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Drug-induced hypothermia in stroke models: does it always protect?

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Abstract

Ischemic stroke is a common neurological disorder lacking a cure. Recent studies show that therapeutic hypothermia is a promising neuroprotective strategy against ischemic brain injury. Several methods to induce therapeutic hypothermia have been established; however, most of them are not clinically feasible for stroke patients. Therefore, pharmacological cooling is drawing increasing attention as a neuroprotective alternative worthy of further clinical development. We begin this review with a brief introduction to the commonly used methods for inducing hypothermia; we then focus on the hypothermic effects of eight classes of hypothermia-inducing drugs: the cannabinoids, opioid receptor activators, transient receptor potential vanilloid, neurotensins, thyroxine derivatives, dopamine receptor activators, hypothermia-inducing gases, adenosine, and adenine nucleotides. Their neuroprotective effects as well as the complications associated with their use are both considered. This article provides guidance for future clinical trials and animal studies on pharmacological cooling in the setting of acute stroke.

Keywords

Brain ischemia; Hypothermic; Neuroprotection; Pharmacological cooling

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Conflict of Interest:

None.

1. Hypothermia and strokes

1.1 Introduction

Therapeutic hypothermia is defined as a 2-6°C reduction of core body temperature [1-3] and is a promising neuroprotective approach against brain injury induced by strokes. In hemorrhagic stroke, hypothermia reduces brain edema [4, 5] and improves neurologic function [4-7]. In ischemic stroke, hypothermia is also protective, which has been proven in randomized clinical trials in human cardiac arrest and in animal studies of focal and global ischemia [8, 9]. However, the impact of hypothermia on patients with acute ischemic stroke still needs to be explored [10]. During the past decades, studies on hypothermia and ischemic stroke have suggested that the required amplitude of protective hypothermia depends on its time of initiation relative to stroke onset, duration, and depth of hypothermia [1, 9, 11]. Optimal protection is typically gained when hypothermia is induced as early as possible after stroke onset, with a mild-to-moderate hypothermia of 32 to 35°C that lasts at least one to two hours [1, 2, 12-14].

1.2 Protective mechanism of hypothermia in ischemic stroke

Although hypothermia is effective in protecting against experimental models of ischemic stroke, the precise protective mechanisms are not yet fully understood. It has been suggested that the protective mechanisms are multifold, including reductions in metabolic rate, cerebral blood flow, blood-brain barrier damage, as well as decreases in excitotoxicity, apoptosis, inflammation and free radical production [15]. On average, hypothermia reduces brain oxygen consumption by approximately 5% per degree Celsius within the range of 22 to 37°C [16]. In regard to cerebral blood flow, there is evidence to support a hypothermia-induced reduction in post-ischemic hyperperfusion and sustained hypoperfusion [13]. This is hypothesized to reduce oxidative metabolism and cytotoxic edema in both experimental studies and clinical settings [17, 18]. Many studies have also shown that mild to moderate hypothermia protects the blood-brain barrier (BBB) by preserving vascular morphology [19] and protecting pericytes [20], which are increasingly recognized to play an important regulatory role in BBB integrity. Detailed neuroprotective mechanisms of hypothermia have been summarized well elsewhere [13, 21].

1.3 Cooling methods

The common methods for inducing therapeutic hypothermia are surface cooling, intravascular cooling and pharmacological cooling; each offers unique advantages and disadvantages [22]. Surface cooling reduces body temperature through the skin by the application of ice packs, ice-cold water, or alcohol spray [23, 24]. These methods are easy to perform, noninvasive and inexpensive. However, surface cooling is intolerable and uncomfortable to awake stroke patients. These methods also require a longer time to achieve target body temperature and do not control body temperature with precision. In addition, muscular blockades are often required to reduce shivering [25]. Intravascular cooling decreases body temperature via blood, by infusing cold fluid through intravenous catheters or devices containing temperature sensors [26-28]. The main advantages of intravascular cooling are shorter time to target temperature and more precise hypothermic control. However, this method is invasive, and patients placed on catheters are more susceptible to

infection and hematoma, especially in thrombolytic patients [12, 21]. Neither surface nor intravascular cooling is practical in emergencies because their application requires full anesthesia. It is more practical and clinically relevant to pharmacologically induce mild hypothermia.

Hypothermia causes side effects or complications, and the severity of them may depend on the cooling methods or hypothermic mechanisms [2, 28]. Cardiovascular suppression, indicated by decreased heart beat rate and blood pressure, is a common side effect of hypothermia and occurs independent of cooling methods. Another side effect is immunosuppression, which may lead to increased rate of infection [2, 28]. A recent study showed that low body temperature reduced the number of circulating lymphocytes, contributing to the immunosuppression [29]. Derangements in blood electrolytes and glucose may also occur if the duration of hypothermia is prolonged. Shivering is very common in surface and intravascular cooling but less common in drug-induced hypothermia.

2. Drug-induced hypothermia in ischemic stroke

To date, eight classes of medicines have been tested for their efficacy in inducing hypothermia and protecting the brain. Most but not all of them are protective against experimental ischemic stroke (Table 1); while there are no available data on hemorrhagic stroke. Therefore, this review will focus the effects of drug-induced hypothermia on ischemic stroke. It should be noted that the protective impact of physical cooling strongly supports a causal link between hypothermia and neuroprotection against ischemia. But it is not always the case in drug-induced hypothermia. It will be evident that a significant reduction in core body temperature is often, but not always, associated with neuroprotection. Because the drugs have multiple effects aside from hypothermia, the association between hypothermia and neuroprotection may or may not be causal. However, if the drug-induced neuroprotection is abolished when core body temperature is maintained at 37 °C with surface heating, the link between hypothermia and neuroprotection is more than a correlation for that compound. On the other hand, some drugs that lower core body temperature can induce unwanted side effects that outweigh the protective impact of the hypothermia. Nevertheless, as outlined below, drug-induced hypothermia is far more practical than physical cooling.

2.1 Cannabinoids

Cannabinoids bind to their specific receptors- CB1 and CB2, and have been scrutinized for their hypothermic and neuroprotective effects [30]. CB1 receptors are widely distributed in the central nervous system, including the striatum, cortex and hypothalamus [31]. CB1 receptors in the preoptic anterior nucleus of the hypothalamus (POAH) are responsible for cannabinoids' hypothermic effects [32, 33]. Direct injection of a selective cannabinoid agonist (WIN 55,212-2) into the POAH elicited significant hypothermia in rats in a rapid and dose-dependent fashion [32, 33]. The effect of WIN55,212-2 could be abolished by CB1 competitive antagonist Rimonabant (SR141716). Independent of their hypothermic effects, CB receptors are also coupled to neuroprotective transduction pathways *per se*. CB1 receptor agonists protect the brain against excitotoxicity by reducing glutamate secretion

from pre-synaptic terminals and blocking the activity of N-methyl-D-aspartate receptors [34, 35]. CB2 receptors are almost exclusively expressed on peripheral immune cells, and demonstrate immunomodulatory effects in brain injury but do not induce hypothermia [36, 37].

The efficacy of CB1 receptor agonists in the treatment of ischemic brain injury has been evaluated in animal studies. WIN 55,212-2 is the most potent and selective water-soluble CB1 receptor agonist; it binds CB1 receptors specifically, with negligible interaction with other neurotransmitter systems and ion channels compared to other cannabinoids [33, 38]. Intramuscular injection of WIN 55,212-2 at a dose of 5 mg/kg significantly reduced rat body temperature within 15 minutes [32]. Peak hypothermia ($3.4 \pm 0.4^{\circ}\text{C}$) occurred 90 min post-injection and the temperatures recovered to normal levels within 5 h post-injection [32]. Intraperitoneal (IP) injection of WIN 55,212-2 (1 mg/kg) did not alter the mean arterial blood pressure, PaO_2 , PaCO_2 , pH, or blood glucose concentration, but significantly increased neuronal survival after global cerebral ischemia and decreased infarct size after focal ischemia [39]. The neuroprotection conferred by WIN 55,212-2 was dependent on its hypothermic effect, because the protection was largely abolished when the body temperature was normalized [40]. Besides WIN 55,212-2, HU-210 and delta 9-tetrahydrocannabinol are also protective against ischemic injury [31, 41]. Although these compounds elicit negative side effects, such as reduction in motility, slight drowsiness, even limb rigidity and hypokinesia [39, 41], CB receptor agonists generally seem to be promising candidates for hypothermic treatment of stroke.

