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A MODERATE DOSE OF CYCLOHEXIMIDE DOES NOT PREVENT THE FEBRILE RESPONSE TO ENDOTOXIN BUT INTERFERE WITH INDUCTION OF PYROGENIC TOLERANCE IN RABBIT.

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The purpose of the present study was to examine the effect of cycloheximide (Cx) — inhibitor of protein synthesis, on the development of pyrogenic tolerance to LPS. It has been observed that Cx at a dose of 1 mg/kg given intravenously 1 h prior to LPS did not prevent fever response, however it modified the induction of pyrogenic tolerance. It was manifested in existence of the second phase of fever after the following administrations of LPS into rabbits pretreated with Cx. In control group of rabbits the induction of pyrogenic tolerance was accompanied with decaying of the second peak of fever visible as early as the second dose of LPS.

Key words: *pyrogenic tolerance; cycloheximide; rectal temperature; rabbit.*

INTRODUCTION

Fever is a regulated increase in body temperature that occurs in response to endotoxins — exogenous pyrogens of bacterial, viral or fungal and other origin (1). These pyrogens activate the production and release of endogenous pyrogen(s) (EP) (2) identified as interleukin 1 (Il-1), interferon, interleukin 6, tumor necrosis factor (3) which in turn raise the thermostatic set-point.

Rabbits treated with endotoxin repeatedly at daily intervals displayed the pyrogenic tolerance to this agent, a process thought to be related in part to diminution of the Il-1 synthesis by host's cells following successive injections of an exogenous pyrogen (4, 5).

Some earlier studies suggested that "*in vitro*" formation and release of EP from blood leucocytes was susceptible to blockade by cycloheximide (Cx) — a protein synthesis inhibitor (6). It has been reported that Cx given at a dose of 5 mg/kg of body weight prevented pyrogen-induced fever in the rabbit (7, 8, 9, 10, 11).

Recent observations, on the other hand, indicate that "*in vitro*" Il-1 production can be enhanced and prolonged in murine macrophages (12) and human monocytes (13) by exposure to a low dose of Cx (of the order of nanogrammes per ml) prior to inclusion of endotoxin into the culture.

The "*in vitro*" effect of Cx on Il-1/EP production suggests that a certain dose of Cx administered systemically before endotoxin may not prevent the febrile response and may desturb the development of pyrogenic tolerance to endotoxin. In this paper we report that a moderate and entirely unlethal single dose of 1 mg/kg Cx given intravenously 1 h prior to endotoxin (LPS, lipopolysaccharide from *Salmonella abortus equi*) did not act as an antypiretic agent, however it prevented a rapid induction of tolerance to the subsequent daily injections of LPS.

MATERIALS AND METHODS

Male New Zealand white rabbits weighing 3—4 kg were used throughout the study. The animals were housed in individual cages at 20°C, with free access to food and water. All experiments were performed at the same time of day, and at ambient temperature of 20 ± 1°C.

In the experiments, a thermistor probe was inserted 10 cm into the rectum, and temperature was recorded using a digital chart recorder. Base-line temperature measurements were recorded for at least 0.5 h prior to injection. All drugs were administered via the marginal ear vein (i.v. injections). A new pool of rabbits which had not been previously injected with any agents was used for experiments. Each rabbit acted as its own control and was pretreated with saline then injected with LPS for four consecutive days (at a dose of 0.3 μg/kg LPS each day) to assess the development of tolerance to this pyrogen. Six weeks later the rabbits were pretreated with cycloheximide (at a dose of 1.0 mg/kg) for 1 h before being injected with pyrogen (LPS, 0.3 μg/kg). Cx was given at the first day of tolerance induction, and in the following days rabbits were treated with LPS only, at a dose as previously.

Lipopolysaccharide *(Salmonella abortus equi)* and cycloheximide were obtained from Sigma Chemical Co., Dorset, UK. These were dissolved in saline (sterile non-pyrogenic 0.9% sodium chloride, Polfa, Poland) immediately before use.

The magnitude of febrile responses were expressed as the change in rectal temperature from a base-line (ΔT°C) and as the fever index, defined as an area under the curve of ΔT for 6 hrs. Data were analysed by the paired Student's t-test and the difference considered to be significant when the probability $p < 0.05$.

