Development Changes in the Febrile Response to Endotoxin in Rabbit

Akira NISHIO and Seizaburo KANOH*

Department of Veterinary Pharmacology, Faculty of Agriculture, Kagoshima University, Kagoshima, 890 Japan
*Department of Pharmacology, National Institute of Hygienic Sciences, Osaka Branch, Osaka, 540 Japan

Abstract The pyrogenicity of E. coli endotoxin (lipopolysaccharide, LPS) was measured at both 25°C and neutral temperatures (Tn) in day -1 to adult rabbits. Intravenous injection of endotoxin (0.5 μg/kg) produced febrile response at Tn in day -1 rabbits, but not at 25°C. A similar response was observed when endotoxin was injected s.c. (1.0 μg/kg) or intracisternally (0.0001 μg/kg). The mean magnitude of the rise of rectal temperature increased with the advance of age. Propranolol abolished the endotoxin-fever in day 3 rabbits. The inhibitory effect of propranolol was incomplete in day 14 rabbits and was not seen in day 28 rabbits. The inhibitory effect of phentolamine was not observed in day 3 and day 28 rabbits, but the blocker reduced the febrile response in day 14 rabbits. No shivering activity was evident in day 3 rabbits after injection of endotoxin. In the day 7 and 14 rabbits, shivering was less significant and of shorter duration than in the case of day 28 rabbits. The endotoxin caused no significant decrease in ear skin temperature in day 3 rabbits. A decrease in ear skin temperature was observed in rabbits older than 14 days. Thus, pyrogenic sensitivity is apparent even in day -1 rabbits. The mechanism of the heat production underlying endotoxin-fever gradually shifted from the nonshivering thermogenesis mediated by catecholamines to shivering thermogenesis during the first month of life.

Although it has been reported that newborn lambs and guinea pigs develop no fever to intravenous (i.v.) injection of endotoxin during their first postnatal week (PITTMAN et al., 1973; BLATTEIS, 1975, 1976), these neonates demonstrated appropriately competent thermoregulatory activities when exposed to a moderately cold environment immediately after birth (ALEXANDER, 1961; BRUCK and WÜNNENBERG, 1965, 1966).

Newborn rabbits, day 0 to 3, developed fever to i.v. or intraperitoneal (i.p.)
injection of endotoxin (SZÉKELY and SZELÉNYI, 1977; SZÉKELY, 1978). On the other hand, SATINOFF et al. (1976) reported that 12 to 72 hr rabbits showed thermoregulatory behavior, but these newborns produced no fever to i.v. or i.p. injection of endotoxin.

The absence of fever in the newborn mammals has been attributed to the immaturity of the central endotoxin-receptive mechanisms, to the insufficient heat-producing and retaining ability to elicit fever, or to the incapacity of leukocytes to produce endogenous pyrogen. Febrile responses are most likely influenced by environmental temperature. In this paper, responses to endotoxin were observed at the respective neutral temperature which varies from day to day in the neonatal stage. The developmental changes in the mechanism of the heat production in the endotoxin-fever was investigated by the use of adrenergic blocking agents. A preliminary report of the results has been presented previously (NISHIO and KANOH, 1977).

MATERIALS AND METHODS

Japanese albino rabbits of both sexes aged -1, 0, 3, 7, 14, 21, and 28 days and adult male rabbits were used. Day -1 rabbits were the neonates obtained with cesarean section one day before delivery (harvested on the 30th day of gestation). All the animals used were born in the laboratory animal room. The neonates remained with their mothers until day 25 when they were weaned. Thereafter, they were fed on a diet of rabbit pellets and tap water, ad libitum. The environmental temperature and humidity in the animal room were maintained at 25±1°C and 50±10%, respectively.

The endotoxin used was a lipopolysaccharide (LPS) extracted from E. coli UKT-b after the method of WESTPHAL and LÜDERITZ (1954). The details of the chemical properties of LPS have been described in a previous report (NISHIO and KANOH, 1972). The LPS was dissolved in the sterile pyrogen-free saline (1 mg/10 ml), and diluted to the appropriate concentration with the sterile pyrogen-free saline. The dose of LPS and the method of injection are mentioned in the figures. Phentolamine mesylate (Regitin, CIBA) and propranolol hydrochloride (Inderal, Ayerst Lab.) were used as α- and β-adrenergic blockers. These chemicals were diluted by pyrogen-free saline (5 mg/ml) and were given subcutaneously (s.c.) (1 ml/kg).

