Journal of Physiology (1990), **430**, *pp.* 595–603
With 4 figures
Printed in Great Britain

EFFECTS OF PHYSICAL TRAINING ON FEBRILE AND ACUTE-PHASE RESPONSES INDUCED IN RATS BY BACTERIAL ENDOTOXIN OR INTERLEUKIN-1

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(*Received 3 April 1990*)

SUMMARY

1. We investigated the effect of physical training on febrile and acute-phase responses induced in rats by intravenous (I.V.) injection of bacterial endotoxin or human recombinant interleukin-1β (IL-1). Physical training was performed by swimming for 1 h per day, 5 days a week. After four weeks of training, animals were used in the experiments.

2. The I.V. injection of endotoxin produced a febrile response in the trained group but not in the control group. However, there were no statistically significant differences between febrile responses induced by the I.V. injection of IL-1 in the control and trained groups.

3. The I.V. injection of endotoxin significantly decreased the plasma concentration of iron and zinc and increased the plasma fibrinogen concentration in both the control and the trained groups. However, the decreases in the plasma iron and zinc concentrations in the trained group were significantly greater than those in the control group. The I.V. injection of endotoxin increased the circulating leucocyte count in the only trained group.

4. The I.V. injection of IL-1 significantly decreased the plasma concentration of iron and zinc and increased the plasma fibrinogen concentration and the circulating leucocyte count in both the control and the trained groups. However, between the two groups, no significant differences in the values of acute-phase reactants were observed.

5. The present results suggest that the ability to produce cytokine(s) to induce febrile and acute-phase responses is enhanced by physical training. However, physical training has no effect on the febrile and acute-phase responses induced by IL-1.

INTRODUCTION

Under infectious or inflammatory conditions, a host reacts with several responses, which include the elevation of body temperature (fever) (Atkins, 1960), stimulation of the cardiovascular system (Skarnes, Brown, Hull & McCraken, 1981) and the

release of several kinds of hormones (Moberg, 1971; Kasting & Martin, 1982; Besedovsky, del Rey, Sorkin & Dinarello, 1986). Moreover, during such conditions, changes in the plasma level of certain trace metals (e.g. reduction of iron and zinc concentration and increase of copper concentration), activation of hepatic protein synthesis (e.g. fibrinogen, ceruloplasmin, C-reactive protein and many others), and an increase in circulating leucocyte count are induced (Kampschmidt, Upchurch, Eddington & Pulliam, 1973), which are termed collectively the acute-phase response (Gordon & Koj, 1985). These are now generally recognized to constitute a primary host defence reaction to microbial invasion (Kluger, Ringler & Anver, 1975; Kluger & Vaughn, 1978; Kluger & Rothenberg, 1979). Furthermore, increasing evidence has revealed that these responses are mediated by endogenous pyrogen (EP) (Kampschmidt *et al.* 1973; Kampschmidt, 1980), which is released by circulating and reticuloendothelial leucocytes in response to a variety of pathogenic stimuli such as bacterial endotoxin.

It is well known that physical training induces organic and functional changes in the body. It is also generally believed that appropriate physical training brings about beneficial changes in the cardiovascular system and hormonal responses. However, little is known as to whether physical training enhances host defence responses, including immune responses to infection or inflammation. Cannon & Kluger (1984) reported that physical training enhances survival rates in mice which have been injected with *Salmonella typhimurium*. Their results lead us to speculate that physical training affects the host defence response to infection.

In the present study, we investigated the effects of physical training on febrile and acute-phase responses induced by bacterial endotoxin or by interleukin-1, which is currently thought to be an active component of the EP (Dinarello, 1984). However, it has recently been shown that, like interleukin-1, the injections of other cytokines such as interferon (Dinarello, Bernheim, Duff, Le, Nagabhushan, Hamilton & Coceani, 1984; Morimoto, Murakami, Takada, Teshirogi & Watanabe, 1987*a*), tumor necrosis factor (Dinarello, Cannon, Wolff, Bernheim, Beutler, Cerami, Figari, Palladino & O'Connor, 1986; Morimoto, Sakata, Watanabe & Murakami, 1989) and interleukin-6 (Helle, Brakenhoff, De Groot & Aarden, 1988), induce fever and some acute-phase responses. These cytokines are also released by leucocytes when stimulated by bacterial endotoxin. Moreover, due to the close interaction of immunoregulatory networks mediated by several cytokines, it is possible that these cytokines contribute mutually to the induction of fever and the acute-phase response. Therefore, we must take into account the possibility that effects of several kinds of cytokines on the febrile and acute-phase responses are modulated by physical training. The main purpose of the present study is to examine whether or not physical training enhances the host defence responses induced by bacterial endotoxin or interleukin-1.

