Bull Yamaguchi Med Sch 29 : 61-66, 1982

Thermoregulatory Behavior in Rats during Induced Fever

Naotoshi Murakami, Takashi Minagawa, Yosuke Kawai, Akio Morimoto, Yoshihiro Sakai, Shigeki Ooki and Yoshitaka Takase

Department of Physiology, Yamaguchi University School of Medicine, Ube, Yamaguchi 755, Japan

(Received July 28; revised November 16, 1982)

Abstract Hypothalamic injection in the rats of bacterial endotoxin or endogenous pyrogen from rabbit's leucocytes induced fever. During the fever thus induced, the rats were exposed to radiant heat. Their behavioral thermoregulation, determined by the bar-pressing rate to escape from the radiant heat, was significantly reduced compared to that in the rats untreated with the pyrogens and exposed to the radiant heat. Thus, the rats with the induced fever prefered high levels of tail-skin and ambient temperatures, signs of the development of behavioral fever.

Key Words: Thermoregulation; hypothalamus, pyrogen, Fever; behavior

Introduction

Temperature regulation in both birds and mammals, the latter including man, is accomplished by two means: behavioral and autonomic. The two thermoregulatory activities seem to be interchangeable in critical situations[1,2]. The elevation of body temperature during fever is usually induced by autonomic thermoregulatory responses[3]. The rat does not raise their body temperature in response to I.V. or I.P. injection of pyrogens. This is the reason why they have not been used for the study of behavior during fever, despite the fact that the rats are one of the most commonly used laboratory animals. However, recent studies revealed that bacterial endotoxin injected into the preoptic/anterior hypothalamic region can induce fever in the rat. Furthermore, it was found that when repeat intravenous injections of bacterial ednotoxin are performed in the rat, the second injection also can induce fever[4]. We induced fever in the rat by hypothalamic injections of pyrogens, and studied the behavioral adjustments of thermoregulation in the development of fever.

Methods

Two series of experiments were carried out. The first series, performed on rats loosely restrained in a cage[5], was undertaken to determine if the rats develop fever after an intracerebral administration of bacterial endotoxin or leukocyte endogenous pyrogen. The second series examined behavioral changes during fever of well trained rats to press a bar to escape radiant heat. Male Wistar rats, 280-400 g, were used in both series. The ambient temperature was maintained at 25±1°C throughout the first series and at 20+1°C for the second series.

A cannula-guide, 1.0 mm in outer diameter and made of stainless steel tube with an obturator, was implanted at least 10 days preceeding the experiment stereotaxically into the left area of the preoptic/anterior hypothalamic region (AP +1.8, L 0.7, H +8.5) of all rats, according to the coordinates of Pellegrino's atlas (1967)[6], under an aseptic condition with pentobarbital sodium anesthesia (0.025 mg/kg). The rectal and tail-skin temperatures were measured with copper-constantan thermocouple probes connected to a potentiometer (RC -5, OHKURA ELECTRIC Co). In the behavioral study, the ambient temperature in the test box, and the floor temperature were concomitantly monitored. All temperatures were recorded with a minute's interval.

A behavior test box, with a plexiglass cylinder mounted at the bottom and fitted with an exhaust fan, was utilized in the study of thermoregulatory behavior. An infrared lamp (250 W), centered over the Plexiglass cylinder, was placed 37 cm over the floor. Two bars were set on the wall of the test box; one, when pressed, turned the lamp off for a period of 8 seconds and activated the exhaust fan which drew room air into the box, and the other was a dummy to ensure that the bar-pressing motion is selective. The frequency of the bar-pressing motion was recorded by a counter and printed every 3 min. After a stable response to escape radiant heat was established by daily training of 7 to 10 days, the cannula-guide was surgically implanted. Following full recovery from the operation, the behavioral study was carried out in the test box. In the study, heat escape response was determined as a bar-pressing rate during a period of 90 min. Temperature measurements were done in various sites of the test box and the test rat.

Lipopolysaccharide from *Salmonella typhosa* (Difco Lab.), dissolved in saline at a concentration of 5 mg/ml, was used as an endotoxin.

