Induction of therapeutic hypothermia by pharmacological modulation of temperature-sensitive TRP channels: theoretical framework and practical considerations

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Abbreviations: DMH, dorso-medial hypothalamus; MPA, medial preoptic area of hypothalamus; rMR, rostral medullary raphe region; ThermoTRPs, Thermosensitive Transient Receptor Potential cation channels; TRP, Transient Receptor Potential; TRPA1, Transient Receptor Potential cation channel, subfamily A, member 1; TRPM8, Transient Receptor Potential cation channel, subfamily M, member 8; TRPV1, Transient Receptor Potential cation channel, subfamily M, member 8; TRPV2, Transient Receptor Potential cation channel, subfamily V, member 1; TRPV3, Transient Receptor Potential cation channel, subfamily V, member 3; TRPV4, Transient Receptor Potential cation channel, subfamily V, member 4

Therapeutic hypothermia has emerged as a remarkably effective method of neuroprotection from ischemia and is being increasingly used in clinics. Accordingly, it is also a subject of considerable attention from a basic scientific research perspective. One of the fundamental problems, with which current studies are concerned, is the optimal method of inducing hypothermia. This review seeks to provide a broad theoretical framework for approaching this problem, and to discuss how a novel promising strategy of pharmacological modulation of the thermosensitive ion channels fits into this framework. Various physical, anatomical, physiological and molecular aspects of thermoregulation, which provide the foundation for this text, have been comprehensively reviewed and will not be discussed exhaustively here. Instead, the first part of the current review, which may be helpful for a broader readership outside of thermoregulation research, will build on this existing knowledge to outline possible opportunities and research directions aimed at controlling body temperature. The second part, aimed at a more specialist audience, will highlight the conceptual advantages and practical limitations of novel molecular agents targeting thermosensitive Transient Receptor Potential (TRP) channels in achieving this goal. Two particularly promising members of this channel family, namely TRP melastatin 8 (TRPM8) and TRP vanilloid 1 (TRPV1), will be discussed in greater detail.

Therapeutic Potential of Hypothermia

Temperature powerfully affects every biological process. Humans and many other mammals maintain their internal temperature at a stable level around 37°C,¹ which appears to be optimal for their functioning under most normal conditions. However, it has been now definitively established that in some pathological states, the endogenously regulated level of internal temperature does not provide the highest chances of survival. In such cases, a more favorable outcome may be achieved by a therapeutic intervention aimed at external modulation of patient’s temperature. In particular, ample basic science and clinical evidence suggests that intentional mild lowering of a patient’s core temperature, also known as induced, or therapeutic hypothermia, has the potential to protect vital organs from damage, preserve their function, and improve survival during acute global ischemia and hypoxia.²,⁶

The therapeutic use of low temperature has been explored probably for as long as medicine exists and has demonstrated clinical benefit in a variety of contexts.⁷ However, only recently has this knowledge been developed into clearly defined clinical protocols of whole-body hypothermia, which have been tested in rigorous clinical trials.⁸,⁹ The first modern seminal trials of therapeutic hypothermia have demonstrated that maintaining the
Temperature

A patient’s temperature in the range of 32-34°C for 12-72 hours improved survival and neurological outcome in patients resuscitated after out-of-hospital cardiac arrest and in neonates with perinatal asphyxial encephalopathy. Based on these positive results, recently there has been a surge of additional clinical investigations of hypothermia in traumatic brain injury, acute ischemic stroke, myocardial infarction, and other conditions. Many of these ongoing studies are expected to further establish therapeutic hypothermia as one of the most promising and effective new treatment methods in acute care medicine.

Unfortunately, however, some of the recently completed studies failed to demonstrate a clear benefit. Thus, while the hopes for clinical potential of the overall concept of hypothermic neuroprotection remain high, these discouraging results highlighted some of the significant challenges for the further progress of this treatment modality. Some challenges may be related to our incomplete basic understanding of the effects of low core temperature on human physiology and disease pathogenesis. More specifically, the optimal depth, duration, and timing of hypothermia have not been yet definitively established.

In regard to the depth of hypothermia, current guidelines recommend the target core temperature of 32-34°C, based on classical animal studies and successful human clinical trials. However, it is not clear if this temperature is the most beneficial due to paucity of studies directly comparing the relative benefit of different target core temperatures. Recently, aggressive cooling to 33°C demonstrated no additional benefit over milder cooling to 35-36°C both in a rat study and in a human clinical trial in cardiac arrest patients. Thus, more comprehensive studies over a wide range of target temperatures are needed to determine the level that will provide the optimal balance between the protective effects of hypothermia and complications in each specific clinical context.

