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NEUROPEPTIDES AND RETINAL DEVELOPMENT

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INTRODUCTION

Besides their multifaceted actions in the mature animal, neuropeptides may play functional roles of fundamental importance during the maturation of the nervous system. Neuropeptides, along with their receptors, are usually expressed at early times, when synaptic connections are still immature. In addition, transient expression of neuropeptides or developmentally regulated peptide expression have been reported in distinct brain regions, suggesting peptides may mediate functional interactions associated with the morphological and functional development of the nervous system. Neuropeptides may affect a variety of parameters, including cell division, neuronal survival, neurite sprouting, growth cone motility, and neuronal and glial phenotype. Neuroprotective and/or neural growth-related actions have been well documented for tachykinin peptides, vasoactive intestinal peptide (VIP), pituitary adenilate cyclase-activating peptide (PACAP), somatostatin (somatotropin inhibiting factor, SRIF), neuropeptide Y and opioid peptides (15, 16, 26, 27, 33, 38). The present paper will focus on the relevant aspects of the expression of some pepdidergic systems during development in a specific region of the central nervous system, the retina. The developmental patterns of VIP, PACAP and SRIF will be summarized, while information concerning the tachykinin peptides will be exposed in greater detail.

NEUROPEPTIDES IN DEVELOPING RETINAS

VIP – VIP is localized to a population of wide-field amacrine cells which constitute a subpopulation of GABAergic amacrine cells (3). In situ hybridization studies showed that VIP expression in the rat retina appears within a few days after birth (4). A quantitative analysis VIP mRNA-containing cells indicated that both their density (labeled cells/mm^2 of retinal area) and total number peak at postnatal day (PND) 15 (eye opening) and decrease to adult values in the following period (4). Since mitotic activity in the rat retina ceases by PND 8 (34) and cell death by PND 13 (18), it appears that some amacrine cells may transiently express VIP at eye opening, when retinal pathways begin the processing of visual information. Other studies show that

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VIP protects retinal neurons from tetrodotoxin-induced death or from glutamate neurotoxicity probably by stimulating cAMP production (20, 28), indicating that VIP may behave as a growth or protecting factor for retinal cells in the developing retina.

PACAP – PACAP and its receptors are expressed early in retinal development (22, 31). Similar to VIP, PACAP protects retinal neurons form cell death. In particular, administration of PACAP to retinal cell cultures from early postnatal rats increases cell survival and counteracts anysomicin- or glutamate-induced cell death (29, 30). These protective actions are mediated by PAC1 receptors through the intracellular cAMP/cAMP-dependent protein kinase pathway (30). These findings indicate that PACAP may represent an important factor modulating cell death/survival in the developing retina.

SRIF – SRIF-containing cells have been detected in embryonic retinas (12, 21). In addition, high levels of SRIF and SRIF mRNA are present at late prenatal/early postnatal periods (13). In rabbit retinas, a population of SRIF containing cells is transiently present in the ganglion cell layer (GCL) from embryonic day 29 to PND 15-20. These cells lack immunostained processes and are distributed in an apparently regular array with peak density in central retina (2). This regular array indicates that they may serve as stable positional markers which may influence the direction of fiber outgrowth in the developing retina and/or the development of retinal mosaics. In addition, a transient appearance of SRIF containing ganglion cells has been documented in rat (14, 36) and cat (21, 35) retinas, suggesting that SRIF expressed by these cells may play a role in the organization of the GCL and/or in the formation of retinofugal projections.

Recent studies in transgenic mice imply developmental roles of specific SRIF receptors (sst_{1-5}, see 25, for references). Indeed, in transgenic retinas, sst_1 and sst_2 receptor expressions have been found to compensate for each other. In particular, sst_1 receptor loss causes an increased expression of sst_2 receptors, while genetic deletion of the sst_2 receptor induces an increased expression of the sst_1 receptor (8, 11). In addition, somatostatinergic system seems to play a role in determining the size of the axonal terminals of rod bipolar cells, thus influencing their function in the retina (8). Indeed, deletion of the sst_1 receptor results in larger axonal endings than in wild type retinas, whereas the deletion of the sst_2 receptor results in smaller terminals. These alterations in transgenic retinas are already evident during postnatal maturation, indicating that these effects are related to SRIF actions during development.

