INTERNATIONAL STUDIES IN THE FIELD OF

PHYSIOLOGY

MART 2026

EDITOR

Prof. Dr. Hamit USLU

gece kitaplığı

İmtiyaz Sahibi / Yaşar Hız
Yayına Hazırlayan / Gece Kitaplığı

Birinci Basım / Mart 2026 - Ankara
ISBN / 978-605-7885-50-0

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CONTENTS

CHAPTER 1

PLANT-DERIVED EXTRACELLULAR VESICLES IN NEURODEGENERATIVE DISEASES

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1. INTRODUCTION

Neurodegenerative diseases (NDDs) comprise a diverse class of progressive neurobiological conditions defined by gradual deterioration of neurons and their functions, frequently accompanied by abnormal protein deposits. Common examples include Alzheimer's disease (AD), Parkinson's disease (PD), and multiple sclerosis (MS) (Li et al., 2026). These disorders cause significant cognitive, motor, and autonomic problems, which reduce patients' physical and mental well-being and place considerable economic and caregiving impacts on families and population (Hu et al., 2026). With the increasing age of populations worldwide, the prevalence of NDDs is increasing quickly, demonstrating the need for alternative treatment options.

In recent years, extracellular vesicles (EVs), nano-sized lipid bilayer vesicles realesed by cells, have become promising options for therapeutic uses and drug delivery systems (Bodala et al., 2026). EVs, including exosomes, enable intercellular interaction by carrying bioactive molecules like proteins, lipids, and RNAs (Nemati et al., 2022). Although mammalian cell-derived EVs have been widely studied for their biocompatibility and targeting abilities, challenges such as low yield and high production costs have led researchers to seek alternative sources.

Plant-derived extracellular vesicles (PDEVs) have attracted growing interest as a natural, abundant, and affordable source of EVs. Like their mammalian counterparts, plant cells release nanosized vesicles that carry bioactive metabolites, often showing anti-inflammatory, antioxidant, and anticancer features (Lian et al., 2022). PDEVs from fruits, vegetables, and other plant tissues including ginger, grapes, and grapefruits have shown promise as carriers for therapeutic agents. For example, grapefruit-derived EVs have been successfully used to deliver anticancer drugs in colon cancer models, while ginger-derived EVs loaded with CD98 siRNA achieved targeted gene silencing in colon tissues (Agrawal et al., 2026). Additionally, ongoing clinical trials are examining PDEVs as platforms for delivering bioactive compounds, emphasizing their potential for translation.

Owing to their unique bioactive cargo, natural abundance, and low immunogenicity, PDEVs represent a potential new avenue for therapeutic intervention in neurodegenerative diseases. By combining targeted drug delivery with intrinsic neuroprotective properties,

PDEVs may provide innovative strategies to mitigate neuronal dysfunction and disease progression in NDDs. This chapter focuses on the use of PDEVs in neurological disorders, highlighting their therapeutic potential and current applications.

2. NEURODEGENERATIVE DISEASES

A variety of neurological conditions are marked by progressive loss of neuronal function, causing cognitive, motor, and sensory impairments. Although these conditions vary in symptoms and affected brain areas, they share common features such as protein buildup, neuroinflammation, mitochondrial issues, and age-related cellular aging (Furtado et al., 2018). Recognizing these shared mechanisms offers a foundation for developing innovative treatments, including PDEVs, which may address multiple aspects of neuronal damage. The following sections describe main neurodegenerative diseases and explore how PDEVs might help in their prevention and treatment.

2.1. ALZHEIMER'S DISEASE

AD constitutes the most prevalent neurological degenerative condition and was initially identified by Alois Alzheimer in 1906. It represents the leading determinant of dementia among older adults, responsible for approximately 60–70% of all dementia patients (Gunes et al., 2022). For many years, cognitive decline in older adults was attributed to normal aging and referred to as "senile dementia," with its prevalence rising alongside an aging population. Today, AD represents the predominant diagnosed form of dementia and a major public health concern. Although amyloid-β plaques and neurofibrillary tangles (NFTs) have long been viewed as the primary drivers of AD, alternative mechanistic pathways including oxidative stress and neuroinflammation have also been proposed to explain its complex and still poorly understood pathology (Mecocci et al., 2018). Senescent astrocytes and microglia are prominently detected in the brains of individuals with AD, accompanied through increased SA-β-gal activity. These aged cells secrete pro-inflammatory cytokines, including TNF-α, IL-6, and IL-8, through activation of the NF-κB and p38/MAPK signaling pathways, and can propagate senescence-related features to neighboring cells via paracrine mechanisms. This inflammatory environment further facilitates amyloid plaque accumulation and tau hyperphosphorylation (Zhu et al., 2024). Consistently, investigations in AD mouse models have shown increased expression of markers related to cellular damage and cell cycle

blockage, particularly p16, p21, and p53, along with inflammatory markers namely IL-6 and TNF-α (Dorigatti et al., 2022).

Exosomes and extracellular vesicles possess unique advantages as drug delivery systems owing to their capacity for targeted transport of bioactive compounds (Witwer & Théry, 2019). Alongside the growing emphasis on food–medicine duality and sustainable healthcare, PDEVs have consequently emerged as promising tools for transversing the blood–brain barrier and addressing AD pathology.

In an AD model, plant-derived glucosylceramides from *Amorphophallus konjac* were shown to enhance extracellular vesicle production, leading to reduced amyloid-β accumulation and plaque burden in APP (amyloid precursor protein) transgenic mice (Yuyama et al., 2019). This EV-mediated clearance of amyloid-β was accompanied by improved cognitive performance, revealing the therapeutic potential of PDEV-related mechanisms in AD. In another study, extracellular vesicles derived from the edible plant *Rheum rhabarbarum* exhibited antioxidant activity, diminished intracellular ROS degree, and adjusted energy metabolism in a cellular model of AD, supporting the potential of PDEVs as nutraceutical tools against oxidative stress–related AD pathology (Calzoni et al., 2025). Similarly, another study demonstrated that exosomes can be employed as natural nanocarriers to enhance the brain delivery of plant-derived bioactive compounds, with exosome-encapsulated neferine improving motor performance and reducing β-amyloid levels in APP/PS1 (amyloid precursor protein/presenilin1) transgenic mice more effectively than the free compound (Tang et al., 2022). Additionally, folic acid–modified ginger-derived extracellular vesicles have been shown to promote a shift in microglial phenotype by selectively targeting activated M1 microglia and regulating the PI3K–AKT signaling pathway (Han et al., 2025). Together, these outcomes suggest that PDEVs may help remodel the pathological microenvironment of AD by modulating neuroinflammatory responses.

2.2. PARKINSON'S DISEASE

PD ranks as the second most common neurodegenerative disorder, impacting roughly 2–3% of people aged 65 and above (Poewe et al., 2017). It is associated with the pathological aggregation of α-synuclein accompanied by the progressive decline of dopaminergic neurons in the substantia nigra pars compacta (SNpc), and hallmark motor symptoms such as resting tremor, bradykinesia, and gait disturbances (Chen et al., 2024). With disease progression, α-

synuclein pathology may extend to cortical regions, and a substantial proportion of patients develop cognitive impairment, which is associated with poorer clinical outcomes and diminished quality of life.

