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Neurochemical changes in dopamine D1, D3 and D1/D3 receptor knockout mice

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Abstract

Neurochemical changes were examined in dopamine D1 receptor knockout ($D1^{-/-}$), dopamine D3 receptor knockout ($D3^{-/-}$) and dopamine D1/D3 receptor double knockout ($D1^{-/-}D3^{-/-}$) mice. The level of dopamine D1- and D2-like receptors and γ-aminobutyric acid ($GABA_A$) receptor was assessed by ligand autoradiography and dopamine D1- and D2 receptor, enkephalin, dynorphin and substance P transcripts measured by in situ hybridization. $D1^{-/-}$ mice had normal $GABA_A$ receptor levels, reduced dynorphin and substance P, and increased enkephalin mRNA and dopamine D2-like binding. $D1^{-/-}D3^{-/-}$ mice evidenced decreased dynorphin and substance P but normal enkephalin expression, whereas dopamine D2-like and $GABA_A$ receptor binding were increased. Major changes occur in substance P and dynorphin expression in $D1^{-/-}$ mice and these changes are unaffected by loss of dopamine D3 receptors. Upregulated dopamine D2-like binding and enkephalin in $D1^{-/-}$ mice may be due to decreased dopamine turnover. Upregulated enkephalin in $D1^{-/-}$ mice is dependent on functional dopamine D3 receptors.

Keywords: Gene targeting; Dopamine receptor; Dynorphin; Substance P; Enkephalin

1. Introduction

The neurotransmitter dopamine plays a pivotal role in the regulation of a diverse array of neural processes that include motor control, reward, neuroendocrine and cardiovascular regulation, cognition (Jaber et al., 1996) and the control of respiration (Mueller et al., 1982). The effects of dopamine on behaviour and drug responses are mediated by two subfamilies of G-protein-coupled receptors (Sibley and Monsma, 1992). The dopamine D1 receptor subfamily consists of dopamine D1 and D5 receptors (also called $D1_A$ and $D1_B$ dopamine receptors, respectively) while the dopamine D2 receptor subfamily consists of dopamine D2, D3 and D4 receptors. High levels of dopamine D1 receptors are detected in the caudate putamen, nucleus accumbens, olfactory tubercle and Islands of Calleja, and lower levels are found in the substantia nigra pars reticulata/entopeduncular nuclear complex and the ventral tegmental area. The localisation of dopamine D1 receptor mRNA generally correlates well with the regional distribution of the dopamine D1 receptor, although dopamine D1 receptor mRNA is not found in the substantia nigra (Meador-Woodruff et al., 1991; Sibley and Monsma, 1992; Gingrich and Caron, 1993). Low levels of dopamine D1 receptor mRNA expression (Le Moine et al., 1991) have been found in striatal cholinergic interneurons (Kawaguchi et al., 1995) and striatal interneurons that make the inhibitory neurotransmitter γ-aminobutyric acid (GABA).

The dopamine D3 receptor has a 52% overall amino acid homology with the dopamine D2 receptor (Sibley and Monsma, 1992). Dopamine D3 receptor mRNA shows a distinctive distribution with relatively high levels in the limbic areas of the brain including the ventral striatal complex consisting of the nucleus accumbens, olfactory tubercle, Islands of Calleja and ventral pallidum as well as

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high level expression in the ventral tegmental area (Sokoloff et al., 1990; Landwehrmeyer et al., 1993); brain regions implicated in the regulation of motivation and emotion. The dopamine D3 receptor is also expressed at low levels in dopaminergic neurons within the substantia nigra, suggesting that it may have a presynaptic function (Sokoloff et al., 1990).

