Thermoregulation as a Switchboard of Autonomic Nervous and Endocrine Control

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Summary: Analyzing the multiple effectors of autonomic temperature regulation has, sometimes unexpectedly, provided insights into the characteristics according to which the non-thermoregulatory functions of these effectors are controlled. This article reviews the sympathetic control of cardiovascular and immune functions, hormonal control of energy balance and neurohormonal control of salt and fluid balance inasmuch as they are challenged by competing demands of thermoregulatory requirements. These interactions are taken as examples for the analytical power of the experimental conception to challenge non-thermoregulatory control systems with thermoregulatory activation, and vice versa. Animal models carrying spontaneous or intentionally produced gene defects and molecular and histochemical techniques of gene identification and neuronal tracing are becoming increasingly important. They are applied, in connection with physiological studies exploiting mutual interactions of autonomic control systems, with the aim to elucidate the cytoarchitecture of neuronal circuits by which specific autonomic regulatory activities are controlled. [Japanese Journal of Physiology, 49, 297–323, 1999]

Key words: temperature regulation, hypothalamus, spinal cord, sympathetic activity, neuro-immunomodulation, energy balance, salt and fluid balance, neurohormone.

Homeothermia is a phylogenetically recent achievement of temperature regulation in the animal kingdom. In the course of evolution, thermoregulation has put into service multiple effector systems controlling the dissipation or generation of body heat. The redundant design of the controlling system in thermoregulation with its multiple sensors, controllers and effectors may be considered as paradigmatic for biological control systems that evolved to stabilize bodily parameters at the organic level [1].

The effectors contributing to heat generation and heat dissipation seem to have attained, at least in part, their thermoregulatory functions only secondarily in the course of evolution towards homeothermy. For several of them, their primary functions are obvious: "Thermoregulatory behavior" is just one out of many adjustments to avoid noxious and search for beneficial environmental circumstances. In conditions of competing behavioral drives, such as search for food or water, behavioral thermoregulation can be replaced by the activities of physiological thermoregulatory effector systems, and thus, the organism has the freedom to weigh the pros against the cons of employing behavior to stabilize its body temperature. "Cold shivering" is a special thermoregulatory state of tremor activity to produce extra heat in the skeletal muscles which primarily serve locomotion. The drive for shivering is generated normally in the hypothalamic thermoregulatory network as a presumably continuous signal [2, 3]. In connection with the discovery of thermoregulatory properties of the spinal cord [4–6], it was shown that the cold-induced tremor produced by the isolated spinal cord was indistinguishable from that of intact animals (Fig. 1) with regard to the frequency distribution of the grouped discharges characterizing the electromyograms of shivering muscles [7, 8]. Thus, spinal neuronal networks — and in an analogous fashion, neurons of the motor brain stem nuclei — were identified as the site of shivering tremor generation. Partial coordination of motor units, as it is typical for shiver-
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Intact rabbits

Spinalized rabbits

Fig. 1. Frequency distribution of grouped discharges in the electromyogram from shivering hindleg and back muscles of conscious rabbits with intact (left) and chronically transected (right) spinal cord (cervico-thoracic level) in response to peripheral cooling (a) and spinal cord cooling (b). Data of Kosaka and Simon [8].

...ing, is related in frequency to body size by neuronal interactions that are still not fully understood [9]. “Thermal panting” is a particular pattern of rapid shallow breathing to ventilate the respiratory mucosal surfaces as sites of evaporative heat dissipation interfering as little as possible with the primary function of respiration to provide gas exchange and to support the stabilization of body fluid pH [10]. “Thermoregulatory salivation” assists the heat loss function of panting in that it provides the moisture necessary for water evaporation. Further, in combination with a particular behavior, it is used with the same goal in that saliva is spread over heat dissipating skin surfaces, but the primary function of saliva obviously consists in the support of chewing, swallowing and initiation of food digestion. “Circulatory convective heat transfer” from the core to the skin is but one aspect of the transport function of the blood, which is essential as the carrier of virtually every extracellular metabolite, and moreover, of hormones as well as the humoral and cellular components of immunological defense. “Water consumption for evaporative cooling” imposes a demand on salt and fluid balance and thereby interferes with the underlying complex endocrine and behavioral control systems, not only because water is required for evaporative heat dissipation but also because of accompanying electrolyte losses, especially during sweating and with saliva.

Even effector activities, which seem, at a first glance, to have evolved specifically for temperature regulation, possess non-thermal functions, perhaps with the exception of “thermal sweating” in humans. Though with some sophistication, “piloerection” may be seen not only as a very effective means to increase external insulation in furred and feathered homeotherms, but also as a component of defensive and aggressive behavior that is even proverbial as a human response to threat and horror. “Non-shivering thermogenesis” (NST) is primarily a specific mode of metabolic cold defense, but it may also support metabolic balance as one of the sites of diet-induced thermogenesis [11]. In mammals, the main site of NST, but not the only one, is the brown adipose tissue (BAT) which is perinatally developed in many species and may increase its heat generating capacity in the course of cold adaptation [12–14]. Heat generation by the BAT is sympathetically driven. Catecholamines acting on adrenoceptors of the \( \beta_3 \) subtype stimulate an intracellular signal chain leading ultimately to the uncoupling of mitochondrial respiration from ATP production by activation of and/or insertion into the inner mitochondrial membrane of uncoupling protein (UCP). In the BAT, the prevailing isoform is UCP1, thermogenin [15], which establishes a proton channel to short-circuit the chemiosmotic mechanism of ATP generation coupled to respiratory chain activity and, as the result, permits the energy of fatty acid oxidation to be liberated as heat. Isoforms (UCP2 and UCP3) are also expressed in other tissues, but it is unclear how they are controlled and whether they are involved in regulatory
Thermogenesis [16–18]. In addition, it has to be considered that there are other sites of NST, for instance skeletal muscle, and a wide scope of thermogenic transmitters and hormones may be relevant depending on the site of heat generation and the species under consideration [19].

Thermoregulation as a Switchboard of Autonomic Thermoregulatory Effectors

Viewing thermoregulation as a control system that has "borrowed" most of its effector mechanisms from other control systems implies, as an analytical conception, that thermoregulatory drives acting on any of these effectors compete with their primary functions. What is important is that this interference is both natural and specific. Thus, analyzing a non-thermal control system by imposing thermal loads has the advantage of a natural challenge in comparison to more artificial experimental interferences such as lesions, drug applications and other experimental stimulations. Indeed, the induction of thermoregulatory activities with the aim to analyze non-thermal control systems has proven to be successful as an experimental approach. The reverse is also true inasmuch as thermal side effects of the control of non-thermal parameters may be useful in the analysis of temperature regulation.

