Memory Enhancers

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Abstract

Polyhydroxyalkanoates (PHAs) constitute a family of naturally-occurring microbial polymers possessing excellent physicochemical properties, non-toxic behavior, biocompatibility and biodegradability which render them distinctive candidates for industrial applications especially in medicinal and pharmaceutical areas. In this review, we deepened in various cellular processes to collect and record information highlighting the biotechnological applications of PHA monomers or derivatives as memory amplifiers. Neurological conditions under the umbrella term of "dementia" are concerning millions of people worldwide, and their prevalence rises exponentially. These diseases are generally defined by gradual loss of cognitive and physical abilities, due to severe dysfunction of important central nervous system (CNS) areas. Neuronal and synaptic degeneration, though not specified in detail, are most probably multi-etiological events, caused by abnormal protein aggregation, neuro-inflammation, oxidative stress, and dys-regulated extracellular or intracellular signaling and energy supply. Therefore, multifunctional polymeric formulations without side effects, such as PHAs (polymers of hydroxy-organic acids), offer numerous applications in the prevention and treatment of various diseases. PHA monomers or derivatives (e.g. 3-hydroxybutyrate, 3-hydroxybutyrate methyl ester, 3-hydroxybutyl-3-hydroxybutyrate) are now proven to act as artificial ketogenic compounds and memory enhancers administered in ketogenic diets. Ketogenic diet (KD) is a well-known alternative for the treatment of neurological conditions, as the produced ketones affect protein modifications, attenuate oxidative and inflammatory stress and modulate signaling pathways contributing to neurogenesis. Collectively, PHAs can ameliorate brain and neuronal activity, improve memory

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recall and even alleviate important pathological features of neurodegenerative problems, such as Alzheimer's disease (AD) – related amyloid plaques.

Keywords
Biopolymers · Polyhydroxyalkanoates · Memory enhancers

7.1 Introduction

PHAs have been established as a complex class of biodegradable homo- and copolymers since the late 1950s. They consist of various hydroxyalkanoic acids (HAs) as monomers whose alteration leads to the development of biopolymers with desirable mechanical and structural properties, biocompatible behavior and favorable degradation kinetics under controlled physiological conditions. PHAs are synthesized by bacteria and gathered in PHA granules as energy and carbon storage composites, in the presence of high carbon concentrations and very low concentrations of growth nutrients, such as sulphur, nitrogen, oxygen or phosphorus (Kumar et al. 2015a, b, c; Patel et al. 2015a, b, 2016; Kalia et al. 2016; Koller et al. 2017). Bacteria are able instead of metabolizing available carbon sources through the Tricarboxylic acid cycle (TCA), to generate energy utilizing the acetylCoA by diverting it towards the polyhydroxyalknoate (PHA) biosynthetic pathway (Singh et al. 2015; Ray and Kalia 2016, 2017). Their high degree of polymerization in combination with their biodegradability, insolubility in water, nontoxic behavior, thermoplastic, piezoelectric and elastomeric properties, render them functional materials for various biotechnological applications in the food and packaging industries, agriculture, pharmacy, medicine, and as basic synthons in chemical processes (Anderson and Dawes 1990).

Poly(3-hydroxybutyric acid) (P(3HB)), one of the most extensively studied constituent of bacterial PHAs, was initially reported to be produced by the bacterium *Bacillus megaterium* in 1926 (Valappil et al. 2006). Two novel HAs, 3-hydroxyhexanoate (3HHx) and 3-hydroxyvalerate (3HV), were identified in the 1960s and 1970s (Valappil et al. 2006). Future research efforts led to the growth of small amounts of 3-hydroxyoctanoate (3HO), and 3HHx units in *Pseudomonas oleovorans* on feed containing *n*-octane (Valappil et al. 2006). By the end of the 1980s, various HA constituents, such as 4-hydroxyalkanoates, and 5-hydroxyalkanoates were discovered, whereas after the late 1980s research was focused on the development of PHA polymers with tailored properties for medical use, through the involvement of cloning and clarification of genes in the biosynthetic procedure (Valappil et al. 2006).

At present, scientists have focused their interest on the specification of tertiary and quaternary structures of the key enzymes in PHA biosynthesis, the PHA synthases. These multifunctional enzymes define the: (i) substrate specificity, (ii) catalytic mechanisms, and (iii) molecular weight of each PHA produced (Valappil et al. 2006).

PHAs are categorized, according to the carbon atoms within a PHA monomer, into the: (i) short-chain length PHAs with 3-5 carbon atoms as *scl*-PHAs, (ii) medium-chain length PHAs with 6-14 carbon atoms as *mcl*-PHAs, and (iii) long-chain length PHAs with ≥15 carbon atoms as *lcl*-PHAs (Khanna and Srivastava 2005). Until nowadays, more than 150 kinds of PHA monomers have been characterized. New species of PHAs arise through the physical or chemical modification of naturally-occurring PHAs (Zinn and Hany 2005), or through recombinant organisms that produce PHAs with specific properties (Escapa et al. 2011).

The discovery of various monomers with numerous useful properties increased interest in PHAs. The first industrial copolymer consisting of 3HB and 3HV was developed by Imperial Chemical Industries (ICI) and sold under the trade name of Biopol® as biodegradable and renewable replacements instead of the petrochemically derived plastics (Valappil et al. 2006). The increasing commercial impact of P(3HB) and P(3HB-*co*-3HV) copolymer, enabled their future assessment as medical biomaterials in biotechnological applications. HAs are responsible for the stimulation of Ca^{2+} channels which in turn act as an aid in amplifying memory especially in AD patients (Xiao et al. 2007; Zou et al. 2009; Chen. 2010b; Magdouli et al. 2015).

Dementia, as a debilitating neurodegenerative disorder, affects memory, orientation, thinking, calculation, learning capacity, comprehension, judgment, and language (Jotheeswaran et al. 2010; Gross et al. 2012). It affects around 35 million people worlwide, and the observed global prevalence for individuals over 60 years old is 4.7%. AD is the most common type of dementia and contributes to 60–70% of the dementia cases (Honjo et al. 2012). Another prevalent type of dementia is vascular dementia, which represents 17% of dementia cases (Ross et al. 2006). Other types of dementia occurring along with AD pathology are Parkinson's disease (PD) and Lewy body dementia (LBD). Since the distinction between these types of dementias is not clear, scientists tend to believe that their main difference lies in the duration of each type (Langa et al. 2004; Plassman et al. 2007).

Dementia is an age-related disease. This fact deposits an enormous social burden in respect of economics and human inflictions, especially in the context of an outstretched aging population. Currently, there is no cure, however, it has been estimated that successful preventive interventions by delaying the symptoms of late-onset AD, will reduce the predicted prevalence and incidence rates of the disease (Jorm et al. 2005).

AD is notably high among patients who are above 65 years of age and it is characterized by a progressive deterioration of cognitive function, ending with severe brain damage (Mattson 2008; Braak and Del Tredici 2012; Revett et al. 2013). Pathological data on the disease imply that oxidative stress is closely associated with tissue damage such as advanced glycation end products (Smith et al. 1994a, b), nitration (Smith et al. 1997), carbonyl-modified neurofilament proteins and free carbonyls (Smith et al. 1991), and products of lipid peroxidation (Montine et al. 1996; Sayre et al. 1997). Moreover, Aβ (amyloid-beta plaque) aggregation, hyperphosphorylation of tau (τ) protein, and reduced synthesis of the neurotransmitter acetylcholine are some of the most prevalent hypotheses on the cause and progression of AD (Zhang et al. 2013).

