

Neuroprotective Effects of 4-phenylbutyric Acid and Its Derivatives: Possible Therapeutics for Neurodegenerative Diseases

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Abstract: The pathogenesis of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases involves the aggregation of denatured and misfolded nascent proteins. Consequently, many pharmacological approaches have been developed to prevent protein aggregation. 4-Phenylbutyric acid (4-PBA) is a chemical chaperone that shows potential as a candidate drug for the treatment of neurodegenerative diseases. The main actions of chemical chaperones are the amelioration of unfolded proteins and the suppression of their aggregation, which result in protective effects against endoplasmic reticulum stress-induced neuronal cell death. Furthermore, 4-PBA exhibits inhibitory activity against histone deacetylases (HDACs). However, owing to the problematically high doses of 4-PBA currently required for therapeutic efficacy, the optimization of 4-PBA is crucial for its effective medicinal application. In the present review, we summarize the recent advances in research on the basic actions of 4-PBA and its derivatives. We also discuss whether these compounds could be viable therapeutic agents against neurodegenerative diseases.

Key words: 4-Phenylbutyric acid (4-PBA), neurodegenerative diseases, chemical chaperone, histone deacetylase (HDAC).

1. Introduction

The accumulation of aggregated proteins is a common feature in the pathogenesis of neurodegenerative diseases. Mutated genes code different amino acids, resulting in protein structures different from those of normal proteins. In general, a protein needs to be folded to work properly. Unfolded proteins are folded through the action of molecular chaperones such as heat shock proteins (HSPs). If a protein is not formed properly, the ubiquitin-proteasome system induces its selective degradation with the 26S proteasome using

mitochondrial ATP, which is vital for selective degradation, and thus for the survival of cells.

Disruption of the protein degradation system causes stagnation of ubiquitinated proteins, which results in the accumulation of aggregated unfolded proteins. These insoluble proteins constitute inclusion bodies in neurodegenerative diseases. Thus, abnormal proteins are thought to be involved in the pathogenesis of neurodegenerative diseases (Fig. 1). From a therapeutic point of view, the prevention of protein aggregation is critical, and recent studies have shown that anti-aggregation molecules may help to prevent aggregation of neurodegenerative diseases-related proteins.

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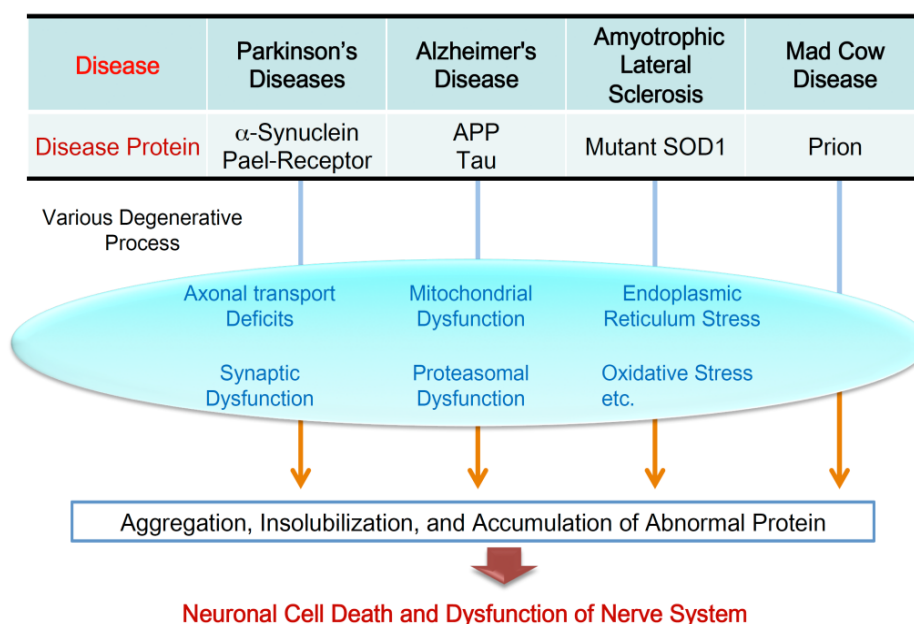


Fig. 1 Molecular mechanisms of neurodegenerative disease.