Of major importance is also the question of when to initiate the hypothermia in relation to the clinical event. An influential classical animal study demonstrated that delay of cooling initiation by as little as 15 min following reperfusion after cardiac arrest in dogs largely offsets the protective effect of hypothermia. This and other findings suggested that cooling should be initiated as soon as possible to provide the most benefit. However, 2 recent clinical trials in cardiac arrest patients have found no improvement in survival or neurological outcome with early pre-hospital cooling initiation compared with standard hospital cooling. Once again, further research is needed to understand what factors related to hypothermia timing and duration play the key role in conferring clinical benefit and to develop protocols that would take full advantage of this information.

Another major obstacle for the further development and wider adoption of therapeutic hypothermia protocols is the technical and logistical difficulty of the current methods of lowering core temperature in human patients. Most methods involve complicated equipment that is available only in a minority of treatment sites. In addition, current protocols require the use of various drugs, which often produce dangerous side effects. Moreover, even these complicated methods do not provide the level of control over the depth and rate of hypothermia required for optimal treatment effect. Therefore, there is a great need in clinical medicine for novel methods of lowering core body temperature, which will be safe, effective, easily administered and widely available.

Fortunately, the basic understanding of the molecular mechanisms of mammalian temperature regulation is rapidly advancing and may provide novel insights and potential solutions to this important problem. Before these advances are discussed, a brief overview of the conceptual approaches to lowering body temperature may be helpful.

Determinants of Body Temperature

In order to effectively manipulate the body temperature of a living organism, it is essential to understand what factors determine it. According to the fundamental principles of thermodynamics, the temperature of an organism is determined by the balance of heat generation within the organism and heat transfer between the organism and its environment (Fig. 1). In most experimental and clinical situations related to hypothermia, ambient temperature is lower than the core body temperature of an organism and thus the heat transfer component can more precisely be described as heat loss to the colder environment.

Heat generation in the body is a natural byproduct of the functional activity of cells. This activity encompasses catabolic, or exergonic, reactions in which complex molecules are converted into simpler substances, releasing energy. The energy released during these reactions is used to power cellular processes and maintain body temperature. The balance between heat generation and heat transfer determines the body's temperature. The body's temperature is regulated by mechanisms that control the rate of heat generation and heat loss, ensuring a stable internal environment for optimal physiological function.
broken down and energy is released, as well as endergonic reactions, which consume energy to perform some useful biochemical, electrochemical or mechanical work. Exergonic reactions are coupled to endergonic reactions, such that the energy liberated in the former drives the latter. However, because this coupling is less than 100% efficient, some energy escapes the transformation, is released into the surrounding body tissues in the form of heat and leads to an increase in their temperature. Thus, the rate of heat generation is proportional to the rates of biochemical reactions within the cell, i.e. to its activity level. Highly active cells, such as brain neurons or contracting skeletal muscle, generate more heat compared to less active cells, such as white fat or skin cells.

Heat loss is driven by evaporation of fluids, radiation and conduction of heat to surrounding air or objects with lower temperature. The latter 2 components are heavily influenced by the gradient of temperature between the body and its environment. The conduction component of heat loss is also dependent on the thermal conductivity of the body-environment interface, which is determined by the properties of barriers, such as clothes, fur, and skin, as well as on the tone of cutaneous blood vessels.

Figure 2. Schematic of the thermoregulatory system and pharmacological targets for its modulation. The thermoregulatory system modifies the heat exchange between the body and the environment to maintain a particular balance level of core body temperature. The thermoregulatory system consists of thermoreceptors, afferent pathways, central thermoregulatory neurons, efferent pathways and effector organs. Strategies for controlling or blocking thermoregulatory responses may be aimed at each of these levels of organization (indicated by green captions). See text for detailed discussion.

Notes: (1) thermoregulatory centers in hypothalamus are actually complex multi-neuronal circuits, but for clarity are presented here as single neurons; (2) only the most important, but not all, thermoeffector processes are shown; (3) the universal role of TRPM8 within the pathway for skin cooling is putative and has been challenged for some cold-defensive responses; (4) only 2 populations of primary sensory neurons are shown, while many others also exist.

In order to modulate the internal temperature of an organism, either or both heat generation and heat loss must be altered (Fig. 1). Because heat generation is necessarily a byproduct of cellular activity, reducing heat production must involve decreasing activity. Incidentally, a decrease in activity of cells during ischemia reduces their metabolic demand and is cytoprotective by itself, independently of decrease in temperature. Thus, direct reduction in cellular activity is a complementary and parallel goal to hypothermia in achieving neuroprotection.

