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Early inflammatory response to carrageenan in the pleural cavity and paw of rats with altered body temperature

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Polymorphonuclear leucocyte (PMNL) migration and oedema induced by carrageenan in the pleural cavity and paw, respectively, of rats made hyper- or hypothermic by physical and chemical means have been investigated. In rats placed in a warm environment to produce a rise in body temperature, carrageenan caused a moderate but significant increase in PMNL migration compared with the control animals. Opposite effects were obtained with hypothermic animals kept in a cold environment. While hyperthermia produced by amphetamine did not alter the PMNL migration, chlorpromazine-induced hypothermia caused a fall in the number of these cells present in the pleural cavity following carrageenan. Both hyper- and hypothermias, whether induced by physical or chemical means, inhibited the carrageenan paw oedema. The observed changes in the PMNL numbers in the pleural cavity did not reflect their numbers in the peripheral circulation. Results indicate that while a rise or a fall in body temperature may have opposite effects on PMNL migration, in carrageenan inflammation both conditions inhibit oedema formation.

In warm-blooded animals, fever is one of the common symptoms of disease caused by infection, neoplastic growth, tissue destruction or by other means. Whether such a febrile response, in moderate intensity (less than 39 °C), is beneficial to the organism is still unclear even though a large body of evidence supports such a view and it has been suggested that the beneficial effects are due to stimulation of the inflammatory and immunological reactions of the host (Roberts 1979; Atkins 1983).

The present studies were undertaken to investigate the effects of changes in body temperature in rats induced by physical and chemical means on polymorphonuclear leucocyte (PMNL) migration and fluid accumulation, the early events of the carrageenan inflammatory response. Leucocyte migration was studied in the pleural cavity and oedema in the paw of the animals. Bernheim et al (1978) have shown that fever in lizards enhances leucocyte migration to the site of infection. If the raised body temperature has a beneficial effect by stimulating the inflammatory response, a defensive reaction (Leme 1981), then the use of antipyretics in moderate fever may be undesirable.

Materials and methods

Male albino rats, 150–190 g, were used in groups of at least 10 animals. Food was withdrawn 16 h before and during the experiments, but there was free access to water.

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Cellular response to carrageenan. Methods for intrapleural injections, collection and total and differential counts of exudate cells were based on general haematological techniques and those of Deporter (1977). Briefly, 50–500 μg of λ-carrageenan in 0·5 ml sterile pyrogen-free saline 0·9% NaCl was injected into the pleural cavity of animals under light ether anaesthesia. Four hours later, the rats were killed, 5 ml of phosphate buffered saline (pH 7·4) was injected intrapleurally and the exudate was withdrawn by suction through an opening in the cavity. Since no measurable volume of exudate was present after carrageenan, the total number of cells was calculated on an exudate volume of 5 ml. A dose of carrageenan that was effective in producing a submaximal response of moderate intensity was selected for further studies.

Carrageenan paw oedema. Saline suspensions of λ-carrageenan (50–500 μg/paw) were injected s.c. into the paw and oedema measured every hour for 4 h according to the method of Winter et al (1962) using a plethysmometer (Ugo Basile). A dose of carrageenan that induced a consistent oedema of moderate degree was chosen for subsequent experiments.

Carrageenan response at different body temperatures induced by physical means. Hyper- or hypothermias were produced in rats by placing them in individual cages in an appropriate hot or cold chamber (approximately 2·0 × 1·0 × 1·0 m) the temperature of which could be controlled. Chamber temperatures of 34, 36 and 38 °C were found to cause suitable graded hyperthermia in the rats. The temperatures for the production of hypothermia were +5, 0 and −5 °C in the cold chamber. Rectal temperatures were measured with thermister probes and a thermocouple.

For cellular studies, the selected dose of carrageenan was injected intrapleurally 1 h after the rats had been in the chamber, by which time their body temperature had been stabilized at the new level. Rectal temperatures were taken every hour for the next 4 h after which the animals were killed for leucocyte counts. The mean temperature change over 4 h was calculated as the average value of the four-hourly differences in temperature compared with their initial temperatures in the normal environment. Results of test and control groups at normal room temperature were then compared. In paw oedema experiments, the previously chosen dose of carrageenan was injected 1 h after placing the rats in the

chambers. Body temperatures and paw volumes were measured hourly for the next 4 h as described before.

