ELSEVIER

Pathophysiology 10 (2003) 7–35

ISP PATHOPHYSIOLOGY

www.elsevier.com/locate/pathophys

Review

Hypothermia and stroke: the pathophysiological background

B. Schaller*, R. Graf

Max-Planck-Institute for Neurological Research, Cologne, Germany

Received 23 June 2003; accepted 25 September 2003

Abstract

Hypothermia to mitigate ischemic brain tissue damage has a history of about six decades. Both in clinical and experimental studies of hypothermia, two principal arbitrary patterns of core temperature lowering have been defined: mild (32–35 °C) and moderate hypothermia (30–33 °C). The neuroprotective effectiveness of postischemic hypothermia is typically viewed with skepticism because of conflicting experimental data. The questions to be resolved include the: (i) postischemic delay; (ii) depth; and (iii) duration of hypothermia. However, more recent experimental data have revealed that a protected reduction in brain temperature can provide sustained behavioral and histological neuroprotection, especially when thermoregulatory responses are suppressed by sedation or anesthesia. Conversely, brief or very mild hypothermia may only delay neuronal damage. Accordingly, protracted hypothermia of 32–34 °C may be beneficial following acute cerebral ischemia. But the pathophysiological mechanism of this protection remains yet unclear. Although reduction of metabolism could explain protection by deep hypothermia, it does not explain the robust protection connected with mild hypothermia. A thorough understanding of the experimental data of postischemic hypothermia would lead to a more selective and effective clinical therapy. For this reason, we here summarize recent experimental data on the application of hypothermia in cerebral ischemia, discuss problems to be solved in the experimental field, and try to draw parallels to therapeutic potentials and limitations.

Keywords: Hypothermia; Ischemia; Stroke; Cerebral blood flow; Neuroprotection

1. Introduction

Acute ischemic stroke represents a leading source of disability and death in major industrialized countries [113,290], that are mostly characterized by large-vessel thromboembolic occlusion and different pathophysiological factors contributing to the subsequent cellular brain tissue damage. To improve neurological outcome, a variety of new pharmacological strategies have been developed, but their neuroprotective influence remains partly controversial [113,271,290,300]. On the other hand, thrombolytic agents have been shown to improve experimentally and clinically the outcome by accelerating reperfusion of the adjacent vascular territory, although there is an elevated risk of additional cerebral hemorrhage [113,187,208,290]. By identifying different other treatment strategies to minimize such unfavorable side-effects [270], increasing interest has been focused on regulated hypothermia as a method of cerebral protection during the past 20 years, representing one of the most effective treatment options in reducing further deterioration of brain tissue after acute ischemic stroke [11,17,24,35,42,52–54,56,59,60,70,152,171,173,203,205,219,231,258,259,269,279,280,331,354,358,361,363], if hypothermia is induced soon after the onset of neurological symptoms and maintained for an adequately long time period [171]. Nevertheless, many questions regarding regulated hypothermia remain unanswered, including the degree of temperature reduction, the time point of application and the time period needed for successful neuroprotection. Both in clinical and experimental studies of regulated hypothermia, two principal patterns of core temperature lowering have been defined: mild (32–35 °C) and moderate hypothermia (30–33 °C) [112]. It is well accepted that hypothermia is remarkably neuroprotective when applied during or after global or focal ischemia. Protracted hypothermia of a few °C can provide sustained behavioral and histological neuroprotection [10,26,33,35], whereas brief or very mild hypothermia only delays neuronal damage [73]. It becomes increasingly clear that an interaction of various pathophysiological factors are involved in ischemia, all of which

* Corresponding author. Present address: Department of Neuroscience, Karolinska Institute, Retzius väg 8, S-17177 Stockholm, Sweden.
E-mail address: bernhard.schaller@neuro.ki.se (B. Schaller).

0928-4680/$ – see front matter © 2003 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.pathophys.2003.09.001

are influenced by hypothermia [117]. However, the exact pathophysiological mechanism of cerebral protection by regulated hypothermia, that means stepwise reduction of core temperature, remains still a matter of debate.On the basis of the increasing interest in clinical application of regulated hypothermia as an additional therapeutic option in the treatment of acute ischemic stroke [272,294], it seems time to summarize the experimental data of hypothermia up to present, to discuss problems to be solved in the experimental field and try to draw parallels to therapeutic potentials and limitations.

2. Pathomechanism and their putative modulation by hypothermia

Several pathophysiological steps may be involved in the neuroprotective effect of hypothermia [333]. These may include: (i) an increase in denaturation and misaggregation of proteins; (ii) a slowing of progression through the cell cycle, with phase G1 typically being the most sensitive; (iii) an inhibition of transcription and translation, leading to a generalized reduction in protein synthesis; (iv) a disruption of cellular cytoskeletal elements; and (v) changes in membrane permeability leading to increased cytosolic Na^+ and H^+ levels. Hypothermia can also produce alterations in the properties of the lipid bilayer, such as phase transitions or changes in the fatty acid composition of the membrane, reflecting a cellular physiological response to cold stress.

The cerebral protection has commonly been attributed to the decreased cerebral metabolic requirements according to the Q10-principle representing the factor by which the rate of a biochemical reaction is increased for a 10 °C rise in temperature [107]. The Q10 is close to a value of 2 for enzymatically catalyzed reactions but greater for non-enzymatic reactions. Michenfelder and Milde [210] have shown that the Q10 of the brain increased from about 3 between 37 and 27 °C to 4.8 between 27 and 18 °C. This means that a 1 °C reduction in core temperature will lead to a 9.6% metabolic reduction, such as in the rate of cellular respiration, oxygen demand or carbon dioxide production. The altered O_2 demand during hypothermia is especially critical in highly aerobic organs such as the brain tissue. Based on these values, if the brain could survive an ischemic event for 5 min at 37 °C, hypothermia to 27 and 17 °C would afford 15 and 72 min neuroprotection, respectively, based solely on decreased $CMRO_2$. While survival times at brain temperatures below 20 °C may be predicted on the basis of increasing Q10 and diminishing $CMRO_2$, some additional physiological mechanisms are required to explain intact survival after prolonged submersion, in which reported core temperature are often above 30 °C.

In recent years, a large amount of experimental data have demonstrated that hypothermia introduced and maintained during the ischemic event (intraischemic hypothermia) diminishes neuronal damage and improves outcome following transient global and focal ischemic events [7,10,16,31,35,71,98,100,107,137,308,345,347,350]. Such experimental findings confirmed substantial evidence that, at least in animal models, intraischemic hypothermia may protect, for a considerably long time, brain tissue that would otherwise undergo delayed cellular death after ischemic event [347]. Fewer experimental studies deal with the pathophysiological alterations of postischemic hypothermia after stroke [7,33,42,50–53,57,58,79,152,171,195–198,201,217,229,277,278,344,352,356] (see Tables 1 and 2). This may be because postischemic hypothermia has only recently been shown to provide lasting benefit [59,178,285]. Suggesting that other pathophysiological mechanisms are involved in the protective effects of postischemic hypothermia than those implicated in intraischemic hypothermic neuroprotection, data of experimental intraischemic hypothermia may not obviously generalize the situations of postischemic hypothermia, particularly, when it is initiated several hours after ischemia, but might broaden the therapeutic window for additional therapeutic intervention.

2.1. Cerebral blood flow

Incongruent experimental data exist on the consequences of hypothermia on cerebral blood flow (CBF). Systemic hypothermia of 25–28 °C is followed by decreased CBF as measured by ^{14}C-butanol [264], whereas selective brain cooling (31 °C) with normal core temperature is followed by increased cortical blood flow as measured by laser-Doppler flowmetry [174]. Thus, the hypothermia-induced hemodynamic changes may depend on the different experimental methods of brain tissue cooling (systemic versus local) as well as the depth of hypothermia [72,120]. External brain cooling produces a centripetal temperature gradient resulting in temperature differences between superficial and deep cerebral structures [97]. Therefore, the pial vessels could be exposed to much lower temperatures than the vessels inside the brain tissue. Profound levels of hypothermia abolish cerebral autoregulation by producing cerebral vasoparalysis [104,126,266]. Thus, an increased cortical blood flow in response to conductive (external) head cooling could be caused by centripetal temperature gradients and would be limited to the outer layers of the cerebral cortex.

In newborn piglets, it could be demonstrated that CBF, as measured by colored microspheres, decreased more than $CMRO_2$, if brain temperature was below 35 °C during systemic hypothermia [232]. The CBF decreased to 72% of the initial value at 35 °C and to 41% at 32 °C, while $CMRO_2$ and CMR_{glc} decreased to 66 and 64% at 35 °C, and to 53 and 46% of the initial value, at 32 °C, respectively. These experimental data indicate that CBF decreased in parallel with $CMRO_2$ and CMR_{glc} down to 35 °C, but decreased more than $CMRO_2$ at brain temperature below 35 °C. Several other studies have established the link between cerebral metabolic rate and CBF. In sheep fetuses, decreased core temperature by 2 °C did not influence CBF [163]. However,

Table 1
Effect of delayed (>30 min) postischemic hypothermia on ischemic CA1 neuronal damage in selected reports of rodent global ischemia models[a]

Authors	Model (min)	Hypothermia			Survival time (days)	Outcome
		Delay (h)	Duration (h)	Temperature (°C)		
Busto and Ginsberg [30]	Rat (10)	0–5	3	30	3	No protection
Carroll and Beek [38]	Gerbil (5)	1.3	6	30	4	Delay 1 h: grade 2; delay 3 h: no protection
Coimbra and Wiesloch [50]	Rat (10)	2	5	33	7	Grade 7
Coimbra and Wiesloch [51]	Rat (10)	2–36	5	33	7	Delay <6 h: grade 3; delay >12 h: no protection
Colbourne and Corbett [58]	Gerbil (3 or 5)	1	12, 24	32	10, 30	3 min: grade 4; 5 min, duration 12 h, 7 days: grade 2; 5 min, duration 12 h, 30 days: grade 1; 5 min, duration 24 h, 30 days: grade 4
Shuaib et al. [278]	Gerbil (repetitive 2 × 3)	0.5, 1	3	34–35	7	No protection
Shuaib et al. [279]	Gerbil (5)	0.5	3	34	7, 29	7 Days: grade 3; 29 days: no protection
Colbourne and Corbett [57]	Gerbil (5)	1, 4	24	32, 34	30, 180	Delay 1 h, 32 °C, 180 days: grade 3; delay 4 h, 32 °C, 180 days: grade 1; delay 1 h, 34 °C, 30 days: grade 3
Nurse and Corbett [227]	Gerbil (3)	1	27	35.5–36.5	4, 10	Delay 4 days: grade 3; 10 days: grade 2
Coimbra et al. [52]	Rat (10)	2	7	33	7, 60	Grade 2

[a] All studies are used by bilateral carotid artery occlusion models. When hypothermia reduced CA1 neuronal injury, the degree of neuroprotective effects are represented by four grades (grade 1: count of normal neuron <25%; grade 2: 25% < count of normal neuron < 50%; grade 3: 50% < count of normal neuron < 75%; grade 4: 75% < count of normal neuron).

Busija and Leffler reported a CBF and $CMRO_2$ decrease of 40–50% at a temperature level of 34–35 °C compared to 39 °C in normal newborn piglets (1–4 days after birth) measured by 15-micro S radioactive microspheres [29]. In adult dogs, every 1 °C the body temperature decreased, CBF and $CMRO_2$ decreased by 6.7% between 35 and 25 °C as measured by autoradiography [265]. Young and McCormick also reported that with each decrease of 1 °C during hypothermia

Table 2
Effect of delayed (>30 min) postischemic hypothermia on cerebral infarction volume in selected reports of rodent focal ischemia models[a]

Authors	Model (min)	Hypothermia			Survival time (days)	Outcome
		Delay (h)	Duration (h)	Temperature (°C)		
Baker et al. [11]	Permanent	0.5	2–3	24	1	Control: 99 mm^3; delay 30 min: 44 mm^3; delay 1 h: 35 mm^3; delay >2 h: no protection
Moyer et al. [217]	Permanent	0.66	1	32	1	No protection
Kader et al. [149]	Permanent	1	1	30–36.5	1	Control: 17%; hypothermia: 8%
Xue et al. [345]	3 h transient	1.5	1.5, 3	32	1	Control: 211 mm^3; duration 1.5 h: 108 mm^3; duration 3 h: 56 mm^3
Zhang et al. [360]	2 h transient	3	3	30	7	Control: 21%; hypothermia: 11%.
Karibe et al. [154]	2 h transient	0.5–1	1–1.5	32–33	1	Control: 160 min^3; delay 30 min: 68 mm^3; delay 1 h: no protection
Markarian et al. [201]	3 h transient	0.5–0.75	3	32–33	3	Control: 211 mm^3; delay 30 min: 179 mm^3; delay 45 min: no protection
Yanamoto et al. [352]	3 h transient	3–21	3–3.5	32–36	1	Control: 197 mm^3; delay 3 h, duration 1 h: no protection; delay 3 h, duration 21 h: 154 mm^3; delay 3.5 h, duration 21 h: no protection
Maier et al. [197]	2 h transient	0.5–2	1–2	33	3	Control: 53%; delay 30 min: no protection; delay 1 h: 84%; delay 2 h: 59%
Colbourne et al. [53]	1.5 h transient	2.5	24	33–35	35–42	Control: 67.5 mm^3 hypothermia: 35.8 mm^3
Maier et al. [195]	2 h transient	1.5–3	2	33	3	Control: 55%; delay 1.5 h: 61%; delay 2 h: no protection; delay 3 h: no protection
Kollmar et al. [169]	2 h transient	3 h	5	33	5	Control: 215 mm^3; hypothermia: 159 mm^3

[a] All studies are used by middle cerebral artery occlusion model. Results are represented by infarct volume (mm^3) or percentage of infarct volume to those in ischemic ipsilateral hemisphere.

of adult humans, CBF and $CMRO_2$ decreased by about 5% as measured by laser-Doppler flowmetry [357].

Another question may be to which degree CBF is metabolically regulated during cerebral hypothermia. Hypothermia changes flow–metabolism coupling in the brain tissue that results in a mismatch of flow and metabolism favoring brain perfusion during moderate and deep cerebral hypothermia [29]. Based on these data, it cannot be decided, if CBF increase relative to metabolism represents a cerebral luxury perfusion or if it is a change in coupling of flow and metabolism. Regardless of this change in flow–metabolism coupling, metabolic regulation remains a main determinant of CBF even during deep cerebral hypothermia.The CBF increase may be caused by factors other than metabolic regulation: moderate and deep hypothermia may exert a direct effect on cerebral resistance vessels, independent of metabolic autoregulation [116,244,323]. In addition, moderate hypothermia dilates cerebral arterioles in vitro [228], suggesting that this effect may in vivo counteract the metabolically mediated vasoconstriction during moderate and deep cerebral hypothermia. From this pathophysiological aspect, it seems that the cerebrovascular response varies, depending on the depth of hypothermia, the method of cooling, the model of ischemia, and the animal species. These differences may represent the key factor in determining the local hemodynamic consequences of hypothermia [72].

2.2. Membrane stability

Disruption of blood–brain barrier (BBB) and brain edema formation are induced by ischemic processes and have been implicated in the progression of ischemic brain tissue damage [134]. In forebrain global ischemia, normothermic (36 °C) but not hypothermic (33 °C) rats display early postischemic BBB disruption to protein tracers after an ischemic event [73]. If intraischemic temperature is maintained at mild hyperthermic levels (39 °C), protein extravagation is much more pronounced compared to normothermic ischemia [73]. Other studies have demonstrated that hypothermia reduce the permission of radiolabeled tracers across the BBB [73], suggesting that decreased formation of brain edema—as induced by hypothermia—is largely mediated by the protection of BBB integrity. Hypothermia dramatically reduces brain edema formation in the basal ganglia at 24 h after induced ischemia [160]. From the pathophysiological point of view, decreased brain edema formation by hypothermia is very likely, when the hypothermic reduction of glutamate surge [214], of Ca^{2+} mobilization [215] and of ATP expenditure [346] helping to maintain brain tissue integrity and therefore reduce brain edema formation [299]. In addition, hypothermia protects the BBB [146,285] and exerts a neuroprotective effect by reducing the passage of potentially harmful endogenous substances across the endothelial barrier. For example, leukotriene B4, an arachidonic metabolite, produced by ischemic degradation of membrane lipids, is followed to the development of ischemia-induced vasogenic edema and is decreased in mild hypothermia [67].

2.3. Energy metabolism

A metabolic reduction has been suggested to be the main pathophysiological mechanism of hypothermia-induced neuroprotection [283,337]. Cerebral metabolism can be divided generally into two compartments [212]: (i) *cellular integrity and structure*, as maintained by the basal compartment and suppressed by hypothermia; and (ii) *cellular function*, as neuronal firing, related to the functional compartment, and suppressed by anesthetic agents, such as barbiturates. Both metabolic states are considered to be more or less independent of each other [330].

Protein synthesis and ion-balancing ATPase are the dominant energy-consuming processes of standard metabolic rate in mammals [262]. Thus, decreased ATP demand, as a consequence of decreased pump activity and lowered membrane permeability, provides evidence as a strategy to down-regulate energy turnover in metabolically depressed states [74,240]: a decline in $CMRO_2$ rate can be demonstrated during regulated hypothermia [103]. During cardiac arrest—lasting 10 min—in dogs with hippocampal temperature of 30 °C, there cannot be demonstrated any difference of cerebral lactate as compared to normothermia [219]. Varying temperature of 27–35 °C induced before initiating global ischemia in cats and maintained during the first 1.5–2.0 h of recirculation reduced intracellular acidosis as assessed by magnetic resonance spectroscopy [47]. Decreased intracellular glucose concentrations together with increased tissue lactate were seen up to 34 °C, but at 31 °C there was significantly less perturbation in both glucose and lactate, indicating a preservative effect of hypothermia on cerebral metabolic rate during ischemia [345].

Decreased $LCMR_{glc}$ follows varying hypothermic temperatures (20 min) at levels of 36 or 30 °C, 2 h after recirculation following bilateral carotid occlusion in rats, but $LCMR_{glc}$ was significantly higher in all cortical and subcortical structures of rats with intraischemic hypothermia as compared to normothermia [101]. In another study, non-ischemic rats under normo- and hypothermic conditions showed decreased $LCMR_{glc}$ at levels of 35–50% below normothermic levels, with considerable regional heterogeneity [203]. The higher tissue concentration of glucose in normoxic brain at 31 °C, in combination with lower tissue lactate, support previous data of adult animals that hypothermia may reduce glucose consumption by inhibiting the enzyme phosphofructokinase [115,192,194]. Hypothermia alone reveals no adverse effect on high-energy phosphate reserves of the immature rat brain tissue [151], but elevated phosphocreatine levels in both the 34 and 31 °C groups have been demonstrated in hypothermic adult rats [192,194]. As homeostasis of phosphocreatine is related to the activity of creatine kinase and to intracellular pH, it is likely that the rise in phosphocreatine may be related to an intracellular

alkaline shift during hypothermia [281]. Further substantiation for this hypothesis has been provided by an increase in intracellular pH of 0.011 units/°C at hypothermic temperatures of 29 °C [147], combined with an increase in the cerebral phosphocreatine/inorganic phosphate ratio. These findings, taken together with the preservation of glycolytic and tricarboxylic acid cycle, support evidence that hypothermia alone may substantially lower $CMRO_2$ [115,281].

Disturbances in cerebral tissue ATP during acute ischemia can lead to brain tissue damage. In vitro and in vivo models of cerebral ischemia have demonstrated that ATP depletion to 25–30% of normal values results in glial cell death [110,335,347]. Preservation of ATP during intraischemic hypothermia of 34–36 °C has been documented in adult rats [22] and in 7-day-old rat pups exposed to a similar ischemic event at 29 and 21 °C [356]. Both of these studies used a focal ischemia model, during which substrate continues to be supplied to the brain tissue, albeit at a reduced rate. In contrast, studies in which complete/near complete ischemia models were used have failed to show an effect of hypothermia on preservation of brain ATP [35,47,329]. Despite the profound effects of hypothermia on lowering the cerebral rate of oxygen consumption, a complete lack of substrate will inevitably result in the failed maintenance of energy stores. This is exemplified by experiments in which cerebral energy utilization was measured using the Lowry decapitation technique, demonstrating that hypothermia of 30 °C reduced the rate of ATP utilization from 0.4 mmol/(kg min) in normothermic animals to 0.23 mmol/(kg min) in the hypothermic groups [212]. However, at the end of short periods of complete cerebral ischemia, ATP stores are not preserved by hypothermia [35,47,299,329], even though the rate of depletion is delayed in some studies [47,329], demonstrating that hypothermia exerted a protective effect despite increased depletion of ATP pool.

By use of magnetic resonance (MR) spectroscopy, moderate hypothermia shunts glucose metabolization from Embden–Meyerhof glycolytic pathway into the potentially neuroprotective pentose phosphate pathway (PPP) [151], playing an important role in maintaining cellular integrity and function during ischemia to maintain membrane potential, and diminish oxidative damage. In normothermic rats, PPP constitutes 2.3% of the whole metabolism of brain glucose [94]. Composed of two reaction sequences, one forming CO_2, D-ribulose-5-phosphate (Ru5P), and the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) and the other regenerating hexose phosphate from Ru5P, it has been suggested that the separate control of these two pathways would allow for the fine regulation of NADPH compared with Ru5P formation [18]. Ru5P is prerequisite of nucleic acid synthesis whereas NADPH serves a number of cell-protective functions [151]. In addition to a depression of cellular metabolism, mild hypothermia results in an apparent decreased steady-state fractional enrichment of ^{13}C label [151], implying that a significant amount of glucose has been metabolized through the PPP rather than through the glycolytic and tricarboxylic acid pathways [151]. In the oxidative branch of the PPP, the ^{13}C-labeled first carbon of glucose is lost as $^{13}CO_2$ during formation and steady-state enrichment of Ru5P [151]. Both glycolysis and the PPP produce lactate, as it can be measured by nuclear MR imaging: glycolysis produces one molecule of ^{13}C-lactate, whereas the PPP produces only unlabeled lactate [151]. Thus, the ratio of labeled lactate produced to labeled glucose administered is less than the expected 1:1. As the activity of the PPP increases, this ratio lowers [151]. Up-regulation of PPP during hypothermia may play a vital role in maintaining cellular integrity and function. The PPP appears to possess a large reserve capacity, and it has been suggested that it may have a specific role in brain metabolism, perhaps being intermittently turned on at a high level of activity to provide large amounts of product as required [13]. Experimental stimulation of the PPP by diethyl maleate or buthionine sulfoximine, which act through glutathione depletion, increases the survival time of animals suffering from ischemia [319].

