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蛋白激酶CK2訊息傳遞在大腦衍生營養因子抗細胞凋亡機制中所扮演的角色及機制探討：對SRF及其下游基因Mcl-1表現的影響(第2年)

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Protein kinase CK2 mediates the signaling pathway of BDNF through phosphorylating NF-κB to increase the anti-apoptotic Bcl-xL gene expression

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Abstract

Protein kinase CK2 (Casein kinase II) is a multifunctional serine/threonine protein kinase that is associated with the anti-apoptosis. The previous studies revealed that CK2 can be elevated and mediate the neurotrophic responses by brain-derived neurotrophic factor (BDNF) or glial cell line derived neurotrophic factor (GDNF). However, its action and the behind mechanisms are rarely focused on the nervous system. In the present studies, we found that BDNF increased the Bcl-xL gene expression correlative with CK2 activity and NF-κB phosphorylation in the hippocampal CA1 region. Transfection of the wildtype CK2α and the catalytically inactive CK2α156A mutant increased and decreased the Ser529 phosphorylation on NF-κB parallel with the anti-apoptotic Bcl-xL gene expression, respectively. Further, inhibition of CK2 would reverse the enhance effects of BDNF on Bcl-xL and NF-κB. Over-expression of mutant NFκBS529A would antagonize the increase effect on Bcl-xL mRNA either by BDNF treatment or by wildtype CK2α transfection. These results together suggest that CK2 might mediate an alternative signaling pathway for BDNF through directly phosphorylating NF-κB to increase the anti-apoptotic Bcl-xL gene expression.

Key words: brain derived neurotrophic factor, protein kinase CK2, NF-κB, Bcl-xL, anti-apoptosis

Introduction

Protein kinase CK2, which is highly conserved during evolution, is a multifunctional, constitutively active and ubiquitous serine/threonine protein kinase (Litchfield, 2003; Pinna and Meggio, 1997). The CK2 holoenzyme consists of subunits α, α' (catalytic) and β (regulatory) which associate to form α2β2, α'2β2, and αα'β2 heterotetramers (Litchfield, 2003). It appears that CK2 has more than 300 substrates those are involved in organelle function, signal transduction, gene transcription in various cells and CK2 also plays a prominent role in ensuring cell survival by counteracting apoptosis (Meggio and Pinna, 2003). Evidence linking CK2 activity to inhibition of apoptosis showed that the inhibition of CK2 activity decreases cell viability and induces apoptosis in cancer cells (Ruzzene et al., 2002; Izeradjene et al., 2005). There are reports indicating that CK2 can directly phosphorylate the pro-apoptotic protein Bid and Bad to inhibit their cleavage by caspase (Desagher et al., 2001; Krippner-Heidenreich et al., 2001) or the ARC protein (apoptosis repressor with caspase recruitment domain) to increase its inhibitory effect on caspase activity (Li et al., 2002). Beside these direct effects, CK2 has also been found to involve in the signaling transduction pathways through phosphorylating Akt, NF-κB or β-catenin to enhance the cell survival (Di Maira et al., 2005; Manna et al., 2007b; Tapia et al., 2006). Many of the anti-apoptotic gene expressions (such as: Bcl-2, IAP, Mcl-1 and *et al*) are found to be activated through the signaling pathways mediated by these molecules (Toruner et al., 2006; Takada et al., 2005; Manna et al., 2007a).

The CK2 protein is much more abundant in the brain and testis than in any other tissues of adult rats and is suggested to play the important roles in specific neural functions (Nakajo et al., 1986; Blanquet 2002). The studies have shown that CK2 can phosphorylate a variety of molecules that are associated with neuronal growth and neuronal plasticity, such as growth-associated protein (GAP-43) and NR2B (Korshunova et al., 2007; Chung et al., 2004). Further, CK2 is shown to associate with long-term potentiation in the hippocampus and spatial memory of rats (Charriaut-Marlangue et al., 1991; Chao et al., 2007). Regarding the CK2 activity regulation, results of studies revealed that CK2 can be elevated and mediate the neurotrophic responses in hippocampal slices following brain-derived neurotrophic factor (BDNF) treatment or in the substaitia nigra by glial cell line derived neurotrophic factor (GDNF) infusion (Blanquet 2000; Chao et al., 2006). Both two neurotrophic factors are shown to increase the neuronal survival under injury. Up to now, there are many studies focusing on the BDNF signaling pathway underlying its neuroprotective mechanisms. Those results indicate that PI-3K/Akt or MAPK/ERK signaling pathway involves in its neuroprotective effects against either trophic

deprivation- or glutamate-induced apoptotic cell death and hypoxic-ischemic injury (Han et al., 2000; Chang et al., 2004; Rossler et al., 2004; Almeida et al., 2005). However, these may not be sufficient to explain BDNF's pharmacological actions.

