Chapter

In Vivo Studies of Protein Misfolding and Neurodegeneration Induced by Metabolic Syndrome Relative to Chronic Cerebral Hypoperfusion: A Critical Review

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Abstract

Metabolic syndrome (MetS) leads to microvascular dysfunction and chronic cerebral hypoperfusion (CCH) in an insidious way. Clinical evidence and several rodent models have contributed to determining the neurodegenerative effect of a sustained decrease in cerebral blood flow (CBF). Protein misfolding and aggregation derived from CCH might account for the establishment of vascular cognitive impairment and dementia (VCID) and Alzheimer's disease (AD). However, the complex and multifactorial etiology of cerebrovascular disease demands the combination of experimental models in scientific research. In this sense, the present work aims at summarizing the differential available rodent paradigms for studying the establishment of cognitive decline resulting from protein misfolding induced by MetS in association with CCH. Revising experimental findings in the field will help further basic research on the pathophysiology of cerebrovascular disease and the future testing of protein-remodeling factors as neuroprotective agents for the prevention of cognitive impairment.

Keywords: metabolic syndrome (MetS), chronic cerebral hypoperfusion (CCH), protein misfolding, experimental models, cognitive impairment

1. Introduction

Metabolic syndrome (MetS) is the resulting condition of specific concurrent maladies, whose common pathogenic component is insulin resistance. Difficult to diagnose in clinical practice, there is consensus on its presence provided a cluster of risk factors be present, including abdominal obesity, hyperglycemia, hypertriglyceridemia, and hypertension [1–3]. Several murine models have contributed to

the knowledge of this vascular risk factors' constellation, which has been studied for over 80 years [4]. The experimental evidence shows that MetS silently, though relentlessly, leads to microvascular dysfunction and chronic cerebral hypoperfusion (CCH) [5]. Clinical findings, including the multivariate association between functional microvascular variables and laboratory-anthropometrical measurements [6], have reinforced the linkage of MetS with CCH [7], which leads to cognitive decline in late middle-aged adults [8]. As much as CCH might explain the considerable overlap between features of vascular cognitive impairment and dementia (VCID) and Alzheimer's disease (AD), it might also underly as a common pathophysiological mechanism [9]. Experimental models of CCH have also contributed to exploring the interplay between hypoperfusion and amyloid β (Aβ) deposition, as it relates to AD [9]. Scientific evidence has underscored the importance of treating dementia comorbid disease conditions, including hypometabolism and diminished cerebral blood flow (CBF) [10]. An alternative target in neuroprotection is the regulation of the proteostasis network since protein aggregates link MetS-induced CCH and sporadic AD late-onset [11]. Therefore, the present work aims at revising different murine models of MetS and CCH, summarizing those experimental findings of relevance in the establishment of cerebrovascular disease. Plus, this overview intends to shed light on the usefulness of experimental models for the study of protein misfolding as a mechanism of neurodegeneration in CCH. Thirdly, this review attempts to discuss the requirement of combining MetS and CCH experimental models in order to resemble multifactorial conditions like VCID and AD and to test protein-remodeling factors as potential neuroprotective mechanisms for cognitive decline in the aging brain [12].

2. Experimental models of MetS and CCH: relevant findings to vascular dementia

Although MetS is a multifactorial and complex condition, several rat strains have been developed to assemble a profile of anomalies described in human subjects that exhibit cerebrovascular disease. Obese Zucker rats constitute the most representative rat strain to study this syndrome since animals present changes similar to those seen in patients [1]. This widely extended model of insulin resistance and obesity was discovered in 1961 by Lois Zucker. The mutation in the leptin receptor fa leads to noticeable obesity from the third week of life [13]. Leptin is synthesized by adipose tissue. This hormone acts in the brain on leptin receptors [14]. Elevated levels of leptin represent the molecular base of the characteristic phenotype of Zucker rats, which includes hyperphagia, deposition of energy in adipose tissue, dyslipidemia, mild glucose intolerance, hyperinsulinemia, and vascular changes [1, 15]. In contraposition to obese Zucker rats, the Wistar Ottawa Karlsburg W (WOKW) rat model is not induced by a single-gene mutation, resembling the context in which this pathology is developed in human subjects. However, these animals exhibit signs of MetS between 8 and 10 weeks of age, much later than Zucker rats [1].

