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Comparison of D1 and D2 Dopamine Receptor mRNAs Expression during Development in Fetal Rhesus Monkey Retinas

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ABSTRACT

Dopamine is a major neurotransmitter detected in mammalian retina. In the retina, dopamine performs various physiological and pharmacological roles that are mediated by several receptor subtypes. Although many reports have focused on the localization and functions of dopamine receptor subtypes in the retina, they have not been elucidated clearly yet. In this study, we report about the spatial distribution and expression pattern of dopamine D1 and D2 receptor mRNAs according to developmental stages in foetal rhesus monkey retina. We have determined mRNA quantities of dopamine receptor subtypes by RNase Protection Assay (RPA). Their localization in retinal tissue was confirmed using *in situ* hybridization.

Our results show that D1 and D2 receptor mRNAs were differentially expressed during development. Two dopamine receptor mRNAs were expressed mainly in the retina, and rarely detected in ciliary body and choroid in adult monkey eyes. During development, a large amount of D2 receptor mRNAs was detected at ED 60 and the level increased slowly throughout the gestation period. However, D1 receptor mRNAs was detected at low level by ED 70, and increased steeply at ED 140. D1 and D2 receptor mRNAs both reached maximum level at ED 140 and slightly decreased in the adult. Dopamine receptor mRNAs were localized using *in situ* hybridization. D1 and D2 receptor mRNAs were both detected in the proximal half of the outer neuroblastic layer at ED 60, extended into the entire region of inner and outer nuclear layers at following stages.

It is supposed that dopamine receptor subtypes play different roles on retinal development because D1 and D2 dopamine receptor subtype mRNAs were expressed differentially according to developing stages in fetal monkey retina.

Key words: Dopamine receptor, development, monkey retina, mRNA expression

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INTRODUCTION

Dopamine, a catecholamine neurotransmitter, have a central role in movement control, expression of sentiment, regulation of neuroendocrine function and cardiac vascular function in mammal (Gingrich and Caron, 1993). The retina is the region in which photoreception occurs, in which exist almost of all neutrotransmitters of brain and relating enzymes including dopamine. Amongst of all, dopamine seems to be a major neurotransmitter in the mammalian retina (Kamp, 1985; Nguyen-Legros et al., 1997) and participates in various functions of the retina (Djagoz and Wagner, 1992). Dopamine was detected in the retina of various animals (Brecha et al., 1984; Mariani and Hokoc, 1988) containing mammals, amphibia and teleost using antiserum directed against tyrosine hydroxylase as a marker.

Dopaminergic neurons of the retina mostly were axonless amacrine cells in inner nuclear layer and contains interplexiform cells and displaced amacrine cells in ganglion cell layer. Dopaminergic amacrine cells are arranged in a row of cell layer in innermost side of inner nuclear layer. The dendritic processes of these cells were extended into inner border of inner plexiform layer (Nguyen-Legros et al., 1983; Brecha et al., 1984; Nguyen-Legros et al., 1984; Tork and Stone, 1979). These dendrites, are studded with fine varicoses or form ring-like structure with some of varicoses, are presynaptic to the somata of AII amacrine cells (Voigt and Wassle, 1987). Distinct morphology of dopaminergic amacrine cells suggests its important role in neural pathway of rod signal to ganglion cells. Through intercellular injection of horseradish peroxidase, it was concluded hypothetically that dopaminergic amacrine cell and interplexiform cell represent a single cell type and that the exons of interplexiform cells reach to inner plexiform layer and outer plexiform layer. Dopaminergic amacrine cells receive synaptic inputs from cone bipolar cells and its varicoses of axon- like processes are connected presynaptic to AII or other amacrine cells (Dacey, 1990). And AII amacrine cells, another amacrine cells, which receive synaptic input from rod bipolar cells and so innervate to cone bipolar cells and ganglion cells by synaptic output in cat and rabbit retina (Hokoc and Mariani, 1988; Sterettoi et al., 1989). Even though, the role of exonless amacrine cell in visual signal transmission processes is not well clarified because of complicate synaptic connections around it.

