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BODY MASS AND SEX AS DETERMINING FACTORS IN THE DEVELOPMENT OF FEVER IN RATS

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SUMMARY

1. We have investigated the effect of a single I.P. injection (20 μg/kg) of bacterial endotoxin on rectal temperature in rats of both sexes and from a wide range of body mass.

2. In male rats, endotoxin produced a monophasic or a biphasic rise in temperature, or a monophasic fall.

3. The extent of the rise in rectal temperature in male rats is related to body mass. There is a statistically significant correlation between body mass and the mean change in temperature measured over 100 min after injection of endotoxin.

4. In the female rats, endotoxin produced a monophasic fall in rectal temperature. The extent of the fall was not significantly correlated with body mass.

5. We suggest that the effect of endotoxin in male rats is determined by the physical relationship between body mass and surface area, because larger animals developed greater fevers.

6. The difference between the effect of endotoxin in male and female rats may have a physiological explanation and may involve differences in susceptibility to cutaneous vasodilation occurring immediately after injection of endotoxin.

INTRODUCTION

Unlike most animals tested, rats do not always develop fever in response to systemic injection of bacterial endotoxin (Van Miert & Frens, 1968; Feldberg & Saxena, 1975). Rats developed fever when endotoxin was injected into the cerebral ventricles, but when endotoxin was injected intravenously, a fall in body temperature, not a rise, was observed (Feldberg & Saxena, 1975). Fevers have been produced in rats by intravenous injections of endotoxin (Avery & Penn, 1974; Szekely, Szelenyi & Sumegi, 1973), but it seems that the dose injected in order to produce fever is critical. More recently, it has been shown that the ambient temperature at which the experiments are carried out, independently of the dose used, is an important factor which determines whether a rise in body temperature occurs. Szekely & Szelenyi (1979) obtained a fever irrespective of the dose, up to 100 μg/kg provided that the ambient temperature was kept at 30 °C or above. Fevers were produced at lower temperatures though hypothermia was more common.

The effect of ambient temperature on the development of fever has been observed

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in other species. The fever produced in dogs by an I.V. dose of endotoxin was less at an ambient temperature of 15 °C than at ordinary laboratory temperatures (Haan & Albers, 1960). It has been suggested that the attenuation of the febrile response by lowering ambient temperature may be related to an unfavourable surface area to body mass ratio (Bligh, 1973). The suggestion implies that body mass is an important factor determining an animal's response to endotoxin.

In the present experiments, we examined the effects of bacterial endotoxin on rats from a wide range of body mass. We found that a clear relationship exists between the body mass of male rats and the mean change in rectal temperature which occurs after an I.P. dose of endotoxin. In our experiments, female rats did not develop fever.

METHODS

Sixty-two albino rats were used in this study; forty males weighing between 180 and 350 g and twenty-two females weighing between 130 and 300 g. Twenty-eight of the males and all the females received a single I.P. injection of bacterial endotoxin (*Salmonella typhosa*, Difco) 20 μg/kg in sterile saline. This dose has been shown to produce fever in rats at an ambient temperature of 30 °C (Szekely & Szelenyi, 1979) and is well below the dose of endotoxin which invariably causes hypothermia in rats (see Discussion). Twelve male rats received control injections of sterile saline.

Rats were placed in a small wire cage and their rectal temperatures measured every 20 min by inserting a copper constantan thermocouple connected to a digital voltmeter (Fluke). The temperatures of individual rats were allowed to stabilize for a period of one hour or more before injection of either endotoxin or saline. In some experiments tail skin temperature was measured with a copper-constantan thermocouple connected to a direct reading thermometer (Bailey Instruments). All experiments were performed at an ambient temperature of 24·5 ± 1 °C.

Two series of experiments were performed. In the first, twenty-four male rats weighing between 260 and 350 g were used. Twelve rats were injected I.P. with endotoxin and twelve rats, matched for weight with those receiving endotoxin, were injected with sterile saline. The changes in temperature produced were followed for 160 min after the injections. All data were subjected to the Student's t test.

In the second series of experiments, sixteen males weighing between 180 and 350 g and the twenty-two females were each injected with endotoxin. The changes in temperature produced were followed for 100 min after the injection and the mean change for each rat (the mean of temperature measurements made every 20 min) was plotted against its body mass. The data obtained from the twelve males receiving endotoxin in the first series of experiments were also included. Regression lines for the male and female rats were determined separately by the method of least squares.