METHODS

Thirty-four male albino rats (Wistar strain) weighing 240–270 g were used in this study. Seventeen rats were subjected to physical training (trained group). The other rats ($n = 17$) were used for the control (control group). The present study was considered and permitted to proceed by the Animal Care Committee of Yamaguchi University, School of Medicine, and the chairman

and members of the Committee inspected the training procedure. In the trained group, the exercise performed was swimming for 1 h per day, 5 days a week. Training was carried out for 4 weeks. During exercise, animals wore a weight of 5 g attached to their necks and swam in a small round pool (50 cm diameter). In comparison with previous studies (Dawson & Horvath, 1970), the weight of

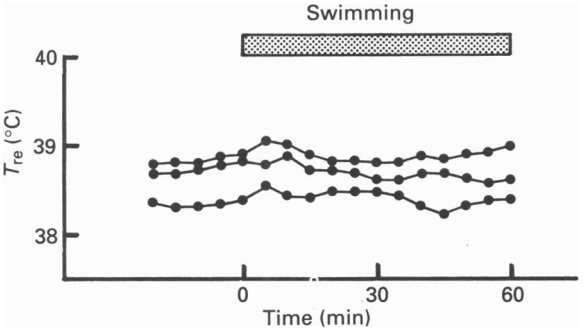

Fig. 1. Changes in rectal temperature (T_{re}) in three individual rats during swimming.

5 g (about 2 % of the body weight) was not considered to be heavy. During the exercise, the trainer carefully watched the rat's condition, and when the rats showed symptoms of fatigue, the exercise was stopped. The water of the pool was circulated and its temperature was maintained at 36 °C. In 36 °C water, as previously reported (Ostman, Sjostrand & Swedin, 1972), animals did not show marked changes in rectal temperature during swimming. Changes in the rectal temperature during a period of swimming in three cases are shown in Fig. 1. Furthermore, it is reported (Dawson & Horvath, 1970) that rats can swim for about 60 h in 36 °C water, indicating that 1 h of swimming is not highly stressful and the water temperature of 36 °C is suitable for rats. To control for the psychological effect of immersion in water, the control animals swam without the weight for 3 min per day, 5 days a week over the course of 4 weeks. Immediately after swimming, rats were rubbed with a towel then dried by an electric hairdryer. The body weight of the animals in both the control and the trained groups was measured every day. During training, there were no significant differences in changes in the body weight between two groups.

Within one week of the end of the training period, all animals were used in the experiments. For blood sampling, all rats were catheterized intravenously with a cannula 3 days before experimentation began. Polyvinyl tubing was inserted into a superior caval vein (SCV) under general anaesthesia (sodium pentobarbitone 40 mg/kg, I.P.) by the method of transjugular cannulation previously described (Harms & Ojeda, 1974).

The bacterial endotoxin used in this study was lipopolysaccharide of *Salmonella typhosa* endotoxin (Difco), which was dissolved in sterile saline at a concentration of 10 μg/ml. Human recombinant interleukin-1β (IL-1), which had been produced by recombinant strains of *Escherichia coli*, was supplied by the Otsuka Pharmaceutical Co. Ltd. The IL-1 was carefully produced and endotoxin-free as confirmed by a *Limulus* amoebocyte lysate test ($< 0{\cdot}05$ pg/μg protein). The biological activity of IL-1 as assayed by thymocyte co-stimulation activity was 2×10^7 U/mg protein. For injection, the recombinant IL-1 was dissolved in sterile saline at a concentration of 2 μg/ml. These solutions were divided into several vials and stored at -40 °C until use. We used vials within 2 days after thawing, and thus avoided repeated freezing and thawing.

