Nuclear Factor-κB Regulates βAPP and β- and γ-Secretases Differently at Physiological and Supraphysiological Aβ Concentrations⁴⁻⁵

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Background: NF-κB regulates BACE1 but there is little data suggesting βAPP and γ-secretase involvement.

Results: NF-κB differentially regulates Aβ production at physiological and supraphysiological Aβ concentrations by modulating transcription of βAPP and γ-secretase promoters, thereby controlling γ-secretase activity.

Conclusion: Under physiological conditions, NF-κB regulates Aβ homeostasis while it contributes in increasing Aβ production in the pathological context.

Significance: NF-κB may be seen as a potential therapeutic target.

Anatomical lesions in Alzheimer disease-affected brains mainly consist of senile plaques, inflammation stigmata, and oxidative stress. The nuclear factor-κB (NF-κB) is a stress-activated transcription factor that is activated around senile plaques. We have assessed whether NF-κB could be differentially regulated at physiological or supraphysiological levels of amyloid β (Aβ) peptides. Under these experimental conditions, we delineated the putative NF-κB-dependent modulation of all cellular participants in Aβ production, namely its precursor βAPP (β-amyloid precursor protein) and the β- and γ-secretases, the two enzymatic machines involved in Aβ genesis. Under physiological conditions, NF-κB lowers the transcriptional activity of the promoters of βAPP, β-secretase (β-site APP-cleaving enzyme 1, BACE1), and of the four protein components (Aph-1, Pen-2, nicastrin, presenilin-1, or presenilin-2) of the γ-secretase in HEK293 cells. This was accompanied by a reduction of both protein levels and enzymatic activities, thereby ultimately yielding lower amounts of Aβ and AICD (APP intracellular domain). In stably transfected Swedish βAPP-expressing HEK293 cells triggering supraphysiological concentrations of Aβ peptides, NF-κB activates the transcription of βAPP, BACE1, and some of the γ-secretase members and increases protein expression and enzymatic activities, resulting in enhanced Aβ production. Our pharmacological approach using distinct NF-κB kinase modulators indicates that both NF-κB canonical and alternative pathways are involved in the control of Aβ production. Overall, our data demonstrate that under physiological conditions, NF-κB triggers a repressive effect on Aβ production that contributes to maintaining its homeostasis, while NF-κB participates in a degenerative cycle where Aβ would feed its own production under pathological conditions.

Alzheimer disease (AD)³ is the first cause of dementia, involving memory and cognitive deficit ultimately leading to the loss of patient autonomy (1). The molecular dysfunctions are not yet elucidated, and actual treatments are only symptomatic. AD-affected brains present extracellular deposits; the senile plaques that are mainly composed of a set of hydrophobic peptides called amyloid β peptides (Aβ) (2). These peptides are at the center of the amyloid cascade hypothesis (3) that proposes Aβ as the main etiological trigger of the neurodegeneration taking place at late stages of the pathology (4). Therefore, a strategy aimed at circumscribing Aβ overload appears as one of the main therapeutic tracks.

Aβ is a normal product of β-amyloid precursor protein (βAPP) processing (5) that results from the sequential cleavage of βAPP by two distinct enzyme activities: the β-secretase β-site APP-cleaving enzyme 1 (BACE1) and the γ-secretase that mainly consists of a high molecular weight protein complex including anterior pharynx defective-1 (Aph1), presenilin enhancer-2 (Pen2), presenilin 1 or 2 (PS1, PS2), and nicastrin (NCT) (6).

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³ The abbreviations used are: AD, Alzheimer disease; AICD, βAPP intracellular domain; Aβ, amyloid β-peptides; Aph1, anterior pharynx defective-1; βAPP, β-amyloid precursor protein; BACE1, β-site APP-cleaving enzyme 1; HEK, human embryonic kidney; wt-βAPP wild-type βAPP; SwβAPP, Swedish mutated βAPP; IκB, inhibitor of NF-κB; lxBSR, lxB superrepressor; MEF, mouse embryonic fibroblasts; NCT, nicastrin; NF-κB, nuclear factor-κB; NSAID, non-steroidal anti-inflammatory drugs; Pen2, presenilin enhancer-2; PS1 and PS2, presenilin-1 and -2.
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Several lines of evidence suggest that inflammation and oxidative stress could contribute to AD pathology (7, 8). This may influence Aβ production to some extent, since BACE1 expression appears to be modulated by stress (9, 10). Oxidative stress and inflammation are characterized by the release of cytokines and reactive oxygen species, known to activate the nuclear factor-κB (NF-κB) (11, 12).