TACHYKININ PEPTIDES AND THEIR RECEPTORS IN THE DEVELOPING RETINA

The family of tachykinin peptides includes substance P, neurokinin A, two neurokinin A-related peptides (neuropeptide K and neuropeptide γ) and neurokinin B. The cellular actions of the tachykinin peptides are mediated by specific, high affi-

nity receptors. Substance P, neurokinin A and neurokinin B are the preferred ligands for the neurokinin receptors, termed NK1, NK2 and NK3, respectively (23). In mammalian retinas, tachykinin immunoreactivity and tachykinin mRNA have been localized to mainly amacrine and displaced amacrine cells. In addition, the presence of tachykinin immunoreactivity in ganglion cells of the rat and rabbit retina has been documented (see 5, 7 for references).

Tachykinin-containing cells are present in the newborn rat retina and they are located both in the inner nuclear layer (INL) and in the GCL (6). In the rabbit retina, tachykinin-immunolabeled cells are present in the GCL of the newborn retina, while immunostained cells in the INL are seen at postnatal day 2 (5). In adult rabbit retinas, many of the tachykinin-containing cells in the GCL are ganglion cells (1), and many of the tachykinin-immunolabeled cells in the GCL of developing retinas are also likely to be ganglion cells. Indeed, in newborn retinas heavily immunolabeled fiber bundles can be seen to run in the NFL and to converge into the optic disk (5). Both in rat and in rabbit retinas, the morphologic development of the tachykinin-containing cells in the INL as well as in the GCL is completed by the time of eye opening (5, 39).

In human retinas, tachykinin-containing cells have been identified at embryonic ages (19). The earliest identification of tachykinin immunoreactivity in human retinas is reported in cells of the neuroblastic layer of 10 week-old fetuses, with subsequent development characterized by the migration of these cells into the inner layers of the retina (37). In addition, the presence of tachykinin immunoreactivity in optic nerves at 13-14 weeks of gestation indicates the presence of tachykinin-expressing ganglion cells in the developing human retina (32).

Together, the studies of the localization of tachykinin peptides in developing retinas indicate that these peptides are expressed at early times of retinal maturation and roughly at the same time when other neurotransmitter systems begin their expression. The subsequent maturation of the tachykinin-containing cell populations follows a pattern that is similar to that of most amacrine cells and that is almost complete at the time of eye opening. SP and/or other tachykinin peptides presumably released in the developing retina may play a developmental role, however it is difficult to hypothesize what kind of actions are performed by tachykinin peptides in developing retinas and whether these actions can be distinguished from those of other neuroactive substances that are present in the retina at the same time. The analysis of the expression of the neurokinin receptors in developing retinas has helped to shed some light onto certain characteristics of the physiologic actions of tachykinin peptides during retinal development.

Of the three neurokinin receptors, NK1 and NK3 are present in the adult as well as in the developing rat retina, while NK2 is absent (24). The main finding about the developmental expression of NK1 and NK3 receptors in the rat retina is that NK1 receptors are expressed at early postnatal ages, while NK3 receptors appear only near the time of eye opening (6, 24), when the main morphologic and functional characteristics of the retinal pathways have reached their maturity. These observations strongly suggest that SP may act at its preferred receptor (the NK1 receptor) in the

retina during the whole period of postnatal development and therefore it may be implicated in developmental functions. In contrast, neurokinin B (the preferred ligand of NK3 receptors) could begin interactions with its receptors only when the retinal circuitry is almost ready to start the processing of visual stimuli, and developmental functions of neurokinin B and NK3 receptors seem unlikely.

In particular, NK1 receptors are expressed in the newborn retina by presumed ganglion cells and by a few amacrine cells in the neuroblastic layer. The subsequent development is characterized by substantial rearrangements of the NK1 expression patterns, which include an increase in the number of NK1-immunopositive amacrine cells, concomitant with a decrease in the number of immunolabeled presumed ganglion cells, and changes in the laminar distribution of NK1-immunostained fibers in the IPL. These fibers are first distributed to laminae 1, 3 and 5 of the IPL from PND 0 to PND 7, then they change to a pattern, seen at PND 12, where the most intensely immunolabeled part of the IPL is lamina 2, as in mature retinas (6). These findings, indicating profound changes in the expression patterns of NK1 receptors during retinal development, suggest that SP and NK1 receptors may mediate processes in the developing retina that are different from those in the retina capable of visual information processing.