2.2 Opioid receptor activators

It has long been held that opioids produce a range of effects on body temperature in mammals [42]. Thermoregulatory responses of opioids are the result of their interaction with three types of opioid receptors— μ , κ , δ receptors [42]. Activation of any of these three receptors can affect body temperature. For instance, U50 488H (a selective κ agonist), morphine (a μ agonist) and Deltorphin II (a selective δ agonist) all induce hypothermia at lower doses [43, 44]. However, at higher doses, they fail to induce hypothermia in restrained animals and even cause hyperthermia in unrestrained animals [43-45].

Increasing attention has been focused on on [D-Ala2, D-Leu5] enkephalin (DADLE), a synthetic 44 kDa protein that acts as a selective δ -opioid receptor agonist. Two features distinguish DADLE: hibernation induction [46, 47] and neuroprotective effects [48-50]. DADLE induces hibernation in ground squirrels in situations where κ and μ agonists have failed [46]. It has also been reported that DADLE treatment protects the brain against focal [48] and global cerebral ischemia [49-51], making DADLE a promising neuroprotective candidate.

It is not clear if hypothermia is essential for the neuroprotective effects of DADLE. In experiments on DADLE in focal ischemia rat body temperature was maintained at 37°C during the surgery [48]. However, body temperature shortly after the surgery was not reported. This is a critical time that encompasses ischemia and early reperfusion. In the case of global ischemia, intracerebral ventricular injection of DADLE attenuated CA1 neuronal loss in experiments that did not monitor post-surgery body temperature [50] or maintained

body temperature at 37°C for the first three hours after surgery [52]. These experiments suggest that DADLE can protect in the absence of hypothermia. In support of this hypothesis, DADLE attenuated ischemic neuronal death in brain slice cultures maintained at 37°C [53]. However, Iwata and colleagues reported that DADLE failed to protect rat brains against global ischemia when the temperature was maintained at 37.5 ± 0.5 °C by surface heating, suggesting that hypothermia is essential for the effect [54]. Iwata and colleagues also reported that DADLE only provided limited neuroprotection to relatively ischemia-resistant regions and not to selectively vulnerable regions [49].

We recently performed a series of experiments to test if DADLE induces hypothermia in non-hibernating animals such as mice and if this was associated with protection against focal ischemia in the brain. We found that DADLE administration quickly decreased core body temperature in mice, reaching into the mild hypothermia range in less than 90 minutes (Figure 1). DADLE-induced hypothermia was linked with protection of the brain against ischemic injury, as indicated by decreased infarct volumes (Figure 2A) and attenuated BBB disruption (Figure 2B).

Although most of the studies support a protective role of DADLE against brain ischemia or neurodegenerative diseases, adverse effects have also been reported. For example, DADLE at 10 mg/kg causes severe vasodilatation and 50% reduction in arterial blood pressure in dogs [55]. DADLE also raises the risk of arrhythmia [56]. These negative side effects may limit the clinical utility of DADLE and warrant further investigation.

2.3 Transient receptor potential vanilloid 1 (TRPV1)

TRPV1 is a nonselective cation channel expressed mainly on a subset of sensory neurons in the peripheral and central nervous systems. TRPV1 plays a thermoregulatory role, probably through peripheral sensors and the POAH [57, 58]. Several endogenous and exogenous TRPV1 agonists have been identified, some of which can modulate body temperature. For instance, dihydrocapsaicin induces a sustainable mild hypothermia in rats, cynomolgus monkeys and young cattle [59]. Rinvanil is a synthetic TRPV1 agonist that possesses ultra-potent activating effects [60]. It is also the only TRPV1 agonist that has been evaluated in the ischemic setting. When IP or intracerebroventricularly (ICV) injected, Rinvanil induced mild hypothermia in rats, decreased infarct size at 24 hours and 7 days after middle cerebral artery occlusion (MCAO) [61]. Notably, Rinvanil did not alter physiological parameters such as blood pressure, PaO₂, PaCO₂, blood pH, and regional cerebral blood flow during ischemia and reperfusion [61]. In this regard, Rinvanil seems superior to other TRPV1 agonists, such as capsaicin and dihydrocapsaicin, which are also reported to cause hypertension and tachycardia [59, 62]. However, several features may still hamper successful translation of Rinvanil from animal studies into the clinic. For example, the hypothermic effect of Rinvanil decayed quickly after one bolus injection. Repetitive and high frequent injections are required to achieve hypothermia by this compound [61]. Second, excessive activation of TRPV1 is neurotoxic, counteracting the hypothermia-dependent ischemic neuroprotection [63, 64].

2.4 Neurotensin (NT) system

Neurotensin is a tridecapeptide neurotransmitter or modulator and is expressed throughout the central nervous system, especially in the hypothalamus [65]. NT elicits a wide range of potent biological effects, including hypothermia and analgesia [66], which are primarily mediated by a G protein coupled receptor - NT receptor type 1 (NTS1). NTS1 is also widely distributed throughout the CNS, with the highest levels in the substantia nigra, ventral tegmental area, lateral septum and bed nucleus of the stria terminalis [65].

Natural NT is unstable once it is in blood because of blood peptidases, therefore it cannot cross the BBB when it is injected intravenously or IP. However, a short synthetic NT analogue, acetyl-neurotensin-(8--13) is stable, able to cross the BBB and has the full binding and pharmacological activities of natural NT [67, 68]. Acetyl-neurotensin-(8--13) and several its analogs demonstrate hypothermic effects. For example, NT69L and KK1-19, two acetyl-neurotensin-(8-13) analogs, can elicit significantly hypothermic responses in rats [68, 69]. ABS-201, a new acetyl-neurotensin-(8--13) analog also induces mild hypothermia in mice [12]. NT-induced hypothermia is predominantly mediated by NTS1, since NTS1 deficient mice were completely insensitive to NT [70], and PD149163, a selective NTS1 agonist, significantly lowered core body temperature in rats [71].

Compelling evidence suggests neuroprotective effects of neurotensin (8-13) analogs against acute ischemic stroke [12, 72, 73]. ICV injection of JMV-449, a pseudopeptide analogue of neurotensin (8-13), quickly induced hypothermia and decreased infarct size in mice after MCAO [72]. ABS-201 lowered core body temperature by 2–5°C in 15-30 min and maintained body temperature below 35°C for six hours in mice [12]. ABS-201 was able to decrease infarct size by 30–40% and to improve long-term neurological function [12].

Despite these promising studies, several limitations of these analogs are apparent. They cause severe hypothermia (< 30°C) and require significant re-warming measures [74-77]. Furthermore, hypothermic tolerance or drug resistance has been reported [74-77]. In general, however, the novel neurotensin (8-13) analogs are promising for the treatment of acute stroke in clinical settings.

2.5 Thyroxine Derivatives

3-iodothyronamine (T₁AM) and thyronamine (T₀AM) are derived from thyroid hormone (TH) through deiodination and decarboxylation *in vivo* [78]. In contrast with TH, T₁AM and T₀AM are not ligands for TH nuclear receptors, but are agonists of a G-protein coupled receptor – trace amine associated receptor 1 (TAAR1) [78, 79]. T₁AM and T₀AM exert functions that are the opposite of TH. IP injection of T₁AM and T₀AM at 50 mg/kg induces hypothermia in mice lasting for 6-10 hours without evidence of shivering or piloerection [78]. It has been suggested that TAMs reset the central temperature set point without eliciting peripheral compensatory responses [80]. The thermoregulatory response to TAMs was originally thought to be mediated by TAAR1 [81]. However, a recent study proposed that the impressive hypothermic response of 3-T₁AM was maintained in TAAR-1 knockout mice to a similar extent as wild type mice [82]. Thus, the receptors responsible for TAMs' thermoregulation are still under debate.

Thyroxine derivatives hold promise as neuroprotective therapies against stroke. Mice treated with T₁AM (50 mg/kg, 2 days before MCAO or 1 hour after MCAO) showed profound reductions in infarct size that depended on hypothermia. Maintaining the body temperature by heating pad or directly applying T₁AM to neurons under oxygen and glucose deprivation *in vitro* abolished the protection of T₁AM. This implies that the neuroprotective effect of T₁AM requires the induction of hypothermia [83]. This is the only study so far evaluating the effect of TAMs in ischemic stroke. Similar to most hypothermia-inducing chemicals, TAMs disturbed the metabolic and cardiovascular systems, as characterized by bradycardia, reduced cardiac output, hyperglycemia, and respiratory depression [84]. As with these other chemicals, this may limit the clinical utility of TAMs unless the side effects can be combated by other means.

