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The Effect of Low Ambient Temperature on the Febrile Responses of Rats to Semi-Purified Human Endogenous Pyrogen

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The febrile responses of Sprague-Dawley rats to semi-purified human endogenous pyrogen were studied at a thermoneutral ambient temperature (26°C) and in the cold (3°C). It was found that while rats developed typical monophasic febrile responses at thermoneutrality, febrile responses were absent in the cold-exposed rats. Experiments were conducted to determine whether this lack of febrile responses in cold-exposed rats was due to an inability of these animals to generate or retain heat in the cold. Thermogenesis and vasoconstriction were stimulated in cold-exposed rats by selectively cooling the hypothalamus, using chronically implanted thermodes. It was shown that, using this stimulus, metabolic rate could be increased by more than 50 percent and body temperature could be driven up at a rate of 5°C/hour in rats exposed to the cold. Therefore, it was concluded that the lack of febrile responses of cold-exposed rats to pyrogen is in no way due to a physical or physiological inability to retain heat. Instead, it appears that in some manner cold exposure suppresses the sensitivity or responsiveness of the rat to pyrogenic stimuli.

INTRODUCTION

A hallmark of the febrile response in mammals is its apparent independence from ambient temperature [1,2]. For example, if rabbits are made febrile in the cold, increases in body temperature are brought about by increases in thermogenesis, since all heat-loss mechanisms are already reduced to minimal values because of the cold environment. On the other hand, if rabbits are made febrile in hot environments, increases in metabolism are minimal or absent, and body temperature is raised by decreasing the active heat losses that are being promoted by peripheral vasodilatation and panting in the heat. Rodents in general, and rats in particular, appear to present a striking exception to this axiom [3,4,5,6]. While there is some debate as to whether rats are capable of generating fevers at all, there seems to be universal agreement that it is not possible to produce fevers in rats at ambient temperatures below 15°C [7,8,9,10,11]. Several reasons have been advanced to account for this inability of rats to become febrile in the cold. For example, it has been suggested that the relatively small body mass and correspondingly large surface-area-to-volume ratio in rats prevents them from storing sufficient heat to raise their body temperatures in cold environments in response to febrile stimuli [7,12]. Since most work on fever in rats has been done using endotoxin pyrogens [3,4,5,8,9,10,12,13], it has also been postulated that separate toxic effects on the peripheral circulation prevent heat storage by small

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rodents in the cold due to an overriding vasodilatation produced by the direct action of the endotoxins on vascular smooth muscle [9]. Finally, on a more general note, it has been suggested that rats do not respond normally to endogenous pyrogen if it is produced in response to endotoxin treatment [10,11] and that endotoxins cannot cross the blood-brain barrier in rats; thus, fevers do not occur in these rodent species [9]. Little information on the effects of endogenous pyrogen on the body temperature of rats is available in the literature. Bodel and Miller [6] showed that mice responded with fevers to intravenous injections of human endogenous pyrogen prepared from blood monocytes, but these febrile responses could only be obtained when the mice were kept at ambient temperatures higher than 34°C. We, therefore, decided to examine the responses of rats to a semi-purified endogenous pyrogen prepared from human monocytes and then ascertain the effect of low ambient temperatures on these responses. We also investigated whether it was physically possible to produce increases in body temperature in cold-exposed rats in order to test the hypothesis that fever in cold-exposed rats was prevented by their inability to store heat because of their adverse surface-area-to-body-mass ratio. It has been shown that specific local cooling of the thermosensitive structures in the preoptic anterior hypothalamic (PO/AH) area of mammals will drive up body temperature by stimulating heat production and heat conservation mechanisms [14,15]. This is achieved by implanting chronic, stainless-steel perfusion thermodes within the PO/AH area. Using a water perfusion system to cool and thermally clamp PO/AH temperature several degrees below its normal value, the conscious animal can be stimulated to raise body temperature by shivering and vasoconstriction.