Several murine models of MetS derive from the spontaneously hypertensive rat (SHR), which represents the of-choice experimental model of essential (or primary) hypertension. While the SHR rats show hypertriglyceridemia and abdominal obesity, corpulent SHR rats are preferable for reproducing MetS [1]. Different strains have been developed, including obese SHR or Koletsky rats, SHR/NIH-corpulent (SHR/N-cp) rats and its subline, the SHR/NDmc-corpulent rats, and stroke-prone-SHR fatty rats. The first strain, originally developed by Koletsky in 1970, shows premature vascular pathology mimicking human atherosclerosis [16]. The SHR/N corpulent model was established to reproduce obesity and

non-insulin-dependent diabetes mellitus (NIDDM) [17]. Spontaneously hypertensive rats (SHR), an animal model of essential (or primary) hypertension, and SHR/NDmc-corpulent rats are also obese, presenting hyperphagia and metabolic alterations, while stroke-prone-SHR fatty rats are characterized by severe hypertension, which induces atherosclerosis and stroke. The spontaneously hypertensive/NIH-corpulent (SHR/N-cp) rat is a genetic model doomed to developing both non-insulin-dependent diabetes mellitus and hypertension.

Low-capacity runner (LCR) rats have been lately described, when cardiovascular risk factors were observed to emerge after artificial selection of low aerobic capacity [18]. These animals are selectively bred according to their performance in a running task. The LCR group is represented by rats capable of running short distances due to their low intrinsic aerobic capacity and bred with each other. After 11 generations, elevated blood pressure, insulin resistance, hyperinsulinemia, and endothelial dysfunction were registered in this strain [1]. Finally, from a translational perspective, both high-fat diet (HFD) and sweet carbonated beverage drinking represent two interesting rodent models of MetS evoking unhealthy dietary habits, increasing cardiovascular risk [19]. The former experimental paradigm reproduces impaired glucose tolerance (IGT) and type 2 diabetes. Rodents fed a HFD containing near 58% of total energy supply from fat develop obesity over the first week of life due to higher energy intake in combination with lower metabolic efficiency [20]. In the latter, 6-month ad libitum coke beverage drinking as the only liquid source results in hyperglycemia, hypertriglyceridemia, hypercholesterolemia, overweight, systolic hypertension, cardiac, renal alterations, and oxidative stress [2, 3, 21–24]. **Table 1** offers a translational overview of the abovementioned experimental models of MetS.

Disruption of CBF has been studied using focal or global ischemia. Focal ischemia models are used for resembling stroke pathophysiology and consist in the occlusion of a specific vessel, which reduces CBF by 70% due to restrictions in the vessel's territory. This condition is generally induced by transient or permanent middle cerebral artery occlusion. Multiple infarcts can be reproduced via intra-arterial injection of emboli (heterogeneous localization) or by inducing spontaneous strokes (SHRSP). Higher reductions of CBF are developed in global ischemia models, which include transient common carotid artery occlusion (TCAO), three- and four-vessel occlusion, and cardiac arrest [9].

Since focal and global ischemia leads to severe reductions in CBF, alternative models have been developed to reproduce CCH, i.e., the subtle yet sustained decrease in CBF relevant to VCID. Early pathological events provoking VCID were studied through the ligation or occlusion of unilateral or bilateral common carotid arteries (two-vessel occlusion) [25]. Bilateral common carotid artery occlusion (BCCAO) was refined to resemble modest reductions in CBF. Bilateral common carotid artery stenosis (BCCAS) was developed to reduce flow to 50% of baseline [26]. However, flow largely recovered 1 month later, which was overcome by establishing a gradual stenosis model. Aneroid devices were used to absorb extracellular fluid and provoke the constriction of arteries, resulting in a slower and progressive onset of hypoperfusion. This experimental condition is known as gradual common carotid artery stenosis [27]. Consequently, murine models of CCH include a wide spectrum of disease severity, ranging from traditional occlusion mechanisms to gradual stenosis methods. Despite these variants, experimental models of CCH induce sustained and moderate blood flow reductions by 30–50%, in contraposition to ischemic models that reduce CBF in 70% acutely [9].