Although the survival effects of CK2 on cancer cells have been studied extensively, its action and mechanisms are rarely focused on the nervous system. According the clinical diagnosis from Alzheimer's disease and schizophrenic patients, results have revealed the correlation of the change of CK2 activity and gene expression profile with the pathogenic processes (Aksenova et al., 1991; Dunckley et al., 2006; Paulson et al., 2003). Since BDNF is thought as one of the therapeutic candidates for neurodegenerative disorders (Schulte-Herbrüggen et al., 2007), it is worthy to investigate the possible mechanisms behind its neurotrophic effects. The aim of the present study was to examine whether CK2-mediated signaling pathway contributes to the neurotrophic action of BDNF on hippocampal neurons.

Materials and Methods

Animals. Adult male Sprague-Dawley rats (250-350 g) bred in the Institution of Biomedical Sciences (IBMS), Academia Sinica, were used. They were maintained on a 12/12 hr light/dark cycle with food and water continuously available. For DNA transfection and drug administration, rats were subjected to stereotaxic surgery. Two 23-gauge, stainless-steel, thin-wall cannulae were implanted bilaterally to hippocampal CA1 area at the following coordinates: 3.5 mm posterior to the bregma; 2.5 mm lateral to the midline; and 3.4 mm ventral to the skull surface. Transfection and drug infusion were made at a rate of 0.4 μl/min and 0.8 μl was delivered to the each side. Animals were sacrificed 48 hr after DNA transfection or at different time points after drug infusion.

Plasmid DNA construction. Plasmid DNA was constructed as described previously (Chao et al., 2006). The full-length RT-PCR products of rat brain CK2α and NF-κB gene were inserted in frame into the pcDNA3 with HA tag or pCMV vector with Flag tag, respectively, and their sequences were verified by DNA sequencing. Mutant plasmid CK2α156A and NFκBS529A were generated by site-directed mutagenesis (Promega). The S529 on NF-κB was identified as the phosphorylation site of CK2α (Wang et al., 2000). Branched polyethylenimine (25 kDa, Aldrich) was used as the transfection agent (Abdallah et al., 1996) and the equivalence of nitrogens per DNA phosphate was 10.

Drug infusion and Plasmid DNA transfection. To assess the dose-response effects of recombinant human BDNF (Pepro Tech LTD.) on CK2 activity, NF-κB phosphorylation and Bcl-xL mRNA expression, 0.2 μg, 0.4 μg, and 1.2 μg of BDNF were bilaterally infused to the CA1. Animals were sacrificed 4 hr after infusion and the CA1 tissue was punched out for further assay.

To prove the role of CK2 in BDNF signaling, CK2α plasmid DNA transfections were first conducted to examine its effects on NF-κB phosphorylation and Bcl-xL mRNA expression. Animals were divided into three groups to receive 1.0 μg of pcDNA3-HA, wildtype CK2αWT and mutant CK2α156A DNA transfection. Animals were sacrificed 48 hr after DNA transfection.

To study the interactive effects of CK2 inhibitor TBB (4,5,6,7-tetrabromobenzotriazole, Calbiochem) and BDNF on NF-κB phosphorylation and Bcl-xL mRNA expression, animals were divided into three groups to receive twice infusion of PBS; PBS followed by 0.4 μg of BDNF, or 10 ng of TBB followed

by 0.4 μg of BDNF. The interval between two infusions was 30 min. Animals were sacrificed 4 hr after the last infusion.

To examine the interaction between BDNF and NFκB on Bcl-xL mRNA expression, animals were divided into three groups to receive pCMV-Flag transfection (1.0 μg) followed by PBS infusion; pCMV-Flag transfection (1.0 μg) followed by BDNF (0.4 μg) infusion; or NFκBS529A transfection (1.0 μg) followed by BDNF (0.4 μg) infusion. The time schedule for these treatments was as follows: DNA transfection was performed on the first day and BDNF (or PBS) was infused 48 hr later. Animals were sacrificed 4 hr after BDNF infusion.

To examine the interaction between CK2α and NF-κB on Bcl-xL mRNA expression, animals were divided into three groups to receive pCMV-Flag and pcDNA3-HA transfection (1.0 μg each); pCMV-Flag+CK2αWT transfection (1.0 μg each); NFκBS529A+CK2αWT transfection (1.0 μg each). The interval between the two transfections was 1 hr. Animals were sacrificed 48 hr after DNA transfection.