2.3.1. Brain oxygenation and tissue acid base status

There is experimental evidence that hypothermia is accompanied by a reduction in $CMRO_2$ leading to relatively improved oxygen supply or to a reduced susceptibility to cellular damage [209]. Mild hypothermia results in a significant (30–40%) depression of tricarboxylic acid (TCA) cycle activity and thus cellular metabolism in rats [151]. This depression is consistent with that seen using indirect measures of metabolism in the non-shivering rat, the $CMRO_2$ decreases linearly from 37 to 22 °C, falling by approximately 5%/°C, which is mirrored by a smaller decrease in CBF [115]. Described by its discoverer as the main energy-yielding process in all of the nature [173], the ubiquitous nature of the TCA cycle would make the control of its activity an ideal mechanism of hypothermic neuroprotection. These data provide further insight into the mechanism by which experimentally induced hypothermia exerts its neuroprotective effect during ischemia indicating a dissociation between the degree of hypothermia and the extent of energy preservation, despite relatively profound sparing of tissue damage at both 31 and 34 °C [320]. This disparity in energy preservation between both animal subgroups suggests that the degree of hypothermia has important implications regarding not only the mode of action, but also the degree of benefit provided by this form of therapy. However, previous studies have shown that in the normal brain tissue, $CMRO_2$ decreases linearly by 5–8% for each 1 °C drop in brain temperature [207].

Hypothermia leads to a left shift of the hemoglobin–O_2 dissociation curve and to a restricted oxygen delivery to tissues [6]. The solubility of oxygen in the blood increases during hypothermia. Under normal conditions the dissolved oxygen in blood plays a minor role in cerebral oxygen metabolism, as only 2.3% of the dissolved oxygen is integrated in the normal $CMRO_2$. During hypothermic cardiopulmonary bypass surgery, $CMRO_2$ obtained from

dissolved oxygen increased far more, proportional to arterial blood [68]. Other studies have shown that there is a decrease in brain tissue oxygenation from the brain surface to deeper tissue because of a decreasing number of capillaries in deeper zones [16]. However, it can be speculated that this shift of the O_2 dissociation curve on HbO_2-binding affinity may be either beneficial or detrimental to the ischemic brain tissue. Because hypothermia increases the affinity of Hb for O_2, release of O_2 into tissue could be reduced. In contrast, when Hb affinity for O_2 is increased, a lower tissue pO_2 is required to release O_2 from Hb preserving O_2 delivery until it is released in more hypoxic tissue. Alternatively, the effect of hypothermia on HbO_2 binding sites may be negligible in the context of other major pathophysiological events initiated by an ischemic event and/or the neuroprotective effect of hypothermia is of such magnitude that the effect of altered O_2 release is negligible in comparison. However, changes of HbO_2 bindings sites caused by mild hypothermia do not alter brain infarct size after experimental focal ischemia so that other mechanisms must account for the benefits observed from hypothermic neuroprotection [324].

2.4. Calcium and intracellular signaling

2.4.1. Calcium signaling

It has been demonstrated in experiments employing Ca^{2+} microfluorometry imaging on slices from gerbil hippocampi preloaded with a Ca^{2+} dye, rhod-2, that $[Ca^{2+}]_{int}$ mobilization is induced within 2–3 min after induction of ischemia; pronounced in CA1 region and more significantly in the stratum radiatum or the stratum oriens than in the stratum pyramidale, leaving CA3 region little affected [215]. Increased $[Ca^{2+}]_{int}$ by ischemic events is related partly to the extracellular space through glutamate receptor channels, more highly attributable to NMDA-type than others like AMPA-type or kainate-type, or through voltage dependent Ca^{2+} channels [284]. The Ca^{2+} originates from intracellular Ca^{2+} stores on the other hand, mediated by ryanodine type receptors or that of inositol triphosphate (IP3) type. As IP3 has been implicated in glutamate metabotropic receptor-mediated events and $[Ca^{2+}]_{int}$ homeostasis, rats have been subjected to 20 min of two-vessel occlusion plus hypotension at intraischemic brain temperatures of either 30 or 37 °C, demonstrating significant decreases of IP3 during normothermic ischemia in hippocampus (74%), striatum (81%), and cortex (66%); thalamic levels remained unchanged [30]. Following intraischemic hypothermia, only the striatum showed a significant, but smaller decrease in IP3 levels [30]. These results demonstrate that decreased IP3 level in vulnerable ischemic brain regions can be at least partially inhibited by intraischemic hypothermia [32].

The NMDA-type receptor channel is one whose function is profoundly affected by ischemic conditions, because glutamate binding to the receptors followed by membrane depolarization induces opening of the channels by removing the Mg^{2+} block, through which Ca^{2+} can penetrate inwardly with monovalent ions. Ca^{2+} permeability of AMPA-type receptors is intrinsically small compared with that of NMDA-type receptors. However, it increases after this permeability changes in ischemic conditions in vivo and in vitro, because a new subtype lacking Glu R2 appears which permeates much Ca^{2+} than Glu R2 containing receptor subtype. The Ca^{2+} mobilization is lethal when it is sustainedly kept high [312], exceedingly a certain levels [5], because Ca^{2+} can induce uncontrolled Ca^{2+} damages mitochondrial respiration or uncontrolled Ca^{2+}-dependent cascade reactions.

2.4.2. Intracellular signaling

The hypothermia-induced effects on ion-exchanges and co-transporters involved in cell regulation are yet poorly understood. Cerebral ischemic events inhibit, by translocation, both the protein kinase C (PKC) and Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) systems [37,40,93], an effect that can be attenuated by intraischemic hypothermia [31]. Recent experiments using gene knockout mice to reduce CaM kinase II activity have shown a doubling of focal ischemic damage compared to their wild littermates [322]. Hippocampal slices taken from CaM kinase II knockout mice yield more robust posttetanic potentiation, a finding that suggests regulation of glutamate release by CaM kinase II [40]. These experimental data suggest that PKC modulate synaptic functions during ischemia. Upon redistribution of the postsynaptic densities after ischemia, CaM kinase and PKC phosphorylate can modulate glutamate receptors [328]. Additionally, Ca^{2+} influx through the NMDA-type ion channel can activate CaM kinase II, thereby regulating AMPA-type glutamate receptor ion channels through their phosphorylation [328].

In support of the deleterious role of PKC in ischemic brain tissue damage, it is reported that non-selective PKC inhibitor prevents neuronal damage [193] and a selective inhibitor prevents cerebellar granule cells apoptosis after depolarization [356]. Moreover, glutamate-induced apoptotic-like cell death is largely prevented by inhibition of NMDA-receptor, PKC, CaM kinase II, and MAP kinase, respectively [146]. Furthermore, NMDA receptor inactivation provides long-term protection to cultured cortical neurons from a variety of cell death signals [90]. These observations support the hypothesis that hypothermia prevents reperfusion-induced neuronal damage and delayed neuronal cell death through inhibition of glutamate receptor-mediated activation of CaM kinase and PKC.

Ubiquitin is a small proteasome binding covalently to abnormal proteins induced by ischemic brain tissue damage and facilitating intracellular protein degradation. Following global ischemia, ubiquitin disappears in the hippocampus and does not subsequently recover. This ischemia-induced loss of ubiquitin may lead to an accumulation of abnormal proteins which affect cell structure and function. In gerbils subjected to 5 min of ischemia, intraischemic hypothermia

was associated with a significant restitution of ubiquitin compared to normothermic animals [349].

2.5. Excitotoxicity

Few reports are available regarding the pathophysiological mechanisms for attenuation of ischemia-induced effluxes of neurotransmitters by brain hypothermia. Activation of synaptic enzymes such as PKC or CaM kinase II facilitates the release of transmitters [280], and PKC is involved in ischemia-induced transmitter release [280]. Therefore, maintenance of presynaptic enzymes may lead to attenuated release of excitatory neurotransmitters (i.e. glutamate, aspartate) [109]. In the early postischemic period, an imbalance between excitatory and inhibitory neurotransmission is apparent [101], and is attenuated by early postischemic hypothermia [100].

Excessive release of glutamate during ischemia represents an important factor in the pathogenesis of ischemic neuronal damage [20,196]. As it is generally accepted that biosynthesis and uptake of neurotransmitters are temperature-dependent, the degree of glutamate surge is largely depressed [34] and the onset of Ca^{2+} mobilization is significantly delayed when ischemic events were carried under hypothermia [217]. Following focal cerebral ischemia, glutamate levels typically peak within 60 min after experimental induction of ischemia and return to baseline [111,137] or decrease substantially [10] by 90–120 min. Hypothermia increases (extracellular) glutamate levels by a delay of up to 20 min [111,137]. A few studies have shown that mild hypothermia is still effective even when applied after glutamate peaks (hypothermia delayed by 60–120 min) [11,200,229,346]. Therefore, there is experimental evidence that mild hypothermia also exerts its protective effects by altering processes down-stream of excitatory amino acid release. Hypothermia between 30 and 33 °C completely inhibits glutamate release [34]. The 30 °C produced a greater degree of protection than 33 °C [35], even though both levels resulted in similar degrees of reduction in excitotoxic transmitter efflux [34]. Therefore, hypothermia effects on ischemic transmitter release per se cannot completely explain its neuroprotective effects.

Altered uptake carrier activity of neurons and glia—as driven by the transmembrane gradients for Na^+/K^+ [34]—is suggested to be the major factor for effluxes of amino acids during brain ischemia. Because hypothermia attenuates ischemic depolarization [34], preservation of ionic gradients by hypothermia may attenuate the reversed uptake mechanism. However, in gerbril models, excitotoxicity is considered to be completely finished with 1 or 2 h after ischemia; while postischemic hypothermia introduced some hours after the event still resuscitates hippocampal neurons [34]. These data indicate that temperature-dependent cellular processes are still going on after the "conclusion" of excitotoxicity and demonstrate potential postexcitotoxic connections. One such connection may be the postischemic, sustained activation of glutamate receptors. If hippocampal slices are subjected to microfluorometric measurement, mobilization of Ca^{2+} in response to the addition of NMDA is selectively visualized in the stratum radiatum and stratum orients of the ischemia-vulnerable CA1 sector [34]. In this model, the Ca^{2+} response to the NMDA addition was found to significantly increase in gerbils that had been subjected to 5 min transient ischemic events 1–9 h before sacrifice, with a peak around 6–9 h [34]. It has been found that virtually no such sustained enhancement of Ca^{2+} mobilization could be detected in slices from gerbils that were subjected to transient ischemia at 33 °C [351]. These findings apparently document sustained activation of NMDA-type glutamate receptors as confirmed by a patch clamp study on CA1 neurons in hippocampal slices of the gerbil [64,215]. In a different experimental paradigm, a delayed activation of both the NMDA-glutamate receptors [227] and the non-NMDA receptors [311] induced by an ischemic event has been reported.

2.6. Concept of acid–base management

During hypothermia, management by acid–base strategy is related to the fact that the pH of electrochemical neutrality varies physiologically in a temperature-dependent fashion [128,169,256,302,303,329]. The increased pH by hypothermia leads to a more alkalotic status relative to normothermic values with a rate of approximately 0.011 pH units/°C [149]. By preserving intracellular pH, poikilothermic animals maintain the histidin-imidazole ionization status at a constant level (referring to the alpha-imidazole ring of the histidin moiety of hemoglobin), that represent primary determinant of the charge state and pH-dependent functions of different proteins. Thus, with so called alpha-stat (pCO_2: 40 mmHg; pH_a: 7.40; without corrections of temperature) acid–base management, changing temperature does not alter histidin ionization, or hinder protein charges, structure, and function [223]. When hypothermic, poikilothermic animals demonstrate constant ionization level of histidin-imidazole by establishing a respiratory alkalosis relative to normothermic (37 °C) blood gas values. Respiratory alkalosis is physiologically appropriate during hypothermia and preserve normal physiological conditions.

These physiological facts indicate that CO_2 management during regulated hypothermia profoundly affects CBF autoregulation. Experimental studies revealed that normothermic rats autoregulated normally [295,325]. CBF values were relatively stable until MABP decreased to values of 55–64 mmHg [325]. Contrary, Kollmar et al. [169] could demonstrate an increased CBF—as measured by ^{14}C-antipyrine—by pH-stat management during the early reperfusion period. These discrepant CBF values may be results of different CBF measurement techniques (laser-Doppler flowmeter versus audiography) or study designs. The variation between normo- and hypothermia has been also demonstrated as an index of CVR, i.e. MABP/CBF

versus MABP [325]. In the normothermic group, CVR decreased steadily until MABP reached 45–54 mmHg [125,246]. Since CVR increased during further hypotension, it is reasonable to conclude that pH-stable conditions were associated with a failure of normal compensatory vasodilatation. CBF values during hypothermia and constant ionization level of histidin-imidazole do not show a distinct plateau phase. Nevertheless, when CBF is expressed as percentage of the baseline value obtained at 110 mmHg, the CVR curve demonstrates a distinct convexity. Examination of the CVR versus MABP curve also demonstrates that, as in normothermic animals, CVR decreased during hypotension [325], indicating cerebral vasodilatory response with constant ionization level of histidin-imidazole and suggesting that autoregulation is at least partially preserved. In addition, Duebener et al. [78] could recently demonstrate that cerebral tissue oxygenation was significantly higher in the pH-stat group at the end of cooling and during early reperfusion after deep hypothermia. One may suggest that CO_2 is counteracting the leftward shift of the Hb–O_2 dissociation curve during hypothermia. Using the pH-stat technique for acid base management in piglets, the return of cerebral ATP and phosphocreatine after reperfusion is more rapid [6]. Near infrared spectroscopy studies have shown an improved redox state of cytochrome aa^3 with pH-stat relative to alpha-stat [349].

The most convincing explanation of the different CBF autoregulation under hypothermia compared to normothermia lies in the fact that pH-stable management results in an unphysiological hypercapnia. It has long been known that hypercapnia impairs or abolishes autoregulation presumably because CO_2-induced vasodilatation limits the vessels' capacity to dilate further [118,122,256]. Since hypothermic animals with pH-stable CO_2-managment are relatively hypercarbic compared with alpha-stable hypothermic animals, it is possible that vasodilatation was maximal under baseline conditions.

2.7. Inflammation/free radicals

Both clinical and animal studies have demonstrated that mild hypothermia is associated with increased risk of subsequent infectious complications [144,245,318], indicating impairment of the immune response. Immunosuppression (reduced numbers of thymocytes, decreased T-cell blastogenesis, and reduced natural killer cell activity) was demonstrated in mice subjected to cold water stress [48]. Mild perioperative hypothermia suppresses mitogen-induced activation of lymphocytes and reduces the production of IL-1β and IL-2 [19]. In animal experiments, hypothermia leads to a decrease in neutrophil phagocytosis and mobility as well as reduced production of reactive oxygen intermediates [2]. Surprisingly, plasma levels of cytokines such as IL-6 and IL-8 represent data that may be explained by indirect influence of hypothermia on immunocompetent cells.

2.7.1. Immunomodulation

Although inflammatory response in development of ischemic infarction has attracted the attention over the last years, little work has been done to assess the effects of hypothermia on leukocyte accumulation in ischemically damaged brain tissue. Recently, the effects of intraischemic moderate hypothermia (30 °C) on neutrophil infiltration following transient (3 h) MCA occlusion demonstrated that, at 24 h after induction of ischemia, hypothermic animals exhibited significantly less myeloperoxidase activity in the pericore region compared with normothermic controls, although no differences were observed in the core [310]. Maier et al. [198] found that 1 h hypothermia is sufficient to reduce neutrophil accumulation by 75% and 2 h of hypothermia decreases it by 72% observing a decrease in neutrophil accumulation within the ischemic core. Thirty minutes of hypothermia reduced polymorphonuclear leukocyte infiltration by 39%, although this was not statistically significant. When normalized for infarct size, only 2 h of mild hypothermia significantly reduced accumulation of polymorphonuclear leukocytes.

Blood-borne monocytes and macrophages infiltrate infarcted brain tissue after the initial invasion of neutrophils, persisting for a long time (>60 days) after maturation of the ischemic infarct [69,147]. At 7 days after the initial event, monocytes and foamy macrophages are present within ischemic regions under normothermic conditions [197]. After arriving in the ischemic area, these cells can contribute to the exacerbation of reperfusion damage by producing cytotoxic factors, such as glutamate, TNF-α, NO, and free radicals [147,167]. Qualitative observations from the study of Maier et al. indicate that levels of monocytes and macrophages are reduced in regions of tissue injury under conditions of hypothermia [198]. Thus, hypothermia may attenuate the inflammatory response that accompanies ischemia-induced cellular damage [121,161]. On the other hand, because hypothermia was only maintained intraischemically, it is possible that the reduction in inflammatory cell infiltration is merely an epiphenomenon. It could, for example, be simply the result of decreased capillary permeability or reduced BBB breakdown, both of which have been demonstrated to occur during hypothermia [74,75,80,148,151]. Such a hypothesis does not adequately account for the increased incidence of systemic infections correlated with hypothermia. Thus, it can be speculated that in addition to local impairment of host defenses, hypothermia may induce a systemic immunosuppression altering endogenous T-cell cytokine production that favors a Th-2 profile.

2.7.2. Cytokines

Short-term mild hypothermia causes only minor changes in cytokine gene expression, with the exception of the *IL-2* gene. Its expression is markedly decreased in hypothermic phytohemagglutinin (PHA)-stimulated mononuclear cells, indicating an immediate response of T-lymphocytes to decrease temperature [19]. Impaired IL-2 secretion in

long-term hypothermic mononuclear cell cultures confirms the findings at the gene expression level and underlines the importance of IL-2 in hypothermia-induced alterations in the immune response [19]. A direct effect of mild hypothermia on T-lymphocytes is also indicated by inhibition of IL-6, IL-10 and TNF-α secretion in PHA-stimulated mononuclear cell cultures at 34 and 32 °C [179], as PHA stimulates primarily mature T-cells. However, in hypothermic peripheral blood mononuclear cell (PBMC) cultures stimulated by LPS, a potent monocyte activator, demonstrates no changes in IL-6, IL-10 and TNF-α release compared with normothermia [179]. Hence, monocyte secretory activity seems not to be impaired by regulated hypothermia [179]. The release of TNF-α, predominantly secreted by monocytes, is even increased in hypothermic non-stimulated PBMC cultures [179]. The absence of a direct effect of hypothermia on IL-6 release or even its decrease in hypothermic PHA-stimulated cultures of PBMC contrast with increased plasma levels of IL-6 under hypothermia [113]. The varying results may be explained either by production of IL-6 by other cell types (fibroblasts, endothelial cells) in vivo or by stimulation of IL-6 secretion by increased catecholamine levels under hypothermia [77]. Induction of hypothermia in vivo activates a broad neuroendocrinologic spectrum [233], which in turn may exert immunoregulatory effects [71].

In vitro examinations allow an estimation of direct influence of hypothermia on cell function in a relatively simple system. Although the primary sensors that trigger the stress response are not known, miscellaneous stress kinases, transcription factors, and heat shock proteins have been shown to participate [268]. In addition to these factors, hypothermia induces the expression of cold shock domain (CSD) proteins [243]. Because of their DNA and RNA binding ability, CSD proteins are involved in transcriptional activation and repression, mRNA packaging, and translational regulation [226]. Although the mechanisms of cytokine gene expression have not been well characterized, repressor elements have been identified in a number of cytokine gene promoters, such as IL-1β, IL-2, IL-3, IL-8, TNF-α and granulocyte macrophage colony-stimulating factor (GM-CSF) [313]. In the case of GM-CSF, CSD proteins have been shown to repress transcriptions from its promoter [61]. It can be presumed that a similar mechanism underlies the hypothermia-induced inhibition of cytokine expression. Repression of cytokine translation may occur also as a result of CSD protein binding to mRNA [226]. On the other hand, it has been reported that low concentrations of CSD protein may be required for maximal translations efficiency [71] demonstrating a possible explanation for the slightly increased TNF-α secretion. Inhibition of transcription factor activated protein (AP-1) expression by hypothermia also may contribute to the depression of IL-2 transcription [164]. Hyperthermia, in contrast, is associated with increased AP-1 activity and IL-2 transcription [179]. Opposite effects of cold and heat shock are observed for TNF-α too [179].