RESULTS

The results of the first series of experiments in which the effect of endotoxin was compared to the effect of sterile saline are shown in Fig. 1. Each rat injected with endotoxin developed a fever. The fever was usually rapid in onset reaching a peak within 60 min. By 160 min the rectal temperatures of all animals were not significantly different either from those of control animals or from their own temperatures at time zero. The pooled results from twelve animals show an apparently monophasic response to endotoxin (Fig. 1). In fact, the responses of individual animals were highly variable. Nine of the twelve rats showed a biphasic fever, the magnitude of the second peak usually greater than that of the first. The latency of the first peak varied from rat to rat, but the peak usually occurred between 20 and 60 min after the injection of endotoxin. The latency of the second peak was also quite variable.

Individual temperature records for three rats are shown in Fig. 2. Two of these rats responded to endotoxin with a biphasic fever. The latency of the first peak and the time between the first peak and the second are different in each case. The record shown in Fig. 2*C* is an example of the records of three animals which responded to endotoxin monophasically.

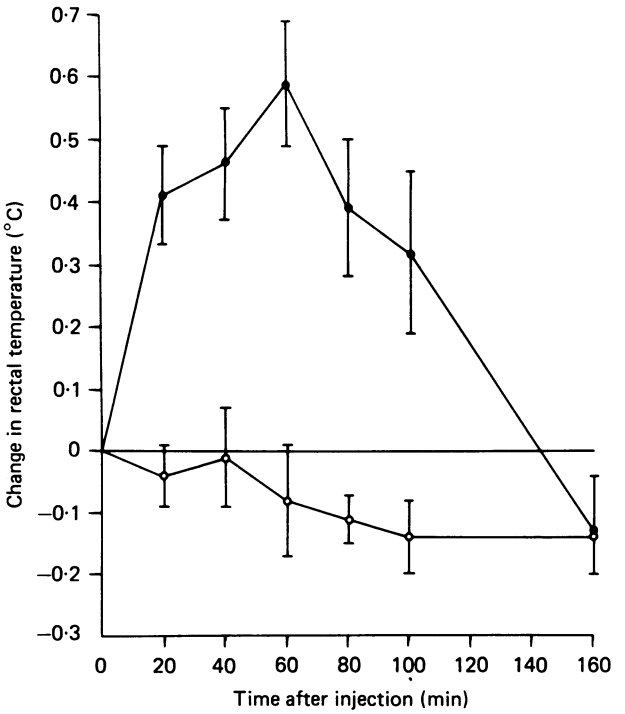

Fig. 1. Changes in rectal temperature, relative to the rectal temperature prevailing at the time of injection, following an intraperitoneal injection of bacterial endotoxin, 20 μg/kg (●—●) and sterile saline (○—○). Each point represents the mean ± S.E. (n = 12).

In part, the variability in response appeared to be related to body mass. We examined the effects of endotoxin on the rectal temperatures of male and female rats from a wide range of body mass. In some experiments we also measured tail skin temperature. Apart from an initial increase in skin temperature immediately following endotoxin injection, skin temperature mirrored the changes in rectal temperature as may be expected. Thus when rectal temperature rose, skin temperature fell. Generally, male rats of mass 260 g or less showed either no change or a monophasic fall in rectal temperature. All the female rats showed a monophasic fall with a corresponding rise in skin temperature. With increasing body mass the effect of endotoxin in males was predominantly a rise in rectal temperature, the magnitude of which was related to body mass. In Fig. 3 the mean change in rectal temperature of each male rat, that is the mean of temperature measurements made every 20 min over the 100 min after injection of endotoxin, is plotted against the individual's

body mass. The correlation between mean temperature change and mass was significant for the male rats ($P < 0{\cdot}001$), but not for the females. The data obtained from female rats are not shown in Fig. 3.