2.6 Dopamine receptor activators

The important role of dopamine receptors (DARs) in thermoregulation has been studied for about 30 years since Barnett and his colleagues reported that DARs may be involved in the hypothermia in mice induced by apomorphine, a non-selective dopamine receptor agonist [85]. There are at least 5 subtypes of DARs (D₁, D₂, D₃, D₄, and D₅), which can be further classified as D1-like receptors (D₁ and D₅) and D2-like receptors (D₂, D₃, and D₅). Stimulation of D1-like receptors results in an increase in body temperature, while D2-like agonists induce hypothermia [86-88].

Both apomorphine (300 mg/kg) and N-propylnorapomorphine (NPA, 18 mg/kg, another nonselective DAR agonists), and quinpirole (LY171555, selective D₂ agonist) can induce a dose- and time-dependent hypothermic effect that is attenuated by pretreatment with sulpiride (200 mg/kg, selective D₂ antagonist) [89]. IP injection of bromocriptine, another D₂ receptor agonist, causes a dose-dependent decrease in core body temperature of mice that can be abolished by administration of reserpine plus alpha-methyl-p-tyrosine before bromocriptine injection [90]. Talipexole (B-HT 920, 0.25-1 mg/kg), an α 2-adrenoceptor agonist and D₂ agonist, also induced hypothermia peaking within 60-90 min after drug administration and lasting about 2 hours [91]. These effects were blocked by D₂ antagonists (haloperidol, 1 mg/kg, or 100 mg/kg, sulpiride) but not α 2-adrenoceptor antagonists (yohimbine, 1 mg/kg, or idazoxan, 1 mg/kg) [91]. Subsequent studies have further elicited the different roles of D2-like receptor subtypes. Millan and colleagues found that the D₃ receptor participates in the hypothermic effects of clozapine and 7-OH-DAPT [92, 93]. Clozapine and 7-OH-DAPT are preferential D₃ versus D₂ receptor agonists whose hypothermic actions can be blocked by AJ76 and S11566 (selective D₃ antagonists) but not by haloperidol and raclopride (mixed D₂/D₃ antagonists) [92, 93]. In an *in vitro* study using Chinese hamster ovary cells transfected with recombinant D₂ or D₃ receptors, Millan and colleagues measured the affinity of eight DAR agonists and nine DAR antagonists for DARs [92, 93], and reported that the D₃ receptor, plays an important role in dopamine-mediated hypothermia [92, 93].

Recent studies have examined the effects of hypothermia induced by D2-like receptor agonists on ischemic outcomes. The core body temperature of rats treated with the D₂ agonist talipexole (B-HT 920, 2 mg bolus injection after MCAO plus 2 mg continuous

infusion for 24 h after MCAO) was reduced by 1.7 °C for 24 hour after MCAO [94]. Infarct volume measured at 7 days after MCAO was reduced by 47% [94]. These results suggest that agonism at the D2 receptor can induce hypothermia and elicit neuroprotection *in vivo*. In addition, bromocriptine, a potent agonist at D2 receptors, induces hypothermia [90] and reduces CA1 neuronal damage after global ischemia in rats [95] [96]. However, it is still unclear if hypothermia is essential for the protective effects of bromocriptine.

2.7 Gaseous hypothermia

Hydrogen sulfide (H₂S) is a colorless gas with the characteristic odor of rotten eggs [97]. It was thought to be toxic to cells because it inhibits cytochrome c oxidase, a component of the oxidative phosphorylation machinery within mitochondria [98, 99]. Breathing H₂S at 80 parts per million by volume (ppm) induces a “suspended animation–like state”, characterized by reduction of core body temperature and respiratory and heartbeat rates [100-102]. Continuous exposure to 70 ppm H₂S induces long-term hypothermia [103, 104]. Post-stroke exposure of rats to H₂S-induced hypothermia for 48 hours reduces infarct volume by more than 50% without causing significant physiological side effects [105]. On the other hand, Qu *et al* found that administration of sodium hydrosulfide, a H₂S donor, increased the H₂S level in brain cortex and enlarged infarct volume in rats [106]. In these studies, body temperature was maintained at 37±0.5°C during anesthesia and not monitored thereafter [106].

There has been a surge of recent interest in the use of inert gases for neuroprotection against ischemic brain injury [107-109]. Helium is one such example; it is a colorless, nontoxic and cost-efficient gas without anesthetic properties [110]. A concentration of 75 % helium in oxygen induces hypothermia in rats [111, 112] and the magnitude of hypothermia correlates positively with the temperature of the gas mixtures [111, 112]. Both intra-ischemic and immediately post-ischemic inhalation of helium alleviates neurological deficits in rats subjected to MCAO [107, 112]. Protection was elicited when the gas temperature was 25 °C but lost when the gas temperature was 33 °C [112]. Furthermore, late helium treatment administered 30- 60 min after reperfusion? did not elicit protection [113]. Physiological parameters such as blood pressure, heart rate were similar between the experimental and control groups [107]. The mechanisms of helium-induced hypothermia and its neuroprotection are not known.

Inhalational anesthetics were also reported to induce hypothermia. For example, sevoflurane induces hypothermia in humans [114] and rabbits [115], probably by inhibiting central thermoregulation. We have also observed that isoflurane, one of most commonly used anesthetics, induces hypothermia in small animals such as mice and rats (Figure 3). Under continuous anesthesia, their body temperature drops quickly to room temperature for about two hours in mice and five hours in rats. Some anesthetics have been shown to be neuroprotective against stroke, such as sevoflurane [116] and isoflurane [117]. However, the role of hypothermia was in the protection is not clear, because body temperature was maintained at 37°C in these studies.

2.8 Adenosine and adenine nucleotides

Adenosine triphosphate (ATP) is the major energy currency of cells. Through one or more steps of dephosphorylation, ATP can be sequentially degraded to adenosine diphosphate (ADP), adenosine monophosphate (AMP), and adenosine. It has long been observed that adenosine induces hypothermia in a dose-dependent manner in mice [118-120]. Furthermore, the adenosine A1 receptor may play a key role because A1 receptor agonists other than adenosine also induce hypothermia [120, 121]. Adenosine is neuroprotective against stroke and elicits multiple protective mechanisms [122-124]. However, it is not clear whether hypothermia is essential for the protection [122-124].

It was recently reported that AMP plays a role in thermoregulation [125]. When animals were housed in constant darkness, an element of hibernation, plasma AMP levels were elevated and this rise was associated with specific patterns of gene expression [125]. In addition, injection of exogenous AMP induces hypothermia in mice [125]. Similarly, IP injection of ADP and ATP also induced hypothermia in mice in a dose-dependent manner [119]. AMP and ATP induce similar hypothermic effects in rats, but at higher doses [126]. However, when AMP- or ATP-induced mild hypothermia was applied to rats with focal strokes, both compounds failed to protect the brain and exacerbated ischemic brain injury [126, 127]. The exacerbation might be attributed to multiple side effects such as hypotension and hyperglycemia [126, 127]. These findings reveal that hypothermia is not always associated with protection and that the means by which hypothermia is elicited play critical roles in determining final ischemic outcome. Some compounds that reduce core body temperature may still cause injury *despite* their hypothermic effects. In other words, the hypothermia that they elicit may still be protective, but not robust enough to counterbalance the negative impact of hypotension and hyperglycemia. It would therefore be interesting to see if ischemic injury is exacerbated even further by maintaining body temperature at 37 °C with a heating pad in ATP-treated animals. The negative impact of some hypothermia-inducing drugs on metabolism and blood pressure are important to consider when testing pharmacological compounds for their neuroprotective properties.

3. Summary and considerations

As discussed above, the majority of experiments support the notion that drug-induced hypothermia is neuroprotective against ischemic stroke, especially cannabinoids, opioid receptor activators (DADLE), TRPV1 (Rinvanil), the novel neurotensins. Drug-induced hypothermia may be preferable to physical means of cooling core body temperature such as ice packs and intravenous infusions of cold liquid. However, before drug-induced hypothermia can be applied to stroke in the clinic, several crucial issues need to be investigated further. First, current data on drug-induced hypothermia were collected from small animals such as mice and rats but not large animals or human beings. Small animals have larger surface area/mass ratios than large animals. For example, the average surface area/mass ratio is 0.33 in mouse and 0.17 in rat (in square meters per kilogram), clearly favoring the loss of heat [97, 128]. In contrast, large animals and humans have small surface area/mass ratios, 0.05 in dogs and 0.026 in humans, favoring heat retention [128]. As expected, body temperature falls quicker in smaller animals (mice) than in larger animals

(rats), as shown in Figure 3. Whether drugs can induce hypothermia in humans as effectively as in small animals is not known. Even if they are able to induce hypothermia in humans, large doses may be required. This increases the potential for complications from negative side effects, which are already of significant concern, as discussed above.

