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Humanin and the Receptors for Humanin

Masaaki Matsuoka · Yuichi Hashimoto

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Abstract Alzheimer's disease (AD) is a prevalent dementia-causing neurodegenerative disease. Neuronal death is closely linked to the progression of AD-associated dementia. Accumulating evidence has established that a 24-amino-acid bioactive peptide, Humanin, protects neurons from AD-related neuronal death. A series of studies using various murine AD models including familial AD gene-expressing transgenic mice have shown that Humanin is effective against AD-related neuronal dysfunction in vivo. Most recently, it has been shown that Humanin inhibits neuronal cell death and dysfunction by binding to a novel IL-6-receptor-related receptor(s) on the cell surface involving CNTFRα, WSX-1, and gp130. These findings suggest that endogenous Humanin [or a Humanin-like substance(s)] may suppress the onset of AD-related dementia by inhibiting both AD-related neuronal cell death and dysfunction.

Keywords Humanin · Humanin receptor · Alzheimer's disease · Neuronal cell death · Neuronal dysfunction

Introduction

There are no curative therapies for Alzheimer's disease (AD). Based on the assumption that amyloid β (Aβ) locates at the top of the cascade leading to AD-relevant neuronal cell death and neuronal dysfunction (Aβ cascade theory), many therapeutic reagents reducing brain Aβ levels have been sought vigorously. Despite predominance of the Aβ cascade theory, accumulating evidence has indicated that AD-relevant neuronal cell death may occur independently of Aβ levels [1, 2], suggesting that Aβ-targeted therapy may modify the course of AD only partially. Another promising therapeutic strategy is to inhibit AD-related neuronal death directly irrespective of Aβ levels. Among various neurotrophic factors, Humanin has been found to inhibit AD-relevant neuronal death in a relatively AD-specific manner [2–6]. A series of in vivo studies have established that Humanin serves as a promising anti-AD therapeutic neurotrophic factor. Despite its potent AD-relevant neuronal cell death-suppressing activity, its receptor(s) on the cell surface mediating AD-relevant neuronal cell death-suppressing activity have remained unknown. A very recent study has indicated that Humanin protects neurons by binding to a complex or complexes involving ciliary neurotrophic factor receptor (CNTFR), IL-27 receptor WSX-1, and gp130 [6]. This review summarizes information on Humanin and Humanin receptors.

M. Matsuoka (✉) · Y. Hashimoto
Department of Pharmacology, Tokyo Medical University,
6-1-1 Shinjuku, Shinjuku-ku,
Tokyo 160-8402, Japan
e-mail: sakimatu@tokyo-med.ac.jp

Atrophy in Hippocampus and Temporal Cortex as a Central Pathological Sign of Alzheimer's Disease

AD is characterized by three pathological hallmarks: senile plaques, neurofibrillar tangles, and neuronal loss. An increase in the number of senile plaques in which aggregated Aβ is deposited or in levels of soluble Aβ oligomers has been hypothesized by the Aβ theory to be the major cause of AD-related dementia [7–14]. In support for this theory, multiple familial AD-causative mutations have been found in amyloid precursor protein (APP), presenilin 1, and presenilin 2 [15–20]. Aβ is cleaved from APP by β-secretase and γ-secretase, the latter of which contains presenilin 1 (or probably presenilin 2) as a subunit

[21, 22]. The expression of these familial AD-causative genes results in an increase in Aβ levels both in vitro and in vivo [18–20]. Important logical predictions by the Aβ theory, which has been recently modified [10–13], are that the neuronal dysfunction, induced by increased levels of soluble Aβ oligomers, should be the earliest and most central manifestation of AD and that neuronal loss occurs as an ultimate result of prolonged neuronal dysfunction [23]. This prediction has been considered valid because most transgenic mice overexpressing familial AD-causative genes suffer from memory impairment, mainly caused by neuronal dysfunction, without easily detectable neuronal loss [24, 25]. Unexpectedly, however, accumulating clinical studies with human AD cases have indicated that this prediction does not appear to hold true in human beings. Recent advance in diagnostic imaging systems has enabled the detection of earliest-phase human AD signs in brains in vivo. Hippocampal atrophy is invariably associated with the early-phase human AD cases and is the most sensitive and specific differentiator of human AD from normal cases and non-AD mild cognitive impairment patients [26–34]. The decline in cognitive function, detected by mental tests, may be preceded by or in parallel to the progression of hippocampal atrophy or atrophy of the temporal cortex, evaluated by magnetic resonance imaging [35]. In contrast, there appears to be no direct evidence indicating that neuronal dysfunction, induced by upregulated levels of Aβ, is the earliest manifestation of human AD. These facts support the idea that neuronal loss may be the earliest human AD sign.

