A study of the role of corticosterone as a mediator in exercise-induced stimulation of murine macrophage phagocytosis

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1. It is generally accepted that physical activity provokes changes in the immune system. Previous studies have demonstrated that the stress of physical activity (swimming until exhaustion) increases the phagocytic activity of peritoneal macrophages. However, the precise mechanisms remain unknown.

2. Two experiments were performed in the present study. (A) Peritoneal macrophages from control mice were incubated with plasma from three different groups of mice: (1) mice subjected to swimming until exhaustion with no previous training, (2) mice subjected to the same activity but with 1 month of training (30 min day^{-1}), and (3) a control (non-exercised) group. The differences in the resulting phagocytic (attachment and ingestion) capacity were measured. (B) Changes in the concentration of plasma corticosterone after exercise were also measured, and the effect of incubation with the postexercise plasma corticosterone level on the phagocytic activity of peritoneal macrophages was then studied *in vitro*.

3. The results were: (A) incubation with plasma from both groups of exercised mice (with and without previous training) led to increased levels of phagocytic capacity (number of *C. albicans* cells ingested per 100 macrophages). (B) Incubation with a corticosterone concentration of 0·72 μmol l^{-1} (similar to that observed in plasma immediately after exercise) raised the phagocytic capacity (144 $\pm$ 12 after incubation with 0·72 μmol l^{-1} *vs.* 93 $\pm$ 19 after incubation with 0·24 μmol l^{-1}). This increase was also significantly greater than that observed with 7·2 μmol l^{-1} corticosterone.

4. It is concluded that corticosterone may mediate the increased phagocytic function of peritoneal macrophages induced by exercise.

It is generally accepted that regular exercise is beneficial to health. However, there is some evidence that athletes may be particularly susceptible to infection (Douglas & Hanson, 1978; Tomasi, Trudean & Czerwinksi, 1982; Fitzgerald, 1988). Since the immune system is responsible for the defence of the organism against pathogens, the study of the influence of exercise upon this system is very important. Although many reports have noted that the immune response of lymphocytes is diminished during or after intense exercise (Eskola *et al.* 1978; Hoffman-Goetz, Thorne, Houston & Young, 1986; Fitzgerald, 1988; Keast, Cameron & Morton, 1988; Ferry, Weil, Amiridis, Laziry & Rien, 1991; Ortega, Galán, De la Fuente & Barriga, 1993*c*), the phagocytic activity of both macrophages (Fehr, Lötzerich & Michna, 1988, 1989; De la Fuente, Martín & Ortega, 1990; Ortega, Collazos, Barriga & De la Fuente, 1992*a*, *b*; Ortega, Forner, Barriga & De la Fuente, 1993*b*) and neutrophils (Lewicki, Tchórzewski, Denys, Kowalska & Golinska, 1987; Rodríguez, Barriga & De la Fuente, 1991), as well as natural killer (NK) activity (Nieman, Miller, Henson, Warren & Gusewitch, 1993), are increased.

Macrophages are an appropriate cell subset in which to study the influence of physical exercise and stress on the status of the immune system. Indeed, Ricken & Kindermann (1986) proposed that this cell population, particularly its phagocytic activity, is the 'first hurdle' for infectious disease within the body.

Strenuous physical exercise increases the plasma concentration of many hormones, including corticosterone, adrenocorticotropic hormone (ACTH), β-endorphins, prolactin, adrenaline and noradrenaline. Such hormones have been shown to have immunomodulating effects (Khansari, Murgo & Faith, 1990; Simon, 1991).

Since possible hormonal mediators, which induce the stimulation of macrophage phagocytosis after exercise or in stress situations (Ortega *et al.* 1993*b*), are transported in

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the blood and thus can be measured in the plasma, in the present study, we initially evaluated the effect that plasma taken from BALB/c mice that had been stressed by physical activity has on the phagocytic function of peritoneal murine macrophages. Subsequently, we investigated the ability of physiological concentrations of corticosterone, the principal glucocorticoid released in the mouse in stress situations, to stimulate phagocytic activity.

METHODS

Animals

The studies were performed on male BALB/c mice (*Mus musculus*), aged 12 ± 4 weeks, maintained at a constant temperature (22 ± 2 °C) on a 12 h light–12 h dark cycle and given food and water *ad libitum*.