Second, the mechanisms of drug-induced hypothermia are more complicated than simple physical cooling; some of the drugs not only elicit hypothermia but also promote other protective pathways, including anti-inflammatory and anti-apoptotic ones [50, 105, 129]. Thus, the neuroprotective effects conferred by these drugs may only be correlated with hypothermia instead of being caused by it. Because drugs act in multiple ways other than just inducing hypothermia, their actions are likely to differ from physical cooling *per se*. This implies that the optimal cooling duration and target temperature may be different for drug-induced cooling and physical cooling. Indeed, shorter durations and higher target temperatures may be effective with drug-induced hypothermia. This may be easier to achieve and more practical in the clinic.

Third, negative side effects must be carefully considered for drugs that induce hypothermia. Some side effects of hypothermic drugs are quite common, such as reduction in mobility, cardiovascular function and decreased metabolism, as they are part of the hypothermic mechanisms [15, 55, 84, 119, 126]. In extreme cases, however, the side effects are so severe that their detrimental effects on ischemia outweigh the protective effects of the hypothermia [126], precluding their use against stroke. Side effects are influenced by the dose, administration method, mechanism of drug action, and target organs, all of which play important roles in determining whether the drugs elicit protection or lead to unfavorable outcomes [125, 126, 130].

Finally, simultaneous use of approved stroke treatments together with hypothermia may elicit synergic protective effects against brain injury. Alteplase, also known as Tissue plasminogen activator is the only FDA-approved medication for the treatment of acute stroke, aiming at lysing blood clots and restoring cerebral blood flow. Currently, the therapeutic window for alteplase in stroke patients is only 4.5 hours after stroke onset [131, 132]. Future studies are warranted to determine whether this temporal window may be extended or whether there are synergic protective effects when alteplase is given in conjunction with hypothermia.

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References

1. Yenari MA, Hemmen TM. Therapeutic hypothermia for brain ischemia: where have we come and where do we go? *Stroke*. 2010; 41(10 Suppl):S72–4. [PubMed: 20876510]
2. Polderman KH, Herold I. Therapeutic hypothermia and controlled normothermia in the intensive care unit: practical considerations, side effects, and cooling methods. *Crit Care Med*. 2009; 37(3): 1101–20. [PubMed: 19237924]

3. Olsen TS, Weber UJ, Kammersgaard LP. Therapeutic hypothermia for acute stroke. *The Lancet Neurology*. 2003; 2(7):410–416.
4. Kollmar R, Staykov D, Dorfler A, Schellinger PD, Schwab S, Bardutzky J. Hypothermia reduces perihemorrhagic edema after intracerebral hemorrhage. *Stroke*. 2010; 41(8):1684–9. [PubMed: 20616317]
5. Abdullah JM, Husin A. Intravascular hypothermia for acute hemorrhagic stroke: a pilot study. *Acta Neurochir Suppl*. 2011; 111:421–4. [PubMed: 21725794]
6. Torok E, Klopotoski M, Trabold R, Thal SC, Plesnila N, Scholler K. Mild hypothermia (33 degrees C) reduces intracranial hypertension and improves functional outcome after subarachnoid hemorrhage in rats. *Neurosurgery*. 2009; 65(2):352–9. discussion 359. [PubMed: 19625915]
7. Fingas M, Penner M, Silasi G, Colbourne F. Treatment of intracerebral hemorrhage in rats with 12 h, 3 days and 6 days of selective brain hypothermia. *Exp Neurol*. 2009; 219(1):156–62. [PubMed: 19445934]
8. van der Worp HB, Sena ES, Donnan GA, Howells DW, Macleod MR. Hypothermia in animal models of acute ischaemic stroke: a systematic review and meta-analysis. *Brain*. 2007; 130(Pt 12): 3063–74. [PubMed: 17478443]
9. van der Worp HB, Macleod MR, Kollmar R. Therapeutic hypothermia for acute ischemic stroke: ready to start large randomized trials? *J Cereb Blood Flow Metab*. 2010; 30(6):1079–93. [PubMed: 20354545]
10. Jacobs S, Hunt R, Tarnow-Mordi W, Inder T, Davis P. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst Rev*. 2007; (4) CD003311.
11. Yager JY, Asselin J. Effect of Mild Hypothermia on Cerebral Energy Metabolism During the Evolution of Hypoxic-Ischemic Brain Damage in the Immature Rat. *Stroke*. 1996; 27(5):919–926. [PubMed: 8623114]
12. Choi KE, Hall CL, Sun JM, Wei L, Mohamad O, Dix TA, Yu SP. A novel stroke therapy of pharmacologically induced hypothermia after focal cerebral ischemia in mice. *FASEB J*. 2012; 26(7):2799–810. [PubMed: 22459147]
13. Yenari MA, Han HS. Neuroprotective mechanisms of hypothermia in brain ischaemia. *Nat Rev Neurosci*. 2012; 13(4):267–278. [PubMed: 22353781]
14. Miyazawa T, Tamura A, Fukui S, Hossmann KA. Effect of mild hypothermia on focal cerebral ischemia. Review of experimental studies. *Neurol Res*. 2003; 25(5):457–64. [PubMed: 12866192]
15. Yenari MA, Iwayama S, Cheng D, Sun GH, Fujimura M, Morita-Fujimura Y, Chan PH, Steinberg GK. Mild hypothermia attenuates cytochrome c release but does not alter Bcl-2 expression or caspase activation after experimental stroke. *J Cereb Blood Flow Metab*. 2002; 22(1):29–38. [PubMed: 11807391]
16. Erecinska M, Thoresen M, Silver IA. Effects of hypothermia on energy metabolism in Mammalian central nervous system. *J Cereb Blood Flow Metab*. 2003; 23(5):513–30. [PubMed: 12771566]
17. Bonifacio SL, Saporta A, Glass HC, Lee P, Glidden DV, Ferriero DM, Barkovich AJ, Xu D. Therapeutic Hypothermia for Neonatal Encephalopathy Results in Improved Microstructure and Metabolism in the Deep Gray Nuclei. *AJNR Am J Neuroradiol*. 2012
18. Cheng G, Sun J, Wang L, Shao X, Zhou W. Effects of selective head cooling on cerebral blood flow and metabolism in newborn piglets after hypoxia-ischemia. *Early Hum Dev*. 2011; 87(2): 109–14. [PubMed: 21144680]
19. Baumann E, Preston E, Slinn J, Stanimirovic D. Post-ischemic hypothermia attenuates loss of the vascular basement membrane proteins, agrin and SPARC, and the blood-brain barrier disruption after global cerebral ischemia. *Brain Res*. 2009; 1269:185–97. [PubMed: 19285050]
20. Duz B, Oztas E, Erginay T, Erdogan E, Gonul E. The effect of moderate hypothermia in acute ischemic stroke on pericyte migration: an ultrastructural study. *Cryobiology*. 2007; 55(3):279–84. [PubMed: 17923122]
21. Choi HA, Badjatia N, Mayer SA. Hypothermia for acute brain injury--mechanisms and practical aspects. *Nat Rev Neurol*. 2012; 8(4):214–22. [PubMed: 22371279]
22. Faridar A, Bershad EM, Emiru T, Iaizzo PA, Suarez JI, Divani AA. Therapeutic hypothermia in stroke and traumatic brain injury. *Front Neurol*. 2011; 2:80. [PubMed: 22207862]