Dopamine receptor and neuropeptide expression in the normal brain is complex, with interactions between receptor systems within neurons and between neurons ultimately impacting on behaviour and responses to drug treatment. The lack of ligands with absolute receptor specificity stimulated the use of gene targeting approaches to examine the in vivo function of the dopamine D1 receptor (Drago et al., 1994, 1996; Xu et al., 1994a,b; Miner et al., 1995; Levine et al., 1996; Moratalla et al., 1996; Crawford et al., 1997; Friedman et al., 1997), dopamine D2 receptor (Baik et al., 1995; Maldonado et al., 1997), dopamine D3 receptor (Accili et al., 1996; Steiner et al., 1997), dopamine D4 receptor (Rubinstein et al., 1997), dopamine D5 receptor (Sibley et al., 1998) and dopamine transporter (Giros et al., 1996). The generation of dopamine receptor mutants offers a powerful opportunity to evaluate the roles of these receptors in dopamine-mediated behaviours and transcriptional regulation. Indeed, studies on both lesioned and genetically manipulated animals suggest that dopamine has a pivotal role in the regulation of gene transcription. Recently, Karasinska et al. (2000) generated dopamine D1/D3 receptor double knockout ($D1^{-/-}D3^{-/-}$) mice and suggested that dopamine D1/D3 receptor interaction was involved in the regulation of exploratory activity in mice. Recent data suggest cellular dopamine D1/D3 receptor interactions (Levavi-Sivan et al., 1998; Ridray et al., 1998; Jung et al., 1999); also, a role for the dopamine D3 receptor in regulating dopamine D1/D2 receptor interactions, particularly in terms of a possible inhibitory effect on dopamine D1/D2 receptor interactions at both electrophysiological and behavioural levels (Xu et al., 1997), has been proposed. While mice with deletion of the dopamine D1 receptor or of the dopamine D3 receptor have been studied, the neurochemical properties of dopamine D1 and D3 receptors and their interactions would be illuminated most powerfully by their co-deletion. We have dopamine D1 receptor knockout ($D1^{-/-}$) and dopamine D3 receptor knockout ($D3^{-/-}$) mice in house and have independently generated a dopamine D1/D3 receptor double knockout ($D1^{-/-}D3^{-/-}$) mouse line. The aim of this study was to characterize the neurochemical changes in $D1^{-/-}$, $D3^{-/-}$ and $D1^{-/-}D3^{-/-}$ mice in an effort to understand the neuroregulatory role of dopamine D1 receptors and any facilitatory role that dopamine D3 receptors may play in dopamine D1 receptor-mediated processes. Quantitative ligand autoradiography was undertaken for striatal dopamine D1- and D2-like receptors, muscarinic acetylcholine receptors, $GABA_A$ receptors and the dopamine transporter. In addition, striatal mRNA expression was also quantified for dopamine D1 receptors, dopamine D2 receptors and the neuropeptides enkephalin, dynorphin and substance P.

2. Materials and methods

2.1. Animals

The generation of $D1^{-/-}$ and $D3^{-/-}$ mutants was as reported previously (Drago et al., 1994; Accili et al., 1996). Heterozygous founders for both the $D1^{-/-}$ and $D3^{-/-}$ genotype were transported from our original colonies at the National Institutes of Health and maintained in a hybrid C57/BL6 × 129/*Sv* genetic background (Drago et al., 1994; Accili et al., 1996). $D1^{-/-}D3^{-/-}$ mice were obtained by crossing $D1^{-/-}$ and $D3^{-/-}$ mice. The resulting F1 heterozygous $D1^{+/-}D3^{+/-}$ mice were bred to produce F2 generation, which were then crossbred to obtain $D1^{-/-}D3^{-/-}$ and wild type (WT) control mice. Homozygous mutants ($D1^{-/-}$, $D3^{-/-}$ and $D1^{-/-}D3^{-/-}$ mice) and WT control mice were bred in the Department of Medicine, Monash University, and the genotype of each animal determined by Southern blotting. Within each genotype, breeding pairs were exchanged frequently to maximize background genetic diversity. Mice were housed in groups of three to five under standard animal housing conditions with food and water available ad libitum, and were maintained at 21 ± 1 °C on a 12:12 h (09:00 on; 21:00 off) light/dark schedule. Procedures involving the use of live animals conformed to the Australian National Health and Medical Research Council code of practice.

2.2. Tissue preparation

Experiments were performed on male mice 70–100 days of age. A total of 23 mice ($n = 5$ for $D1^{-/-}$, $n = 5$ for $D3^{-/-}$, $n = 7$ for $D1^{-/-}D3^{-/-}$ and $n = 6$ for WT mice) were killed by overdose of sodium pentobarbitone (600 mg/kg, i.p.); brains were removed, snap-frozen in isopentane cooled in dry ice and stored at − 70 °C before use. A series of 20-μm frozen coronal sections were cut and then thaw-mounted onto 3-aminopropyltriethoxysilane (Sigma, St. Louis, MO, USA) coated slides for in situ hybridization and gelatin-coated slides for ligand binding studies. Alternate sections derived from the same animals were used in ligand autoradiography and in situ hybridization studies.

