

Physiological Characteristics of Pika (*Ochotona rufescens rufescens*) as a Weak Heat Tolerant Animal

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Abstract: Since 1985, Afghan pika (*Ochotona rufescens rufescens*) provided by the Central Institute for Experimental Animals, Japan, has individually been reared in P₂-facilities at 22 °C, in the Animal Research Center for Infectious Tropical Diseases, Institute of Tropical Medicine, Nagasaki University. The morphological characteristics and thermoregulatory functions of Afghan pikas such as the constitutionally short rounded ears, a small tail, extremely thin abdominal skin, moderate LPS-pyrogenic response, high metabolic rate, poor heat dissipation and high body temperature, as compared to albino rabbits, enable them to ecologically adapt to cold- and high altitude-environments. However, recent studies have reported that the pika has weak heat tolerance and is sensitive to heat. The present study, therefore, was designed to elucidate weak heat tolerance in the Afghan pika through cellular, cardiovascular, thermophysiological and morphological approaches to reveal that the mechanism of weak heat-tolerance in Afghan pika might be closely related to lack of autonomic heat loss response, such as thermal panting and salivation, as well as difficulty of induction of heat-tolerant substances, the so-called heat shock proteins (HSPs). Also, these morphological, cardiovascular findings were compared to those of the wild Chinese pika and discussed from the viewpoint of thermal physiology.

Key words: Pika (whistle rabbit), Thermoregulation, Thermal panting, Life span, Heat shock proteins (HSPs), Interleukin–1 (IL–1)

INTRODUCTION

The Afghan pika (*Ochotona rufescens rufescens*) is a herbivorous animal belonging to the Family Ochotonidae of the Order Lagomorpha (Fig. 1). Members of the Genus *Ochotona* live above timber line or in cold zone or in high mountain ridges such as the Himalayas, Alaska, Manchuria, Rocky Mountains and Ural Mountains (Goodwin, 1968). In Japan, the Yezo pika

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All pikas were reared and bred at the Animal Research Center for Infectious Tropical Diseases, Institute of Tropical Medicine, Nagasaki University.

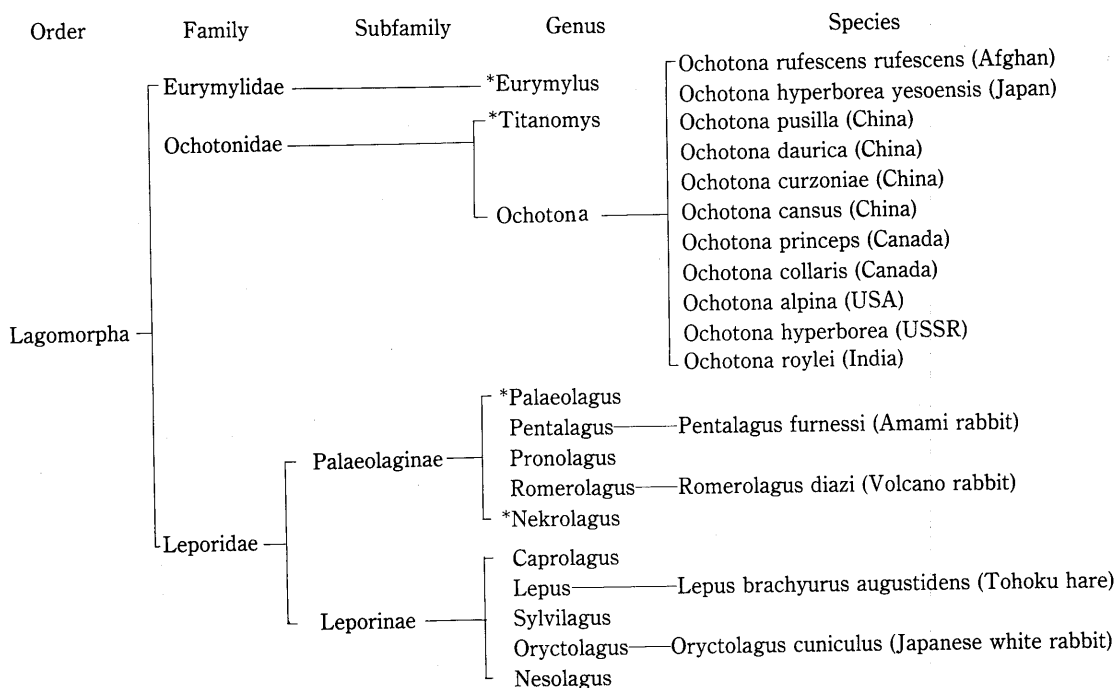


Fig. 1. Classification of the Order Lagomorpha. Cited from Fox (Fox, 1974) and partially modified. *These genres of rabbits were extinction