Elevated SA-β-gal expression and increased numbers of senescent cells have been observed in the brains of patients with PD and are closely associated with α-synuclein accumulation. PD brain tissues also exhibit upregulation of senescence markers such as $p16^{INK4a}$ and several SASP factors, including MMP-3 and pro-inflammatory cytokines like IL-6, IL-1α, and IL-8, suggesting a contributory role of cellular senescence in dopaminergic neurodegeneration (Chinta et al., 2018). In parallel, mitochondrial dysfunction is a key feature of PD pathogenesis, with PTEN-induced kinase 1 (PINK1), a mitochondria-associated kinase linked to early-onset PD, accumulating in damaged mitochondria (Van Duijn et al., 2001).

PDEVs have also started to attract attention in the context of PD. In a recent study, vesicles isolated from *Gardenia jasminoides* (GDEVs) were shown to protect dopaminergic neurons in rotenone-induced cellular and *Caenorhabditis elegans* models by improving mitochondrial function, reducing α-synuclein levels, and limiting apoptosis, ultimately alleviating PD-like features (Chen et al., 2025). Moreover, extracellular vesicles isolated from *Salvia sclarea* and *Salvia dominica* hairy roots were shown to enter neuronal cells, suppress apoptosis, and reduce oxidative stress in a 6-hydroxydopamine–induced cellular model of PD, supporting the potential of PDEVs as neuroprotective agents in PD (Vestuto et al., 2024). Further supporting the therapeutic potential of PDEVs in PD, Xu et al. reported that intranasal application of extracellular vesicles isolated from *Pueraria lobata* enhanced PINK1–Parkin–dependent mitophagy, improved mitochondrial function and ATP production, and promoted dopaminergic neuron survival, leading to an attenuation of PD-related symptoms (Xu et al., 2024).

2.3. MULTIPLE SCLEROSIS

MS is a chronic autoimmune inflammatory condition affecting the central nervous system (CNS). It is the leading cause of non-traumatic neurological impairments in young adults, influencing nearly 2.5 million individuals worldwide (Liu et al., 2019). The disease is characterized by immune-mediated demyelination attributable to the complex crosstalk between the immune system and the central nervous system. Aberrantly activated autoreactive T cells target myelin antigens, triggering inflammatory responses involving both innate and

adaptive immune cells, which lead to myelin loss, astrocyte activation, disruption of the blood–brain barrier (BBB), oligodendrocyte apoptosis, and ultimately axonal degeneration and neuronal loss within the CNS (Friese et al., 2014).

Aging and age-related cellular senescence are increasingly recognized as key contributors to MS. With advancing age, the aggregation of senescent cells and the release of senescence-associated secretory factors, particularly pro-inflammatory cytokines from senescent microglia, can promote neurodegenerative processes associated with MS. In demyelinated white matter lesions from MS patients, lipofuscin-positive aged glial cells and neurons are frequently observed, in contrast to normal-appearing white matter. Moreover, cellular senescence restricts the remyelination capacity of neural progenitor cells, thereby accelerating disease progression in MS (Nicaise et al., 2019).

Alongside these mechanisms, plant-derived exosome-like nanovesicles (PELNs) have emerged as promising candidates in MS research. Exosomes isolated from *Petasites japonicus* were shown to regulate immune activity by suppressing Th1 cell hyperactivation, reducing pro-inflammatory cytokine secretion, and enhancing cytotoxic T cell–mediated neuroprotective effects, thereby attenuating neuroinflammation and supporting neural repair in MS (Han et al., 2021). These findings underscoring the relevance of EV-based approaches in MS; however, the limited number of PDEV studies suggests the need for further investigation into PDEVs as safe and sustainable alternatives for MS therapy.

3. CONCLUSION AND FUTURE DIRECTIONS

PDEVs have become a versatile and promising tool for neurodegenerative diseases. Growing evidence shows that PDEVs can effectively deliver bioactive molecules, modulate neuroinflammation, improve mitochondrial function, and decrease pathological protein aggregation in models of AD, PD, and MS. The remarkable benefits of PDEVs, including their natural abundance, low immunogenicity, and intrinsic bioactive cargo, make them a promising alternative to traditional drug delivery systems and mammalian-derived EVs.

In spite of these promising findings, multiple obstacles still exist before PDEVs can be broadly applied as clinical therapies. Standardized methods for isolating, characterizing, and producing PDEVs on a large scale are still being developed. Additionally, the mechanisms behind tissue targeting, blood–brain barrier penetration, and intracellular cargo delivery need

further clarification. Safety, biodistribution, and long-term effects of PDEVs also require systematic investigation in preclinical and clinical studies.

Future research should concentrate on optimizing PDEV production and engineering methods to improve their therapeutic effectiveness and targeting accuracy. Comparative studies among various plant sources may help identify the most potent vesicles for specific neurodegenerative diseases. Incorporating PDEVs with existing pharmaceutical or gene therapies could also create new possibilities for combined strategies to slow down or reverse neurodegenerative processes.

In conclusion, PDEVs are a promising advancement in the development of safe, efficient, and natural nanocarriers for neurological treatments. With ongoing progress in isolation methods, mechanistic insights, and clinical testing, PDEVs have significant potential to become a key element for future interventions in neurodegenerative disorders

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CHAPTER 2

THERMOREGULATION PHYSIOLOGY AND CHANGES OCCURRING WITH AGING

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Introduction

Organisms that can maintain their internal temperature nearly constant regardless of changes in the external environment are called homeothermic (warm-blooded), while organisms that cannot regulate their body temperature internally and whose vital processes are entirely dependent on external temperature conditions are called poikilothermic (cold-blooded) organisms (Precht et al., 1973). Temperature variations affect physiological processes such as metabolism, molecular control, and reaction rates. Normal living requires the maintenance of a steady internal temperature. Humans, being homeothermic organisms, also maintain their core body temperature within a narrow range (36.1–37.8 °C), with a normal core body temperature of approximately 37 °C (Osilla et al., 2018). Numerous thermoregulatory systems work together to maintain this equilibrium. However, body temperature can vary depending on the time of day, whether you are hungry or full, the type of food consumed, the ambient temperature, whether clothing is thin or thick, the stage of the menstrual cycle in women, the person's activity level and age, and the presence of infection or feverish illness (Ağar, 2021). For example, there is a circadian rhythm in which body temperature fluctuates by approximately 0,5°C over a 24-hour period, reaching a minimum in the early morning hours and a maximum in the late afternoon. Women who menstruate also have a monthly rhythm; body temperature can increase by up to 1°C after ovulation (Figure 1) (Campbell, 2008). Cellular physiological functions decrease below 36°C. A core body temperature of 42°C or higher is associated with cellular death and protein denaturation, whereas temperatures around 40°C to 41°C are typically observed during fever or intense physical activity (Kuht and Farmery, 2021). The ideal physiological function is ensured by controlling the temperature around 37°C since cellular enzyme reaction rates are temperature-dependent. The body's temperature homeostasis is regulated by the equilibrium between variations in heat production and variations in heat loss (Ağar, 2021). Energy produced through metabolic processes is released primarily as body heat, while a portion is converted into external mechanical work. This heat is transferred to the environment through radiation, convection, and conduction; it is also lost through evaporation mechanisms during sweating and respiration. Conductive, convective, and radiant heat exchanges are considered positive when heat is acquired from the environment and negative when heat is relinquished to the environment. Thermal equilibrium is achieved when the amount of heat produced by the body is equal to the amount exchanged with the environment. Conversely, an imbalance between heat production and heat loss leads to heat storage or heat loss in body tissues, resulting in an increase or decrease in core body temperature. The human

body's heat balance is maintained by physiological thermoregulation mechanisms (Weller, 2005).

