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Research report

Selective increase of Nurr1 mRNA expression in mesencephalic dopaminergic neurons of D2 dopamine receptor-deficient mice

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Abstract

The orphan nuclear receptor Nurr1 is critical for the survival of mesencephalic dopaminergic precursor neurons. Little is known about the mechanisms that regulate Nurr1 expression in vivo. Other members of this receptor family have been shown to be activated by dopamine. We sought to determine if Nurr1 expression is also regulated by endogenous dopamine through dopamine receptors. Consequently, we investigated the expression of Nurr1 mRNA in genetically modified mice lacking both functional copies of the D2 dopamine receptor gene and in their congenic siblings. Quantitative in situ hybridization demonstrated a significant increased expression of Nurr1 mRNA in the substantia nigra pars compacta and the ventral tegmental area of D2 dopamine receptor −/− mice. No change in Nurr1 expression was detected in other brain regions, such as the habenular nuclei and temporal cortex. Among the cell groups studied, mesencephalic dopaminergic neurons are unique in that they express both Nurr1 and the D2 dopamine receptor, and synthesize dopamine. Thus, it seems plausible that the selective increase in Nurr1 expression observed in D2 receptor-deficient mice is the consequence of an impaired dopamine autoreceptor function.

Theme: Neurotransmitters, modulators, transporters and receptors

Topic: Receptor modulation, up- and down-regulation

Keywords: Dopamine receptors; Nurr1; Substantia nigra

1. Introduction

The transcription factor Nurr1 (also known as RNR-1, HZF-3, TINUR and NOT) is an orphan member of the nuclear steroid/thyroid hormone receptor superfamily, which is expressed predominantly in the central nervous system [13]. 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 dopamine (DA) neurons of the substantia nigra pars compacta (SNpc) and ventral tegmental area (VTA) [20,28].

Genetically modified mice lacking both copies of the Nurr1 gene fail to generate mesencephalic DA neurons [3,22,29]. Recent in vitro studies showed that Nurr1 is able to activate tyrosine hydroxylase gene transcription [19]. Despite these interesting findings, little is known about the mechanisms that regulate Nurr1 expression in DA neurons. DA is able to selectively activate some members of the steroid receptor superfamily [18]. Since D2-class DA autoreceptors are important modulators of mesencephalic

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DA cell function [6,9,14,16], it seems plausible that activation of D2-class autoreceptors will modify Nurr1 expression in DA neurons. To test this hypothesis, we evaluated the expression of Nurr1 mRNA in mesencephalic DA neurons and other neuronal groups, in mice lacking both functional copies of the D2 receptor gene and their congenic wild-type siblings [11], using quantitative in situ hybridization. Furthermore, we studied the expression of D2 and D3 DA receptors in the SNpc and VTA by ligand autoradiography.

2. Materials and methods

2.1. Animals and tissue processing

The original F_2 hybrid strain (129/Sv×C57BL/6J) of mice expressing the mutated D2 DA receptor allele was obtained as previously described [11]. For the experiments reported below we used congenic N_5 mice of both sexes (wild-type: $n=6$ and D2 DA receptor $-/-$: $n=8$), generated by back-crossing D2 DA receptor $+/-$ mice with wild-type C57BL/6J mice for five generations, as reported elsewhere [11]. Mice were decapitated 15 weeks after birth and their brains quickly removed and frozen in isopentane. Coronal 15 μm-thick tissue sections were cut in a cryostat, thaw-mounted onto cold, gelatin-coated glass slides, and stored at −80°C until processing.

