Restorative Neurology and Neuroscience 16 (2000) 5–22

Abstracts of the 14th Annual Meeting of the Japanese Society for Neural Growth, Regeneration and Transplantation

1. Oxidized Galectin-1 Regulates Initial Repair in Peripheral Nerves after Axotomy (II) – Initial Regulation of Neural Regeneration in Sciatic Nerves After Axotomy

Hidenori Horie[1], Yoshimasa Inagaki[2], Yoshiaki Sohma[2], Risa Nozawa[2], Mitsuhiro Hasegawa[3], Naoki Muramatsu[3], Hitoshi Kawano[4], Masao Horie[4], Hiromichi Koyama[5], Ikuko Sakai[1], Kaori Takeshita[1], Yuki Kowada[1], Masahiko Takano[6], Toshihiko Kadoya[2]
Departments of [1]Physiology and [6]Ophthalmology, Yokohama City University, School of Medicine and [5]College of Nursing, Yokohama City University, Yokohama 236-0004, [2]Pharmaceutical Research Laboratory, Kirin Brewery Co. Ltd., Takasaki 370-1295, [3]Department of Neurosurgery Kanazawa University, School of Medicine, Kanazawa, 920-0934, [4]Anatomical Development, Tokyo Metropolitan Neuroscience Institute, Tokyo, 183-8526, Japan

Oxidized galectin-1 (GAL-1/Ox) regulates axonal regeneration in the DRG explant. These *in vitro* results suggest that GAL-1/Ox may regulate nerve regeneration *in vivo* after axotomy. Here we used two kinds of acellular neural regeneration models, first a crush injury of the nerve combined with freezing of the distal stump, and second, a transection of the nerve with suturing of the proximal stump into a silicon tube with one end closed off. These acellular models are efficacious for the analysis of the effects of GAL-1/Ox on neural regeneration because the neural regeneration process is slow in the models. At 14 days after the operation, coronal sections at a distance of 6 mm distal to the crush site were analyzed under the electron microscope (EM). The number of the reactive Schwann cells was significantly higher in the GAL-1/Ox group than in the control group and most of them engulfed regenerating axons in the GAL-1/Ox treatment group. These *in vitro* nerve crush injury experiments suggest that GAL-1/Ox promoted axonal regeneration by the activation of Schwann cell migration into the acellular nerve. Further analysis of the effect of GAL-1/Ox on neural regeneration used the *in vivo* nerve-transection plus tubulation model. At 10 days after the operation, double immunostaining of both longitudinal and cross sections of frozen regenerated tissues taken from the silicone tubes was performed in the presence of anti-neurofilament and anti-S-100 antibodies. The results from the longitudinal sections show that the numbers and the migrating rate of Schwann cells together with regenerating axons were increased by the application of GAL-1/Ox and strongly reduced by that of anti-galectin-1 antibody. These results were confirmed by the cross sectional analysis. Since galectin-1 is expressed in the regenerating sciatic nerve and can be secreted, these experiments suggest that oxidized galectin-1 may regulate initial repair after axotomy.

2. Intraspinal Implants of Fibrin Glue Containing GDNF Enhance Dorsal Root Regeneration into the Host Spinal Cord

Masaya Iwakawa, Yasunobu Itoh, Naoyuki Kuwahara, Kohki Iwaya, Tohru Ohta, Kazuo Mizoi
Department of Neurosurgery, Akita University School of Medicine, Akita 010-8543, Japan

We have previously reported that BDNF, NT-3, and CNTF mediated dorsal root regeneration into the host spinal cord (Iwaya, K., et al., *Neurosurg*. **44** (1999) 589–96), however it is as yet unknown whether glial cell line-derived neurotrophic factor (GDNF) enhances dorsal root regrowth into host spinal cord. Rats received intraspinal implants of fibrin glue (Tisseel; Nippon Zoki Pharmaceutical, Japan) containing GDNF (R&D System Inc., 2.5 μg) into the left dorsal quadrant cavities aspirated in the lumbar enlargement where the transected L5 dorsal root stump was sandwiched between fibrin glue ball and the spinal cord. Three months later the inserted dorsal root was cut distal to the entry site and labeled with 40 % aqueous horseradish peroxidase (HRP) solution. HRP-labeled axons crossed the interface between the dorsal root and host spinal cord and arborized within the host spinal cord. Among these axons, myelinated axons could be found, probably remyelinated within the host spinal cord. Parts of regenerated axon terminals labeled for HRP formed synaptic contacts with dendritic profiles of the host spinal cord neurons. Regenerated dorsal root axons were also immunohistochemically labeled for calcitonin gene-related peptide (CGRP). CGRP-immunore-

