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Thermophysiology Research in Czechoslovakia

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The first thermophysiology experiments in our country were performed by E. Babák and collaborators in 1909–1910. They concerned the effect of temperature on the respiration rate of insects and urodeles. The effect of temperature on the metabolic rate of different species was intensively studied by J. Bělehrádek, professor of General Biology at the Faculty of Medicine (Charles University in Prague) in the years 1925–1935. The main aim of J. Bělehrádek was to explain differences in temperature coefficients (Q_{10}) and in the heat of activation, occurring due to the adaptation to different temperatures, age of the animals and action of drugs, using a number of models, mostly isolated frog and fish hearts. He proposed a new formula explaining the effect of temperature on biological processes and presented a theory that the intensity of metabolic rate at lowered temperatures is limited by a low diffusion velocity in viscous parts of the protoplasm, the physical properties of fats and phospholipids being mainly responsible for these processes. He summarized these views in his monograph "Temperature and Living Matter" published in Berlin (Bělehrádek 1935). This book not only dealt with the effect of temperature on biological processes and on chemical and physical properties of living systems, but also with the resistance to heat or cold and with heat and cold injury.

New interest on thermophysiology arose in 1950s at the Department of Comparative Physiology, Faculty of Science, Charles University in Prague. This research, which is still continuing, was performed by L. Janský and his collaborators (J. Mejsnar, S. Vybíral, B. Štefl, J. Moravec, R. Bartůňková-Novotná). Originally, the aim of this work was to explain the effect of lowered temperatures on thermoregulatory efficiency of muscular work. These studies revealed different responses in warm and cold adapted individuals. Subsequently, further studies were oriented towards clarifying the mechanisms of cold adaptation in mammals. In collaboration with J.S. Hart from the National Research Council in Ottawa, Canada, it was discovered that the main mechanism of cold adaptation in small mammals is their increased capacity of heat production due to the development of a new thermogenic mechanism called nonshivering thermogenesis (for reviews see Janský 1973, 1988). Nonshivering thermogenesis supplements heat production due to shivering by acting at environmental temperatures immediately below the thermoneutral zone and thus represents the first line of defence against cold. Only severe cold stress induces both nonshivering thermogenesis and shivering in animals adapted to cold. Simultaneous activation of these heat production mechanisms shifts

the survival limit to lower temperatures and substantially increases the resistance to cold.

Nonshivering thermogenesis is also of considerable significance for newborn mammals, including neonates. In species which are born immature, the amount of nonshivering thermogenesis first increases and then decreases after about three weeks of postnatal development. In other species, which are born mature, the amount of nonshivering thermogenesis decreases immediately after birth. It can be induced again in adults by long-term cold exposure. The magnitude of nonshivering thermogenesis depends on body size, being greatest in small mammals, where it may surpass the basal metabolic rate by almost 800 %. Species greater than 10 kg do not usually possess nonshivering thermogenesis in adulthood.

The magnitude of nonshivering thermogenesis also depends on the intensity of cold stimuli. Every temperature below the thermoneutral zone can induce a certain amount of nonshivering thermogenesis. In rats, there exists an inverse relation between the environmental temperature and the magnitude of nonshivering thermogenesis – the lower the environmental temperature, the greater the magnitude of nonshivering thermogenesis. At all environmental temperatures it takes about 3–4 weeks of continuous cold exposure to induce the corresponding amount of nonshivering thermogenesis. The magnitude of nonshivering thermogenesis is generally used as an indicator of the level of cold adaptation in animals and man.

Nonshivering thermogenesis is based on the thermogenic action of hormones, mainly on that of noradrenaline released from the sympathetic nervous system. Other hormones (adrenaline, glucagon, thyroid hormones) may also contribute to some extent to nonshivering heat production.

It is well documented that most of the heat during nonshivering thermogenesis is liberated from the brown adipose tissue (Foster and Frydmann 1978). Our data on the total cytochrome oxidase activity in this organ have indicated that about 70 % of total nonshivering thermogenesis can be covered by heat produced in the brown fat. Noradrenaline induces a unique process of regulation of metabolism in brown adipose tissue cells. Brown adipose tissue mitochondria possess a proton conductance pathway that permits them to become reversibly uncoupled and thus to oxidize substrates at an extremely high rate independently of the need to phosphorylate ADP. When activated by fatty acids released by the action of noradrenaline, this pathway provides a mean for short-circuiting the usual proton cycling, which is normally associated with oxidative phosphorylation. When this short-circuiting occurs, the rate of electron transport, no longer restrained by the proton gradient, rises to a maximum and available substrates are oxidized. In the resting brown adipose tissue cell, this pathway is inhibited by the binding of purine nucleotides to the specific protein of the mitochondrial inner membrane. This protein is known as "thermogenin" or "uncoupling protein" (Nichols and Locke 1984).