2.2. Synthesis of ^{35}S-labeled riboprobes

In order to generate a Nurr1 cDNA clone, total RNA was extracted from mouse mesencephalic tissue samples following the method described by Chomczynski and Sacchi [5]. A 600-bp fragment of the Nurr1 gene was amplified using specific primers (5'-GCAAACCCTGA CTATCAGATGAG-3'; 5'-ACCAAGTCTTCCAATTTC-AGG-3') by RT-PCR (Access RT-PCR system, Promega). The RT-PCR product was subcloned into a homemade Bluescript vector (Stratagene) following the manufacturer's instructions. Clones bearing the right Nurr1 probes were isolated and their orientation was determined by restriction analysis. For the synthesis of antisense and sense ^{35}S-labeled riboprobes, plasmids were linearized using restriction sites (HindIII and BahmHI), and transcribed in vitro by T3 and T7 polymerases, respectively (Promega), following the method described by Vila et al. [27].

2.3. In situ hybridization and binding assay

The protocol for in situ hybridization with ^{35}S-riboprobes has already been published [27]. Briefly, slide-mounted sections were post-fixed, acetylated, treated with Tris–glycine and dehydrated. After air-drying, sections were covered with 50 μl of hybridization solution containing 2.5×10^6 cpm of ^{35}S-labeled probe, and incubated for 3.5 h at 50°C in humid chambers, 30 min at 37°C with RNAse A, rinsed at 50°C in 50% formamide/2×SSC, and left overnight in 2×SSC at room temperature. Finally, the sections were dehydrated, delipidated in xylene, rinsed in 100° ethanol, and air dried. Autoradiograms were generated by exposing the slides to X-ray sensitive films (Hyperfilm β-max, Amersham, Buckinghamshire, UK) for 2 to 7 days at 4°C. The slides were then dipped in Kodak NTB2 emulsion diluted 1:1, air dried, and stored at 4°C for 2 weeks.

For ^{125}I–sulpiride autoradiography [1], slide-mounted sections were washed in 'Tris-ions buffer' (50 mM Tris–HCl buffer containing 120 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5.7 mM ascorbic acid, pH 7.4), and then incubated in the same buffer containing 0.2 nM ^{125}I–sulpiride (Amersham, specific activity 2000 Ci/mmol) for 30 min at room temperature. Non-specific binding was determined on adjacent sections incubated in the same solution containing 25 μM apomorphine, and accounted for less than 5% of the signal. Similarly, for ^{125}I–7-hydroxy-propyl-iodo-propenyl-aminotetralin (^{125}I–OH-PIPAT) autoradiography [12], the slices were washed in 50 mM HEPES containing 1 mM EDTA and 0.005% ascorbic acid, pH 7.4, and then incubated in the same buffer containing 0.2 nM ^{125}I–7-OH-PIPAT (Amersham, specific activity 2200 Ci/mmol) and 1 mM di-tolylguanidine (to prevent binding of ^{125}I–7-OH-PIPAT to sigma sites) for 45 min at room temperature. Dopamine (1 μM) was used for non-specific binding. Labeled tissue sections were apposed to tritium-sensitive films (Hyperfilm-^{3}H, Amersham) and placed in cassettes for 7 days for ^{125}I–sulpiride and 24 h for ^{125}I–7-OH-PIPAT.

2.4. Data analysis

Autoradiographic signals were measured by densitometry with a computer image analyzer (Biocom, Les Ulis, France). The system yielded an integrated mean optical density (OD) (gray levels measured on a 0–255 range scale) per unit area. For a given structure OD was determined in both hemispheres from at least three different sections, and averaged to obtain a single value per animal. Grain density at the cellular level was quantified by a computer-assisted semiautomatic image analysis system (Biocom), as previously described [27]. The number of silver grains over a neuronal profile was estimated under polarized light by measuring the mean optical density of the neuronal profile. The optical density value was converted to a grain density value following a calibrated standard curve. For each brain section, all labeled neuronal profiles were analyzed in the SNpc and averaged to obtain a single mean grain density value per brain. Total counts of Nurr1-labeled nigral profiles were obtained at the same time. Background labeling was determined from a region lacking specific signal and

subtracted from total labeling. All experiments were run including sections hybridized with the sense probes in order to test the specificity of labeling.