Rats were acclimated to a 12 h light–dark cycle (light on at 7.00 h and off at 19.00 h) in a room temperature of 26 ± 1 °C. On the day of the experiments, rats were minimally restrained in conventional wooden stocks in which they could freely move their extremities, except their tails which were fixed by adhesive plaster (1 cm in width) to the rods horizontally attached to the board. Throughout the experiment, rectal temperature was measured every minute with a copper–constantan thermocouple. The rectal temperature of each rat was allowed to stabilize for a period of 1 h before injections. Injections of endotoxin or IL-1 were given at 11.00 h through the cannulae inserted into the SCV. To minimize the effect of restraint-induced stress on acute-phase response (Morimoto, Watanabe, Myogin & Murakami, 1987*b*), animals were released 2 h after injection. To measure the blood cell counts and the plasma concentration of iron and zinc, about

0·6 ml of blood was withdrawn through the cannulae inserted into the SCV. The blood samples were taken three times: 1 h before and 8 and 24 h after injection of bacterial endotoxin or IL-1. To measure the plasma concentration of fibrinogen, about 0·4 ml of blood was taken 1 h before and 24 h after the injection. Both white and red blood cell counts were made with an automatic cell

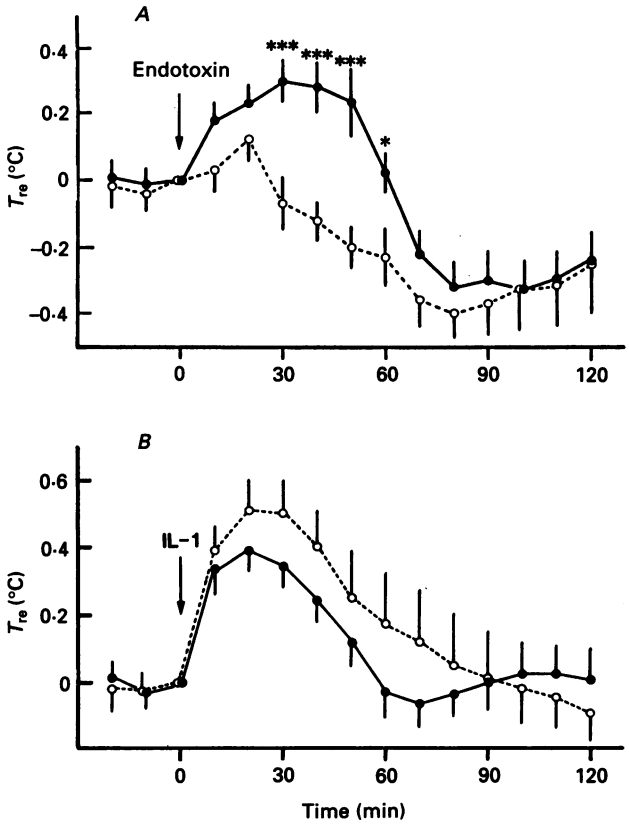

Fig. 2. *A*, mean changes (mean ± S.E.M.) in rectal temperature (T_{re}) in ten rats of the control (○) and the trained (●) groups after intravenous injection of bacterial endotoxin (10 μg/kg). $*P < 0{\cdot}05$, $***P < 0{\cdot}001$. *B*, mean changes (mean ± S.E.M.) in rectal temperature in seven rats of the control (○) and the trained (●) groups after intravenous injection of interleukin-1β (IL-1, 2 μg/kg).

counter (Coulter, Model S plus II). The remaining blood was centrifuged at 2000 r.p.m. for 15 min at 4 °C, and the plasma was collected in test tubes and stored at −40 °C until the measurement was made of iron, zinc and fibrinogen concentrations. The methods for measuring the concentrations of iron, zinc and fibrinogen have been previously described in detail (Morimoto, Murakami, Myogin, Takada, Teshirogi & Watanabe, 1987*c*). The data were analysed for statistical significance by Student's *t* test or by ANOVA.

