

PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/32917>

Please be advised that this information was generated on 2017-12-05 and may be subject to change.

Reduced *Aph-1b* expression causes tissue- and substrate-specific changes in γ -secretase activity in rats with a complex phenotype

Marcel W. Coolen,* Karen M.J. van Loo,* Nick N.H.M. van Bakel,*
Bart A. Ellenbroek,[†] Alexander R. Cools,[†] and Gerard J. M. Martens*¹

*Department of Molecular Animal Physiology-Nijmegen Center for Molecular Life Sciences (NCMLS) and Institute for Neuroscience-Radboud University of Nijmegen, The Netherlands;

[†]Department of Psychoneuropharmacology-UMC St. Radboud, Nijmegen, The Netherlands

 To read the full text of this article, go to <http://www.fasebj.org/cgi/doi/10.1096/fj.05-4337fje>;
doi: 10.1096/fj.05-4337fje

SPECIFIC AIMS

The γ -secretase enzyme is a tetrameric protein complex consisting of presenilin-1 (or -2), nicastrin, PEN-2, and *Aph-1a* (or -1b), and displays intramembrane catalytic activity toward a number of type I transmembrane proteins that are involved in a variety of (neuro)developmental signaling pathways. The aim of this study was to examine the functional consequences of a gene-dosage imbalance of a γ -secretase component, namely the effect of the presence of only one copy or three copies of the rat *Aph-1b* gene on the level of γ -secretase cleavage activity toward various endogenous substrates.

PRINCIPAL FINDINGS

1. Reduced *Aph-1b* expression levels are not compensated by its paralog *Aph-1a*

Using Northern blot analysis, we detected high levels of *Aph-1b* mRNA in most tissues from rats with three copies of the gene encoding the γ -secretase component *Aph-1b* (III/III rats), whereas in the same tissues from rats with one *Aph-1b* gene copy (I/I rats) the levels were not or hardly above background. In contrast, the mRNA levels of the paralogs *Aph-1aS* and *-1aL* did not differ between I/I and III/III rat tissues, also not in the tissues with large differences in *Aph-1b* expression. Furthermore, the ratios between the *Aph-1b* and *Aph-1a* mRNA levels greatly varied among the III/III rat tissues. Tissues such as the olfactory bulb, pons/medulla, testis and lung showed high *Aph-1b/-1a* ratios, while the cortex, hippocampus, spinal cord, stomach, large intestine and thymus had moderate ratios, and the cerebellum, striatum, (hypo)thalamus, eye, heart, muscle, small intestine, spleen, liver and pancreas displayed low ratios (Fig. 1). Real-time quantitative RT-PCR analysis revealed significant reductions in *Aph-1b* mRNA levels in cerebellum, olfactory bulb, cortex, and lung from I/I

compared with III/III rats ($P < 0.05$), with the largest difference observed for the olfactory bulb (~8-fold reduction). In contrast, the *Aph-1a* mRNA levels did not differ between the two rat lines in the tissues analyzed. The *Aph-1b/-1a* ratios determined by PCR were similar to those obtained through Northern blot analysis (high *Aph-1b/-1a* ratio in olfactory bulb and lung, moderate ratio in cortex and low ratio in cerebellum).

2. Tissue- and substrate-specific alterations in γ -secretase cleavage activity in rats with reduced *Aph-1b* levels

Since the *Aph-1* protein is an essential component of the γ -secretase complex, we were interested in the effect of the differential mRNA expression of *Aph-1b* on the proteolytic cleavage activity of the complex in I/I and III/III rat tissues. To this end, the levels of cleavage products of various γ -secretase substrates were examined by Western blot analysis using antibodies directed against the C-terminal regions of the substrates. In general, the proteolytic processing of a γ -secretase substrate starts with shedding of its extracellular domain, leaving a C-terminal fragment (CTF) that is subsequently cleaved by γ -secretase to its intracellular domain (ICD). One of the best known substrates of γ -secretase is the Alzheimer's disease-linked amyloid- β precursor protein (APP). APP is part of the APP superfamily that in mammals includes the two APP-like proteins APLP1 and APLP2. We compared the γ -secretase cleavage activities toward the APP superfamily members in tissues of III/III rats having different *Aph-1b/-1a* ratios (high: olfactory bulb and lung; moderate: spinal cord and cortex; low: [hypo]thalamus, cerebellum and spleen) with the activities in the corre-

