DIFFERENT CHARACTERISTICS OF THE INBRED FOK RAT IN ADDITION TO GENOTYPIC RESISTANCE ADAPTATION TO A SEVERE HOT ENVIRONMENT

Furuyama, F[1]., Kuroshima A[2]., Shido O[3]., Yahata T[4]., Tanaka, E[5]., Ikemoto A[6]., Okuyama H[6]., and Nishino H[1].

[1]Dept. Physiol., Nagoya City Univ. Med. Sch., Nagoya, Japan; [2]Dept. Physiol., Asahikawa Med. Coll., Asahikawa, Japan; [3]Dept. Physiol., Shimane Med. Univ., Izumo, Japan; [4]Dept. Nurs., Nayoro City Coll., Nayoro, Japan; [5]Dept. Physiol., Tokai Univ. Sch. Med., Isehara, Japan.[5]Dept. [6]Biol. Chem., Fac. Pharmaceut. Sci., Nagoya City Univ., Nagoya, Japan;

Adaptive changes contribute to reduce physiological strain produced by severe environments, to expand areas to live comfortably and to build up health in various environments. Although physiological strains produced by stressful components of environments within a lifetime is not inherited (phenotypic adaptation), changes as results of selection by environments for generations are inherited by descendants and genetically fixed (genotypic adaptation) (11). New experimental animals had been expected for research in this area. Using animal models with specific congenital trait of adaptation/resistance to a severe environment, it will be studied what changes are induced in intracellular events, cellular interactions, tissue and organ functions, and systemic integration, and what genes link to each change.

We were especially interested in resistance adaptation (10) to ambient heat in rats. We aimed to develop an animal model in the early days and recently developed an inbred FOK rat, which has characteristics of genotypic resistance adaptation to heat (4). The FOK rat has been kept in thermoneutral zone in all lifetime except one day for heat selection. The FOK rat resists ambient heat longer than the other 18 existent strains by evaporative heat loss, especially heat-induced saliva spreading on body surface (5). The FOK rat endures the ambient temperature (Ta) of 42.5°C for 2.5 – 5 times longer than the other rat strain. It was known rats secret saliva in response to heat (6). The heat-induced salivation is not special in rats but common response in various animals. However, the amount of saliva in FOK was large and estimated to be 14 % of body weight in total (4).

The body temperature response to ambient heat (BTRAH) is a predominant indicator of thermoregulatory ability. There are 3 types of BTRAH among rats. Monophasic BTRAH shows a continuous rise up to the final temperature. Diphasic BTRAH is composed of an initial convex-shaped rise and the final rise. Triphasic BTRAH is composed of a rapid rise followed by an steady-state equilibrium near to 40°C and the further rise (9, 8, 3). Heat-resistant individuals respond with a typical triphasic BTRAH (3, 4).

We report on direct correlation between core temperature (Tc) and thermal salivation, and the other multiple characteristics of the FOK rat in broad viewpoint.

Animals

Male ACI, Donryu, WKAH, Std: Wistar, and FOK rats were used at the age of 13weeks. ACI, Donryu, WKAH, Std: Wistar are heat intolerant (1, 2). SD rat is the most heat-tolerant among existent rat strains, although majority of it is heat intolerant (1). All rats were dissected under anesthesia after experiments, and congenital normality was confirmed in major organs. Research was conducted according to the Guideline for the Care and Use of Laboratory Animals of each university.

Experimental Designs:

Experiment 1: Threshold in core temperature (Tc) for onset of saliva spreading in heat exposed rats. Rats were individually transferred into a climatic room. Tc was continuously monitored using a telemetric system. Rats were left at rest 3 hours after the transfer, and then room temperature was started gradually to increase up to 40°C. The Tc at which saliva was observed between lips was determined in each rat.

Experiment 2: ACI, Donryu, SD and FOK rats were anesthetized by 110 mg/kg ketamineHCl. Tc was continuously monitored using a copper-constantan thermocouple. Rats were transferred into a climatic room with a constant Ta of 38.0°C. Rats were kept in a prone position on the wire-net slope. The head was placed lower than the tail. Each rat was oriented with its face protruding over the edge of the wire-net with a beaker containing mineral oil directly below. Saliva dropped into the beaker. Amount of saliva in terms of mg/ 100 g body weight/ min was determined for each period taken for Tc to increase by an increment of 0.5°C from 37.0°C to 43.0°C.

Experiment 3: Cell suspension was prepared from brown adipose tissue (BAT) of WKAH and FOK rats. Heat production in brown adipocyte was measured in presence of 10^{-9}M isoproterenol using microcalorimeter at 37.0°C and 39.9°C.