0922-6028/00/$8.00

active axons regenerated into spinal cord and some of them extended into host motoneuron pool, but only a few axons regenerated into the spinal cord of animals with fibrin glue without GDNF. Our results indicate that GDNF enhances the regeneration of injured dorsal roots into the host spinal cord and supports synapse formation with host spinal cord neurons. GDNF may therefore provide a strategy for restoring injured spinal reflex arcs.

Supported by Grant-in-Aid No.10671280 for Scientific Research from the Ministry of Education, Sports, Science and Culture, Japan.

3. Restoration of Somatotopic Organization in the Regenerated Rubrospinal Tract in Adult Rats

Ken Kojima, Saburo Kawaguchi, Toshiro Hasebe, Takeshi Nishio
Department of Integrative Brain Science, Graduate School of Medicine, Kyoto University, Kyoto 606-8501, Japan

To investigate whether regenerated axons of the adult mammalian central nervous system (CNS) can correctly find path and target, we examined the somatotopic organization of the rubrospinal tract after transection in adult rats. A complete transection of the tract was made unilaterally by hemisection of the spinal cord at the level of C4 and embryonic spinal cord tissue was grafted in the lesion to promote axonal regeneration. The tract was examined anterogradely or retrogradely. For the anterograde labeling wheat germ agglutinin conjugated horseradish peroxidase was injected into the red nucleus contralateral to the lesion. For the retrograde labeling two kinds of tracers, namely fluorescein dextran amine (FDA) and Texas Red dextran amine (TDA) were injected into the cervical and lumbar enlargement, respectively. The anterograde labeling revealed the occurrence of fibers that crossed over the lesion, that is, regenerated fibers. The regenerated fibers mostly extended the length of the spinal cord as a compact bundle in the dorsal part of the lateral funiculus in a manner very similar to normal and terminated normally, whereas a small proportion of fibers regrew aberrantly for a short distance. The retrograde labeling revealed that neurons single-labeled with FDA occurred mostly in the dorsomedial part of the red nucleus, and neurons single-labeled with TDA mostly in the ventrolateral part. Such a somtotopic organization is similar to normal although the number of labeled neurons indicative of the extent of regeneration differed greatly among the animals. The ratio of double-labeled neurons with both FDA and TDA, however, was larger in the operates than in intact animals. In the operates the ratio was larger in poor regeneration than in marked regeneration. The present findings indicate that regenerated CNS axons in adult animals have potentials of path and target finding which deteriorate as the axonal environment for regeneration becomes worse.

4. Neurotrophic Effect of Cytokine-Activated Astrocytes for Rat Brainstem Neurons

Kenji Shimazaki, Kazunari Yoshida, Hisatsugu Ishimori, Arata Kondo, Takeshi Kawase, Shigeo Toya
Department of Neurosurgery, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

We have previously reported that astrocytes activated by several cytokines, which are considered to be supplied during brain injury, have neurotrophic effects on cholinergic brainstem neurons. The effects were assayed by measuring the activity of choline acetyltransferase (ChAT), which is synthetic enzyme of acetylcholine. In order to elucidate neurotrophic effects for other brainstem neurons, effects of cytokine-activated astrocytes on the expression of synthesized neurotransmitter enzymes were measured by RT-PCR. Fetal rat (E16) brainstem neurons were cultured in N2 medium, newborn rat hippocampal astrocytes (C-ACM) conditioned medium and cytokine-activated astrocytes (S-ACM) conditioned medium. RNA was extracted, then tyrosine hydroxylase (TH) and glutamic acid decarboxylase (GAD) mRNAs were detected by RT-PCR. Semi-quantitative PCR was performed, comparing the mRNAs with the amount of beta actin mRNA as an internal control. Expression of TH and GAD mRNA was increased in neurons cultured in C-ACM and S-ACM. Expression tended to be greater in neurons cultured in S-ACM than C-ACM. It was suggested that cytokine-activated astrocytes have neurotrophic effects on brainstem neurons during regeneration after brain injury, by up-regulation of the expression of synthesized neurotransmitter enzymes and reconstruction of the neural network.