1. Autonomic nervous control

Differential cardiovascular innervation. This mode of sympathetic control discovered in the course of experimental efforts trying to overcome the long-standing discrepancy between evidence for independent vasomotor control of functionally different vascular regions, as suggested by blood flow analysis, and the notion of the directionally uniform responsivity of sympathetic efferent innervation concluded mostly from the results of electrostimulation experiments. Accordingly, vasoconstrictor innervation was previously assumed to vary only quantitatively in different vascular beds due to the non-uniformity of baroreflex inhibition of regional sympathetic outflows. This impasse was overcome by two experimental approaches. One studied the influence of competing demands exerted by non-cardiovascular regulatory activities on cardiovascular control (120) for references), initially of those imposed by temperature regulation on regional blood flow (21) and its vasomotor control [22]. These studies had been undertaken primarily to prove the equivalence of the thermosensory function of the spinal cord with that established for the preoptic and anterior hypothalamic (POAH) region [23]. In accordance with antagonistic blood flow changes monitored with electromagnetic flow probes in functionally different, cutaneous and visceral vascular beds, multifiber recordings from sympathetic efferents innervating these vascular regions clearly demonstrated antagonistic changes of efferent sympathetic activity (Fig. 2). The fine tuning of this regional antagonism as a primary thermoregulatory response was convincingly demonstrated in baroreceptor-denervated dogs, in which hypothalamic heating and cooling produced the expected cutano-visceral blood flow antagonism (Fig. 3) without major changes in arterial pressure [24]. An alternative approach to sympathetic regional differentiation proceeded from the thorough
analysis of baroreflex patterns [25] (for references). Both approaches were successful in disclosing regionally diverse changes of sympathetic cardiovascular innervation. In addition, the analysis of reflex responses of autonomic, peripheral and visceral efferents to sensory inputs of different modalities revealed non-uniform changes of activity in sympathetic efferents serving different vasomotor and sudomotor functions [26-30]. By analyzing integrated cardiovascular adjustments to various physiological and pathophysiological challenges such as hypoxia, hypercapnia and fever, function-specific response patterns of regional sympathetic innervation were disclosed [31].

**Active vasodilatation.** Reduction of intrinsic smooth muscle tone in terminal arterial vessels due to the local release of relaxing transmitters, occurs in various tissues in response to certain states of stress like physical exercise, fight-and-flight conditions and heat defense. As a thermoregulatory adjustment in humans, active vasodilatation is mainly associated with sweat gland activation by sympathetic efferents releasing acetylcholine (ACh) as the sudomotor transmitter [32]. In animals, early evidence for the thermoregulatory function of vasodilatory, non-sudomotor autonomic efferents of sympathetic origin in heat dissipation was provided by skin blood flow measurements (Fig. 4) in catecholamine-depleted dogs [33]. In the paw skin of the cat, partially overlapping working ranges of vasoconstrictor and vasodilator innervation as a function of spinal cord temperature (Fig. 5) could be clearly demonstrated [34], and vasodilators and sudomotor are clearly discernible [28]. In humans, vasointestinal peptide (VIP) seems to be involved as a vasorelaxant transmitter co-released from the sudomotor cholinergic nerve endings [35], but a special, non-cholinergic non-adrenergic (NANC) vasodilatory innervation of arterioles has not been excluded [36, 37]. “Sensory” peptides acting locally as vasodilators [38] like substance P (SP) and calcitonin gene-related peptide (CGRP) have, so far, not been identified as transmitters of autonomic efferents mediating thermoregulatory local blood flow adjustments.

Nitric oxide (NO), as the agent representing the endothelium-derived-relaxing-factor (EDRF) stimulated by ACh [39-41], may be involved in thermoregulation as indicated by coordinated thermolytic actions of NO donors administered peripherally [42] and centrally [43] to rabbits. However, hyperthermic actions have been ascribed to NO as well; especially, the role as a central nervous interneuronal messenger is certainly
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complex and has to be further clarified [44]. A heuristic working hypothesis suggests that NO, when acting at sites of thermointegration, tends to decrease deep-body temperature but has a hyperthermic effect when it acts at sites of thermo- (and pyrogen) perception. The latter idea is supported by the prevailing inhibitory NO action on warm sensitive spinal cord neurons in lamina II, the presumed site of ascending temperature signal generation [45], and generally on POAH neurons (Fig. 6). In the periphery, NO acts as a vasodilator, but it is not certain that it is physiologically involved as a thermoregulatory vasodilator considering that ACh is not established as the transmitter of active vasodilator fibers innervating skin vessels [46].

The interplay between vasoconstrictors and -dilators. The contributions of both modes of vasomotor control to thermoregulatory adjustments of blood flow are characterized by different working ranges [34] as illustrated by Fig. 5. For the analysis of NANC transmission in the vascular control of heat dissipating surfaces, the dog, as a panting animal, turned out to be a most suitable experimental model. For the tongue, as an important heat sink during panting, a strong parasympathetic component was identified in the control of mucosal blood flow. A high degree of synchrony was seen in conscious dogs between the respiratory rate and vasodilatory bouts in the mucosal layer of the tongue (Fig. 7), indicating functional specificity [47]. In this animal model, vasodilatation was characterized by the precise tuning of naso-facial and lingual blood flows in relation to the state of panting (Fig. 8); graded vasodilation in the nasal mucosa paralleled the increasing rate of closed-mouth panting, but lingual mucosal blood flow increased suddenly when the animal turned to open-mouth panting [48]. Pharmacological analysis showed that thermoregulatory vasodilatation in the nasal mucosal surfaces followed from the reduction of α-adrenergic sympathetic activity, but was due to the activation of NANC vasodilatory innervation of the lingual mucosal surface [49] and presumably involved VIP as the transmitter (co-)released from parasym pathetic efferents [50]. Thus, thermoregulatory vasomotor control reveals an intricate degree of differentiation with respect to both efferent neuronal activity and transmitter distribution in vascular regions serving as avenues for heat dissipation [51, 52].

Local mechanisms of skin vasodilatation. A multitude of functions are served by locally resident vasoactive compounds. In response to antidromic excitation or direct local stimulation of certain non-myelinated, afferent nerve terminals, "sensory" neu-

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Fig. 5. Relationship between the discharge activity of 4 spontaneously active vasoconstrictor single fibers (open symbols) and 3 vasodilator single fibers (filled symbols), the latter being silent at normal temperature, both innervating the paw of an anesthetized cat. Data are plotted against spinal cord temperature which was changed with a percutaneously located water perfused thermode. The normal (100%) vasoconstrictor discharge rate at 40°C, in the original data presentation, was equalized in this diagram with the corresponding average discharge rate of 1.3 imp/s. Vasoconstrictor activity was reduced incrementally with increasing spinal cord temperature. The vasodilators became active above 41°C and were strongly activated with rising spinal cord temperature. Data from Gregor et al. [34].