NK3 receptors in adult rat and mouse retinas are expressed by populations of OFF-type cone bipolar cells (6, 17, 24) and in dopaminergic amacrine cells (10). The same bipolar cells that express NK3 receptors can also be labeled with antibodies directed to the calcium binding protein recoverin (6, 17). A parallel immunohistochemical investigation of NK3 and recoverin immunolabelings in the developing rat retina indicated that recoverin-containing bipolar cells have reached an advanced degree of morphologic maturation when they start expressing NK3 receptors, at a time just before eye opening (6). This observation suggests that the timing of NK3 receptor expression is set to allow certain types of bipolar cells to initiate their functions in visual information processing, and that NK3 expression in these cells is not required for developmental functions.

Recent investigations on the expression of NK1 receptors in developing rabbit retinas seem to allow new hypotheses on the roles played by SP in the developing retina. NK1 receptors in mature rabbit retinas are expressed by a population of ON-type cone bipolar cells and by the population of dopaminergic amacrine cells identified by tyrosine hydroxylase immunoreactivity (7). This adult pattern is achieved around the time of eye opening after a drastic rearrangement during the postnatal developmental period. In particular, before eye opening, NK1 receptors are expressed by cholinergic amacrine cells, while NK1 expression in dopaminergic amacrine cells and in bipolar cells is detected only at later ages (9). Consistent with these immunocytochemical data, SP or a SP agonist have been observed to increase the intracellular Ca^{2+} levels in individual cholinergic amacrine cells of the newborn rabbit retina (Fig. 1). In addition, as shown in Figure 2, SP is ineffective in stimulating dopamine release in the developing retina, while it stimulates dopamine release in the mature retina (9). It is easy to hypothesize that this surprising switch in NK1 expression patterns and SP functional actions may be related to developmental

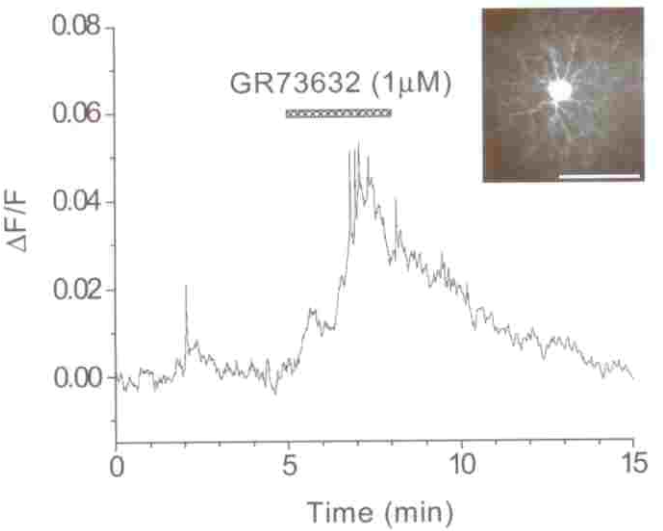

Fig. 1. - *Imaging from cholinergic amacrine cells in perinatal rabbit retinal flat-mounts identified by their typical morphology (see inset).* Changes in fluorescence intensity measured from a cholinergic cell loaded with the calcium-sensitive dye Oregon Green BAPTA-1, showing a rise in intracellular Ca^{2+} evoked by a short (3 min) application of the NK1 receptor-specific agonist GR73632 (1 μM). Inset: fluorescence image of a cholinergic amacrine cell in a newborn retina. The cell was loaded with Oregon Green BAPTA-1 by means of ballistic delivery of indicator-coated particles. The image was taken with a cooled CCD camera under a 40x water immersion objective lens. Scale bar: 50 μm. From reference 9, modified.

events. In particular, the onset of NK1 expression in dopaminergic amacrine cells and in a population of ON-type cone bipolar cells at eye opening is consistent with actions played by SP on vertical retinal pathways and on dopaminergic amacrine cells for visual information processing, and probably including modulation of light adaptation (see ref. 7). In contrast, the expression of NK1 receptors by cholinergic amacrine cells in the immature retina is likely to be related to a role of SP in modulating cholinergic neurotransmission in the developing retina. Since cholinergic amacrine cells have been shown to directly participate in the spontaneous rhythmic

