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ARTICLE

Different approaches, one target: understanding cellular mechanisms of Parkinson's and Alzheimer's diseases

Andréa S. Torrão,[1] Cecilia C. Café-Mendes,[1] Caroline C. Real,[1] Marina S. Hernandes,[1] Ana F. B. Ferreira,[1] Taisa O. Santos,[1] Gabriela P. Chaves-Kirsten,[1] Caio H. Y. Mazucanti,[2] Emer S. Ferro,[3] Cristoforo Scavone,[2] Luiz R. G. Britto[1]

[1] Department of Physiology and Biophysics, Institute of Biomedical Sciences, Universidade de São Paulo (USP), Brazil
[2] Department of Pharmacology, Institute of Biomedical Sciences, Universidade de São Paulo (USP), Brazil
[3] Department of Cell and Developmental Biology, Institute of Biomedical Sciences, Universidade de São Paulo (USP), Brazil

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Abstract
Neurodegenerative disorders are undoubtedly an increasing problem in the health sciences, given the increase of life expectancy and occasional vicious life style. Despite the fact that the mechanisms of such diseases are far from being completely understood, a large number of studies that derive from both the basic science and clinical approaches have contributed substantial data in that direction. In this review, it is discussed several frontiers of basic research on Parkinson´s and Alzheimer´s diseases, in which research groups from three departments of the Institute of Biomedical Sciences of the University of São Paulo have been involved in a multidisciplinary effort. The main focus of the review involves the animal models that have been developed to study cellular and molecular aspects of those neurodegenerative diseases, including oxidative stress, insulin signaling and proteomic analyses, among others. We anticipate that this review will help the group determine future directions of joint research in the field and, more importantly, set the level of cooperation we plan to develop in collaboration with colleagues of the Nucleus for Applied Neuroscience Research that are mostly involved with clinical research in the same field.

Corresponding author: Luiz R. G. Britto. Department of Physiology and Biophysics. Institute of Biomedical Sciences, Universidade de São Paulo. Av. Prof. Lineu Prestes, 1524, CEP: 05508-900. São Paulo, SP, Brazil. Phone: (+55 11) 3091-7242, Fax: (+55 11) 3091-7426. E-mail address: britto@icb.usp.br

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Introduction

This review addresses the two most common neurodegenerative diseases: Parkinson's and Alzheimer's diseases, highlighting some aspects related to their etiological and therapeutical hypothesis from a basic research perspective. In this regard, it is mainly discussed a well-established animal model of Parkinson's disease. We also comment on a recently proposed animal model of Alzheimer's disease, which has also been the object of investigation in our laboratories.

Parkinson's disease

Parkinson's disease (PD), first described in 1817 by James Parkinson in "An assay on the shaking palsy",[1] is the second most common neurodegenerative disorder, affecting around 1-2% of the population above 60 years and up to 6 million people around the world.[2] This disease is clinically characterized by motor dysfunctions, such as resting tremor, bradykinesia, rigidity, and postural instability, due to a decrease of dopaminergic inputs to the striatum, as a result of neuronal degeneration of the substantia nigra pars compacta (SNc), in a rate of ca. 5% per year. In addition, there are cognitive and vegetative disturbances.[3] Loss of dopamine concentration in the projection area promotes a reduction of thalamic activation, resulting in an excessive inhibition of motor responses.[4] The motor deficits manifest after a 40-60% of dopaminergic neuron loss and dopamine levels in the striatum.[5]

In addition to the neuronal degeneration of SNc, there is also a progressive neuronal loss in several other brain regions, such as the brainstem, locus coeruleus, the reticular nucleus of the brainstem and the dorsal motor nucleus of the vagus, as well as in the Meynert basal nucleus, amygdala and hippocampal CA2 region. Another characteristic of the disease includes the presence of inclusions known as Lewy bodies or Lewy neurites, depending on its location (cytoplasm vs. neuronal processes), which are basically composed of α-synuclein. These protein inclusions are caused by a failure in the degradation system of the cell and are composed by normal protein aggregates, truncated proteins, and by proteins with conformational alterations, in addition to ubiquitin.[2,6] Alpha-synuclein belongs to a family of proteins composed by α, β e γ-synucleins, widely expressed in the brain, with uncertain physiological function, but apparently presenting roles in neurotransmission, such as regulating the size of synaptic vesicles, recycling and plasticity processes.[7]

The cognitive deficits were neglected for several years. However, they affect almost 60% of the PD patients[8] and may be due to Lewy bodies.[9] Furthermore, approximately 40% of PD patients exhibit anxiety and depression disorders symptoms[10], as well as memory deficits as a consequence of alterations in the fronto-striatum-thalamic circuit after dopamine decrease[11] and the death of the noradrenergic neurons in the locus coeruleus.[12,13]

The 6-hydroxydopamine animal model of Parkinson's disease

Since nigrostriatal neurodegeneration was recognized as a pathological hallmark of PD, research on the pathogenesis of the disease has relied on the development of animal models that reproduce the loss of dopaminergic neurons in the SNc. The first animal model of PD was generated in 1968, when Ungerstedt demonstrated that the injection of 6-hydroxydopamine (6-OHDA) into striatum or SNc it was able to deplete dopamine content in nerve terminals and cell bodies, respectively.[14] Ever since, several other animal models to study PD were developed, employing distinct compounds capable to produce selective dopaminergic lesions accompanied by parkinsonian symptoms, such as the heroin contaminant 1-methyl-4-phenyl-1,2,3,6-tetrahidropiridine (MPTP), pesticides (rotenone, paraquat and maneb), lipopolysaccharide, and manganese.[15] However, the 6-OHDA model is still the most commonly used to produce nigrostriatal lesions.[16]