The various functions of dopamine in the retina are mediated through five or more dopamine receptor subtypes. Dopamine receptor subtypes are grouped into D1 and D2 subfamilies. D1 subfamily is positively coupled to adenylate cyclase but not D2 subfamily (Civelli et al., 1993; Choi et al., 1995). D1 subfamily has D1-like pharmacological profile and composed of D1R, D5R subtypes. D2 subfamily has D2-like pharmacological profile and contains three subtypes such as D2R, D3R and D4R. With the use of a sensitive ribonuclease protection assay and *in situ* hybridization, we analysed regional distribution of D1 and D2 dopamine receptor subtype mRNAs in the foetal monkey eye.

The mRNAs encoding the D1 and D2 receptors are similarly distributed in many region of the brain, with the exception that D1 receptor mRNA are not detected in some dopamine containing cell regions where D2 receptor mRNA are found to be expressed (Fremeau et al., 1991; Meador-Woodruff et al., 1991). D2R mRNA was localized in rat retina by *in situ* hybridization. The labelling was presented in a narrow rim of perikaryon in cells of INL at all level and in rare, scattered cells in the ganglion layer. Cells of ONL and inner segment of photoreceptors and OPL were labelled not above the background (Derouiche and Asan, 1999).

The retina serves as a research model for development and differentiation of neural system (Harris and Christine, 1990). Retinal histogenesis takes weeks in mammals with laminar and cell-type gradient. Ganglion cells are the first to be born, followed subsequently by cone and horizontal cells, while rods are the last (Holt et al., 1988). Several papers reported recently that neural cells in development activate suicide program, results in apoptosis and this process can be inhibited by some nerve growth factors, so on, suggested that nerve growth factors make a important role in development of retina. During development the apoptosis, natural cell death, is occurred in the retina. Within 2 weeks *post natum*, more than 50% of ganglion cells enter in apoptotic process in the rat. Neuronal cells in inner nuclear layer and outer

nuclear layer also suffer apoptosis in a considerable proportion. After all, the apoptosis have an important role in process of retina development (Versaux-Botteri et al., 1992).

Dopamine, an important neuromodulator in the retina, controls the balance of rod con photoreceptor activity and influences on the activity of several interneurons (Koulen, 1999). Nevertheless, its function is not cleared yet. To elucidate its function, it needs in priority more precise morphological informations about synaptic network of dopaminergic neurons. The expression and distribution of dopamine receptor subtype mRNA during development are important to comprehend dopaminergic system in asmuchas tracking out the birth of dopaminergic neuron and its differentiation in the retina. Immunohistochemical approaches using anti-dopamine receptor specific antibodies were not successful and inappropriate for accurate determination of the receptor in other investigations. We applied fine quantitative measurement using RPA for detection of dopamine receptor mRNA and *in situ* hybridization of RNA-anti sense RNA for localization study.

MATERIALS AND METHODS

Experimental animals

Animal care and use were in accordance with our institutional guidelines based on NIH standards, Adults female rhesus monkeys (*Maccaca mulata*) were paired with fertile males for 3 days beginning on day 9～18 of their menstrual cycle based on analysis of their previous menstrual cycle lengths. Pregnancy was determined by RIA analysis of oestrogen (>100 pg/ml) and progesterone (>2.5 ng/ml) in blood sample obtained at day 13～17 after pairing. The second day of pairing was considered as the day of conception and gestation times calculated from the point. The total gestation period in the rhesus monkey is about 165 days.

Tissue preparation

The foetuses were delivered by caesarean section at Day 60, Day 70 and Day 130. For adult eye preparation one 7 years female rhesus monkey was used. Foetus eyes and adult eyes were dissected under dissecting microscope: each eye was, if necessary, dissected 4 portions composed of retina, iris, ciliary body and process, choroid/ retina pigment epithelium. Subsequently, the eye tissues were snap frozen in liquid isopentane (2-methylbutane) chilled to -55°C on dry ice, and stored in liquid nitrogen for RPA assay. For *in situ* hybridization experiments the whole eyes were fixed in 4% NBP containing of DEPC and infiltrated with 20% sucrose solution overnight. The following day, the eyes were frozen, mounted, and cryosected. Then, the sections were stored at -80°C.