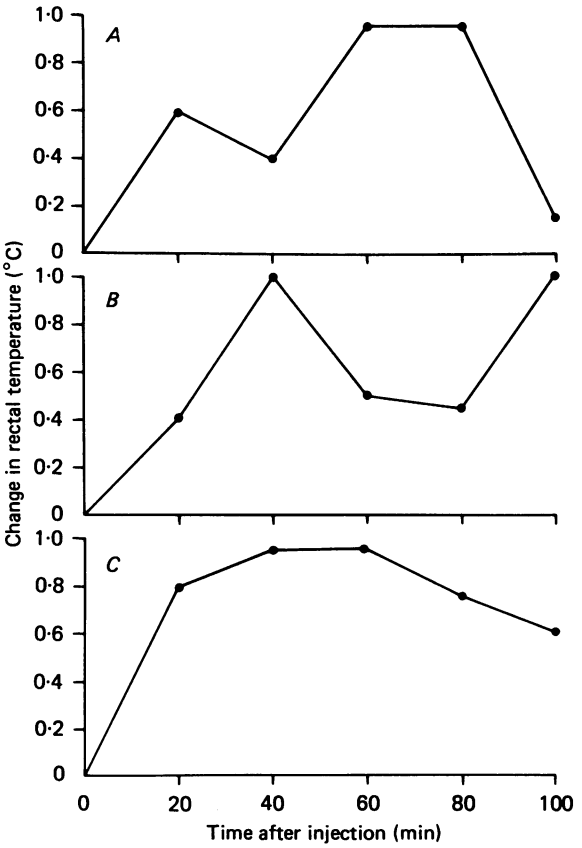

Fig. 2. Typical reponses of rats to bacterial endotoxin, showing biphasic fevers of different time course (*A*, *B*) and a monophasic fever (*C*). Each panel, *A*, *B*, and *C*, is the record obtained from a single animal.

In male rats, the increasing extent of the rise in temperature with increasing body mass is explained by a change from a short latency, small magnitude, short-lasting monophasic response to a biphasic response in which a larger increase in temperature followed the first. Similar biphasic responses have been observed in many other species (see, for example, Myers, Rudy & Yaksh, 1974).

DISCUSSION

Feldberg & Saxena (1975) found that rats developed fever after an intracerebral injection of endotoxin, but not after an I.V. injection: then, body temperature fell. They suggested that endotoxin did not cross the blood brain barrier in rats. An alternative explanation has been put forward by Splawinski, Zacny & Gorka (1977):

after a single injection, endotoxin is rapidly detoxified in the blood, a process which apparently does not take place subsequently, because a second dose of endotoxin caused fever. Neither explanation is now tenable. The results of the present study and those of others (see Table 1) indicate that rats do indeed develop fever in response to a single I.V. or I.P. dose of endotoxin.

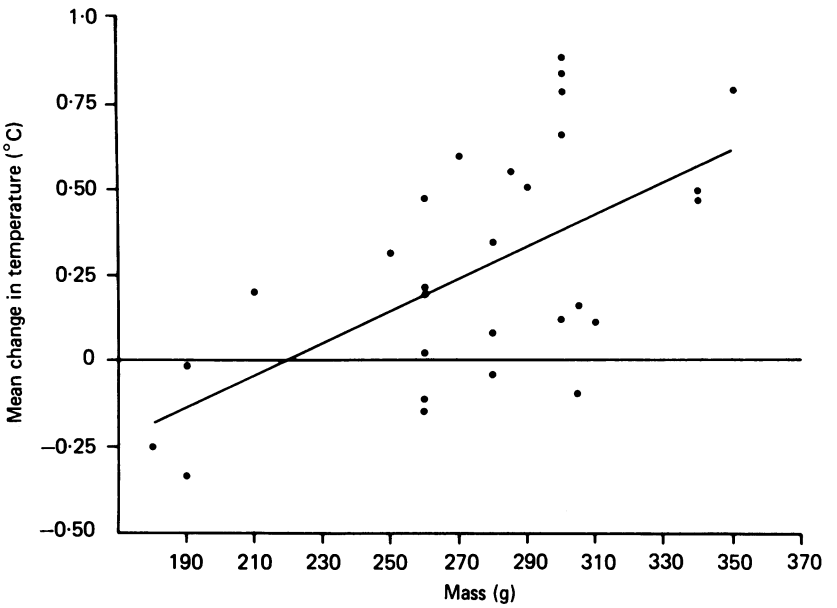

Fig. 3. Relationship between the mean change in temperature of twenty-eight male rats plotted against body mass. Each point represents the mean of five temperature measurements taken every 20 min after the injection of endotoxin in an individual. The regression line was calculated by the method of least squares. Its equation is given by: $y = 0{\cdot}0046x - 1{\cdot}005$. Correlation coefficient = 0·59; $P < 0{\cdot}001$.

The dose of endotoxin injected to produce fever is important. In rats, doses in excess of 150–200 μg/kg invariably cause hypothermia (Filkins & DiLuzio, 1968, using male rats of mass 250–300 g; Holmes & Miller, 1963, using male rats of mass 300 g and greater; Lytle, Taam & Wurtman, 1973, using male rats of mass 250–280 g; Pohorecky, Wurtman, Taam & Fine, 1972, using female rats of mass 200–250 g). With doses below 100 μg/kg, ambient temperature may be the more important factor (Szekely & Szelenyi, 1979). But our results lead us to believe that body mass and sex may be principal factors determining the direction and the extent of the temperature change. Table 1 summarises our data and that of others. We list body mass, sex and the effect of endotoxin and it is apparent that a relationship exists between body mass, sex and either rises or falls in body temperatures.