2.3. In situ hybridization histochemistry

The sequence of oligonucleotide probes (synthesized by Life Technologies, Melbourne, Australia) for detection of dopamine D1 receptor, dopamine D2 receptor, enkephalin, dynorphin and substance P mRNA transcripts were detailed in our previous study (Drago et al., 1998b). The mouse antisense dopamine D1 receptor oligonucleotide (D1.3) was as described (Drago et al., 1994). The D1.3 oligonucleotide

hybridizes with mRNA transcribed from genomic sequence excluded from the targeting vector and therefore absent from the disrupted dopamine D1 receptor allele. The oligonucleotide probes were 5′-end labelled using a standard kinase protocol (Wong et al., 1997) with [γ-^{33}P] ATP (NEN Life Science Products, Boston, USA) and T4 polynucleotide kinase (New England Biolabs, Beverly, MA, USA). In situ hybridization was performed according to the protocol as reported in Wong et al. (1997). Specificity of mRNA expression was determined by using a 100-fold excess of unlabelled antisense oligonucleotide which was added to the in situ hybridization reactions to competitively inhibit probe hybridization. Slides were then exposed, together with laboratory-prepared ^{33}P standards, to Hyperfilm (Amersham International, Amersham, UK). The density of mRNA expression was quantified using a Microcomputer Imaging Device (MCID) M4 image analysis system. Standardization was achieved by comparing autoradiographic images with standards exposed with each film. All values are expressed as specific cpm/mm^2 for mRNA expression (mean ± S.E.M.) calculated by subtracting background counts from the total counts.

2.4. Ligand binding studies

A single concentration of each ligand was used, at a concentration of a similar order of magnitude to K_D values in the described methods, because of the limited amount of brain tissue available from mice and limited availability of knockouts, particularly of the D1$^{-/-}$D3$^{-/-}$ double knockout, which made it difficult to conduct saturation analyses for determination of K_D and B_{max} values. D1$^{-/-}$D3$^{-/-}$ double knockout mice were in short supply because, like the D1$^{-/-}$ mice, they failed to thrive and many died suddenly in the first few months of life.

2.4.1. Dopamine D1-like receptor binding

Sections were incubated for 60 min at room temperature in a 50 mM Tris–HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 2 nM [*N*-methyl-^{3}H]SCH23390 (NET-930, New England Nuclear, Boston, MA) and 1 μM mianserin hydrochloride (Research Biochemicals International, MA) to block serotonin receptors; 1 μM R(+)SCH23390 hydrochloride (Research Biochemicals International) was used to determine non-specific binding (Wong et al., 2000). For all autoradiographic studies and unless otherwise stated, slides were rinsed twice in fresh 4 °C buffer before drying.

2.4.2. Dopamine D2-like receptor binding

Sections were incubated for 30 min at room temperature in a 50 mM Tris–HCl buffer (pH 7.4) containing 120 mM NaCl and 20 nM [methoxy-^{3}H]sulpiride (NET-775, New England Nuclear). Haloperidol (1 μM) (Searle, Sydney, Australia) was used to determine non-specific binding (Wong et al., 2000).

2.4.3. Dopamine transporter binding

Sections were incubated for 60 min at room temperature in a 50 mM Tris–HCl buffer (pH 7.4) containing 300 nM NaCl, 5 mM KCl, 0.2% bovine serum albumin and 4 nM [^{3}H] mazindol (NET-816, New England Nuclear); 1 μM mazindol (Sigma) was used to determine non-specific binding (Padungchaichot et al., 2000).

2.4.4. Muscarinic acetylcholine receptor binding

It has been reported that [^{3}H]quinuclidinyl benzilate ([^{3}H]quinuclidinyl benzilate, NET-656, New England Nuclear) binds to all muscarinic acetylcholine receptors in human and rat (Wamsley et al., 1981, 1984; Bonner et al., 1987; Vanderheyden et al., 1990; Svensson et al., 1992). Sections were incubated for 60 min at room temperature in a 0.2 M sodium phosphate buffer (pH 7.4) and 1 nM [^{3}H] quinuclidinyl benzilate; 1 μM atropine sulphate (Delta West, Australia) was used to determine non-specific binding (Padungchaichot et al., 2000).

2.4.5. $GABA_A$ receptor binding

Frozen slide-mounted tissues were thawed at room temperature before pre-incubation in 50 mM Tris–citrate buffer (pH 7.4) containing 100 mM $MgCl_2$. Sections were cooled in ice-cold buffer for 5 min before incubation in the same buffer containing [butyryl-^{3}H]SR95531 (NET-946, New England Nuclear) for 30 min, and then washed in ice-cold buffer (3 × 5 s) and rinsed in distilled water (2 × 10 s) before drying. GABA (10 mM) (Research Biochemicals International) was used to determine non-specific binding (Padungchaichot et al., 2000).