RESULTS

In preliminary experiments (data not shown) we observed that i.v. pretreatment with Cx at a dose of 5 mg/kg for 1 h before the LPS administration did indeed abolish the febrile response in rabbits. A base-line of rabbit colonic temperature dropped by 0.8°C soon after the Cx administration, accompanied with tachypnea and enormous ear vasodilation. This dose of Cx,

however, appeared to be lethal to 6 of 8 treated rabbits within 24—48 h of administration. Decreasing the dose of Cx to 1 mg/kg resulted in survival of all treated rabbits within an experimental and longer period, although it had still been proved to cause a drop in normal colonic temperature, approximately by 0.5°C, and this has been considered as a base-line for ΔT changes assessment following LPS injection.

Cx at a dose of 1 mg/kg administered i.v. did not prevent the febrile response to LPS given i.v. 1 h afterwards. It been shown to modulate, however, the time-course of the post-endotoxin changes of rectal temperature during the first-day injection of LPS and, furthermore, this single dose of Cx transiently eliminated the development of pyrogenic tolerance to LPS during the following days *(Fig. 1)*.

Both in saline (control) and in cycloheximide (experimental) pretreated rabbits the LPS (0.3 μg/kg) produced a biphasic fever with the increase in body temperature beginning with 20 min of injection. Cx pretreatment, however, delayed the peak temperature of respective phases, and the first peak was significantly lower ($p < 0.05$) than that of observed for saline pretreatment *(Fig. 1/I)*. Nevertheless, this modulatory effect of Cx did not result in significant changes in the magnitude of febrile responses, i. e., in fever indexes calculated for 6 hrs of fever duration *(Fig. 2/I)*. As shown in *Fig. 2,* a mean fever index of the first-day response to LPS was even higher after the Cx pretreatment than that of saline, although the difference was statistically insignificant.

If the dose of 0.3 μg LPS per b. w. was i. v. administered repeatedly at 1-day intervals into the saline pretreated rabbit, the febrile response was attenuated *(Fig. 2)*. Already after the 2 nd injection the second phase of fever disappeared and the time-course of rectal temperature elevation coincided with the first phase of fever *(Fig. 1)*.

Cycloheximide (1 mg/kg) pretreatment for 1 h at the first day of LPS administration, significantly modified the response to endotoxin given repeatedly in the following days. The second phase of fever still persisted after the 2 nd and the 3 rd dose of LPS injected into the Cx-treated rabbits, and was on the decline after the 4 th injection *(Fig. 1)*. The fever indexes of the 2 nd and 3 rd day-response of Cx-treated rabbits approximated to that of the 1 st day, and were significantly higher ($p < 0.01$) than fever indexes assessed for respective days of saline pretreated rabbits *(Fig. 2)*.

DISCUSSION

Our study reports that cycloheximide at a dose of 1 mg/kg, administered 1 h prior to LPS is not antipyretic in the rabbit, in spite of the drop of normal rectal temperature ascertained soon after the administration of Cx. However in

