Effect of age on hypothalamic prostaglandin E₂ production and fever in response to tumour necrosis factor (cachectin) and endotoxin in rats

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SUMMARY

1. Decreased febrile responses to interleukin-1 and endotoxin have been noted in a number of species with ageing.

2. The present study extends these observations by examining the pyrogenic response to intravenous recombinant human tumour necrosis factor-α (50 μg/kg) using conscious rats aged 7, 20 and 80 weeks.

3. The febrile response decreased in magnitude and duration with age. Fevers of 0.9 °C and of 5 h duration were observed in the youngest rats, whereas those aged 80 weeks were afebrile. The depression in serum zinc level and the elevation in liver zinc level, which occurred 7 h after injection, were unaffected by age.

4. The mechanism of the reduced pyrogenic response was examined by assessing prostaglandin E₂ production in vitro from hypothalami of rats, aged 10 and 24 weeks, in response to Escherichia coli endotoxin and tumour necrosis factor.

5. Whereas the production of prostaglandin E₂ increased by 47% and 52%, respectively, in hypothalami from 10-week-old rats, no response to either pyrogen was obtained in tissue from rats aged 24 weeks.

6. Maturity brings about a decreased responsiveness of hypothalamic prostaglandin E₂ production to pyrogens, which may explain the decreased febrile responses observed.

Key words: fever, hypothalamus, prostaglandin E₂.

Abbreviations: IL-1, interleukin-1; MEM, minimal essential medium; PGE₂, prostaglandin E₂; TNF, tumour necrosis factor.

INTRODUCTION

The development of fever is an important component of the response of animals to invasion by pathogens. Current views on the precise mechanism for the development of fever have recently been reviewed by Dinarello et al. [1]. In essence, the interaction of pathogens with the cells of the immune system leads to the production of cytokines, such as interleukin-1 (IL-1), interleukin-6 and tumour necrosis factor (TNF; cachectin). The mechanism by which cytokines induce fever has been most precisely delineated for IL-1. Cytokines may act upon a richly vascularized part of the hypothalamus called the organum vasculosum laminae terminalis, which lies close to the preoptic/anterior region.

Prostaglandins are subsequently produced from endothelial cells of the organum vasculosum laminae terminalis and either directly stimulate temperature-sensitive neurons nearby or stimulate the production of neurotransmitters which act likewise. Signals emanating from the preoptic/anterior region lead to the generation of fever.

The ability to generate a fever has been shown to decline with age. Aged rats, rabbits and squirrel monkeys were found to produce reduced fevers in response to endotoxin. The pyrogenicity of IL-1 was also reduced in aged mice and rabbits [2–6]. This latter finding, together with the observation that changes in serum zinc and iron levels in response to endotoxin were normal in aged rats, suggests that reduced pyrogenicity was not due to an impaired ability to produce cytokines in aged animals. The mechanism might therefore be related to changes in the hypothalamus associated with ageing.

Many lines of evidence suggest that prostaglandin E₂ (PGE₂) may be a major pyrogenic eicosanoid which arises as the result of interaction of cytokines with the hypothalamus, since it is produced before and during fever in vivo, can induce fever on central administration, and is
produced by exposure of hypothalamic preparations to cytokines and endotoxin. PGE$_2$ injected into the cerebral ventricles and the preoptic nucleus of rabbits produced fever [7, 8] as did PGE$_2$ given by the intracerebroventricular route to rats [9]. Intravenous and intracerebroventricular administration of endotoxin or IL-1 increases cerebrospinal fluid concentration of PGE$_2$ both before and during fever in cats [10], and intravenous administration of either pyrogen has been demonstrated to raise hypothalamic PGE$_2$ concentrations \textit{in vivo} in the same species [11]. Intravenous TNF raised PGE$_2$ concentrations in the cerebrospinal fluid of rats and rabbits [12]. A variety of studies \textit{in vitro} have also shown that a range of pyrogens stimulate PGE$_2$ production by the hypothalamus. Endotoxin increases PGE$_2$ production by murine and rabbit hypothalami [13]. Interferon, IL-1 and TNF do likewise in the rabbit [14, 15, 16].