Preparation of total RNAs

Total RNAs from these tissues were isolated by a modification of Chirgwin method (Berger and Chirgwin, 1989). Briefly, retinal tissues were homogenized in extraction media (4 M guanidium isothiocyanate, 10 mM EDTA, 2% sodium N-laurylsarcosine, 1% β-mercaptoethanol, 10 mM tris, 10 mM vanadyl ribonucleoside complexes). The homogenized extract was then layered over a 5.7 M cesium chloride gradient and centrifuged overnight at 35,000 RPM with a Beckman SW55TI rotor. The pellet was resuspended in Tris-EDTA buffer containing 0.1% SDS, purified by phenol- chloroform extraction and precipitated with 100% ice-cold ethanol. Total RNA was dissolved in sterile RNase-free water. The obtained RNA was aliquoted and stored at -80°C.

Preparation of cRNA probe and reference RNA

The rhesus monkey dopamine D1 and D2 receptor cDNA segments were subcloned previously (Choi et al., 1995). A 500 bp segment of the D1 receptor was subclonedd into pGEM3Zf(+) and a 337 bp PCR product ofthe D2 receptor cDNA was subcloned into pGEM4Z. The D1 and D2 constructs were linealized using *Eco*R1 and *Hind*III. The 32P-UTP (NEN)-labelled antisense cRNAs were synthesized from respective recombinants using Sp6 RNA polymerase. After reaction the remaining DNA template was digested with DNase I (10 U) at 37°C for 20 min. Then, cRNA probe was subjected to phenol-chloroform extraction and precipitated with ice-cold ethanol precipitation. Obtained probe was separated by 6% polyacrylamide electrophoresis (250 V, 2 h) and eluted in elution buffer. The radioactivity of probe was determined with Beckman β-counter.

Nuclease protection assay (RPA)

The quantities of dopamine receptor subtypes were determined using ribonuclease protection assay as described previously (Choi et al., 1995). Briefly, appropriate quantity of sample RNA (5 or 20 ìg) or reference RNA (sense RNA) was hybiridized with 32P-labeled antisense probes (5×105 cpm) for 16 h at 45°C in a solution (60% formamide, 0.9 M NaCl, 6 mM EDTA, 60 mM Tris-HCl (pH 7.4), 50 ìg of yeast tRNA). Sample RNA was denatured for 5 min at 95°C before hybridization. Non hybridized RNA was digested 37°C for 1 h with RNase T1 solution which was composed of 0.3 M NaCl, 30 mM sodium acetate (pH 4.8), 3 mM ZnCl2 and RNase T1 (Gibco/BRL) of 700 U/tube.

Hybridized RNA was precipitated in 300 ìl of RNase inactivating precipitation solution (Ambion kit) on dry ice for 20 min and pelletized by microcentrifuge (4°C, 20 min, 13,000 RPM). The pellets was resuspended in 8 ìl of loading buffer and denatured at 95°C for 5 min and subsequently subjected to electrophoresis with 6% denatured polyacrylamide gel for 2 h at 250 V. Autoradiograph was prepared by exposing X-ray film on the dried gel for 14～40 h at -80°C. Densitometric scanning values were normalized corresponding to cyclophilin index and were calculated by linear regression analysis of autoradiograph using sense RNA standard curves. Estimated numerical data were expressed by a bar chart.

In situ hybridization localization

Radiolabelled cRNA probes were synthesized using *in vitro* transcription kit (Promega). Template DNA was linearized by appropriate restriction enzyme and transcribed using T7 or SP6 RNA polymerase in the presence of 35S-UTP. First of all, prepared template DNA was transcribed at 37°C for 1 h in transcription reaction media. Remaining DNA template was digested at 37°C for 20 min after adding 1 ìl of RNase-free DNase I. Thereafter, cRNA probe was separated using Sephadex G-50 Nick Column (Pharmacia) and determined radioactivity with β-counting (Beckman). Purified radiolabelled cRNA probes marked the radioactivity of 1.0 ×109 cpm/ìg specificity.