In the present study we have shown that, for male rats, there is a statistically significant correlation between mass and mean change in rectal temperature. This correlation may be explained on the basis of the surface area to mass ratio. During the rising phase of fever, heat produced is in excess of heat loss. Thus the extent of the rise in temperature must be determined by the extent to which heat production

exceeds heat loss. With increasing body mass there is a corresponding decrease in area to mass ratio and a corresponding decrease in heat loss through the skin per unit of mass. Thus larger rats developed greater fevers than did smaller ones.

TABLE 1. Summary of results showing the effect of a single I.V. or I.P. dose of bacterial endotoxin (100 μg/kg or less) on the body temperature of rats

Sex of animal used	Mass range (g)	Major effect	Reference
Male	150–170	No change	Splawinski *et al.* (1977)
	180–260	No change or fall	Present work
	260–350	Rise	Present work
	300–400	Rise	Horwitz & Hanes (1976)
	350–400	Rise	Avery & Penn (1974)
Both sexes	170–380	Fall or rise*	Szekely & Szelenyi (1979)
	230–250†	No change or fall	Feldberg & Saxena (1975)
Both sexes (70% female)	175–315	No change or fall	Van Miert & Frens (1968)
Female	130–300	Fall	Present work

* Exact effect dependent on the ambient temperature.
† Over a period of several weeks, the mass increased by up to 100 g.

Why male rats of less than 260 g in mass and all the females tested showed either no change in rectal temperature or a fall is less clear. The explanation usually given for this effect on body temperature in small animals is that suggested by Bligh (1973). With a large area to mass ratio, heat loss, resulting from whole body movements associated with shivering, exceeds heat production. This, however, cannot be the entire explanation because of the differences in response which we obtained between male and female rats of the same mass. In our experiments, endotoxin produced an initial increase in skin temperature, indicating cutaneous vasodilatation in rats of both sexes. The consequence of this for heat loss is likely to be greater in smaller animals than in larger ones, owing to the greater area to mass ratio of the smaller animals. In small male rats heat loss is presumably greater than heat production and body temperature falls as a result. This imbalance is progressively reversed with increasing mass, as the area to mass ratio is reduced. Why then did the females not develop fever when males of similar mass did? There are two possible explanations. Tail vasodilatation was more prolonged in female rats than in males of similar mass. This prolongation determines the extent and duration of hypothermia in female rats. It does not explain, however, why the rectal temperatures of female rats began falling at a time when male rats showed tail vasodilatation but a rise in rectal temperature. It is known that female rats have more body fat than males of the same mass (Young & Cook, 1955). Presumably in common with other species, they have a thicker sub-cutaneous insulating fat layer. If this is so, female rats may be more susceptible than their male counterparts to heat transfer, mediated by cutaneous vasodilatation, outside their thermal insulation.

We might expect rats to be less susceptible to the attenuating influence of cutaneous vasodilatation at ambient temperatures at or near thermoneutrality, regard-

less of sex or body mass of the rat. This appears to be the case. Szekely & Szelenyi (1979) have studied the effects of ambient temperature on the response to endotoxin. Direct comparison of their data with ours is not possible, since they pooled the results obtained from rats of both sexes and from a wide range of body mass (Table 1). Nevertheless, they have shown that while fevers are produced in rats at an ambient temperature of 30 °C or above, hypothermia is more common at 20 °C.

It is possible that maturity, rather than body mass, determines the response to endotoxin (Van Miert & Frens, 1968). However, it has been shown that endotoxin may produce fever in the new-born guinea-pig (Szekely, 1978). As with adult rats, the precise effect depended upon the ambient temperature. Although one should be wary of extrapolation from one species to another, it seems likely that age as a determining factor is of no particular importance.

In this study, we have provided quantitative evidence in support of the hypothesis that the effect of endotoxin on body temperature in male rats is determined by body mass. We suggest that the explanation is a physical rather than a physiological one. The difference in response between male rats and female rats of the same mass, however, may have a physiological explanation and may involve differences in susceptibility to cutaneous vasodilatation occurring immediately after injection of endotoxin. Of course, we cannot exclude the possibility of a subtle hormonal influence on the action of endotoxin, an influence which has been unsuspected hitherto.

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