¹ Correspondence: Department of Molecular Animal Physiology-Nijmegen Center for Molecular Life Sciences, Radboud University of Nijmegen, Geert Grooteplein Zuid 28, 6525 GA Nijmegen, The Netherlands. E-mail: g.martens@ncmls.ru.nl

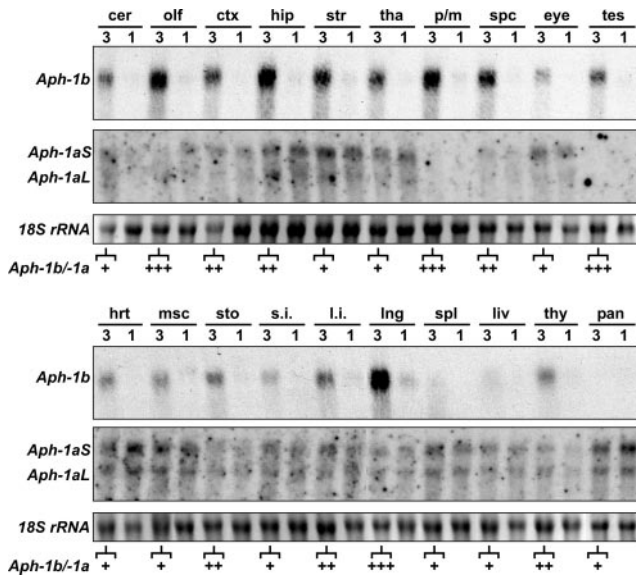


Figure 1. Northern blot analysis of *Aph-1a* and *-1b* mRNA expression in various tissues of I/I and III/III rats. The *Aph-1a* probe detected both the short and long isoform (*Aph-1aS* and *-1aL*, respectively). The *Aph-1b/-1a* ratio was determined for every III/III rat tissue and categorized as high ($Aph-1b/-1a > 2.0$; +++), moderate ($1.0 < Aph-1b/-1a \leq 2.0$; ++) or low ($Aph-1b/-1a \leq 1.0$; +). Tissues used were cerebellum (cer), olfactory bulb (olf), cortex (ctx), hippocampus (hip), striatum (str), (hypo)thalamus (tha), pons/medulla (p/m), spinal cord (spc), eye, testis (tes), heart (hrt), muscle (msc), stomach (sto), small intestine (s.i.), large intestine (l.i.), lung (lng), spleen (spl), liver (liv), thymus (thy), and pancreas (pan) of PND9 III/III (3) or I/I (1) rats.

sponding I/I rat tissues. The levels of the direct γ -secretase substrates (CTFs) were significantly increased in the olfactory bulb (APP: 1.6-fold [$P < 0.01$]; APLP1: 2.0-fold [$P < 0.05$]; APLP2: 2.1-fold [$P < 0.05$]), the lung (APP: 2.2-fold [$P < 0.05$]; APLP2: 1.3-fold [$P < 0.05$]), the spinal cord (APP: 1.6-fold [$P < 0.02$]; APLP1: 1.6-fold [$P < 0.05$]; APLP2: 1.8-fold [$P < 0.05$]) and the cortex (APP: 1.3-fold [$P < 0.05$]; APLP2: 2.2-fold [$P < 0.02$]) of I/I compared with III/III rats. No significant differences in the CTF levels were observed in the (hypo)thalamus, cerebellum, and spleen of I/I and III/III rats, i.e., in tissues with a low *Aph-1b/-1a* ratio (Fig. 2).

We next analyzed the cleavage products of the γ -secretase substrates neurotrophin receptor p75, the neuregulin receptor ErbB4, and neuregulin-2 (NRG2). The levels of p75-CTF were similar in all I/I and III/III rat tissues tested, whereas p75-ICD showed significantly reduced levels only in the olfactory bulb of the I/I rats (1.8-fold [$P < 0.05$]). The olfactory bulb was also the only tissue with significantly reduced ErbB4-ICD levels when comparing I/I and III/III rat tissues (1.3-fold [$P < 0.05$]). The CTF levels of NRG2 were similar in all tissues examined.

