Korean J Biol Sci 1: 497-505, 1997

Localization and Developmental Changes of Dopamine D_1 and D_2 Receptor mRNAs in the Rat Brain

Myeong Ok Kim, Wan Sung Choi*, Bong Hee Lee, Kyung Jae Cho, Sook Jae Seo[1], Sung Goo Kang[2], Kyungjin Kim[3], and Sang Ho Baik[4]

Department of Anatomy and Neurobiology, College of Medicine, [1]Department of Biology, College of Natural Sciences, Gyeongsang National University, Chinju 660-280, Korea; [2]Department of Biology, College of Natural Sciences, Inje university, Kimhae 621-749, Korea; [3]Department of Molecular Biology and Research Center for Cell Differentiation, College of Natural Sciences, [4]Department of Anatomy, College of Medicine, Seoul National University, Seoul 110-799, Korea

Key Words:
Ontogeny
Dopamine receptor
In situ hybridization
Immunohistochemistry
Rat brain

Dopamine plays diverse roles in the fetal brain development and differentiation. However, the development of the dopaminergic neurons and its receptors has not been fully understood. In our studies, *in situ* hybridization and immunohistochemical methods were used to investigate the ontogeny of dopaminergic neurons and its receptor subtypes during the fetal development of the rat. *In situ* hybridization data showed that dopamine D_1 and D_2 receptor mRNAs were expressed in the ventricular and subventricular zones of ganglionic eminence, thalamus, hypothalamus, and cortical neuroepithelium on gestational day 13. Expression of dopamine D_1 and D_2 receptors during gestational days 17 and 19 reached the same or similar level of that in the adult brain. Expression of D_1 receptor mRNA preceded that of D_2 receptor mRNA in the early developmental stage, although this pattern was reversed with the sharp increase of D_2 receptor mRNA soon after. D_2 receptor mRNA was expressed in substantia nigra of mesencephalon of the fetal rat brain. However, D_1 receptor mRNA was not detected in substantia nigra. Our results indicate that dopamine might function in the fetal brain during the early gestational period.

Dopamine, a catecholaminergic neurotransmitter, plays crucial roles in the early stage of differentiation and development of the rat fetus (Olson and Seiger, 1972; Seiger and Olson, 1973; Lindvall et al., 1978; Schlumpf et al., 1980). The dopaminergic neurons are first observed from the ventricular and subventricular zones of differentiating neuroepithelium and mesencephalic A9~A10 dopaminergic areas in the rat fetus on gestational day (GD) 12.5 at very early stages of the brain development (Spect et al., 1978; 1981). The development of the dopamine receptors depends upon the development of the presynaptic terminal containing the neurotransmitter (Deskin et al., 1981). Increasing evidence shows that the dopamine receptors can be functional at very early stages during development, while there exist contradictary results on the development of dopamine receptors during ontogeny.

Dopamine receptors can be divided into five distinct types that cluster into two families (Bunzow et al., 1988; Monsma et al., 1990; Sibley and Monsma, 1992). The D_1-like dopamine receptors consist of the D_1 and D_5 receptors and the D_2-like family consists of the D_2, D_3, and D_4 receptors. The development of dopamine receptors during fetal ontogeny has been studied with different biochemical approaches. For instance, using ligand binding autoradiography, the D_1 receptor was observed in the developing striatum on postnatal day (PD) 1 during the early postnatal period (Murrin and Zeng, 1989; 1990). However, the D_2 receptors was observed in the developing stritum between GD17.5 and 18 (Bruinink et al., 1983; Noisin and Thomas, 1988). Using *in situ* hybridization technique, the D_1 dopamine receptor mRNA was shown in the developing striatum on GD17 (Guennoun and Bloch, 1992), and D_2 dopamine receptor mRNA was expressed in the striatal primodium on GD14 (Guennoun and Bloch, 1991). Signals for these receptors increased during the following days of prenatal development. Recently, by *in situ* hybridization using cRNA probes, dopamine D_1 and D_2 receptor mRNAs were first observed in the ventricular, subventricular and intermediate zone of ganglionic eminence on GD14 (Schambra et al., 1994), but not at earlier times. The earliest detection of dopamine receptors was

* To whom correspondence should be addressed.
Tel: 82-591-50-8716, Fax: 82-591-759-0779

confirmed by polymerase chain reaction. D_1, D_{1b}, and D_3 receptor mRNAs were first detected between GD11 and GD14. The D_1 receptor mRNA was marginally expressed from GD11, while the D_{1b} receptor was not detectable before GD12. The D_3 receptor mRNA was slightly expressed at GD11 and clearly present at GD14 (Cadoret et al., 1993). Thus, the first appearance of dopamine receptors was different depending on methods employed.

During the prenatal development, the dopamine receptor mRNAs were differentially observed according to the many discrete brain areas. Recently, Schambra et al. (1994) found that dopamine receptor mRNAs were expressed in the caudate putamen, olfactory tubercle, nucleus accumbens, inferior colliculus, cerebral cortex, hypothalamus, and thalamus during the rat prenatal period. In the present study, we used *in situ* hybridization and immunohistochemistry to investigate the ontogenic expression of catecholaminergic neurons and dopamine receptor subtype mRNAs in the rat fetus.

Materials and Methods

Animals and tissue preparation

Timed pregnant [day of insemination equals to GD (gestational day) 0] Sprague Dawley rats, sperm-positive on specific days, were sacrificed by decapitation on GD9, 11, 13, 15, 17, 19, and 21, and postnatal day (PD)3, and then embryos and fetuses were removed by cesarean section. Whole fetuses on GD9, 11, 13, 15, and 17, the heads of GD19 and the brains of GD21 and PD3 were removed and freed from the ammiotic membranes. The embryos were dissected, fixed by immersion in 4% paraformaldehyde for 24 h, and cryoprotected by immersion in 20% sucrose phosphate buffer for 24 h. Ten micrometer sections were cut in sagittal (mid, para, and lateral) planes on a cryomicrotome. Sections were thaw-mounted on the probe-on plus charged slides (Fisher) at room temperature, placed in the cryostat, and then stored at -70℃ until use.