3. Results

As expected, binding of ^{125}I–sulpiride to the SNpc and VTA was significantly lower in D2 DA receptor −/− mice than in their wild-type siblings. OD was reduced to 28.3% of the wild-type value in the SNpc and to 14.2% in the VTA (Fig. 1, $P<0.001$ for each structure, Student's *t*-test). No differences were found between D2 DA receptor −/− and wild-type mice for total ^{125}I–7-OH-PIPAT binding (Fig. 1).

Sections hybridized with the Nurr1 antisense probe showed specific labeling in the ventral mesencephalon, hippocampus, habenula, and deep layers of the cerebral cortex (mainly the temporal cortex) (Fig. 1), as previously described [20,28]. No labeling was observed in sections hybridized with the sense probe (insert Fig. 1). Dopaminergic neurons showed a moderate expression of Nurr1 mRNA in wild-type mice. A regional evaluation of Nurr1 mRNA expression in the brain, performed on autoradiograms, showed that Nurr1 was significantly increased in D2 receptor −/− mice, both in the SNpc and the VTA when compared with wild-type mice (Fig. 1, Table 1). In contrast, Nurr1 mRNA expression was not different between wild-type and D2 receptor −/− mice in any other brain region studied (Table 1). The study of Nurr1 expression at the cellular level in the SNpc, showed a significant increase of both the number of Nurr1 labeled neuronal profiles and the mean grain density per cell in D2 receptor −/− mice relative to wild-type mice (Fig. 1, Table 2).

4. Discussion

Our study demonstrates that genetically modified mice lacking both functional copies of the D2 DA receptor gene display a selective increase of Nurr1 mRNA expression in mesencephalic DA neurons. The reduced number of Nurr1 mRNA labeled neurons in the SNpc of D2 +/+ animals compared to their knock-out counterparts may be due to the sensitivity of the method used, which cannot detect the presence of very low quantities of mRNA. No change in Nurr1 expression was observed in any other studied brain region. Mesencephalic DA neurons are unique among the cell groups studied in that they express both Nurr1 and D2 receptors, and also synthesize DA. Thus, it seems plausible that Nurr1 expression is selectively modulated by D2 autoreceptors on DA neurons, but not by post-synaptic D2 receptors in non-DA cells. Alternatively, Nurr1 expression in DA neurons could be modulated by complex circuits involving D2 receptors localized to other neuronal groups. This latter possibility, although less likely, cannot be excluded on the basis of our results.

DA regulates, mainly through actions mediated by D2 DA autoreceptors, DA neuronal excitability and firing rate [9,25], DA uptake [7,14], and release [6,17], tyrosine hydroxylase activity and expression [16,19] and probably, neurite morphogenesis [23]. Our results suggest that some of these effects could be related to changes in Nurr1 expression, which is known to be essential for the acquisition of a mature dopaminergic phenotype during development.

The mechanisms involved in D2 receptor regulation of Nurr1 expression are not known. The expression of Nurr1 is increased by potassium-induced membrane depolarization in PC12 cells [13], indicating a selective sensitivity of Nurr1 to membrane potential. Mesencephalic DA neurons are inhibited by D2 agonists through D2 autoreceptors [9,25]. Thus, if D2 autoreceptor function is impaired in D2 receptor −/− mice, an increased expression of Nurr1 mRNA in mesencephalic DA neurons could be expected. At least two lines of evidence suggest that D2 autoreceptor function is impaired in D2 receptor-deficient mice: (i) binding of ^{125}I–sulpiride to the SNpc/VTA is reduced in D2 receptor −/− mice, and no compensatory change seems to occur in D3 receptor expression (see also Refs. [11,25]; (ii) a lack of autoreceptor-mediated inhibition of mesencephalic DA neurons was observed in another strain of D2 receptor-deficient mice [15]. Recent reports suggest that D2 receptors could also regulate Nurr1 expression by other mechanisms as well. The presence of consensus binding sites for CREB in the Nurr1 gene suggests a mechanism for cyclic AMP-mediated induction of Nurr1 [2,10,21,26]. Activation of D2 DA autoreceptors, which are inhibitory to adenylyl-cyclase [4,24], can consequently be expected to inhibit Nurr1 expression in mesencephalic DA neurons.

