Fever in Rats after Intravenous E. coli Endotoxin Administration

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Summary. In conscious unrestrained rats, at an ambient temperature of 22°C, oesophageal temperature was measured and temperature effect of single and repeated intravenous injection of E. coli endotoxin was examined. The first injection of endotoxin in a dose of 10.0 μg/rat did not change the rat body temperature. The second injection of this dose in the same animals repeated after 48 h produced fever. With following injections the fevers observed were less pronounced. The absence of fever after a single injection of endotoxin was accompanied by the rapid loss of pyretic activity of the rat plasma samples (bioassayed in rabbits). When fever was observed (48 h interval between endotoxin injections) the pyretic activity of the rat plasma remained unchanged for 90 min following endotoxin injection. It was concluded that after a single injection endotoxin is rapidly detoxified in the rat circulation while this process does not take place after the second endotoxin injection (48 h interval). The process of endotoxin detoxification can be depressed by the pretreatment with nitrogen mustard. Analysis of changes of skin temperature following endotoxin injections and the influence of aspirin on endotoxin-induced fever suggest that the fever observed was of central origin.

Key words: Endotoxin — Rat — Fever — Anti-endotoxin system.

INTRODUCTION

Intravenous (i.v.) injections of endotoxin into rats resulted in a fall in their body temperature, in contrast to the endotoxin-induced fever observed in other species of mammal (van Miert and Frens, 1968). Injections of endotoxin into the cerebral ventricles of the rat produce fever in this species (Feldberg and Saxena, 1975). The absence of fever following i.v. endotoxin injection cannot be explained by the inability of the rat to produce leucocyte pyrogen (Kampschmidt and Upchurc, 1969); it is generally accepted that fever induced by endotoxin in other mammals results from the action of leucocyte pyrogen as well as endotoxin (Raskova and Vanecek, 1964).

To explain the absence of fever in rats following i.v. endotoxin it was postulated that endotoxin in this species does not pass through the blood-brain barrier (Feldberg and Saxena, 1975). There is no evidence that it crosses this barrier in other mammalian species (Milton, 1976). Alternatively, the rat’s exceptional response to i.v. endotoxin may depend on the presence of mechanism(s) effectively reducing the availability of injected endotoxin at the target sites.

METHODS

Male Wistar rats, 150–170 g, were used throughout. Lyophilized E. coli endotoxin, type Kroeger 08 (Biomed) dissolved in apyrogenic physiological saline (Polfa), or the solvent alone, was injected i.v. in a volume of 0.2 ml per rat through the dorsal vein of the tail. In some experiments nitrogen mustard (Nitrogranulogen, Polfa), aspirin (Polfa), and heparin (Polfa) were used. Glassware, syringes, and needles were sterilized. Injections of endotoxin or saline were repeated after 48 h (second injection) and then at 5-day intervals.

During the experimental session the rats were kept in plastic cages at an ambient temperature of 22 ± 1°C. Following an adaptation period (90 min) oesophageal (Te) and skin (Tsk) temperatures were measured at 30 min intervals, using an Ellab TE-3 thermometer (OR-5 and H-2 probes). The average of the last three measurements of Te before injection was accepted as the initial body temperature providing that no steady trend in Te was observed.

For assay of the pyretic activity of the rat plasma rabbits of either sex, 2–4 kg, were used. Blood samples, 0.3 ml, were withdrawn from rats through the lateral vein of the tail into syringes containing 0.01 ml heparin. The blood was immediately centrifuged and 0.1 ml samples of plasma were injected into the carotid of restrained, intact rabbits whose rectal temperature was continuously monitored. The body temperature of rabbits was not changed after injection of 0.1 ml of plasma from rats which had not received endotoxin. For quantitative determination of pyretic potency of the rat plasma presumably due to the presence of endotoxin the inverse re-
relation between the dose of endotoxin and the latency of onset of fever in rabbits (van Miert and Frens, 1968) was used. Fever was arbitrarily assumed when the rise in the rabbits' rectal temperature was equal to or exceeded 0.2°C. Five rabbits were injected with three different doses of endotoxin and the results obtained did not differ from those of van Miert and Frens (1968). The slope of the regression line of inverse dose vs. latency relation was 1.5 times higher than the slope of the regression line of dose vs. maximum ΔT relation.

RESULTS

The first i.v. injection of 10.0 μg endotoxin per rat did not change T<sub>es</sub> as compared with saline, while the second injection—repeated in the same animals 48 h later—produced fever (Fig. 1). The following injections produced less pronounced fever although the rise in T<sub>es</sub> was still statistically significant in comparison with saline. Lower doses of endotoxin, i.e. 0.1 and 1.0 μg/rat, injected according to the same experimental procedure did not induce fever. The changes in T<sub>s</sub> following i.v. injections of different doses of endotoxin are shown in Figure 2. When endotoxin in a dose of 100.0 μg/rat was injected i.v. for the first time it significantly depressed T<sub>es</sub> with a maximum fall of -2.0 ± 0.24°C. After the second injection (48 h interval) this dose produced an insignificant rise (0.27 ± 0.16°C) in T<sub>es</sub>.

Blood samples were withdrawn 2, 5, 15, and 90 min following i.v. injection of 10.0 μg/rat of endotoxin and the pyretic activity of the rat plasma was bioassayed in rabbits. When endotoxin was injected into rats for the first time the pyretic activity of the rat plasma samples assayed in rabbits decreased proportionally to the time of endotoxin circulation in rats and 90 min following endotoxin injection more than 50% of the initial pyretic potency of plasma was lost. When endotoxin was injected into rats for the second time (48 h interval) the pyretic potency of their plasma samples remained almost unchanged during endotoxin circulation for 90 min (Fig. 3).

In rats made leucopenic (leucocyte count, 48.8 ± 6.8% of the initial value) by s.c. injection of nitrogen mustard (2.0 mg/kg, 48 h before endotoxin injection) the first i.v. injection of endotoxin in a dose of 10.0 μg/rat produced fever (Fig. 4).

Aspirin, 300 mg/kg p.o., given 30 min after the second injection of endotoxin (10.0 μg/rat) depressed the development of fever and induced hypothermia (Fig. 5). Rats receiving saline and aspirin showed only a small and insignificant hypothermia (-0.42 ± 0.28°C).

DISCUSSION

Our results show that although rats do not respond with fever to a single dose of 10.0 μg/rat of endotoxin, the same dose given to the same animals 48 h later is capable of producing fever. The absence of fever following the first i.v. injection may be related to the