We have examined, in a study \textit{in vitro}, whether the same phenomenon occurs in response to TNF and endotoxin in rats and whether ageing is associated with a decreased ability of the hypothalamus to generate PGE$_2$ in response to these pyrogens.

A preliminary study on the ability of TNF to produce fever and changes in serum and liver zinc concentrations in rats of various ages was also carried out to see whether a general decline in the metabolic response to this cytokine occurs with ageing.

**METHODS**

**Animals and tissues**

Male Wistar rats were used. In the preliminary study, animals aged 7, 20 and 80 weeks were studied. In the study \textit{in vitro}, hypothalami from male Wistar rats aged 10 and 24 weeks were studied. Rats were fed standard laboratory chow (CRMX; Labsure, Manea, Cambs., U.K.) throughout the course of the study.

**Experimental procedure**

(i) Preliminary study. Rats received either an intravenous injection of human recombinant TNF-\(\alpha\) (6.63 \times 10^6 units/mg of protein; 50 \(\mu\)g/kg body weight; endotoxin content <0.137 ng/mg of protein; BASF/Knoll A.G., Ludwigshaven, Germany) or sterile non-pyrogenic saline (150 mmol/l NaCl). Rectal temperatures were measured, using an electronic thermometer and plastic covered probe, before injection and at hourly intervals thereafter for 7 h. At this time the animals were killed and the serum and liver were subsequently analysed for zinc content by atomic absorption spectroscopy as described by Tocco-Bradley & Kluger [17]. In previous studies we have demonstrated that the response of serum zinc concentration to TNF is maximal at around this time [18]. Animals were caged separately at an ambient temperature of 26°C, and were acclimatized to the rectal probe for 1 week before the study.

(ii) Study \textit{in vitro}. The method of tissue incubation for measurement of PGE$_2$ production in response to stimulation with endotoxin or TNF is derived from the protocol of Bernheim & Dinarello [14] and Dinarello et al. [16]. Rats were killed by stunning and neck dislocation. The brain was rapidly removed and was placed on ice while the hypothalamus was dissected out. The hypothalamus was placed in a pre-weighed 3.5 cm diameter plastic Petri dish (Sterilin Ltd, Feltham, Surrey, U.K.), covered with 2 ml of ice-cold minimal essential medium (MEM; Gibco) and its weight was calculated. The hypothalamus was finely chopped with a razor blade and additional ice-cold MEM was added to produce a suspension of 15 mg/ml. The Petri dish remained on ice throughout this procedure. Portions (1 ml) of the suspension were pipetted into 12 \times 75 mm polypropylene tubes (Luckham Ltd, Burgess Hill, Sussex, U.K.) and were incubated in a shaking water bath at 37°C for 30 min to remove PGE$_2$ generated by tissue processing. MEM (3 ml) at 37°C was added to each tube followed by centrifugation at 3000 \text{ g} for 5 min. The supernatant was discarded. The pellet was resuspended in 1 ml of MEM (37°C) containing various amounts of \textit{Escherichia coli} endotoxin (strain 0127:B8; butanol extract; Sigma) or recombinant human TNF-\(\alpha\) (BASF/Knoll A.G.). The incubates contained TNF at concentrations of 0, 0.25, 2.5 and 25 \(\mu\)g/ml, or endotoxin at concentrations of 0.05, 0.5, 5.0 and 50 \(\mu\)g/ml. A series of incubations of hypothalami from rats aged 10 weeks was also carried out in the presence of the various doses of endotoxin and indomethacin (20 \(\mu\)g/ml). Incubation was carried out at 37°C for 1 h in a shaking water bath. After centrifugation at 4°C and 3000 \text{ g} for 5 min, the supernatants were frozen in liquid nitrogen and stored at −70°C until analysed for PGE$_2$ by \textit{i.r.a.}

PGE$_2$ \textit{i.r.a.}. The assay used was that described by Hillier et al. [19] using a specific anti-PGE$_2$ antibody obtained from Dr K. Hillier (Department of Clinical Pharmacology, Southampton University Medical School, Southampton, U.K.).

