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Review article

Hibernation-like neuroprotection in stroke by attenuating brain metabolic dysfunction

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ABSTRACT

Many mammalian species naturally undergo hibernation, a process that is associated with drastic changes in metabolism and systemic physiology. Their ability to retain an undamaged central nervous system during severely reduced cerebral blood flow has been studied for possible therapeutic application in human ischemic stroke. By inducing a less extreme 'hibernation-like' state, it has been hypothesized that similar neuroprotective effects reduce ischemia-mediated tissue damage in stroke patients. This manuscript includes reviews and evaluations of: (1) true hibernation, (2) hibernation-like state and its neuroprotective characteristics, (3) the preclinical and clinical methods for induction of artificial hibernation (i.e., therapeutic hypothermia, phenothiazine drugs, and ethanol), and (4) the mechanisms by which cerebral ischemia leads to tissue damage and how the above-mentioned induction methods function to inhibit those processes.

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Abbreviations: AQP, aquaporin; AQP-4, aquaporin 4; AQP-9, aquaporin 9; ASIC, acid sensing ion channel; BBB, blood-brain barrier; ETC, electron transport chain; GLUT, glucose transporter; GLUT1, glucose transporter 1; GLUT3, glucose transporter 3; HIE, hypoxic-ischemic encephalopathy; ICSI, intracarotid cold saline infusion; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MCA, middle cerebral artery; MCAO, middle cerebral artery occlusion; MMP, matrix metallopeptidase; MMP-2, matrix metallopeptidase 2; MMP-9, matrix metallopeptidase 9; NADPH, nicotinamide adenine dinucleotide phosphate; NMDA, *N*-methyl-D-aspartate; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOX, nicotinamide adenine dinucleotide phosphate oxidase; PFK, phosphofructokinase; ROS, reactive oxygen species; SBC, selective brain cooling; t-PA, tissue plasminogen activator; TNF-α, tumor necrosis factor alpha; TRP, transient receptor potential.

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1. Introduction

1.1. What is hibernation?

Hibernation is an altered physiological state seen in a number of mammals. It is a survival tool utilized by a variety of species to endure the harsh seasons when resources are scarce. Ordinarily, mammals can maintain a constant body temperature of about 37 °C regardless of ambient temperatures due to their high metabolic rate generating heat in their bodies (Geiser, 2013; Heldmaier et al., 2004). Animals that are capable of heat generation and maintenance are called tachymetabolic endotherms. However, heat generation comes at a large cost. An immense amount of energy is required for endotherms to maintain their ideal body temperature; therefore, food is crucial. There are periods of time when tachymetabolic endotherms undergo hypometabolism. This hypometabolism, also termed torpor, can be separated into two categories in mammals. The first, shallow daily torpor, follows circadian rhythm of activity and rest (Geiser, 2013; Geiser and Ruf, 1995; Ruf and Geiser, 2014). A 0.5–2 °C decrease in body temperature is observed during resting phase in mammals and this is associated with a 20% decrease in metabolic rate (Geiser, 2013; Heldmaier et al., 2004; Storey and Storey, 2010). The second category, hibernation, is characterized by a much larger decrease in both body temperature and metabolic rate, lasting several days or weeks (Drew et al., 2009; Geiser and Ruf, 1995; Ruf and Geiser, 2014; Storey and Storey, 2010). During hibernation, body temperature can be as low as −2.9 °C; spontaneous arousals occur regularly and mammals return to active core temperature of about 37 °C from their minimal body temperature (Carey et al., 2003; Drew et al., 2007). Hibernators rely heavily on metabolic inhibition, such that their metabolic rate during prolonged torpor can be reduced to as low as 1% of active state basal metabolic rate (Carey et al., 2003; Drew et al., 2007; Geiser, 2004; Storey and Storey, 2010). Decreased rates of gas exchange are also observed in hibernators, accompanied by severely depressed ventilation rates (Milsom and Jackson, 2011). They may only breathe episodically or even exclusively by passive diffusion through their skin and airways. This is achieved partially through the low body temperature increasing hemoglobin's affinity for oxygen. Additionally, hibernators experience significantly decreased cardiovascular parameters, such as heart rate and blood pressure (Horwitz et al., 2013).

Hibernation is an incredible phenomenon that still leaves us with many unanswered questions. In addition to surviving immense temperature drops and metabolic rate decreases, hibernators in deep torpor also withstand drastic fluctuations in cerebral blood flow without causing brain damage (Dave et al., 2012; Drew et al., 2009; Zhou et al., 2001). Cerebral ischemia, a common result of cerebral trauma, stroke, and heart failure, can lead to brain damage within seconds and permanent brain dysfunction or death within minutes if not treated in time (Dave et al., 2012; Frerichs et al., 1994). While humans and other mammals suffer from cerebral ischemia, animals that hibernate tolerate the extensive decrease in cerebral blood supply without experiencing devastating damages to the brain. In this systematic review, we aim to provide information on the background of hibernation as a natural neuroprotective mechanism for cerebral ischemia, summarize possible methods for induction of a hibernation-like state, and discuss the mechanisms by which they are thought to be neuroprotective.

1.2. Hibernation as a natural neuroprotectant

Hibernation is a natural survival mechanism during times of food shortage and is characterized by drastic decreases in both body temperature and metabolic rate (Dave et al., 2012; Drew et al., 2009, 2007, 2001). Animals typically enter a deep torpor state with depressed body temperature and metabolism; intermittently, animals wake and return to their euthermic body temperature by endogenous heat production for about one day before returning to deep torpor. This cycle of deep torpor and wake is called a hibernation bout (Geiser, 2004; Geiser and Ruf, 1995; Heldmaier et al., 2004; Ruf and Geiser, 2014). Hibernators actively regulate the characteristics of deep torpor and the rhythm of hibernation bouts (Drew et al., 2007).

Studies suggest that hibernation is neuroprotective. In a paper by Zhou et al. (2001), histological slides of traumatic brain injury in both hibernating and non-hibernating arctic ground squirrels showed that there was less cell death and phagocytosis in the slides of the hibernating tissue. In another study, Frerichs (1999) demonstrated that during hibernation, the weighted average cerebral blood flow dropped to less than 10% of active state levels and that glucose utilization was decreased by approximately 98%. Along with core body temperature, the central nervous system also has a significant decrease in temperature during hibernation. This temperature depression has even been associated with functional changes in the hippocampus, most notably, the addition of control over hibernation bout (Arant et al., 2011). Furthermore, hippocampal slices from hibernating animals showed a higher tolerance for in vitro hypoxia/aglycemia (Frerichs, 1999), presumably due to the down regulation of cellular metabolism in hibernating brain tissue (Carey et al., 2003).

Another area of research is exploring a number of methods to induce a 'hibernation-like' state in non-hibernators and studying the neuroprotective effects of the altered physiology. Several physiological changes during hibernation are suggested as potential neuroprotective mechanisms, such as hypothermia, metabolism depression, inflammatory response suppression, and increased resistance to oxidative stress. These have been artificially

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induced and associated with neuroprotection in preclinical and clinical studies, thereby implicating a possible therapeutic role of artificial hibernation in ischemic stroke.

2. Methods for induction of hibernation-like state

2.1. Artificial hibernation induction

In order for hibernation-like therapy to be relevant in the clinical treatment of stroke, there must be a method that has high efficacy, minimized adverse effects, and realistic economic feasibility. There are several proposed methods currently being investigated, and some of them are even being studied in combination with one another. A number of these possible treatment options have support from clinical studies, but a majority of current experiments studying their respective roles in hibernation and neuroprotection are largely preclinical.

The remainder of this section will review some of the current methods being investigated for their abilities to induce neuroprotective hibernation-like states. Although additional studies are necessary for all of these approaches before they can be considered for use in the clinical treatment of stroke, the present body of supporting data emphasizes the significance of conducting those studies.

