Pyrogenic Responses of the Pika, *Ochotona rufescens rufescens*

Takeo SAKAI, Yukio KODAMA, and Shigetomo HORIUCHI*

Department of Preventive Veterinary Medicine, College of Agriculture and
Veterinary Medicine, Nihon University, 1866 Kameino, Fujisawa-shi,
Kanagawa 252 and Hamamatsu Research Center, 95-15
Aoi-cho, Hamamatsu-shi, Shizuoka 433, Japan

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In 3-month-old male and female pikas, *Ochotona rufescens rufescens*, reared at an ambient temperature of 25°C, the mean within-day body temperature was 39.2°C without significant variance. At an ambient temperature of 35°C it increased markedly in males while decreased slightly at 5°C. The pikas showed lower sensitivity than Japanese white rabbits to a pyrogenic *E. coli* endotoxin. — KEY WORDS : body temperature, endotoxin, pika, pyrogen test, thermometer

Puget [9, 10] described the successful breeding of the pika, *Ochotona rufescens rufescens* from the mountains of Afghanistan. After serial breeding by Puget, the pikas were introduced into Japan, and their usefulness as a new laboratory animal species has been evaluated [6-8, 13, 15, 16]. We studied the histology and enzymatic histochemistry of pika skin [12] and its response to primary irritants [11]. We also investigated the experimental induction of gastric ulcers in pikas [5]. This paper deals with findings regarding the pyrogenic responses of pikas.

Materials and Methods

Animals : Eight male and 6 female laboratory-born pikas from the Biological Safety Research Center of the National Institute of Hygienic Sciences, aged 3 months and weighing between 200 and 300g, were subjected to a pyrogen test. Five each of male and female Japanese adult white rabbits aged 12 to 14 weeks and weighing 2.5 to 3.2 kg were purchased from a commercial breeder (Funabashi Farm, Chiba) and were used for similar tests.

Rearing : The pikas were kept in individual cages (26W × 33D × 22 H cm) within feeding chambers (CR-M type, Nihon Airtech, Tokyo) kept at 23 ± 1°C and 55 ± 5% relative humidity

with a total of 70 air changes per hour and 12 hours illumination per day. The rabbits were housed in aluminum cages (R-2 A type, Okazaki Sangyo, Tokyo) kept at 25 ± 1°C and 55 ± 5% relative humidity with a total of 18 air changes per hour and 12 hours illumination per day. Pikas and rabbits were freely fed pika (CLEA-177 type, CLEA Japan) and rabbit pellets (RM-1 type, Funabashi Farm), respectively.

Measurement of body temperature : As pillory-type immobilizers and thermistor-type automatic recorders (Iio electric, Tokyo) normally used for rabbit pyrogen tests are not appropriate for pikas, an electronic thermometer (Omron, Tokyo) was used. The thermometer was inserted into the rectum of the pikas restrained with a rolled cloth, 15 cm wide. The depth of the insertion was marked as 0.5, 1.0, 1.5, 2.0, and 3.0 cm from the proximal end of the sensor to the anal sphincter. Preliminary information concerning the depth of insertion and recorded temperature was obtained. Then, an optimum depth was selected and measurements were performed at 1 to 1.5 hr intervals.

The within-day variance in body temperature of 8 male and 6 female pikas was studied, and changes in body temperature were subsequently effected in 8 male pikas placed in wooden incubators (60W × 40D × 50 H cm) at 5, 25, or

35°C.

Pyrogen test: The test was performed according to the methods of Kanoh *et al.* [4] and Itami *et al.* [3]. An *E. coli* endotoxin (5 ng/ml) provided by the Osaka Branch of the National Institute of Hygienic Sciences was injected subcutaneously, intramuscularly, or intraperitoneally into pikas at 25, 50, or 100 ng/kg, and into rabbits at 10 ng/kg.

Fig. 1. Within-day variance of the body temperature of pikas aged 13 weeks ●—●: males (n = 8) ○—○: females (n = 6) Each circle with a vertical bar represents the mean $\pm$ SE.

Fig. 2. Changes in the body temperature of male pikas in different ambient temperatures ■—■: 5°C ●—●: 25°C ▲—▲: 35°C Each point represents the mean of 8 animals.

Results

The men body temperatures of 5 adult male pikas in relation to the depth of insertion from the proximal end of the sensor to the anal sphinctor were 37.5°C at 0.5 cm, 38.2°C at 1.0 cm, 39.0°C at 1.5 cm, 39.2°C at 2.0 cm, and 39.3°C at 3.0 cm. In the following study, the depth between 1.5 to 2.0 cm was selected.

Fig. 3. Changes in body temperature of male (n = 8) and female (n = 6) pikas after subcutaneous injection of *E. coli* endotoxin ○—○: saline ■—■: 20 ng/kg ▲—▲: 50 ng/kg ●—●: 100 ng/kg Each point represents the mean of 14 animals.

