

THE FEBRILE RESPONSES IN RABBITS AND RATS TO LEUCOCYTE PYROGENS OF DIFFERENT SPECIES

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SUMMARY

1. We have investigated the effects on body temperature of rats and rabbits of leucocyte pyrogen derived from the blood of rat, rabbit, ox, pig and baboon.

2. In the rabbit intravenous injections (3.5 ml.) of solutions containing leucocyte pyrogen derived from ox, pig and rabbit blood produced fevers with short latencies; no fevers resulted from injections of similar solutions derived from rat or baboon blood.

3. In the rat intraperitoneal injections (2.0 ml.) of solutions containing leucocyte pyrogen derived from ox blood caused a fever, while pig leucocyte pyrogen produced a marked hypothermia. Neither rabbit, baboon, nor rat leucocyte pyrogen had any significant effect on rectal temperature of the rats.

4. Our results show that there is a variability of response in the rat and the rabbit to injection of leucocyte pyrogen of different species; leucocyte pyrogen may be species specific.

INTRODUCTION

Fever is caused by an action on the brain of endogenous pyrogen, a protein released from leucocytes and cells of the reticuloendothelial system in response to challenge by exogenous pyrogens, including bacterial endotoxin (Hellon, 1975). Leucocyte pyrogen has been manufactured experimentally from the blood of man (Bodel & Atkins, 1966), rabbit (Bornstein & Woods, 1969), cat (Jackson, 1967; Bornstein & Woods, 1969), dog (Petersdorf & Bennett, 1957*a*; Bornstein & Woods, 1969) and goat (Van Miert & Atmakusuma, 1970). However, pure leucocyte pyrogen has never been identified chemically, and its mode of action remains obscure.

With the possible exception of mice, rats and guinea-pigs, the action of bacterial endotoxin appears to be the same in all species tested (Van Miert & Frens, 1968; Hellon, 1975). Fever develops in all cases, the amplitude and time course varying with dose and mode of application of the pyrogen. The same is not true for leucocyte pyrogen. For example, rabbit leucocyte pyrogen caused fever in rabbits and cats, but not in dogs (Bornstein & Woods, 1969). The variability of response to leucocyte pyrogen suggests a degree of species specificity. Nevertheless, endogenous pyrogen produced from different human and rabbit cell-types appeared to have similar molecular weights and chemical characteristics (Rafter, Cheuk, Krause & Wood, 1966; Kozak, Hahn, Lennarz & Wood, 1968; Bodel, Wechsler & Atkins, 1969).

In this paper we report the effects on body temperature of rats and rabbits, of leucocyte pyrogen derived from the blood of rat, rabbit, ox, pig and baboon.

The rabbit was chosen as one recipient animal because of its frequent use in experimental work on fever. The rat was chosen as the other recipient because of its anomalous febrile response (Van Miert & Frens, 1968; Kampschmidt & Upchurch, 1969; Hellon, 1975; Feldberg & Saxena, 1975).

METHODS

Preparation of leucocyte pyrogens. Leucocyte pyrogens were prepared using a modification of the method described by Allison, Cranston, Duff, Luff & Rawlins (1973).

Samples of whole blood from ox, pig, baboon, rat and rabbit were collected into sterile bottles containing a volume of citrated saline (1 ml. per 3 ml. blood). To the blood, 30 μ g of the endotoxin of *Salmonella typhosa* (Difco) was added for each 100 ml. whole blood collected, and the mixture was incubated at 37 °C. After 1 hr, the mixture was centrifuged at 2000 *g* for 15 min. The supernatant was discarded and the remaining cells washed by adding a volume of dextrose saline equal to that of the discarded plasma. The centrifugation was then repeated. After three such washes, the cells were incubated in a similar volume of dextrose saline for 3 hr at 37 °C. Then, after a final centrifugation, the cells were discarded and the supernatant containing the leucocyte pyrogen was collected and stored in sealed sterile containers at 4 °C until used.

For control experiments, blood samples from the various animal species were treated in the manner described above, except that the endotoxin was not added.

Temperature measurements. Rabbits were conscious and restrained in conventional stocks throughout each experiment. Rectal temperature was measured using an indwelling thermistor probe (YSI), inserted about 100 mm into the rectum. The thermistors were connected to an appropriate bridge circuit (YSI Telethermometer, model 47), and the output continuously recorded on a chart recorder (Kipp BD5). Rats were placed in a large wooden cage and their rectal temperatures were recorded every 10 min by inserting a rapid-reading portable thermistor (Anmitherm).