Multiple clinical studies have suggested that increased levels of Aβ are not directly related to neuronal cell death. A long-term follow-up report of a phase II clinical study with active immunization with Aβ indicated that prominent Aβ antibody-mediated reduction of brain Aβ levels did not preclude the progression of neurodegeneration in advanced AD cases [36]. Recently, Savva et al. compared the extent of three AD-related pathological manifestations, neurofibrillar tangles, amyloid plaques, and brain atrophy in AD patients with those in age-matched normal cases aged 70–90 years [37]. There were significant differences in the extent of the three manifestations between AD and non-AD cases at 70 years. On the other hand, there are no significant differences in the extent of neurofibrillar tangles and senile plaques between AD and non-AD cases at 90 years while there was a significant difference in the extent of brain atrophy between AD and non-AD cases at 90 years. In addition, there are many studies indicating that formations of senile plaques and neurofibrillar tangles may occur in aged non-dementia cases [38–42]. A straightforward interpretation of these findings is that the brain atrophy, mainly caused by neuronal loss or neuronal cell death, is linked directly to AD while the other two manifestations may be linked indirectly to AD.

An AD-Related Neuronal Death-Suppressing Factor Humanin Simultaneously Inhibits AD-Related Neuronal Dysfunction

Humanin has been identified as an endogenous peptide that inhibits AD-relevant neuronal cell death [3]. A series of in vitro studies have established that Humanin inhibits neuronal death relevant to AD [2, 4, 5]. A series of in vivo studies have established that Humanin ameliorates cognitive impairment, caused by the dysfunction of cholinergic neurotransmission, in AD-related mouse models including transgenic mouse models [43–50]. However, because there are no AD animal models with detectable brain atrophy, it remains to be shown whether Humanin inhibits neuronal death in vivo. In addition, Humanin inhibits non-AD-related neuronal cell death, including mutant *SOD1* gene-induced motor neuronal death [51], serum-deprived induced death of PC12 pheochromocytoma cells [52], and DRPLA polyglutamine peptide-induced death [53], and neuronal death induced by brain ischemia [54]. Regarding the underlying mechanism for Humanin-induced amelioration of AD-related cholinergic dysfunction, Chiba et al. have demonstrated that Humanin upregulates cholinergic neurotransmission by increasing presynaptic acetylcholine levels and postsynaptic muscarinic M1 receptor-mediated signal transductions, which ultimately leads to the suppression of cognitive impairment caused by the dysfunction of cholinergic neurons [50]. All of these Humanin-induced anti-AD effects in vitro and in vivo have been shown to depend on the activation of STAT3 [6, 50, 55].

Humanin Receptors

Humanin is a secreted protein and inhibits AD-related death in vitro by binding to a receptor(s) on the cell membrane [3]. Guo et al. has shown that Humanin inhibits staurosporine-induced death by intracellularly binding to and inhibiting Bax [56]. These findings have suggested that the protective activity of Humanin is due to its binding to two types of receptors; one receptor on the cell membrane and another receptor in the cytoplasm or mitochondria. However, it remains unknown whether the latter mechanism involves AD-related neuronal cell death. In regards to the receptor(s) on the cell membrane, Ying et al. demonstrated that Humanin inhibited Aβ-induced death of PC12 cells by binding to formyl-peptide receptor-like receptor 1 [57]. Hashimoto et al. have recently shown that a Humanin receptor on the cell membrane that mediates neuroprotective activity belongs to the IL-6 receptor family, involving CNTFR, IL-27 receptor WSX1, and gp130 [6].