The rectal and ear temperatures were measured with copper-constantan thermocouples. Rabbits were enclosed in a restrainer consisting of meshed hemicylinders of varying diameter, designed to accommodate rabbits of different sizes. The restrainers were placed in the temperature-controlled box. Animals were left for at least 90 min before the injection of endotoxin to confirm the normal rectal temperature of the individual rabbit. Temperatures were recorded at 30 sec intervals for a period of 5 hr after the injection of endotoxin on a multichannel
pen-recorder. The experiments were conducted under two ambient thermal conditions; at a temperature of 25°C, and at a neutral temperature corresponding to age; day -1 (35.5°C), 0 (35), 1 (34.5), 3 (33), 7 (32), 14 (29), 21 (27), 28 (25) (Hull, 1965).

Shivering activity was recorded by two needle electrodes inserted into the flanks of the animal. All the glasswares and needles used in the experiments were sterilized by dry heating at 250°C for 2 hr. The student t-test was used for statistical analysis. The level of significance adopted in this paper was $p<0.05$.

RESULTS

*Effect of ambient temperature on the rectal temperature of newborn rabbits*

Newborn rabbits have little fur and a large surface area relative to the body weight, so that they are quite poor in thermal insulation (Hull, 1965). Accordingly, ambient temperature is one of the important factors in evaluating the pyrogenic response to endotoxin in newborn rabbits.

The steady-state rectal temperatures of rabbits exposed to the two different environmental temperatures (25°C and $T_n$) are compared in Fig. 1. The values represent the mean ± S.D. of the individual measurements on 20 to 30 rabbits. Stable levels were usually obtained within 60 min at 25°C and within 90 min in $T_n$, after the onset of exposure.

Fig. 1. Changes in the rectal temperature ($T_{re}$) of rabbit exposed to the room ($T_r$) and neutral ambient temperature ($T_n$) after birth. Each point represents the mean of 20 to 30 rabbits, and vertical bars indicate the standard deviation. ●, constant $T_{re}$ of the rabbit exposed to $T_n$; ○, constant $T_{re}$ of the rabbit exposed to $T_r$. 

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During the first 7 days of life, the rectal temperature of the rabbit was significantly lower at 25°C than at Tn. However, after day 14, there was no difference in the two environments. At Tn, the rectal temperature did not significantly differ according to age. As shown in Fig. 1, body weight increased rapidly after day 3 of life.

Fever in the newborn rabbits and its developmental changes

The time courses of the rectal temperature following the injection of endotoxin at Tn are illustrated in Fig. 2. In day –1 to 1 rabbits, i.v. injection of endotoxin (Fig. 2, upper trace) induced a monophasic febrile response, and their rectal temperature started to rise after a latency of about 15 min and reached a peak about 90 min after the injection. In the adults, the biphasic febrile response was observed, and their rectal temperature started to rise after a latency of about 15 min, reaching the first peak at about 90 min and the second about 180 min after the injection. Intracisternal injection of endotoxin (Fig. 2, lower trace) induced a monophasic febrile response both in the newborn and in the adult rabbits. At 25°C, no fever was observed in the newborn rabbits younger than day 3.

In Fig. 3, it was noteworthy that the febrile response was significantly lower in the newborn rabbits day –1 to 3 than in the adult rabbits. The dose-response relationship was compared in both the newborn and the adult rabbits at Tn. The response was lower in the newborn than in the adult rabbits, especially in the case of i.v. injection.

Fig. 2. Effect of LPS on the rectal temperature in the newborn* (solid lines) and adult rabbits (broken lines) at the neutral ambient temperature. Each curve represents the mean of 6 to 10 animals. Vertical bars show the standard deviation. * –1 to 1 day-old.

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Fig. 3. Dose-response of febrile response in the newborn* (●) and the adult rabbits (○) at the neutral ambient temperature. Each point represents the mean of 10 to 20 rabbits and vertical bars indicate the standard deviation. i.c., intracisternal injection; i.v., intravenous injection; s.c., subcutaneous injection. *—1 to 3 day-old.