Both types of thermometer were calibrated against a mercury-in-glass thermometer, by immersion in water in a vacuum flask. The YSI thermistor measured its own temperature to a precision of about 0.1 °C and the Anmitherm to about 0.2 °C.

Experimental procedure. Experiments were performed on twelve New Zealand White rabbits weighing between 2.5 and 3.0 kg, and twelve albino rats weighing between 250 and 300 g.

In one series of experiments rats and rabbits were divided into two groups, test and control. Test animals received injections of leucocyte pyrogens, no animal receiving more than one injection of a particular pyrogen. Control animals received injections of the appropriate control solutions. Injections of the various pyrogens and their control solutions were given to the rats and rabbits in the same experimental sequence.

Solutions were given to rabbits in a volume of 3.5 ml, injected into an ear marginal vein. Rats received 2.0 ml. of the solutions intraperitoneally.

In another series of experiments, and using a separate batch of ox leucocyte pyrogen prepared as described above, intravenous injections were made of (a) 3 ml. of the solution of ox leucocyte pyrogen (five rabbits), (b) 0.3 ml. ox leucocyte pyrogen in 3 ml. sterile dextrose saline (three rabbits), (c) 0.03 ml. ox leucocyte pyrogen in 3 ml. sterile dextrose saline (three rabbits). Control rabbits ($n = 3$) received 3 ml. sterile dextrose saline. Rectal temperatures were recorded as described above.

All experiments took place at an ambient temperature of between 20 and 23 °C. Rectal temperatures were monitored for 1 hr before injection of solutions.

All data was subjected to the Student's *t* test and values of *P* equal to or less than 0.01 were considered significant.

RESULTS

The results are expressed as the change in rectal temperature (mean \pm S.E.) relative to the rectal temperature prevailing at the time of injection.

Effects of injection of various leucocyte pyrogens in rats and rabbits

Rectal temperature changes for the groups of rats and rabbits are shown in Figs. 1 and 2.

Ox leucocyte pyrogen produced a rise in rectal temperature in both rats and rabbits. The elevation in rectal temperature following pyrogen injection was significantly different ($P < 0.01$) from that following the control injection after 40 min for the rats, and after 60 min for the rabbits. The rise in rectal temperature persisted for the duration of the experiment. Vasoconstriction and piloerection were observed in both species.

Pig leucocyte pyrogen produced opposite responses in rabbits and rats. In rabbits, a hyperthermia ensued, and reached a plateau level of 1 °C after 60 min. The temperature change was significantly different to that following the control injections after 20 min. A profound hypothermic response was observed in the rat. The mean maximum fall in rectal temperature was 2.0 ± 0.45 °C, which was reached in about 60 min, after which the rectal temperature began to revert to pre-injection levels. Vasoconstriction of the ear and piloerection was observed in the rabbit, but was more intense in the rat. The rats were also observed to shiver.

Baboon leucocyte pyrogen had no effect on the rectal temperature of the rats or rabbits.

Rabbit leucocyte pyrogen had no effect in the rat, but produced the usual febrile response in rabbits. The rectal temperature change following leucocyte pyrogen injection was significantly different to that following control injections after 40 min ($P < 0.01$).

Rat leucocyte pyrogen had no effect in rabbits, and produced only a slight hyperthermia when injected into rats. The rise in rectal temperature in the rats was not significantly different from that which followed injection of the control solution made from rat blood.

Effects of injection of control solutions

Fig. 1 shows that injection of control solutions into rabbits had no significant effect on rectal temperature. A small rise in temperature followed the injection of the control solution made from pig blood.

In rats (Fig. 2), injection of all control solutions resulted in a fall in rectal temperature. This was pronounced after injection of baboon control solution, and, in the case of the rat control solution, the hypothermia was followed by a small, delayed rise in temperature.

Effects of injection of various concentrations of leucocyte pyrogen

Fig. 3 shows the effects, on rectal temperature of rabbits, of injection of a full dose (3 ml.), of 1/10th and of 1/100th of this dose of ox leucocyte pyrogen. Control injections of dextrose saline had little effect on rectal temperature. Significant, dose-dependent fevers resulted from injection of the solutions of ox leucocyte pyrogen.