The endogenous pyrogen was prepared by the method of Cranston et al.[7] with slight modifications. In the first, rabbit whole blood was stimulated by an addition of lipopolysaccharide from *Salmonella typhosa* at a concentration of 5 μg/ml, and incubated at 37°C for 2 h. After centrifuging the blood at 2,000 rpm for 20 min, the buffy coat layer was collected, repeatedly washed with 0.9% saline containing 100 μg/ml penicilline G, 100 μg/ml streptomycine and 0.1% glucose, resuspended in 0.9% saline at a concentration of $1\text{-}2\times10^7$ leukocytes/ml, and incubated at 37°C for 18 h. The suspension was centrifuged at 2,000 rpm for 20 min. The supernatant, containing the endogenous pyrogen, was used for the experiment. Its pyrogenicity was confirmed by intravenous injection in the rabbit. The supernatant heated at 60°C for 40 min did not cause fever in the rabbit, thus excluding the possibility of contamination by exogenous pyrogen.

The injection of bacterial endotoxin, leukocyte endogenous pyrogen, or saline was performed using a cannula injector, 0.5 mm in outer diameter, connected by a polyethylene tube to a microsyringe (10 μl, Thermo Co.) and placed in a cannula-guide. The insert extended 0.5 mm beyond the tip of the cannula-guide. In practice, 1.0 μl of endotoxin containing 5 μl bacterial lipopolysaccharide, 1.0 μl endogenous pyrogen, or 1-2 μl saline was injected at a rate of 1.0 μl/min. Aseptic precautions were observed throughout the preparation and injection of the pyrogen solutions.

Following the injection, 1.0 μl of Indian ink was injected through the cannula-insert for verification of cannula-guide placement and injection site. The rats were anesthetized with 40% formalin-saline solution. The brains were removed from the cranium and placed in a formalin solution. After sectioning (100 μm), the injection site was identified.

Results

1. Development of fever in the rat

The rectal temperature response to the hypothalamic injection of bacterial endotoxin was studied in eight rats, and that to saline injection was recorded in another five rats (Fig. 1). The rectal temperature in the study group kept to increase during the threehour observation period, while that in the control group did not. The difference between the two groups became significant one hour after the injection. The hypothalamic injection of leukocyte endogenous pyrogen was followed by a rapid increase of the rectal temperature (Fig. 2). The temperature in the study group became significantly higher than the control saline group soon after the pyrogen injection, and it needed

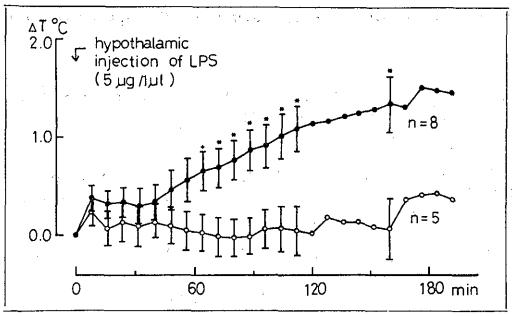

Fig. 1 Changes of the rectal temperature in the rats after a hypothalamic injection of 5 μg bacterial endotoxin and those after a saline injection. Each value represents mean ± S.E. The solid circles represent the test group, and the empty circles indicate the control group.
+: $P<0.1$; *: $P<0.05$

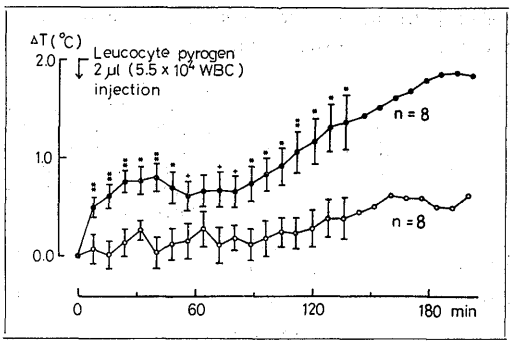

Fig. 2 Changes of the rectal temperature in the rats after a hypothalamic injection of 2 μl leukocyte endogenous pyrogen.
**: $P<0.01$

more than 3 h to reach the peak. Although the endogenous pyrogen used in the study was derived from the rabbit, a species distant from the rat, it was apparently capable of inducing fever in the rat. Figure 3 shows histological reconstruction of injection sites verified in the 14 rats which could make successively histological preparations in the study.

