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MECHANISM OF ANTI-INFLAMMATORY ACTION OF GLUCOCORTICOIDS: RE-EVALUATION OF VASCULAR CONSTRICTION HYPOTHESIS

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1 The question whether constriction of local vessels is essential for the anti-inflammatory action of glucocorticoids in carrageenin-induced granulomatous inflammation was studied.

2 The vasodilator prostaglandin E_1 injected into the granuloma pouch fluid increased the exudation of plasma protein into the granuloma tissue.

3 Noradrenaline significantly reduced plasma exudation, possibly through α-adrenoceptor stimulation.

4 Cortisol and dexamethasone in doses sufficient to inhibit vascular permeability were without effect on the blood content in the granuloma tissue.

5 The results suggest that constriction of local vessels does not play an essential role in the anti-exudative effect of glucocorticoids in chronic proliferative inflammation.

Introduction

Glucocorticoids are the most potent anti-inflammatory drugs used clinically and experimentally. The mechanisms responsible for their anti-inflammatory action are still uncertain. Schayer (1964) suggested that these effects of the glucocorticoids could be explained by the ability to counteract vasodilatation by histamine-like substances. Vasoconstrictor responses of human skin to glucocorticoids have been used as a method for evaluation of the anti-inflammatory activity of the steroids (McKenzie & Atkinson 1962; Reid & Brookes, 1968; Kaidbey & Kligman 1974). However, these studies did not establish whether glucocorticoids in doses too low to produce vasoconstriction still produced anti-inflammatory effects.

The present study was undertaken to determine whether the anti-exudative effect of glucocorticoids is accompanined by local vasoconstriction. The carrageenin-induced granuloma pouch was used as a model of chronic proliferative inflammation (Tsurufuji, Sugio & Endo 1977).

Method

Carrageenin-induced granulomatous inflammation (granuloma pouch)

Male rats of the Donryu strain, 6 – 7 weeks old and weighing 140 to 160 g, were used. They were maintained on laboratory food (Funabashi Farm, Chiba, Japan) and tap water *ad libitum* in a well-controlled environment. The carrageenin granuloma pouch was induced as described previously (Fukuhara & Tsurufuji 1969; Tsurufuji *et al.*, 1977) with slight modifications. The rats were injected with 8.0 ml of air subcutaneously on the dorsal surface under light ether anesthaesia to make an oval air sac. After 24 h, 4.0 ml of 2% (w/v) heat-sterilized solution of carrageenin in 0.9% NaCl solution was injected into the air sac (day 0). Drug effects were tested on day 8.

Vascular permeability in the granuloma pouch was measured by the method described by Tsurufuji *et al.* (1977) using ^{125}I-HSA and ^{131}I-HSA. Both ^{125}I-HSA and ^{131}I-HSA were purified before use by Sephadex G-100 column chromatography in order to remove radioactive low molecular weight impurities. About 1 μCi of purified ^{125}I-HSA in 0.2 ml of 0.9% NaCl solution (saline) was injected into the femoral vein. After 30 min, 1.0 ml of inflammatory exudate was withdrawn from each granuloma pouch to measure the leakage of ^{125}I-HSA into the pouch fluid. After administration of the drug, about 1 μCi of purified ^{131}I-HSA was injected into the femoral vein. After 30 min, 1.0 ml of the pouch fluid was again withdrawn to measure the concentration of ^{131}I-HSA. In the experiments with noradrenaline, isoprenaline and prostaglandin E_1 which may be unstable in the inflammatory tissue, exudate was sampled 20 min after the injection of ^{125}I-HSA and of ^{131}I-HSA. The animals were killed immediately after the second sampling and the total volume of the pouch

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fluid measured. The radioactivity of ^{125}I and ^{131}I was counted separately in a well-type scintillation spectrometer (Aloka JDC-751) as described by Tsurufuji *et al.* (1977). The ratio of $^{131}I/^{125}I$ was taken as an index of the vascular permeability change induced by the drug treatment. In the control rats, the ratio was very close to 1.0, reflecting no change in vascular permeability.

Measurement of blood content in granuloma tissue

^{51}Cr-labelled red blood cells were prepared according to the method of Albert, Eccleston, Rafli, Hurter, Henley & Albert (1959) with slight modifications. Approximately 150 μCi of $Na_2{}^{51}CrO_4$ solution was added to 10 ml of whole blood collected from rats and incubated at room temperature for 30 min with occasional shaking in a sterile 50 ml centrifuge tube containing 5 ml of citrate-dextrose solution. Then 50 mg of ascorbic acid was added to the solution which was left for 10 min. Tagged red cells were washed eight times to remove free chromate and then resuspended in a suitable volume of saline. At an appropriate time after administration of test drugs to the animals, approximately 0.3 μCi of ^{51}Cr-labelled red blood cells in 0.2 ml of saline was injected into the femoral vein. After 20 min, the entire granuloma tissue was surgically removed from the rats under light ether anaesthesia, washed with saline and blotted. After removal of fat, muscle and non-granulomatous tissues, the granuloma was cut into 1 to 2 mm pieces. The radioactivity of ^{51}Cr in 1.0 g of the tissue was counted in a well-type scintillation spectrometer (Aloka JDC-751). The blood content in the granuloma was expressed in terms of the percentage of the radioactivity injected.