NF-κB is a dimeric transcription factor, the family of which is composed by the proteins p50, p52, p65(RELA), RelB, and c-Rel (13, 14). Two pathways have been delineated for NF-κB activation, the canonical and the alternative pathways. They are generally associated with distinct triggers, involve different signaling proteins, and are linked to structurally and functionally varying DNA-binding dimers (14) (Fig. 1). Interestingly, an increase of NF-κB expression is observed in the hippocampus and entorhinal cortex of AD patients, two cerebral areas altered in this pathology (15).

Several in vitro studies suggested that NF-κB could be activated by Aβ peptides in primary cultured neurons (16, 17). This observation is indirectly consistent with the fact that NF-κB has been observed in cells surrounding or within the amyloid plaques (16–19). These studies report on an activation of the canonical pathway (16–19) involved in a feedback control by which Aβ activates NF-κB, which, in turn, regulates the production of Aβ peptides (17, 19).

Interestingly, the inhibition of NF-κB activation reduces Aβ secretion in vitro (20–22), and it was suggested that this could occur by interfering with BAPP processing (22–27). In this context, it is noteworthy that we and others previously showed that NF-κB mediated the Aβ-associated increase of BACE1 transcriptional activity (28, 29) but to date, there is relatively little data concerning the putative control of the γ-secretase build-up and activity or on the expression of BAPP by NF-κB; and, if so, if this control may be dependent on Aβ.

Here we show an Aβ concentration-dependent control of BAPP and of the members of the γ-secretase complex by NF-κB. We establish that under physiological conditions, NF-κB lowers Aβ production by repressing the protein BAPP and the β- and γ-secretase activities at a transcriptional level. Conversely, at supraphysiological concentrations of Aβ aimed at mimicking the pathological situation, NF-κB activates Aβ production by increasing BAPP and processing enzyme activities. This set of data delineates a differential control of BAPP, and β- and γ-secretases by NF-κB that depends upon physiological or supraphysiological conditions of Aβ production. Thus, our results indicate that NF-κB could normally control Aβ homeostasis in physiological conditions or contribute to a degenerative cycle by which Aβ feeds its own production in the pathological context.

**MATERIALS AND METHODS**

**Cell Culture and Transfections**—Human embryonic kidney 293 (HEK293) cells stably overexpressing empty pcDNA3.1 vector, wild-type (wt-βAPP), or BAPP harboring the Swedish double mutation (K670N/M671L; Sw-βAPP) were obtained and cultured as described previously (30). Human SH-SY5Y neuroblastoma cells (CRL-2266, ATCC) were cultured following the manufacturer's instructions. SH-SY5Y cells stably expressing pcDNA3.1 or Sw-βAPP were generated following standard protocols and maintained in the presence of 400 μg of genetin (Invitrogen). Mouse embryonic fibroblasts (MEF) cells devoid of the βAPP gene (βAPP−/−) were kindly provided by Dr. U. Müller (31). Transient and stable transfections of cDNAs were obtained with jetPRIME reagent (Polyplus) with an optimized protocol (half quantities of cDNAs, buffer, and reagent recommended by the manufacturer were used). Cells were collected 30 h or 48 h after transient transfection, or selected with genetin antibiotic (Invitrogen) to obtain stable transfection.

**Activation and Inhibition of NF-κB**—NF-κB was activated by transfection of HA-tagged-IKK1S>EG cDNA (IKK1SE, mutations S176E and S180E on the IKK1 kinase) and Flag-tagged-IKK2S>EG cDNA (IKK2SE, mutations S177E and S181E on the IKK2 kinase), and inhibited by transfection of IκB superrepressor (Myc-tagged IκBβ cDNA, mutations S32A and S36A on IκB) (32, 33). Their expression was verified by Western blot using mouse monoclonal anti-HA (Eurogentec), anti-Flag (Sigma), and anti-Myc 9E10 (given by Dr. Luc Mercken) antibodies, respectively (Fig. 1B). Their functionality was verified by cotransfection with κB-luciferase, a consensus sequence of fixation of the various dimers of NF-κB in-frame with luciferase (Fig. 1, A–C). Cells were then lysed and assayed as described below for transactivation of promoters.

**Exogenous Aβ Treatment**—Twenty-four hours after transfection of the κB-luciferase construct, the medium was replaced with Opti-MEM medium (Sigma) containing 2% fetal bovine serum and 10 μM phosphoramidon to prevent Aβ degradation. Cells were treated for 48 h with 0.3, 1, or 3 μM synthetic Aβ42 (Bachem).