Six-OHDA is a hydroxylated analogue of dopamine found in the brains of PD patients.[17] Since this neurotoxin does not cross the blood brain-barrier, it is directly injected in the central nervous system, specifically into the striatum, SNc or even in the medial forebrain bundle. As a result of its uptake by dopamine and noradrenergic transporters, 6-OHDA selectively destroys the catecholaminergic systems, and promotes a PD-like loss of dopaminergic neurons that initiates immediately after the injection, becoming stable after two weeks.[18,19] The injection of 6-OHDA in the central-lateral portion of the striatum is the animal model that most resembles the human disease[20,21], as it produces a slow evolution of symptoms, and seems to be more suitable for studies that aim therapeutic strategies.[16]

The effects of 6-OHDA are mainly related to the massive oxidative stress caused by the toxin that, once accumulated in the cytosol, seems to be auto-oxidated, promoting a high rate of free radical generation[21] and interruption of the mitochondrial respiratory chain (complexes I and IV).[1,21] The oxidation of 6-OHDA directly generates hydrogen peroxide and superoxide, both critical in propagating its oxidation, and para-quinones, which seem to inactivate critical enzymes such as catechol-*O*-methyltransferase and tyrosine hydroxylase.[22] Furthermore, 6-OHDA oxidation is associated with the production of the hydroxyl radical, a powerful oxidizing agent that can react at a high rate with organic and inorganic molecules.[23]

Associated to neurochemical and molecular analysis, behavioral tests are usually employed to evaluate the extent of the 6-OHDA injury site in that animal model. A classical test applied to rats with unilateral lesion of the nigrostriatal pathway is the rotational behavior, induced by the dopamine agonist apomorphine, which induces rotation contralaterally to the injury side.[16]

NADPH oxidase and Parkinson's disease

The NADPH (nicotinamide adenine dinucleotide phosphate-oxidase) oxidases (Nox) represent a family of multi-subunit enzymes that transfer electrons across biological membranes and produce superoxide via a single electron reduction. All the seven Nox isoforms described so far (Nox1-5 and Duoxes 1-2) contain at least six transmembrane domains and cytosolic FAD (flavin adenine dinucleotide) and NADPH-binding domains. Each Nox family member has specific cytosolic components, activation mechanisms, subcellular localizations, and tissue distribution.[24] Nox2 was the first to be discovered and still represents the most extensively studied Nox isoform, being essential to innate host defense.

It is comprised of subunits localized in the cell membrane ($p22^{phox}$ and $gp91^{phox}$ – forming the heterodimeric flavoprotein cytochrome b558) and in the cytoplasm ($p40^{phox}$, $p47^{phox}$, and $p67^{phox}$). Activation of a low-molecular weight G protein (Rac1 or Rac2) and phosphorylation of $p47^{phox}$ initiate migration of the cytoplasmic elements to the plasma membrane, where they associate with cytochrome b558, generating the functional enzyme. The electron from cytoplasmic NADPH travels first to FAD, then through the Nox heme groups, and finally across the membrane and it is transferred to oxygen.[25] Similar to Nox2, Nox1 interacts with $p22^{phox}$, $p47^{phox}$ and $p67^{phox}$, or its homologs, NoxO1 and NoxA1, respectively. Nox3 activation is less well-defined, but it seems to involve Rac, $p47^{phox}$, and NoxA1. Nox4 is constitutively active, requiring only $p22^{phox}$. Nox5 and Duoxes are regulated by calcium through its EF-hand domains in the cytosol.[26] Among the Nox isoforms described in the nervous tissue are: Nox1, Nox2, Nox 3 and Nox4.[27,28] However, Nox2 appears to play a predominant role in neurodegenerative conditions.

Under physiological conditions, Nox-derived reactive oxygen species (ROS) are signaling molecules that influence many physiological processes. However, several studies using animal models and human *post-mortem* brains have consistently implicated Nox proteins over-activation in a wide variety of neurodegenerative conditions such as Alzheimer's disease and PD, but the mechanisms involved are poorly understood. Here, we review the most recent studies regarding Nox activation in the 6-OHDA-PD model. Most of the data was provided by *in vitro* observations and indicate a major involvement of Nox2 in dopaminergic neurotoxicity. For instance, it has been demonstrated in rat primary mesencephalic cultures that 6-OHDA induced a significant increase of $gp91^{phox}$ and $p47^{phox}$ immunolabeling, indicating increased activation of Nox2. Microglial activation and $O_2^{\cdot -}$ generation in dopaminergic neurons were also significantly reduced by apocynin, a Nox inhibitor.[29] By using the same *in vitro* model, the same authors were also able to show a significant increase of the mRNA levels of $gp91^{phox}$ as well as $p47^{phox}$, 12h after cell treatment with 6-OHDA.[30] In another study, it was shown that 6-OHDA also induced increase of $gp91^{phox}$ expression in human dopaminergic neuroblastoma cells.[31] These results are in consonance with our *in vivo* observations. In fact, the membrane protein levels of $p67^{phox}$ were markedly elevated in the SNpc of 6-OHDA-lesioned mice, which is suggestive of Nox2 activation. Tyrosine hydroxylase immunolabeling indicated that $gp91^{phox-/-}$ mice appear to be protected from dopaminergic cell loss in the SNc and from dopaminergic terminal loss in the striatum. Moreover, wild type mice treated with apocynin and $gp91^{phox-/-}$ mice all exhibited significantly ameliorated apomorphine-induced rotational behavior after 6-OHDA lesion. Therefore, despite the established autooxidation-derived ROS and the contribution of mitochondrial inhibition mechanisms to dopaminergic neurodegeneration in the 6-OHDA-induced PD model, altogether, the above data indicate that Nox-derived ROS are also importantly involved in that PD model.

