Altered expression of Nurr1 in mice lacking dopamine D2 receptor

Thesis by

Kyou Chan Choi

Department of Medical Science

The Graduate School, Yonsei University

Altered expression of Nurr1 in mice lacking dopamine D2 receptor

Directed by Professor Jong-Eun Lee

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Kyou Chan Choi

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This certifies that the Master's Thesis of Kyou Chan Choi is approved.

[Thesis Supervisor : Jong-Eun Lee]

[Thesis Committee Member]

[Thesis Committee Member]

The Graduate School

Yonsei University

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ABSTRACT

Altered expression of Nurr1 in mice lacking dopamine D2 receptor

Kyou Chan Choi

Department of Medical Science

The Graduate School, Yonsei University

(Directed by Professor Jong-Eun Lee)

The dopamine D2 receptor (D2R) is highly expressed in the central nervous system and is crucial for the regulation of various neurophysiological functions. In mice lacking D2R (D2R -/-), the number of mesencephalic tyrosine hydroxylase (TH)-positive at embryonic stage was significantly low, when compared to that of wild-type (WT) mice, indicating an alternation in dopaminergic (DAergic) neuronal development in the absence of D2R. I have

investigated the expression of Nurr1, an orphan nuclear receptor is known to be essential for development of midbrain DAergic cells. RT-PCR and immunohistochemical anlaysis revealed that Nurr1 expression was selectively decreased in D2R -/- mice at embryonic stage, which was rescued in the adulthood. These data suggest that D2 receptor-mediated signaling is important in the development of midbrain do DAnergic neurons and probably in association with Nurr1.

Key words: Dopamine D2 receptor, Nurr1, Dopaminergic neuron, Differentiation

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Ⅰ. Introduction

Dopamine (DA) is the predominant catecholamine neuro-transmitter in the mammalian brain, where it controls diverse functions such as locomotor activity, neuroendocrine hormone release[1]. DA is synthesized from tyrosine hydroxylase (TH), the rate limiting enzyme in the catecholamine biosynthesis. The

majority of neurons that produce dopamine originate in the ventral midbrain in the substantia nigra (A9) and the ventral tegmental area (A10). The importance of proper DAergic system function is evident when any of these systems become compromised such as in Parkinson's disease[2], Schizophrenia[3]. The mechanism of DAergic neuronal development might be useful in directing commitment of DAergic neuronal cells *in vitro* before grafting in therapies for Parkinson's disease[21,22,23]. It is known that factors are often critical as components of developmental regulator, and transcription factors such as Nurr1[8,9], Ptx3[29,30,31], and Lmx1b[32] were known to be critical in development of DAergic neuron .

The transcription factor Nurr1 is an orphan member of the nuclear steroid/thyroid hormone receptor superfamily, which is expressed predominantly in the central nervous system[4].

Genetically modified mice lacking both copies of the Nurr1 gene fail to generate mesencephalic neurons[7,8]. The exogenous expression of Nurr1 into stem cells enhances differentiation and maturation into DAergic neurons. Recent in vitro studies showed that Nurr1 is able to activate TH gene transcription[9]. Despite these interesting findings, little is known about the mechanisms that

regulate Nurr1 expression in DAergic neurons.

Nurr1 can also activate gene transcription independent of ligands, possibly being influenced by other signaling pathways acting via cell-membrane-bound receptors.

DA acts through membrane receptors of the seven transmembrane domain G-protein coupled receptor family. Molecular cloning of the dopamine receptor family revealed five receptor subtypes, D1 through D5. They have been classically divided into two groups based on ligand specificity and effector coupling. The D1-like receptors, comprising D1 and D5 receptors, are positively coupled to adenylyl cyclase by the G protein Gs, whereas the D2-like receptors, comprising D2, D3 and D4 receptors, whose activation results in inhibition of adenylyl cyclase and suppression of cAMP production[11,12]. The D2 subtype of DA receptor represents the main autoreceptor of the DAergic system[16,17,18].

Recently, it has been reported that the signaling through D2 receptor (D2R) was found to be crucial for anti-proliferative effects and cell death in pituitary tumor cells[13]. Indeed, it was found that the DAergic agonists could induce the anti-proliferative effects and

cell death via D2R. I investigated that how the absence of D2R affects the neuronal cell death and development in mice lacking D2R (D2R -/-). I found that in the absence of D2R, development of DAergic neuron was blunted in association with altered expression of Nurr1 in these mice.