2.2. Therapeutic hypothermia

2.2.1. History and current clinical utility

Reports of the therapeutic effects of hypothermia have been documented throughout history, and it was even used clinically as a neuroprotectant in the 1960s. These patients, however, had serious systemic complications because the hypothermia was as low as 28 °C and poorly managed. Interest subsequently declined, and it was not popularized again until neuroprotective effects were found in even mild hypothermic temperatures (Karnatovskaia et al., 2014; Yenari and Hemmen, 2010). Hypothermia-based research for brain ischemia generally aims for a target temperature within the range of 28–36 °C in the ischemic tissue. This range is commonly divided for the distinction between mild (36–34 °C) and moderate (28–34 °C) hypothermia, although these ranges can vary slightly throughout the literature (Schwab et al., 1998). Hypothermic temperatures are known to have depressive effects on neuronal metabolic activity (Dehaes et al., 2014; Polderman, 2009; Yenari and Han, 2012). This is thought to be neuroprotective because acute cerebral ischemia promotes cellular pathways affecting excitotoxicity, inflammation, free radical production, apoptosis, cerebral blood flow, and blood-brain barrier integrity (Dehaes et al., 2014; Jiang et al., 2014; Yenari and Han, 2012). Hypothermia induced soon after stroke is thought to inhibit ischemia-mediated tissue damage, but when applied later, it is thought to primarily control cerebral edema and intracranial pressure (Dirnagl et al., 1999; Lyden et al., 2014). The therapeutic objective is preventing physiological and morphological damage to the affected tissue, while minimizing adverse effects and cost.

2.2.2. Preclinical findings

There are now a number of preclinical studies to support the robust neuroprotective effects of therapeutic hypothermia in brain ischemia. One of the studies brought it back into the spotlight by demonstrating that mild (34 °C) hypothermia in rats significantly reduced ischemia-mediated histopathological changes in neurons (Busto et al., 1987). Additionally, reductions in infarct volumes of up to 90% were observed in MCAO rodent models treated with mild hypothermia (Burk et al., 2008; Florian et al., 2008; Wang et al., 2010). Inflammation, edema, and thus, intracranial pressure are also reduced by hypothermic treatment in rats (Darwazeh and Yan, 2013; Gu et al., 2014; Maier et al., 1998; Murtha et al., 2014; Song and Lyden, 2012; Yenari and Han, 2012; Yuan et al., 2014). Animal models and *in vitro* models showed that mild and moderate hypothermia both reversibly reduce O_2 consumption, glycolysis, and electrophysiological activity (Astrup et al., 1981; Zager and Ames, 1988), which are some of the defining characteristics of hibernation, and thus, a hibernation-like state (Zhou et al., 2001). It should be noted that true hibernators are capable of inducing temperatures around 0 °C, but this level of hypothermia can actually increase tissue damage in non-hibernating species (Drew et al., 2001; Wass and Lanier, 1996). Instead, an optimal depth of hypothermia for rats was determined to be 33 °C in transient (2-h) MCAO (Maier et al., 1998). These preclinical studies of *in vitro* and *in vivo* therapeutic hypothermia have provided enough promising evidence to warrant clinical studies.

2.2.3. Clinical cooling methods

In many previous cases of clinical hypothermia, patients have undergone sedation, neuromuscular blockage, and mechanical ventilation. More recent developments, however, allow patients to remain awake during cooling (Kammersgaard et al., 2000; Lyden et al., 2014; van der Worp et al., 2014). The methods in which hypothermia is induced clinically are quite variable. The most common of these has been whole-body surface cooling, which utilizes cooling blankets and ice packs. Clinical sedatives and paralytics are typically used to prevent shivering (Lyden et al., 2014), but it has also been achieved in awake patients (Kammersgaard et al., 2000). Many studies have used surface cooling in the past because it is the simplest and most cost-effective option (Feigin et al., 2003). Some limitations include long cooling times (Kammersgaard et al., 2000; Krieger et al., 2001) and risk of rebound hyperthermia (>38 °C) after rewarming (Felberg et al., 2001). Another popular classification of cooling methods is whole-body endovascular cooling, which is done by inserting a catheter into a large vein (e.g., inferior vena cava) and infusing cold saline into the patient. The main advantages of endovascular cooling over surface cooling are shorter cooling times, better temperature feedback and regulation, and the ability to keep patients awake (De Georgia et al., 2004; Delhaye et al., 2012; Georgiadis et al., 2001; Guluma et al., 2006; Lyden et al., 2005, 2014; Ovesen et al., 2013; Sherman and Wang, 2014).

In addition to surface and endovascular cooling procedures, there are alternative cooling methods that exist or are in development. Selective brain cooling (SBC) methods aim to reduce cooling times by focusing temperature reduction on just the brain. Some of the most common of these are cooling caps and helmets, but there are more in development such as intracarotid cold saline infusion (Choi et al., 2010; Ji et al., 2012; Konstas et al., 2007; Neimark et al., 2008). Further research is needed to support the efficacy of these SBC methods.

2.2.4. Clinical findings

To date, the clinical efficacy of therapeutic hypothermia in stroke has only been reported in small pilot studies (Jeon et al., 2014; Krieger et al., 2001; van der Worp et al., 2010; Zgavc et al., 2011). The outcomes have been mixed, leaving its clinical utility inconclusive. This discrepancy among the findings can be partially attributed to the diversity in methodologies and experimental designs between studies. Various surface and endovascular cooling procedures have been used most commonly, and some use a combination of the two. Additionally, target temperatures have ranged between mild and moderate hypothermia, and treatment durations have ranged from hours to as long as 22 days (Milhaud et al., 2005; Mourand et al., 2012). More limitations include low numbers of participants and different inclusion criteria.

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There is clearly a need for large-scale clinical trials before therapeutic hypothermia can be considered clinically effective for the treatment of ischemic stroke. Two of these are currently being conducted. EuroHYP-1 (van der Worp et al., 2014) is an international phase III clinical trial of therapeutic hypothermia. The primary measure is three-month functional outcome in 1,500 patients with acute ischemic stroke. The patients will either receive optimal medical treatment or therapeutic hypothermia in addition to optimal medical treatment. The target temperature is between 34 °C and 35 °C, achieved by either surface or endovascular cooling depending on the treatment center. Hypothermia treatment is planned to begin less than 6 h after stroke onset and maintained for 24 h. The second clinical trial is the Intravascular Cooling in the Treatment of Stroke 2/3 (ICTuS 2/3) trial (Lyden et al., 2014). This study includes a phase II component ($n = 400$) and a subsequent phase III component ($n = 1200$) if phase II meets appropriate outcomes. Hypothermia will be induced only by endovascular cooling within 2 h of thrombolysis. The target temperature of 33 °C is planned to be maintained for 24 h.

2.2.5. Limitations of therapeutic hypothermia

The clinical utility of hypothermia-based stroke therapy is limited by multiple factors. One of these limitations is its inhibitory interaction with recombinant tissue plasminogen activator (t-PA). The efficacy of t-PA is temperature-dependent, and thus, temperatures below 37 °C reduce the amount of clot lysis by t-PA (Yenari et al., 1995). This is an important consideration because administration of intravenous (IV) t-PA within 3 h of symptom onset is currently the only the Food and Drug Administration (FDA)-approved treatment for ischemic stroke. Another complication of therapeutic hypothermia is the potential to mask the detection of fever resulting from infection (Jeon et al., 2014). Some of the known adverse effects associated with therapeutic hypothermia include cardiac arrhythmia, hemodynamic instability, bleeding, electrolyte shift, shivering, and pneumonia (Chen et al., 2009; Liu and Chen, 2012). The robust neuroprotective effects of mild and moderate hypothermia are strongly supported by preclinical data, but clinical data has been inconclusive. Future studies, including the two ongoing large clinical trials EuroHYP-1 and ICTuS 2/3, will provide important information regarding the safety and efficacy of therapeutic hypothermia in the treatment of stroke.

2.3. Phenothiazine drugs

2.3.1. History and current clinical utility

The most significant event for the phenothiazine class of drugs dates back to Paul Charpentier's original synthesis of chlorpromazine on December 11, 1951. It was first explored as a supplement to surgical anesthesia, but research quickly shifted to psychiatry after reports of chlorpromazine's calming effect on highly agitated psychiatric patients. Less than one year following its synthesis, chlorpromazine was released as a prescription drug in France, and in the subsequent three years, countries across the world did the same (Ban, 2007). The discovery of chlorpromazine not only initiated the development all subsequent first-generation antipsychotics, it also revolutionized the treatment of psychiatric disorders such as schizophrenia. To this day, chlorpromazine continues to be one of the few antipsychotics listed as an essential drug by the World Health Organization (2014). Its utility in the treatment of schizophrenia is particularly notable because it helped define the heterogeneity of the disease (Ban, 1987, 2007), and it is still one of the most common and effective treatments to date (Adams et al., 2014; Samara et al., 2014).

