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Molecular mechanism of the neurotrophic effect of GDNF on DA neurons: role of protein kinase CK2

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Abstract

Glial cell line-derived neurotrophic factor (GDNF) is suggested as a specific neurotrophic factor for midbrain dopamine (DA) neurons, but the molecular mechanism underlying the neuroprotective action of GDNF is not well known. In the present study, we have shown that GDNF increased protein kinase CK2 activity in rat substantia nigra (SN) in a dose-dependent and time-dependent manner. This effect is prevented by prior treatment of the receptor Ret blocker K-252b. Immunostaining results also revealed that CK2 is expressed in TH-positive neurons in mesencephalon culture. Transfection of the wildtype CK2α DNA increased, whereas transfection of the catalytically inactive CK2αA^{156} mutant DNA decreased CK2 activity in the SN. CK2αA^{156} mutant DNA also antagonized the enhancing effect of GDNF on CK2 activity. It also antagonized the enhancing effects of GDNF on tyrosine hydroxylase (TH) protein level in the SN, DA turnover in the striatum and rotarod performance in rats. Further, CK2α wildtype DNA increased, whereas CK2αA^{156} mutant DNA decreased TH activity in the SN without altering the TH protein level. On the other hand, the DA neuron toxin 1-methyl-4-phenylpyridinium iodide (MPP$^+$) markedly decreased the number of TH-positive neurons and TH protein level in the SN, decreased DA level in the striatum and impaired rotarod performance in rats. Over-expression of the CK2α wildtype DNA partially, but significantly, prevented the deteriorating effect of MPP$^+$ on these measures. Prior administration of MPP$^+$ also antagonized the enhancing effect of GDNF on CK2 activity. These results together suggest that the CK2 signaling pathway contributes to the neuroprotective action of GDNF on DA neurons.

Keywords: Glial cell line-derived neurotrophic factor; Protein kinase CK2; Substantia nigra; Striatum; Tyrosine hydroxylase; Dopamine; MPP$^+$

1. Introduction

Parkinson's disease is characterized by progressive degeneration of dopamine (DA) neurons in the substantia nigra (SN) that innervates the striatum (ST) [3,33]. Current treatments are aimed at elevation of striatal DA level but which do not prevent the continued neuronal loss. Neurotrophic factors that specifically prevent this degeneration and increase DA neuron activity become important.

Glial cell line-derived neurotrophic factor (GDNF), a 134-amino acid glycosylated polypeptide, has been shown to specifically increase the survival, outgrowth, uptake and function of DA neurons in rat midbrain culture [37]. Further experimental results have revealed that GDNF protects DA neurons from continuous cell death caused by 1-methyl-4-phenylpyridiniumiodide (MPP$^+$), a neurotoxin that depletes DA and produces a parkinsonism-like syndrome in humans and experimental animals [22,33]. A single injection of GDNF (0.1–100 μg, i.c.v.) also increases locomotor activity in rats [40]. The presence of a relatively higher GDNF mRNA level in neurons of the nigrostriatal and mesolimbic dopaminergic pathways than that of other areas suggests that GDNF may play an important role in regulation of midbrain DA neuronal function [58]. Therefore, GDNF treatment may be considered as one of the therapeutic strategies for Parkinson's disease. Actually, clinical results have shown that direct infusion of GDNF to the putamen of Parkinson's patients im-

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proves DA uptake and motor performance in these patients [18].

Although the pharmacological actions of GDNF on DA neurons have been studied extensively, the mechanisms underlying the neurotrophic and neuroprotective actions of GDNF on DA neurons are less known. We have previously reported that GDNF increases various antioxidant enzyme activities in midbrain DA neurons [9]. GDNF also protects mesencephalic neurons against apoptosis by up-regulation of bcl-2 and bcl-x through PI3 kinase activation [56]. Further, MAPK/ERK pathway activation is necessary for GDNF to protect against NMDA-induced neuronal death through reduction of Ca^{2+} influx [45]. However, these may not be sufficient to explain GDNF's pharmacological actions.

Protein kinase CK2, which is highly conserved during evolution, is a multifunctional, constitutively active and ubiquitous serine/threonine/tyrosine protein kinase [39,48]. The CK2 holoenzyme consists of subunits α, α′ (catalytic) and β (regulatory) which associate to form α2β2, α′2β2, and αα′β2 heterotetramers [12]. The highest activity of CK2 was found in the brain and testis of adult rats [44]. CK2 was thought to be unregulated in cells for many years; however, a number of more recent studies have shown that post-translational modulation of CK2 activity is present in both neuronal and non-neuronal cells. For example, elevated CK2 activity has been found in various tissue extracts following a variety of hormone and growth factor treatments [8]. CK2 has also been shown to mediate neurotrophic responses in hippocampal slices following brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4) treatments [6]. Further, CK2 is shown to associate with long-term potentiation in rat hippocampus [11]. There appears to be more than 300 substrates for protein kinase CK2 that are involved in signal transduction, protein synthesis, cytoskeleton structure, cell–cell adhesion, gene transcription, and DNA synthesis in various cells [42]. Many of these substrates have clear implications in development of neuronal tissue, neuritogenesis, synaptic transmission, synaptic plasticity, and neuronal survival [7]. These data therefore suggest the importance of identifying the signal transduction pathways that are coupled to CK2 in regulation of these functions. The aim of the present study was to examine whether CK2 contributes to the neurotrophic action of GDNF on DA neurons, and whether over-expression of CK2 protects DA neurons against MPP^+ toxicity.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (250–350 g) bred in the Institution of Biomedical Sciences (IBMS), Academia Sinica, Taiwan were used in the present study. They were maintained on a 12/12 h light/dark cycle with food and water continuously available. For in vivo administrations, rats were subjected to stereotaxic surgery under sodium pentobarbital anesthesia (40 mg/kg, i.p). Twenty-three gauge stainless-steel thin wall cannulae (15 mm long) were implanted bilaterally into the SN alone or into both the SN and ST in separate animals, and were affixed on the skull with dental cement. The coordinates for SN were: AP – 5.4 mm from bregma, ML ± 2.0 mm from midline and DV – 8.3 mm below the skull surface. The coordinates for ST were: AP – 1.0 mm from bregma, ML ± 2.9 mm from midline and DV – 4.5 mm below the skull surface. The tooth bar was set at 2.4 mm. The animals were allowed to recover for a week after the surgery. Experimental procedures follow the Guidelines of IBMS, Academia Sinica.

2.2. GDNF and drug interaction study

To assess the dose–response effects of recombinant human GDNF (Pepro Tech EC Ltd., London, England) on protein kinase CK2 enzyme activity, 0.5, 1.0 and 1.5 μg GDNF were bilaterally infused to the SN ($n = 6$ each group). Animals were sacrificed 2 h after infusion and the SN tissue was punched out for enzyme activity assay. Based on the results from the dose–response study, 1.0 μg GDNF was chosen for the time-course study of CK2 activity. Animals were sacrificed at 1, 2, 4 and 8 h after infusion, separately ($n = 5$–6 each group). All infusions were made in a volume of 0.8 μl each side.

Receptor protein kinase inhibitor K-252b was shown to abolish Ret receptor-mediated effects of GDNF on DA neurons [49]. The interaction study of GDNF and K-252b was therefore adopted to examine whether GDNF increases CK2 activity through GDNF receptor Ret. Animals were divided into five groups of five rats each. The PBS group received two PBS infusions in the SN; the PBS + GDNF group received PBS followed by GDNF (1 μg) infusions in the SN; and the other three groups received different concentrations of K-252b (0.75, 1.5 and 3 μg) followed by GDNF (1 μg) infusions in the SN. Each infusion volume was 0.8 μl and the interval between two infusions was 30 min. Animals were sacrificed 4 h after GDNF infusion and the SN tissue was punched out for protein kinase CK2 enzyme activity assay.