MATERIALS AND METHODS

The rats used in this study were male Sprague-Dawley rats (Camm Laboratories, NJ) weighing between 250 and 350 g. They were conditioned to accept minimal restraint in a conventional neck stock. About five to seven days prior to the start of the experiments, a polyvinyl catheter was implanted into the femoral vein of each animal under general anesthesia. The catheter was passed subcutaneously to the dorsal thoracic region and exteriorized at the mid-scapular region, behind the neck. The catheter was kept patent by flushing it with a dilute solution of heparinized 0.9 percent saline (100 U/ml) twice a week, and all intravenous injections were administered by this route.

The endogenous pyrogen (EP) used in this study was made from activated human monocytes derived from freshly banked blood. The techniques used to prepare and purify the pyrogen have been reported previously [16]. Each ml of the semi-purified pyrogen contained the supernatant product of 2×10^6 stimulated monocytes, and a dose of 0.5 ml/kg (intravenously) was sufficient to raise the body temperature of bioassay test rabbits by 0.7°–0.9°C. A single batch of pyrogen was used throughout the study. It was stored under refrigeration at 4°C, and aliquots were dispensed using rigidly sterile techniques. All experiments were conducted between 0800 and 1400 hours on the minimally restrained conscious rats in an indirect calorimeter [17]. Rectal and tail skin temperatures, metabolic rate, and an index of tail skin blood flow [18] were measured continuously and recorded once per minute, by an on-line computerized data acquisition system [17]. Each rat sat calmly in neck stocks, enclosed in a flat cylindrical plexiglas chamber (30.5 cm in diameter, 23 cm high, and 8.7 l in volume) through which air at ambient temperature was drawn at a rate of 10 l/minute to permit

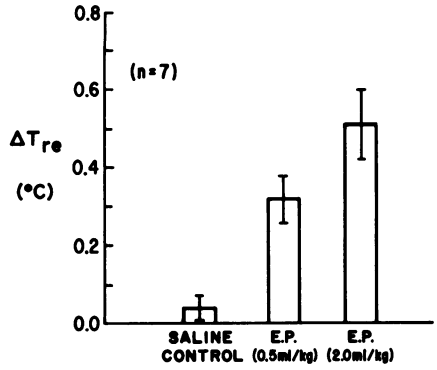

FIG. 1. Body temperature increases elicited by EP, at two dose levels, when injected intravenously into rats exposed to an ambient temperature of 26°C.

continuous oxygen consumption measurements. Experiments were conducted at two ambient temperatures, 26°C (thermoneutrality for rats) and 3°C. After the rat was placed in the calorimeter, a period of 60 minutes was permitted to elapse in order to allow the animal to attain a thermal steady state. Rectal temperature was always between 38° and 39°C at the start of each experiment. Each rat was injected with EP on three separate occasions, once at a dose of 0.5 ml/kg (intravenously), once at a dose of 2 ml/kg (intravenously) at an ambient temperature of 26°C, and once at a dose of 2 ml/kg (intravenously) at an ambient temperature of 3°C. The order of these latter two exposures was determined in a random fashion. At least five days were allowed to elapse between EP injections.

The animals that were used to investigate the ability of rats to raise their body temperatures in the cold were prepared by implanting a pair of thermocouple and thermode re-entry tubes in the preoptic anterior hypothalamic area, using sterile stereotaxic techniques under general anesthesia, at least 15 days prior to the start of the study. The methods and techniques used to implant animals have been described previously [17]. Both tubes were manufactured from blind-ended, stainless-steel tubes (1 mm outer diameter, 0.8 mm inner diameter). They were placed 1.2 mm on either side of the mid-line at AP: 7.5 and V: −1.5 according to the stereotaxic coordinates of deGroot [19]. Dental cement secured the tubes to two jewellers' screws anchored to the calvaria of the skull. When these rats were to be tested, they were placed in the calorimeter at an ambient temperature of 6°C and a thermocouple was inserted into the thermocouple re-entrant tube. A water perfusion system was connected to the thermode re-entrant tube by means of coaxial polyvinyl tubes, so that water of a desired temperature could be perfused down the thermode to alter, and hold clamped, the temperature of the hypothalamus to several degrees lower than normal. In this manner, thermogenesis could be stimulated in the animal and its effect on body temperature could be studied.