4-Phenylbutyric acid (4-PBA) is a small-molecular-weight fatty acid with a terminal aromatic group, and its sodium salt (sodium 4-phenyl butyrate) has been used for the treatment of urea cycle disorders [1]. This molecule is also used for the treatment of sickle cell diseases and thalassemia owing to its ability to activate β -globin transcription [2]. Moreover, 4-PBA is employed as an orally bioavailable agent for the treatment of spinal muscular atrophy (SMA) [3] and tumors [4]. Recently, the use of 4-PBA as a novel therapeutic agent for type 2 diabetes has been reported [5], and it has also been used as a treatment for familial hypercholesterolemia [6].

4-PBA has two main modes of action: as a chemical chaperone, and as a histone deacetylase (HDAC) inhibitor. Many studies have been published on the effects of 4-PBA in various biological models, and recently, the therapeutic effects of 4-PBA have been thoroughly classified and reviewed by Kolb *et al.* [7].

2. 4-PBA as a Chemical Chaperone

Molecular chaperones such as HSPs provide cellular protection by preventing nascent proteins

from misfolding, and reducing protein-protein aggregation. For example, Hsp70 is an intracellular chaperone that affects the amounts of misfolded and aggregated α -synuclein (α -syn) both *in vivo* and *in vitro* [8]. The aggregation of nascent or denatured proteins is considered to be a major factor in the pathogenesis of neurodegenerative diseases, as this aggregation can induce endoplasmic reticulum (ER) stress. For further details, the reader is directed to Hosoi *et al.* (2015), who thoroughly reviewed the relationship between Alzheimer's disease (AD) and endoplasmic reticulum stress [9].

Chemical chaperones mimic the functions of intracellular molecular chaperones. 4-PBA appears to stabilize nascent proteins and HSPs, which are known to interact with mutated or unfolded proteins (Fig. 2). 4-PBA has been reported to restore mutated cystic fibrosis transmembrane conductance regulator (CFTR), a phenomenon that may be exploited for therapeutic effect [10-13]. Furthermore, mutant α_1 -antitrypsin (α_1 -ATZ), which is retained in the ER of liver cells rather than being secreted into the blood and body fluids [14], can be folded by 4-PBA and may consequently be secreted. Moreover, 4-PBA exhibits

