THE ROLE OF CERTAIN STEROIDS IN THE ADRENAL-HYALURONIDASE RELATIONSHIP*

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It has been reported previously that the dermal spread of India ink with hyaluronidase is markedly influenced by the hormones of the adrenal cortex.[5,6] Removal of the adrenals resulted in an enhancement of the spreading reaction, while the administration of a potent extract of the adrenal cortex either in the adrenalectomized or normal animal inhibited spreading. However, desoxycorticosterone acetate was found to be without effect.

These observations indicated some specific relationship between hyaluronidase inhibition and chemical structure of the adrenal cortex steroids. In the present study several pure steroids have been investigated in an attempt to define more accurately the structural or hormonal factors that are related to the inhibition of the hyaluronidase-enhanced spreading phenomenon.

The primary object of this study was to determine which steroids would cause an inhibition of the spreading reaction when conditions were provided that permit adrenal cortical extracts to exert maximal effects. It was hoped that a better understanding of the adrenal-hyaluronidase relationship would be obtained in this way, although it was recognized that conditions of the experiments should be altered in order to obtain complete information regarding the influence of steroids on the spreading reaction.

Materials and methods

The general methods and precautions have been described in some detail.[5,6] CBA mice, 10 to 12 weeks old and with equal distribution of sex, were used in all experiments, with the exception of the local intradermal injection studies. The volume of the steroid solution injected was either 0.5 or 1.0 cc. except in the local injections. In the latter a total volume of 0.5 cc. was employed and this volume included steroid and India ink either with or without hyaluronidase. The hyaluronidase preparation used in the majority of the studies was the standardized preparation W-108-A and was supplied by Dr. Joseph Seifter of the Wyeth Institute.

In the mouse experiments, bilateral intradermal injections of India ink were made. During the early observations a saline control injection was made on one side and hyaluronidase mixture was injected on the other side. However, it became evident that none of the steroids caused definite responses in the non-enzyme control area.

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Received for publication November 1, 1949.

Therefore, in the majority of experiments presented here, enzyme was used in both injections, thus doubling the number of observations for a given substance.

The steroids were dissolved in sesame oil, dissolved in absolute ethanol and then diluted with water to make a suspension in 10% alcohol, or a suspension was made by thorough grinding and diluting with 0.9% sodium chloride solution. The alcohol-water suspensions were very poor, but the plain saline suspensions appeared fine and well dispersed, although the steroids settled out rapidly since no suspending agent was used.

Lipo-Adrenal Extract (Upjohn) and adrenal cortical extract (Wilson) were used as positive controls, for both of these preparations had been shown in previous experiments to inhibit the spreading reaction. The method of handling these preparations has been described in detail in earlier publications.

The general plan of the experiments was to inject the steroid into normal mice. After an interval of from one to six hours, bilateral intradermal injections of India ink and hyaluronidase in a total volume of 0.05 cc. were made. One hour later the mice were killed by fracturing the spinal cord at the cervical level; the skins were then removed. The area of spreading was measured as described in previous reports.

Experimental

The results obtained with six steroids together with control studies with Lipo-Adrenal Extract and adrenal cortical extract are presented in Tables 1 and 2. Interpretation of activity in the spreading phenomenon is made from the last column which shows the percentage change in the hyaluronidase-enhanced area of spread that has resulted from the administration of the substance under investigation. It will be seen that Compound E given intraperitoneally, intramuscularly, subcutaneously, or locally caused a marked and consistent inhibition of the spreading reaction. The inhibition of spread by Compound E when given locally at the site of hyaluronidase and India ink injection has been confirmed in several preliminary experiments with mice. The local effects produced by the other steroids when tested in mice were equivocal and, therefore, have not been included here.

Compound A inhibited spreading when administered systemically, but no effect was noted on local injection. The results with Compound A were not so definite as those with Compound E, but since a consistent inhibition was noted, there can be no doubt that this steroid was qualitatively similar to Compound E.

In the four experiments with testosterone, no effect on spreading was noted. An analysis of results in males and females separately revealed no differences between the sexes. It has been observed consistently that there is no sex difference in the spreading phenomenon and this finding was reported in an earlier publication.[5]

TABLE 1

THE INFLUENCE OF CERTAIN STEROIDS ON THE DERMAL SPREADING OF INDIA INK WITH HYALURONIDASE: I. ADRENAL CORTICAL HORMONES.