Parkinson's disease and neuroprotective effects of exercise

Exercise and behavioral stimulation can trigger plasticity processes in the nervous system. Animal data have shown that exercise can increase neuronal survival and resistance to brain insult, promotes angiogenesis and neurogenesis, enhances learning, and contributes to cognitive function during aging.[32] Thus, there is an agreement that a possible neuroprotection can be achieved by physical exercise.

Physical exercise has been shown to be inversely related to neurodegenerative diseases, because it contributes to the functional process that involves recovery, maintenance, and prevention against brain damage in animals and humans.[33,34] Studies with animal models of PD employing distinct paradigms of exercise have attempted to explain the molecular mechanisms of exercise-induced changes in the pathophysiology of PD, as the extent of the lesion and the type of the exercise (voluntary or forced) may affect the degree of neuroprotection and behavioral improvement.[33,34] These studies have shown angiogenesis,[35] increased anti-inflammatory[36] and decreased inflammatory responses,[37] improvement of mitochondrial functions,[38] neurogenesis in the striatum[39] and in the SN.[40]

The plasticity responses improve the neurochemical deficits, especially tyrosine hydroxylase levels, and both cognitive and motor symptoms.[37-39,41,42] For instance, the treadmill exercise for 14 consecutive days during 30 minutes in rats submitted to 6-OHDA model of PD is capable of improving the tyrosine hydroxylase expression in striatum and SNc and motor performance in the rotation test with apomorphine.[41] On the other hand, voluntary exercise in wheel running protocol that began 2 1/2 weeks before intracerebral 6-OHDA infusion in rats, and continued for up to 4 weeks after the neurotoxin infusion, improved the animals' performance in behavioral tests related to forelimb asymmetry without tyrosine hydroxylase and dopamine transporter changes.[42]

In addition to the data derived from animal models, clinical studies have shown exercise-dependent improvement of motor control and equilibrium, which results in decreased falling frequency of the patients, and increase of life quality and gait.[33,34,43]

It is possible that the neuroprotective effects of exercise in 6-OHDA injected rats described above are promoted by neurotrophins, such as the brain-derived neurotrophic factor (BDNF) and glial derived neurotrophic factor (GDNF).[38,39] In fact, Nguyen and collaborators[44] showed that BDNF has neuroprotective effects against a neurotoxic stimulus *in vitro* that activates apoptotic pathways. *In vivo* data obtained with a treadmill exercise protocol for 4 weeks before the injection of the lipopolysaccharide (LPS) in mice showed that exercise completely prevented the LPS-induced loss of DA neurons, the reduction of dopamine levels and dysfunction of motor movement loss, as well as restored the LPS-reduced BDNF signaling.[37] In addition, the blockade of the BDNF receptor abolished the exercise-induced protection against LPS-induced dopamine neuron loss. Furthermore, BDNF is capable of indirectly activating the antioxidant enzymes and, thus, decreasing the oxidative damages induced by 6-OHDA.[1,45]

Parkinson's disease and cannabinoid system

The cannabinoid system consists of the lipophilic endogenous compounds such as N-arachidonoylethanolamide (anandamide) and 2-arachidonoylglycerol (2-AG), their synthetic and degradation enzymes, and their receptors CB_1 and CB_2. Most of central cannabinoid effects are believed to be mediated by CB_1 receptors which have a predominant presynaptic localization, suggesting their retrograde signaling in axon terminals by modulating neurotransmitter release. More recently, it has also been shown that cannabinoids can bind to other kinds of receptors, such as transient receptor potential vanilloid-1 (TRPV1)[46] and peroxisome proliferator-activated receptor (PPAR) family of nuclear receptors.[47]

CB_1 receptor is one of the most abundant metabotropic receptor in the central nervous system[48] and they are especially found in high amounts in the basal ganglia,[49] located mainly in the terminal of the γ-aminobutyric acid (GABA)-ergic and glutamatergic neurons. High densities of CB_1 are observed in the striatum, external and internal globus pallidus and substantia nigra pars reticulata (SNpr).[50] Moreover, both endocannabinoids, anadamide and 2-AG, are largely concentrated in the striatum.[49]

In the last decade, much attention has been given to the involvement of the cannabinoid system in numerous pathologies, as the constitutive elements of this system have been found to be altered in numerous pathologies, either in the central nervous system or in the periphery.[51] In the nervous system, the cannabinoid system has also been implicated in neuronal death/survival processes.[52] In this context, several groups have focused efforts in understanding the relation between PD and the cannabinoid system, which has led to prospects for cannabinoid therapies.