The significance of the difference between treated and control groups was calculated according to the F test.

Drugs

Carrageenin (Seakem No. 202; Marine Colloid Inc., Springfield, N.J., U.S.A.), ^{125}I-labelled human serum albumin (^{125}I-HSA, specific activity: 2.5 μCi/mg albumin; Kaken Kagaku Co., Tokyo, Japan), ^{131}I-human serum albumin (^{131}I-HSA, sp. act. 5 μCi/mg albumin; Dainabot Co., Tokyo, Japan), $Na_2{}^{51}CrO_4$ (specific activity: 20 mCi/mg: Daiichi Radioisotope Institute, Tokyo, Japan), dexamethasone and prostaglandin E_1 (Sigma Chemicals Co., St. Louis, Mo., U.S.A.), cortisol (Wako Pure Chemicals Ltd., Tokyo, Japan), noradrenaline (Sankyo Co., Tokyo, Japan) and isoprenaline (Nikken Chemicals Ltd., Tokyo, Japan) were used.

Drug treatments of animals

Cortisol and dexamethasone were dissolved in ethanol and diluted with saline to 25 and 5% (v/v) ethanol. Noradrenaline was dissolved in saline. Prostaglandin E_1 was also dissolved in ethanol and then diluted to 5% (v/v) ethanol with saline. The drugs were injected directly into the exudate fluid in the granuloma pouch.

Results

Effect of noradrenaline

Vascular permeability and blood content in the granuloma tissue were reduced by 10 μg/kg and 100 μg/kg of noradrenaline but not by 1 μg/kg (Figure 1). The effect appeared to be due to α-adrenoceptor stimulation, since isoprenaline (100 μg/kg) injected into the pouch fluid produced only a non-significant reduction in the ratio $\frac{^{131}I\text{-HSA}}{^{125}I\text{-HSA}}$.

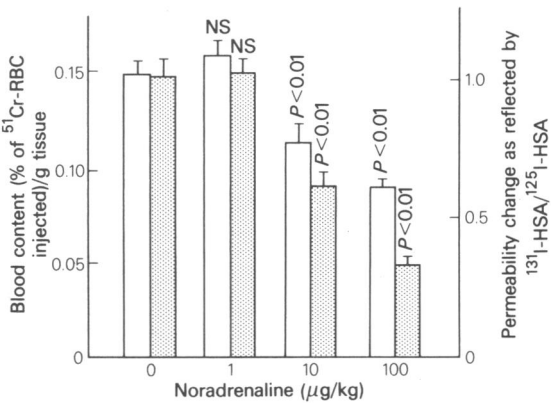

Figure 1 Effect of noradrenaline on plasma exudation and blood content in the granuloma tissue. Open columns: mean blood content determined 20 min after administration of noradrenaline. In each group $n = 6-7$ rats. Vertical lines show s.e.mean. NS = not significant. Stippled columns: mean change of plasma exudation, as reflected in the ratio, ^{131}I-HSA/^{125}I-HSA. Plasma exudation as assayed immediately before, and 23 min after the injection of noradrenaline, with ^{125}I-HSA and ^{131}I-HSA, respectively. In each group $n = 7-8$ rats. Vertical lines show s.e.mean.

Effect of prostaglandin E_1

Both vascular permeability and blood content of the tissue were increased when 5 μg of the vasodilator, prostaglandin E_1 was injected into the granuloma pouch, while 0.5 μg had no significant effect (Figure 2).

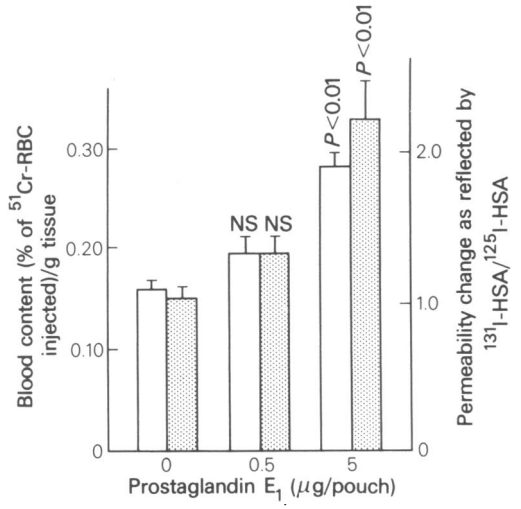

Figure 2 Effect of prostaglandin E_1 on plasma exudation and blood content in the granuloma tissue. Open and stippled columns as in Figure 1. Blood content was measured 20 min after the injection of prostaglandin E_1 and plasma exudation immediately before and 23 min after the injection of prostaglandin E_1. In each group $n = 7-8$ rats.