Experimental animal	*Route of administration*	*Dose*	*Type of diluent*	*Time of steroid injection*	*Area of spreading with hyaluronidase and India ink*		
					Control injected with diluent†	*Experimental injected with steroid*†	*% Change from control*
		mg.		*hours*	*sq. mm.*	*sq. mm.*	
	COMPOUND E (11-dehydro, 17-hydroxycorticosterone)						
Mouse	i.p.	1.0	saline	1	374 (12)	223 (6)	—40
"	i.p.	1.0	saline	3	374 (12)	164 (10)	—56
"	i.m.	1.0	saline	3	366 (12)	248 (12)	—32
"	s.c.	1.0	oil	3	310 (6)	223 (8)	—28
Rabbit	local	0.5	saline	0	3000 (2)	2000 (2)	—33
	COMPOUND A (11-dehydrocorticosterone)						
Mouse	i.p.	5.0	saline	3	380 (8)	254 (4)	—33
"	i.p.	1.0	10% ETOH	3	351 (8)	197 (8)	—44
"	i.p.	0.5	10% ETOH	3	374 (6)	339 (4)	— 8
"	i.m.	5.0	saline	3	346 (8)	310 (4)	—10
"	s.c.	1.0	oil	1	261 (4)*	220 (4)*	—16
"	s.c.	0.5	oil	1	185 (2)*	155 (2)*	—16
"	s.c.	0.5	oil	3	180 (4)*	173 (4)*	— 4
"	s.c.	0.5	oil	3	235 (4)*	222 (4)*	— 6
Rabbit	local	0.5	saline	0	2800 (1)	3100 (1)	+11
	LIPO-ADRENAL EXTRACT (UPJOHN)						
Mouse	s.c.	0.25 cc.	oil	1	375 (4)	239 (4)	—36
"	s.c.	0.25 cc.	oil	24	331 (4)	287 (4)	—13
	ADRENAL CORTICAL EXTRACT (WILSON'S)						
Mouse	i.m.	0.5 cc.	saline	3	346 (8)	271 (6)	—22

* Lower potency hyaluronidase than employed in the other studies here reported.

† Numbers in parentheses represent the number of animals employed. Since each animal was bilaterally injected with the hyaluronidase-India ink mixture, the actual number of observations is double the number of animals.

TABLE 2

THE INFLUENCE OF CERTAIN STEROIDS ON THE DERMAL SPREADING OF INDIA INK WITH HYALURONIDASE: II. RELATED STEROIDS.

Experimental animal	*Route of administration*	*Dose*	*Type of diluent*	*Time of steroid injection*	*Area of spreading with hyaluronidase and India ink*		
					Control injected with diluent†	*Experimental injected with steroid†*	*% Change from control*
		mg.		*hours*	*sq. mm.*	*sq. mm.*	
	TESTOSTERONE						
Mouse	i.p.	5.0	saline	3	380 (8)	403 (7)	+ 6
"	i.p.	1.0	saline	3	374 (12)	370 (4)	— 1
"	i.m.	1.0	saline	3	366 (12)	386 (19)	+ 5
Rabbit	local	0.5	saline	0	1800 (1)	1800 (1)	0
	ESTRADIOL BENZOATE						
Mouse	i.p.	5.0	saline	3	380 (8)	379 (8)	0
"	i.p.	1.0	saline	3	374 (12)	359 (4)	— 4
"	i.m.	1.0	saline	3	366 (12)	304 (13)	—17
Rabbit	local	0.5	saline	0	1500 (1)	1500 (1)	0
	PROGESTERONE						
Mouse	i.p.	5.0	saline	3	380 (8)	362 (8)	— 3
"	i.p.	1.0	saline	3	374 (12)	387 (4)	+ 3
"	i.m.	1.0	saline	3	366 (12)	306 (10)	—16
"	i.m.	1.0	oil	3	309 (8)	296 (10)	— 4
Rabbit	local	0.5	saline	0	1250 (2)	1650 (2)	+28
	PREGNENOLONE						
Mouse	i.p.	5.0	saline	3	380 (8)	398 (8)	+ 5
"	i.p.	1.0	saline	3	374 (12)	388 (4)	+ 4
"	i.m.	5.0	saline	1	330 (4)	334 (5)	+ 1
"	i.m.	5.0	saline	3	330 (4)	275 (6)	—16
"	i.m.	5.0	saline	6	330 (4)	330 (6)	0
"	i.m.	1.0	saline	3	366 (12)	317 (6)	—13
Rabbit	local	0.5	saline	0	2300 (1)	2650 (1)	+15

† Numbers in parentheses represent the number of animals employed. Since each animal was bilaterally injected with the hyaluronidase-India ink mixture, the actual number of observations is double the number of animals.

Estradiol benzoate, progesterone, and pregnenolone gave equivocal results, but it appears justifiable to conclude that under the experimental conditions employed, these steroids did not affect definitely the spreading reaction.

