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THE EFFECT OF CORTICOSTEROIDS ON ALVEOLAR MACROPHAGE ACTIVITY*

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SUMMARY

The effect of corticosteroids on macrophage activity in rat lung was studied by determining the clearance rate of inhaled $^{59}Fe_2O_3$ particles and the number of alveolar macrophages recovered by lung washing.

The results indicate that the structure relationship for the action on alveolar phagocytosis is different from the well known potencies in terms of glucocorticoid activity. The endogeneous glucocorticoids corticosterone† and cortisol† were found to be the most potent compounds tested while some synthetic steroids such as triaminocolone acetonide and dexamethasone produced no significant changes in the parameters examined.

From these and other studies a reduced macrophage mobility by elevated corticosterone or cortisol is postulated. This reduction can be prevented by inhibition of corticosteroid biosynthesis.

INTRODUCTION

In the past much attention has been paid to the influence of corticosteroids on the reticulo-endothelial system [1]. This interest was promoted by the large fluctuations of these hormones *in vivo* [2, 3] and their pharmacological applications [4]. Unwanted side effects, such as reduced resistance to many kinds of infection [5], impairment of the immune response [6] and depression of phagocytosis [7] are related to macrophage function. In our study on the elimination of inhaled particles [8] therefore the effect of corticosteroids on alveolar macrophage activity was of primary interest [9].

EXPERIMENTAL

Animals. Groups of 12–16 male Sprague–Dawley rats weighing 200–250 g were used in these experiments.

Chemicals. The steroids were applied s.c. in a solution of propylenglycol–saline 2:1 (v/v). Metopirone (supplied by Ciba–Geigy) was administered either s.c. or orally mixed with the food.

Inhalations. The inhalations were carried out with $^{59}Fe_2O_3$ aerosols of particle size 1–5 μm and a S.A. of 5–7 μCi g^{-1}. The radioactivity of the lung at the end of the exposure lay between 0.1 and 0.2 μCi. Details of the methods and the inhalation apparatus are described in a previous article [10]. After inhalation of the ^{59}Fe-haematite aerosol, the whole-body radioactivity of each rat was measured with a γ-spectrometer for several weeks.

Macrophage isolation. Alveolar macrophages were isolated according to the method of Brain and Frank[11].

Determination of radioactivity. The distribution of the radioactivity in different organs, body fluids and isolated macrophages was determined by scintillation counting [12]. The lung clearance was estimated by fitting the whole-body retention data to a two compartment system [13].

RESULTS

Stress. It has been postulated by Schild and Löw[14] that the post-traumatic depression of phagocytosis is caused by the corresponding increase in plasma corticosterone. If this correlation is valid in stress situations in general we should expect that our inhalation procedure should also result in a reduced clearance rate.

When we adapted the animals for 3 weeks to the inhalation procedure (Fig. 1), we found after the exposure an increased elimination of radioactivity about 30% greater than the values for rats that had not been adapted ($p < 0.05$). A small additional effect could be obtained by blocking corticosteroid biosynthesis [15].

Inhibition of corticosteroid biosynthesis. In our experiments we used Metopirone (2-methyl-1,2-bis-(pyridyl)-1-propanone) a relatively non-toxic inhibitor of 11β-hydroxylation [16]. The effect of Metopirone depends on dose [17], route and time of administration relative to the inhalation and to the time of day [18]. In our investigations (Fig. 2) the alveolar

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† Systematic names of steroids mentioned in this paper: aldosterone: 11β,21-dihydroxy-4-pregnene-3,20-dion-18-al; corticosterone: 11β,21-dihydroxy-4-pregnene-3,20-dione; cortisol: 11β,17,21-trihydroxy-4-pregnene-3,20-dione; dexamethasone: 11β,17,21-trihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione-9α-fluoro; DOC: 21-hydroxy-4-pregnene-3,20-dione; prednisolone: 11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione; spirolactone: 3-(3-OXO-7α-acetylthio-17β-hydroxy-4-androsten-17α-yl) propionic acid-γ-lactone; triaminocolone acetonide: 21-tetrahydroxy-9α-fuoro-11β,16α,1,4-pregnadiene-3,20-dione-16,17-acetonide.