CK2 activity assay. CK2 activity assay was described elsewhere (Chao et al., 2007). Briefly, CK2 activity was measured in 5 μg protein aliquots from each sample by using the protein kinase CK2 assay kit (Upstate). The synthetic peptide and [γ-^{32}P]-ATP were used as the substrates and a kinase inhibitor cocktail was added to block the activity of other serine/threonine kinases. Total CK2 activity was calculated by subtracting the blank (without the peptide substrate) from the ^{32}P radioactivity incorporated in the presence of the substrate.

Real-time PCR analysis. Real-time PCR analysis of *Bcl-xL* mRNA was performed by using the Applied Biosystems 7500 Real-Time PCR System with standard TaqMan method. The house-keeping enzyme hypoxanthine phosphoribosyl transferase (HPRT) was used as an internal control. The cDNA was synthesized from 0.5 μg total RNA with 0.5 μg of oligo-dT by Superscript II reverse transcriptase (Invitrogen Inc.) and the commercial primers and fluorogenic probes for Bcl-xL and HPRT were purchased from Applied Biosystem Inc. The quantities of Bcl-xL and HPRT from the same sample were analyzed simultaneously but in separate tubes. Each assay was carried out in duplicate. The thermal conditions were: 2 min at 55 ℃ and 10 min at 95 ℃; followed by 40 cycles at 95 ℃ for 15 s and 60 ℃ for 1 min.

Western blot analysis. Fifteen μg of protein extract was subjected to SDS-PAGE and transferred to the PVDF membrane. The membrane was preincubated with 0.05%

TBS-T containing 2% BSA followed by anti-pS529NFκB (1:1000; Stressenge), anti-NFκB (1:1000; Santa Cruz Biotechnology), or anti-β-actin (1:10000, Chemicon) antibody and then with HRP-conjugated secondary antibody (1:8000, Amersham). The signals were detected by chemiluminescence and the density of each band was quantified by using the NIH Image J software.

Immunohistochemistry. The immunohistochemistry procedures were described in detail elsewhere (Chao et al., 2007). The paraformaldehyde-fixed brains were cut into series of 20-μm coronal sections through the CA1 region. Sections were incubated with mouse anti-HA antibody (1:400, Upstate) and then with anti-mouse FITC-conjugated IgG (1:1000, Sigma). Image acquisition was obtained by using a confocal microscope (Bio-Rad).

Statistical analysis. Data for enzyme activity, western blot analysis and real-time PCR determination were evaluated by Student's t-test or one-way analysis of variance (ANOVA) followed by Dunnett's t-test or the Newman-Keul's method.

Results

Anti-apoptotic effects of BDNF on CA1 neurons

The effects of different doses of acute BDNF treatment on Bcl-xL mRNA expression in the CA1 are shown in Fig. 1A. There was an overall increase in Bcl-xL mRNA expression by BDNF treatments ($F_{3,17}$ = 11.44, p<0.01). Further analyses indicated that BDNF at 0.4 μg and 1.2 μg both markedly enhanced Bcl-xL mRNA expression (tD = 3.91 and 9.06, respectively, both p<0.01 by Dunnett's t-test). Next we examine whether protein kinase CK2 and transcription factor NFκB were influenced by BDNF treatments. Statistical analyses showed that there was an overall significant effect of BDNF on CK2 activity ($F_{3,17}$ = 23.3, p<0.01) (Fig. 1B). Further analyses indicated that CK2 activity significantly enhanced by BDNF both at 0.4 μg and 1.2 μg (tD = 4.12 and 8.19, respectively, both p<0.01 by Dunnett's t-test). The Ser529 phosphorylation on NF-κB was also found to increase significantly by BDNF treatment ($F_{3,17}$ = 92.70, p<0.01) (Fig. 1C). Further analyses indicated that BDNF both at 0.4 μg and 1.2 μg significantly enhanced Ser529 phosphorylation (tD = 6.37 and 15.90, respectively, both p<0.01 by Dunnett's t-test). Fig. 1D is a representative illustration showing the needle placement and dye distribution in the CA1.

Effects of manipulation CK2α in CA1 neurons

The above results showed the correlations between CK2 activity, NF-κB phosphorylation and Bcl-xL mRNA expression by BDNF treatment. We further examined whether manipulations of CK2α indeed influences the NF-κB phosphorylation and Bcl-xL mRNA expression in CA1. The results from Fig. 2A revealed that Ser529 phosphorylation on NF-κB was significantly increased by wildtype CK2αWT DNA transfection ($F_{2,22}$ = 69.91, p<0.01; tD = 8.12, p<0.01 by Dunnett's t-test) while decreased by mutant CK2α156A DNA transfection (tD = 4.71, p<0.01). The expression of Bcl-xL mRNA was also significantly increased by wildtype CK2αWT DNA transfection ($F_{2,22}$ = 127.6, p<0.01; tD = 10.12, p<0.01 by Dunnett's t-test) while decreased by mutant CK2α156A DNA transfection (tD = 7.10, p<0.01) (Fig. 2B). Immunohistochemistry staining against HA confirmed the transfection and expression of CK2αWT in CA1 neurons (Fig.2C).