In the intrapleural experiments, blood was also collected from some of the groups before the rats were killed, to determine peripheral leucocyte counts using standard histological techniques.

Carrageenan response at different body temperatures induced by chemicals. (±)-Amphetamine (CEME) and chlorpromazine (CEME) both at 2·5–10·0 mg kg^{-1} i.p., were tested for their effects on body temperature and doses of those which altered the temperature by approximately 2 °C were selected. Pleural or paw injections were made 1 h after the temperature modifying agent. In the intrapleural studies, temperatures were measured hourly following the carrageenan and at the end of 4 h, animals were killed and cell counts in the pleural exudate and blood determined as before. In the oedema tests, paw volumes and rectal temperatures were evaluated at hourly intervals.

Results

Cellular response to carrageenan. Carrageenan injection produced a dose-dependent increase in PMNL migration in 4 h while the mononuclear population remained similar to control values ($8·5 \pm 0·5 \times 10^6$) except for the largest dose of 500 μg in which case it was increased to $13·1 \pm 0·5 \times 10^6$ cells. The dose of 100 μg of carrageenan per rat was selected for further studies as it gave a large PMNL migration ($16·0 \pm 0·5 \times 10^6$) without causing mononuclear cell migration. The values in parenthesis are mean ± s.e.m. of at least 10 animals.

Carrageenan paw oedema. Subcutaneous administration of carrageenan into the paw produced a dose-related increase in oedema which was marked as early as 1 h and which reached a maximum in 3 to 4 h. The dose of 100 μg/paw, which caused between 25–35% increase in paw volume in the first hour and a maximum of 50–60% increase in 3 to 4 h, was selected for subsequent experiments.

Effects of hyper- or hypothermia induced by physical means on carrageenan response in the pleural cavity and in the paw. The results of altering the body temperature by placing the rats in warm or cold environment are presented in Table 1. Hyperthermia resulted in a moderate but statistically significant increase in the number of cells, principally of PMNL, in the pleural cavity. The maximum effect of 26·7% was attained when the animals were kept at 36 °C to produce a mean increase in temperature of 1·9 °C. The effect was less (14·0%) when the body temperature was further raised. Lowering the body temperature by 1·88, 2·1 and 2·84 °C, caused statistically significant reductions of 19·5, 23·2 and 27·0%, respectively, in the number of PMNL and reduced the population of resident mononuclear cells. Neither hyper- nor hypothermia induced any significant changes in the blood white cell counts which were similar in untreated and in those animals receiving intrapleural saline or carrageenan injections.

Carrageenan oedema was evaluated at chamber temperatures ranging from 0 to 36 °C designed to cause hypo- or hyperthermia in the rats. Both conditions significantly inhibited the oedema during the 4 h of evaluation. For example, an ambient temperature of 0 °C produced a mean fall in body temperature of 2·4 °C and inhibited the carrageenan oedema by 39·3, 59·3, 45·3 and 41·7% when compared with the oedema measured at hourly intervals for 4 h in animals kept at room temperature of 22–24 °C. Keeping the rats at 36 °C raised their body temperature by 2·15 °C on an average, the corresponding inhibitions of oedema being 20·3, 34·3, 41·2 and 45·9%.

Carrageenan response with alteration of body temperature by chemical means. Chlorpromazine, 5·0 mg kg^{-1} i.p., caused a fall in body temperature of 2·5 °C and a

Table 1. Effects of hyper- and hypothermia induced by physical means on carrageenan (100 μg) cellular response in the rat pleural cavity. Results are mean ± s.e.m. of the number ($\times 10^6$) of polymorphonuclear (PMNL) and mononuclear leucocytes (MNL) present in 5 ml of the pleural fluid. Leucocyte counts (10^3 mm^{-3}) in the blood of some groups of rats are also given. T = temperature.