In general, oxidative stress is affected by a lack of balance in the antioxidant defence systems and the production of reactive oxygen species (ROS) (Jung et al. 2009). The cytopathological significance of the damage induced by oxidative stress is observed through the up-regulation of the antioxidant enzyme heme oxygenase-1 (Smith et al. 1994a, b; Premkumar et al. 1995), associated with the alteration of amyloidogenic deposits of tau (τ) (Takeda et al. 2000), found in the neurofibrillary tangles of mild AD patient brains (Nday et al. 2015; Halevas et al. 2016). AD symptoms often commence with loss of ability to create novel memories, leading to confusion. Eventually, the inability for self-care often leads patients to their committal in institutionalization (Allen et al. 2013).

The ketogenic diet (KD) is considered a putative preventive treatment for various types of brain diseases, such as AD or epilepsy. During KD, carbohydrates are replaced by fats resulting in the increase of blood-borne ketone bodies (KBs) levels. Since drug development for AD is based on the acetylcholinesterase inhibition or the antibody of Aβ with no satisfactory results on the prevention or reversion of the disease progress, scientists have focused their research interest on the development of drugs that confront the dysfunction of mitochondria (Colell et al. 2009; Swerdlow et al. 2010; Du et al. 2010; Zhu et al. 2013), and the impairment of energy metabolism (Liang et al. 2008; O'Connor et al. 2008).

KBs have been reported as the only alternative to energy supplement for the brain. The major component of KBs, 3-hydroxybutyrate (3HB), and a degradation product of microbial, natural and biocompatible biopolymer P(3HB) (Williams 2008, 2009), have been proved to possess neuroprotective properties. However, due to its charged nature and acidity, 3HB is not considered an ideal drug candidate (Zhang et al. 2013). On the other hand, 3-hydroxybutyrate methyl ester (HBME), the esterification product of 3HB, has a lower polarity with a neutral pH, providing better evidence on its bioavailability and ability to go through the blood-brain barrier (BBB) more efficiently than 3HB (Zhang et al. 2013). Consequently, HBME constitutes a more effective drug candidate for developing CNS biotechnology, especially anti-AD and neuroprotective pharmaceutical prevention and treatment.

7.2 Production of PHA Monomers, Their Biotechnological Applications, and Economic Impacts

PHAs are a category of polyesters consisting of a great number of chiral R-hydroxyalkanoic acids (R-HAs), constituting an abundant source of optically pure chiral compounds, bi-functional hydroxyl acids of R(−) configuration that can be utilized as starting materials for several biotechnological processes such as the synthesis of many pharmacological bioactive compounds including drugs, vitamins, antibiotics and antifungal agents, hormones, pheromones, β-peptides, enzymes inhibitors, siderophores, biofuels, aromatics as well as chiral synthons for several fine chemicals (Ren et al. 2010). Therefore, an emphasis has been given on the

utilization of PHA degradative pathway products and their chemical modifications, which provide unique properties for biomedical applications (Hazer et al. 2012; Martinez et al. 2014; Ke et al. 2017).

PHA monomers can be obtained by several strategies such as chemical de novo synthesis of R-HAs, or chemical degradation of PHAs, biotransformation, microbial de novo biosynthesis, in vitro enzymatic degradation of purified PHAs, enzymatic in vivo depolymerization attributed to intracellular PHA depolymerases of wild-type bacteria or R-HA production by metabolic pathway engineering by use of recombinant microorganisms (Ren et al. 2010).

It is remarkable that all these follow-up compounds possess higher economic value in the commercial market than PHAs themselves. However, PHA degradation and recovery of the yielded monomers or oligomers are highly energy-consuming processes. To resolve this problem research studies were directed towards the purification of PHA hydrolyzing enzymes such as depolymerases (Jendrossek et al. 1996; Jendrossek and Handrick 2002; Kadouri et al. 2005; Kim et al. 2007; Jendrossek 2007; Papaneophytou et al. 2009, Papaneophytou and Pantazaki, 2011), and the development of effective methods to activate these enzymes in vivo within the cells (Cheng et al. 2005**;** Ren et al. 2010) or extracellularly (Papaneophytou et al. 2009**,** Papaneophytou and Pantazaki, 2011). Additionally, other strategies aimed at the decrease of the market price of the initial PHAs products by using low-value sources such as industrial wastes (Pantazaki et al. 2009) or by the simultaneous production of other useful biotechnological products (Pantazaki et al. 2011), in order to benefit from multipotent advantageous applications such as the production of monomers and oligomers.

7.3 Ketone Bodies, Ketosis, Ketogenic Diet

In order to understand in depth, the basis and the extent of PHAs and their monomers biomedical applications, it is important to elucidate the involvement of the principal of them, 3HB, their natural counterpart, in various cellular processes. Analytically, 3HB is one of the main natural endogenous circulating KBs, as acetoacetate and acetone.

In the mitochondrial milieu, 3HB constitutes the precursor of acetoacetate, which is produced during a catabolic reaction catalyzed by the enzyme 3HB dehydrogenase 1 (BDH1) and directly linked to the reduction of nicotinamide adenine dinucleotide (NAD) to $NADH^{+}$. Subsequently, acetoacetate is converted to acetoacetyl-CoA and subsequently to acetyl-CoA, which is a component of the citric acid (TCA) cycle (Liu et al. 2014), as presented in Fig. 7.1.

KBs are products of the catabolism of fatty acids in the liver and are used as an alternative energy source (fuel) instead of blood glucose, when glucose availability is insufficient, by enhancing the fatty acid-beta oxidation levels in the brain cells (Panov et al. 2014). The maximal concentrations of circulating KB can be up

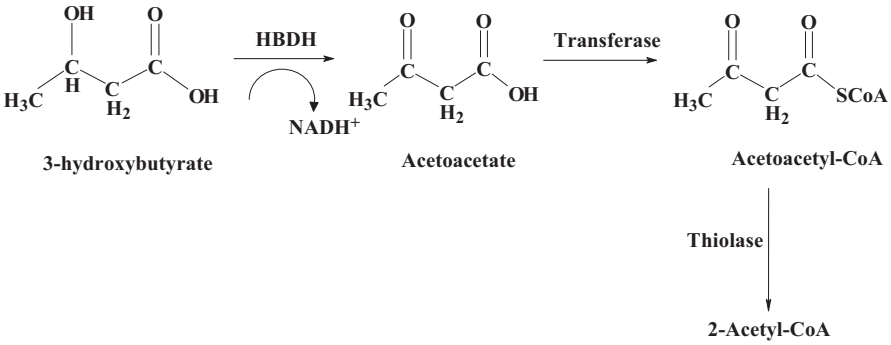


Fig. 7.1 Enzymatic steps involved in the 3HB catabolic process to TCA cycle entry as acetyl-CoA

regulated by adhering to a ketogenic diet (KD). It is considered that a high-fat content and adequate-protein diet, in which carbohydrate intake is drastically reduced and replaced with body dietary sources of glucose, exhorts a metabolic transition towards the consumption of fatty acids (Hammami 1997; LaManna et al. 2009).

Accumulation of KBs in the blood circulation leads to a metabolic situation known as ketosis. It is a survival mechanism functioning as a metabolic state activated during starvation, or due to lack of carbohydrate digestion (Brownlow et al. 2013). It should be noted that the plasma concentration of 3HB is estimated in the range of 0.02–0.09 $\mu mol \cdot mL^{-1}$ (Blomqvist et al. 2002). In addition, the metabolism of KBs mimics some actions of insulin and overcomes insulin resistance indicating a useful therapeutic potential in the alleviation of the pathological hallmarks of cognitive impairment (Sato et al. 1995).