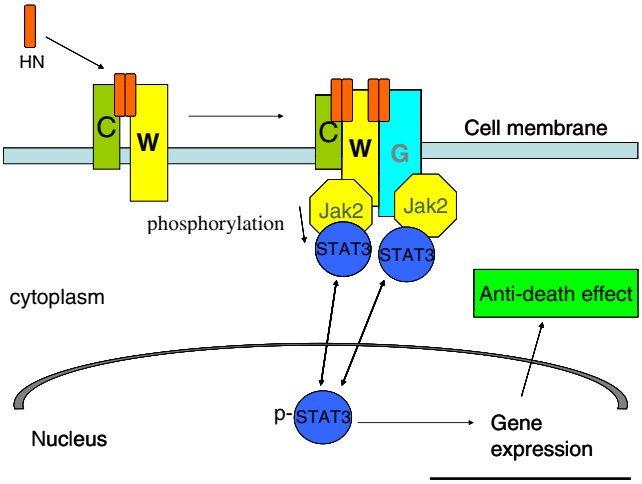

Fig. 1 The sturucture of the Humanin receptor and the Humanin signal transduction pathway. The Humanin receptor is composed of three IL-6 receptor-related subunits, CNTFR, WSX-1, and gp130. Humanin initially binds to CNTFR and WSX-1 and then induces the heterooligomerization of CNTFR, WSX-1, and gp130 [6]. Humanin-induced heterodimerization of the intracellular domains of WSX-1 and gp130 mediates the Humanin signals mainly via JAK2 and STAT3 [6, 50, 55]. STAT1 is also phosphorylated by Humanin treatment [6]

The Humanin Receptor Consisting of CNTFR/WSX-1/gp130 as a Mediator for Inhibition of Neuronal Cell Death

There are two types of receptors belonging to IL-6 receptor family [58, 59]. Gp130 is the common subunit for all receptors belonging to the IL-6 receptor family. IL-6 (IL-11) binds to an IL-6 (IL-11) receptor composed of two single-spanning transmembrane subunits, the IL-6 (IL-11) receptor α subunit without intracellular signaling modules and gp130. Upon ligand binding, induced homodimerization of the intracellular domains of gp130 mediates intracellular signaling. The other receptors belonging to the IL-6 receptor family have two subunits with a long intracellular domain mediating intracellular signaling. The receptor for leukemia-inhibiting factor (LIF) is composed of the LIF receptor (LIFR) and gp130 while receptors for oncostatin M, cardiotropin-1, and cardiotropin-like protein are composed of the oncostatin M receptor and gp130. Upon ligand binding, induced heterodimerization of the intracellular domains of LIFR (or oncostatin M receptor) and gp130 mediates intracellular signaling for these cytokines. The receptor for IL-27 is composed of WSX-1 and gp130 [60]. Upon IL-27 binding, induced heterodimerization of WSX-1 and gp130 is thought to mediate intracellular signals, followed by the activation of STAT1 and STAT3 [61–65]. The receptors for ciliary neurotrophic factor (CNTF), cardiotropin-like cytokine, and neuropoietin consist of three subunits. It consists of CNTFR (or CLF= the soluble receptor cytokine-like factor-1), LIFR, and gp130. CNTFR (or CLF) does not have intracellular signaling modules. Upon ligand binding, induced heterodimerization of the intracellular domains of LIFR and gp130 mediates intracellular signaling for these cytokines [66–69].

The Humanin receptor is another receptor with three subunits consisting of CNTFR, WSX-1, and gp130 (Fig. 1). Upon Humanin binding, it is likely that induced heterodimerization of the intracellular domains of WSX-1 and gp130 mediates intracellular signals [6]. WSX-1 is highly expressed immunological cells. It is also expressed in most neuronal cells [6] and some non-neuronal cells. CNTFR is highly expressed in neuronal cells. It is also expressed to lower extents in non-neuronal cells such as heart, kidney, muscle, and mammary gland. Gp130 is universally expressed in many tissues. Altogether, these facts suggest that the Humanin receptor CNTFR/WSX-1/gp130 may exist in neuronal cells and some non-neuronal cells, although there is no information regarding the function of this Humanin receptor in non-neuronal cells.

Using primary cortical neurons, Niikura et al. showed that IL-6 together with soluble IL-6 receptor mimicked Humanin activity while only IL-6 did not [70]. This finding suggests that upregulated homodimerization of the intracellular domains of gp130 results in Humanin-like activity. In agreement, Hashimoto et al. have recently shown that soluble WSX-1 lacking intracellular signaling modules could behave as a functional subunit for Humanin signaling in the absence of wild-type WSX-1 at least when soluble WSX-1 is overexpressed [71] (Fig. 2). Because this alternative Humanin receptor presumably consists of CNTFR, soluble WSX-1, and gp130, upregulated homodimerization of the intracellular