(*Ochotona hyperborea yesoensis*) is found near the summit of Mt. Daisetsu in Hokkaido (Haga, 1958). Pikas are paleontologically extremely primitive, and are considered to be "living fossils" of the Eocene epoch in the Tertiary period (65–25 million years ago).

Since 1985, Afghan pikas supplied from the Central Institute for Experimental Animals, Japan, were reared and bred in the Animal Research Center for Infectious Tropical Diseases, Institute of Tropical Medicine, Nagasaki University (Kosaka *et al.*, 1985, 1987, 1988; Yang *et al.*, 1988). Originally, they were collected in Afghanistan by Puget in 1969 (Puget, 1973a, b). The Afghan pika lives mainly in Afghanistan and Iran, and the distribution area is mainly located at high altitudes: 1,800–3,600m.

A photograph of an adult pika (female, 6 months old, 240g) is shown in Fig. 2. Adult pikas weigh 250g, and exceptionally 300g. They have silky and short fawn fur. The very short neck supports a globulous head with black, prominent eyes and short, rounded ears. The vibrissae are relatively long. The tail composed of 8 vertebrae, is not visible from the outside (Puget, 1973a).

The morphological characteristics and thermoregulatory functions in pikas, such as the short rounded ears, a short tail, moderate pyrogenic response to LPS, high metabolic rate, high body temperature and poor heat dissipation ability, as compared to albino rabbits, were previously thought to be ecological adaptations to cold-environment (Kosaka *et al.*, 1985, 1987; Yang *et al.*, 1988).

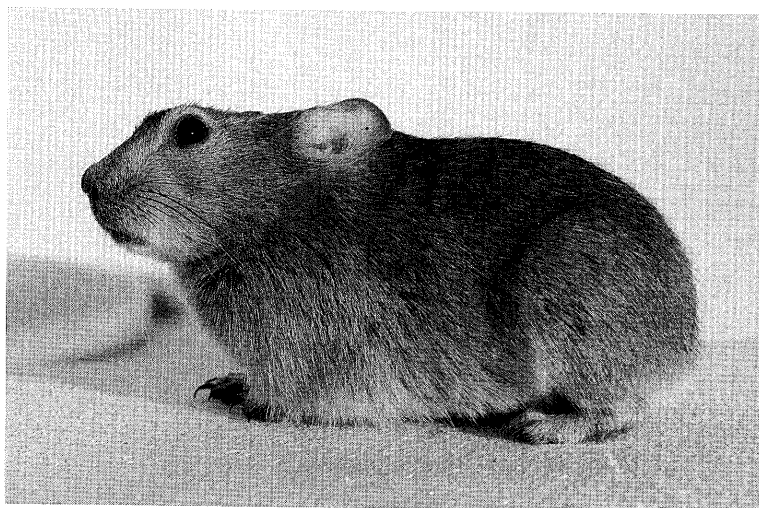


Fig. 2. An Afghan pika (female, 6 months old, 240g). (For details see text).

On the other hand, pikas' distribution ranges widely from low to extremely high altitudes (about 10 to 6,100m above sea level). The pika is thought to be a high-altitude adapted animal (Sakai *et al.*, 1988). Biological data of pikas as new laboratory animals is reviewed by Saitoh (1989).

Recent reports revealed that the heat shock protein 70KD family (HSP70) induced by heat relates to thermotolerance (Alahiotis, 1983; Hatayama *et al.*, 1985, 1986; Ohtsuka *et al.*, 1986). It is very interesting to examine the induction of HSPs in pikas after heat exposure to clarify the physiological mechanisms of weak heat-tolerance in pikas. A lack of induction of HSPs might be a disadvantage to survival in hot environment.

In the present study, therefore, in order to elucidate the mechanisms of strong cold- and weak heat-tolerance, several characteristics of pikas were examined from the viewpoints of cellular, cardiovascular and thermal physiology.