Frozen sections were digested with proteinase K at 37°C for 5 min before prehybridization. The slides were dipped into prehybridization buffer and standed at room temperature for 4 h with gently agitation. Then, Hybridization buffer, which was prepared by adding the cRNA probe into prehybridization buffer, was covered over the section in a moistened dark box and proceeded to the reaction at 60°C for 24 h. After reaction the slides were rinsed with several changes of 2× SSC at room temperature for 5 min and followed by digetion of non-hybridized RNA with RNase A for 1 h at 37°C. Then, the slides were washed stringently with gradually decreasing concentrations of 2× SSC to 0.1× SSC under gradually increasing temperatue

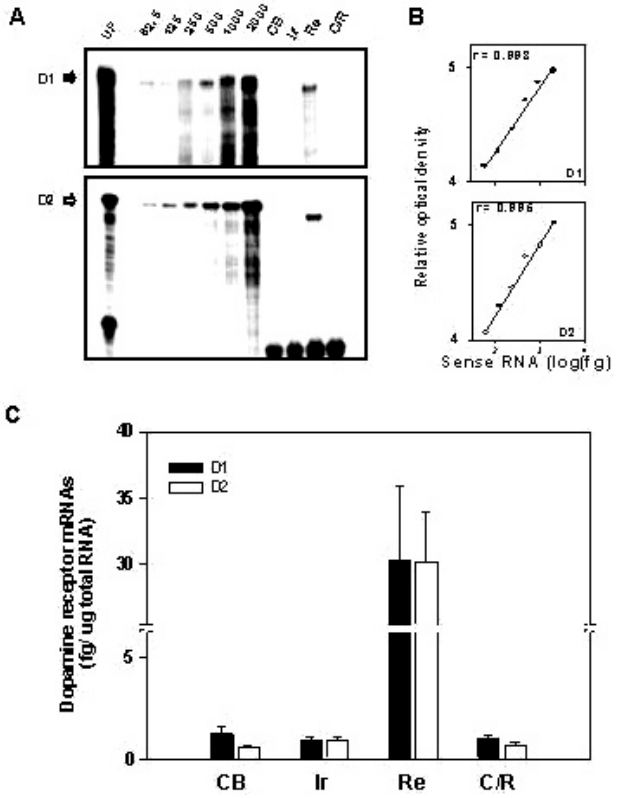

Fig. 1. RPA analysis to determine the distribution of D1, D2 dopamine receptor subtype mRNAs in different tissues of adult monkey eye. Experiment was carried in triplicate for each group. (A): Representative X-ray for D1, D2 mRNAs For the standards, sense RNAs from 62.5 to 2000 fg were used and cyclophilin was used as a standard for normalization. (B): Standard curves for D1 (left panel) and for D2 receptor mRNA (right panel) were presented corresponding to the autoradiographs in (A). Numerical values calculated from each standard curve and are expressed by a bar chart (C) for D1 mRNA (black column) and for D2 mRNA (white column). Standard errors are indicated by bars Abbreviations: CB (ciliary body and processes), Ir (iris), Re (retina), C/R (choroid and retinal pigmented epithelium), UP (undigested probe).

of 37°C to 65°C. After dehydration the slides were coated with autoradiographic emulsion (NTB2, Kodak) and rested in a dark box at 4°C for 14 d to permit autoradiographic reaction and were developed with D-19 developer and fixed with rapid fixer. The developed slides were counterstained with cresyl violet or methyl green and observed with dark field and light field microscopy to analyse dopamine receptor localization patterns.

RESULTS

Regional distribution of D1 and D2 dopamine receptor mRNAs in adult monkey eye

Regional distribution of D1 and D2 dopamine receptor mRNAs in 4 different eye parts (ciliary body and process, iris, retina, choroid/retinal pigmented epithelium) of adult monkey was analysed by ribonuclease protection assay (RPA) with specific cRNA probes. D1 and D2 dopamine receptor mRNAs were detected commonly in high levels in the retina (Fig. 1). But they were detected also in ciliary body, iris and choroid, albeit commonly in a basal levels. Both types were detected in a similar amount in the adult retina. The two mRNAs were detected in ciliary body and processes in similar amount. In iris, D2 mRNAs were detected in a significant measure (1 fg/g total RNA) but for D1 receptor mRNA in a negligible amount, interestingly (Fig. 1C).