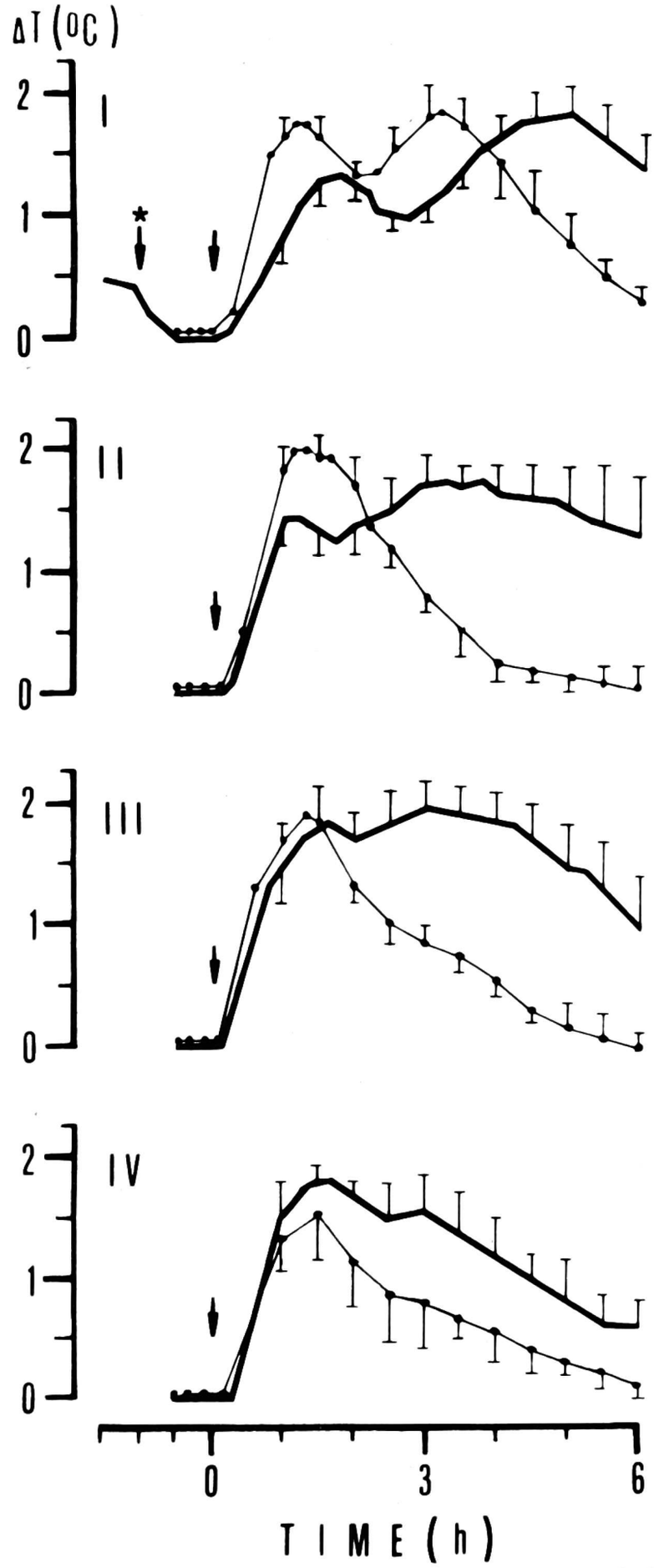

Fig. 1. Changes of the rectal temperature (ΔT, mean $\pm$ SD, n = 6) during induction of tolerance to LPS i. v. (S. abortus eq., 0.3 μg/kg) in rabbits pretreated with cycloheximide i. v. (1 mg/kg) (heavy line) or its vehicle (fine line) on the first day of experiment. Respective days of experiment are as follows: first (I); second (II; third (III); fourth (IV). Pyrogen was injected in time "0" and cycloheximide 1 h prior to pyrogen. The first peak of temperature curve on the 1 st and 2 nd day of experiment is significantly different ($p < 0.05$) in Cx pretreated from vehicle injected animals.