To our knowledge our results constitute the first evidence to support the hypothesis that endogenous DA can regulate the expression of Nurr1. The activity of other nuclear receptors is also modulated by DA in a ligand-independent manner. Thus, the chicken ovalbumin upstream promoter transcription factor can be activated in a ligand-independent manner by physiological concentrations of DA [18]. Furthermore, DA-dependent activation of the human estrogen receptor seems to involve D1 DA receptors, and requires nuclear receptor phosphorylation via a cyclic AMP and protein-kinase A-dependent mechanism [8].

In summary, our present results add a new dimension to the known interactions between the orphan nuclear receptor Nurr1 and the mesencephalic DA system. It seems plausible that Nurr1 is involved in negative feedback mechanisms through which DA regulates the expression and/or function of DA markers in mesencephalic DA neurons. A disruption of these mechanisms could be involved in the etiology or pathogenesis of Parkinson's

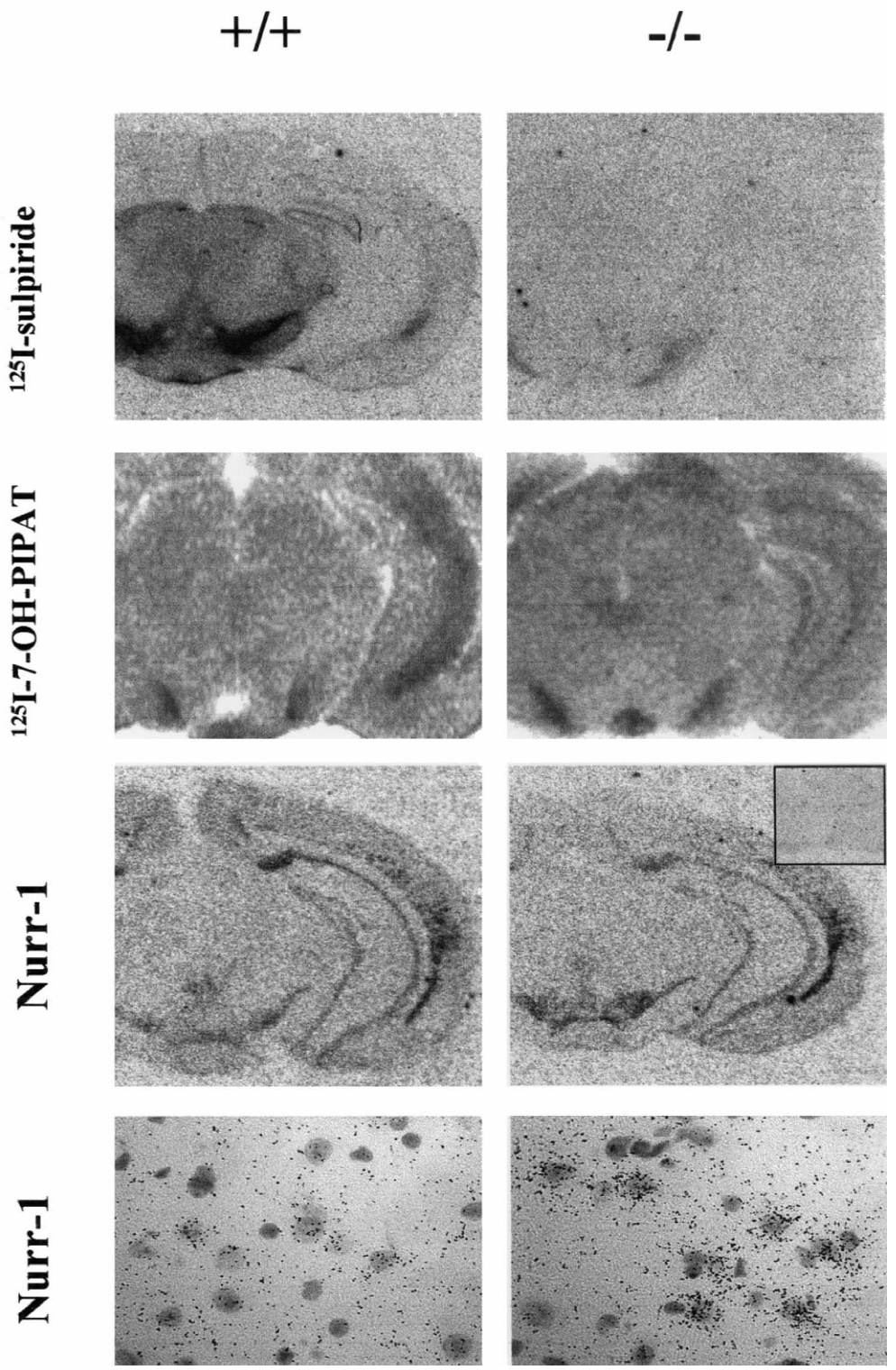

Fig. 1. Bright-field images of ^{125}I–sulpiride autoradiograms, ^{125}I–7-OH-PIPAT autoradiograms and Nurr1 mRNA in situ hybridization, of wild-type (+/+) and D2 (−/−) dopamine receptor-deficient mice. Note the decreased binding of ^{125}I–sulpiride in the mesencephalon of D2 receptor-deficient mice, and the absence of changes in ^{125}I–7-OH-PIPAT binding. The increased expression of Nurr1 mRNA in the mesencephalon of D2 receptor-deficient mice is evident both at the regional (films) and cellular (high power microphotographs of emulsion-coated sections) levels. Inset: mesencephalic section from a D2 dopamine receptor-deficient mouse hybridized with the Nurr1 sense probe.

Table 1