Although stenosis represents a better theoretical approach from a clinical perspective, it involves difficult techniques, rendering BCCAO the most commonly used model [28]. An alternative experimental model of CCH comprises the

Name of the model	Experimental induction of MetS	Characteristic phenotype	Translational advantages
Obese Zucker rats	Mutation in leptin receptor fa causes obesity in rats from the 3rd week of life.	Hyperphagia, energy deposition in adipose tissue, dyslipidemia, mild glucose intolerance, hyperinsulinemia, and vascular changes.	Reproduces phenotypic changes resembling those in patients with MetS.
Wistar Ottawa Karlsburg W (WOKW) rats	Derived from a Wistar rat outbred strain, WOKW rats first exhibit signs of MetS at 8–10 weeks of age.	Obesity, moderate hypertension, dyslipidemia, hyperinsulinemia, and glucose intolerance.	Resembles MetS in a polygenetic context, as in humans.
Spontaneously hypertensive rats (SHR)	Rats bred with high blood pressure develop hypertension around 5–6 weeks of age.	Hypertension, hypertriglyceridemia, and abdominal obesity. The phenotype varies according to the respective corpulent strain: a. Obese SHR or Koletsky rats b. SHR/NIH-corpulent (SHR/N-cp) rats c. SHR/NDmc-corpulent rats d. Stroke-prone-SHR fatty rats	The of-choice experimental model of essential (or primary) hypertension. Corpulent SHR rats are preferable for reproducing MetS. Some strains resemble human-like atherosclerosis (a,d) or non-insulin-dependent (type II) diabetes mellitus (NIDDM) (b,c), respectively.
Low-capacity runner (LCR) rats	Rats capable of running short distances due to their low intrinsic aerobic capacity are selectively bred with each other along various generations.	Elevated blood pressure, insulin resistance, hyperinsulinemia, and endothelial dysfunction.	Represents metabolic dysfunction associated with low aerobic capacity.
High-fat diet (HFD)	Rodents fed a diet containing near 58% of total energy supply from fat become obese over the 1st. week of life due to higher energy intake.	Obesity and low metabolic efficiency.	Evokes impaired glucose tolerance (IGT) and type-2 diabetes due to unhealthy dietary habits.
Sweet carbonated beverage drinking	6-month ad libitum coke beverage drinking as the only liquid source causes metabolic dysfunction in rats.	Hyperglycemia, hypertriglyceridemia, hypercholesterolemia, overweight, systolic hypertension, cardiorenal alterations, and oxidative stress.	It mimics MetS derived from unhealthy dietary habits.

Table 1.
Experimental models of MetS: summary of phenotypic features from a translational perspective.

asymmetric common carotid artery surgery. Differential procedures are used for each common carotid artery (CCA), allowing interesting comparisons between both hemispheres. Gradual occlusion of the right artery lasts 1 month, while the

Name of the model	Experimental induction of CCH	Characteristic phenotype	Translational advantages
Bilateral common carotid artery occlusion (BCCAO)	Both common carotid arteries are ligated.	Cerebral blood flow (CBF) CBF rapidly decreases.	Represents a widely used model of CCH, characterized by its feasibility.
Bilateral common carotid artery stenosis (BCCAS)	Microcoils are placed on both CCAs.	CBF decreases and gradually recovers.	Mimics the clinical scenario of modest reductions in CBF.
Gradual common carotid artery stenosis (GCCAS)	Aneroid devices are used to absorb extracellular fluid and provoke the constriction of arteries.	CBF gradually decreases without recovery.	Reproduces a progressive onset of hypoperfusion, slower than induced by BCCAS.
Asymmetric common carotid artery surgery (ACCAS)	An aneroid constrictor is placed on the right common carotid artery (CCA), inducing a gradual occlusion for 1 month. The left CCA undergoes 50% stenosis by placing a micro-coil.	CBF decreases to different extents between the right and left CCAs.	Resembles differential reductions in CBF between both hemispheres.