The role of phenothiazine drugs in creating a hibernation-like state is not a new concept. In fact, the French surgeon originally studying chlorpromazine in 1952 wanted to investigate its body-cooling and sedative effects to induce artificial hibernation as a form of surgical anesthesia (Ban, 2007; Laborit et al., 1952). Phenothiazine-induced neuroprotection during brain ischemia (Poteet et al., 2012; Yu et al., 1992), on the other hand, is a more recent direction of artificial hibernation research. Chlorpromazine and promethazine are the two most studied phenothiazine drugs in hibernation-like stroke therapy, and will therefore be the main topics of this section.

2.3.2. Chlorpromazine

As stated above, chlorpromazine immediately became a point of interest for artificial hibernation research when it became available. There were actually many scientists that continued to study this topic through the years of popular psychiatric research. While not directly focused on neuroprotection, they reported findings that are still important to consider for achieving a hibernation-like state. For instance, in 1954, a study was published that investigated the processes by which chlorpromazine facilitates cooling during hypothermia induction. Their findings suggest that the drug promotes vasodilation while simultaneously inhibiting shivering (Dundee et al., 1954). By acting in opposition to two innate thermoregulatory mechanisms, chlorpromazine allows for a smoother and more rapid fall in temperature. Higher doses (10 mg/kg) have even been reported to independently induce hypothermia in rats (Yehuda and Carasso, 1982).

This property of chlorpromazine to supplement body temperature lowering procedures has significant implications for hibernation-like neuroprotection during stroke. Although therapeutic hypothermia has powerful neuroprotective effects (Yenari and Han, 2012), its clinical application is limited in treating focal ischemic stroke. This is partially due to lengthy cooling times, which directly conflict with the time-dependent prognosis of stroke treatment (Chen et al., 2009; Lee et al., 2009). Thus, there is a need for future studies to address whether chlorpromazine could reduce cooling durations, making therapeutic hypothermia a more feasible clinical treatment for brain ischemia. This hypothesis is rather new in the area of hibernation-like neuroprotection, but the combination of chlorpromazine and hypothermia has been effective in the treatment of both renal (Jayachandran et al., 1985) and myocardial (Thomas et al., 1983) ischemia.

Chlorpromazine has been shown to independently protect against ischemic insult in a number of tissue types, such as those of the liver (Chien et al., 1977), kidney (Jayachandran et al., 1985), spinal cord (Sadanaga and Ohnishi, 1989; Sader et al., 2002; Zivin et al., 1989), and brain (Bastianetto et al., 2006; Li et al., 2014; Liu et al., 2015; Zivin et al., 1989). A recent study of its neuroprotection in cerebral ischemia was conducted using MCAO rat models, and two relevant findings were reported (Li et al., 2014). First, after intraperitoneally injecting concentrations ranging from 0.5 mg/kg to 20 mg/kg at the onset of MCAO, cerebral infarct volumes were lowered in every treatment group, with the greatest reduction from 10 mg/kg. Additionally, chlorpromazine treatment (10 mg/kg) at 1 hr after the onset of MCAO still significantly reduced infarct volumes. A different study demonstrated that a chlorpromazine dose administered over the course of two injections was more beneficial than injecting it all at once in spinal cord ischemia (Sader et al., 2002). These reports demonstrate the dose- and time-dependent efficacy of chlorpromazine in the treatment of neuronal ischemia. Further research is needed to determine an optimum dose and establish a reliable therapeutic window.

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2.3.3. Promethazine

The widespread clinical use of chlorpromazine has made phenothiazine-derivative drugs most commonly associated with antipsychotic effects. However, other members of this drug class possess additional important properties, and promethazine is no exception. In fact, it was first popularized as a potent antihistamine in 1946, five years before the original synthesis of chlorpromazine (Kopera and Armitage, 1954). As more effects of promethazine were subsequently reported, studies began to explore its potential application in other areas of medicine. For instance, early research of its utility in anesthesia (Burn, 1954; Hopkin et al., 1957; Laborit and Huguenard, 1951; Pitcher, 1959) has translated into promethazine currently being one of the most common clinical treatments for nausea and vomiting, even in pregnant (Furyk et al., 2014; Furyk, 2014) and postoperative (Deitrick et al., 2014; Hasaniya et al., 2001) patients.

Even though promethazine is already FDA-approved and mass-produced, it continues to be assessed for novel therapeutic uses. The involvement of promethazine in artificial hibernation has been researched for over 60 years (Laborit and Huguenard, 1951), and it has been shown to promote specific physiological processes observed in hibernation-like states. More recent studies of promethazine and other compounds (e.g., the so-called "lytic cocktail": promethazine, chlorpromazine, and pethidine) have attempted to elicit neuroprotection in cerebral ischemia by utilizing previously documented hibernation-like effects on the brain. Hypothermia, for example, can be independently induced by promethazine (Burn, 1954), but reduced cerebral blood flow and oxygen consumption require use of the lytic cocktail (Berntman and Carlsson, 1978).

The efficacy of promethazine-based neuroprotection is still not completely established, and this is partially due to the multitude of possibilities leading to neuronal cell damage. Additionally, it was not even classified as a neuroprotectant until the 2004 publication of the NINDS screening program of over 1000 FDA-approved drugs and bioactive compounds. After they highlighted promethazine's ability to provide neuroprotection within clinically reasonable doses (Stavrovskaya et al., 2004), it became the topic of many subsequent studies on neurodegenerative conditions. With regards to stroke, promethazine has been shown to provide notable attenuation of ischemia-induced neurotoxicity both *in vitro* and *in vivo*. When primary cerebrocortical neuron cultures were subjected to an oxygen-glucose deprivation chamber, promethazine significantly reduced cell death in a dose-dependent manner (Narayanan et al., 2003; Stavrovskaya et al., 2004). Promethazine-mediated neuroprotection was also demonstrated in rats that had undergone transient 2-h MCA occlusions. Two 10 mg/kg intraperitoneal injections (one at 1 h before MCAO and the other 12 h later) significantly reduced both infarct volumes and improved neurological scores (Narayanan et al., 2003; Stavrovskaya et al., 2004). Furthermore, the neuroprotective properties of promethazine are not isolated to just ischemic brain injury. Rats have also exhibited reduced neurotoxicity in models of neurodegenerative disorders such as Huntington's disease (Cleren et al., 2010) and Parkinson's disease (Cleren et al., 2005).

2.3.4. Limitations and summary

The phenothiazine class of neuroleptic drugs has been used worldwide for over 60 years. Chlorpromazine and promethazine, in particular, are very common pharmaceutical agents for their applications in schizophrenia, anesthesia, nausea, and more. There are significant advantages in exploring new therapeutic uses for drugs already approved by the FDA. One of the most important of these is the preexisting data supporting drug safety. When administered at the appropriate doses, both chlorpromazine and promethazine are considered to be safe treatments options for most patients, even those who are pregnant (Einarson and Boskovic, 2009; Lacasse et al., 2006). However, adverse effects still exist, and some of these are reduced blood pressure, sedation, dizziness, weight gain, and increased risk for acute movement disorders (Adams et al., 2014). Additionally, the FDA has implemented a "boxed warning" contraindicating use of promethazine in children less than two years of age (Starke et al., 2005). This information, when studying new uses for established drugs, allows them to be more easily and safely used in clinical trials. There is also preexisting data on drug mechanisms of action, which is helpful in developing new therapies.

Administration of phenothiazines for inducing a state of hibernation-like physiology has been studied for almost as long as they have been available. This concept was originally explored for use in anesthesia, but it was not until decades later that its application in stroke-related neuroprotection was considered. Chlorpromazine and promethazine have both been shown to independently produce certain parts of artificial hibernation, such as reductions in body temperature and cerebral energy metabolism (Burn, 1954; Larsson, 1961). These phenothiazines have generated neuroprotection in ischemic conditions both *in vitro* and *in vivo*. These data, when combined with the understanding of their physiological effects on other body systems, suggest that their neuroprotective properties result from both cellular and systemic changes. To reach the level of hibernation-like conditions, however, some of these studies used doses too high for clinical translation. In response, a recent study demonstrated that clinically realistic doses of chlorpromazine and promethazine (1 mg/kg each), injected in combination, improved the neuroprotective effects of mild hypothermia therapy (Liu et al., 2015). Chlorpromazine has also enhanced hypothermic protection of other ischemic tissues (Jayachandran et al., 1985; Thomas et al., 1983). Further research is necessary to determine the exact role of phenothiazines in treating stroke with a hibernation-like state, but the positive results from previous studies warrants these efforts.