Experiment 4: *a*) WKAH, Std: Wistar and FOK rats were exposed to 0°C and Tc was monitored for 3 hours using copper-constantan thermocouple. *b*) Conscious rats from the 3 strains were kept at a Ta of 25°C. Norepionephrine was injected and then oxygen consumption was measured. *c*) Oxygen consumption of rats from the 3 strains was measured at a Ta of 5°C. *d*) UCP1 content was determined in BAT of the 3 strains. *e*) Shivering activity was assessed for 90 min using electromyogram

Experiment 5: Thermode was chronically implanted into main vein of rats from strains of WKAH, Donryu and FOK. Ten days after, hypothalamic temperature was continuously monitored. Rats were heated and then cooled by the thermode. Threshold hypothalamic temperatures for onset of vasodilatation in tail skin and of increase in thermogenesis were determined. Hypothalamic temperature was determined in conscious rats when they were allowed to select preferred Ta,

Experiment 6. Difference in psychological stress-induced fever among 5 strains was determined using telemetric system. Transfer into new type cage induced stress.

Experiment 7: *a*) Fatty acid composition of phospholipid was determined in BAT of Wistar and FOK rats kept in thermoneutral zone. *b*) Difference in amount of tryglyceride, phopholipid and free fatty acids among strains were determined in rats at a Ta of 25°C and starved at a Ta of 2°C.

Threshold Tc for thermal salivation varied in a Tc range of 38.40 – 40.60°C among conscious individuals from 5 strains in the experiment 1. The FOK rat (38.72 ± 0.14°C) was one of 3 strains that had low threshold. WKAH and ACI had higher threshold than the other 3 strains. Although threshold of thermal salivation was reported to be 38.4 - 38.5°C and 40.0 - 40.3°C in submandibular glands and in the parotid glands (7), respectively, saliva was not secreted from parotid glands (5). Tc of conscious rats increases immediately just after the beginning of heat exposure, because of no effective heat loss system which functions in the Tc range of 36.5 – 39.0°C. Tc soon attains at the threshold for saliva secretion. Tc is kept at steady state equilibrium near to 40°C in heat-resistant rats. Difference in heat resistance between FOK rats and the other 2 strains with low threshold thought to depend on salivation at Tc higher than threshold.

In the experiment 2, Tc increased gradually in anesthetized rats and saliva was secreted in response to Tc higher than the threshold. Amount of saliva was null or small at a Tc lower than 40.0°C. Small amounts of saliva were secreted by Donryu rats in all

Tc range from 39.0°C to 42.5°C. SD rats secreted rather than Donryu rats. However, the amount did not enoughly increase with increase in Tc from 40.0 to 42.5°C. ACI rats secreted only 3.0 ± 3.1 mg/100g/min at a Tc of 40.0 - 41.0°C. It increased up to 19.8 ± 11.1 mg/100g/min at hyperthermia of 41.5 - 42.0°C. FOK rats secreted far larger amounts of saliva in response to heat than the other rat strains. The amount increased to 16.7 ± 20.7 mg/100g/min and 40.8 ± 15.5 mg/100g/min at a Tc of 40.0 - 40.5°C and 41.5 -42.0°C, respectively. Heat resistance of FOK is attributable to remarkable increase in saliva secretion in proportion to Tc increase. This evidence shows contribution of a negative feedback system between thermoregulation center in hypothalamus and salivary system to heat resistance in FOK rats. Efferent neural signals from hypothalamus to salivary system via superior salivatory nucleus and chorda tympanic nerve increase in response to Tc increase. The more efferent firing rate increases, the more submandibular glands secret saliva in Tc range of 39.0 – 42.5°C. The saliva spreads on wide body surface and therefore saliva evaporation contributes to powerful heat dissipation even if Ta was higher than Tc. Temperature lowered by salivary cooling is the afferent inhibitory signal from salivary system to thermoregulation center in hypothalamus. Finally heat lost mainly by evaporation cancels out with heat produced in the body and gained from environments. Thus, Tc steadies near to 40°C in heat tolerant rats especially in FOK rats.

In the experiment 3, basal heat production by brown adipocyte of FOK rats was not different from that of WKAH rats. Although a increase in heat production in WKAH was caused by 10^{-9}M isoproterenol at 37.0°C and 39.9°C, no change was caused in FOK rats. At 39.9°C and in the presence of 10^{-9}M isoproterenol, heat production in FOK rats was significantly lower than that in WKAH rats. The low heat production under hyperthermia and in presence of beta-agonist partly contributes to heat resistance in FOK rats.

