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Regulation of Proteolytic Processing of the Amyloid β-Protein Precursor by First Messengers

A novel potential approach for the treatment of Alzheimer's disease

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Summary

Amyloid deposits in Alzheimer's disease brains consist of aggregated amyloid β-peptides(Aβ) which are derived by proteolytic processing of the amyloid β-protein precursor (APP). Proteolytic APP processing can be regulated by the activity of neuronal cell surface receptors including the muscarinic m1 and m3, the serotoninergic 5-HT_2 and 5-HT_{1C}, vasopressin and bradykinin receptor subtypes. Receptor stimulation with appropriate agonists rapidly increases the rates of release of the α-secretase processing product APP^s which is cleaved within the Aβ domain and thus is a non-amyloidogenic derivative. Moreover, stimulation of m1 receptors also decreases the formation of Aβ, a secreted potentially amyloidogenic and possibly neurotoxic APP fragment. Similar biochemical events occur in stimulation experiments of fresh rat brain slices suggesting that neuronal activity may be involved in regulating APP processing in mammalian brain. Activation of non-amyloidogenic APP processing and inhibition of amyloidogenic processing pathways by subtype-specific agonists of muscarinic, serotoninergic or peptidergic receptors provides a novel approach for the pharmacological modulation of APP processing in Alzheimer's disease.

Zusammenfassung

Regulation der proteolytischen Spaltung des Amyloid-β-Protein-Precursors durch First Messenger / Eine neuartige Methode für die Behandlung der Alzheimer-Krankheit

Amyloid-Ablagerungen im Gehirn von Alzheimer-Patienten bestehen aus Amyloid β-Peptid(Aβ)-Aggregationen, die bei der Proteolyse von Amyloid-β-Protein-Precursors (APP) entstehen. Die APP-Proteolyse kann durch die Aktivität von Rezeptoren an der neuronalen Zelloberfläche wie zum Beispiel serotoninergen 5-HT_2 und 5-HT_{1C}, Muscarin m1- und m3-, Vasopressin- und Bradykinin-Rezeptorsubtypen reguliert werden. Eine Rezeptorstimulierung mit geeigneten Agonisten steigert die Freisetzung des α-Sekretase-Produkts APP^s, das innerhalb der Aβ-Domaine gespalten ist und so ein nicht-amyloidogenes Derivat darstellt. Außerdem verringert eine Stimulation von m1-Rezeptoren auch die Bildung von Aβ, einem sezernierten, potentiell amyloidogenen und möglicherweise neurotoxisch wirksamen APP-Fragment. Ähnliche biochemische Ergebnisse konnten bei Stimulationsversuchen an frischen Rattengehirnschnitten beobachtet werden, was darauf hindeutet, daß neuronale Aktivität an der Regulation der APP-Umsetzung im Säugetiergehirn beteiligt sein könnte. Aktivierung der nicht-amyloidogenen APP-Umsetzung und Hemmung amyloidogener Synthesewege durch Subtypen-spezifische Agonisten von serotoninergen, peptidergen oder muskarinergen Rezeptoren stellt eine neuartige Methode für die pharmakologische Regulierung der APP-Umsetzung bei der Alzheimer-Krankheit dar.

Key words: *Alzheimer's disease · β-Amyloid precursor protein, regulation of proteolytic processing · Muscarinic receptors · Serotoninergic receptors*

