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Lack of Interaction between Glucocorticoids and the Kallikrein–Kinin System[1] (37870)

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The kallikrein–kinin system has been implicated as playing a significant role in a variety of inflammatory disease states (1) including circulatory shock (2). Thus, either the inflammatory reaction or the shock activates plasma or tissue kallikrein which then hydrolyzes kininogen to release kinins locally. The primary kinin released is bradykinin which has several actions that could propagate the inflammatory process or exacerbate the shock condition (e.g., vasodilation, increasing capillary leakage of fluid, promoting leukotaxis) (3).

One of the primary actions of pharmacological concentrations of glucocorticoids is to suppress inflammatory reactions. Moreover, very high doses of two synthetic glucocorticoids, dexamethasone phosphate and methylprednisolone sodium succinate, have been found to improve survival in several types of circulatory shock (4). Although the mechanisms of this protective effect are not fully known, attention has recently focused on membrane stabilizing actions (5) or metabolic actions (6) rather than on hemodynamic effects. The purpose of this study was to determine whether methylprednisolone or dexamethasone, in concentrations comparable to those which protect in shock, antagonize the kallikrein–kinin system. We studied the influence of glucocorticoids on three facets of this system: (a) kinin formation, (b) bradykinin action, and (c) rate of inactivation of kinins (e.g., by kininases).

Methods. The system used for the bioassay of bradykinin is the isolated cat jejunal strip preparation of Ferreira and Vane (7). Segments of jejunum were isolated from adult cats and washed free of internal contents. Strips (2–3 cm long, 5–7 mm wide) were dissected from the jejunum and placed in oxygenated (i.e., 95% O_2 and 5% CO_2) low-bicarbonate Krebs–Henseleit solution (8) at 37°. The strips were stretched to a resting tension of about 1.4 g. After an equilibration period of 60–90 min, the spontaneous contractions of the strips became very regular in amplitude and frequency. At this time, samples could be added to the 20-ml bath for the bioassay of bradykinin. Bradykinin standards (1 and 10 ng/ml) were added to each strip for purposes of calibration. Strips were used from 2 to 48 hr after removal from the animal.

Kinin system compounds. Synthetic bradykinin (Sandoz BRS-640) was diluted in Krebs–Henseleit solution. Lyophilized pancreatic kallikrein (Bayer, Padutin) was dissolved in Krebs–Henseleit solution in appropriate concentrations. These substances were freshly prepared for each experiment. Cat plasma kininogen was prepared similarly to the method of Webster and Prado (9). Fresh heparinized cat blood was drawn in plastic tubes under ice and the plasma collected by centrifugation at 2500*g* for 15 min. The plasma was heated at 60° for 40 min to inactivate prekallikrein. The plasma was then cooled and dialyzed in Nojax (20 mm flat width) dialysis tubing against distilled water at 4° for 24 hr to remove preformed kinin. The plasma was then pipetted into 1-ml tubes and frozen until use. Samples

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of plasma were thawed once and used; unused plasma was discarded.

In the experiment designed to test the action of bradykinin, bradykinin was added to the bath in volumes of 0.1–0.5 ml 10 min after the addition of steroid or its vehicle to the bath. In experiments in which the formation of kinin was studied, 0.1 ml of plasma kininogen was added to the bath 2–5 min before the addition of steroid or its vehicle (0.1–0.2 ml). Ten minutes later, the kallikrein (0.1–0.25 ml) was added.

Steroid preparations. Dexamethasone phosphate (Decadron, Merck) was freshly diluted in Krebs–Henseleit solution for each experiment. Lyophilized methylprednisolone sodium succinate (Solu-Medrol, Upjohn) was mixed with its diluent just before use in each experiment. Hydrocortisone sodium succinate (Solu-Cortef, Upjohn) was dissolved directly in Krebs–Henseleit solution and used in a few experiments. Volumes of 0.1–0.2 ml of steroid or its vehicle were added directly to the bath.

The final concentrations of glucocorticoids in the bath were between 5×10^{-5} and 1×10^{-3} *M*. These concentrations correspond closely to the doses of the steroids that are generally found to protect animals and patients in circulatory shock (i.e., 30 mg/kg of methylprednisolone, 8 mg/kg dexamethasone).

Results. The cat jejunal strip preparation is very sensitive to bradykinin. Concentrations of 0.1–0.2 ng/ml exerted consistent increases in developed force of 0.25–0.50 g. Moreover, the specificity of this smooth muscle preparation is very high for kinins. Other agents which are known to stimulate smooth muscle either do not influence the strips at all (e.g., vasopressin, oxytocin) or stimulate the strips only at concentrations about 1000 times that of kinins (10). Massive concentrations of the glucocorticoids used, methylprednisolone, dexamethasone, or hydrocortisone, and their vehicles were totally without effect on the cat jejunal strip, thus simplifying the calculations for the kinin responses.

