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Strategies for therapeutic hypometabothermia

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Abstract

Although therapeutic hypothermia and metabolic suppression have shown robust neuroprotection in experimental brain ischemia, systemic complications have limited their use in treating acute stroke patients. The core temperature and basic metabolic rate are tightly regulated and maintained in a very stable level in mammals. Simply lowering body temperature or metabolic rate is actually a brutal therapy that may cause more systemic as well as regional problems other than providing protection. These problems are commonly seen in hypothermia and barbiturate coma. The main innovative concept of this review is to propose thermogenically optimal and synergistic reduction of core temperature and metabolic rate in therapeutic hypometabothermia using novel and clinically practical approaches. When metabolism and body temperature are reduced in a systematically synergistic manner, the outcome will be maximal protection and safe recovery, which happen in natural process, such as in hibernation, daily torpor and estivation.

Keywords

Hypothermia; metabolic suppression; cold adaption; thermoregulation; neuroprotection

1. Introduction

Neuroprotective means for ischemic stroke is desperately needed in clinical settings because thrombolytic treatment can only be delivered to a very limited fraction of stroke patients. Therapeutic metabolic suppression and hypothermia are troubled with severe complications. While investigators are desperately in searching for an effective and safe neuroprotective means for critical illness, nature has already provided a solution for these problems millions years ago.In hibernators body temperature can reach −1.97 °C, metabolic rate can be reduced to 1.22% of euthermic base levels,(Buck and Barnes 2000; Karpovich *et al* 2009)[1,2](Buck and Barnes 2000; Karpovich et al. 2009)(Buck and Barnes 2000; Karpovich et al. 2009)and cerebral blood flow can drop below ischemic threshold, (Frerichs *et al* 1994) which are followed by complete recovery. The efficacy and safety in therapeutic hypometabothermia can be greatly improved by utilizing the strategies that hibernators use for surviving extreme living conditions. This is supported by: 1) hibernation is associated with differential expression of conserved genes, rather than novel hibernation specific genes, (Zhao et al 2010) human shares similar genome with hibernating mammals;(Andrews 2007) 2) human have the capability for enduring extremely low temperature; successful recovery from accidental hypothermia with body temperature reaching 16 °C has been reported, (Wollenek et al 2002) 3) human can also enter into some kind of “pseudo-hibernation” status; *loska*, “winter sleep”, was reported to be a common practice among ancient Russian

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Conflict of Interest
None

peasants in the Pskov Government, where Russian peasants were alleged to spend half year in sleep for dealing with famine; (BMJ1900 2000) practicing meditation can lower metabolic rate and enter a “pseudo-hibernation” status; Indian yogis being studied under laboratory conditions demonstrated their ability to drastically reduce metabolic rate and survive air-tight confinement for up to 8 days without injury. (Young and Taylor 1998)Although it is not possible to directly put human into hibernation, but what we have learnt from hibernation can make a difference in treating ischemic strokes.

2. Current problems and barriers in therapeutic hypometabothermia

2.1. Therapeutic hypothermia provides robust protection but it is troubled by thermoregulatory defenses

Hypothermia therapy for patients is almost always counteracted by thermoregulatory defenses,(Sessler 2001) which include both visible (such as shivering and vasoconstriction) and invisible (metabolic rate increase in non-muscular organs) thermogenic responses. A decrease of 1.3 °C of body temperature provokes a 3-fold increase in circulating catecholamine concentrations(Frank et al 1997). These thermoregulatory defenses need to be blunted for efficiently lowering body temperature and for avoiding complications.(Frank et al 1997; Greif et al 2003) Visible thermoregulatory defenses can be attenuated through drug-induced tolerance to cold. There is no single drug can induce therapeutic hypothermia to 33 to 34°C in human. A combination of meperidine and buspirone can reduce shivering threshold by 2.3°C.(Mokhtarani et al 2001) This temperature is far from the much lower body temperatures observed in hibernators,(Buck and Barnes 2000; Heldmaier *et al* 2004) although some optimization can be achieved through isolated core cooling, surface warming, (Kimberger et al 2007) or combined use of meperidine and buspirone.