2.4. Ethanol

2.4.1. Brief introduction

Ethanol consumption is common in many parts of the world, and it has been studied extensively for its effects on disease processes. The results have been mixed, showing dose-dependent increases and decreases in risk for a number of diseases. Some diseases that have shown significantly increased risk from alcohol consumption are liver cirrhosis, hypertension, and atrial fibrillation (Corrao et al., 2002; Frost and Vestergaard, 2004). On the other hand, a number of studies have associated light-to-moderate alcohol consumption with lower risk for cardiovascular disease (Gordon and Kannel, 1983; Kitamura et al., 1998) and ischemic stroke (Iso et al., 2004; Zhang et al., 2014). Preclinical studies have demonstrated the neuroprotective properties of ethanol, but as of now, its clinical efficacy and safety as a therapeutic agent in ischemic stroke have yet to be determined.

2.4.2. Preclinical findings

Some studies have shown preconditioning with ethanol to be neuroprotective against future ischemic damage (Phillis et al., 1998; Wang et al., 2007). However, this does not support the development of a therapy for patients who have already presented with stroke. Thus, studies would be more relevant to stroke therapy if they illustrate neuroprotection when ethanol is administered following the onset of acute ischemic stroke. A 2-h MCAO rat model treated with 1.5 g/kg ethanol showed significantly reduced infarct volumes and behavioral dysfunction up to 4 h after the onset of occlusion (Wang et al., 2012). Another study of the same animal model reported significantly reduced neuronal cell

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death and apoptotic proteins when 1.5 g/kg ethanol was injected 2 h after the onset of MCAO (Fu et al., 2013). In an investigation of mechanism and efficacy, ethanol (1.5 g/kg) reduced brain glucose metabolism and reactive oxygen species (ROS), which were attributed to its neuroprotective mechanism (Kochanski et al., 2013). An intraperitoneal injection of 1.5 g/kg ethanol appears to be an optimal dose for preclinical neuroprotection, and it leads to rat blood alcohol levels equivalent to the legal driving limit (Fu et al., 2013). Other preclinical animal studies have supported the enhanced neuroprotective effects of ethanol when combined with therapies such as normobaric oxygenation (Geng et al., 2015a; Geng et al., 2013a; Geng et al., 2013b; Geng et al., 2015b) and low-dose caffeine (caffeinol) (Aronowski et al., 2003; Belayev et al., 2004; Martin-Schild et al., 2009; Piriyawat et al., 2003; Strong et al., 2000; Zhao et al., 2005).

2.4.3. Clinical findings

Low and moderate alcohol intake have been defined in clinical studies as less than 15 g/day and 15–30 g/day, respectively (Zhang et al., 2014). A recent meta-analysis found that low alcohol intake was associated with risk reductions for total stroke, ischemic stroke, and stroke mortality (Reynolds et al., 2003). No significant association was determined between low dose alcohol intake and risk of hemorrhagic stroke, but a different meta-analysis did find an increased risk (Corrao et al., 1999). In support of possible neuroprotective mechanisms, a human study of regional brain metabolism reported that ethanol induced a hibernation-like change in brain glucose metabolism. These metabolic changes were similar to those induced by benzodiazepine anesthetics (Wang et al., 2000). Another clinical study demonstrated a dose-dependent effect of ethanol on brain glucose metabolism. A dose of 0.25 g/kg reduced whole-brain metabolism by 10%, while a dose of 0.5 g/kg elicited a 23% reduction (Volkow et al., 2006). A 0.5 g/kg dose of ethanol also yielded a reduction in whole-brain metabolism greater than that of lorazepam and induced less sedation and cognitive impairment. The same study found that the 0.5 g/kg dose of ethanol also reduced whole-brain metabolism by 30%, similar to reductions by anesthetics that induce unconsciousness (Volkow et al., 1995).

There have been a very limited number of clinical trials investigating the therapeutic effects of ethanol in ischemic stroke. A small pilot study on the combination of caffeine and ethanol (caffeinol) was unable to demonstrate neuroprotection, but supported the safety of its administration at certain doses (Piriyawat et al., 2003). Another small pilot study of caffeinol combined with 24 h mild hypothermia was unable to demonstrate neuroprotection, but showed that the administration of the combination was feasible (Martin-Schild et al., 2009). Before ethanol can be determined as a safe and effective treatment for ischemic stroke, there is a need for more clinical trials with larger patient populations.

2.5. Additional methods for induction of hibernation-like state

Isoflurane is also under investigation as a possible method for inducing artificial hibernation. This anesthetic is administered via inhalation, and has been associated with depressed metabolism (Bosel et al., 2012), which is also found in artificial hibernation. Subsequently, it has been studied as a possible neuroprotective agent in ischemic stroke. Both pretreatment and post-treatment with isoflurane have been implicated in neuroprotection of preclinical stroke models. Long-term neurological outcomes in mice were better in those pretreated with isoflurane (McAuliffe et al., 2007). Neuroprotection was also found in rat stroke models post-conditioned with isoflurane (Segal et al., 2012). However, using this volatile anesthetic in ischemic brain injury has some associated complications, making its clinical translation difficult (Bosel et al., 2012). Further studies on its safety and efficacy compared to pre-existing treatment options are necessary before the start of clinical trials.

Another possible method of inducing a hibernation-like state is ischemic preconditioning. This is the process by which a prior mild ischemic event serves to epigenetically alter the affected tissue, causing it to express new protective mechanisms upon a subsequent ischemic event. These new phenotypes are the result of changes in activator and repressor proteins for transcription, along with DNA methylation (Thompson et al., 2013). Some of the newly expressed neuroprotective mechanisms are similar to those found in true hibernation and a hibernation-like state, which include tolerance to long-term decreases in cerebral blood flow, oxygen delivery, and glucose supply (Stenzel-Poore et al., 2003). Ischemic preconditioning is still being understood at the preclinical stage, requiring a great deal of research to be done before it can be clinically investigated as a treatment for ischemic stroke.

3. Application of hibernation-like treatment in the setting of ischemia

3.1. Ischemia, the primary phase

Occlusion of cerebral arteries causes either a transient or permanent decrease in oxygen perfusion to brain tissue. This hypoxic-ischemic encephalopathy (HIE) is a dynamic process that can be divided into two main phases: a primary phase involving the HIE event and a latent phase following reperfusion to the damaged area (Drury et al., 2010). In the primary phase, the lack of oxygen leads to the release of excitatory neurotransmitters and large increases of intracellular calcium leading quickly to necrosis. The latent phase is slower, occurring anywhere from hours to days after the primary insult, with neuronal death due to a secondary energy failure that ultimately leads the controlled cell death mechanism of apoptosis rather than the necrosis seen in the primary phase. Before discussing all the different ways through which hypothermia plays a neuroprotective role by inducing a hibernation-like state, the underlying mechanisms of ischemia need to be talked about. During the primary phase there is a large reduction in oxygen and glucose to neural tissue that leads to many simultaneous events, quickly resulting in necrotic cell death (Broughton et al., 2009). The neurons and glia of this affected area, the ischemic core, are unable to maintain their transmembrane ion gradients because of their inability to produce ATP at an appropriate rate (Posada-Duque et al., 2014). The disruption of ion channel gradient is due to glutamate channels, purinergic receptors, pannexin channels, transient receptor potential (TRP) channels, and acid sensing ion channels (ASIC).

