The Chemistry of Cold: Mechanisms of Torpor Regulation in the Siberian Hamster

Siberian hamsters use spontaneous daily torpor, a state of hypometabolism and hypothermia, to save energy during winter. Multiple neuroendocrine signals set the scene for spontaneous torpor to occur, and several brain areas have been identified as potential sites for torpor regulation. Here, we summarize the known mechanisms of a fascinating physiological state in the Siberian hamster.

Living at temperate and polar latitudes means living in an extremely variable environment. Whereas summer is pleasant with its long days, high ambient temperatures ($T_a$), and blooming plants, the surroundings become uncomfortable in winter. Day length substantially decreases, temperature falls, and vegetation, hence food sources, run short. This is a particular problem for endotherms, since they need enormous amounts of energy to maintain a high body temperature ($T_b$) when $T_a$ and food availability are at their lowest. Mammals living in this environment show multiple adaptations to deal with these annual variations. Changes in fur properties, reproduction, growth, body weight, metabolism, and $T_b$ are common parameters they use to adjust energy balance over the course of the year. The most drastic way of saving energy during times of food shortage is the use of torpor, a hypometabolic state during which all physiological functions are decreased to a minimum. Torpid mammals appear very strange: they are hunched in a typical posture, appear stiff, react only slowly to external stimuli, and, most oddly, are cold to the touch. But although they appear stiff and lethargic, they are far from just being “switched off”! Torpor is a very precisely regulated physiological state during which metabolic rate can be suppressed to 96% below basal metabolic rate (33). Subsequently, $T_b$ drops to values that can approach $T_a$ when $T_a$ and food availability are at their lowest. Torpor has been described in species from most mammalian orders, but its regulatory mechanisms have only begun to be understood. A species whose seasonal adaptations and use of daily torpor have been well studied is the Siberian hamster (*Phodopus sungorus*, also known as Djungarian hamster) (24). Here, we review the mechanisms known to be involved in the regulation of daily torpor in this species.

Siberian hamsters originate from the steppes of South-West Siberia and North-East Kazakhstan. Their habitat is characterized by a dry and sharply continental climate with a $T_a$ range from $-45^\circ$C in winter to $30^\circ$C in summer. Since the environmental conditions are highly variable, Siberian hamsters use changes in day length (photoperiod) to predict the season. When days are getting shorter and a critical photoperiod of 13.5 h of light and 10.5 h of darkness is reached in September, they start to morph into their winter phenotype that is completed after ca. 12 wk (43). The winter phenotype can be induced under natural as well as laboratory photoperiod changes and does not necessarily require cold exposure or food restriction (35, 83). Low $T_a$, however, is able to alter critical photoperiod, and promotes onset and duration of torpor season as well as incidence and depth of torpor episodes (72, 82). During the winter adaptation period, Siberian hamsters change fur, cease reproduction, and substantially decrease body weight (75). After 10 wk of short photoperiod
exposure, they start to spontaneously display bouts of daily torpor. Spontaneous winter torpor is initiated by a decrease of metabolic rate to ~25% of resting metabolic rate at the beginning of the animals’ circadian resting phase, and consequently $T_b$ drops to minimal values of 15°C (34). Torpor is maintained for an average duration of 6 h and terminated by an arousal during which normothermia is reached within 30 min by nonshivering and shivering thermogenesis. The energy saving of a single torpor bout approximates 30% over a 24-h period but can add up to 65% over a period of 15 days when torpor is used frequently (71). The frequency of spontaneous winter torpor varies between individuals and can range from random single torpor bouts to 6–7 bouts/wk despite food ad libitum and without any obvious environmental challenge (35, 83). Hence, spontaneous winter torpor must at least partly rely on mechanisms that are linked to intrinsic long-term physiological adaptations.