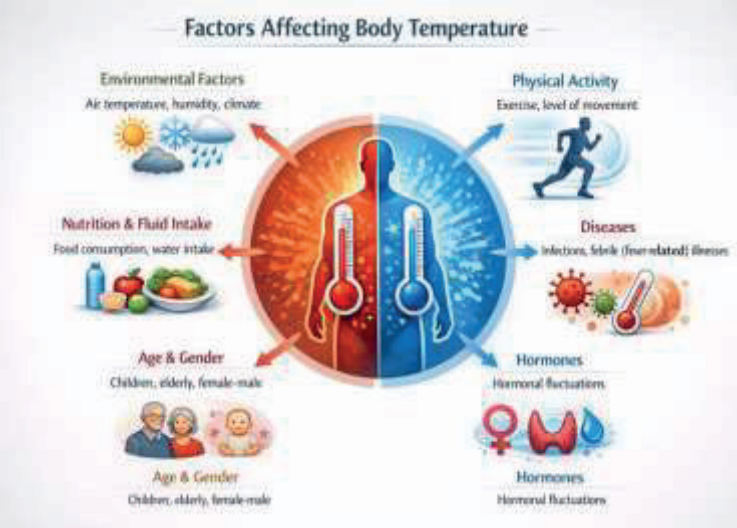

Figure 1. Factors causing changes in body temperature

Conduction, convection, radiation, and evaporation are the ways that the body surface transfers heat to the surroundings. Heat loss or gain through radiation occurs via electromagnetic waves due to the temperature difference between the body surface and surrounding objects. Convective heat loss occurs as a result of the movement of air or liquid in contact with the body surface. Conductive heat loss occurs when the body comes into direct contact with a surface at a lower temperature. Evaporative heat loss occurs through the evaporation of water on the body's surface during sweating and breathing. It is the body's primary heat loss mechanism, especially in high ambient temperatures. In high humidity conditions, reduced evaporation can lead to an increase in body temperature (Figure 2) (Kuht and Farmery, 2021; Werner, 1998).

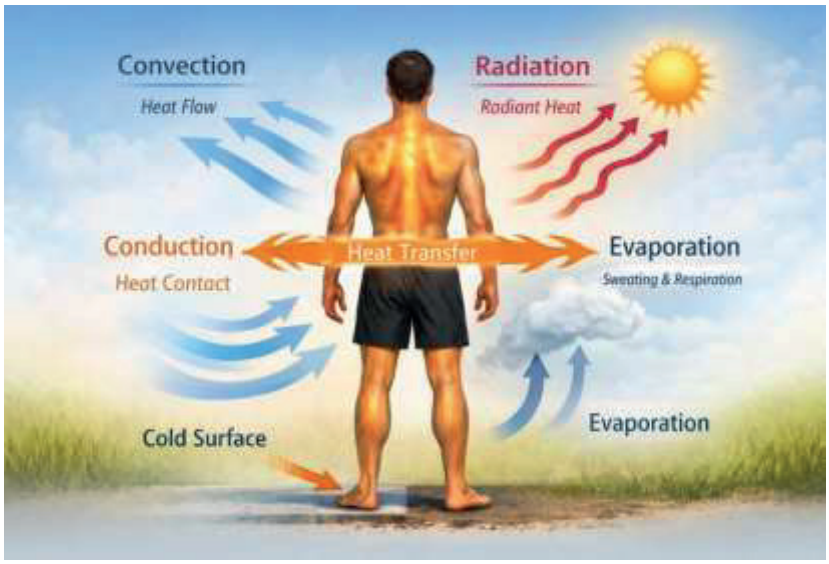

Figure 2. Physiological Mechanisms of Heat Exchange Between the Body and the Environment

The role of the hypothalamus in thermoregulation and changes associated with aging

Environmental and internal body temperature changes are detected by thermal receptors in the skin, internal body tissues, and hypothalamus, which respond to potential temperature changes. The hypothalamus is considered the central control unit for physiological thermoregulation. Hypothalamic control processes like vasodilation, vasoconstriction, shivering, and sweating are triggered to maintain the ideal body temperature (Tonge et al., 2025).

When examining the regions of the hypothalamus involved in thermoregulation, the preoptic area, anterior hypothalamic nucleus, posterior hypothalamic nucleus, dorsomedial hypothalamic nucleus, and paraventricular nucleus stand out. The preoptic area is rich in heat-sensitive neurons and sends excitatory signals to brain regions involved in heat loss mechanisms and inhibitory signals to regions involved in heat production mechanisms. Thermosensitive neuronal populations within the hypothalamus receive afferent signals from sensory neurons responsible for monitoring body temperature. The majority of these sensory neurons have their cell bodies situated in peripheral ganglia, and their axons project to peripheral tissues that play a critical role in maintaining thermal homeostasis (Tan, 2018). At the peripheral ends of somatosensory neurons, thermal transmission occurs over a broad temperature range due to heat-sensitive transient receptor potential ion channels (thermo-TRPs). Today, numerous thermo-TRPs are known, including TRPV1, TRPV4, TRPM2, TRPM3, TRPM8, TRPC5, and TRPA1 (Kashio and Tominaga, 2022). Among these, TRPM8 primarily functions as the

peripheral cold sensor. In vitro studies indicate that this channel becomes activated in response to moderate decreases in temperature (approximately 26–28°C). TRPM8 is present in nearly all neurons that are responsive to cold stimuli, and inhibition of TRPM8-expressing cells abolishes both behavioral and neuronal reactions to cold exposure (Tonge et al., 2025; Bautista et al., 2007; Dhaka et al., 2007). Experiments conducted on mice have shown that TRPM8 deficiency causes significant impairments in the perception of environmental cold (Winter, 2017; Reimúndez et al., 2018). Body temperature regulation primarily exhibits differences depending on hot or cold conditions. In cold environments, the afferent inputs received by free nerve endings (Aδ and C fibers) (as mentioned earlier, especially TRPM8 channels) enter the spinal cord (via the neospinothalamic tract) from the dorsal root, synapse in Lamina I (Lamina marginalis), cross over in the anterior commissure, and ascend to reach the parabrachial nucleus in the brainstem via the anterolateral tract. They then reach the preoptic area of the hypothalamus. Stimulation of this pathway triggers tremor and vasoconstriction responses. In the perception of heat, the TRPV1–V3 receptor family and mostly C fibers are involved. The sensation of heat essentially reaches the brain via two different pathways. If the sensation of heat can be localized, it enters the spinal cord via the dorsal horn through fast Aδ fibers and synapses in lamina I - lamina V. It crosses over at the anterior commissure, turns upward, and terminates in the ventral posterolateral nucleus of the thalamus within the neospinothalamic tract, where it is transmitted to the somatosensory cortex. If the sensation of heat cannot be localized, it enters the spinal cord via unmyelinated C fibers with slow conduction, then synapses in lamina II and lamina III (substantia gelatinosa), crosses over to the opposite side via the anterior commissure, turns upward, and synapses again in the intralaminar nucleus of the thalamus within the paleospinothalamic tract. From here, it projects to the amygdala, hypothalamus, and reticular formation structures. As a result of all this, discomfort, restlessness, and sweating autonomic responses are triggered (Hall and Hall, 2020; Isa and Chetty, 2021; Craig, 2004).