2.5. Autoradiography

Autoradiographic detection was carried out by exposing slide-mounted sections, together with laboratory-prepared ^{33}P standards and [^{3}H]microscales (RPA 510, Amersham International) to Hyperfilm (RPN12, Amersham) for 42 days (dopamine D1 receptor mRNA), 32 days (dopamine D2 receptor mRNA, $GABA_A$ receptor), 12 days (enkephalin mRNA and dopamine transporter), 35 days (substance P and dynorphin mRNA) and 14 days (dopamine D1-like receptor, dopamine D2-like receptor and muscarinic acetylcholine receptor binding). The films were developed using Kodak D-19 Photo Developer.

2.6. Quantitation of autoradiographic studies

Binding densities were measured using a MCID with software (Imaging Research, Brock University, St. Catherine's, Ontario, Canada). For all studies, a minimum of three coronal sections from each animal were used for quantitation. The level of the striatal sections used was between the region corresponding to levels 0.14 and 1.10 mm, rostral to the bregma line of the mouse brain (Franklin and Paxinos, 1997). This anatomical region is contained between Figs. 22

and 30 of Franklin and Paxinos (1997). Standardization was achieved by comparing binding densities with standards exposed with each film. Standard curves were created from ^{33}P-standards produced in the laboratory and [^{3}H]microscales (RPA 510, Amersham) for in situ hybridization and ligand binding studies, respectively. All values are expressed as cpm/mm^2 for mRNA expression and fmol/mg tissue for ligand binding studies (mean ± S.E.M.).

2.7. Statistical analysis

The statistical significance of differences between groups was analysed using one-way analysis of variance (ANOVA) (SigmaStat 2.0, Jandel Scientific) and appropriate post hoc tests (Dunn's method).

3. Results

3.1. Dopamine D1 receptor mRNA expression

Dopamine D1 receptor mRNA was identified in the striatum, nucleus accumbens, Islands of Calleja and olfactory tubercle of WT mice (Fig. 1A) but was not detected in the striatum of D1$^{-/-}$ mice (Fig. 1G) or D1$^{-/-}$D3$^{-/-}$ mice (Fig. 1S). The signal obtained using both labelled and unlabelled excess D1.3 oligonucleotide in normal mice was identical to the signal obtained when labelled D1.3 oligonucleotide was hybridized to D1$^{-/-}$ or D1$^{-/-}$D3$^{-/-}$ mice, suggesting that this signal intensity represented non-dopamine D1 receptor background hybridization. There was a 44% ($P<0.05$) reduction of striatal dopamine D1 receptor mRNA in D3$^{-/-}$ mice (Figs. 1M and 2A).

3.2. Dopamine D1-like receptor autoradiography

There was a typical distribution of dopamine D1-like receptor binding sites in the striatum, nucleus accumbens, Island of Calleja and the olfactory tubercle of WT mice (Fig. 1A′). As expected, dopamine D1-like binding was not detected in the striatum of D1$^{-/-}$ and D1$^{-/-}$D3$^{-/-}$ mice (Fig. 1G′ and S′). Despite a significant reduction in dopamine D1 receptor mRNA levels (Fig. 2A), dopamine D1-like binding in D3$^{-/-}$ mice was not significantly different from WT control (Fig. 2B).

3.3. Dopamine D2 receptor mRNA expression

Dopamine D2 receptor mRNA was identified in all genotypes (Fig. 1B,H,N and T), with expression being 9% greater ($P<0.05$) in D1$^{-/-}$ than in WT mice. Dopamine D2 receptor mRNA expression in D3$^{-/-}$ and D1$^{-/-}$D3$^{-/-}$ mice did not differ from that in WT (Fig. 2C).

3.4. Dopamine D2-like receptor autoradiography

In contrast to dopamine D1 receptor autoradiography, dopamine D2-like receptor binding was detected in all genotypes (Fig. 1B′,H′,N′,T′). Levels in both D1$^{-/-}$ and D1$^{-/-}$D3$^{-/-}$ mice were significantly greater than in WT mice (23%, $P<0.05$), but were unaltered in their D3$^{-/-}$ counterparts (Fig. 2D).