ANIMALS AND METHODS

In the present investigation, a total number of 131 Afghan pikas (*Ochotona rufescens rufescens*) were used for morphological and biological estimations and thermoregulatory experiments. The pikas were reared in individual stainless steel cages at 22°C with a 12:12 h light-dark photoperiod, with lights on at 0800 a.m. Tap water and special chow (Matsuzaki *et al.*, 1980) were provided ad libitum. Each pika was used for experiment only once.

Life span of pika. For an estimation of the life span, 101 pikas, 45 male and 56 female, which naturally died were used. Pikas which died within 4 weeks after birth were excluded since newborn pikas were separated from their mothers 4 weeks after birth. Pikas which died due to experiments were also excluded from the data.

Morphology of pika. Five pikas and 35 male Wistar rats (200–350g) were supplied for the cardiovascular system experiments. The ventricular weight was measured according to Fulton's method (Fulton *et al.*, 1952) i. e., the ventricle was separated into the left ventricle and septum (LVW) and the right ventricular free wall (RVW), and each weight was measured by an electro-balance (JL-180, Chyo Balance Corporation, Japan). Then the ratio of RVW/LVW was calculated and used as an index of right ventricular hypertrophy. Hematocrit values (Hct) were determined in some of these pikas, and in rats by an ordinal method.

The ratio of ear surface area to body surface area, which is closely related to heat loss ability in rabbits, was measured in 15 pikas and 4 rabbits (male Japanese white rabbits weighing 2.5–3.5kg). After sacrifice, the skin was removed from the body and the areas of ear skin and whole body skin were measured.

Pyrogenic response to IL-1. In an environmental control room of 28°C and 60% relative humidity, 2.0 µg/kg of recombinant human Interleukin-1 α (IL-1 α) was intravenously administered to unanesthetized freely moving pikas (n=3) through the marginal ear vein. Rectal temperatures were measured with thermistor probes (TF-DN, Terumo, Japan), and respiratory rates were measured by visual observation every 10 minutes.

Thermoregulatory responses to general heating. Changes concerning thermoregulatory responses such as respiratory rate, rectal and ear skin temperatures due to general heat load were compared between pikas (n=2) and rabbits (n=2). The unanesthetized animals were lightly restricted in an environmental control room. After each parameter was stabilized to an ambient temperature of 28°C, ambient temperature was stepwise raised to 33°C and further to 37°C. Relative humidity was constant at 60% throughout the experiment. Rectal and ear skin temperatures were measured every minute with thermistor probes (K923 TAKARA, Japan) connected to a personal computer (PC-9801 NEC, Japan), and respiratory rate was detected by a strain gauge transducer which was attached around animal's abdomen and counted with a data analyzer (ATAC 450, Nihon Kodan, Japan).

Induction of HSPs. Induction of heat shock proteins (HSPs), after whole body hyperthermia, was examined by SDS-PAGE in pikas (n=5) and rats (n=5). A weak anesthetized animal (Sodium pentobarbital 25mg/kg, i. p.) in a stainless box was immersed in a water bath at 43–45°C and the rectal temperature was kept at 41.8–43.0°C for 15 min. Animals were sacrificed 20 hours after heat shock and were exsanguinated from the inferior caval vein, and perfused with normal saline solution through a cannula inserted into the thoracic aorta. The liver was removed, homogenized and centrifuged at 15,000xg for 30min and 75,000xg for 90 min at 4°C. Finally the cytosol fraction was separated and liver tissue proteins were analyzed by 10% SDS-PAGE.

The results obtained in this study were compared to those of wild Chinese pikas (*Ochotona curzoniae*, n=36 and *Ochotona cansus*, n=2) (unpublished data).

Data were analyzed for statistical significance by unpaired Students' *t*-test and Mann-Whitney test at 0.05 level.

RESULTS

Life span of pika. Pikas have been reared in our laboratory since 1985. Up to now, a total of more than 200 pikas (F_9 of pika) were reared in the Animal Research Center. The histogram of the life span of pikas ($n=101$) is shown in Fig. 3. Average pika's life span in our laboratory was 56.4 ± 3.3 weeks (mean \pm SE), with a maximum of 144.0 weeks. Mean life span was 59.1 ± 5.6 weeks in male ($n=45$), 54.3 ± 3.9 weeks in female ($n=56$). There was no sex differences ($P > 0.05$) in life span of pikas as shown in Fig. 4. A shortening tendency of life span through breeding was not observed (data are not shown).