When the central nervous system is stimulated by afferent inputs, different pathways are activated depending on whether the processed data is hot or cold. The initial response to a cold stimulus is the activation of the vasoconstriction mechanism. At this point, sympathetic noradrenergic stimulation increases vascular tone, causing the blood vessels to constrict. As a result, skin blood flow is reduced, thereby attempting to conserve existing heat. Another method of increasing body temperature is to activate involuntary muscle contractions, which consume ATP to produce heat. Additionally, perhaps one of the most important pathways is the heat

production pathway known as shivering-free thermogenesis (Castellani and Young, 2016; Brychta and Chen, 2017). Brown adipose tissue, which is extremely rich in mitochondria, contains uncoupling protein 1 (UCP1), a molecule located in the inner mitochondrial membrane that plays a crucial role in heat production. Indeed, when UCP1 is activated, guanosine diphosphate (GDP) binding sites are exposed, initiating the free flow of protons across the inner mitochondrial membrane. This mechanism allows protons to bypass the ATP synthase and return to the matrix. During this process, ATP is not produced; instead, energy is rapidly released as heat (Sidossis and Kajimura, 2015; Symonds et al., 2015; Cypess et al., 2009; Klingenspor and Fromme, 2011). It should also be noted that hormonal regulation is used to increase basal metabolic rate in order to boost heat production during adaptation to cold.

If the central nervous system is activated by a warm stimulus, sympathetic vasoconstrictor tone is suppressed first. Naturally, widespread peripheral vasodilation occurs, and skin blood flow increases. At this time, heat loss through radiation and convection is facilitated. Of course, shivering is suppressed during this time, and the activity of brown adipose tissue is reduced to try to prevent heat production. These events are often followed by behaviors such as increased cardiac output, moving to a cool environment, and stimulating thirst to replace fluids lost through sweating (McAllen and McKinley, 2018; Smith and Johnson, 2016; Charkoudian and Morrison, 2023).

Studies have shown that norepinephrine released from sympathetic nerves is largely responsible for cold-induced vasoconstriction, while other substances play a lesser role. Norepinephrine exerts its vasoconstrictor effect by binding to α-adrenergic receptors located on vascular smooth muscle cells. Similarly, Neuropeptide Y (NPY), released from sympathetic nerve endings during sympathetic activation in a cold environment, also causes constriction in vascular smooth muscle cells (Figure 3) (Alba et al., 2019; Holowatz et al., 2010). On the contrary, in response to an increase in body temperature, there is a significant increase in skin blood flow. This is because the cholinergic vasodilator system is activated in response to the increase in body temperature. Acetylcholine binds to M3 receptors located in the vascular endothelium, causing an increase in calcium in endothelial cells, activation of endothelial nitric oxide synthase (eNOS), and nitric oxide (NO) production, leading to vasodilation in vascular smooth muscle. Acetylcholine also stimulates the release of vasodilator substances such as prostacyclin (PGI_2) and vasoactive intestinal peptide (VIP) from endothelial cells. It should also be noted that acetylcholine binds to M3 receptors in sweat glands, facilitating heat loss through sweating (Figure 3) (Johnson et al., 2014; Holowatz et al., 2005; Holowatz and Kenney, 2010).

In the elderly, decreased levels of acetylcholine and nitric oxide, particularly in hot conditions, as well as impaired vascular endothelial function, lead to disruptions in vasodilation mechanisms (Johnson et al., 2014; Holowatz and Kenney, 2010). The situation is somewhat more complex in cold conditions. In young individuals, norepinephrine accounts for 60% of reflex cutaneous vasoconstriction, while sympathetic cotransmitters (neuropeptide Y and ATP) account for 40%. In older individuals, the secretion of co-transmitters and, consequently, their contribution to vasoconstriction is almost completely eliminated. In this case, the reflex vasoconstriction mechanism becomes almost entirely dependent on norepinephrine. However, the fact that neuronal norepinephrine secretion is also reduced in this process results in a significant disruption of the vasoconstriction mechanism (Holowatz et al., 2010; Thompson and Kenney, 2004; Thompson-Torgerson et al., 2008). Age-related attenuation of sympathetic neurotransmission and co-transmitter contribution during cold exposure is accompanied by a relative increase in endothelin-1 (ET-1) signaling and Rho-kinase (ROCK)–dependent vasoconstrictor mechanisms. Although enhanced ET-1/ROCK activity may partially preserve vasoconstrictor tone in aged skin, this compensation appears to be less finely regulated than adrenergic control. Increased ROCK-mediated calcium sensitization and reduced nitric oxide bioavailability may promote sustained and exaggerated vasoconstriction, potentially impairing microvascular perfusion. Therefore, while ET-1 and ROCK pathways may contribute to maintaining vascular tone during cold stress in older adults, they are unlikely to fully restore effective thermoregulatory homeostasis and may instead shift the response toward a more rigid and maladaptive vascular phenotype (Figure 3) (Holowatz and Kenney, 2010; Thompson and Kenney, 2004; Thompson-Torgerson et al., 2008).

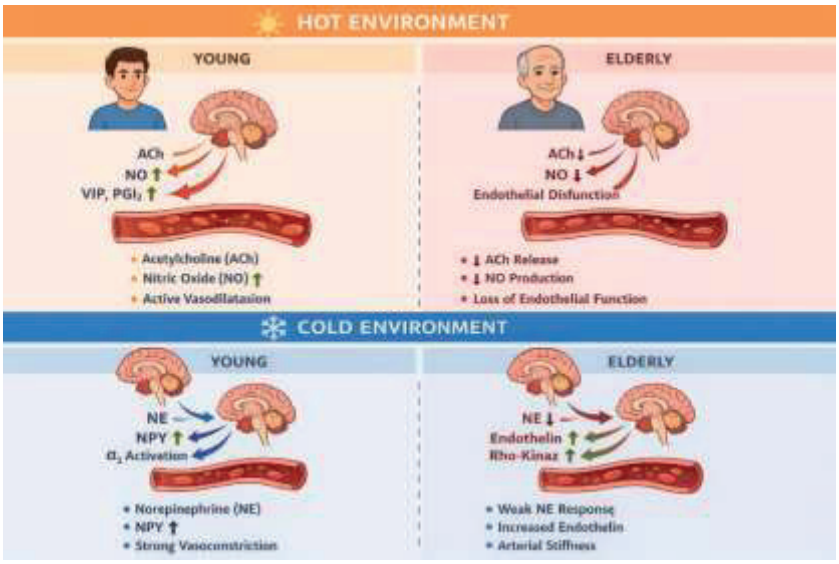

Figure 3. Factors affecting vasoconstriction and vasodilation in response to heat and cold in young and elderly individuals.