5. Neurotrophic Effects of Human Amniotic Epithelial Cell Conditioned Medium

Saiko Uchida[1], Yukiko Inanaga[1], Syouei Hurukawa[2], Norio Sakuragawa[1]
[1]Department of Inherited Metabolic Disease, National Institute of Neuroscience, NCNP, 4-1-1, Ogawahigashi, Kodaira, Tokyo 187-8502, [2]Laboratory of Molecular Biology, Gifu Pharmaceutical University, 5-6-1, Mitahora-higashi, Gifu 502-8585, Japan

Previous reports from our laboratory have demonstrated that human amniotic epithelial cells (hAEC) express genes of neurons, astrocytes and oligodendrocytes. More recently we provided evidence for synthesis and release of acetylcholine and catecholamines by hAEC. In this study, we investigated the neurotrophic effects of hAEC cultured medium on rat neurons *in vitro* compared with several other nerve growth and neurotrophic factors. We also examined the release of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) as well as their gene expression in hAEC. Human amniotic ep-

ithelial membrane was peeled from the chorion of a placenta obtained from an uncomplicated elective cesarean section. hAEC cultures were carried out as previously described. Neuronal cells were cultured using rat cerebral tissue from 18-day-old embryos. Neuronal cells were seeded at $8.5-14.0 \times 10^4$ cells/cm^2. Cells were cultured in a serum-free medium (Neurobasal) supplemented with N2 for 24 hrs. The medium was then changed to the conditioned medium which was prepared from 3-day culture of hAEC in N2-supplemented medium. At one day, almost all of cells were stained positively against MAP-2 antibody. Most of neuronal cells died at the 8th day of culture in the N2-supplemented medium without any treatment. However, the conditioned medium dramatically promoted rat neuronal survival. In addition, the following growth and trophic factors were examined to test the trophic effects on rat neurons: BDNF (20 ng/ml), NT-3 (20 ng/ml), NGF (10 ng/ml), EGF (10 ng/ml), basic FGF (10 ng/ml), cilliary neurotrophic factor (CNTF; 10 ng/ml), platelet-derived growth factor (PDGF; 10 ng/ml), macrophage colony stimulating factor (MCSF; 100U/ml), hepatocyte growth factor (HGF; 10 ng/ml), IL-2 (10 ng/ml) and IL-6 (10 ng/ml). Among these growth or trophic factors, NGF and EGF demonstrated a promotional effect on rat neuron survival, which was not superior to the conditioned medium. We next investigated the synthesis and release of nerve growth and trophic factors by hAEC. Enzyme immunoassay revealed the detection of BDNF and NT-3 in cultured hAEC medium (BDNF, 610 ± 540 ρg/ml; NT-3, 600 ± 279 ρg/ml). However, NGF and EGF were not detected by this method. The targeted RT-PCR products, obtained from hAEC, demonstrated the presence of BDNF, NT-3 and EGF mRNA. These results provide evidence that hAEC conditioned medium promotes survival of rat neurons in E18 embryos. Judging from comparative studies showing that the neurotrophic effect of conditioned medium was superior to NGF and EGF, we interpret these data as suggesting that hAEC may produce unidentified neurotrophic factors.

6. Neural Stem Cells

Yasuhiro Tomooka
Department of Biological Science and Technology, Science University of Tokyo, 2641, Yamazaki, Noda, Chiba 278-8510, Japan

The neural tube consists of neural precursors or stem cells which express intermediate filament nestin. They proliferate and differentiate into various types of neurons and glia, from which the cental nervous system (CNS) develops. To analyze the mechanisms controlling the CNS development *in vivo*, precursors isolated from fetuses have been used in defining in what and how growth factors are involved, for isolation of unknown genes, and for following their lineages. Recently, in adult mouse, rat and human brain, such precursors have also been isolated, from which neurons and glia are born, and the precursors seem to be supplied to the olfactory bulb and hippocampal granule layer. Migration, terminal differentiation and plasticity of the newly-born neurons are under investigation. From recent studies, we have just begun to understand the diversity of neuron and glia, the switch from symmetric to asymmetric division, and the interaction between genetic and epigenetic factors determining the fates of stem cells.

Clonal cell lines of the CNS have been established from brain tumors developed in transgenic mice or by introduction of oncogenes into precursors. We found another simple method to establish clonal cell lines: enzymatically dissociated cells of p53-/-mouse tissues are cultured, and then the subculture at low density gives rise to clones. By this method we have established many cell lines from various tissues including brains, among which we focus our interest on a stem cell like line (2y6f1) established from a cerebellum of an adult male. This line has an epithelial morphology and produces sublines of neuron and/or glia. Further analyses are under investigation.