Besides the brown adipose tissue, some heat is being produced in other organs, namely in muscles. It was found that under *in vivo*, *in situ* and *in vitro* conditions skeletal muscles increase heat production by 50–100 % after administration of noradrenaline. The mechanism of noradrenaline thermogenetic action in muscles is completely different from that in the brown adipose tissue, since

it increases with increased blood flow. In contrast to muscles, heat production in the liver, kidney and gut is not affected by noradrenaline (Mejsnar and Janský 1976).

The discovery that the brown adipose tissue has a great thermogenic capacity stimulated biochemical research in many laboratories in the world, as well as in our country. Z. Drahota, J. Houštěk, J. Kopecký, P. Svoboda and others from the Institute of Physiology (Czechoslovak Academy of Sciences in Prague) studied the role of free fatty acids in regulating cell metabolism, the enzyme pattern in inner and outer membranes of mitochondria, the glycerol-3-phosphate shuttle, biochemical characteristics of the ATPase and of the "uncoupling" protein as well as the H^+ and Cl^- conducting pathways in membranes of the brown adipose tissue.

In recent years the scientific interest at the Department of Comparative Physiology (Charles University in Prague) has been oriented towards hibernation and the role of humoral substances in modulating the activity of hypothalamic thermoregulatory centers. Additionally, the adaptation of neuromuscular transmission to the hibernation state was also studied electrophysiologically (Moravec and Vyskočil 1976). The results indicate that changes in junctional membranes allow the neuromuscular junction to perform more effectively at lowered temperatures.

Furthermore, it was shown in hibernating animals that the absence of gonadal steroids is a necessary prerequisite for changing the activity of thermoregulatory centers and for inducing hibernation hypothermia. On the other hand, gonadal steroids do not play a crucial role in termination of the hibernation state, and neither does melatonin from the pineal gland (Janský 1986). The same applies to the intrahypothalamic injections of noradrenaline or serotonin which also do not influence body temperature control of hibernators significantly (Janský 1978).

It was therefore suggested that hibernation might be induced by a peptide called the "hibernation trigger". This idea stimulated further research on the effect of neuropeptides on body temperature control in normotherms. It was shown that neuropeptides induce specific changes in body temperature control and thus participate in thermoregulation under physiological conditions. They influence the temperature sensitivity of control centers in the hypothalamus as well as the cooperation between neuronal pathways in the brain stem conveying warm and cold signals from different parts of the body to the central controller. This results in changes of threshold body temperature for induction of individual thermoregulatory effectors (cold thermogenesis, peripheral vasomotor tone, respiratory evaporative heat loss) and in changes of their efficacy (hypothalamic thermosensitivity).

Further studies on the effect of neuropeptides on hypothalamic neurones under *in vitro* conditions revealed that the hypothalamic thermosensitivity is not a fixed property of individual cold or warm receptors but that it is rather dependent on the humoral status of the organism. Thus neuropeptides act as modulators of neural activity and may induce changes in their thermosensitivity.

Since some neuropeptides induce similar changes as pyrogens, temperature regulation has also been studied during fever. It was found that during the early phase of fever, the threshold body temperature for induction of activity of all thermoregulatory effectors is shifted upwards to higher body temperatures, while the thermosensitivity of hypothalamic control centers remains unchanged (Fig. 1). On the other hand, in the late phase of fever only the threshold for induction of cold