To appreciably decrease heat generation, activity must be reduced at the level of whole organs or even of the whole organism. Incidentally, patients are typically already in the resting state, characterized by close to minimal physiological activity and near basal metabolic rate. The metabolic rate can be further reduced by general anesthesia. Thus, deeply anesthetized and mechanically ventilated humans have a metabolic rate at ~65% of the basal level. However, even at this low level of heat generation and assuming typical room ambient temperatures of 22-24°C, the rate of the drop in core temperature is still suboptimal for a neuroprotective strategy.
It is tempting to speculate if even lower levels of metabolism may be achieved in humans. A promising opportunity may be related to the phenomenon of hibernation that occurs in several mammalian species. During hibernation, an animal is able to substantially decrease its metabolism and energy demands to survive a period of low nutrient supply. For example, black bear, a species of comparable size to humans, has 50% lower levels of metabolism in hibernation compared to pre-hibernation levels measured at the same level of core temperature. If a similar hypothetical state of “hibernation” could be induced in humans, the same 50% reduction in their metabolic rate and heat generation may be reasonably expected. However, this reduction would be only slightly larger than the previously mentioned reduction in metabolism of anesthetized humans (~3%), and still would not provide the therapeutically sufficient rate of hypothermia at typical ambient temperatures. Moreover, even this seemingly marginal benefit is currently not feasible, because no strategies for inducing hibernation in humans are known. Thus, the overall strategy of decreasing heat generation through anesthesia, hibernation or other methods without an increase in heat loss doesn’t appear to have the potential to achieve hypothermia within the timeframe consistent with neuroprotection.

At the same time, these methods aimed at decreasing heat generation do lower cellular activity and metabolic demand even at normal levels of core temperature, and provide cytoprotection independently from any possible temperature-lowering effect. Therefore, as mentioned earlier, they may be utilized independently or in synergy with other hypothermic strategies. Induction of the putative “hibernation” state in humans is a particularly promising and actively explored area, which is beyond the scope of the current review.

A more straightforward alternative to decreasing heat generation in achieving hypothermia is increasing heat loss. As mentioned earlier, 2 out of 3 possible mechanisms of heat loss, namely radiation and conduction, strongly depend on the gradient between core and ambient temperatures. Thus, heat loss may be greatly enhanced by lowering environmental temperature, or in other words, by application of physical cooling. This strategy has become the basis of the current clinical protocols of therapeutic hypothermia. A variety of practical implementations of the physical cooling strategy have been created, and new and improved methods are constantly being developed.

Physical cooling methods can be broadly divided into surface and core cooling methods. The earliest and simplest application of surface cooling involved covering the patient’s body with ice packs. The same principle is used in more advanced surface cooling devices of various designs, which typically utilize cold water-perfused cooling blankets and pads and include additional features such as a feedback temperature control and gradual active rewarming capabilities.

Core cooling methods employ intravascular heat-exchanging catheters, which drain the heat not from the surface, but directly from the core component of the body. Intravascular devices allow higher rates of cooling, yet they have higher requirements for skilled staff and higher possibility of complications. A special case of core cooling is a rapid high-volume infusion of cold fluids. This strategy is often used as the very first step for a rapid induction of hypothermia, which is then maintained by other methods. Ultimately, all physical cooling methods are capable of inducing substantial heat transfer away from the body and a drop in core temperature.

However, in contrast to passive inanimate objects, complex living systems possess a critical property of homeostasis, or the ability to maintain key physiological variables at stable levels. In particular, body temperature is one of the regulated variables in homeothermic (or warm-blooded) animals, such as mammals. The structures of the body responsible for this regulation are organized into the highly complex and powerful thermoregulatory system (Fig. 2). When mammalian body temperature is challenged, the thermoregulatory system initiates a number of behavioral and physiologic responses, which modulate the thermal balance of the organism. Thus, a fully conscious mammal exposed to cold and increased heat loss responds with warm-seeking/cold-avoiding behaviors, an increase in heat generation through shivering and non-shivering thermogenesis, a decrease in heat conductivity through peripheral vasoconstriction, as well as a number of other mechanisms. As a result, the heat loss caused by cold exposure may be minimized and offset by the increased heat generation. In this case, at least initially, hypothermia does not develop.

An experimental illustration of this theoretical model may be found, for instance, in the experiment by Lampert et al., in which core temperature of nude men exposed to the cold air at 10°C and wind of 10 mph for 2 hours dropped by merely ~0.2°C from the pre-exposure levels. Similarly, Adolph et al. reported that core temperature of nude men exposed to cold air at temperatures down to 0°C and wind speeds up to 15 mph for 4 hours did not drop below 35.8°C. Moreover, compensatory thermoregulatory responses prevent the clinically meaningful drop in core temperature not only during surface cooling, but also during core cooling. For instance, a study by Moore et al. demonstrated that rapid high-volume infusion of 4°C cold saline lowered core temperature of healthy awake volunteers by only 1°C and did not achieve the conventionally defined therapeutic plane of hypothermia.