Ⅱ. Materials and Methods

1. Animals and dissections

The D2R-/- mice and wild type littermates originated from the mating of heterozygous D2R-/- mice identified by Southern hybridization analyses as described by Baik et al16. The D2R-/- mice had a mixed 129SV/C57BL/6 genetic background, with a 75% constribution of C57BL/6. Heterozygous D2R-/- mice were mated from 18:00h to 8:00h. Insemination was confirmed by vaginal plug and considered as E0. Pregnant mothers were killed in accordance with Society for Neuroscience guidelines. At E15 days, embryos were excised, brains were removed from the skull and the striatum and the ventral mesencephalon were dissected by mild mechanical trituration in ice-cold Ca^{++} and Mg^{++} free Hank`s balanced salt solution with a supplement of 4.2 mM sodium bicarbonate.

2. Mesencephalic and striatal culture

Cells were dissociated by mechanically dispersing tissue pieces with a 1 ml pipet. Subsequently, cells were centrifuged at 200 g for 5min and resuspended in Minimum essential medium (MEM, SIGMA) containing 2 mM L-glutamine, 1 g/L glucose, 5 % heat

inactivated fetal bovine serum, 5 % heat inactivated horse serum. Dissociated cells were plated in 35 mm culture dishes onto pre-coated 24 mm glass cover slips with 0.1 mg/ml ploy-D-lysine (SIGMA) and 2 μg/ml laminin (SIGMA). After 2 days in cultures, 5 μM cytosine β-D-arabinofuranoside (SIGMA) was added to inhibit glial cell proliferation. Cultures were maintained at 37 ℃ in a humidified atmosphere of 5 % CO_2 and 95 % air for 6 days. The Exposure to MPP^+ was begun on Day 7 *in vitro*. The medium was removed and replaced with serum-free growth medium. MPP^+I^-(Research Biochemicals, Inc.) was dissolved in culture medium and added at the concentration specified for 24 hr.

3. Immunocytochemistry

The cultures were processed for TH immunocytochemistry using the biotin-avidin peroxidase method. Cultures grown on cover slips were rinsed with 0.1 M phosphate buffered saline (PBS, pH 7.4) and fixed for 30 min with 4 % paraformaldehyde in 0.1 M PBS at 4 ℃ overnight. After washing (3X) with PBS, the fixed cultures were treated for 30 min with blocking solution (1 % BSA, 0.2 % Triton X-100, and 10 % normal serum in PBS). The cultures were

then incubated overnight at 4 ℃ with a monoclonal antibody against TH (1:1000, Diasorin Stillwater, MN, USA). After washing, the cells were exposed to a biotinylated anti-mouse IgG (1:200, Vector lab.) for 1 hr at room temperature. Cultures were rinsed with PBS, then treated for 1 hr at room temperature with avidin-biotinylated horseradish peroxidase complex (Vectastain Elite ABC kit, Vector lab. Burlingame, CA) The peroxidase was visualized with diaminobenzidine (DAB) and hydrogen peroxide. Specimens were dehydrated through a graded ethanol series and mounted on slide glasses with Permount® solution (Fisher Scientific, New Jersey, USA). The number of TH-positive neurons were evaluated under a microscope and counted in 20 randomly selected fields at 100 X magnification. A 2.5 mm^2 square grid was inserted into the microscopic field.

For immunohistochemical analysis of tissues, brains were removed, rinsed in ice-cold PBS fixed 4% paraformaldehyde, cryoprotected in 30% sucrose in 0.1 M PBS (pH 7.4) overnight at 4℃, placed in OCT Tissue Tek embedding medium (Sakura®, CA, USA) and frozen on dry ice. Thin section (10㎛) were cut on a microtome and the kept at -80 ℃ until they were processed for

immunohistochemistry.

4. RT-PCR reaction

Tissues were removed from freshly killed mice and homogenized by polytron homogenizer and lysis buffer containing 3 M $LiCl_2$, 6M urea, 0.1 % SDS, 10 mM NaOAc (pH 5.0), and 0.2 mg/ml heparin. After overnight precipitation at 4℃, the precipitates was washed with buffer containing 4 M $LiCl_2$, 8 M urea and 10 mM NaOAc (pH 5.0) and then extracted with acidic phenol. Total RNA was prepared from isolated mesencephalon of mice brain using LiCl RNA extraction buffer. First strand cDNAs were generated from total RNA using reverse-transcription with random primer by denaturing at 90 ℃ for 4 min, annealing at room temperature for 10 min and extending at 42 ℃ for 50 min. The following primers were used to amplify target cDNA: Nurr1, 5′TAAAAGGCCGGAGAGGTCGTT3′,5′CTCTCTTGGGTTCCT TGAGCC3′ β-actin, 5′GATGACGATATCGCTGCGCT3′, 5′GCTCATTGCCGATAGTGATGACCT3′. Conditions for PCR amplifications were as follows: 94 ℃ for 5 min, 30 cycles at 94 ℃ for 1 min, 60 ℃ for 1 min, 72 ℃ for 1 min, and final extension at

72 ℃ for 7 min. The PCR reaction products were run on 1.5 % agarose gels containing ethidium bromide (0.5 μg/ml), to mark and visualize the PCR products.