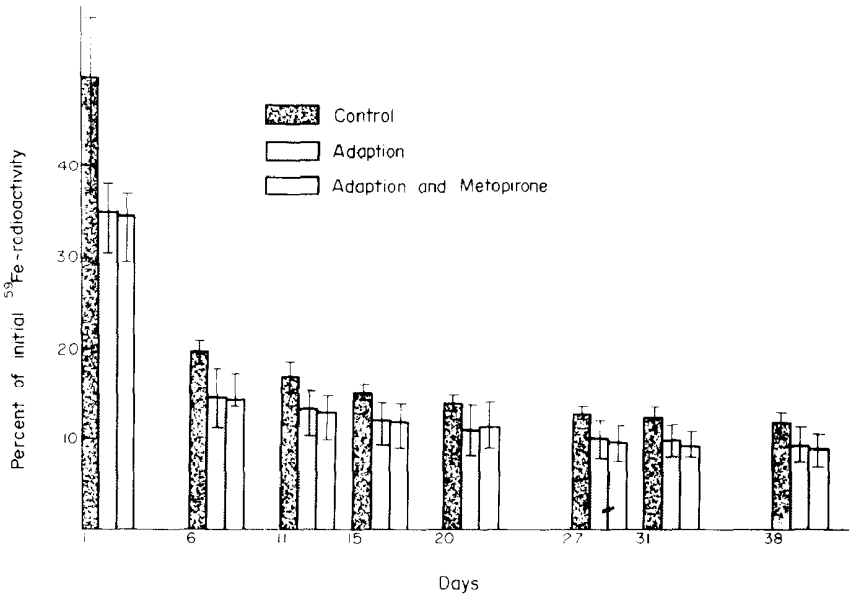

Fig. 1. Stress influence on clearance-rate of inhaled Fe_2O_3.

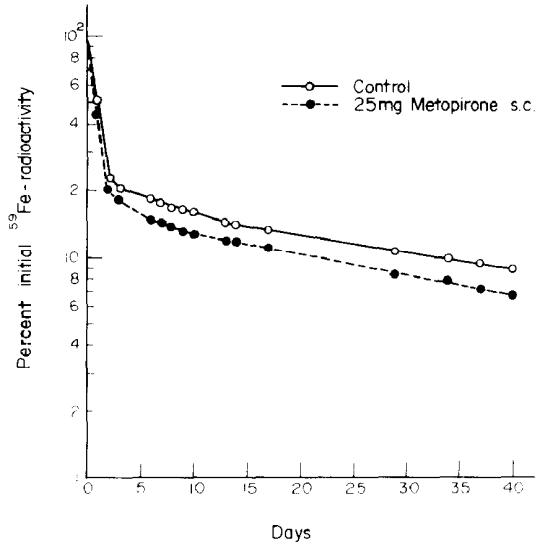

Fig. 2. Lung clearance of inhaled $^{59}Fe_2O_3$ particles after metopirone treatment.

load of inhaled haematite could be reduced by Metopirone to 60–70% of that found in controls [19]. In the example given, 25 mg Metopirone was injected s.c. 30 min after the beginning of inhalation ($p < 0.05$). We conclude that Metopirone prevents the depression of phagocytosis which is provoked by increased adrenal hormone levels.

Mineralocorticosteroids. Nicol *et al.* [20] have reported that desoxycorticosterone (DOC) stimulates phagocytosis. Therefore an additional direct effect of DOC in studies with Metopirone could not be excluded. Under our experimental conditions however we found a suppression of phagocytosis ($p < 0.02$) (Fig. 3). The dose of 2 mg DOC was higher than the *in vivo* level found after Metopirone.

When we gave 2 mg spirolactone, a substance which blocks the action of mineralocorticosteroids on the kidney, the clearance rate of ^{59}Fe was also reduced ($p < 0.05$).

With 0.1 mg aldosterone no statistically significant difference could be detected.

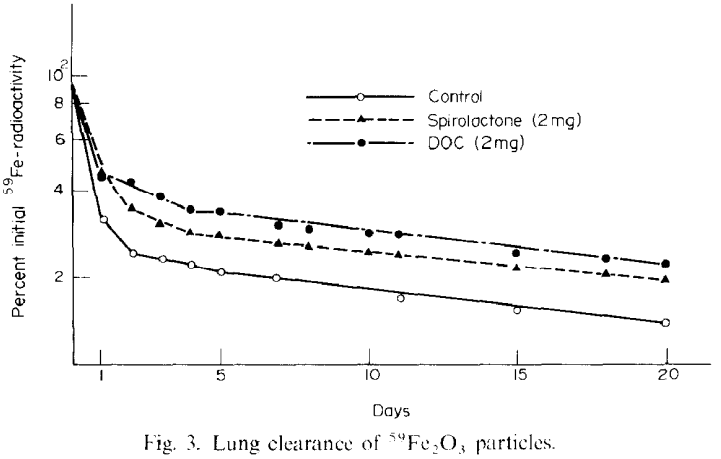

Fig. 3. Lung clearance of $^{59}Fe_2O_3$ particles.