2.2. Severe adverse effects of therapeutic hypothermia

Moderate hypothermia (28–32°C) and deep hypothermia (<28 °C) are associated with more complications.(Matthew et al 2002)Major problems with therapeutic hypothermia include cardiac arrhythmia, hemodynamic instability, bleeding, electrolyte shift (such as hypokalemia), shivering, and pneumonia. A comparative analysis of comatose survivors after cardiac arrest shows increased rate of arrhythmia, pneumonia, sepsis, and electrolyte disorder in therapeutic hypothermia (74%) group than in standard treatment group (71%). Of these increased adverse effects, electrolyte disorder only happens in therapeutic hypothermia.(Holzer 2010; Merchant et al 2006; Sagalyn et al 2009) Severe hypokalemia, hypophosphatemia and hypomagnesemia happen during the cooling phase(Mirzoyev et al 2010; Polderman et al 2001) and hypokalemia is significantly associated with the development of polymorphic ventricular tachycardia.(Mirzoyev et al 2010) Hypothermia-induced hypokalemia is probably caused by a shift of potassium from the extracellular to intracellular or extra vascular spaces. Potassium therapy is associated with hyperkalemia during rewarrming phase.(Koht et al 1983; Sprung et al 1991) These hypothermia-induced electrolyte shift and arrhythmia are attributable to increased blood catecholamine levels associated with hypothermia.(Frank et al 1995; Wood et al 1980) This is further supported by the evidence that adrenaline administration results in hypomagnesemia, hypokalemia, hypocalcemia and hyponatremia, which can be prevented by pretreatment of carvedilol, (Nahar and Akhter 2009) a non-selective beta blocker and alpha-1 blocker. Local use of epinephrine also causes hypokalemia and ECG changes.(Hahn and Lofgren 2000; Kubota et al 1993)

2.3. Severe complications of pharmacological suppression of metabolic rate

Although metabolic suppression (Koerner and Brambrink 2006) has shown robust neuroprotection in experimental brain ischemia, drug-related systemic

complications(Coupey 1997) have limited their use in treating acute stroke patients. Therapeutic barbiturate coma is troubled with complications, in which hepatic dysfunction, hypokalemia, respiratory complications and hypotension occur in 87%, 82%, 76%, and 58% patients, respectively.(Schalen et al 1992) Severe life-threatening hypokalemia refractory to potassium therapy and rebound hyperkalemia have also been reported associated with barbiturate coma therapy.(Cairns et al 2002; Jung et al 2009; Neil and Dale 2009)Other anesthetics also have been reported to cause hypokalemia, such as lignocaine,(van Heerden and Chew 1996) and pentobarbital(Robson et al 1981). Many anesthetics, including isoflurane, sevoflurane, ketamine-medetomidine-atropine, ketamine/xylazine, avertine, have been reported to induce hyperglycemia.(Brown et al 2005; Saha et al 2005; Zuurbier et al 2008) The hyperglycemic response in ketamine- or pentobarbital-anesthetized rats can be abolished by adrenergic blockade.(Reyes Toso et al 1995)

3. Strategies for therapeutic hypometabothermia

*3.1.*Blocking cold/nociceptive cold signals

Hibernators in natural environment have already acclimated to cold weather before they undergo hibernation or torpor. Therefore, cold tolerance may play a role in reducing cold stress and thermoregulatory responses during hibernation and therapeutic hypothermia. Cold signal generation, transduction and processing are the first step for initiation of thermoregulatory responses. Even when cold perception is blocked or attenuated such as in comatose or anesthetic conditions, subconscious cold signal generation and processing are still functioning and leading to thermodefenses. Blunting or eliminating cold and nocicold signals will theoretically reduce stress and thermoregulatory responses during therapeutic hypothermia for acute ischemic stroke.