GDNF and MPP^+ interaction study was conducted to examine whether GDNF-induced CK2 activation protects DA neurons against MPP^+ toxicity. Intranigral MPP^+ infusion, instead of systemic MPTP administration, was adopted because rat is shown to be more susceptible to MPP^+ toxicity [19]. Animals were divided into three groups of five rats each. The PBS group received two PBS infusions; the PBS + GDNF group received PBS followed by GDNF (1 μg) infusions; and the MPP^+ + GDNF group received MPP^+ (15 μg) followed by GDNF (1 μg) infusions. Each infusion volume was 0.8 μl and the interval between two infusions was 30 min. Animals were infused for 2 days and were sacrificed 4 h after the last GDNF infusion. The SN tissue was dissected out for protein kinase CK2 enzyme activity assay.

2.3. CK2α plasmid DNA construction

The complete coding sequence of the rat CK2α gene was generated by RT-PCR from rat brain RNA. The RT product was synthesized with Oligo-dT. The sense primer (5′-CGGAATTCTCGGGACCCGTGCCAAGCAG-3′) contains sequence corresponding to the 5′-end of rat CK2α (the ATG start coden was removed) with an EcoRI cutting site (underlined). The antisense primer (5′-GCTCTAGATTACTGCTAGCGCCAGCGG-3′) contains sequence corresponding to the 3′-end of rat CK2α (the stop coden TAA was maintained) with an XbaI cutting site (underlined). The PCR product was then inserted in frame into the pcDNA3 vector (Invitrogen, CA) between EcoRI and XbaI sites, and with the hemagglutinin (HA) tag at its 5′-end between KpnI and BamHI cutting sites. The coding sequence for the influenza HA epitope was MAYPYDVPDYAS. The sequence of the HA-CK2α hybrid was verified by DNA sequencing.

To investigate the possible role of CK2α in GDNF signaling, two CK2α mutant plasmids were constructed. One is the CK2αA^{156} mutant in which the key amino acid Asp 156 in the active center of CK2α is mutated to Ala. This mutant has been shown to be inactive but still able to bind the CK2β subunit and to compete effectively with the wildtype subunit α and α′ [13,30]. The other is the CK2αF^{182} mutant in which the autophosphorylation site Tyr182 of CK2α is mutated to Phe [15]. This mutant showed impaired catalytic activity due to a dramatic increase in the K_m value for ATP [55]. These mutants were constructed based on the HA-CK2α plasmid obtained above with the use of GeneEditor in vitro site-directed mutagenesis system (Promega, WI). The protocol provided by the manufacturer was followed without modification. The mutagenic oligonucleotides used to generate the CK2αA^{156} and CK2αF^{182} mutants were 5′-ATGCACAGAGCAGTGAAACCGC-3′ and 5′-GCAGAGTTTTTCCATCCTGGCC-3′ with the mismatch base(s) underlined. Enzyme activity of these mutants expressed in *E. coli* was evaluated with commercial CK2 kinase assay kit (Upstate).

2.4. CK2α plasmid DNA and drug interaction study

Total CK2 kinase activity was assayed 48 h after animals received intranigral CK2α plasmid DNA transfection. This time point was chosen because transient transfection was shown to have optimal efficiency 48–72 h later [1]. Branched polyethylenimine (PEI, 25 kDa, Aldrich)/DNA complex (6 equivalent nitrogens per DNA phosphate) was used as the transfection agent because it produced satisfactory results from our previous studies [59,66]. Animals were divided into three groups to receive 1.0 μg of pcDNA3-HA, wildtype CK2α, and mutant CK2αA^{156} DNA transfections (n = 9–11 each group). The SN tissue was dissected out for CK2 kinase activity assay.

For the interaction study between CK2α mutant and GDNF, animals were divided into three groups (n = 7–8 each group). The control group received pcDNA3-HA plasmid DNA (1.0 μg) transfection followed by PBS infusion; the GDNF group received pcDNA3-HA plasmid DNA (1.0 μg) transfection followed by GDNF (0.75 μg) infusion; and the CK2αA^{156} group received CK2αA^{156} mutant DNA (1.0 μg) transfection followed by GDNF (0.75 μg) infusion. All manipulations were carried out in the SN. Each infusion volume was 0.8 μl. The time schedule adopted for these treatments was as follows: DNA transfection was given on the first day and every 2 days afterwards. GDNF (or PBS) infusion was first given on the third day and lasted for 5 days consecutively. Animals were subjected to the behavioral test next day after the last infusion. They were sacrificed after the behavioral test and tissues of SN and ST were collected for further analysis.

In evaluation of the protective effect of CK2 overexpression on MPP^+ toxicity, CK2α plasmid DNA transfection combined with MPP^+ infusion was adopted. Animals were divided into three groups (n = 6–9 each group): the control group received pcDNA3-HA plasmid DNA (1.0 μg) transfection to the SN followed by PBS infusion to the ST; the MPP^+ group received pcDNA3-HA plasmid DNA (1.0 μg) transfection to the SN followed by MPP^+ (15 μg) infusion to the ST; the CK2α-WT+MPP^+ group received wildtype CK2α plasmid DNA (1.0 μg) transfection to the SN followed by MPP^+ (15 μg) infusion to the ST. The time schedule adopted for CK2α transfection was the same as that of the above experiment, and the schedule used for MPP^+ infusion was the same as that of GDNF infusion.

To assess the effect of CK2α DNA transfection on DA neuronal function, animals were divided into three groups (n = 5 each group): the control group received pcDNA3-HA plasmid DNA (1.0 μg) transfection to the SN; the CK2α-WT group received CK2α-WT plasmid DNA (1.0 μg) transfection to the SN, and the CK2αA^{156} group received CK2αA^{156} mutant DNA (1.0 μg) transfection to the SN. The time schedule used for CK2α transfection was the same as that of the above experiment. Animals were sacrificed next day after the last infusion and the SN tissue was punched out for CK2 kinase activity assay.

2.5. Western blot analysis

Effects of the above treatments on tyrosine hydroxylase (TH) protein level *and* CK2α protein level in the SN were determined by Western blot analysis as described previously [10]. Briefly, the SN tissue was sonicated in ice-cold extraction buffer and centrifuged at 14,000 × g at 4 °C for 10 min. Equal amounts of sample extracts (15 μg protein) were subjected to 8% SDS-PAGE and transferred to PVDF membrane by semi-dry transfer method (Millipore, MA). The membrane was preincubated with blocking buffer (TBS buffer containing 2% BSA and 0.05% Tween-20) for 1 h, followed by TH (1:5000, Chemicon), CK2α (1:1000, Chemicon) or β-actin (1:10,000, Chemicon) antibodies in blocking buffer

at 4 °C overnight. After washing with TBS containing 0.1% Tween 20, the membrane was incubated with horseradish peroxidase-conjugated secondary antibody (1:8000, Amersham) at room temperature (RT) for 1 h. After extensive wash, the peroxidase signals were detected using chemiluminescence (ECL plus, Amersham). The protein bands were visualized on the X-ray film and quantified by using the NIH Image J software.

2.6. Enzyme activity assay

For CK2 enzyme activity assay, CK2 protein in the transformed *E. coli* or in the SN tissue was extracted as described previously [20]. Briefly, tissue was sonicated in ice-cold lysis buffer and centrifuged at 10,000 × *g* at 4 °C for 30 min. The pellet was resuspended in lysis buffer, centrifuged again, and the supernatants from two centrifugations were pooled. CK2 activity was measured in 5 μg protein aliquots from each sample using the protein kinase CK2 assay kit (Upstate Biotechnology). Briefly, the assay was carried out at 30 °C for 10 min by using a specific synthetic peptide as substrate and a kinase inhibitor that blocks the activity of other serine/threonine kinases. An amount of 5 μCi of [γ-^{32}P] ATP and equal amount of protein extract were added to each reaction mixture. The reaction was stopped by the addition of 20 μl of 40% TCA. A volume of 25 μl of the reaction mixture was spotted onto P81 papers, and washed five times with 400 ml of 0.75% phosphoric acid. Paper pieces were then rinsed with acetone, dried, and transferred to scintillation vials for radioactivity counting. The enzyme activity was calculated by subtracting the blank (determined in identical assays from which the peptide substrate was omitted) from the ^{32}P radioactivity incorporated in the presence of the substrate.