Besides spontaneous winter torpor, a distinct form of torpor can be induced by a period of food restriction at any time of year. When induced in animals that are not winter adapted, however, fasting-induced torpor requires an initial loss of body weight of ~30–35% (67, 81). A recent study by Diedrich et al. could show that spontaneous winter torpor differs from fasting-induced torpor in several parameters, such as duration, depth, and arousal properties (16). Detailed analysis of respiratory quotient (RQ) revealed that fasting-induced torpor occurs from a state of lipid-based metabolism (RQ = 0.79 ± 0.01), whereas spontaneous winter torpor is entered from a state of glucose metabolism (RQ = 0.88 ± 0.02). Moreover, fasting-induced torpor loses its circadian rhythmicity and rather depends on feeding schedule than on the light-dark cycle (60, 67, 81). Thus fasting-induced torpor differs from spontaneous winter torpor, which has to be carefully taken into account when data are interpreted (18) (FIGURE 2).

Melatonin

In Siberian hamsters, photoperiod is the most important cue for annual physiological changes (80). Day length is perceived by the eye and forwarded to the master circadian clock in the suprachiasmatic nuclei (SCN) of the hypothalamus, which in turn controls nocturnal production and release of melatonin from the pineal gland. When day length decreases toward winter, duration as well as amplitude of melatonin secretion extend and this eventually initiates the physiological changes (29). Melatonin has an indirect effect on torpor: Melatonin release from the pineal gland is required for torpor readiness, since spontaneous winter torpor is largely prevented in Siberian hamsters that are pinealectomized prior to short day exposure (83). No inhibitory effect occurs, however, when direct or functional pinealectomy is carried out during the torpor season in animals that are already winter adapted (64, 65). Also, constant release of melatonin has no acute effect on spontaneous winter torpor (47). Hence, melatonin regulates a series of seasonal physiological changes, which in turn control the expression of spontaneous winter torpor.
Prolactin

The most obvious transition Siberian hamsters show in response to photoperiod is a change in fur color and composition (24, 49, 59). The key hormonal signal that has been linked to fur changes is the hormone prolactin. Prolactin secretion is controlled by endocrine neurons of the arcuate nucleus (ARC) of the hypothalamus, produced by the pars distalis of the pituitary gland and controlled by pineal melatonin secretion (2). Prolactin serum concentrations in Siberian hamsters are high during summer (200 U/ml), when the animal carries its brownish summer coat, and decreasing prolactin concentrations (30 U/ml) are required for changing into their white and insulating winter fur (22, 87). Decreased serum prolactin correlates with the onset of the torpor season, and infusion of prolactin from osmotic minipumps in winter-adapted animals decreases the incidence of spontaneous winter torpor (66). Importantly, however, prolactin infusions do not inhibit fasting-induced torpor in summer animals. Thus, despite its inhibitory effect on spontaneous winter torpor, a strong fasting-induced signal can override the prolactin message if energetically necessary.

FIGURE 2. Body temperature, respiratory quotient, and metabolic rate of a Siberian hamster
Body temperature (black line), respiratory quotient (gray line), and metabolic rate (gray field) of a Siberian hamster showing spontaneous winter torpor (A) or fasting-induced torpor (B).

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Testosterone

One of the energetically most expensive processes is reproduction. Siberian hamsters entirely cease reproduction during winter. Gonadotropin concentrations (FSH, LH) decrease, followed by a regression of testes and uteri, with a consequent drop in gonadal hormones (41, 42, 76, 87).

Generally, spontaneous winter torpor only occurs when testes are regressed and circulating testosterone is low (30, 83). The torpor season ends when testes size increases and testosterone levels rise. When Siberian hamsters are castrated, the onset of the torpor season is accelerated and prolonged (55, 56).

The importance of testosterone for torpor becomes clear when testosterone is supplemented: Testosterone infusions potently block spontaneous winter as well as fasting-induced summer torpor (5, 55, 66, 83). Very low testosterone concentrations after castration increase torpor frequency in winter-adapted animals; however, castrated summer animals do not undergo spontaneous torpor (83). Hence, low testosterone alone is not sufficient to induce torpor.

Testosterone levels correlate with vasopressin immunoreactivity in the lateral septum of the brain, which has been suggested to mediate the inhibitory effects of testosterone on torpor in Siberian hamsters (56). Taken together, testosterone has a potent inhibitory effect on spontaneous as well as fasting-induced torpor, possibly mediated by the lateral septum of the brain. However, low testosterone alone without a short day signal cannot induce torpor.