A classical model of stress induced by physical activity was employed: swimming until exhaustion (Sudakov, 1980; Sudo, 1985; Ferry *et al.* 1991; Ortega *et al.* 1992*a*, *b*). Animals were separated into three experimental groups as follows.

Acute physical activity (APA group). Mice were put into individual tanks (25 × 25 × (height) 15 cm) containing 7 l water (at 25 ± 2 °C) and required to maintain continuous swimming action until exhaustion (De la Fuente *et al.* 1990). Exhaustion was defined as that moment when the animal stopped making rapid spontaneous swimming movements and was immediately removed from the tank. The mean duration of exercise was 30 ± 5 min. This study was approved by the Ethical Committee of the University of Extremadura (Spain).

Acute physical activity after training (APAT group). Animals from this group were kept swimming under similar conditions to the APA group, for 25 min a day for 30 days. On the final day each mouse was subjected to the same acute physical exercise stress as the APA group.

Controls. Control animals were maintained under similar environmental conditions to the previous two groups but not subjected to exercise stress.

Immediately after finishing the physical exercise, animals were killed by cervical dislocation. Assays were then performed in parallel on samples taken from the three groups.

Plasma

Immediately after cervical dislocation the head was removed and blood collected from neck blood vessels and centrifuged at 300 *g* in order to obtain plasma. A pool of plasma samples from each experimental group (APA and APAT) and control mice was divided into aliquots and stored deep frozen (−20 °C) until required for assay.

Collection of peritoneal exudate cells (PECs)

The abdomen was cleansed with 70% ethanol, the abdominal skin carefully dissected without opening the peritoneum, and 4 ml of Hank's solution (Sigma Chemical Company), adjusted to pH 7·4, injected I.P. The abdomen was massaged and the PECs removed, with recovery of 90–95% of the injected volume of fluid. The cells (macrophages and lymphocytes) were counted and adjusted to a final of concentration of 5×10^5 macrophages per millilitre in Hank's medium. Cell viability was 98 ± 1% as measured by the Trypan Blue exclusion method (De la Fuente, 1985).

Effect of the plasma from exercised animals on the attachment and ingestion capacity of control peritoneal macrophages

Aliquots of 160 μl of a macrophage suspension (5×10^5 macrophages per millilitre of medium) together with either 40 μl of a *pool* of plasma from control mice (Control values), or 40 μl of plasma from exercised mice, in both the absence and the presence of the previous training programme, were incubated at 37 °C in a shaking water bath for 30 min. After incubation, an assay for attachment and ingestion of *Candida albicans* was carried out following a method previously described by Ortega *et al.* (1993*b*). Briefly, 200 μl aliquots of each sample (macrophages and plasma) were incubated at 37 °C on migration inhibitory factor (MIF) plates (Sterilin, Greiner Labor. technik, Teddington, Middlesex, UK) for 30 min and the adherent monolayers were washed with phosphate-buffered saline solution (PBS) at 37 °C. Following this, 200 μl of a suspension of *C. albicans*, adjusted to 5×10^6 cells ml^{-1} Hank's medium, was added to the MIF plates and incubated with the adherent monolayer of peritoneal macrophages for 60 min. After incubation, the plates were washed with PBS, fixed and stained, and the number of *C. albicans* cells attached (Attachment index) or ingested (Phagocytosis index) per 100 macrophages counted. Analysis was also performed of the percentage exhibiting phagocytosis and phagocytic efficiency (number of *C. albicans* cells phagocytosed by each active macrophage).

Corticosterone assay

Plasma corticosterone levels of control mice and mice stressed by physical exercise were measured using a radioimmunoassay kit (Cambridge Medical Technology Corporation, Billerica, MA, USA). All samples were assayed in a single assay to avoid interassay variation.