5. Cell Culture

HEK (Human Embryonic Kidney) 293 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Gaithersbrug, Maryland, USA) supplemented with 10% fetal bovine serum, penicillin G (100 units/ml), streptomycin sulfate (100 μg/ml) and amphotericin B (250mg/ml).

6. Transfection and Luciferase reporter gene assay

HEK (Human Embryonic Kidney) 293 cells were grown to confluence in 6-well plates. Transient transfections of the HEK293 were performed using PEI (polyethylenimine)-mediated transfection reagent, jetPEI™ (Qbiogene, Inc., Carlsbad, CA, USA). Briefly, 70% ~ 80% confluent monolayers in 6-well plates were incubated at 37°C with transfection mixture containing 1.5 μg of D2R, 1.5 μg of Nurr1, 1.5 μg of a reporter plasmid regulated by three copies of the NurRE (Nur response element), 0.5 μg of

pCH110 carrying the β-galactosidase gene. After 3 h, the transfection mixture was replaced with fresh growth medium. Assays were performed 48 h after transfection. Cells were preincubated overnignt in serum free DMEM medium before cells were treated with dopamine. The cells were pretreated with Haloperidol (1μM) for 5min or Pertussis toxin (100ng/ml) for 12h, followed by treatment with various concentrations of dopamine for a further 6 hr at 37 °C. HEK293 cells were assayed for luciferase activity using the luciferase assay system (Promega), and luminescence was measured using a 96-well luminometer (Microlumat; EG & Berthold, Bad Wilbad, Germany). The expression of the reporter gene was normalized using β-galactosidase activity[19,20]. Transfection in the control group was performed under the transfection conditions above with 1.5 μg of D2R, 1.5 μg of Nurr1, 1.5 μg of a reporter plasmid regulated by three copies of the NurRE (Nur response element), 0.5 μg of pCH110 carrying the β-galactosidase gene, but without stimulating by dopamine. The mean values of the data obtained were fitted to a sigmoid curve with a variable slope factor using the nonlinear squares regression in a GRAPHPAD PRISM®. EC_{50} (nM) values

were described as mean ± S.E. All of the luciferase reporter gene activity assays were performed at least four independent.

Ⅲ. Results

1. The Role of D2R in cell death and development

To investigate the regulation of DAergic neuronal cell death and development in WT and D2R-/- mice, I used primary neuronal cells of the mesencephalon from WT and D2R-/- mice. The selective neurotoxin 1-methyl-1,2,3,6-tetra-hydropyridine (MPTP) has been widely used to generate animal models of Parkinson's disease. When administered in vivo, MPTP crosses the blood–brain barrier and is converted, mainly in glial cells, into its effective form, 1-methyl-4-phenylpyridinium (MPP+), by monoamine oxidase B[33]. MPP+ finally enters mitochondria by an energy-dependent mechanism inhibiting the activity of this organelle and leading to a drop in cellular ATP levels and subsequent DAergic neuronal cell death[34].

TH immunohistochemistry showed that treatments of MPP+ (10 μM) for 24hr induced DAergic neuronal cell death (Fig. 1 C and D). After 24hr exposure of the MPP+ (1-10 μM) to the cells, significant decrease of the DAergic neuronal cells was observed in a dose dependant manner (Fig 2). D2R-/- mice was more susceptible to neurotoxin, when compared to WT (Fig 3B). Interestingly, the

number of mesencephalic TH-positive neurons at embryonic stage in D2R-/- mice was significantly low, when compared to that of WT mice (Fig. 2, and 3A). However, comparison of total number of neurons in E15 days mesencephaon of WT and D2R-/- showed no significant differences between the two genotypes (Fig. 3C). Tyrosine hydroxylase (TH) expression level was monitored in SNc (substantia nigra compacta) and VTA of D2R-/- and wild-type (WT) mice. These results suggest that absence of D2Rs may be involved in the development of mesencephalic neuron.