7. Molecular Control of Proliferation and Differentiation of Neural Stem Cells During Development and Regeneration of the Nervous System

Masato Nakafuku
Department of Neurobiology, The University of Tokyo, Graduate School of Medicine, Bunkyo-ku, Tokyo 113-0033, Japan

Multipotential neural stem cells serve as the origin of diverse cell types during genesis of the mammalian central nervous system (CNS). During early development, stem cells continue proliferation and increase their total cell numbers without overt differentiation. At subsequent stages, the cells withdraw from this self-renewal mode and begin to commit themselves to generating both neurons and glia. This lineage commitment proceeds with distinct kinetics and manner among different areas of the brain, finally contributing to the highly organized and complex morphogenesis of the CNS. Thus, the cell fate determination of neural stem cells should be under strict control at the molecular and cellular levels; details of this, however, remain poorly understood. Here we show that the expression and function of the neural-specific transcription factors Mash1 and Prox1 positively regulate differentiation of CNS stem cells. Our study has also demonstrated that signals from the cell surface receptor Notch negatively regulate differentiation of stem cells through inhibiting the activity of Mash1. Thus, we propose that early cell fate choices of neural stem cells are under both positive and negative controls involving multiple regulatory molecules. The potential significance of our findings in the context of future applications of stem cells for regeneration of damaged nervous tissues will be discussed.

8. Identification of Neural Precursor Cells in the Adult Human Brain – Clonal Analysis in Vitro

Takaaki Kato, Osamu Honmou, Yukinori Akiyama, Kazuo Hashi
Department of Neurosurgery, Sapporo Medical University School of Medicine, Sapporo, Hokkaido 060-8543, Japan

It has generally been assumed that the adult brain is incapable of significant self-repair because of lack of neurogenesis in the adult mammalian central nervous system (CNS). In the last three decades, however, several studies have reported that the adult mammalian brain harbors precursor cells that retain potential for both neural production and differentiation in experimental animal models, although the identity of neural precursors in the brains of adult mammals is still unknown. These findings offer the prospect of the presence of neural precursors in the adult human brain. The objective of this study was to identify neural precursors in the adult human CNS and examine the potential for self-renewal and differentiation *in vitro*.

We established cell-lines derived from periventricular subependymal zone (SEZ) in the adult human brain. Single-cell clonal analysis demonstrated that both neuronal and glial lineages could be induced from a single cell *in vitro*, which were characterized immunophenotypically and electrophysiologically. These results provide *in vitro* evidence that the adult human brain contains precursor cells which have the properties reminiscent of neural stem cells and exhibit both neuronal and glial differentiations; thus, these cells may be a potential source for investigations of human CNS neuronal and glial development and differentiation, and could be used as a source of cells for neural transplantation therapy.

9. The Effect of Glutamate Receptor Antagonists on the Expression of Immediate Early Gene mRNAs after Mechanical Injury in Cultured Glial Cells

Hiroyuki Katano[1,2], Kaori Fujita[3], Taiji Kato[3], Kiyofumi Asai[3], Yasuhiro Kawamura[2], Atsuo Masago[2], Kazuo Yamada[2]
[1]Department of Neurosurgery, Nagoya City Higashi General Hospital, Departments of [2]Neurosurgery and [3]Bioregulation Research, Nagoya City University Medical School, Nagoya, Japan

It is still unclear whether reactive gliosis suppress or promotes neural regeneration. To investigate molecular glial reaction following injury, we confirmed the expression of immediate early gene (IEG) mRNAs by *in situ* hybridization (ISH) using an *in vitro* mechanical injury model. IEG mRNA has been reported to be induced by glutamate influx to cytosol in brain injury models *in vivo*. Several glutamate receptors have also been detected on glial cells in recent years. We studied the relationship of glutamate receptors with IEG mRNA expression in injured glial cells using glutamate receptor antagonists. Cortical glial cells were prepared from embryos of Wistar rats. Glutamate receptor antagonists, MK-801, NBQX and MCPG, were added to growth medium 3 hours before scratch injury to monolayer glial cells on chamber slides. Glial cells were fixed after 30 min after the injury when the IEG mRNA expression was maximum. ISH was performed with [α-^{35}S]dATP-labeled *c-fos*, *c-jun* mRNA oligonucleotide probes. IEG mRNAs were strongly detected along the wound on control cultures and no significant changes were observed on the cultures treated with MK-801 and NBQX. The expression of both c-*fos* and c-*jun* mRNAs along the injury was attenuated on the cultures adding MCPG. The present study indicates that the metabotropic glutamate receptor might have some relation to the IEG expression following mechanical injury on cultured glial cells, effecting successive molecular events.