Chamber T (°C)	Change (mean ± s.e.m.) in body T (°C) over 4 h	Treatment	Number of cells		Blood cell counts	
			PMNL	MNL	PMNL	MNL
Control (22–24)	0.0	Saline	3·3 ± 0·5	9·5 ± 0·4	1324 ± 62	9781 ± 231
Control (22–24)	0·0	Carageenan	15·0 ± 0·7	8·3 ± 0·2	1112 ± 45	9558 ± 181
34	1·0 ± 0·11	Carageenan	16·3 ± 0·9	8·9 ± 0·3	—	—
36	+1·9 ± 0·17	Carageenan	19·0 ± 0·9*	9·7 ± 0·2	1103 ± 80	9513 ± 148
38	+2·5 ± 0·09	Carageenan	17·1 ± 0·8	9·5 ± 0·4	—	—
Control (22–24)	0·0	Saline	15·9 ± 0·6	8·3 ± 0·3	1430 ± 65	10090 ± 210
5·0	−1·88 ± 0·14	Carrageenan	12·8 ± 0·6*	6·7 ± 0·2*	—	—
0·0	−2·1 ± 0·10	Carrageenan	12·2 ± 0·5*	6·8 ± 0·2*	—	—
−5·0	−2·84 ± 0·11	Carrageenan	11·6 ± 0·5*	6·3 ± 0·2*	1365 ± 51	9607 ± 134

Student's two tail *t*-test. *$P < 0·05$.

significant reduction of 25·5% ($P < 0·05$) in the number of migrating PMNL. The values were $10·2 \pm 0·3 \times 10^6$ cells for the treated and $13·7 \pm 0·5 \times 10^6$ cells for the control groups of 12 rats. In the same series of experiments, amphetamine, 10·0 mg kg^{-1} i.p. while raising the body temperature by 1·8 °C had no effect on PMNL migration, the number of cells migrated being $14·2 \pm 0·5 \times 10^6$. The drugs did not modify the total and PMNL counts in the blood of the animals.

In spite of their opposing effects on body temperature, both chlorpromazine and amphetamine in the above doses inhibited the paw oedema significantly ($P < 0·05$) during 4 h after the carrageenan injection. Thus chlorpromazine which reduced the body temperature by an average of 2·36 °C inhibited the 1, 2, 3 and 4 h carrageenan oedema by 24·9, 22·7, 22·0 and 16·8%, respectively. The inhibitory values for amphetamine were 32·6, 46·0, 40·0 and 42·6%, respectively, for similar time intervals after carrageenan, even though amphetamine raised the body temperature by 1·7 °C.

Discussion

Formation of oedema and appearance of leucocytes at the site of injury is a mechanism employed by the organism to eliminate the noxious agent or to limit and repair the damage caused by it. Fever which occurs as a common symptom of injury of a wide nature is also considered to be beneficial (Roberts 1979; Atkins 1983). It is possible that such different protective mechanisms interact to assist the survival of the individual. An example is the recent observation that interleukin 1 not only stimulates the proliferation of T lymphocytes but also causes fever; the higher temperature in turn was found to promote T cell functions (Duff & Duran 1982; Atkins 1983).

To observe whether changes in body temperature alter the early inflammatory response to carrageenan, the comparatively shorter period of 4 h was chosen as it is easier to maintain the temperature changes for such a duration and also is the period of maximum oedema formation and of PMNL migration with carrageenan application. Raising the body temperature by keeping the rats in a warm environment stimulated PMNL migration, the maximum effect occurring with a rise of 1·9 °C. On the other hand a comparable febrile response to amphetamine failed to increase leucocyte accumulation. Hypothermia induced by exposing the animals to a cold environment or by administering chlorpromazine in doses which do not affect cell migration (Saxena 1960) inhibited the carrageenan-provoked cell migration. The changes in PMNL numbers in the pleural cavity after carrageenan in hyper- or hypothermic animals were independent of their numbers in peripheral circulation. Since blood leucocytes are the source of the migrating PMNL (Spector et al 1965), the lack of changes in the blood may indicate compensatory mechanisms in operation involving sites of production, storage and release of these cells in the body.

It was reported that carrageenan oedema is not affected by variations in ambient temperature and humidity (see Swingle 1974). However the inhibitory potency of phenylbutazone in this oedema is dependent on such factors (Green et al 1971). In the present experiments, a rise or fall in body temperature induced by either physical or chemical means always suppressed carrageenan oedema.

Whether PMNL migration and oedema formation, are enhanced in fever is difficult to answer from the results obtained as the oedematous and cellular reactions to carrageenan were found to have no obvious relation to changes in body temperature in rats. Interpretation of the present results are further complicated by the observations that in general rats respond to inflammation and infection with no fever or even with hypothermia (Bennett & Cluff 1957), and there is experimental evidence indicating that fever is detrimental, not beneficial to rabbits, rats, and mice when they are injected with bacterial endotoxins (Atwood & Kass 1964).

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