A key feature that increases the lifespan of many organisms is dietary restriction (Greer and Brunet 2009). The ketone moiety 3HB functions as a Dietary Restriction (DR) mimetic compound (Newman and Verdin 2014), by increasing in the plasma during DR, and additionally by decreasing the levels of oxidative stress after exogenous administration (Shimazu et al. 2013).

In general, the KD is regarded as an effective non-pharmacological treatment providing symptomatic avail, and effective disease-ameliorating activity of various neurological disorders typified/provoked by the death of neurons, including epilepsy, AD, Huntington's disease (HD), PD, stroke and brain injury (Gasior et al. 2006). Studies on 3HB functionality have proved its utilization as an energy substrate in hemorrhage shock rats (Katayama et al. 1994), and head trauma patients (Hiraide et al. 1991).

7.3.1 Ketotherapeutic Intervention

KBs serve as alternative energy sources, and they can be increased as liver glycogen supplies get consumed after prolonged fasting (Cotter et al. 2013; Newman and

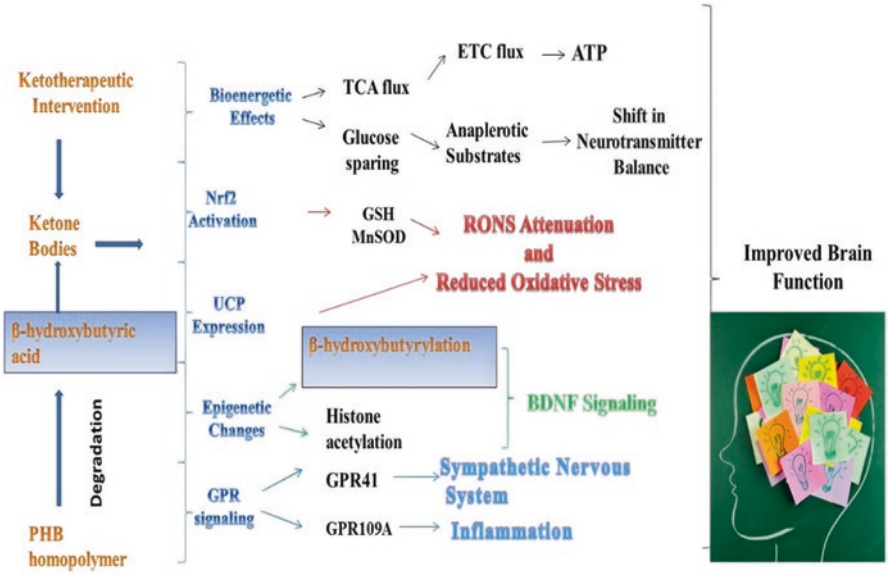


Fig. 7.2 Ketotherapeutic interventions through KB influence on brain function. *Nrf2* nuclear factor erythroid 2-related factor, *UCP* uncoupling protein, *GPR* G-coupled protein receptor, *TCA* tricarboxylic acid, *GSH* glutathione, *MnSOD* manganese superoxide dismutase, *ETC* electron transport chain, *RONS* reactive oxygen-nitrogen species, *ATP* adenosine triphosphate, *BDNF* brain derived neurotrophic factor (Koppel and Swerdlow 2017)

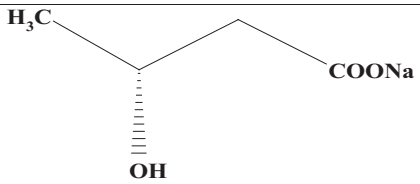
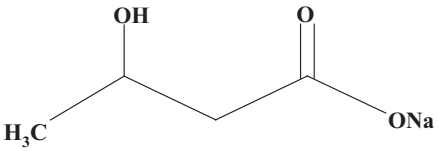
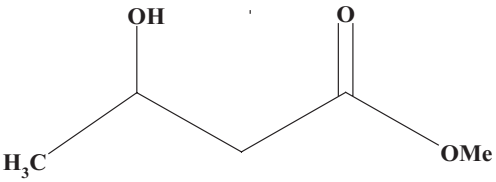
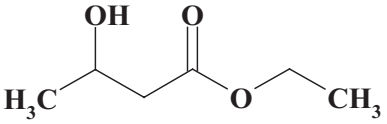
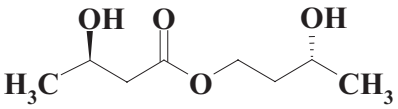
Verdin 2014). It has been proved that recent ketotherapeutic approaches influence the CNS through a variety of mechanisms (Fig. 7.2). More specifically, the action of KBs includes: (a) post-translational modifications of proteins, (b) attenuation of RONS production, (c) enhancement of the anti-oxidant stress response pathway expressions, (d) modulation of G-coupled protein receptor (GPR) and the signaling pathways brain derived neurotrophic factor (BDNF), (e) contribution to substrates replacement and (f) exhibition of anti-inflammatory effects (Brownlow et al. 2013; Koppel and Swerdlow 2017).

7.4 The Case of the Ubiquitous (or Omnipresent) 3-Hydroxybutyrate

Hydrolysis of PHB yields 3HB and its oligomers. 3HB is a physiological constituent of tissues and blood (Reusch et al. 1992; Malm et al. 1992; Peng et al. 1996), primarily originated by the long chain fatty acids catabolism in the liver and liberated to the peripheral tissues and plasma, where it diffuses rapidly, and subsequently, penetrates cell membranes serving as a lipogenic precursor and an oxidative combustible material (fuel) (Kesl et al. 2016). Sodium derivatives of D-3-hydroxybutyrate (D-3HB), DL-3-hydroxybutyrate (DL-3HB), and Methyl 3-hydroxybutyrate

(3HBME), ethyl (±)-3-hydroxybutyrate, 3-hydroxybutyl-(R)-3-hydroxybutyrate/ketone monoester (Table 7.1) are derivatives of 3HB, some are KBs produced in vivo in animals and humans, significantly useful for applications associated with tissue engineering (Chen and Wu, 2005), mainly due to the inhibition of cell death and the increase in cytosolic Ca^{2+} levels (Chen et al. 2010).

Table 7.1 Structures of (i) Sodium (S)-3-hydroxybutyrate (D-3HB), (ii) Sodium DL-3-hydroxybutyrate (DL-3HB), (iii) Methyl 3-hydroxybutyrate (3HBME), (iv) Ethyl (±)-3-hydroxybutyrate, and (v) 3-hydroxybutyl-(R)-3-hydroxybutyrate/ketone monoester

Structures	Compound
	**Sodium (S)-3-hydroxybutyrate (D-3HB)**
	**Sodium DL-3-hydroxybutyrate (DL-3HB)**
	**Methyl 3-hydroxybutyrate (3HBME)**
	**Ethyl (±)-3-hydroxybutyrate**
	**3-hydroxybutyl-(R)-3-hydroxybutyrate/ketone monoester**

7.4.1 Therapeutic Applications of 3-Hydroxybutyrate as Memory Enhancer

7.4.1.1 3-Hydroxybutyrate as an Anti-aging Agent Against Cognitive Impairment

Aging is one of the primary risk factors that progressively deteriorate the decline of cell and tissue function in neurodegenerative diseases such as PD and AD. The development of premature aging is directly related to mitochondrial dysfunction (Braeckman et al. 1999; Hansen et al. 2005; Dell'agnello et al. 2007; Copeland et al. 2009), whilst the increase in the mitochondrial reactive oxygen species (ROS) generation provokes the progressive damage of cellular macromolecules (Harman 2009). AD specific characteristics also include early and region-specific impairments of cerebral glucose metabolism. KBs are considered intra-corporeal glucose deprivation products and are metabolized by the brain. Recent scientific research results in animal models and human trials have indicated that 3HB, as a DR mimetic compound, delays the onset and progression of age-related neurodegenerative diseases, such as AD (Pasinetti et al. 2007**;** Mercken et al. 2012), by protecting cultured hippocampal neurons from $A\beta_{1-42}$ mediated toxicity and increased inflammation due to increased ROS levels (Kashiwaya et al. 2000; Van der Auwera et al. 2005; Tamagno et al. 2008). Research results on 3HB supplementation in *C. elegans* nematodes led to the increase of worm thermotolerance, whilst it partially hindered glucose toxicity, delayed AD Aβ toxicity and decreased PD alpha-synuclein aggregation. Additionally, 3HB extended the lifespan of *C. elegans* nematodes through histone deacetylases (HDACs) inhibition and via the activation of conserved stress response pathways (Edwards et al. 2014).