2. Behavioral study during induced fever

The second series of study dealt with the behavior of the rats, with or without fever

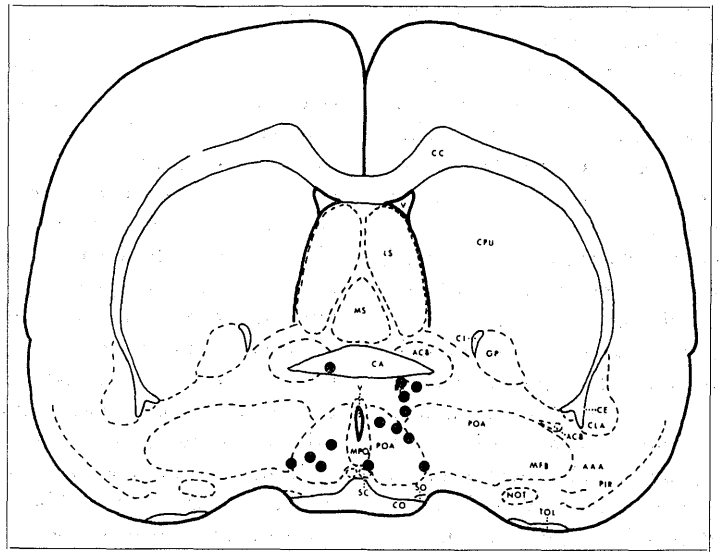

Fig. 3 The sites of pyrogen injection in 14 rats which could make successively histological preparations in Fig. 1 and 2. Drawn according to Pellegrino (1967).

Table 1 Changes of bar-pressing rate, and rectal, tail-skin and ambient temperature in pyrogen-injected and radiant heat-exposed rats

	No. of animals studied	Pyrogen	Interval between pyrogen injection and start of radiant heat (min)	Cumulative bar-pressing rate during first 60 min of radiant heat exposure. Difference from control	Temperature at 60 min after starting radiant heat exposure. Difference from control (°C)		
					Rectal temperature	Tail-skin temperature	Ambient temperature
Group 1	8	Bacterial endotoxin	0	−12.75±3.23**	0.16±0.22	0.70±0.70	0.30±0.54
Group 2	8	〃	30	−17.38±4.79**	0.45±0.11**	2.10±0.54**	3.18±0.68**
Group 3	8	Leukocyte endogenous pyrogen	9	−15.75±3.86**	0.51±0.16*	1.50±0.58*	3.31±1.07*

All values are the means±S.E.
*; $P<0.05$ **; $P<0.01$

after a pyrogen injection, under radiant heat. Three groups of study were performed. 1) The first group of rats (n=8) were simultaneously exposed to both radiant heat and hypothalamic endotoxin injection. 2) The second group of rats (n=8) first received the hypothalamic endotoxin injection, followed 30 min later by the exposure to the radiant heat. 3) The third group of rats (n=8) recieved hypothalamic injection of endogenous pyrogen, followed 9 min later by the exposure to the radiant heat. The rats used in the above experiments also received saline injection and were exposed to the radiant heat in the same manner as the study groups. They served as controls.

Table 1 shows the changes in the rectal, tail-skin and ambient temperature between the test and control groups, 60 min after the initiation of the heat exposure, and cumulative bar-pressing rates. The cumulative bar-pressing rates in the test three groups all decreased significantly compared with those in the control groups. The magnitude of the difference between the test and control groups varied depending upon the interval in the test groups between the pyrogen injection and initiation of the radiant heat, and the quality of the pyrogen; endotoxin or endogenous pyrogen. The rectal, tail-skin and ambient temperatures rose significantly in the second and third test groups. No significant rise of the temperatures, on the other hand, was observed in the first group, in which both the pyrogen and radiant heat were given simultaneously and the behavioral study was performed over the latent period of the fever.

