

EFFECTS OF WATER-SOLUBLE ADRENOCORTICAL STEROIDS ON PASSIVE CUTANEOUS ANAPHYLAXIS OF THE GUINEA PIG

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THAT CORTISONE has no effect on passive cutaneous anaphylaxis was shown in 1952¹ and these observations were confirmed in 1956² using threshold amounts of antibody. Later, however, Prochazka-Fisher and Cooke³ have reported that the administration of water-soluble hydrocortisone intravascularly to guinea pigs in which passive cutaneous anaphylaxis is elicited has resulted in a reduction of the intensity of the reaction and an increase in the threshold concentration of antibody required for effective reaction. Osler and his co-workers⁴ have emphasized that, in order to demonstrate inhibition of the passive cutaneous anaphylactic reaction, one must employ not only a threshold amount of antibody, but also that corresponding minimal amounts of antigen should be used to elicit the reaction. Recently, moreover, more potent adrenocortical steroid preparations have become available, and it, therefore, seemed important to re-evaluate the effect of steroids on passive cutaneous anaphylaxis in the guinea pig using these preparations and threshold levels of reactants.

MATERIALS AND METHODS

a. *Antibody*.—Rabbit antbovine plasma gamma-globulin (RaaBGG) containing 48.0 μ g antibody N per milliliter was used. The concentrations employed are given in Table I.

b. *Antigen*.—Bovine plasma gamma-globulin, fraction II (BGG),* was diluted to give a final concentration of 15 μ g protein N per milliliter.

c. *Histamine*.—A solution of histamine containing 10 μ g per milliliter was employed.†

d. *Compound 48/80*.—A solution of Compound 48/80‡ was used which contained 40 μ g per milliliter.

e. *Steroids*.—The steroids used in this study were hydrocortisone hemisuccinate and methylprednisolone hemisuccinate.§

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*From the Armour Pharmaceutical Company, Kankakee, Ill. (lot # R30005).

†Histamine dihydrochloride, Fisher.

‡From the Burroughs Wellcome & Co., Inc., Tuckahoe, N. Y.

§Supplied as Solu-Cortef and Solu-Medrol, respectively, by the Upjohn Company, Kalamazoo, Mich.

f. *Dye*.—Evans blue dye was used as a 1 per cent solution in 0.15 M NaCl.

g. *Diluent*.—All materials requiring further dilution were prepared with 0.15 M NaCl.

h. *Animals*.—Albino guinea pigs (Hartley strain), weighing approximately 250 grams, were used in this study without regard to sex distribution.

The technique of passive cutaneous anaphylaxis (PCA) as described previously by Ovary^{5, 6} was used in this study. All animals received intradermal injections of antibody (RaaBGG), histamine, and Compound 48/80 parasagittally on carefully shaved areas of the back (each of the two latter compounds were injected immediately before challenge), each solution injected in a constant volume of 0.1 ml. Histamine and Compound 48/80 were included in this experiment as controls in order to study the effects of these steroids on the permeability of the minute blood vessels and on histamine liberation, respectively. A three-hour latent period was provided to allow fixation of the antibody, after which time 1.0 ml. challenging doses of an antigen-dye-steroid mixture (antigen-dye-saline in control animals) were administered intravenously through the veins of the foot.

At the time of intradermal preparation, animals in the experimental series received intraperitoneal injections of steroid: 50 mg. of hydrocortisone hemisuccinate, or 10 mg. of methylprednisolone hemisuccinate, in a volume of 1 ml. Control animals received 1.0 ml. of physiologic saline by the same route.

Preliminary studies with the RaaBGG showed that less than 0.08 μ g antibody N per intradermal site gave poor reactions after a challenging dose of 15 μ g antigen N. On the other hand, 0.08 to 0.12 μ g antibody N per intradermal site gave poor reactions when less antigen was used for challenge. It thus seemed reasonable to employ 15 μ g antigen N per guinea pig in the challenging intravenous injection. At the time of challenge, each control animal received a mixture of 0.5 ml. dye and 15 μ g antigen N in 0.5 ml., and each experimental animal received 15 μ g BGG N, 0.5 ml. dye, and either 25 mg. of the hydrocortisone preparation or 10 mg. of the methylprednisolone preparation in a total volume of 1.0 ml. per guinea pig.

All animals were killed and skinned fifteen minutes after the challenging injection. Reactions were measured on the internal surface of the skin.

RESULTS

Results are given in terms of the diameter of the colored spot in millimeters.⁶ The time after elicitation of the reaction did not appear to be a factor, both the control and experimental groups showing the appearance of the reaction to the same extent at the same time after challenge. The data in Table I show a representative experiment.