Morphology of pika. Fig. 5 shows the ratio of right ventricular weight to left ventricular weight, RVW/LVW, in pikas and rats. There was no significant difference in body weight between pikas and rats, 256.0 ± 51.3 g (mean \pm SD) in pikas ($n=5$) and 266.3 ± 28.0 g in rats ($n=35$). However, RVW/LVW in pikas (0.248 ± 0.013) was significantly lower than that in rats (0.305 ± 0.017), $P < 0.001$.

In rabbits, heat radiation from a large ear surface and thermal panting are major heat loss mechanisms. However pikas have fairly small rounded ears. The ratio of ear surface area to body surface area, ear/body surface ratio, of pikas ($7.2 \pm 0.7\%$) was significantly small compared to that of rabbits ($17.0 \pm 0.5\%$) as shown in Table 1.

Pyrogenic response to IL-1. Changes in rectal temperature and respiratory rate after i. v. administration of recombinant human IL- 1α ($2\mu\text{g/kg}$) in three pikas were demonstrated in Fig. 6. Fevers up to 1.5°C were induced by IL-1 administration. Respiratory rate responses, however, were not coordinated with the elevation of core temperature.

Thermoregulatory responses to general heating. Thermoregulatory responses during general heating in pikas were compared to those in rabbits. Typical recordings in a rabbit and a pika were shown in Figs. 7 and 8. At 28°C of ambient temperature, respiratory rate in

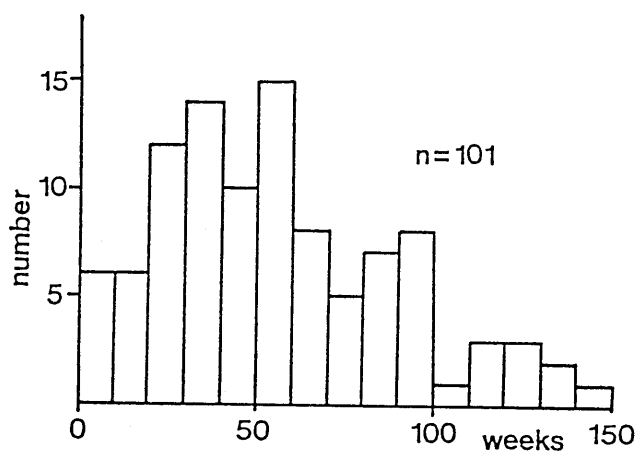


Fig. 3. The histogram of life span of the Afghan pikas ($n=101$) in our laboratory. Mean and SE of life span was 56.4 ± 3.3 weeks with a maximum of 144.0 weeks.

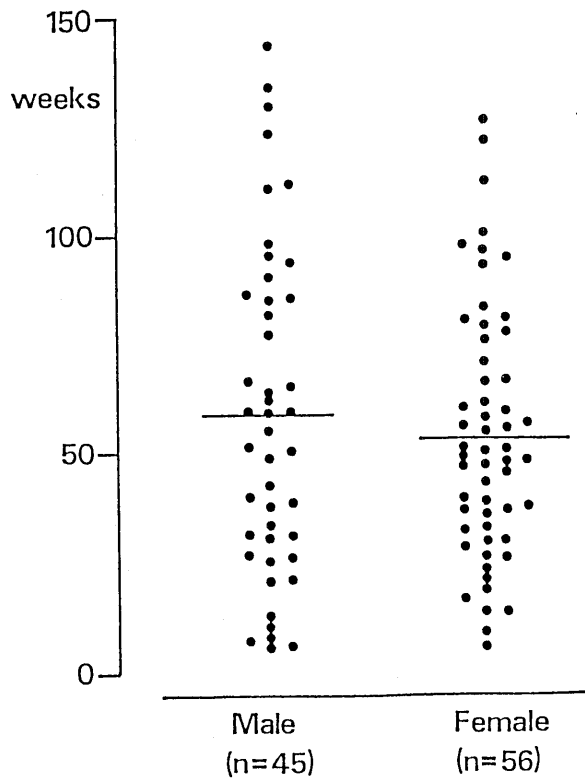


Fig. 4. Life span of the Afghan pika domesticated in our laboratory. Mean and SE of life span was 59.1 ± 5.6 weeks in male, 54.3 ± 3.9 weeks in female. (For details see text).