**Inhibition of γ-Secretase Activity**—Thirty hours after transfection of the κB-luciferase construct, cells were treated for 18 h with 100 μM DFK167 (34) or a corresponding amount of Me6SO.

**Transactivation of Promoters**—cDNA encoding human promoters of βAPP (given by Dr. D. Lahiri), Aph1, Pen2, NCT (given by Dr. X. Xu), PS1 (given by Dr. M. Vittek), PS2 (given by Drs. P. Renbaum and E. Levy-Lahad), and rat promoter of BACE1 (given by Dr. S. Rossner) in-frame with luciferase were co-transfected with β-galactosidase transfection vector, to normalize transfection efficiency. CMV-β-galactosidase construction was used to assess putative artificial effect of IKK1SE, IKK2SE, and IκB constructions on the CMV promoter. 30 h after transfection, cells were harvested with phosphate-buffered saline/EDTA (5 mM), pelleted by centrifugation 5 min at 1000 × g, lysed with 50 μl of lysis buffer (luciferase kit Promega), centrifuged for 5 min at 2000 × g. Luciferase and β-galactosidase activities were then analyzed as previously described (35), and protein concentration determined to normalize the luciferase activity.

**Western Blotting Analysis and Antibodies**—Cells were collected 48 h after transfection and lysed with the following buffer (Tris-HCl 1 mM pH 7.5, NaCl 150 mM, EDTA 5 mM, Triton X-100 0.5%, deoxycholate 0.5%). Equal amounts of proteins (70 μg) were separated on 10% (BAPP, BACE1, PS1, PS2, NCT) Tris/glycine gel acrylamide, and 16.5% Tris/tricine gels (Aph1, Pen2), and were transferred to Hybond-C membranes (Amer-
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sham Biosciences Pharmacia Life Science). Membranes were blocked with nonfat milk and incubated overnight with the following antibodies: anti-βAPP 2H3 (provided by Dr. D. Schenk, Elan Pharmaceuticals), anti-BACE1 (Abcam), anti-Aph1 O2C2 (36), anti-Nter Pen2 (Calbiochem), anti-Nter PS1 Ab14 (36), anti-C-terminal fragment-PS2 (37), anti-Nicar8 (Sigma), anti-β-tubulin, and anti-β-actin (Sigma). Immunological complexes were revealed with anti-mouse peroxidase (Amersham Biosciences Pharmacia Life Science; βAPP, BACE1) or anti-rabbit peroxidase (Immunotech; γ-secretase members proteins) antibodies. Electrochemiluminescence (Amersham Biosciences) was recorded as described (35), and data were processed with Multi Gauge software (Fujifilm).

BACE1 Fluorimetric Assay—Cells were collected 30 h after transfection and lysed with 10 mM Tris-HCl, pH 7.5, then homogenates were monitored for their BACE1 activity as described previously (38, 39). Briefly, samples (30 μg of proteins in 25 mM acetate buffer, pH 4.5) were incubated in a final volume of 100 μl of the above acetate buffer containing BACE1 substrate (10 μM (7-methoxycoumarin-4-yl)acetyl-SEVNLDAEFR K(2,4-dinitrophenyl)-RRNH2; R&D Systems) in the absence or presence of β-secretase inhibitor I (50 μM, Promocell). BACE1 activity corresponds to the β-secretase inhibitor-sensitive fluorescence recorded at 320 and 420 nm as excitation and emission wavelengths, respectively. The slopes of the initial linear phase were calculated and expressed as fluorimetric units/mg/h.

In Vitro γ-Secretase Assay—48 h after transfection, cells were used for an in vitro γ-secretase assay developed in the laboratory (40). Briefly, cells were lysed with Tris 10 mM, pH 7.5, and membranes were isolated by centrifugation (22,000 × g for 1 h). An equal amount of membranes was incubated overnight with a Flag-tagged quenched fluorimetric substrate (IMV2650) mimicking the βAPP sequence targeted by γ-secretase. The products Aβ and AICD-Flag were detected by Western blotting on a 16.5% Tris/tricine gel and revealed simultaneously with anti-Aβ 2H3 and anti-Flag antibodies, respectively.