2.7.3. Free radicals

In vitro studies have shown that brain macrophages are able to release glutamate [204] and other neurotoxins, including reactive oxygen species [62,92]. Therefore, the above-mentioned inflammatory process that follows neuronal damage [99] may represent a source of neurotoxic substances, such as superoxide or NO, which could induce a rise in temperature quite late after the ischemic episode [150,287]: LPS induces the inducible NO synthetase (iNOS) enzyme in microglial cell, which produce NO molecules [189]. The production of NO was reduced to one fifth at 30 °C in contrast to that at 37 °C, which was significantly low in comparison with the proliferation of astroglial cells and fibroplasts at 30 °C. The response to NO is prolonged by hypothermia, suggesting a reduced degradation of NO. Superoxide anions inactivate NO [98], and production of the anions is expected to be reduced at hypothermia. NO-mediated nerve function is protected by endogenous superoxide dismutase (SOD) from NO degradation by superoxide anions [102,212]. SOD increases and prolongs the action of exogenous NO, but only slight or no potentiation of the response to nicotine is induced by SOD [98]. Whether differential effectiveness of SOD and hypothermia on NO responses is due to a different accessibility of these interventions to the site of NO and superoxide generation or to other mechanism remains to be clarified. It has been reported that possible mechanisms of action of hypothermia include a decreased generation of reactive oxygen radicals [98] that are formed by incomplete reduction of O_2 to superoxide and hydrogen peroxide or a reduction of stimulated NO synthesis [348], besides the above mentioned prevention of the Ca^{2+}/CaM kinase II inhibition. SOD cannot prevent the ischemia-induced inhibition of the neurogenic response; this may not exclude an involvement of superoxide in the inhibition, because of a possible barrier for the access of SOD to the site of generation of superoxide and NO. Therefore, the prevention of hypoxic inhibition of the neurogenic relaxation by hypothermia expected to derive mainly from an inhibition of superoxide generation. These experimental findings lead to the notion that neuron-aggravating characteristics of microglial cells, such as their proliferation and production of O^{2-}, NO or TNF-α, are significantly depressed by mild hypothermia. Microglial proliferation, seen at 2 months of recovery, suggests that the initial tissue damage occurring during the first days after ischemia may trigger an inflammatory response that becomes a vicious cycle enhancing a slowly progressive neurodegeneration [99]. Since cooling depresses and hyperthermia enhances inflammation [95], hypothermia may interrupt this cycle, and controversially, increasing core temperature would enhance or reactivate this process.

There is evidence that oxygen free radicals, or more specifically hydroxyl radicals, may play a significant role in the development of microvascular brain damage and subsequent breakdown of the BBB [288]. By reducing the

$CMRO_2$ and depressing the rates of all enzymatic reactions, hypothermia may blunt the generation of free radicals and protect the BBB [105]. Since stimulated leukocytes release oxygen free radicals and increased vascular permeability [75], the hypothermia-induced protection of BBB integrity is partly mediated by attenuation of leukocyte accumulation. The direct relationship between reduced brain edema formation and less permeability of the BBB by hypothermia suggests that one part of the neuroprotective effect of hypothermia is mediated by restoration of BBB [286].

2.8. Spreading depression

Spreading depressions (SDs) has thought to contribute to the growth of ischemic area due to increased Ca^{2+} influx through glutamate activated Ca^{2+} channels [301] and propagation through gap-junctions [28,45,196–198,234,235]. There are only few reports on SDs and the effects of hypothermia. After topical applications of KCl, hypothermia does not affect the brain's ability to depolarize, but mild hypothermia reduces the duration of SDs [301]. Following focal cerebral ischemia [47,332], SDs is increased in number, and their onset is delayed with lower temperature. The reasons for these observations are not clear, but lower temperatures are known to alter membrane fluidity [3] and ion channel [160]. These results could be explained, particularly, as temperature affects the function of proteins involved in regulating cellular fluid and ion homeostasis. Other reports of temperature-dependent SD changes describe slowing and reduced numbers of propagated waves [47]; however, amplitude is not altered [304].

Hypothermia of 30 °C has been shown to reduce the incidence of transient recurring DC potential shifts measured with cortical electrodes during MCA occlusion [47]. Such shifts are believed to be caused by transient cellular depolarization that occurs during SD. These results are consistent with the observation that hypothermia attenuated changes are associated with SD. However, in a recent study done with electrode measurement of $[K^+]_{ex}$ in cortical penumbra, hypothermia did not affect the number of peaks in $[K^+]$ during acute focal ischemia [281]. Such peaks are believed to be caused by SD [47].

Reduced incidence of SD during ischemia could be an important component of the neuroprotective effect of hypothermia, since SD is believed to increase the size of necrotic regions during focal ischemia [28]. Cerebral perfusion (CPP) levels in a region depolarized by SD increase in response to the increased energy demand necessary to restore ionic gradients [135]. SD in the absence of marked perfusion deficit does not produce permanent injury. However, in the partially ischemic region, where CBF is restricted, CPP cannot increase to meet the increased energy demand. This hastens energy failure, which allows Ca^{2+} to cross the cell membrane. High levels of $[Ca^{2+}]_{int}$ activate enzymes that cause necrosis [236,263,282].

2.9. Gene-related mechanism

Four pathophysiological mechanisms have been identified by which hypothermia produce changes in gene expression: (i) generalized cold-induced inhibition of transcription and translation [241]; (ii) inhibition of RNA degradation, which is used by bacteria to increase cold shock protein expression [348]; (iii) increased transcription, mediated by a cold response element in the promoter region of cold-inducible RNA-binding protein (CIRP), a cold shock protein; and (iv) an enhanced efficiency of translation at lower temperatures that is mediated by specialized regions within the mRNA 5′-leader sequences (internal ribosome entry sites (IRESs)) of RBM3, another cold shock protein [41].

Three mechanisms have been postulated by which changed gene expression might occur after return to normothermia following hypothermia [334]. First, severe hypothermia activates signals for a cell stress response interfering sufficiently with processes such as transcription and translation as to preclude stress protein expression until rewarming occurs. This could explain the HSF-1 mediated induction of HSP-70 and HSP-90 expression by human fibroplasts after cold shock at 4 °C [186]. In these experiments, cold-induced trimerization of HSF-1, binding of HSF-1 to the HRE, and increases in HSP expression occurred not during the period of hypothermia but rather after rewarming to 37 °C. A second hypothesis is that rewarming after hypothermia leads to generation of free radicals and other toxic metabolites that are capable of inducing a stress response. The finding that HSF-1 activation following hypothermia only occurs on rewarming can also be explained by this hypothesis [184]. A third hypothesis may be required to explain the peculiar induction pattern of APG-1 (a HSP) and HSP-105 in mouse NIH/3T3 fibroplasts. In these cells, induction of APG-1 and HSP-105 did not occur after a conventional heat shock (by raising the temperature from 37 to 42 °C); rather, it was required that cells first were incubated at 32 °C and then heated to 39 °C [21]. Temperature shifts both from 32 to 42 °C and from 32 to 39 °C increased the binding of HSF-1 to HSEs in the APG-1 promoter. However, whereas both temperature shifts induced HSP-70 expression, only the latter (32–39 °C) leads to APG-1 over-expression. A possible (although untested) explanation for these findings would be that the APG-1 and HSP-105 promoters are under dual control of both HSF-1 and a hypothetical repressor that becomes inactive at hypothermia and whose rate of reactivation is substantially faster at 42 °C than at 39 °C.

2.9.1. Cold shock proteins

Cold shock proteins are thought to be induced during hypothermia at 25–33 °C. The best-characterized cold shock protein is CIRP, a 172-amino acid protein containing an RNA-binding domain [224]. A *CIRP* gene has been detected in animal and human cells, and its sequence is highly conserved in these species [224]. The mRNA encoding this

protein is expressed constitutively in most tissue of adult mice at relatively low levels. However, CIRP is strongly induced in a cell culture model (BALB/3T3 mouse fibroblasts) within 3 h after reduced ambient temperature of 32 °C, with maximal expression detected between 6 and 24 h of exposure [224]. CIRP shares structural similarity with a number of other known RNA-binding proteins, speculating that one of its physiological functions is to protect and restore native RNA conformations during stress [351]. Experimental data suggest that CIRP enhances translation of its target RNA species, at least in part through stabilization of the mRNA. Importantly, cells transfected with an antisense CIRP vector demonstrated decreased CIRP expression and a diminished ability to survive after ultraviolet irradiation, illustrating a the stress response [352].

CIRP may also play a role as a suppressor of mitosis, in the maintenance of differentiated states [91], and in the cold-induced cell cycle arrest, as cells that over-express CIRP exhibit reduced growth rates at 37 °C and a prolonged G1 phase [224]. Conversely, inhibition of CIRP induction at 32 °C (by addition of antisense *CIRP* mRNA to cultured BALB/3T3 cells) attenuates the slowing of cellular growth that occurred at this temperature in normal cells [224].

Another well-characterized cold shock protein is RBM3 that is structurally similar to CIRP. The expression of its mRNA is increased by hypothermia at 32 °C [66]. However, unlike CIRP, RBM3 does not appear to be involved in the cold-induced growth suppression [65]. The tissue distribution of RBM3 appears to be more limited than that of CIRP, as RBM3 is not detected (by Northern blot analysis) in either heart or thyroid, both of which expressed CIRP [66]. Interestingly, the *RBM3* mRNA 5′-leader sequence contains a number of specialized sequences that allow initiation of translation independently of the methylated G nucleotide 5′-cap that is typically used by cells to tag an mRNA molecule for initiation of protein synthesis. These IRESs appear to facilitate translation at 33 °C [41], a temperature that decreases protein synthesis. Other genes known to contain IRESs that enhance translation of reporter constructs at 33 °C are c-*myc* and sequences from poliovirus [41].

2.9.2. Changes in expression of other genes

Many different kinds of transcription factors encoded by the immediate early genes such as the *fos* and *jun* families are induced after cerebral ischemic events [130,153], as demonstrated by in situ hybridization, immunohistochemistry or DNA binding activity. Enhanced expression of a number of immediate early genes in brain by ischemic events has been demonstrated [77,343]: biphasic expression of c-*fos*, *fosB*, *fra-1*, *fra-2*, c-*jun*, *junB* [156,175,178,267] and *junD* [154] in the dentate gyrus in an early period and in hippocampal CA1 and CA3 in a late period after ischemia. Intraischemic hypothermia alters temporal profiles of their expression, suggesting their functional significance as cytosolic signal responses and stress response lining to delayed neuronal death. Marked enhancement of the DNA binding ability of a transcription factor, AP-1, has been demonstrated in gerbil hippocampus of the CA1, CA3 and dentate gyrus including CA4 (hereafter, dentate gyrus) in temporal profiles of the DNA binding activities [154]. Time course of the activity enhancement is prolonged from 1 to 9 h in the CA3 or the dentate gyrus, both being comparatively resistant to ischemia. A rather short-lived enhancement is observed in the CA1, the area extremely vulnerable to ischemia [154]. However, gerbils with prior ischemia at 32 °C showed a prolonged period of binding activation, becoming closer to the profiles for the CA3 and dentate gyrus [154]. Similar prolongation can be seen in gerbils which are rendered resistant to ischemic events by a prior induction of a short ischemic event for 2 min [321,355]. The protein that is synthesized after AP-1 binds to an upstream region of the corresponding gene is still unidentified, but may represent a possible to link to protection or resuscitation of neurons. Different other genes and proteins like zinc finger immediate early genes [168], cycle AMP-responsive element binding protein [327], nuclear factor-kappa B [49,51], heat shock protein [268], heme-oxygenase-1 protein [221,225], cyclin D1 [335], stat 3 [247,251], nerve growth factor, brain derived neurotropic factor and neurotrophin 3 [64,170,188], have been reported, but they have not been analyzed in the state of hypothermia.

2.10. Apoptosis

Recently, suppression of apoptotic neuronal cell death has been suggested as a possible mechanism of the protective effect of hypothermia [108]. Experimental data demonstrated that at 3 days after an ischemic event, 1 and 2 h of mild hypothermia are sufficient to decrease the number of apoptotic cells, as determined by TUNEL staining and morphology, by 78 and 99%, respectively [198]. Thirty minutes of hypothermia, however, revealed no protective effect [166]. This is of particular interest, because the only other study demonstrating reproducible effects of hypothermia on the number of apoptotic cells (in transient global ischemia) used a 12 h hypothermia period following the ischemic event [81]. In that study, mild hypothermia (35 °C) revealed no effect on the cellular fraction undergoing necrosis, although the fraction of apoptotic cells was significantly reduced, suggesting that hypothermia may specifically inhibit apoptotic cellular mechanisms [55]. For this reason, Maier et al. [198] speculate that, although the trigger of programmed cell death may occur during ischemia, cellular commitment to death may follow later, thus allowing hypothermia to interrupt the biochemical cascade leading to apoptosis.

The underlying molecular biological effect of temperature on apoptotic cell death is a matter of debate, mainly depending on the nature and severity of the underlying ischemic event [119]. Using a rat model of mild focal cerebral ischemia (1 h MCA occlusion), qualitative differences in Bcl-2 (antiapoptotic action), Bax (proapoptotic action), and cytochrome *c* release from mitochondria (triggering

cell death) immunostaining could be demonstrated after 1 h of mild hypothermia [252], suggesting that mild hypothermia decreases the density and intensity of staining for Bax and cytochrome *c*, but increases Bcl-2 staining [23]. Similarly, mild hypothermia (33 °C) did not alter Bcl-2 or Bax expression in cultured neurons exposed to serum deprivation, an apoptosis-specific ischemic event, cytochrome *c* release and caspase activity [355]. A major difference in cytochrome *c* release between mild hypo- and normothermic rats 5 h after induction of MCA occlusion (3 h after hypothermic treatment was terminated) was observed [355], a time period, when reactive oxygen species and $[Ca^{2+}]_{int}$ are known to be increased after cerebral ischemia [141]. These data suggest that, although mild hypothermia transiently inhibits cytochrome *c* release, its protective effect does not appear to be due to alterations in other apoptosis-related proteins.

PARP has been suggested to augment ischemia-related neuronal damage because of high-energy substrate needs [84], so that its increased breakdown by hypothermia may lead to neuroprotection. Immunoblots of PARP, a caspase-3 substrate, showed that caspases not involved in apoptotic pathways increasingly cleave PARP to an inactive 89 kDa cleavage product by mild hypothermia [172]. Mild hypothermia leads to an interruption of a necrotic process such as mitochondrial swelling, potentially also interfering with other apoptotic pathways. The extrinsic pathway involves cell surface-receptor activation by ligands, such as TNF-α or fas-ligand, with subsequent activation of caspase-8 and Bid, another proapoptotic Bcl-2-related protein [9], and may be influenced by mild hypothermia. However, both the intrinsic and extrinsic pathways ultimately lead to activation of caspase-3. It has already been reported that caspase-3 activation and apoptosis do not occur after focal cerebral ischemia [57,182].

After 2 h of intraischemic mild hypothermia (33 °C), the number of TUNEL-positive cells in a model of rat transient focal cerebral ischemia decreased [355]. Postischemic moderate hypothermia (35 °C) significantly reduced the number of apoptotic cells compared to normothermia (39 °C), although the number of necrotic cells did not differ between the two groups [81]. Although the presence of TUNEL-positive cells does not necessarily demonstrate that apoptotic cell death is the predominant form of injury in experimental ischemic events, it suggests that mild hypothermia is also associated with decreased DNA laddering [94,123]. However, apoptosis may also occur without caspase activation, as demonstrated that Bax and other factors can induce mitochondrial damage and apoptosis even if caspases are blocked [191]. In this scenario, apoptosis-inducing factor is released from the mitochondria, and is translocated to the nucleus, resulting in chromatin condensation and DNA fragmentation [110,191]. Whether this occurs during cerebral ischemia is not yet known [110], but it may be a potential explanation for the observation that caspase activity remarkably lacks after such events. Maier et al. [198] have found that TUNEL staining is absent before 6 h of reperfusion, but is clearly visible at 24 h. By 72 h after the ischemic event, TUNEL staining is very prominent in hypothermic animals, although to a significantly different degree [166]. Results from DNA fragmentation analysis confirm the latter findings. After 3 days of reperfusion, there is evidence of DNA laddering in the cortex and striatum (ipsilateral to MCA occlusion) of hypothermic animals [199]. Investigation of the temporal profile of in situ DNA fragmentation following 2 h of MCA occlusion, apoptosis first appears within 30 min of reperfusion, peaks at 24–48 h, and persists for 4 weeks after the ischemic event [186]. The differences observed in these studies may be attributed to variations in the ischemic model, as well as in the duration of vessel occlusion. It is possible that apoptosis is triggered by various mechanisms that themselves may have unique temporal profiles and be differentially activated depending on infarct severity. On the other hand, the variability between studies may simply reflect differences in the sensitivity of the assays used in each study. The anatomical distribution of TUNEL-stained cells is another pathophysiological issue that deserves attention. Apoptotic cells are primarily localized in the inner boundary zone of the ischemic infarct and that they appear earlier in the preoptic area and striatum than in the cortex, being consistent with the idea that apoptosis is an active process that requires energy, so the pericore regions of the affected hemisphere may provide the necessary conditions for the induction of programmed cell death [182,183].

3. Management of hypothermia and different modes of outcome control

Permanent histological protection has been demonstrated (see Tables 1 and 2) either by immediate or delayed application of hypothermia under numerous experimental conditions. An important example of temperature-related alterations in postischemic outcome have come from Warner et al. [328] using a model of focal cerebral ischemia in rats, and Wass and Lanier [329] who used a model of complete ischemia in dogs. These authors demonstrated that temperature changes of only 1.2 °C [328] and 1.0 °C [329], respectively, altered both functional and histological outcome. Although hypothermia, in comparison with normothermia, causes a stable reduction of cerebral infarct volume, the possibility cannot be excluded that this effect is only transient and vanishes after longer periods [305]. Experimental data on long-term neuroprotective effects of hypothermia are inconclusive for global as well as for focal cerebral ischemia in the experimental setting [73,157]. However, such data of possible slow maturation of neuronal degeneration may be relevant in the clinical setting, in the sense that in patients in whom progressive neuropsychological changes cannot be detected within weeks after ischemia, may be evident years after initial stroke.

3.1. Neural mechanism of thermoregulation

Physiological thermoregulation in mammalian species is complex, as different interactions with other physiological processes of the central and peripheral nervous system are additionally involved [107]: the principal function of the thermoregulatory system contains the maintenance of a stable core temperature of internal tissues and organs by allowing temperature of skin and peripheral tissues to vary with the temperature of the environment. A reference temperature point, generated by a network of warm, cold, and thermal insensitive neurons in the preoptic area and hypothalamus, is compared with feedback informations from the thermoreceptors in the skin and core. The hypothalamus temperature depends mainly on the arterial blood temperature. A negative feedback activates the appropriate thermoeffector pathways that control the balance between heat production and heat loss.

General anesthetic agents impair the body's ability to regulate core temperature by inhibiting temperature control and peripheral temperature conservation mechanisms. Isoflurane produces a dose-dependent decrease in vasoconstriction by 0–1.5 minimum alveolar concentration [298] and 3 °C/% of end-tidal isoflurane concentration. NO depresses the vasoconstriction threshold less than equal minimum alveolar concentrations of potent inhalation agents [88]. Most general anesthetics also cause direct vasodilatation, increasing skin blood flow, skin temperature, and surface heat loss. Induction of anesthesia by propofol causes greater peripheral vasodilatation than induction with an inhalation agent. This in turn results in a more rapid decrease in core temperature [142]. However, an induction with ketamine, a vasoconstrictor, has the opposite effect [143].

3.2. Cooling technique and temperature measurement sites in hypothermia

It has been theorized that brain temperature is mainly related to three physiological factors: (i) CBF; (ii) CMR; and (iii) heat exchange with the environment [122,327].

The quantities of *cerebral blood flow* to the temperature gradient between brain tissue and blood determines the magnitude and direction of heat exchange [12,122]. The temperature of blood perfusing the brain represents the primary determinant of brain temperature [274]. Thus, brain temperature is generally in good accordance with core temperature in the normal, non-ischemic brain tissue [274].

Cerebral tissue has one of the highest metabolic rates of the entire body, and, as a result of this metabolism, heat can be produced [274]. This probably explains why the temperature of the resting cerebral tissue can be 0.1–0.9 °C warmer than core temperature in some species [12,274].

The temperature of the *environment* immediately surrounding the normal animal brain (i.e. bone, muscle, skin) will normally be at or slightly lower than core temperature [327]. However, if the temperature of these surrounding tissues is lowered further, the temperature of the brain should decrease, beginning within the outermost layers [327].

3.2.1. Cooling technique

Several cooling techniques for inducing and maintaining hypothermia have been developed. To reduce core temperature for prolonged periods below 30 °C, extracorporal circulation is required due to major circulatory instability caused by cardiac arrhythmias. An extracorporal circulation is unnecessary to reach a target core temperature between 32 and 34 °C. Cooling to mild to moderate hypothermia is usually performed by conductive (e.g. liquid-circulating water mattress) or convective (forced air cooling via full body blankets or airbeds) surface cooling, cold infusions, gastric lavage, or simply by leaving the anesthetized animal uncovered in a cool environment [307]. Cooling from inside the body, through either the intravenous or intraarterial route, represents an alternative method that has the potential to induce hypothermia more rapidly than surface cooling [326]. Insertion of an intravenous catheter combined with balloons perfused with cold saline solutions is a novel technique that avoids volume overload. Selective cooling of the major intracranial arteries by means of transfemoral catheterization, which has been performed only in baboons, may be an attractive method of inducing hypothermia. The use of pharmacological hypothermic agents may offer a method to reduce core temperature [158].

Achieving and maintaining a desired target core temperature is a challenging procedure. For example, using a canine model, core temperature is passively decreased to 32.4 ± 0.3 °C following discontinuation of active hypothermia at a target core temperature of 33 °C [177].

3.2.2. Temperature measurement sites

Typical temperature measurement sites are tympanic membrane, pulmonary artery catheter, nasopharyngx, esophagus, rectum, and skin [276]. Intracranial temperature can be measured via epidural or intraparenchymal probes. The focus of interest with regard to the neuroprotective potential of hypothermia is certainly the intraparenchymal temperature of brain tissue and the temperature gradients. Such temperature gradients of 0.4–1 °C have been observed in men between the ventricular system and the epidural space, even during normothermia, without temperature manipulation [209] or in cases of cerebral ischemia [35]. Tympanic temperature closely correlates with epidural space temperature [209]. Because of the easily accessible of rectal, esophageal, and nasopharyngeal locations, there is a main interest in the gradients of brain temperature between these sites. For core temperature measurements, esophageal and pulmonary artery temperature have been found to be well suited [308].