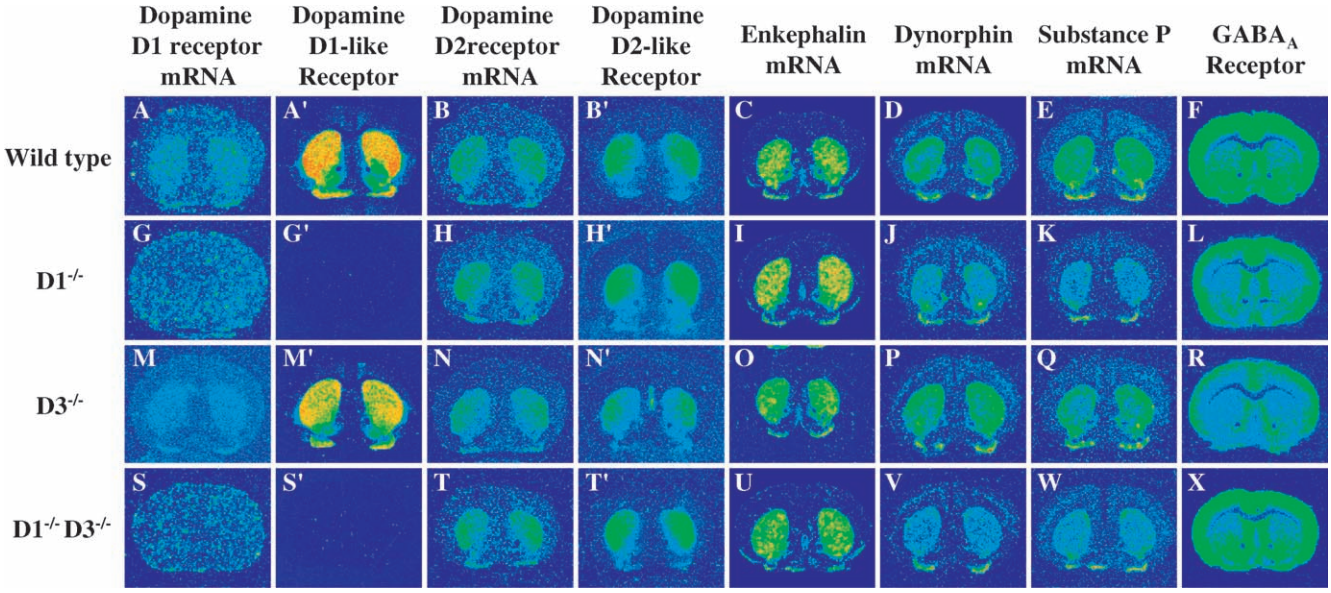

Fig. 1. Autoradiographs showing dopamine D1 receptor mRNA expression (A, G, M and S), dopamine D1-like receptor binding (A′, G′, M′ and S′), dopamine D2 receptor mRNA expression (B, H, N and T), dopamine D2-like receptor binding (B′, H′, N′ and T′), enkephalin (C, I, O and U), dynorphin (D, J, P and V), substance P (E, K, Q and W) mRNA expression, and GABA$_A$ receptor (F, L, R and X) binding in the striatum of wild type (A, A′, B, B′, C, D, E, and F), D1$^{-/-}$ (G, G′, H, H′, I, J, K and L), D3$^{-/-}$ (M, M′, N, N′, O, P, Q and R) and D1$^{-/-}$D3$^{-/-}$ (S, S′, T, T′, U, V, W and X) mice.

3.5. Enkephalin mRNA expression

Enkephalin mRNA was detected in all genotypes (Fig. 1C,I,O and U), with expression significantly greater in $D1^{-/-}$ than in WT mice (25% increase, $P<0.05$), but unchanged in $D3^{-/-}$ and $D1^{-/-}D3^{-/-}$ mice (Fig. 2E).

3.6. Dynorphin mRNA expression

Dynorphin mRNA was detected in all genotypes (Fig. 1D,J,P and V), with expression in dopamine $D1^{-/-}$ and dopamine $D1^{-/-}D3^{-/-}$ receptor mice being, respectively, 50% and 49% ($P<0.05$) less than in WT, but unaltered in $D3^{-/-}$ mice (Fig. 2G).

3.7. Substance P mRNA expression

Substance P mRNA was detected in all genotypes (Fig. 1E,K,Q and W), with expression in $D1^{-/-}$ and $D1^{-/-}D3^{-/-}$ mice being, respectively, 43% and 50% ($P<0.05$) less than in WT, but unaltered in $D3^{-/-}$ mice (Fig. 2I).