3.1.1. **Cold sensing receptors—**Cold signal is generated through transient receptor potential (TRP) channels A1 and M8.(McKemy 2005) TRPA1 is co-expressed in some neurons with the heat-gated channel TRPV1(Kobayashi et al 2005; Story et al 2003) and is also activated by the pungent ingredients in mustard and cinnamon. TRPA1 mediates perception of noxious cold temperatures below 15°C, (Kwan and Corey 2009; Story et al 2003) and its activation merges both noxious cold and noxious heat due to the co-expression of TRPV1. (Story et al 2003) Non-painful cool temperatures in the range of 30–15°C is mediated through TRPM8 channel,(McKemy et al 2002; Peier et al 2002) which also mediates noxious cold perception. TRPA1-deficient mice show reduced sensitivity to cold nociception and noxious cold induced behavioral response.(Karashima et al 2009; Kwan et al 2006) TRPM8-deficient mice show strikingly reduced avoidance of cold temperatures, lack behavioral response to unpleasant cold stimulus, but have normal nociceptive-like responses to subzero centigrade temperatures.(Dhaka et al 2007)The transduction of cold signals could be different between somatic and visceral sensory neurons and TRPA1 may be the major mediator of cold-evoked responses in vagal visceral neurons. (Fajardo et al 2008)TRPA1 can be inhibited by ruthenium red,(Brignell *et al* 2008)camphor, HC03001[2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7*H*-purin-7-yl)-*N*-(4-isopropylphenyl)acetamide],(Fajardo et al 2008) Gentamicin, Amiloride, and Gadolinium. (Garcia-Anoveros and Nagata 2007) TRPM8 can be inhibited by 5-benzyloxytryptamine (5-BT),(Defalco et al 2010)*N*-(p-Amylcinnamoyl)anthranilic Acid (ACA),(Harteneck et al 2007) N-(3-aminopropyl)-2-([(3-methylphenyl)methyl]oxy)-N-(2-thienylmethyl)benzamide hydrochloride salt (AMTB),(Lashinger et al 2008) ruthenium red, (Brignell *et al* 2008) BCTC, thio-BCTC, capsazepine, and protons.(Andersson et al 2004; Behrendt et al 2004)

3.1.2. **Substances inhibiting cold signals—**There are many substances that can inhibit TRPM8 and TRPA1 channels.(Cahusac 2009) The selection of a starting antagonist

depends on their availability, delivery approach and toxicity. 5-benzyloxytryptamine and ruthenium red have very goodwater solubility and very low known half maximal inhibitory concentration (IC50) values. The IC50 of 5-benzyloxytryptamine (TRPM8 antagonist) and ruthenium red (TRPA1 antagonist) are 0.34μM (Defalco et al 2010) and 3.4 μM,(Farris et al 2004; Garcia-Anoveros and Nagata 2007; Jordt et al 2004) respectively. If a 10 time the IC50 concentration is to be reached in in vivo condition, doses of 1.03 mg/kg and 26.72 mg/kg for 5-benzyloxytryptamine and ruthenium red will be needed, respectively, assuming they are evenly distributed in body fluid. Similar dose of ruthenium red has been used in rats and proved effective for blocking capsaicin induced artery response,(Bari and Jancso 1994) but a dose range of 0.026-0.26 mg/kg is not effective in blocking cold-evoked activities in cutaneous primary afferents. (Dunham et al 2010)