23. Chen H, Chopp M, Zhang ZG, Garcia JH. The effect of hypothermia on transient middle cerebral artery occlusion in the rat. *J Cereb Blood Flow Metab.* 1992; 12(4):621–8. [PubMed: 1618941]
24. Maier CM, Ahern Kv, Cheng ML, Lee JE, Yenari MA, Steinberg GK, Kirsch JR. Optimal Depth and Duration of Mild Hypothermia in a Focal Model of Transient Cerebral Ischemia : Effects on Neurologic Outcome, Infarct Size, Apoptosis, and Inflammation • Editorial Comment: Effects on Neurologic Outcome, Infarct Size, Apoptosis, and Inflammation. *Stroke.* 1998; 29(10):2171–2180. [PubMed: 9756600]
25. Testori C, Sterz F, Behringer W, Spiel A, Firbas C, Jilma B. Surface cooling for induction of mild hypothermia in conscious healthy volunteers - a feasibility trial. *Crit Care.* 2011; 15(5):R248. [PubMed: 22018242]
26. Guluma KZ, Oh H, Yu SW, Meyer BC, Rapp K, Lyden PD. Effect of endovascular hypothermia on acute ischemic edema: morphometric analysis of the ICTuS trial. *Neurocrit Care.* 2008; 8(1): 42–7. [PubMed: 17922082]
27. Hinz J, Rosmus M, Popov A, Moerer O, Frerichs I, Quintel M. Effectiveness of an intravascular cooling method compared with a conventional cooling technique in neurologic patients. *J Neurosurg Anesthesiol.* 2007; 19(2):130–5. [PubMed: 17414000]
28. Groyzman LI, Emanuel BA, Kim-Tenser MA, Sung GY, Mack WJ. Therapeutic hypothermia in acute ischemic stroke. *Neurosurg Focus.* 2011; 30(6):E17. [PubMed: 21631218]
29. Bouma HR, Kroese FG, Kok JW, Talaei F, Boerema AS, Herwig A, Draghiciu O, van Buiten A, Epema AH, van Dam A, Strijkstra AM, Henning RH. Low body temperature governs the decline of circulating lymphocytes during hibernation through sphingosine-1-phosphate. *Proc Natl Acad Sci U S A.* 2011; 108(5):2052–7. [PubMed: 21245336]
30. Piomelli D, Giuffrida A, Calignano A, Rodriguez de Fonseca F. The endocannabinoid system as a target for therapeutic drugs. *Trends Pharmacol Sci.* 2000; 21(6):218–24. [PubMed: 10838609]
31. Hayakawa K, Mishima K, Nozako M, Hazekawa M, Ogata A, Fujioka M, Harada K, Mishima S, Orito K, Egashira N, Iwasaki K, Fujiwara M. Delta9-tetrahydrocannabinol (Delta9-THC) prevents cerebral infarction via hypothalamic-independent hypothermia. *Life Sci.* 2007; 80(16):1466–71. [PubMed: 17289082]
32. Rawls SM, Cowan A, Tallarida RJ, Geller EB, Adler MW. N-methyl-D-aspartate antagonists and WIN 55212-2 [4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrrolo[3,2,1-i,j]quinolin-6-one], a cannabinoid agonist, interact to produce synergistic hypothermia. *J Pharmacol Exp Ther.* 2002; 303(1):395–402. [PubMed: 12235276]
33. Rawls SM, Cabassa J, Geller EB, Adler MW. CB1 receptors in the preoptic anterior hypothalamus regulate WIN 55212-2 [(4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrrolo[3,2,1-i,j]quinolin-6-one)-induced hypothermia. *J Pharmacol Exp Ther.* 2002; 301(3):963–8. [PubMed: 12023525]
34. Gerdeman G, Lovinger DM. CB1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. *J Neurophysiol.* 2001; 85(1):468–71. [PubMed: 11152748]
35. Hampson AJ, Grimaldi M. Cannabinoid receptor activation and elevated cyclic AMP reduce glutamate neurotoxicity. *Eur J Neurosci.* 2001; 13(8):1529–36. [PubMed: 11328347]
36. Klein TW. Cannabinoid-based drugs as anti-inflammatory therapeutics. *Nat Rev Immunol.* 2005; 5(5):400–11. [PubMed: 15864274]
37. Croxford JL, Miller SD. Immunoregulation of a viral model of multiple sclerosis using the synthetic cannabinoid R+WIN55,212. *J Clin Invest.* 2003; 111(8):1231–40. [PubMed: 12697742]
38. Showalter VM, Compton DR, Martin BR, Abood ME. Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification of cannabinoid receptor subtype selective ligands. *J Pharmacol Exp Ther.* 1996; 278(3):989–99. [PubMed: 8819477]
39. Nagayama T, Sinor AD, Simon RP, Chen J, Graham SH, Jin K, Greenberg DA. Cannabinoids and neuroprotection in global and focal cerebral ischemia and in neuronal cultures. *J Neurosci.* 1999; 19(8):2987–95. [PubMed: 10191316]
40. Bonfils PK, Reith J, Hasseldam H, Johansen FF. Estimation of the hypothermic component in neuroprotection provided by cannabinoids following cerebral ischemia. *Neurochem Int.* 2006; 49(5):508–18. [PubMed: 16730099]

41. Leker RR, Gai N, Mechoulam R, Ovadia H. Drug-induced hypothermia reduces ischemic damage: effects of the cannabinoid HU-210. *Stroke*. 2003; 34(8):2000–6. [PubMed: 12829867]
42. Adler MW, Geller EB, Rosow CE, Cochin J. The opioid system and temperature regulation. *Annu Rev Pharmacol Toxicol*. 1988; 28:429–49. [PubMed: 2837979]
43. Baker AK, Meert TF. Functional effects of systemically administered agonists and antagonists of mu, delta, and kappa opioid receptor subtypes on body temperature in mice. *J Pharmacol Exp Ther*. 2002; 302(3):1253–64. [PubMed: 12183687]
44. Spencer RL, Hraby VJ, Burks TF. Body temperature response profiles for selective mu, delta and kappa opioid agonists in restrained and unrestrained rats. *J Pharmacol Exp Ther*. 1988; 246(1):92–101. [PubMed: 2839673]
45. Handler CM, Geller EB, Adler MW. Effect of mu-, kappa-, and delta-selective opioid agonists on thermoregulation in the rat. *Pharmacol Biochem Behav*. 1992; 43(4):1209–16. [PubMed: 1361992]
46. Oeltgen PR, Nilekani SP, Nuchols PA, Spurrier WA, Su TP. Further studies on opioids and hibernation: delta opioid receptor ligand selectively induced hibernation in summer-active ground squirrels. *Life Sci*. 1988; 43(19):1565–74. [PubMed: 2904105]
47. Borlongan CV, Wang Y, Su TP. Delta opioid peptide (D-Ala 2, D-Leu 5) enkephalin: linking hibernation and neuroprotection. *Front Biosci*. 2004; 9:3392–8. [PubMed: 15353366]
48. Borlongan CV, Hayashi T, Oeltgen PR, Su TP, Wang Y. Hibernation-like state induced by an opioid peptide protects against experimental stroke. *BMC Biol*. 2009; 7:31. [PubMed: 19534760]
49. Iwata M, Inoue S, Kawaguchi M, Nakamura M, Konishi N, Furuya H. Effects of delta-opioid receptor stimulation and inhibition on hippocampal survival in a rat model of forebrain ischaemia. *Br J Anaesth*. 2007; 99(4):538–46. [PubMed: 17704092]
50. Duan YL, Wang SY, Zeng QW, Su DS, Li W, Wang XR, Zhao Z. Astroglial reaction to delta opioid peptide [D-Ala2, D-Leu5] enkephalin confers neuroprotection against global ischemia in the adult rat hippocampus. *Neuroscience*. 2011; 192:81–90. [PubMed: 21745540]
51. Gao CJ, Li JP, Wang W, Lu BC, Niu L, Zhu C, Wei YY, Zhang T, Wu SX, Chai W, Li YQ. Effects of intracerebroventricular application of the delta opioid receptor agonist [D-Ala2, D-Leu5] enkephalin on neurological recovery following asphyxial cardiac arrest in rats. *Neuroscience*. 2010; 168(2):531–42. [PubMed: 20167252]
52. Su DS, Wang ZH, Zheng YJ, Zhao YH, Wang XR. Dose-dependent neuroprotection of delta opioid peptide [D-Ala2, D-Leu5] enkephalin in neuronal death and retarded behavior induced by forebrain ischemia in rats. *Neurosci Lett*. 2007; 423(2):113–7. [PubMed: 17689189]
53. Zheng YJ, Wang XR, Chen HZ, Wu XJ, Zhao YH, Su DS. Protective Effects of the Delta Opioid Peptide [D-Ala2, D-Leu5]enkephalin in an Ex Vivo Model of Ischemia/Reperfusion in Brain Slices. *CNS Neurosci Ther*. 2012; 18(9):762–6. [PubMed: 22943142]
54. Iwata M, Inoue S, Kawaguchi M, Kurita N, Horiuchi T, Nakamura M, Konishi N, Furuya H. Delta opioid receptors stimulation with [D-Ala2, D-Leu5] enkephalin does not provide neuroprotection in the hippocampus in rats subjected to forebrain ischemia. *Neurosci Lett*. 2007; 414(3):242–6. [PubMed: 17207574]
55. Drabek T, Han F, Garman RH, Stezoski J, Tisherman SA, Stezoski SW, Morhard RC, Kochanek PM. Assessment of the delta opioid agonist DADLE in a rat model of lethal hemorrhage treated by emergency preservation and resuscitation. *Resuscitation*. 2008; 77(2):220–8. [PubMed: 18207625]
56. Sigg DC, Coles JA Jr, Oeltgen PR, Iaizzo PA. Role of delta-opioid receptor agonists on infarct size reduction in swine. *Am J Physiol Heart Circ Physiol*. 2002; 282(6):H1953–60. [PubMed: 12003798]
57. Shimizu I, Iida T, Horiuchi N, Caterina MJ. 5-Iodoresiniferatoxin evokes hypothermia in mice and is a partial transient receptor potential vanilloid 1 agonist in vitro. *J Pharmacol Exp Ther*. 2005; 314(3):1378–85. [PubMed: 15947039]
58. Gavva NR, Bannon AW, Surapaneni S, Hovland DN Jr, Lehto SG, Gore A, Juan T, Deng H, Han B, Klionsky L, Kuang R, Le A, Tamir R, Wang J, Youngblood B, Zhu D, Norman MH, Magal E, Treanor JJ, Louis JC. The vanilloid receptor TRPV1 is tonically activated in vivo and involved in body temperature regulation. *J Neurosci*. 2007; 27(13):3366–74. [PubMed: 17392452]