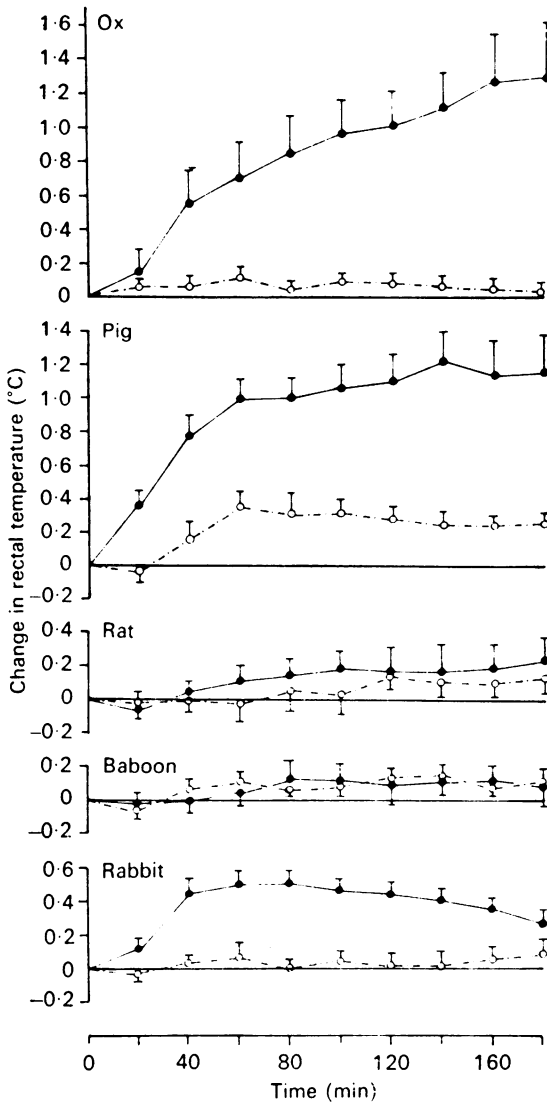


Fig. 1

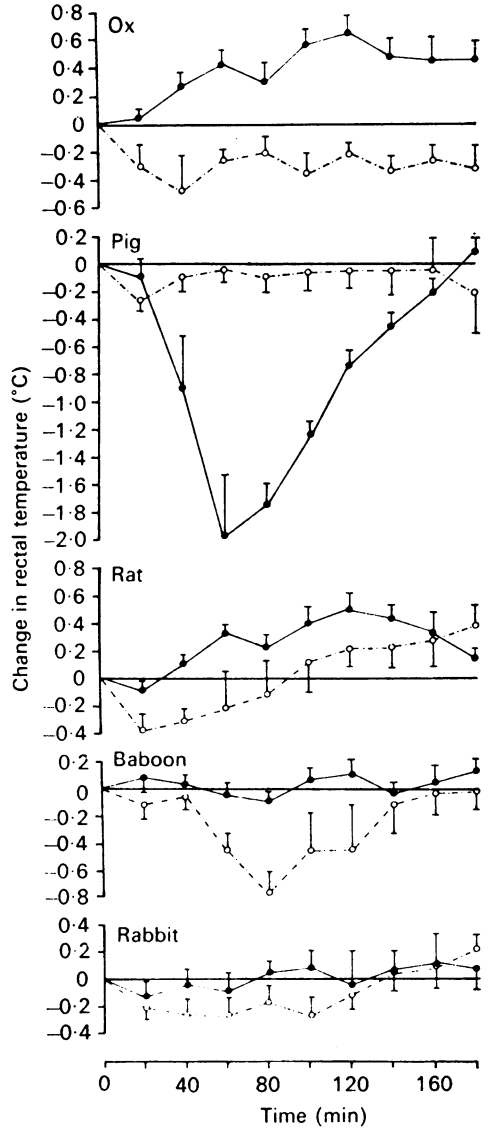


Fig. 2

Fig. 1. Changes in rectal temperature of the rabbit following intravenous injection of solutions containing different leucocyte pyrogens (●—●) and control solutions (○---○). Significant differences between control and test injections were found for ox leucocyte pyrogen (from 60 to 180 min), for pig leucocyte pyrogen (from 20 to 180 min) and for rabbit leucocyte pyrogen (from 40 to 140 min). No significant differences were found between the responses to baboon or rat leucocyte pyrogen and their controls. Each point represents the mean ± 1 s.e. ($n = 6$).

