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THE ACTIVATION OF LYMPHOID TISSUE LYSOSOMAL ENZYMES BY STEROID HORMONES

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SUMMARY

Injections of 1 or 2 mg doses of cortisone, hydrocortisone, corticosterone or prednisolone into mice cause, 24 h after the injection a large decrease in weight of spleen and thymus which is accompanied by very large increases in the activity of the lysosomal acid phosphatase in these tissues. Prednisolone and hydrocortisone were most effective. Injection of testosterone, 11-hydroxyprogesterone and 17-hydroxyprogesterone had very little effect on the weight of the lymphoid tissues and caused only very small changes in the activity of lysosomal enzymes. These effects of the activating hormones are very similar to those previously observed following irradiation of the spleen or thymus with X-rays and it is considered that the lysosomal activation observed is mediated by lymphocytolysis and not by a direct effect of the steroids on the lysosomes

INTRODUCTION

Some steroid hormones, such as cortisone, stabilise lysosomes of rat liver *in vitro* [1, 2] and injections of cortisone can prevent the augmented release of acid hydrolases from rabbit ear cartilage following administration of Vitamin A [3].

However, increased activity of lysosomal acid phosphatase has been demonstrated after injection of cortisone acetate [4] and increased hyaluronidase activity of rat kidney lysosomes is caused by injection of hydrocortisone [5]. Furthermore, Shewell[6] and Dougherty *et al.*[7] demonstrated that cortisone injection causes a large loss of thymus weight in young animals. Aikman and Wills[8] showed that the loss of thymus weight which resulted from an injection of hydrocortisone was accompanied by a greatly increased activity of lysosomal acid phosphatase. X-irradiation also caused a loss of thymus weight and lysosomal enzyme activation. Progesterone caused no lysosomal activation.

In view of the apparent discrepancies between the actions of steroids on lyososomes reported from experiments carried out *in vitro* and *in vivo*, we have now extended our study of the effects of steroid hormones on lysosomes of thymus, spleen and some other tissues. A wide range of steroid hormones has been studied and we have compared effects observed *in vitro* to those observed *in vivo* and attempted to relate the effects of the hormones to their structure. A quantitative histochemical technique which we described recently [9, 10] has been used to gain more precise information about the behaviour of lysosomes in their natural environment.

EXPERIMENTAL

Male, SAS 4 strain mice, 5–10 weeks old and weighing 25–30 g, bred from the strain maintained at St. Bartholomew's Hospital Medical College were used in nearly all experiments. In a few experiments female mice of the same strain were used.

The sources of most materials used in this investigation have been previously described [8, 9].

Cortisone acetate was obtained from Roussel Laboratories, Ltd. London or from Sigma Chemical Co. Ltd., Kingston, Surrey, England who also supplied prednisolone, aldosterone and hydrocortisone. Corticosterone, 11 hydroxyprogesterone and 17 hydroxyprogesterone were supplied by Koch-Light Ltd., Colnbrook, Bucks, England and testosterone propionate was supplied by Organon Laboratories, Morden, Surrey, England.

A quantitative histochemical method, described by Aikman and Wills[9] and Reynolds and Wills[10] was used in all the investigations of lysosomal acid phosphatase. For this method slices of spleen or thymus, 3 mm thick, were rapidly frozen in a bath of hexane kept at $-60°$ to $-70°$C. Slices, 7 μm thick were then cut in a cryostat at $-40°$ and transferred to glass slides. The slices were then incubated for periods up to 90 min in a medium containing acetate buffer (pH 5.0), lead nitrate and sodium β-glycerophosphate as substrate. Slides were removed at intervals, rinsed, and treated with a saturated solution of hydrogen sulphide. After mounting, the absorbance of the lead sulphide stain produced was measured using a M85 Vickers Scanning and Integrating Microdensitometer. The progress of absorbance of the stain was plotted against time of incubation of the slices in the substrate medium to give a measurement of enzyme activity.