Figure 1 summarizes the influence of methylprednisolone and dexamethasone on the responsiveness of the cat jejunal strips to bradykinin. Separate experiments were conducted for each glucocorticoid and its vehicle. The upper log concentration–response line is for dexamethasone and its vehicle. No significant difference was observed between the steroid and its vehicle at any of the five bradykinin concentrations. Similarly, there was no difference between methylprednisolone or its vehicle on bradykinin

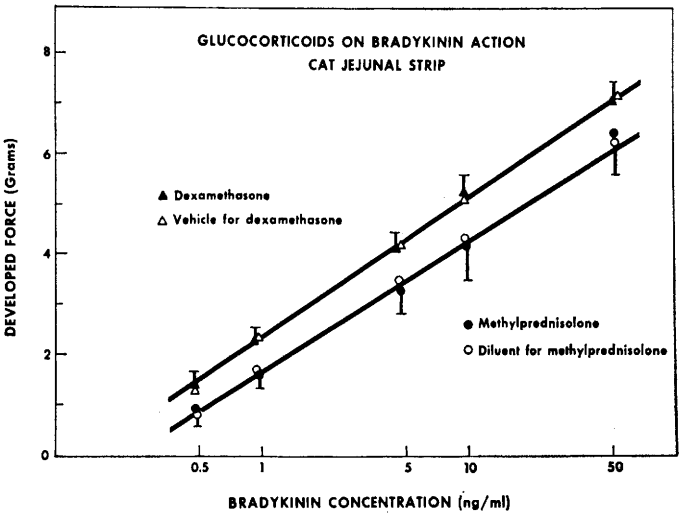

FIG. 1. Log concentration–response graph of the influence of glucocorticoids on the action of bradykinin on the isolated cat jejunal strip. No significant difference exists between either glucocorticoid or their vehicle, or between either steroid. Concentration of glucocorticoid in the bath was 5×10^{-4} *M*. Each point represents a mean of six experiments ± SEM.

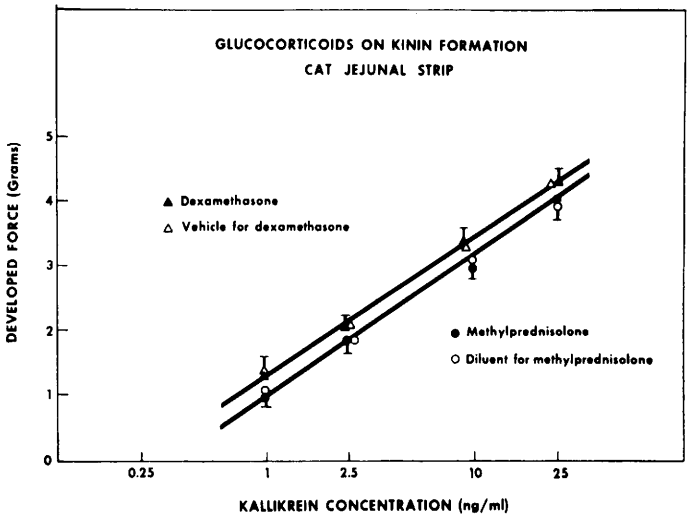

FIG. 2. Log concentration–response graph of the influence of glucocorticoids on kinin formation in the isolated cat jejunal strip. Bradykinin formation by the action of hog pancreatic kallikrein on cat plasma kininogen is plotted on the ordinate vs log concentration of kallikrein on the abscissa. Concentration of glucocorticoid in the bath was $5 \times 10^{-4} M$. Each point represents a mean of six experiments ± SEM. No significant difference exists between either glucocorticoid and their vehicle or between either steroid.

responsiveness as shown in the lower line. Thus, neither glucocorticoid altered the amplitude of the jejunal strip response to bradykinin. Neither was there an effect on the duration of the response. Similar results were obtained with jejunal strips from shock animals.

Although the glucocorticoids did not affect the action of bradykinin, these agents were then tested for their ability to alter the formation of kinins. Figure 2 summarizes the effects of methylprednisolone and dexamethasone on kallikrein-induced formation of kinins from plasma kininogen. Neither synthetic glucocorticoid modified the amount or the rate of kinin formation. This lack of effect was consistent over the entire concentration range of kallikrein used (i.e., from 1 to 25 ng/ml), and also in tissue isolated from cats in hemorrhagic shock. In addition, the time of steroid administration was varied from between 30 min to 1 min before the kallikrein with the same results. In additional experiments, pharmacologic concentrations of hydrocortisone (i.e., 5×10^{-5} to $1 \times 10^{-3} M$) were also without effect on the formation of plasma kinins. These results provide data that antishock concentrations of glucocorticoids do not significantly influence the plasma kinin system. It is highly unlikely, although possible, that these agents could alter the tissue kinin system in some critical organ or tissue. However, even if this occurred, it would probably not be sufficient to account for the systemic anti-inflammatory action or the antishock properties of the glucocorticoids. Moreover, there was no significant effect of any of the glucocorticoids on the rate of degradation of the kinins formed. The response to the highest kallikrein concentration (i.e., 25 ng/ml) declined 85 ± 5% within 40 min in the dexamethasone-treated strips compared with 87 ± 4% for the vehicle-treated strips. Similarly, the methylprednisolone-treated strips declined 87 ± 4% in 40 min compared with 89% for the vehicle-treated strips. Thus, the glucocorticoids did not appear to influence the actions of plasma kininases or the rate of metabolism of the kinins.