Nurr1 mRNA expression in different central nervous system structures of wild-type and D2 dopamine receptor-deficient mice[a]

Group	Habenular nuclei			Cortex		Mesencephalon	
	Medial	Lateral	Subiculum	Perirhinal	Temporal	SNpc	VTA
+/+	28.1±2.3	11.3±0.7	14.0±1.2	13.3±0.9	10.5±0.5	6.7±1.1	5.0±0.6
−/−	33.4±3.6	12.8±0.9	14.7±1.4	14.7±1.9	11.8±1.4	11.8±1.5[b]	8.9±0.9[c]

[a] Experimental conditions are indicated in the text. Optical density (OD) on autoradiograms was determined by densitometry. Data represent the mean OD×100±S.E.M. of values obtained in three different sections, from six wild-type (+/+), and eight D2 DA receptor-deficient mice (−/−).
[b] $P=0.019$ considered significant compared with corresponding structures in wild-type mice, Student's *t*-test.
[c] $P=0.005$ considered significant compared with corresponding structures in wild-type mice, Student's *t*-test.

Table 2
Cellular expression of Nurr1 mRNA in the substantia nigra of wild-type and D2 dopamine receptor-deficient mice[a]

Group	Substantia nigra pars compacta	
	Number of labeled neuronal profiles per section	Mean grain density on neuronal profiles (grains/μm^2)
+/+	43.2±17.6	0.29±0.07
−/−	160.2±18.4[b]	0.81±0.11[b]

[a] Date are mean±S.E.M. One section of the substantia nigra pars compacta was analyzed per animal. Values were obtained from five different animals in each group. +/+ (wild-type), −/− (D2 dopamine receptor-deficient mice).
[b] $P<0.001$ considered significant compared with corresponding cells in wild-type, Student's *t*-test.

disease and schizophrenia. The elucidation of the precise signaling pathways involved in DA-dependent regulation of Nurr1 expression, and of the role of Nurr1 in the regulation of mesencephalic DA neuronal function in adult animals, will be an important target of future work.