Table 1. Effect of incubation with plasma from exercised animals upon percentage exhibiting phagocytosis and phagocytic efficency for *Candida albicans* by peritoneal macrophages from BALB/c control mice

	Percentage exhibiting phagocytosis	Phagocytic efficiency
Control	20 ± 4	1·9 ± 0·4
PS	39 ± 6***	1·7 ± 0·2
PST	36 ± 8***	2·0 ± 0·5

Each value is the mean ± S.D. of 10 experiments performed in duplicate. *** $P < 0{\cdot}001$ with respect to control values. PS, macrophages incubated with plasma from acutely stressed mice. PST, macrophages incubated with plasma from acutely stressed mice after training.

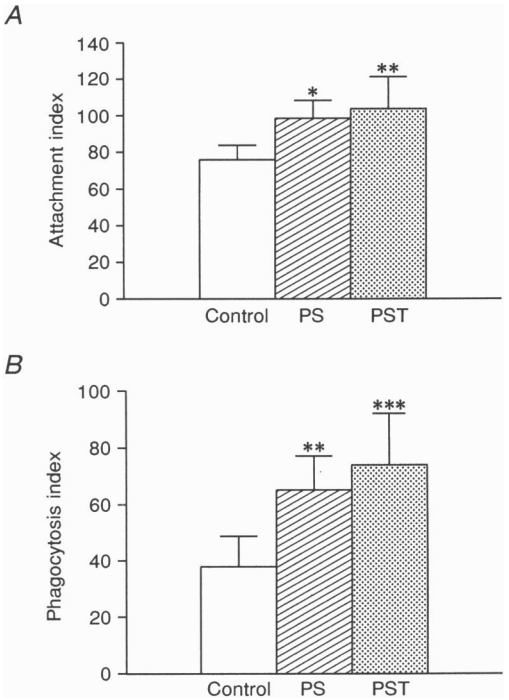

Figure 1. Effect of incubation with plasma from exercised animals on attachment (*A*) and ingestion (*B*) of *Candida albicans* by peritoneal macrophages from BALB/c control mice

Each value is the mean ± S.D. of 10 experiments performed in duplicate. * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$ compared with control values. PS, macrophages incubated with plasma from acutely stressed mice. PST, macrophages incubated with plasma from acutely stressed mice after training.

Effect of corticosterone on attachment and ingestion capacity of peritoneal macrophages

In order to study the effect of corticosterone on the attachment and ingestion capacity for *C. albicans* by peritoneal macrophages, aliquots of 200 μl of macrophage suspension (5×10^5 macrophages per millilitre of medium) were incubated with 40 μl of a *pool* of plasma from control mice and 20 μl of corticosterone (Sigma) at the following final concentrations: (i) 0·24 μmol l^{-1}, corresponding to the concentration observed in plasma from control animals (see Fig. 2); (ii) 0·72 μmol l^{-1}, corresponding to the concentration in the plasma of both APA and APAT groups (Fig. 2); (iii) 7·2 μmol l^{-1}, a concentration 10-fold greater than that observed in groups APA and APAT; or (iv) 20 μl of PBS alone. After 30 min of pre-incubation with shaking, the suspension was incubated at 37 °C on MIF plates, and the method described previously for the attachment and ingestion of *C. albicans* was followed.

Statistical analysis

Results were analysed by using the non-parametric ANOVA–Scheffe's *F* test; $P < 0{\cdot}05$ was taken as the minimum significance level.

RESULTS

The effects of incubation with plasma from exercised mice on the attachment and ingestion capacity for *C. albicans* by peritoneal macrophages are shown in Fig. 1*A* and *B* respectively. Peritoneal macrophages incubated with plasma from APA mice after acute physical activity (PS) showed greater attachment ($P < 0{\cdot}05$) and ingestion ($P < 0{\cdot}01$) indices than those which were incubated with plasma from control mice (see Fig. 1*A* and *B*, respectively). Similar effects were observed when macrophages were incubated with plasma taken from APAT mice that exercised acutely after training (PST) (Fig. 1*A* and *B*). No differences were observed between the APA and APAT groups.