Example of a warm sensitive POAH neuron

TC = 0.6 imp/sec/°C  SNP 10⁻⁴ M

Sample properties
n = 17

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Fig. 6. Extracellular recording in a tissue slice from a POAH neuron identified as warm-sensitive by step- and rampwise temperature changes of the incubation medium (lower trace). The NO donor sodium nitroprusside (SNP) inhibits the neuron. The pie chart shows that 7 warm-sensitive and 10 thermo-insensitive POAH neurons were uniformly inhibited by NO. Unpublished observation of Schmid et al. [44].
ropeptides, especially SP and CGRP, are released as vasodilators in association with the phenomenon of neurogenic inflammation [53–56]. Thermally induced release is consistently observed if local heating becomes intense enough to activate heat nociceptors, but non-noxious heating of the skin may also increase local blood flow in this way [38]. The latter response, however, occurs independently of centrally mediated thermoregulatory adjustments of the circulation [57]. Particularly important for the phenomenon of cold vasodilation [58] is the direct thermal control of the arteriovenous anastomoses (AVA). Using the respiratory surfaces of panting animals as a model for a multifunctional vascular bed, local thermal control of capillary blood flow was shown to differ from that of AVA blood flow in protective local vasodilatory reactions of dogs breathing air at temperatures ranging from as hot as 45°C to as cold as −50°C [59, 60].

**Sympathetic neuro-immuno-modulation.**

This is a recently disclosed functional component of differential sympathetic control, especially of the efferent fibers innervating lymphoid tissue; in particular, the spleen [61–63]. Changes in local efferent activity contribute to adjustments of cellular, phagocytic and secretory components of the immune response under the influence of pyrogens and their consecutive endogenous mediators [64–67]. Local catecholamine actions may be supplemented by those of co-localized neuropeptides [68]. The “fever syndrome” [69] displays, during the first fever phase, the differential sympathetic response pattern of cold defense (Fig. 9), though with some deviations. Moreover, it is characterized by intricate interactions between differential, autonomic nervous and humoral-endocrine components in the control of metabolism, circulation and specific as well as non-specific immune defense activities [70–74]. Specifically, pyrogens seem to activate sympathetic efferents innervating the spleen [62], different from other visceral efferents in which the inhibition of activity prevails in the phase of rising fever (Fig. 10). Sympathetic activation of the spleen (Fig. 11), and possibly other lymphatic organs, also seems to contribute to stress-induced decreases in immune defense in addition to the activation of the hypothalmo-adenohypophysial-adrenocortical axis, as demonstrated for the spleen in immobilization-induced stress [75].

2. **Energetics**

Homeothermic temperature regulation may interfere with the control mechanisms for the incorporation and storage of chemical energy. This applies especially to increased metabolic heat production to stabilize deep-body temperature under cold load conditions if the rate of heat loss exceeds the prevailing state of metabolic activity characterized as “standard,” “basal,” “resting,” “field,” etc. metabolic rate [76]. Thermoregulatory changes of energy expenditure, en-
Fig. 9. Typical autonomic fever response to intracerebroventricular injection of the ultimate central mediator of fever, prostaglandin E₂ (PGE₂), in a rabbit [74]. At the onset of rising deep-body temperature (Tₑ), the decrease in ear skin temperature (Tₑₛ) indicates skin vasoconstriction, whereas direct recorded activity in a renal sympathetic branch (RSNA) is reduced. Changes in mean arterial pressure (MAP) and heart rate (HR) are not distinct. Iniki and Saigusa [74].

![Graph showing fever response to PGE₂ injection](image)

Fig. 11. Upper diagram: Immobilization stress induces an increase of sympathetic noradrenaline (NE) release in the rat spleen (filled circles) but this response is strongly attenuated after spleen denervation (open circles). Lower diagram: Activity of splenic natural killer (NK) cells determined at different ratios of NK and target cells used to demonstrate cytotoxicity. Filled circles: control conditions. Open circles: spleenic denervation, but no stress immobilization. Suppression of NK activity is pronounced after stress immobilization with intact spleen innervation (filled squares), but much less pronounced when the spleen is denervated (open squares). Data of Shimizu et al. [75].

![Graph showing NK cell activity](image)

energization and energy storage, especially under cold ambient conditions, may be interrelated differently depending on the species and food availability.

Extra energy expenditure. Balance of increased heat loss by enhanced release of metabolic heat requires adequate energy supply from energy stores or by food intake. This is experienced by humans living in cold climates and, although life under these conditions is often physically strenuous, healthy subjects may increase rather than decrease their body mass and energy stores if the cold load is not too severe and food is available ad libitum [77]. As side effects of physical activity and large meals, increases in resting metabolic rate [78] and postprandial extra heat generation [76] may contribute considerably to thermal balance in the cold.

Energy saving. Reduced metabolism associ-
ated with nycthemeral or seasonal periodicities may become essential in climates with large day-night temperature fluctuations or with long-lasting cold seasons. Large, well-insulated animals in which little, if any, extra thermoregulatory heat production is required under even the coldest conditions of their natural environment, may depend for months mainly on their fat stores while minimizing their energy expenditure but maintaining normal deep-body temperature [79, 80]. Small homeotherms may temporarily undergo torpor, i.e., they become moderately hypothermic in nycthemeral states of inactivity, and thus, preserve their energy stores for the daily phases of activity [81, 82]. The most extremely advanced means as a mode of saving energy in mammals during periods of low ambient temperatures and food scarcity is hibernation. It is characterized by repeated and extended phases of deep torpor, lasting for a few to many days as a means to get along with limited fat stores throughout the entire cold season [81, 83]. Active recovery of mammals from both daily torpor and hibernation primarily employs NST, but cold shivering is a supplementary mode of extra heat generation.

**Modes of energy balance.** Reference to thermoregulation was first recognized as helpful in elucidating mechanisms of metabolic control, when diet-induced thermogenesis due to sympathetic NST activation was identified as a means to limit energy storage under conditions of a surplus of food [11, 84]. The malfunction of this mode of energy dissipation is discussed as a contribution, but certainly not the main causative factor for the frequent occurrence of adiposity in humans living with an abundance of food in the developed countries of the world. Why control of energy stores fails in some individuals and not in others is an unresolved but most relevant question. Excessive adiposity is not only an esthetical and psychological problem but, in particular, a medically serious risk factor in many cardiovascular and metabolic diseases. The development of adiposity in the adult is due to the mismatch between food intake and energy expenditure. Genetic predisposition seems to be important in many cases and it often becomes manifest in early childhood.

In studies on animal models for genetic obesity, the altered action or production of a humoral factor had been recognized early as a causative factor, a conclusion drawn from experiments on obese/diabetic mice that had been made parabiotic with normal mice [85]. This key factor was recently identified as the peptide hormone leptin that is produced mainly by white fat cells as they undergo fat deposition. Basic insights into the molecular mechanisms of leptin action and its disturbances have recently been obtained with the analytical tools of molecular genetics applied to animal models of genetic obesity: The ob/ob mouse is leptin deficient due to a gene defect, while in the fa/fa, or "Zucker", rat a missense point mutation is responsible for the (almost) completely abolished transducer function of the long isoform of the leptin receptor [86–88].