A recent study with humans has shown a marked decrease of CB_1 in the SN of PD patients, concomitant with a slight increase in dopaminergic projection areas.[53] Studies with the 6-OHDA model of PD also reinforce the idea of the variation in the CB_1 expression, as well as in the concentration of endogenous cannabinoids in the basal ganglia. For example, CB_1 receptor-mRNA levels were increased in the striatum of rats 7-10 weeks after unilateral 6-OHDA injection, although no significant changes in CB_1 receptor binding was found.[54] On the other hand, a more recent study showed a decrease in the CB_1 receptor in the SNpr, only when the 6-OHDA was injected in the striatum and not in the medial forebrain bundle.[55] In contrast, Casteels and colleagues[56] did not observe any changes in CB_1 expression into SN after 6-OHDA injection. Those heterogeneous results may depend on the 6-OHDA injection site or, more importantly, on the periods analyzed after the lesion.[57]

Increased levels of anandamide were observed in 6-OHDA-injected rats, accompanied by a reduction of the enzyme that metabolizes endocannabinoids (mainly anandamide), the fatty acid amide hydrolase and the anandamide transporter.[58] Furthermore, a study with the PC12 cell line exposed to 6-OHDA and treated with anandamide described a neuroprotective effect which was CB_1-independent.[59] It has been suggested, for example, that enhancement of the endocannabinoid tone provides an anti-levodopa-induced dyskinesia effect in the 6-OHDA model.[60]

Exogenous cannabinoids have also demonstrated potential neuroprotective effect, and, more recently, special attention has been given to the antioxidant properties described for some cannabinoid compounds. For example, the Δ(9)-tetrahydrocannabinol (THC), the main psychoactive constituent in *Cannabis*, promotes a reduction in the dopaminergic neuron death and reverts decrease in the dopaminergic transmission in the basal ganglia of rats lesioned by 6-OHDA.[61] The same study also observed a neuroprotective effect of cannabidiol, which was attributed to its antioxidant properties. Another study supports the idea that only the cannabinoids with antioxidant properties, such as AM 404, unlike those with affinities for cannabinoid receptors, reduces the toxicity caused by 6-OHDA.[62] We cannot exclude, however, the participation of CB_2 receptors in protective effects observed in the 6-OHDA model, as an induction/upregulation of these receptors, mainly in reactive microglia, which can contribute to the neuroprotective properties of the cannabinoid system in basal ganglia disorders.[61]

Therefore, regardless of the heterogeneous and sometimes apparently conflicting results described above, it seems that the cannabinoid system plays a role in compensatory mechanisms that counteract the imbalance in the physiology of basal ganglia which occurs in PD.[50]

Parkinson's disease, proteomics and peptidomics

Despite the several already identified mechanisms involved in PD, such as oxidative stress, mitochondrial dysfunction, abnormal protein aggregation, ubiquitin-proteasome dysfunction, glial proliferation, inflammatory responses and so on, its diagnosis is still dependent on the appearance of symptoms. In this context, biological markers are of great interest for early diagnosis and prevention, a field which has been explored using a proteomic approach.[2,63]

During a proteomic approach of PD using human cerebrospinal fluid, the expression of a protein that regulates the lipid metabolism and possibly protein deposition as observed for Lewy bodies, Apo A-I, was found to be down-regulated in PD patients in comparison to control groups.[64] Other studies using A53T α-synuclein Drosophila model of PD highlighted the importance of α-synuclein in membrane transport and synaptic membrane biogenesis. Besides, heat shock protein cognate 3 (Hsc3p), which regulates protein folding and degradation, was also increased, indicating higher concentrations of misfolded proteins and, consequently, leading to endoplasmatic reticulum stress.[65] In a proteomic study of 6-OHDA rat model of PD, more than 70 proteins were shown to be changed in the striatum and SN. Some of these altered proteins include 14-3-3 protein beta/alpha (Ywhab; upregulated), and other downregulated proteins such as calretinin (Calb2), NADH dehydrogenase 1 alpha (NDUFA10), ubiquitin carboxyl-terminal hydrolase isozyme L3 (UCHL3) and prohibitin.[66] Prohibitin is connected to mitochondrial complex I subunits, in particular with the NDUFS3 subunit (NADH-ubiquinone oxidoreductase 30kDa), which is involved in senescence mechanisms and also acts as a chaperone protecting complex I subunits before its generation. Co-immunoprecipitation assays demonstrated the interaction between prohibitin and NDFUS3, and immuno-hystochemistry assays demonstrated that they are increased in dying dopamine neurons. Besides, the absence of prohibitin in SH-SY5Y cells induced 6-OHDA cell death. All these data suggest their potential role in regulating mitochondrial function in dopaminergic cells.[66]

Another approach using the 6-OHDA model revealed five altered proteins, namely αB-crystalin, gamma-enolase, guanidoacetate methyltransferase, vinculin, and proteasome α-2 subunit. These proteins are related to the upsurge of L-DOPA induced dykinesias, a side effect of chronic use of L-DOPA in PD treatment.[67]

A two-dimensional electrophoresis, in combination with MALDI-TOF MS study of hemiparkinsonian rats induced by 6-OHDA, revealed five upregulated proteins, namely amyloid precursor-like protein 2 (APLP2), kininogen, glucokinase (GK), tropomyosin alpha chain, type brain-1 (TMBR) and calpactin I light chain. APLP2 presented a 5.35-fold increase at 2-week post-lesion. This protein disappeared from the SNc after 6-OHDA lesion and increased in striatal APLP2-positive neurons, which may indicate its presence in pre- and postsynaptic neurons of the nigrostriatal system.[68] This finding suggests the role of this protein in synaptogenesis and/or re-organization of synapses in the striatum. The authors also discuss that the increase of APLP2 may be the result of a higher number of cells expressing the protein (neurogenesis) or a differentiation of these neurons in response to injury.[68]