Differential expression of D1, D2 dopamine receptor mRNAs in the retina during development

In order to quantify D1 and D2 receptor subtype mRNAs in the monkey foetus retina during development by RPA analysis, we prepared foetal tissues from ED (embryonic day) 60, ED 70 and ED 130 foetuses by caesarean section. In addition, we obtained adult tissue from 7 yr old monkey for the control. D1 receptor mRNA was expressed in a little amount in ED 60 and ED 70 but arrived at the peak (1250 fg/5 ìg total RNA) at ED 140 and sustained high level in 7 yr old adult (Fig. 2C). We

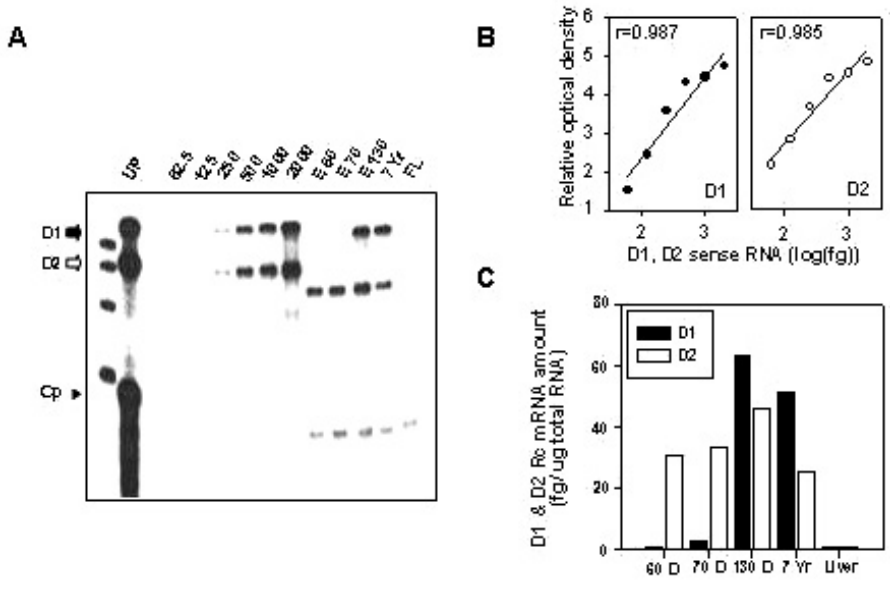

Fig. 2. RPA analysis of D1, D2 dopamine receptor subtype mRNAs corresponding to development in monkey retinas (at ED60, ED70, ED130 and 7 yr old adult). Only one sample for each group was analysed. (A): X-ray autoradiographs for D1, D2 mRNAs are showed. Liver tissue was used for negative control. For the standards, twofold serial diluted sense RNAs from 62.5 to 2000 fg were used and cyclophilin was used as a standard for normalization. (B): Standard curves were prepared for D1 mRNA (upper panel) and D2 mRNA (under panel). (C): Numerical values that were calculated by linear regression are expressed by a bar chart for D1 mRNA (black column) and for D2 mRNA (white column). Abbreviations: UP (undigested probe), FL (foetal liver), Cp (cyclophilin).

can deduct, therefore, that D1 receptor expression rapidly increased at a time point between ED 70 and ED 130 and the peak may sustain till parturition. Contrawise, the expression level in the adult was decreased slightly comparing to the one of ED 130 (Fig. 2C). D2 dopamine receptor mRNA was already expressed profoundly high level (630 fg/ 5 ìg total RNA) at ED 60. And, the level was increased gradually in the later stages at ED 70, ED 130 and decrease slightly in adult (Fig. 2A, C). D2 receptor expression, therefore, shows constitutive expression pattern. Nevertheless, the tendency that the level in the adult diminished slightly below the level of ED 130 can be observed like as D1 dopamine receptor RNA.

These results can be abstracted as follows: Firstly, it can be suggested that D2 dopamine receptor mRNA is the major type dopamine receptor in early developmental stages. Secondly, after ED130, Expression of D1 receptor mRNA is much than D2 dopamine receptor mRNA expression. Finally, D1 and D2 dopamine receptor mRNA expression in the adult were fallen down slightly below the levels of ED 130.