1. Brain amyloid in Alzheimer's disease and processing of the amyloid β-protein precursor

Brain amyloid deposits are invariant neuropathological hallmarks of Alzheimer's disease (AD) and Down's syndrome, and are sometimes also found in lesser amounts in brains of non-demented aged human subjects. AD-type brain amyloid consists of aggregated amyloid β-(Aβ) peptides which are hydrophobic and self-aggregating molecules of 39-43 amino acid residues in length. Aβ is derived, by proteolytic processing, from a larger amyloid β-protein precursor (APP). APP is a transmembrane glycoprotein that constains a single membrane spanning domain, a large N-terminal ectodomain and a short cytoplasmic C-terminal tail. The hydrophobic C-terminal region of the amyloidogenic Aβ domain is located within the transmembrane domain, and its N-terminus extends 28 residues into the ectodomain. APP exists in various forms encoded by alternatively spliced mRNA derived from a single gene that maps to chromosome 21 [1, 2]. The APP gene was highly conserved during evolution and is expressed at remarkably high levels in brain but is also expressed in many peripheral tissues. The biological function of APP is unclear, and it is possible that individual proteolytic derivatives of APP have distinct biological consequences. For instance, accumulating evidence suggests that the full-length protein and its secreted N-terminal derivatives can promote cell adhesion [3], stimulate neurite outgrowth [4], and protect cultured neurons from excitotoxic damage [5]. In contrast, Aβ and its aggregates can be cytotoxic, and induce cell death in cultured neurons [6, 7]. Moreover, Aβ inhibits the normal function of a potassium channel which appears to be impaired in fibroblasts obtained from AD patients [8, 9]. These initial data support a bimodal

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model for the biologic roles of APP with trophic functions of large portions of its ectodomain, and toxic properties of its Aβ domain. The biochemical mechanisms involved in the regulation of local brain tissue concentrations of distinct proteolytic APP derivatives may thus play an important role in determining the actual functions of APP.

Mature full-length APP is normally processed by various alternative proteolytic pathways to yield both secreted and cell-associated derivatives. The secreted forms include the 97-110kDa N-terminal ectodomain (APP^s) derived from α-secretase cleavage within the Aβ region [10, 11] and secreted p3, a 3kDa Aβ fragment possibly generated by cleavage at the α-secretase site, and the γ-secretase site at the C-terminus of the Aβ domain [12]. Additionally, ~4KDa Aβ-peptides derived from two cleavage events both at the N-terminus (β-secretase) and at the C-terminus of the Aβ domain can be produced by normal cellular metabolism of APP [13–16]. Similar secretory APP processing pathways are likely to occur in human brain tissues because APP^s and Aβ are present in human cerebrospinal fluid at concentrations of 0.5–2.5 μg/ml and 5–25 ng/ml, respectively [17, 18]. Besides the secretory processing pathways, full-length APP can be targeted to the endosomal system, either directly from the Golgi, or by re-internalization from the plasma membrane [19]. Within the endosomal-lysosomal compartment, APP can be hydrolyzed to yield multiple fragments some of which contain the intact Aβ domain and thus are potentially amyloidogenic [20]. Simlar Aβ-comprising C-terminal APP fragments have also been found in human brains [21].

In summary, α-secretase processing precludes the processing of APP into fragments that are found to accumulate as amyloid in human brains. In contrast, full-length APP as well as its proteolytic derivatives generated either by endosomal-lysosomal proteinases or by the β-secretase processing pathway are potentially amyloidogenic. It is therefore important to understand the cellular mechanisms that regulate the relative activities of the proteolytic events involved in APP processing.