**Statistical analysis**

Analysis of variance was used. When examining for differences in rectal temperature and hypothalamic PGE$_2$ production, two-way analysis of variance was employed. One-way analysis of variance was used for examining differences in serum and liver zinc content.

**RESULTS**

**Preliminary study**

Rectal temperature changes are shown in Fig. 1(a-c), and liver and serum zinc concentrations are given in Table 1. The mean starting temperature did not vary significantly between the rats of various ages and was 37.7 \pm 0.1°C. TNF caused significant fever 2 h after injection in rats aged 7 and 20 weeks, but not in those aged 80 weeks (Fig. 1). The latter were afebrile throughout the period of temperature measurement \((F=8.64, P<0.01; F=5.44, P<0.01; F=1.48, P>0.05, \text{ respectively})\). The intensity and duration of fever was greater in
Fig. 1. Effects of intravenous injection of TNF (50 μg/kg body weight, filled symbols) or sterile non-pyrogenic saline (open symbols) in rats aged 7 weeks (a), 20 weeks (b) or 80 weeks (c). Values are means ± SEM. n = 8, n = 6 and n = 4 for (a), (b) and (c), respectively. TNF raised temperatures significantly from 2 h to 6 h after injection in animals aged 7 weeks and from 2 h to 4 h after injection in animals aged 20 weeks, but had no effect in animals aged 80 weeks.

Table 1. Effects of TNF on serum and liver zinc concentrations in rats of various ages

Results were obtained 7 h after injection of TNF (50 μg/kg) or saline. Values are means ± SEM. Statistical significance (one-way analysis of variance): *P < 0.05, **P < 0.01 compared with saline-injected rats of the same age.

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Serum zinc concn. (μg/ml)</th>
<th>Liver zinc concn. (μg/g wet wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>TNF</td>
</tr>
<tr>
<td>7 (n=8)</td>
<td>1.94 ± 0.12</td>
<td>0.86 ± 0.12**</td>
</tr>
<tr>
<td>20 (n=6)</td>
<td>1.94 ± 0.24</td>
<td>0.83 ± 0.10**</td>
</tr>
<tr>
<td>80 (n=4)</td>
<td>1.69 ± 0.53</td>
<td>0.52 ± 0.04**</td>
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the 7-week-old rats than in those aged 20 weeks. In the former a maximum mean temperature rise of 0.9°C and a fever duration of 5 h was observed, while in the latter the temperature rise was 0.5°C and the fever duration was 3 h. Mean rectal temperatures in the saline-injected controls tended to fall throughout the 7 h of the study (Fig. 1). Values were significantly lower than starting values from 2 to 7 h after injection in animals aged 7 weeks, 4–6 h after injection in 20-week-old animals and 6 h after injection in rats aged 80 weeks. Serum zinc concentration was depressed in all rats exposed to TNF, irrespective of age (Table 1). There was a reciprocal increase in liver zinc concentration (Table 1).

Study in vitro

The results are shown in Figs. 2 and 3. In young rats, a dose-related increase in PGE\textsubscript{2} production occurred in response to TNF and endotoxin (for TNF: age, F = 6.93, P < 0.05; dose, F = 6.72, P < 0.01; interaction, F = 3.16, P < 0.05; for endotoxin: age, F = 18.19, P < 0.01; dose, F = 4.15, P < 0.05; interaction, F = 6.10, P < 0.01). A maximum increase of approximately 50% occurred with each pyrogen. The appearance of PGE\textsubscript{2} in the incubation medium can be assumed to be due to increased synthesis, since the inclusion of indomethacin (20 μg/ml) in the incubation medium prevented the response to endotoxin and reduced concentrations below basal levels. While 25, 40 and 55% increases above basal production occurred in response to 0.5, 5 and 50 μg of endotoxin/ml, respectively, no increase occurred in the presence of indomethacin and production rates were less than 30% of the amount produced in the absence of either endotoxin or indomethacin. In rats aged 24 weeks no significant increase occurred in response to either pyrogen.