Fig. 2. Changes in rectal temperature of the rat following intraperitoneal injections of solutions containing different leucocyte pyrogens (●—●), and control solutions (○---○). Significant differences between control and test injections were found for ox leucocyte pyrogen (40–180 min) and for pig leucocyte pyrogen (from 40 to 100 min). No significant differences were found between the responses to rat, baboon or rabbit leucocyte pyrogens and their controls. Each point represents the mean ± 1 s.e. ($n = 6$).

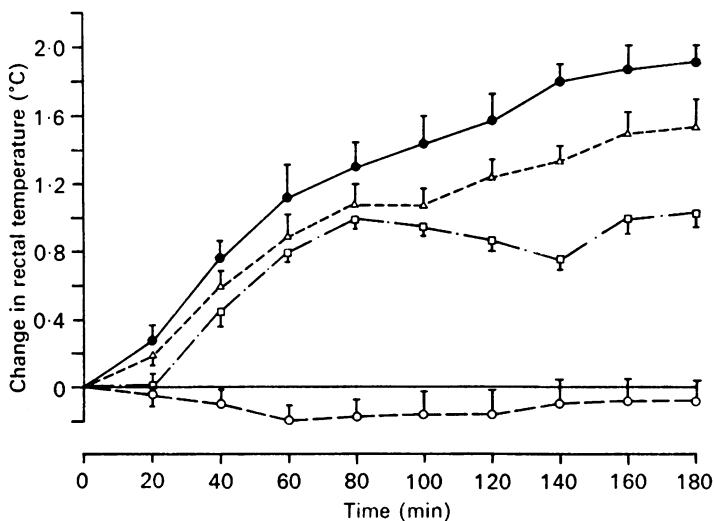


Fig. 3. Changes in rectal temperature of rabbits following injection of a full dose (●—●), 1/10th (△---△), and 1/100th (□-·-·-□) of this dose of ox leucocyte pyrogen. Control rabbits (○-·-·-○) were administered sterile dextrose saline. Each point represents the mean \pm 1 s.e. ($n = 3$ for all experiments, except for full dose experiments, where $n = 5$).

DISCUSSION

Our results show that leucocyte pyrogens derived from the blood of various animal species have varied effects on the rectal temperatures of rats and rabbits. In particular, rabbits responded with fever to leucocyte pyrogen derived from rabbit, ox and pig blood, but showed no response to rat or baboon pyrogen. Rats, on the other hand, developed fever in response to ox leucocyte pyrogen only, while exhibiting hypothermia to pig leucocyte pyrogen, and no change in rectal temperature following injection of baboon, rabbit or rat leucocyte pyrogen.

Several other workers have reported variability of the response of rectal temperature after injection of endogenous pyrogens into species other than that from which the pyrogen was derived (see Table 1). From these and our observations two important issues arise. The first concerns the nature of leucocyte pyrogen, and the reason for such variability of response. The second concerns the effect of the pyrogens in rats, a species in which homologous leucocyte pyrogen appears to have no effect on body temperature.

Variability of response in different animal species to leucocyte pyrogens

At least three possible mechanisms could account in general for the variability of the thermal response to leucocyte pyrogen. The first is that fixed quantities of endotoxin may produce different amounts of the same endogenous pyrogen per unit volume of blood in different animal species. However, quantitative variations in the amount of leucocyte pyrogen generated certainly could not account for the entirely different responses of rats and rabbits to the same solution of pig leucocyte pyrogen. We have shown that even low doses of ox pyrogen will still cause a significant fever in the rabbit, suggesting that different concentrations of leucocyte pyrogen would

only account for the differences in the magnitude of the response, and not for differences in the direction of the response.

The second possibility is that different animal species display variable sensitivity to the same endogenous pyrogens. Cats are reported to be three to four times more sensitive than are rabbits to their own leucocyte pyrogen, and dogs are less sensitive than rabbits to cat leucocyte pyrogen (Bornstein & Woods, 1969). If variations in

TABLE 1. Summary of results showing cross-species responses to endogenous pyrogens