3.3. Hypothermia and time-window of effectiveness

Previous studies have indicated a possible therapeutic window of up to 2 h after ischemia [33,52,346]. During

this time period, hypothermia can be initiated demonstrating neuroprotective effects [242,275]. However, in many reported animal studies, hypothermia was initiated at the onset of MCA occlusion, whereas in only a few investigations hypothermia was initiated at a later time point. In this context, the experimental investigated rats were subjected to 3 h of focal ischemia and cooled to 32 °C in various regimens [346]. Hypothermia, lasting 3 h and beginning at the time of MCA occlusion, led to 92% reduction in cortical infarct volume. Hypothermia for 1.5 h was demonstrated equally effective if begun at the onset of MCA occlusion or delayed by 1.5 h (45–49% cortical rescue), whereas a 3 h hypothermia period delayed by 1.5 h achieved greater protection (73% cortical salvage). In other experimental studies [11,127,157], a delay of up to 2–3 h in initiating hypothermia is considered, but in none of these studies longer durations of hypothermia systematically is evaluated.

The time-window of successful therapeutic opportunity for transient focal ischemia appears to be shorter than that for brief global ischemia. In a model of transient focal cerebral ischemia, a significant reduction (32%) in the volume of infarction was obtained, when mild hypothermia was established immediately after reperfusion and was maintained for a prolonged time period. Delayed induction of hypothermia (30 min) induction following reperfusion failed to achieve a significant reduction of infarct volume [353]. This observation leads to the assumption that mild hypothermia affects some of the cellular injury mechanisms that occurs early in the reperfusion process. Further amelioration might be achieved by starting the hypothermic treatment 30 min before reperfusion and continuing it for prolonged periods into the postischemic phase [162], underlining the hypothesis that the time-window of effectiveness may be very small and from the experimental point of view it seems that it makes sense to begin the hypothermia treatment as fast as possible after the ischemic event, but not more than 3 h after. This assumption may be related to the possible favorable effects initiated by hypothermia within this small time-span.

3.4. Depth of hypothermia

Using hypothermia as cerebroprotective therapeutic approach, its optimal depth is currently discussed. Since standard measures for outcome are still missing in animal models, this discussion continues up to now. The available experimental data demonstrate the necessity of careful postischemic temperature assessment in pharmacological studies of cerebral ischemia, because differences of only 1.0–2.0 °C, if prolonged, can significantly affect outcome.

In an immature rat-model of induced intraischemic hypothermia at 37, 34 or 31 °C for 3 h and histopathological brain examination at 23 days of recovery, intraischemic hypothermia of 31 °C completely protected the brain tissue from damage, 34 °C produced neuronal injury in 30% of animals, only one of which demonstrated a cerebral infarction and 90% of the animals exposed to ischemia at 37 °C exhibited brain tissue damage [347]. However, hypothermia at either 34 or 31 °C for 3 h immediately following the ischemic event at 37 °C showed no effect on the extent of brain tissue damage when compared with data in normothermic control group. In contrary to these findings without any difference between the two hypothermic group (29 and 33 °C), temperature-dependent reduction in volume of cerebral infarction have been reported [107]. In that study, infarct volume was reduced by 22.4% in mild hypothermia (33 °C) and by 49.5% in moderate hypothermia (29 °C), suggesting that a more intensive ischemic challenge may require larger temperature reductions to obtain significant neuroprotection. In fact, the 3 h model of reversible ischemia may be more analogous to permanent focal ischemia, since the size of infarction obtained is not different from what others have found using permanent MCA occlusion models [210]. Providing similar degree of neuroprotection, 33 °C is associated with more stable intraoperative hemodynamic and respiratory status as well as with improved recovery from anesthesia compared with 30 °C [198]. In addition, average postanesthesia recovery time showed that animals in the 33 °C group recovered at a significantly faster rate than normothermic controls, whereas animals in the 30 °C group showed no improvement in recovery time [198].

From the experimental point of view, there is no question that moderate intraischemic hypothermia following global ischemia delays the onset of ischemic histopathological alterations leading to cell death. The question remains whether postischemic hypothermia alone is useful in delaying the deleterious effects of ischemia.

3.5. Duration of hypothermia

Studies in experimental global ischemia have shown that, although shorter durations of postischemic moderate hypothermia are not permanently neuroprotective [73,360], prolonged reductions in body temperature confer sustained behavioral and histological neuroprotection [58,59]. Prolonged postischemic hypothermia in focal ischemia has been less extensively investigated [203,353,357,359]. One such study compared 1 and 3 h periods of hypothermia to 30 °C, which were initiated after a 2 h MCA occlusion [358]. Hypothermia for 3 h reduced cortical infarct volume by 84%, whereas cooling for 1 h was not significantly protective. Recent experimental data comparing 30 min and 2 h hypothermia periods to 33 °C in a rat MCA occlusion model, similarly led to the conclusion that longer hypothermia duration enhanced the degree of neuroprotection [199]. From the few experimental studies available, there is a neuroprotective effect of hypothermia, when it is maintained for 3–22 h [58,59,73,140,197,203,353,357,359], but the volume of protected cerebral infarction depends on the duration of lowering the brain temperature.

It is obvious that a prolonged period (48 h) of moderate hypothermia (29 °C) has severe deleterious effects in different animals [294]. This is in concordance with a previous study

in which 48 h of hypothermia was found to aggravate rather than ameliorate the effects of regional cerebral ischemia in monkeys [213]. These data would seem to conflict with the many favorably reports describing the beneficial effects of mild of moderate hypothermia initiated in the postischemic period. To our knowledge, all these reports have been concerned with short-term application of hypothermia only (up to 24 h). Other experimental studies following hypothermia of longer duration have been equivocal or even negative [211,220,299]. The presumed basis for such protection is the known direct effect of temperature on metabolic rates [340]. It is suggested that prolonged hypothermia may hinder the phase of protective hypermetabolism. However, although it may not be necessary to maintain intraischemic hypothermia for more than 1 h, if started within 30 min of the focal ischemic onset in animal models, longer periods of hypothermia may be needed to achieve neuroprotection following permanent vessel occlusion, if initiated in a delayed fashion after ischemic onset, as it is only available in clinical conditions.

Conflicting data exist regarding hypothermia that is started after 3–6 h of induction of ischemic infarction: mild hypothermia has been shown to lead to significant reduction of cerebral cortical infarct volume, if it was induced directly within 3 h after induction of ischemic event, but not, if it was induced later [98,140,171]. These data provide evidence that, within limits, increasing either the depth or the duration of hypothermia should improve histological outcome. Nevertheless, animal data suggest that mild, in contrary to moderate, postischemic hypothermia may not translate to permanent neuroprotection, even if the duration of hypothermia is prolonged (i.e. several days). In studies in which survival times have been increased from 7 days to 1 month, pharmacological neuroprotection has been lost [165]. The cascade of events resulting in neuronal necrosis may only be temporarily interrupted during the hypothermic period, and once temperature is normalized, these processes may resume, albeit at a slower rate. The severity of ischemia will probably determine the length of time that is necessary to regulate postischemic temperature and prevent hypothermia from confounding histological outcome.

3.6. Rewarming

The rewarming period has been considered as a time of high risk for brain tissue damage, because metabolic needs may outstrip O_2 release. Hence, rewarming is generally considered a critical phase of hypothermic therapy [297]. The exact pathophysiological background of this rebound after rewarming is not known, but it might be due to a proposed hypermetabolic response after induced hypothermia. Normally, protective thermoregulatory mechanisms that increase core heat are impaired after cessation of hypothermia because of the anesthetics and opioids administered.

During rewarming the most striking observation is a further reduction of cardiac output [14]. A maintained low cardiac output during and after rewarming is associated with fatal outcome [207]. In addition, substantial depression of LV-myocardial function has been reported [14]. Based on these studies, cellular Ca^{2+} overload, disturbed Ca^{2+} homeostasis, changes in myocardial myofilament responsiveness to $[Ca^{2+}]_{int}$ as well as impaired high energy phosphate homeostasis could all be proposed as important factors leading to the changes observed in the hypothermic heart and contributing to failure of functional recovery during rewarming.

Reduced plasma volume after long-term hypothermia is generally observed [46]. Plasma volume in posthypothermic rats was reported to be 23% lower than that of normothermic control rats [315]. Although the reported reduction in total blood volume did not reach significance, when compared to base levels, on average 12% of circulating blood volume was missing after rewarming. Thus, hypovolemia can be ruled out as an important factor to explain posthypothermic cardiac output reduction, as a 12% reduction of circulating blood volume is within the range handled by an integrated circulatory regulation [315]. Despite the reduction of plasma volume during hypothermia, an increase of plasma volume to above prehypothermic levels has been reported after rewarming from short-term hypothermia [86].

The integrity of sympathetic nervous function and peripheral vascular tone has been studied after rewarming [316]. Spectral analysis of the low frequency component of the diastolic blood pressure power spectrum has suggested that the reduction in sympathetic peripheral vascular tone found after rewarming is partly responsible for the fall in arterial blood pressure. In the same study, a lack of variability of posthypothermic vascular tone was observed during low cardiac output and blood flow disturbances [316]. Alterations in sympathetic peripheral vascular control could be an important mechanism for the development of rewarming shock.

3.7. Hypothermia and side-effects

Prolonged hypothermia is known to have several side-effects [254,273], limiting its use not only in experimental, but also in clinical condition and affecting every organ system. However, it is well accepted that moderate hypothermia is much safer than the classic deep hypothermia [138,190,213,293]. Nevertheless, the benefit of hypothermia exceeded its possible adverse effects [309].

Table 3.

3.7.1. Central nervous system

Increasing degree of hypothermia results in a progressive reduction in neuronal function that may manifest as altered consciousness (disorientation, stupor or coma) (see Table 4) and is clearly correlated with the duration of deep hypothermia [82]. In common with many central nervous system depressants, hypothermia causes a transient increase in minute ventilation and oxygen uptake during mild hypothermia [145] before producing the more characteristic

Table 3
Genes whose expression is affected by hypothermia

Functional	Gene	Change	Exposure model	Time of change	Proposed mechanism/comments	References
Cell cycle	*p53*	Up	Cell culture	During and after hypothermia	Inhibition cell cycle progression	[206,230]
	WAF1/p21	Up	Cell culture	During and after hypothermia	Induction in cells carrying wild-type but not mutant *p53* genes	[206,230]
Heat shock proteins	*HSP-70*	Up	Cell culture	After hypothermia	Activation of HSF-1 on rewarming	[129,155,176,185]
	HSC-90	Up	Cell culture	After hypothermia	Activation of HSF-1 upon rewarming	[129,185]
	HSP-105	Up	Cell culture	After hypothermia	Dual control: HSF-1 binding to HSE in the promoter plus a resetting of the set point of a cellular temperature sensor	[155]
	APG-1	Up	Cell culture	After hypothermia	Same as for *HSP-105*	[155]
RNA binding	*CIRP*	Up	Cell culture	During hypothermia	Putative cold response element in the promoter	[222–224]
	RBM3	Up	Cell culture	During hypothermia	Enhanced efficiency of translation through IRESs in the 5′-leader sequence	[41,65,66]
Signal transduction	*NF-1* variant	Up	Cell culture	During hypothermia	Alternative splicing, leading to mRNA with an additional exon	[8]
Unknown	*KIAA0058*	Up	Cell culture	During hypothermia		[91]

depression below 35 °C [87]. Both respiratory rate and tidal volume are reduced and appear to result from central nervous system effects as experimentally selective rewarming of the brain stem reverses these respiratory effects.

Thermoregulatory shivering is the most common effect of hypothermia beginning at levels of 36.5–38.5 °C, whereas a decrease by 1 °C increased the probability of shivering by 35% [87]. More recent carefully controlled studies suggest that total body oxygen consumption increases by 40–100% during shivering, resulting systemic hypoxemia and metabolic acidosis [273]. Nevertheless, such increases in oxygen consumption could still have adverse effects on organs such as the heart or brain which may have fixed obstructions to their arterial blood flow [87].

At temperatures different from 37 °C, the optimum level of ventilation, and hence pCO_2 and pH, for non-hibernating homeotherms is yet unknown. It has been established that poikilotherms do not adjust ventilation with a decrease in temperature and thus pCO_2 remains constant (uncorrected for temperature) [253]. If this is appropriate for homeotherms (with abnormal pCO_2 of 35 mmHg) then at 29 °C the appropriate corrected pCO_2 would be 24 mmHg. Such a relative respiratory alkalosis would further shift the O_2–Hb dissociation curve to the left (hypothermia per se results in a leftward shift of the curve) and might aggravate tissue hypoxia by limiting off-loading of O_2. On the other hand, hibernating animals tend to maintain a constant pH with reduction in body temperature [261]. If this is appropriate for homeotherms, then corrected pCO_2 at 29 °C should remain close to 35 mmHg (equivalent to a pCO_2 of about 50 mmHg uncorrected) and the leftward shift of Hb–O_2 dissociation would be reduced. An additional important variable altered by CO_2 is the CBF. In normal hypothermic rat, CBF remains responsive to CO_2. CBF is reduced to 15% of control at a pCO_2 of 15 mmHg (corrected) and a temperature of 22 °C [115]. They observed no adverse metabolic effects resulting from this low CBF state. Again, the possible effects on CBF in areas of regional ischemia is unknown. The maintenance of a constant uncorrected pCO_2 (as in poikilotherms) might, by combined effect on oxygen–hemoglobin dissociation and CBF, aggravate regional ischemia, thus accounting for the untoward effects of hypothermia, so that, survival in animals maintained at a corrected pCO_2 of 35 mm Hg was not improved [189].

Table 4
Clinical scale for temporary neurological dysfunction

Grade		Duration
1	Simple confusion	Short (<48 h)
2	Confusion and lethargy	Short
3	Confusion and agitation	Short (<72 h)
4	Overt psychosis	Long (>72 h)
5	Psychosis and parkinsonism	Long

3.7.2. Cardiovascular system

Hypothermia results in activation of the sympathic nervous system as is evident by the huge increase in circulating norepinephrine and the associated vasoconstriction. The vasoconstrictive threshold begins only about 0.2 °C below normal core temperature in awake individuals. There is also minimal increase in adrenal hormones. The sympathetic effects both through peripheral vasoconstriction and coronary vasoconstriction may cause with the subsequent problems of cardiac arrhythmia, hypertension and myocardial ischemia. Cardiac function is impaired at temperatures below 32 °C. Left ventricular dysfunction is found during prolonged, but not during brief periods of hypothermia [314]. The threshold for arrhythmia seems to be about 31 °C, and as temperature falls below 30 °C ventricular fibrillation becomes

increasingly likely [88,89]. The cardiac conduction system is cold-sensitive, leading to symptoms like bradycardia, prolonged PR intervals, widening of the QRS-complex, and prolongation of the QT interval resulting in the typical "J wave" after hypothermic therapy [88,89].

Hemoconcentration and low microcirculatory flow contribute to the known increase in blood viscosity of 4–6% for each 1 °C in temperature reduction [34]. Cold-induced diuresis caused by suppression of ADH and shunting of peripheral blood volume centrally leads to further depletion of intravascular volume. Hypothermia plays, therefore, a greater risk for hypovolemia when compared to normothermic individuals, particularly, once rewarming is started.

3.7.3. Gastrointestinal tract

Pancreatitis with high serum amylase and lipase levels is observed after hypothermic therapy [83,336]. This association between hypothermia and pancreatitis is poorly understood.

3.7.4. Renal tract

Increased depth of hypothermia results in the progressive depression of renal tubular cell function and a reduction in the reabsorptive function. Sympathetic stimulation and vasoconstriction may also contribute to the altered diuresis. These pathophysiological changes explain the severe polyuresis after induction of hypothermia that can cause severe electrolyte depletion [248]. This is important because even brief episodes of hypovolemia can affect CPP [249] and electrolyte disorders can cause severe arrhythmias, which decrease CBF [248]. Severe disruption of fluid balance, electrolyte levels and CPP are most likely to occur in the phase during which core temperature is falling [248].

As hypothermia-induced hypokalemia is due to a combination of increased urinary loss and intracellular shift, there is a risk for hyperkalemia during rewarming due to reversal of the intracellular shift, i.e. mobilization of potassium from the cells.

3.7.5. Immune system and infection

Leukocyte mobility and phagocytosis are decreased during mild hypothermia [273]. Reversible pancytopenia has been described in association with moderate hypothermia of 31 °C [273]. Mild hypothermia under halothane anesthesia led to increased bacterial counts and a larger area of induration after a standard bacterial dermal inoculation [273]. Immunologic depression combined with decreased cutaneous blood flow during mild hypothermia [273] and surgical manipulations may, therefore, increase the risk of wound infection. Immune suppression associated with hypothermia may thus account for the trend toward higher sepsis and pneumonia rates in recent trials employing mild hypothermia. Hypothermia has been demonstrated to reduce the functioning of neutrophils, lymphocytes, and macrophages [273], viewed as experimental curiosity. However, a recent study of patients undergoing colon surgery found a statistically significant higher incidence of wound infections in patients who has experienced mild intraoperative hypothermia [273].

Mild hypothermia induces a number of hormonal changes, such as an increase in plasma corticosterone and thyroid-stimulating hormone, a decrease in plasma prolactin, and a decrease in hypothalamic thyrotropin-stimulating hormone (TSH), a decrease in hypothalamic thyrotropin-releasing hormone (TRH). It is suggested that mild hypothermia causes prolactin release through a central dopaminergic mechanism and that the increased TSH levels are due to release of TRH [273]. These findings indicate that mild hypothermia is associated with widespread physiological alterations, the full extent and implications of which have yet to be defined.

3.7.6. Hematological effects

Many of the effects of hypothermia on blood viscosity and coagulation have been undetected in the majority of studies because laboratories typically perform their studies at 37 °C. In vitro and animal data show coagulation abnormalities including reversible platelet sequestration and dysfunction, enhanced fibrinolytic activity and slowing of bleeding times (aPTT, PT, TT) of hypothermic plasma (<37 °C) are prolonged if the assay temperature is hypothermic [35]. At 34 °C, PT and PTT increased by 9% compared to 37 °C [101], interfering with the enzymatic steps in the clotting cascade, independent of clotting factor level [100,101,255].

Platelet function appears to be adversely affected by mild hypothermia [238]. Surface cooling to 32 °C can produce reversible platelet dysfunction [317]. In hypothermic (30 °C) pigs, Oung et al. [237] found that bleeding time was nearly doubled. Thrombocytopenia has only rarely been reported in environment-induced mild hypothermia [39]. Coagulation system effect was explained by temperature-induced reduction of enzyme activity in the humoral and cellular coagulation system [43,44]. Data regarding platelet count and function indicate that decreasing temperature first reduces platelet function followed by reduction of platelet count by a reversible sequestration into the spleen. The coagulopathy associated with hypothermia is the result of reduced clotting factor activity as temperature declines, rather than any reduction in the amount of clotting factors present. Clotting ability will recover as the patients warms up.

3.7.7. Altered pharmacokinetics and pharmacodynamics

Hypothermia increases the effects of central nervous system depressant drugs and prolongs the duration of action of drugs dependent on enzyme systems for their clearance [139,181]. This is at least partially due to an additional decrease in hepatic blood flow during hypothermia [131]. For example, the MAC of inhaled anesthetics is progressively reduced by hypothermia and the duration of action of non-depolarizing neuromuscular blockers is prolonged. The duration of action of vecuronium neuromuscular block (10% recovery if first twitch) is prolonged by 120% at a body temperature of 34.5 °C. The plasma clearance of vecuronium is

reduced by 11.3% for each degree C decrease in core temperature (more in women than in men).

3.7.8. Interference with diagnostic and monitoring methods

Monitoring neuromuscular blockade by compound electromyogram may be more misleading during mild to moderate hypothermia, compared with monitoring mechanical twitch tension [271]. Furthermore, mild hypothermia to 32 °C markedly prolonged the rate at which serum concentration of D-tubocararine and twitch tension equilibrated, resulting in an apparent marked delay in onset of paralysis which could be interpreted as decreased sensitivity. Hypothermia decreases the adductior pollicis twitch response, prompting the recommendation to maintain the temperature of this muscle above 35 °C [271] for optimal monitoring of neuromuscular block.

Hypothermia reduces carbon dioxide production and may lead to excessive respiratory alkalosis if appropriate ventilatory adjustments are not made. Furthermore, owing the altered gas solubilities, blood gas tensions and pH are affected by hypothermia, although the clinical significance of these changes is controversial. With each °C decrease, pH increases by 0.05, pCO_2 decreases by 4.4%, and pO_2 decreases by 7.2% [260]. Misinterpretation of "corrected" blood gas results may result in relative brain tissue acidosis and abolition of CBF autoregulation.

3.8. Electrophysiological monitoring and hypothermia

It is difficult to determine to what extent the histologically demonstrated protection against ischemia is followed of pure preservation of neuronal function or reflects only brain tissue recovery without functional relevance. While hypothermia largely prevents cellular death, neuronal function could still be abnormal, albeit masked by recovery/compensatory processes. Indeed, neurons salvaged by postischemic hypothermia are not always entirely normal with respect to ultrastructural features. One way to avoid these interpretative pitfalls is to assess the functional state of neurons by characterizing their electrophysiological properties.

3.8.1. Electroencephalogram

Hypothermia is generally followed by a progressive reduction of both frequency and amplitude in EEG [85]. Increases in beta-activity during moderate hypothermia may be related to a neuronal hyperresponsiveness. However, the combined effects of hypothermia and anesthetics have to be taken into account, as most of the studies are performed during presence of various background anesthetic drugs. The brain tissue during rewarming seems to have its own speed for regeneration of the EEG following a slower time course than the core temperature [257]. When normothermia has been restored, residual EEG changes may persist until the neurons recovered to a normal functional status.