***3.1.3*. Potential pitfalls and alternative strategies—**The TRPM8 blocker 5-benzyloxytryptamine (5-BT) is a tryptamine derivative that also activates the 5-HT_{1D}, 5-HT_2 and 5-HT_6 serotonin receptors.(Boess et al 1997; Buzzi et al 1991; Cohen et al 1992; Peroutka et al 1991)Ruthenium red is polycationic cell biology reagent that tightly binds to tubulin dimers and ryanodine receptor and inhibits intracellular calcium release.(Ma 1993) Ruthenium red is membrane-impermeant, (Bari and Jancso 1994) so it may not pass blood-brain barrier and block TRPA1 channels in central venous system. 5-BT is a most recently discovered TRPM8 channel blocker; its optimal doses for mice and rats are not clear. Infusion of RR at a dose of 10umol in rats weighing 300–420g for 10min prior to the infusion of 100pmol capsaicin inhibited the vasodilatory response. The effects of these blockers are temporary, which is good for short-term treatment and recovery. The inhibition lasted for at least 15 min and the vasodilatatory response was restored after 30 min. Considering the above mentioned factors, dose adjustment may be needed for achieving maximal efficacy and reducing potential side effects. Other antagonists that may serve as alternatives.

4. Inhibiting glycolysis and mitochondrial respiration chain

Observation showed that hibernators deliberately suppress their metabolic rate before entering hibernation, torpor or estivation(Wilz and Heldmaier 2000) which are followed by a decline of body temperature. During hibernation and torpors glucose consumption(Frerichs et al 1995) and mitochondrial respiration(Brown et al 2007; Staples and Brown 2008) are significantly suppressed. Therefore, it is reasonable to hypothesize that active metabolic suppression facilitates reaching targeted temperature and reduce thermoregulatory responses during therapeutic hypothermia for ischemic stroke. Glucose utilization can be inhibited by 2-DG; and mitochondrial respiration can be reversely inhibited by amobarbital. Decreasing energy demand by metabolic suppression is the classic method for achieving neuroprotection. Metabolic rate could be drastically reduced by hypothermia,(Astrup et al 1981; Berger et al 1998; Mori et al 1998) anesthetics and sedatives.(Astrup et al 1981; Warner et al 1996) Hypothermia seems to have its unique effect in delaying the time to terminal depolarization (Nakashima et al 1995) than metabolic suppression alone.

4.1. Using 2-deoxy-D-glucoseas a glycolysis inhibitor

2-Deoxy-D-glucose (2-DG) has been recognized as an antagonist of glucose metabolism for 60 years and its biological effects and working mechanisms have been widely studied. (Kurtoglu et al 2007) 2-DG is rapidly absorbed when being administered orally (Tmax0.5–1h) with a half-life of 5–10h.(Raez et al 2007) 2-DG has a similar structure to D-glucose, is taken up through the glucose transporters (GLUTs) and phosphorylated by hexokinase (HK) to form 2-DG-6-phosphate (2-DG-6-P), which is slowly utilized at a rate of less than 4% of its natural substrate, glucose-6-phosphate (G6P). 2-DG accumulates within the cell,

competes with glucose for phosphoglucose isomerase (PGI), and noncompetitively inhibits HK. The LD50 of 2-DG in mice by i.v. injection is 8000 mg/kg.(Vijayaraghavan et al 2006) It has been used in a range of 125–2000mg/kg in in vivo studies for treating convulsion, (Gasior et al 2010) tumor(Boutrid et al 2008; Gupta et al 2005) and for inducing torpor. (Dark et al 1994)

4.2. Metabolic suppression

About 87% of brain energy consumption reflects function-related activities,(Magistretti 2002) and could be suppressed to conserve energy. Slowing and isoelectric changes of electroencephalogram (EEG) occur during hibernation(Frerichs et al 1994; Walker et al 1977) and anesthesia.(Esmaeili et al 2007) EEG burst suppression provides neuroprotection. (Doyle and Matta 1999) Barbiturates have been used for such burst suppression with proven efficacy.(Astrup et al 1981; Warner et al 1996)