RESULTS

The effect of the I.V. injection of endotoxin or IL-1 on rectal temperature is shown in Fig. 2. Changes in the rectal temperature are expressed as the deviation from the baseline recorded at the time of the injection. In Fig. 2*A*, the I.V. injection of endotoxin (10 μg/kg) produced a febrile response in the trained group but not in the

control group. However, as shown in Fig. 2*B*, the I.V. injection of IL-1 (2 μg/kg) produced fever in both the control and the trained groups. The mean rise in rectal temperature in the control group was greater than that in the trained group, however, there was no statistically significant difference in the magnitude of the febrile responses induced by IL-1. between the control and the trained groups.

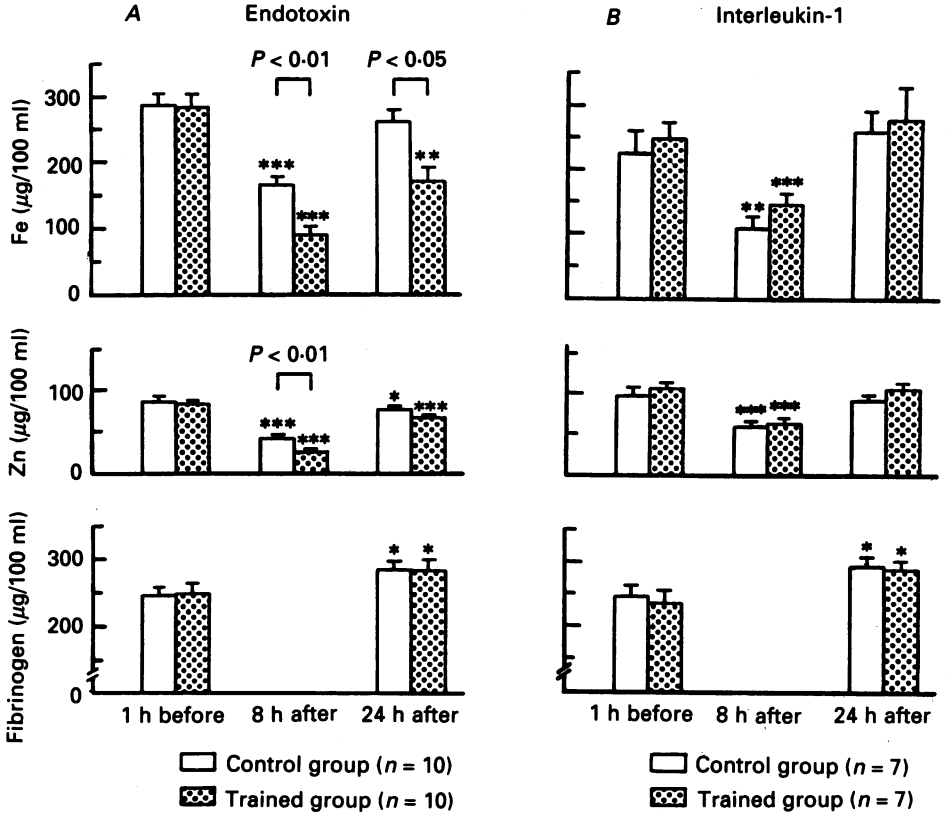

Fig. 3. Mean changes (mean ± S.E.M.) in the plasma concentration of iron, zinc and fibrinogen 1 h before and 8 and 24 h after intravenous injection of bacterial endotoxin (*A*) or interleukin-1 (*B*). $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$.

Figure 3 summarizes the changes in the plasma level of iron and zinc 1 h before and 8 and 24 h after I.V. injections of endotoxin (10 μg/kg; Fig. 3*A*) or IL-1 (2 μg/kg; Fig. 3*B*). Also changes in the plasma concentration of fibrinogen 1 h before and 24 h after injection are shown in Fig. 3. In Fig. 3, the changes in each parameter represent the mean ± S.E.M. The values at 1 h before injection were statistically compared with those at each respective time after injection. In addition, the values of each parameter were statistically compared between two groups. In both the control and the trained groups, the I.V. injection of endotoxin significantly decreased the plasma concentration of iron and zinc 8 and 24 h after injection, and increased the plasma fibrinogen concentration 24 h after injection. The decreases in the plasma iron and zinc concentrations in the trained group were significantly greater than those in the

control group. The plasma concentration of iron and zinc in both the control and the trained groups decreased 8 h after injection of IL-1. The plasma concentration of fibrinogen significantly increased 24 h after the injection of IL-1. However, there was no significant difference between the control and the trained groups in the values of each parameter before and after the injection of IL-1.