Subcloning of dopamine D_1 and D_2 receptor cDNAs

The full length cDNA clones for the rat dopamine D_1 and D_2 receptors were obtained from Dr. David Grandy (Vollum Institute, OR) (Bunzow et al., 1988; Zhou et al., 1990). The partial fragment of D_1 and D_2 receptor was 500 bp (from 1563 to 2063) and 309 bp (from 2008 to 2317) in size. A 500 bp partial D_1 receptor fragment containing sequences encoding C-terminus was cloned into the RNA synthesizing vector pGEM3Z. A 309 bp partial D_2 receptor fragment containing sequences encoding C-terminus was cloned into the same vector. There was no sequence homology between them. The vector contains a polylinker and the promoters for T7 and SP6 polymerase. The DNA constructs were confirmed by sequencing (Sequenase 2.0; USB).

Synthesis of cRNA probes

The dopamine D_1 and D_2 receptor cRNA probes were synthesized from the pGEM3Z recombinant subclones. Antisense D_1 receptor cRNA probe was transcribed by SP6 RNA polymerase from the D_1 construct linearized with *Sac*I restriction enzyme, while sense D_1 receptor cRNA probe was transcribed by T7 RNA polymerase. Antisense D_2 receptor cRNA probe was transcribed by T7 RNA polymerase from the D_2 construct linearized with *Apa*I, while sense D_2 receptor cRNA probe was transcribed with SP6 RNA polymerase. The [^{35}S]-UTP-labelled probes were prepared using Promega *in vitro* Transcription Kit (Promega) to a specific activity of 1.0×10^9 cpm/μg. The antisense and sense cRNA probes were purified by Sephadex G-50 DNA grade column and eluted with SET buffer (0.1% SDS, 1 mM EDTA, 10 mM Tris, 10 mM dithiothreitol). Polyacrylamide gel analysis of purified probes revealed that >90% of the probes were of the expected length. The activity of the cRNA probes were approximately 1×10^9 cpm/μg.

In situ hybridization

The specific activity of the probes was greater than 1×10^8 cpm/μg. Before *in situ* hybridization, the tissue sections were fixed in ice-cold 4% paraformaldehyde in phosphate-buffered saline, and washed in 2× sodium chloride-sodium citrate buffer (SSC; 0.5 M NaCl; 0.3 M sodium citrate, pH 7.0). Subsequently, the sections were covered with prehybridization buffer [50% formamide, 0.6 M NaCl, 10 mM Tris-HCl (pH 7.5), 0.02% Ficoll, 0.02% polyvinyl pryollidone, 0.1% bovine serum albumin, 1 mM EDTA (pH 8.0) and dextran sulfate] and incubated at 37℃ for 1 h. After removal of the prehybridization buffer, slides were covered with hybridization buffer. Hybridization with the antisense or sense probes were carried out in this same solution with the addition of 50 μg/ml yeast tRNA, 10 mM dithiothreitol, 10% and 6×10^5 cpm of RNA probe per μl of solution. The slides were coverslipped and incubated at 60℃ for 24 h. Tissue slides were then posthybridized in a posthybridization buffer. Subsquently, following a wash in 2×SSC for 30 min, the sections were treated with RNase A (50 μg/ml), washed twice in warmed (50℃, high-stringency) 2× SSC buffer, transferred to a wash buffer containing 0.1 ×SSC at 65℃ for 15 min, and dehydrated in alcohol solutions with ascending concentrations. Slides were apposed to autoradiography X-ray film (Amersham) for one to six days in light-tight cassettes at -70℃. The slides were then dipped in Kodak NTB 2 emulsion (1:1 dilution), exposed for two weeks at 4℃, developed in Kodak D19 developer (1:1 dilution, 15℃) and counterstained with methyl-green and cresyl violet. The slides were observed under a dark and a bright fielded

microscope, and then photographed.

Immunohistochemistry

For the localization of immunoreactive tyrosine hydroxylase (TH), avidin-biotin complex (ABC) method was used (Hsu et al., 1981). Tissue sections on slides were air-dried, dipped twice in 0.02 M phosphate buffered saline (PBS, pH 7.4) for 5 min. Before the primary antibody application, the tissue sections were incubated for 30 min with 40 μl of normal goat serum diluted with 1:20 to eliminate the nonspecific binding. Slides were then applied with 50 μl of the primary antibody, rabbit-derived anti TH (Instar Co.) with a final dilution of 1:2000 for overnight at 4℃. The sections were washed with PBS for 10 min twice, incubated with 0.5% periodic acid to exclude the endogenous peroxidase, and then applied with 50 μl of biotinylated goat anti-rabbit IgG (Vector) for 30 min at room temperature. After washing with PBS, the sections were incubated with 0.5% 3,3'-diaminobenzidine (DAB) in 0.01% hydrogen peroxide in PBS for 5 min, washed, dehydrated, mounted in a synthetic mounting medium, and then observed under a light microscope.

Results

Expression of dopamine D_1 and D_2 receptor mRNAs was detected in the fetal rat brains with *in situ* hybridization using ^{35}S-labeled cRNA probes. Dopamine D_1 and D_2 receptor mRNAs were not found in the fetal rat brains from GD9 and GD11. Dopamine D_1 and D_2 receptor mRNA signals were observed in neural tissues from the GD13.

Ontogeny of dopamine D_1 receptor mRNA

On GD13, dopamine D_1 receptor hybridization signals were detected in discrete brain regions including the ventricular and subventricular zone of ganglionic eminence (basal ganglia) (Fig. 1A, B, and C), and the thalamic, and hypothalamic neuroepithelium (Fig. 1A and B), cortical area (neocortex, cingulate cortical neuroepithelium) and hippocampal neuroepithelium (Fig. 1B).

Dopamine D_1 receptor mRNA was detected in the midsagittal section of fetuses on GD15. D_1 receptor mRNA was found in the hippocampus, neocortex, cingulate cortex, pallidal neuroepithelium, superior and inferior colliculus, epithalamus, thalamus, hypothalamus (posterior, anterior, and intermediate zone) and pontine neuroepithelia. Dopamine D_1 receptor mRNAs were expressed in the anterior pontine neuroepithelium, anterior pons differentiating field, and posterior pontine neureoepithelium. At this stage, in the pontine area, pattern of expression for D_1 mRNAs was similar to the pattern of that for D_2 mRNAs (Fig. 5A and B). At the same stage, the expression of dopamine D_1 receptor mRNA decreased in the ventricular and subventricular zone of ganglionic eminence and increased in the caudate putaman (Fig. 2A).