The chronological changes of the ambient temperature, the tail-skin and rectal temperatures, and the bar-pressing rate are recorded for the second group (Fig. 4) and the third group (Fig. 5). In both groups, a reduction of the bar-pressing rate was noted, although it was not always significantly different from the controls. The rectal temperature increased with passing time. The tail-skin and ambient temperatures kept increasing during the experiments. In the first group, not illustrated and in which both the environmental heat and hypothalamic injection were simultaneously given, no significant rise was observed in the rectal and ambient temperatures. The tail-skin temperature tended to increase and the bar-pressing rate tended to decrease, especially dur ing the latter half of the experiment, but the changes were not statistically significant.

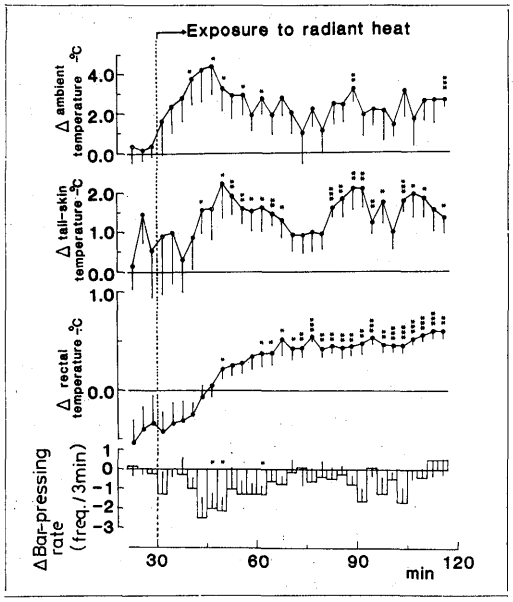

Fig. 4 The second group. Hypothalamic injection of bacterial endotoxin followed with a 30 min interval by continuous exposure to radiant heat, interruped 8 sec by each bar-pressing motion by the rat.

*: $P<0.05$; **: $P<0.01$, ***: $P<0.001$.
Changes of the rectal, tail-skin and ambient temperature, as well as those of the bar-pressing rate expressed as means of differences between the study and control pairs.

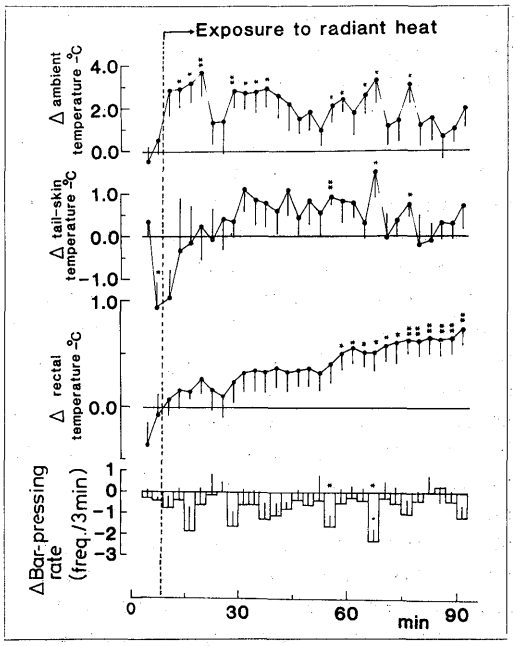

Fig. 5 The third group. Injection of leukocyte endogenous pyrogen followed with a 9 min interval by continuous exposure to heat, interrupted 8 sec by each bar-pressing motion.

Discussion

Our study confirmed the previous reports that the hypothalamic injection of bacterial endotoxin in the rat is capable of inducing fever[4], with a latency period of around one hour[8]. The hypothalamic injection of leukocyte endogenous pyrogen was also followed by fever, but with a short latency period. The absence of febrile response in the rat to intravenous or intraperitoneal injections of pyrogens, described in the literature, may be due to the presence of a blood-brain barrier that inhibits the penetration of the pyrogens to the hypothalamic region.