Effects of CK2 inhibitor TBB and BDNF on CA1 neurons

The results above do not reveal whether the CK2 signaling pathway contributes to the anti-apoptotic effect of BDNF on CA1 neurons. We addressed this issue in the present experiment. The concentration of CK2 inhibitor TBB used in the present study only slight altered CK2 basal activity in the CA1 ($t_{1,7}$ = 1.78, p>0.05) (Fig. 3A). Results from Figure 3B revealed that BDNF consistently increased Ser529

phosphorylation on NFκB ($F_{2,17}$ = 7.47, p<0.01; q = 5.46, p<0.01 by Newman-Keul's method), but this effect is significantly blocked by prior TBB treatment (q = 3.10, p<0.05). BDNF also consistently increased Bcl-xL mRNA expression ($F_{2,17}$ = 20.84, p<0.01; q = 9.02, p<0.01 by Newman-Keul's method), while prior administration of TBB markedly antagonized this BDNF's effect (q = 5.75, p<0.0) (Fig. 3C).

Interactive effects of mutant NFκBS529A *with BDNF or CK2αWT on Bcl-xL expression in CA1 neurons*

The above results showed that CK2 mediates the anti-apoptotic signaling of BDNF. We therefore examine whether this signaling pathway through the NF-κB molecule. In the study of mutant NFκBS529A transfection with BDNF, results revealed that BDNF markedly increased the Bcl-xL mRNA expression ($F_{2,11}$ = 26.58, p<0.01; q = 10.25, p<0.01 by Newman-Keul's method), while the NFκBS529A transfection significantly antagonized this effect of BDNF (q = 6.11, p<0.01) (Fig. 4A). In analyzing the effects of co-transfection of CK2αWT and NFκBS529A, the expression of Bcl-xL mRNA was shown to increase significantly by CK2αWT transfection ($F_{2,12}$ = 7.79, p<0.01; q = 5.12, p<0.05 by Newman-Keul's method), whereas NFκBS529A transfection blocked the effect of CK2αWT (q = 4.58, p<0.05) (Fig. 4B).

Discussion

In the present study, we have shown that BDNF increased the Bcl-xL gene expression correlative with CK2 activity and NF-κB phosphorylation in the hippocampal CA1 in a dose-dependent manner. The MAPK/ERK or PI-3K/Akt signaling pathways have been indicated to mediate the neuroprotective effects of BDNF (Han et al., 2000; Chang et al., 2004; Rossler et al., 2004; Almeida et al., 2005). CK2 was revealed to be regulated by B-Raf/MAPK/ERK pathway possibly through PP1 or PP2A in hippocampal slices by BDNF combined with pharmacological drugs treatment (Blanquet, 2000). However, a previous study had shown that CK2 directly phosphorylates PP2A and up-regulates its activity (Heriche et al., 1997). Further, a recent study also identified that CK2 contributes in kinase suppressor of Ras (KSR1) complex by functioning as Raf N-region serine kinase (Ritt et al., 2007). Disruption of the KSR1/CK2 interaction or inhibition of CK2 activity significantly reduces the growth-factor-induced phosphorylation of B-Raf and correlates with impaired Raf, MEK, and ERK activation. In addition, Ck2 was found to partially involve in PI-3K/Akt signaling pathways by directly phosphorylating Akt on ser473 (Di Maira et al., 2005). It seems that CK2 might play a cross-talk role between different signaling pathways and mediate an alternative signaling pathway for BDNF (see the below discussion about NF-κB).

The transcription factor NF-κB is indicated to participate in the anti-apoptotic mechanisms in many cell types (Burstein and Duckett, 2003; Janssens and Tschopp, 2006). After activation, the phosphorylated NF-κB will translocate into the nucleus and bind to the cognate DNA sequences present in gene promoters. The decrease in NF-κB/RelA phosphorylation by PI-3K or MAPK/ERK inhibitor was revealed to increase the cell death in HTLV-1-transformed cells or pancreatic cancer cell line (Jeong et al., 2005; Rengifo-Cam et al., 2007). The inhibition of CK2 phosphorylation on NF-κB/RelA by chemical compound P_3-25 was also shown to increase the sensitivity of apoptosis in constitutive NF-κB-expressing cells (Manna et al., 2007b). Further, the NF-κB/RelA is indicated to be phosphorylated on Ser529 by CK2 and can be responsible for increased NOSII gene transcription (Wang et al., 2000; Chantome et al., 2004). Our present studies also found that transfection of wildtype CK2α increased, whereas mutant CK2α156A decreased, the Ser529 phosphorylation on NF-κB. It is though that NF-κB plays a key role in the protection of cells against apoptosis possibly through the activation of the anti-apoptotic genes. In addition, two functional NF-κB DNA binding sites are found to cluster in the promoter region of murine bcl-x (Glasgow et al., 2001). In the previous studies, the inhibition of NF-κB was found to reverse the enhance effects of Inhibitor of apoptosis protein hRFI or