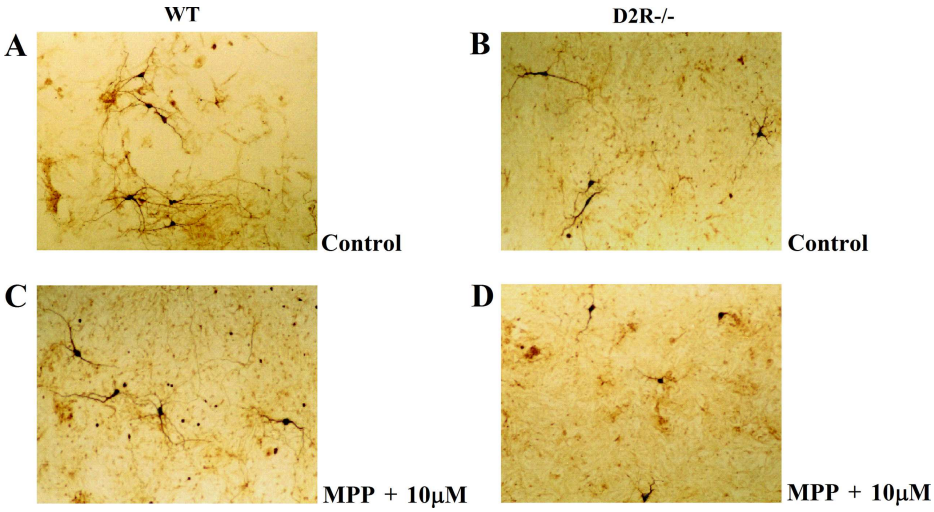


Fig 1. The representative Photomicrographs of TH-positive neurons in ventral mesencephalic-striatal co-culture on day 8. (A) Wild-type (WT), control (B) D2R-/- , control (C) Wild-type (WT), exposure to 10 μM of MPP+ for 24hr (D) D2R-/-, exposure to 10 μM of MPP+ for 24hr.

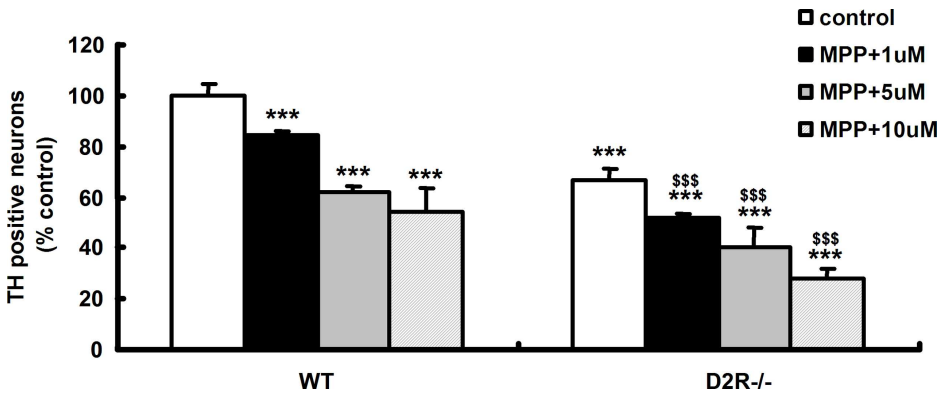


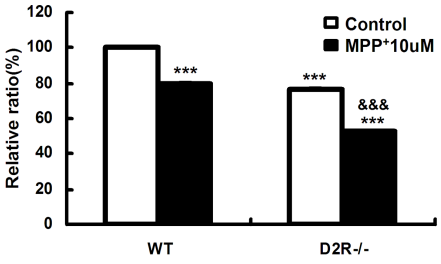
Fig 2. TH staining in mouse mesencephalic culture. TH+ cells counting in mesencephalic cultures established from WT and D2R-/- E15 days mice after treatment with MPP+ (1-methyl-4-phenylpyridinium). WT (n=5) and D2R-/- (n=7) embryonic mice that were treated 1-10 μM with MPP+ (24hr exposure) were performed by immunocytochemistry. * $p < 0.001$ compared wild type control. $ $p < 0.001$ compared with D2R-/- control.

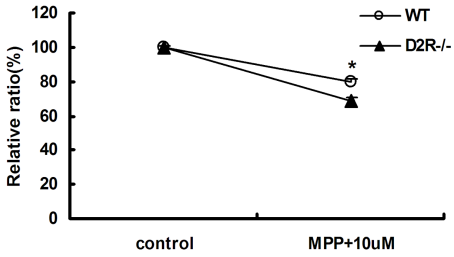
A

Ratio	control	MPP+10uM
WT	100	80.1
D2R-/-	76.4	52.5

B

Ratio	control	MPP+10uM
WT	100	80.1
D2R-/-	100	68.7





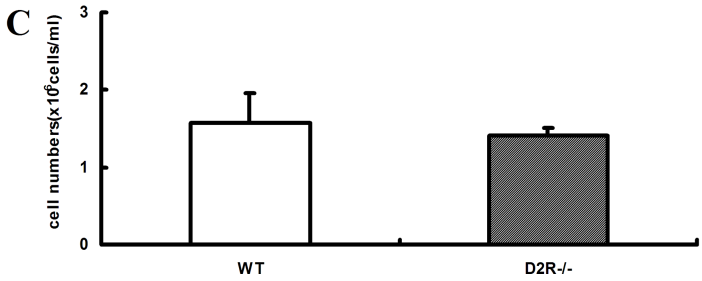


Fig 3. TH staining in mouse mesencephalic-striatal co-culture. (A), (B) TH-positive cells counting in mesencephalic-striatal co-cultures established from wild-type (WT) and D2-/- E15 days mice after treatment with MPP+ (1-methyl-4-phenylpyridinium). WT (n=4) and D2R-/- (n=5) embryonic mice that were treated with 10 μM MPP+ (24hr exposure) were performed by immunocytochemistry. * $p < 0.001$ compared wild type control. $ $p < 0.001$ compared with D2R-/- control. (C) The number of mesencephalic neurons of E15 days WT and D2R-/- mice.