RESULTS

Effects of various doses of cortisone on lysosomal enzyme activities in spleen and thymus

Sixteen mice were used for the experiment. Four

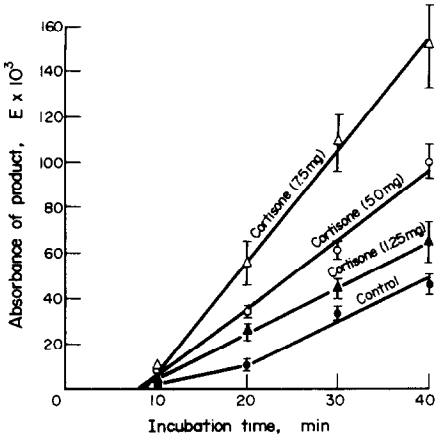

Fig. 1. The effect of cortisone on the acid phosphatase activity of mouse thymus lysosomes measured 24 h after injection. Slices of thymus were incubated in the β-glycerophosphate substrate medium for the times shown and the absorbance of the stain produced measured as described [9, 10]. The values shown for each incubation time is the mean of 5 fields measured in 3 different slices. Four mice were injected with each dose of cortisone. Mean values and standard errors are shown.

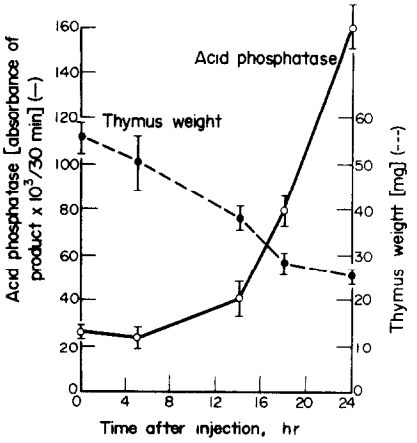

Fig. 2. The effect of time after cortisone injection (7.5 mg) on the acid phosphatase activity and weight of mouse thymus. Six mice were used in each experiment and the experimental procedure was as described for Fig. 1. Mean values and standard errors are shown.

were injected with 1.25 mg, four with 5.0 mg and four with 7.5 mg cortisone acetate. An equal vol. of 0.9% sodium chloride was injected into four remaining mice as controls. The mice were killed 24 h after the injection, spleens and thymuses were removed, weighed and sections prepared for measurement of lysosomal acid phosphatase activity by the quantitative histochemical method [9, 10].

The largest doses of cortisone (5.0 mg and 7.5 mg) caused a very large increase in enzyme activity in the thymus macrophage lysosomes. The smallest dose (1.25 mg) caused the point of inflexion observed for control sections to disappear but did not cause an increased rate of enzyme activity as determined by the slope (Fig. 1). The disappearance of the point of inflexion is belived to be caused by a greatly increased permeability of the lysosomal membrane to the substrate [8]. Increased enzyme activity was also observed in lysosomes of the spleen and in both organs weight decreases accompanied the enzyme activation (Table 1).

Time response to cortisone injection

During investigations of the effect of irradiation on lymphoid tissue lysosomes Aikman and Wills[8] observed a delay of 1–3 h before activation of the enzyme was measurable.

Table 1. The effect of different doses of cortisone acetate on mouse spleen and thymus weight 24 h after injection

Cortisone acetate (mg)	Spleen weight (mg)	Thymus weight (mg)
—	98.6 ± 8.0	59.2 ± 3.1
1.25	89.8 ± 10.4	30.8 ± 2.4
5.0	66.8 ± 5.2	21.2 ± 1.1
7.5	59.6 ± 8.7	13.6 ± 1.8

Four animals were used for each steroid dose in each of three experiments. Mean values and standard errors are shown.

The time of the response to a steroid hormone, cortisone, was studied by injecting 30 mice with 7.5 mg cortisone acetate. Six mice were killed immediately after injection, six after 3 h, six after 14 h, six after 18 h and the remaining six, 24 h after the injection. After killing, thymuses were removed, weighed, sections prepared and incubated in the glycerophosphate medium for determination of acid phosphatase.

Decreases of thymus weight were measurable 3 h after injection and the organ weight fell steadily for 18 h. Acid phosphatase activity was not increased 3 h after injection but increased activity was very marked after 14 h and it increased rapidly with time up to 24 h (Fig. 2).