With regard to the maintenance and aggravation of obesity in adults, the functional consequences of molecular defects underlying obesity are being analyzed in great detail, but it has proven very difficult to separate primary from secondary pathogenetic mechanisms. From the viewpoint of preventive medicine, however, it would be of key importance to identify the earliest disturbances leading from genetic predisposition to the early manifestation of obesity. Interestingly, perinatal subtle and temporary impairments of thermoregulatory energy expenditure [89] in an animal model, the fa/fa rat, were among the first observations, well prior to the identification of leptin and its receptors, that paved the way for the elucidation of the early pathogenetic mechanisms of excess fat deposition. The decisive observation was that suckling rats of the Zucker strain that became subsequently obese as homozygous carriers of the fa-gene (fa/fa) were less effective in defending their core temperature than their lean littermates (+/+)[90–92]. When this deficit was compensated for by rearing rat litters at thermoneutrality, the enhanced fat deposition of the fa/fa rat pups could be postponed for the first two postnatal weeks [93]. The reduced thermoregulatory energy expenditure of the fa/fa rat sucklings was due to a reduced responsivity of BAT thermogenesis [94]. On the other hand, the BAT itself could be excluded as the site of the functional defect since postnatal excess fat deposition in fa/fa rat pups could be delayed temporarily when BAT thermogenesis was driven by exogenous application of the sympathetic transmitter, noradrenaline [95]. These observations clearly identified a central nervous functional inhibition of sympathetically driven energy expenditure as the primary cause for excess fat deposition of homozygous carriers of the fa-gene in the early postnatal phase, while the growth of fat-free dry body mass remained unaffected. Quantitatively, the minute restriction of metabolic cold defense during the postnatal period was shown to be, indeed, the major factor that enabled the homozygous carrier of the gene defect to save energy for excess fat deposition. However, in order to demonstrate this effect, the suckling rats had to be nourished artificially to ensure identical energy supply while they were modestly cold-exposed similar to their natural rearing conditions [92].
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Fig. 12. Metabolism of phenotypically normal rat pups reared artificially and identically from day 4 to day 17 under a moderate cold load corresponding to about 50% of their heat-producing capability, which increases with age as indicated by the increase in weight-specific metabolic rate. Compared to the control group (open circles), rat pups treated with leptin (bar) by 2 daily injections (filled circles, 5.3 μg/g/d) showed reduced torpor-related decreases in metabolism without major differences in the non-torpor phase. Inset: At the end of the leptin treatment, body fat content was reduced by 40% (black columns) compared to the non-treated controls (hatched columns). Fat-free dry mass (FFDM) did not differ. Data of Steinhil et al. [100].

The availability of recombinant leptin following the cloning of the obese gene in the mouse [86] and the identification of molecular markers for the fatty locus, the site of the leptin receptor gene [87, 88], have provided new experimental tools. Intracerebroventricular leptin injections in rats confirmed the role of leptin as a central inhibitory modulator of food intake, suggesting that the missense mutation in the fa-gene may greatly inhibit but not completely abolish the transducer function of the long leptin receptor isoform [96]. On the other hand, it was shown in suckling fa/fa rats that peripherally administered leptin could not disinhibit the centrally imposed suppression of metabolic energy dissipation [97]. The resulting conservation of metabolic energy was shown to account for excess fat deposition at a time when the control of food intake was not yet relevant [98] and when secondary hormonal disturbances like hyperinsulinemia had not yet developed [99]. Under the natural rearing conditions, which correspond to a moderate cold load, leptin treatment induced comparable reductions in fat deposition in both naturally reared and artificially reared wild-type rat pups (Fig. 12) by attenuating the nychthemeral decrease in metabolic heat generation underlying the torpor phase [100]. In these early stages, torpor was shown to be controlled by an endogenous circadian pacemaker that is perinatally active in rats [101, 102]. Whether leptin was able to reduce torpor in the adult stage, as it typically occurs in small animals as an energy-saving measure, was found to depend on the energy supply when studied in wild-type mice (Fig. 13). Only enhanced torpor, which developed in food-restricted mice, was abolished by leptin treatment. Interestingly, in free-feeding mice, the same leptin treatment had no effect on energy expenditure but reduced food intake. As the result, both food-restricted and free-feeding mice reduced their fat stores during a 10-d leptin treatment by about 40%, although the free-feeding animals started from a higher fat content, while fat-free dry mass remained unaffected [103, 104]. Interestingly, even in a marsupial lacking thermogenetically active BAT, leptin reduced energy savings during the torpor phase (Fig. 14) resulting in shortened and less pronounced hypothermic phases [105].

 Obviously leptin has been functioning early in the mammalian phylogeny as a hormone that signals the size of energy stores. Depending on the circumstances, when food is scarce, the expenditure of metabolic energy is reduced below that required to maintain homeothermia in phases of rest and inactivity, but food intake is decreased under conditions of food abundance. Both are centrally mediated effects. According to recent evidence, leptin exerts inhibitory actions on hypothalamic neurons [106] to upregulate the melanocortin system that is considered an inhibitory component in the control of food intake [107] and is assumed to oppose neuropeptide Y (NPY) in its central action as a stimulator of food intake [108]. On the
other hand, orexin, a recently discovered neuropeptide stimulating food intake [109] and gastric digestive enzymes [110], facilitates neuronal excitation [111] and seems to activate NPY containing neurons [112]. Indeed, for the arcuate nucleus, where the blood-brain barrier is not completely tight [113], and thus, accessible to circulating leptin, most recent in vitro studies have demonstrated inhibition by leptin of those neurons that were activated by orexin [114]. More insights may be expected from corresponding studies in a recent gene knock-out model, a mouse that is devoid of a functioning melanocortin-4 receptor and becomes very obese [115, 116]. There is evidence for a role of spontaneous defects at this gene locus in human obesity of early onset [117].

Food abundance is a rare event in nature. Thus, the disinhibitory action of leptin on thermoregulatory energy dissipation may be regarded as one of its primordial functions in addition to its putative actions as an antagonist of insulin, both of its secretory control [118] and peripheral actions [119], as well as an inhibitor of cortisol release [120] and an endocrine factor controlling fertility [121]. On the other hand, viewing leptin as an inhibitor of food ingestion concentrates on an aspect that became physiologically relevant only late in the progress of human civilization when large populations gained access to surplus food, a condition that applies, by the way, also to most experimental animals living in the artificial conditions of a laboratory. Although obesity related to the fa-gene is essentially a recessive disturbance, several recent studies indicate that heterozygous carriers of the defective gene temporarily display traits of altered fat deposition and leptin regulation in their postnatal phase [97, 122-124]. With respect to human pathophysiology, notwithstanding that leptin receptor defects are extremely rare in human populations, the early minute deviations from normality seen in the heterozygous Zucker (+/fa) rat may be considered as an example for genetic risk factors. Thus, genes that might be associated with obesity in animal models deserve thorough analysis because combinations of several subliminal genetic traits may become effective in altering the phenotype. This may also be relevant for the most likely polygenic predisposition to obesity in humans: certain combinations might generate a locus minoris resistantiae favouring the onset of obesity when the relationship between food availability and energy expenditure is inadequate.

3. Salt and fluid balance

Water evaporation for heat defense may severely compromise salt and fluid balance. Not only water has to be replenished, but also salt that is lost with the sweat. Volume reductions of body fluid compartments impair thermoregulatory heat dissipation. Because of the importance of this topic in work physiology, the interactions between thermoregulation and body fluid control and the adaptive adjustments following sustained heat exposure were thoroughly analyzed ([125-127] for references).