2. Nurr1 mRNA expression during midbrin development in WT and D2R-/- mice.

As mentioned above, the transcription factor/nuclear receptor Nurr1 is essential for the development of mesencephalic DAergic neuron[7,8]. Nurr1 mRNA is expressed in substantia nigra pars compacta (SNC) and the ventral tegmental area (VTA). By using semiquantitative RT-PCR and immunohistochemistry, I analysed the Nurr1 gene expression during ontogeny of the mesencephalon. RT-PCR showed that Nurr1 expression was selectively decreased in D2R -/- mice at embryonic stage and was rescued in the adulthood (Fig 4). From embryonic 15 days to post-natal 1day, Nurr1 immunoreactivity was selectively decreased in D2R-/- mice. However in the adult, there was no obvious difference in the Nurr1 immunoreactivity of ventral tegmental area (VTA) in WT and D2R-/- mice (Fig 5B and C). These data suggest that in the absence of D2R, the development of DAergic neuron is blunted in association with altered expression of Nurr1.

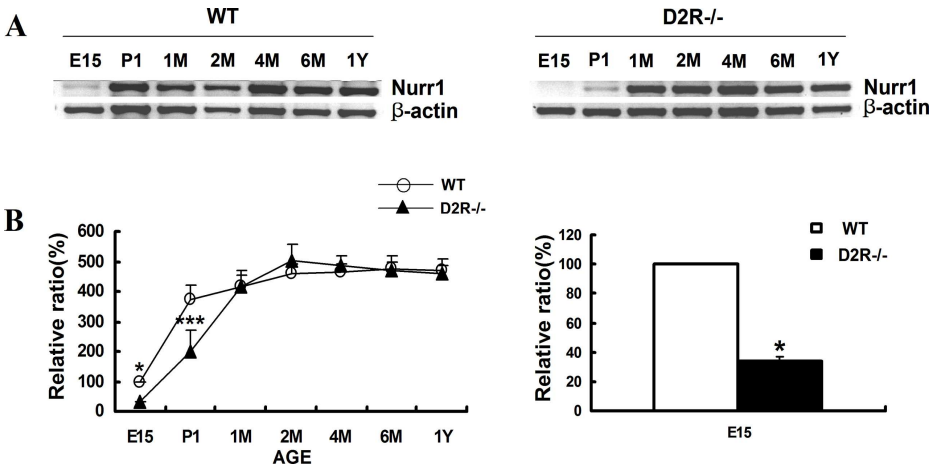


Fig 4. (A) Developmental expression of Nurr1 mRNA in wild-type (WT) and D2R-/- mice by RT-PCR analysis. Total RNAs from midbrain of WT and D2R-/- mice were analyzed by reverse transcription PCR (RT-PCR) for Nurr1 transcripts. RT-PCR analysis was performed at various stage of development. The developmental stages analyzed were embryonic 15 days (E15), postnatal 1 day (P1), M (1, 2, 4, 6 months) and Y (1 year). * $p < 0.001$ compared with wild-type E15 days. (B) Data were plotted (in %) for Nurr1 mRNA levels, respectively, in relation to mRNA levels of the β-actin gene, as an internal standard.