CONCLUSIONS AND SIGNIFICANCE

The γ -secretase enzyme complex requires at least four protein components to display cleavage activity: prese-

nilin (PS-1 or -2), nicastrin, PEN-2, and Aph-1 (*Aph-1aS*, *-1aL*, or *-1b*). We examined the mRNA expression levels of *Aph-1aS*, *-1aL*, and *Aph-1b* and the effects of the differential *Aph-1b* expression on the cleavage activity of the γ -secretase complex in rats with one or three *Aph-1b* gene copies (I/I and III/III rats, respectively). In III/III rat tissues, ratios between the *Aph-1b* and *-1a* mRNA levels varied greatly. A reduction in *Aph-1b* gene copy numbers in the I/I rats was accompanied by a large drop in *Aph-1b* mRNA expression levels, not compensated for by its paralog *Aph-1a*. The reduced expression

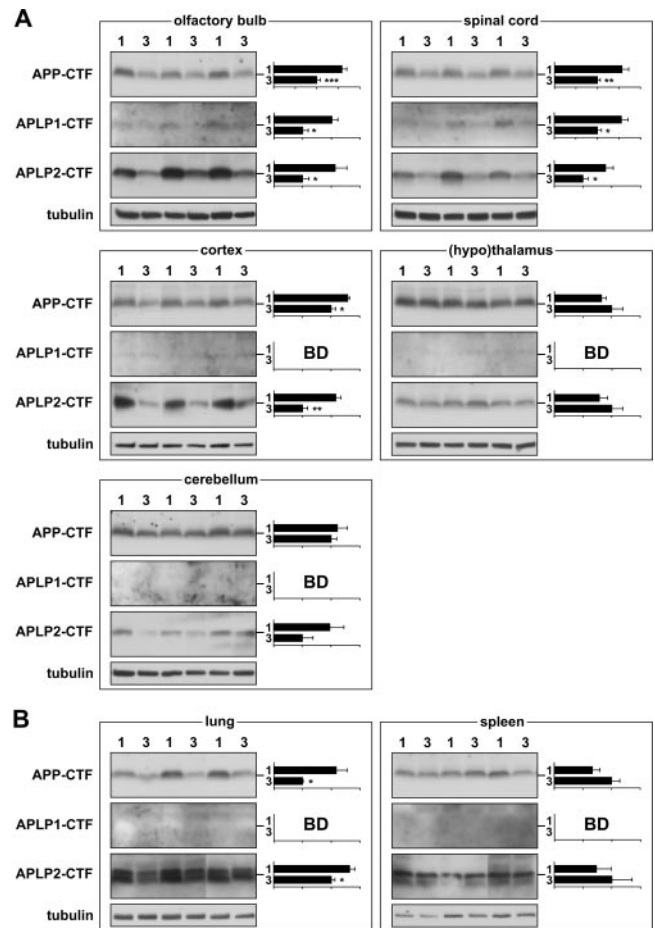


Figure 2. Western blot analysis of the γ -secretase cleavage products derived from the APP, APLP1 and APLP2 proteins in various tissues of I/I and III/III rats. *A*) Levels of C-terminal fragments (CTFs) of amyloid- β precursor protein (APP), and the APP-like proteins APLP1 and APLP2 (sizes of all 3 CTFs ~ 10 kDa) were analyzed in neuronal tissues of PND13 I/I (1) and III/III (3) rats using specific antibodies. Tissues used were the olfactory bulb (high *Aph-1b/-1a* ratio), spinal cord, and cortex (moderate ratio), and (hypo)thalamus and cerebellum (low ratio). *B*) Levels of APP-CTF, APLP1-CTF and APLP2-CTF were analyzed in the lung (high *Aph-1b/-1a* ratio) and spleen (low ratio) of PND13 I/I and III/III rats. In each case, tubulin (~ 55 kDa) was used for normalization. Bars represent quantifications in arbitrary units of normalized CTF signals of 5 tissue samples with the average level in III/III rat tissues set to 1. The levels of the APP, APLP1 and APLP2 holoproteins were similar in the I/I and III/III rat tissues. * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; $n = 5$, with the 5 rats per genotype from different nests; plus SEM; BD: below detection.