59. Fosgerau K, Ristagno G, Jayatissa M, Axelsen M, Gotfredsen JW, Weber UJ, Kober L, Torp-Pedersen C, Videbaek C. Increased susceptibility to cardiovascular effects of dihydrocapsaicin in resuscitated rats. Cardiovascular effects of dihydrocapsaicin. *BMC Cardiovasc Disord*. 2010; 10:39. [PubMed: 20807439]
60. Appendino G, De Petrocellis L, Trevisani M, Minassi A, Daddario N, Moriello AS, Gazzieri D, Ligresti A, Campi B, Fontana G, Pinna C, Geppetti P, Di Marzo V. Development of the First Ultra-Potent “Capsaicinoid” Agonist at Transient Receptor Potential Vanilloid Type 1 (TRPV1) Channels and Its Therapeutic Potential. *Journal of Pharmacology and Experimental Therapeutics*. 2005; 312(2):561–570. [PubMed: 15356216]
61. Muzzi M, Felici R, Cavone L, Gerace E, Minassi A, Appendino G, Moroni F, Chiarugi A. Ischemic neuroprotection by TRPV1 receptor-induced hypothermia. *J Cereb Blood Flow Metab*. 2012; 32(6):978–82. [PubMed: 22434066]
62. Chanda S, Mould A, Esmail A, Bley K. Toxicity studies with pure trans-capsaicin delivered to dogs via intravenous administration. *Regul Toxicol Pharmacol*. 2005; 43(1):66–75. [PubMed: 16051405]
63. Kim SR, Chung YC, Chung ES, Park KW, Won SY, Bok E, Park ES, Jin BK. Roles of transient receptor potential vanilloid subtype 1 and cannabinoid type 1 receptors in the brain: neuroprotection versus neurotoxicity. *Mol Neurobiol*. 2007; 35(3):245–54. [PubMed: 17917113]
64. Shirakawa H, Yamaoka T, Sanpei K, Sasaoka H, Nakagawa T, Kaneko S. TRPV1 stimulation triggers apoptotic cell death of rat cortical neurons. *Biochem Biophys Res Commun*. 2008; 377(4):1211–5. [PubMed: 18996081]
65. Caceda R, Kinkead B, Nemeroff CB. Neurotensin: role in psychiatric and neurological diseases. *Peptides*. 2006; 27(10):2385–404. [PubMed: 16891042]
66. Bissette G, Nemeroff CB, Loosen PT, Prange AJ Jr, Lipton MA. Hypothermia and intolerance to cold induced by intracisternal administration of the hypothalamic peptide neurotensin. *Nature*. 1976; 262(5569):607–9. [PubMed: 8728]
67. Granier C, van Rietschoten J, Kitabgi P, Poustis C, Freychet P. Synthesis and characterization of neurotensin analogues for structure/activity relationship studies. Acetyl-neurotensin-(8–13) is the shortest analogue with full binding and pharmacological activities. *Eur J Biochem*. 1982; 124(1):117–24. [PubMed: 7084220]
68. Kokko KP, Hadden MK, Price KL, Orwig KS, See RE, Dix TA. In vivo behavioral effects of stable, receptor-selective neurotensin[8-13] analogues that cross the blood-brain barrier. *Neuropharmacology*. 2005; 48(3):417–25. [PubMed: 15721174]
69. Cusack B, Boules M, Tyler BM, Fauq A, McCormick DJ, Richelson E. Effects of a novel neurotensin peptide analog given extracranially on CNS behaviors mediated by apomorphine and haloperidol. *Brain Res*. 2000; 856(1-2):48–54. [PubMed: 10677610]
70. Remaury A, Vita N, Gendreau S, Jung M, Arnone M, Poncelet M, Culouscou JM, Le Fur G, Soubrie P, Caput D, Shire D, Kopf M, Ferrara P. Targeted inactivation of the neurotensin type 1 receptor reveals its role in body temperature control and feeding behavior but not in analgesia. *Brain Res*. 2002; 953(1-2):63–72. [PubMed: 12384239]
71. Feifel D, Goldenberg J, Melendez G, Shilling PD. The acute and subchronic effects of a brain-penetrating, neurotensin-1 receptor agonist on feeding, body weight and temperature. *Neuropharmacology*. 2010; 58(1):195–8. [PubMed: 19596358]
72. Torup L, Borsdal J, Sager T. Neuroprotective effect of the neurotensin analogue JMV-449 in a mouse model of permanent middle cerebral ischaemia. *Neurosci Lett*. 2003; 351(3):173–6. [PubMed: 14623134]
73. Babcock AM, Baker DA, Hallock NL, Lovet R, Lynch WC, Peccia JC. Neurotensin-induced hypothermia prevents hippocampal neuronal damage and increased locomotor activity in ischemic gerbils. *Brain Res Bull*. 1993; 32(4):373–8. [PubMed: 8221127]
74. Tyler-McMahon BM, Boules M, Richelson E. Neurotensin: peptide for the next millennium. *Regul Pept*. 2000; 93(1-3):125–36. [PubMed: 11033059]
75. Tyler-McMahon BM, Stewart JA, Farinas F, McCormick DJ, Richelson E. Highly potent neurotensin analog that causes hypothermia and antinociception. *European journal of pharmacology*. 2000; 390(1-2):107–11. [PubMed: 10708713]

76. Katz LM, Young A, Frank JE, Wang Y, Park K. Neurotensin-induced hypothermia improves neurologic outcome after hypoxic-ischemia. *Crit Care Med*. 2004; 32(3):806–10. [PubMed: 15090966]
77. Smith KE, Boules M, Williams K, Fauq AH, Richelson E. The role of NTS2 in the development of tolerance to NT69L in mouse models for hypothermia and thermal analgesia. *Behav Brain Res*. 2011; 224(2):344–9. [PubMed: 21718721]
78. Scanlan TS, Suchland KL, Hart ME, Chiellini G, Huang Y, Kruzich PJ, Frascarelli S, Crossley DA, Bunzow JR, Ronca-Testoni S, Lin ET, Hatton D, Zucchi R, Grandy DK. 3-Iodothyronamine is an endogenous and rapid-acting derivative of thyroid hormone. *Nat Med*. 2004; 10(6):638–42. [PubMed: 15146179]
79. Bunzow JR, Sonders MS, Arttamangkul S, Harrison LM, Zhang G, Quigley DI, Darland T, Suchland KL, Pasumamula S, Kennedy JL, Olson SB, Magenis RE, Amara SG, Grandy DK. Amphetamine, 3,4-methylenedioxymethamphetamine, lysergic acid diethylamide, and metabolites of the catecholamine neurotransmitters are agonists of a rat trace amine receptor. *Mol Pharmacol*. 2001; 60(6):1181–8. [PubMed: 11723224]
80. Klieverik LP, Foppen E, Ackermans MT, Serlie MJ, Sauerwein HP, Scanlan TS, Grandy DK, Fliers E, Kalsbeek A. Central effects of thyronamines on glucose metabolism in rats. *J Endocrinol*. 2009; 201(3):377–86. [PubMed: 19273499]
81. Tan ES, Naylor JC, Groban ES, Bunzow JR, Jacobson MP, Grandy DK, Scanlan TS. The molecular basis of species-specific ligand activation of trace amine-associated receptor 1 (TAAR(1)). *ACS Chem Biol*. 2009; 4(3):209–20. [PubMed: 19256523]
82. Panas HN, Lynch LJ, Vallender EJ, Xie Z, Chen GL, Lynn SK, Scanlan TS, Miller GM. Normal thermoregulatory responses to 3-iodothyronamine, trace amines and amphetamine-like psychostimulants in trace amine associated receptor 1 knockout mice. *J Neurosci Res*. 2010; 88(9):1962–9. [PubMed: 20155805]
83. Doyle KP, Suchland KL, Ciesielski TM, Lessov NS, Grandy DK, Scanlan TS, Stenzel-Poore MP. Novel thyroxine derivatives, thyronamine and 3-iodothyronamine, induce transient hypothermia and marked neuroprotection against stroke injury. *Stroke*. 2007; 38(9):2569–76. [PubMed: 17690312]
84. Piehl S, Hoefig CS, Scanlan TS, Kohrle J. Thyronamines--past, present, and future. *Endocr Rev*. 2010; 32(1):64–80. [PubMed: 20880963]
85. Barnett A, Goldstein J, Taber RI. Apomorphine-induced hypothermia in mice; a possible dopaminergic effect. *Arch Int Pharmacodyn Ther*. 1972; 198(2):242–7. [PubMed: 5054733]
86. Costentin J, Duterte-Boucher D, Panissaud C, Michael-Titus A. Dopamine D1 and D2 receptors mediate opposite effects of apomorphine on the body temperature of reserpinized mice. *Neuropharmacology*. 1990; 29(1):31–5. [PubMed: 1968237]
87. Sanchez C. The effects of dopamine D-1 and D-2 receptor agonists on body temperature in male mice. *Eur J Pharmacol*. 1989; 171(2-3):201–6. [PubMed: 2575999]
88. Salmi P, Jimenez P, Ahlenius S. Evidence for specific involvement of dopamine D1 and D2 receptors in the regulation of body temperature in the rat. *Eur J Pharmacol*. 1993; 236(3):395–400. [PubMed: 8102971]
89. Meller E, Hizami R, Kreuter L. Hypothermia in mice: D2 dopamine receptor mediation and absence of spare receptors. *Pharmacol Biochem Behav*. 1989; 32(1):141–5. [PubMed: 2567521]
90. Zarrindast MR, Mahmoudi M. Bromocriptine-induced hypothermia: D-2 receptor involvement. *Arch Int Pharmacodyn Ther*. 1989; 298:38–49. [PubMed: 2569299]
91. Verma A, Kulkarni SK. Dopamine receptor mediated hypothermic action of B-HT 920 in rats. *J Pharm Pharmacol*. 1991; 43(6):421–4. [PubMed: 1681056]
92. Millan MJ, Audinot V, Melon C, Newman-Tancredi A. Evidence that dopamine D3 receptors participate in clozapine-induced hypothermia. *Eur J Pharmacol*. 1995; 280(2):225–9. [PubMed: 7589191]
93. Millan MJ, Peglion JL, Vian J, Rivet JM, Brocco M, Gobert A, Newman-Tancredi A, Dacquet C, Bervoets K, Girardon S, et al. Functional correlates of dopamine D3 receptor activation in the rat in vivo and their modulation by the selective antagonist(+)-S 14297: 1. Activation of postsynaptic