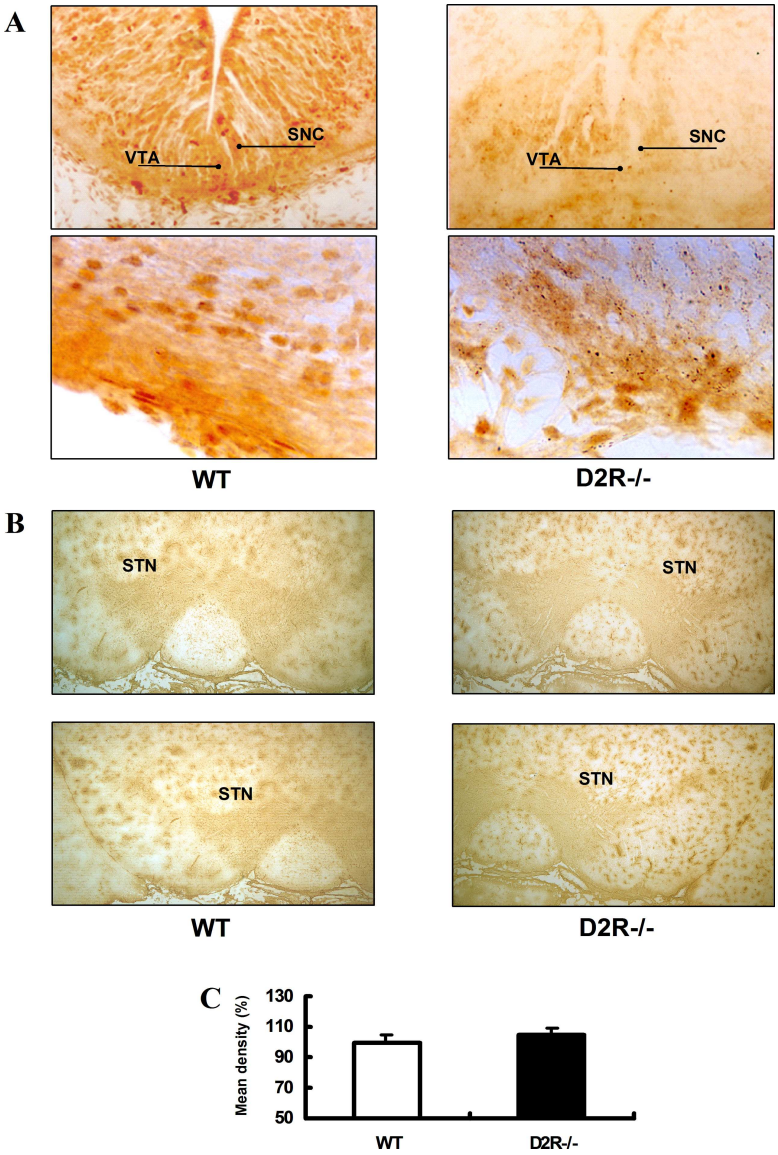


Fig 5. Immunohistochemistry for Nurr1 on coronal sections of ventral midbrain in WT and D2R-/- mice. (A) Nurr1 immunoreactivity in ventral tegmental area (VTA) of E15 days in WT and D2R-/- mice. (B) Nurr1 immunoreactivity in ventral tegmental area (VTA) of adult in WT and D2R-/- mice. (C) The relative Nurr1 immunoreactivity in ventral tegmental area (VTA) of adult in WT and D2R-/- mice.

3. Transactivation of Nurr1 by dopamine D2 receptor.

To investigate whether the activation of Nurr1 is involved in signaling pathway mediated by D2R, the DNAs encoding D2R, Nurr1, and a reporter plasmid regulated by three copies of the NurRE (Nur response element) were transiently transfected into HEK 293 cells. When increasing concentrations dopamine were treated, a typical dose-dependant and saturable induction of luciferase activity was observed. However, I detected no significant effect of dopamine on cells transfected with either D2R or Nurr1 alone (Fig 6A). To confirm activation of Nurr1 is specific to the stimulation of D2R, the cells were pretreated with D2R antagonist, haloperidol for 5 min, followed by the stimulation of D2R with various concentrations of dopamine. D2R-mediated reporter gene activation was completely inhibited by the treatment of haloperidol (Fig 6B), suggesting that the activation of Nurr1 is mediated by stimulation of D2R.

It is now generally accepted that numerous GPCRs can also activate MAPK to influence diverse cellular processes, ranging from the regulation of neuronal survival to cell differentiation and gene expression. D2R-mediated MAPK activation involves

predominantly Gßγ subunit-mediated signaling[27]. Treatment of PTX caused complete inhibition of Nurr1 activation in HEK 293 cells by stimulation of D2R with DA (Fig 6C), suggestion that the activation of Nurr1 is involved in G protein dependant signaling.

The synthetic compound PD98059 has been characterized as a selective inhibitor of the MAPK pathway by preventing the activation of MEK (MAPK kinase)-1[28]. Pretreatment of cells with 20 μM of PD98059 for 30 min completely inhibit the NurRE-dependent transcriptional activation of luciferase reporter gene by the stimulation of D2R (Fig 6E), suggesting that D2R-medited MAPK activation is essential for the activation of Nurr1.

It was recently suggested that GPCR-mediated activation of induction of Nurr1 in corticotrophs was protein kinsase A (PKA) dependant. The role of PKA on the D2R-mediated activation of Nurr1 was investigated. Treatment of a PKA inhibitor, H-89 did not affect the activation of Nurr1 by the stimulation of D2R (Fig 6D).

These results suggest that the signaling through D2R induces the activation of Nurr1 by PKA-independent and Ras-dependant MAPK pathaway.