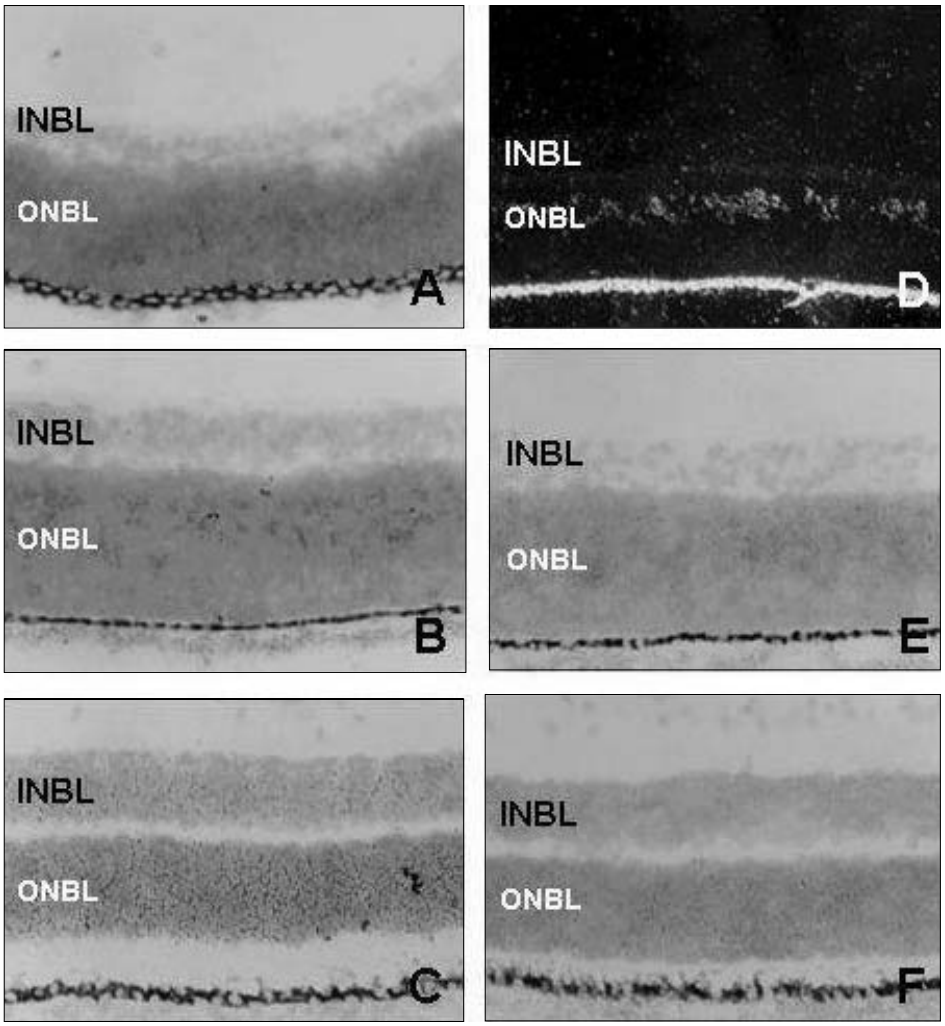

Fig. 3. *In situ* hybridization localization of D1, D2 dopamine receptor mRNAs in developing monkey retina. Autoradiographes reflect visualized image captured by NTB2 emulsion and the tissue slides were counter-stained with 0.5% cresyl violet. Upper panel: D1 mRNA localization in foetal monkey retina (A: ED60, B: ED70, C: ED130). Under panel: D2 mRNA localization in foetal monkey retina (*D: ED60, E: ED70, F: ED130). *D is only presented as a dark field image. Abbreviations: INBL (inner neuroblastic layer), ONBL (outer neuroblastic layer).

Localization of D1 and D2 receptor mRNAs in the developing monkey retina using in situ hybridization

To investigate cellular localization of dopamine receptor subtype mRNAs in retina, autoradiography was applied on *in situ* hybridized slides of which radioemission could be captured using superposed NTB2 emulsion. Visualized signals were observed using dark field microscopy and bright field microscopy after counterstaining. Under the dark field microscope we could identify systemic distribution of the signals that is shown by representative image (Fig. 3D). The bright band superposed on pigmented epithelial layer seems to be false signal generated by diffraction of light and this estimation was confirmed by light microscopic observation.