Tyrosine hydroxylase activity was measured by the tritium release method as described earlier [60]. The final reaction volume of 100 μl potassium phosphate buffer (50 mM, pH 6.0) contains 30 μl SN sample extract and the following components: 100 μM L-tyrosine, 1 μCi L-[3,5-^{3}H]-tyrosine, 1 mM ascorbic acid, 1 mM tetrahydrobiopterin, 10 μM ferrous ammonium sulfate, 7500 U catalase. The reaction was carried out at 37 °C for 15 min and was terminated by adding trichloroacetic acid. The $^{3}H_2O$ was separated from other reaction components by passing the entire mixture through a column of Dowex 50-H^+ (3 cm) overlaid with activated charcoal (0.5 cm) and Dowex 1-acetate (0.5 cm). The elute was collected in a scintillation vial to which 5 ml of Beckman ready safe liquescent was added and analyzed by liquid scintillation spectrometry. Blank values were obtained from identically prepared samples that did not contain tissue lysate. The assays were performed in duplicate.

2.7. Immunostaining

For immunohistochemistry experiments, rats were deeply anesthetized with sodium pentobarbital and were transcardially perfused with 300 ml cold PBS followed by 300 ml 4% paraformaldehyde in PBS. The brains were dissected out and post-fixed in the same fixative overnight and transferred into 25% sucrose solution (in PBS) for 24 h at 4 °C. The fixed brains were cut on a freezing microtome into series of 40-μm coronal sections through the SN and processed for HA or TH staining.

Brain sections were rinsed with PBS at RT for 10 min and permeabilized with pre-cold EtOH/CH_3COOH (95%:5%) at −20°C for 10 min, followed by PBS for 10 min for three times. The sections were preincubated in a blocking solution containing 3% normal goat serum, 3% BSA, and 0.05% Triton X-100 in PBS at RT for 1 h, and then incubated with mouse anti-HA antibody (1:100, Upstate) or mouse anti-TH antibody (1:1000, Chemicon) in blocking buffer at 4 °C overnight. For HA staining: after three washes in PBS, tissue sections were incubated with goat anti-mouse FITC-conjugated IgG (1:1000, Sigma) in PBS at RT for 1 h. Sections were washed three times in PBS and mounted with mounting medium containing 20 mM DABCO in 90% glycerol/50 mM Tris–HCl (pH 8.0). Image acquisition was obtained by using a confocal microscope equipped with fluorescence and phase contrast microscopy (Bio-Rad). For TH staining: tissue sections were incubated with biotin-conjugated goat anti-mouse IgG antibody (1:2000, Vector Laboratories). ABC reaction kit and peroxidase DAB substrate kit (Vector Laboratories) were used to visualize TH-positive neurons. Image acquisition was obtained by using phase contrast microscopy.

For visualization of the co-localization of CK2α and TH, primary mesencephalic culture (DIV-5) was used and immunocytochemistry was performed as described previously [10]. Culture cells were stained with primary mouse anti-TH antibody (1:1000, Chemicon) and rabbit anti-CK2 antibody (1:100, Stressgen) at 4 °C for 36 h. The secondary antibodies used were anti-mouse IgG-AF486 and anti-rabbit IgG-Cy3 (both 1:1000, Molecular Probes), and were applied at RT for 1 h. Images were obtained by using confocal microscope.

2.8. Quantification of DA neurons in the SN

Animals were transfected with wildtype CK2α plasmid DNA combined with MPP^+ infusion, and their brain tissues were processed for TH staining. The number of TH-positive neurons in the SN pars compacta was counted by a systemic, unbiased method as described earlier [51]. Briefly, TH-positive cells, situated lateral to a vertical line through the medial tip of the cerebral peduncle, were counted under light microscope (400×) in a series of five sections (one section was selected among three consecutive sections) near the transfection area bilaterally.

2.9. HPLC–EC detection of DA and DOPAC

The concentration of DA and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in the striatum was assayed by using high performance liquid chromatography–

electrochemical (HPLC–EC) detection according to that described previously [10]. Briefly, the striatal tissue was sonicated in 20 vol of 0.1 N $HClO_4$ containing 4 mM $Na_2S_2O_4$. The homogenate was centrifuged at 12,000 × *g* for 15 min and 10 μl of the supernatant was directly injected into the HPLC system. The working electrode was maintained at an oxidation potential of +0.75 V relative to the Ag–AgCl reference electrode. Dopamine and DOPAC were separated by using an ODS reverse phase column. The mobile phase consisted of 70 mM H_3PO_4, 0.25% triethylamine, 430 mM acetonitrile, 0.01% EDTA, and 0.175% heptanesulfonic acid at pH 2.1 and was delivered at a flow rate of 0.4 ml/min. DA and DOPAC concentrations were calculated in comparing with a known amount of DA and DOPAC. Dopamine turnover was expressed as the ratio of DOPAC/DA.

2.10. Rotarod treadmill performance

Rats were subjected to the rotarod treadmill endurance measure. In this experiment, the speed was set at eight turns per minute and the rat was placed on the center grid of the rotarod. The timer was stopped automatically when the animal fell off the grid and the total time the rat stayed on the grid was recorded as the endurance measure.

2.11. Statistical analysis

Statistical analysis was evaluated by Student's *t*-test, one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test or the Newman–Keul's method.

3. Results

3.1. Effects of GDNF on protein kinase CK2 activity

Effects of different doses and time courses of acute GDNF treatment on CK2 enzyme activity in the SN are shown in Fig. 1A and B, respectively. Statistical analyses revealed that there was an overall significant effect of GDNF on CK2 activity ($F_{3,20} = 2.69$, $p < 0.01$) (Fig. 1A). Further analyses indicated that GDNF at 1.0 and 1.5 μg both significantly enhanced CK2 activity (tD = 3.52 and 4.35, both $p < 0.01$ by Dunnett's *t*-test). CK2 activity at different time points after 1.0 μg GDNF treatment is shown in Fig. 1B. Results revealed an overall significant and time-dependent increase in CK2 activity in the SN ($F_{4,21} = 2.13$, $p < 0.01$). Further analyses indicated that GDNF markedly increased CK2 activity at 1, 2, 4 and 8 h after GDNF infusion (tD = 3.10, 3.42, 3.58 and 2.76, respectively; $p < 0.05$ or $p < 0.01$) with the effect peaked at 4 h. The receptor protein kinase inhibitor K-252b was then applied to examine whether GDNF increases CK2 activity through GDNF receptor Ret. As shown in Fig. 1C, GDNF consistently increased CK2 enzyme activity in the SN ($p < 0.01$ by Newman–Keul's method), but this effect was abolished by prior K-252b infusion in a dose-dependent manner ($F_{4,20} = 1.85$, $p < 0.05$). Further analyses indicated that 1.5 and 3.0 μg K-252b produced a significant effect (both $p < 0.05$ when compared with GDNF group). Fig. 1D revealed that the concentration of K-252b used in the present study only slight altered CK2 basal activity in the SN ($t_{1,8} = 0.76$, $p > 0.05$).

3.2. Co-expression of CK2α and TH in cultured mesencephalon neurons

The above results revealed that GDNF increased CK2 activity in the SN. However, these results do not reveal whether the CK2α protein is actually expressed in DA neurons. Because the soma and neuronal processes can be clearly visualized in dissociated cells, immunostaining of CK2α and TH in cultured mesencephalon neurons was carried out to examine their cellular distributions. Fig. 2A showed a representative TH-positive neuron in mesencephalon culture. Fig. 2B revealed that CK2α is mainly distributed in the cytoplasma of various cells in the same culture. The superimposed image from A and B revealed that CK2α is expressed in TH-positive neurons in mesencephalon culture (Fig. 2C). The phase contrast photograph of these cells was shown in Fig. 2D. A higher-magnification of the double-labeled cell in Fig. 2C is shown in Fig. 2E.

3.3. Effects of CK2α DNA transfection on CK2 activity

In vitro kinase assay for different CK2α DNA constructs from the bacteria lysates was carried out. As shown in Fig. 3A, the wildtype CK2α effectively phosphorylated the peptide substrate. Enzyme activity for both $CK2\alpha A^{156}$ and $CK2\alpha F^{182}$ mutant constructs was extremely low when compared with that of the CK2α-WT. The $CK2\alpha A^{156}$ mutant was therefore chosen for further experiments because of its ability to trap the β subunit to form an inactive complex [13]. Effects of intranigral CK2-WT and $CK2\alpha A^{156}$ DNA transfection on CK2 activity is shown in Fig. 3B. CK2-WT DNA transfection increased total CK2 activity (tD = 4.25, $p < 0.01$), whereas $CK2\alpha A^{156}$ mutant DNA transfection decreased CK2 activity in the SN (tD = 4.88, $p < 0.01$). Fig. 3C and D indicated that the $CK2\alpha A^{156}$ DNA is indeed transfected to the SN area and to individual neurons in the SN.