Peptides also play an important role in neurological disorders and are considered to be good biomarkers. In the rat 6-OHDA PD model, the L-DOPA-induced dyskinesia was related to increased levels of Dyn B (dynorphin B) and aNeo (alpha-neoendorphin) in SN. MALDI imaging analysis revealed that the dynorphin metabolite Tyr-Gly-Gly-Phe-Leu-Arg was high and Dyn B peak intensities were low in SN, where there is high receptor binding specificity for delta opioid receptors.[69]

PACAP (pituitary adenylate cyclase activating polypeptide) acts as a neurotransmitter and neuromodulator and is present in the amygdala, thalamus and spinal cord. PACAP has been shown to present neuroprotective effects in the 6-OHDA-induced rat model, decreasing dopaminergic neuronal loss by 50% as well as preventing the resultant hypokinesia due to neurotoxicity of 6-OHDA.[70] Another important peptide is VIP (vasoactive intestinal peptide), which presents potent antioxidant, anti-inflammatory and anti-apoptotic effects. Beneficial effects have also been demonstrated in motor function, probably due to increased GABA levels in the thalamus. VIP reduces lipid peroxidation, DNA fragmentation and NO production.[71]

Therefore, the role of peptides in neurological disorders such as Parkinson's disease has become a prominent research field that should be carefully investigated when searching for biological markers.

Alzheimer's disease

Alzheimer's disease (AD) is the leading cause of dementia in elderly people and is associated with progressive damage in brain functions including memory, language, spatial orientation, behavior, and personality. It is estimated that there are currently 36 million people worldwide living with AD, and this number is expected to increase dramatically over the next decades.[72] AD is a multifactorial pathology and about 99% of the cases have a sporadic occurrence (SAD), in opposition to the less common familial form of the disease,[73] with advanced age being the main risk factor. Other important risk factors are metabolic and vascular parameters which comprise the so called 'metabolic syndrome', such as dyslipidaemia and hypertension, as well as hyperglycaemia. In addition, type II diabetes mellitus is associated with increased risk of both AD and vascular dementia.[74]

Clinically, AD is characterized by progressive memory loss and a progressive decline in cognitive function, culminating in premature death of the individual, on average 10 years after diagnosis.[72] Additionally, AD is accompanied by non-cognitive neuropsychiatric symptoms, including anxiety, aggression, delirium, excitement or apathy, disinhibition or depression.[75] Characteristic neuropathological hallmarks of AD include: neuronal loss, accumulations of abnormal neurofibrillary tangles (NFT) corresponding to intracellular deposits of hyperphosphorylated Tau protein and dystrophic fibers, and increased expression and abnormal processing of amyloid-beta precursor protein (APP), leading to the deposition of amyloid beta (Aß) peptide, and, therefore, the formation of senile plaques.[73] Another hallmark of AD is cerebral amyloid angiopathy. In fact, cerebrovascular dysfunction may precede cognitive decline and onset of AD. Cerebral hypoperfusion and impaired Aß clearance across the blood-brain-barrier may contribute to the onset and progression of dementia of the AD type. There is also evidence of microglia playing important roles throughout these pathological processes.[76]

Even though AD is multifactorial, its etiology is still unknown. Although most studies have suggested that the Aß peptide ('amyloid cascade hypothesis') may initiate and/or contribute to the pathogenesis of AD, the mechanisms through which it causes neuronal loss and Tau abnormalities still remain poorly understood. Therefore, in the last few years, several other new hypotheses have emerged, in an attempt to contribute to the knowledge of neurodegenerative processes of AD. Bellow we briefly discuss recent data on AD, with a focus on the roles of insulin signaling and glucose metabolism disorders as a possible factor in the etiology of AD.

Mitochondrial deficiency, Ca2+ signaling and Alzheimer's disease

While more than 20 years have been dedicated to the 'amyloid cascade hypothesis', many other hypotheses remain as possible causes of the onset and progression of AD, such as oxidative stress, Tau protein, prion, and environmental causes.[77] It is believed by some authors that AD is initiated by a deficiency of enzymes of the tricarboxylic acid cycle, reduced cytochrome oxidase activity and mitochondrial DNA damage. The production of reactive oxygen species, for example, seems to be involved in triggering and maintaining the degeneration cycle of AD, aggravating mitochondrial DNA damage and altering other complexes of the electron transport chain, which leads to increased production of those reactive species.[78]

Learning and memory deficits in the onset of AD may also be a result of alterations of Ca^{2+} signaling. Oligomers of Aß peptide enhance Ca^{2+} entry and extra Ca^{2+} is pumped into the endoplasmic reticulum. Increased reticulum Ca^{2+} enhances the sensitivity of ryanodine receptors (RyR) which, in turn, release more Ca^{2+} from the internal stores. There is evidence that various AD mutations can induce changes of Ca^{2+} signaling. Another observation is that spines and dendrites of neocortical pyramidal neurons which are close to Aß deposits had higher resting Ca^{2+} levels. However, the question of which occurs first, activation of amyloidogenic pathway or changes in Ca^{2+} signaling, still remains open.[79]

Insulin signaling and glucose metabolism deficiency and Alzheimer's disease

Functional studies have shown disorders in both cerebral glucose mobilization and energy metabolism either preceding or accompanying the initial stages of cognitive impairments in SAD.[80-81]