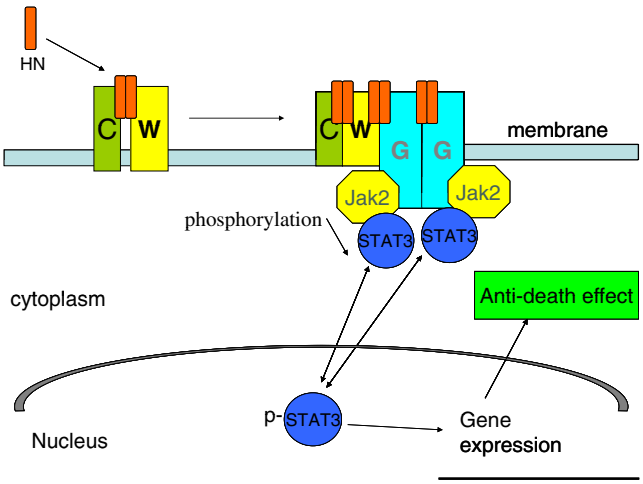

Fig. 2 The alternative Humanin receptor. Soluble WSX-1 lacking the intracellular domain of the wild-type WSX-1 replaces wild-type WSX-1 as a functional subunit of the Humanin receptor at least when it is overexpressed [71]. Humanin-induced upregulated homodimerization of the intracellular domains of gp130 is thought to mediate the intracellular Humanin signals. STAT3 and possibly JAK2 appear involved in signals by the alternative Humanin receptor

domains of gp130 is thought to mediate intracellular signaling upon Humanin binding to the alternative Humanin receptor.

Humanin as a Putative Physiological Defense Factor Against AD

Immunological studies have indicated that a small peptide, recognized by an antibody to Humanin, is expressed in testis [72]. In AD brains, reactive astrocytes in the hippocampus contain higher levels of a substance(s) recognized by the antibody to Humanin. Although several nuclear genes encoding Humanin-like peptides have been recently reported to be transcribed endogenously [73], there has been no direct evidence indicating that the Humanin peptide exists in vivo. However, recent discovery of the specific receptor for Humanin in neuronal cells [6, 70] indirectly but strongly supports the idea that Humanin [or a Humanin-like substance(s)] exists in vivo and suppresses AD-related neuronal cell death as a putative physiological defense factor against AD. In addition, an insufficiency in Humanin activity may contribute to the development of AD in addition to the increase in neurotoxic substances.

Humanin-Based Therapy

Multiple studies have indicated that there are Aβ-dependent and Aβ-independent mechanisms underlying AD-related neuronal death and dysfunction [2, 5]. Given Aβ-independent mechanisms for AD-related dementia, therapy reducing Aβ levels in the brain may be only partially effective against AD. In reality, clinical studies with such reagents have been rather disappointing [14, 36, 74]. In contrast, stimulation of the Humanin receptor may be an excellent therapeutic strategy to inhibit AD-relevant neuronal death as well as neuronal dysfunction because it is effective against Aβ-dependent and Aβ-independent neuronal death and dysfunction [2, 5]. Many Humanin peptide derivatives with stronger activity have been developed and tested on their in vivo activity using several AD mouse models [43–50]. Intranasal administration of the most potent Humanin derivative named Colivelin is very effective against all AD models tested [47, 50]. The intraperitoneal administration of Colivelin and another Humanin derivative with potent activity, S14G-Humanin, had been shown to be effective against AD models [44, 45, 49]. The latter result indicates that a substantial portion of Humanin derivatives cross the blood–brain barrier. For the future development of an ideal Humanin therapy, the identification of Humanin-mimetic small chemicals that can be administered orally would be required in addition to these injection-based Humanin therapies.

Future Perspectives

Wild-type Humanin has relatively weak neuroprotective activity (EC_{50}=1–10 μM). Therefore, Humanin may need to be structurally modified to be sufficiently active in vivo. One possible mechanism for the potentiation of Humanin activity is isomerization of the 14th serine of Humanin [75]. Replacement of L-serine with D-serine at this position increases Humanin activity by a thousand-fold. This possibility should be further tested carefully in vitro and in vivo. It is also possible that an unidentified Humanin-like molecule(s) with stronger Humanin activity than wild-type Humanin is the endogenous player in Humanin-induced neuroprotection. In addition to Humanin or Humanin-like molecules consisting of 20–30 amino acids [73], multiple larger peptides (10–45 kD) have been shown to be recognized by the antibody to Humanin (unpublished finding). The identification and functional examination of these peptides may clarify this issue.

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