The effects of cortisone on liver and kidney lysosomes

Eight mice were injected with 2.5 mg cortisone acetate, and killed after 24 h. Sections of liver and kidney were prepared for measurement of lysosomal acid phosphatase using exactly the same technique as described for spleen and thymus [9].

No increase in lysosomal acid phosphatase activity was detected in the liver but small changes in activity are difficult to detect in this tissue on account of the large amount of non-specific staining [8, 9].

This problem does not exist in kidney [8] and for this tissue a clear increase of acid phosphatase activity was discernible after injection of cortisone but quantitatively much smaller than was observed in thymus lysosomes (Fig. 3).

A comparison of the effects of several different steroid hormones on lysosomal acid phosphatase activity in thymus

Aikman and Wills[8] observed that although

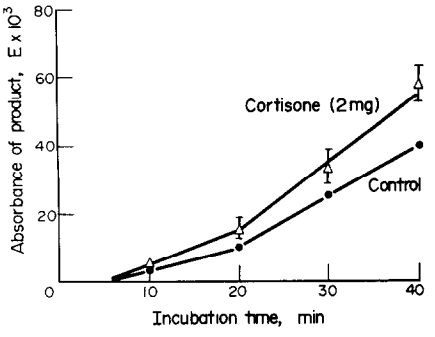

Fig. 3. The effect of cortisone on the acid phosphatase activity of mouse kidney. Four mice were used for the experiment and each point is a mean value of 5 fields of 3 sections. S.E.M.'s are shown.

marked lysosomal enzyme activation in spleen and thymus was caused by injection of hydrocortisone, progesterone was without effect. These experiments have now been extended to include a wide range of steroid hormones.

2 mg of cortisone acetate, hydrocortisone, corticosterone, testosterone, 17 hydroxyprogesterone, 11 hydroxyprogesterone, prednisolone or aldosterone, suspended in 0.9% sodium chloride were injected into groups of four mice. A control group was injected with a similar volume of 0.9% sodium chloride.

24 h after each injection the mice were killed, thymuses were removed and sections prepared for measurement of acid phosphatase activity. Activity of acid phosphatase observed after each hormone injection was related to the values obtained for control animals.

With the exception of 11-hydroxyprogesterone, all hormones tested increased the activity of lysosomal acid phosphatase in the thymus. Very strong activation was caused by injection of hydrocortisone or prednisolone and weak activation by testosterone or 17 hydroxyprogesterone. Activating effects of corticosterone, cortisone and aldosterone were intermediate and between these extremes (Table 2).

Table 2. The effect of steroid hormones on the acid phosphatase activity of mouse thymus

Injection	% control
Saline	100
Cortisone acetate (2 mg)	290 ± 27
Hydrocortisone (2 mg)	420 ± 23
Corticosterone (2 mg)	168 ± 9
Prednisolone (1 mg)	425 ± 22
Aldosterone (1 mg)	200 ± 9
Testosterone (2 mg)	114 ± 5
11-OH Progesterone (2 mg)	91 ± 11
17-OH Progesterone (2 mg)	121 ± 10

1 mg or 2 mg of each hormone was injected into groups of four mice and 0.9% sodium chloride into four mice as a control group. Acid phosphatase activity was measured in thymus sections as described for Fig. 1. Sections of thymus, three from each mouse, were incubated for 40 min and the absorbance measured as described [8, 9]. Mean values of acid phosphatase activity are shown related to values obtained for control animals and standard errors are shown.

Effect of a combination of irradiation and steroid hormone treatments

Aikman and Wills[8] had shown that the regression of thymus and spleen after irradiation with whole body doses of 100–1000 rads was accompanied by a marked increase in lysosomal acid phosphatase. In view of the fact that certain steroid hormones caused very similar effects to irradiation, we attempted to elucidate whether the hormones imitated irradiation by combining the two treatments.

Hormones were selected from those showing strong activation, intermediate activation and weak or no activation. Cortisone, corticosterone, prednisolone, testosterone and 11-hydroxyprogesterone were studied.