In agreement with earlier studies, a definite inhibition was obtained with the two adrenal extracts. It is interesting to note that there appears to be some decrease in activity in the 24 hours following the subcutaneous administration of the Lipo-Adrenal Extract.

Discussion

The results presented here, together with the earlier observation of a lack of inhibition by desoxycorticosterone, are very suggestive of a correlation between the C-11 oxygenated adrenal steroids and inhibitory activity in the hyaluronidase-enhanced spreading phenomenon. Of seven steroids that have been studied only two have definitely shown activity. Furthermore, these compounds, E and A, are the only ones in this series that have the carbohydrate metabolism characteristics of the C-11 oxygenated adrenal steroids. Additional evidence in support of this correlation is the observation that A is less active than E. Although quantitative interpretation cannot be drawn from these studies, the difference in activity between compounds E and A appears to be rather definite. A similar difference in the carbohydrate metabolism influence of these two steroids is well established.

The experiments described here were designed so as to obtain comparable data with the different steroids. In order to accomplish this, it was necessary to test all compounds under identical conditions. For example, there was some indication that the adrenal cortical extracts exerted their maximal influence on the spreading reaction within a period of from one to six hours following administration of the extract. Since it was the primary objective of this investigation to study compounds that might exhibit the adrenal cortical effect, the steroids were all administered within this time interval prior to the injection of the hyaluronidase-India ink mixture.

The lack of activity observed for testosterone, estradiol benzoate, progesterone, and pregnenolone must be interpreted cautiously. The dosages employed for the hormonally active steroids were large enough to give the conventional end-organ responses. Actually the dosage of estradiol benzoate and of progesterone was much larger than is required for maximal hormonal responses. However, the time of action was far too short for these hormonal effects fully to be manifested. In dealing with these sex hormones, days rather than hours are required for complete anatomical responses to the

hormones. On the other hand, adrenal effects such as glycogen storage are obtained within a matter of hours following administration of the hormone. It is possible that different results would have been obtained with the sex hormones if a longer time-interval had been used. However, this variation in conditions was outside the scope of this study. In the case of the adrenal steroids, the time relationship was compatible with that required for a primary hormonal response.

Other investigators have observed an inhibition of the spreading reaction by estrogens. For example, Sprunt[7,8] found that both estrone and estradiol-dipropionate inhibited India ink spreading in normal and in castrate rabbits when rather large doses were administered daily over long periods of time. Lurie and Zappasodi[3] have found that as compared with female rabbits, the males showed a decreased spread of India ink. As noted above, this observation has not been substantiated for mice.

Contrary to the effects with estrogens, Lurie and Zappasodi[4] found that progesterone increased the area of India ink spreading in the rabbit. Because of the difference in the time factor, neither the estrogen nor the progesterone studies of these investigators can be compared directly with the experiments presented here.

Regardless of the obvious importance of varying the time interval for the different steroids, the conclusion is justified that under conditions necessary for the C-11 oxygenated adrenal steroids to exert a pronounced inhibition of hyaluronidase-enhanced spread of India ink, testosterone, estradiol benzoate, progesterone, and pregnenolone appear to be inactive.

The recent observations that Compound E and the pituitary adrenocorticotrophic hormone (ACTH) are active agents for the treatment of rheumatoid arthritis and possibly for other diseases that are relieved by pregnancy[1,2] have made it essential to develop laboratory assay procedures that can be used to study the fundamental biochemical properties of this field of chemotherapy. As it is possible that hyaluronic acid metabolism is involved in the manifestation of certain of the arthritides, it is logical to examine the hyaluronidase inhibiting activity of compounds that influence the clinical condition in rheumatoid arthritis. At the present time this comparison is impossible since completely documented results of clinical investigations are very meager. Nevertheless, the general technique employed in the present study should be extended so that correlations can be made when clinical results become available. In establishing this correlation, the time factor emphasized in the preceding discussion should be given due consideration, for it is possible that steroids having beneficial clinical effects

will exhibit these effects after varying time intervals. One of the remarkable features of the Compound E treatment of rheumatoid arthritis is the rapidity of action. This characteristic is apparent not only in certain primary hormonal responses such as glycogen storage, but also in its inhibition of hyaluronidase-enhanced spreading.

Conclusions

1. Compound E and, to a lesser extent, Compound A inhibit the spreading of intradermally injected India ink with hyaluronidase.

2. Testosterone, estradiol benzoate, progesterone, and pregnenolone, when tested under conditions necessary for the optimal inhibitory activity of adrenal cortical extracts, did not have a definite influence on the spreading phenomenon.

3. The evidence suggests that inhibition of the spreading reaction by the adrenal hormones is restricted to steroids that have an oxygen at Carbon-11.

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