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A protective action of an anti-inflammatory steroid on collagen synthesis in rat carrageenan granuloma *in vitro*

Administration of anti-inflammatory steroids *in vivo* has been repeatedly shown to inhibit protein syntheses in experimentally inflamed tissues (Robertson & Sanborn, 1958; Bavetta, Bekhor & others, 1962; Bavetta & Nimni, 1964; Mikkonen, Lampiaho & Kulonen, 1966; Oronsky & Nocenti, 1967; Fukuhara & Tsurufuji, 1969; Nakagawa, Fukukara & Tsurufuji, 1971). In the present study, the effect of an anti-inflammatory steroid on the synthesis of collagen and non-collagen protein was investigated *in vitro* by incubating minced carrageenan granuloma in a medium containing the steroid.

A granuloma pouch was induced in male rats of the Donryu strain, 110–140 g, by injecting (s.c.) a 2% solution of Seakem 202 carrageenan according to Fukuhara & Tsurufuji (1969). On day 8 after carrageenan injection, granulation tissue was taken immediately after death and minced into 1–2 mm pieces 3 g of which was incubated with or without betamethasone disodium phosphate at 37° under an atmosphere 5% CO_2 in oxygen in 10 ml of Krebs saline serum substitute (Krebs, 1950) containing 10 mg each of potassium penicillin G and dihydrostreptomycin sulphate. After the

incubation of 2 or 6 h, 30 μCi of L-[^{3}H]proline (in 0·3 ml of the medium; 63 Ci mmol^{-1}, generally labelled) was added to the incubation mixture. The incubation was continued for further 30 min and the reaction was stopped by addition of 2·5 ml of 50% trichloroacetic acid containing 1% proline to the final concentrations of 10% trichloroacetic acid and 0·2% proline. Collagen and non-collagen protein were extracted from the incubated granuloma and their specific activities were determined according to Nakagawa & others (1971). When minced granuloma was incubated in the presence of a high concentration (2×10^{-3} M) of betamethasone disodium phosphate, the incorporation of [^{3}H]proline into collagen and non-collagen protein was markedly inhibited, agreeing with the *in vivo* effect of the steroid. On the other hand, the steroid significantly enhanced the incorporation of [^{3}H]proline into collagen hydroxyproline at the concentrations of 2×10^{-6} and 2×10^{-5} M, while the [^{3}H]proline incorporation into non-collagen protein was unaffected. Since free L-proline released through the degradation of tissue proteins gradually accumulates in the medium with lapse of incubation time, the specific activity of free [^{3}H]proline in the medium may be lowered with time. Therefore, to investigate the possibility that the increase by the steroid of [^{3}H]proline incorporation into collagen might be caused by the change of free L-proline concentration in the medium, series of incubations were made with various concentrations of L-proline. The results are summarized in Table 1. Free L-proline at 0·05 and 0·5 mM did not affect the incorporation rate of

Table 1. *Effect of betamethasone disodium phosphate on [^{3}H]proline incorporation into collagen and non-collagen protein under incubation* in vitro *of carrageenan granuloma in the presence of varying amounts of* L-*proline in the medium.* Incubation conditions were the same as described in the text except that the medium contained various amounts of L-proline and 30 μCi (Expt 1 and 2) or 70 μCi (Expt 3) of [^{3}H]proline.

Concn of L-proline	No. of flasks	Sp. act. of collagen (d min^{-1} μg^{-1} hyp.)		Sp. act. of non-collagen protein (d min^{-1} μg^{-1} prot.)	
		Betamethasone		Betamethasone	
		None	2×10^{-5} M	None	2×10^{-5} M
Expt 1 (6 h-incubation):					
None	5	2·235 ± 0·044	3·875 ± 0·063* (173·4%)	22·430 ± 0·097	23·257 ± 0·521
0·05 mM	5	2·211 ± 0·074	3·839 ± 0·156* (173·6%)	21·912 ± 0·318	22·524 ± 0·388
0·5 mM	5	2·142 ± 0·108	3·587 ± 0·042* (167·5%)	19·366 ± 0·213	19·898 ± 0·380
Expt 2 (6 h-incubation):					
None	5	2·025 ± 0·038	3·162 ± 0·097* (156·2%)	17·772 ± 0·227	18·008 ± 0·365
5 mM	5	1·097 ± 0·015	1·685 ± 0·072* (153·6%)	6·096 ± 0·131	6·774 ± 0·164† (111·1%)
10 mM (2 h-incubation)	5	2·240 ± 0·061	—	4·960 ± 0·049	—
10 mM (6 h-incubation	5	1·055 ± 0·029	1·403 ± 0·036* (133·0%)	4·037 ± 0·092	4·199 ± 0·092

Data are shown as means ± s.e. Per cent of control (the group incubated without the steroid) is shown in parentheses. Significantly different from control; *P <0·001, †P <0·02.

[^{3}H]proline into collagen and non-collagen protein. At these concentrations of L-proline the stimulation of the steroid for [^{3}H]proline incorporation into collagen was also maintained. Incubation with 5 mM L-proline resulted in a marked decrease in the specific activities of the two proteins. However, the stimulatory effect of the steroid upon collagen synthesis was still evident. The incorporation of [^{3}H]proline into non-collagen protein, on the contrary, was not significantly affected by the steroid, though there was a slight increase in the presence of 5 mM L-proline.

On the basis of these results, the incubation medium containing a large excess (10 mM) of L-proline was applied in an attempt to avoid a possible influence of free L-proline released by the tissues under incubation. Consequently, differences between 2 and 6 h-incubation groups in specific activities of collagen and non-collagen protein were considerably diminished (Expt 3 in Table 1) compared with the results obtained by incubation without L-proline (specific activity of collagen at 6 h-incubation was one quarter of that at 2 h-incubation). The specific activities of these two proteins of 6 h-incubation group were still lower than those of 2 h-incubation group, suggesting that protein syntheses by granulation tissue were progressively decreased with incubation time. As shown in Expt. 3, this decrease was much greater in collagen than in non-collagen protein. The biosynthesis of collagen is a complex process which involves several discrete steps such as transcription, translation, modifications of a precursor, and secretion and maturation of collagen. Accordingly, it seems probable that the biosynthesis of collagen is depressed more than that of non-collagen protein during the incubation of granulation tissues, since the conditions *in vitro* must be a poor environment for the cells compared with those *in vivo*. Thus, it is likely that the steroid exerts a protective action on the biosynthesis *in vitro* of collagen which is more sensitive to various deteriorating factors than non-collagen protein biosynthesis. The results seem to be consistent with the findings that glucocorticoids prolong the lifespan of human fibroblasts *in vitro* (Macieira-Coelho, 1966) and that hydrocortisone tends to induce a round-up of fibroblasts and such cells appear to be more resistant to fibroblastolytic stimuli (Dougherty & Schneebeli, 1955).

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