Analysis of Aβ40 Production—30 h after transfection, cells were allowed to secrete Aβ in Optim-MEM medium (Sigma) for 16 h, in the presence of 10 μM phosphoramidon to prevent Aβ degradation. The culture media were collected, supplemented with RIPA buffer (10 mM Tris-HCl, pH 8, EDTA 5 mM, NaCl 150 mM), and incubated overnight with a 100-fold dilution of FCA3340 (41). Aβ40 was precipitated with protein A-Sepharose (Invitrogen), Western blotted on a 16.5% Tris/tricine gel, and revealed with anti-Aβ 2H3 (provided by Dr. D. Schenk, Elan Pharmaceuticals). Cells were collected, and protein concentration was determined in order to normalize Aβ secretion.

Statistical Analysis—Statistical analysis was performed with Prism software (Graphpad, San Diego, CA) using either the unpaired Student’s t test for pairwise comparison or the Tukey multiple comparison test for one-way analysis of variance.

RESULTS

NF-κB Reduces Aβ Secretion, βAPP and Secretase Expressions and Activity in Physiological Conditions—Two pathways have been described for the activation of the transcription fac-
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FIGURE 2. NF-κB inhibition of the transactivation of βAPP, BACE1, and γ-secretase promoters in transiently transfected HEK293 cells. HEK293 cells were transiently transfected with NF-κB activating (IKK1SE and IKK2SE) or inhibitory (IkBSR) cDNA and the promoters of βAPP (A), BACE1 (B), and γ-secretase protein components (C–G). 30 h after transfection, luciferase and β-galactosidase activities were measured as described under "Material and Methods." Bars correspond to luciferase activity expressed as percent of that observed in mock-transfected cells and are the means ± S.E. of 9–22 independent determinations. *, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, not statistically significant.

pared with control, n = 20, Fig. 1C) via constitutive phosphorylation/inactivation of IkB. Conversely, IkB superrepressor (IkBSR) does not undergo phosphorylation and therefore, its overexpression lowers NF-κB activity (−46.8 ± 3.2% compared with control, n = 16, Fig. 1C). By means of these constructions, we establish that NF-κB activation reduces endogenous Aβ secretion (−37.5 ± 6.3% and −35.9 ± 6.5% for IKK1SE and IKK2SE-expressing cells, respectively, n = 5–6, Fig. 1D) while IkBSR did not alter Aβ secretion (Fig. 1D). Our results indicate that both canonical and alternative NF-κB-mediated pathways exert a negative modulation of physiological Aβ secretion in HEK293 cells.

The above data do not delineate the targets of NF-κB underlying this phenotype. At first sight, it was reasonable to envision that either the precursor of Aβ was lowered or alternatively, that the βAPP-cleaving enzymes expression and/or activities yielding Aβ could have been down-regulated. In this context, we examined whether modulation of NF-κB could influence the promoter transactivation, expression, and activity of βAPP, BACE1, as well as the components of the γ-secretase high molecular weight complex (Aph-1, Pen-2, nicastrin, PS1, or PS2).

NF-κB activation by IKK1SE and IKK2SE reduces the transactivation of the promoters of βAPP (−41.9 ± 2.8% and −73.6 ± 2.8%, respectively, n = 9–10, Fig. 2A), BACE1 (−30.7 ± 13.4% and −50 ± 8.6%, n = 10–16, Fig. 2B), Aph1 (−38.4 ± 13.7% and 88.0 ± 2.4%, n = 10–16, Fig. 2C) and PS2 (38.3 ± 4.4% and 53.4 ± 3.4%, n = 12–15, Fig. 2F) while the promoters of Pen2 (79.1 ± 3.4%, n = 12, Fig. 2D) and nicastrin (56.2 ± 2.8%, n = 13, Fig. 2G) are only selectively altered by IKK2SE overexpression. PS1 promoter activity (Fig. 1E) is not affected by NF-κB activation but drastically potentiated by IkB repression (+166.2 ± 64.6% of control value, n = 15, Fig. 2E) as is observed for βAPP (+167.9 ± 82.9%, n = 14, Fig. 2A), BACE1 (+82.4 ± 15.3%, n = 11, Fig. 2B), Aph1 (+47.9 ± 11.9%, n = 11, Fig. 2C), Pen2 (+66.2 ± 18.4%, n = 11, Fig. 2D) and PS2 (+36.0 ± 14.6% n = 10, Fig. 2F), while nicastrin promoter remains unchanged (Fig. 2G).