3.4. Effects of $CK2\alpha A^{156}$ mutant DNA transfection and GDNF on DA neurons

Next we examined whether protein kinase CK2 contributes to the neurotrophic effect of GDNF on DA neurons. $CK2\alpha A^{156}$ mutant DNA was transfected to the SN 2 days before GDNF infusion. Results from Fig. 4A revealed that GDNF markedly increased CK2 enzyme activity in the SN ($p < 0.01$ by Newman–Keul's method), but this effect was abolished by prior $CK2\alpha A^{156}$ mutant DNA transfection ($p < 0.01$ when compared with GDNF group). In addition, GDNF also markedly increased TH protein level in the

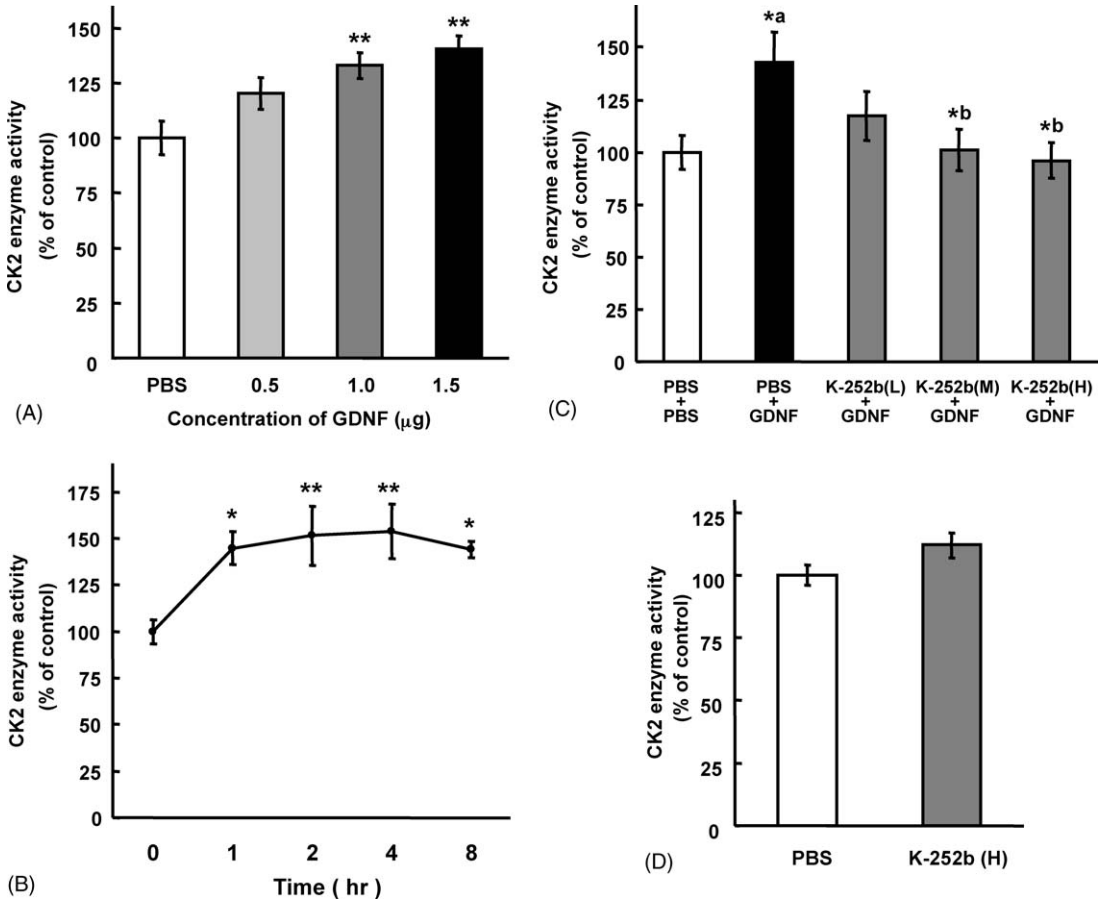

Fig. 1. Effects of GDNF and K-252b on protein kinase CK2 activity in the SN. (A) GDNF dose-dependently increased CK2 activity in the SN. Animals received bilateral infusions of 0.5, 1.0, or 1.5 μg GDNF in the SN and were sacrificed 1 h later; $n = 6$ each group. (B) GDNF increased CK2 activity in the SN in a time-dependent manner. Animals received bilateral infusions of 1.0 μg GDNF in the SN and were sacrificed 1, 2, 4 and 8 h after infusion. Data are expressed as mean ± S.E.M.; $n = 5$–6 each group. Statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test; $^*p < 0.05$, $^{**}p < 0.01$. (C) Prior K-252b treatment dose-dependently inhibited GDNF-induced CK2 activity in the SN. Animals received bilateral infusions of different concentrations of K-252b (0.75, 1.5 and 3.0 μg) 30 min prior to 1.0 μg GDNF infusion to the SN, and were sacrificed 4 h after GDNF infusion. Data are mean ± S.E.M.; $n = 5$ each group. Statistical significance was evaluated by one-way ANOVA followed by Newman–Keul's method; $^*p < 0.05$; (a) compared with the PBS + PBS group; (b) compared with the PBS + GDNF group. (D) Basal CK2 activity in the SN was not altered by K-252b treatment alone. Animals received bilateral infusions of 3.0 μg K-252b to the SN, and were sacrificed 30 min later. Data are mean ± S.E.M.; $n = 5$ each group. Statistical significance was evaluated by Student's *t*-test.

SN ($p < 0.05$) and this effect was similarly blocked by prior CK2αA^{156} mutant DNA transfection ($p < 0.05$ when compared with GDNF group) (Fig. 4B).

The striatal tissue from these animals was subjected to DA and DOPAC analyses. Results revealed that GDNF significantly increased DOPAC level and DA turnover in the ST (both $p < 0.01$ by Newman–Keul's method) (Table 1), but these effects were antagonized by prior CK2αA^{156} mutant DNA transfection (both $p < 0.01$ when compared with GDNF group). To further investigate the role of CK2 in contributing to the biological effect of GDNF on DA neurons, we measured rotarod activity in these animals. Results from Table 1 revealed that GDNF markedly enhanced rotarod treadmill performance in rats ($p < 0.01$), but this effect was similarly antagonized by prior CK2αA^{156} mutant DNA transfection ($p < 0.05$ when compared with GDNF group).

3.5. Effects of wildtype CK2α-WT DNA transfection on MPP$^+$-induced DA neuron toxicity

The above results revealed that CK2α is involved in the GDNF signaling pathway, but they do not reveal whether CK2α protects DA neurons against MPP$^+$-induced toxicity. The present experiment examined this hypothesis. Results from Fig. 5A revealed that MPP$^+$ decreased CK2 activity *by* approximately 10% ($p > 0.05$), but prior CK2α-WT DNA transfection markedly reversed the effect of MPP$^+$ on CK2 activity ($p < 0.05$ when compared with MPP$^+$ group by Newman–Keul's method). MPP$^+$ also markedly decreased TH protein level in the SN ($p < 0.01$), and this effect was partially, but significantly prevented by prior CK2α-WT DNA transfection ($p < 0.05$ when compared with MPP$^+$ group) (Fig. 5B).