An uncontrolled release of presynaptic glutamate causes an over-stimulation of its postsynaptic *N*-methyl-D-aspartate (NMDA) receptors. The opening of NMDA receptors leads to opening of Na^+ and Ca^{2+} channels, allowing excessive influx and membrane depolarization (Weilinger et al., 2013). This membrane depolarization is further promoted by the reduced function of Na^+/K^+ ATPase on the plasma membrane of the neuron. Function is reduced because of the decreased ability of mitochondria to produce ATP with low oxygen levels. The depolarization of the membrane opens voltage gated Na^+ and Ca^{2+} channels causing further depolarization. A lack of energy results in a less than optimal Ca^{2+}-ATPase on the plasma membrane that cannot keep up with the Ca^{2+} influx. This overload of Ca^{2+} alters intracellular processes by a variety of mechanisms. In mitochondria, the negative membrane potential is the driving force for Ca^{2+} to be taken up from the cytoplasm via an electrophoretic uniporter. The high Ca^{2+} concentration within mitochondria inhibits oxidative

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phosphorylation, reducing ATP synthesis. This altered rate of ATP synthesis results in less energy for energy-dependent Ca^{2+} pumps used to remove Ca^{2+} and causes a positive feedback loop to occur. Increased cytoplasmic Ca^{2+} leads to reduced mitochondrial function, leading to reduced ATP synthesis. This subsequently leads to less removal of the excess cytoplasmic Ca^{2+}, which then accumulates in mitochondria and damages them by increasing their membrane permeability. The overall effect of cation entry through the plasma membrane is a secondary influx of Cl^- and H_2O leading to cellular swelling and necrosis (Won et al., 2002).

Glucose metabolism also plays an important role in the primary phase. After the HIE, reduced intracellular oxygen concentration shifts the energy production from aerobic ETC to anaerobic glycolysis to compensate for ATP loss. Hyperglycolysis is not nearly as efficient as aerobic metabolism and cannot sustain the energy demands of the cell. As a result of the increased production of lactic acid via glycolysis, there is a rise in H^+ concentration. This proton concentration then reaches levels detrimental to the cell because there is no blood flow to the ischemic area to remove the acidic byproducts of glycolysis. Acidosis, along with the lack of oxygen to act as the final electron acceptor of the ETC, promote even more glycolysis, further acidifying the cytosol (Harada et al., 2012). The acidic intracellular environment affects cellular processes through three mechanisms. Free protons in cytosol go through a series of H^+-dependent reactions that increase ROS. The high acid content also activates endonucleases that fragment DNA. Lastly, it activates signal transduction pathways that alter protein synthesis (Siesjö et al., 1996). Reperfusion of cerebral blood flow and restoration of glucose can actually mediate more damage, which is discussed later.

3.2. Reperfusion, the latent phase

The latent phase follows reperfusion to the peri-infarct zone, an area called the penumbra. (Drury et al., 2010). Many of these neurons die as a secondary deterioration event, but they have the capability of surviving. Therefore, it is seen as a window of opportunity for protection from further damage, given an effective intervention. One of the major issues following an HIE is that many neurons of the penumbra die as a result of reperfusion to the area. The largest influence on the rate of this secondary neuronal death is due to mitochondrial failure (Drury et al., 2010). Injury occurs through the production of free radicals. Reperfusion to the ischemic neurons creates a transient burst in the ROS production by mitochondria due to the large increase in oxygen availability and oxidative phosphorylation. These ROS have the same actions as they did during the primary phase, oxidation of lipids of plasma and mitochondrial membranes, DNA, and proteins. As blood flow returns to the area following an HIE, edema is created at the site due to inflammatory mediators entering the newly created vessels bringing cells of the immune system to remove the necrotic tissue. Free nucleotides in the interstitial fluid volume cause an up-regulation of phagocytosis by innate cells of the immune system that will remove necrotic tissue but also lead to more injury in the reperfused area. The blood may also carry proteins toxic to the surviving neurons that influence apoptosis such as TNF-α and Fas. The physical pressure by the cerebral blood flow to the area may further the damage. Vasodilation to the area may lead to hyperemia, which would enhance the mechanisms of damage created by reperfusion (Nour et al., 2013).

Glucose metabolism is restored following reperfusion. With oxygen restoration to the tissue, normal glycolytic rates and ETC can resume. However, reperfusion in the presence of glucose appears to activate the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) system. Originally discovered in immune cells for the killing of foreign microbes, NOX is an important pathway in generating ROS and is also found in non-phagocytic cells such as neurons. NOX is a multicomponent enzyme whose subunits normally existing in the cell membrane and cytosol. The key subunit that activates NOX once phosphorylated, $p47^{phox}$, translocates the other subunits to the plasma membrane to form an active complex. NOX then produces superoxide (O_2^-) by transferring an electron from NADPH to O_2. The phosphorylation of $p47^{phox}$ is regulated by kinases, kinases that are upregulated during an HIE. Once activated by these kinases, NOX cannot generate ROS because glucose is required to produce NADPH via the hexose monophosphate shunt. Following reperfusion the glucose rich blood can now provide the necessary substrate for NADPH and subsequent superoxide synthesis. Also, because many victims suffering an HIE also have diabetes mellitus, the greater glucose content leads to more severe damage to the penumbra (Tang et al., 2012).

Unlike the ischemic core which undergoes necrotic cell death within minutes of an HIE, neurons of the penumbra undergo a regulated cell death by apoptosis from several hours to days after the event due to activation of several different intrinsic and extrinsic pathways (Broughton et al., 2009). Apoptosis is tightly regulated by a balance of pro-apoptotic and anti-apoptotic proteins with the pro-apoptotic proteins including: Bax, Bcl-xs, Bak, Bad, and Bid. The main factors that initiate and directly influence the balance of these proteins toward more pro-apoptotic proteins are Fas expression, decreased pH, and increased Ca^{2+}. A decreased pH and increased Ca^{2+} activate the mitogen-activated protein kinases (MAPKs) c-Jun N-terminal kinases (JNK) and p38 MAPK. JNK and p38 may mediate apoptosis in two ways. It may prevent the anti-apoptotic actions of Bcl-2 and also activate pro-apoptotic Bax proteins to move into mitochondria. Both of these actions ultimately cause cytochrome c of the ETC to be released from the mitochondrial inner membrane into the cytosol. This mediates the cleavage and activation of caspase-9. In general, Fas and Fas ligand expression is increased in stressed cells. The hypoxic conditions seen in the penumbra favor an up-regulation of Fas and Fas ligand. Activation of the Fas ligand on a neuron activates caspase-8, which can cleave and activate other procaspases. The activation of caspases initiates a cascade effect with activation caspase-3 ultimately resulting in neuronal death through apoptosis (Won et al., 2002).

3.3. Therapeutic Hypothermia

The mechanisms by which ischemia causes neuronal cell death is very complicated involving many of pathways described above plus many more all working together simultaneously to result in neuronal death in the primary and latent phase of an HIE event, but hypothermia acts in a variety of ways to help limit the extent of the damage by salvaging the neurons of the latent phase penumbra. Hypothermia can reduce cerebral metabolism by 5% for every degree (°C) of temperature reduction (Yenari and Han, 2012). In the adult gerbil, hypothermia suppressed the actions of ROS on mitochondria, helping to preserve them. Because mitochondrial failure is the major event leading to apoptosis of neurons in the penumbra, it is important to preserve the function of mitochondria during this reperfusion phase (Drury et al., 2010).

Hypothermia is not able to reduce calcium influx and not able to inhibit the release of excitatory glutamate unless initiated immediately after the HIE during the primary phase. Therefore, inhibiting ion overload is not a necessary component of neuroprotective effects for latent phase cooling (Drury et al., 2010). However, hypothermia does modulate activity of calpains, calcium activated proteases that disrupt cytoskeletal and nuclear proteins. The down-regulation of these enzymes helps keep the intracellular structures intact (Yenari and Han, 2012).

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Although the exact mechanism is unknown, it has been shown in near-term fetal sheep that hypothermia suppresses activity of caspase-3, the key mediator for the execution of apoptosis. It has also been shown in rat neurons that the lowered cellular metabolism induced by hypothermia effectively inhibited the production of hypoxia-associated proteins; by inhibiting the synthesis of these factors, it suppressed the multiple pathways through which apoptosis occurs (Askalan et al., 2010; Drury et al., 2010; Liu and Yenari, 2007; Yenari and Han, 2012).