platelet activating factor on the Bcl-2 and Bcl-xL mRNA levels (Konishi et al., 2006; Heon Seo et al., 2006). These above studies can support our present finding that the over-expression of mutant NFκBS529A would antagonize the increase effect on Bcl-xL mRNA either by BDNF treatment or by CK2αWT transfection. The present findings also suggest that NF-κB might exist in an alternative signaling pathway for the neuroprotective mechanism of BDNF. The classical pathway for NF-κB activation requires the presence of IKKβ and NEMO and leads to phosphorylation of IκBα, liberating an active NF-κB heterodimer (Janssens and Tschopp, 2006). The present finding from us and the results of Chantome *et al.* (2004) provide the evidence that NF-κB can be directly phosphorylated by CK2 to trigger the gene expression.

The neuroprotective effects of BDNF are well characterized in many studies (Schulte-Herbrüggen et al., 2007). The up-regulation of anti-apoptotic Bcl-2 family members is one of the actions for BDNF to promote the cell survival. Among those studies, BDNF was found to increase the Bcl-xL protein level under excitotoxicity- or ischemia-induced injury, which could counter the translocation of apoptotic protein Bax and Bad (Almeida et al., 2005; Perez-Navarro et al., 2005; Miyawaki et al., 2008). However, the increase degree of Bcl-xL protein in those studies is not enormous as that of mRNA level in our present study. This diversity might involve in the post-transcriptional regulation for this protein. Recently, the study about the repeated unpredictable stress has shown that Bcl-xL mRNA, not Bcl-2 mRNA, was significantly decreased in hippocampal subfields (Kosten et al., 2008). Our study also found that the increase degree in Bcl-2 mRNA (~1.4 folds) was not as enormous as that of Bcl-xL by BDNF treatment (unpublished data). How to increase the Bcl-xL protein level by BDNF might be one of the considerations to improve its potential therapeutic effects.

In summary, our results suggest that CK2 might mediate an alternative signaling pathway for BDNF through directly phosphorylating NF-κB to increase the anti-apoptotic Bcl-xL gene expression. This sheds light on new therapeutic strategies for BDNF application.

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Figure Legends

Fig. 1 BDNF treatments increased the Bcl-xL expression, CK2 enzyme activity and NFκB phosphorylation. BDNF dose-dependently increased (A) Bcl-xL mRNA expression, (B) CK2 enzyme activity and (C) phosphorylation of Ser529 on NFκB in the CA1. Animals received bilateral infusions of 0.2, 0.4, or 1.2 μg BDNF in the CA1 and were sacrificed 4 hr later. Data are expressed as mean±SEM. n=4-6 each group. Statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's t-test. * $p<0.05$, ** $p<0.01$. (D) A representative illustration showed the needle placement and dye distribution (0.3% methylene blue) in the CA1.

Fig. 2 Effects of CK2α DNA transfection on NFκB phosphorylation and Bcl-xL expression. CK2αWT DNA transfection increased whereas CK2α156A mutant DNA transfection decreased (A) phosphorylation of Ser529 on NFκB and (B) Bcl-xL mRNA expression in the CA1. Animals separately received different plasmid DNA transfection in the CA1 and were sacrificed 2 days later. Data are expressed as mean±SEM. n=8-10 each group. Statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's t-test, ** $p<0.01$. (C) Immunohistochemical staining showing CK2αWT DNA transfection to the CA1. Anti-HA tag antibody and FITC-conjugated IgG secondary antibody were used. Arrow indicates the area of transfection, scale bar equals 300 μm.

Fig. 3 CK2 inhibitor TBB blocked the effects of BDNF on NFκB phosphorylation and Bcl-xL expression. (A) Basal CK2 activity was not significantly altered by CK2 inhibitor TBB treatment alone. BDNF treatments increased (B) phosphorylation of Ser529 on NFκB and (C) Bcl-xL mRNA expression but these effects were antagonized by TBB. Animals received bilateral infusions of 10 ng TBB (or PBS) followed by 0.4 μg BDNF in the CA1 and were sacrificed 4 hr after the last infusion. The interval between two infusion was 30 min. Data are expressed as mean±SEM. n=6-7 each group. Statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by Newman-Keul's method. * $p<0.05$, ** $p<0.01$; a: compared with the PBS+PBS group, b: compared with the PBS+BDNF group.