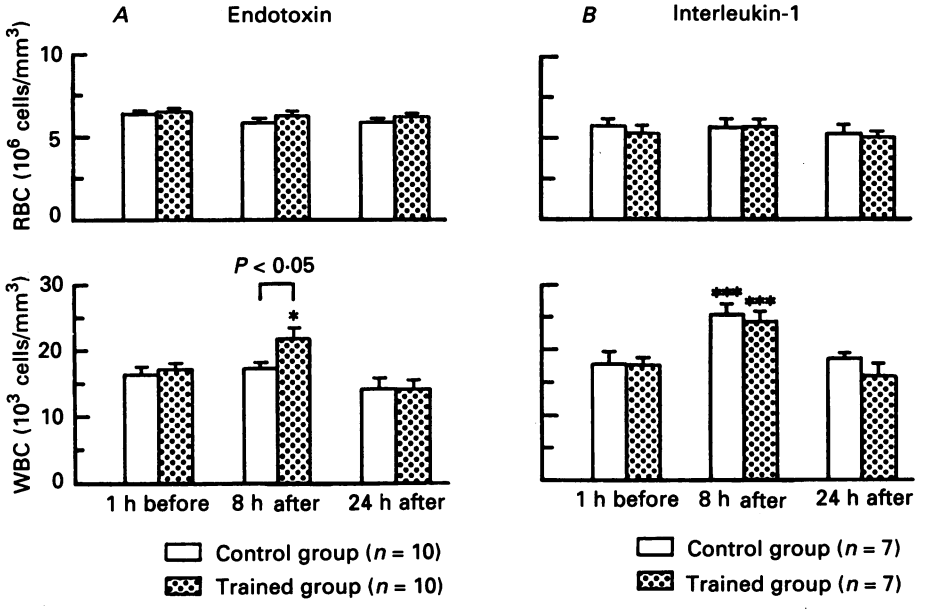

Fig. 4. Mean changes (mean ± S.E.M.) in the red blood cell count (RBC) and the white blood cell count (WBC) 1 h before and 8 and 24 h after intravenous injection of bacterial endotoxin (*A*) or interleukin-1 (*B*). $*P < 0.05$, $***P < 0.001$.

In Fig. 4, changes in the erythrocyte and the leucocyte counts in the blood after the I.V. injection of endotoxin (Fig. 4*A*) or IL-1 (Fig. 4*B*) are shown. Neither the injection of endotoxin not that of IL-1 affected the number of erythrocytes in either the control or the trained groups. The I.V. injection of endotoxin increased the number of circulating leucocytes 8 h after injection. However, in the control group, no significant increase in the leucocyte count was observed. The I.V. injection of IL-1 increased the number of circulating leucocytes, although no significant differences between two groups were observed.

DISCUSSION

It has been frequently reported (Kjellberg, Rudhe & Sjostrand, 1949; Stromme & Hammel, 1967; Chin, Seaman & Kapileshwarker, 1973) that the calorigenic response to cold exposure is enhanced by physical training. Therefore, before the start of the present study, we speculated that the febrile response would also be enhanced after physical training by an increased capacity for heat production. However, the present results showed that for both groups, the pattern and magnitude of the febrile

responses induced by I.V. injection of IL-1 was almost the same. It is generally believed that fever is caused by the action of EP/IL-1 on the central nervous system (CNS) (Atkins, 1960; von Siegert, 1981; Morimoto, Murakami, Nakamori & Watanabe, 1987*d*). Therefore, our results may indicate that the febrile response induced by IL-1 is controlled by the CNS, although the capacity for heat production might be enhanced by physical training.