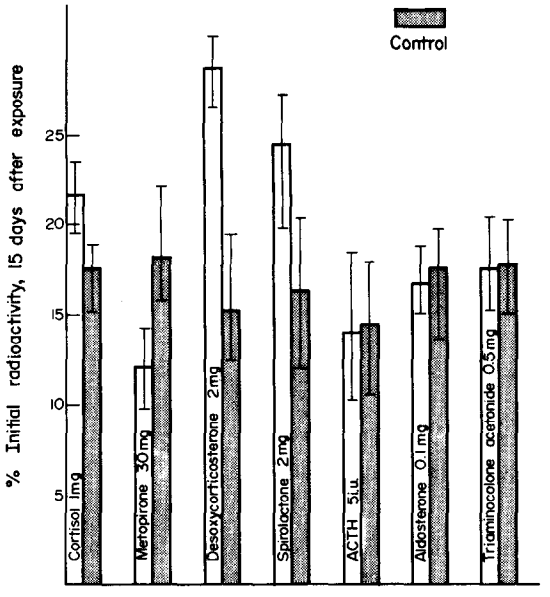

Fig. 4. Corticosteroid effect on the elimination of inhaled $^{59}Fe_2O_3$ in the rat.

Glucocorticosteroids. Studying the influence of glucocorticosteroids on phagocytosis of inhaled haematite aerosols (Fig. 4), we found a reduction of the clearance rate after s.c. injection of 0.1 mg corticosterone ($p < 0.05$). A statistically significant depressive effect on the elimination of radioactivity was also found after application of cortisol. The treatment with ACTH, however, did not impair the clearance rate (Fig. 5), possibly because the adrenals were already stimulated by the manipulation stress.

The application of 0.5 mg triaminocolone acetonide (Fig. 4) and also of dexamethasone did not result in a significant reduction of the clearance rate.

The influence of corticosteroids on alveolar macrophage yield. In order to elucidate the mechanism of the corticosteroid effect on alveolar macrophage activity we investigated the influence of these hormones on the proportion of alveolar macrophages eluted with saline. All steroids were administered 20 min before cell isolation.

The most important result (Fig. 6) was the reduction of macrophage yield after treatment with corticosterone ($p < 0.02$), the main adrenal hormone secreted by the rat adrenal cortex. Lung washing with Hanks solution had the same tendency, taking in account the smaller number of cells isolated by this solution. The same findings were observed in both male and female rats. The high total number of macrophages found in females may be due to the influence of endogeneous estrogens [21]. These results could be reproduced with rabbits.

After cortisol application a similar decrease in macrophage yield was observed. The effect of the other corticosteroids tested were not significant.

DISCUSSION

The results presented indicate that the phagocytic activity in the rat lung can be depressed by corticosterone and cortisol. Synthetic glucocorticosteroids did not produce this effect or at least to a lesser extent. Therefore it can be deduced that their action on alveolar macrophages does not parallel their glucocorticoid effect. Observations in this direction have been reported in the literature. For instance Fauve[22] found that a hemisuccinate group at position 21 in the steroid molecule prevents the decreased resistance to infection and the inhibition of macrophage spreading on glass—two parameters for phagocytosis by unesterified corticosteroids. The leucocyte mobilization and the phagocytosis activity index, which were measured by Jungi *et al.*[23] decreased after prednisolone administration. Dexamethasone, however, did not impair these mechanisms.

From these observations and our own results it seems that the 11β-hydroxy- and the 17α-hydroxy-group are not essential for the inhibition of phagocytosis. The introduction of a fluoratom in the 9α-position and a semi-succinate in position 21 on the other hand can abolish this effect. After corticosteroid treatment an analogy between the influence on the clearance rate and on cell elution could be observed.