In fact, the biological mechanisms underlying the processes related to aging are not fully known today. Therefore, there is no consensus among researchers on a functional definition of the concept of aging. Nevertheless, if we want to define aging by considering the biological literature, it is possible to define it as the gradual deterioration of the physiological functions of the living being after the maturity period (Florez-Duquet and Mcdonald, 1998).

Today, many theories have been put forward about the occurrence of aging.

- With each cell division, the protective sheath at the ends of the telomere disappears a little more, resulting in errors in DNA replication.
- Decrease in maintenance and repair mechanisms over time and consequently delayed repair of cellular damages and their accumulation (autophagy disorders, mitochondrial dysfunction, etc.)
- Accumulation of free radicals formed as a result of metabolism over time and damage to cells
- The organism's use of energy and energy resources for reproductive functions rather than for longevity
- Accumulation of DNA mutations over time
- Depletion of stem cells
- Changes in intercellular communication
- Irregularities in the perception of food

- Genes that are beneficial for survival in early life become harmful later in life.

The theories mentioned above are the main theories put forward about the formation of aging (Preston and Biddell, 2024; Guo et al., 2022). Research conducted for a long time has clearly demonstrated that many diseases such as diabetes (Yamada et al., 2023), Alzheimer's disease (Cortes-Canteli and Iadecola, 2020), Parkinson's disease (Levy, 2020), cardiovascular diseases (Yamada et al., 2023; Bolton and Rajkumar, 2011), chronic obstructive pulmonary disease (Brandsma et al., 2017), osteoporosis (Ginaldi et al., 2005), and osteoarthritis (Valdes and Stocks, 2018) are associated with aging (especially after the age of 60). All these pathological conditions that may arise with aging are, of course, due to the deviation of physiological mechanisms from the equilibrium state.

Body temperature regulation is a vital event for human health and is closely related to the physical condition and fitness of the living being. Just as expected, fitness decreases with age, and body temperature regulation functions deteriorate accordingly (Shibasaki et al., 2013; McKenna et al., 2023). Although problems related to the deterioration of thermoregulation mechanisms in the elderly are not a new topic, it is clear that they remain an unresolved issue. As a matter of fact, it is still controversial whether this situation in the elderly is due to the weakening of their ability to perceive temperature, the restriction of thermoregulation mechanisms, or whether their physiology is less tolerant to extreme changes in temperature (Van Someren, 2011). It is known that the high air temperatures recently observed worldwide have significantly increased the mortality rate in the elderly (Ginaldi et al., 2005; Valdes and Stocks, 2018). Disorders in thermoregulation mechanisms in the elderly cause deaths due to cold as well as heat-related deaths (even more frequently) (Xi, 2025).

In 1869, Wunderlich, who is known for determining the ranges of normal body temperature, defined normal body temperature as 36.2°C to 37.5°C with an average of 37°C. Wunderlich reported that the temperature values reach the lowest level between 2 and 8 o'clock in the morning and the highest level between 4 and 9 o'clock in the evening (Lu, 2010). Today, with the development of modern thermometers, this range can be determined more precisely. Nevertheless, the data obtained and currently accepted are quite close to the values reported by Wunderlich. The body temperature of the human being, which is a homeothermic and warm-blooded creature, is currently accepted to be between 36.1°C and 37.8°C under normal conditions. Of course, although this temperature varies in different individuals under physiological conditions at different times of the day and depending on different behavioral

conditions, it is tried to be kept within a constant range (Campbell, 2008). As is well known and has already been mentioned, the control center of thermoregulation is located mainly in the preoptic nucleus of the hypothalamus. However, the hypothalamus has to work in coordination with the skin, muscles, sweat glands, cardiovascular system, endocrine system, and nervous system to effectively control body temperature (Osilla et al., 2018; Weller, 2015). Studies have shown that both metabolism and vasomotor responses decrease in the elderly, and accordingly, decreases in ambient temperature reduce body temperature more than in younger individuals (Kenney and Munce, 2003). In addition, as it is known, during cold stress, the body tries to minimize heat loss by causing constriction in peripheral vessels as a physiological response and to increase heat production by causing shivering in skeletal muscles (DeGroot et al., 2006). Studies have also shown that the skin tissue of the elderly has decreased vasoconstriction ability not only in cold but also in heat (Florez-Duquet and Mcdonald, 1998; Kenney and Munce, 2003; Rida et al., 2014). It has been stated by many researchers that the thermal physiology, control of thermoregulatory mechanisms, and control of basal metabolic rate are different between young adults and the elderly (Rida et al., 2014; Schellen et al., 2010; Van Hoof, 2008). Indeed, Vassilieff and colleagues (1995) suggested that the threshold for hypothermia-induced shivering during spinal anesthesia decreases with age. According to the details of this study, it was reported that patients under 80 years of age started shivering at 36.1 ± 0.6°C, while patients over 80 years of age started shivering at 35.2 ± 0.7°C. According to another study, anesthetic drugs impair thermoregulation in all patients, young or old. Disruption of thermoregulation results in hypothermia. Excessive hypothermia in the elderly may occur mainly due to deficiencies in thermoregulatory mechanisms controlled by the central and peripheral nervous systems (Sessler, 2017). Studies show that half of all deaths due to cold exposure occur in the elderly over the age of 65, who are more prone to hypothermia (Statistics, 2005; Hajat et al., 2007).

Studies have shown that the elderly not only have a decreased reaction to cold but also a decreased sensitivity to high temperature and humidity. Because of these conditions, elderly individuals tend to neglect cooling the environment against high temperature and humidity, fluid intake, and protection from heat. In this case, their body fluid volume decreases, their homeostatic balance is disturbed, and their cardiovascular functions slow down, sometimes without even realizing it. For this reason, heat-related diseases are much more common in the elderly compared to young people. As a matter of fact, the condition of the majority of elderly people who apply to health institutions with complaints of heat-related discomfort is quite

serious. If circulatory failure and multiple organ failure occur, the patient may die within 2 days of admission to hospital. Dysfunctions in the central nervous system, liver, and kidneys, and diffuse intravascular coagulation are characteristic findings of heat-related diseases (Miyake, 2013). An increase in body temperature above 40.5°C is defined as hyperthermia. Disruption of thermoregulation mechanisms may cause fatigue, cramps, tetany, oedema, and fainting, as well as life-threatening heat strokes, which are usually caused by extremely high body temperature. It has also been reported that the most susceptible group to heat stroke is the elderly (Savioli et al., 2022). One of the biggest problems in the elderly is the decreased ability to distribute and remove the heat generated in the body. This increases the risk of heat-related complications in the elderly, especially during physical activity in hot environments (Balmain et al., 2018). A study has shown that elderly people with cardiovascular diseases who have two or more chronic health problems simultaneously have excessive decreases in blood pressure in hot environments and increases in blood pressure in cold environments before thermal regulation mechanisms are activated (Schlader et al., 2018).