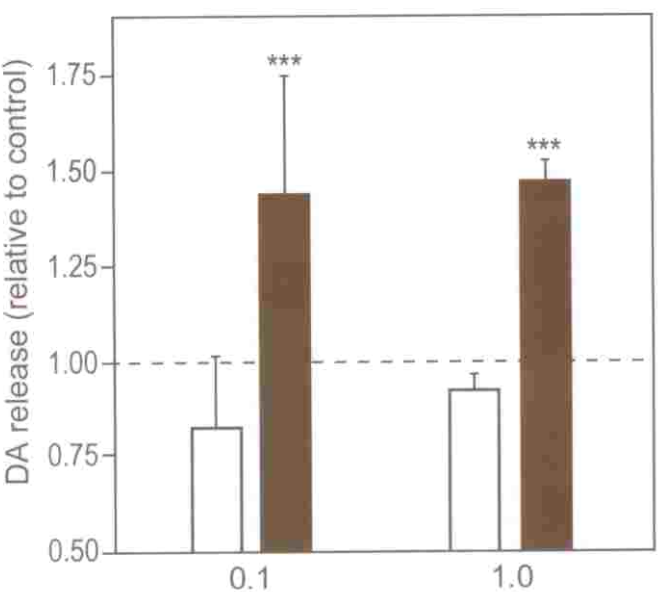

Fig. 2. - *Dopamine (DA) levels in the bath solution of retinas at PND 10 (open columns) and at PND 35 (filled columns) treated with two different doses of the NK1 receptor agonist GR73632.*
The histograms represent DA values relative to controls; the dashed line represents 100% of control values. Statistically significant differences from control values were only observed in PND 35 (adult) retinas. *** $p < 0.001$. From reference 9, modified.

activities in the developing rabbit retina (40), SP may be a factor implicated in the regulation of this important mechanism.

SUMMARY

Different peptidergic systems have been investigated with some detail during retinal development, including substance P (SP), vasoactive intestinal polypeptide (VIP), pituitary adenylate cyclase activating polypeptide (PACAP) and somatostatin (SRIF). Concerning possible developmental actions of neuropeptides, VIP and PACAP exert protective and growth-promoting actions that may sustain retinal neurons during their development. In addition, the presence of transient SRIF expressing cells and recent observations in SRIF receptor knock out mice indicate variegated roles of this peptide in the development of the retina and of retinofugal projections. Finally, recent studies have shown that, in the developing rabbit retina, changes in the expression pattern of SP receptors are accompanied by modifications of SP physiological effects, indicating that retinal circuits where SP is involved are likely to function in a substantially different manner before the retina becomes involved in the processing of visual stimuli. SP neurotransmission in the immature retina may subserve developmental events, and SP is likely to represent an important developmental factor for the maturation of retinal neurons and circuitries.

REFERENCES

1. Brecha, N.C., Johnson, D., Bolz, J., Sharma, S., Parnavelas, J.G. and Lieberman, A.R. Substance P-immunoreactive retinal ganglion cells and their central axon terminals in the rabbit. *Nature*, **327**: 155-158, 1987.
2. Brecha, N.C., Casini, G. and Rickman, D.W. Organization and development of sparsely distributed wide-field amacrine cells in the rabbit retina. In: Bagnoli, P. and Hodos, W. (Eds.) *The Changing Visual System: Maturation and Aging in the Central Nervous System*. London: Plenum Press 1991, pp. 95-117.
3. Casini, G. and Brecha, N.C. Colocalization of vasoactive intestinal polypeptide and GABA immunoreactivities in a population of wide-field amacrine cells in the rabbit retina. *Vis. Neurosci.*, **8**: 373-378, 1992.
4. Casini, G., Molnar, M. and Brecha, N.C. Vasoactive intestinal polypeptide/peptide histidine isoleucine messenger RNA in the rat retina: adult distribution and developmental expression. *Neuroscience*, **58**: 657-667, 1994.
5. Casini, G., Trasarti, L., Andolfi, L. and Bagnoli, P. Morphologic maturation of tachykinin peptide-expressing cells in the postnatal rabbit retina. *Dev. Brain. Res.*, **99**: 131-141, 1997.
6. Casini, G., Brecha, N.C., Bosco, L. and Rickman, D.W. Developmental expression of neurokinin-1 and neurokinin-3 receptors in the rat retina. *J. Comp. Neurol.*, **421**: 275-287, 2000.
7. Casini, G., Sabatini, A., Catalani, E., Willems, D., Bosco, L. and Brecha, N.C.