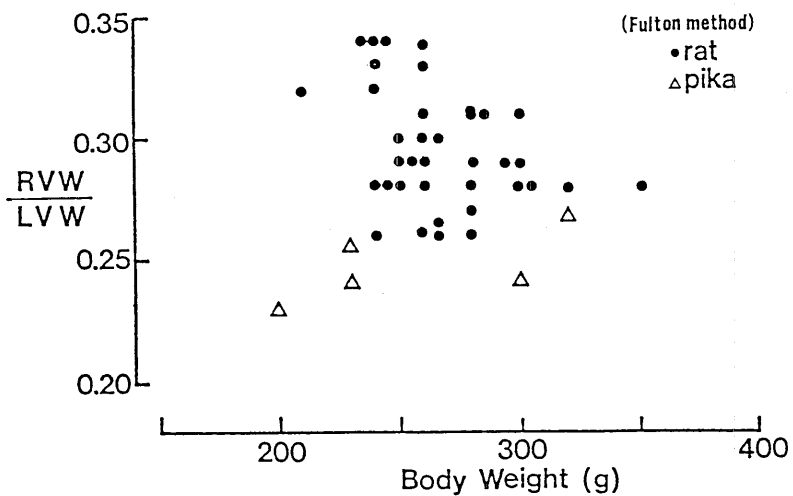


Fig. 5. Ratio of right ventricular weight (RVW) to left ventricular weight (LVW), RVW/LVW , of Afghan pikas ($n=5$) and Wistar rats ($n=35$) in relation to body weight. (For details see text).

Table 1. Comparison of ear to body surface area ratio between pikas and albino rabbits

Ear/Body surface ratio (%)	
pika (n=15)	7.2 ± 0.7
rabbit (n=4)	17.0 ± 0.5
Mean \pm SD	

pikas was $120-140 \text{ min}^{-1}$, however, that in rabbits was 300 min^{-1} . In rabbits, marked increase in respiratory rate (thermal panting), up to 500 min^{-1} , was observed during general heating at 37°C of ambient temperature. In pikas, however, no change in respiratory rate during 33°C general heating and a slight increase, less than 270 min^{-1} during 37°C general heating, was noted. Although rectal temperatures in rabbits were regulated below 40°C , those in pikas rapidly elevated to above 42°C during 37°C general heating, therefore heating was stopped. Though rectal temperature elevated above 42°C , thermal panting was not induced in

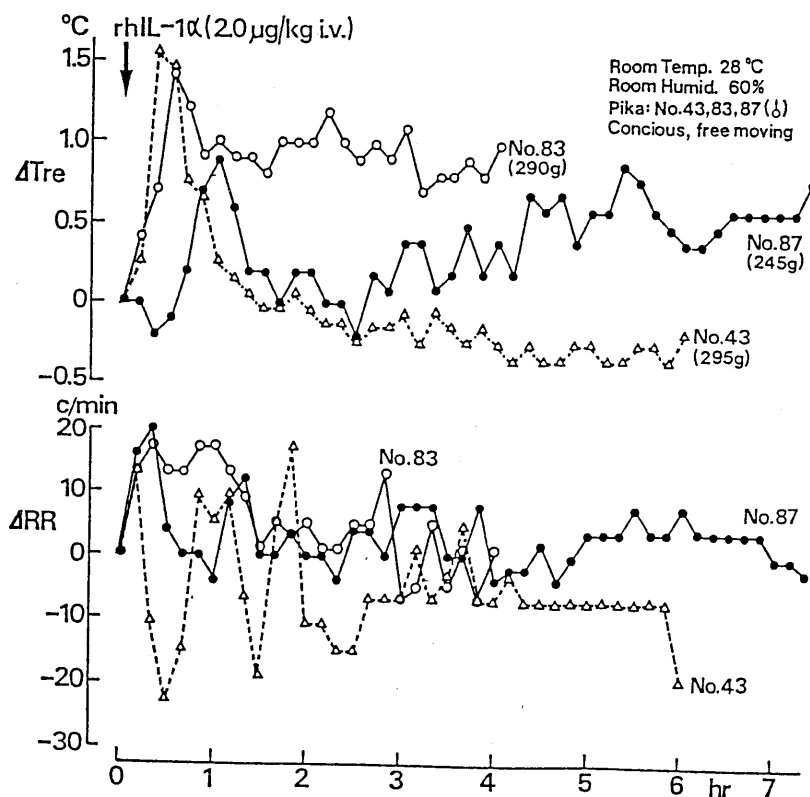


Fig. 6. Pyrogenic response to IL-1. Recombinant human interleukin- 1α ($2.0 \mu\text{g/kg}$) was intravenously administrated in three pikas. Tre: rectal temperature, RR: respiratory rate. (For details see text).