4.3. Partial mitochondrial respiratory chain inhibition

Inhibiting different sites on mitochondrial electron transporting chain will result in significantly different effect on free radical production. For examples, block of electron transport at complex I by rotenone reduces superoxide production on complex I, (Grivennikova and Vinogradov 2006) preserves electron transport chain and reduces cytochrome c loss during ischemia.(Lesnefsky et al 2004) Antimycin A (AMA) inhibits mitochondrial electron transport chain between cytochrome b and c.(You and Park 2010) This inhibition results in the production of reactive oxygen species (ROS), which can be attenuated by rotenone.(Chen et al 2003) Different Complex I inhibitors also have different effect on ROS production. Rotenone, piericidin A and rolliniastatin increase ROS production whilst stigmatellin, mucidin, capsaicin and coenzyme Q2 prevent ROS production.(Fato et al 2009) Transient and partial mitochondrial inhibition reduces ROS production and protects ischemia/reperfusion related injuries.(Anderson et al 2006; Chen et al 2006; Stewart et al 2009) Rotenone is a widely studied potent irreversible inhibitor of complex I that can be used for modeling Parkinson disease, therefore not compliant with the purpose of neuroprotection in the proposed study.

4.4. Using amobarbital for metabolic suppression and partial mitochondrial respiratory chain inhibition

Amobarbital is a short-acting barbiturate that (like all barbiturates) works by potentiating GABA-ergic effect and inhibiting glutamate receptors. Amobarbital weakly inhibits complex I at the same site that rotenone works. Inhibition of respiration through complex I by amobarbital is rapidly reversible.(Chen et al 2006) When being used at 2.5 mM in perfused rat heart, amobarbital inhibits complex I, reduces free radical production and protects heart mitochondria.(Chen et al 2006; Stewart et al 2009) For anesthesia amobarbital can be used at a dose of 80 mg/kg in rats.(Cohn et al 1976) Amobarbital is also the standard drug used in clinical for diagnosing hemisphere functional preference in Wada test, (Baxendale 2009; Kim et al 2007) during which the mean aphasic time is around 1.5 minutes, and EEG slowing time is of 4 minutes.(Kim et al 2007)Mouse subcutenous route LD50 for amobarbital is 212 mg/ kg. EC50 of amobarbital on the inhibitory postsynaptic currents (IPSCs) in neocortex is 0.103 mM.(Mathers et al 2007) Amobarbital is also a weak inhibitor for complex I with an IC50 of 1.2 mM.(Fato et al 2009)

4.5. Potential pitfalls and alternative strategies

2-DG causes reversible decrease of phosphocreatine (PCr) and increase ofADP levels, and an ireversible reduction of the cytosolic adenine nucleotidepool. (Kupriyanov et al 1991) Intravenous administration of 2-DG (250–1000 mg/kg) in anaesthetised rats may cause

hypotension. (Vijayaraghavan et al 2006) We may need to do dose adjustment for further reducing side effect and maximizing therapeutic potential. Other similar glucose analogs may also be considered as alternative choices. 2-fluoro-deoxy-D-glucose (2-FDG) is more closely similar to glucose structure and more potent in glycolytic inhibition, and also more cytotoxic than 2-DG.(Kurtoglu et al 2007) 3-methyl-glucose (3-MG) interferes with glucose uptake, but does not inhibit glycolysis.(Gasior et al) 3-MG has showed protective effects in hepatocytes cryopreservation.(Sugimachi et al 2006)

Amobarbital potentiates GABA-ergic effect, blocks AMPA-selective glutamate receptor, and inhibits mitochondrial complex I. Alternative method for metabolic suppression without inhibiting mitochondrial respiration can be considered. Pentobarbital may be one of these choices. Measurement of ADP to O_2 (ADP/O) ratio (Takaki et al 1997) or ATP/ADP ratio (Schwenke et al 1981) indicates that pentobarbital doesn't inhibit mitochondrial respiratory function.