On GD17, strong dopamine D_1 receptor hybridization

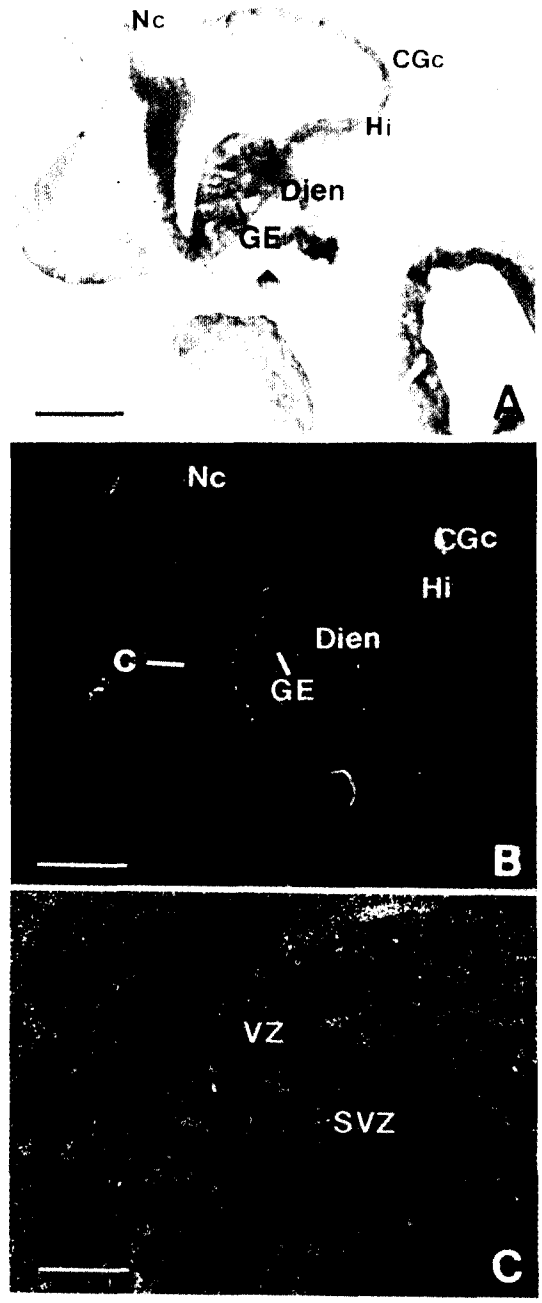

Fig. 1. Parasagittal sections of the whole fetus for D_1 receptor mRNA on GD13. A, Signals for D_1 receptor mRNAs were localized in the ventricular (VZ) and subventricular zone (SVZ) of ganglionic eminence, thalamic, and hypothalamic neuroepithelium of diencephalon (Dien), neocortex (Nc), cingulate (CGc) cortex neuroepithelium, and hippocampal neuroepithelium (Hi). Black silver grains represent D_1 receptor mRNA in the bright-field photomicrograph. B, The clusters of white grains represent the D_1 receptor mRNA in the dark-field photomicrograph, corresponding to the dark-field photomicrograph of Fig. A. C, Higher magnification of indicated area in Fig. B. D_1 receptor mRNAs are strongly expressed in the ventricular and subventricular zone of ganglionic eminence. Scale bars=600 μm (A, B) and 100 μm (C).

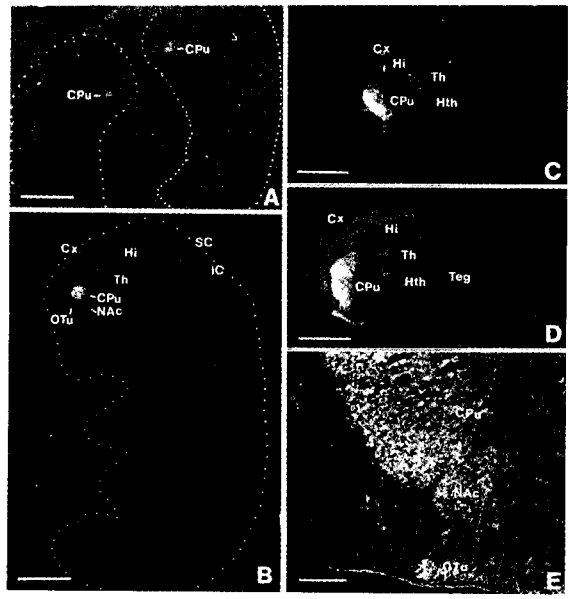

Fig. 2. Film autoradiography (A, B, C, and D) and dark-field photomicrograph (E) for D_1 receptor mRNA in the lateral sagittal sections of whole embryos on GD15 (A) and GD17 (B), and fetal brains on GD21 (C) and PD3 (D and E). A, D_1 receptor mRNAs were clearly found in the caudate putamen (CPu) of striatum in the x-ray film autoradiography. B, On GD17, strong positive signals were localized in the CPu, olfactory tubercle (OTu), nucleus accumbens (NAc), frontal, insular, retrosplenial and cingulate cortex (Cx), hippocampus (Hi), thalamus (Th) and hypothamus. Note that the D_1 dopamine receptor mRNA are increased in the CPu of striatum. C, On GD21, positive signals were increased in CPu, Th, frontal cortex (Cx), and Hi. D, On PD3, positive signals were increased in the CPu, NAc, OTu, thalamus, and hypothalamus. E, Higher magnification of CPu area of fig(D). Scale bars=1500 μm (A, B, C, D) and 300 μm (E).

signals were observed in the caudate putamen, and lower levels of signals were detected in the olfactory tubercle, nucleus accumbens, frontal, insular, retrosplenial and cinglulate cortex, hippocampus, thalamus, and hypothalamus. Dopamine D_1 receptor mRNA increased gradually in the caudate putamen of striatum (Fig. 2B).