Modes of interaction in thermo- and osmoregulatory control. Peripheral interactions contribute to the fluid shifts between the extra- and intravascular compartments that are induced by heat exposure. They vary in degree and direction, depending on posture, on the amount of accompanying exercise and on blood flow redistribution. The increase in skin blood flow in support of convective heat transport to the body surface is blunted by the limitation of cardiac
output if reduction of central venous pressure (CVP) passes a critical level (Fig. 15). This effect becomes more pronounced at reduced intravascular filling, especially as a consequence of dehydration. Hyperthermia per se also reduces CVP. Blood flow redistribution as well as alterations in colloid osmotic pressure may contribute to reduced vascular compliance in hyperthermia and to compensatory fluid shifts from the extra- to the intravascular compartment [127].

Interactions of afferent information about the state of salt and fluid balance with the control of body temperature are most obvious for the inputs from high- and low-pressure receptors in the central sections of the cardiopulmonary vascular system. Their modulatory actions account for the negative relationship between the degree of hydration and the rise of deep-body temperature necessary to induce a certain degree of evaporative heat dissipation. Similarly, hyperosmolarity inhibits evaporative heat loss. Osmosensors located near the third cerebral ventricle are involved, and possibly hepatic portal osmosensors as well. Thus, with increasing dehydration and/or hyperosmolarity, greater degrees of hyperthermia were required to induce a certain degree of evaporative water loss by panting and sweating [128–133].

The reverse interaction, i.e., heat-induced afferent signals as modulators of fluid balance, is less well documented. Stimulation of thirst in humans exposed to heat, especially in connection with physical exercise, seems to require some degree of dehydration. Therefore, voluntary ingestion of more fluid than stimulated by the sensation of thirst and, in addition, adequate mineral replacement is recommended for humans doing heavy work or being engaged in high-performance sport activities [133–135]. Yet the increase in plasma volume observed in heat-stressed humans indicates adaptive adjustments in salt and fluid balance [136–138]. Although heat acclimation was associated with physical exercise in these studies, it was assumed that repeated deep-body hyperthermia was the causative factor initiating the endocrine adjustments in support of enhanced water and electrolyte retention [139]. In animal experiments, fluid intake was shown to be adaptive to heat. In a warm environment, guinea pigs ingested water to a degree that tended to decrease their body fluid osmolality and initiate a state of diuresis [140]. In rats, there is evidence of a “feed-forward” system by which heat exposure supports hyperhydration [141], in agreement with early observations [142]. Heat-induced hyperhydration was also seen in pregnant goats [143].

Central “meshing” of the control systems was most convincingly demonstrated when heat-stressed experimental animals could choose between two modes of heat defense; thermal panting, which consumes water, and instrumental cooling behavior, which saves water but interferes with other behavioral states that may normally have priority. As shown in studies on heat-exposed pigeons, well-hydrated animals clearly preferred to pant in hot air, but turned to instrumental requests for cold air when salt and fluid balance was challenged by dehydration [144] or hyperosmolality [145], especially in the brain (Fig. 16). Interestingly, the osmotically induced transition from evaporative to behavioral heat defense occurred with no change or even a drop in deep-body temperature. Thus, the positive correlation seen frequently between the degree of dehydration and the level of steady state hyperthermia during sustained exercise does not seem to be due to a dehydration- or hyperosmolality-induced rise of the setpoint of temperature regulation. It rather reflects the greater load error necessary to drive cardiovascular and evaporative heat loss activities against the inhibitory signals originating in the osmoregulatory system that tend to reduce skin blood flow and water loss [131]. Thus, physiological heat defense is automatically switched, if possible, from modes of autonomic cooling that challenge circulation or water balance, to-
Fig. 16. Study on a pigeon exposed to hot ambient air but trained to control the external heat load (shaded field in lower diagram) by activating a light gate to receive one puff of cold air at a time (reinforcement event, R. E., small vertical bars above abscissa). Initially, the well hydrated pigeon pants (upper diagram) at about 47°C ambient temperature. Under intracarotid infusion of 0.2 ml/min of 4% saline (hatched bar), the pigeon switches from panting to increased cold reinforcement decreasing air temperature to about 28°C, resulting in decreases of back skin temperature (open circles) and deep-body temperature (filled circles). Data of Brummermann and Rautenberg [145].

Towards modes of thermoregulatory behavior by which these challenges can be avoided during states of increasing hyperosmolality and/or dehydration.

The hypothalamus as the site of central interaction. Controller functions residing in the rostral brain stem establish links between thermo- and osmoregulation due not only to converging neuronal and humoral signals but also to mutual interactions in the process of signal integration. The brain-intrinsic vasopressinergic system which releases, as an intracerebral synaptic transmitter, the same neuropeptide, arginine vasopressin (AVP), that is secreted as the antidiuretic hormone by the posterior pituitary, mediates antipyresis by neurons innervating the septal region of the forebrain [146–148]. Increases in AVP plasma concentration due to dehydration were shown to be paralleled by increased release from the septal vasopressinergic system and to reduce the febrile response [149]. In rabbits, peripheral AVP release was also stimulated in fever but the increase in plasma concentration remained temporarily restrained during the initial phase in which cold defense was activated [150]. In guinea pigs [140], sustained cold exposure continually elevated plasma AVP, whereas chronic external heat exposure that did not produce hyperthermia tended to decrease plasma AVP (Fig. 17). Angiotensin II (AngII), as the most important systemically produced osmoregulatory hormone, stimulates aldosterone secretion by the adrenal cortex to enhance renal sodium reabsorption and acts centrally to increase water and salt intake. However, AngII also induces hypothermia [151], presumably in part by acting on a central nervous target [152]. Central nervous AngII microinjections indicate that the brain-intrinsic renin angiotensin system may also mediate hypothermic adjustments of temperature regulation [153–155]. In support of this idea, abolishing central AngII actions in heat-stressed rats by intracerebroventricular injections of the AngII receptor blocker losartan was found to reduce visceral sympathetic activation [156] as a typical component of the differential vasomotor heat-defense response, and it elevated the thermoregulatory salivation threshold [157]. Thus, osmo- and thermoregulatory control interact centrally in complex ways.

