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Alpha synuclein is transported into and out of the brain by the blood–brain barrier

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ABSTRACT

Alpha-synuclein (α-Syn), a small protein with multiple physiological and pathological functions, is one of the dominant proteins found in Lewy Bodies, a pathological hallmark of Lewy body disorders, including Parkinson's disease (PD). More recently, α-Syn has been found in body fluids, including blood and cerebrospinal fluid, and is likely produced by both peripheral tissues and the central nervous system. Exchange of α-Syn between the brain and peripheral tissues could have important pathophysiologic and therapeutic implications. However, little is known about the ability of α-Syn to cross the blood–brain barrier (BBB). Here, we found that radioactively labeled α-Syn crossed the BBB in both the brain-to-blood and the blood-to-brain directions at rates consistent with saturable mechanisms. Low-density lipoprotein receptor-related protein-1 (LRP-1), but not p-glycoprotein, may be involved in α-Syn efflux and lipopolysaccharide (LPS)-induced inflammation could increase α-Syn uptake by the brain by disrupting the BBB.

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Introduction

Parkinson's disease (PD) is among the most common neurodegenerative disorders affecting 1–2% of the aging population [56]. Pathologically, the disease involves multiple brain regions, including progressive loss of dopaminergic (DA) neurons, particularly in the substantia nigra pars compacta (SNpc) and in the locus ceruleus [27]. The histological hallmark of PD is the presence of inclusion body formation called Lewy bodies (LBs) in remaining neurons.

Alpha-synuclein (α-Syn) is the major component of LBs, and accumulations of α-Syn aggregates are also found in dementia with Lewy bodies (DLB) and multiple system atrophy (MSA) [54]. Physiologically, α-Syn in the brain at multiple cellular locations, including presynaptic terminals. Substantial evidence has shown association between α-Syn and familial and sporadic PD. For example, mutations in the α-Syn gene (SNCA) can cause familial PD [35,49,62] and multiplication of the normal wild-type *SNCA* can also increase the risk of sporadic PD [16,28,43,52].

Although α-Syn is considered an intracellular protein, a large body of literature has found the presence of α-Syn in biological fluids including human cerebrospinal fluid (CSF) and blood plasma [12,23,38,40,45,58]. Most studies showed decreased CSF levels of total α-Syn in patients with PD compared to controls [30,44,58], whereas the levels of oligomeric forms were increased in PD patients compared to controls [24,45,57]. Defective transport mechanisms of α-Syn from parenchyma to the CSF may account for the elevated load of α-Syn. However, little is known about the mechanisms responsible for α-Syn transportation.

The blood–brain-barrier (BBB) is an important regulator of the CNS environment, controlling the exchange of substances between the central nervous system (CNS) and the peripheral circulation, determining the concentration of molecules in the CNS, and contributing to the normal function of the brain. The vascular BBB is formed by brain endothelial cells that are joined by tight junctions, giving the BBB the ability to transport ions, peptides, and regulatory proteins selectively between the brain and the periphery [14]. Recently, evidence has shown that a dysfunctional BBB may

Abbreviations: α-Syn, alpha-synuclein; Aβ, amyloid-beta protein; BBB, blood–brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; DA, dopaminergic; ICV, intracerebroventricular; LBs, Lewy bodies; LPS, lipopolysaccharide; LRP-1, low-density lipoprotein receptor-related protein-1; PD, Parkinson's disease; SN, substantia nigra.

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play a role in the pathogenesis and progression in PD [1,34,37,46]. Altered transport mechanisms, such as defective expression of efflux pumps, altered transporter affinities, and receptor dysfunctions are all potential pathologies of the BBB in PD. By analogy, defective efflux of amyloid beta protein (Aβ) by low-density lipoprotein receptor-related protein-1 (LRP-1) and p-glycoprotein occurs with both Alzheimer's disease and with inflammation and is thought to be a major factor in the accumulation of Aβ by brain [25].

Levels of α-Syn in brain and blood are likely responsive to disease onset and progression. A mechanism that has not been understood is the possibility that α-Syn could cross the BBB. In this study, we examined the transport of α-Syn across the BBB in both the blood-to-brain and the brain-to-blood directions by injecting radioactively labeled α-Syn into mice and investigating the roles of LRP-1 and p-glycoprotein in its transport.

Materials and methods

Animal use

All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) and performed at a facility that is approved by AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care International). CD-1 male mice (8-week old) were used for all studies (Charles River, Wilmington, MA). Mice were given *ad lib.* access to food and water and kept on a 12/12 h light/dark cycle until study.

Radioactive labeling

α-Syn (rPeptide, Athens, GA) and murine amyloid beta peptide$_{1-42}$ (Aβ_{1-42}) (Bachem) were radioactively labeled with Na ^{125}I (Perkin Elmer, Waltham, MA) by the chloramine-T (Sigma–Aldrich, St. Louis, MO) method. Albumin (Sigma–Aldrich, St. Louis, MO) was radioactively labeled with Na^{131}I (Perkin Elmer) by the chloramine-T method. Radioactively labeled α-Syn (I-Syn), radioactively labeled Aβ_{1-42} (I-Aβ), and radioactively labeled albumin (I-Alb) were purified on a column of Sephadex G-10 (Sigma–Aldrich, St. Louis, MO). The I-Syn eluted as a single peak with a molecular weight of 20,000 by autoradiographic gel immediately, 72 h, and 11 days after labeling with greater than 90% of the radioactivity precipitating with acid.