4.6. Alternatives for mitochondrial inhibition

Other potent complex I inhibitors that may also be considered for alternative choices, which include pyridaben, rotenone, piericidin A, and fenpyroximate(Schuler and Casida 2001). ComplexI inhibitors can be grouped into three classes. A-type includes fenazaquin, fenpyroximate, fyrimidifen, piericidin A, rolliniastatin, 2-decyl-4-quinazolinyl amine, and AE F117233; B-type includes rotenone epirotenone, amobarbital; and C-type includes capsaicin and 4-(p-tert-butylphenoxy)benzoic acid-3,4-dimethoxybenzylamide.(Okun et al 1999). Rotenone and piericidin A are 50,000–100,000 times more potent than amobarbital for inhibiting complex I.(Okun et al 1999; Schuler and Casida 2001). Hydrogen sulfide (H_2S), inhibiting cytochrome c oxidase,(Collman et al 2009; Truong et al 2006) which is also reversibly inhibited during hibernation,(Muleme et al 2006) can be considered as an alternative mitochondrial inhibitor. H_2S is able to make mice entering severe hypothermia or suspended animation at a low dosage of 80 ppm.(Blackstone et al 2005)

5. Preemptive suppression of thermogenic defense

The phenomenon that hibernators enter into hibernation rapidly and recover from hibernation without causing injury is attributable to their suppressed, balanced, and tightly regulated thermogenesis. During entrance and in deep hibernation, plasma catecholamines (dopamine, norepinephrine and epinephrine) are significantly lower than cold-adapted levels. When a hibernator arouses from hibernation, catecholamines markedly increase. (Florant et al 1982) In addition, administration of norepinephrine and epinephrine may cause arousal from hibernation.(Lyman and O'Brien 1988) The hypothalamus-pituitary-adrenal (HPA) axis is least active during hibernation season, maintains a stable level during hibernation bouts and fluctuates in association with arousals.(Hudson and Wang 1979) Glucocorticoids are important for enduring and surviving hypothermia,(Musacchia 1988) and are closely balanced during hibernation.(Musacchia and Deavers 1978) During hibernation, significant decreases in thyrotropin-releasing hormone (TRH) occurs in many regions of central nervous system including hypothalamus and preoptic area and fluctuates in different phase of hibernation.(Stanton et al 1982) Furthermore, administration of TRH during the entrance and maintenance phases of hibernation causes body temperature elevation(Tamura et al 2005). Central nervous system thyrotropin-releasing hormone is also reduced during estivation.(Kreider et al 1990) In dormant phase of hibernation total serum T3 (trioodo-L-thyronine) and T4 (L-thyroxine) are elevated but free T3 and T4 are decreased over active levels because of increased serum binding capacity and affinity. (Magnus and Henderson 1988; Tomasi et al 1998) Short term cold exposure activates the sympathoadrenal system (SAS), HPA axis, and hypothalamus-pituitary-thyroid (HPT) axis; it also increases cellular levels of TRH mRNA and CRH mRNA in neurons of the

paraventricular nucleus (PVN). The neurally mediated central effect of cold can override the inhibitory effects of circulating hormones.(Leppaluoto et al 2005; Zoeller et al 1990)Theoretically, preemptive suppression of these systems will reduce thermoregulatory defenses and facilitate reaching target temperature during therapeutic hypometabothermia.

5.1. Major thermodefensing systems

The sympathoadrenal system (SAS), hypothalamus-pituitary-adrenal (HPA) axis, and hypothalamus-pituitary-thyroid (HPT) axis are the major systems that mediate thermoregulatory responses, which are suppressed and tightly regulated during hibernation. We will preemptively suppress these systems by preadministration of reserpine, metyrapone, and iodine solution, which are all up-to-date clinical medications with proved efficacy but have not been used in therapeutic hypometabothermia yet. We will use the same methodologies and time frame for inducing acute middle cerebral artery occlusion, for delivering therapeutic hypometabothermia, for evaluating neurological function and infarction volume, for monitoring metabolic rate and core temperature, and for blood sampling and assays of electrolyte homeostasis, glucose, thyroid hormones, and catecholamine levels.