2. Muscarinic acetylcholine receptors can regulate proteolytic processing of APP: increase in APP^s and decrease in Aβ

G protein-coupled cell-surface receptors can regulate the rates at which APP is hydrolyzed by individual proteolytic processing pathways (for review, see [22]). For example, stimulation of muscarinic acetylcholine receptor (mAChR) subtypes m1 and m3 with carbachol increases the release of APP^s 6- to 8-fold within minutes in HEK 293 cell lines which were stably transfected with cDNA expression constructs encoding these receptors [23, 24]. Increased APP^s secretion was paralleled by a decrease in levels both of cell-associated full-length APP and of C-terminal fragments. Carbachol also increased APP^s secretion in the presence of the translation inhibitor cycloheximide, indicating that muscarinic receptors can accelerate the cleavage of pre-existing, full-length APP, independently of possible effects on the rate of APP synthesis. Moreover, carbachol caused a 60 % decrease in the production and the secretion of Aβ from cell lines that overexpress the m1 receptor subtype [25]. Together these data suggest that muscarinic receptors can accelerate α-secretase processing of APP and concurrently inhibit the β-secretase processing pathway. It is possible that stimulated α-secretase processing and the β-secretase processing pathway simply compete for APP as a substrate to be metabolized. Alternatively, the (unknown) proteases involved in generating either APP^s or Aβ may be regulated differentially by signalling events that are initiated by muscarinic receptor activation. Secretion of Aβ is increased 4- to 5-fold in cell lines expressing an APP gene that encodes the double mutation found in the APP gene of a Swedish familial Alzheimer's disease kindred [26, 27]. These results suggested a causal role of this mutation in contributing to amyloid formation in affected individuals. In cells transfected with cDNAs encoding both the muscarinic m1 receptors and this mutated APP695, receptor stimulation with carbachol blocked the increased Aβ secretion [25]. This result shows that the cleavage events regulated by m1 receptors are able to process both normal APP and the APP associated with the Swedish mutation. It also implies that even pathologically high Aβ secretion associated with a disease-causing mutation can be suppressed by the activity of muscarinic m1 receptors. A clinical study in this Swedish family is needed to test whether specific m1-receptor agonists can decrease Aβ levels and slow amyloid formation in brains of affected family members.

3. Serotonin 5-HT_2 and 5-HT_{1c} receptors can increase the secretion of APP^s

Muscarinic m1 and m3 receptors are members of a large family of structurally and functionally related neurotransmitter and hormone receptors characterized by 7 transmembrane domains and 3 cytoplasmic loops [28]. These receptors are coupled to G proteins and to signalling pathways that involve activation of phospholipases, and the consequent generation of diacylglycerol along with inositol trisphosphate. Additional members of this receptor family include the serotoninergic 5-HT_2 and 5-HT_{1C} receptors (for review, see [29]). To test whether such serotonin (5-HT) receptors can regulate proteolytic APP processing pathways, we used NIH 3T3 fibroblast lines that were stably transfected with cDNA expression constructs encoding 5-HT_2 and 5-HT_{1C} receptors [30,31], stimulated them with 5-HT, and measured the release of APP^s into the culture medium. 5-HT caused a dose-dependent 3- to 4-fold increase in APP^s secretion from both cell lines (5-HT_2: EC_{50} = 300 nmol/l; 5-HT_{1C}: EC_{50} = 10 nmol/l). In control experiments with the untransfected parent NIH 3T3 cell line, 5-HT (100 pmol/l–100 μmol/l) did not change the release of APP^s. Increased APP^s secretion in the transfected cell lines was blocked by the 5-HT receptor antagonists ritanserin (20 μmol/l), mianserin (50 μmol/l), and ketanserin (20 μmol/l). Hence stimulation of 5-HT_2 and 5-HT_{1C} receptor can also regulate rates of APP^s secretion. The data obtained so far imply that stimulation of these serotonin receptor subtypes accelerates α-secretase processing of APP.

4. Increase in APP^s secretion by the neuropeptides vasopressin and bradykinin

Vasopressin (V1a) [32] and bradykinin (B2) [33] receptors also belong to the 7 transmembrane domain receptor family. To test whether these neuropeptide receptors could also regulate APP processing, we cloned a rat kidney fibroblast (NRK 49F) line, and a PC-12 cell line to express functionally intact receptors for vasopressin and bradykinin, respectively, as affirmed by agonist-induced acceleration (10-fold) of PI-turnover. Arginine-vasopressin caused a rapid 2- to 5-fold dose-dependent increase in APP^s secretion (EC_{50} = lnmol/l) in NRK 49F cells. This increase was blocked by the vasopressin receptor [diamino-Pen^1, Val^4, Arg^8]-vasopressin (100 nmol/l), indicating that the vasopressin had increased APP^s release by stimulating endogenously expressed vasopressin receptors coupled to PI-turnover. Similarly, bradykinin caused a dose-dependent (EC_{50} = 500 nmol/l) increase in APP^s release that was blocked by D-Arg-Arg-Pro-

Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg, a bradykinin receptor antagonist. These data show that bradykinin also can regulate APP processing via endogenous bradykinin receptors. Bradykinin stimulated APP^s secretion both in undifferentiated and in differentiated (50 μg/ml 7S-NGF for 3 days prior to stimulation) PC-12 cells, suggesting that both the undifferentiated and differentiated cell lines used in these experiments expressed sufficient levels of functionally intact bradykinin receptors to modulate APP processing pathways.

5. Redundancy in cellular signalling pathways that couple receptor activation to the regulation of APP metabolism

Control experiments with transfected muscarinic m2 and m4 receptor subtypes showed that activation of these receptor subtypes did not change APP^s secretion [23]. Stimulation of m2 and m4 receptors with carbachol inhibits adenylyl cyclase activity and thereby decreases intracellular formation of cAMP [34]. It appears likely that this signalling pathway is not involved in regulating APP metabolism. In contrast, muscarinic m1 and m3, serotoninergic 5-HT_2 and 5-HT_{1C}, and the peptidergic V1a and B2 receptors are coupled to activation of phospholipase C, and to the generation of diacylglycerol (which activates protein kinase C) and inositol triphosphate (which releases Ca^{++} from the endoplasmic reticulum) [28, 33]. Additionally, m1 and m3 receptors as well as 5-HT2 receptors stimulate the activities of phospholipase A_2 [35, 36] and muscarinic receptors stimulate phospholipase D activity [37], and open a receptor-operated calcium channel at the plasma membrane [38]. Initial evidence showed that staurosporine, a protein kinase inhibitor, blocked the increase in the rates of APP^s secretion induced by m1- and m3-receptor [23]. This finding suggested that receptor-coupled stimulation of protein kinases may be involved in the cellular signalling pathways that couple surface receptor activity to APP processing events. Moreover, phorbol myristate acetate (PMA), a potent activator of protein kinase C (PKC), also increases APP^s secretion from many cell lines [39, 40] including these described in this report, underscoring the posssibility that PKC might be involved in the signalling pathways that couple receptor activation to APP processing pathways. However, our data clearly indicate that down-regulation PKC by chronic treatment with PMA, which blocked the PMA-mediated increase in APP^s secretion, did not inhibit the increase in APP^s secretion caused by 5-HT, for example, in 3T3 cells expressing 5-HT_2 or 5-HT_{1c} receptors. Moreover, the protein kinase inhibitors chelerythrine (1 μmol/l) and staurosporine (1 μmol/l) did not block the increase in APP^s secretion caused by 5-HT in either cell line. These results demonstrate that stimulation of PKC can be sufficient but may not be necessary to increase APP^s release. Our data imply redundancy in cellular signalling pathways that couple 5-HT receptors to the regulation of APP metabolism.

The downstream targets of PKC-dependent and PKC-independent signalling with respect to the regulation of APP processing are unknown but may include additional kinase steps as well as changes in intracellular Ca^{++} concentrations. Phosphorylation of APP, however, is not required in order for PMA to stimulate the release of APP^s as suggested by mutagenesis experiments with APP that lacked the potential phosphorylation sides but still responded to PMA with increased rates of APP^s secretion. Moreover, deletions of the entire endodomain, which contains some of the potential phosphorylation sites, did not change rates of PMA-stimulated APP^s release [41], suggesting that the endodomain is not necessary to support stimulated ectodomain cleavage. Future identification and characterization of the APP-secretases is likely to provide the tools required for the study of these endoproteinases and their regulation by PKC-dependent and PKC-independent mechanisms that regulate APP processing pathways.