On GD19, distinct hybridization signals were observed in the caudate putamen, olfactory tubercle, nucleus accumbens, frontal, insular, retrosplenial and cinglulate cortex, hippocampus, thalamus, and hypothalamus. The dopamine D_1 receptor mRNA first observed in the olfactory bulb on GD19. At the same day, positive signals were also detected in posterior nuclear thalamus complex and anterior ventral thalamic nucleus of thalamus (Fig. 3).

Just before birth and on PD3, intensity of signals for D_1 receptors was increased. Positive signals for D_1 receptor were localized in the caudate putamen, olfactory tubercle, nucleus accumbens, olfactory bulb, thalamus, superior and inferior colliculus, frontal cortex, hippocampus, and pons in the parasagittal and midsagittal section of fetal brains (Fig. 2D). On GD21, positive signals were observed in the olfactory bulb, superior and inferior collicullus differentiating field, cortical plate, medulla and interpedunclar nucleus,

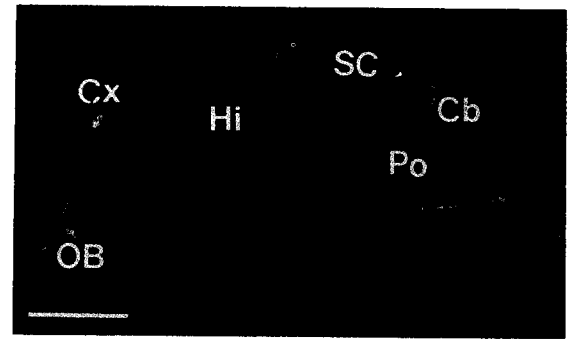

Fig. 3. Midsagittal section of fetal brains for D_1 receptor mRNA on GD19. Positive signals were localized in the olfactory bulb (OB), thalamus (Th), superior colliculus (SC) and cerebellar area (Cb), frontal cortex (Cx), hippocampus (Hi), and pons (Po). Scale bar=1500 μm.

hypothalamus, and hippocampus (Fig. 2C). On PD3, positive signals were increased in the caudate putamen, olfactory tubercle, nucleus accumbens, thalamus, hypothalamus, hippocampus, and frontal, insular, and cingulate cortex (Fig. 2D and E).

Ontogeny of dopamine D_2 receptor mRNA

On GD13, strong dopamine D_2 receptor hybridization

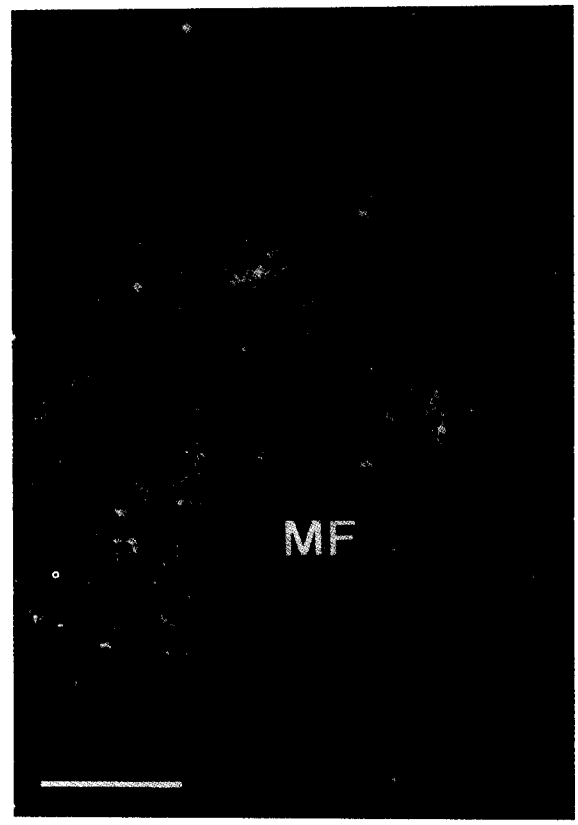

Fig. 4. Dark-field photomicrograph of parasagittal sections of GD13 brains through mesencephalic flexure (MF) of ventral mesencephalon. Strong signal for D_2 receptor mRNA are observed in cells in the mesencephalic dopaminergic nuclear complex of tegmental differentiating field. Scale bar=300 μm.

signals were detected in discrete brain regions including the ventricular and subventricular zone of ganglionic eminence (basal ganglia), thalamic, hypothalamic (intermediate, anterior and posterior) neuroepithelium. At this stage, dopamine D_2 receptor mRNA signals were more weakly labeled than that of D_1 receptor mRNA in the cortical neuroepithelium. The hybridization signal of the D_2 dopamine receptor mRNA was also found in the tegmental differentating field (Fig. 4). Mesencephalic dopaminergic nuclear complex was developed in this tegmental differentiating field. However D_1 mRNA was not detected in this area.

Dopamine D_2 receptor mRNAs were observed in the subventricular zone and intermediate zone of the developing caudate putaman on GD15. At this stage, the expression of dopamine receptor mRNAs decreased in the ventricular and subventricular zone of ganglionic eminence and increased in the caudate putaman. D_2 receptor mRNA was also expressed in the neocortex, pons, superior and inferior colliculus, thalamus, and hypothalamus. However, D_2 receptor mRNA was less intense than D_1 receptor mRNA in the cortical area. Dopamine D_2 receptor mRNA in the A9~10 appeared from this age. In the pons, dopamine D_2 receptor mRNAs were expressed in the anterior pontine neuroepithelium, anterior pons differentiating field, and posterior pontine neureoepithelium (Fig. 5A and C).

On GD17, dopamine D_2 receptor mRNAs were expressed on parasagittal sections of fetal brain. On GD17, cells in the tegmentum and ventral tegmentum area of the ventral mesencephalon were intensely labeled for the D_2 dopamine receptor. Also positive signals were observed in the caudate putamen, nucleus accumbens, olfactory tubercle, tegmentum, and ventral tegmental area (Fig. 6A and B).

On GD19, dopamine D_2 receptor mRNA was expressed on parasagittal and midsagittal section of embryos. Positive cells were seen in the caudate putamen, nucleus accumbens, olfactory tubercle, pars compacta of substantia nigra, ventral tegmental area, olfactory bulb (neuroepithelium, subventricular zone, glomerular layer of external plexform cortex, granular layer, and accessory olfactory bulb), differentiating field of olfactory bulb (Fig. 6C and D). D_2 mRNA was observed in cells of the mesencephalic A9~10 complex, which is separated into the ventral tegmental area and substantia nigra after GD19 (Fig. 7B). On GD21 and PD3, dopamine D_2 receptor mRNA signals were increased in the caudate putamen, nucleus accumbens, olfactory tubercle, pars compacta of substantia nigra, ventral tegmental area, and olfactory bulb.