Table 2.
Experimental models of CCH: Summary of phenotypic features from a translational perspective.

left artery undergoes 50% stenosis by placing a micro-coil. Further investigation is necessary to assess CBF reductions at longer time points, discarding the complete occlusion of carotid arteries in the long term [9]. **Table 2** summarizes the main features of the described models of CCH. For more details regarding experimental paradigms of CCH, including primate models, see [29].

Recent evidence using the abovementioned experimental models of CCH has shown that disruption of CBF leads to vascular cognitive impairment (VCI). Because of BCCAS induction in mice, selective recognition alterations were encountered in the novel object recognition (NOR) test, together with damage to the perirhinal cortex [30]. When CBF was gradually reduced, progressive motor impairment and working memory decline were found in the rotarod and Y-maze tests, respectively. Loss of oligodendrocytes in the white matter might underlie these behavioral deficits, suggesting that the GCCAS model could closely replicate the clinical pathogenesis of hypoperfusive vascular dementia in humans [31]. After implanting an aneroid constrictor on the left CCA and provoking stenosis on the right CCA, mice exhibited sustained motor, learning, and memory dysfunction, inferred from the balance beam maze, a fear conditioning task, and the NOR test. Histopathological analysis showed neurodegeneration in the cerebral cortex, dorsal striatum, and hippocampus. These findings support the usefulness of the ACCAS experimental model for reproducing the effect of microvascular occlusions on cognitive impairment [32].

3. Experimental findings supporting protein misfolding as a neurodegenerative mechanism in CCH

The causative role of CCH in cognitive impairment and AD has been reported in several studies using the BCCAO model, an experimental paradigm of easy application [28]. Differential mechanisms have been proposed as a potential link between CCH and neurodegeneration, including synaptic dysfunction, oxidative stress, neuronal loss, white matter lesion, neuroinflammation, and protein misfolding [28].

The latter appears as a novel target for neuroprotection, according to cumulative evidence [11]. **Figure 1** illustrates how CCH alters proteostasis network, leading to protein misfolding and neurodegeneration.

Degeneration of hippocampal neurons was attributed to proteostasis network destruction and protein aggregation induced by BCCAO [33]. This murine model has also led to a sustained increase in autophagy related-proteins Beclin-1, light chain 3B (LC3-B), and P62, suggesting a defensive reaction against protein misfolding. In this study, although CBF returned to baseline, cognitive failure was irreversible and attributed to Aβ aggregation in the hippocampus [34]. This brain region is characterized by its vulnerability to CCH [35] and its association with memory and learning dysfunction in AD [36]. Hippocampal degeneration has also been related to BCCAO-induced macroautophagy and endoplasmic reticulum (ER) stress, as it was inferred from the expression of light chain 3 II (LC3-II), Beclin 1, CCAAT/enhancer-binding protein, and C/EBP homologous protein [37].

Besides BCCAO, oxygen-glucose deprivation (OGD) provokes autophagy upregulation and apoptosis [35]. Recent evidence extended these findings, reporting CCH-induced high levels of LC3-II and Beclin-1 along with ultrastructural markers of apoptosis in CA1 neurons, including nuclear pycnosis, autophagosomes, and autolysosomes. ER fragmentation and spatial working memory impairment appeared as subcellular and functional correlates [38]. In addition to autophagy [34, 35, 38] and macroautophagy [37], ubiquitin-proteasome system (UPS) appears as another proteostatic pathway altered as a consequence of experimental CCH, which leads to CA1 degeneration. Long-term decrease peptidase activity and accumulation of ubiquitinated protein aggregates were observed after ligating the left vein and draining the transverse sinus and the bilateral external carotid arteries [39]. Previous studies from this laboratory had suggested the removal of misfolded proteins was impaired by UPS downregulation [40], and cognitive decline might be associated with long-term potentiation inhibition [41].