EEG recordings have been used to establish objective criteria to consistently identify a safe level of regulated hypothermia [67]. Electrocerebral slices are taken as an end-point in which no electrical activity of cerebral origin could be detected as this state is thought to be consistent with cerebral metabolic inactivity during hypothermia, occurring in a temperature range of 20–22 °C [342]. Woodcock et al. [341] conclude that hypothermia of 26–30 °C, which does not extinguish EEG activity, provides more cerebral metabolic depression than does pharmacological EEG suppression at 37 °C. EEG suppression and reduction in metabolic rate may be produced at moderate hypothermia using thiopental, isoflurane or propofol [180]. As cause of the sensitivity of the EEG to a variety of physiologic variables, EEG changes during hypothermia may be difficult to interpret.

3.8.2. Evoked response

Recording of evoked responses is used for the assessment of peripheral and lemniscal pathways up to the sensory cortex and may provide information about cerebral function, even when the EEG is completely suppressed by anesthetic agents [76]. Changes in the evoked responses of all modalities induced by hypothermia are due to two basic effects on neuronal activity: progressive slowing of axonal conduction and increased synaptic delay [164,263]. This accounts for increases in latencies and decreases in amplitude of major EP components with more profound effects on later components [199].

3.8.2.1. Auditory evoked responses (AEPs). BAEP amplitudes increase during progressive hypothermia with a maximum at 28–32 °C [202] and cannot be reproducibly recorded at temperatures above 25 °C, independent to differences in species (e.g. poikilotherm versus homeotherm) [146]. It seems that the rate of temperature change during hypothermia appears to be important in BAEP response [36]. BAEP amplitudes increase, when temperature is rapidly shifting and decrease with stabilized temperature [339]. Temperature fluctuations, especially in the cuneate nucleus, significantly affect BAEP amplitudes [4]. Other experimental data suggest that membrane potential and membrane resistance in cat spinal motoneurons follow different time courses when temperature is shifting [250]. These changes in membrane characteristics may explain differential changes in amplitude, in that hypothermia-induced depolarization may result in smaller action potentials, whereas increased resistance may result in larger spikes once cells have exceeded firing thresholds [146,280]. Another possible explanation for discrepancies among studies is the difference in the stimulus levels used. Hypothermia related BAEP amplitudes increase at stimulus levels of 75–90 dB, but have not been seen at lower stimulus levels [38]. With lowering brain temperature, latencies of BAEP waves I, III and V are progressively increased being smallest for wave I and greatest for wave V [202] and the effect of amplitude may be related to the time course of temperature changes [146]. With rewarming, all of these changes occur in reverse order [202]. Experimental

data suggest that during hypothermia from up to 27 °C alterations in BAEP are due to disturbances induced both in axonal propagation and synaptic transmission by hypothermia [290]. It was concluded that the hypothermic effect on synaptic transmission is 1.3–1.7 times greater than on axonal propagation.

3.8.2.2. Somatosensory evoked responses (SEPs). Being less influenced by anesthetics and hypothermia [25,265,362], recovery of SEPs may be related to the degree of damaged subcortical afferent white matter tract [159]. After 4 h of permanent focal ischemia in rabbits, SEP was measured at 37, 33 and 30 °C [192] and SEP recovered significantly in the hypothermic compared to the normothermic group and has been related to a better neurological outcome following transient postischemic hypothermia after experimental cerebral ischemia [13]. However, other studies have shown that there may be a poor correlation between neuronal activity and neurological recovery [190].

During hypothermia, peripheral and central conduction times are significantly prolonged [106,114,216]. A more profound increase in the early cortical response is probably caused by an additional suppression of synaptic transmission in the lemniscal–thalamic pathway [27]. Later cortical components, which involve an increasing number of synaptic transmissions, are slowed progressively. This may be explained by earlier findings showing that prolonged latencies of potentials from presynaptic structures were the result of slowed conduction velocity [23]. Components from structures with interposed synapses showed a more pronounced prolongation of latencies. From the interpretation of changes in SEP components, it has to be taken into account that the temperature–latency relationship and peripheral/central conduction time during hypothermia is different form that during rewarming [166]. The more profound increase in the early cortical component may be explained by additional suppression of synaptic transmission in the lemniscal–thalamic pathway. The later cortical SEP components were slowed to a greater extent, because an increasing number of synapses are involved in the generation of these peaks.

3.8.2.3. Visual evoked responses (VEPs). VEP latencies increase by 10–20% at a temperature of 33–34 °C when compared to normothermia [73]. This is consistent with data during normal sleep when core temperature is below 20 °C daytime normothermia BAEP latencies are increased by 6–7% [169]. However, hypothermia to the level of 33.5 °C seems to be at the borderline of significant effects upon VEP [73].

4. From experimental towards clinical use

There are limitations to the above-mentioned animal model studies. Hypothermia is effective in reducing neuronal injury when applied intraischemically, but a more clinically relevant issue is whether hypothermia can protect if applied hours after ischemia or once reperfusion has occurred. Some studies [9,343,358] on focal ischemia have shown that delaying hypothermic onset up to 2 h after induction can still be beneficial, provided hypothermia is maintained for at least 1–3 h. Longer durations of mild hypothermia with 2–3 h of delay have not yet been studied in animal models. Secondly, since injury has been assessed at 24 and 72 h postischemia, the possibility cannot be excluded that mild hypothermia simply delays neuronal damage. Indeed, the work on global ischemia has shown that mild hypothermia (applied for 3 h at reperfusion onset) is very effective at reducing neuronal damage 3 days after ischemia, but the protective effect is less impressive at 7 days [73]. By 2 months, cytoprotection is lost unless hypothermia is applied intraischemically.

Even though it is unrealistic to expect similar results in all brain regions (e.g. therapeutic windows may be different), a few investigators focus on hippocampal CA1 region. Additionally, several studies rely solely on histopathology, such as CA1 cell counts or infarct volume measurements, to measure therapeutic efficacy even though functional outcome is a clinically more relevant end point. Notably, several factors complicate the relationship between histopathology and behavior, such as innate variation in performance, presence of undetected damage, selective protection by a treatment, and recovery of function [96]. Neurons may also appear normal in Nissl stains, but not function properly [133]. Function thus cannot be precisely gauged by histology alone.

Animal studies are sometimes limited by the use of too short follow-up time [61] or lack of functional assessment, or both. The use of short survival times is problematic because cell death has been shown to continue for days or weeks after mild ischemic events [77]. This condition is analogous to that created by a partially effective neuroprotective intervention. For example, brief duration (for example, 2–3 h) of postischemic hypothermia may appear quite effective with short survival times (that is, <1 week), but in reality may only postpone cell death as has been noted in global ischemia [77]. A previous study [61] addressed the tissue of long-term benefit after prolonged (that is, >24 h) postischemic hypothermia because a persistent reduction in cortical, and to a lesser extent striatal, infarct size was seen at a survival time of 60 days. A persistent histological and functional protection in a standardized stroke model is an important advance over previous efficacy studies, especially given that hypothermia was delayed 2.5 h after the onset of ischemia [53].

The ideal hypothermic therapy is unknown. In global ischemia, brief cooling is either ineffective or only transiently beneficial [71], whereas longer bouts of hypothermia convey substantial protection [54]. Furthermore, longer durations of hypothermia not only increase protection but also extend the possible therapeutic window to at least several hours postischemia. Because no study has compared diverse periods of

hypothermia in focal ischemia (for example, 12 h versus 24 h versus 48 h), it is unknown whether the currently suggested period of experimental hypothermia of about 24 h is necessary to achieve a sufficient neuroprotection or whether a more prolonged therapy would be more efficacious. There is also a variability in the degree of physiological regulation (e.g. temperature, glucose, blood pressure). Surprisingly, many investigators continue to use rectal and/or skull temperature measurements even though they do not reliably reflect brain temperature [29]. Thus, unknown variations in brain temperature make the quantification of the severity of the ischemic damage difficult. Careful physiological control (e.g. blood gases, shivering) during hypothermia is also important. However, few studies closely mimic the manner in which hypothermia is produced in humans. This is especially true in rodents because of the technical limitations (e.g. limited blood sampling) of prolonged physiological control under anesthesia.

The majority of experimental studies that investigate the potential benefits of hypothermia have been performed in mice, gerbils, and rats. There are species, sex, and strain differences in tolerance to ischemia [15,22,99,195,239] and species differences in tolerance to hypothermia [1,124,132]. Lack of protection in one model, therefore, does not necessarily mean lack of protection in other models or in humans. However, differences in thermoregulatory response between rodents and humans subjected to ischemic events hampers the extrapolation process. Many experiments use young adult animals, whereas most strokes occur in the middle-aged to elderly. In fact, young animals have been shown to be more resistant to ischemia [298], and they may also tolerate hypothermia better than old animals [1].

Rodents have relatively large surface area/body mass ratios and are capable of rapidly lowering core temperature. As body mass increases, the surface area/body mass ratio decreases resulting in greater thermal stability. Few studies suggest that the rate and overall spontaneously change in brain tissue temperature after an ischemic event is far smaller in humans as compared with rodents. This inability to reduce body temperature in large species may be a result of their changed thermal sensitivity. On the other hand, the attenuation in stroke induced changes of brain temperature in large species may reflect different physiological strategies. That is, large species may have evolved alternative mechanism to respond and recover to ischemic events because they are unable to mount a regulated hypothermic response that is characteristic in small mammals.

Next to reperfusion, hypothermia is clearly the most potent therapeutic approach to reduce cerebral infarction in experimental cerebral ischemia to date. Certainly, clinical evaluation of hypothermia in human stroke is warranted by these data [291,292,296,364]. In particular, hypothermia is dramatically protective in conditions of temporary focal ischemia, and even mild hypothermia needs to be more detailed investigated. Hypothermia should be started as soon as possible following a focal stroke, before thrombolytic therapy in acute incomplete stroke, during temporary focal cerebrovascular occlusion in certain neurosurgical procedures [136,289], following head injury [218], and immediately postresuscitation from cardiac arrest [63,306,338]. Indeed, hypothermia has been shown to be the mechanism involved in the neuroprotective effects of drugs in some models of cerebral ischemia [22], underlying the hope that the future will provide the opportunity for routinely combining hypothermia with pharmacotherapy to optimize neuroprotection in stroke patients. This combination of hypothermia with neuroprotective drugs as a therapeutic approach has already been demonstrated to improve the outcome in experimental cerebral ischemia [142,277]. Finally, preischemic therapeutic interventions may be also effective in limiting ischemic damage [225,269], but its value in the clinical setting clearly remains to be established. Taken together preischemic hypothermia could be of value as a preconditioning stimulus for inducing delayed tolerance, such as it is needed in a variety of therapeutical procedures [271]. However, the improved understanding of the pathophysiological mechanism underlying regulated hypothermia is likely to lead to significant therapeutic benefits in the future for a majority of patients with first ever cerebral ischemic events.

5. Conclusion

To date no other single therapeutic approach has been developed that can reduce ischemic neuronal damage to the extent that is observed with hypothermia except thrombolysis. Postischemic hypothermia provides powerful and long-lasting protection in animal studies, if cooling is maintained for an adequate time period and not excessively delayed. As it is suggested in the present review, mild hypothermia's neuroprotective benefit may be secondary to attenuation of several detrimental processes involved in both necrotic and apoptotic cell death. Furthermore, hypothermia may be a cost-effective therapy that is easily implemented, and may prove to be of value by itself or in combination with more traditional pharmaceutical approaches. Two key issues that need to be addressed are the optimal depth and duration of mild hypothermia. Although further work must be accomplished to optimize its use in humans, clinical studies have already been started to test its efficacy in the treatment and prevention on stroke.

References

[1] E.F. Adolph, Responses to hypothermia in several species of infant mammals, Am. J. Physiol. 166 (1951) 75–91.
[2] V. Akriotis, W.D. Biggar, The effects of hypothermia on neutrophil function in vitro, J. Leukoc. Biol. 37 (1985) 51–61.
[3] M.T. Almeida, J. Ramalho-Santos, C.R. Oliveria, et al., Parameters affecting fusion between liposomes and synaptosomes, role of proteins, lipid peroxidation, pH and temperature, J. Membr. Biol. 142 (1994) 217–222.

[4] P. Anderson, L. Gjerstad, E. Pasztor, Effect of cooing on synaptic transmission through the cuneate nucleus, Acta Physiol. Scand. 84 (1972) 433–447.
[5] P. Andine, I. Jacobson, H. Hagberg, Calcium uptake evoked by electrical stimulation is enhanced postischemically and precedes delayed neuronal death in CA1 of rat hippocampus: involvement of NMDA receptors, J. Cereb. Blood Flow Metab. 8 (1988) 799–807.
[6] M. Aoko, F. Nomura, M.E. Stromansik, et al., Effects of pH on brain energetics after hypothermic circulatory arrest, Ann. Thorac. Surg. 55 (1993) 1093–1103.
[7] N. Araki, J.H. Greenby, J.T. Sladky, et al., Effects of mild hypothermia on the changes of intracellular calcium in focal ischemia, J. Cereb. Blood Flow Metab. 11 (Suppl.) (1991) 843.
[8] E. Ars, E. Serra, S. de la Luna, et al., Cold shock induces the insertion of a cryptic exon in the neurofibromatosis type 1 (*NF1*) mRNA, Nucleic Acids Res. 28 (2000) 1307–1312.
[9] A. Ashkenazi, V.M. Dixit, Death receptors: signaling and modulation, Science 281 (1998) 1305–1308.
[10] C.J. Baker, A.J. Fiore, V.I. Frazzini, Intraischemic hypothermia decreases the release of glutamate in the cores of permanent focal cerebral infarcts, Neurosurgery 36 (1995) 994–1001.
[11] C.J. Baker, S.T. Onesti, R.A. Solomon, Reduction by delayed hypothermia of cerebral infarction following middle cerebral artery occlusion in the rat: a time-course study, J. Neurosurg. 77 (1992) 438–444.
[12] M.A. Baker, P.A. Stocking, J.P. Meehen, Thermal relationships between tympanic membrane and hypothalamus in conscious cat and monkey, J. Appl. Physiol. 32 (1972) 739–742.
[13] W.A. Baldwin, J.R. Kirsch, P.D. Hurn, Hypothermic cerebral perfusion and recovery from ischemia, Am. J. Physiol. 261 (1992) H774–H781.
[14] E. Balir, A.V. Montgomery, H. Swan, Posthypothermic circulatory failure. I. Physiologic observations on the circulation, Circulation 13 (1956) 909–915.
[15] N.Z. Baquer, J.S. Hothersall, P. McLean, Function and regulation of the pentose phosphate pathway in brain, Curr. Topics Cell Regul. 29 (1988) 265–289.
[16] F.C. Barone, G.Z. Feuerstein, R.F. White, Brain cooling during transient focal ischemia provides complete neuroprotection, Neurosci. Behav. Rev. 21 (1997) 31–44.
[17] F.C. Barone, D.J. Knudsen, A.H. Nelson, et al., Mouse strain differences in susceptibility of cerebral ischemia are related to cerebral vascular anatomy, J. Cereb. Blood Flow Metab. 13 (1993) 683–692.
[18] H. Baumgärtl, U. Heinrich, W.D. Lübbers, Oxygen supply of the blood-free perfused guinea-pig brain in normo- and hypothermia measured by the local distribution of oxygen pressure, Pflügers Arch. 414 (1989) 228–234.
[19] B. Beilin, Y. Shavit, J. Razumovsky, et al., Effects of mild perioperative hypothermia on cellular immune response, Anesthesiology 89 (1998) 1133–1140.
[20] H. Beneviste, J. Drejer, A. Schousboe, et al., Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracranial microdialysis, J. Neurochem. 43 (1984) 1369–1374.
[21] K. Bergstedt, B.R. Hu, T. Wieloch, Postischemic changes in protein synthesis in the rat brain: effects of hypothermia, Exp. Brain Res. 95 (1993) 91–99.
[22] L. Berntman, F.A. Welsh, J.R. Harp, Cerebral protective effect of low-grade hypothermia, Anesthesiology 55 (1981) 495–498.
[23] C. Bossenmeyer-Pourie, V. Koziel, J.L. Daval, Effects of hypothermia on hypoxia-induced apoptosis in cultured neurons from developing rat forebrain: comparison with preconditioning, Pediatr. Res. 47 (2000) 385–391.
[24] S. Brint, M. Jacewicz, M. Kiessling, et al., Focal brain ischemia in the rat: methods for reproducible neocortical infarction using tandem occlusion of the distal middle cerebral and ipsilateral common carotid arteries, J. Cereb. Blood Flow Metab. 8 (1988) 474–485.
[25] J.L. Browing, M.L. Heizer, D.S. Baskin, Variations in corticomotor and somatosensory evoked potentials: effects of temperature, halothane anaesthesia, and arterial partial pressure of CO_2, Anesth. Analg. 74 (1992) 643–648.
[26] A. Buchan, W. A, Hypothermia but not *N*-methyl-D-aspartate antagonist MK-801 attenuates neuronal damage in gerbils subjected to transient global ischemia, J. Neurosci. 10 (1990) 311–316.
[27] B. Budnick, K.L. McKeown, W.C. Wiederholt, Hypothermia-induced changes in rat short latency somatosensory evoked potentials, Electroencephalogr. Clin. Neurophysiol. 15 (1963) 238–245.
[28] E. Busch, M.L. Gyngell, M. Eis, et al., Potassium-induced cortical spreading depressions during focal cerebral ischemia in rats: contribution to lesion growth assessed by diffusion-weighted NMR and biochemical imaging, J. Cereb. Blood Flow Metab. 16 (1996) 1090–1099.
[29] D.W. Busija, C.W. Leffler, Hypothermia reduced cerebral metabolic rate and cerebral blood flow in newborn pigs, Am. J. Physiol. 253 (1987) 869–873.
[30] R. Busto, M.D. Ginsberg, The influence of altered brain temperature in cerebral ischemia, in: M.D. Ginsberg, J. Bogousslavsky (Eds.), Cerebrovascular Disease: Pathophysiology, Diagnosis, and Management, Blackwell Scientific Publications, Malden, MA, 1998, pp. 287–307.
[31] R. Busto, M.Y.T. Globus, J.T. Neary, et al., Regional alterations of protein kinase C activity following transient cerebral ischemia: effects of intraischemic brain temperature modulation, J. Neurochem. 63 (1994) 1095–1103.
[32] R. Busto, M.Y.T. Globus, E. Martinez, et al., Effect of intraischemic hypothermia on ischemia-induced changes in regional levels of inositol 1,4,5-triphosphate (IP3), Soc. Neurosci. Abstr. 19 (1993) 1669.
[33] R. Busto, D. Dietrich, M.Y.T. Globus, et al., Postischemic moderate hypothermia inhibits CA1, hippocampus ischemic neuronal injury, Neurosci. Lett. 101 (1989) 299–304.
[34] R. Busto, M.Y.T. Globus, W.D. Dietrich, et al., Effect of mild hypothermia on ischemia-induced release of neurotransmitters and free fatty acids in rat brain, Stroke 20 (1989) 904–910.
[35] R. Busto, W.D. Dietrich, M.Y.T. Globus, et al., Small differences in intraischemic brain temperature critically determine the extent of ischemic neuronal injury, J. Cereb. Blood Flow Metab. 7 (1987) 729–738.
[36] R.A. Butler, T. Konishi, C. Fernandez, Temperature coefficients of cochlear potentials, Am. J. Physiol. 199 (1960) 688–692.
[37] M. Cardell, T. Wieloch, Time course of the translocation and inhibition of protein kinase C during complete cerebral ischemia in the rat, J. Neurochem. 61 (1993) 1308–1314.
[38] M. Carroll, O. Beek, Protection against hippocampus CA1 cell loss by post-ischemic hypothermia is dependent on delay of initiation and duration, Metab. Brain Dis. 7 (1992) 45–50.
[39] K.M. Chan, K. Beard, A patient with recurrent hypothermia associated with thrombocytopenia, Postgrad. Med. J. 79 (1993) 227–229.
[40] P.F. Chapman, B.G. Freguelli, A. Smith, et al., The alpha Ca^{2+}/calmodulin kinase II: a bidirectional modulator of presynaptic plasticity, Neuron 14 (1995) 591–597.
[41] S.A. Chappell, G.C. Owens, V.P. Mauro, A 5′-leader of Rbm3, a cold stress-induced mRNA, mediates internal initiation of translation with increased efficiency under conditions of mild hypothermia, J. Biol. Chem. 272 (1997) 26803–26806.
[42] C. Charriaut-Marlangue, I. Margaill, M. Plotkine, et al., Early denonuclease activation following reversible focal ischemia in the rat brain, J. Cereb. Blood Flow Metab. 15 (1995) 385–388.
[43] R.Y.Z. Chen, S. Chien, Hemodynamic functions and blood viscosity in surface hypothermia, Am. J. Physiol. 235 (1978) H136–H143.
[44] R.Y.Z. Chen, S. Chien, Plasma volume, red cell volume, and thoracic duct lymph flow in hypothermia, Am. J. Physiol. 233 (1977) H605–H612.