Table 1
Effects of GDNF and CK2αA^{156} mutant DNA transfection on DA and DOPAC levels, DA turnover in the striatum as well as on rotarod performance in rats

	DA (%)	DOPAC (%)	DA turnover (DOPAC/DA)	Rotarod performance (s)
pcDNA3 + PBS	100.0 ± 2.0	100.0 ± 4.2	0.071 ± 0.002	47 ± 5
pcDNA3 + GDNF	85.1 ± 2.0	121.7 ± 6.3^{**,a}	0.099 ± 0.002^{**,a}	83 ± 5^{**,a}
CK2αA^{156} + GDNF	98.1 ± 5.7	107.2 ± 5.2^{**,b}	0.080 ± 0.006^{**,b}	59 ± 9^{*,b}

Data are expressed as mean ± S.E.M. (n = 6–10 each group). DA and DOPAC levels are expressed as percentage of the control group. Control group received pcDNA3 transfection (1.0 μg) and PBS infusion in the SN; GDNF group received pcDNA3 transfection (1.0 μg) and GDNF infusion (0.75 μg) in the SN; CK2αA^{156} + GDNF group received CK2αA^{156} mutant DNA transfection (1.0 μg) and GDNF infusion (0.75 μg) in the SN. Statistical significance was evaluated by one-way ANOVA followed by Newman–Keul's method.

* $p < 0.05$.

** $p < 0.01$.

[a] Compared with the control group.

[b] Compared with the GDNF group.

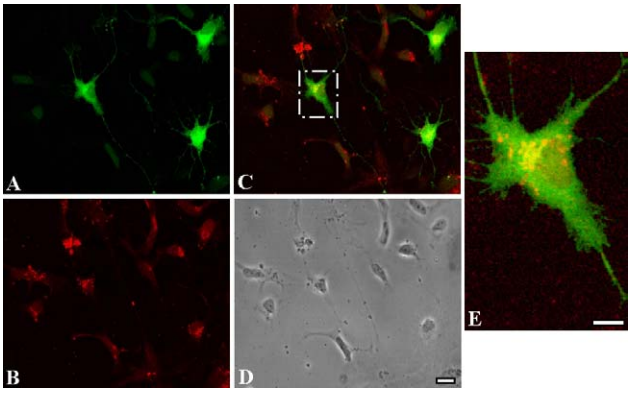

Fig. 2. Co-expression of CK2α with TH in mesencephalon culture. Culture cells were double stained with CK2α and TH at DIV 5. The detailed procedure was described in Section 2. (A) A TH-positive neuron was identified in the culture cell. (B) CK2α was mainly expressed in the cytoplasma of culture cells. (C) The superimposed image from (A) and (B) showed that CK2α is co-localized with TH in the same neuron. (D) The phase contrast photograph of the same cells. (E) Higher magnification of the double-labeled cell indicated by broken lines in (C). Scale bar equals 25 and 15 μm in (D) and (E), respectively.

In analyzing the effect of CK2α-WT DNA transfection and MPP$^+$ on DA neuron activity, results from Table 2 revealed that MPP$^+$ infusion markedly decreased the concentration of DA and DOPAC (both $p < 0.01$ by Newman–Keul's method), and increased DA turnover in the ST ($p < 0.01$), whereas CK2α-WT DNA transfection partially, but significantly, antagonized the effect of MPP$^+$ on DA and DA turnover measure ($p < 0.05$ and $p < 0.01$, respectively, when compared with MPP$^+$ group). We next examined the effect of CK2α-WT DNA transfection on the physiological function of DA neurons. Results from Table 2 revealed that MPP$^+$ significantly impaired rotarod performance in rats ($p < 0.01$). Prior CK2α-WT DNA transfection partially, but significantly, reversed this behavioral deficit ($p < 0.01$ when compared with MPP$^+$ group).

To determine whether recovery of impaired DA neuronal function by CK2α-WT DNA transfection is associated with increase in DA neuron survival, we examined the number of TH-positive neurons in control, MPP$^+$ and CK2α-WT + MPP$^+$-treated animals. Fig. 6 showed the photographs taken in the SN area from these three groups of animals (lower magnification in the upper panel and higher magnification in the lower panel). Statistical analyses revealed that MPP$^+$ significantly decreased the number of TH-positive neurons by approximately 40% ($p < 0.01$ by Newman–Keul's method). Prior CK2α-WT DNA transfection partially, but significantly, reversed the effect of MPP$^+$ on this measure ($p < 0.05$ when compared with pcDNA3 + MPP$^+$ group).

3.6. Effects of GDNF and MPP$^+$ on CK2 activity

The above results suggested that GDNF increased CK2 activity in the SN. However, these results do not reveal whether the CK2 signaling pathway contributes to the neuroprotective effect of GDNF on DA neurons. We addressed this is-

Table 2
Effects of MPP$^+$ and CK2α wildtype (WT) DNA transfection on DA and DOPAC levels, DA turnover in the striatum as well as on rotarod performance in rats

	DA (%)	DOPAC (%)	DA turnover (DOPAC/DA)	Rotarod performance (s)
pcDNA3 + PBS	100.0 ± 7.2	100.0 ± 5.2	0.199 ± 0.015	74 ± 12
pcDNA3 + MPP$^+$	45.6 ± 2.7^{**,a}	64.5 ± 4.7^{**,a}	0.309 ± 0.030^{**,a}	12 ± 5^{**,a}
CK2α-WT + MPP$^+$	65.9 ± 6.2^{*,b}	70.7 ± 6.7	0.133 ± 0.019^{**,b}	44 ± 5^{**,b}

Data are expressed as mean ± S.E.M. (n = 6–9 each group). DA and DOPAC levels are expressed as percentage of the control group. Control group received pcDNA3 transfection (1.0 μg) and PBS infusion in the SN; MPP$^+$ group received pcDNA3 transfection (1.0 μg) in the SN and MPP$^+$ infusion (15 μg) in the striatum; CK2α-WT + MPP$^+$ group received CK2α-WT DNA transfection (1.0 μg) in the SN and MPP$^+$ infusion (15 μg) in the striatum. Statistical significance was evaluated by one-way ANOVA followed by Newman–Keul's method.

* $p < 0.05$.

** $p < 0.01$.

[a] Compared with the control group.

[b] Compared with the MPP$^+$ group.

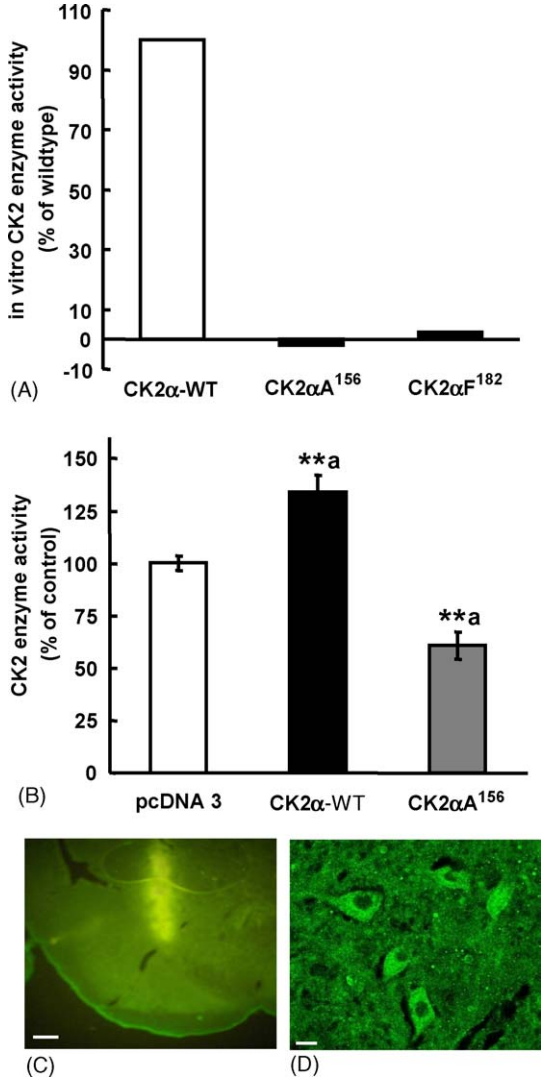

Fig. 3. Effects of CK2α DNA transfection on CK2 activity in the SN. (A) In vitro kinase assay showed that both the CK2αA^{156} mutant DNA and CK2αF^{182} mutant DNA completely lose enzyme activity when compared with the CK2α wildtype DNA. Cell lysates were prepared from transformed *E. coli* and the procedures are described in Section 2. Results are the average of duplicate determined from two independent experiments. (B) CK2α-WT DNA transfection increased whereas CK2αA^{156} mutant DNA transfection decreased total CK2 activity in the SN. Animals received different plasmid DNA transfection to the SN. SN tissue extraction and total CK2 activity assay were described in Section 2. Values are expressed as percentage of vector transfection group. Data are expressed as mean ± S.E.M.; $n = 9$–11 each group. Statistical significance was evaluated by one-way ANOVA followed by Dunnett's *t*-test; $^{**}p < 0.01$; (a) compared with the pcDNA3 group. (C and D) Immunohistochemical staining showing CK2αA^{156} mutant DNA transfection to the SN. Anti-HA tag antibody and FITC-conjugated IgG secondary antibody were used. (C) The whole visual field of transfected SN. Scale bar equals 300 μm. (D) At a higher magnification it showed that the CK2αA^{156} DNA was transfected into individual neurons in the SN. Scale bar equals 25 μm.