We have confirmed this data in stably transfected cells. First, as expected, stable expression of IKK1SE and IkBSR (Fig. 3A),
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FIGURE 3. NF-κB inhibition of βAPP, BACE1, Pen2, PS1, and nicastrin protein expression in stably transfected HEK293 cells. A, HEK293 cells were stably transfected with NF-κB activating (IKK1SE) or inhibitory (IκBSR) cDNA as described under "Material and Methods." The efficiency of the transfection is established by Western blot, and the functional effect of the transfection is measured by co-transfection with λ-b-luciferase in-frame with luciferase. Bars correspond to luciferase activity expressed as percent of that observed in mock-transfected cells and are the means ± S.E. of 18 independent determinations. **, p < 0.01. Expression of βAPP (B), BACE1 (C), and indicated members of the γ-secretase complex (D–H) were measured in those stably transfected HEK293 cells by Western blot as described under "Material and Methods." Bars correspond to protein expression expressed as percent of that observed in mock-transfected cells and are the means ± S.E. of 5–11 independent determinations. *, p < 0.05; **, p < 0.01, n.s, not statistically significant.

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NF-κB induces (283.8 ± 62.9%, n = 18, Fig. 3A) and reduces (−37.5 ± 4.7% n = 18, Fig. 3A) NF-κB activity, respectively. It should be noted here that our attempt to stably overexpress IκB2SE failed due to transfectant lethality upon selection procedure. Most of the proteins examined are inhibited by NF-κB in stably transfected cells (Fig. 3, B–H). Thus, IKK1SE reduces the expression of βAPP (−23.3 ± 6.9%, n = 10, Fig. 3B), Pen2 (−40.4 ± 9.8%, n = 8, Fig. 3E), and NCT (−40.9 ± 6.2%, n = 10, Fig. 3H) while conversely, the inhibition of NF-κB by IκBSR increases the expression of βAPP (+63.9 ± 22.5%, n = 6, Fig. 3B), BACE1 (+143.8 ± 73.2%, n = 6, Fig. 3C) and PS1 (+38.2 ± 10%, n = 6, Fig. 3F). Aph-1 and PS2 expression are unchanged upon NF-κB modulation in HEK293 cells (Fig. 3, D and G).

The above set of data obtained in transiently and stably transfected cells overall show that βAPP, BACE1, Pen2, PS1, and NCT expression is negatively regulated by NF-κB indicating that both substrates and components of enzymes involved in Aβ production are turned down by NF-κB in physiological conditions. However, our data also show that there exists a distinct NF-κB-mediated regulation of some members of the γ-secretase complex. It was therefore of prime interest to establish the functional consequence of NF-κB modulation on β-secretase and γ-secretase activities. We have taken advantage of BACE1-directed fluorimetric assay (38) and of a recently developed in vitro γ-secretase assay in reconstituted membranes (40) that allows monitoring of Aβ and AICD produced from an exogenous recombinant substrate. This permits us to directly assess whether the reduction of NF-κB on protein expression converts into an enzymatic deficiency without putative “artifactual” modulation of Aβ production via the alteration of the secretory process, βAPP trafficking, or secretase mislocalization.

Fig. 4 shows that fluorimetric recording of β-secretase activity is increased by stable transfection of IκBSR (+100.6 ± 40.7%, n = 10, Fig. 4A) while stable transfection of the alternative pathway kinase IKK1SE did not alter it. Furthermore, AICD and Aβ productions are reduced by IKK1SE (−27.8 ± 12.8% and −26.1 ± 4.2% respectively, n = 6–8, Fig. 4, B–D) and increased by IκBSR (+77.2 ± 36.2% and +26.4 ± 7.4%, respectively, n = 6–9, Fig. 4, B–D). Overall, these data show that in physiological conditions, NF-κB-linked negative modulation of β- and γ-secretase compo
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FIGURE 4. NF-κB inhibition of β- and γ-secretase activities in stably transfected HEK293 cells. A, HEK293 cells were stably transfected with NF-κB activating (IKK1SE) or inhibitory (IkBSR) cDNA and the β-secretase activity was fluorimetrically recorded as described under "Material and Methods." Bars correspond to β-secretase activity expressed as percent of that observed in mock-transfected cells (Ct) and are the means ± S.E. of 7–10 independent determinations. *, p < 0.05, ns, not statistically significant. β–D, in vitro γ-secretase assays were performed in stably transfected HEK293 cells as described under "Material and Methods." The products AICD and Aβ were detected by Western blot (B) and quantified (C and D) as described under "Materials and Methods." Bars correspond to AICD and Aβ protein expression as percent of that observed in mock-transfected cells (Ct) and are the means ± S.E. of 6–7 independent determinations. *, p < 0.05.