- D3 receptors mediates hypothermia, whereas blockade of D2 receptors elicits prolactin secretion and catalepsy. *J Pharmacol Exp Ther.* 1995; 275(2):885–98. [PubMed: 7473180]
94. Johansen FF, Jorgensen HS, Reith J. Prolonged drug-induced hypothermia in experimental stroke. *J Stroke Cerebrovasc Dis.* 2003; 12(2):97–102. [PubMed: 17903912]
 95. Liu XH, Kato H, Chen T, Kato K, Itoyama Y. Bromocriptine protects against delayed neuronal death of hippocampal neurons following cerebral ischemia in the gerbil. *J Neurol Sci.* 1995; 129(1):9–14. [PubMed: 7751851]
 96. O'Neill MJ, Hicks CA, Ward MA, Cardwell GP, Reymann J-M, Allain H, Bentué-Ferrer D. Dopamine D2 receptor agonists protect against ischaemia-induced hippocampal neurodegeneration in global cerebral ischaemia. *European journal of pharmacology.* 1998; 352(1):37–46. [PubMed: 9718265]
 97. Wagner F, Asfar P, Calzia E, Radermacher P, Szabo C. Bench-to-bedside review: Hydrogen sulfide—the third gaseous transmitter: applications for critical care. *Crit Care.* 2009; 13(3):213. [PubMed: 19519960]
 98. Khan AA, Schuler MM, Prior MG, Yong S, Coppock RW, Florence LZ, Lillie LE. Effects of hydrogen sulfide exposure on lung mitochondrial respiratory chain enzymes in rats. *Toxicol Appl Pharmacol.* 1990; 103(3):482–90. [PubMed: 2160136]
 99. Dorman DC, Moulin FJ, McManus BE, Mahle KC, James RA, Struve MF. Cytochrome oxidase inhibition induced by acute hydrogen sulfide inhalation: correlation with tissue sulfide concentrations in the rat brain, liver, lung, and nasal epithelium. *Toxicol Sci.* 2002; 65(1):18–25. [PubMed: 11752681]
 100. Blackstone E, Morrison M, Roth MB. H₂S induces a suspended animation-like state in mice. *Science.* 2005; 308(5721):518. [PubMed: 15845845]
 101. Volpato GP, Searles R, Yu B, Scherrer-Crosbie M, Bloch KD, Ichinose F, Zapol WM. Inhaled hydrogen sulfide: a rapidly reversible inhibitor of cardiac and metabolic function in the mouse. *Anesthesiology.* 2008; 108(4):659–68. [PubMed: 18362598]
 102. Blackstone E, Roth MB. Suspended animation-like state protects mice from lethal hypoxia. *Shock.* 2007; 27(4):370–2. [PubMed: 17414418]
 103. Joseph C, Buga A-M, Vintilescu R, Balseanu AT, Moldovan M, Junker H, Walker L, Lotze M, Popa-Wagner A. Prolonged gaseous hypothermia prevents the upregulation of phagocytosis-specific protein Annexin 1 and causes low-amplitude EEG activity in the aged rat brain after cerebral ischemia. *J Cereb Blood Flow Metab.* 2012; 32(8):1632–1642. [PubMed: 22617647]
 104. Florian B, Vintilescu R, Balseanu AT, Buga A-M, Grisk O, Walker LC, Kessler C, Popa-Wagner A. Long-term hypothermia reduces infarct volume in aged rats after focal ischemia. *Neuroscience Letters.* 2008; 438(2):180–185. [PubMed: 18456407]
 105. Joseph C, Buga AM, Vintilescu R, Balseanu AT, Moldovan M, Junker H, Walker L, Lotze M, Popa-Wagner A. Prolonged gaseous hypothermia prevents the upregulation of phagocytosis-specific protein Annexin 1 and causes low-amplitude EEG activity in the aged rat brain after cerebral ischemia. *J Cereb Blood Flow Metab.* 2012; 32(8):1632–42. [PubMed: 22617647]
 106. Qu K, Chen CP, Halliwell B, Moore PK, Wong PT. Hydrogen sulfide is a mediator of cerebral ischemic damage. *Stroke.* 2006; 37(3):889–93. [PubMed: 16439695]
 107. Pan Y, Zhang H, VanDeripe DR, Cruz-Flores S, Panneton WM. Heliox and oxygen reduce infarct volume in a rat model of focal ischemia. *Exp Neurol.* 2007; 205(2):587–90. [PubMed: 17467695]
 108. Gruss M, Bushell TJ, Bright DP, Lieb WR, Mathie A, Franks NP. Two-pore-domain K⁺ channels are a novel target for the anesthetic gases xenon, nitrous oxide, and cyclopropane. *Mol Pharmacol.* 2004; 65(2):443–52. [PubMed: 14742687]
 109. Hobbs C, Thoresen M, Tucker A, Aquilina K, Chakkarapani E, Dingley J. Xenon and hypothermia combine additively, offering long-term functional and histopathologic neuroprotection after neonatal hypoxia/ischemia. *Stroke.* 2008; 39(4):1307–13. [PubMed: 18309163]
 110. Hess DR, Fink JB, Venkataraman ST, Kim IK, Myers TR, Tano BD. The history and physics of heliox. *Respir Care.* 2006; 51(6):608–12. [PubMed: 16723037]
 111. Tapper D, Arensman R, Johnson C, Folkman J. The effect of helium-oxygen mixtures on body temperature. *J Pediatr Surg.* 1974; 9(5):597–603. [PubMed: 4419541]