Preparation and administration of lipopolysaccharide (LPS)

LPS from *Salmonella typhimurium* (Sigma) was dissolved in 0.9% NaCl and 3 mg/kg given in a volume of 0.2 ml by intraperitoneal (i.p.) injections at $t = 0$, $t = 6$ h, $t = 24$ h. Mice were studied at 28 h after the first injection.

Efflux-competition

An ICV method was used to assess brain-to-blood efflux rates [4,6]. Mice were anesthetized with an i.p. injection of 0.15 ml of 40% urethane (Sigma–Aldrich, St. Louis, MO). The scalp was removed and a hole was made into the skull (0.5 mm posterior and 1 mm lateral to the bregma) and 1 μl of lactated Ringers solution containing 2.5×10^4 cpm of I-Syn was injected into the lateral ventricle of the brain (icv) using a 1.0 μl Hamilton syringe. For efflux kinetics studies, mice were decapitated at 2, 5, 10, 20, or 60 min. For self-inhibition studies, 2.5×10^4 cpm of I-Syn with or without 1 μg of unlabeled α-Syn was injected icv and mice were decapitated 5 min after icv injection. Other mice received an injection of I-Syn with or without 1 μg of unlabeled murine Aβ_{1-42}. The whole brain was removed and weighed. The level of I-Syn available for transport at $t = 0$ was estimated by repeating this procedure in mice that had been overdosed with urethane and had been dead for 10–20 min [3,5]. The levels of radioactivity in the brain samples were counted for 3 min in a gamma counter and divided by brain weight in grams to yield cpm/g. The amount of I-Syn that was transported out of the brain (%T) was determined with the equation:

$$\%T = \frac{100\,(\text{cpm/g})_0 - (\text{cpm/g})_5}{(\text{cpm/g})_0}$$

where $(\text{cpm/g})_0$ is the mean level of cpm/g for the $t = 0$ group and $(\text{cpm/g})_5$ is the individual mouse's level of cpm/g at $t = 5$ min.

Other mice were prepared as above but received an icv injection of I-Aβ with or without 1 μg unlabeled α-Syn. Because of the slower efflux rate of I-Aβ, these mice were studied 10 min rather than 5 min after receiving the icv injection.

Brain perfusion

A brain perfusion method [2] was used to assess whether I-Syn was a substrate of the p-glycoprotein efflux transporter. Mice were anesthetized with an i.p. injection of urethane (40%). The heart was exposed by opening up the thorax. The descending aorta was clamped and both jugular veins were severed. A 23-gauge butterfly needle was injected into the left ventricle of the heart. Zlokovic buffer (7.19 g/l NaCl. 0.3 g/l KCl, 0.28 g/l $CaCl_2$, 2.1 g/l $NaHCO_3$, 0.16 g/l KH_2PO_4, 0.17 g/l anhydrous $MgCl_2$, 0.99 g/l D-glucose, and 10 g/l bovine serum albumin added on the day of perfusion) containing 200,000 cpm/ml I-Syn and 200,000 cpm/ml and I-Alb with or without 10 μg/ml cyclosporine was infused at a rate of 2 ml/min. After 2 min of perfusion, the mouse was decapitated and the brain was removed and weighed. The brain was then placed into a gamma counter for 3 min to determine the levels of radioactivity. Brain/perfusion ratios were calculated, expressed as μl/g, and the ratios for I-Syn corrected for vascular space by subtracting the ratios for I-Alb.

Influx

Multiple-time regression analysis was used to measure blood-to-brain uptake of I-Syn [11,47]. Mice were anesthetized with i.p. urethane (40%) and a 200 μl injection of lactated solution containing 3×10^5 cpm I-Syn was injected into the jugular vein. Between 1 and 10 min after the injection, arterial blood was collected from the carotid artery and the brain was removed and weighed. Levels of radioactivity in the brain and 50 μl of serum were in a gamma counter for 3 min. Brain/serum ratios were calculated to yield units of μl/g and plotted against exposure time. The linear portion of the slope of the resulting correlation measures the unidirectional influx rate in units of μl/g-min and the Y-intercept measures the distribution space in brain at $t = 0$ in units of μl/g.

Statistical analysis

Regression analysis and other statistical analyses were performed with the use of Prism 5.0 (GraphPad Software Inc., San Diego, CA). Means are reported with their SE and compared by one way analysis of variance (ANOVA) followed by Newman–Keuls post-test; t-test was used only in studies that compared two means. Slopes of the lines were compared with the regression package contained in Prism 5.0.

In vivo stability of I-Syn in brain and blood

Arterial serum and whole brain were obtained 10 or 60 min after the iv injection or 2 and 10 min after ICV injection of