Molecular evidence raised the assumptions that trafficking of the amyloid precursor protein (APP) is under control of insulin signaling and insulin receptor tyrosine kinase,[82,83] and that insulin regulates phosphorylation of Tau protein via glycogen synthase kinase-3 activity (GSK-3).[84,85] In addition, insulin affects brain functions, such as cognition and memory, as shown by *in vivo* studies.[85] Consequently, impairment of glucose metabolism and of insulin signaling has been proposed as a probable etiology of SAD.[86] Insulin affects numerous brain functions including cognition, memory and synaptic plasticity through complex insulin/insulin receptor (IR) signaling pathways. Insulin binds to the extracellular subunit of its receptor, which results in the autophosphorylation and activation of the intracellular ß-subunit. Activated insulin receptor phosphorylates several intracellular substrates, including the insulin receptor substrate.The phosphorylation of intracellular substrates then leads to the recruitment and activation of multiple proteins and the initiation of several signaling cascades, amongst the most abundant of which are the phosphoinositide 3-kinase (PI3K) and the mitogen-activated protein kinase (MAPK) signaling pathways.[86-88] Activation of PI3K pathway, in turn, mediates the activation of the serine-threonine kinase Akt (also known as protein kinase-B, PKB), promoting neuronal survival by directly inactivating the pro-apoptotic machinery.[89] In addition, activated PI3K/Akt phosphorylates and, therefore, inhibits both cytosolic forms of glycogen synthase kinase 3 (GSK3),[90] which is known to regulate the formation of the Aß peptide. Therefore, insulin regulates soluble APP release via a PI3K-dependent pathway.[91]

Several studies confirmed that cerebral metabolism declined before the deterioration of cognitive functions, suggesting that energy failure is one of the earliest reversible hallmarks of SAD.[92] Predominant abnormalities in cerebral glucose metabolism and its control by the neuronal insulin signal transduction system have been found in SAD,[93,94] leading to the hypothesis that SAD is the brain's type II diabetes mellitus.[92] A mismatch between the insulin action and insulin receptor function, including downstream signaling pathways, has been proposed to be involved in brain insulin system dysfunction in SAD.[95]

Intracerebroventricular injection of streptozotocin as a model for sporadic Alzheimer's disease

Considering the presence of insulin and its receptors in the brain, an experimental rat model was developed by using streptozotocin (STZ) to induce a brain insulin system dysfunction.[93] STZ (glycosamine derived from nitrosourea) is a drug selectively toxic to insulin producing/secreting cells and is used to induce both insulin-dependent and non-insulin-dependent diabetes mellitus (DM) after intravenous or intraperitoneal administration in rats.[96] The intracerebroventricular injection of streptozotocin (icvSTZ) in low doses does not alter, however, plasma glucose levels and does not induce DM, but it alters the brain glucose metabolism.[97] Considering the important roles of insulin and insulin receptors in the brain, and the fact that insulin deficiency and resistance are related to both SAD and DM, icvSTZ has been considered by many authors as a model for SAD.[74,94,97,98]

In the periphery, the toxicity of STZ starts when this drug is taken up by pancreatic B cells via the glucose transporter GLUT2 and induces cell death by alkylation of DNA and activation of poly ADP-ribosylation.[99] Since STZ is a nitric oxide (NO) donor, participation of NO in the cytotoxic effect of STZ has also been observed, as well as the generation of reactive oxygen species, which also contributes to DNA fragmentation and evokes other deleterious changes. STZ action on mitochondria results in the formation of superoxide anions, inhibition of the tricarboxylic acid cycle and substantial decrease in oxygen consumption by mitochondria, strongly limiting mitochondrial ATP production.[96] The mechanism of action of STZ in the nervous system, however, has not been totally elucidated.

Behavioral and molecular findings which follow glucose metabolism and insulin signaling disruption in the nervous system seem to mimic SAD, at least in some aspects. Here it is presented a short summary of the results obtained, until the present moment, by authors studying icvSTZ-injected rats, all resulting in changes similar to what is observed in AD patients. In general, the icv administration of STZ has been associated with morphological, molecular and behavioral changes in animals.

As mentioned earlier, the major hallmarks of AD are the formation of senile plaques due to Aß accumulation and the formation of NFT due to Tau hyperphosphorylation.[73] In many regions of the icvSTZ rat brain, there is an increase of Tau phosphorylation,[83,87,88,100] of neurofibrillary tangles,[80] and of the expression of Aß peptide,[80,86,100] even though these studies do not report the formation of senile plaques.

Corroborating the pathological hallmarks mentioned before, behavioral data have been very consistent in showing cognitive deficits, compromised learning and short and long-term memory after icvSTZ administration. For instance, memory deficits were observed in the Morris water maze as early as 3 hours following the injection and persist for at least 30 days.[100] STZ injection has also been shown to lead to cognitive impairments in memory tasks, including the passive avoidance, and the elevated plus-maze,[101-103] which seem to be independent of how many injections or what dose of STZ was administered.[86] There are, however, some studies that demonstrate that smaller doses result in less cognitive deficit.[104]

Even though the cholinergic system has been described to be one of the first to be affected in AD,[73] no changes were observed in the number or morphology of cholinergic neurons of the basal forebrain nuclei, medial septum, diagonal band, the nucleus basalis magnocellularis or the hippocampus, one week after a single icv STZ injection.[105] Furthermore, the choline acetyltransferase (ChAT) did not vary in several brain areas even after one month post-injection.[100] However, there was an increase of acetylcholinesterase (AChE) activity[86,105] and a reduction of ChAT activity,[106,107] which may explain the reduced synaptic function, learning ability, and memory deficits observed in these animals.[102]

Other neurotransmitter systems seem to be also modified in the icvSTZ model. For example, there is a downregulation of the dopamine receptor D1 and an upregulation of GABA-A receptor α-1 subunit.[108]