The compensatory capabilities of the body are nonetheless finite. If the cold exposure is of sufficient magnitude and duration, these capabilities may be overwhelmed by the heat loss, and core temperature will ultimately decrease. Thus, it is possible to achieve hypothermia even in a conscious non-medicated mammal with an intact thermoregulatory system. But along with the intended and beneficial consequence of lowered core temperature, such a forcible intervention will trigger a maximal compensatory effort by the body. This compensatory response puts a considerable load on cardiovascular, respiratory, and other homeostatic systems, and is maladaptive for a patient undergoing the hypothermic protocol. In addition, vigorous shivering, which is a major part of the compensatory cold defense response in humans, is very uncomfortable. This scenario demonstrates that targeting only one component of heat balance in the presence of the highly robust and redundant thermoregulatory system will result in many unintended effects and is unlikely to achieve...
hypothermia in a safe and specific manner. Thus, in order for the physical cooling strategy to work, it must be combined with some measures for inactivation of the thermoregulatory system.

**Targets for Modulation within the Thermoregulatory System**

The thermoregulatory system incorporates a series of structures, including thermoreceptors, afferent neural pathways, integrative brain centers, efferent neural pathways and effector organs such as skeletal muscle, blood vessels and brown adipose tissue, which may be amenable to inactivation or modulation (Fig. 2). Theoretically, inactivation on any of these levels has a potential to alter compensatory responses and induce tolerance to hypothermia. Accordingly, pharmacological strategies targeting various parts of the thermoregulatory system have been developed and have enabled the successful achievement of hypothermia in clinical trials. However, these strategies also have important limitations, which hinder the full therapeutic potential of induced hypothermia.

The most important targets for inactivation in the thermoregulatory system are the integrative centers in the hypothalamus. A broad suppression of the central nervous system is a natural consequence of a comatose state, which is a typical occurrence in patients resuscitated after out-of-hospital cardiac arrest. Further inactivation of the thermoregulatory system may be achieved by general anesthetic drugs, such as midazolam and fentanyl. Other common targets for inactivation in the thermoregulatory system are the integrative centers in the hypothalamus (MPA). MPA neurons are postulated to be tonically active at thermoneutral temperatures and intrinsically sensitive to local brain temperature. In addition, they are affected by skin temperature through excitatory and inhibitory inputs from peripheral thermoreceptors. On the efferent side, MPA neurons send inhibitory projections to primary effector neurons in the dorso-medial hypothalamus (DMH) and rostral medullary raphe region (rMR).

Functionally, MPA neurons are inhibited in response to skin or core cooling. Inhibition of MPA neurons disinhibits, i.e., activates, primary effector neurons in the DMH and rMR. Activation of primary effector neurons triggers cold defense responses, such as BAT thermogenesis, peripheral vasconstriction, and shivering.

In addition to anatomical location of the relevant groups of neurons, their receptor inputs have been also identified. Pharmacological modulation of these receptor inputs allows abolishing cold defense responses at the level of central circuits. For instance, it was shown that blocking GABA-ergic inhibitory input to the MPA neurons with the GABA antagonist bicuculline suppresses cold defense responses to skin cooling. The same effect can be achieved by pharmacological inhibition of DMH and rMR effector neurons by the GABA agonist muscimol.

If these mechanisms, identified in rodents, are also relevant in humans, such interventions are predicted to effectively induce hypothermia in patients cooled by physical methods. However, specific modulation of these compactly localized neuronal populations by pharmacological modulators of the ubiquitous central nervous system receptors is possible only through spatially specific delivery of pharmacological agents. In experimental rodent studies, this is typically achieved by intra-cerebral nano-injections of drugs to the well-defined areas of the brain. However, the clinical use of this delivery route in human patients has not been well established and at present appears impractical. Thus, the pharmacologically feasible strategy of controlling thermoregulatory system has to rely on molecular, rather than spatial, specificity of the targets.

Other common targets for inactivation within the thermoregulatory system are the effector organs. The most important cold defense response in humans is the shivering response of the skeletal muscles. Importantly, the shivering response is not always fully abolished even in comatose and deeply anesthetized patients.
patients, and in many cases must be managed additionally. Motor activity of the skeletal muscles, including shivering, may be effectively blocked using neuromuscular blocking pharmacological agents, or muscle paralytics. Accordingly, neuromuscular blockers are an essential component of most hypothermia protocols. However, effector organs, including skeletal muscles, may be involved in other critical functions of the body besides thermoregulation. In particular, muscle paralysis interferes with such a vital function of skeletal muscles as respiration. Thus, this strategy is also limited only to anesthetized and mechanically ventilated patients and is not amenable for use in other patient populations, which may benefit from hypothermic treatment.