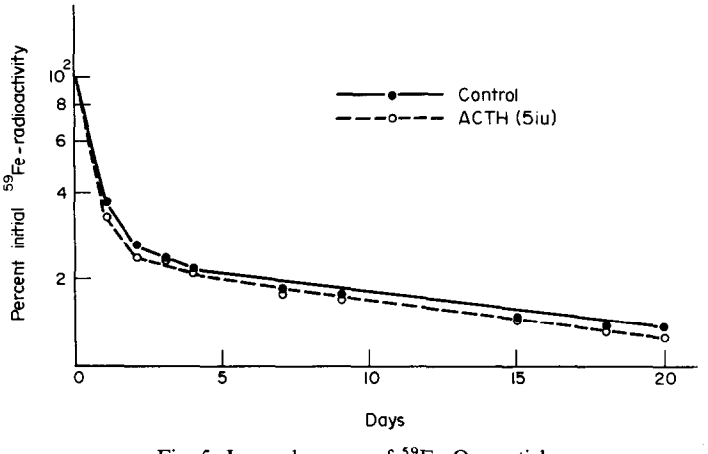

Fig. 5. Lung clearance of $^{59}Fe_2O_3$ particles.

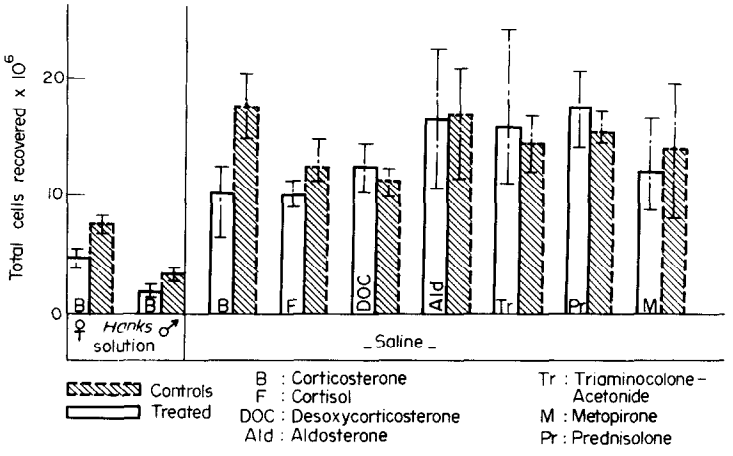

Fig. 6. Influence of corticosteroids on alveolar macrophage yield in rats.

The reduction of macrophage yield after treatment with cortisol and corticosterone cannot be explained by cell loss, because the total radioactivity in the lung did not change under these conditions. Furthermore it was found that most of the radioactivity in the lung was bound to alveolar macrophages [24]. If we compare the fraction of macrophages eluted with the ratio of radioactivity of these cells to the radioactivity remaining in the lung these two values are directly proportional (Fig. 7). Therefore we postulate that the reduced number of alveolar macrophages recovered by lung washing after corticosteroid treatment is caused by reduced macrophage mobility.

These results are in accordance with the findings of Thompson and van Furth[25]. After administration of glucocorticosteroids they found a decreased number of circulating monocytes in mice. With [^{3}H]-thymidine it could be demonstrated that the influx of mononuclear phagocytes from the circulation into the peritoneal cavity was greatly reduced. The same group could not detect an influence on the engulfment and the digestive step of phagocytosis by cortisol [26]. A decrease in circulating monocytes [27] and an impaired differentiation of monocytes to macrophages during cortisol treatment [28] was also suggested by other investigators.

The alveolar macrophages are derived mainly from peripheral blood monocytes [29]. Therefore it is not clear if the action of corticosteroids in our experiments is restricted to the lung [30]. In any case a good agreement was found between the decreased number of alveolar macrophages eluted and the reduction of ^{59}Fe elimination.

In conclusion we always have to take into account that in inhalation accidents a stress reaction will occur with a corresponding increase in cortisol level. The elevated concentration of corticosteroids will result in a reduced clearance rate of inhaled particles. This reduction can be prevented by avoidance of stress and blockage of corticosteroid biosynthesis.

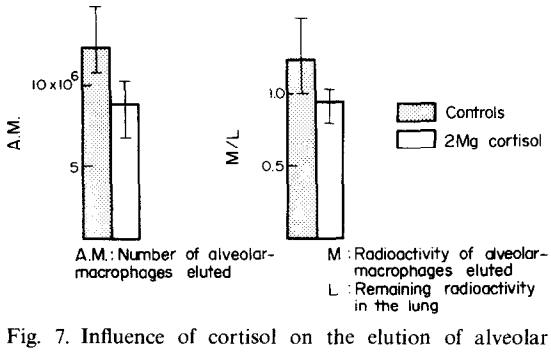

Fig. 7. Influence of cortisol on the elution of alveolar macrophages.

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