Fig. 4 Transfection of mutant NFκBS529A antagonized the effects of BDNF or CK2αWT on Bcl-xL expression. (A) The Bcl-xL mRNA expression in the CA1 was increased by BDNF infusion, but this effect was blocked by mutant NFκBS529A Transfection. Animals received bilateral infusion of 0.4 μg BDNF following 48 hr after 1 μg of NFκBS529A DNA transfection in the CA1 and were sacrificed 4 hr after

BDNF infusion. (B) The Bcl-xL mRNA expression in the CA1 was increased by CK2αWT DNA transfection, but this effect was also antagonized by mutant NFκBS529A Transfection. Animals bilaterally received NFκBS529A DNA transfection followed by CK2αWT DNA transfection (1 μg each) in the CA1 and were sacrificed 48 hr later. The interval between two transfections was 1 hr. Data are expressed as mean±SEM. n=4-5 each group. Statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by Newman-Keul's method. * $p<0.05$, ** $p<0.01$; a: compared with the corresponding control group, b: compared with the (A) BDNF or (B) CK2αWT group.

Fig 1

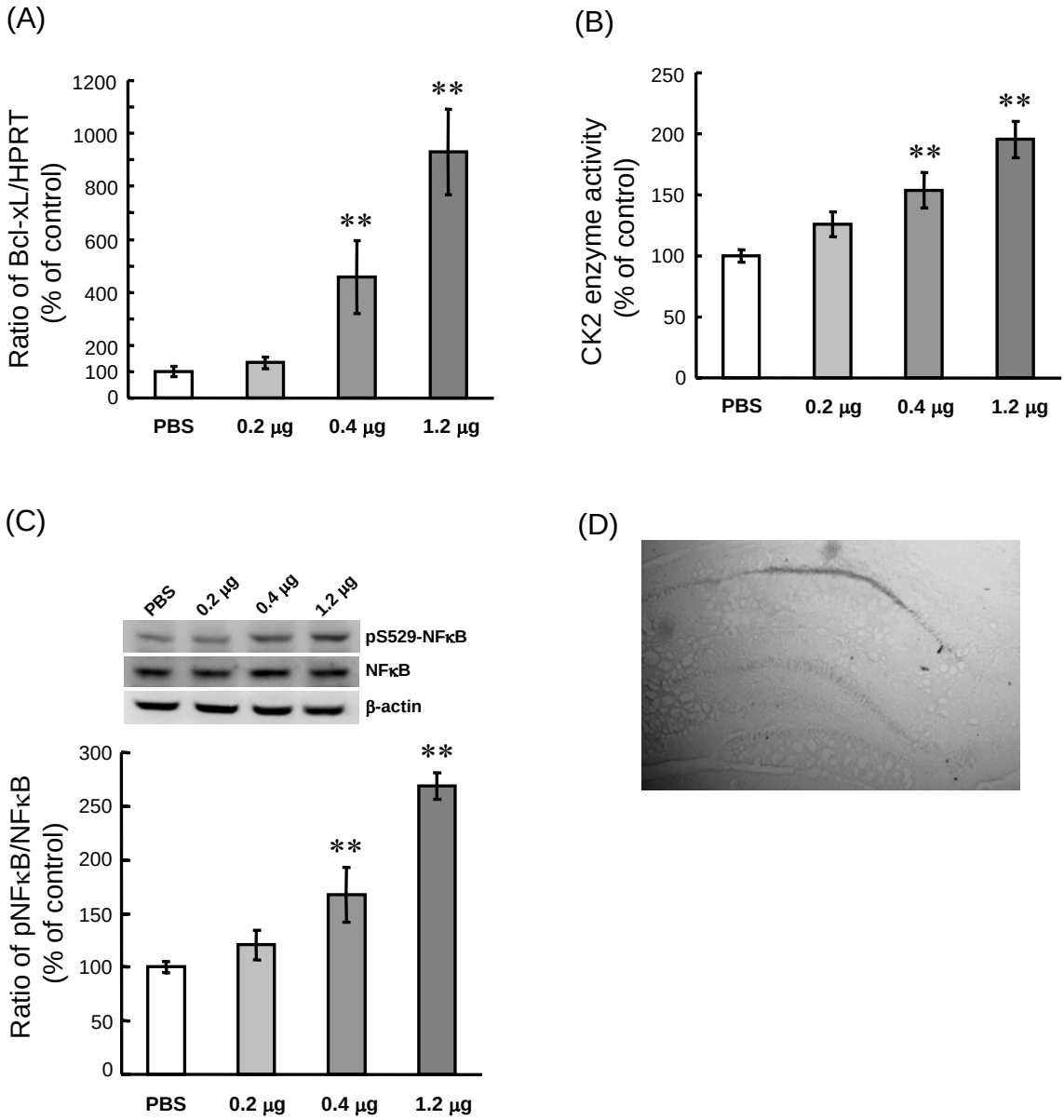
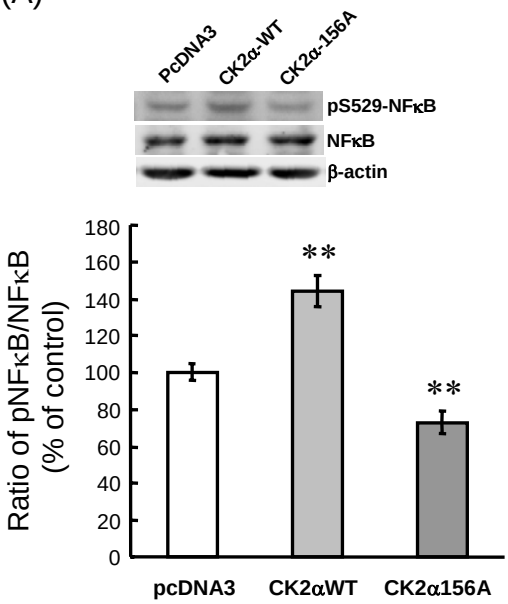
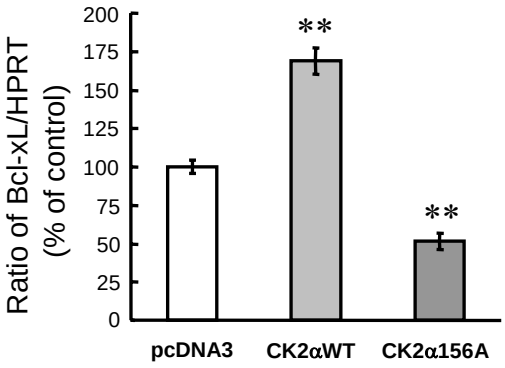