D1 dopamine receptor subtype mRNA signal was observed in the central part of outer neuroblastic layer at ED 60. The signal was concentrated in inner half of outer neuroblastic layer at ED 70. The retina showed three layer structure composed of inner and outer nuclear layers and ganglion cell layer at ED 130 when D1 signal was detected diffusely in entire region containing inner and outer nuclear layers but not detected in ganglion cell layer. The signal is more intensive in outer nuclear layer than in inner nuclear layer (Fig. 3C, F). Localization pattern of D2 dopamine receptor subtype mRNA signal was similar to that of D1 mRNA (Fig. 3E, F). After all, D1, D2 receptor mRNAs were localized in the similar regions in all stages. But it could not be identified whether the both colocalize on the same cell types during development or not.

DISCUSSION

Identification and localization of dopamine receptors in retina were investigated by many reseachers (Firth et al., 1997; Nguyen-Legros et al., 1997; Hirano et al., 1998). Because there are discripancy between reports depending to experimental methods, animal species and researchers, it remains in discussion. In this report, we could determinate quantitatively dopamine receptor subtype mRNAs in the monkey eyes using ribonuclease protection assay (RPA). On the other hand, we localized D1, D2 receptor mRNAs in developing foetal monkey eye and in adult using autoradiographic *in situ* hybridization.

We investigated the regional distribution of D1 and D2 dopamine receptor mRNAs in different tissues of adult monkey eye by RPA. Because of high sensitivity of RPA, we can measure accurately the mRNA quantities of dopamine receptor subtypes. D1 and D2 dopamine receptor mRNAs were detected overwhelming majority in retina in comparison to other parts of the adult monkey eye. This fact is coincident with various functions of dopamine in retina.

We were able to detect D1 and D2 mRNAs also in ciliary body, iris and choroid in a basal level. Dopamine receptors are identified in ciliary body. D2 dopamine receptors were identified on postganglionic sympathetic nerves in the ciliary bodies of normal rabbits (Chu et al., 1999) and sympathetically denervated rabbits. D2 dopamine receptor agonists have been shown to lower intraocular pressure (Potter, 1997; Chu et al., 1999). D1 dopamine receptor was localized within the epithelium of ciliary body and involved to increase of the ocular pressure through an increase of aqueous humour formation rather than by an inhibition of the outflow of aqueous humour (Manchino et al., 1992). In iris, D2 mRNA was detected in a significant measure but not D1 receptor mRNA. Although catecholaminergic neurons innervate in iris, this discripancy of two dopamine receptor mRNAs remains to be discussed. We detected weak signals of D1 and D2 mRNAs in optic nerve by in situ hybridization, too.

We determined quantities of D1 and D2 receptor subtype mRNAs in the monkey fetus retina corresponding to developmental stages by RPA analysis. D1 and D2 receptors showed distinct expression patterns. D1 receptor mRNA was expressed in a little amount in ED 60 and ED 70 but showed a dramatic rise at ED 130. Contrawise, D2 dopamine receptor mRNA shows comparatively constitutive expression pattern. D2 mRNA was already expressed profoundly high level at ED 60. And, the level was increased gently in the later stages at ED 70 and ED 130. In the adult, the two mRNAs was fallen down slightly under the level of ED 130.

At ED 60, D1 and D2 receptor mRNAs were already detected in foetal monkey retina. The onset of dopamine synthesis in human embryo takes place in early stage of development, assumed to be at 6 weeks post ovulation by detection of tyrosine

hydroxylase immunoreactivity in retinoblasts in the peripheral retina (Versaux-Botteri, 1992). Thereupon, the first detection of TH-immunoreactivity is temporally coincident with the beginning of synaptogenesis in the IPL in the chick retina development (Sheffields and Fischman, 1970; Hughes and LaVelle, 1974). Although the birth of dopamine receptors is not verified in our experiment, it is assumed inferring from above references that dopamine receptors may appear in a similar stage with first detection of dopamine in retina.