Furthermore, the role of oxidative stress in AD is recently well-accepted (Chen and Zhong 2014; Huang et al. 2016; Liu et al. 2017). In this frame, 3HB represents a potent protective agent against oxidative stress conditions, through its inhibitory activity of both classes of HDACs (I and IIa), in the increase of the expression of genes that encode antioxidant stress response factors, such as the forkhead box O3 (FOXO3A), the transcriptional factor DAF16, the mammalian orthologue of the stress-responsive lifespan regulator in worms, and the MT2 (Shimazu et al. 2013). Additionally, the 3HB-mediated inhibition of HDACs and the activation of the well-conserved antioxidant stress response mechanisms, result in lifespan extension and protection against metabolic, proteotoxic and thermal stress conditions in the cellular milieu (Edwards et al. 2014). Recently, new research orientations are based on the thinking that acute elevation of serum 3HB concentration via the oral administration of medium chain triglycerides (MCTs) meliorates memory and attention in AD individuals setting the stage for the development of an innovative therapeutic approach towards the effective confrontation of neurodegenerative disorders (Reger et al. 2004).

7.4.1.2 The Protective Role of 3-Hydroxybutyrate Against Parkinson's and Huntington's Diseases

PD main clinical characteristics are bradykinesia, muscle rigidity, and tremor of the distal extremities, whilst its pathological features include eosinophilic cytoplasmic Lewy-body inclusions consisted of: (i) ubiquitin, (ii) α-synuclein nucleoprotein,

and (iii) death of essential nigral dopaminergic neurons (Dunnett and Bjorklund 1999). Most important causes of PD are infections, environmental toxins or genetic irregularities, and it can be temporarily treated by L-3,4-dihydroxyphenylalanine (L-dopa) administration (Dunnett and Bjorklund 1999). Experimental results on the protective role of ketones against amyloid $A\beta_{1-42}$ or 1-methyl-4-phenylpyridinium (MPP^+) exposure of either hippocampal or mesencephalic neurons, respectively, suggest that mitochondrial dys-operation is the key factor in both of these common neurological disorders (Dunnett and Bjorklund 1999; Kashiwaya et al. 2000), despite the pathophysiological and genetic diversity of AD and PD etiology. AD and PD share a common dysfunction in the protein degradation process, possibly due to the defective mitochondrial energy generation. Thus, increase of ketones may promote protection of neurons by contributing in prevention and in treatment against memory decline related diseases AD and PD, in cases where cure with L-dopa must be period restricted. However, alternative biotechnologically produced dietary sources of ketones are beneficial without displaying the harmful side effects of contemporary KDs, although the high-fat KD is not considered appropriate for adults due to its atherogenic capacity (Gerngross 1999).

Taken into account all these biochemical experimental data, it is noteworthy to add that regulation of 3HB amounts can constitute a targeted neuroprotective approach for the cure against PD. More specifically, 3HB has been proved to confer protection through oxidative phosphorylation enhancement, in a stereo-specific and dose-dependent way, against the structural and functional toxic activities of the parkinsonian toxin 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), such as degeneration of dopaminergic substantia nigra pars compacta (SNpc) striatal fibers and neurons, PD-like motor abnormalities, and loss of striatal dopamine (Duan and Mattson 1999; Przedborski et al. 2003; Tieu et al. 2003).

HD is a genomic abnormalities-derived neurological disease provoked by the expansion of CAG (cytosine-adenine-guanine) trinucleotide repeats in the gene coding for polyglutamine domain for the protein huntingtin. HD main characteristics are protrusive perdition of medium-size spiny neurons and constitution of protein assemblages in the striatum and its cortical connections and the cerebral cortex (Vonsattel et al. 2011). Epigenetic modifications and bioenergetic defects play also a decisive role in the onset of the disease. To that end, in vitro and in vivo experimental results have demonstrated that the bioenergetic and epigenetic effects of 3HB confer neuroprotection in two animal models, a genetic one of HD and a murine toxic one of striatal neuronal perdition, by hindering the histone deacetylation stimulated by an erroneous form of huntingtin, the mutant huntingtin (mhtt) (Lim et al. 2011).

7.4.1.3 The Beneficial Role of 3-Hydroxybutyrate in Cell Proliferation and Prevention of Cell Death

Apart from the various potential therapeutic applications of 3HB in the amelioration of tissue damage, protein catabolism, and metabolic dysfunction (Cheng et al. 2006), experimental studies have proved that 3HB promotes cell proliferation in cell cultures (Cheng et al. 2005). The results at hand indicated that 3HB either speeds up

cell cycle progression by stimulating impermanent increases in intracellular calcium (Ca^{2+}) levels, which subsequently activate signal transduction pathways responsible for the regulation of the cell cycle (Berridge 1995), or decreases cell apoptosis, by enhancement of the mitochondrial respiration, bypassing glycolysis, while they are also introduced in The Citric Acid (TCA) cycle (Zou et al. 2002; Nakamura et al. 2003). In general, 3HB could supplement Adenosine Triphosphate (ATP) production, thereby hindering necrosis at high levels of cell growth, intervened by cell-to-cell contacts.

Recent experimental results on new drug formulations with various benefits such as enhanced bio-distribution and bioavailability, prolonged drug release and decreased cytotoxicity, have shown that conjugation of ibuprofen, a Non-steroidal anti-inflammatory drug (NSAID), linked to PHA oligomers, and particularly oligo(3-hydroxybutyrate) (O3HB), exhibited significantly increased inhibitory ability against proliferation of HT-29 and HCT 116 colon cancer cells in comparison with unconjugated ibuprofen. Collectively, the observed results indicated that conjugation enhances the cellular uptake of the conjugate, decreases cytotoxicity and improves the biopharmaceutical properties of the drugs (Zawidlak-Węgrzyńska et al. 2010).

7.4.1.4 The Role of 3-Hydroxybutyrate in Neuronal Death

Macro-autophagy is considered an intracellular destructive mechanism exclusively associated with the hydrolytic potency of lysosomes and responsible for the disassembly of damaged or dysfunctional organelles and proteins (Mizushima et al. 2008; Tanida 2011). It is considered a highly conserved process stimulated under various types of stress, such as nutritional stress, providing energy and sustaining cell survival (Ogata et al. 2006; Kroemer et al. 2010; Alirezaei et al. 2010). The autophagic flux is a process initiated by the recruitment of a multi-component proteinic complex, important for the formation of autophagosomes or double membrane vesicles, which then join with lysosomes to produce autophagolysosomes, where degradation of damaged proteins and intracellular components occur by hydrolytic enzymes. A dysfunction of the autophagic flux can result in increase in formation of autophagosomes leading to neuronal cell death (Kulbe et al. 2014; Sarkar et al. 2014). It has been shown that 3HB stimulates the autophagic flux by decreasing the formation rate of autophagosomes under conditions of energy deficiency and glucose withdrawal and minimizes neuronal death stimulated by glucose restriction (GD) (Camberos-Luna et al. 2016).