Discussion. We tested the acute effects of one naturally occurring and two synthetic glucocorticoids on three aspects of the kinin system: (a) kinin formation, (b) kinin action, and (c) kinin inactivation. None of

these glucocorticoids significantly influenced any of these parameters of the kallikrein–kinin system. Since these glucocorticoids are known to have considerable protective effects in shock, it is highly unlikely that this antishock action is mediated via the antagonism of the kinin system. Similarly, these data argue against antagonism of kinins as the mechanism of the anti-inflammatory effect of these glucocorticoids.

Several reports appeared in the literature between 1965 and 1966 claiming that glucocorticoids exert a significant antagonism or impairment of some facet of the kallikrein–kinin system (11–14). In this connection, Cline and Melmon (11) showed that cortisol appeared to prevent the release of kinin from kininogen (activated by glass contact) and partially inhibited the release of kinin by kallikrein. These workers measured plasma kininogen levels as an index of kinin formation, which may not be totally accurate, since alterations in protein synthesis, degradation, or transport of kininogen can exert nonspecific alterations in plasma kininogen levels. In another study, Suddick (12) showed that glucocorticoids (i.e., cortisol, cortisone) protected against the lethal effects of submaxillary gland extracts in adrenalectomized rats. The inference was that the bradykinin contributed to this toxic effect. However, substances in the extract other than bradykinin may have contributed to the lethality. In general, one must interpret data obtained in adrenalectomized animals with some degree of caution since these animals are very sensitive to a wide variety of noxious stimuli and trauma. Moreover, adrenalectomized animals have a deficit of mineralocorticoids as well as of glucocorticoids. In this regard, Geller *et al.* (13) have shown that adrenalectomy results in decreased excretion of kallikrein, but that deoxycorticosterone (DOC), by regulation of sodium metabolism, can counteract this process. Mineralocorticoids such as DOC do not possess much intrinsic anti-inflammatory or antishock activity. Thus, one should not use the term corticosteroids in generalities, but refer specifically to either glucocorticoids or mineralocorticoids as the case may be.

Zweifach (14) reported that cortisone markedly counteracted the vasodilator action of bradykinin in the cutaneous or mesenteric microcirculation of rats. However, the cortisone was given chronically over three days, and the steroid could have exerted an indirect effect on the cardiovascular system over that time period. Therefore, these data fail to definitively show a direct antagonism between glucocorticoids and bradykinin, and the chronic nature of the effect would not explain the acute antishock properties of the glucocorticoids. Finally, Dryud *et al.* (15) showed that high doses of cortisol exerted an inhibitory effect on kininase activity thus prolonging the effects of bradykinin. If this were the case, cortisol would enhance kinin action, an effect which would be of negative survival value, if kinins mediate inflammatory and lethal mechanisms in shock.

In contrast to the above-cited studies, several other investigators have failed to obtain evidence for an inhibitory effect of glucocorticoids on the kallikrein–kinin system (16–19). One of these studies (16) represents very carefully controlled experiments conducted in three independent laboratories. Thus, Eisen *et al.* (16) showed that glucocorticoids did not inhibit the activation of kinin-forming enzymes, the action of kallikrein on kininogen, or the capacity of human plasma, obtained from patients receiving high doses of glucocorticoids, to form kinins. Our data are consistent with those of Eisen *et al.* (16) and extend them to concentrations of glucocorticoids used in the therapy of circulatory shock. Moreover, we have tested three possible sites of interaction in the kallikrein–kinin system using one standardized biological preparation. No influence of glucocorticoid was found at any of these three sites.

It appears at present that acute administration of glucocorticoids in pharmacological doses does not result in a significant inhibition of the kallikrein–kinin system. In those studies in which an inhibitory effect was reported, either the effect was an indirect one or chronic administration of steroid was required to obtain an effect. Thus, anti-inflammatory or antishock actions of glucocorticoids which occur acutely cannot be explained by an anti-kinin effect. This does

not preclude indirect actions of glucocorticoids on other biological systems which may eventually modulate the kallikrein–kinin system. However, a direct anti-kinin action of sufficient magnitude to alter the severe pathological disturbances observed in inflammation and shock seems unlikely.

Summary. We found no significant modifying action of acute administration of pharmacological doses (5×10^{-5}–1×10^{-3} *M*) of either methylprednisolone, cortisol, or dexamethasone on the kallikrein–kinin system. Thus, there was no modifying effect of these glucocorticoids on (a) bradykinin action, (b) formation, or (c) kininase activity as determined by the rate of inactivation of the kinin response in the bioassay system. A similar lack of response occurred in jejunal strips isolated from control cats or in cats in hemorrhagic shock. These results argue against a direct inhibitory action of the glucocorticoids on the kallikrein–kinin system as the major mechanism of their anti-inflammatory or antishock properties.

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