Table 1 summarizes the values obtained for percentage exhibiting phagocytosis and phagocytic efficency. With respect to percentage exhibiting phagocytosis, both the plasma taken from APA and that taken from APAT had greater effects ($P < 0{\cdot}001$) than that taken from control

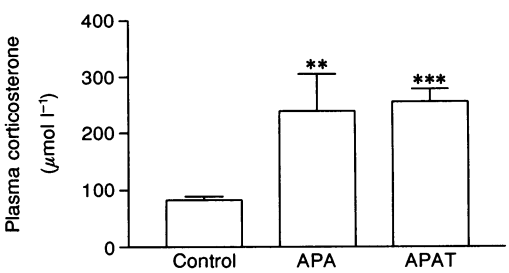

Figure 2. The effects of acute physical exercise on plasma corticosterone levels without and with previous training

Each value is the mean ± S.D. of 10 determinations performed in duplicate. ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$ with respect to control values. APA, acute physical activity. APAT, acute physical activity after training.

Table 2. Effect of incubation with corticosterone (μmol l^{-1}) upon percentage exhibiting phagocytosis and phagocytic efficiency for *Candida albicans* by peritoneal macrophages from BALB/c mice

	Percentage exhibiting phagocytosis	Phagocytic efficiency
Control	40 ± 6	2·3 ± 0·5
0·24 μmol l^{-1}	46 ± 7	2·0 ± 0·5
0·72 μmol l^{-1}	47 ± 9	3·1 ± 0·4
7·2 μmol l^{-1}	45 ± 11	2·4 ± 0·6

Each value is the mean ± S.D. of 10 experiments performed in duplicate.

mice. No statistical differences were found between the groups for phagocytic efficiency.

Plasma corticosterone concentrations (μmol l^{-1}) are shown in Fig. 2. After physical activity there was an increase in corticosterone concentration both in APA ($P < 0{\cdot}01$) and in APAT ($P < 0{\cdot}001$) mice compared with values recorded in control animals. However, no difference was found between APA and APAT groups. After incubation of macrophages with a concentration of corticosterone similar to that observed in plasma after exercise, i.e. 0·72 μmol l^{-1}, in both APA and APAT groups there was an increase in the ingestion index but not in the attachment index (Fig. 3) compared with values recorded for macrophages incubated with PBS and for macrophages incubated with the basal concentration of corticosterone in the plasma of control animals (0·24 μmol l^{-1}). The percentages exhibiting phagocytosis and phagocytic efficiency are listed in Table 2. After incubation of macrophages with corticosterone at 0·72 μmol l^{-1}, there tended to be an increase both in the percentage exhibiting phagocytosis and in the phagocytic efficiency, but this did not reach statistical significance.

After incubation with 7·2 μmol l^{-1} corticosterone, macrophages demonstrated a similar phagocytic index to macrophages incubated with 0·24 μmol l^{-1} corticosterone or PBS, but a lower ($P < 0{\cdot}01$) phagocytic index than cells incubated with 0·72 μmol l^{-1} (Fig. 3*B*). However, after incubation with 7·2 μmol l^{-1} corticosterone, the attachment index was only significantly greater than that for macrophages incubated with PBS ($P < 0{\cdot}05$; Fig. 3*A*).

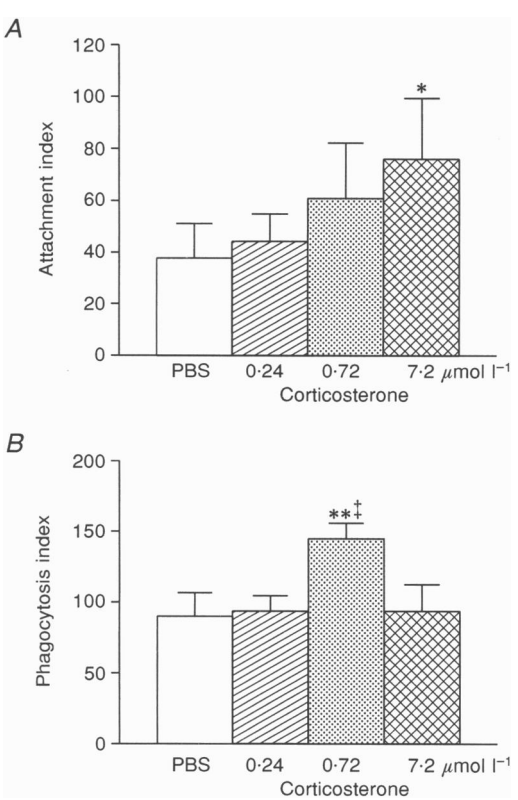

Figure 3. Effect of incubation with corticosterone on attachment (*A*) and ingestion (*B*) of *Candida albicans* by peritoneal macrophages from BALB/c mice

Each value is the mean ± S.D. of 10 experiments performed in duplicate. * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$ with respect to control values (without corticosterone). ‡ $P < 0{\cdot}01$ with respect to corticosterone levels from control mice group (0·24 μmol l^{-1}) and 7·2 μmol l^{-1} values.