Expression of the neurokinin 1 receptor in the rabbit retina. *Neuroscience*, **115**: 1309-1321, 2002.

8. CASINI, G., DAL MONTE, M., PETRUCCI, C., GAMBELLINI, G., GROUSELLE, D., ALLEN, J.P., KREIENKAMP, H.-J., RICHTER, D., EPELBAUM, J. AND BAGNOLI, P. Altered morphology of rod bipolar cell axonal terminals in the retinas of mice carrying genetic deletion of somatostatin subtype receptor 1 or 2. *Eur. J. Neurosci.*, **19**: 43-54, 2004.
9. CASINI, G., DAL MONTE, M., FORNAI, F., BOSCO, L., WILLEMS, D., YANG, Q., ZHOU, Z.J. AND BAGNOLI, P. Neurokinin 1 receptor expression and substance P physiological actions are developmentally regulated in the rabbit retina. *Neuroscience*, **124**: 147-160, 2004.
10. CHEN, L.W., WEI, L.C., LIU, H.L., DUAN, L., JU, G. AND CHAN, Y.S. Retinal dopaminergic neurons (A17) expressing neuromedin K receptor (NK(3)): a double immunocytochemical study in the rat. *Brain Res.*, **885**: 122-127, 2000.
11. DAL MONTE, M., PETRUCCI, C., VASILAKI, A., CERVIA, D., GROUSELLE, D., EPELBAUM, J., RICHTER, D., KREIENKAMP, H.J., HOYER, D. AND BAGNOLI, P. Genetic deletion of somatostatin receptor 1 alters somatostatinergic transmission in the mouse retina. *Neuropharmacology*, **45**: 1080-1092, 2003.
12. FERRIERO, D.M. AND SAGAR, S.M. Development of somatostatin immunoreactive neurons in rat retina. *Brain Res.*, **431**: 207-214, 1987.
13. FERRIERO, D.M., HEAD, V.A., EDWARDS, R.H. AND SAGAR, S.M. Somatostatin mRNA and molecular forms during development of the rat retina. *Dev. Brain Res.*, **57**: 15-19, 1990.
14. FONTANESI, G., CASINI, G., THANOS, S. AND BAGNOLI, P. Transient somatostatin-immunoreactive ganglion cells in the developing rat retina. *Dev. Brain Res.*, **103**: 119-125, 1997.
15. GOZES, I. AND BRENNEMAN, D.E. A new concept in the pharmacology of neuroprotection. *J. Mol. Neurosci.*, **14**: 61-68, 2000.
16. HANSEL, D.E., EIPPER, B.A. AND RONNETT, G.V. Neuropeptide Y functions as a neuroproliferative factor. *Nature*, **410**: 940-944, 2001.
17. HAVERKAMP, S., GHOSH, K.K., HIRANO, A.A. AND WÄSSLE, H. Immunocytochemical description of five bipolar cell types of the mouse retina. *J. Comp. Neurol.*, **455**: 463-476, 2003.
18. HORSBURGH, G.M. AND SEFTON, A.J. Cellular degeneration and synaptogenesis in the developing retina of the rat. *J. Comp. Neurol.*, **263**: 553-566, 1987.
19. JOTWANI, G., ITOH, K. AND WADHWA, S. Immunohistochemical localization of tyrosine hydroxylase, substance P, neuropeptide-Y and leucine-enkephalin in developing human retinal amacrine cells. *Dev. Brain Res.*, **77**: 285-289, 1994.
20. KAISER, P.K AND LIPTON, S.A. VIP-mediated increase in cAMP prevents tetrodotoxin-induced retinal ganglion cell death in vitro. *Neuron*, **5**: 373-381, 1990.
21. MITROFANIS, J., ROBINSON, S.R. AND PROVIS, J.M. Somatostatinergic neurones of the developing human and cat retinae. *Neurosci. Lett.*, **104**: 209-216, 1989.
22. OLIANAS, M.C., INGIANNI, A., SOGOS, V. AND ONALI, P. Expression of pituitary adenylate cyclase-activating polypeptide (PACAP) receptors and PACAP in human fetal retina. *J. Neurochem.*, **69**: 1213-1218, 1997.
23. OTSUKA M. AND YOSHIOKA K. Neurotransmitter functions of mammalian tachykinins. *Physiol. Rev.*, **73**: 229-308, 1993.
24. OYAMADA, H., TAKATSUJI, K., SENBA, E., MANTYH, P.W. AND TOHYAMA, M. Postnatal development of NK1, NK2, and NK3 neurokinin receptors expression in the rat retina. *Dev. Brain Res.*, **117**: 59-70, 1999.
25. PATEL, Y.C. Somatostatin and its receptor family. *Front. Neuroendocrinol.*, **20**: 157-198, 1999.
26. RAFFA R.B. Possible role(12) of neurokinins in CNS development and neurodegenerative or other disorders. *Neurosci. Biobehav. Rev.*, **22**: 789-813, 1998.