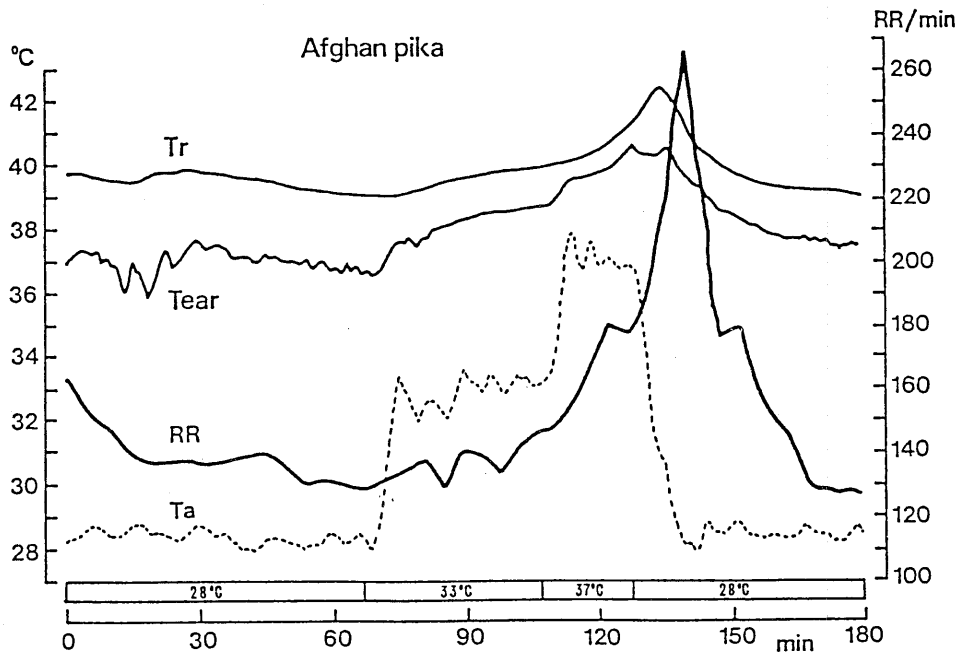


Fig. 7. Thermoregulatory responses to stepwise general heat load (28°C–33°C–37°C, 60 %rh) in an Afghan pika. Tr: rectal temperature, Tea: ear skin temperature, RR: respiratory rate, Ta: ambient temperature. (For details see text).