10. Overexpression of bcl-2 does not Improve Axonal Regeneration of Mature Retinal Ganglion Cells after Peripheral Nerve Transplantation

Tetsu Inoue, Mizuho Hosokawa, Yutaka Fukuda
Department of Physiology, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

In adult mammals, retinal ganglion cell (RGC) axons can regrow along a peripheral nerve (PN) segment after optic nerve transection, but the regeneration is very poor. The enhancement of regeneration is necessary to make a good functional reprojection to the visual center. Previousely, many kinds of molecules such as neurotrophic factors have been shown to promote RGC survival or axonal regeneration. Recently, Maffei and his collaborators have revealed that overexpression of the bcl-2 gene powerfully promotes *in vivo* survival of mature axotomized RGCs (Cenni et al., 1996). We questioned whether the promoted survival also contributes to massive axonal regeneration. We made PN transplantation in transected optic nerve in bcl-2 transgenic and wild mice (Martinou et al., 1994) and evaluated RGC survival and their axonal regeneration 4 weeks after the transplantation. First, we confirmed the rescuing effect of bcl-2 overexpression on RGC survival; a mean proportion of the survived RGCs was estimated at 53 % (n = 3D2), being 9 fold higher than that in the wild mice (6 %, n = 3D3). Next, we found that the number of regenerated RGCs in the transgenic mice (median = 3D148, n = 3D6) was not significantly higher than that in the wild mice (median = 3D109, n = 3D9). Because the survival ratio was higher in the transgenic mice, a proportion of regenerated RGCs in the survived cells was much lower in the transgenic mice than in the wild mice. Although Chen et al. (1997) have shown that overexpression of bcl-2 promotes *in vitro* axonal regeneration of mature RGCs, our study suggestes that it is not enough to enhance *in vivo* axonal regeneration in adult mammals.

The bcl-2 transgenic mice were provided from Nippon Glaxo Ltd. Tsukuba Research Laboratories.

11. Temporal Pattern of Argyrophilic Neurons after Diffuse Axonal Injury in Rats

Noritaka Aihara[1], Hiroaki Komatsu[1], Akira Iwata[1], Takashi Matsumoto[1], Kazuo Yamada[1], Hitoo Nishino[2]
[1]Department of Neurosurgery and [2]Physiology, Nagoya City University Medical School, 1-Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8602, Japan

In the present study, to elucidate the temporal pattern of histological changes in rats after diffuse axonal injury, we used silver staining for the selective demonstration of collapsed neurons according to the method of Gallyas. The diffuse axonal injury model in rats developed by Marmarou was used with slight modifications. Eighteen adult male Wistar rats weighing 350–400 g were placed in the prone position on a foam bed. Impact-acceleration brain injury was produced with a weight-drop device. A brass weight (450 g) was dropped freely by gravity from a height of 1.5 m onto a metalic helmet fixed to the skull vertex of the rat. Animals were sacrificed 1 day, 3 days, 7 days, 30 days, 60 days and 180 days after injury. Dark argyrophilic axons were mainly seen in the corpus callosum, fimbria of the hippocampus, internal capsule, cerebral peduncle and commissure of the superior colliculus 1 day after the injury. However, cell bodies were usually not stained at any designated post-injury time points. Only a few neurons were darkly stained at the CA1 area and CA3 area in the hippocampus, which is known to be vulnerable to ischemia. There were no dark neurons at the cortex or the striatum, which are also vulnerable to ischemia, at any time points. Quantitative evaluation revealed that the number of dark axons at the cerebral peduncle increased during the experimental period. There was a significant difference between the number of dark axons 180 days after the injury and 1, 3, 7, 30 or 60 days after the injury. This suggests that a chronically progressive degeneration of axons may be initiated by the injury. In other words, degeneration may not be effected until at least 180 days after the injury.

