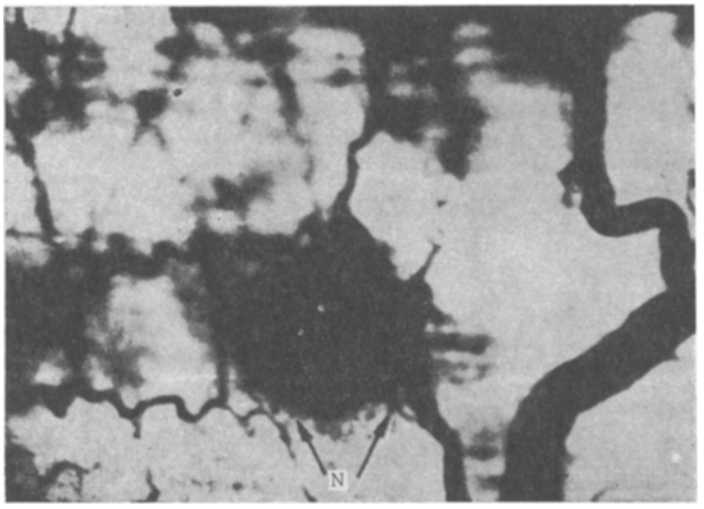

Fig. 1. Zone of necrosis (N) of mucous membrane on 5th day after UV-irradiation. Intravital microscopy, 80×.

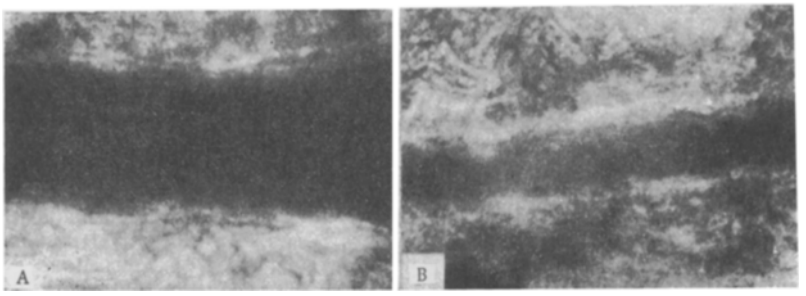

Fig. 2. Reaction of 45-μ arteriole to application of serotonin (1×10^{-6} g/ml): A) before exposure; B) after exposure. Intravital microscopy, 160×.

stasis (2nd-3rd day), and migration of blood cells from the vessels (3rd-4th day) followed by their lysis were observed. By the 5th day irreversible stasis had developed in all microvessels of the region of the burn, with necrosis in the center (Fig. 1). In 2.1% of cases the disturbances of the microcirculation described above affected the whole mucous membrane, in 1.5% an ulcer developed in the region of the UV-burn, and in 4.3% no visible changes could be detected.

When the antagonists were chosen it was considered that the vessels of the intact mucous membrane of the retrobuccal pouch (as preliminary experiments showed) constricted sharply in response to the application of serotonin (Fig. 2) but responded hardly at all to histamine.

After application of the antagonists directly to the mucous membrane no visible changes were observed in the microcirculation. When UV-irradiation of the mucous membrane was carried out after application of morphine hydrochloride (1×10^{-8} g/ml) constriction of the arterioles and venules developed, whereas D-LSD-25 (0.05 ng/ml) prevented such constriction. If tipindole was applied in a concentration of 2×10^{-5} g/ml, corresponding to its D-antiserotonin properties [3], constriction of the vessels developed in 52% of cases, but if applied in a concentration of 2×10^{-6} g/ml (its T-activity [3]) – it developed in 98% of cases. Diphenhydramine in a concentration of 2×10^{-4} g/ml, with antihistamine activity [8], did not prevent the change in diameter of the arterioles and venules.

The drugs used had no effect on the microcirculation of the unirradiated mucous membrane of the hamster retrobuccal pouch when injected daily into the implanted chamber.