5.2. SAS suppression

The SAS structural components have different preferences in responding to stimuli. The adrenal medulla responds very rapidly to single stress exposure; the sympathetic nervous system responds to HPA axis activation; adrenocorticotropic hormone (ACTH) may directly stimulate sympathetic ganglia; the locus coeruleus-noradrenergic system that supplies norepinephrine throughout the central nervous system responds to repeated stress exposures. (Sabban 2007) The SAS system can be targeted at different levels by various methods for therapeutic purposes. Reserpine is well known to be a depletor for norepinephrine (NE), dopamine (DA) and 5-hydroxytryptamine (5-HT). Reserpine inhibits ATP/Mg^{2+} pump, which is responsible for sequestering neurotransmitters into storage vesicles located in the presynaptic neuron, resulting in reduction or depletion of catecholamines and serotonin from central and peripheral axon terminals in many organs, including the brain and adrenal medulla. It has been used as an antihypertensive and an antipsychotic as well as a research tool. This depletion in the adrenal medulla is slower and less complete than in other tissues. Reserpine LD_{50} in rats is 420 mg/kg by oral route; 44 mg/kg by i.p. injection; 15 mg/kg by i.v. injection; its LD_{50} in mice is 200 mg/kg by oral route; 52 mg/kg by subcutaneous injection. In experimental studies reserpine can be used in single i.p. injections at a dose range of 0.25-6 mg/kg for inducing gastric mucosal lesions in SD rats,(Ma et al 2010) and at 2.5 mg/kg i.p. 16 to 20 hr before experiments for its effect on nociceptive testing.(Nakazawa et al 1991).

5.3. HPA axis suppression

The HPA axis functions through hypothalamic corticotropin-releasing hormone (CRH), pituitary adrenocorticotropic hormone (ACTH) and arginine vasopressin (AVP), and adrenal glucocorticoids (GCs). HPA is a well-known stress response system,(Papadimitriou and Priftis 2009) having a close interaction with adrenomedulla.(Goldstein and Kopin 2008) The HPA axis can be targeted at different levels by various methods for therapeutic purposes. Metyrapone(Metopirone) reduces cortisol and corticosterone production by inhibiting the 11-β-hydroxylation reaction in the adrenal cortex, resulting in elevated ACTH level if pituitary gland functions normally. It is used as an HPA functional diagnostic test with urinary 17-OHCS measured as an index of pituitary ACTH responsiveness, and is also used for treatment of Cushing's syndrome. Metyrapone oral LD_{50} in rats is 521 mg/kg. In clinical settings metyrapone is used at a dose of 30mg/kg at midnight per oral route and the plasma cortisol and 11-deoxycortisol are measured the next morning between 8:00 and 9:00 am. In

many species, including amphibians, reptiles, rodents and birds, corticosterone is the main glucocorticoid hormone. It has been used in a dose range of 50–150 mg/kg in 0.5% carboxymethylcelluloseat 30-min(Lowery et al 2010) to 4-h before experiments(Krugers et al 2000) for reducing corticosterone levels. In rats, metyrapone at dose of 150 mg/kg decreases locomotion.(Canini et al 2009)