Fig. 4. The magnitude of rectal temperature rises to intravenous and intracisternal injections of LPS in the developing rabbit at the neutral ambient temperature. Each point represents the mean of 6 to 10 rabbits and vertical bars indicate the standard deviation.

Developmental change in the fever-height induced by endotoxin is shown in Fig. 4. The fever-heights induced by the i.v. injection of endotoxin were lower in the rabbits younger than day 7 and the fever curves were characterized by the single peak. In the rabbits over day 14, the biphasic fever curves were observed. The degree of fever-heights induced by intracisternal injection showed no significant difference through all the ages examined, but younger rabbits showed weaker responses than in the case of adult rabbits.

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Shown in Fig. 5 are the changes of ear skin temperature during fever at different developing stages. At $T_n$, a significant decrease in ear skin temperature was produced by the endotoxin in day 14 and 21 rabbits. The decrease in ear skin temperature was associated with the increased rectal temperature. But in the rabbits younger than day 7, the ear skin temperature was elevated with the increased rectal temperature.

Effect of $\alpha$- and $\beta$-blocker on endotoxin-fever in developing rabbits

Cold-induced thermogenesis in the newborn rabbits is initiated and controlled by the sympathetic nervous system, and norepinephrine is the mediator at the nerve endings (HULL and SEGALL, 1965). To determine whether endotoxin-
fever in newborn rabbits was mediated by the noradrenergic sympathetics, propranolol (β-adrenergic antagonist) or phentolamine (α-adrenergic antagonist) was administrated s.c. immediately after the injection of endotoxin. As shown in Fig. 6, propranolol prevented the endotoxin-fever in the rabbits at day 3. The inhibitory effects of propranolol were incomplete in day 14 rabbits and not seen in day 28 rabbits. Phentolamine showed no significant reduction of endotoxin-fever in the rabbits at day 3, but a significant reduction in the rabbits aged day 7 to 14. No effect was observed in the rabbits of 28 days of age.

Developmental changes of shivering thermogenesis during endotoxin-fever
Since propranolol or phentolamine did not alter the endotoxin-fever in the rabbits at day 28, developments of shivering thermogenesis during endotoxin-fever were studied in day 3, 7, 14 and 28 rabbits. As shown in Fig. 7, the endotoxin
administration did not produce shivering at $T_a$ in rabbits aged day 3. Shivering activities were associated with the increased rectal temperature in rabbits of 7, 14 and 28 days of age. The degree and duration of shivering activity tended to be augmented according to the advance of age.

**DISCUSSION**

The present results indicate that the newborn rabbits obtained with cesarean section one day before delivery are able to produce fever to endotoxin at the thermoneutral temperature. This agrees with the findings of SZEKELY (1978) and SZEKELY and SZELÉNYI (1977), but not with the results of SATINOFF et al. (1976) who reported that newborn rabbits injected with endotoxin were unable to develop fever at $T_a$ of 32°C. This $T_a$ (ambient temperature) seems to be low for their newborn rabbits to maintain normal body temperature. In newborn rabbits younger than day 3 of age, no shivering and no vasoconstriction were observed (Figs. 5, 7). Non-shivering thermogenesis (NST) in the brown adipose tissue was controlled by the sympathetic nervous system (HULL, 1965; HULL and SEGALL, 1965; HEIM and HULL, 1966). The temperature of the brown adipose tissue was elevated by endotoxin-administration in young guinea pigs (BLATTEIS, 1976). NST in rabbits younger than day 3 of age seems to be the prevailing mechanism of heat production in endotoxin-fever. Usually, no newborn rabbits showed a biphasic fever curve after i.v. injection of endotoxin. These findings may be attributed to the inability of leukocytes to produce endogenous pyrogen or to the immaturity of the system involved in heat production. The present experiments suggest that the latter case is probable. However, the question whether endogenous pyrogen is present or not in the bloodstream of the newborn rabbits during endotoxin-fever remains obscure at present.

The present results show that the newborn rabbits younger than day 6 of age differ from the newborn guinea pigs and lambs of corresponding ages which produce no fever to endotoxin injected into the vein or into the hypothalamus (PITTMAN et al., 1973, 1977; BLATTEIS, 1975; BLATTEIS and SMITH, 1979). The cause of these species differences in the neonatal febrile response is unknown.

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**REFERENCES**