(A)
Ratio of Bcl-xL/HPRT (% of control)
1200
1000
800
600
400
200
100
0
**
**
PBS
0.2 μg
0.4 μg
1.2 μg
(B)
CK2 enzyme activity (% of control)
250
200
150
100
50
0
**
**
PBS
0.2 μg
0.4 μg
1.2 μg
(C)
PBS
0.2 μg
0.4 μg
1.2 μg
pS529-NFκB
NFκB
β-actin
Ratio of pNFκB/NFκB (% of control)
300
250
200
150
100
50
0
**
**
PBS
0.2 μg
0.4 μg
1.2 μg
(D)

Fig 2

(A)


PcDNA3
CK2α-WT
CK2α-156A
pS529-NFκB
NFκB
β-actin
Ratio of pNFκB/NFκB (% of control)
180
160
140
120
100
80
60
40
20
0
**
**
pcDNA3
CK2αWT
CK2α156A

(B)


Ratio of Bcl-xL/HPRT (% of control)
200
175
150
125
100
75
50
25
0
**
**
pcDNA3
CK2αWT
CK2α156A

(C)

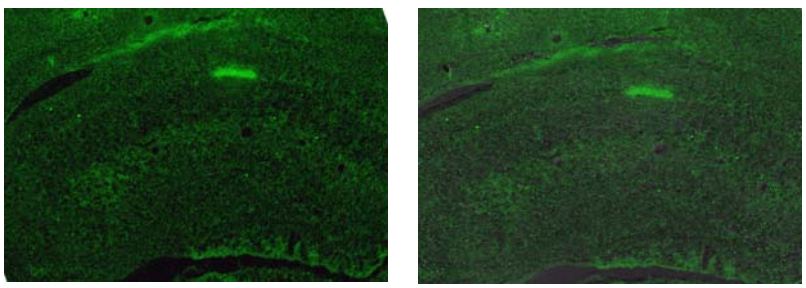
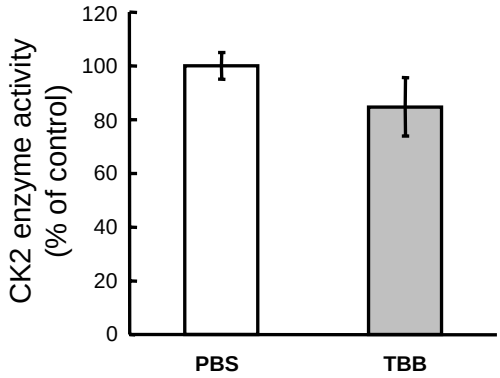
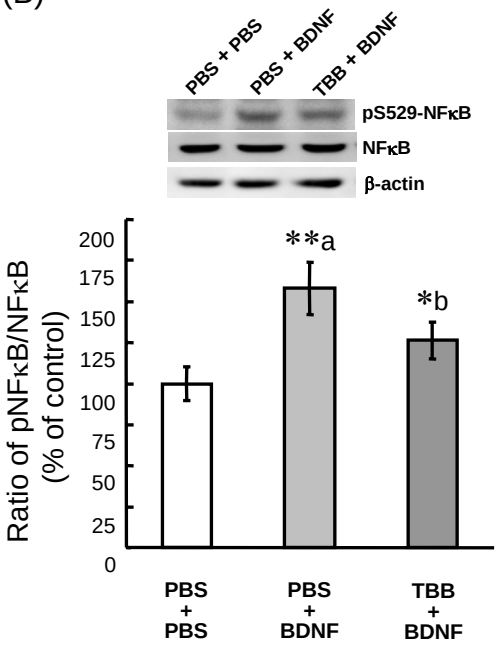