Ontogeny TH-containing cells

TH was immuno-stained in the fetal rat in order to correlate the protein level. TH-containing cells were first detected in the neuroepithelium of intermediate zone. TH positive neurons from GD11 and GD13 were migrating and fetuses of these cells were immature

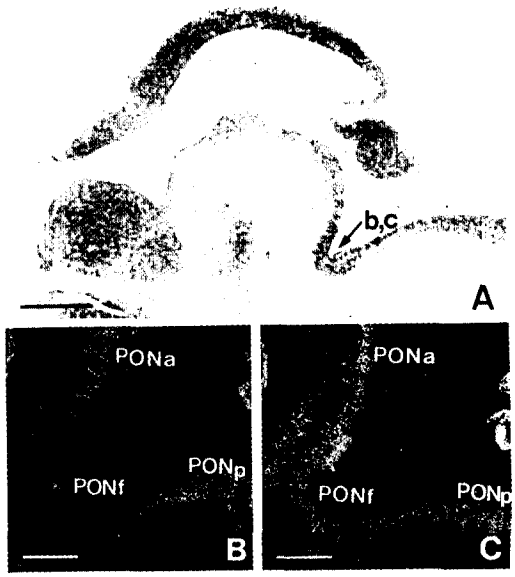

Fig. 5. Expression of D_1 and D_2 receptor mRNAs in the pontine area of GD15 embryos. A, Bright-field photomicrograph in the midsagittal section of fetal brain. B and C, Dark-field photomicrograph for the D_1 receptor mRNA (B) and the D_2 receptor mRNA (C) in the corresponding area of Fig. A. Notes the location of D_1 and D_2 receptor mRNAs in the anterior pontine neuroepithelium (PONa), anterior pons differentiating field (PONf), and posterior pontine neuroepithelium (PONp). Scale bars=600 μm (A) and 300 μm.

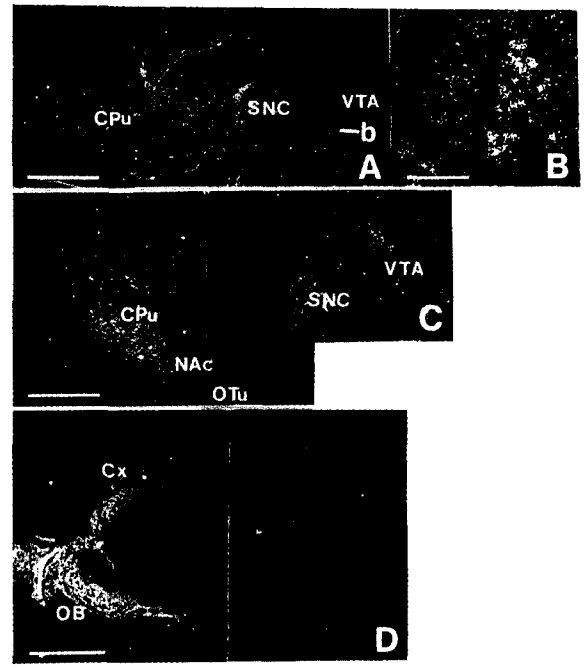

Fig. 6. Dark-field photomicrographs for D_2 receptor mRNA on sagittal section of GD17 (A and B) and GD19 (C and D) embryos. A, B, and C are parasagittal planes and D is midsagittal section through olfactory bulb. B, Higher magnification of the indicated area in Fig. A. Labeled cells are shown in the CPu, NAc, OTu, pars compacta of substantia nigra (SNC), ventral tegmental area (VTA) (A, B, and C), glomerular layer external plexform layer, granular layer, and accessory olfactory bulb differentiating field of olfactory bulb (OB) (D). Scale bars=600 μm (A, C, D) and 100 μm (B).

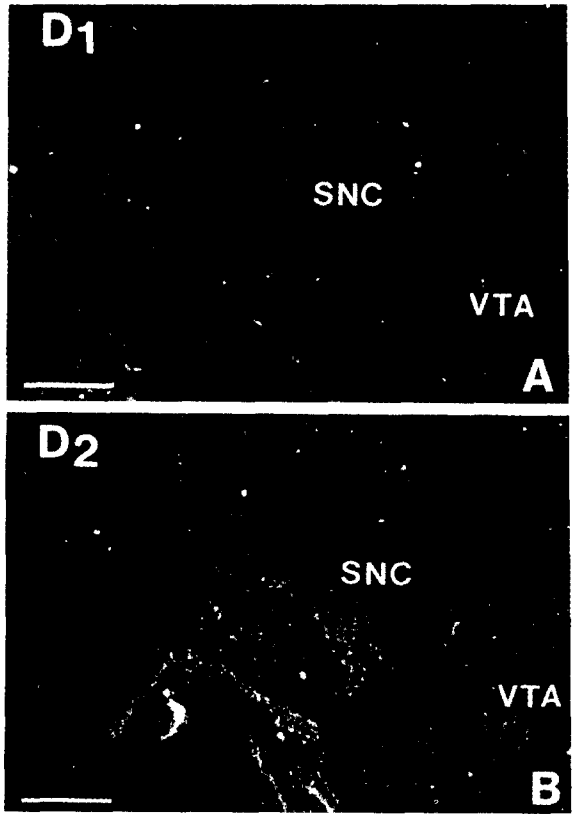

Fig. 7. Comparison between D_1 and D_2 receptor mRNA in the substantia nigra of mesencephalon on GD19. A, D_1 receptor mRNA was not detected in the substantia nigra. B, D_2 receptor mRNA was observed in cells in the mesencaphalic A9~10 complex, which is separated into the ventral tegmental area (VTA) and substantia nigra (SNC). Scale bars=300 μm.