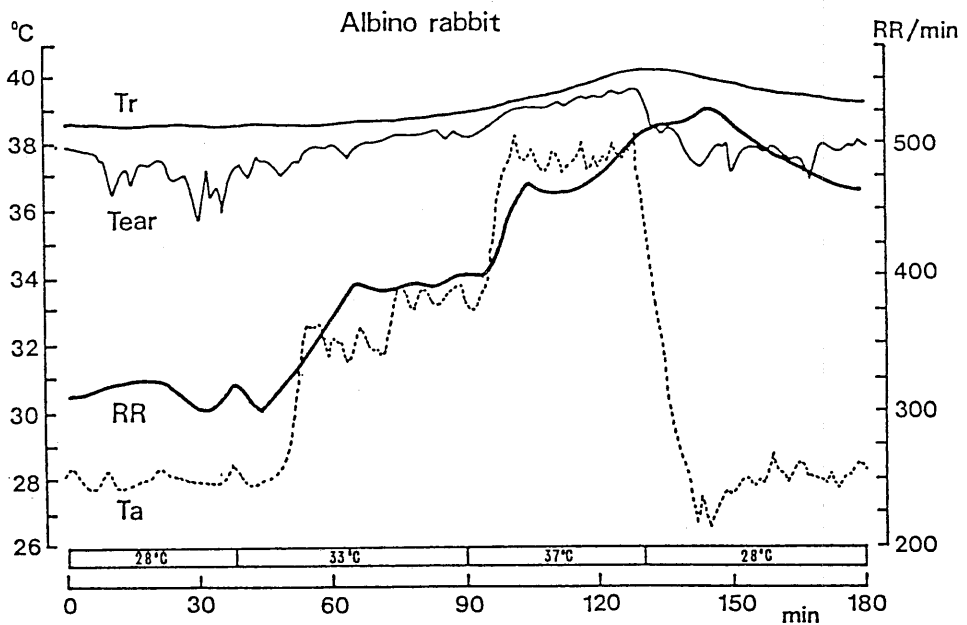


Fig. 8. Thermoregulatory responses to stepwise general heat load (28°C–33°C–37°C, 60 %rh) in an albino rabbit. Tr: rectal temperature, Tea: ear skin temperature, RR: Respiratory rate, Ta: ambient temperature. (For details see text).

pikas. Furthermore, salivation which is a major heat dissipation mechanism in rats, was not observed in pikas.

Induction of HSPs. The induction of HSPs after whole body hyperthermia (rectal temperature: 42°C for 15 min) was determined by 10% SDS-PAGE in pikas and rats. In rats, HSP70 was clearly induced after heat shock. In pika, however, changes in liver proteins were not observed at least by 10% SDS-PAGE after heat shock (Fig. 9).

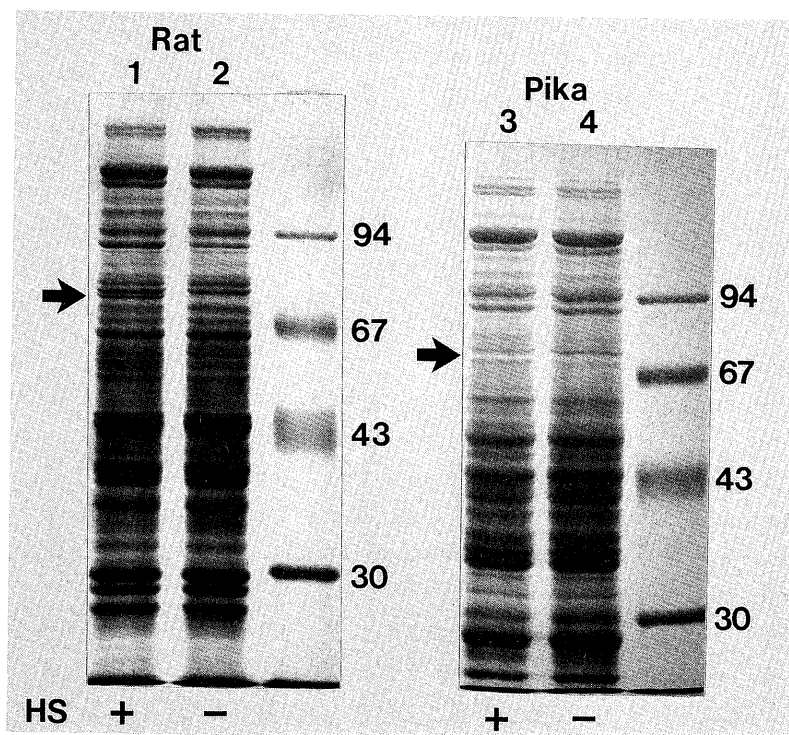


Fig. 9. 10% SDS-PAGE of liver proteins of rats and Afghan pikas. Lane 1, 2: Wistar rat, Lane 3, 4: Afghan pika. HS+: with heat shock, HS-: without heat shock. (For details see text).

DISCUSSION

Since 1985, Afghan pikas have been reared and bred in our laboratory at 22°C in accordance with Puget *et al.* (1973a). Gestation period was 27–28 days as same as Puget (1973a, b) and Matsuzaki *et al.* (1980). Mean life span of pikas in our laboratory was 56.4 weeks with a maximum of 144 weeks, which is identical to the previous report that pikas live for at least 2.5 years (Puget, 1973a). Neither differences in life span between both sexes nor a shortening tendency of life span through breeding was observed. Because of low adult weight, high reproduction rate and relatively short life span, pikas are considered to be suitable for laboratory animals.