Recipient	Donor	Response	Reference
Rabbit	Rabbit	Fever	Bornstein, Bredenberg & Wood (1963)
	Rabbit	Fever	Cranston, Hellon & Mitchell (1975)
	Rabbit	Fever	Present work
	Ox	Fever	Present work
	Pig	Fever	Present work
	Goat	Fever	Van Miert & Atmakusuma (1970)
	Man	Fever	Bodel & Atkins (1966, 1967)
	Man	Fever	Cranston, Luff & Rawlins (1971)
	Man	Fever	Allison, Cranston, Duff, Luff & Rawlins (1973)
	Cat	Fever	Bornstein & Woods (1969)
	Dog	Nil	Petersdorf & Bennett (1957 <i>a</i>)
	Dog	Fever	Bornstein & Woods (1969)
	Rat	Nil	Present work
	Baboon	Nil	Present work
	Rat	Rat	Nil
Rat		Nil	Present work
Ox		Fever	Present work
Rabbit		Biphasic	Kampschmidt & Upchurch (1969)
Rabbit		Biphasic	Present work
Baboon		Nil	Present work
Pig		Hypothermia	Present work
Cat	Cat	Fever	Bornstein & Woods (1969)
	Rabbit	Fever	Bornstein & Woods (1969)
	Dog	Fever	Bornstein & Woods (1969)
Dog	Dog	Fever	Bornstein & Woods (1969)
	Dog	Fever	Petersdorf & Bennett (1957 <i>a, b</i>)
	Rabbit	Nil	Petersdorf & Bennett (1957 <i>a</i>)
	Rabbit	Nil	Bornstein & Woods (1969)
	Cat	Fever	Bornstein & Woods (1969)
Goat	Goat	Fever	Van Miert & Atmakusuma (1970, 1971)
	Goat	Fever	Bornstein & Woods (1969)
	Rabbit	Fever	Bornstein & Woods (1969)
	Rabbit	Slight hypothermia	Van Miert & Atmakusuma (1970)
	Dog	Fever	Bornstein & Woods (1969)
	Sheep	Dog	Fever
Mouse	Mouse	Fever	Bodel & Miller (1976)
	Man	Fever	Bodel & Miller (1976)
Squirrel	Squirrel	Fever	
monkey	monkey		Lipton & Trzcinka (1976)
	Cat	Fever	Lipton & Trzcinka (1976)

sensitivity were the sole cause of differences in response, a particular species should exhibit the same response to leucocyte pyrogen of whatever origin. Table 1 shows that in most animal species tested, but especially in the rat, there appear to be different responses to pyrogens of different origins.

The third and most likely explanation for the variable effects of different leucocyte pyrogens when injected into various animal species is that endogenous pyrogens are indeed species specific. In some species, the differences between pyrogens could be sufficiently small for cross-reactivity to occur. Alternatively, some species may have the capacity to react to several different leucocyte pyrogens. Although the gross structure and composition of some leucocyte pyrogens have been elucidated (Murphy, Chesney & Wood, 1971; Atkins & Bodel, 1974), and all appear to be similar, it would require only subtle differences in structure or composition to impose species specificity. The isolation and detailed analysis of leucocyte pyrogens is clearly needed.

In our experiments, only two pyrogen solutions failed to affect both rat and rabbit. These were the solutions supposedly containing leucocyte pyrogens derived from rat and baboon blood. The question arises whether the solutions actually contained pyrogen. There is some evidence (Kampschmidt, Pulliam & Upchurch, 1973) which suggests that rat peritoneal granulocytes and macrophages have the capacity to produce leucocyte pyrogen. We know of no such evidence in the case of the baboon. An endogenous substance released by ox, rabbit and pig blood in the conditions of our experiments had specific effects on body temperature of the experimental animals. This substance was presumably released in response to endotoxin, because the control solutions derived from blood to which no endotoxin had been added had little or no effect on rectal temperature of the rats and rabbits. We know of no reason why the identical procedure should fail to release leucocyte pyrogen from rat and baboon leucocytes.

The effect of pyrogens in the rat

Our observations on the effect of rat pyrogen injected into rats are of particular interest in that the rat appears to be the only species which does not develop fever in response to its own endogenous pyrogen. Rats also have been reported not to develop fever in response to intraperitoneal injections of endotoxin (Feldberg & Saxena, 1975). The failure to respond to endotoxin may reflect tolerance to endotoxin as a result of chronic infection, common in rats. However, such a mechanism could not account for the failure to respond to endogenous pyrogen, since animals do not ever become tolerant to their own endogenous pyrogen (Hellon, 1975).

Because intraventricular injection of endotoxin does result in fever in the rat, Feldberg & Saxena (1975) suggested that endogenous pyrogen released after systemic endotoxin challenge does not pass the blood-brain barrier. Our findings would appear not to support this idea because the rats developed fever in response to I.P. injection of a solution containing leucocyte pyrogen made from ox blood. Why rats are refractory to parenteral endotoxin and their own endogenous pyrogen remains unknown.

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