We did find that the I.V. injection of endotoxin caused fever in the trained group but not in the untrained group. This finding suggests that the capacity of rats to produce pyrogenic cytokines, such as IL-1 or tumor necrosis factor, is enhanced after physical training. Depending on the experimental conditions, the injection of endotoxin can cause fever (Morimoto, Murakami, Ono & Watanabe, 1986*a*) or hypothermia (Feldberg & Saxena, 1975) in rats. Under the conditions of this experiment, we consistently observed a drop in body temperature in the control group after the endotoxin was administered. The mechanism of this hypothermia is not known, but is presumably the result of an antipyretic agent. One possible explanation for our findings is that both groups of animals produce equal amounts of pyrogenic cytokines, but untrained rats produce a larger amount of the antipyretic than do trained rats, and that this substance counteracts the pyrogenic action of these cytokines. We believe that this is unlikely, however, since the other aspects of the acute-phase response that are enhanced in trained rats (decreases in iron and zinc, leucocytosis) so strongly resemble the effect of the injection of IL-1. Therefore, it is likely that the untrained rats do not release enough endogenous pyrogens (pyrogenic cytokines) to cause fever in response to endotoxin (Morimoto, Watanabe, Ono, Sakata & Murakami, 1986*b*) but release small amounts of cytokines which are sufficient to cause some changes in acute-phase reactants. The possibility that exercise-induced changes in body temperature were responsible for the observed changes in the febrile and acute-phase responses is ruled out by the fact that body temperature of the rats did not change during swimming. Hence, it is not known whether training by other kinds of exercises which cause changes in the body temperature alters these parameters.

The I.V. injection of IL-1 significantly decreased the plasma concentration of zinc and iron, and increased the circulating leucocyte count. Moreover, after I.V. injection of IL-1, the plasma level of fibrinogen significantly increased. Since the measurement of fibrinogen requires a relatively large blood sample (0·4 ml), we measured this protein only twice at 1 h before and 24 h after injection. Previous studies in rabbits have shown a change in fibrinogen concentration 24 h after the injection of EP (Morimoto *et al.* 1987*c*).

Cannon & Kluger (1983) suggested that endogenous pyrogen-like activity increases during exercise, and that subsequently acute-phase responses are induced. If adaptations in the acute-phase responses are induced by physical training, acute-phase responses induced by IL-1 may be reduced in the trained group. However, these data show that there are no significant differences in the changes in the acute-phase reactants after I.V. injection of IL-1 between the trained and the control groups. In other words, the capacity for acute-phase responses is well maintained in the trained animals. This point is significant, because if the ability to produce cytokines that induce febrile and acute-phase responses is enhanced after physical

training, then the trained group can react with greater acute-phase responses. The present results clearly support this possibility. After I.V. injection of endotoxin, the plasma concentrations of iron and zinc in the trained group decreased significantly more than those in the control group. Moreover, the number of circulating leucocytes increased in the trained group. It is generally believed that bacterial endotoxin causes febrile and acute-phase responses by inducing circulating and reticulo-endothelial leucocytes to synthesize and release cytokines. Therefore, it is likely that physical training enhances the ability of leucocytes to produce cytokines in response to endotoxin.

As for the mechanisms of acute-phase response induction, it has been recently shown that EP/IL-1 induces the acute-phase response direction through its action on several peripheral organs (muscle, bone marrow, liver and many others) as well as indirectly via neural output originating from its action on the CNS (Morimoto *et al.* 1987*c*; Morimoto *et al.* 1989). Since both groups of rats showed similar responses to IL-1, we believe that physical training by swimming had no effect on the central and peripheral mechanisms involved in acute-phase response production. It has recently been shown that IL-1 as well as other cytokines are released by various classes of leucocytes. Some of these cytokines, like IL-1, also have the ability to induce fever and some acute-phase responses (Morimoto *et al.* 1987*a*; Morimoto *et al.* 1989). Therefore, our results suggest that physical training may enhance the capacity of leucocytes to produce such cytokines in response to pathogenic stimuli. Furthermore, these observations suggest that physical training enhances the capacity to survive microbial invasion.

We are grateful to the Otsuka Pharmaceutical Company for supplying us with human recombinant interleukin-1β. This work was partly supported by Meiji Welfare Foundation grant in Japan. We are grateful to Dr N. C. Long for her critical reading of the manuscript.

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