Some effector organs may be more selectively involved in thermoregulation. In particular, brown adipose tissue has a well-established function in compensatory heat generation during cold exposure in small mammals and has been recently implicated in the same role in humans. At the same time, this tissue doesn’t appear to be acutely involved in other vital physiological functions. Thus, its pharmacological inactivation may lead to relatively safe and specific decrease in compensatory heat generation during physical cooling. However, no such strategies in humans have been reported so far. Furthermore, because the effector systems are at least partially redundant, inactivating any one of them may not be sufficiently effective.

**Targeting Thermoreceptors**

Lastly, the largely untapped part of the thermoregulatory system which may be targeted in order to control thermal balance is thermoreception. Temperature in the body is sensed by central thermoreceptors in hypothalamus, spinal cord and viscera, as well as by peripheral receptors in the nerve endings of the primary sensory neurons. The central hypothalamic thermoregulatory neurons integrate all cold- and warm-sensitive peripheral and central temperature inputs, and generate an appropriate output signal determining the activity of the thermoregulatory processes. Here, an opportunity may be recognized that modulation of the neural activity of the thermoreceptors may enable deliberate control of the heat-transferring activity of the thermoeffector.

Up to this point, it has been proposed that achieving hypothermia requires increasing heat loss by physical cooling with concomitant elimination of the compensatory responses. Accordingly, this discussion has focused on strategies for inactivation of the thermoregulatory system. However, given the capacity of the thermoregulatory system to induce net heat gain as well as net heat loss, pharmacological manipulation of thermoreceptors offers an intriguing and potentially more effective alternative for altering core temperature. In contrast to merely inactivating the thermoregulatory system, manipulating the signal from the thermoreceptors should allow shifting the thermal balance in the hypothemic direction by reducing heat retention, inhibiting thermogenesis, and facilitating active heat loss. In this scenario, the thermoregulatory system may be driving, instead of defending against, the drop in core temperature, which should result in hypothermia with less time, energy costs, and side effects. Importantly, active physical cooling thus becomes merely an auxiliary, and not a necessary measure. The fulfillment of the described strategy critically requires specific and pharmacologically accessible molecular targets within central or peripheral thermosensory neurons that would allow modulation of the activity of these neurons.

The molecular mechanisms responsible for thermosensitivity of the central neurons have not yet been clearly established. There is some evidence that this thermal sensitivity is achieved by a particular combination of common neuronal ion channels, such as hyperpolarization-activated cyclic nucleotide-gated channels (HCN), voltage-activated A-type potassium channels (Kv4.1), and tandem pore domain, or background leak potassium channels (Kv2p), rather than by a single and unique receptor protein. Because these common channels are broadly involved in nervous system functions, they have potential as therapeutic targets.

Until recently, the molecular markers of peripheral thermoreceptors were also unknown. In a series of major advances, several members of the Transient Receptor Potential (TRP) superfamily of non-specific cation channels were shown to be directly gated by temperature. These channels are collectively known as thermoTRPs and include cold-sensitive TRPM8 and TRPA1, as well as as warm-sensitive TRPV1, TRPV2, TRPV3, and TRPV4.779 Thermoreceptors are expressed, with variable selectivity, in primary sensory neurons and therein fulfil one of the critical requirements for useful pharmacological targets for controlling thermoregulation. Importantly, in agreement with the model described above, modulation of at least 2 of these channels, TRPV1 and TRPM8, produced hypothermia in experimental animal studies. 65-71 Next, the possible mechanism for the hypothermic action of these drugs and some practical aspects of their potential use as hypothermic agents in human patients will be discussed.

**Targeting TRPM8, a molecular sensor of cold**

As mentioned earlier, the activity of the thermoeffector is controlled by an integrated signal from anatomically distinct cold- and warm-sensitive inputs. These inputs have a reciprocal influence on the thermoeffector activity, with cold inputs favoring heat gain responses, and warm inputs favoring heat loss responses. Thus, the net heat loss state of the thermoregulatory system, required for achieving hypothermia, may be induced either by increasing warm input, or by blocking the cold input. With the establishment of the cold-sensitive ion channel TRPM8 as an in vivo molecular cold sensor, its inhibition seemed likely to provide the latter opportunity, namely, to block the cold signals from reaching the thermoregulatory system and enable the drop in core temperature during exposure to subneutral ambient temperatures.