Effects of dexamethasone and cortisol

The anti-inflammatory effect of glucocorticoids becomes evident 1 to 2 h after their application and reaches a maximum at 3 to 12 h (Tsurufuji & Sugio, 1978). When assayed at 5 h after the treatment, 0.03 to 0.3 mg/kg of dexamethasone reduced vascular permeability in a dose-dependent manner (Figure 3a). Cortisol was effective at doses of 0.5, 1.5 and 5 mg/kg (Figure 3b). The blood content in the granuloma tissue was not significantly changed.

Discussion

Since anti-inflammatory steroids constrict local blood vessels when applied to human skin, it has been suggested that a vasoconstrictor action is responsible for the anti-exudative effect of these steroids (McKenzie & Atkinson, 1962; Reid & Brookes, 1968; Kaidbey & Kligman, 1974). Constriction of local blood vessels may reduce blood content of the tissue and lower pressure in the capillaries and venules, thereby decreasing plasma leakage through the vessel walls. In fact, noradrenaline exerted a potent anti-exudative effect and reduced blood content of the tissue in a dose-dependent manner (Figure 1). Similar observations were made by Spector & Willoughby, (1960; 1964) and by Sensch, Zeiller & Raake, (1979). This inhibition of vascular permeability was blocked by α-adrenoceptor blocking agents but not by β-blockers (Edlund & Juhlin, 1954). In contrast, the anti-inflammatory effect of catecholamines in carrageenin-induced oedema appears to be mediated by β-adrenoceptors, since it was antagonized by β-adrenoceptor blocking agents (Green, 1972). As the present study shows, the β-adrenoceptor agonist, isoprenaline, did not inhibit the vascular permeability in proliferative inflammation. It is likely, therefore, that constriction of vessels by the α-adrenoceptor stimulant action of noradrenaline is responsible for the inhibition of the vascular permeability. In similar fashion, colchicine injected into the carrageenin granuloma pouch also reduced both blood content and plasma exudation (Tsurufuji, Min & Mizuno, 1979).

In contrast to the vasoconstrictors, vasodilators may be expected to enhance vascular permeability in inflamed tissues. Among various substances, including acetylcholine, isoprenaline, phentolamine, sodium nitrate and prostaglandin E_1, only the last

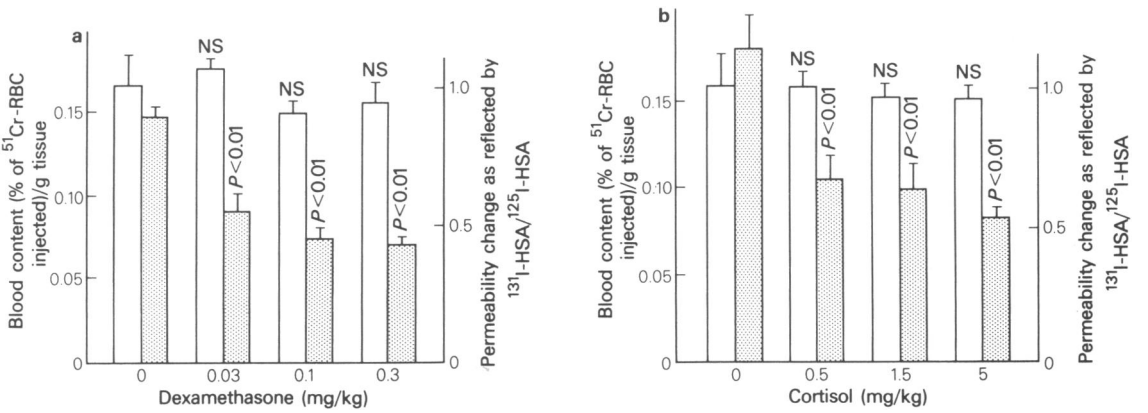

Figure 3 Effects of dexamethasone (a) and cortisol (b) on plasma exudation and blood content in the granuloma tissue. Open and stippled columns as in Figure 1. Blood content was determined 5 h after administration of the corticoids and plasma exudation immediately before and 5 h after administration. In each group $n = 7-8$ rats.

compound increased the blood content in the carrageenin-induced granuloma tissue. Potent vasodilator activity of prostaglandin E_1 and prostaglandin E_2 has been well documented (Bergström, Duner, von Euler, Pernow & Sjovall, 1959; Horton, 1963; Williams & Peck, 1977). E-type prostaglandins produce vasodilatation with little effect on vascular permeability in non-inflamed skin (Williams & Peck, 1977). In the granuloma tissue (Figure 2) prostaglandin E_1 increased the blood content and this was associated with enhanced exudation. The results of the present experiments indicate that the blood content in the local vessels exerts a definite influence on plasma exudation in inflamed tissues. However, suppression of the exudation by anti-inflammatory steroids was not accompanied by changes in the blood content of the granuloma tissue (Figure 3) and does not seem to depend on vasoconstrictor effects.

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