FIGURE 5. Influence of Aβ levels on NF-κB in MEF and HEK293 cells. A and B, MEF cells devoid of the βAPP gene (A), wt-βAPP and Sw-βAPP HEK293 cells (B) were transiently transfected with κB-lucerase. 30 h after transfection, luciferase and β-galactosidase activities were measured as described under "Material and Methods." Bars correspond to luciferase activity expressed as percent of that observed in HEK293 mock cells (Ct) and are the means ± S.E. of respectively 3 or 16 independent determinations, respectively.**, p < 0.001. C, 24 h after transient transfection with κB-lucerase, HEK293 cells were treated for 48 h with the indicated concentrations of synthetic Aβ42. Luciferase and β-galactosidase activities were measured as described under "Material and Methods." Bars correspond to luciferase activity expressed as percent of that observed in non-treated cells and are the means ± S.E. of four independent determinations. *, p < 0.01; **, p < 0.001; ns, not statistically significant. D, mock or Sw-βAPP HEK293 cells were transiently transfected with κB-lucerase and treated 18 h with γ-secretase inhibitor DFK167 (100 μm). 48 h after transfection, luciferase and β-galactosidase activities were measured as described under "Material and Methods." Bars correspond to luciferase activity expressed as percent of that observed in nontreated HEK293 mock cells (Ct) and are the means ± S.E. of eight independent determinations, respectively. ***, p < 0.001, ns, not statistically significant.

NF-κB Activates Aβ Secretion and Secretases Activities in Sw-βAPP HEK293 Cells—The above data led us to envision that, at supraphysiological levels of Aβ, NF-κB could function differently than under physiological conditions. We therefore manipulated NF-κB activity by transfection of its modulators in Sw-βAPP-expressing cells. First, we established that these cells display expected responsiveness to NF-κB modulation, since IKK1SE or IKK2SE expression (Fig. 6A) increases κB activity that is reduced by IkBSR (Fig. 6B). Second, unlike in naïve human cells where IKK1SE and IKK2SE reduce Aβ production (see Fig. 1D), these two kinases enhance Aβ production in Sw-βAPP cells (Fig. 6, C and D). We therefore examined whether this NF-κB-mediated increase of Aβ levels could be accounted for by a modulation of βAPP or secretase expression and/or activities. The promoter activities of βAPP, BACE1, and PS1 are activated by NF-κB (Fig. 7, A, B, E). IKK1SE and IKK2SE expression increases the promoter activity of βAPP (+30.1 ±
**FIGURE 6. NF-κB activation of Aβ secretion in transiently transfected Sw-βAPP HEK293 cells.** A and B, Sw-βAPP HEK293 cells were transiently transfected with NF-κB activating (IKK1SE and IKK2SE) or inhibitory (IκBBSR) cDNA as described under “Material and Methods.” The efficiency of the transfection is established by Western blot (A), and the functional effect of the transfection is measured by co-transfection with κB-luciferase in-frame with luciferase (B). Bars correspond to luciferase activity expressed as percent of that observed in mock-transfected cells and are the means ± S.E. of 9–18 independent determinations. **, p < 0.001. C and D, Sw-βAPP HEK293 cells were transiently transfected with IKK1SE, IKK2SE, or IκBBSR, and the secreted Aβ was detected after immunoprecipitation with FCA3340 antibody and Western blotting as described under “Material and Methods.” Bars in D correspond to the densitometric analysis of secreted Aβ immunoreactivity expressed as percent of that observed in mock-transfected cells (Ct) and are the means ± S.E. of eight independent determinations. *, p < 0.05; ns, not statistically significant.

**FIGURE 7. NF-κB activation of βAPP, BACE1, and PS1 promoter transactivations in Sw-βAPP HEK293 cells.** Sw-βAPP HEK293 cells were transiently co-transfected with IKK1SE, IKK2SE, or IκBBSR and the promoters of βAPP (A), BACE1 (B), and γ-secretase proteins (C–G). 30 h after transfection, luciferase, and β-galactosidase activities were measured as described under “Material and Methods.” Bars correspond to luciferase activity expressed as percent of that observed in mock-transfected cells (Ct) and are the means ± S.E. of 9–23 independent determinations. *, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, not statistically significant.
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**FIGURE 8. NF-κB activation of βAPP, BACE1, and PS1 expression in Sw-βAPP HEK293 cells.** Sw-βAPP HEK293 cells were transiently transfected with NF-κB activating (IKK1SE and IKK2SE) or inhibitory (IκBSR) cDNA and the protein expression of βAPP (A), BACE1 (B), and γ-secretase members (C–G) were measured by Western blot as described under “Material and Methods.” Bars correspond to protein expression expressed as percent of that observed in mock-transfected cells (Ct) and are the means ± S.E. of 6–8 independent determinations. *, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, not statistically significant.