(Fig. 8A). TH-containing neurons were continuously observed on GD11, 13, 15, 17, 19, 21, and PD3. The most striking change in the TH-labeled neurons during developmental process was the increase in number of cells, bundles of detectable axons and in terminal aborization (Fig. 8B). TH-containing cells were observed in the neuroepithelium of subventricular zone and mesencephalic dopaminergic A8~10 complex through the brain on GD13. Also, TH-containing cells were observed in several different areas which is substantia nigra, pons, thalamus, and A1~A15 zones through the whole brain on GD15, 17, and 19 (Fig. 8B and C).

Discussion

Striatum

We observed both dopamine D_1 and D_2 receptor mRNAs in the intermediate, ventricular and subventricular zone of ganglionic eminence from which the striatum developed as early as GD13. Cardoret et al. (1993) observed that dopamine D_1 receptor mRNA was expressed on GD11 by reverse transcription polymerase chain reaction. Schambra et al. (1994) reported the expression of dopamine D_1 and D_2 receptor

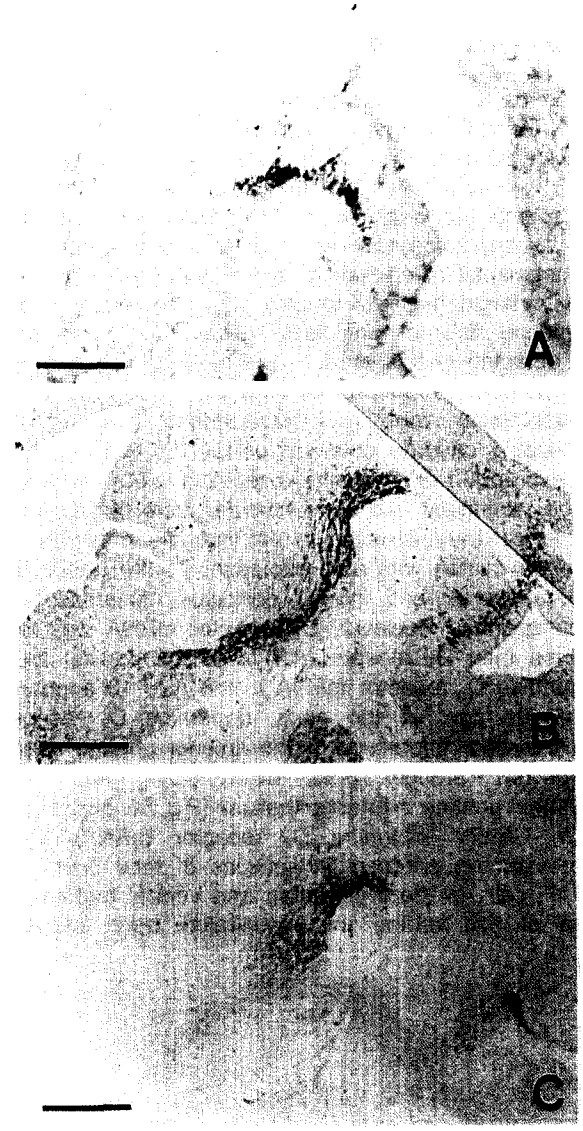

Fig. 8. Immunohistochemical localization of TH-containing cells in the mesencephalic dopaminergic A8~10 complex through the brain on GD13 (A), several different areas through the whole brain on GD15 (B), and several different areas through the substantia nigra and ventral tegmental area of whole brain on GD17 (C). Scale bars=650 μm.

mRNAs on GD14 in the rat fetal brain. Guennoun and Bloch (1991) observed that dopamine D_2 receptor is expressed on GD14 by using autoradiography and oligonucleotide probe. These data suggested that the origin of the cells associated with the dopamine D_1 and D_2 receptor expression lies shortly before GD13. Our results suggest that neuroblasts from this region, which expresses dopamine receptor mRNA, have migrated to the deep intermediate zone in the final step of cell division and will participate in the dopaminergic innervation as neurons of the first differentiation.

Two days later, on GD15, most cells showing the signal for the dopamine D_1 and D_2 receptor mRNA were localized in the deeper intermediate zone which

contains maturing neurons in the caudate putamen of striatum. But a few cells were still labeled with the receptor mRNA in the ventricular zone/subventricular zone. Bayer (1984) reported that most striatal neurons are born between GD12 and 15, with a peak of development of large neurons on GD13 and of medium-size neurons on GD15. Earlier developing medium size neurons of the rostral striatum have been also observed to settle in clusters with a ventrolateral to dorsomedial gradient (Marchard and Lajoio, 1986).

The localization of dopamine D_1 and D_2 receptor mRNAs on GD17 was similar to that shown in the adult brain (Weiner and Brann, 1989; Fremeau et al., 1991; Weiner et al., 1991). We also observed that intense signals for the dopamine D_1 and D_2 receptor mRNAs were mostly localized in the lateral caudate putamen cell groups, but at birth, strong signals were detected in the medial putamen cell clusters. Before birth, expression of dopamine receptors is related to the neuronal function according to their subtypes. Schambra et al. (1994) reported that at birth, clusters of cells were additionally expressed in the medial caudate putamen. Such clusters of cells were intensely labeled with the dopamine D_1 and D_2 receptor mRNAs. This labelling continued until PD1. In the present study, however, both dopamine receptor messages increased in the caudate putamen during the development.

On GD19 and GD21, it is conceivable that these cell clusters are representative of developing patches in the striatum. Such patches are thought to be formed from groups of earlier developing cells that become separated by cells generated later (Fishell and van der Kooy, 1987; Van der Kooy and Fishell, 1987; Mack et al., 1991). Acetylcholinesterase, enkephalin, substance P, opiate receptors neurotensin, and dopamine have been observed in these patches (Murrin, 1982; Graybiel and Ragsdale, 1983; Murrin and Ferrer, 1984; Lanca et al., 1986; Graybiel, 1990). Therefore, dopamine receptors and dopaminergic transmission are supposed to be connected with neuropeptide secretion.

Substantia nigra and tegmentum.