D1 dopamine receptor subtype mRNA was already detected at ED 60, albeit in a substantial level, and maintained in a constant level until ED 70. D2 dopamine receptor subtype mRNA expressed in a large amount at early developmental stages and maintained its level dissimilar to D1 type. D2 mRNA expression overwhelmed D1 mRNA expression by quantity in early stages (ED 60 and ED 70). Although there are discrepancy between reports concerning to birth of D1 and D2 receptors, the birth of D2 may be appeared at the same time or at slightly retarded time point comparing to D1 expression inferring from the obtained data in chick embryo by others. D1 dopaminergic receptor system differentiates very early in the development of chick embryo retina. Dopamine-mediated cyclic AMP accumulation in the tissue is already observed on ED 7, a stage when no synapses are found in retina (De Mello, 1978). D2 receptor, in contrast, differentiate several days later, by the time synapses are found in the tissue and concomitantly with the differentiation of photoreceptor cells (Ventura et al., 1984). The tendency of continual increase of D1 expression in our result is agreed with increasing pattern as a function of the maturation of the chick embryo retina (Ventura and De Mello, 1990). It is presumed that D1 receptor expression increases in proportion to development of neuronal circuitry in the retina.

Since ED 130, Expression of D1 receptor mRNA exceeds D2 dopamine receptor mRNA expression. D1 and D2 dopamine receptor mRNA expression in the adult were fallen down slightly under the levels of ED 130. The descent of dopamine receptor level might be caused primarily by apoptotic destruction of dopaminergic cells. Perinatal stage is the period when takes place the ceasing of retinal cell proliferation, completion of neuronal circuit and massive apoptotic extinction of dopaminergic neurons. During perinatal period, the activation of apoptotic program brings forth the destruction of significant proportion (nearly to 50 percent) of dopaminergic neurons during organization of neuronal circuitry. As a result, fully differentiated dopaminergic neurons in the adult exist in lower density but have a large soma and complicated network comparing to perinatal, immature retina.

D1 and D2 receptor mRNAs were localized using *in situ* hybridization in the monkey retina corresponding to developmental stages. Retina originates from the inner layer of optic cup. Cells migrate toward the interior of the eye from primitive neuroepithelial layer and form inner neuroblastic layer and remained cells forms outer neuroblastic layer. The inner neuroblastic layer subsequently splits, giving rise to ganglionic cell layer and inner nuclear layer. Cells of outer neuroblastic layer differenciate into photoreceptors. IPL and OPL are formed subsequently. Just as in the brain, cell differentiation occurs first in the farthest from the germinal layer (McIlwain, 1996). At ED 60, inner neuroblastic layer existed in a thin band of loosely organized cells and the thickness of it increases at subsequent stages (ED 70 and ED 130). And three cell-layered structures were distinct in retina at ED 130.

A group of cell mass expressing D1 or D2 mRNAs localized in the central region of outer neuroblastic layer at ED 60. The boundary of the signals was confined in inner half part of outer neuroblastic layer at ED 70. This displacement seems to be caused dynamic cell proliferation in outer neuroblastic layer and migration of the cells from inner margin of outer neuroblastic layer into inner neuroblastic layer succeedingly until ED 130. At ED 130 the signal was ditected diffusely in entire region of inner and outer nuclear layer and the signal is more intensive in outer nuclear layer than in inner nuclear layer. Because we localized dopamine receptor mRNAs in our experiment, the localization pattern of mRNAs can not be applied directly on dopamine receptor localization and the radiolabelling was accumulated on perikaryon of neuron but not on neuronal processes. Even though, we determined accurately quantities of dopamine receptor subtypes mRNAs and localized mRNAs of

dopamine receptors, according to different regions and developmental stages, in the monkey eye.

Although our presentation missed out data of certain group in Fig. 3, we applied originally a precise mRNA quantitative RPA analysis on the dopamine receptor study in monkey retina. Because of the similarity between monkey eye and human eye, the resembling studies in primates would offer important clues to comprehend the role of dopamine in human eye. In this context, the study of dopamine receptor subtypes expression and its distribution in the retina might give insight to comprehend development of dopaminergic system. As a consequence, we observed that D1 and D2 dopamine receptor mRNAs express differentially during development and show different pattern of regional distribution in foetal eye retina.

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