Following reperfusion, inflammatory cytokines can enhance neuronal destruction. Hypothermia can reduce the production of these toxic inflammatory mediators due to effects on neural support cells, microglia, by inhibiting their activation and proliferation. Through this inhibition, microglia will not express cytokine-mediated inducible nitric oxide synthase (iNOS) whose role is to produce NO. This helps prevent mitochondrial failure because there is less NO to oxidize the lipids of the mitochondrial membrane (Gu et al., 2014; Han et al., 2002; Karabiyikoglu et al., 2003; Stefanutti et al., 2005; Yenari and Han, 2006). Also, hypothermia inhibits the accumulation of polymorphonuclear leukocytes in penumbra thereby limiting the extent of phagocytosis that can occur and cause damage (Drury et al., 2010; Kira et al., 2005; Kollmar et al., 2007; Prandini et al., 2005). The reduction of leukocytes and edema is further promoted by reduced blood-brain barrier disruptions and decreased vascular permeability (Baumann et al., 2009; Polderman, 2009). By reducing the number of leukocytes, there is a reduction in the amount of TNF-α released, a potent cytokine that inhibits Complex-I of the ETC (Drury et al., 2010). It has been shown that there is also significant reduction in tumor necrosis factor receptor-1 that further limit the effects of TNF-α (Webster et al., 2009; Yenari and Han, 2006; Yenari and Han, 2012). These immune and inflammatory responses do not occur until relatively late following the HIE and because they take time to develop there is a clear therapeutic window; for these reasons, hypothermia is especially effective in for dealing with immune and inflammatory responses (Polderman, 2009).

Hypothermia seems to be a promising therapy because it has a positive impact on many of the pathways seen in this multifactorial, dynamic event. Although the exact mechanisms are not completely known, it seems to plays key roles in suppressing

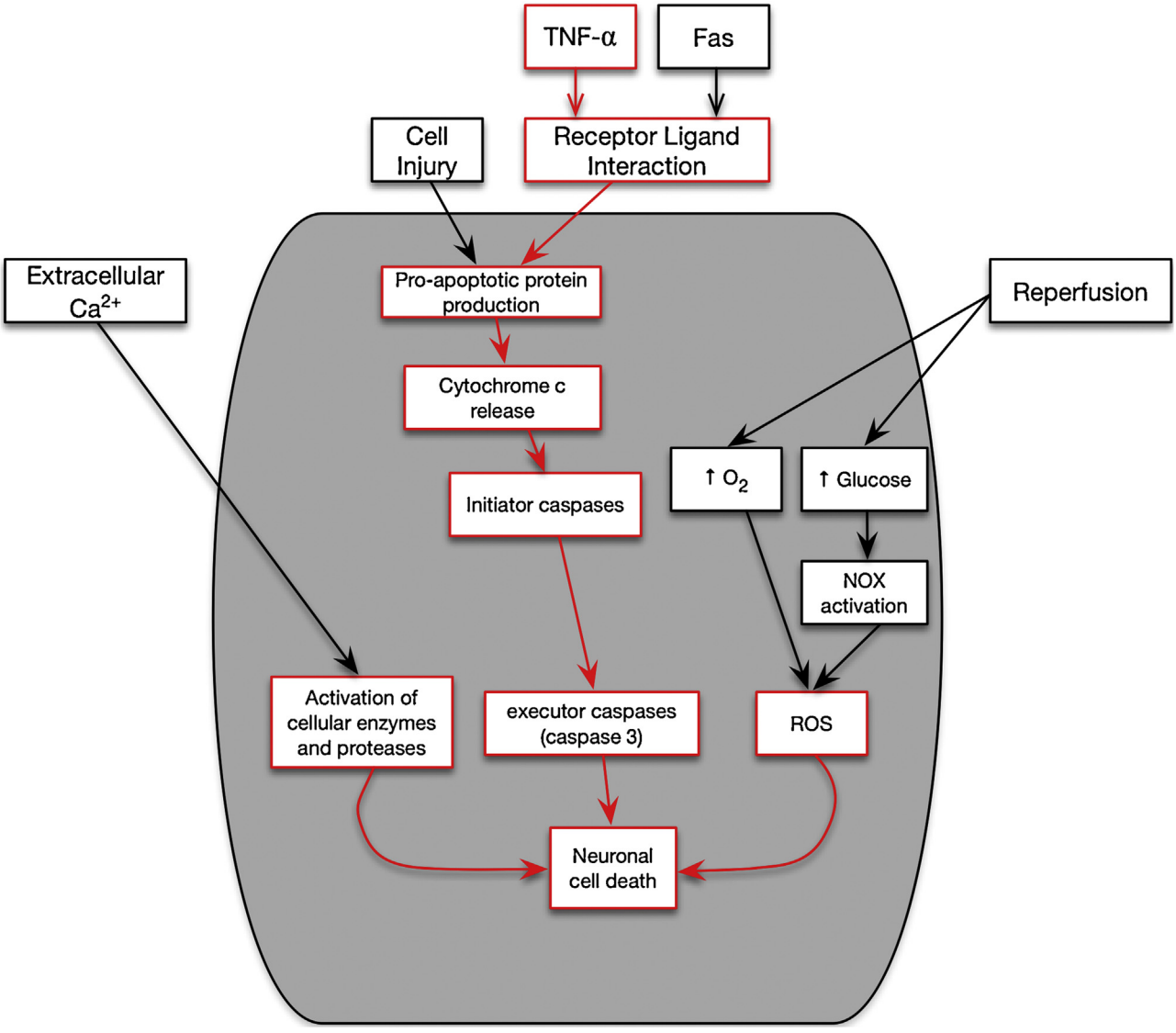

Fig. 1. Hypothermia's protective effects. The boxes highlighted in red are pathways suppressed due to the actions of hypothermia. Hypothermia affects both the intrinsic and extrinsic cell death pathway. It suppresses activity of caspase-3 and expression of tumor-necrosis factor receptor-1. Hypothermia also reduces activation of cellular enzymes triggered by calcium influx. Damage is limited following reperfusion by reducing the amount ROS production.

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apoptosis, inflammation, and extrinsic cell death pathways (see Fig. 1) (Drury et al., 2010).

In addition to creating an induced hibernation-like state via hypothermia, there are also some other agents that can be given to help further mitigate the damage in the penumbra of the latent phase by down regulating metabolism. Ethanol and phenothiazines, a class of drugs that have been used since the 50s for psychiatric disorders and sedative effects (Burn, 1954), have shown to exhibit neuroprotective qualities following an HIE.

3.4. Phenothiazine drugs

Phenothiazines, specifically chlorpromazine and promethazine, have showed promise as drugs that could be repurposed to treat victims suffering an HIE. The phenothiazines are ideal for for HIE events because of their prominent membrane effects. They have the ability to stop free radicals and reduce the uptake of Ca^{2+} and K^{+} leakage of the plasma membrane. They also have the ability to reduce swelling of the cell and prevent the release of acidic hydrolyses from the lysosome. It is also considered that the phenothiazines protect the membrane by inhibiting phospholipase activation (MacMillan, 1982).

Phenothiazines can alter metabolism. They slow metabolism not by affecting O_2 consumption but by uncoupling oxidative phosphorylation via inhibition of oxidation of glucose (MacMillan, 1982). They decrease glucose utilization and even inhibit glucose uptake. Mode of action was looked at by testing hexokinase activity. These drugs were used to see the effect on uptake of a methylated form of glucose that is not phosporylated by hexokinase. Phenothiazines largely reduced glucose uptake, suggesting that the target of these drugs was involved in the transport pathway and not in reducing the glucose metabolism within the neuron. Their mechanism is still up for debate, but it was shown that there is a marginal effect as antagonists to dopaminergic receptors (D2 dopamine receptors) mediates the reduced uptake of glucose. Because of their marginal effect, it is also hypothesized that these drugs act on Ca^{2+} channels (Dwyer et al., 1999). They have been shown to inhibit apoptosis, reducing activation of caspases and cytochrome c release from mitochondria; these effects are most likely a result of protecting the