The role of brown adipose tissue in thermoregulation and changes occurring during aging

Brown adipose tissue, found only in mammals, is estimated to have emerged approximately 150 million years ago (Enerbäck, 2010). Brown adipose tissue, which contains numerous mitochondria and iron-containing cytochromes within these mitochondria, has a brown appearance due to its high blood supply. Brown adipose tissue is found in newborns, particularly in the interscapular region, paravertebral area, paraesophageal area, neck, and perirenal region, and in adults in the supraclavicular, paravertebral, and, to a lesser extent, cervical regions (Figure 4) (Sidossis and Kajimura, 2015; Symonds et al., 2015; Cypess et al., 2009; Enerbäck, 2010). Brown adipose tissue is unique in that it separates the respiratory process from ATP synthesis. In these mitochondria-rich brown adipose cells, uncoupling protein 1 (UCP1) is located in the inner mitochondrial membrane. When UCP1 is activated, it exposes guanosine diphosphate (GDP) binding sites and thus initiates the free flow of protons across the inner mitochondrial membrane. This mechanism allows protons to bypass the ATP synthase and return to the matrix. During this process, ATP is not produced; instead, energy is rapidly released as heat. This process is known as adaptive thermogenesis, facultative thermogenesis, or non-shivering thermogenesis (Sidossis and Kajimura, 2015; Symonds et al., 2015; Cypess et al., 2009; Klingenspor and Fromme, 2011). Brown adipose tissue is important for humans, but it is even more important for newborns. Because newborns do not yet have the ability to shiver,

brown adipose tissue is essential for maintaining thermoregulation (Zoico et al., 2019). Studies have shown that brown adipose tissue decreases with advancing age. Indeed, it has been reported that while it is over 50% in people in their 20s, this percentage drops below 10% in people in their 50s and 60s (Yoneshiro et al., 2011). It has been frequently reported that brown adipose tissue is closely related to aging, the development of metabolic and cardiovascular diseases, obesity, and the associated development of type 2 diabetes, and that its levels decrease in these conditions (Darcy and Tseng, 2019; Becher et al., 2021; Cypess and Kahn, 2010).

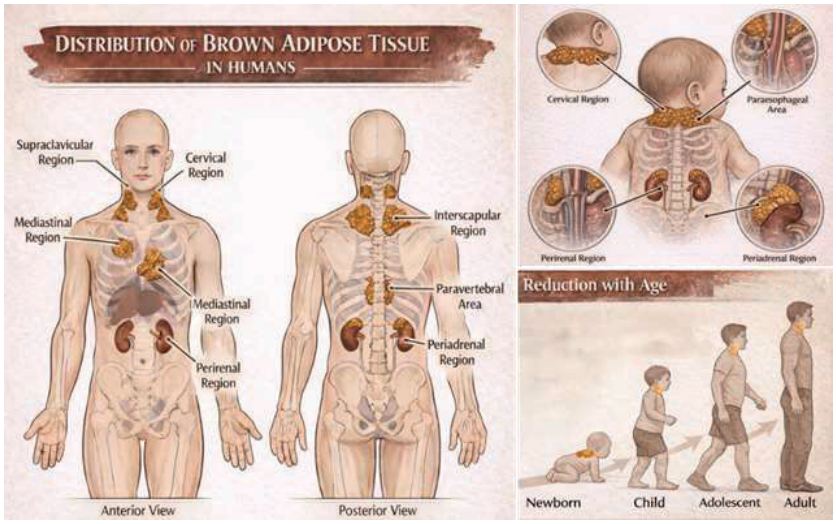

Figure 4. Distribution of brown adipose tissue in humans and infants.

Based on the information available to date, it is possible to say that brown adipose tissue gradually decreases throughout life. However, some researchers have identified an unexplained increase in the prevalence and volume of brown adipose tissue during adolescence. Furthermore, the fact that brown adipose tissue activity is more frequently observed in both children and adults with lower body mass index suggests that it may have a protective effect against obesity (Rogers, 2015). The presence of brown adipose tissue has been associated with less obesity and metabolic dysfunction (Franssens et al., 2017). Data obtained from experimental animals has shown that an increase in and/or activation of brown adipose tissue prevents disorders such as diet-induced weight gain and type 2 diabetes. Unlike white adipose tissue, brown adipose tissue can expend energy by producing heat instead of storing it as triglycerides (Lidell and Enerbäck, 2010). Based on this information, increasing or activating brown adipose tissue rather than white adipose tissue may be a new strategic approach for preventing and/or treating obesity and obesity-related diseases.

Conclusion

This review demonstrates that aging is associated with a marked decline in thermoregulatory capacity due to reduced hypothalamic sensitivity, impaired vasomotor and sweating responses, and decreased metabolic heat production. Comorbidities, polypharmacy, and environmental stressors further exacerbate this condition, rendering older adults more vulnerable to both hyperthermia and hypothermia. Considering increased life expectancy and the rise in extreme temperature events related to climate change, age-related thermoregulatory alterations represent a significant clinical and public health concern, underscoring the need for further elucidation of underlying mechanisms and the development of preventive intervention strategies.

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CHAPTER 3

EARLY BRAIN INJURY IN SUBARACHNOID HEMORRHAGE

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1. INTRODUCTION

Subarachnoid hemorrhage (SAH) is a serious cerebrovascular condition involving the blood accumulation within the subarachnoid space, commonly arising from the rupture of an intracranial artery (Suzuki et al., 2026). While traumatic causes are well known, most spontaneous cases involve the rupture of saccular aneurysms, making SAH one of the most urgent vascular emergencies affecting the central nervous system (Jabbarli et al., 2020; Marbacher et al., 2018). The highest incidence occurs between ages 40 and 60; however, its annual occurrence varies greatly by region, ranging from 9 to 12 per 100,000 people (Son et al., 2026).

Despite advances in diagnostic imaging, neurosurgical techniques, and intensive care management, SAH still has significant mortality and lasting functional deficits (Fujii et al., 2013). About 12% of patients die before reaching medical care, one-third pass away within the first 48 hours, and up to half do not survive beyond the first month after the hemorrhage (Long et al., 2017). Survivors often face ongoing neurological and cognitive problems that impact their independence and overall well-being, leading to a significant long-term disease burden (Hu et al., 2026).

The prognosis of SAH is associated with various clinical factors, including rapid neurological decline within the first 24 hours, older age, larger aneurysm size, early development of cerebral edema, and the extent of blood infiltration into the brain tissue (Park et al., 2004). Another concern is the probability of rebleeding, which peaks during the first 24 hours after the first hemorrhagic episode and is estimated at 4%. Collectively, these factors emphasize the urgent need for early recognition and prompt medical intervention.

