Table of contents

1		Introdu	ction1
	1.1	1 Alzl	neimer's Disease1
	1.2	2 The	APP gene family3
		1.2.1	Structure and processing
		1.2.2	Physiological functions of the APP gene family in CNS and PNS9
		1.2.3	Dimerization and metal ion interaction12
	1.3	3 Ast	rocyte-mediated synaptogenesis14
	1.4	4 The	function of thrombospondins in CNS and PNS15
2		Aim of t	he study
3		Results.	
	3. 1	1 Ast	rocyte-derived thrombospondin-1 accelerates <i>trans</i> -directed dimerization of APP 18
		3.1.1	The factor of interest mediating APP dimerization is an astrocyte-secreted protein
		3.1.2 aggrega	Heparan sulfate proteoglycans do not modulate APP dimerization in a bead tion assay
		3.1.3	Establishment of an elution strategy to enrich the factor of interest
		3.1.4 astrocyt	Identification of APP-interacting proteins derived from conditioned medium of es
		3.1.5	APP forms dimers in conditioned medium of HEK293 cells that secrete TSP-1 26
		3.1.6	TSP-1 is the astrocyte-secreted protein in ACM that drives APP dimerization
		3.1.7	Astrocyte-derived TSP-1 interacts with the ectodomain of APP and induces
		dimeriz	ation of its subdomains E1 and E2 when overexpressed
		3.1.8	TSP-1 has no impact on APP dimerization in a <i>trans</i> co-immunoprecipitation36
		3.1.9	Analysis of TSP-1-mediated presynaptic differentiation in dependence on APP 37 $$
	3.2	2 Neg	atively charged cyclic molecules modulate APP <i>trans</i> dimerization
		3.2.1 dimeriz	Cyclic peptides carrying a negatively charged phosphonate group induce ation of APP40



	3	.2.2 verexp	The cyclic peptide SK-28 does not modulate <i>trans</i> -oriented dimerization of A ressed in HEK293 cells	.PP
	3	.2.3	Cyclization of SK-28 is a prerequisite to promote APP dimerization	.43
	2	24	APP dimerization is regulated by molecules that consist of negatively charge	har
	g	roups l	pound to a cyclic backbone	,cu .45
	3	.2.5	Heparin inhibits SC4 mediated but not SK-28 mediated APP dimerization	47
	3.3	Zind	ions bind to the APP E1 domain, thereby inhibiting copper binding	49
4	D	liscussi	۵n	52
•	4.1 dim	Ider	ntification of thrombospondin-1 as an astrocyte-secreted factor that promotes A	.PP 52
	4 d	.1.1 irected	Astrocytes secrete a proteinaceous factor other than an HSPG that facilitates <i>tra</i>	ns- 52
	4	.1.2	Identification of astrocyte-derived APP-interacting proteins	54
	4 d	.1.3 imeriz:	Thrombospondin-1 is the astrocyte-secreted factor that promotes <i>trans</i> -direct	ted 55
	4	.1.4	TSP-1 binds to the E1 and E2 domain of APP and facilitates their dimerization	57
	4	.1.5	The physiological function of an interaction between TSP-1 and APP	58
	4.2	Cycl	lic molecules carrying negatively charged groups modulate APP dimerization <i>in-vi</i>	tro
	4.3	Zinc	ions bind with a nanomolar affinity to the E1 domain and modulate the dimerizati	on
	of A	PP		61
	4.4	Out	00k	62
5	М	laterial	S	64
	5.1	List	of materials used	64
	5.2	Reci	ipes for buffers and solutions	69
	5.3	Cell	lines, bacterial strains, yeast strains and mouse strains	72
	5.4	Cult	ivation media	73
	5.5	Anti	bodies	75
	5.6	Plas	mids	75
	5.7	Olig	onucleotides and siRNAs	76
6	М	ethods		78

Table	of cor	itents	VI
6.1	Мо	lecular biology	
6.	1.1	Restriction digestion of DNA	
6.	1.2	Agarose gel electrophoresis	
6.	1.3	PCR purification	78
6.	1.4	Gel extraction	78
6.	1.5	DNA ligation	78
6.	1.6	Retransformation	79
6.	1.7	Transformation of ligated DNA	79
6.	1.8	Site directed mutagenesis	79
6.	1.9	Small scale (Miniprep) DNA preparation	79
6.	1.10	Large scale (Maxiprep) DNA preparation	80
6.	1.11	DNA sequencing	80
6.2	Pro	tein biochemistry	80
6.	2.1	Expression of APP E1 in Pichia pastoris	80
6.	2.2	Purification of APP E1	81
6.	2.3	Expression and purification of APPex-Fc	81
6.	2.4	Expression and purification of APLP2ex-Fc	82
6.	2.5	SDS-polyacrylamide gel electrophoresis (SDS-PAGE)	82
6.	2.6	Coomassie staining	83
6.	2.7	Colloidal Coomassie staining	83
6.	2.8	Silver staining	83
6.	2.9	Western blot	83
6.	2.10	Mass spectrometry and data analysis	
6.	2.11	Isothermal titration calorimetry (ITC)	85
6.	2.12	Bead aggregation assay with purified APPex-Fc or APLP2ex-Fc	
6.	2.13	Bead aggregation assay with transiently expressed APPex-Fc	
6.	2.14	Pulldown assay with coated paramagnetic protein A beads	
6.	2.15	Cis and trans co-immunoprecipitation (co-IP)	
6.	2.16	Immunocytochemistry	

Ta	Table of contents V		
	6.2.17	Quantification of synapses in neuronal cultures	
	6.2.18	Statistical analysis	
e	5.3 Cell	biology	
	6.3.1	Cultivation of cell lines	
	6.3.2	Preparation and cultivation of astrocytes	
	6.3.3	Preparation and cultivation of primary cortical neurons	
	6.3.4	Freezing of cells90	
	6.3.5	Thawing of cells	
	6.3.6	DNA or siRNA transfection using jetPRIME90	
	6.3.7	DNA transfection using PEI90	
7	Referen	ces	
8	Append	ix 116	
List of Abbre		viations	
List of Figures			
List	of Tables		
Dan	ıksagung.		
Leb	enslauf		
Erk	lärung zu	r Beteiligung Dritter	
Eidesstattliche Erklärung			