In the experiment 4, fall in Tc during cold exposure was unexpectedly smaller in conscious FOK than those in the other strains. Shivering in FOK was less active than those in the other strains. Nevertheless, in vivo oxygen consumption was higher in FOK than the other strains in a cold environment. This is the same as the typical trait of phenotypic cold adaptation. It can be expected that non-shivering thermogenesis (NST) is up-regulated through increase in energy expenditure by uncoupling between electron transport and ATP synthesis in mitochondria of BAT. However, such evidence was not obtained in relation to BAT. Especially, injection of norepinephrine did not induce enough increase in vivo oxygen consumption as acute cold exposure, and amount of

UCP1 was not congenitally larger than those in control rats. These evidences suggested that the mechanisms of NST in the FOK are different from the case of phenotypic cold adaptation. Thus, it was at least shown that the FOK rat is an inbred for cross-adaptation to cold environment as results of experimental heat selection for generations.

In the experiment 5, the FOK rat had lower threshold hypothalamic temperatures for tail skin vasodilatation and for cold-induced thermogenesis than control rats. In addition, Tc of the FOK rat was significantly lower than controls, when they stayed at the preferred Ta. Thus, Tc of FOK is set lower than the other strains.

In the experiment 6, psychological stress-induced fever lasted 3 hours in FOK and WKAH rats. These fevers were larger than those in the other 3 strains. It possibly came from difference in stress mechanisms and/or thermogenic mechanisms. Because purpose of the present study is to get first evidence, answer takes us further studies in near future.

In the experiment 7 – *a*, docosahexaenoic acid (DHA), an unsaturated fatty acid, was increased in phospholipid of BAT in FOK rats at a Ta of 25°C. In the experiment 7 – *b*, tryglyceride in blood plasma of FOK was remarkably lower than control strain. Arachidonic acid and DHA was higher in tryglyceride of FOK than of control rats. Both unsaturated fatty acids increased as results of fasting and cold exposure for 84 hours. Phospjolipid level was higher in blood plasma of FOK than those of controls at the end of the fasting and cold exposure. Evidence was still fragmentary and insufficient for understanding lipid metabolism in FOK. Values in FOK were at least different from control rats, suggesting specific lipid metabolism.

It is hereafter worthy to study further on different characteristics of the FOK rat. The sdudies will concern circulating system including blood pressure, body fluid and electrolyte metabolism, neuronal cell injury, brain functions (emotion, sleep, learning etc.) and genes linked to these problems. The studies are based on actual status of FOK rats in hot environments and will provide people with evidences, which will arise in people and animals from possible global warming of the earth.

In conclusion, the FOK rat developed by heat selections resists heat using a negative feedback system between thermoregulation center and evaporation system. FOK inherits multiple characteristics in addition to heat tolerance. Improved cold resistance means that the FOK rat had acquired a cross-adaptation as a result of heat selection for generations.

REFERENCES

1. Furuyama, F. Strain difference in thermoregulation of rats enduring extreme heat. J. Appl. Physiol. : Respirat. Environ. Exercise Physiol. 52: 410-415, 1982.
2. Furuyama, F.; Yoshida, T.; Kumazaki, M.; Ohara, K. Thermal salivation and body water economy among Wistar rat strains. Jpn. J. Vet. Sci. 50: 415-423, 1988.
3. Furuyama, F., Kumazaki, M., Nishino, H. Overshoot and high equilibrium in body temperature responses of rats to ambient heat: relation to thermoregulatory ability. J. Vet. Med. Sci. 54: 915-921, 1992.
4. Furuyama, F. Ohara, K. Genetic development of an inbred rat strain with increased resistance adaptation to a hot environment. Am. J. Physiol. 265 (Regulatory Integrative Comp. Physiol. 34): R957-R962, 1993.
5. Furuyama, F., Murakam, M., Oiwa, T., Nishino, H. Differences in thermal salivation between the FOK rat (a model of Genotypic heat adaptation) and three other rat strains. Physiol. Behav. 63: 787 – 793, 1998.
6. Hainsworth, F. R., Epstein, A. N.. Severe impairment of heat-induced saliva spreading in rats recovered from lateral hypothalamic lesions. Science 9: 1255-1257, 1966.
7. Hainsworth, F. R., Stricker, E. M.. Evaporative cooling in the rat: Differences between salivary glands as thermoregulatory effectors. Can. J . Physiol. Pharmacol. 49: 573-580; 1971
8. Gordon, C. J. Temperature Regulation in Laboratory Rats. Cambridge University Press, Cambridge, 1993.
9. Ohara, K., Isobe, Y., Furuyama, F. Prediction of survival time of rats in severe heat. J. Appl. Physiol. 38: 724-729, 1975.
10. Prossor, C. L. Theory of adaptation. In: Biological adaptation. edited by G. Hildebrandt and H. Hensel. Stuttgart: Georg Thieme Verlag, 1982, p. 2-22.
11. The IUPS Commission for Thermal Physiology. Glossary of terms for thermal physiology. Pfluger's Arch. 410: 567 – 587, 1987.