10.3% and +108.7 ± 26.4% respectively, n = 14–23, Fig. 7A), while IκBSR inhibits the promoter activities of BACE1 (−17.2 ± 7.3%, n = 9, Fig. 7B) and PS1 (−32.8 ± 4.1%, n = 9, Fig. 7E). The activation of BACE1 promoter was confirmed with the human construction (kindly provided by Dr. W. Song, data not shown). Aph1 promoter activity is reduced by IKK1SE (−16.2 ± 3.7%, n = 12, Fig. 7C), Pen2 promoter activity is reduced by IKK2SE (−43.3 ± 7.8%, n = 9, Fig. 7D) and increased by IκBSR (+25.0 ± 7.6%, n = 12, Fig. 7D), niascimerin promoter activity is increased by IκBSS (+33.7 ± 22.2% compared with control, n = 6, Fig. 7G) while PS2 promoter transactivation is not affected by the modulation of NF-κB activity (Fig. 7F). The examination of the changes in protein expression that could have resulted from their transcriptional modulation shows that only βAPP and Pen-2 expressions are controlled by NF-κB in Sw-βAPP-expressing cells (Fig. 8). Thus, βAPP levels are increased by IKK1SE and IKK2SE (+32.9 ± 2.8% and +106.7 ± 28.7%, respectively, n = 6, Fig. 8A) while Pen2 expression is inhibited by IKK1SE (−30.7 ± 7.0%, n = 8, Fig. 8D). The protein expression of BACE1, Aph1, PS1, PS2, and NCT is not affected by the transient transfection of the constructions in Sw-βAPP HEK293 cells. We ruled out any putative artifactual effect of IKK1SE, IKK2SE, and IκBSR on the CMV promoter driving the Sw-βAPP used for the stable transfection by means of a CMV-β-galactosidase construct. Thus, none of the above constructs affects galactosidase activity (supplemental Fig. S1, A and B).

The rather complex set of above data led us to examine as a functional integrated readout, whether in fine, β- and γ-secretase activities were modulated by NF-κB in Sw-βAPP-expressing cells. The β-secretase activity is inhibited by IκBSS (−34.1 ± 13.9%, n = 4, Fig. 9A) while the γ-secretase activity is activated by IKK2SE, as measured by the increase of AICD and Aβ expression (+22.0 ± 6.4% and +23.0 ± 11.9% respectively, n = 4–5, Fig. 9, B–D).

**DISCUSSION**

We previously established that BACE1 promoter transactivation could be triggered by overexpression of wild-type βAPP, and to a higher extent, Sw-βAPP in human cells. This effect could be accounted for by enhanced production of Aβ, since the pharmacological inhibition and mutational inactivation of γ-secretase activity abolished the increase of BACE1 promoter transactivation while conversely, exogenous application of synthetic Aβ42 promoted this effect (29). Our study showed that Aβ42-mediated increase of BACE1 transactivation was NF-κB-dependent (29). This agreed well with a study showing that
NF-κB could regulate neuronal BACE1 promoter activation (28). These data delineate a deleterious dysfunction by which overproduction of Aβ feeds its own production.

In vivo studies have suggested an NF-κB-dependent regulation of Aβ production. In transgenic mice NF-κB increases βAPP levels (43), BACE1 promoter activity (44), expression (25, 45), and enzymatic activity (25, 43), γ-secretase activity (43); and Aβ production (25, 43, 46). Furthermore, the inhibition of NF-κB reduced the plaque burden in mice (25, 44) and increased the learning and memory deficits of mice (43). However, to modulate NF-κB activity, those studies used non-steroidal anti-inflammatory drugs (46), natural compounds (25, 43) or modulation of upstream receptors of NF-κB activation pathway (44, 47), which are nonspecific to NF-κB activation. In the present study, we focused on the mechanisms of NF-κB regulation of Aβ production; thus we transfected mutant construction of the proteins that directly activate or inhibit NF-κB dimers and are commonly activated by the various stimuli activating NFκB.

BACE1 produces C99 that requires subsequent cleavage by γ-secretase to yield Aβ (5). However, although NF-κB-dependent regulation of γ-secretase has been suggested (43, 48, 49), the direct regulation of the γ-secretase components by NF-κB has not been detailed. Furthermore, although two NF-κB binding sites have been delineated on the βAPP promoter, no data concern its putative control by NF-κB in pathological conditions. Our study shows that in supraphysiological conditions, NF-κB activation up-regulates some components of the high molecular weight γ-secretase complex and the βAPP protein in human cells from both renal and neuronal origin.