For decades, delayed-onset cerebral vasospasm was considered the main contributor to secondary neuronal deterioration following SAH. However, growing evidence has shifted the focus toward early brain injury (EBI), a complex set of disease-related processes that begin within minutes of aneurysm rupture and continue over the first 72 hours (Li et al., 2026). EBI encompasses a cascade of events, including transient global cerebral ischemia, oxidative stress, blood–brain barrier impairment, excitotoxicity, neuroinflammation, and programmed cellular loss (Tan et al., 2026). These processes interact to exacerbate neuronal damage long before vasospasm develops, suggesting that EBI may play an even more decisive role in determining patient outcomes (Rass & Helbok, 2019).

Although significant advances have been made in understanding the mechanisms behind SAH, many aspects of its pathogenesis remain unresolved. No single treatment has yet been proven to reliably improve both survival and functional recovery, and current therapies mainly focus on supportive care and preventing complications. Recognizing the central role of EBI has opened new research avenues, with a growing emphasis on developing interventions targeting the earliest stages of brain injury (Lauzier et al., 2023). Such strategies hold promise not only for reducing the high mortality linked to SAH but also for improving long-term neurological outcomes and easing the substantial socioeconomic burden on patients, caregivers, and healthcare systems.

2. PATHOPHYSIOLOGY OF EBI

EBI occurring within the first 72 hours after SAH is a key factor in patient prognosis. Among the various elements contributing to EBI, neuroinflammation is a primary pathological process. The inflammatory response begins with the discharge of blood components into the subarachnoid area, which activates resident immune cells and stimulates the production of proinflammatory factors. Besides causing direct neuronal damage, this cascade promotes the development of cerebral edema, further worsening secondary injury (Miller et al., 2014; Sehba et al., 2012).

One important proinflammatory mediator involved in this process is tumor necrosis factor-alpha (TNF-α). It is a versatile cytokine primarily produced by activated macrophages but also secreted by CD4+ T lymphocytes, natural killer cells, and neurons. TNF-α plays a multifaceted role in modulating immune responses by inducing fever, cachexia, apoptosis, and inflammation. It acts as an endogenous pyrogen and can stimulate the production of other cytokines namely IL-1β and IL-6, thus amplifying the inflammatory environment. Mechanistically, TNF-α promotes the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a transcription factor essential for gene expression involved in cell survival, proliferation, and angiogenesis (Hanafy et al., 2010; Wu et al., 2016). In terms of EBI, elevated TNF-α levels during the early phase contribute to increased blood-brain barrier (BBB) permeability and trigger neuronal apoptosis through inflammatory cascades (Chou et al., 2012). Studies show that the activation of microglia is strongly linked with the secretion of TNF-α, IL-1β, and IL-6, which collectively intensify astrocyte toxicity and promote the progression of neuronal damage.

Interleukin-6 (IL-6) is a essential cytokine produced by various cell types, including vascular endothelial cells, mononuclear phagocytes, fibroblasts, and astrocytes. It is involved not only in systemic immune responses but also in neuroinflammation and vascular conditions such as aneurysm formation. Elevated IL-6 levels in EBI patients are linked to increased inflammation; however, the exact molecular mechanisms controlling IL-6 signaling in this setting remain unclear (Simon & Grote, 2021). Treatments like resveratrol (RES) have been shown to lower IL-6 expression and other inflammatory cytokines by inhibiting NF-κB activity, demonstrating potential anti-inflammatory effects (Shao et al., 2014; Zhang et al., 2016a).

Similarly, interleukin-1 beta (IL-1β) serves as a powerful mediator of the innate immune response, evolved to combat pathogens and facilitate tissue repair. It plays a prominent role in neuroinflammation across various CNS diseases. In SAH models, blocking IL-1β signaling has demonstrated neuroprotective effects, reducing cerebral edema and neuronal apoptosis (Wu et al., 2017).

The NF-κB signaling pathway is critically involved in these inflammatory processes because it transmits signals from pattern recognition receptors, notably Toll-like receptor 4 (TLR4), and proinflammatory cytokines. NF-κB activation initiates the transcriptional activity of several genes involved in immunity, inflammation, and apoptosis. After SAH, NF-κB promotes microglial activation, which amplifies inflammatory responses and worsens neuronal damage (Pawlowska et al., 2018; You et al., 2016). Modulating this pathway with microRNAs like miR-195-5p, which increase endothelial nitric oxide synthase (eNOS) and decrease inducible NOS (iNOS), has shown promise in reducing vasospasm and apoptosis, thereby protecting brain tissue.

TLR4 is a fundamental receptor that detects both microbial components and endogenous danger signals released during brain injury, including high mobility group box 1 (HMGB1) and heat shock proteins. When TLR4 is engaged, it activates MyD88-dependent signaling pathways that lead to NF-κB activation and subsequent cytokine production. Notably, TLR4 expression increases in microglia after SAH, and inhibiting it has been shown to decrease neuroinflammation and EBI. Pharmacological agents like fluoxetine exert anti-inflammatory effects partly by affecting the TLR4/MyD88/NF-κB pathway (Liu et al., 2018).

Alongside inflammation, apoptosis is a carefully controlled programmed cell death, a process that is critically involved in neuronal loss after EBI. The tumor suppressor protein p53 plays a pivotal role in starting apoptosis by responding to cellular stress signals, including those mediated by TNF-α. Stabilization and phosphorylation of p53 in the cytoplasm promote the stimulation of mitochondrial apoptotis mechanisms through regulation of the Bcl-2 protein family (Nijhawan et al., 2000; O'Brate & Giannakakou, 2003). p53 helps release cytochrome c from mitochondria, triggering the apoptosome formation and activating initiator caspase-9, which then activates effector caspases like caspase-3 (Antonsson & Martinou, 2000). Caspase-3 is a main executor of apoptosis, cleaving various cellular substrates and leading to DNA fragmentation and cellular demise (Yu et al., 2014). Additionally, p53 influences the release of apoptosis-inducing factor (AIF), supporting caspase-independent apoptotic pathways (Cregan et al., 2002).

Beyond apoptosis, p53 also contributes to blood-brain barrier disruption by upregulating matrix metalloproteinase-9 (MMP-9), responsible for extracellular matrix degradation, thereby promoting cerebral edema and further neural injury (Zhou et al., 2005). Thus, p53 serves as a critical molecular link between apoptotic and inflammatory damage following EBI.

Apoptosis occurs through two interconnected pathways: extrinsic and intrinsic. The extrinsic pathway begins when death receptor ligands like TNF-α, TRAIL, and Fas ligand bind to their receptors, leading to the activation of caspase-8 (Peter & Krammer, 1998). The intrinsic pathway, mainly regulated by mitochondrial signals, is strictly regulated by Bcl-2 family proteins, which include both proapoptotic and antiapoptotic members. Antiapoptotic proteins like Bcl-2 are located on mitochondrial membranes and prevent apoptosis by maintaining mitochondrial integrity and blocking cytochrome c release (Reed, 2000). In contrast, proapoptotic proteins particularly Bax and Bak enhance mitochondrial outer membrane permeation, enabling secretion of apoptogenic factors and activation of caspases (Edlich, 2018; Wong & Puthalakath, 2008).