[45] Y. Chen, G. Chopp, G. Bodzin, et al., Temperature modulation of cerebral depolarization during focal cerebral ischemia in rats: correlation with ischemic injury, J. Cereb. Blood Flow Metab. 13 (1993) 389–394.
[46] G.J. Cheng, J.L. Morrow-Tesch, D.I. Beller, et al., Immunosuppression in mice induced by cold water stress, Brain Behav. Immun. 4 (1990) 278–291.
[47] M. Chopp, R. Knight, C.D. Tidwell, et al., The metabolic effects of mild hypothermia on global cerebral ischemia and recirculation in the cat: comparisons of normothermia and hyperthermia, J. Cereb. Blood Flow Metab. 9 (1989) 141–148.
[48] D. Chyatte, J. Elefteriacles, B. Kim, Profound hypothermia and circulatory arrest for aneurysm surgery, J. Neurosurg. 70 (1989) 489–491.
[49] J.A. Clemens, D.T. Stephenson, E.P. Dixon, et al., Global cerebral ischemia activates nuclear factor-κB prior to evidence of DNA fragmentation, Mol. Brain Res. 48 (1997) 187–196.
[50] C. Coimbra, T. Wiesloch, Hypothermia ameliorates neuronal survival when induced 2 hours after ischemia in the rat, Acta Physiol. Scand. 146 (1992) 543–544.
[51] C. Coimbra, T. Wiesloch, Moderate hypothermia mitigates neuronal damage in the rat brain when initiated several hours following transient cerebral ischemia, Acta Neuropathol. 87 (1994) 325–331.
[52] C. Coimbra, M. Drake, F. Boris-Müller, et al., Long-lasting neuroprotective effect of postischemic hypothermia and treatment with an anti-inflammatory/antipyretic drug. Evidence for chronic encephalopathic processes following ischemia, Stroke 27 (1996) 1578–1585.
[53] F. Colbourne, D. Corbett, Z. Zhao, et al., Prolonged but delayed postischemic hypothermia: a long-term outcome study in the rate middle cerebral artery occlusion model, J. Cereb. Blood Flow Metab. 20 (2000) 1702–1708.
[54] F. Colbourne, H. Li, A.M. Buchan, Indefatigable CA1 sector neuroprotection with mild hypothermia induced 6 hours after severe forebrain ischemia in rats, J. Cereb. Blood Flow Metab. 19 (1999) 742–749.
[55] F. Colbourne, G.R. Sutherland, R.N. Auer, Electron microscopic evidence against apoptosis as the mechanism of neuronal death in global ischemia, J. Neurosci. 19 (1999) 4200–4210.
[56] F. Colbourne, G. Sutherland, D. Corbett, Postischemic hypothermia. A critical appraisal with implications for clinical treatment, Mol. Neurobiol. 14 (1997) 171–201.
[57] F. Colbourne, D. Corbett, Delayed postischemic hypothermia. A six-month survival study using behavioral and histological assessments of neuroprotection, J. Neurosci. 15 (1995) 7250–7260.
[58] F. Colbourne, D. Corbett, Delayed and prolonged post-ischemic hypothermia is neuroprotective in the gerbil, Brain Res. 623 (1993) 1–5.
[59] L.S. Coles, P. Diamond, F. Occhiodoro, et al., Cold shock domain proteins repress transcription from the GM-CSF promoter, Nucleic Acids Res. 24 (1996) 2311–2317.
[60] C.A. Colton, D.L. Gilbert, Production of superoxide anion by a CNS macrophage, the microglia, FEBS Lett. 223 (1987) 284–288.
[61] D. Corbett, M. Hamilton, F. Colbourne, Persistent neuroprotection with prolonged postischemic hypothermia in adult rats subjected to transient middle cerebral artery occlusion, Exp. Neurol. 163 (2000) 200–206.
[62] J.S. Coselli, E.S. Cranford, A.C. Beall, et al., Determination of brain temperature for safe circulatory arrest during cardiovascular operation, Ann. Thorac. Surg. 45 (1988) 638–642.
[63] E.S. Crawford, J.S. Coselli, H.J. Safi, Partial cardiopulmonary bypass, hypothermic circulatory arrest, and posterolateral exposure for thoracic aortic aneurysm operation, J. Thorac. Cardiovasc. Surg. 94 (1987) 824–827.
[64] R.A. Crozier, I.B. Black, M.R. Plummer, Blockade of NR2B-containing NMDA receptors prevents BDNF enhancement of glutamergic transmission in hippocampal neurons, Learn. Mem. 6 (1999) 257–266.
[65] S. Danno, K. Itoh, T. Matsuda, et al., Decreased expression of mouse Rbm3, a cold-shock protein, in Sertoli cells of cryptorchid testis, Am. J. Pathol. 156 (2000) 1685–1692.
[66] S. Danno, H. Nishiyama, H. Higishitsuji, et al., Increased transcript level of RBM3, a member of the glyicne-rich RNA-binding protein family, in human cells in response to cold stress, Biochem. Biophys. Res. Commun. 236 (1997) 804–807.
[67] R.J. Dempsey, D.J. Combs, M.E. Maley, et al., Moderate hypothermia reduces postischemic edema development and leukotriene production, Neurosurgery 21 (1987) 177–181.
[68] F. Dexter, F. Kern, B.J. Hindmann, et al., The brain uses mostly dissolved oxygen during profoundly hypothermic cardiopulmonary bypass, Ann. Thorac. Surg. 63 (1997) 1725–1729.
[69] P. Diamond, M.F. Shannon, M.A. Vadas, Cold shock domain factors activate the granulocyte-macrophage colony-stimulating factor promoter in stimulated Jurkat T cells, J. Biol. Chem. 276 (2001) 7943–7951.
[70] W.D. Dietrich, R. Busto, M.Y. Globus, et al., Brain damage and temperature: cellular and molecular mechanisms, Adv. Neurol. 71 (1996) 177–194.
[71] W.D. Dietrich, R. Busto, O. Alonso, et al., Intraischemic but not postischemic brain hypothermia protects chronically following global forebrain ischemia in rats, J. Cereb. Blood Flow Metab. 13 (1993) 541–549.
[72] W.D. Dietrich, M. Halley, I. Valdes, et al., Interrelationships between increased vascular permeability and acute neuronal damage following temperature-controlled brain ischemia in rats, Acta Neuropathol. 81 (1991) 615–625.
[73] W.D. Dietrich, R. Busto, M. Halley, et al., The importance of brain temperature in alterations of the blood–brain barrier following cerebral ischemia, J. Neuropathol. Exp. Neurol. 49 (1990) 486–497.
[74] P.H. Donohoe, T.G. West, R.G. Boutiller, Factors affecting membrane permeability and ionic homeostasis in the cold-submerged frog, J. Exp. Biol. 203 (2000) 504–514.
[75] M. Dragunow, H.A. Robertson, Brain injury induces c-fos protein(s) in nerve and glial-like cells in adult mammalian brain, Brain Res. 455 (1988) 295–299.
[76] J.C. Drummond, M.M. Todd, U.H. Sang, The effects of high dose sodium thiopental on brain stem auditory and median nerve somatosensory evoked responses in humans, Anesthesiology 63 (1985) 249–254.
[77] C. Du, R. Hu, C.A. Csernansky, et al., Very delayed infarction after mild focal cerebral ischemia: a role for apoptosis? J. Cereb. Blood Flow Metab. 16 (1996) 195–201.
[78] F.L. Duebener, I. Hagino, T. Sakamoto, et al., Effects of pH management during deep hypothermic bypass on cerebral microcirculation: alpha-stat versus pH-stat, Circulation 106 (12 Suppl. 1) (2002) I-103–I-108.
[79] A.D. Edwards, X. Yue, M.V. Squier, et al., Specific inhibition of apoptosis after cerebral hypoxia–ischemia by moderate post-insult hypothermia, Biochem. Biophys. Res. Comm. 217 (1995) 1193–1199.
[80] I.J. Elenkov, R.L. Wilder, G.P. Chrousos, et al., The sympathetic nerve—an integrative interface between two supersystems: the brain and the immune system, Pharmacol. Rev. 52 (2000) 595–638.
[81] M.J. Eliasson, K. Sampei, A.S. Mandir, et al., Poyl(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia, Nat. Med. 3 (1997) 1089–1095.
[82] M.A. Ergin, J.D. Galla, S.L. Lansman, et al., Hypothermic circulatory arrest in operations on the thoracic aorta. Determinants of operative mortality and neurological outcome, J. Thorac. Cardiovasc. Surg. 107 (1994) 788–797.
[83] J.C. Escolar, R. Hoo-paris, C. Castex, et al., Effect of low temperature on glucose-induced insulin secretion and glucose metabolism in isolated pancreatic islets of the rat, J. Endocrinol. 125 (1990) 45–51.

[84] E.J. Fedor, B. Fisher, Simultaneous determination of blood volume with Cr-51 and T-1824 during hypothermia and rewarming, Am. J. Physiol. 196 (1959) 703–705.
[85] T. FitzGibbon, J.S. Hayward, D. Walker, et al., EEG and visual evoked potentials of conscious man during moderate hypothermia, Electroencephalogr. Clin. Neurophysiol. 58 (1984) 48–54.
[86] S.M. Frank, L.A. Fleisher, M.J. Breslow, et al., Perioperative maintenance of normothermia reduces the incidence of morbid cardiac events, J. Am. Med. Assoc. 277 (1997) 1127–1134.
[87] S.M. Frank, C. Beattie, R. Christopherson, et al., Unitentional hypothermia is associated with postoperative myocardial ischemia, Anesthesiology 78 (1993) 468–476.
[88] K. Frei, C. Siepl, P. Groscurth, et al., Antigen presentation and tumor cytotoxicity by interferon-y-treated microglial cells, Eur. J. Immunol. 17 (1987) 1271–1278.
[89] P. Friedlander, Y. Haupt, C. Prives, et al., A mutant p53 that discriminates between p53-responsive genes cannot induce apoptosis, Mol. Cell. Biol. 16 (1996) 4961–4971.
[90] B.A. Freeman, J.D. Crapo, Biology of disease: free radicals and tissue injury, Lab. Invest. 47 (1982) 412–426.
[91] J. Fujita, Cold shock response in mammalian cells, J. Mol. Microbiol. Biotechnol. 1 (1999) 243–255.
[92] H. Fujishima, Y. Yagi, L. Toda, et al., Increased comfort and decreased inflammation of the eye by cooling after cataract surgery, Am. J. Ophthalmol. 119 (1995) 301–306.
[93] K. Fukunaga, T.R. Soderling, E. Miyamoto, Activation of Ca^{2+}/calmodulin-dependent protein kinase II and protein kinase C by glutamate in cultured rat hippocampal neurons, J. Biol. Chem. 267 (1992) 22527–22533.
[94] M.K. Gaitonde, E. Evison, G.M. Evans, The rate of utilization of glucose via hexosemonophasphate shunt in brain, J. Neurochem. 41 (1983) 1253–1260.
[95] Y. Garnier, D. Pfeiffer, A. Jensen, et al., Effects of mild hypothermia on metabolic disturbances in fetal hippocampal slices after oxygen/glucose deprivation depend on depth and time delay of cooling, J. Soc. Gynecol. Invest. 8 (2001) 198–205.
[96] J. Gehrmann, P. Bonnekoh, T. Miyazawa, et al., Immunocytochemical study of an early microglial activation in ischemia, J. Cereb. Blood Flow Metab. 12 (1992) 257–269.
[97] B. Gelman, C.L. Schleien, J.W. Kuluz, Regional brain temperatures in infant piglets during resuscitation form cardiac arrest: selective brain cooling vs. total body cooling, J. Cereb Blood Flow Metab. 17 (Suppl. 1) (1997) S45.
[98] M.D. Ginsberg, R. Busto, Y. Castella, et al., The protective effect of moderate intra-ischemic brain hypothermia is associated with improved postischemic glucose utilization and blood flow, J. Cereb. Blood Flow Metab. 9 (Suppl.) (1989) 380.
[99] M.Y.T. Globus, O. Alonso, D.W. Dietrich, et al., Glutamate release and free radical production following brain injury: effects of posttraumatic hypothermia, J. Neurochem. 65 (1995) 1704–1711.
[100] M.Y.T. Globus, R. Busto, W.D. Dietrich, et al., Intraischemic extracellular release of dopamine and glutamate is associated with striatal vulnerability to ischemia, Neurosci. Lett. 91 (1988) 36–40.
[101] M.Y.T. Globus, R. Busto, E. Martinez, et al., Early moderate postischemic hypothermia attenuates the rise in excitotoxic index in the hippocampus—a possible mechanism for the beneficial effects of early postischemic moderate cooling, J. Cereb. Blood Flow Metab. 11 (Suppl. 2) (1991) S10.
[102] R.J. Glyglewski, R.M.J. Palmer, S. Moncada, Superoxide anion is involved in the breakdown of endothelium-derived relaxing factor, Nature 320 (1986) 454–456.
[103] T.C. Gray, Reflections on circulatory control, Lancet 1 (1957) 383–389.
[104] W.J. Greeley, F.H. Kern, J.N. Meliones, et al., Effects of deep hypothermia and circulatory arrest on cerebral blood flow and metabolism, Ann. Thorac. Surg. 56 (1993) 1464–1466.
[105] C.J. Gordon, The therapeutic potential of regulated hypothermia, Emerg. Med. J. 18 (2001) 81–89.
[106] T. Goto, T. Matsukawa, D.I. Sessler, et al., Thermoregulatory thresholds for vasoconstriction in patients anesthetized with various 1-minimum alveolar concentration combinations of xenon, nitrous oxide, and isoflurane, Anesthesiology 91 (1999) 626–632.
[107] Y. Goto, N.F. Kassell, K. Hiramatsu, Effects of intraischemic hypothermia on cerebral damage in a model of reversible focal ischemia, Neurosurgery 32 (1993) 980–984.
[108] S.H. Graham, J. Chen, Programmed cell death in cerebral ischemia, J. Cereb. Blood Flow Metab. 21 (2001) 99–109.
[109] S.H. Graham, K. Shiraishi, S.S. Panter, et al., Changes in extracellular amino acid neurotransmitters produced by focal cerebral ischemia, Neurosci. Lett. 110 (1990) 124–130.
[110] G.A. Gregory, F.A. Welsh, A.C.H. Yu, et al., Fructose-1,6-biphospahte reduces ATP loss from hypoxic astrocytes, Brain Res. 516 (1990) 310–316.
[111] J. Grunenfelder, G. Zund, A. Schoeberlien, et al., Expression of adhesion molecules and cytokines after coronary artery bypass grating during normothermic and hypothermic cardiac arrest, Eur. J. Caridothorac. Surg. 17 (2000) 723–728.
[112] L.M. Gentilello, Advances in the management of hypothermia, Surg. Clin. North Am. 75 (1995) 243–256.
[113] W. Hacke, M. Kaste, C. Fieschi, et al., Intravenous thrombolysis with recombinant tissue plasminogen activation for acute hemispheric stroke. The European Cooperative Acute Stroke study (ECASS), J. Am. Med. Assoc. 274 (1995) 1017–1025.
[114] M. Hagerdal, J. Harp, B.K. Siesjö, The effect of induced hypothermia upon oxygen consumption in the rat brain, J. Neurochem. 24 (1975) 311–316.
[115] M. Hagerdal, J. Harp, B.K. Siesjö, Effect of hypothermia upon organic phosphates, glycolytic metabolites, citric acid cycle intermediates and associated amino acids in rat cerebral cortex, J. Neurochem. 24 (1975) 743–748.
[116] E. Häggendal, B. Johansson, Effects of arterial carbon dioxide tension and oxygen saturation on cerebral blood flow autoregulation in dogs, Acta Physiol. Scand. 66 (Suppl. 258) (1965) 27–53.
[117] J. Haher, V. Hashinski, Hypothermia as a potential treatment for cerebral ischemia, Cerebrovasc. Brain Metab. Rec. 5 (1993) 277–300.
[118] E.D. Hall, P.K. Adnrus, K.E. Pazara, Protective efficacy of a hypothermic pharmacological agent in gerbil forebrain ischemia, Stroke 24 (1993) 711–715.
[119] E.D. Hall, K.E. Pazara, K.L. Linseman, Sex differences in postischemic neuronal necrosis in gerbils, J. Cereb. Blood Flow Metab. 11 (1991) 292–298.
[120] A.M. Harper, H.I. Glass, Effect of alterations in the arterial carbon dioxide tension on the blood flow through the cerebral cortex at normal and low arterial blood pressures, J. Neurol. Neurosurg. Psychiatry 28 (1965) 449–452.
[121] R.S. Hansen, A.W. Braithwaite, The growth-inhibitory function of p53 is separable form transactivation, Oncogene 13 (1996) 995–1007.
[122] J.N. Hayward, M.A. Baker, Role of cerebral arterial blood in the regulation of brain temperature in the monkey, Am. J. Physiol. 215 (1968) 389–403.
[123] D.D. Heistad, H.A. Kontos, Cerebral circulation, in: Handbook of Physiology: Cerebral Circulation. Section 2. The Cardiovascular System, vol. III, American Physiological Society, Bethesda, MD, 1983, pp. 137–182.
[124] G.R. Hervey, Hypothermia, Proc. R. Soc. Med. 66 (1973) 1053–1058.
[125] B. Hindfelt, The prognostic significance of subfebrility and fever in ischaemic cerebral infarction, Acta Neurol. Scand. 53 (1976) 72–79.
[126] B.J. Hindman, F. Dexter, B.S. Cutkomp, et al., Brain blood flow and metabolism do not decrease at stable brain temperature during

cardiopulmonary bypass in rabbits, Anesthesiology 77 (1992) 342–350.
[127] K. Hiramatsa, N.F. Kassell, K.S. Lee, Thermal sensitivity of hypoxic responses in neocortical brain slices, J. Cereb. Blood Flow Metab. 13 (1993) 395–401.
[128] T. Hiramatsu, T. Mirua, J.M. Forbess, et al., pH strategies and cerebral energetic before and after circulatory arrest, J. Thorac. Cardiovasc. Surg. 109 (1995) 948–957.
[129] D.B. Holland, S.G. Roberts, E.J. Wood, et al., Cold shock induces the synthesis of stress proteins in human keratocytes, J. Invest. Dermatol. 101 (1993) 196–199.
[130] J. Honkaniemi, B.A. States, P.R. Weinstein, et al., Expression of zinc finger immediate early genes in rat brain after permanent middle cerebral artery occlusion, J. Cereb. Blood Flow Metab. 17 (1997) 636–646.
[131] N. Hori, D.O. Carpenter, Functional and morphological changes induced by transient in vivo ischemia, Exp. Neurol. 129 (1994) 279–289.
[132] S.M. Horvath, G.E. Folk, F.N. Draig, et al., Survival time of various warm-bloods animals in extreme cold, Science 197 (1948) 171–172.
[133] K.A. Hossmann, Periinfarct depolarization, Cerebrovasc. Brain Metab. Rev. 8 (1996) 195–208.
[134] K.A. Hossmann, Treatment of experimental cerebral ischemia, J. Cereb. Blood Flow Metab. 2 (1982) 275–297.
[135] F.P. Huang, L.F. Zhou, G.Y. Yang, Effects of mild hypothermia on the release of regional glutamate and glycine during extended transient focal cerebral ischemia in rats, Neurochem. Res. 23 (1998) 991–996.
[136] L. Hubert, L. Rosomoff, Hypothermia and cerebral vascular lesions. I. Experimental interruption of the middle cerebral artery during hypothermia, J. Neurosurg. 13 (1986) 244–255.
[137] P.W. Huh, L. Belayev, W. Zhao, et al., Comparative neuroprotective efficacy of prolonged moderate intraischemic and postischemic hypothermia in focal cerebral ischemia, J. Neurosurg. 92 (2000) 91–99.
[138] C. Iadecola, Bright and dark sides of nitric oxide in ischemic brain injury, Trends Neurosci. 20 (1997) 132–139.
[139] T. Ikeda, T. Kazama, D.I. Sessler, et al., Induction of anesthesia with ketamine reduces the magnitude of redistribution hypothermia, Anesth. Analg. 93 (2001) 934–938.
[140] T. Ikeda, D.I. Sessler, M. Kikura, et al., Less core hypothermia when anesthesia is induced with inhaled sevofluare than with intravenous propofol, Anesth. Analg. 88 (1999) 921–924.
[141] T. Ikeda, T. Kazama, D.I. Sessler, et al., Induction of anesthesia with ketamine reduces the magnitude of redistribution hypothermia, Anesth. Analg. 93 (2001) 934–938.
[142] C. Ikonamidou, J.L. Mosinger, J.W. Olney, Hypothermia enhances protective effect of MK-801 against hypoxic/ischemic damage in infants rats, Brain Res. 487 (1989) 184–187.
[143] R. Janssen, B.E. Hetzler, J.P. Creason, et al., Differential impact of hypothermia and pentobarbital on brain-stem auditory evoked responses, Electroencephalogr. Clin. Neurophysiol. 80 (1991) 412–421.
[144] W.C. Jean, S.R. Spellman, E.S. Nussbaum, et al., Reperfusion injury after focal cerebral ischemia: the role of inflammation and the therapeutic horizon, Neurosurgery 43 (1998) 1382–1397.
[145] B. Just, E. Delva, Y. Camus, et al., Oxygen uptake during recovery following naloxone, relationship with intraoperative heat loss, Anesthesiology 76 (1992) 60–64.
[146] J.Y. Jiang, B.G. Lyeth, M.Z. Kapasi, et al., Moderate hypothermia reduces blood–brain barrier disruption following traumatic brain injury in the rat, Acta Neuropathol. 84 (1992) 495–500.
[147] D.C. Johnson, M. Nishimura, P. Okunieff, et al., Effects of hypothermia on rat brain pH and phosphate metabolite regulation by ^{31}P NMR, J. Appl. Physiol. 67 (1989) 2527–2534.
[148] G.J. Jurkovich, R.M. Pitt, P.W. Curreri, et al., Hypothermia prevents increased capillary permeability following ischemia–reperfusion injury, J. Surg. Res. 44 (1988) 514–521.
[149] A. Kader, M.H. Brisman, N. Maraire, et al., The effect of mild hypothermia on permanent focal ischemia in the rat, Neurosurgery 31 (1992) 1056–1061.
[150] A. Kader, V.I. Frazzini, C.J. Baker, et al., Effect of mild hypothermia on nitric oxide synthesis during focal cerebral ischemia, Neurosurgery 35 (1994) 272–277.
[151] T. Kaibara, G.R. Sutherland, F. Colbourne, et al., Hypothermia: depression of tricarboxylic acid cycle flux and evidence for pentose phosphate shunt upregulation, J. Neurosurg. 90 (1999) 339–347.
[152] F. Kamme, T. Wieloch, Induction of *junD* mRNA after transient forebrain ischemia in the rat. Effect of hypothermia, Mol. Brain Res. 43 (1996) 51–56.
[153] F. Kamme, K. Campell, T. Wieloch, Biphasic expression of the fos and jun families of transcription factors following transient forebrain ischemic in the rat. Effect of hypothermia, Eur. J. Neurosci. 7 (1995) 2007–2016.
[154] H. Karibe, J. Chen, G.J. Zarow, et al., Delayed induction of mild hypothermia to reduce infarct volume after temporary middle cerebral artery occlusion in rats, J. Neurosurg. 80 (1994) 112–119.
[155] Y. Kaneko, H. Nishiyama, K. Nonoguchi, et al., A novel hsp-110 related gene, *apg-1*, that is abundantly expressed in the testis respond to a low temperature heat shock rather than the traditional elevated temperatures, J. Boil. Chem. 272 (1997) 2640–2645.
[156] S.E. Kaiser, T. Wean, P. Piriyawat, et al., Acetaminophen for altering body temperature in acute stroke. A randomized clinical trial, Stroke 33 (2002) 130–135.
[157] K. Katakoa, R. Graf, G. Rosner, et al., Experimental focal ischemia in cats. Changes in multimodality evoked potentials as related to local cerebral blood flow and ischemia brain edema, Stroke 18 (1987) 188–194.
[158] K. Katsura, H. Minamisawa, A. Ekhol, et al., Changes of labile metabolites during anoxia in moderately hypo- and hyperthermic rats: correlation to membrane fluxes of K^+, Brain Res. 590 (1992) 6–12.
[159] H. Kato, K. Kongrue, Biochemical and molecular characteristics of the brain with developing cerebral infarction, Cell. Mol. Neurobiol. 19 (1999) 93–108.
[160] N. Kawai, M. Kawanishi, M. Okauchi, et al., Effects of hypothermia on thrombin-induced brain edema formation, Brain Res. 895 (2001) 50–58.
[161] S. Kawamura, A. Suzuki, H. Hadeishi, et al., Cerebral blood flow and oxygen metabolism during mild hypothermia in patients with subarachnoid hemorrhage, Acta Neurochir. Wien 142 (2000) 1117–1121.
[162] Y. Kawamura, K. Yamada, A. Masago, et al., Hypothermia modulates induction of *hsp70* and c-*jun* mRNA in the rat brain after subarachnoid hemorrhage, J. Neurotrauma 17 (2000) 243–250.
[163] Y. Kawamura, R.D. Gibert, G.G. Power, Effects of cooling and heating on the regional distribution of blood flow in fetal sheep, J. Dev. Physiol. 8 (1986) 11–21.
[164] N.K. Keenan, M.J. Taylor, J.E. Coles, et al., The use of VEPs for CNS monitoring during continuous cardiopulmonary bypass and circulatory arrest, Electroencephalogr. Clin. Neurophysiol. 68 (1987) 241–246.
[165] Q. Kiang, Z. Gu, G. Zhang, et al., *N*-Methyl-D-aspartate receptor activation results in regulation of extracellular signal-regulated kinases by protein kinases and phophatases in glutamate-induced neuronal apoptotic-like death, Brain Res. 887 (2000) 285–292.
[166] H.Y. Kil, J. Zhang, C.A. Piantadose, Brain temperature alters hydroxyl radical production during cerebral ischemia/reperfusion in rats, J. Cereb. Blood Flow Metab. 16 (1996) 100–106.
[167] Z. Kokaia, Q. Zhao, M. Kokaia, et al., Regulation of brain-derived neurotrophic factor gene expression after transient middle cerebral artery occlusion with and without brain damage, Exp. Neurol. 136 (1995) 73–88.