The TRPM8 channel, also known as the cold and menthol receptor, is directly gated by temperatures below ~27°C in vitro.74-77 Within the nervous system, TRPM8 is located in a subset of primary sensory neurons of the dorsal root and trigeminal ganglia within the afferent pathway for skin cooling. In the context of a neuron, this ion channel converts the physical stimulus of lowered temperature into a depolarizing current, thus
endowing a select population of primary neurons with cold sensitivity. Because cold-sensitive neurons transmit the signal about cold exposure to the higher-order thermoregulatory centers, TRPM8 is responsible at least for some part of this signal. Accordingly, pharmacological inhibition of TRPM8 abolishes cold-sensitivity of a subset of primary neurons and leads to a decrease in the overall cold-dependent input to the thermoregulatory system, shifting it to a less heat-generating and more heat-dissipating state. On the level of individual thermoeffector, pharmacological inhibition or genetic deletion of TRPM8 decreases whole body energy production, thermogenesis in brown adipose tissue, peripheral vasoconstriction, and cold-avoidance behaviors. As mechanistically predicted, the net effect of these changes has been a decrease in core temperature (Fig. 3). Importantly, such a hypothemic effect has been demonstrated for at least 3 unrelated TRPM8 antagonists and in the case of one of them - in both mice and rats. These findings have provided an initial conceptual support for the use of pharmacological TRPM8 inhibition to induce therapeutic hypothermia. However, further development of this idea reveals several important limitations.

The first thing to consider is that TRPM8-independent pathways for cold sensing exist, and TRPM8-dependent neurons carry only some part, albeit a substantial one, of the cold signal from the skin. Even more importantly, the thermoregulatory system receives cold-generated input not only from peripheral tissues, but also from the body core, mediated at least in part by the decreased activity of the warm-sensing neurons in MPA. Owing to this partial redundancy, the thermoregulatory system can compensate for the failure of skin-dependent receptor input, such as that induced by TRPM8 inhibition. Thus, the use of physical cooling to achieve a neuroprotective range of hypothermia. Nevertheless, TRPM8 inhibition may not be entirely free of complications. Because there have been no studies reporting the use of TRPM8 antagonists in humans, this prediction awaits experimental confirmation.

The effects of TRPM8 antagonists also depend on ambient temperature, and thus may be increased by exposing the subject to physical cooling. However, as outlined above, inhibition of TRPM8 does not fully inactivate the thermoregulatory system, which still retains the ability to mount cold defense responses to prevent a further drop in core temperature. Therefore, physical cooling will still produce unwanted physiological thermoregulatory responses. Moreover, it is being increasingly recognized that individual thermoeffector mechanisms may be under the control of functionally, anatomically and molecularly distinct afferent pathways, and thus may be regulated by TRPM8 to a different extent. In particular, there is evidence that shivering and tachycardic responses to external cooling are not significantly controlled by TRPM8-mediated signals and are not abolished by TRPM8 inhibition. Thus, the use of physical cooling to enhance the hypothemic effect of TRPM8 inhibitors is expected to produce the same complications as physical cooling alone, namely, almost full activation of shivering and other cold defense responses.

Finally, pharmacological modulation of TRPM8 may be problematic because of the potential “off-target” effects. Although the field of thermoregulation is justifiably focused on TRPM8 in sensory neurons, this channel is also expressed in a number of other tissues, including prostate, liver, bladder and vascular smooth muscle, as well as in various types of tumors. The effects of TRPM8 modulation in these tissues are largely unknown, but may complicate its therapeutic utility.

To summarize, despite the initial encouraging prospects, both empirical data and mechanistic understanding of the functions of the TRPM8 channel in the thermoregulatory system argue against its clinical usefulness as a sole target for safe and effective hypothermia. Nevertheless, TRPM8 inhibition may not be entirely...
Figure 3. For figure legend, see page 252.
without clinical value in hypothermic protocols. Because TRPM8 antagonists seem to have at least some effects on the thermoregulatory system, their use as adjuvants may be considered and deserves further investigation. Along these lines, it was shown that TRPM8 inhibition can enhance the hypothermic effect of the TRPV1 agonist dihydrocapsaicin, and additional such interactions may be uncovered in future studies.

The relative lack of success of the putative universal cold sensor TRPM8 in hypothermia should not be generalized to the overall strategy of blocking cold input to the thermoregulatory system. Because this input is probably mediated by additional cold-sensitive mechanisms, identification of such mechanisms and development of the pharmacological methods for their inhibition may ultimately make this strategy more effective and promising.

Targeting TRPV1, a molecular sensor of heat

As an alternative to inhibiting cold-sensitive pathways, the net heat loss state of the thermoregulatory system may be achieved by activating the warm-sensing pathways. The conceptual validity of this idea has been confirmed in a series of studies, demonstrating that application of physical skin counter-warming techniques is able to suppress the cold defense responses to hypothermia. However, the effect of skin counter-warming alone is not sufficient to decrease core temperature into the therapeutic range. There is some evidence that a more efficient implementation of this strategy may be provided by pharmacological activation of the TRPV1 ion channel.