The interplay between Bcl-2 and Bax is crucial in determining neuronal survival or death. After SAH, multiple studies show an increase in Bax and caspase-3 expression alongside a substantial decline in Bcl-2, indicating a shift toward apoptosis and neuronal loss (He et al., 2018; Zhang et al., 2022). Experimental models also confirm that interventions restoring Bcl-2 levels or inhibiting caspases can reduce apoptosis, improve neurological

function, and decrease brain edema, emphasizing apoptosis as a promising therapeutic target (Gao et al., 2008).

Another key regulator of apoptosis and cellular stress responses is sirtuin 1 (SIRT1), a NAD+-dependent deacetylase involved in controlling oxidative stress, immune function, mitochondrial biogenesis, and autophagy. SIRT1 is abundantly expressed in the CNS and has shown neuroprotective impact in various injury models. Its activation results in deacetylation and inhibition of p53, thereby reducing proapoptotic signaling (Yan et al., 2008). Resveratrol, a natural activator of SIRT1, has been demonstrated to lessen EBI after SAH by boosting SIRT1 activity, suppressing NF-κB-mediated inflammation, and preventing apoptosis (Qian et al., 2017; Zhang et al., 2016b).

In conclusion, EBI following SAH results from a complex interaction of neuroinflammatory and apoptotic processes. The coordinated activation of proinflammatory cytokines including TNF-α, IL-6, and IL-1β, through pathways involving NF-κB and TLR4, triggers and sustains brain inflammation. At the same time, apoptotic regulators including p53, caspases, and the Bcl-2 family control neuronal cell death. Modulators like SIRT1 provide natural neuroprotection by integrating metabolic and stress signals. Targeting these molecular pathways presents promising therapeutic strategies to reduce neuronal damage and improve clinical outcomes after EBI.

3. ANIMAL MODELS FOR EBI

Understanding the pathophysiology of EBI following SAH requires using experimental models, as research in humans is inherently limited. An ideal model should closely mimic the clinical condition, be highly reproducible, allow for adjustments in severity, provide appropriate controls, and be practical to implement in many experimental animals. Various species have been used for EBI research; however, rats are increasingly preferred due to their relatively low cost and the extensive biological knowledge available about them (Prunell et al., 2002). In current experimental practice, two main methods are commonly used to induce SAH in rats: one involves mechanically rupturing an intracranial vessel to cause bleeding (Bederson et al., 1995; Veelken et al., 1995), while the other involves injecting autologous blood into the cisterns surrounding the brain (Delgado et al., 1985; Zhao et al., 1999). Both approaches are widely used today, each offering unique advantages and limitations that influence their selection based on the specific goals and design of the study.

The endovascular perforation model is created by penetrating an intracranial artery and is considered to most closely resemble the pathophysiology of SAH. One of its main advantages in rats is that bleeding can be induced without damaging the skull. Additionally, because SAH in humans usually results from the rupture of intracranial vessels, this method effectively mimics the clinical condition. However, regulating the severity of bleeding in this model is not always possible, leading to variability in the amount of hemorrhage both within and across experimental groups. For studies that require precise measurement of extravasated blood, intracisternal injection of autologous arterial blood is generally preferred. Overall, the inability to reliably control hemorrhage severity (Schwartz et al., 2000), the high mortality rate associated with it (Bederson et al., 1995), and the lack of an appropriate placebo procedure have limited the use of the endovascular perforation technique in experimental research.

Blood injection models are implemented in two different ways. The initial and frequently utilized SAH model in rats, involves injecting blood into the subarachnoid space through the cisterna magna. This method is relatively simple to perform and yields highly consistent results; however, it has certain limitations. Unlike the typical clinical presentation of SAH, the injected blood in this model tends to accumulate mainly in the posterior cranial fossa and spinal canal. Additionally, the sudden increase in intracranial pressure does not reach the degrees of mean arterial pressure typically seen in aneurysmal SAH. To overcome these limitations, a second blood injection model was developed (Prunell et al., 2002).

The SAH model entails injecting blood into the prechiasmatic cistern, leading to its accumulation along the basal subarachnoid area and, to a lesser degree, across the cerebral hemispheres (Delgado et al., 1985; Solomon et al., 1985). The main benefit of this method is its close similarity to clinical SAH. First, the blood distribution mimics that of the clinical condition, where over 90% of cases result from anterior circulation aneurysm rupture (Kassell et al., 1990; Velthuis et al., 1998). Second, the sudden increase in intracranial pressure to levels near mean arterial pressure closely replicates the hemodynamic changes seen in aneurysmal SAH. Third, this technique reproduces the simultaneous rise in mean arterial pressure that occurs during blood infusion (Prunell et al., 2002). Due to these benefits, the prechiasmatic cistern blood injection model is now extensively used in experimental research.

4. CONCLUSION

SAH remains one of the most severe cerebrovascular conditions, marked by high mortality and significant long-term neurological disability. Over the past decades, there has been considerable progress in diagnostic imaging, neurosurgical procedures, and intensive care management. Despite these advances, clinical outcomes are still subpar, mainly because many therapeutic strategies focus on secondary complications rather than the initial pathological events that happen immediately after hemorrhage. Increasing evidence shows that EBI, which develops within the first 72 hours after aneurysm rupture, plays a pivotal role in shaping both short- and long-term neurological outcomes.

The pathophysiology of EBI involves a complex and interconnected network of mechanisms, including neuroinflammation, oxidative stress, mitochondrial dysfunction, disruption of the BBB, and apoptosis. Among these processes, inflammatory signaling pathways mediated by cytokines namely TNF-α, IL-1β, and IL-6, along with regulators like NF-κB and TLR4, have become vital contributors to neuronal damage and cerebral edema. Simultaneously, apoptotic pathways involving p53, caspases, and the Bcl-2 protein family further worsen neuronal loss. The interaction between inflammatory and apoptotic cascades highlights the multifactorial nature of EBI and emphasizes the need for therapeutic approaches that can target multiple molecular pathways at once.

Recently, evidence has emerged on identifying novel neuroprotective strategies aimed at reducing EBI. Pharmacological agents with antioxidant and anti-inflammatory properties, including natural compounds like resveratrol, have shown promising results in experimental models by modulating pathways like SIRT1 and NF-κB. Likewise, interventions targeting mitochondrial health, apoptosis control, and blood–brain barrier stabilization are actively being explored as potential therapeutic options. Advances in molecular biology and gene regulation, including the modulation of microRNAs and signaling molecules involved in neuronal survival, could also offer innovative approaches for improving outcomes after SAH.

Future research should aim to translate these experimental findings into clinically useful therapies. Standardizing experimental models, deepening understanding of molecular signaling networks, and developing targeted pharmacological treatments are crucial steps toward this goal. Additionally, combining neuroprotective therapies with established clinical management methods, such as early aneurysm repair, optimal hemodynamic control, and prevention of delayed cerebral ischemia, may improve overall treatment outcomes.

In conclusion, a better understanding of the underlying mechanisms of EBI has shifted SAH research focus from addressing delayed complications alone to targeting the earliest stages of brain damage. Ongoing multidisciplinary research combining experimental neuroscience, molecular biology, and clinical studies is important for developing effective treatments. Such progress not only has the potential to lower mortality but also promote functional recovery and enhance patients' quality of life in EBI following SAH.

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