Glutamate is an important neurotransmitter which acts excitatory in the brain of mammals. Nevertheless, increase in extracellular levels activates neuronal death through the excitotoxicity process (Olney 1971; Choi 1992). Further scientific studies have estimated the contribution of oxidative damage in the promotion of excitotoxicity derived from inhibition of glycolytic metabolism, highlighting the neuroprotective potentiality of 3HB in the confrontation of the in vivo excitotoxic oxidative impairment (Mejía-Tober et al. 2006).

The results at hand indicate that systemic administration of 3HB prevents neuronal damage and lipoperoxidation, and stimulates two cellular processes occurred in isolated mitochondria, ATP generation and oxygen depletion (Tieu et al. 2003),

implying that administration of KBs, such as 3HB, in the mitochondrial metabolism, provides in the cells the ability to confront the neurotoxic effect originated from the activation of glutamate receptor. In accordance are studies performed in vitro, demonstrated the benefit of 3HB administration during hypoxia promoting the mitochondrial membrane durability and survival of hippocampal neuronal cell cultures, by preventing the generation of free radicals and the liberation of apoptotic molecules (Masuda et al. 2005). Furthermore, various in vitro studies indicate that 3HB opposes MPP^+ (the heroin analogue 1-methyl-4-phenylpyridinium), and β-amyloid toxicity (Kashiwaya et al. 2000) affecting memory, sustains creatine-phosphate and ATP at the initial amounts during glucose deprivation (Wada et al. 1997), and maintains synaptic communication as well as neuronal integrity (Izumi et al. 1998). In addition, evaluation of the in vitro effect of 3HB and its derivatives, DL-3HB and methyl D-3-hydroxybutyrate (M-D-3HB) on cellular apoptotic death and cytosolic Ca2+ levels of mouse glial cells indicated that the proportion of apoptotic cells significantly decreased when they are exposed to the aforementioned compounds (Xiao et al. 2007).

7.4.1.5 The Role of 3-Hydroxybutyrate as a Blocker of NLRP3 Inflammasome Activation in AD

The NLRP3 inflammasome is a significant multiprotein assemblage acting as a sensor of the innate immunity system found in macrophages with a fundamental role in the control of caspase-1 activation and the secretion of pro-inflammatory cytokines IL-1β and IL-18 (Martinon et al. 2009; Lamkanfi and Dixit 2014; Wen et al. 2013). The activation of inflammasome derives as a response to various damage-linked structural features, including among them amyloids, called DAMPs (damage-associated molecular patterns) (Masters et al. 2010; Heneka et al. 2013). Ablation of NLRP3 attenuates AD and age-related functional decline (Heneka et al. 2013; Youm et al. 2013).

Since the particular nutritional conditions are related to the modified functions of immune cells, recent experimental studies have proved that 3HB specifically affects the activated state of inflammasome that is also ATP-stimulated as well ordinary mechanisms of signal transduction in response to different structurally NLRP3 activators (Youm et al. 2015). Thus, under DR conditions, the role of 3HB as a metabolic signal can reduce the responses of the innate immune system, consuming sparingly ATP to assure the proper operation of organelles, which are ketone-dependent, like the heart and brain. Thus, the use of 3HB, in this case, is to set the stage for new pharmacological approaches to confronting chronic inflammatory diseases, where NLRP3 is involved (Youm et al. 2015).

7.4.1.6 Cerebro-Protective Effect of 3-Hydroxybutyrate on Anoxia, Hypoxia and Ischemia

The brain is an active tissue, whose basic source of energy supplies is glucose oxidation. Although glucose provision limitation and metabolism under the situations of ischemia and hypoxia, lead to brain injuries such as infarct and cerebral edema, it have been shown in epidemiological studies that hyperglycemia was linked with greater dangers of both cerebral ischemia and strokes occurrence (Asplund et al.

1980; Riddle and Hart 1982; Pulsinelli et al. 1983). Moreover, studies in several animal models have verified that the brain injury, provoked by cerebral ischemia, was accelerated by glucose administration (Siemkowicz and Hansen 1978; Ibayashi et al. 1986; Natale et al. 1990). The elevated levels of anaerobic glucose metabolism, during such conditions as hypoxia and ischemia, cause in tissues an overproduction of lactate leading to augmented intracellular quantities of proton H^+ (Rehncrona et al. 1981; Raichle 1983; Siesjo 1988). It has been reported that fasting under such conditions as hypoglycemia and ketosis, increased 3HB levels in the blood, preventing rats from displaying brain infarction after ischemia and hypoxia (Go et al. 1988).

Furthermore, studies carried out for elucidating the effect of 3HB administration showed the cerebroprotective activity and the notable protraction of the survival time under hypoxia, anoxia, and ischemia in rats and mice, subjected to bilateral common carotid artery ligation (BLCL). These results were obtained by reducing cerebral edema formation, maintaining higher ATP levels and inhibiting lipid peroxidation and anaerobic lactate accumulation (Suzuki et al. 2001). These research data indicate that 3HB has the potential to be utilized as a clinical nutritional treatment of acute cerebrovascular disorders (Suzuki et al. 2002).

7.4.1.7 The Boosting Role of 3-Hydroxybutyrate in the Brain Synthesis of Kynurenic Acid

Kynurenic acid (KYNA) is produced endogenously along the kynurenine pathway and constitutes a neuroactive intermediate product of tryptophan metabolism. Its formulation is involved in a process catalyzed by the enzymes kynurenine aminotransferases (KAT I–III) (Guidetti et al. 1997, 2007). Brain KYNA is synthesized intracellularly in astrocytes and its secreted extracellular amounts reach the nanomolar levels (Moroni et al. 1988; Turski et al. 1989; Németh et al. 2006). KYNA possesses enhanced anticonvulsant and neuroprotective activities, especially this related to the inhibition of strychnine-insensitive site of N-methyl-D-aspartate receptors (NMDA) (Urbanska et al. 1991; Stone et al. 2001; Schwarcz and Pellicciari 2002; Németh et al. 2006), the sufficiency in the noncompetitive reduction of $\alpha 7$ nicotinic receptors and the enhancement of the expression of $\alpha 4\beta 2$ nicotinic receptors by increasing the presynaptic liberation of glutamate (Carpenedo et al. 2001; Hilmas et al. 2001; Luccini et al. 2007).

Experimental data have proved that relatively low concentrations of a racemic mixture of 3HB stimulate the brain biosynthetic pathway of KYNA in primary glial cells as well as in cortical slices as effectively as during a KD or ketosis in diabetes (Gilbert et al. 2000; Fukao et al. 2004). Moreover, in vitro studies confirmed that high levels of circulating 3HB accompanied by acidosis or mild to severe hypoglycemic conditions inhibited KYNA synthesis in rat cortical tissue pieces. Furthermore, data from cultures of glial cells demonstrated that 3HB enhances the KYNA biosynthetic enzymes (KAT I and II) expression (Chmiel-Perzyńska et al. 2011). In general, the action of 3HB is to augment the levels of KYNA synthesis in the brain that via interaction with presynaptic NMDA receptors, may cause a reduction of the glutamate liberation and minimize the effects of the postsynaptic glutamate receptors contributing to their neuroprotective potency (Chmiel-Perzyńska et al. 2011).