Simultaneous neuronal osmo- and thermoresponsiveness, as demonstrated for the hypothalamus, may contribute to the interactions of temperature regulation with salt and fluid balance irrespective of the putative sensory or merely interneuron functions ascribed to these neurons in either of the two control
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Fig. 18. Plasma arginine vasopressin (AVP, left abscissa) increasing in rabbits infused intravenously with hypertonic saline for 3 h (plasma osmolality: shaded curve, right abscissa). Chronically implanted, water-perfused thermodes were used to raise (filled circles) or lower (open circles) hypothalamic temperature (T \text{th}) increasing and decreasing, respectively, plasma AVP. Data of Keil et al. [161].

systems. Thus, the positive temperature coefficients of many hypothalamic neurons would predict direct temperature effects on both thermo- and osmoregulatory control. Indeed, the positive correlation between hypothalamic temperature and the “gain” of thermoregulatory effector control was confirmed in studies on goats and rabbits [158–160]. Similarly, the osmotically stimulated increase of plasma AVP concentration in rabbits was positively correlated with hypothalamic temperature (Fig. 18) in studies with hypothalamic heating and cooling [161]. Thus, the temperature dependence of hypothalamic-neurohypophysial AVP release and the resulting changes in renal tubular water absorption may be one of the factors responsible for cold-induced diuresis and heat-induced antidiuresis. An overall positive temperature coefficient of the neurons controlling osmotic thirst may also contribute to heat-induced drinking, as suggested by the observation that local increases in hypothalamic temperature stimulated thirst in goats [162] and sheep [163]. These data would suggest basically equidirectional effects of temperature and osmolality on hypothalamic neurons, and indeed, corresponding observations were made with single unit recordings [164, 165]. On the other hand, opposing effects of rising temperature and osmolality on the same hypothalamic neuron were reported as well [166, 167], and this combination of effects would explain the well-known inhibition of heat-induced panting and sweating by hyperosmotic stimuli. The fact that neuronal POAH osmosensitivity is directionally mixed was indeed confirmed; especially, neurons with high spontaneous firing rates that were in all likelihood also warm-sensitive, were in part excited by hypoosmotic and hyperosmotic stimulation in vitro [168]. It might be hypothesized that the latter neurons drive the thirst response to hyperosmolarity and/or hyperthermia, while the former neurons inhibit body water utilization for evaporative heat dissipation. However, it is obvious that the observation of bimodally sensitive neurons is not sufficient to draw a connection between osmo- and thermoregulation, and that substantial information about neuronal interconnections is required.

4. Correlates of central integration: thermo- and osmoregulation as examples

Knowledge about the neuronal cytoarchitecture of the central osmoregulatory system is much more advanced than that of the thermoregulatory system. The greater difficulties encountered in the structural analysis of the neuronal basis of thermoregulation may have a phylogenetic background. Control of salt and fluid balance is an early accomplishment in vertebrate phylogeny. This may be the reason why the central neuroendocrine functions are organized in well-defined structural entities. The magnocellular components of the supraoptic and paraventricular nuclei (SON, PVN) were identified long ago as the neurosecretory antidromic hypothalamo-neurohypophysial effector system. They provide morphological and histochemical landmarks to elucidate the cytoarchitecture of osmoregulatory neuroendocrine control. Knowledge about the location of sensory elements is also well advanced, with the subfornical organ (SFO) and organum vasculosum laminae terminalis (OVLT) as circumventricular structures, where the blood-brain barrier is leaky to permit the perception of circulating hormones, and where at least part of the osmosensors are located. The anterior third ventricular region (AV3V), as a periventricular entity, and the median preoptic nucleus (MnPO) were identified as important relay stations establishing reciprocal connections between the neurosecretory and sensory structures of the osmoregulatory system. Much of this evidence rests on the functional deficits seen after microsurgical tract dissections ([169, 170] for references).

For the thermoregulatory system, early attempts to identify rostral brain stem structures as sites of temperature signal generation and processing in lesioning experiments were limited by the rather coarse spatial resolution of the methods [171, 172]. With special respect to signal perception, topographical analysis with multi-barrel thermodes in the rather large hypothalamus of the goat convincingly demonstrated the diffuse, medio-lateral and rostro-caudal distribution of

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the thermosensory function within the POAH region [173] as part of an even more distributed system extending caudally to the medulla oblongata and spinal cord [174]. The OVLT was earlier identified as a putative sensor for circulating pyrogens, but it may not be the only site of endogenous pyrogen perception [175–178]. Sites responsive to one or the other pyrogen or fever mediator were identified at the ventromedial hypothalamic [179], periaqueductal [180], rhombencephalic [181] and medullary [72] levels. The role of vagal afferents transmitting the fever signal is also receiving increasing interest [148, 178]. For those components of the brain-intrinsic vasopressinergic system on which central antipyretic activities are assumed to converge [148], pyrogen-induced changes of activity, as derived from immunocytochemistry [176, 182, 183] and local peptide release [184], have identified sites serving this specific function in thermoregulation.

Analyzing neuron discharges, neuropeptides and pathways. The cellular basis of central thermoregulatory functions is currently analyzed with electrophysiological approaches mainly by studying the intrinsic osmosensitivity of the magnocellular neurons (MNCs) of the hypothalamo-nephrophysial antidiuretic system. Although neurons in the OVLT, SFO and MnPO deliver excitatory outputs in response to local hyperosmotic stimulations, functional specificity, as concluded from the efficiency to produce osmoregulatory responses, seems presently to be attributable only to the OVLT and SON [185]. For the MNCs, stretch sensitive, poorly selective cation channels currently appear as the most likely transducers of the osmotic information [186]. Although this mechanism is also considered for neuronal osmosensitivity in the lamina terminalis structures, reports on larger samples of neurons analyzed in the rat SFO [187] and OVLT [188] describe inhibitory as well as excitatory effects of hyper- or hypoosmolality, sometimes bi-directional sensitivity, and the observed responses require rather large osmotic stimuli. The importance of reciprocal connections between the OVLT and AV3V region on one hand, and the magnocellular sections of the SON and PVN on the other, for appropriate osmoregulation, shed light on the existing uncertainty about the mode of adequate signal generation, either by specific sensors or by neuronal networks utilizing neuronal osmosensitivity in a more general fashion. Thus, the idea of signal generation in an osmoreceptor complex [189] still seems to hold as a working hypothesis. An important input might originate in addition from periventricularly located osmosensitive neurons displaying cation sensitivities, as suggested by drinking responses to microinjections of hypertonic solutions of different composition into the hypothalamus of sheep [190]. Concurrent effects of osmotic stimulation on renal water excretion in vivo and on the activity of a histologically defined [191] periventricular population of hypothalamic neurons in vitro have been found, so far, only in studies on birds, both osmoregulatory and neuronal responses being in accordance with the assumption of a primary cation sensitivity of periventricular osmosensors [192, 193].

Tracing inputs and projections pertinent for salt and fluid balance from hypothalamic to extrahypothalamic brain and spinal regions greatly profited from the immunocytochemical and autoradiographical detection of the two major brain-intrinsic osmoregulatory hormones, AVP [194–197] and AngII [198, 199], and their receptors, respectively. Characterizing atrial natriuretic peptides as messengers acting mainly as a functional antagonist of AngII, both as circulating messengers and as brain-intrinsic mediators [200], has contributed to understanding the roles played by peptideergic systems in central osmoregulatory control.

The cellular basis of central thermoregulatory functions has been assessed for a long time by making efforts to obtain clues about the neuronal organization at the hypothalamic level from the electrophysiological characteristics displayed by hypothalamic neurons that are directly thermosensitive or responsive to remote thermal stimuli. However, the properties that might discriminate sensory from integrative or effector neurons have remained equivocal [201–204], not least because it is not clear whether or not temperature signals are provided by specific thermosensors or originate in specifically designed modules within which neurons exhibit basically non-specific positive temperature coefficients [205]. Attempts to characterize thermosensitive neurons according to the orientation of their dendritic ramifications [206] have remained anecdotal. Possibly because homeothermia developed comparatively late in vertebrate phylogeny, defined thermoregulatory control functions may not be associated with classical structural entities like brain nuclei and brain tracts.

