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, 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.

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 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 hypothemic 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’ hypothemic 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 hypothemic 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 presynaptic 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±0.4°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 hypothemic 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 hypothemic 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...
maintained at 37°C [53]. However, Iwata and colleagues attenuated ischemic neuronal death in brain slice cultures hypothermia. In support of this hypothesis, DADLE suggests that DADLE can protect in the absence of the first three hours after surgery [52]. These experiments temperature [50] or maintained body temperature at 37°C for loss in experiments that did not monitor post-surgery body ventricular injection of DADLE attenuated CA1 neuronal reperfusion. In the case of global ischemia, intracerebral is a critical time that encompasses ischemia and early temperature shortly after the surgery was not reported. This temperature during the surgery [48]. However, body temperature 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 (Fig. 1). DADLE-induced hypothermia was linked with protection of the brain against ischemic injury, as indicated by decreased infarct volumes (Fig. 2A) and attenuated BBB disruption (Fig. 2B).

![Fig. (1)](Image)

**Fig. (1).** DADLE induces mild hypothermia in mice. DADLE was intraperitoneally injected and core body temperature was measured rectally using an Omega 450 AET Thermocouple Thermometer. n=5.

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, cynomologus monkeys and young cattle [59]. Rinvanil is a synthetic TRPV1 agonist that possesses ultra-potent activating effects [60]. It is also

### Table 1. Drug-Induced Hypothermia that Shows Neuroprotection Against Stroke

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

DADLE, [D-Ala2, D-Leu5] enkephalin; H2S, Hydrogen sulfide; IP, Intraperitoneal; IV, Intravenous; MCAO, middle cerebral artery occlusion; T1AM, 3-iodothyronamine; WIN, (4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrrolo[3,2,1ij]quinolin-6-one].

Increasing attention has been focused 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].

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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 K11-19, 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

Fig. (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.


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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 derivates 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 hypothymia. 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 D4). 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-propionylapomorphine (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 α2-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 α2-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-DAP (92, 93). Clozapine and 7-OH-DAP 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 (Fig. 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.

Fig. (3). Isoflurane anesthesia induces hypothermia in mice and rats. Mice (20.2±6 g) and rats (285±10g) 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.

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 Fig. (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.

**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  
T9AM = 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]

**CONFLICT OF INTEREST**

The authors confirm that this article content has no conflict of interest.

**ACKNOWLEDGEMENTS**

This work was supported by grants from the National Institutes of Health/NINDS (NS36736, NS43802 and NS45048 to J.C.), the American Heart Association (10SDG2560122 to F.Z.), and grants from the National Natural Science Foundation of China (81020108021 and 81171149 to Y.G.; 81150110494 to P.V.; and 81228008 to J.C.).

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