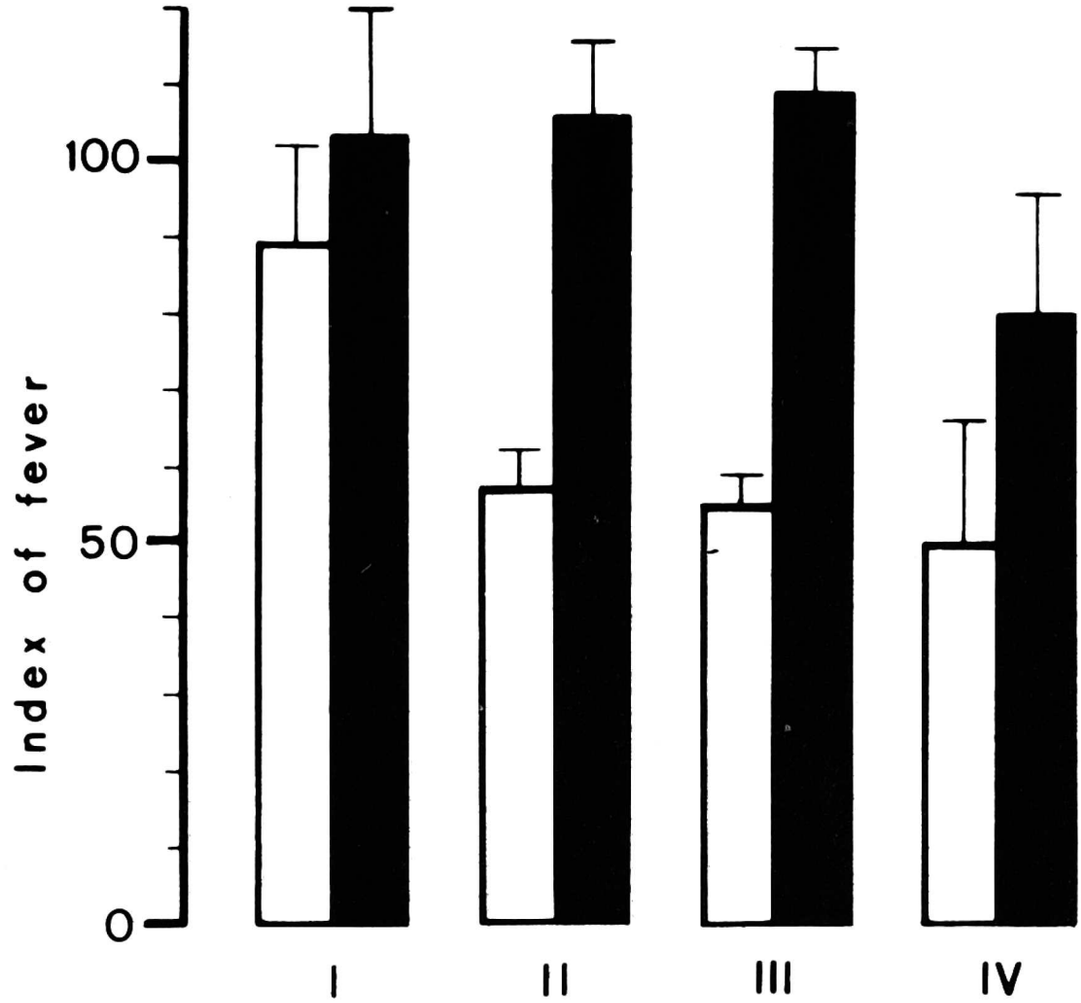

Fig. 2. Changes of the fever indexes (mean ± SD, n = 6) during induction of tolerance to LPS i. v. (S. abortus eq., 0.3 μg/kg in rabbits pretreated with cycloheximide i. v. (1 mg/kg) (closed bars) or its vehicle (open bars). For symbols description see fig. 1. The fever indexes on the 2nd and 3rd day-response of Cx pretreated rabbits are significantly different ($p < 0.01$) from vehicle injected animals.

conflict with studies by Siegert et al. (7, 8) our results are similar to those published by Stitt (14). He has found that although Cx at a dose 5 mg/kg impaired cold-induced thermogenesis in rabbits but like in our study it failed to prevent the febrile response to intravenous E. coli endotoxin. Therefore he concludes that fever production pathway per se is unaffected by Cx treatment (14).

The observations reported by Stitt (14) are consistent with those of Barney et al. (15) that cycloheximide has a general depressant effect on temperature regulation in the rat. Alternatively, and perhaps more likely, Cx may have a general debilitating effect on the organism as a whole, interfering with the ability to regulate body temperature. A well known adverse effects of Cx including lethality observed by us after 5 mg/kg dose of this protein synthesis inhibitor are, for the most part, dose dependent (16, 17). However, a dose dependent effects of Cx on temperature regulation and fever response have not been studied so far.

The most striking observation in our studies, however, is the effect of cycloheximide on tolerance induction to LPS in rabbits. Presented results confirmed the earlier findings that repeated daily injections of LPS produces a refractory state to this pyrogen, the process characterized by loss of the second peak of fever (5, 18, 19). Rabbits pretreated with Cx failed to develop a pyrogenic tolerance, which was manifested by keeping the second phase of fever in response to LPS given for three consecutive days. A mode of action of Cx in inhibition of pyrogenic tolerance induction is a matter of speculation so far, owing to lack of relevant experimental data. It may be related to "*in vivo*" superinducing effect of a low dose of Cx on Il-1 production. It might also be considered, on the other hand, in terms of cycloheximide-induced corticosteroidogenesis inhibition. It was observed that the blood level of corticosteroids increased during induction of tolerance (20, 21), and it has been postulated that these hormones play role in processing of membranes for adaptation to repeated doses of exogenous pyrogen (5). It has been shown that cycloheximide pretreatment abolished steroidogenesis in ACTH-stimulated adrenals (22).

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