1 mg or 2 mg of each hormone in an equal volume of 0.9% sodium chloride was injected into eight mice and 4 h later four mice were irradiated with 850 rads X-rays. 24 h after the hormone injection and 20 h after the irradiation the mice were killed and sections of thymuses prepared for determination of lysosomal enzyme activity. The experiment with cortisone was repeated using groups of female mice in addition to males.

After an injection of cortisone acetate very marked activation of lysosomal acid phosphatase was observed which was slightly less than that following a dose of 850 rads (Table 3) A similar result was obtained using the group of female mice.

In view of the fact that lysosome stabilisation has frequently been reported following cortisone injection [11] the experiment was repeated using a much smaller dose (0.1 mg) of cortisone acetate. This dose when given alone did not affect the lysosomal enzyme activity of the unirradiated animals and did not alter the labilising effect of irradiation.

Prednisolone injection caused a large lysosomal enzyme activation and was more effective than a whole body dose of 850 rads (Table 3). The combined effect of irradiation and hormone treatment was identical to that of hormone treatment alone (Table 3).

Corticosterone, however, was less effective than irradiation but did not modify the effect of irradiation when the combined treatment was given (Table 3).

11-hydroxyprogesterone (1 mg) when injected did not alter the lysosomal enzyme activity and did not change the effect of irradiation but both testosterone and 17-hydroxyprogesterone enhanced the enzyme activation effect when given with irradiation (Table 3).

DISCUSSION

In studies of the effect of whole body irradiation on lysosomal enzyme activity in spleen and thymus, Aikman and Wills[8, 9] demonstrated that during the initial incubation period of tissue slices, the enzyme activity was limited by the permeability of the lysoso-

Table 3. The effect of steroid hormones and irradiation on lysosomal acid phosphatase activity in thymus

Hormone	Acid phosphatase activity (increase of absorbance in 30 min incubation)	
(Control)	34 ± 6	
(850 rads)	122 ± 10	
	Hormone alone	Hormone + 850 rads
Cortisone (1 mg)	105 ± 5	138 ± 10
(0.1 mg)	39 ± 4	123 ± 6
Corticosterone (2 mg)	56 ± 7	119 ± 4
Prednisolone (1 mg)	151 ± 10	153 ± 12
Testosterone (2 mg)	35 ± 6	183 ± 20
11-OH Progesterone (2 mg)	30 ± 5	127 ± 5
17-OH Progesterone (2 mg)	42 ± 7	154 ± 6

Groups of four mice were either treated with an injection of hormone alone or by hormone injection followed 4 h later with a dose of 850 rad X-rays. A group was given a dose of X-rays with no hormone injection and controls were injected with 0.9% sodium chloride. The mice were killed 24 h after hormone injection and sections of thymus prepared for measurement of acid phosphatase activity [8, 9]. The acid phosphatase activity is expressed as the increase of absorbance during 30 min incubation, between the 10th and 40th min when the rate was linear. S.E.M.'s are shown.

mal membrane to the substrate. This was shown by the existence of a point of inflexion in the plot of absorbance against incubation time [8, 9]. The disappearance of the inflexion point after small doses of whole body irradiation [9] could occur without increase in the rate of enzyme activity as indicated by the slope of the absorbance–incubation time plot but if slightly larger doses of irradiation were used, large increases in the rate of enzyme activity occurred [9]. This hypothesis is supported by other investigations [12] and these concepts are used in the interpretation of the effects of steroid hormones.

No changes were discernible in the activity of the enzymes of the liver lysosomes after injection of cortisone. Stabilisation of lysosomes is more difficult to detect than labilisation using the quantitative histochemical method but stabilisation would be expected to produce an extended latent period before enzyme activity was affected. This was not observed and thus neither stabilisation nor labilisation could be demonstrated. In the kidney, however, the point of inflexion of the plot of absorbance against incubation time tended to disappear following cortisone injection and the rate of enzyme action, as shown by the slope, increased (Fig. 1). It would thus appear that cortisone has a small activating effect on kidney lysosomes *in vivo*, unlike its stabilising effect on liver lysosomes *in vitro* [1, 2].