Fig 3

(A)


CK2 enzyme activity
(% of control)
120
100
80
60
40
20
0
PBS
TBB

(B)


PBS + PBS
PBS + BDNF
TBB + BDNF
pS529-NFκB
NFκB
β-actin
Ratio of pNFκB/NFκB
(% of control)
200
175
150
125
100
75
50
25
0
**a
*b
PBS + PBS
PBS + BDNF
TBB + BDNF

(C)

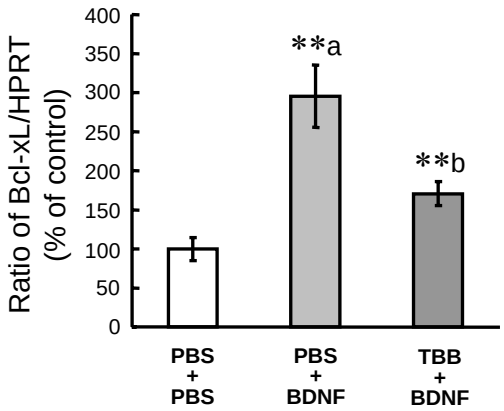
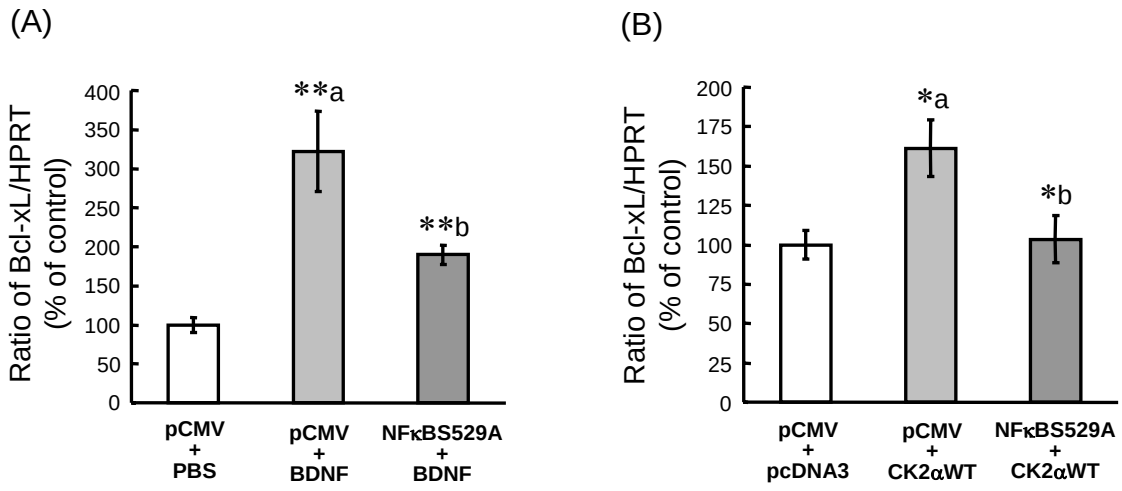

Ratio of Bcl-xL/HPRT
(% of control)
400
350
300
250
200
150
100
50
0
**a
**b
PBS + PBS
PBS + BDNF
TBB + BDNF

Fig 4


(A)
Ratio of Bcl-xL/HPRT
(% of control)
400
350
300
250
200
150
100
50
0
**a
**b
pCMV
+
PBS
pCMV
+
BDNF
NFκBS529A
+
BDNF
(B)
Ratio of Bcl-xL/HPRT
(% of control)
200
175
150
125
100
75
50
25
0
*a
*b
pCMV
+
pcDNA3
pCMV
+
CK2αWT
NFκBS529A
+
CK2αWT