On GD13, the dopamine D_2 receptor mRNA was localized in the cells of mesencepalic dopaminergic A9~10 complex, which had already migrated from the ventricular zone of the mesencephalic area. Since the development of those cells peaks between GD12 and GD13 (Olson and Seiger, 1972; Lauder and Bloom, 1974; Schlumpf et al., 1980), it appears that dopamine D_2 receptor mRNA detected on GD13 is probably in the ventricular zone. Further studies with GD12 fetuses are required to determine whether dopamine D_2 receptor mRNA is expressed in the neuroblasts producing the cells for the A8~10 complex. It is of importance to note that the intermediate zone produces dopamine D_2 receptor protein as shown by autoradiography (Sales et al., 1989).

Cortex

Dopamine D_1 receptor mRNA was expressed in ventricular cells of the insular, cingulate, frontal and parietal cortices on GD13. Expression of dopamine receptor in the cortex on GD13 is earlier than any other report. By GD13, dopaminergic fibers, identified with an antibody to TH, reached to the ventral ganglionic eminence and by GD15, they arrived at the lateral cortex through the ganglionic eminence. Then, these dopaminergic fibers were observed in the frontal cortex and cortical subplate at GD17 (Voorn, 1988). Schambra et al. (1994) observed dopamine receptor mRNAs in the cells of the ventricular zone from GD14 to 16, after then in neurons of layers. This is consistent with the appearance of neurons in the deep cortical layers. In the adult rat brain, dopamine terminals are prominent in the prefrontal, frontal, retrosplenial, cingulate, piriform and parietal cortices (Lindvall et al., 1978; Descarries, 1987; Van Eden et al., 1987; Kalsbeek et al., 1988). In the present study, the most intense cortical label of the dopamine D_1 and D_2 receptor mRNAs were observed in the frontal, cingulate, and parietal cortical areas. Especially, in this cortical areas, the mRNAs were first expressed on GD13. From GD13 to 15, dopamine D_1 and D_2 receptors were mainly expressed in the cells of cortical layers. The cortical projections from the mediodorsal thalamic nucleus and the mesencephalic dopaminergic nuclei suggests their functional roles in affective, cognitive, and emotional behaviors and locomotor activity (Van Eden et al., 1987). This distribution of the dopamine D_1 and D_2 receptor mRNAs may be related to the TH-immunoreactive fiber bundle in the marginal zone and a larger bundle within the subplate. Specht et al. (1978) observed cells stained with the antibody to TH in the ventricular zone of the ganglionic eminence and cortex between GD12 and 17.

Other brain regions

There are signals of dopamine D_1 receptor mRNA in the intermediate zone of the hypothalamus, thalamus, epithalamus, pons, and spinal cord on GD13. These results indicate that these cells are under the process of migrating to their final destination. The early expression of dopamine D_1 and D_2 receptor mRNAs in the suprachiasmatic nucleus of the hypothalamus may be required to receive projections from the retinohypothalamic tract. The retinohypothalamic tract arisen from the ganglion cells in the retina or from the substantia nigra expressed dopamine receptor transiently in the hypothalamus (Moore and Lenn, 1972; Reisert et al., 1990). Later, dopamine D_1 and D_2 receptor were also detected in the pons, olfactory bulb, inferior, and superior colliculus on GD15. By GD17, strong signals for the dopamine D_1 and D_2 receptor mRNAs were detected in similar structures of the adult rats. When dopamine is no longer needed as

neurogenetic signal and synapses become functional, reciprocal feedback, and self-regulation would establish the number of dopaminergic receptor for adult functional needs (Noisin and Thomas, 1988).

In conclusion, dopamine D_1 and D_2 receptors were expressed in various brain areas during fetal development. It appears that dopamine might function in the fetal brain during the early gestational period.

Acknowledgments

This work was supported by grants from the Korea Science and Engineering Foundation (KOSEF 951-0709-050-2 and HRC-97-0102). M.O.K. was supported by the KOSEF postdoctoral fellowship.

References

Bayer SA (1984) Neurogenesis in the rat neostriatum. *Int J Dev Neurosci* 2: 163-175.

Bruinink A, Lichtensteiger W, and Schlumpf M (1983) Pre- and postnatal ontogeny and characterization of dopaminergic D_2, serotonergic S_2, and spirodecanone binding sites in rat forebrain. *J Neurochem* 40: 1227-1236.

Bunzow JR, Van Tol HHM, Grandy DK, Albert P, Salon J, Christie MC, Machida CA, Neve KA, and Civelli O (1988) Cloning and expression of a rat D_2-dopamine receptor cDNA. *Nature* 336: 783-787.

Cadoret MA, Jaber M, and Bloch B (1993) Prenatal D_1, D_{1b}, and D_3 dopamine receptor gene expression in the rat forebrain: detection by reverse polymerase chain reaction. *Neurosci Lett* 155: 92-95.

Descarries L, Lemay B, Doucet G, and Berger B (1987) Regional and laminar density of the dopamine innervation in adult rat cerebral cortex. *Neuroscience* 21: 807-824.

Deskin R, Seidler FJ, Whitmore WL, and Slotkin TA (1981) Development of alpha-noradrenergic and dopaminergic receptor systems depends on maturation of their presynaptic nerve terminals in the rat brain. *J Neurochem* 36: 1683-1690.

Fishell G and van der Kooy D (1987) Pattern formation in the striatum: developmental changes in the distribution of striatonigral neurons. *J Neurosci* 7: 1969-1978.

Fremeau RT, Jr, Duncan GE, Fornaretto MG, Dearry A, Gingrich JA, Breese GR, and Caron MG (1991) Localization of D_1 dopamine receptor mRNA in brain supports a role in cognitive, affective, and neuroendocrine aspects of dopaminergic neurotransmission. *Proc Natl Acad Sci USA* 88: 3772-3776.

Graybiel AM and Ragsdale CW, Jr (1983) Biochemical anatomy of the striatum. In: Emson PC (ed), Chemical Neuroanatomy, Raven Press, New York, pp 27-504.

Graybiel AM (1990) Neurotransmitters and neuromodulators in the basal ganglia. *Trends Neurosci* 13: 244-254.

Guennoun R and Bloch B (1991) D_2 dopamine receptor gene expression in rat striatum during ontogeny: an *in situ* hybridization study. *Dev Brain Res* 60: 79-87.

Guennoun R and Bloch B (1992) Ontogeny of D_1 and DARPP-32 gene expression in the rat striatum: an *in situ* hybridization study. *Mol Brain Res* 12: 131-139.