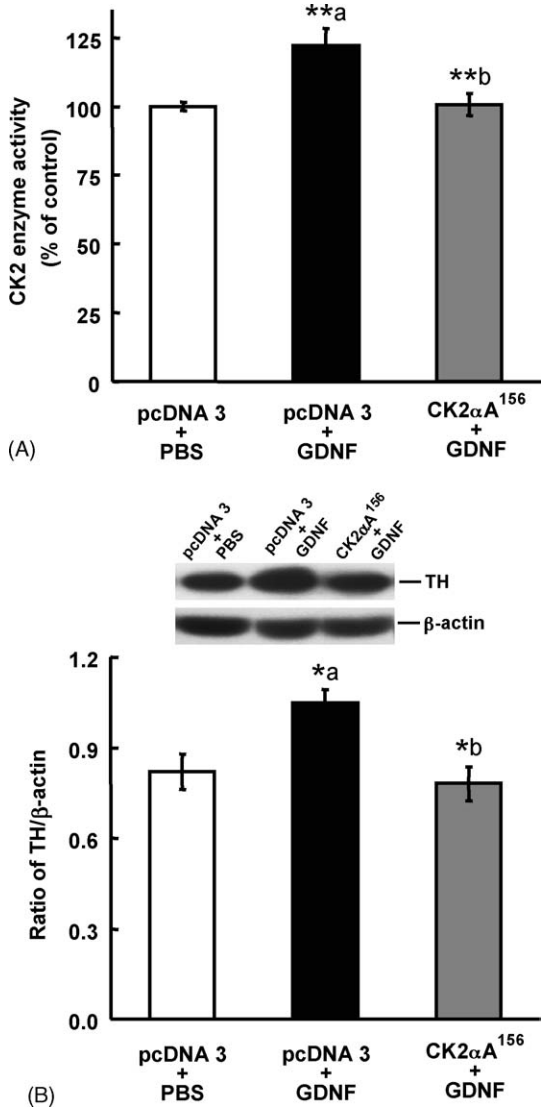

Fig. 4. Effects of GDNF and CK2αA^{156} mutant DNA on CK2 activity and TH protein level in the SN. The details of GDNF and CK2αA^{156} DNA treatments were described in Section 2. (A) CK2 activity assay showed that GDNF markedly increased CK2 activity in the SN and this effect was antagonized by prior CK2αA^{156} mutant DNA transfection. Values are expressed as percentage of pcDNA3 + PBS group. (B) Western blot analysis and representative gel pattern showed that GDNF significantly increased TH protein level in the SN and this effect was also blocked by prior CK2αA^{156} mutant DNA transfection. Data are mean ± S.E.M.; $n = 7$–8 each group. Statistical significance was evaluated by one-way ANOVA followed by Newman–Keul's method; * $p < 0.05$; (a) compared with the pcDNA3 + PBS group; (b) compared with the pcDNA3 + GDNF group.

sue in the present experiment. Results from Fig. 7A revealed that GDNF consistently increased CK2 activity in the SN ($p < 0.01$ by Newman–Keul's method). This effect is partially, but significantly, blocked by prior MPP$^+$ treatment ($p < 0.01$ when compared with MPP$^+$ group). On the other hand, neither GDNF treatment alone nor the combined MPP$^+$ and GDNF treatment affected CK2 protein level in the SN (Fig. 7B).

3.7. *Effect of CK2α DNA transfection on TH activity*

The above results suggested that CK2α is involved in the neuroprotective action of GDNF on DA neurons and it par-

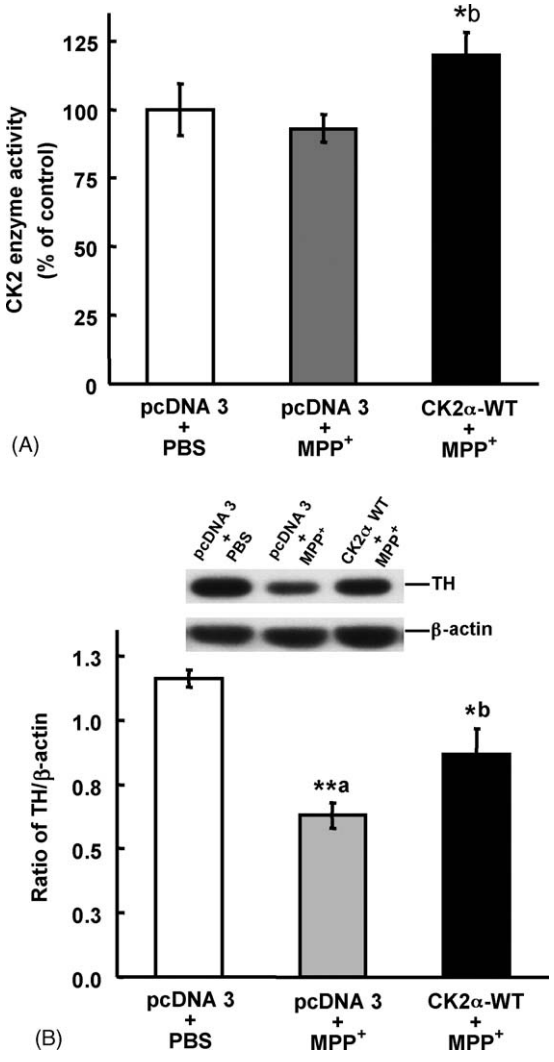

Fig. 5. Effects of CK2α-WT DNA and MPP^+ on CK2 activity and TH protein level in the SN. The details of CK2α-WT DNA and MPP^+ treatments were described in Section 2. (A) CK2 activity assay showed that MPP^+ decreased CK2 activity by approximately 10%, but this effect was reversed by prior CK2α-WT DNA transfection. Values are expressed as percentage of the pcDNA3 + PBS group. (B) Western blot analysis and representative gel pattern showed that MPP^+ significantly decreased TH protein level in the SN and this effect was partially, but significantly, antagonized by prior CK2α-WT DNA transfection. Data are mean ± S.E.M.; $n = 6$–9 each group. Statistical significance was evaluated by one-way ANOVA followed by Newman–Keul's method; $*p < 0.05$, $**p < 0.01$; (a) compared with the pcDNA3 + PBS group; (b) compared with the pcDNA3 + MPP^+ group.

tially protects DA neurons against MPP^+ toxicity. But how does CK2α exert this protective effect is unknown. In this experiment, we examined whether alteration in CK2α signaling may alter TH activity in the SN. Results from Fig. 8A revealed that CK2α-WT DNA transfection significantly increased TH activity in the SN ($tD = 5.23$, $p < 0.01$), whereas $CK2\alpha A^{156}$ DNA transfection decreased TH activity in the same area ($tD = 2.12$, $p < 0.05$). The TH protein level was unaffected by either the CK2α-WT or the $CK2\alpha A^{156}$ mutant DNA transfection ($p > 0.05$, Fig. 8B).