12. Acidic Glycosphingolipids (b-Series Gangliosides) Promote Regeneration of the Axotomized Hypoglossal Nerve

M. Itoh, N. Baba, R.H. Cho, Y. Kuga, A. Mizuno, S. Fukumoto[1], K. Furukawa[2]
The First Department of Oral and Maxillofacial Surgery, [1]Department of Pediatric Dentistry, Nagasaki University School of Dentistry, 1-7-1, Sakamoto, Nagasaki 852-8588, Japan, [2]The Second Department of Biochemistry, Nagoya University School of Medicine, 65, Tsurumai, Shouwaku, Nagoya, 466-8550, Japan

Acidic glycosphingolipid (gangliosides) have been postulated to play a variety of important function in neurons, since they are richly present in the central nervous system in many vertebrates. The purpose of the present study was to determine *in vivo* the effects of exogenous and endogenous gangliosides on nerve fiber regeneration after experimental lesion. We investigated nerve fiber regeneration following local application of mixed and pure gangliosides (GM1a, GD1a, GD1b, GT1b, GQ1b) after a 5 mm resection of the right hypoglossal nerve in rat, and following transection of the right hypoglossal nerve in transgenic and knockout mice with gangliosides GM2/GD2 synthesis gene (β1,4-N-acetylgalactosaminyltransferase). At 10 weeks after the operation, the number of horseradish peroxidase(HRP)-labeled motor neurons indicating the repaired axonal processes dramatically increased in the ganglioside GT1b (*b* series)-injected group as compared with the 5 mm-resected group in the rats. Further, the number of HRP-labeled motor neurons in transgenic and in knockout mice decreased slightly as compared with that in wild type mice. Thus, it was suggested that gangliosides might play important roles in the regeneration of severed motor nerve fiber and that gangliosides might promote axonal regeneration after a lesion, leading to an improved functional recovery *in vivo*. The shift of the ganglioside synthetic pathway to *a* series in the transgenic and knockout mice resulted in a decrease of motor nerve recovery, suggesting the importance of the *b* series in axonal regeneration.

13. Oxidized Galectin-1 Regulates Initial Repair in Peripheral Nerves after Axotomy (I) – Structure and Axonal Regeneration-Promoting Activity

Toshihiko Kadoya[1], Yoshimasa Inagaki[1], Yoshiaki Sohma[1], Risa Nozawa[1], Hidenori Horie[2]
[1]Pharmaceutical Research Laboratory, Kirin Brewery Co. Ltd., Takasaki 370-1295, Japan, [2]Department of Physiology, School of Medicine, Yokohama City University, Yokohama 236-0004, Japan

The regulating factors of axonal regeneration are not well understood and we hypothesize that their identification will help to develop effective strategies for nerve repair. We introduced an *in vitro* model for axonal regeneration, which consists of a dorsal root ganglion explant with the associated nerve fiber bundles. The cultured medium of COS1 cells derived from kidney tissue had the property of promoting axonal regeneration from the transected-nerve site of these explant cultures. This COS1-secreted activity was purified by a combination of ultrafiltration, ion-exchange chromatography, gel filtration and reversed phase chromatography. This activity could be eluted from a SDS-PAGE gel slice corresponding to an apparent molecular weight of around 14 kDa. The analysis of an internal partial amino acid sequence indicated that it was identical with galectin-1. Galectin-1 is a member of the family of β-galactoside-binding lectins distributed in the animal kingdom and shows lectin activity in a reduced state. However, our study revealed that galectin-1 promoted

the axonal regeneration only in the oxidized form containing three intramolecular disulfide bonds, not in the reduced form. Oxidized rhGAL-1 enhanced axonal regeneration from the transected nerve terminals of DRG explants in a dose-dependent manner (0.5 to 500 ρg/ml), but it lacked lectin activity. These results suggested that oxidized galectin-1 functions more like a cytokine or chemokine than as a lectin.