RESULTS

Figure 1 illustrates the body temperature responses of rats to intravenous injections of endogenous pyrogen at a thermoneutral environment. It can be seen that at a dose of 0.5 ml/kg EP, significant elevations in body temperature were elicited. However, rats appear to be relatively insensitive to EP, since doses of 2 ml/kg produced an average increase in body temperature of only 0.51°C. By contrast, the same pyrogen given to rabbits produced an average increase in body temperature of more than 0.8°C, at a

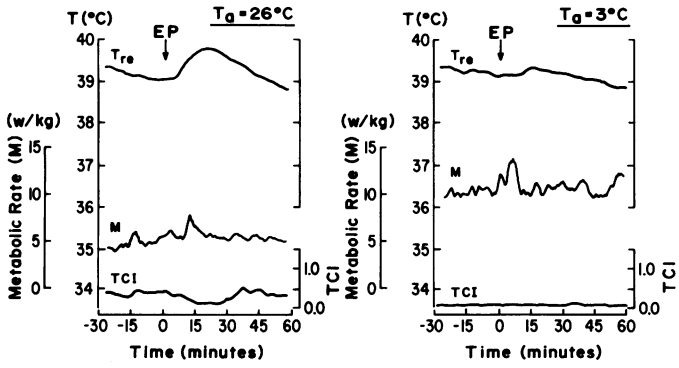

FIG. 2. The left panel illustrates the febrile response exhibited by a rat exposed to T_a = 26°C when EP (2 ml/kg intravenously) was injected. The right panel shows the response of the same animal after the same amount of EP was injected when the ambient temperature was 3°C.

dose level of 0.5 ml/kg. Consequently, all succeeding experiments were conducted using the largest dose possible, 2 ml/kg. This required an injection volume of 0.5 to 0.7 ml. Figure 2 compares the febrile responses of a typical rat when the EP was administered first at an ambient temperature (T_a) = 26°C and on a subsequent occasion at T_a = 3°C. At T_a = 26°C, a prompt, monophasic fever was elicited within ten minutes of the injection. The peak elevation of rectal temperature (T_{re}) occurred between 20 and 30 minutes after the injection, and body temperature returned to normal within 60 minutes. The increase in body temperature was brought about by a combination of peripheral vasoconstriction and a slight elevation in metabolism. By contrast, when the animal was exposed to T_a = 3°C, although metabolism appeared to increase immediately after the injection, there was no significant elevation in body temperature during the ensuing 60-minute period. The average changes in body temperature in a group of seven rats, treated with endogenous pyrogen at T_a = 26°C and 3°C in random order, are compared in Fig. 3. It can be seen that, while the body temperature of the animals treated at T_a = 26°C rose by about 0.5°C, there was no significant change in body temperature when the animals were treated with pyrogen at T_a = 3°C. Finally, we wished to test the hypothesis that the inability of rats to exhibit fevers in cold environments is due to a physical or physiological inability to store heat at low ambient temperatures. Rats which had been implanted with a re-entrant thermode and thermocouple tube in the PO/AH area 15 days prior to the test were used. When tested, they were exposed to an ambient temperature of 6°C and allowed to

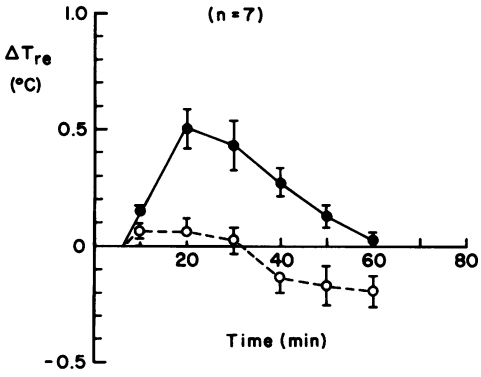

FIG. 3. A comparison of the body temperature changes obtained after EP (2 ml/kg intravenously) was injected into a group of seven rats at T_a = 26°C and T_a = 3°C.