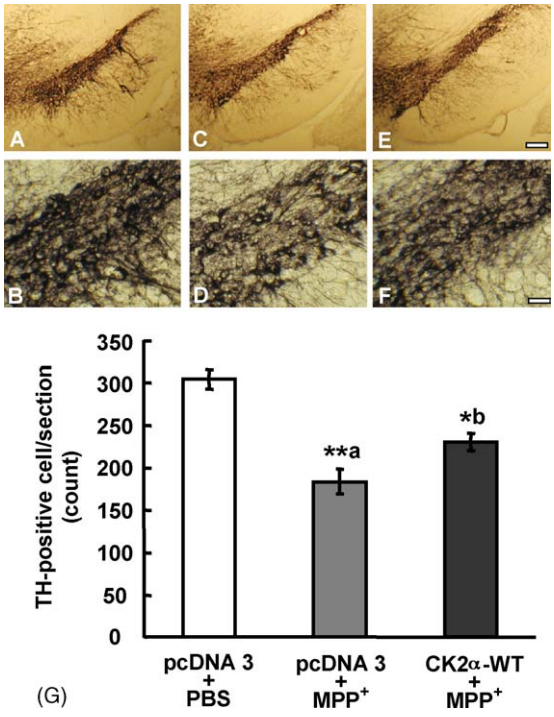

Fig. 6. Effects of CK2α-WT DNA and MPP^+ on TH-positive neurons in the SN. The details of MPP^+ and CK2α-WT DNA treatments were described in Section 2. The whole visual field (upper panel) and higher magnification (lower panel) photographs of the SN area of control (A and B, $n = 5$), MPP^+ (C and D, $n = 3$), and CK2α-WT + MPP^+ (E and F, $n = 4$) group, respectively. Scale bar equals 300 and 50 μm in the upper and lower panel, respectively. (G) Statistical analysis revealed that MPP^+ significantly decreased the number of TH-positive neurons in the SN ($p < 0.01$). Prior CK2α-WT DNA transfection partially, but significantly, reversed this effect ($p < 0.05$). Data are mean ± S.E.M. Statistical significance was evaluated by one-way ANOVA followed by Newman–Keul's method; $*p < 0.05$, $**p < 0.01$; (a) compared with the pcDNA3 group; (b) compared with pcDNA3 + MPP^+ group.

4. Discussion

The present study revealed that GDNF increased CK2 activity in the SN in a dose-dependent and time-dependent manner. The same concentration of GDNF has also been shown to promote DA neuronal function in vivo [10]. The effect of GDNF on CK2 occurred as soon as 1 h after acute GDNF treatment, peaked at 4 h and sustained for more than 8 h. Further, this activation was abolished by prior treatment of the receptor protein kinase inhibitor K-252b. In addition, K-252b has been shown to block GDNF-mediated DA uptake, DA neuron differentiation, and Ret phosphorylation in DA neurons [49]. Moreover, GDNF signaling pathway was demonstrated to be mediated through receptor Ret phosphorylation [16,26]. Therefore, the observation that K-252b blocked the effect of GDNF on CK2 activity suggests that CK2 is involved in receptor Ret-mediated GDNF signaling pathway. Our results are also consistent with a previous report showing that CK2 activity in rat hippocampal slice can be stimulated by BDNF and NT-4 in a similar dose-dependent and time-dependent manner, with BDNF showing a more potent effect

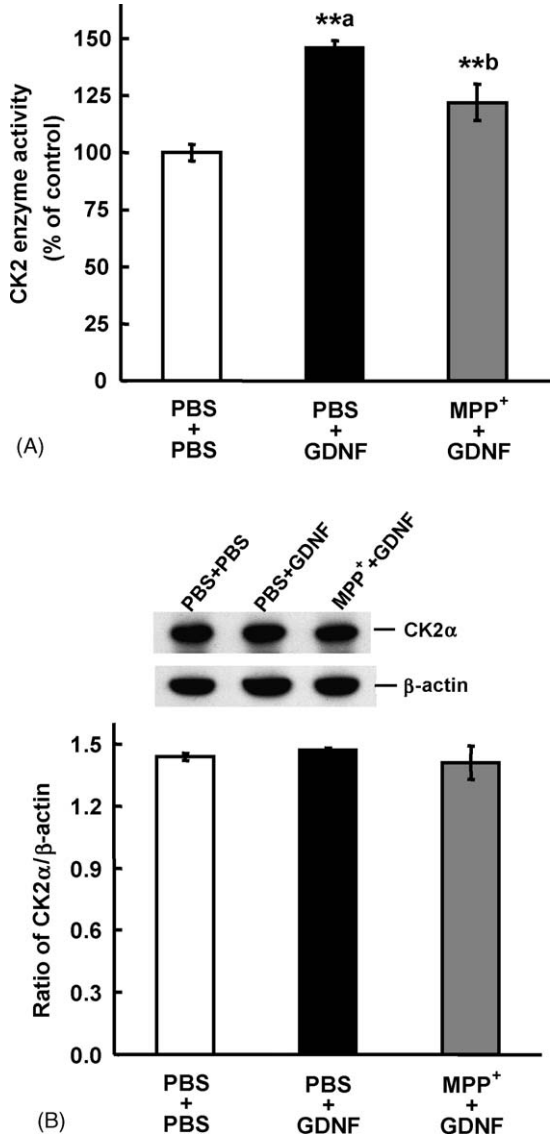

Fig. 7. Effects of GDNF and MPP^+ on CK2 activity and CK2α protein level in the SN. The details of GDNF and MPP^+ treatments were described in Section 2. (A) Enzyme activity assay showed that GDNF markedly increased CK2 activity in the SN and this effect was partially antagonized by prior MPP^+ treatment. Values are expressed as percentage of the PBS group. (B) Western blot analysis and representative gel pattern showed that CK2α protein level in the SN was not altered by either GDNF or MPP^+ + GDNF treatment. Data are mean ± S.E.M.; $n = 5$ each group. Statistical significance was evaluated by one-way ANOVA followed by Newman–Keul's method; $^{**}p < 0.01$; (a) compared with PBS group; (b) compared with GDNF group.

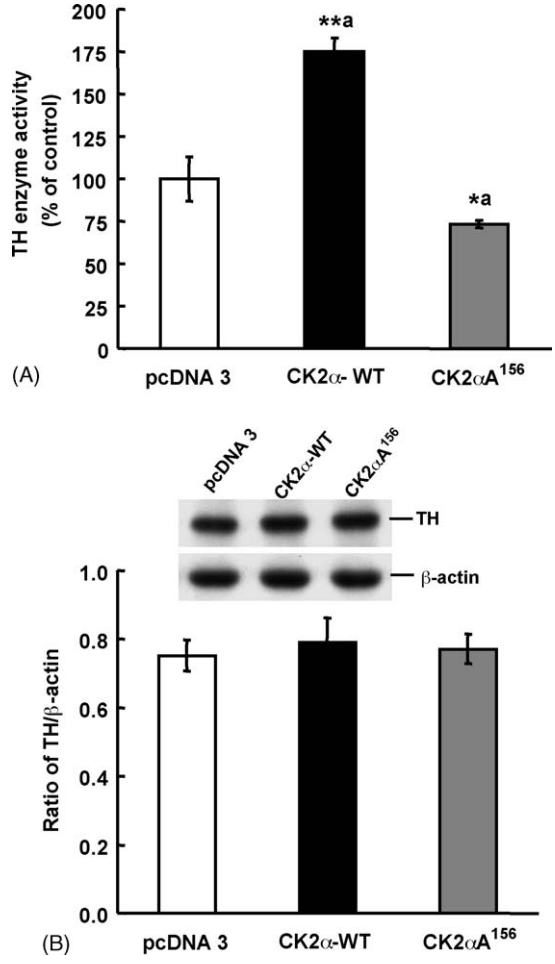

Fig. 8. Effects of CK2α DNA transfection on TH activity and TH protein level in the SN. Animals received different CK2α plasmid DNA transfection to the SN. (A) CK2α-WT DNA transfection markedly increased, whereas $CK2\alpha A^{156}$ mutant DNA transfection markedly decreased TH activity in the SN. (B) Western blot analysis and representative gel pattern showed that neither CK2α-WT nor $CK2\alpha A^{156}$ mutant DNA transfection altered TH protein level in the SN. Values are expressed as percentage of vector transfection group. Data are mean ± S.E.M; $n = 5$ each group. Statistical significance was evaluated by one-way ANOVA followed by Dunnett's *t*-test; $^{*}p < 0.05$, $^{**}p < 0.01$; (a) compared with pcDNA3 group.

than NT-4 does [6]. Because BDNF is also known as a neurotrophic factor for midbrain DA neurons [24], these results together suggest that CK2 is probably involved in the trophic action of neurotrophic factors on DA neurons.