14. Growth and Development of Aortic Nerve Fibers in Rats Raised in Outer Space

Masao Yamasaki, Tsuyoshi Shimizu, Tadanori Nagayama
Department of Physiology, Fukushima Medical University School of Medicine, 1-Hikari-ga-oka, Fukushima 960-1295, Japan

To study growth and development of the aortic nerve under conditions of microgravity, we examined cross section of the left aortic nerve, which is the afferent of the aortic baroreflex, in neonate rats aged 25 days raised on the space shuttle Columbia (flight: FLT group) for 16 days. Two kind of ground control groups were compared with the FLT group; one was the asynchronous ground control (AGC) group where the rats were housed in the same cage as that on the shuttle, and the other was vivarium ground control (VIV) group where the rats were housed in a commercial cage. We reported previously that the number of unmyelinated fibers of the left aortic nerve in FLT was significantly less than that of both control groups and the proportion of the aortic nerve fibers in FLT was different from AGC and VIV. In the present study, the axon diameter and myelin thickness of the nerve fibers of five left aortic nerves in each group were measured on the montages of transverse sections of the left aortic nerve, magnified approximately × 10000, and mean values of those in each nerve trunk were calculated. The averages of axon diameters (μm, mean ± SD) of unmyelinated fibers in the FLT, AGC and VIV groups were 0.61 ± 0.08, 0.59 ± 0.13 and 0.56 ± 0.07, and those of the myelinated fibers were 1.44 ± 0.18, 1.32 ± 0.24 and 1.43 ± 0.19, respectively. The myelin thickness (μm, mean ± SD) of the myelinated fibers were 0.27 ± 0.03 in FLT, 0.26 ± 0.04 in AGC and 0.26 ± 0.02 in VIV. None of these sets of average values demonstrated any significant difference among the three groups. The results suggest that the microgravitational environment in space has no effect on the growth and development of each nerve fiber in the aortic nerve.

This study was supported by Grant A 2:08407004 from the Ministry of Education, Science and Culture of Japan, and by NASA, NASDA and JSF.

15. Neurotoxicity of Acrylamides and their Molecular Orbital Calculations

Kojun Torigoe
Department of Anatomy, Fukui Medical School, Mastuoka, Fukui 910-1193, Japan

After receiving repeated doses of acrylamide (AAM), N-methyl acrylamide (NMA), N-ethyl acrylamide (NEA) and methacrylamide (MAA) at 500 ppm concentration in drinking water for 2 weeks, mice manifested clinical symptoms of functional impairment such as a loss of body weight, a muscular atrophy and an exaggerated response to a hind-limb spray reflex. Acrylamide intoxication markedly impairs neural regeneration. To assess the degree of acrylamide-neurotoxicity quantitatively, growth retardation was analyzed using an *in vivo* film model: following transection of the common peroneal nerve, the proximal stump was sandwiched between two sheets of thin plastic film and kept for 5 days. The degree of toxicity in acrylamides examined was obtained in accordance with the length of the regenerating axons on the fifth day after axotomy when the regenerating axons stopped growing. The degree of neurotoxicity was most manifested in mice intoxicated with AAM followed by NMA, MAA and NEA. The lowest unoccupied molecular orbit (LUMO) of these acrylamides was analyzed by computer. The LUMO electric potential (x) was the negative coefficient of the degree of neurotoxicity (y), and the equation was expressed as $y = 3D0.054/(x-0.148)$. The structure and localization of LUMO were the same among the acrylamides examined. A pair of orbits was localized at the nitrogen unit of the acrylamides and two pairs of the greater orbits were at –C = 3DC-CO. Received by the acrylamides in accordance with the LUMO electric potential, the electrons in situ, for example in the nervous tissue, are attracted to the LUMO at the carbon unit of the carbonyl group, and then move toward the LUMO at the acryl group.

16. Diabetes-Induced Changes of Neural Regeneration from Adult Mouse Sensory Ganglia and Retinal Tissue in Vitro

Kazunori Sango[1,2], Hidenori Horie[3], Masahiko Takano[4], Haruhiro Saito[5], Yoshiaki Ishigatsubo[5], Shigeaki Ohno[4], Yoshihiro Ishikawa,[3] Shuji Inoue[2]
[1]Department of Anatomy and Embryology, Tokyo Metropolitan Institute for Neuroscience, [2]Division of Geriatric Health and Nutrition, National Institute of Health and Nutrition, Tokyo, Departments of [3]Physiology, [4]Ophthalmology and [5]Internal Medicine, Yokohama City University School of Medicine, Yokohama, Japan

We examined the effects of experimental diabetes on neural regeneration from adult mouse nodose ganglia (NG), dorsal root ganglia (DRG), and retinas in culture. The numbers of regenerating neurites from transected nerve terminals of NG and DRG at 3 and 7 days in culture were significantly larger in streptozotocin-induced diabetic C57BL/6 mice than those in normal control mice. On the other hand, the numbers of regenerating neurites from retinal explants at 3, 6 and 10 days in culture were significantly smaller in the diabetic mice than in the controls. These results suggest that experimental diabetes could enhance the regenerative capability of

vagal and spinal sensory nerves and simultaneously impair the capability of the retina after axotomy.