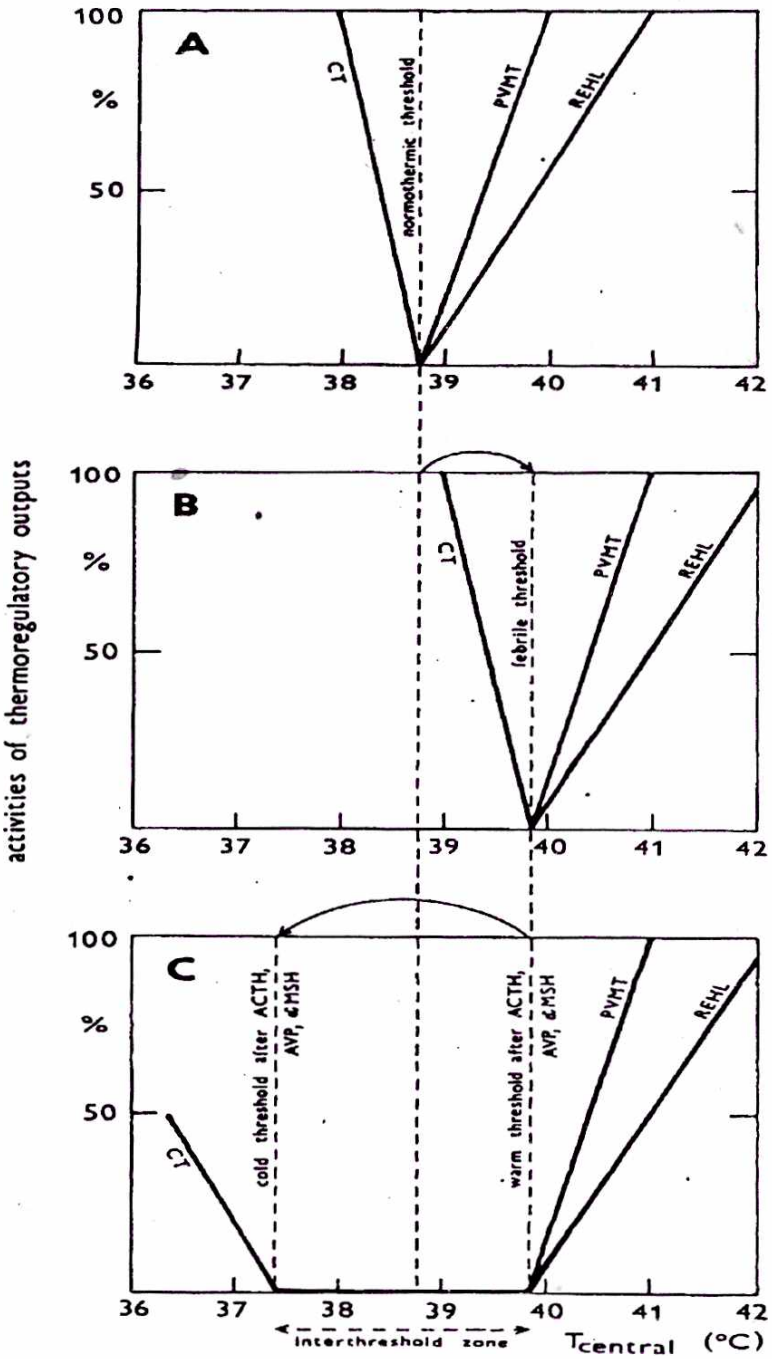

Fig. 1

Scheme of activation of thermoregulatory outputs (cold thermogenesis – CT, peripheral vasomotor tone – PVMT, respiratory evaporative heat loss – REHL) due to changes in central body temperature in normal (A), in febrile rabbits (B), or in rabbits treated by ACTH, AVP, alpha-MSH during the second phase of the fever (C).

thermogenesis is shifted to lower body temperatures, thus preventing the induction of shivering. Thermosensitivity to cold is lowered. This results in a widening of the interthreshold zone between activation of warm and cold defence mechanisms and in relative inertia of thermoregulatory centers to cold stimuli. The finding that some neuropeptides (ACTH, AVP, alpha-MSH), when injected into the anterior hypothalamus or into the septal area of the brain, induce similar changes in activity of thermoregulatory centers as those occurring during the late phase of fever led to the conclusion that these neuropeptides may play a role as natural antipyretic substances. Recently, this research was reviewed by Janský (1990).

Czechoslovak thermophysiologists also paid attention to applied thermophysiology, namely to cryobiology. As early as 1952, the first tissue bank in Europe was founded at the Faculty of Medicine in Hradec Králové, under the leadership of R. Klen. The laboratory was used for preparing seven kinds of tissue grafts using cooling, freezing and lyophilization.

At the Institute of Hygiene and Epidemiology in Prague, K. Luštinec studied the limit values of physiological functions during heat and work loads. Several other researchers from different institutes studied the healing effect of locally applied heat (J. Ipser, J. Kadeřávek, J. Raušer, A. Hlaváček) and the physiological effect of heat stress (J. Štverák, M. Hubač, L. Novák, K. Blažek, O. Štros and others).

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