5.4. HPT axis suppression

The HPT axis functions through hypophysiotropic thyrotropin-releasing hormone (TRH), pituitary thyroid stimulating hormone (TSH), and thyroid hormones T3, T4. The HPT axis is well-known to be stimulated by cold exposure.(Fuzesi et al 2009) The central nervous system norepinephrine (NE) potently stimulates the biosynthesis and proteolytic processing of proTRH.(Perello et al 2007) Induced hyperthyroidism is associated with activation of the HPA axis.(Johnson et al 2005)When being exposed to cold, TRH deficient mice cannot maintain their body temperatures.This is associated with hypothalamic TRH depletion and reduction in thyroid hormone.(Nillni et al 2002) The HPT axis can be targeted at different levels by various methods for therapeutic purposes. Iodine solution in pharmacologic doses produces rapid remission of symptoms by inhibiting the release of thyroid hormone into the circulation. It is used for emergency management of thyroid storm and for preoperative preparation of hyperthyroid patients for thyroidectomy. Many iodine solution formulae are available. The usual dosage in clinical settings is 2 to 3 drops (100 to 150 mg) of a saturated K iodide solution p.o. tid, or 0.5 to 1 g Na iodide in 1 L 0.9% saline solution given i.v. slowly q 12 h. In animal studies iodine solution can be added into drinking water by adding 1 to 5 drops of fresh Lugol's solution in 100ml,(Boatman and Moses 1951) or using 0.05% sodium.(McLachlan et al 2005) Iodine solution can be used with a high dose safely (160 mg/kg in rats) by i.p. injection.(Sharp et al 1982) Mouse has a greater surface area/body weight ratio that is approximately 12–16 times of the ratio in human. When converted from human dose by this ratio, the dose will be 228–457 mg/kg/day for mice.

5.5. Potential pitfalls and alternative strategies

Reserpine is non-selective for monoamine neurotransmitters. It depletesNE,DA and 5-HT. Reserpine at high dose may cause gastric ulceration, hypotension, bradycardia, and drowsiness. For these reasons, selective degeneration of noradrenergic nerves can be considered as alternative approaches for suppressing the sympathoadrenal system. Pharmacological choices include N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4, i.p.)(Jonsson et al 1981) or intrathecal (i.t.) 6-hydroxydopamine (6-OHDA).(Nakazawa et al 1991) DSP-4 can pass through the blood-brain barrier and is effective at 50–100 mg/kg.

Alternative approaches for inhibiting HPA axis include siRNA for corticotropin-releasing hormone (CRH) through intracerebralventricular delivery, the nonselective CRF receptor antagonist a-helical CRF, the selective CRF2R agonist Urocortin-3, the glucocorticoid receptor type I antagonist mifepristone (RU38486), the selective CRF1R antagonist, CP-154,526 (butyl-[2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-7H-pyrrolo [2,3-d]pyrimidin-4-yl]-ethylamine). (Lowery et al 2010)

Iodine solution is effective in suppressing the release of T3 and T4, but may also cause complications, which include inflammation of the salivary glands, conjunctivitis, and rash. Propylthiouracil and methimazole can be used as alternatives for suppressing HPT axis. Propylthiouracil in high doses also inhibits the peripheral conversion of T4 to T3. Another choice would be 3-Iodothyronamine (T1AM), which is a natural derivative of thyroid hormone. T1AM opposites the biological effects of T3 and T4,(Scanlan et al 2004; Scanlan 2009) induces profound hypothermia and bradycardia within minutes in mice,(Scanlan et al 2004) depresses metabolism via a rapid interruption of carbohydrate utilization followed by

a compensatory rise in lipid utilization. (Braulke et al 2008) siRNA against prepro-TRH can be used through intracerebralventricular delivery for reducing TRH secretion.(Guissouma et al 2006; Landa et al 2007a; Landa et al 2007b)

The beta adrenergic receptor antagonist propranolol can be used as an alternative method for reducing the effects of epinephrine (adrenaline) and other stress hormones. Propranolol is also effective in inhibiting peripheral conversion of T4 to T3.

6. Conclusion remarks

To realize the thermogenically optimal and synergistic reduction of core temperature and metabolic rate, we propose novel strategies and practical approaches for therapeutic hypometabothermia: 1) blunting cold-sensing transient receptor potential (TRP) channels A1 and M8 so that to minimize the input signal that initiates thermogenic defenses; 2) delivering active metabolic suppression by amobarbital and 2-DG so that hypothermia will have reduced counteraction from metabolic process; 3) preemptively suppresssympathoadrenal system (SAS), hypothalamus-pituitary-adrenal (HPA) axis, and hypothalamus-pituitary-thyroid (HPT) axis by preadministration of reserpine, metyrapone, and iodine solution so that to defeat thermogenic outputs.

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