Our data demonstrate a coordinated regulation of the three principal elements of Aβ biogenesis, i.e. its precursor βAPP, and the two enzymatic activities β- and γ-secretases responsible for its production. We also show, strikingly, that NF-κB-dependent control of Aβ production depends on pathophysiological context, unlike the above experimental conditions aimed at mimicking the pathology. In physiological conditions, Aβ-mediated NF-κB-dependent pathway results in a consistent lowering of βAPP expression as well as β-secretase and γ-secretase members expression, ultimately leading to reduced enzymatic activity. Thus, in physiological conditions, NF-κB contributes to maintain Aβ homeostasis at a physiological level while in pathological conditions, NF-κB contributes to a vicious cycle by which Aβ self-feds its own production (Fig. 11).

It has been demonstrated that NF-κB inhibits BACE1 promoter activity in neuronal and resting glial cells while it activates BACE1 promoter in Aβ-exposed neuronal and activated glial cells (28). This can be explained by the nature of the NF-κB dimers involved since p52/c-Rel occurs in resting conditions while p50/p65, p52/p65, and p52/c-Rel are responsible for NF-κB-mediated BACE1 transactivation in activated neurons and glia (28). In this context, it is noteworthy that Valerio et al. (17) demonstrated that Aβ40 could activate NF-κB by favoring the nuclear translocation of p50 and p65 subunits. Interestingly, it has been demonstrated that the recruitment of NF-κB components and the build-up of the distinct heterodimeric complex could be related to Aβ levels. Thus, Arevalo et al. showed that a low concentration of Aβ40 triggers NGF-like phenotype while a 40-fold higher concentration prevents NGF-induced activation of NF-κB (50). Accordingly, it was reported that only low concentrations of Aβ could trigger NF-κB-dependent protective phenotype (18). This set of data support our conclusion that physiological and supraphysiological levels of Aβ could differentially contribute to its own production by
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modulating its precursor and secretases via an NF-κB-dependent mechanism.

Our data do not establish whether NF-κB-associated increase in promoter transcription is direct or involves intracellular intermediates. One or several consensus sequence sites have been identified on the βAPP promoter (51), human (19), and rat (28) BACE1 promoters as well as on the PS2 promoter (52) that could mediate a physical interaction between NF-κB and these promotors. An additional possibility lies on the control of the tumor suppressor p53 by NF-κB. In this matter, the literature indicates that NF-κB could either activate p53 (53) or antagonize its effect (54), leading to either antiapoptotic and/or proapoptotic phenotypes (55). Interestingly, several studies indicated that p53 represses PS1 (56–59), and we have shown that, at physiological concentrations of Aβ, p53 also lowers Pen-2 promoter transactivation (60, 61). If one accepts the view that NF-κB activates p53 (53), this agrees with our observation that in a physiological context, p53-mediated NF-κB–linked reduction of PS1, Pen-2, and other contributors of Aβ genesis (see Figs. 2 and 3) ultimately leads to decreased γ-secretase expression and activity. Alternatively, if one considers NF-κB as a repressor of p53, then one can envision that NF-κB-mediated p53-dependent effect could only occur in pathological conditions where PS1 promoter transactivation is enhanced (see Fig. 7). It is actually difficult to resolve this issue in the absence of data concerning the influence of Aβ concentration on the control of p53 by NF-κB. But there again, this presumes a differential NF-κB-p53-dependent control of Aβ in physiological and supraphysiological conditions.

NF-κB is an ubiquitous transcription factor activated by inflammation, oxidative, and others cellular stresses (11, 13). This activation results in a protective response aimed at restoring cellular homeostasis, but can be deleterious when becoming chronic. This can be compared with NF-κB-mediated control of Aβ production that protects against Aβ overload in physiological conditions while it contributes to perpetuate and even increase Aβ levels in a more pathological context.

Besides classical strategies aimed at reducing Aβ production by directly modulating either β- or γ-secretases with pharmacological probes or by neutralizing Aβ-associated effects by a vaccinal approach (62, 63), alternative therapeutic tracks targeting NF-κB could be theoretically envisioned. One of these concerns is non-steroidal anti-inflammatory drug (NSAID) treatment. In these pathological conditions, our data show that NSAID-associated down-regulation of NF-κB could have beneficial effects on Aβ load even if deleterious side effects of interfering with NF-κB pathways should not be underestimated.

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