[168] R. Kollmar, W.R. Schäbitz, S. Heiland, et al., Neuroprotective effect of delayed moderate hypothermia after focal cerebral ischemia. An MRI study, Stroke 33 (2002) 1899–1904.
[169] R. Kollmar, T. Frietsch, D. Georgiadis, et al., Early effects of acid–base management during hypothermia on cerebral infarct volume, edema, and cerebral blood flow in acute focal cerebral ischemia in rats, Anesthesiology 97 (2002) 868–874.
[170] D.J. Kominskiy, R.J. Bickel, K.L. Tyler, Retrovirus-induced apoptosis requires both death receptor- and mitochondrial-mediated caspase-dependent pathways of cell death, Cell Death Differ. 9 (2002) 926–933.
[171] H. Kraibe, J. Chen, G.J. Zarow, et al., Delayed induction of mild hypothermia to reduce infarct volume after temporary middle cerebral artery occlusion in rats, J. Neurosurg. 80 (1994) 112–119.
[172] R.S. Kramer, A.P. Sanders, A.M. Lesage, et al., The effect of profound hypothermia on preservation of cerebral ATP content during circulatory arrest, J. Thorac. Cardiovasc. Surg. 56 (1968) 699–709.
[173] H.A. Krebs, The history of the tricarboxylic acid cycle, Perspect. Biol. Med. 14 (1970) 154–170.
[174] J.W. Kuluz, R. Prado, J. Chang, et al., Selective brain cooling increases cortical cerebral blood flow in rats, Am. J. Physiol. 265 (1993) H825–H827.
[175] K. Kumar, X. Wu, A.T. Evans, Expression of c-fos and fos-B proteins following transient forebrain ischemia: effect of hypothermia, Mol. Brain Res. 42 (1996) 337–343.
[176] E. Laios, I.M. Rebeyka, C.A. Prody, Characterization of cold-induced heat shock protein expression in neonatal rat cardiomyocytes, Mol. Cell. Biochem. 173 (1997) 153–159.
[177] W.I. Lanier, P.A. Iaizzo, M.J. Murray, The effect of convective cooling and rewarming on systematic and central nervous system physiology in isoflurane-anesthetized dogs, Resuscitation 23 (1992) 121–136.
[178] G.J.A. Laycock, I.M. Mitchell, R.D. Paton, et al., EEG burst suppression with propofol during cardiopulmonary bypass in children: a study of the hemodynamic, metabolic and endocrine effects, Br. J. Anaesth. 69 (1992) 356–362.
[179] S.L. Lee, F.D. Battistella, K. Go, Hypothermia induces T-cell production of immunosuppressive cytokines, J. Surg. Res. 100 (2001) 150–153.
[180] H. Leotscher, O. Niederhause, J. Kemp, et al., Is caspase-3 inhibition a valid therapeutic strategy in cerebral ischemia? Drug. Discov. Today 6 (2001) 671–680.
[181] K. Leslie, D.I. Sessler, A.R. Bjorksten, et al., Mild hypothermia alters propofol pharmocokinetics and increases the duration of action of atracrium, Anesth. Analg. 80 (1995) 1007–1014.
[182] Y. Li, M. Chopp, N. Jiang, et al., Temporal profile of in situ DNA fragmentation after transient middle cerebral artery occlusion in the rat, J. Cereb. Blood Flow Metab. 15 (1995) 389–397.
[183] Y. Li, V.G. Sharov, N. Jiang, et al., Ultrastructural and light microscopic evidence of apoptosis after middle cerebral artery occlusion in the rat, Am. J. Pathol. 146 (1995) 1045–1051.
[184] A.Y. Liu, H. Bian, L.E. Huang, et al., Transient cold shock induces the heat shock response upon recovery at 37 °C in human cells, J. Biol. Chem. 269 (1994) 14768–14775.
[185] A.Y. Liu, H. Bian, L. Huang, et al., Transient cold shock induces the heat shock response upon recovery at 37 °C in human cells, J. Biol. Chem. 269 (1994) 14768–14775.
[186] O. Lindvall, P. Ernfors, J. Bengzon, et al., Differential regulation of mRNAs for nerve growth factor, brain-derived neurotrophic factor, and neurotrophin 3 in the adult rat brain following cerebral ischemia and hypoglycemic coma, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 648–652.
[187] H. Link, G. Lebram, I. Johansson, et al., Prognosis in patients with infarction and tia in carotid territory during and after anticoagulant therapy, Stroke 10 (1979) 522–529.
[188] D.M. Little, Hypothermia, Anesthesiology 20 (1959) 842–877.
[189] M. Loeffler, E. Daugas, et al., Dominant cell death induction by extramitochondrially target apoptosis-inducing factor, FAESB J. 15 (2001) 758–767.
[190] E.H. Lo, G.K. Steinberg, Effects of hypothermia on evoked potentials, magnetic resonance imaging, and blood flow in focal ischemia in rabbits, Stroke 23 (1992) 889–893.
[191] O.H. Lowry, J.V. Passonneau, A Flexible System of Enzymatic Analysis, Academic Press, New York, NY, 1972.
[192] O.H. Lowry, J.V. Passonneau, F.X. Hasselberger, et al., Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain, J. Biol. Chem. 239 (1964) 18–30.
[193] K.P. Madden, W.M. Clark, A. Kochar, et al., Effect of protein kinase C modulation on outcome of experimental CNS ischemia, Brain Res. 547 (1991) 193–198.
[194] P.J. Magistretti, L. Pellerin, D.L. Rothman, et al., Energy on demand, Science 283 (1999) 496–497.
[195] C.M. Maier, G.H. Sun, D. Kunis, et al., Delayed induction and long-term effects of mild hypothermia in a focal model of transient cerebral ischemia: neurological outcome and infarct size, J. Neurosurg. 94 (2001) 90–96.
[196] C.M. Maier, D. Onley, M.A. Yenari, et al., Long-term effects of mild hypothermia on SOD2 expression following transient focal cerebral ischemia, Stroke 30 (1999) 275 (Abstract).
[197] C.M. Maier, K.V. Ahern, M.L. Cheng, et al., Optimal depth and duration of mild hypothermia in a focal model of transient cerebral ischemia: effects on neurologic outcome, infarct size, apoptosis, and inflammation, Stroke 29 (1998) 2171–2180.
[198] C.M. Maier, M.A. Yenari, G.K. Steinberg, Delayed induction of mild hypothermia in experimental focal cerebral ischemia: effects on neurological outcome and infarct size, Ann. Neurol. 44 (Suppl.) (1998) 440 (Abstract).
[199] O.N. Markand, C. Warren, G.S. Mallik, et al., Temperature-dependent hysteresis in somatosensory and auditory evoked potentials, Electroencephalogr. Clin. Neurophysiol. 77 (1990) 425–435.
[200] O.N. Markand, C.H. Warren, S.S. Moorthy, et al., Monitoring of multimodality evoked potentials during open heart surgery under hypothermia, Electroencephalogr. Clin. Neurophysiol. 59 (1984) 432–440.
[201] G.Z. Markarian, J.H. Lee, D.J. Stein, et al., Mild hypothermia: therapeutic window after experimental cerebral ischemia, Neurosurgery 38 (1996) 540–546.
[202] N.K. Marshall, E. Donchin, Circadian variation in the latency of brainstem responses and its relation to body temperature, Science 212 (1981) 356–358.
[203] J. McCullock, He. Savaki, J. Jehle, et al., Local cerebral glucose utilization in hypothermic and hyperthermic rats, J. Neurochem. 39 (1982) 255–258.
[204] D. Maclean, D. Emslie-Smith, Accidental hypothermia, Blackwell Scientific Publications, Melbourne, 1977.
[205] J. Mahrer, V. Hachinsik, Hypothermia as a potential treatment for cerebral ischemia, Cerebrovasc. Brain Rev. 5 (1993) 277–300.
[206] Z. Matijasevic, J.E. Snyder, D.B. Ludlum, Hypothermia causes a reversible, p53-mediated cell cycle arrest in cultured fibroblasts, Oncol. Res. 10 (1998) 605–610.
[207] P. Mellegard, C.H. Nordstrom, Epidural temperature and possible intracerebral temperature gradients in man, Br. J. Neurosurg. 4 (1990) 31–38.
[208] J. Memezasa, M.L. Smith, Penumbral tissues salvaged by reperfusion following middle cerebral artery occlusion in rats, Stroke 23 (1992) 552–559.
[209] C. Metz, M. Holzschuh, T. Bein, et al., Moderated hypothermia in patients with severe head injury: cerebral and extracerebral effects, J. Neurosurg. 85 (1996) 533–541.
[210] J.D. Michenfelder, J.H. Milde, The relationship among canine brain temperature, metabolism, and function during hypothermia, Anesthesiology 75 (1991) 130–131.

[211] J.D. Michenfelder, J.H. Milde, Failure of prolonged hypocapnia, hypothermia, or hypertension to favorably alter acute stroke in primates, Stroke 8 (1977) 88–91.
[212] J.D. Michenfelder, R.A. Theye, The effects of anesthesia and hypothermia on canine cerebral ATP and lactate during anoxia produced by decapitation, Anesthesiology 33 (1970) 430–439.
[213] A. Mitani, S. Namba, K. Ikemune, et al., Postischemic enhancements of NMDA and non-NMDA receptor-mediated responses in hippocampal CA1 pyramidal neurons, J. Cereb. Blood Flow Metab. 18 (1998) 1088–1098.
[214] A. Mitani, K. Kataoka, Critical levels of extracellular glutamate mediating gerbil hippocampal delayed neuronal death during hypothermia: brain microdialysis study, Neuroscience 42 (1991) 661–670.
[215] A. Mitani, F. Kayoda, K. Kataoka, Temperature dependence of hypoxia-induced calcium accumulation in gerbil hippocampal slices, Brain Res. 562 (1991) 159–163.
[216] J.J.A. Mooij, A. Buchthal, M. Belopavlovi, Somatosensory evoked potential monitoring of temporary middle cerebral artery occlusion during aneurysm operation, Neurosurgery 21 (1987) 492–496.
[217] D.J. Moyer, F.A. Welsh, E.L. Sager, Spontaneous cerebral hypothermia diminished focal infarction in rat brain, Stroke 23 (1992) 1812–1816.
[218] S. Mullan, A.J. Raimondi, C. Suwanwela, Effect hypothermia upon cerebral injuries in dogs, Arch. Neurol. 5 (1961) 545–551.
[219] J.E. Natale, C.G. D'Alecy, Protection form cerebral ischemia by brain cooling without reduced lactate accumulation in dogs, Stroke 20 (1989) 270–277.
[220] E.E. Nattie, The alpha-stat hypothesis in respiratory control and acid–base balance, J. Appl. Physiol. 69 (1990) 1201–1207.
[221] T. Nimura, P.R. Weinstein, S.M. Massa, et al., Heme oxygenase-1 (HO-1) protein induction in rat brain following focal ischemia, Mol. Brain Res. 37 (1996) 201–208.
[222] H. Nishiyama, S. Danno, Y. Kaneko, et al., Decreased expression of cold-inducible RNA-binding protein (CIRP) in male germ cells at elevated temperature, Am. J. Pathol. 152 (1998) 289–296.
[223] H. Nishiyama, H. Higiashitsuji, H. Yokoi, et al., Cloning and characterization of human CIRP (cold-inducible RNA binding protein) cDNA and chromosomal assignment of the gene, Gene 204 (1997) 115–120.
[224] H. Nishiyama, K. Itoh, Y. Kneko, et al., A glycine-rich RNA-binding protein mediating cold-inducible suppression of mammalian cell growth, J. Cell Biol. 137 (1997) 899–908.
[225] S. Nishio, M. Yunoki, Z.F. Chen, et al., Ischemic tolerance in the rat neocortex following hypothermic preconditioning, J. Neurosurg. 93 (2000) 845–851.
[226] T.S. Nowak, W.A. Pusinelli, Delayed hypothermic protection after transient focal ischemia in spontaneously hypertensive rats, Stroke 30 (1999) 246 (Abstract).
[227] S. Nurse, D. Corbett, Neuroprotection after several days of mild, drug-induced hypothermia, J. Cereb. Blood Flow Metab. 16 (1996) 474–480.
[228] M. Takayasu, R.G. Dacey Jr., Effects of hypothermia and hyperthermia on the reactivity of rat intracerebral arterioles in vitro, J. Neurosurg. 75 (1991) 433–439.
[229] K. Oguro, T. Miyawaki, H. Cho, et al., Cyclic changes in NMDA receptor activation in hippocampal CA1 neurons after ischemia, Neurosci. Res. 29 (1997) 273–281.
[230] T. Ohnishi, X. Wang, K. Ohnishi, et al., p53-dependent induction of WAF-1 by cold shock in human glioblastomas cells, Oncogene 16 (1998) 1507–1511.
[231] C. Okuda, M. Miyazaki, K. Kuriyama, Hypothalamic control of pituitary and adrenal hormones during hypothermia, Psychoneuroendocrinology 11 (1986) 415–427.
[232] K. Okubo, S. Itoh, K. Isobe, et al., Cerebral metabolism and regional cerebral blood flow during moderate systemic cooling in newborn piglets, Pediatr. Int. 43 (2001) 496–501.
[233] H.S. Oliff, E. Weber, G. Eilon, et al., The role of strain/vendor differences on the outcome of focal ischemia induced by intraluminal middle cerebral artery occlusion in the rat, Brain Res. 675 (1995) 20–26.
[234] K. Ohta, R. Graf, G. Rosner, W.D. Heiss, Profiles of cortical tissue depolarization in cat focal cerebral ischemia in relation to calcium ion homeostasis and nitric oxide production, J. Cereb. Blood Flow Metab. 17 (1997) 1170–1181.
[235] K. Ohta, R. Graf, G. Rosner, W.D. Heiss, Calcium ion transients in peri-infarct depolarization may deteriorate ion homeostasis and expand infarction in focal cerebral ischemia in cats, Stroke 32 (2001) 535–543.
[236] K. Ohta, R. Graf, G. Rosner, E. Kumura, W.D. Heiss, Early nitric oxide increase in depolarized tissue of cat focal cerebral ischemia, Neuroreport 8 (1996) 143–148.
[237] C.M. Oung, M.S. Li, D. Shum-Tim, R.C. Chiu, E.J. Hinchey, In vivo study of bleeding time and arterial hemorrhage in hypothermic versus normothermic animals, J. Trauma 35 (1993) 251–254.
[238] A. Patt, B.L. McCroskey, E.E. Moore, Hypothermia-induced coagulopathies in trauma, Surg. Clin. North Am. 68 (1988) 775–785.
[239] H.M. Payan, J.R. Conrad, Carotid ligation in gerbils. Influence of age, sex, and gonads, Stroke 8 (1977) 194–196.
[240] L.C. Pettigrew, J.C. Grotta, H.M. Rhoades, et al., Regional depletion of adenosine triphosphate, Metab. Brain Dis. 3 (1988) 185–198.
[241] S. Phadtare, J. Alsian, M. Inouye, Cold shock response and cold-shock proteins, Curr. Opin. Microbiol. 2 (1999) 175–180.
[242] D. Piani, K. Frey, K.Q. Do, et al., Murine brain macrophages induce NMDA receptor mediated neurotoxicity in vitro by secretin glutamate, Neurosci. Lett. 133 (1991) 159–162.
[243] D.L. Paterson, W.H. Staplefeldt, M.M. Wagener, et al., Intraoperative hypothermia is an independent risk factor for early cytomegalovirus infection in liver transplant recipients, Transplantation 67 (1999) 1151–1155.
[244] O.B. Paulson, S. Strandgaard, L. Edvinsson, Cerebral autoregulation, Cerebrovasc. Brain Metab. Rev. 2 (1990) 161–192.
[245] C.K. Petito, E. Feldmann, W.A. Pusinelli, et al., Delayed hippocampal damage in humans following cardiorespiratory arrest, Neurology 37 (1987) 1281–1286.
[246] F.K. Pierua, M.R. Klee, F.W. Klussman, Effects of local hypo- and hyperthermia on mammalian spinal motoneurons, Fed. Proc. 28 (1969) 1006–1010.
[247] A.M. Planas, M.A. Soriano, M. Berruezo, et al., Induction of Stat3, a signal transducer and transcription factor, in reactive imbroglio following transient focal cerebral ischemia, Eur. J. Neurosci. 8 (1996) 2612–2618.
[248] K. H, A.R.J. Girbes, S.M. Egeler-Peerdeman, Hypomagnesia and hypophosphatemia induced by cooling in patients with severe head injury, Intensive Care Med. 25 (1999) S96 (Abstract).
[249] K.H. Polderman, R. Tjong Tijn Joe, S.M. Peerdeman, et al., Effects of therapeutic hypothermia on intracranial pressure and outcome in patients with severe head injury, Intensive Care Med. 28 (2002) 1563–1573.
[250] P. Prakasa Babu, Y. Yoshida, M. Su, et al., Immunohistochemical expression of Bcl-2, BAX and cytochrome *c* following focal cerebral ischemia and effect of hypothermia in rat, Neurosci. Lett. 29 (2000) 196–200.
[251] H. Rahn, Body temperature and acid–base autoregulation, Pneumologie 151 (1974) 87–94.
[252] H. Rahn, R.B. Reeves, B.J. Howell, Hydrogen ion regulation, temperature, and evolution, Am. Rev. Respir. Dis. 112 (1975) 165–175.
[253] C.E. Rapela, H.D. Green, Autoregulation of canine cerebral blood flow, Circ. Res. 14 (Suppl. I) (1964) I-205–I-211.
[254] T. Rekand, I.A. Sulg, L. Bjaertnes, Neuromonitoring in hypothermia and in hypothermic hypoxia, Arch. Med. Res. 50 (Suppl. 6) (1991) 32–36.