Studies of the descending pathways at the hypothalamic level by which thermoregulatory effectors are controlled have remained, for a long time, limited mainly to shivering ([3] for references) and vasoconstriction and -dilatation ([49] for references). Probing of descending pathways has been resumed recently by applying topical stimulations and lesions at the hypothalamic level [207]. The concept underlying this approach assumes genuine warm signals as the prevailing source of thermosensory outputs from the POAH
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Fig. 19. Schematic presentation of the degrees and levels of signal transmission from one side of the POAH region across the midline in descending pathways controlling different autonomic thermoregulatory effectors. Pathways were identified by combining hypothalamic thermal stimulation with unilateral anterior hypothalamic transection. Modified presentation of Kanosue et al. [208].

region (Fig. 19). The additional postulate of hypothalamic thermosensory control of the diverse thermoregulatory effectors by individual sets of descending neurons is derived from the observations that their signals cross the brain midline to different degrees at different brain stem levels [208]. Strictly unilateral are the drives for thermoregulatory salivation [209], whereas signal crossing was described at the POAH level for vasomotion [210] and at a subhypothalamic level for shivering [211]. At the midbrain level, reticulospinal and rubrospinal neurons were identified as carriers of the drive for shivering [212], but the courses of the controlling neurons that are assumed to transmit inhibitory signals from the POAH region and excitatory signals from the posterior hypothalamus have not been identified. For non-shivering thermogenesis (NST), neurons providing excitatory drives are located in, or their axons pass through, the ventromedial hypothalamus (VMH) and are under tonic inhibitory control of POAH neurons that may carry locally generated warm signals [213]. Signals from POAH neurons driving skin vasodilatation pass through an area ventrolateral to the periaqueductal grey, but those inhibiting skin vasoconstriction run in the ventral tegmental area [214]. POAH efferents carrying the locally generated warm signals for vasomotor control and possibly also the thermoregulatory drive for salivatory run in the medial forebrain bundle but seem to cross the midline to different degrees. A more lateral position in the zona incerta of neurons carrying sudomotor drives has not yet been established [207].

Histochemical indicators for neuronal activity and efferent connections. Tracing with phytoagglutinins [170, 215] and neurotropic toxins [216] successfully supplemented lesioning studies in revealing many details of the connectivities underlying osmoregulatory integration in the lamina terminalis and adjacent anterior hypothalamic regions. More recently, immediate early gene products as indicators for altered neuronal function [217–219] have been increasingly used to analyze central components of salt and fluid balance. Mostly acute [217, 220, 221] but also chronic [222] challenges were applied to stimulate c-fos expression and identify the mRNA message or the gene product, the Fos protein. Proceeding from the known functions, sensory for the OVLT and SFO, integrative for the MnPO and neuroendocrine for the SON and PVN, Fos immunocytochemistry is providing new insights on how the osmoregulatory and other autonomic control systems are interconnected. Especially, the relationship to cardiovascular control has been clarified by showing that the SON, PVN and SFO are preferred targets activated at the hypothalamic level by medullary inputs carrying information about the intravascular states of filling and pressure [223–229].

Tracing c-fos mRNA and its product has also proven useful in detecting altered states of neuronal activity in response to fever-inducing agents, from endotoxins to prostaglandin E2 (PGE2) as the presumably ultimate mediator of fever [230]. Not surprising and in accordance with the numerous autonomic, motor, endocrine and neuroimmune adjustments of the fever syndrome, endotoxin administration induced widespread activation of c-fos transcription, including sites known to respond generally to stress. With special respect to interference with the osmoregulatory system, the OVLT seems to be a candidate for interactions because of its dual function as an osmosensor and a target for pyrogens [231, 232]. Activation of SON and PVN neurons, including the magnocellular division of the latter, in fever are in accordance with the known stimulation of AVP release and the brain-intrinsic vasoressinergic systems with the septal region as the target for vasopressin-mediated antipyresis [232–234]. Fever-induced Fos expression in the preoptic area (POA), especially in its ventromedial section, may relate to presumed humoral modulation of thermosensory and thermointegrative hypothalamic neurons leading to the well-controlled febrile hyper-
Fig. 20. Immunocytochemically identified Fos-positive cell nuclei in various hypothalamic areas of the rat representative for differential activation by dehydration and external heat loading (3V: third cerebral ventricle) [242]. The conditions in which sections were taken for Fos identification were, from left to right: control at room temperature (21°C), 24 h of water deprivation at 21°C, 48 h of exposure to 34°C ambient temperature, the same heat exposure with additional water deprivation during the last 24 h.

A–D: The medial preoptic area (MPA) with its ventromedial portion (VMPO) shows little Fos expression in control conditions (A) and dehydration alone (B). Heat exposure (C) moderately increased Fos expression, which became more pronounced when dehydration was superimposed on heat exposure (D).

E–H: The dorsal portion of the median preoptic nucleus (dMnPO) extending beyond the anterior commissure (ac; f: fornix) shows little Fos expression under control conditions (E), distinct expression after both dehydration (F) and heat exposure (G), and a further augmentation when superimposing dehydration on heat exposure (H).

I–L: The hypothalamic paraventricular nucleus, in particular the magnocellular portion (mPVN) but less so the parvocellular portion (pPVN), is a structure in which only dehydration (J) and dehydration superimposed on heat exposure (L) induce enhanced Fos expression in comparison to control (I) and selective heat exposure (K).

thermia [232, 235]. Centrally applied PGE2 seems to generate patterns of enhanced Fos expression similar to those induced by endotoxin, perhaps with the exception of a lesser involvement of the OVLT [236, 237]. In summary, c-fos expression was shown to provide additional cytoarchitectural information supplementing knowledge about the pathophysiological involvement of the vasopressinergic system in fever.

The comparative evaluation of Fos expression in rats after acute cold exposure for 2 h, leading to intense cold defense response with no hypothermia and, respectively, heat exposure for 2 h producing hyperthermia and activation of heat defense, was followed by a differential pattern of Fos activation [238]. After heat exposure, the medial preoptic area (MPA, MPO), a region of distributed neurons immediately lateral to the OVLT, was a prominent site of enhanced Fos expression with the highest density in the anterodorsal parts. These areas belong to the POAH region as a functionally identified site of thermoreception and temperature signal integration. Short-term cold exposure that did not change deep-body temperature did not enhance Fos expression in this region. Thus, Fos-induced activation would correspond with the view that warm perception and activation of descending pathways driving heat defense and inhibiting of cold defense is the primary function of the POAH region in normal temperature regulation [207]. More detailed insight was provided by a study in which rats were subjected to similar degrees and durations of cold and warm challenges while more extended brain stem sections were analyzed [239]. Fos activations induced by both challenges were comparable in the lateral septum (LS), POA and supramammillary nucleus. Further, structures responding to both challenges, however, with a more pronounced effect of heat, were restricted to the lateral preoptic area (LPO, or LPA), whereas the effect of cold prevailed in the parvocellular PVN (pPVN) in the zona incerta (ZI), in the posterior hypothalamic area (PH), in neurons of the lateral dorsal and ventral central (periaqueductal) grey and in several more caudally located brainstem and spinal structures, often with no significant heat-induced Fos activation. Especially, some of these latter structures were identified as sites where descending cold defense information is conducted [207]. Regions showing sustained Fos activation after 14 d of chronic cold exposure included the POA, LPO, ZI and the periaqueductal grey, and Fos activation in the ventromedial hypothalamic nucleus (VMH) had become even more pronounced.
[240].