The pika is one of the species native to high altitudes. The small degree of right ventricular hypertrophy is thought to be an adapted characteristic and a merit at high altitude-environment. In the present study, RVW/LVW in pikas was significantly small compared to that of rats although there was no significant difference in body weight. Sakai *et al.* (1988) examined the hemodynamics of pikas and rats in relation to altitude, from 610m to 4,460m, and revealed that RVW/LVW, pulmonary artery pressure, Hct of pikas were lower compared to those of rats at any altitude, and that the extent of increase in RVW/LVW with altitude in pikas was less than that of rats. Du and Li (1982) showed that chronic hypoxia (5,000m level) induces elevated Hct, cardiac hypertrophy and decline of P_{aO_2} , S_{aO_2} , and O_2 -utilized percentage in rats, but neither cardiac hypertrophy nor decline of P_{aO_2} , S_{aO_2} , and O_2 -utilized percentage with lesser elevation of Hct in pikas. These findings suggest that pikas which lived not only in high but also in low altitudes have a strong positive adaptability to high altitude-environment.

The mean Hct value of $36.7 \pm 0.6\%$ in wild Chinese pikas (personal communication from Prof. M. Kosaka) is almost identical to the value of 36–37% of Afghan pikas. Mean RVW/LVW of 0.27 in the wild Chinese pikas is quite near to 0.248 ± 0.013 of Afghan pikas in this study.

Another principal finding of the previous investigations on the physiological characteristics of pikas is weak heat- and strong cold-tolerance. Pikas have a higher metabolic rate, higher rectal temperature compared to rabbits (Kosaka *et al.*, 1985). Thermogenesis through brown adipose tissue for cold is present throughout the year and have seasonal variation in wild Chinese pikas (*Ochotona curzoniae*) (Wang and Wang, 1989). Furthermore, Yang *et al.* (1988) showed the pika's poor heat loss ability during heat exposure and suggested this was due to lack of thermal panting in pikas. In rabbits, a marked increase of respiratory rate and typical thermal panting were induced by heat load. In pikas, however, little increase in respiratory rate was observed even at 35°C of ambient temperature except a spasmodic tachypnea which resulted in death by heat. In this study, changes in respiratory rates due to stepwise general heating were continuously recorded and compared between pikas and rabbits to disclose heat loss ability through breathing (thermal panting) in pikas.

Although an increase in respiratory rate proportional to high ambient temperatures was observed and core temperature was kept under regulation in rabbits, pikas' respiratory rate did not increase against high ambient temperatures, and rapid elevation of rectal temperature above 42°C at 37°C exposure was considered to be out of regulation in pikas. Radiation from the ear pinna is another main heat dissipation mechanism in rabbits, however, the ratio of ear surface area to body surface area was considerably smaller in pikas compared to that in rabbits. These results indicate pikas' weak heat tolerance due to poor heat loss ability through a difficulty of thermal panting and a less effectiveness of radiation from ear pinnas.

Several thermoregulatory data obtained from wild Chinese pika (*Ochotona curzoniae*) living at Qinghai district in China are available (personal communication from Prof. M. Kosaka) for comparison to the present data of Afghan pika. Wild Chinese pikas were captured in a 3,260m high land district in Qinghai and were used for experiments in a laboratory room with

ambient temperature of 15.5°C and the same altitude of 3,260m. The mean rectal temperature of $39.8 \pm 1.2^\circ\text{C}$ of wild Chinese pikas ($n=17$) is identical to that of 39.6°C of Afghan pikas (Kosaka *et al.*, 1985). Mean respiratory rate of $110.5 \pm 14.1 \text{ min}^{-1}$ of wild Chinese pikas is almost the same as that of Afghan pikas. Further, the wild Chinese pika is extremely heat sensitive and is weak heat tolerance. (personal communication from Prof. M. Kosaka).

The similarity of all data of wild Chinese pikas to those of Afghan pikas indicates that the hemodynamic and thermoregulatory characteristics of these different species of pikas are both well adapted to high altitude- and cold-environment.

Today, in the research field of Hyperthermic Oncology, heat shock proteins (HSPs) in the 70KD family are thought to be closely related to thermo-tolerance in some kinds of cells (Hatayama *et al.*, 1985, 1986). However, in the present study, HSPs70 was almost not induced by systemic heat shock in pikas. This finding suggests that the lack or difficulty of induction of HSPs is somehow related to the characteristic of weak heat tolerance in pikas. Further investigation on the relationship between HSPs and heat tolerance not only in the cellular but also in the whole body level is necessary in the near future.

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