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References

[1] M.L. Bouthenet, M.P. Martres, N. Sales, J.C. Schwartz, A detailed mapping of dopamine D2 receptors in rat central nervous system by autoradiography with [^{125}I]–iodosulpiride, Neuroscience 20 (1987) 117–155.
[2] S.O. Castillo, Q. Xiao, M.S. Lyu, C.A. Kozak, V.M. Nikodem, Organization, sequence, chromosomal localization, and promoter identification of the mouse orphan nuclear receptor Nurr1 gene, Genomics 41 (1997) 250–257.
[3] S.O. Castillo, J.F. Baffi, M. Palkovits, D.S. Goldstein, I.J. Kopin, J. Witta, M.A. Magnuson, V.M. Nikodem, Dopamine biosynthesis is selectively abolished in substantia nigra/ventral tegmental area but not in hypothalamic neurons in mice with targeted disruption of Nurr1 gene, Mol. Cell. Neurosci. 11 (1998) 36–46.
[4] O. Civelli, J.R. Bunzow, D.K. Grandy, Molecular diversity of the dopamine receptors, Ann. Rev. Pharmacol. Toxicol. 32 (1993) 281–307.
[5] P. Chomczynski, N. Sacchi, Single step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction, Anal. Biochem. 162 (1987) 156–159.
[6] S.J. Cragg, S.A. Greenfield, Differential autoreceptor control of somatodendritic and axon terminal dopamine release in substantia nigra, ventral tegmental area, and striatum, J. Neurosci. 17 (1997) 5738–5746.
[7] S.D. Dickinson, J. Sabeti, G.A. Larson, K. Giardina, M. Rubinstein, M.A. Kelly, D.K. Grandy, M.J. Low, G.A. Gerhardt, N.R. Zahniser, Dopamine D2 receptor-deficient mice exhibit decreased dopamine transporter function but no changes in dopamine release in dorsal striatum, J. Neurochem. 72 (1999) 148–156.
[8] E.A. Gangolly, O.M. Conneely, B.W. O'Malley, Neurotransmitters activate the human estrogen receptor in a neuroblastoma cell line, J. Steroid Mol. Biol. 61 (1997) 1–9.
[9] P.M. Groves, C.J. Wilson, S.J. Young, G.V. Rebec, Self inhibition by dopaminergic neurons, Science 190 (1975) 522–529.
[10] H. Ichinose, T. Ohye, T. Suzuki, C. Sumi-Ichinose, T. Nomura, Y. Hagino, T. Nagatsu, Molecular cloning of the human Nurr1 gene: characterization of the human gene and cDNAs, Gene 230 (1999) 233–239.
[11] M.A. Kelly, M. Rubinstein, T.J. Phillips, C.N. Lessov, S. Burkhart-Kasch, G. Zhang, J.R. Bunzow, Y. Fang, G.A. Gerhardt, D.K. Grandy, M.J. Low, Locomotor activity in D2 dopamine receptor-deficient mice is determined by genetic dosage, genetic background, and developmental adaptations, J. Neurosci. 18 (1998) 3470–3479.
[12] M.P. Kung, S. Chumpradit, D. Frederick, S. Garner, K.D. Burris, P.B. Molinoff, H. Kung, Characterization of binding sites for [125I]R(+)trans-7-OH-PIPAT in rat brain, Naunyn Schmiedebergs Arch. Pharmacol. 350 (1994) 611–617.
[13] S.W. Law, O.M. Conneely, F.J. DeMayo, B.W. O' Malley, Identification of a new brain specific transcription factor, Nurr1, Mol. Endocrinol. 6 (1992) 2129–2135.
[14] S.M. Meiergerd, T.A. Patterson, J.O. Schenk, D2 receptors modulate the function of the striatal transporter for dopamine: kinetic evidence from studies in vitro and in vivo, J. Neurochem. 61 (1993) 764–767.
[15] N.B. Mercuri, A. Saiardi, A. Bonci, R. Picetti, P. Calabresi, G. Bernardi, E. Borrelli, Loss of autoreceptor function in dopaminergic neurons from dopamine D2 receptor deficient mice, Neuroscience 79 (1997) 323–327.
[16] C.M. O'Hara, A. Uhland-Smith, K.L. O' Malley, R.D. Todd, Inhibition of dopamine synthesis by dopamine D2 and D3 but not D4 receptors, J. Pharmacol. Exp. Ther. 277 (1996) 186–192.
[17] E.N. Pothos, S. Przedborski, V. Davila, Y. Schmitz, D. Sulzer, D2-like dopamine autoreceptor activation reduces quantal size in PC12 cells, J. Neurosci. 18 (1998) 5575–5585.
[18] R.F. Power, J.P. Lydon, O.M. Conneely, B.W. O'Malley, Dopamine