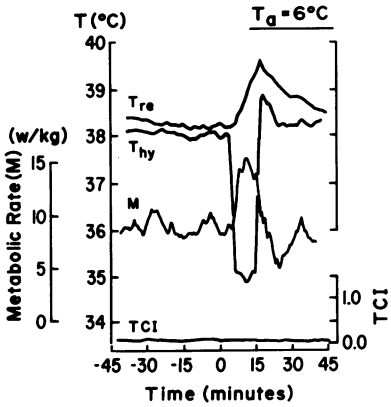

FIG. 4. Body temperature and thermogenesis responses induced when hypothalamic temperature (T_{hy}) was lowered from 38°C to 35°C when the rat was exposed to an ambient temperature of 6°C.

attain a thermal steady state. Hypothalamic temperature was then reduced from 38°C to 35°C and maintained at that level for 15 minutes. Figure 4 illustrates such an experiment. It can be seen that when hypothalamic temperature (T_{hy}) was lowered and clamped at 35°C, metabolic rate rose from 9 watts/kg to around 13.5 watts/kg and body temperature rose by 1.2°C within 15 minutes. This fact demonstrates that there is no physical or physiological impediment to rats increasing their body temperatures at low ambient temperatures. If adequately stimulated, the rat possesses a sufficient metabolic reserve and vasomotor integrity enabling it to raise its body temperature at a rate of ~5°C/hour; clearly there is no physical reason why rats fail to become febrile in low ambient temperatures.

DISCUSSION

This study demonstrates that rats possess the capacity to produce fevers in response to exogenously administered human endogenous pyrogen, at least within their zone of thermoneutrality. However, there are several marked differences between the responses of rats and rabbits to this endogenous pyrogen. First, rats appear to be considerably less sensitive to the pyrogen, in that it took a dose of 2.0 ml/kg of EP to raise body temperature about 0.5°C in rats. By contrast, the same pyrogen, administered at a dose of 0.5 ml/kg to rabbits, produced increases in body temperature of between 0.7° and 0.9°C. Second, when EP was administered to rats exposed to an ambient temperature of 3°C, considerably colder than their thermoneutral temperature of 26°C, febrile responses failed to develop. Rabbits on the other hand develop fevers at low ambient temperatures that appear no different from those obtained at higher ambient temperatures [2]. In this respect, our work confirms and extends the findings of Bodel and Miller [6] using mice. Examination of the responses of the cold-exposed rats to pyrogen administration, such as those illustrated in Fig. 2 or Fig. 3, led us to suspect that the failure of body temperature to rise after pyrogen administration in the cold was not due to any inability on the part of the animal to generate or retain heat in response to the pyrogenic stimulus. It can be seen that vasoconstriction of the tail (thermal circulatory index) was maximal before, during, and after the period of pyrogen administration and action. Metabolic rate (10 watts/kg) was not at its maximal levels during the cold exposure and could certainly have gone higher if required. In order to prove this suspicion, we artificially stimulated thermogenesis in cold-exposed rats, by cooling the PO/AH region when rats were

exposed to an ambient temperature of 6°C. From the example of the response obtained, shown in Fig. 4, it can be seen that metabolic rate, initially about 9 watts/kg, was driven up to near 14 watts/kg during hypothalamic cooling, and body temperature rose by 1.2°C during the 15-minute period of stimulation. Clearly, the lack of febrile response in cold-exposed rats is not due to a physical or physiological inability to generate or retain heat at these low temperatures.

Thus, we are left with the conclusion that rats in some manner become less sensitive to pyrogen stimuli when they are cold-exposed. This reduced sensitivity to febrile stimuli, coupled to their innately low responsiveness to pyrogens, even in thermoneutral environments, seems to account for the absence of any increase in body temperature when pyrogen is administered to rats in cold environments. For this reason one is tempted to speculate that if an adequately large pyrogenic stimulus is applied to cold-exposed rats, a febrile response would be elicited, albeit a lesser response than one would obtain with the same stimulus if it were appled to the rats in a thermoneutral environment.

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