Along with aggregation of extracellular Aβ, intracellular phosphorylated tau protein deposition constitutes a hallmark of AD [42]. Tau hyperphosphorylation was observed as a result of unilateral common carotid artery occlusion (UCCAO),

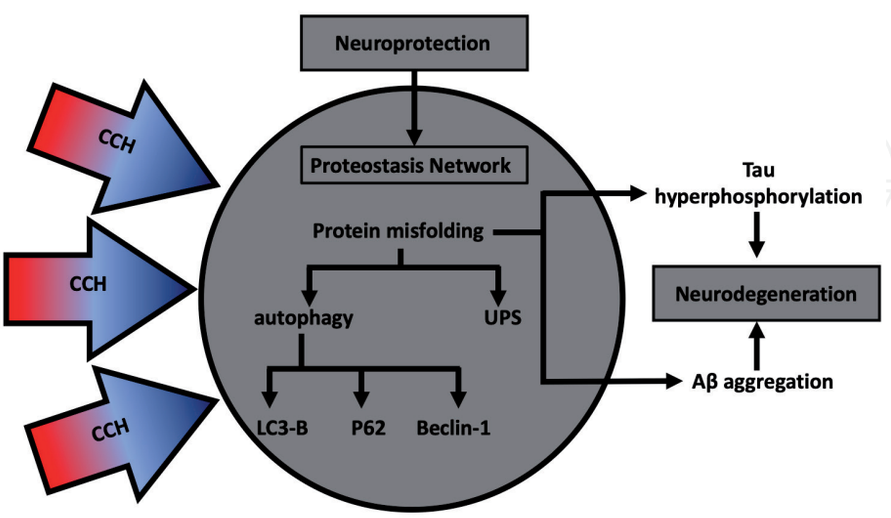

Figure 1.
Protein misfolding as a neurodegenerative mechanism and novel neuroprotective target in CCH. Chronic cerebral hypoperfusion impairs proteostasis network, inducing protein misfolding. Under cell stress, proteostasis network surveillance systems degrade proteins through different mechanisms. Depending on the nature of misfolded proteins, different systems are activated such as ubiquitin-proteasome system or macroautophagy. Protein aggregates, are recognized by molecular chaperones, ubiquitinated and delivered to the autophagosome via Beclin-1 complex. Neuroprotective agents, which target proteostasis network, emerge as promising treatments for cognitive impairment following CCH.

together with decreased post-translational tau O-GlcNAcylation by β-N-acetylglucosamine, dysregulation of synaptic proteins, and memory deficits [43]. According to previous findings, brain glucose metabolic dysfunction might down-regulate tau O-GlcNAcylation mediated by tau hyperphosphorylation [44–46]. Recent studies have confirmed and extended this finding, suggesting CCH might exacerbate tau hyperphosphorylation in AD-rodents, either after UCCAO in mice [47] or BCCAO in rats [48]. Similarly, previous evidence prompted CCH might precipitate AD neuropathology since BCAS seemed to accelerate Aβ aggregation, the same process found in amyloid protein precursor (APP)-transgenic (APP-Tg) mice [49]. In fact, aberrant processing of APP has been reported after BCCAO [50].

4. Combining experimental models

Since MetS is associated with an increased risk of cerebral ischemia, recent investigations developed murine models of MetS combined with experimental paradigms of ischemia-reperfusion injury to study the impact of ischemia associated with MetS. Wistar rats fed a high-fat diet for 20 weeks were more susceptible to BCCAO-reperfusion than normal diet (ND)-fed animals, showing worsening in microvascular dysfunction and oxidative stress. These results show that MetS increases the vulnerability of the ischemic brain to damage, whereby BCCAO exacerbates cerebrovascular disease previously induced by HFD [51]. Similarly, BCCAO, followed by reperfusion, aggravated microvascular alterations in obese Zucker rats compared with lean Wistar controls. Lesions in the cortex and striatum were largely more pronounced in obese Zucker rats, suggesting MetS could increase the risk of adverse outcomes following a brain hypoperfusion-reperfusion event [52].