27. SCHWARTZ, J.P., JI, Z. AND EPELBAUM, J. Somatostatin as a neurotrophic factor. Which receptor/second messenger transduction system is involved? *Perspect. Dev. Neurobiol.*, **5**: 427-435, 1998.
28. SHOGE, K., MISHIMA, H.K., SAITOH, T., ISHIHARA, K., TAMURA, Y., SHIOMI, H. AND SASA, M. Protective effects of vasoactive intestinal peptide against delayed glutamate neurotoxicity in cultured retina. *Brain Res.*, **809**: 127-136, 1998.
29. SHOGE, K., MISHIMA, H.K., SAITOH, T., ISHIHARA, K., TAMURA, Y., SHIOMI, H. AND SASA, M. Attenuation by PACAP of glutamate-induced neurotoxicity in cultured retinal neurons. *Brain Res.*, **839**: 66-73, 1999.
30. SILVEIRA, M.S., COSTA, M.R., BOZZA, M. AND LINDEN, R. Pituitary adenylyl cyclase-activating polypeptide prevents induced cell death in retinal tissue through activation of cyclic AMP-dependent protein kinase. *J. Biol. Chem.*, **277**: 16075-16080, 2002.
31. SKOGLOSA, Y., TAKEI, N. AND LINDHOLM, D. Distribution of pituitary adenylate cyclase activating polypeptide mRNA in the developing rat brain. *Mol. Brain Res.*, **65**: 1-13, 1999.
32. WADHWA, S., RIZVI, T.A. AND BIJLANI, V. Substance P-immunoreactivity in the developing human retinogeniculate pathway. *Neurosci. Lett.*, **89**: 25-30, 1988.
33. WASCHEK, J.A. Multiple actions of pituitary adenylyl cyclase activating peptide in nervous system development and regeneration. *Dev. Neurosci.*, **24**: 14-23, 2002.
34. WEBSTER, M.J. AND ROWE, M.H. Disruption of developmental timing in the albino rat retina. *J. Comp. Neurol.*, **307**: 460-474, 1991.
35. WHITE, C.A. AND CHALUPA, L.M. Ontogeny of somatostatin immunoreactivity in the cat retina. *J. Comp. Neurol.*, **317**: 129-144, 1992.
36. XIANG, Z., JIANG, L. AND KANG, Z. Transient expression of somatostatin mRNA in developing ganglion cell layers of rat retina. *Dev. Brain Res.*, **128**: 25-33, 2001.
37. YEW, D.T., LUO, C.B., ZHENG, D.R., GUAN, Y.L., TSANG, D. AND STADLIN, A. Immunohistochemical localization of substance P, enkephalin and serotonin in the developing human retina. *J. Hirnforsch.*, **32**: 61-67, 1991.
38. ZAGON, I.S., VERDERAME, M.F. AND MCLAUGHLIN, P.J. The biology of the opioid growth factor receptor (OGFr). *Brain Res. Rev.*, **38**: 351-376, 2002.
39. ZHANG, D. AND YEH, H.H. Substance-P-like immunoreactive amacrine cells in the adult and the developing rat retina. *Dev. Brain Res.*, **68**: 55-65, 1992.
40. ZHOU, Z.J. The function of the cholinergic system in the developing mammalian retina. *Prog. Brain Res.*, **131**: 599-613, 2001.