Fig. 4. Changes in body temperature of male (n = 5) and female (n = 5) rabbits after subcutaneous injection of 10ng of *E. coli* endotoxin/kg ○—○: saline ●—●: *E. coli* endotoxin Each point represents the mean of 10 animals.

The body temperature of pikas at a room temperature of 25°C ranged from 38.8°C to 40.3°C with a mean of 39.2°C in both sexes and there was no significant within-day variance (Fig. 1). Body temperature fluctuations at 5°C, 25°C, and 35°C were 0.2–0.4°C, 0.1–0.2°C and 0.5–0.9°C (Fig. 2).

After subcutaneous administration of 100 ng of endotoxin/kg, the body temperature of the pikas, both sexes, increased by 0.7°C (Fig. 3). No effect was seen at 20 or 50 ng/kg. In rabbits, an increase in body temperature of 1°C or more was observed at a 10 ng/kg dose (Fig. 4) regardless of the method of administration.

Discussion

Rabbits have been widely used in pyrogen testing due to their stable body temperature and high sensitivity. In particular, the Dutch rabbit has been considered useful for such testing [3, 14] because of its high resistance to diseases, and easy handling [1].

The body temperature of the pika, which is much smaller than rabbits, was shown to be sufficiently stable at low ambient temperatures, decreasing only slightly at 5°C but increasing markedly at 35°C. This latter phenomenon may be due to the fact that the species originated from the Afghan highlands. On the other hand, the sensitivity of pikas to *E. coli* endotoxin was lower than that in rabbits. Further studies are needed before using this species in pyrogen tests.

The pika, the smallest species of the order of rabbits, has been determined to be useful as an experimental animal [5–8, 11–13, 15, 16]. Matsuzaki *et al.* [7] reported that the optimal environmental conditions for breeding pikas, were 22±2°C, 55±5% relative humidity, 10–15 times ventilation per hour, and 16 hours illumination per day. At our laboratory, with pikas bred in a conventional facility together with mice and rats, the breeding data (shown below) were not markedly different from that reported by Puget [9, 10] and Matsuzaki *et al.* [7].

For our purposes, the breeding was carried out according to the methods of Matsuzaki *et al.* [7]. Three-month-old sexually mature male and female pikas were placed in a cage overnight. In cases of unsuccessful cage-mating, females were mated with another male. The

day of copulation was regarded as day 0 of pregnancy. Pregnant pikas were placed in wooden cages (21W×17D×13H cm) with wood shavings as bedding. The animals were weighed twice a week. Most neonates were weaned within 3 weeks after birth and males and females were reared separately. By 6 weeks of age, the animals were maintained individually. Twenty males and females were weighed monthly for 15 months after birth.

During breeding, copulation was observed in a total of 63 females, of which 45 (71.4%) became pregnant. The gestation period was 25, 26, 27, and 28 days in 2, 14, 19, and 10 animals, respectively, with a mean gestation period of 26.8 days. The mean weight on day 24 of gestation was about 5 times greater than that on day 3. The mean litter size was 4.9, ranging 1 to 9, and the weaning rate was 65.3% within 3 weeks after birth. The major causes of death in pups were compression and cannibalism by the dams, the latter being frequently observed immediately after parturition. The mean body weight of the pups was 8.9±1.5 g immediately after birth, and increased almost linearly thereafter to 43.5±5.5 g at 2 weeks of age.

Since about 30% of inseminated pikas fail to gestate, females should be checked early for pregnancy and be mated again to increase breeding efficiency. Indeed, body weight gain within 7 days after copulation may be an effective parameter with which to confirm gestation. Matsuzaki *et al.* [7] reported that cannibalism shortly after parturition was not as frequent in pikas as in rabbits. In this study, however, many neonates were cannibalized, resulting in a mean of 1.8 dead pups per litter while nursing continued. Such cannibalism occurred mostly in the first parturition of the dams. The weaning rate of pikas in this study (65.3%) was not different from that observed in rabbits. The postnatal body weight gains were nearly the same as those reported by Puget [10] and Matsuzaki *et al.* [7], and individual variations were much smaller in this study, indicating successful rearing and breeding for nearly 10 years.

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ナキウサギの発熱反応

井 健 夫・児 玉 幸 夫・堀 内 茂 夫*

日本大学農獣医学部獣医衛生学研究室

*生物科学研究所

3ヵ月齢のナキウサギ (*Ochotona rufescens rufescens*) の昼間の体温は、25℃環境下では雌雄とも平均 39.2℃で、変動は小さかった。ナキウサギ雄の体温は、35℃の環境下では大きく上昇し、5℃の環境下では小さ

く下降した。25℃環境下での発熱物質である *E. coli* の endotoxin に対するナキウサギの感受性は、ウサギ (日本白色種) に比べて低かった。