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Gene Expression as a Molecular Mechanism in the Anti-inflammatory Action of Glucocorticoids

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During the last 25 years, glucocorticoids have been extensively used in the treatment of rheumatoid arthritis. Recently, however, because of the undesirable side effects of the steroids, a number of non-steroid anti-inflammatory agents have been developed in an attempt to meet the needs of clinical medicine. Parallel to findings of new anti-inflammatory substances, many models of inflammation have been introduced in the experimental pharmacology for evaluating anti-inflammatory activity of drugs. So far, however, these methods are not successful in accurately predicting the effect of drugs in human diseases. Most of the methods hardly reveal differences in the mode of action between the steroid and the non-steroid drugs, in spite that certain definite differences have been recognized in the clinical medicine. The difficulty seems to have been derived mainly by the application of inadequate experimental models. Since rheumatoid arthritis is a chronic inflammatory desease of proliferative nature, models of proliferative inflammation should be preferred in the assay rather than those of acute inflammation. Moreover, assay methods should be rather based on testing therapeutic effect of the drug, in place of testing prophylactic effect which has been done commonly. Based on these considerations we have developed carrageenin granuloma pouch method as an experimental model of human chronic inflammatory diseases of proliferative nature, and we have investigated not only prophylactic but also therapeutic effects of anti-inflammatory drugs[1]. In the prophylatic manner, the steroid and the non-steroid drugs are both potently inhibitory against exudation of fluid and formation of granuloma. In the therapeutic manner, however, the steroid only is effective in inducing involution of the pre-formed granuloma and in inducing absorption of the existing fluid.

We have been also working in the attempt to elucidate mechanism of strong anti-inflammatory actions of the steroid. In previous papers we mainly dealt with both the changes of granuloma tissue components and the biochemical aspects of involution of the pre-formed granuloma tissue under the influence of glucocorticoids. We reached a conclusion that involution of the granuloma was induced from inhibition by the steroid in biosyntheses of protein

and DNA[2~4]. Degradation of DNA and tissue protein was not affected significantly. Collagen biosynthis was interfered with more markedly than that of non-collagen protein[4,5]. In this case protocollagen proline hydroxylase was not affected in a single administration of the steroid[5,6], but protocollagen biosynthesis was inhibited and the inhibition was significantly stronger than that of non-collagen protein. On the other hand, restoration from steroid-induced suppression of the biosynthetic activity for collagen was attained much more rapidly than that of non-collagen protein as for the case of a single administration of the steroid[7]. These facts suggest that granuloma involution caused by the steroid results from its anti-anabolic action in the manner of repressing biosynthesis of DNA and mRNA and that life span of mRNA for collagen is much shorter than average life span of mRNA for non-collagen proteins of the granuloma tissue[7]. Interference with translational phase of protein synthesis, if any, seemed to be insignificant, because there occurred a difference in the time course of inhibition between collagen and non-collagen protein syntheses.

With respect to the biochemical pathway for the manifestation of physiologic functions of various steroid hormones involing glucocorticoids, a concept suggesting hormonal induction of specific gene expression has been steadily growing in recent years[8,9]. Therefore, further experiments have been undertaken in an attempt to examine whether or not inhibition of DNA-dependent synthesis of RNA interferes with the manifestation of anti-exudative action of the steroid. Actinomycin D was used in this respect. We also used puromycin to inhibit mRNA-dependent synthesis of protein, attempting to investigate the possibility of participation of newly synthesized protein in anti-exudative action of the steroid. Carrageenin granuloma pouch method was again used in this experiment. Rats bearing 8-day-old granuloma pouch were taken for testing drug effect of vascular permeability in the proliferative inflammation. Vascular permeability was measured according to the method described in a previous report with the aid of radioiodinated human serum albumins[10]. Cortisol dissolved in 25% ethanol was injected locally into the exudate of the granuloma pouch. Control rats were given the vehicle only. The time course for the change of vascular permeability by cortisol were done in the first instance. One hour after the injection of cortisol at a dosage of 5 mg/kg body weight no inhibition of the permeability was observed; a moderate, though not significant, suppression (10% inhibition) was at 1.5 hr; statistically significant changes of 22, 47, 65 and 58% inhibition were attained at 2, 3, 5 and 12 hr respectively. Dose-response correlation was also confirmed over the dose range of 0.5, 1.5, 5.0 and 15 mg/kg. Thereafter all the experiments were done at 5 mg/kg whenever cortisol was given. In the experiments that either actinomycin D or puromycin was given, the antibiotics were always injected into the exudate in the granuloma pouch. We fixed the dose for actinomycin D at 2 mg/kg and for puromycin at 30 mg/kg respectively, referring to the data from literatures[11,12] in which the antibiotics had been used as suppressors against hormonal induction of specific proteins.

When actinomycin D was given 20 min before or 1 hr after the administration of cortisol, suppressive effect of the steroid on vascular permeability was counteracted, while administration of actinomycin D at 3 hr after cortisol injection could not counteract the vascular perme-

ability-suppressive action of cortisol. Moreover, puromycin also gave results very close to those of actinomycin D. When puromycin was given 30 min before or 1 hr after cortisol administration vascular permeability-suppressive action of cortisol disappeared, while the anti-exudative effect of cortisol was still apparent in the case that time of puromycin injection was delayed until 2hr after the cortisol injection.

The facts that the anti-exudative effect of cortisol was blocked or not blocked in accordance with the change of time for the injection of the antibiotics are very consistent, as explained below, with the concept that gene expression is required in the manifestation of the cortisol action. The data from the time course studies for the effect of a single injection of cortisol which have been mentioned above are also agreeable with this concept. Namely, a latent period of 1.5 hr which exists prior to significant lowering in vascular permeability is considered to be a time assigned for nuclear uptake of the steroid and for induction of specific mRNA and protein. Agreeing with the results in present experiments, the latent period in inducing hepatic enzymes with glucocorticoids through gene activation has been reported in the literatures to be about 2hr[11,12]. Moreover, actinomycin D and puromycin were shown to block the vascular permeability-suppressive action of cortisol when administered during the latent period of the cortisol action, while they did not block if these antibiotics had been given at a time beyond the end of the latent period. These facts are also agreeable with the concept that blocking of the synthesis of mRNA or protein to be induced by cortisol before its accumulation in sufficient quantity blocks anti-exudative effect of the steroid, while neither actinomycin D nor puromycin interfers with the effect of cortisol in the cases that they are given at an interval enough for suitable quantity of the mRNA and protein to be synthesized under the influence of the steroid.

In conclusion, all the results in present experiments seem to be quite consistent with the concept that the steroid exerts its vascular permeability-suppressive action through activating specific gene(s) to induce certain mRNA(s) and then to produce certain functional protein(s).

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