Leptin and Growth Hormone

Like many other small mammals, Siberian hamsters substantially decrease body weight before winter (up to 40%) (32). This is very different from many larger seasonal mammals that increase their body and fat mass to survive the meager winter season. The Siberian hamster, however, uses a different strategy: it tries to rather minimize its body mass and weight reduction results from voluntarily decreased food intake and comprises loss of fat as well as lean mass (21, 74, 84).

Spontaneous winter torpor is not initiated before fat and lean mass have substantially decreased. In line with the decrease in white adipose tissue, serum leptin concentrations in Siberian hamsters decline in short photoperiod (20). The peptide leptin is mainly produced in white adipose tissue and provides a feedback about endogenous fat stores to the brain (44). Reduced leptin levels seem to be a prerequisite for the onset of torpor season, and spontaneous winter torpor can be inhibited by exogenous leptin from subcutaneous minipumps in most (60%) but not all hamsters (25). In the animals that continue torpor upon leptin treatment, neither depth, duration, nor frequency is different from saline-treated controls. Hence, reduced leptin concentrations seem to be important for torpor readiness but are not sufficient to induce torpor.

Also, changes in lean mass contribute to the body weight cycle of Djungarian hamsters (9, 21, 74). There is strong evidence that the growth axis is shut down during winter, with decreased levels of circulating growth hormone and insulin like growth factor 1, likely mediated by increased somatostatin expression in the hypothalamus (38, 46). Paseriotide, a long-acting somatostatin receptor agonist with high affinity to SST 5, is able to decrease body weight of summer-adapted hamsters and to retard body weight increase from winter to summer state consistent with reduced serum insulin-like growth factor 1 (21). When paseriotide is given in winter, it is able to strongly increase torpor frequency as well as torpor bout duration (73). The somatostatin receptor agonist ocreotide with high affinity to SST2, however, does not affect torpor, suggesting that it is rather the activation of specific somatostatin receptors than downregulation of the growth hormones axis per se that is involved in the regulation of torpor.

Glucose and Fatty Acids

It is obvious that torpor is related to energy balance. During daily torpor, cellular metabolism is reduced, so it is likely that a metabolic signal is involved in its induction (34, 35). Glucose and fatty acids are the main endogenous metabolizable fuel sources, and a series of studies has investigated the role of these substances.

One possibility to decrease cellular glucose availability is the application of 2-deoxy-D-glucose (2DG), a glucose analog that disrupts glucose oxidation. The idea of glucoprivation to regulate torpor is tempting, since endogenous plasma glucose concentrations decline during spontaneous winter torpor (12). When 2DG is injected ip in very large doses (2–2.5 mg/kg) into summer-adapted Siberian hamsters, it induces torpor within 1 h after injection and decreases 24 h after food intake (13, 14). Glucoprivation by 2DG even counteracts the inhibitory effect of physiological testosterone concentrations on torpor (13). However, in winter-adapted animals, induction of torpor by 2DG is less effective, and, when plasma glucose levels are decreased by insulin within a physiological range,
they fail to induce torpor (12, 79). So it is likely that the effects of 2DG in summer-adapted hamsters result from the extreme glucoprivation of a pharmacological dose and resembles fasting-induced torpor. Spontaneous winter torpor, however, appears to be unrelated to acute glucoprivation, which is also supported by RQ values of ~1 at torpor entry (17).

A role for fatty acids in torpor regulation has been discussed, since polyunsaturated fatty acids (PUFAs) have been shown to be beneficial for torpor in deep hibernators (68). In Siberian hamsters, fatty acid composition of tissues, in particular brown adipose tissue and heart, has been shown to change significantly after short-day adaptation independent of changes in dietary fat, but no clear increase of torpor frequency can be found upon substituting essential PUFAs (28, 71). Not the absolute PUFA content but rather the ratio of different PUFAs might influence torpor behavior. A slight increase in torpor frequencies occurs in hamsters fed a diet rich in omega-6 PUFAs (19, 27).