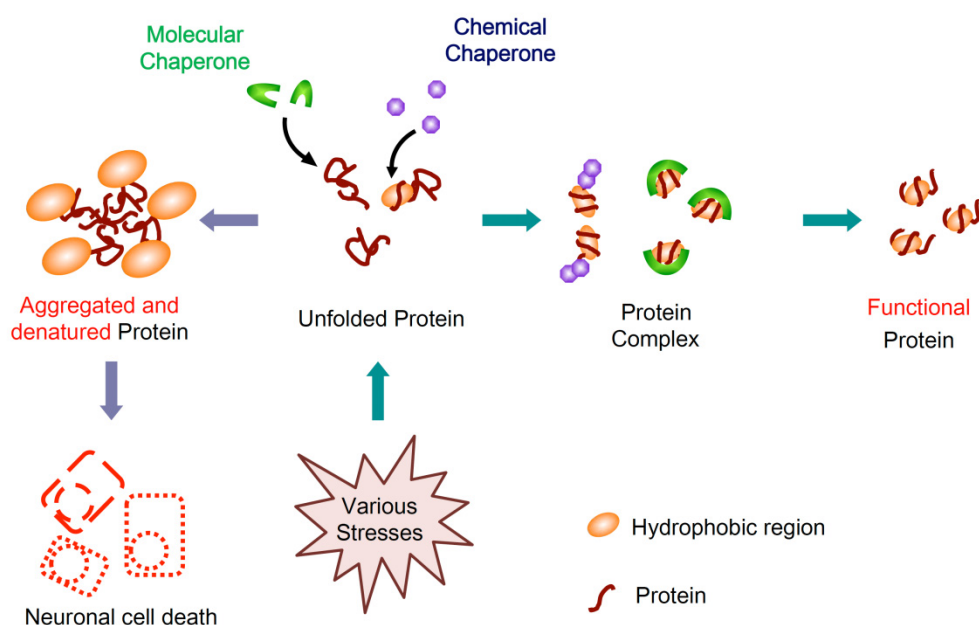


Fig. 2 4-PBA working as a molecular chaperone.

chemical chaperone activity, and has been reported to prevent the aggregation of denatured α -lactalbumin and bovine serum albumin (BSA) *in vitro* [15]. 4-PBA has also been reported to restore normal expression levels of Parkin-associated endothelin receptor-like receptor (Pael-R), which is one of the genes responsible for Parkinson's disease (PD), and to suppress Pael-R-induced ER stress [16-18]. Thus, 4-PBA is an effective compound against a wide variety of aggregated-protein conditions. Furthermore, 4-PBA ameliorates palmitate-induced inhibition of glucose-stimulated insulin secretion (GSIS) [19], and has been reported to improve cataracts caused by mutant γ D-crystallin in cultured cells [20].

3. 4-PBA as an HDAC Inhibitor

In general, histone acetyltransferases (HATs) and HDACs catalyze the acetylation and deacetylation, respectively, of histones. Epigenetic gene expression is regulated by the balance of HATs and HDACs, and by the methylation of DNA. The acetylation of histones promotes their partial loosening, so that transcription factors can easily bind to the gene. This induces epigenetic transcription (which regulates

cellular homeostasis) and the expression of a variety of genes. In many types of cancer, related proteins are constitutively overexpressed, and HDACs are thought to remediate this aberrant gene expression to its correct state. Thus, HDAC inhibitors have been developed as anticancer agents. These studies have already been summarized in several other excellent reviews [21-23].

Unlike cancer, neurodegenerative conditions are accompanied by collapsed homeostasis and declined turnover of proteins. However, both cancer and neurodegenerative diseases are accompanied by abnormal gene expression. In general, neurodegenerative diseases are presumed to be caused, or at least exacerbated, by the low acetylation of histones. Thus, HDAC inhibitors have recently become thought of as candidate therapeutic agents to improve the balance of gene expression, as reviewed by Das *et al.* (2016) [24].

Eighteen HDACs have been identified and classified into four categories by their homology, as reviewed by Zhang *et al.* (2015) [25]. Crystal structure analysis of several types of HDACs has been performed to facilitate the discovery of HDAC

inhibitors [26-28]. To function as a catalytic enzyme, almost all HDACs require a metal ion, except for class III HDACs. 4-PBA is thought to chelate zinc ions, forming a complex that subsequently acts as an HDAC inhibitor. Butyrate also acts via the same mechanism as 4-PBA owing to its similar structure, as short chain fatty acids are involved in both [29]. Butyrate inhibits many HDACs, except for HDAC6, HDAC10, and class III HDACs. Furthermore, Valproic acid, a similar compound, is thought to enhance the anticonvulsant activity of GABA in suppressive synapses [30, 31].

HDACs have been reported to act upon the central nervous system in disorder models, and the inhibitory effects of HDACs are likely to play an important role in the treatment of mood disorders. For example, 4-PBA is reported to ameliorate cognitive deficit and reduction of tau pathologies in an AD mouse model [32]. Moreover, systemic administration of 4-PBA has been reported to reinstate fear learning in the Tg2576 AD mouse model [33], and 4-PBA has been shown to exert significant neuroprotective effects in a PD mouse model [34-36]. It has also been reported that 4-PBA has a neuroprotective effect in the N171-82Q transgenic mouse model of Huntington's diseases (HD) [37]. Administration of 4-PBA increased brain histone acetylation and decreased histone methylation levels, as assessed by both immunocytochemistry and Western blots. Moreover, 4-PBA was shown to significantly extend survival and improve clinical and neuropathological phenotypes in G93A transgenic amyotrophic lateral sclerosis (ALS) mice [38], and the effectiveness of 4-PBA against SMA has been frequently reported [3, 39, 40]. Improvement of these conditions is suggested to be mediated by the inhibitory activity and/or chemical chaperone activity of HDAC inhibitors [3, 38-40].