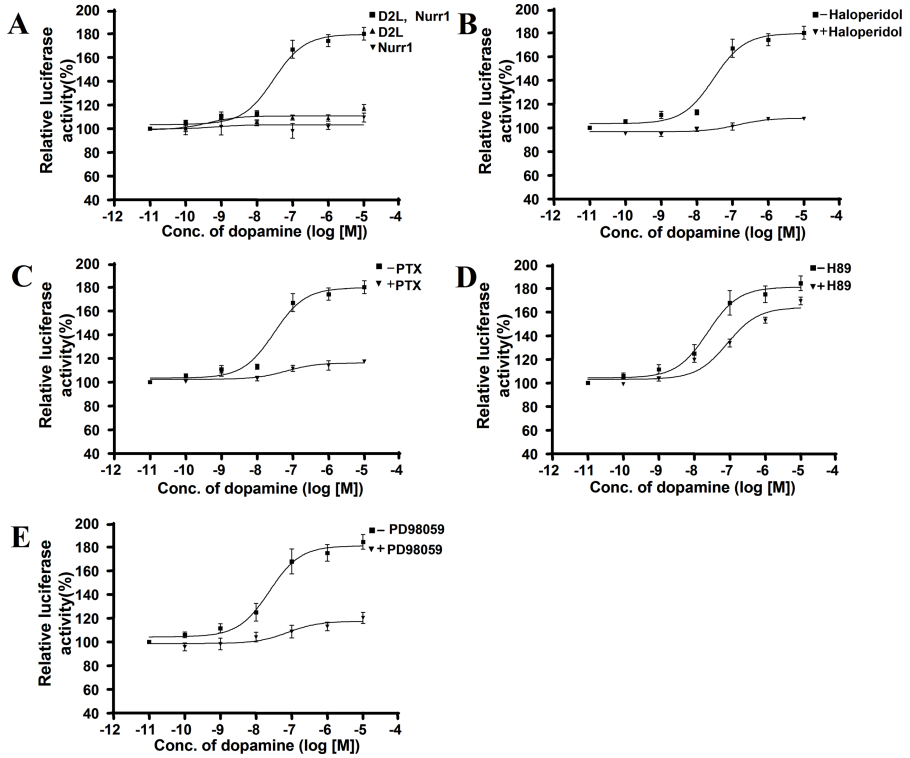


Fig 6. (A) NurRE-dependent transcriptional activation of luciferase reporter gene upon stimulation of D2R in HEK293 cells. HEK293 cells were transiently transfected with the D2R and Nurr1 together or either Nurr1/ D2R alone followed by the stimulation of D2 receptor with various concentrations of dopamine for 6 h at 37 °C. Effect of dopamine D2 receptor antagonist, Haloperidol (1μM for 5min) (B), (C) an inhibitor of Gi proteins, Pertussis toxin (PTX) (100ng/ml for 12h), (D) PKA inhibitor, H-89 (1μM for 20min), and (E) MAP Kinase Kinase inhibitor, PD98059 (10μM for 30 min) on NurRE-dependent transcriptional activation of luciferase reporter gene upon stimulation of D2R.

Expression/Treatment	EC_{50} (*nM*)
D2L, Nurr1	**58.8 ± 29.24**
D2L only	**ND**
Nurr1 only	**ND**
+Haloperidol	**ND**
+PTX	**ND**
+PD98059	**ND**
+H89	**75.4 ± 9.67**

Table1. Estimated EC50 values of dopamine for D2 receptor in HEK293 cells. EC50 values were determined for the Nur response element luciferase reporter gene activity stimulated by dopamine. Data are mean ±S.E. from at least four independent experiments.

Ⅳ. Discussion

As mentioned above, the mechanism of DAergic neuronal development might be useful in directing commitment of DAergic neuronal cells *in vitro* before grafting in therapies for Parkinson's disease[21,22,23]. It is known that factors are often critical as components of developmental regulator, and transcription factors such as Nurr1[7,8], Ptx3[29,30,31], and Lmx1b[32] were known to be critical in development of DAergic neuron . The transcription factor Nurr1 is an orphan member of the nuclear steroid/thyroid hormone receptor superfamily, which is expressed predominantly in the central nervous system[4]. Nurr1 mRNA is expressed across many regions of the developing central nervous system but, in the adult rodent brain, its expression is restricted to the temporal cortex, hippocampus, habenular nuclei, some thalamic nuclei and to DAergic neurons of the substantia nigra pars compacta (SNpc) and ventral tegmental area (VTA)[5,6]. The onset of Nurr1 expression in the ventral midbrain occurs at embryonic day 10.5 before the appearance of the DAergic marker enzyme, TH, at embryonic day 11.5. Expression of Nurr1 continues in mature DAergic neurons during adulthood, suggesting that Nurr1 may also be required for

normal function of mature DAergic neurons.

Little is known about the mechanisms that regulate the development of DAergic neurons and involvement of Nurr1 in this regulation. The importance of Nurr1 in developing and mature DAergic neurons has focused interest on Nurr1 as a potential drug target.