TRPV1, also known as the heat and capsaicin receptor, is another temperature-sensitive member of the TRP superfamily, activated by temperatures above ~42°C in vitro. It is mainly expressed in primary and higher-order sensory neurons and in numerous structures in the central nervous system. Although the gene encoding this channel was identified only in 1997, it has been known for decades that systemic administration of the TRPV1 agonist capsaicin, the pungent ingredient of chili peppers, leads to pronounced hypothermia in numerous mammalian species.

Despite numerous studies, the site of action and the exact mechanism responsible for the hypothermic effect of TRPV1 agonists have not been definitively established. The existing findings may be largely accounted for by a model wherein TRPV1 agonists activate the skin-warming pathway. According to this model, pharmacological TRPV1 activation provides “false,” or spurious, information about the exposure of skin to high temperatures to the central thermoregulatory neurons of the hypothalamus and effectively “tricks” them into sensing heat in the absence of a real temperature challenge. In an effort to offset the consequences of the presumed overheating, central thermoregulatory neurons would be predicted to respond to such a signal with inhibition of heat-generating thermoeffector mechanisms, like warm-seeking and non-thermoregulatory locomotion, shivering, and brown adipose tissue activity, and activation of heat-dissipating mechanisms, such as peripheral vasodilation and species-dependent saliva-spreading, panting, and sweating. Such a coordinated shift in the activity of the thermoeffectors was indeed demonstrated in experimental studies and may explain the generation of a substantial heat flow away from the body resulting in a rapid decline in body temperature. In addition, it has been speculated that TRPV1-containing neurons responsible for these effects are most likely anatomically located in the median preoptic nucleus of the hypothalamus, and functionally belong to the afferent pathway for skin warming. However, whether TRPV1 agonists actually act on the hypothalamic neurons or increase the afferent signal within the skin warming pathways has yet to be conclusively demonstrated. Until then, the proposed model remains largely speculative.

Importantly, TRPV1 activation by agonists not only abolishes compensatory responses to core cooling, which actually enables a profound decrease in core temperature, but also substantially blunts the shivering and tachycardic responses to ambient cooling. This finding suggests that application of external cooling on the background of pharmacological TRPV1 activation has a substantially diminished effect on the overall integrated afferent thermoregulatory drive and does not trigger a significant compensatory increase in heat generation. Therefore, the rate and depth of hypothermia, induced by TRPV1 activation, may be enhanced by increasing heat loss through ambient physical cooling.

A number of additional favorable pharmacological properties further augment the practical value of TRPV1 agonists in achieving hypothermia. Importantly, it is possible to continuously deliver the agonists via intravenous or subcutaneous infusion routes, producing a long-lasting (at least 6-12 hours) and stable decrease of core temperature with rapid onset and recovery of normal temperature after discontinuation of drug delivery. In addition, the hypothermic effect is sufficiently large to reach the clinically relevant range and dependent on the agonist dose, which potentially allows fine control over the depth of hypothermia. Furthermore, the hypothermic effect was reproduced in a number of species, including mice, rats, monkeys and cattle, and showed minimal desensitization. Taking advantage of these favorable properties, 2 studies have demonstrated that the TRPV1 agonists dihydrocapsaicin and rinvanil decreased infarct size and improved neurological recovery in 2 different mouse models.

Figure 3 (See previous page). The effects of the genetic deletion and pharmacological inhibition of TRPM8 channels on core body temperature. (A) Core temperature of rats after intravenous infusion (AA) or mice after intraperitoneal infusion (AB) of the TRPM8 antagonist M8-B (6 mg/kg) at an ambient temperature of 19°C (AA) or 26°C (AB). Time of infusion is indicated by a gray bar. Adapted from Almeida et al. The American Physiological Society. Permission to reuse must be obtained from the rightsholders.
models of acute ischemic stroke (Fig. 4).92,93 These findings serve as a proof-of-principle for the use of TRP channel modulators for therapeutic hypothermia.

At the same time, despite the significant promise of TRPV1 agonists in inducing hypothermia in animal models, some aspects of targeting TRPV1 still create major obstacles for the clinical use of these agents in humans. TRPV1 was found to be expressed and functionally involved not only in thermoregulatory pathways, but also in a plethora of other physiological functions.94 Therefore, TRPV1 activation may lead to numerous “off-target” effects. Perhaps the most salient side effect is related to TRPV1 expression in nociceptors, a subset of neurons responsible for triggering pain.95 Accordingly, TRPV1 agonists, such as capsaicin, have the potential to cause, alongside the beneficial hypothermic effect on the thermoregulatory system, a conscious experience of burning pain, such as the one caused by eating chili peppers. Indeed, in addition to the widely familiar culinary experience, burning pain was reported after topical, intradermal and intramuscular administration of capsaicin in humans.96,97

Overall, although considerable further study is needed to determine the feasibility of using TRPV1 agonists as hypothermic agents in human patients, their dramatic hypothermic effect makes it seem worthwhile to continue basic and translational research in this direction even in the face of these challenges.