112. David HN, Haelewyn B, Chazalviel L, Lecocq M, Degoulet M, Risso JJ, Abraini JH. Post-ischemic helium provides neuroprotection in rats subjected to middle cerebral artery occlusion-induced ischemia by producing hypothermia. *J Cereb Blood Flow Metab.* 2009; 29(6):1159–65. [PubMed: 19384333]
113. Pan Y, Zhang H, Acharya AB, Cruz-Flores S, Panneton WM. The effect of heliox treatment in a rat model of focal transient cerebral ischemia. *Neurosci Lett.* 2011; 497(2):144–7. [PubMed: 21539894]
114. Ikeda T, Sessler DI, Kikura M, Kazama T, Ikeda K, Sato S. Less Core Hypothermia when Anesthesia Is Induced with Inhaled Sevoflurane Than with Intravenous Propofol. *Anesthesia & Analgesia.* 1999; 88(4):921. [PubMed: 10195549]
115. Guzel O, Inal G, Cirakli ZT, Eraslan E, Aktas M. Effects of propofol and sevoflurane anesthesia on some physiological and biochemical parameters in rabbits. *Med Weter.* 2006; 62(12):1383–1386.
116. Adamczyk S, Robin E, Simerabet M, Kipnis E, Tavernier B, Vallet B, Bordet R, Lebuffe G. Sevoflurane pre- and post-conditioning protect the brain via the mitochondrial KATP channel. *British Journal of Anaesthesia.* 2010; 104(2):191–200. [PubMed: 20086064]
117. Lee JJ, Li L, Jung HH, Zuo Z. Postconditioning with isoflurane reduced ischemia-induced brain injury in rats. *Anesthesiology.* 2008; 108(6):1055–62. [PubMed: 18497606]
118. Mehta AK, Kulkarni SK. Effect of purinergic substances on rectal temperature in mice: involvement of P1-purinoceptors. *Arch Int Pharmacodyn Ther.* 1983; 264(2):180–6. [PubMed: 6314920]
119. Swoap SJ, Rathvon M, Gutilla M. AMP does not induce torpor. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology.* 2007; 293(1):R468–R473.
120. Iliff BW, Swoap SJ. Central adenosine receptor signaling is necessary for daily torpor in mice. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology.* 2012; 303(5):R477–R484.
121. Anderson R, Sheehan MJ, Strong P. Characterization of the adenosine receptors mediating hypothermia in the conscious mouse. *Br J Pharmacol.* 1994; 113(4):1386–90. [PubMed: 7889296]
122. Schubert P, Kreutzberg GW. Cerebral protection by adenosine. *Acta Neurochir Suppl (Wien).* 1993; 57:80–8. [PubMed: 8380674]
123. Sweeney MI. Neuroprotective Effects of Adenosine in Cerebral Ischemia: Window of Opportunity. *Neuroscience & Biobehavioral Reviews.* 1997; 21(2):207–217. [PubMed: 9062944]
124. Cunha RA. Neuroprotection by adenosine in the brain: From A(1) receptor activation to A (2A) receptor blockade. *Purinergic Signal.* 2005; 1(2):111–34. [PubMed: 18404497]
125. Zhang J, Kaasik K, Blackburn MR, Lee CC. Constant darkness is a circadian metabolic signal in mammals. *Nature.* 2006; 439(7074):340–3. [PubMed: 16421573]
126. Zhang F, Wang S, Luo Y, Ji X, Nemoto EM, Chen J. When hypothermia meets hypotension and hyperglycemia: the diverse effects of adenosine 5'-monophosphate on cerebral ischemia in rats. *J Cereb Blood Flow Metab.* 2009; 29(5):1022–34. [PubMed: 19319149]
127. Zhang M, Li W, Niu G, Leak RK, Chen J, Zhang F. ATP induces mild hypothermia in rats but has a strikingly detrimental impact on focal cerebral ischemia. *J Cereb Blood Flow Metab.* 2012
128. Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. *Cancer Chemother Rep.* 1966; 50(4):219–44. [PubMed: 4957125]
129. Wang S, Duan Y, Su D, Li W, Tan J, Yang D, Wang W, Zhao Z, Wang X. Delta opioid peptide [D-Ala2, D-Leu5] enkephalin (DADLE) triggers postconditioning against transient forebrain ischemia. *European journal of pharmacology.* 2011; 658(2-3):140–4. [PubMed: 21349267]
130. Tao Z, Zhao Z, Lee CC. 5' - Adenosine monophosphate induced hypothermia reduces early stage myocardial ischemia/reperfusion injury in a mouse model. *Am J Transl Res.* 2011; 3(4):351–61. [PubMed: 21904655]
131. Hacke W, Kaste M, Bluhmki E, Brozman M, Davalos A, Guidetti D, Larrue V, Lees KR, Medeghri Z, Machnig T, Schneider D, von Kummer R, Wahlgren N, Toni D. Thrombolysis with

alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med.* 2008; 359(13):1317–29. [PubMed: 18815396]

132. Sandercock P, Wardlaw JM, Lindley RI, Dennis M, Cohen G, Murray G, Innes K, Venables G, Czlonkowska A, Kobayashi A, Ricci S, Murray V, Berge E, Slot KB, Hankey GJ, Correia M, Peeters A, Matz K, Lyrrer P, Gubitz G, Phillips SJ, Arauz A. The benefits and harms of intravenous thrombolysis with recombinant tissue plasminogen activator within 6 h of acute ischaemic stroke (the third international stroke trial [IST-3]): a randomised controlled trial. *Lancet.* 2012; 379(9834):2352–63. [PubMed: 22632908]

List of Abbreviations

ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
BBB	blood brain barrier
CB	cannabinoid
DA	Dopamine
DADLE	[D-Ala2, D-Leu5] enkephalin
DAR	dopamine receptor
H₂S	hydrogen sulfide
ICV	intracerebroventricular
MCAO	middle cerebral artery occlusion
NT	neurotension
NTS1	NT receptor type 1
POAH	preoptic anterior nucleus
ppm	parts per million
T₀AM	thyronamine
T₁AM	3-iodothyronamine
TAAR1	trace amine associated receptor 1
TH	thyroid hormone
TRPV1	Transient receptor potential vanilloid 1
WIN	[(4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl)-carbonyl)-6H-pyrrolo[3,2,1ij]quinolin-6-one]

Figure 1.

DADLE induces mild hypothermia in mice.

DADLE induced mild hypothermia in mice. DADLE was intraperitoneally injected and core body temperature was measured rectally using an Omega 450 AET Thermocouple Thermometer. n=5.

Figure 2.

DADLE-induced mild hypothermia protects brain against ischemic injury in mice.

DADLE was intraperitoneally injected in mice to induce hypothermia and transient focal ischemia was induced by MCAO for 60 minutes followed by 72 hours of reperfusion. (A) TTC staining showed that DADLE-induced hypothermia decreased infarct volume. $n=6$, $**p<0.01$ vs vehicle group. (B) DADLE-induced hypothermia also attenuated blood brain barrier (BBB) damage. Following MCAO and DADLE injection, mice were intravenously injected with Evan's blue (EB), brains were then collected for the measurement of EB content. $n=6$, $*p<0.05$ vs vehicle group.

Figure 3.

Isoflurane anesthesia induces hypothermia in mice and rats.

Mice (20.2 ± 6 g) and rats (285 ± 10 g) were anesthetized with 1% isoflurane in a mixture of 30% oxygen and 70% nitric oxide. Animals were maintained at room temperature (22°C) without any heating and core body temperature was continuously monitored via a rectal probe, $n=3$. Isoflurane caused hypothermia in both mice and rats, and the effect was more rapid in the former.

Table 1

Drug-induced hypothermia that shows neuroprotection against stroke

Drugs	Administration	Treatment time	Dosage	Animal models	Results	References
WIN 55212-2	IP	Pre- or post-ischemia	1 mg/kg	MCAO in rats	Protective	Nagayama, 1999
DADLE	IP	Pre-ischemia	4 mg/kg every 2 h	MCAO in rats	Protective	Borlongan, 2009
Rinvanil	IP	Immediately after MCAO	25 mg/kg	MCAO in mice	Protective	Muzzi, 2012
ABS-201	IP	Immediately after MCAO	2 mg/kg	MCAO in mice	Protective	Choi., 2012
T₁AM	IP	2 days before or 1 hour after ischemia	50 mg/kg	MCAO in mice	Protective	Doyle, 2007
Talipexole	IV	Immediately after MCAO	2 mg bolus plus 2 mg continuous infusion for 24 h	MCAO in rats	Protective	Johansen, 2003
H₂S	Inhalation	1 hour after ischemia	80 ppm	MCAO in rats	Protective	Joseph, 2012
Helium	Inhalation	Intra-ischemia or immediately post-ischemia	75 % in volume	MCAO in rats	Protective	David, 2009; Pan, 2007

DADLE, [D-Ala², D-Leu⁵] enkephalin ; H₂S, Hydrogen sulfide; IP, Intraperitoneal; IV, Intravenous; MCAO, middle cerebral artery occlusion; TIAM: 3-iodothyronamine; WIN, [(4,5-dihydro-2-methyl-4-(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrazolo[3,2,1-i]quinolin-6-one]