Hsu SM, Raine L, and Fanger H (1981) The use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase technique: a comparison between ABC and unlabled antibody (PAP) procedure. *J Histochem Cytochem* 29: 577-580.

Kalsbeek A, Voorn P, Buijs RM, Pool LW, and Uylings HBM (1988) Development of the dopaminergic innervation in the prefrontal cortex of the rat. *J Comp Neurosci* 20: 773-795.

Lanca AJ, Boyd S, Kolb BE, and Van der Kooy D (1986) The development of a patchy orgnization of the rat striatum. *Dev Brain Res* 27: 1-10.

Lauder JM and Bloom FE (1974) Ontogeny of monoamine neuron in the locus coeruleus, raphe nuclei and substantia nigra of the rat. I. Cell differentiation. *J Comp Neurol* 155: 469-482.

Lindvall O, Bjorklund A, and Divac I (1978) Organization of catecholamine neurons projecting to the frontal cortex in the rat. *Brain Res* 142: 1-24.

Mack KJ, O'Mally KL, and Todd RD (1991) Differential expression of dopaminergic D_2 receptor messenger RNAs during development. *Dev Brain Res* 59: 249-251

Marchard R and Lajoie L (1986) Histogenesis of the striopallidal system in the rat: neurogenesis of its neurons. *Neuroscience* 17: 573-590.

Miller JC and Friedhoff AJ (1986) Development of specificity and stereoselectivity of rat brain dopamine receptors. *Int J Dev Neurosci* 4: 21-26.

Monsma FJ, Jr, Mahan LC, McVittie LD, Gerfen CR, and Sibley DR (1990) Molecular cloning and expression of a D_1 dopamine receptor linked to adenylate cyclase activation. *Proc Natl Acad Sci USA* 87: 6723-6727.

Moore, RY and Lenn NJ (1972) A retino-hypothalamic projection in the rat. *J Comp Neurol* 146: 1-14.

Murrin LC (1982) *In vivo* studies of dopamine receptor ontogeny. *Life Sci* 31: 971-980.

Murrin LC and Ferrer JR (1984) Ontogeny of the rat striatum: correspondence of dopamine terminals, opiate receptors and acetylcholinesterase. *Neurosci Lett* 47: 155-160.

Murrin LC and Zeng W (1989) Dopamine D_1 receptor development in the rat striatum: early localization in striosomes. *Brain Res* 480: 170-177.

Murrin LC and Zeng W (1990) Ontogeny of dopamine D_1 receptors in rat forebrain: a quantitative autoradiographic study. *Dev Brain Res* 57: 7-13.

Noisin EL and Thomas WE (1988) Ontogeny of dopaminergic function in the rat midbrain tegmentum, corpus striatum and frontal cortex. *Dev Brain Res* 41: 241-252.

Olson L and Seiger O (1972) Early prenatal ontogeny of central monoamine neurons in the rat: fluorescence histochemical observations. *Z Anat Entwicklungsgesch* 137: 301-316.

Reisert I, Schuster R, Zienecker R, and Pilgrim C (1990) Prenatal development of mesencephalic and diencephalic dopaminergic systems in the male and female rat. *Dev Brain Res* 53: 222-229.

Sales N, Martres MP, Bouthenet ML, and Schwartz JC (1989) Ontogeny of dopaminergic D-2 receptors in the rat nervous system: characterization and detailed autoradiographic mapping with [^{125}I] iodosulpride. *Neuroscience* 28: 673-700.

Schambra UB, Duncan GE, Breese GR, Fornaretto. MG, Caron MG, and Fremeau RT, Jr (1994) Ontogeny of D_{1A} and D_2 dopamine receptor subtypes in rat brain using *in situ* hybridization and receptor binding. *Neuroscience* 62: 65-85.

Schlumpf M, Lichtensteiger W, Shoemaker WJ, and Bloom FE (1980) Fetal monoamine systems: early stages and cortical projections. In: Rarvez H and Parvez S (eds), Biogenic Amines in Development, Elsevier/North Holland Biomedical Press, New York, pp 567-590.

Seiger A and Olson L (1973) Late prenatal ontogeny of central monoamine neurons in the rat: fluorescence histochemical observations. *Z Anat Entwicklungsgesch* 140: 81-318.

Sibley DR and Monsma FJ (1992) Molecular biology of dopamine receptors. *Trends Pharm Sci* 13: 61-69.

Specht LA, Pickel VM, John TH, and Reis D (1978) Immunocytochemical localization of tyrosine hydroxylase in processes within the ventricular zone of prenatal rat brain. *Brain Res* 156: 315-321.

Specht LA, Pickel VM, John TH, and Reis D (1981) Light-microscopic immunocytochemical localization of tyrosine hydroxylase in prenatal rat brain. I. Early ontogeny. *J Comp Neurol* 199: 233-253.

Van der Kooy D and Fishell G (1987) Neuronal birthdate underlies the development of striatal compartments. *Brain Res* 401: 155-161.

Van Eden CG, Hooreman EMD, Buijs RM, Mattijssen MAH,

Geffard M, and Uyling HBM (1987) Immunocytochemical localization of dopamine in the prefrontal cortex of the rat at the light and electron microscopical level. *Neuroscience* 22: 849-862.

Voorn P, Kalsbeek A, Jorritsma-Byham B, and Groenewegen H (1988) The pre-and postnatal development of the dopaminergic cell groups in the ventral mesencephalon and the dopaminergic innervation of the striatum of the rat. *Neuroscience* 25: 857-888.

Weiner DM and Brann MR (1989) The distribution of a dopamine D_2 receptor mRNA in the rat brain. *FEBS Lett* 253: 207-213.

Weiner DM, Levey AI, Sunahara RK, Niznik HB, O'Dowd BF, Seeman P, and Brann MR (1991) D_1 and D_2 dopamine receptor mRNA in rat brain. *Proc Natl Acad Sci USA* 88: 1859-1863.

Zhou Q-Y, Grandy DK, Thambi L, Kushner JA, Van Tol HHM, Cone R, Pribnow D, Salon J, Bunzow JR, and Civelli O (1990) Cloning and expression of human and rat D1 dopamine receptors. *Nature* 347: 76-79.

[Received March 7, 1997; accepted May 17, 1997]