7.4.1.8 Accumulation of 3HB Induced by Physical Exercise Promotes Brain-Derived Neurotrophic Factor Production

Exercise is beneficial for both physical and mental health. Corporeal exercise may influence the production of valuable proteins in the brain, particularly the levels of a significant protein called brain-derived neurotrophic factor (BDNF). Recent experimental studies demonstrated that 3HB accumulation induced by the physical exercise in the hippocampal milieu acts as an energy source and as an inhibitor of HDACs (class I) in the induction of BDNF expression (Fig. 7.3).

It has been proved that BDNF causes enhancement of mental competences, counteracting depression and anxiety as effectively in mice as in humans in a similar manner (Sleiman et al. 2016). In general, BDNF is necessary in order to survive susceptible populations of neurons. Furthermore, it plays an important role in dendritic and axonal neuronal cell growth as well as in synaptogenesis (Bibel and Barde 2000; Alsina et al. 2001). It has been proved that low levels of BDNF provoke depression, whereas they are elevated after administration of antidepressant treatment (Duman and Monteggia 2006; Martinowich et al. 2007).

In addition, exercise increases BDNF levels in the CNS promoting amelioration of cognitive ability and of comportment resembling depression (Russo-Neustadt et al. 2000; Marais et al. 2009), as it has been shown in animals and in patients suffering from mental disorders with depressive behavior (Sleiman et al. 2016), and neurodegenerative disorders such as PD (Frazzitta et al. 2014) or AD (Crabb et al. 2014).

Previous work indicated the neuroprotective effect of 3HB in HD (Lim et al. 2011) and PD (Kashiwaya et al. 2000; Tieu et al. 2003), on dopaminergic and striatal neurons, respectively (Autry and Monteggia 2012). Moreover, recent studies proved that the induction of 3HB in the brain after treatment of AD mice with structural analogs of glucose (e.g. 2-deoxy-D-glucose), delayed the occurrence/or advancement of bioenergetic deficiencies and the correlated β-amyloid

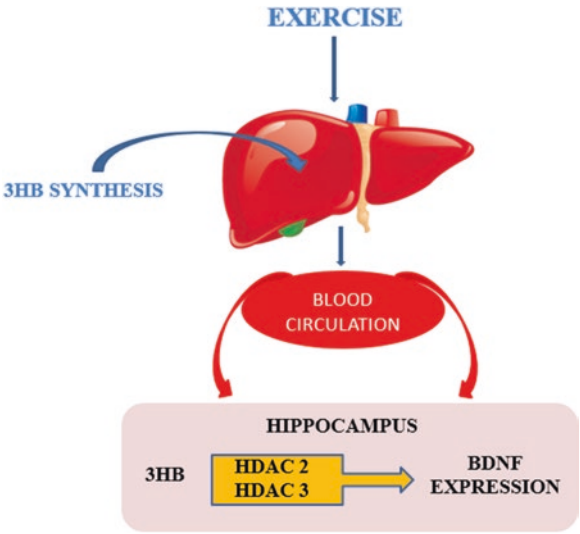


Fig. 7.3 A model mechanism indicating the effect of exercise on the induction of BDNF expression in the hippocampal milieu. Exercise provokes 3HB synthesis within the liver. Subsequently, 3HB is transferred through blood circulation to the hippocampus, where it induces BDNF expression through HDAC inhibition

encumbrance (Ralser et al. 2008; Yao et al. 2011). Therefore, since 3HB represents an endogenous molecule with the ability to cross the BBB, the experimental results at hand suggest that through physical exercise and peripheral metabolism 3HB upregulates BDNF transcription in the hippocampus, affecting the gene expression and epigenetic monitoring in the brain and the synaptic transmission (Sleiman et al. 2016).

7.5 Modified PHA Monomers. The Cases of 3-Hydroxybutyrate Methyl Ester and 3-Hydroxyalkanoate Methyl Esters

7.5.1 The 3-Hydroxybutyrate Methyl Ester

The 3-hydroxybutyrate methyl ester (3HBME) or other 3-hydroxyalkanoate methyl esters (3HAME) (Fig. 7.4) derive as products of the methyl esterification of *scl*-PHAs such as PHB (and PHV for 3HVME), or *mcl*-PHAs, including all HAs with equal and higher than six carbon atoms (Fig. 7.2) (Pantazaki et al., 2003). They have been considered as a basic source for PHA-based biofuels production (Zhang et al. 2009; Chen et al. 2010a). Modified PHA monomers such as 3HBME can also be employed as drugs against mitochondrial damage (Zhang et al. 2013).

Ex vivo production of 3HBME can be easily achieved by acidic methanolysis of PHB in a chloroform solution, at 67 °C for 60 h (de Roo et al. 2002; Wang et al. 2010; Zhang et al. 2009). Direct degradations of PHB to 3HB and 3HBME have also been reported (Seebach et al. 2003). To our knowledge, no in vivo data have been reported regarding the existence of 3HBME in biological systems. However, several microbial species, plants and possibly mammals are capable of producing both 3HB and PHB, with the latter proved to be carried by blood lipoproteins and

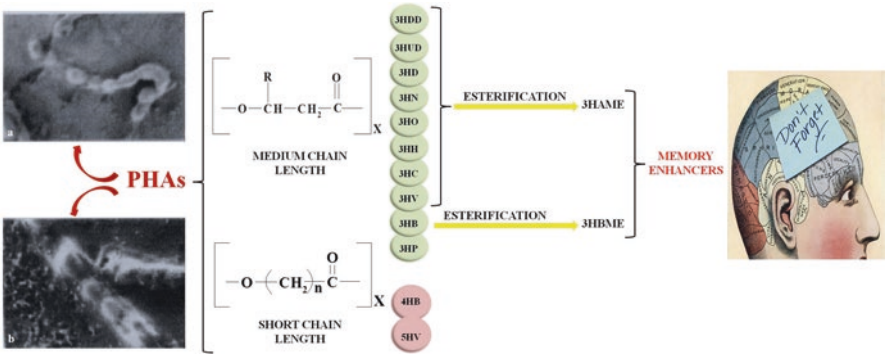


Fig. 7.4 Memory enhancers derived from PHAs like 3-hydroxybutyrate methyl ester (3HBME) or other 3-hydroxyalkanoate methyl esters (3HAME). Scanning electron microscopy (SEM) images of PHAs produced by the thermophilic bacterium *Thermus thermophilus* HB8. (Pantazaki et al. 2003)

albumin and intracellularly located in mitochondria and microsomes (Reusch et al. 1992; Reusch 2015). So, it is possible that some esterases or special de-esterases may yield 3HBME.

7.5.2 Bioavailability and Pharmacokinetics of 3-Hydroxybutyrate Methyl Ester

As it has been previously stated extensively, 3HB has been reported to possess strong neuroprotective properties (Kashiwaya et al. 2000; Reger et al. 2004; Cheng et al. 2013). 3HB is transported through the BBB via the monocarboxylate transporter family SLC16 and the sodium-dependent transporters SMCT and SLC5A with a relatively good rate (K_M of 6.03 mM in cultured rat astrocytes) (Tildon et al. 1994; Achanta and Rae 2017). Additionally, it has been proved that the bioavailability of 3HB in the brain is related to various factors including diet, age and species (Regen et al. 1983; Leino et al. 2001; Ito et al. 2011).