Markers for apoptosis are increased in the icvSTZ model and an atrophy of oligodendrocytes has been noted, probably due to the decreased cellular density observed in the periventricular region and to the ensuing inflammation.[86] In addition, many authors have described increased expression of glial fibrillary acidic protein (GFAP), mainly in peri and paraventricular regions, such as septum, fornix, striatum,[105] and in the hippocampus.[100,105] There is also an enlargement of the third ventricle after STZ injection, consistent with the hypothesis of neuronal loss.[109]

Considering proteins related to glucose metabolism and the insulin signaling pathway, a decreased expression of IRS1 and IRS2, IR, AKT/PKB, glucose transporter type 1 (GLUT1), GLUT3, and GSK-3β has been observed in the icvSTZ model, which is also observed in SAD patients.[86,87] Moreover, after icv administration of STZ, severe brain abnormalities of glucose/energy metabolism occurred,[110] such as the reduction of glucose utilization in 17 brain areas. Finally, the activities of key glycolytic enzymes decreased sharply after the icv injection of STZ.[111]

Therefore, energy metabolism and insulin signaling impairment, the reduction of ChAT activity and the increased activity of AChE, may all be part of the biological basis for the marked reduction of learning ability and memory, as well as the increased histopathological hallmarks of AD in the icvSTZ model.[102] Further studies, however, are necessary to fully understand the effects of STZ on the central nervous system. The events that trigger AD neurodegeneration have yet to be fully elucidated in order to generate an adequate model for this devastating multifactorial disease.

The intracerebroventricular streptozotocin model and therapeutic approaches

Oxidative stress is an important contributor to the development of neurodegenerative disorders as demonstrated previously in the present review (please see section on **NADPH oxidase and Parkinson's Disease)**. Similar mechanisms also seem to be part of the AD etiology.[112] Some of this oxidative damage include lipid peroxidation and protein degradation, leading to alterations on enzyme activity, causing cell membrane disruption and ultimately cell death.

One of the upsurging therapeutics for Alzheimer's disease treatment is the use of curcumin. *Curcuma* (Curcuma Longa L.) presents 3-5% of curcuminoids, including 50-60% of curcumin and also oils and resins (ca. 5%). Curcumin is composed by two monomers of ferulic acid, and presents free-radical scavenger properties.[113] In contrast to the increased levels of free radicals induced by the icv injection of STZ in rats, curcumin was able to revert this process and also the activity of Na^+/K^+-ATPase in the hippocampus and cerebral cortex. The treatment induced the activity of antioxidant enzymes such as glutathione peroxidase (GPx) and glutathione reductase (GR), and also increased reduced glutathione (GSH) and oxidized glutathione (GSSG) levels in both brain structures. Besides, the treatment also counteracted the decreased levels of acetylcholine induced by the STZ injection, which also contributed to ameliorate memory and learning deficits.[101]

Other studies evaluated the influences of curcumin treatment on glucose and glycogen metabolism, which are notably reduced in the STZ model in cerebral cortex and hippocampus, including a decreased level of insulin receptors (IR). Intraperitoneal injection of curcumin in rats improved the performance in passive avoidance task and in the Morris water maze test and increased levels of IGF-1 in these brain structures. IGF-1 has been related to tau phosphorylation and its impairment leads to tau hyper-phosphorylation and consequently to mitochondrial dysfunction and cell death.[114] Corroborating these data, the oral treatment with curcumin restored the IR levels in both structures, as well as the performance in behavioral memory and learning tests.[115] Other studies in SHSY5Y cells demonstrated the role of curcumin on activating the Wnt/β-catenin signaling pathway through inhibition of GSK-3β, which is responsible for phosphorylating β-catenin and also plays a part as a β-catenin substrate. In addition to that, curcumin then induces the expression of β-catenin and cyclin D1. All these signaling pathways cross-talk interferes with the amount of free PS1 (presenilin 1) and, consequently, with the activity of γ-secretase involved in the cleavage of APP.[116] Another plant extract, *Centella asiatica* (Umbelliferae), has also presented effects similar to curcumin.[117]

The Wnt and the MAP kinase-signaling pathways are also involved in other approaches involving exercise protocols, which are beneficial to the Parkinson's disease as demonstrated previously in the present review (please see the section on **Parkinson's disease and neuroprotective effects of exercise**). Similar studies have been undertaken in the STZ model of SAD. Treadmill running (5-week) significantly reverted cognitive decline observed in water maze task, and probably this effect is due to alterations in insulin-like signaling pathways and also MAP kinases and Wnt pathways.[118] MAP kinases have been shown to play important roles in neurotrophic signaling and synaptic plasticity[119] and Wnt is also involved with plasticity, learning, memory, neurogenesis and LTP.[120]

A recent study has used a flavonoid named rutin, which has demonstrated antioxidant and anti-inflammatory effects, such as suppressing microglial activation.[121] The same study demonstrated the attenuation of thiobarbituric acid reactive substances (TBARS), which indicates lipid damage, and also other beneficial effects involving oxidative stress-induced enzymes GPx, GR, and catalase. The anti-inflammatory effects were demonstrated by the decrease in the nuclear translocation of NF-κB, production of IL-8, and GFAP and COX-2 immunoreactive neurons.[121] In the same research field, statins are used to evaluate the effects of anti-inflammatory and anti-oxidative interventions on AD. A conjugated model of celecoxib (nonsteroidal anti-inflammatory drug)/STZ-induced sporadic dementia has been used to test pitavastatin (3-hydroxy-3-methyl glutaryl co-enzyme A (HMG-CoA), a reductase inhibitor, and donepezil (a cholinesterase inhibitor), and these treatments presented successful results in neuroprotection.[122]