There have been many reports indicating that in mammals changes in the autonomic function, i.e. vasoconstriction and increased heat production etc., mainly contribute to the development of fever. During fever, the body temperature is reset at a new level. In a neutral or warm environment, this shift is accomplished by the inhibition of heat loss, rather than an increase of heat production[3]. Once the body temperature reaches the newly set level, thermal equilibrium remains at this level.

The importance of behavioral regulation of the body temperature was discovered more than 15 years ago[2]. It has since been clarified that the behavioral and autonomic thermoregulation complement each other and that the deep body temperature and the skin temperature play the most powerful inputs for both thermoregulation. Studies on cold-blooded animals[8] and some infant mammals[9]

revealed that they also increase their internal temperature by behavioral means when infected with pathogenic organisms. However, as mentioned earlier, no reports have been published on behavioral thermoregulation in the rat. Our study indicated that the rat, even during the exposure to environmental heat, may raise its body temperature after a hypothalamic injection of bacterial endotoxin or leukocyte endogenous pyrogen. The increase of the rectal temperature and the high level of the tail-skin temperature, due to the exposure to heat, may be expected to activate the heat escape response, an indicator of the thermoregulatory behavior. Because they are powerful inputs for the behavioral thermoregulation as mentioned above. However, their heat escape response was suppressed not only during the elevated rectal temperature but also during the initial 60 min of the study when the increase of the rectal temperature was not significant (Table 1). Therefore, it was assumed that the hypothalamic injection of pyrogens affects the behavioral thermoregulation through an alteration of the activities of the thermoregulatory neuronal network. It was also clarified that the alteration of the activity in the CNS already started even during the latent period of the fever.

The thermoregulatory behavioral change after the injection of leukocyte endogenous pyrogen was similar to, but less marked than that after the bacterial endotoxin injection. The two pyrogens also differed in their latency period: that with bacterial endotoxin was longer than that with leukocyte endogenous pyrogen. This long latent period following an injection of endotoxin are explained to be related to the phagocytosis of endotoxin and the subsequent production of endogenous pyrogen.

The suppression of the heat escape response and the preference of raised ambient and skin temperatures appear to be a feature characteristic not only of the rat but also of other mammals[10], under developing of behavioral fever.

Supported by a Grant-in-Aid for Scientific Research from the Ministry of Culture and Education of Japan.

References

1) Corbit, J.D.: Behavioral regulation of body temperature. In J.D. Hardy, A.P. Gagge and J.A.J. Stolwijk (eds.), Physiological and behavioral temperature regulation, Ch. C. Thomas, Springfield, Ill, 1970, p. 777–801.
2) Cabanac, M.: Temperature regulation. *Ann. Rev. Physiol.*, 37 : 415–439, 1975.
3) Stitt, J.T.: Fever versus hyperthermia. *Fed. Proc.*, 38 : 39–43, 1979.
4) Splawinski, J.A., Zacny, E. and Gorka, Z.: Fever in rats after intravenous E. coli endotoxin administration. *Pflügers Arch.*, 368 : 125–128, 1977.
5) Bollman, J.L., Cain, J.C. and Grindlay, J.H.: Technique for collection of lymph from liver, small intestine or thoracic duct of the rat. *J. Lab. Clin. Med.*, 33 : 1349–1352, 1948.
6) Pellegrino, L.J. and Cushman, A.J.: A stereotaxic atlas of the rat brain. Appleton-Century-Crofts, New York, 1967.
7) Cranston, W.I., Luff, R.H., Rawlins, M.D. and Rosendorff, C.: The effects of salicylate on temperature regulation in the rabbit. *J. Physiol.*, 208 : 251–259, 1970.
8) Kluger, M.J.: Fever, its biology, evolution and function. Princeton University Press, Princeton ,1979, p. 106–128.
9) Satinoff, E., McEwen, Jr., G.N. and Williams, B.A.: Behavioral fever in newborn rabbits. *Science*, 193 : 1139–1140, 1976.
10) Crawshaw, L.I. and Stitt, J.T.: Behavioral and autonomic induction of prostaglandin E_1 fever in squirrel monkeys. *J. Physiol.*, 244 : 197–206, 1975.