When diphenhydramine (2×10^{-4} g/ml) and morphine hydrochlorides (1×10^{-8} g/ml) were injected into the chamber before UV-irradiation, and when injected daily for 10 days after irradiation, the inflammatory reaction developed just as in the control. D-LSD-25 (0.05 ng/ml) completely abolished the pathological

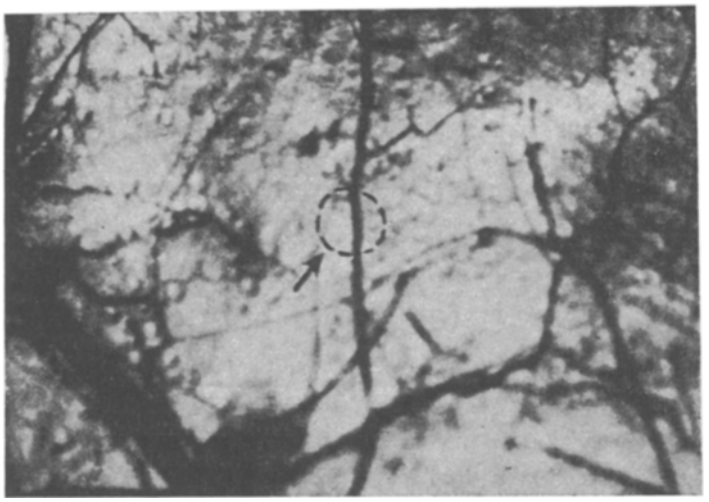

Fig. 3. Zone of UV-irradiation in an animal receiving D-LSD-25 in a dose of 0.05 ng/ml on the 5th day (inside circle marked by arrow). No inflammatory reaction present. Intravital microscopy, 80×.

changes (Fig. 3). When tipindole (2×10^{-5} g/ml) was injected daily into the chamber; inflammation developed in the mucous membrane after UV-irradiation in 32% of cases.

Injection of the histamine and serotonin antagonists into the chamber 15 min after UV-irradiation and thereafter daily for 10 days showed that morphine hydrochloride (1×10^{-8} g/ml) and diphenhydramine (2×10^{-4} g/ml) did not affect the dynamics of inflammation. When D-LSD-25 (0.05 ng/ml) was injected into the chamber the blood flow in the mucous membrane was increased and dilatation of the microvessels reduced considerably in 21% of cases. Necrosis of the irradiated area occurred only on the 7th day. The same picture was observed in 13% of cases after injection of tipindole.

The focus of inflammation was always limited to the area of irradiation whether the serotonin D-antagonists were injected before or after UV-irradiation. No ulcers developed in the mucous membrane.

These results indicate that type I histamine receptors evidently do not play an important role in the onset and development of inflammation in the mucous membrane of the hamster retrobuccal pouch in response to local exposure to UV rays. On the other hand, the arterioles and venules of the mucous membrane definitely constrict in response to the application of serotonin (1×10^{-6} g/ml), and this occurs also during local UV irradiation. Prevention of vasoconstriction in both cases by application of serotonin antagonists of the D-type points to a role of serotonin and of D-serotoninergic receptors in the mechanism of development of the initial phase of inflammation. The effectiveness of the serotonin D-antagonists in acute experiments may be connected with the comparatively high serotonin concentration in the blood and mast cells of hamsters [7, 11]. Meanwhile, the anti-inflammatory activity of D-LSD-25 and tipindole in concentrations corresponding to their D-antiserotonin properties, revealed in chronic experiments, cannot be explained entirely by blocking of the D-serotoninergic structures, for serotonin is a "mediator" of acute inflammation [12]. The possibility cannot be ruled out that these preparations, which are indole derivatives, possess an action similar to that of indometacin.

Serotonin antagonists of M- and T-types do not affect the course of inflammation of the mucous membrane of the hamster retrobuccal pouch in local UV burns.

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