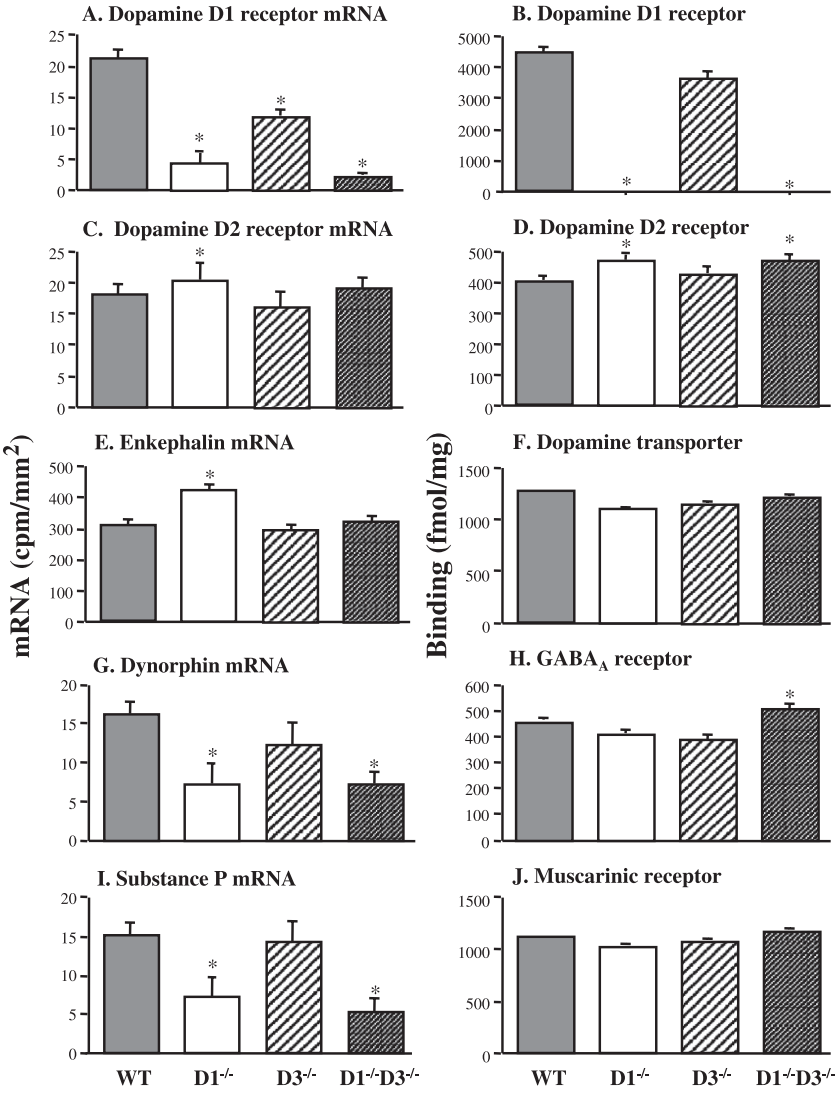

Fig. 2. Dopamine D1 receptor mRNA expression (A), dopamine D1-like receptor binding (B), dopamine D2 receptor mRNA expression (C) and dopamine D2-like receptor binding (D), enkephalin mRNA expression (E), dynorphin mRNA expression (G), substance P mRNA expression (I), dopamine transporter binding (F), $GABA_A$ receptor binding (H) and muscarinic acetylcholine receptor binding (J) in the striatum of wild type, $D1^{-/-}$, $D3^{-/-}$ and $D1^{-/-}D3^{-/-}$ mice. Values are cpm/mm^2 for mRNA expression, or fmol/mg tissue for receptor binding. Data are means ± S.E.M. for $n=6$ wild type, $n=5$ $D1^{-/-}$, $n=5$ $D3^{-/-}$ and $n=7$ $D1^{-/-}D3^{-/-}$ mice per group. *$P<0.05$ vs. wild type. In (A), the signal obtained using both labelled and unlabelled excess D1.3 oligonucleotide in normal mice was identical to the signal obtained when labelled D1.3 oligonucleotide was hybridized to $D1^{-/-}$ or $D1^{-/-}D3^{-/-}$ mice, suggesting that this signal intensity represented non-dopamine D1 receptor background hybridization. Dopamine D1 receptor mRNA expression observed in $D1^{-/-}$ and $D1^{-/-}D3^{-/-}$ mice indicated total signal, not specific signal.

3.8. Dopamine transporter

Dopamine transporter binding did not differ significantly between the genotypes (Fig. 2F; autoradiographs not shown).

3.9. Muscarinic acetylcholine receptor

Muscarinic acetylcholine receptors were found to be widely distributed, with specific receptor binding evident in the striatum, nucleus accumbens, olfactory tubercle, septum and throughout the cortex (autoradiographs not shown). There were no significant differences in distribution or level of expression among the three knockout lines (Fig. 2J).

3.10. $GABA_A$ receptor

$GABA_A$ receptors were evident in the striatum, nucleus accumbens, septum and cortex (Fig. 1F,L,R and X). Although $GABA_A$ receptor binding was unaltered in $D1^{-/-}$ and $D3^{-/-}$ mice, $D1^{-/-}D3^{-/-}$ mice exhibited a 14% increase ($P<0.05$) in striatal binding levels (Fig. 2H).