6. Neuronal activation modulates APP processing in mammalian brain

Compared to the regulation of processing events in tissue culture experiments, regulation of APP processing in brain tissue may be much more complex in that multiple surface receptors may interact simultaneously with various APP processing pathways. In an attempt to study receptor-coupled regulation of APP processing in brain tissue, we prepared fresh tissue slices from the hippocampus, the cortex, the striatum, and the cerebellum of young adult rats, and incubated them in superfusion chambers that were equipped with electrical field stimulation electrodes [42]. Electrical depolarization with 10 to 30 Hz (individual pulse duration 1ms) caused 3- to 10-fold increases in the release of such endogenous hippocampal neurotransmitters as glutamate and acetylcholine. We controlled for the structural integrity of cells within the slices by monitoring the release of lactate dehydrogenase, an intracellular marker enzyme, which was unchanged before, during and after the electrical stimulation periods. The increased release in endogenous neurotransmitters was paralleled by an averaged 2-fold increase in the rates of APP^s release during a 50-min stimulation period with 30 Hz. Individual brain regions varied slightly with respect to the magnitute of the increase in APP^s formation: in brain cortex, hippocampus and striatum stimulation increased APP^s secretion 2-fold, and in cerebellum 1.5-fold. The depolarization-induced increase in APP^s secretion was blocked by the sodium channel blocker tetrodotoxin, indicating that this release resulted from the formation of action potentials, and, probably, from depolarization-generated neurotransmitter release. The effect of electrical stimulation on APP^s secretion from hippocampal slices was frequency-dependent in a range from 0 to 30 Hz, and reached its maximum at 30 Hz. Together, these data suggest that APP processing pathways in brain may be regulated by neuronal activity. Pharmacological experiments showed that the muscarinic receptor agonist atropine partially blocked the depolarization-induced increase in the rates of APP^s release. Moreover, receptor agonists which specifically stimulate m1 and m3 receptor subtypes (as opposed to m2 and m4) stimulated rates of APP^s release from cortical slices in a dose-dependent manner. These data show that muscarinic receptors in the mammalian brain cortex can regulate APP processing pathways and imply that muscarinic agonists can stimulate non-amyloidogenic α-secretase processing of APP in brain.

7. Implications for Alzheimer's disease

Results from tissue culture experiments demonstrate that cell surface receptors for neurotransmitters and neuromodulators can modify APP processing by accelerating the formation of non-amyloidogenic α-secretase products (APP^s) which have been shown to have neurotrophic and neuroprotective activities in cell culture. Such receptors include muscarinic M1 and M3, serotoninergic 5-HT_2 and 5-HT_{1C}, and the peptidergic vaspressin and bradykinin receptors. Concurrently, rates of β-secretase processing apear to be decreased by receptor stimulation as indicated by a decrease in the formation of Aβ in response to carbachol in cells expressing muscarinic ml receptors. Brain tissue slice experiments suggest that

similar biochemical events – initiated by the release of endogenous neurotransmitters and neuromodulators – can regulate the metabolism of APP in mammalian brain. The concept of receptor-coupled APP processing has two possible implications: First, the normal metabolims of APP and its processing into various non-amyloidogenic and amyloidogenic derivatives in AD brain may be compromized as a result of impaired neuronal signalling caused by a combination of diminished availability of chemical neurotransmitters, decreased number in synaptic receptors, and impaired postsynaptic signal transduction. Together, these abnormalities may result in changes of the relative rates of individual APP processing pathways in favour of amyloidogenic processing and the increased generation of possibly neurotoxic Aβ peptides, which might eventually aggregate to form amyloid. Second, the neurotransmitter and neuromodulator systems that are capable of regulating brain metabolism of APP may provide potential targets for drugs designed to increase non-amyloidogenic APP processing pathways. Human studies will be necessary to test whether this treatment approach can decrease the amyloid burden in brain, slow the rate of progression of dementia, and ameliorate the clinical symptoms of Alzheimer's disease.

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