Extensive activation of thymus lysosomal acid phosphatase occurred when mice were treated with doses of cortisone ranging from 1.25 to 7.5 mg (Fig. 1). It is note worthy that the earliest change observable after injection of the lowest dose used (1.25 mg) was the disappearance of the point of inflexion when the absorbance, which is dependent on product formation, begins to increase rapidly with time. This demonstrates that the great increase of lysosomal membrane permeability which follows the steroid injection is similar to that caused by irradiation. Increased permeability of the lysosomal membrane is then followed by enzyme activation when the dose of hormone or irradiation is increased.

Irradiation is known to cause destruction of lymphocytes and factors liberated from lymphocytes are believed to play an important role in activation of the macrophage lysosomal enzymes (Aikman & Wills [8]). The nature of these factors is not yet established but lymphocyte debris is inactive and it is likely that an enzyme activator, possibly a proteolytic enzyme is involved (Reynolds and Wills[13]).

The effect of the hormones which cause a greatly increased activation of lysosomal acid phosphatase was likely to be mediated initially by lymphocytic destruction. The effects of the steroid on thymus and spleen weight (Table 1) are caused by destruction of lymphocytes and by activation of macrophage lysosomal enzymes.

In lymphoid tissues a time delay must elapse before lysosomal activation occurs and components released from damaged lymphocytes appear to be much more important in macrophage lysosomal enzyme activation than the direct action of steroids on the lysosomes.

It is for this reason that no correlation has been found between the effects observed on lymphoid tissue lysosomes after injection of steroids and the effects reported on liver lysosomes *in vitro* [1, 2]. Cortisone has a protective effect *in vitro* [1, 2] but it has a strong activating effect *in vivo* (Fig. 1) and so also does corticosterone (Table 3). Direct effect of steroids on the lysosomal membrane may therefore be less important *in vivo* because other indirect effects which they cause could override them.

If the measurement of the lysosomal activation in

spleen and thymus can be regarded as a direct measurement of lymphocytic destruction, the determination of lysosomal enzyme activation enables us to establish the structural configuration which is most effective in causing lymphocytolysis. Of special importance appears to be the existence of hydroxyl groups in positions 11 and 17 of the steroid nucleus, both of which are contained in hydrocortisone and prednisolone, the most active steroids. Replacement of the 11 OH by :O reduces the lysosomal activation effect as may be seen by a comparison of the effects of hydrocortisone and cortisone (Table 2). Loss of the OH in either the 11 position such as in testosterone, or in the 17 position such as in corticosterone, leads to a marked drop in activity. A hydroxyl group in position 21 could also be important in the lympholytic action because all the most active componds possess a OH group in this position and corticosterone (11-OH, 21-OH) is much more active than 11-hydroxyprogesterone which has no OH group in the 21 position.

Aikman and Wills[8] demonstrated that extensive lymphocytolysis accompanied by activation of lysosomal enzyme was caused by irradiation of the thymus and spleen.

By combining irradiation with hormone treatment we compared lymphocytolysis caused by each treatment. No hormone tested offered any protection against lysosomal labilisation in thymus and spleen (Table 3) and the majority of the hormones did not affect the response of lymphoid tissues to irradiation although several steroid hormones have been described as lysosomal stabilisers *in vitro* [1–3]. Some hormones, such as testosterone and 17-hydroxyprogesterone, even enhanced the effect of irradiation.

These hormones therefore increase the lymphocytolysis initiated by irradiation by enhancing the activity of the lymphoid factors released which activate the lysosomal enzymes or by sensitising the lysosomes to irradiation or to the effects of the activating lymphocytic components released after damage.

The mechanism by which corticosteroids cause lysis of lymphoid tissue is not yet established but there is good evidence that the release of free fatty acids from adipose tissue may be of primary importance. These free fatty acids are believed to attack the nucleus which in turn causes cellular lysis [14, 15]. Such a proposed mechanism would be in accord with the slow time response to steroid injection (Fig. 2) but then the great variation of steroid hormone effects observed (Table 3) would be entirely dependent on their ability to release fatty acids from adipose tissue. If irradiation also causes nuclear damage to lymphocytes as is generally believed [16] then the subsequent stage of lysosomal activation would be the same whether the lymphocytic tissue was damaged by irradiation or indirectly by steroid hormones.

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