4. Discussion

At a neuroanatomical/neurochemical level, the dual pathway model of basal ganglia circuitry assumes the segregation of dopamine D1 and dopamine D2 receptors (Albin et al., 1989; Kawaguchi et al., 1990). In situ hybridization studies have shown that dopamine D1 receptors are preferentially expressed on substance P/dynorphin positive neurons which project directly to the substantia nigra pars reticulata/entopeduncular nuclear complex. The same studies also revealed that enkephalin-positive dopamine D2 receptor neurons project to the substantia nigra pars reticulata/entopeduncular nuclear complex via the external segment of the globus pallidus and subthalamic nucleus (Gerfen et al., 1990; Graybiel, 1990, 1991). Striatopallidal and striatonigral medium spiny projection neurons are also known to produce the inhibitory neurotransmitter GABA (Kawaguchi et al., 1995). Analysis of mice generated by targeted expression of an attenuated form of the diphtheria toxin gene to dopamine D1 receptor-positive neurons by *Cre* mediated site-specific recombination provides further evidence for the anatomical segregation of dopamine D1 and D2 receptors (Drago et al., 1998a,b). However, this dual pathway model is challenged by a number of studies revealing that a substantial subpopulation of the striatal projection neurons contain both dopamine D1-like and D2-like receptors (Meador-Woodruff et al., 1991; Surmeier and Kitai, 1994; Surmeier et al., 1996). Dopamine D3 receptor mRNA is present and abundant in neurons expressing substance P but not enkephalin. About 50% of these substance P-positive neurons were found to have levels of dopamine D3 receptor mRNA detectable by single-cell reverse transcriptase polymerase chain reaction, all of which also expressed the dopamine D1 receptor (Surmeier et al., 1996), providing an intracellular molecular substrate for a direct interaction between the dopamine D1-like and D2-like receptor systems within the striatonigral projection pathway. These anatomical studies may be important in understanding the neurochemical sequelae of targeted deletion of specific dopamine receptor subtypes.

Dopamine D1-like receptor expression by either dopamine D1-like receptor autoradiography or in situ hybridization was absent in $D1^{-/-}$ or $D1^{-/-}D3^{-/-}$ mice. In addition, striatal substance P and dynorphin mRNA levels in $D1^{-/-}$ and $D1^{-/-}D3^{-/-}$ mice were substantially reduced. There was a 44% reduction in dopamine D1 receptor mRNA level in $D3^{-/-}$ mice, while substance P mRNA levels remained unchanged. A 15% reduction in substance P expression was also observed in dopamine D2 receptor knockout mice, although the expression of dynorphin remained unaltered (Baik et al., 1995), suggesting that substance P and dynorphin are regulated independently. These quantitative observations are consistent with earlier studies showing a lack of dopamine D1-like binding and significant reduction of both substance P and dynorphin mRNA in the striatum of $D1^{-/-}$ mice (Drago et al., 1994, 1996). Reduction in dynorphin immunoreactivity was also demonstrated in an independently-generated dopamine D1 receptor knockout line (Xu et al., 1994b), thus confirming that dopamine regulates neuropeptide expression in dopamine D1 receptor-positive striatonigral projection neurons (Gerfen et al., 1990). Dopamine D1-like binding was normal in $D3^{-/-}$ mice despite the reduction in dopamine D1 receptor mRNA. This may be due to altered regulation at the protein level, upregulated striatal dopamine D5 receptor expression or upregulation of other as yet uncharacterized dopamine D1-like receptors recognized by the same ligand; dopamine D1-like receptors which mediate accumulation of inositol phosphate have been postulated (Friedman et al., 1997; Clifford et al., 1999; Waddington et al., 1998a,b).

Previously, a moderate increase in striatal enkephalin mRNA levels was identified in vehicle-injected $D1^{-/-}$ mice (Drago et al., 1996). A comparable increase in enkephalin mRNA levels was seen in the present study in unchallenged $D1^{-/-}$ mice but not in $D1^{-/-}D3^{-/-}$ mice, suggesting that a functional dopamine D3 receptor is required for this downstream effect of dopamine D1 receptor gene ablation.

Dopamine D2-like receptor binding was upregulated in both $D1^{-/-}$ and $D1^{-/-}D3^{-/-}$ mice. Theoretically, this may be due to increased dopamine D2 receptor expression on striatal postsynaptic sites and/or presynaptic sites on nigrostriatal neurons. Our data suggest that increased dopamine D2-like binding seen in $D1^{-/-}$ mice is likely to be due, at least in part, to increased expression of striatal dopamine D2 mRNA. In contrast, striatal dopamine D2 mRNA levels are not significantly increased in $D1^{-/-}D3^{-/-}$ mice suggesting that upregulated presynaptic dopamine D2 receptor expression may potentially contribute to the increased striatal dopamine D2-like binding seen in this line. Although

upregulated striatal dopamine D3 expression is excluded in $D1^{-/-}D3^{-/-}$ mice, there is also a possibility that upregulated dopamine D4 receptor expression may contribute because a significant proportion of striatal substance P/dopamine D1 receptor positive neurons co-express functional D4 receptors (Surmeier et al., 1996).