It has been suggested that a membrane phospholipid composition with high omega-6-to-omega-3 PUFA ratio is beneficial for torpor expression since it increases the activity of the Ca\(^{2+}\)-Mg\(^{2+}\) pump in the sarcoplasmic reticulum of the heart (SERCA). This mechanism may protect the heart from arrhythmia at low tissue temperatures (68). Overall, PUFAs rather seem to protect organs at low \(T_b\) than being directly involved in torpor induction. Indeed, shutting down fatty acid oxidation by injections of mercaptoacetate is not able to initiate torpor bouts in *Phodopus sungorus* (14, 79).

**Neuronal Structures**

Spontaneous winter torpor requires an intact noradrenergic signaling of the sympathetic nervous system, so neuronal structures controlling autonomic functions are likely to be involved (8). Within the brain, the sympathetic nervous system is controlled by the hypothalamus, which has received particular attention in torpor research. One of the difficulties in torpor research is the unpredictability of spontaneous winter torpor bouts, which is why pharmacological torpor induction by 2DG has been used to investigate which parts of the brain are generally involved in the regulation of metabolism and \(T_b\).

Screening of c-fos immunoreactivity, a marker for neuronal activation, revealed that various structures of forebrain, hindbrain, thalamus, and hypothalamus are activated during 2DG-induced hypothermia in *Phodopus sungorus* (57). Neuronal activity increases in five hypothalamic structures upon torpor induction by 2DG: SCN, paraventricular nucleus (PVN), median preoptic area (MPOA), ARC, and supraoptic nucleus (SON). This suggests that these structures may be important for timing (SCN), regulation of \(T_b\) and metabolism (PVN, MPOA, SON, ARC) (1, 50, 52, 63). Lesion studies have investigated the importance of most of these nuclei.

Spontaneous winter torpor is a strictly circadian phenomenon that is controlled by the circadian clock in the SCN, and it has been suggested that the SCN might regulate torpor onset (45). SCN lesions, however, only disrupt the temporal organization of all daily rhythms, including torpor onset, but do not entirely prevent spontaneous winter torpor (67). Also, fasting-induced torpor can be provoked in SCN-lesioned winter and summer animals, and timing of torpor can be reinstated by feeding schedules (60). Thus the SCN does not seem to directly regulate the induction of hypometabolism but rather indirectly by controlling feeding times. Moreover, SCN lesions lead to hyperprolactinemia, which, in turn, might interfere with the torpor response (7, 65).

The PVN is an important relay station for SCN input to the pineal gland and thereby involved in the regulation of melatonin release. Moreover, subpopulations of PVN neurons project to control various pituitary functions. Yet other PVN neurons directly regulate appetite and autonomic functions in the brain stem and spinal cord. When the PVN is ablated in winter-adapted Siberian hamsters, body weight increases, and the majority (67%) of animals does not express spontaneous winter torpor (64). This inhibitory effect is unlikely to be mediated by PVN-controlled changes in pineal melatonin secretion, since pinealectomy does not prevent torpor once the hamsters are adapted to short days and spontaneous winter torpor is already expressed (65). So it is more likely that PVN ablations disrupt spontaneous winter torpor via neuroendocrine or metabolic mechanisms like increased prolactin concentrations or body weight gain (7, 64).

The ARC of the hypothalamus is well known for its role in the regulation of energy balance (1). Two neuronal populations sense and balance energy input and outflow: Neurons co-expressing neuropeptide Y (NPY) and Agouti-related peptide (AgRP) potently stimulate food intake and decrease energy expenditure when activated, whereas POMC/CART neurons reduce food intake and increase energy expenditure (11, 48, 54, 86). When the ARC is ablated with monosodium glutamate injections, spontaneous winter torpor is prevented. However, torpor-like hypothermia can still be induced by fasting and 2DG injections, but with reduced frequency and depth (62). Hence, ARC mechanisms are likely to be involved in the regulation of
Neuropeptide Y

Since the ARC seems to be involved in torpor control, the underlying mechanisms are of substantial interest. NPY in the ARC of the hypothalamus is a neurotransmitter that is well established to regulate several aspects of the orexigenic response in rodents (11). When NPY is injected into the ventricles of the brain, it increases food intake and decreases energy expenditure by reducing metabolic rate and nonshivering thermogenesis (6, 23).