The minimum doses of TNF and endotoxin required to produce a response in young rats were 2.5 μg/ml and 5 μg/ml, respectively. On the assumption that the M\textsubscript{r} of endotoxin is 10\textsuperscript{6} [20] and that TNF acts as a trimer of M\textsubscript{r} 51 000 [21], then human TNF proved to be 10 times as potent as endotoxin on a molecular basis in the rat.

The responsiveness of rat hypothalamus to human TNF is considerably less than that shown by rabbit hypothalamus. Dinarello et al. [16] found that 5 ng of TNF/ml produced a 30% stimulation of PGE\textsubscript{2} production from rabbit hypothalamus. In the present study, 2.5 μg of TNF/ml was required to produce a similar increase in rat tissue.
DISCUSSION

In a previous study we have shown that 50 µg/kg is the minimal intravenous dose of TNF required to produce a fever of several hours duration in the 8-week-old Wistar rat [18]. At this dosage circulating concentrations would have rapidly fallen to those observed in rats given E. coli endotoxin (see [18]). The present study indicated that the pyrogenic response had declined considerably by 20 weeks of age and had disappeared by 80 weeks. In the study another age-related temperature phenomenon occurred. In the saline-injected animals temperatures fell by between 0.25 and 0.5°C over the 7 h study, with the largest decline occurring in the youngest animals. There are a number of explanations for this phenomenon. Measurements were made on the animals commencing at 08.00 hours. Daylight hours are the quiescent part of a rat’s daily life cycle. The temperatures of small rodents may fall slightly during the quiescent period [22]. The effect may be more noticeable in younger rats, who have a larger surface-to-mass ratio than older rats. Long et al. [23] noted that rodent temperatures are sensitive to stress or novel stimuli. Although our rats were acclimatized to temperature measurements by a rectal probe for 1 week before the study, a longer acclimatization period may have been necessary for the younger rats. If this were the case, the starting temperatures would have been slightly elevated due to a degree of unacclimatization in the younger animals. It is possible that the decreased febrile response observed in the present study is part of a general phenomenon of reduced responsiveness to pyrogens.

A lack of a febrile response to endotoxin has been previously reported in aged rats by Tocco-Bradley et al. [2]. Whereas 8-week-old rats developed fever, those aged 76–112 weeks did not. Aged rats also had a diminished fever when infected with Salmonella typhimurium. As endotoxin initiates fever by the production of pyrogenic cytokines, a reduced ability to produce cytokines might explain the reduced responses observed. However, Kauffmann [24] showed that IL-1/endogenous pyrogen production in rats, stimulated with Staphylococcus epidermidis, was unaffected with age. No data on the effect of age upon TNF production per se were published; however, since TNF is also pyrogenic, the rabbit bioassay used to detect IL-1 production may also have detected TNF. Bradley et al. [25], in a more recent study, reported that peritoneal macrophages from elderly rats aged 24 months had a reduced ability to produce IL-1 and TNF when stimulated with S. epidermidis. At 14 months of age, a differential effect was noted: while the ability to produce TNF was reduced, the ability to produce IL-1 remained intact.

A number of studies have shown no effect of ageing on the febrile response to pyrogens. The febrile response to IL-1 and endogenous pyrogens were the same in young and old rats [26]. Subsequent work demonstrated that aged mice [3] and rabbits [4] produced diminished fevers in response to IL-1. Therefore there may be some species variation in the effects of age on the pyrogenic effects of IL-1.

The results of the present study show that the ability of the hypothalamus to produce PGE₂ in response to a cytokine and to a cytokine-inducing agent declines as the rat matures. Insofar that PGE₂ production is an important determinant of the pyrogenicity of cytokines, this phenomenon may explain why reduced febrile responses have been observed in animals as they mature. The observation that the reciprocal changes in serum and liver zinc concentrations are unaffected by ageing indicates that a general decline in responsiveness to cytokines is not a normal feature of the ageing process.

ACKNOWLEDGMENTS

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REFERENCES