The MAPK signaling cascade is a prominent cellular pathway used by many growth factors, hormones and neurotransmitters to regulate diverse physiological functions[25]. It has also been observed that the ↓° subunits of G-proteins are able to mediate Ras-dependent MAPK activation[26]. It has been recently shown that the agonist-stimulated D2R activates MAPK activity Pertussis toxin (PTX) treatment completely revoked stimulation of MAPK mediated by D2R, demonstrating that D2R couple to pertussis toxin-sensitive G proteins in this signaling[27].

DA is able to selectively activate some members of the steroid receptor superfamily[10]. Nurr1 can also activate gene transcription independent of ligands, possibly being influenced by other signaling pathways acting via cell-membrane-bound receptors such as dopamine D2 receptor.

These results suggest that signaling through D2R in association with Nurr1 plays a dominant role in development of midbrain DAergic neuron. Future experiments will be required to assess the effect of D2R antagonist, haloperidol, on DAergic neuronal cell death in primary mesencephalic neuron culture system. The identification of interaction between the orphan nuclear receptor Nurr1 and D2R will allow us to elucidate the key molecular mechanism responsible for the DAergic neuronal development and to provide a possible therapeutic strategy to several pathologies with DAergic neuronal degeneration.

Ⅴ. Conclusion

This present study showed role of D2R on cell death and development. These results have led me to the following conclusions.

1. **TH immunohistochemistry showed that treatments of MPP+ induced DAergic neuronal cell death. D2R-/- mice was more susceptible to neurotoxin, when compared to WT. The number of TH-positive neurons in ventral tegmental area was significantly low, when compared to that of WT mice.**
2. **From embryonic 15 days to post-natal 1day, Nurr1 immunoreactivity was selectively decreased in D2R-/- mice. However in the adult, there was no obvious difference in the Nurr1 immunoreactivity of ventral tegmental area (VTA) in WT and D2R-/- mice.**
3. **Treatment of increasing concentrations dopamine induced a typical dose-dependant and saturable Nurr1 activity, while D2R-mediated reporter gene activation was completely inhibited by the treatment of haloperidol. The activation of Nurr1 is involved in G protein dependant signaling and Ras-MAPK**

singnaling.

Taken together, these data suggest that absence of D2Rs may be involved in the development of mesencephalic neuron. DA can activate orphan neuclear receptor Nurr1. It may be possible that signaling through D2R in association with Nurr1 plays a dominant role in development of midbrain DAergic neuron.

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국문요약

도파민 D2 수용체 결여 마우스에서 Nurr1 의 발현 조절

(지도교수 이종은)

최 규 찬

연세대학교 대학원

의과학과

도파민(dopamine)은 중추 신경계에 가장 많이 존재하고 있는 카테콜아민(catecholamine)의 하나로 운동기능이나 그 조절, 호르몬 분비 조절 등 여러 가지 생리적 기능에 관여한다. 도파민 수용체는 그 구조와 약리학적 성질에 의해 D1과 D2로 나눌 수 있는데, D2는 뇌의 여러 부분에 발현되며 여러 가지 도파민성 신경기능에 관여하는 것으로 알려져 있다. 본 연구에서는 도파민 D2 수용체가 결여된 마우스와 정상적인 마우스를 이용하여 도파민 D2 수용체의 중뇌 도파민성 신경세포의 발달에서의 기능을 알아보고자 도파민 D2 수용체가 결여됨으로 인해 중뇌 도파민성 신경세포의 발달에 결함이 있는 지를 in vitro culture model인 일차신경세포배양을 통해 확인하였다. 그 결과 도파민 D2 수용체가 결여된 마우스의 발달단계에 있는 중뇌의 일차신경세포배양체에서 도파민성 신경세포의 표지 단백

인 TH의 면역양성반응성을 갖는 신경세포의 수가 정상적인 쥐에 비해 현저히 저하되어 있는 것을 관찰하였다. 또한 도파민 D2 수용체가 결여된 마우스와 정상적인 마우스의 중뇌에서 중뇌 도파민성 신경세포의 분화와 발달에 중요한 역할을 하는 전사조절인자로 알려진 Nurr1의 면역활성도를 면역세포화학분석을 통해 발생단계에서 측정하고, 배아시기부터 성인기까지 각 단계별 Nurr1의 mRNA 발현 수준을 측정한 결과 도파민 D2 수용체가 결여된 마우스가 정상적인 마우스에 비해 배아시기에서 낮은 Nurr1의 mRNA 발현 수준을 보였다. 이는 도파민 D2 수용체가 결여됨으로 인해 중뇌 도파민성 신경세포의 성장과 분화에 문제가 있는 것을 의미한다. 이러한 연구 고찰은 도파민 D2 수용체의 기능과 신호전달 체계를 이해하는 데뿐만 아니라 도파민성 신경세포로의 분화과정을 인위적으로 조절할 수 있는 가능성을 제시할 수 있을 것이라 생각된다.

핵심되는 말 : 도파민 D2 수용체, **Nurr1**, 도파민성 신경세포, 분화