Modulation of dopamine turnover in $D1^{-/-}$ mice may explain the small changes seen in dopamine D2-like binding and enkephalin expression. Measurements of striatal dopamine and dehydroxyphenylacetic acid (DOPAC) concentrations in the same line of $D1^{-/-}$ mice have indicated that dopaminergic activity (DOPAC/dopamine ratio) is markedly decreased when compared to WT mice (Parish et al., 2001). Upregulated dopamine D2-like binding seen in $D1^{-/-}$ mice may relate to relative dopamine D2 receptor understimulation. The plasticity of dopamine receptors in response to changes in extracellular dopamine concentrations is well documented in dopamine transporter knockout mice which have decreased dopamine D1 and D2 receptor mRNA levels (Fauchey et al., 2000). Understimulation of postsynaptic dopamine D2 receptors may also underlie the increased enkephalin expression seen in $D1^{-/-}$ mice (Gerfen et al., 1990).

An earlier study showed no difference in binding in an independently generated dopamine D1 receptor mouse line using another dopamine D2-like ligand, [^{3}H]spiroperidol (Xu et al., 1994b). There are several possible explanations for the upregulated dopamine D2-like binding seen in our line of $D1^{-/-}$ mice. Fundamental biological differences may underlie variability of knockout phenotype in mice generated in the two different laboratories. Although both groups back-crossed chimeric mice with C57BL/6 mice to generate heterozygous mice with a hybrid 129 and C57BL/6 genetic background, there were differences in the ancestral origin of the embryonic stem cells used to produce the knockout lines, as well as differences in the configuration of the targeting vectors. Extensive genetic variation among 129 substrains is well documented (Simpson et al., 1997), and adds to the degree of potential phenotype variability between knockout mice generated in different laboratories. Despite differences in dopamine D2-like binding, dopamine transporter binding sites remained unchanged in the striatum of both $D1^{-/-}$ mouse lines (Moratalla et al., 1996).

We also investigated the distribution of $GABA_A$ receptor levels in the striatum and cortex. There was no difference between genotypes in cortical $GABA_A$ receptor levels but there was a 14% increase in striatal $GABA_A$ receptor binding restricted to dopamine $D1^{-/-}D3^{-/-}$ receptor mice. As GABA receptors are expressed on striatal projection neurons (Yung and Bolam, 2000), this change would have the effect of decreasing striatal output. In addition, we found no significant change across the knockout mice examined in muscarinic acetylcholine receptor binding using [^{3}H]quinuclidinyl benzilate, a non-selective ligand which binds all muscarinic acetylcholine receptors. This may reflect a lack of sensitivity in the assay used in the study.

Compensatory changes have also been documented in other dopamine receptor and dopamine transporter knockout mice. Dopamine D2 receptor knockout mice had an unexpected downregulation of baseline expression of substance P and increased expression of glutamic acid decarboxylase in the striatum and cortex (Baik et al., 1995). Elevation of baseline levels of the dopamine metabolite DOPAC may reflect compensatory dopaminergic turnover in dopamine D4 receptor knockout mice (Rubinstein et al., 1997). The most dramatic downstream changes seen in gene expression occur in dopamine transporter knockout mice (Giros et al., 1996), where impaired dopamine re-uptake is thought to result in increased dopaminergic neurotransmission. The level of tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, was decreased and dopamine degradation was enhanced (Jones et al., 1998). There was significant downregulation of dopamine D1 and D2 receptor mRNA levels to compensate for enhanced presynaptic dopamine resulting from impaired re-uptake. Significant changes are now described in $D1^{-/-}$, $D3^{-/-}$ and $D1^{-/-}D3^{-/-}$ mice. As in the dopamine transporter knockout model, the effects seen in dopamine receptor knockout mice can be understood within the context of adaptive changes in dopamine neurotransmission both with respect to the striatal input pathways and the regulatory role of dopamine receptors on the transcription of striatal neuropeptides. Patterns are also emerging in relation to the differential regulation of neuropeptides expressed in defined pathways. The observation that dopamine receptor knockout mice show compensatory effects (Drago et al., 1998a), together with the complicating issue of their hybrid genetic background, may temper conclusions regarding the direct effects of the targeted mutation on the neurochemical and behavioural phenotype.

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