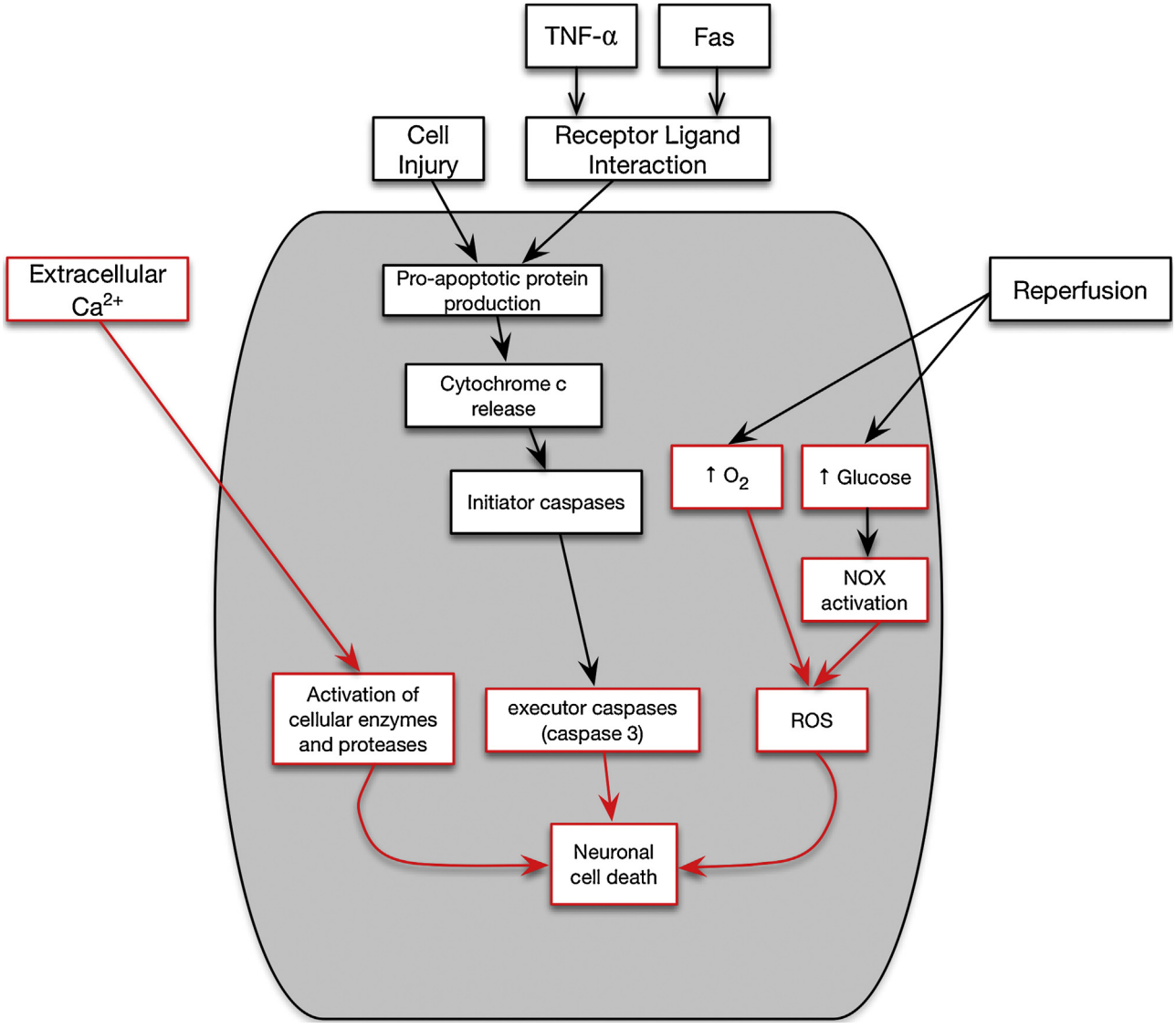

Fig. 2. Phenothiazine's protective effects. The boxes highlighted in red are pathways suppressed due to the actions of phenothiazine drugs. Phenothiazines affect the intrinsic cell death pathway by preventing cytochrome c release. They reduce calcium uptake and therefore limit the downstream effects. They also decrease glucose utilization, which limits the amount of ROS produced following reperfusion.

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mitochondria membrane from ROS and lipases that try to disrupt it (Narayanan et al., 2003). When animal brains were pretreated with promethazine and chlorpromazine, free radical induced lipid peroxidation was significantly reduced in vitro (MacMillan, 1982). Additionally, once phenothiazines are administered, cerebral blood flow is markedly reduced, and oxygen consumption is consequently reduced as well (see Fig. 2) (Berntman and Carlsson, 1978).

3.5. Ethanol

Ethanol's positive effects are due to its ability to decrease brain metabolism. Imaging studies have shown that acute low doses of ethanol can significantly reduce cerebral glucose metabolism along with regional differences based on dose (Volkow et al., 2006). The expression of the two main glucose transporters (GLUT) of cerebral tissue, GLUT1 and GLUT3, has been shown to be attenuated. There is also a down regulation of phospofructokinase (PFK), a major rate-limiting step of glucose metabolism. The reduction in glucose metabolism following reperfusion is important because activated NOX can utilize the glucose to produce ROS. In rat models, ethanol reduced the elevation of lactic acid. This is most likely due the decrease in expression of lactate dehydrogenase, an important enzyme of glycosis that converts pyruvate to lactate (Kochanski et al., 2013). Acidosis is also attenuated by a down regulation of monocarboxylate transporters that regulate lactic acid levels; which is important because acidosis in the penumbra exacerbates damage following reperfusion (Geng et al., 2015b). Acidosis and NOX activation is further ameliorated by ethanols effect on the pyruvate dehydrogenase complex (PHDC). PHDC is the key link between aerobic and anaerobic energy metabolism and because of its strict regulation, it's very sensitive to inactivation and down regulation during stroke. However, ethanol treatment upregulated expression of PDHC and it's key modulators thus bringing PDHC activity back to a normal level (Geng et al., 2015a). The energy balance can be expressed as a ratio, ADP/ATP, and post-ischemic ethanol decreases the elevated ADP/ATP ratio (Geng et al., 2013a).

Ethanol also affects other pathways. It decreases the activity of NOS and also the expression of $gp91^{phox}$ and $p22^{phox}$, two subunits of the multi-subunit enzyme complex (Geng et al., 2013a). When gerbils were preconditioned with ethanol treatment prior to an HIE, oxidative DNA damage was reduced and improved neurologic