activation of an orphan of the steroid receptor superfamily, Science 252 (1991) 1543–1546.
[19] K. Sakurada, M. Ohshima-Sakurada, T.D. Palmer, F.H. Gage, Nurr1, an orphan nuclear receptor, is a transcriptional activator of endogenous tyrosine hydroxylase in neural progenitor cells derived from the adult brain, Development 126 (1999) 4017–4026.
[20] O. Saucedo-Cardenas, O.M. Conneely, Comparative distribution of Nurr1 and Nurr77 nuclear receptors in the mouse central nervous system, J. Mol. Neurosci. 7 (1996) 51–63.
[21] O. Saucedo-Cardenas, R. Kardon, T.R. Ediger, J.P. Lydon, O.M. Conneely, Cloning and structural organization of the gene encoding the murine nuclear receptor transcription factor Nurr1, Gene 187 (1997) 135–139.
[22] O. Saucedo-Cardenas, J.D. Quintana-Hau, W.D. Le, M.P. Smidt, J.J. Cox, F. De Mayo, J.P. Burbach, O.M. Conneely, Nurr1 is essential for the induction of the dopaminergic phenotype and the survival of ventral mesencephalic late dopaminergic precursor neurons, Proc. Natl. Acad. Sci. USA 95 (1998) 4013–4018.
[23] B.C. Swarzenski, L. Tang, Y.J. Oh, K.L. O'Malley, R.D. Todd, Morphogenetic potentials of D2, D3 and D4 dopamine receptors revealed in transfected neuronal cell lines, Proc. Natl. Acad. Sci. USA 91 (1994) 649–653.
[24] L. Tang, R.D. Todd, A. Heller, K.L. O'Malley, Pharmacological and functional characterization of D2, D3 and D4 dopamine receptors in fibroblast and dopaminergic cell lines, J. Pharmacol. Exp. Ther. 268 (1994) 495–502.
[25] J.M. Tepper, B.-C. Sun, L.P. Martin, I. Creese, Functional roles of dopamine D2 and D3 autoreceptors on nigrostriatal neurons analyzed by antisense knockdown in vivo, J. Neurosci. 17 (1997) 2519–2530.
[26] T. Torii, T. Kawarai, S. Nakamura, H. Kawakami, Organization of the human orphan nuclear receptor Nurr1 gene, Gene 230 (1999) 225–232.
[27] M. Vila, R. Levy, M.-T. Herrero, M. Ruberg, B. Faucheux, J.A. Obeso, Y. Agid, E.C. Hirsch, Consequences of nigrostriatal denervation on the functioning of the basal ganglia in human and non-human primates: an in situ hybridization study of cytochrome oxidase subunit I mRNA, J. Neurosci. 17 (1997) 765–773.
[28] R.H. Zetterström, R. Williams, T. Perlmann, L. Olson, Cellular expression of the immediate early transcription factors Nurr1 and NGFI-B suggests a gene regulatory role in several brain regions including the nigrostriatal dopaminergic system, Mol. Brain Res. 41 (1996) 111–120.
[29] R.H. Zetterström, L. Solomin, L. Jansson, H.J. Hoffer, L. Olson, T. Perlmann, Dopamine neuron agenesis in Nurr1-deficient mice, Science 276 (1997) 248–250.