DISCUSSION

Recently we have reported that macrophage phagocytic function increases after strenuous physical activity (De la Fuente *et al.* 1990; Ortega *et al.* 1992*a*,*b*; De la Fuente, Martín & Ortega, 1993; Ortega *et al.* 1993*b*). Similar results have been obtained by other groups (Fehr *et al.* 1988, 1989; Jäpel, Lötzerich & Appell, 1992). It has been suggested that the effects of exercise and stress cannot be distinguished (Caren, 1991; Hoffman-Goetz & Pedersen, 1994) and that increases in the plasma concentrations of hormones such as glucocorticoids are responsible for the modifications observed in the immune system following exercise (Khansari *et al.* 1990; Hoffman-Goetz & Pedersen, 1994). If this is so then the stimulation of macrophage phagocytic function by exercise may be mediated by those 'stress hormones'. In agreement with this idea the present results demonstrate that incubation with plasma taken from exercised mice stimulates the attachment and ingestion indices of peritoneal macrophages. Moreover, no differences were found between the effect of plasma from acutely exercised mice (APA group) and from mice that had exercised acutely after a 1 month training programme (APAT group). This implies that phagocytic function increases after acute physical activity, and the effect is independent of whether or not the animals are partially adapted to the exercise. A similar finding has been made by others for neutrophils from humans (Fehr *et al.* 1989; Ortega, Barriga & De la Fuente, 1993*a*). The stimulation of peritoneal macrophage phagocytosis which we observed after incubation with plasma from both APA and APAT groups was due to an increase in the percentage of cells exhibiting phagocytosis (macrophages that phagocytosed at least one *C. albicans* cell) and not to any difference in phagocytic efficiency (number of cells phagocytosed by each active macrophage).

Corticosterone, the principal glucocorticoid released in the mouse in situations of stress such as strenuous exercise (Khansari *et al.* 1990; Ferry *et al.* 1991; Simon, 1991), increased to a similar extent in the plasma of both the APA and APAT groups (to about 0·72 μmol l^{-1}). A similar finding was made in trained animals (Bernton, Bryand & Holaday, 1991). Accordingly, this physiological concentration was subsequently used to evaluate its *in vitro* effect on the attachment–ingestion capacity of macrophages. Classically, both stress and glucocorticoids have been implicated as immunosupressors. Nevertheless, studies of the effects of glucocorticoids upon the immune system have usually been carried out using pharmacological and not physiological concentrations. In fact, the stimulation of macrophage phagocytosis induced by the physiological concentrations of corticosterone reported here (0·72 μmol l^{-1}; similar to that found in the blood after exercise) is in agreement with a recent hypothesis that low levels of glucocorticoids may stimulate immunity rather than suppress it (Sharp & Parry-Billings, 1992; Ortega, 1994). Other results also support this hypothesis. For example, prolonged incubation (3 days) of monocytes with glucocorticoids has been observed to induce an enhancement of their phagocytic capacity (Kurlander, 1981).

Following the observation that the concentration of corticosteroids found in the plasma after swimming until exhaustion stimulated the phagocytic capacity of macrophages, the effect of a 10-fold concentration of this hormone (7·2 μmol l^{-1}) was studied. Results showed that the phagocytosis index was lower than that obtained with 0·72 μmol l^{-1}, and similar to that obtained after incubation with 0·24 μmol l^{-1} or PBS. This finding may indicate that there is an 'optimum' physiological range at which glucocorticoids can induce the stimulation of phagocytic cells.

In summary, it is concluded that corticosterone is at least partly responsible for the increased phagocytic function of peritoneal macrophages induced by acute physical activity.

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