Targeting other TRP channels

Besides TRPM8 and TRPV1, other thermoTRP channels have also been implicated in thermoregulation, which suggests that modulation of their activity may similarly allow controlling thermoeffectors and altering core temperature. Although evidence for this so far is either lacking or controversial, further research is still warranted to resolve the existing issues.

For example, TRPA1 is another cold-sensitive channel, which shares some properties with TRPM8. It is activated by temperatures below ~17°C, is expressed in sensory neurons and is required for avoidance behaviors in response to painfully low temperatures.98,99 These findings have initially suggested that, by analogy with TRPM8, TRPA1 may be
responsible for autonomic cold defense responses and that its inhibition may be utilized to enable a therapeutic drop in core temperature during physical cooling. However, although the physiological role of TRPA1 was the subject of considerable controversy in the literature, a recent comprehensive study has convincingly demonstrated that pharmacological inhibition of TRPA1 has no effect on thermoafferent activity and core temperature, and thus does not appear to be useful in the context of hypothermia. Warm-sensitive thermoTRPs, in addition to TRPV1, also include closely related members TRPV2, TRPV3 and TRPV4. These channels are activated by above-neutral temperatures with distinct thresholds (52, 33 and 27°C respectively), and are expressed in primary sensory neurons as well as in skin keratinocytes, which may signal to sensory neurons. Based on these properties, similarly to TRPV1, these warm-sensitive thermoTRPs were initially thought to be responsible for thermosensation, and thus were likely candidates for mediating afferent inputs to the thermoregulatory system. However, studies in knockout animals failed to reach definitive conclusions about their roles as thermosensors, providing both supporting and opposing evidence. Nevertheless, interpretation of these studies is complicated by limitations associated with the knock-out technology, such as an influence of genetic background and the possibility of developmental compensation. Moreover, the involvement of these channels in autonomic thermoregulatory responses, as opposed to behavioral responses, has not been thoroughly explored. Finally, even if it had been definitively established that warm-sensitive thermoTRPs did not serve as thermosensors, it still would not preclude the possibility that they are wired to and affect the activity of the central thermoregulatory neurons. One way to test such a possibility involves determining the specific effects of channel modulation on core temperature. However, this is complicated by the absence of specific pharmacological agents suitable for in vivo administration. Presently only one such study has been reported, which found no effect of intragastric administration of the TRPV3 agonists thymol and ethyl vanillin on core temperature of mice. However, the use of only a single agonist dose delivered by only an intragastric route precludes making definitive conclusions based on this study. Therefore, whether modulation of other warm-sensitive TRP channels has the potential of inducing hypothermia is not fully resolved at this point and may become a focus of future investigations.

It should also be noted that while this review was focused on pharmacological modulators of the TRP channels for hypothermia induction, a number of other agents have been shown to affect body temperature and have been proposed for therapeutic hypothermia, including agonists for neurotensin, dopamine, serotonin and adenosine receptors. Without a doubt, these agents have an important place in the general framework described herein and present more opportunities for controlling thermoregulation.

Conclusions

Induced hypothermia has proven to be an exceptionally powerful means to preserve brain function in conditions of hypoxia and promises to find an increasingly wider application in clinics. However, body temperature is regulated by an incredibly complex array of physical and biological processes. Therapeutic modulation of core temperature critically relies on the ability to finely tune these processes. As the study of thermoregulation has entered the molecular era, the discovery of novel specific and pharmacologically accessible molecular targets may provide just that possibility, and therein pave the way for the promising field of “thermopharmacology,” aptly named so by one of its pioneers. ThermoTRP channels represent a considerable advance in this regard, but they have important limitations that still stand in the way of producing safe and effective therapeutic hypothermia in human patients.

The fascinating advances in science and technology allow us to transcend the natural homeostatic capabilities of the human body and induce states and conditions that offer unprecedented resistance to pathological processes. However, with great power comes great responsibility. As these powerful tools increasingly enter clinical practice, it is incumbent upon us to have a deep and comprehensive understanding of

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their effects on physiology and interactions with the body’s natural defenses. Therefore, further research is warranted and highly needed either to circumvent the limitations of the current strategies, or to discover entirely novel approaches to therapeutic temperature modulation.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest are disclosed.

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