4. Development of 4-PBA in our Research

4-PBA protects against cerebral ischemic injury and ER stress-induced neuronal death [41], which

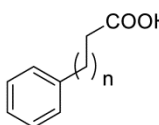
indicates its chemical chaperone activity. It also prevents the aggregation of reduced α -lactalbumin with denatured BSA [15].

Unfortunately, 4-PBA is required in high doses to prevent protein aggregation. Consequently, in order to improve the efficiency of the chemical chaperone activity of 4-PBA, we investigated the structure-activity relationship (SAR) in 4-PBA analogs with different numbers of carbon atoms in the fatty acid chain (Fig. 3) [42]. The aggregation inhibitory effect *in vitro* increased relative to the number of carbons from three to six. The longer carbon chain molecules were effective against ER stress-induced cell death by tunicamycin in SH-SY5Y neuroblastoma cells, whereas 3-phenylpropanoic acid (3-PPA) and 4-PBA only protected against Pael-R-induced cell death. Moreover, localization of the overexpressed Pael-R was shifted from the ER to the cytoplasmic membrane by 3-PPA and 4-PBA.

As another strategy to optimize 4-PBA, we investigated synthetic methoxy-substituted 4-PBA derivatives (Fig. 4) [43]. 4-(4-Methoxyphenyl) butanoic acid (4-MPB) showed protective effects against ER stress-induced neuronal cell death. However, further optimization is necessary for clinical use, since the required dose of 4-MPB was not significantly smaller than that of 4-PBA.

It is important to determine whether it is the chemical chaperone or HDAC inhibitor activity of 4-PBA that reduces ER stress-induced cell death. We showed that 4-PBA protects against neuronal cell death by primarily acting as a chemical chaperone rather than as an HDAC inhibitor using three simple 4-(*p*-substituted phenyl) butyric acids [44]. 4-(4-Aminophenyl) butanoic acid (4-APB) exhibited high affinity an HDAC7/4-PBA-derivative binding model. In fact, 4-APB showed a stronger HDAC inhibitory effect than the other derivatives. However, 4-APB did not exhibit protective effects against ER stress-induced cell death or chemical chaperone activity *in vitro*, whereas 4-MPB exhibited slightly

(A)



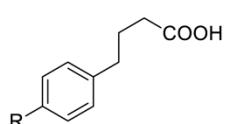
3-PPA: n=1
4-PBA: n=2
5-PVA: n=3
6-PHA: n=4

(B)

	Chemical chaperone activity	Protective effects against cell death (tunicamycin)	Protective effects against cell death (Pael-R overexpression)	HDAC inhibition
3-PPA	+	+	++++	+++
4-PBA	++	++	++++	++++
5-PVA	++	+++	++	++
6-PHA	++++	++++	++	+

Fig. 3 Activity comparison of 4-PBA derivatives with different numbers of carbons in the fatty acid chain. (A) 4-PBA derivatives. (B) The results of our study (Ref. [42]). Relative efficacy is indicated by the number of plus (+) symbols.

(A)



4-NPB: R=NO₂
4-APB: R=NH₂
4-MPB: R=OCH₃
4-PBA: R=H

(B)

	Chemical chaperone activity	HDAC inhibition	Protective effects against cell death (tunicamycin)
4-NPB	+++	++	+++
4-APB	+	+++++	+
4-MPB	++++	+++	+++
4-PBA	+++	++++	+++

Fig. 4 Activity comparison of the 4-PBA derivatives replaced with the para position. (A) 4-PBA derivatives replaced with the para position. (B) The results of our study (Ref. [44]). Relative evaluation using the number of plus (+) symbols.

stronger chemical chaperone activity than that of 4-PBA. Conversely, 4-MPB exhibited weaker HDAC inhibitory activity than that of 4-PBA. These data suggest that the inhibitory effect of 4-PBA on cell death is due to its aggregation-inhibitory activity (i.e.,

in vitro chemical chaperon activity) (Fig. 4).