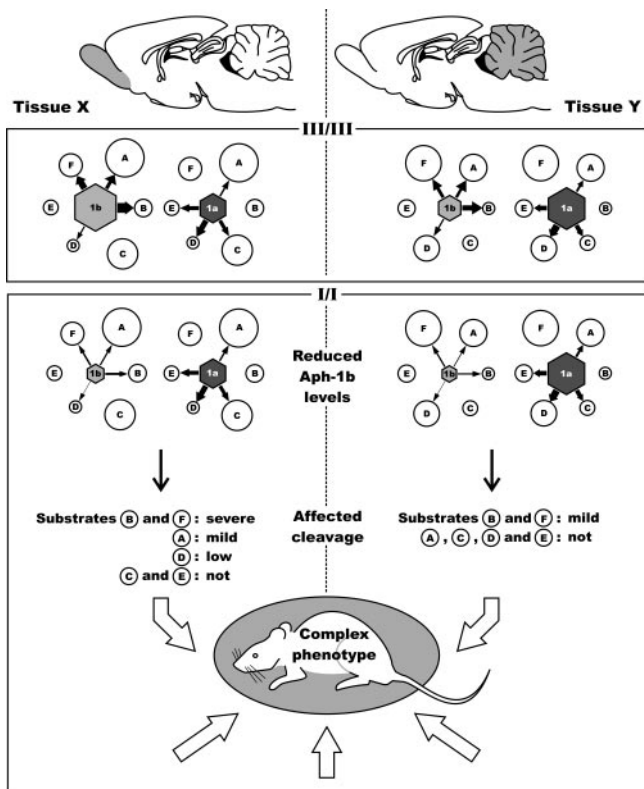


Figure 3. Schematic representation of the tissue- and substrate-specific changes in γ -secretase activity upon reduced expression of Aph-1b, affecting a great variety of signaling pathways and resulting in a complex phenotype. Reduction of Aph-1b levels (in I/I compared with III/III rats) causes changes in γ -secretase complex compositions; complexes containing Aph-1b (termed *1b*) become less available (smaller hexagonal sizes) and Aph-1a containing complexes remain unaffected (*1a*; same hexagonal sizes). These changes affect γ -secretase cleavage activity (indicated by changes in arrow thickness), but toward a limited number of substrates and only in certain tissues. Circle sizes reflect the different substrate levels in the various tissues and the thickness of the arrows corresponds to activity toward a specific substrate. For clarity, only 2 types of γ -secretase complexes (*1a* and *1b*) and 6 substrates (A–F) are shown; in mammals, 6 γ -secretase complexes with different subunit compositions can be formed; > 15 substrates are known so far. The reduced expression of a single gene (*Aph-1b*) results in a complex phenotype, thought to have a multigenic origin.

resulted in substrate- and tissue-specific alterations in γ -secretase cleavage activity (Fig. 3). Tissues that normally have a high *Aph-1b*/*-1a* ratio displayed clear differences in γ -secretase cleavage activity, whereas the activity in tissues with a low ratio was either hardly affected or not at all. Within a particular tissue, substrate processing was not affected to the same extent,

showing that γ -secretase complexes with different subunit compositions are not functionally redundant and that a specific complex is involved in the preferential cleavage of a subset of γ -secretase substrates.

We earlier found that the *Aph-1b* rat genotypes segregated with behavioral phenotypes, and rats with a natural *Aph-1b* knockdown (APO-SUS) display alterations in brain information processing (prepulse inhibition and latent inhibition), locomotor activity in response to novelty, fleeing, and problem-solving behavior, and HPA-axis response to stress. The APO-SUS phenotype partially overlaps with the behavioral phenotypes of a number of mouse models with altered expression of a γ -secretase substrate. For example, heterozygous *ErbB4* or *NRG1* knockout mice (with an overall ~50% reduction of mRNA expression) are also hyperactive in an open field and show an impaired prepulse inhibition, whereas reduced expression of Notch has no effect on the open field behavior. Conversely, overexpression of human APP751 or APP-CTF caused a general hypoactivity in mice.

In our studies, we further found that of the brain tissues examined, the olfactory bulb of the I/I rats displayed the most severely affected γ -secretase cleavage activity. Removal of the olfactory bulb from normal rats resulted in a model for agitated depression. These rats displayed alterations in behavior, and in the functioning of the HPA-axis, the immune system, thymus and spleen weight, and self-administration of drugs. These phenotypic features are also observed in APO-SUS rats. Furthermore, human neurological disorders such as Alzheimer's disease and schizophrenia are characterized by olfactory dysfunction. Still, it is not clear to what extent differences in γ -secretase cleavage activity found in tissues other than the olfactory bulb, such as the cortex, spinal cord, and lung, have also contributed to the complex phenotype of the APO-SUS rats.

In conclusion, the differential expression of *Aph-1b* in the I/I and III/III rats caused subtle, substrate-specific alterations in γ -secretase cleavage activity toward a number of γ -secretase substrates, especially in tissues with a relatively high *Aph-1b* level (Fig. 3). Thus, a single gene defect may affect a great variety of (neuro)developmental signaling pathways, resulting in a complex phenotype that is generally thought to have a multigenic origin. Furthermore, the γ -secretase complex, generally known because it is linked to Alzheimer's disease (a neurodegenerative and ageing disorder), may also be associated with (neuro)developmental disorders that become apparent much earlier in life. [F]