Intracerebroventricular (ICV) injections of NPY are able to induce torpor-like hypothermia in cold-acclimated summer Siberian hamsters (58). NPY-induced torpor patterns resemble those of fasting-induced torpor rather than spontaneous winter torpor bouts, in line with its role as a strong metabolic signal in rodents. Six receptor subtypes for NPY are known, two of which, Y1 and Y5, appear to be the main mediators of food intake and energy expenditure. ICV injections of NPYY1 receptor agonist ([D-Arg²⁵]-NPY), but not NPYY5 agonist ([D-Trp³⁴]-NPY), induce torpor-like hypothermia, whereas NPYY1 receptor antagonist prevents NPY-induced torpor (15, 61). This implies that NPY-induced torpor in Siberian hamsters is mainly mediated via Y1 receptors.

Thyroid Hormones

Thyroid hormones have long been known to be potent modulators of metabolic rate and energy balance in mammals. Tri-iodothyronine (T3) is considered the bioactive thyroid hormone, and it is well known to increase metabolic activity by regulating adaptive thermogenesis (78). Classically, the effect of T3 has been attributed to its action in peripheral tissues: T3 increases noradrenergic activation of uncoupling proteins in mitochondria of brown adipose tissue that uncouple substrate from phosphorylation of ADP to ATP so that the energy from the proton motive force dissipates as heat. The first indication that thyroid hormones underlie a seasonal regulation in Phodopus sungorus was found in 1987, when Seidel et al. showed a seasonal variation of thyroid hormone concentrations in blood plasma (77). Levels of T4 and T3 were higher during the winter months, consistent with the increased thermogenic capacity of Siberian hamsters during the torpor season (36). Thyroid hormones have regained massive attention in the field of seasonal physiology since it could be shown that melatonin-controlled thyroid hormone availability, specifically to the hypothalamus, is a key driver of seasonal adaptations in birds and mammals (4, 31, 40, 53, 85). In the brain of Siberian hamsters, deiodinase enzymes controlling T3 metabolism are regulated by photoperiod as well as fasting and are potentially able to integrate long- and short-term metabolic challenges (37, 38, 40). Because of their well known effects on metabolism and Tb, thyroid hormones are conceivable candidates for being involved in the regulation of torpor. Indeed, thyroid hormones have a pronounced effect on torpor: When T3 is supplemented via drinking water to winter-adapted Siberian hamster, torpor is inhibited within a few days (2a). Moreover, silastic implants releasing T3 into the hypothalamus are just as efficient in blocking torpor (51). In contrast, when T3 serum concentrations are lowered by giving methimazole and perchlorate in the drinking water, torpor frequency, depth and duration increase (2a). Peripheral injections of 3-iodothyronamine (T1AM), a thyroid hormone derivative, induce torpor-like hypothermia in summer animals and, to a lesser extent, in winter animals (10). Whether the effects of thyroid hormones on torpor are driven by central or peripheral mechanisms remains unclear. Gene expression data from Bank...
et al. (2a) indicate changes in uncoupling proteins in brown adipose tissue, but the hypothalamic systems looked at were largely unaffected. However, expression data of deiodinase type 2 would suggest a downregulation of T3 production in the hypothalamus during torpor. Given the significance of the hypothalamic thyroid hormone system in seasonal adaptations per se, a central T3-driven mechanism is likely but still has to be revealed.

Conclusions

The expression of spontaneous winter torpor requires adjustment of many endocrine systems (FIGURE 3). All of these systems are able to affect the torpor response, but none of them has been identified as the exclusive signal for torpor. The acute induction mechanism of spontaneous winter torpor remains unclear. The pharmacological approaches that have been used target metabolic pathways and rather induce a hypothermia that resembles fasting-induced torpor. Caution should be taken in drawing conclusions from these experiments about the regulation of spontaneous winter torpor, given that the torpor-like states induced by the pharmacological methods used thus far seem to be distinct from spontaneous torpor in winter-acclimated animals. Since the use of spontaneous winter torpor is irregular and unpredictable with high individual variability, investigation of natural torpor induction mechanisms is difficult. The hypothalamus appears to be a key brain area involved in torpor induction, and molecular as well as in vivo investigations and manipulations of this brain area are promising to nail down torpor regulatory mechanisms.

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