We are currently conducting drug discovery research focusing on the chemical chaperone activity of the benzene ring of 4-PBA, which can interact with other benzene rings through π - π interactions (or

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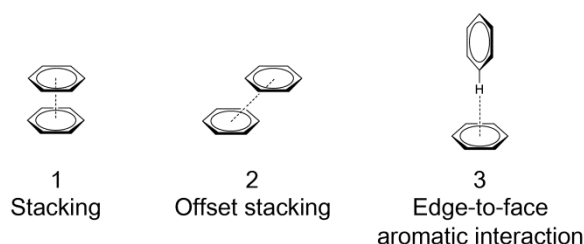


Fig. 5 Possible interactions of benzene rings.

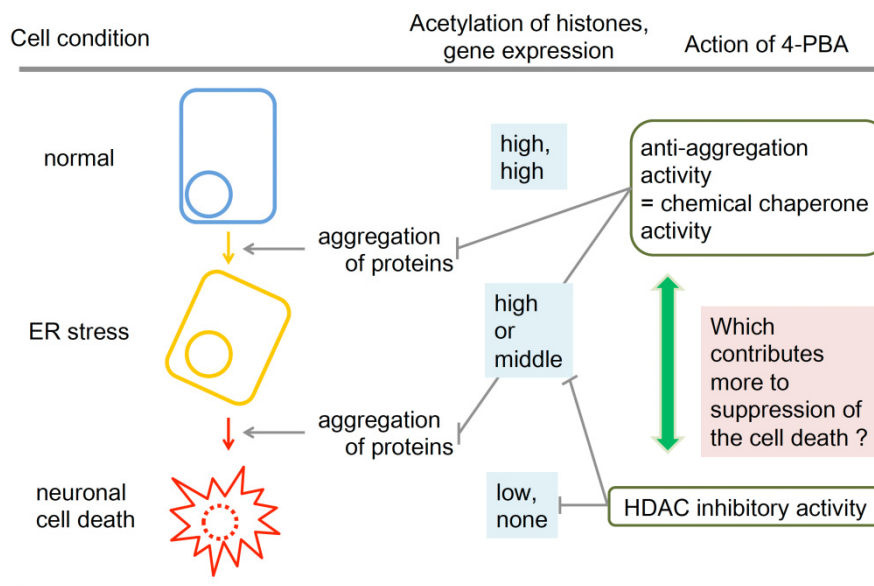


Fig. 6 Hypothetical mechanism of 4-PBA against cell death.

stacking). The possible interactions are shown in Fig. 5. The electron cloud area may be directly related to the anti-aggregation activity. This model appears to require a large amount of 4-PBA for the assembly of denatured proteins. We assume that the electron clouds of the benzene rings interact with the aggregates of denatured proteins. Thus, we attempted to expand the electron cloud area by synthesizing naphthalene-based 4-PBA analogs [45]. The chemical chaperone activity increased relative to that of 4-PBA. However, the synthesis of these analogs entailed the use of highly toxic reagents, so further work on 4-PBA analogs with different frameworks to naphthalene is underway in our group.

5. Conclusions

4-PBA is a biologically active small molecule that has already been demonstrated to be safe, and is

approved by the FDA. However, further study is required in order to fully understand this interesting compound. We believe that the main effects of 4-PBA are its protein-aggregation inhibitory activity and its HDAC inhibitory activity (Fig. 6). Histones are thought to be over-deacetylated in neurodegenerative conditions; therefore, the control of deacetylation is a promising therapeutic strategy for their treatment.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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