There is a set of reports focusing on the regulation mechanism of CK2 by point mutation or deletion of the α subunit sequence [13,15,55]. These CK2 mutants were shown to lose their enzyme activity, but some inactive forms may recover their enzyme activity when associated with the β subunit in vitro [55]. The $CK2\alpha A^{156}$ mutant is known to not recover its enzyme activity when associated with the β subunit because it traps the β subunit to form an inactive complex [13]. This is also shown in the present study that $CK2\alpha A^{156}$ mutant DNA completely lost CK2 enzyme activity in vitro. However, in vivo transfection of either the wildtype CK2α-WT DNA or mutant $CK2\alpha A^{156}$ DNA to the SN area only altered total CK2 activity for approximately 40%. A major explanation for this discrepancy is probably due to the limitation of transfection efficiency in vivo. However, although $CK2\alpha\beta$ and $CK2\alpha'\beta$ activity is inhibited in the presence of $CK2\alpha A^{156}$ in vitro [30], free forms of CK2α and CK2α′ still express enzyme activity in vivo [14,41]. It is possible that $CK2\alpha A^{156}$ mutant DNA does not inhibit free form CK2α and CK2α′ activity that contributes to total CK2 activity measured in the present study. This explanation is supported by the finding that trans-

fection of the CK2αA^{156} mutant DNA to COS-7 cells only slightly decreased total CK2 activity [31].

On the other hand, although CK2αA^{156} mutant DNA transfection alone decreased CK2 activity by only 40% in the SN, it completely antagonized the enhancing effect of GDNF on CK2 activity. Furthermore, GDNF increased TH protein level in the SN. This observation parallels with the findings that intrastriatal GDNF infusion increased TH activity in the mesencephalon of neonatal rats and intranigral GDNF infusion increased TH immunoreactivity in the adult brain [4,10]. However, there are also some reports showing the opposite result that GDNF decreased TH protein level in the SN [52,54]. We do not know the explanation for this discrepancy yet. However, in those studies, either higher dose of GDNF (100 μg) was used or the assay was performed 30 days after GDNF treatment. The observed decrease in TH protein level may reflect a compensatory effect in response of continuous GDNF stimulation in order to maintain normal DA neuronal function. CK2αA^{156} mutant DNA transfection similarly blocked the effect of GDNF on TH protein level. In the present study, GDNF also markedly increased DA turnover in the ST and rotarod performance in rats. These results are consistent with other reports also showing enhanced DA turnover and motor function in rats treated with GDNF [10,40]. Meanwhile, the dominant-negative mutant of CK2α also antagonized these neurotrophic effects of GDNF on DA neurons. These results together suggest that the CK2 signaling pathway contributes to GDNF's pharmacological actions. Then, what might be the underlying mechanism involving CK2? Many studies have shown that CK2 could phosphorylate a variety of molecules that are associated with neuronal growth and guidance, such as neural cell adhesion molecule (NCAM), microtubule-associated protein and vitronectin [43,57,64]. In parallel with these findings, we have recently found that GDNF enhanced the expression of integrin and NCAM in TH-positive neurons [10]. Moreover, CK2 may be required for microtubule-associated protein for microtubule assembly-ing, a process associated with neurite outgrowth [61]. But the exact mechanism of how CK2 activation contributes to the neurotrophic effect of GDNF on DA neurons requires further investigation.

The phosphorylation status of TH is known to correlate with its enzyme activity [17,62]. Phosphorylation of some amino acid residues through different kinase-mediated pathway is identified to increase TH activity [21,38,50]. In the present study, CK2α-WT DNA transfection increased, whereas CK2αA^{156} mutant DNA transfection decreased TH activity without altering the TH protein level. In another study, we have similarly found that transfection of the focal adhesion kinase (FAK) mutant DNA significantly decreased FAK activity without altering FAK protein level in the hippocampus [66]. Whether CK2 activates TH directly and the possible phosphorylation site for CK2 activation of TH awaits to be determined. In addition, CK2α DNA transfection alone did not alter TH protein level in the SN, but CK2αA^{156} mutant DNA transfection prevented GDNF-induced increase in TH protein level. One possible explanation for this is that although alteration of CK2α only alters TH activity instead of TH protein level, CK2α may still regulate TH gene expression induced by GDNF. Moreover, there may be other pathways that mediate the effect of GDNF on TH expression. For example, NCAM has recently been identified as an alternative signaling receptor, independent of Ret, for GDNF family ligands [46]. TH gene expression is also regulated by the transcription factor CREB through the cAMP response element on promoter region of the TH gene [36,47]. Recently, up-regulation of CK2 phosphorylation accompanied with increased CREB phosphorylation was found to attenuate TNF-α induced cell death [29]. Thus, enhanced CREB phosphorylation by CK2 may increase TH gene expression upon neuronal damage.

The above results suggest that CK2 contributes to the neurotrophic effect of GDNF on DA neurons. GDNF is shown to protect DA neurons against toxin-induced damage [9,22]. We hypothesized that CK2 activation should also protect DA neurons against toxin-induced damage. Over-expression of CK2α combined with MPP^+ treatment was carried out to examine this hypothesis. Our results revealed that transient transfection of the CK2α gene partially, but significantly, antagonized the deteriorating effect of MPP^+ on DA neurons and on rotarod performance in rats. It also partially antagonized the deteriorating effect of MPP^+ on the number of TH-positive neurons. Although the protective role of CK2 in the nervous system has not been studied before, the present results are consistent with the observation that CK2 expression is associated with bone repair [63]. But the present results do not reveal the cellular mechanism of CK2 in protecting DA neurons against MPP^+ toxicity. MPP^+ toxicity on DA neurons has been similarly protected by over-expression of other kinases, such as Akt/PKB [53]. Adenoviral gene transfer of the JNK-interacting protein-1, which was indicated to promote Akt activation [28], has also been shown to block DA neuronal death in MPP^+-treated mice [65]. In addition, MPP^+ has been shown to activate caspase-mediated apoptosis in DA neurons [5,27], and protein ARC inhibits this apoptosis by selectively interacting with caspase [32]. Recent study has further demonstrated that it is necessary for ARC to be phosphorylated by protein kinase CK2 to prevent this apoptosis [35]. Whether CK2 signaling and Akt signaling activate the same downstream genes, such as ARC, that are necessary for DA neuron protection is currently under investigation.

In the present study, MPP^+ produced approximately 50% decrease in TH protein level in the SN and DA level in the ST. MPP^+ infusion for 2 days also produced approximately 35% decrease in the number of TH-positive neurons in the SN. However, CK2 activity was not significantly decreased by MPP^+ treatment in the present study. One explanation for this discrepancy is that the surviving DA neurons may have a higher CK2 activity to compensate for the loss of DA neurons produced by MPP^+. In another study,

we have similarly found that chronic MPTP treatment produced a compensatory increase in manganese superoxide dismutase activity in the SN [23]. Moreover, other studies have revealed that both CK2 activity and CK2 concentration were decreased in the cortex of Alzheimer's disease and schizophrenic patients [2,25]. Whether CK2 activity is also decreased in Parkinson's disease patients awaits further investigation.

In the present study, the CK2 plasmid DNA was transfected only to a limited area in the SN. The exact transfection efficiency is difficult to be estimated. But by counting the number of HA-tagged cells versus total cells in the same section of the SN tissue, it is probably less than 5% (unpublished observations). Yet, significant biological and behavioral changes were observed. We do not know the explanation for this observation yet; but in other studies transient transfection of the serum and glucocorticoid-inducible kinase gene to a limited area in the hippocampus also produced significant changes in spatial learning performance in rats [34,59]. It is possible that molecular alteration of the CK2 kinase may alter the downstream signaling necessary for normal CK2 function.

In summary, our results suggest that protein kinase CK2 is involved in the neuroprotective action of GDNF on DA neurons. Over-expression of the CK2α mutant DNA antagonized the neurotrophic effect of GDNF on DA neurons, but over-expression of the CK2α gene partially protected DA neurons against MPP^+-induced toxicity. Further, GDNF-induced increase in CK2 activity was abolished by prior MPP^+ treatment. The present results not only reveal a novel molecular mechanism for GDNF's action, but also shed light on new therapeutic strategies for Parkinson's disease.

Acknowledgement

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