Novel findings support the hypothesis that the brain under obesity's conditions is more vulnerable to ischemic injury. A brief episode of transient ischemia (TI) was provoked in obese gerbils, commonly known as desert rats. After 12 weeks of HFD, the rodents underwent a 2-min experimental TI. Hyperglycemia, cholesterolemia, and triglyceridemia observed in gerbils fed with a HFD were associated with a massive loss of pyramidal neurons in the hippocampal CA1 region 5 days after TI, indicating that a short-lived episode of TI could evoke neuronal damage along with pre-existing MetS. Increased levels of dihydroethidium, 4-hydroxynonenal, tumor necrosis factor-α, and interleukin-1β indicated brain injury was mediated by oxidative stress and neuroinflammation. On the contrary, ND gerbils did not exhibit neuronal death as a consequence of acute TI. In addition, these control animals could develop cerebral ischemic tolerance against a subsequent severer episode of TI [53]. Previous studies from the same laboratory have deepened the role of mammalian target of rapamycin (mTOR) in the pathogenesis of metabolic and neurological diseases and demonstrated that obesity and its related metabolic dysfunction might exacerbate the impact of TI in certain brain areas, including cerebral cortex, striatum, and hippocampus (CA1-3 regions) [54–56].

Conversely, cerebral ischemia itself might lead to glucose deregulation, a pathognomonic feature of MetS. Experimental evidence combining rodent models of ischemia and MetS shows that ischemic hippocampal neuronal death hampers glucose homeostasis, decreasing insulin secretion, which is later exacerbated by a HFD. In this study, gerbils were subjected to an 8-min BCCAO or a sham operation followed by either 11 or 40% fat diet for 7, 14, or 28 days. Although the initial occlusion provoked a 70% decrease in CA1 neurons, only HFD and longer ischemic periods resulted in larger hippocampal cell death. Similarly, glucose intolerance measured through the oral glucose tolerance test (OGTT) was overrated in gerbils

fed a HFD as the ischemic periods became longer. During the OGTT, insulin levels were significantly lower in gerbils subjected to BCCAO than in sham-operated controls. In addition, insulin secretion decrease was elevated the most after 28 days of HFD in ischemic gerbils [57]. These interesting findings using a combination of rodent models suggest that ischemic damage is a risk factor for glucose homeostasis, which might be worsened by the experimental induction of MetS in a HFD paradigm.

5. Future directions

Scientific findings combining rodent models have shown that chronic MetS is associated with poor stroke outcomes following experimental cerebrovascular events [58] since HFD or the way of inducing MetS modulates ischemic mechanisms of brain damage [59]. Also, experimental CBF restriction seems to hamper glucose homeostasis, posing a risk factor for developing MetS. When artery occlusion models are followed by the experimental induction of metabolic dysfunction features, the resulting MetS might exacerbate previously ischemia-induced glucose deregulation [57]. Therefore, combining experimental models offers an interesting scientific paradigm for elucidating the complexity of pathophysiological mechanisms underlying chronic cerebrovascular disease. In terms of Zhao and Gong [28], differential clinical scenarios may coexist in chronic pathologies where risk factors rarely exist alone or may even exert a causative role in some patients while acting as a consequence in others. In this regard, experimental studies using the combined application of murine models could help to close the gap between rodent models and human disease [9].

However, this complex translational perspective is still necessary for studying the interaction between MetS and CCH inducing neurodegeneration. Although brain microvascular dysfunction has been confirmed in several murine models of MetS, including HFD [60, 61], Zucker [62], and SHR rats [63], whether MetS causes cognitive impairment due to a decrease in CBF has not been fully addressed yet [64]. In addition, since protein misfolding is a hallmark of neurodegenerative diseases [65], dissecting the exact role of MetS in association with CCH in protein aggregation represents a relevant challenge in the field. Detailed studies on the time-dependent proteostatic changes after experimental MetS and CCH will shed light on the roles and mechanisms of these clinical conditions in the establishment of VCID and AD. Furthermore, these studies will contribute to testing protein-remodeling factors [12] as putative neuroprotective agents for the prevention of cerebrovascular disease and cognitive decline.

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