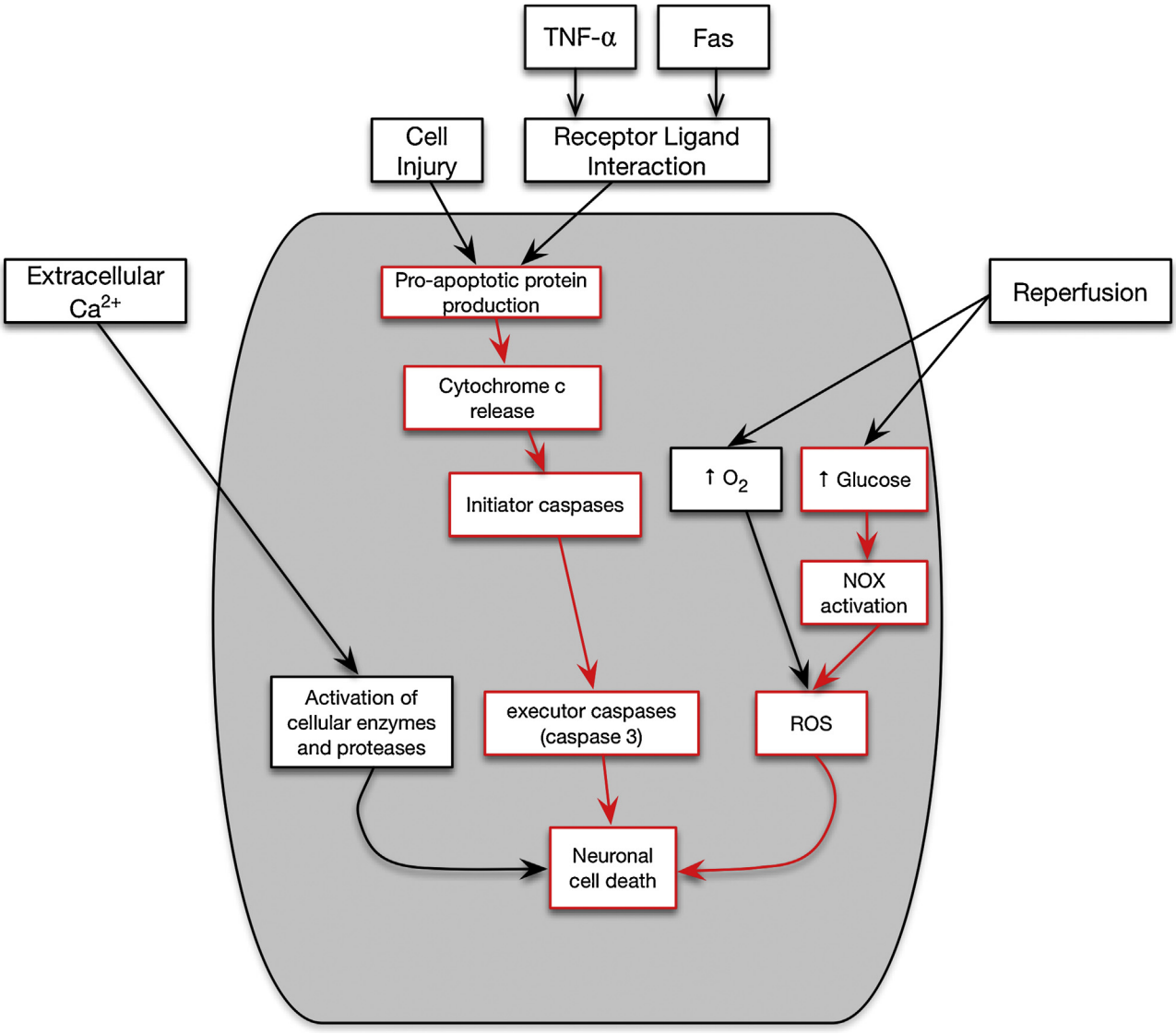

Fig. 3. Ethanol's protective effects. The boxes highlighted in red are pathways suppressed due to the actions of ethanol. Ethanol affects the intrinsic cell death pathway by down regulating the expression of pro-apoptotic proteins and up regulating the expression of anti-apoptotic proteins. Ethanol reduces ROS production by decreasing glucose utilization following reperfusion.

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deficits were seen (Wang et al., 2007). The down regulated expression of matrix metallopeptidases (MMP) MMP-2, MMP-9, aquaporins (AQP) AQP-4, and AQP-9 lead to a reduction in edema to the penumbra (Zeng et al., 2012). Also contributing to the reduced edema is the upregulated expression of endothelial proteins zona occludens-1 and the basal lamina protein, laminin which decrease the permeability of the BBB (Peng et al., 2013). Ethanol also modulates apoptotic pathways by decreasing expression of pro-apoptotic proteins such as Bax and increasing expression of anti-apoptotic proteins such as Bcl-2 and Bcl-xL (see Fig. 3) (Yuan et al., 2012). These effects where enhanced when ethanol was given in combination with normobaric oxygen (NBO) treatment (Geng et al., 2013b). Other factors such protein kinase C delta (PKC-δ) and Akt are also affected. PKC-δ and Akt are protein kinases that act as modulators in the signal transduction pathway of intrinsic apoptosis with PKC-δ being a pro-apoptotic and Akt being anti-apoptotic. Ethanol simultaneously decreases PKC-δ expression and increases Akt expression (Hafeez et al., 2014).

4. Summary

A number of mammalian species have the natural ability to undergo true hibernation in times of scarce food and harsh seasonal conditions. Some hibernators can achieve hypothermic temperatures as low as −2.9 °C (Carey et al., 2003; Drew et al., 2007) and metabolic activity as low as 1% of their active-state basal metabolic rate (Carey et al., 2003; Drew et al., 2007; Geiser, 2004). These are partially attributed to how they can have such a significant reduction in cerebral blood flow without damaging the central nervous system (Zhou et al., 2001). This innate neuroprotection, if induced to a lesser degree in non-hibernating species (i.e., humans), has been proposed as a possible therapy for ischemic stroke.

Non-hibernators are unable to survive the extreme physiological changes that true hibernators exhibit, but mimicking hibernation to a lesser extent is better tolerated. This so-called 'hibernation-like' state has been induced to varying degrees by a number of procedures and pharmaceutical agents. Administration of therapeutic hypothermia, for instance, has been achievable in both preclinical and clinical studies. However, the robust neuroprotective effects during cerebral ischemia have only been conclusive in animal models (Karibe et al., 1994; Xue et al., 1992). Previous clinical studies of therapeutic hypothermia and stroke have been small pilot studies with small patient populations, leading to unconvincing findings (Jeon et al., 2014). There are currently two large-scale clinical trials being conducted (EuroHYP-1 and ICTuS 2/3) to obtain reliable evidence on the efficacy and safety of therapeutic hypothermia in the treatment of ischemic stroke (Lyden et al., 2014; van der Worp et al., 2014).

Another proposed method of creating a neuroprotective hibernation-like state is through the use of phenothiazine drugs, specifically chlorpromazine and promethazine. These FDA-approved drugs have been used in medicine since the 1950s for their sedative and antipsychotic effects (Ban, 2007). They have both independently reduced infarct volumes in MCAO rat models (Li et al., 2014; Narayanan et al., 2003; Stavrovskaya et al., 2004), and a recent study showed that when given together, and in combination with mild hypothermia, MCAO rat models had reduced infarct volumes and neurological deficits (Liu et al., 2015). Their clinical efficacy has not been established for treatment of stroke, but the results from animal studies and their established clinical safety (Einarson and Boskovic, 2009; Lacasse et al., 2006) both support conducting clinical trials.

Low alcohol intake has been associated with decreased risk of total stroke, ischemic stroke, and stroke mortality in humans (Zhang et al., 2014). However, even though a pretreatment with ethanol has been found to be neuroprotective (Phillis et al., 1998; Wang et al., 2007), studies supporting its neuroprotection after the onset of stroke (Fu et al., 2013; Kochanski et al., 2013; Wang et al., 2012) are more relevant to its possible therapeutic application. Clinical trials for ethanol-mediated stroke therapy are lacking, but human studies have demonstrated advantages of ethanol in reducing brain glucose metabolism, similar to that of a hibernation-like state (Wang et al., 2000).

As more becomes known about the mechanisms underlying an HIE, it has been revealed that it is a dynamic, multifactorial process with many processes occurring simultaneously. From what research has shown so far, it seems that hypothermia would be an appropriate treatment but further research is needed to find the multiple mechanisms through which hypothermia provides protection. Hypothermia suppresses the activity of caspase-3, the final mediator for apoptosis (Drury et al., 2010). Although hypothermia has been shown reduce apoptosis by suppressing activity of the final key protein mediator, the mechanism behind it is not well understood. Understanding the upstream intracellular mechanisms of action by which hypothermia decreases apoptosis will be essential to improve future neuroprotective strategies. Outside of the neuronal cell, a key role of hypothermia is to limit inflammation. Neuroprotection is provided by reducing the extent of edema, and therefore, reducing the number of leukocytes to the penumbra.

Phenothiazines and ethanol seem like promising agents because of the pathways they affect. Phenothiazines reduce uptake of Ca^{2+}, which reduces the extent of the many downstream consequences of intracellular Ca^{2+} accumulation. Ethanol down-regulates the expression of the expression of proteins that facilitate edema and up-regulate endothelial proteins that help to decrease BBB permeability (Peng et al., 2013). Both phenothiazines and ethanol alter metabolism by uncoupling oxidative phosphorylation via inhibition of glucose utilization (MacMillan, 1982). Using both in combination may prove effective due to their ability to inhibit glucose utilization by different means. Phenothiazines affect the transport of glucose into the neuron by acting as a dopaminergic antagonist or blocking Ca^{2+} channels (Dwyer et al., 1999), whereas ethanol reduces expression of glucose transporters and PFK (Kochanski et al., 2013). These actions are ideal for ischemia because by inhibiting the uptake of glucose they can reduce the amount of ROS generated by the activated NOX system (Tang et al., 2012). Also because of this uncoupling, metabolism is of the cell is slowed, limiting the stress following the HIE (MacMillan, 1982). Like hypothermia, ethanol and phenothiazines have also been shown to directly alter the apoptotic pathway. A further understanding of mechanisms by which hypothermia, phenothiazines, and ethanol alter processes during ischemia will provide a strong basis for improving future neuroprotective strategies and for finding other similar drugs that can add to hypothermia treatment.

The current body of research on artificially induced hibernation-like states for neuroprotection in ischemic stroke has shown promise in preclinical *in vitro* and *in vivo* studies. The three different hibernation-like therapies mentioned all demonstrate protective effects through similar, but slightly different, mechanisms. The end result is a reduction of the following: activation of executor caspases, activation of cellular enzymes and proteases, and ROS (Figs. 1–3). However, there is not enough support from previous clinical trials to conclude whether its efficacy and safety will translate to human stroke patients. There are a small number of ongoing large-scale clinical trials, but more are needed to determine a safe, effective, and cost-efficient induction procedure.

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