Alzheimer's disease, proteomics and peptidomics

Peptides have also been shown to be important in Alzheimer's disease. Somatostatin was recently associated to the onset of the disease once the formation of the β-amyloid plaques impairs this neuropeptide transmission. The ß-amyloid plaques are key targets for the insulin-degrading enzyme (IDE) and are regulated by the presence of somatostatin, functioning as a substrate and allosteric modulator for the enzyme.[123] Another peptide is substance P (SP), which has been shown to present an effect in the proteolytic pathway of amyloid precursor protein (APP) due to an increased activity of α-secretase and less availability of APP to ß-secretases.[124]

Furthermore, peptidases are also important in the ß-amyloid generation. Increasing evidence demonstrates the importance of neprilysin in the clearance of ß-amyloid peptide due to a decrease of this enzyme level with aging.[125] In addition, there are increased levels of peptidases such as the endopeptidase EP 24.15[126] and prolyl oligopeptidase (POP) in Aß-treated rat hippocampus.[127] EP 24.15 was also shown to be increased in AD brain tissue in comparison to controls, probably acting in the clearance of this peptide as well.[128]

There is an upcoming research field that involves the identification of intracellular peptide alterations, called peptidomics, and that does not use digestive enzymes, and analyzes the native form of the peptides and their post-translational modifications (PTMs).[129,130] Some of them are hemoglobin fragments (six from α-chain and three from ß-chain)[131] called hemorphins (LVV hemorphin-7, VV hemorphin-7, LVV hemorphin-6, and VV hemorphin-6). They were found to be significantly elevated in the temporal lobe of Alzheimer's disease, but not in frontal lobe, occipital lobe, or hippocampus.[132] These hemorphins present opioid receptor function.[133,134]

There are also other fragments of hemoglobin with intracellular function. They are called hemopressins (hemoglobin α1-chain) (PVNFKFLSH; HP) and presented a hypotensive effect.[126] Other studies demonstrated antinoceptive properties on inflammatory pain,[135] and its activity on cannabinoid CB_1 receptors as a selective antagonist.[136] Hemopressin seems to be part of the now called non-classical peptide secretory pathway characterized by "on demand" synthesis and no vesicle storage.[137] These effects shed light over the endocannabinoid system, which presents important roles in neurodegenerative disorders, such as Alzheimer's disease.[138]

One possible origin for these intracellular peptides seems to be the proteasome. Our group's recent studies using mass spectrometry demonstrated that the epoxomycin inhibition of the proteasome in HEK293T cells significantly altered the intracellular peptide composition.[139] Preliminary results also demonstrated changes in the intracellular peptide profile, as well as in some peptidases mRNA expression, such as EP24.15 and aminopeptidase B (unpublished data).

Altogether, the data indicate that these peptides may function in several cellular mechanisms, including the modulation of protein-protein interactions.[140,141] The natural generation and degradation of these intracellular peptides may be an important part of the mechanisms involving neurological disorders.

Conclusion

The data presented above illustrate some of the recent outcomes of several basic science approaches aimed at understanding the cellular and molecular mechanisms involved in Parkinson´s and Alzheimer´s diseases. The animal models discussed here have been very useful for that purpose, aside from several other models that are available, including transgenic mice. Some of the approaches discussed here have disclosed the relevance of oxidative stress, endocannabinoids, physical exercise/neurotrophic factors and peptides as sources of potential neuroprotective strategies, which largely remain to be tested in humans. The multidisciplinary environment provided by the Nucleus for Applied Neuroscience Research will hopefully stimulate the expansion of these ideas in the years to come.

Disclosures

Andréa S. Torrão
Employment: *Department of Physiology and Biophysics, Institute of Biomedical Sciences, Universidade de São Paulo (USP), Brazil.*
Cecilia C. Café-Mendes
Employment: *Department of Physiology and Biophysics, Institute of Biomedical Sciences, Universidade de São Paulo (USP), Brazil.*
Caroline C. Real
Employment: *Department of Physiology and Biophysics, Institute of Biomedical Sciences, Universidade de São Paulo (USP), Brazil.*
Marina S. Hernandes
Employment: *Department of Physiology and Biophysics, Institute of Biomedical Sciences, Universidade de São Paulo (USP), Brazil.*
Ana F. B. Ferreira
Employment: *Department of Physiology and Biophysics, Institute of Biomedical Sciences, Universidade de São Paulo (USP), Brazil.*
Taisa O. Santos
Employment: *Department of Physiology and Biophysics, Institute of Biomedical Sciences, Universidade de São Paulo (USP), Brazil.*
Gabriela P. Chaves-Kirsten
Employment: *Department of Physiology and Biophysics, Institute of Biomedical Sciences, Universidade de São Paulo (USP), Brazil.*
Caio H. Y. Mazucanti
Employment: *Department of Pharmacology, Institute of Biomedical Sciences, Universidade de São Paulo (USP), Brazil.*
Emer S. Ferro
Employment: *Department of Cell and Developmental Biology, Institute of Biomedical Sciences, Universidade de São Paulo (USP), Brazil.*
Cristoforo Scavone
Employment: *Department of Pharmacology, Institute of Biomedical Sciences, Universidade de São Paulo (USP), Brazil.*
Luiz R. G. Britto
Employment: *Department of Physiology and Biophysics, Institute of Biomedical Sciences, Universidade de São Paulo (USP), Brazil.*
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** Modest*
*** Significant*
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