[255] R.L. Reed 2d, A.W. Bracey Jr., J.D. Hudson, et al., Hypothermia and blood coagulation: dissociation between enzyme activity and clotting factor levels, Circ. Shock 32 (1990) 141–152.
[256] R.B. Reevers, Role of body temperature in determining the acid–base state of vertebrates, Fed. Proc. 28 (1969) 1204–1208.
[257] H. Reith, H.S. Jorgensen, H. Nakayama, et al., Seizures in acute stroke: predictors and prognostic significance. The Copenhagen Stroke Study, Stroke 28 (1997) 1585–1589.
[258] J. Reith, H.S. Jorgenseon, P.M. Pederseon, et al., Body temperature in acute stroke: relation to stroke severity, infarct size, mortality, and outcome, Lancet 347 (1996) 422–425.
[259] J.B. Reuler, R.A. Parker, Peritoneal dialysis in the management of hypothermia, J. Am. Med. Assoc. 240 (1978) 2289–2290.
[260] J.B. Reuler, Hypothermia: pathophysiology, clinical settings, and management, Ann. Intern. Med. 89 (1978) 519–527.
[261] M.J. Rohrer, A.M. Natale, Effect of hypothermia on the coagulation cascade, Crit. Care Med. 20 (1992) 1402–1405.
[262] D.F. Rolfe, G.C. Brown, Cellular energy utilization and molecular origin of standard metabolic rate in mammals, Physiol. Res. 77 (1997) 731–758.
[263] A.H. Ropper, Evoked potentials in cerebral ischemia, Stroke 17 (1986) 3 (Editorial).
[264] G. Rosner, R. Graf, K. Kataoka, et al., Selective vulnerability of cortical neurons following transient MCA-occlusion in the cat, Stroke 17 (1986) 76–82.
[265] H.L. Rosomoff, D.A. Holaday, Cerebral blood flow and cerebral oxygen consumption during hypothermia, Am. J. Physiol. 179 (1954) 85–88.
[266] T. Sakamoto, W.W. Monafo, Regional blood flow in the brain and spinal cold of hypothermic rats, Am. J. Physiol. 247 (1989) H785–H790.
[267] O. Sanz, A. Estrada, I. Ferrer, et al., Differential cellular distribution and dynamics of Hsp70, cyclooxygenase-2, and c-fos in the rat brain after transient focal ischemia or kainic acid, Neuroscience 80 (1997) 221–232.
[268] K.D. Sarge, Egulation of HSF1 activation and Hsp expression in mouse tissues under physiological stress conditions, Ann. N. Y. Acad. Sci. 30 (1998) 112–116.
[269] B. Schaller, New pathophysiological and therapeutic aspects. Part 1. The treatment in the primary phase, Swiss Surg. 8 (2002) 123–137.
[270] B. Schaller, R. Graf, Cerebral ischemic preconditioning: an experimental phenomenon or clinical important entity of stroke prevention, J. Neurol. 11 (2002) 1503–1511.
[271] B. Schaller, R. Graf, A.H. Jacobs, Invited commentary. Ischemic tolerance: a window to endogenous neuroprotection? Lancet 362 (2003) 1007–1008.
[272] K. Schiene, C. Bruehl, K. Zilles, et al., Neuronal hyperexcitability and reduction of GABA A-receptor expression in the surround of cerebral photothrombosis, J. Cereb. Blood Flow Metab. 16 (1996) 906–914.
[273] A. Schubert, Should mild hypothermia be routinely used for human cerebral protection? The flip side, J. Neurosurg. Anesth. 4 (1992) 616–620.
[274] S. Schwab, D. Georgiadis, J. Berrouschot, et al., Feasibility and safety of moderate hypothermia after massive hemispheric infarction, Stroke 32 (2001) 2033–2035.
[275] W.R. Selman, A.J. Ricci, R.C. Crumrine, et al., The evolution of focal ischemic damage: a metabolic analysis, Metab. Brain Dis. 5 (1990) 33–44.
[276] K. Shiraki, S. Sagawa, F. Tajima, et al., Independence of brain and tympanic temperatures in an anaesthetized human, J. Appl. Physiol. 65 (1988) 482–486.
[277] L.H. Short, R.E. Peterson, P.D. Mongan, Physiologic and aesthetic alterations on spinal-sciatic evoked responses in swine, Anesth. Analg. 76 (1993) 259–265.
[278] A. Shuaib, D. Trulove, M.S. Ijaz, et al., The effect of post-ischemic hypothermia following repetitive cerebral ischemia in gerbils, Neurosci. Lett. 186 (1995) 165–168.
[279] A. Shuaib, T. Waqar, T. Wishart, et al., Post-ischemic therapy with CGS-19755 (alone or in combination with hypothermia) in gerbils, Neurosci. Lett. 191 (1995) 87–90.
[280] Q.S. Si, Y. Nakamura, K. Kataoka, Hypothermic suppression of microglial activation in culture: inhibition of cell proliferation and production of nitric oxide and superoxide, Neuroscience 81 (1997) 223–229.
[281] T.J. Sick, R. Tang, M.A. Perez-Pinzon, Cerebral blood flow does not mediated the effect of brain temperature on recovery of extracellular potassium ion activity after transient focal ischemia in the rat, Brain Res. 821 (1999) 400–406.
[282] B.K. Siesjo, Pathophysiology and treatment of focal cerebral ischemia. Part I. Pathophysiology, J. Neurosurg. 77 (1992) 169–184.
[283] B.K. Siesjö, Brain Energy Metabolism, Wiley, Chichester, UK, 1978.
[284] I.A. Silver, M. Erecinska, Ion homeostasis in rat brain in vivo: intra- and extracellular (Ca^{2+}) and (H^{+}) in the hippocampus during recovery form short-term, transient ischemia, J. Cereb. Blood Flow Metab. 12 (1992) 759–772.
[285] E.S. Sirimanne, R.M. Blumberg, D. Bossano, et al., The effect of prolonged modification of cerebral temperature on outcome after hypoxic–ischemic brain injury in the infant rat, Pediatr. Res. 39 (1996) 591–597.
[286] S. L, E.D. Hall, Mild pre- and posttraumatic hypothermia attenuates blood–brain barrier damage following controlled cortical impact injury in the rat, J. Neurotrauma 13 (1996) 1–9.
[287] S.L. Smith, P.K. Andrus, J.R. Zhang, et al., Direct measurement of hydroxyl radicals., lipid peroxidation, and blood–brain barrier disruption following unilateral cortical impact head injury in the rat, J. Neurotrauma 11 (1994) 393–404.
[288] T.R. Soderling, CaM-kinase: modulators of synaptic plasticity, Curr. Opin. Neurobiol. 10 (2000) 375–380.
[289] R.A. Solomon, C.R. Smith, E.C. Raps, Deeps hypothermic circulatory arrest for the management of complex anterior and posterior circulation aneurysms, Neurosurgery 29 (1991) 732–738.
[290] H. Sohmer, S. Gold, M. Cahani, et al., Effects of hypothermia on auditory brain-stem and somatosensory evoked responses. A model of a synaptic and axonal lesion, Electroencephalogr. Clin. Neurophysiol. 74 (1989) 50–57.
[291] R.F. Spetzler, M.N. Hadley, D. Regamoti, Aneurysms of the basilar artery treated with circulatory arrest, hypothermia and barbiturate cerebral protection, J. Neurosurg. 68 (1988) 868–879.
[292] C. Stapf, J.P. Mohr, Ischemic stroke therapy, Ann. Rev. Med. 53 (2002) 453–475.
[293] P. Steen, J. Milde, J.D. Michenfelder, The detrimental defects of prolonged hypothermia and rewarming in the dog, Anesthesiology 52 (1980) 224–230.
[294] P.A. Steen, E.H. Soule, J.D. Michenfelder, Detrimental effect of prolonged hypothermia in cats and monkey with and without regional cerebral ischemia, Stroke 10 (1979) 522–529.
[295] P.A. Steen, J.D. Michenfelder, J.H. Milde, Incomplete versus complete cerebral ischemia: improved outcome with minimal blood flow, Ann. Neurol. 6 (1979) 389–398.
[296] T. Steiner, T. Friede, A. Aschoff, et al., Effect and feasibility of controlled rewarming after moderate hypothermia in stroke patients with malignant infarction of the middle cerebral artery, Stroke 32 (2001) 2825–2833.
[297] R. Stoen, D.I. Seesler, The thermoregulatory threshold is inversely proportional to isoflurane concentration, Anesthesiology 72 (1990) 822–827.
[298] S. Strack, S. Choi, D.M. Lovinger, et al., Translocation of autophosphorylated calcium/calmodulin-dependent protein kinase II to the postsynaptic density, J. Biol. Chem. 272 (1997) 13467–13470.

[299] M.J. Strong, A.S. Keats, Induced hypothermia following cerebral anoxia, Anesthesiology 28 (1967) 920–923.
[300] G.R. Sutherland, G.A. Dix, R.N. Auer, The effect of age in rodent models of focal and forebrain ischemia, Stroke 27 (1996) 1663–1668.
[301] G.R. Sutherland, H. Lesiuk, P. Hazendonk, et al., Magnetic resonance imaging and 31P magnetic resonance spectroscopy study of the effect of temperature on ischemic brain injury, Can. J. Neurol. Sci. 19 (1992) 317–325.
[302] H. Swan, The importance of acid–base management for cardiac and cerebral preservation during open heart operations, Surg. Gynecol. Obstet. 158 (1984) 391–414.
[303] K. Takano, L.L. Latour, J.E. Formato, et al., The role of spreading depression in focal ischemia evaluated by diffusing mapping, Ann. Neurol. 39 (1996) 308–318.
[304] T. Tanaka, T. Okumura, J. Handa, et al., Neurogenic vasodilatation mediated by nitric oxide in porcine cerebral arteries, J. Cardiovasc. Pharmacol. 33 (1999) 56–64.
[305] T.K. Tatemichi, M. Paik, E. Bagiella, et al., Risk of dementia after stroke in a hospitalized cohort: results of a longitudinal study, Neurology 44 (1994) 1885–1891.
[306] D.C. Tharion J, J.M. Celermajer, et al., Profound hypothermia with circulatory arrest. Nine years' clinical experience, J. Thorac. Cardiovasc. Surg. 84 (1982) 66–72.
[307] M.A. Therd, R. Tempelhoff, C.M. Crowder, et al., Convection versus conduction cooling for induction of mild hypothermia during neurovascular procedures in adults, J. Neurosurg. Anesthesiol. 9 (1997) 250–255.
[308] The Hypothermia After Cardiary Arrest Study Group, Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest, NEJM 346 (2002) 549–556.
[309] N. Toda, T. Okamura, Mechanism underlying the response to vasodilator nerve stimulation in isolated dog and monkey cerebral arteries, Am. J. Physiol. 259 (1990) H1511–H1517.
[310] T. Toyoda, S. Suzuki, N.F. Kassell, et al., Intraischemic hypothermia attenuates neutrophil infiltration in the rat neocortex after focal ischemia–reperfusion injury, Neurosurgery 39 (1996) 1200–1205.
[311] H. Tsubokawa, K. Oguro, T. Masuzawa, et al., Ca^{2+}-dependent non-NMDA receptor-mediated synaptic currents in ischemic CA1 hippocampal neurons, J. Neurophysiol. 71 (1994) 1190–1196.
[312] H. Tsubokawa, K. Oguro, H.P.C. Robinson, Abnormal Ca^{2+} homeostasis before cell death revealed by whole cell recording of ischemic CA1 hippocampal neurons, Neuroscience 49 (1992) 807–817.
[313] J. Tsukada, K. Saito, W.R. Waterman, et al., Transcription factors NF-IL-6 and CREB recognized a common essential site in the human prointerleukin 1beta gene, Mol. Cell. Biol. 14 (1994) 7285–7297.
[314] T. Tveita, K. Ytrehus, E.S.P. Myhre, et al., Left ventricular dysfunction following rewarming from experimental hypothermia, J. Appl. Physiol. 85 (1998) 2135–2139.
[315] T. Tveita, K. Ytreshus, M. Skandfer, et al., Changes in blood flow distribution and capillary function after deep hypothermia in rat, Can. J. Physiol. Pharmacol. 74 (1996) 376–381.
[316] T. Tveita, E. Traasdal, K. Ytreshus, Alteration of autonomous cardiovascular control after rewarming from experimental hypothermia in rat, Acta Anaesthesiol. Scand. 43 (1999) 1–2.
[317] M. Ueda, N. Watanabe, Y. Ushikubo, et al., The effect of hypothermia on CSD propagation in rats, No Shinkei Geka 25 (1997) 523–528.
[318] A. Uusaro, J.A. Russell, Could anti-inflammatory actions of catecholamines explain the possible beneficial effects of supranormal oxygen delivery in critically ill surgical patients? Intensive Care Med. 26 (2000) 299–304.
[319] C.R. Valeri, H. Feingold, G. Cassidy, et al., Hypothermia-induced reversible platelet dysfunction, Ann. Surg. 205 (1987) 175–181.
[320] M. van Lookeren Campagne, R. Gill, Ultrastructural morphological changes are not characteristic of apoptotic cell death following focal cerebral ischemia in the rat, Neurosci. Lett. 13 (1996) 111–114.
[321] A. Vanella, C. Di Giacomo, V. Sorrenti, et al., Free radical scavenger depletion in post-ischemic reperfusion brain damage, Neurochem. Res. 18 (1993) 1337–1340.
[322] R.C. Vannucci, J.Y. Yager, S.J. Vannucci, Cerebral glucose and energy utilization during the evaluation of hypoxic–ischemic brain damage in the immature rat, J. Cereb. Blood Flow Metab. 14 (1994) 279–288.
[323] A.T. Vanrheineck Leyssius, C.J. Kalkman, et al., Influence of moderate hypothermia on posterior tibial nerve somatosensory evoked potentials, Anesth. Analg. 65 (1986) 475–480.
[324] M.J.J. Verhaegen, M.M. Todd, B.J. Hindma, et al., Cerebral autoregulation during moderate hypothermia in rats, Stroke 24 (1993) 407–414.
[325] B. Walter, R. Bauer, G. Kuhnen, et al., Coupling of cerebral blood flow and oxygen metabolism in infant pigs during selective brain hypothermia, J. Cereb. Blood Flow Metab. 20 (2000) 1215–1224.
[326] M.S. Wainwright, H. Sheng, Y. Sato, et al., Pharmacological correction of hypothermic p50 shift does not alter outcome from focal cerebral ischemia in rats, Am. J. Physiol. Heart Circ. Physiol. 282 (2002) H1863–H1870.
[327] M. Walton, E. Sirimanne, C. Williams, The role of the cyclic AMP-responsive element binding protein (CREB) in hypoxic–ischemic brain damage and repair, Mol. Brain Res. 43 (1996) 21–29.
[328] W.A. Warner, A.H. Anton, T.W. Andersen, et al., Ventricular fibrillation and catecholamine responses during profound hypothermia in dogs, Anesthesiology 33 (1970) 43–51.
[329] C.T. Wass, W.L. Lanier, Hypothermia-associated protection form ischemic brain injury: implications for patient management, Int. Anesthesiol. Clin. 34 (1996) 95–111.
[330] M.N. Waxham, J.C. Grotta, A.J. Silva, et al., Ischemia-induced neuronal damage: a role for calcium/calmodulin-dependent protein kinase II, J. Cereb. Blood Flow Metab. 16 (1996) 1–6.
[331] F.A. Welsh, R.E. Sims, V.A. Harris, Mild hypothermia prevents ischemic injury in gerbil hippocampus, J. Cereb. Blood Flow Metab. 10 (1990) 557–563.
[332] T. Westermaier, S. Zausinger, A. Baethmann, et al., No additional neuroprotection provided by barbiturate-induced burst suppression under mild hypothermic conditions in rats subjected to reversible focal ischemia, J. Neurosurg. 93 (2000) 835–844.
[333] F. White, A comparative physiological approach to hypothermia, J. Thorac. Cardiovasc. Surg. 82 (1981) 821–831.
[334] R. Widmann, T. Miyazawa, K.A. Hossmann, Protective effect of hypothermia on hippocampal injury after 30 min of forebrain ischemia in rats is mediated by postischemic recovery of protein synthesis, J. Neurochem. 61 (1993) 200–209.
[335] C. Wiessner, I. Brink, P. Lorenz, et al., Cyclin D1 messenger RNA is induced in microglia rather than neurons following transient forebrain ischemia, Neuroscience 72 (1996) 947–958.
[336] D.C. Wilford, E.P. Hill, W.Y. Moores, Theoretical analysis of oxygen transport during hypothermia, J. Clin. Monit. 2 (1986) 30–43.
[337] G.D. Williams, J. Twofighi, M.B. Smith, Cerebral energy metabolism during hypoxia–ischemia correlates with brain damage: a ^{31}P NMR study in unanesthetized immature rats, Neurosci. Lett. 170 (1994) 31–34.
[338] M.D. Williams, W.G. Rainer, H.G. Fieger, et al., Cardiopulmonary bypass, profound hypothermia, and circulatory arrest for neurosurgery, Ann. Thorac. Surg. 52 (1991) 1069–1075.
[339] J.S. Williston, D.L. Jewett, The Q10 of auditory brain stem responses in rats under hypothermia, Audiology 21 (1982) 457–465.
[340] L.R. Wolin, L.C. Massopust Jr., R.J. White, Behavioral effects of autocerebral perfusion, hypothermia and arrest of cerebral blood flow in the rhesus monkey, Exp. Neurol. 39 (1973) 336–341.
[341] T.E. Woodcock, J.M. Murkin, J.K. Farrar, et al., Pharmacologic EEG suppression during cardiopulmonary bypass: cerebral hemodynamic

and metabolic effects of thiopental or isoflurane during hypothermia and normothermia, Anesthesiology 67 (1987) 218–224.
[342] B. Woodhall, W. Sealy, K. Hall, et al., Craniotomy under conditions of quinidine-protected cardioplegia and profound hypothermia, Ann. Surg. 152 (1960) 37–42.
[343] P.F. Worley, A.J. Cole, D.W. Saffen, et al., Regulation of immediate early genes in brain: role of NMDA receptor activation, Prog. Brain Res. 587 (1990) 66–72.
[344] J.S. Wyatt, A.D. Edwards, D. Azzopardi, et al., Magnetic resonance and near infrared spectroscopy for investigation of perinatal hypoxic-ischaemic brain injury, Arch. Dis. Child 64 (1989) 953–963.
[345] D. Xue, Z.G. Huang, K.E. Smith, et al., Immediate or delayed mild hypothermia prevents focal cerebral infarction, Brain Res. 587 (1992) 66–72.
[346] J.H. Xue, K. Nonoguchi, M. Fukumoto, et al., Effects of ischemia and H_2O_2 on the cold stress protein CIRP expression in rat neuronal cells, Free Radic. Biol. Med. 27 (1999) 1238–1244.
[347] J.Y. Yager, J. Asselin, Effect of mild hypothermia on cerebral energy metabolism during the evolution of hypoxic–ischemic brain damage in the immature rat, Stroke 27 (1996) 919–926.
[348] J.Y. Yager, G. Kala, L. Hertz, et al., Correlation between content of high-energy phosphates and hypoxic–ischemic damage in immature and mature astrocytes, Dev. Brain Res. 82 (1994) 62–68.
[349] J.Y. Yager, R.M. Brucklacher, R.C. Vannucci, Cerebral energy metabolism during hypoxia–ischemia and early recovery in immature rats, Am. J. Physiol. 262 (1992) H672–H677.
[350] K. Yamanaka, Cold shock response in *Escherichia coli*, in: M. Inoue, K. Yamanaka (Eds.), Cold Shock: Response and Adaptation, Horizon, Norfolk, VA, 2000, pp. 5–26.
[351] K. Yamashita, Y. Eguchi, K. Kajiwara, et al., Mild hypothermia ameliorates ubiqutin synthesis and prevent delayed neuronal death in the gerbil hippocampus, Stroke 22 (1991) 1574–1581.
[352] H. Yanamoto, S.C. Hong, S. Soleau, et al., Mild postischemic hypothermia limits cerebral injury following transient focal ischemia in rat neocortex, Brain Res. 718 (1996) 207–211.
[353] H. Yanase, A. Mitani, K. Kataoka, Postischemic enhancement of calcium mobilization to NMDA, and the effect of hypothermia, J. Neurochem. 65 (1995) 17 (Abstract).
[354] C. Yang, F. Carrier, The UV-inducible RNA-binding protein A18 (A18 hnRNP) plays a protective role in the toxic stress response, J. Biol. Chem. 276 (2001) 47277–47284.
[355] M.A. Yenari, S. Iwayama, D. Cheng, et al., Mild hypothermia attenuates cytochrome *c* release but does not alter Bcl-2 expression or caspase activation after experimental stroke, J. Cereb. Blood Flow Metab. 22 (2002) 29–38.
[356] Y. Yoneda, K. Ogita, K. Inoue, et al., Rapid potentiation of DNA binding activates of particular transcription factors with leucine-zipper motifs in discrete brain structures of the gerbil with transient forebrain ischemia, Brain Res. 667 (1994) 54–66.
[357] W.L. Young, P.C. McCormick, Perioperative management of intracranial catastrophes, Crit. Care Med. 5 (1989) 821–848.
[358] R.S.K. Young, T.P. Olenginski, S.K. Yagel, et al., The effect of graded hypothermia on hypoxic–ischemic brain damage: a neuropathologic study in the neonatal rat, Stroke 14 (1983) 929–934.
[359] E.Z. Llonga, P.R. Weinstein, S. Carlson, et al., Reversible middle cerebral artery occlusion without craniectomy in rats, Stroke 20 (1989) 84–91.
[360] Z.G. Zhang, M. Chopp, H. Chen, Duration dependent post-ischemic hypothermia alleviates cortical damage after transient middle cerebral artery occlusion in the rat, J. Neuronal Sci. 117 (1993) 240–244.
[361] R.L. Zhang, M. Chopp, H. Chen, et al., Postischemic (1 h) hypothermia significantly reduces ischemic cell damage in rats subjected to 2 h of middle cerebral artery occlusion, Stroke 24 (1993) 1235–1240.
[362] J. Zeitlhofer, M. Steiner, K. Bousek, et al., The influence of temperature on somatosensory-evoked potentials during cardiopulmonary bypass, Eur. Neurol. 30 (1990) 284–290.
[363] H. Zhang, E.T. Tong, Protection of mild hypothermia beginning at different time intervals on cerebral ischemia/reperfusion injury, J. Neurochem. 1 (Suppl. 1) (2002) 108–111.
[364] J.M. Zimmermann, F.C. Spencer, The influence of hypothermia on cerebral injury resulting from circulatory occlusion, Surg. Forum 9 (1959) 216–218.