DISCUSSION

The results given in Table I show that the use of intravenously administered water-soluble adrenocortical steroids do not significantly alter the size of the cutaneous reaction elicited at threshold concentrations of antibody and antigen

TABLE I

INTRAVENOUS DOSE	ANIMAL NUMBER	INTRADERMAL INJECTIONS (DIAMETER OF REACTION IN MM.)				
		RaaBGG N/0.1			HISTAMINE	COMPOUND 48/80
		0.12	0.08	0.02	1 μ g	4 μ g
<i>Control Series</i>	1	20	20	nd	20	25
15 μ g BGG N and dye	2	10	0	nd	20	25
	3	20	18	nd	18	25
	4	15	13	nd	20	22
	5	12	tr	nd	20	18
	6	16	15	nd	18	16
	7	15	12	nd	16	16
	8	15	14	nd	18	16
	9	nd	17	tr	22	17
	10	nd	17	tr	18	17
	11	nd	16	tr	18	16
	12	nd	15	0	20	15
	13	nd	13	0	17	17
	14	nd	15	0	18	15
	15	nd	16	0	20	17
	16	nd	15	tr	20	14
Average		14.1	13.5	NC	18.3	18.4
<i>Experimental Series A</i>	17	20	20	nd	20	25
15 μ g BGG N, dye, and 25 mg. hydrocortisone	18	10	0	nd	20	25
	19	20	18	nd	18	20
	20	13	13	nd	20	22
	21	10	10	nd	14	8
	22	13	0	nd	15	12
	23	0	0	nd	16	15
	24	15	13	nd	20	18
Average		12.6	11.7	nd	19.1	18.1
<i>Experimental Series B</i>	25	0	0	nd	20	20
15 μ g BGG N, dye, and 10 mg. methylprednisolone	26	16	20	nd	15	25
	27	15	13	nd	15	20
	28	13	10	nd	15	20
	29	13	tr	nd	16	16
	30	16	16	nd	15	15
	31	0	0	nd	12	16
	32	16	16	nd	16	16
	33	nd	20	tr	17	15
	34	nd	15	0	16	15
	35	nd	17	tr	20	17
	36	nd	16	0	17	16
	37	nd	23	17	20	15
	38	nd	0	0	18	17
	39	nd	20	0	19	17
	40	nd	15	0	15	14
Average		11.1	12.3	NC	16.7	17.1

Note: BGG = bovine gamma globulin; RaaBGG = rabbit antiovine gamma globulin; nd = not done; NC = not calculated.

by PCA in the guinea pig; moreover, these drugs do not alter the temporal development of the reaction after challenge, the permeability of the minute blood vessels under the influence of locally injected histamine, or the release of histamine by a well-known histamine liberator (Compound 48/80). The slight alterations in reactivity observed in this study in the experimental animal series were of no significance and were within the limits of differential reactivity observed in the control animal series. These variations are expected even in an animal population comprised of the same strain and weight.

It has been established that ACTH and corticoids do not prevent the union of antibody and antigen in the guinea pig, nor do these agents alter the subsequent effects of such a union.⁷ Furthermore, other workers have demonstrated that reactions leading to the release of active histamine in vivo in the guinea pig are not impeded by steroid preparations. Reactions investigated include active anaphylaxis,⁸⁻¹¹ passive anaphylaxis,⁸ and exogenous histamine intoxication administered by aerosol.¹¹ Dragstedt, Mills, and Mead,¹² Carryer and Code,¹³ and Spain, Fontana, and Strauss¹⁴ have each used the appearance of histamine in biologic and test-tube systems as an indication of antigen-antibody reaction and have found that the appearance and final concentration of histamine and histamine-activity is unrelated to the steroid concentration of their respective systems. Kass and Finland¹⁵ have recently reviewed the action of steroids and have concluded that they have no effect on those cutaneous reactions which develop rapidly and are associated with the release of histamine or histamine-like substances.

Germuth, Ottinger, and Oyama¹⁶ have shown that steroids offer more protection to rabbits than guinea pigs against the Arthus reaction, but have confirmed that steroids do not protect the guinea pig against active or passive anaphylactic shock. The reasons for the decreased Arthus reaction in cortisone-treated guinea pigs are not known.

Prochazka-Fisher and Cooke³ have reported finding an appreciable inhibition of PCA in the guinea pig after the administration of one dose of water-soluble hydrocortisone at the time of intravenous challenge. The importance of using minimal amounts of antigen was recently stressed as essential for inhibition studies on PCA.¹⁷ It has been established that cortisone, when given in doses and for periods of time greater than those required to modify delayed hypersensitivity of the tuberculin type, does not effect PCA, even when threshold amounts of antibody are used.² Using more potent adrenocortical steroid preparations in high, repeated dosage with the sensitive technique of minimal antigen doses, we did not observe any inhibition under the experimental conditions described.

SUMMARY

Hydrocortisone and methylprednisolone, both as the water-soluble hemisuccinate, have no effect on the permeability of the minute blood vessels produced by histamine, histamine release by Compound 48/80, and do not inhibit the passive cutaneous anaphylactic reaction in the guinea pig when administered at the time of passive sensitization and again three hours later at the time of intravenous challenge.

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