It is interesting that the bioavailability of 3HB in animals treated with 3HBME is higher than the ones treated with analogous shots of 3HB (7.5 times higher than basal levels compared to 1.6 times, respectively) (Uchida et al. 2011). Although the particular behavior has yet to be clarified, researchers have speculated that the amphiphilicity and small size of 3HBME could assist its easy transport through the BBB and its better distribution and transformation to 3HB in the brain (Zhang et al. 2013).

7.5.3 3-Hydroxybutyrate Methyl Ester and 3-Hydroxybutyrate Ethyl Ester as Neuroprotective Agents and Memory Enhancers

Memory function and learning action are highly energy-demanding processes. In so, if the brain is supplied with an excess of suitable metabolic substrates, an enhancement of these processes should be expected. Research results on neuroglial cells cultures supplemented with 3HB or its derivatives [(3-hydroxybutyrate ethyl ester (HBEE) and HBME] demonstrated that the neuroglial cell metabolic activity was remarkably elevated ($p < 0.05$) after 1 and 2 days of cultivation. In all cases, HBME-treated cells were more active than both HBEE and 3HB. Moreover, intracerebral expression of the receptor for the 3HB uptake into the cells, a protein upregulated in macrophages by interferon-g (PUMA-G), occurred after 3HBME administration in mice (Zou et al. 2009).

Furthermore, it was also affirmed that cerebral proteins such as phosphorylated extracellular-signal-regulated kinase 2 (ERK2) and connexin 36 were upregulated after 3HBME administration, but in statistically not significant way. Moreover, mice performed significantly better in the Morris water-maze than non-treted controls or mice administered with the neuronal stimulator acetyl-l-carnitine.

Additionally, 3HBME could increase the gap junctional intercellular communication between neurons (Zou et al. 2009).

In vivo studies were also performed on double transgenic mice models of AD that exhibited age-dependent amyloidal plaques aggregation in the cerebral cortex and the hippocampus and declining learning and memory ability. After training and daily treatment with different doses of 3HBME for 2.5 months, animals scored better in water-maze than non-treated controls and animals treated with AXONA–an FDA approved supplement for AD (Zhang et al. 2009). Significant alleviation of amyloidal burden was also demonstrated in HBME treated mice, accompanied by inhibition of brain atrophy around ventricles. Additionally, transcription of two genes encoding apolipoprotein E (ApoE) that promotes amyloidal aggregation, and caspase-3, an important protease in cell death, were down-regulated in mice treated with 3HBME (Zhang et al. 2013).

In the same study, PC12 cells were treated with NaN_3 to inhibit mitochondrial electron chain, ATP production and to induce oxidative stress. Interestingly, cells supplemented with 3HBME significantly increased ATP levels and inhibited ROS production. Additionally, 3HBME treatment restored NADH/NAD^+ ratio to the normal levels, after Rotenone treatment. Furthermore, 3HBME proved capable of protecting PC-12 cells from starvation-induced apoptosis, and carbonyl cyanide m-chlorophenyl hydrazine (CCCP)-induced dissociation of mitochondrial membrane potential (Zhang et al. 2013).

7.5.4 (R)-3-Hydroxybutyl (R)-3-Hydroxybutyrate/Ketone Monoester

(R)-3-hydroxybutyl (R)-3-hydroxybutyrate (D-3HB), also known as ketone monoester (KME), is a synthetic oil consisted mainly from (R)-3-hydroxybutyl (R)-3-hydroxybutyrate (94%) and other small-chain organic esters or non-esters. The purpose of this product, firstly synthesized at the University of Oxford, is to induce artificial mild ketosis, by its enzymatic hydrolysis to 3HB and butane-1,3-diol, with the latter being enzymatically turned also to 3HB (Clarke et al. 2012a, b).

7.5.4.1 Bioavailability and Pharmacokinetics of Ketone Monoester

Experimental studies have proved that KME is metabolized quickly, shortly after injection of a single dose of 714 $mg \cdot Kg^{-1}$ in adult humans and mice, as it cannot be traced in the systemic circulation. At the same time, blood concentrations of 3HB and acetoacetate increased, reaching a plateau after 1.5–2.5 h. In addition, it was verified that KME is hydrolyzed to its components (i.e. D-3HB and R-1,3-butanediol) and by that an increase in the levels of ketones in systemic circulation is achieved, as R-1,3-butanediol is hepatically metabolized to D-β-hydroxybutyrate and acetoacetate. Also, ingestion of the KME over a period of 5 days did not impose any significant adverse effects, even in repeated

doses of 2142 mg·Kg^{-1}bw/day. There were also no effects noticed on the development of mice embryos (Clarke et al. 2012a, b).

7.5.4.2 Ketone Monoester as a Possible Treatment for Alzheimer's Disease

Human studies on the employment of KME on a 51-aged male patient suffering from the early-onset AD, after three doses of 21.5 g daily for 3 days and three doses of 28.7 g daily thereafter, showed a remarkable improvement in the mood even after the first dose. Moreover, an improvement in the patient's impaired linguistic skills was noticed. After increased dosages, the patient also began to perform everyday activities without prompting or assistance, activities that he could not perform prior to the medication. After 6 weeks, memory retrieval has also been enhanced. It is interesting that the patient's physician noted that elevated cognitive performance paralleled plasma ketone levels and induced a decline in interaction skills and mood as ketone levels fell gradually toward basal levels. Additionally, a decline in total LDL and HDL cholesterol was recorded after 20 months of supplementation. No other noteworthy biochemical alteration was noticed in blood tests (Newport et al. 2015).

A synoptic presentation of all the in vitro, ex vivo, in vivo and clinical studies of the memory- enhancing activity of PHAs monomers was recorded in Table 7.2.

7.6 Conclusions

Microbes, as cellular factories exploiting low-cost carbon sources, can produce PHAs. PHA derivatives constitute high-value products, important curative agents and memory amplifiers. Memory decline, on the cognitive level, is a major issue of AD and related neurodegenerative diseases, from which millions of people suffer in the world and is set to grow rapidly in the coming decades. Although PHAs possess unique properties such as biodegradability and biocompatibility, their high cost at the moment, acts as a deterrent for their large-scale utilization. The newly developed field of PHA-based drugs functioning as memory enhancing moieties will help in the development of novel treatments against neurodegenerative disorders. The applicability of PHAs in those pharmaceutical and medical areas is more than plausible in the near future. However, at present only 3HB and its related products have been demonstrated for therapeutic activities, but more PHA monomers need to be evaluated for their medical efficacy in the near future. These memory enhancing monomers could be then utilized on their own, or even as encapsulating agents of multi-functional therapeutic compounds for their effective delivery through the BBB against AD.