More specific information about many still unknown physiological interactions between thermoregulation and osmoregulation may be expected from analyzing the close functional interdependence to be expected when salt and fluid balance compete with evaporative heat dissipation. Accordingly, Fos immunocytochemistry was used to identify hypothalamic sites of integration in studies on the short-term, heat-acclimated Sabra rat as a suitable animal model [241]. As thermo- and osmoregulatory loads known not to exceed the animal’s physiological capacities, 24 h of water deprivation and exposure to 34°C ambient temperature for 48 h (designated as short-term heat acclimation, STHA) were imposed, or the two challenges were combined during the last 24 h of heat exposure [242], leading to moderate dehydration, modest hyperthermia or more pronounced combined states of hyperthermia and dehydration. Although Fos expression in response to these sustained challenges may represent states of neuronal activity indicating adaptive rather than primary regulatory adjustments in the central nervous system, the patterns of Fos expression were remarkably similar to those seen with short exposures. As an exception, Fos expression did not increase in the paraventricular nucleus of the thalamus (PV), contrary to short-term heat exposure as reported in the above-mentioned studies, and would indicate the absence of a sustained, generalized stress response in the heat-loaded, water-deprived Sabra rat. Fos expression was shown to be quite differential, both in distribution (Fig. 20) and quantitative interaction (Fig. 21). The lateral hypothalamic area (LHA), the LS and the MPA were identified as structures exclusively activated by heat stress. Only slight activation was seen in the SON, PVN, OVLT and SFO. Selective dehydration distinctly increased Fos immunoreactivity in the SON, PVN, SFO and OVLT, and to a slight degree in the ventromedial portion of the MPA (VMPO) immediately adjacent to the OVLT. The only structure being equally activated by either heat exposure or dehydra-

![Fig. 21. Quantitative evaluation of Fos expression in the hypothalamic structures illustrated in Fig. 20. Only the MnPO shows an approximately additive activation by combined heat exposure and water deprivation.](image)

![Fig. 22. Immunocytochemical detection of pseudorabies virus protein in a rat at the level of the paired hypothalamic paraventricular nuclei seen on either side of the cavity of the third cerebral ventricle [243]. The tracing period was about 72 h following virus injection into the left submandibular salivary gland. Virus distribution at this stage of infection is mainly ipsilateral.](image)
Fig. 23. Immunocytochemical detection of pseudorabies virus protein in a rat at the preoptic level comprising structures of the lamina terminalis as the rostral border of the third cerebral ventricle [243]. Located centrally, immediately dorsal to the chiasma opticum and to the rostral extension of the third ventricular recessus supraopticus is the organum vasculosum laminae terminalis. Bilaterally adjacent are the medial preoptic area extending laterally into the lateral preoptic area. The median preoptic nucleus extends dorsally in the midline towards the anterior commissure. The tracing period was about 92h following unilateral virus injection into the sublingual salivary gland. Virus distribution at this stage of infection is bilateral.

Fig. 24. Quantitative analysis of POAH structures in a representative rat for neurons containing the pseudorabies virus protein 3 d after unilateral virus injection into the submandibular salivary gland. Structures: OVL, organum vasculosum of the lamina terminalis; MnPO, median preoptic nucleus; SFO, subfornical organ (not shown in Figs. 22 and 23); MPA/LPA, medial (and lateral) preoptic area; PVN, hypothalamic paraventricular nucleus; and LHA, lateral hypothalamic area (not shown in Figs. 22 and 23). Data of Hübschle et al. [243].

pseudorabies virus strain [243]. Using a virus-specific antiserum, the retrograde and transynaptic progress of infection could be detected within 1 to 4 d after infection. In the parasympathetic secretomotor pathway, the virus appeared 55h after infection in the ipsilateral salivatory nuclei of the ponto-medullary brain stem. In the forebrain, larger numbers of infected neurons considered as third-order neurons projecting to the salivatory nuclei were detected after 3 d. They included the amygdala ventrolaterally and the bed nucleus of the stria terminalis dorsolaterally, as well as the LHA and PVN, and initially the virus appeared mostly ipsilaterally (Fig. 22). Infection reached the preoptic level at about the same time as affecting the MPA as well as the LPA. In the midline lamina terminalis structures (OVL, MnPO and SFO), the virus was detected slightly later with no distinct difference in lateral distribution (Fig. 23). Thus, up to the anterior hypothalamus, the infections were more pronounced ipsilaterally (Fig. 24) in accordance with the presumed high crossing at the preoptic level of the thermoregulatory descending pathways controlling salivation [207]. The sequence of ascending infections
identify the LHA and parvoacellular PVN as relay stations within the descending efferent pathways transmitting control outputs generated at higher levels of osmo-/thermoregulatory integration.

Conclusions and Perspectives
Starting with the early elucidation of competing demands on circulation and respiration, the analytical conception to exploit the challenges imposed by thermoregulation on non-thermoregulatory autonomic and behavioral control has proven fruitful in disclosing basic control characteristics of the latter. While the neurophysiological aspects of integrative control of thermal and non-thermal homeostatic systems prevailed for a long time, the endocrine aspects have become more important recently, as demonstrated especially for the control of energetics and salt and fluid balance. In the assessment of central nervous signal perception, signal integration and neuro-endocrine effector control, electrophysiological analysis is increasingly supplemented by bio-/histochemical approaches. This includes the detection of mRNA signals for specific peptides, molecular receptors, or of the products themselves. Further elucidation is provided by molecular indicators for the state of activity of central neurons and by marking their afferent and efferent connections by means of advanced tracing techniques. The assessment of the cytoarchitecture of central autonomic and neuroendocrine systems by which the multiple homeostatic control systems are mutually adjusted will expand increasingly from the integration of in vivo approaches with the multitude of in vitro approaches available. Animals which carry spontaneous gene defects, or in which interesting genes are knocked out, will become increasingly important as experimental models. However, it has to be kept in mind that the construction of such a model is only a precondition. Ultimately, understanding such an animal’s bodily functions in vivo will be decisive. It is becoming increasingly clear that the most refined multidisciplinary approaches, as they were developed during decades of integrative physiology, will be required to identify the often minute or sometimes unexpected traits left by the loss of a seemingly important and well-defined gene. Possible reasons are that not only genetic background variations but also the often marvelous redundancy of biological control systems may largely compensate in vivo for the theoretically most serious gene defects.

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