Table 7.2 Synoptic presentation of the in vitro, ex vivo, in vivo and clinical studies of the memory enhancing activity of PHAs monomers

Compound	In vitro studies	Ex vivo studies	In vivo studies	Clinical studies	Results-conclusions	References
3HB			Male ddY mice and Wistar rats		Remarkable protective effects against hypoxia, anoxia and ischemia-induced metabolic change	Suzuki et al. (2001)
					Decrease of cerebral water and sodium contents	
					Maintenance of high ATP and low lactate levels	
3HB			Male Wistar rats with permanent (p)-occlusion and transient (t)-occlusion of middle cerebral artery (MCA)		Decrease of cerebral edema formation and infarct area	Suzuki et al. (2002)
					Improvement of cerebral energy metabolism during ischemia	
					Inhibition of lipid peroxidation after reperfusion	
3HB		Rat brain slices from adult male rats			Stimulation of brain CO_2 production by reduced oxygen availability in the presence of ketones	Kirsch and D'Alecy (1984)
3HB	*C. elegans* nematodes				Extension of mean lifespan by approximately 20% through inhibiting HDACs and through the activation of conserved stress response pathways	Edwards et al. (2014)
					Increase of worm thermotolerance and partial prevention of glucose toxicity	
					Upregulation of 3HB dehydrogenase activity and increase of oxygen consumption in the worms	
					Delay of AD amyloid-beta toxicity	
					Decrease of PD alpha-synuclein aggregation	

(continued)

Table 7.2 (continued)

Compound	In vitro studies	Ex vivo studies	In vivo studies	Clinical studies	Results-conclusions	References
3HB				20 individuals with probable AD or amnestic mild cognitive impairment	Oral doses of medium chain triglycerides (MCTs) increase serum 3HB levels	Reger et al. (2004)
					Improvement of memory and attention in individuals with AD or mild cognitive impairment	
3HB	Lipopolysaccharide (LPS)-primed mouse bone marrow–derived macrophages (BMDMs)		*Oxct1*$^{fl/fl28}$ and *Atg5*$^{fl/fl}$ mice crossed with *LysM*-Cre (B6.129P2-Lyz2tm1(cre)Ifo/J) animals and breeded with Tamoxifen-inducible Cre mice (B6.Cg-Tg(CAG-cre/Esr1*)5Amc/J)		3HB affects ATP-induced inflammasome activation or common signaling mechanisms in response to structurally diverse NLRP3 activators	Youm et al. (2015)
	$CD14^+$ monocytes sorted from cryopreserved peripheral blood mononuclear cells				3HB as a metabolic signal reduces the innate immune responses	
3HB	Immature primary cortical neurons from C57BL/6 mice	Hippocampal slices	Male mice		High BDNF levels in the CNS promote improvement in cognitive ability and depressive-like behavior	Sleiman et al. (2016)
					Through physical exercise and peripheral metabolism 3HB upregulates BDNF transcription in the hippocampus, affecting the epigenetic control and gene expression in the brain and the synaptic transmission	

3HB	Glial cultures from Wistar rats of both sexes	Cortical slices of brain tissue obtained from adult male Wistar rats			3HB enhances brain synthesis of kynurenic acid (KYNA) BHB reduces the activity of postsynaptic glutamate receptors and contributes to neuroprotective effects of KYNA	Chmiel-Perzyńska et al. (2011)
D-3HB	Cortical cultured neurons from Wistar rat embryos				D-3HB stimulates the autophagic flux preventing autophagosome accumulation and neuronal death induced by glucose deprivation (GD)	Camberos-Luna et al. (2016)
D-3HB			Male Wistar rats		D-3HB supplementation of mitochondrial metabolism prevents neuronal damage and lipoperoxidation	Mejía-Tober et al. (2006)
D-3HB	Human embryonic kidney cells (HEK293)	Mouse serum after a 24-h fast	Mice on calorie restriction (CR)		D-3HB is an endogenous and specific inhibitor of class I histone deacetylases (HDACs) D-3HB induced histone acetylation and gene expression promote stress resistance in the kidney	Shimazu et al. (2013)
D-3HB	Brains of male C57BL mice		Male C57BL mice		Infusion of D-3HB confers partial protection against dopaminergic neurodegeneration and motor deficits induced by 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxin D-3HB improves mitochondrial respiration and ATP production	Tieu et al. (2003)

(continued)

Table 7.2 (continued)

Compound	In vitro studies	Ex vivo studies	In vivo studies	Clinical studies	Results-conclusions	References
D-3HB	Brains of male C57BL mice		Male C57Bl/6 mice		D-3HB attenuates motor deficits, striatal lesions, and microgliosis in mice treated with 3-nitropropionic acid (3-NP) neurodegenerative toxin	Lim et al. (2011)
	Pheochromocytoma cells (PC12 cells)		Female hemizygous R6/2 breeders bred with male B6CBAFI/J mice		D-3HB extents life span, attenuates motor deficits, and prevents striatal histone deacetylation in transgenic R6/2 mice	
					D-3HB prevents histone deacetylation in PC12 cells	
D-3HB	Primary serum-free culture of 14-day embryonic mesencephalic cells				D-3HB protects cultured mesencephalic neurons from MPP1 toxicity and hippocampal neurons from $Ab_{1–42}$ toxicity	Kashiwaya et al. (2000)
	Hippocampal cells were dissected from embryonic rats				D-3HB corrects defects in mitochondrial energy generation	
Sodium D-3-hydroxybutyrate			Bal/c mouse newborn primary dissociated glial cell cultures		Cell apoptosis decreased significantly in the presence of 3HB and its derivatives	Xiao et al. (2007)
Sodium DL-3-hydroxybutyrate					3HB derivatives enhanced cytosolic Ca^{2+} concentration	
Methyl-D-3-hydroxybutyrate						

DL-3HB	Murine fibroblast L929 cells				3HB stimulates cell cycle progression mediated by a signaling pathway dependent upon increases in $[Ca^{2+}]_i$	Cheng et al. (2005, 2006)
	Human umbilical vein endothelial cells					
	Rabbit articular cartilages				3HB promotes proliferation in high-density cultures of L929 cells by preventing apoptotic and necrotic cell death	
Ibuprofen-oligo(3-hydroxybutyrate) conjugates (Ibu-OHB)	HT-29 and HCT 116 colon cancer cells		WIST rats		More effective cellular uptake of Ibu-OHB than free Ibu	Zawidlak-Węgrzyńska et al. (2010)
					Conjugation increases anticancer potential of Ibu	
					Conjugation improves antiproliferative activity, bioavailability and toxicity compared to free Ibu	
3-hydroxybutyrate methyl ester (3HBME)	Neuroglial cells of BALB/c mice				Metabolic activity was remarkably elevated	Zou et al. (2009)
					Enhanced gap junctional intercellular communication between neurons	
3-hydroxybutyrate methyl ester (3HBME)			BALB/c mice		Brain 3HB receptor PUMA-G, connexin 36 protein and phosphorylated ERK2 were upregulated	Zou et al. (2009)
					Mice performed significantly better in the Morris water maze than either the negative controls (no treatment) or positive controls	
3-hydroxybutyrate methyl ester (3HBME)	PC12 pheochromocytoma cell line				HBME inhibited cell apoptosis under glucose deprivation	Zhang et al. (2013)
					HBME rescued activities of mitochondrial respiratory chain complexes and stabilized the mitochondrial membrane potential	
					HBME decreased the generation of ROS	

(continued)

Table 7.2 (continued)

Compound	In vitro studies	Ex vivo studies	In vivo studies	Clinical studies	Results-conclusions	References
			C57/BL6 mice		HBME crossed the blood brain barrier easier compared with charged 3HB	Zhang et al. (2013)
					AD mice treated with HBME performed significantly better in the Morris water maze compared with other groups	
					Reduced amyloid-β deposition in mouse brains after intragastrical administration of	
(R)-3-hydroxybutyl (R)-3-hydroxybutyrate (D-3HB) or Ketone monoester (KME)				A 51-year old AD patient	Remarkable improvement in patients's mood and his ability to participate in conversations	Newport et al. (2015)
					Improvement in cognitive and everyday skills like writing, memory retrieval, personal care	
					Total cholesterol fell from 244 to 163 mg/dl, HDL cholesterol fell from 85 to 68 mg/dl, and LDL cholesterol from 145 to 81 mg/dl	

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