17. Analysis of Migration and Differentiation of Mouse Hindbrain Oligodendrocyte Progenitor Cells in a Flat Culture System

Tamaki Wada[1], Tetsushi Kagawa[1], Anna Ivanova[1], Ryuichi Shirasaki[2], Fujio Murakami[2], Shunichiro Iemura[3], Koji Shibuya[3], Naoto Ueno[3], Bernard Zalc[4], Kazuhiro Ikenaka[1]
[1]National Institute for Physiological Sciences, Okazaki, Aichi 444-8585, Japan, [2]Faculty of Engineering Science, Osaka University, Toyonaka, Osaka 560-8531, Japan, [3]National Institute for Basic Biology, Okazaki, Aichi 444-8585, Japan, [4]INSERM-U495, 75651 Paris Cedex 13, France

Oligodendrocytes (OLs) are the myelinating cells in the central nervous system. We found that the earliest OLs appeared in the cervical spinal cord and hindbrain, however, the developmental profile of OLs in this region is not well understood. There are two early OL markers, mRNAs for DM-20, a product from the myelin proteolipid protein (PLP) gene, and the alpha subunit of the receptor for platelet–derived growth factor (PDGFRα). There is no overlapping in the distribution of PLP^+ cells and $PDGFR\alpha^+$ cells in this hindbrain region, indicating that there might be dual origins of OLs in the developing mouse hindbrain.

To investigate which population is a main source for OLs in the hindbrain region, PLP^+ cells or $PDGFR\alpha^+$ cells, we have established the flat explant culture system of mouse embryonic hindbrain and cervical spinal cord. At E12+1DIV, both PLP^+ cells and $O4^+$ cells, another marker for immature OLs, aligned along the ventral midline, and then spread out in the caudal region. On the other hand, $PDGFR\alpha^+$ cells had already widely spread in the ventral half of the explants at E12+1DIV and reached to the dorsal edge of the hindbrain at 3DIV. Thus, since OLs in the explants showed similar appearance and distribution pattern to that *in vivo*, this culture system is useful for investigating the effect of various growth factors on OL development in an environment similar to that *in vivo*. When the caudal midline region was isolated and cultured by itself, the number of $O4^+$, PLP^+ and $PDGFR\alpha^+$ cells increased dramatically.

When only one side of the dorsal region was removed from the flat culture, the number of $O4^+$ cells increased specifically in the ablated side suggesting that some factors from dorsal region repress OL development. The addition of BMP-4 which is known as a dorsalizing signal caused drastic decrease in the $O4^+$ cell number.

These data suggest that caudo-ventral midline region is the origin of OLs for both PLP^+ cells and some of the $PDGFR\alpha^+$ cells *in vivo* and their proliferation and/or their differentiation are suppressed by the dorsally located BMPs.

18. The Blood-Brain-Barrier Formation of Grafted Human Umbilical Vein Endothelial Cells in the Athymic Mouse Brain

Akiyama Hideyuki, Takeshi Kondoh, Takashi Kokunai, Norihiko Tamaki
Department of Neurosurgery, Kobe University School of Medicine, Kusunoki-cho 7-5-1, Chuou-ku, Kobe 650-0017 Japan

The contribution of blood vessels in the grafting of cell suspension of neuronal tissue cell suspensions has been widly studies and it has been thought that grafted cells are predominantly vascularized by host-dirived endothelial cells. Human umbilical vein endothelial cells (HUVECs) are the most widly used human-origin endothelial cells for experimental purpose which are known express MHC class I, intracellular adhesion molecule-1, and form angiogenesis *in vitro*. In this study, HUVECs were transplanted in athymic mouse brain and neovascularization of the grafted endothelial cells was studied. HUVECs were transfected by reporter gene pEGFPE-N1 *in vitro* and grafted stereotactically in the unilateral striatum of adult nude mice. Histological studies were performed four weeks after surgery. The grafted HUVECs formed a dense cell accumulation along the needle tract. The border between the grafted cell and host brain was obscure and grafted cells were well-integrated in the host brain environment, demonstrated by Nissl staining. There were radiating cells clusters from the needle tract suggesting that grafted HUVECS migrated far from the tract. Sections treated by DAB demonstrated host blood vessels as well as vessels in and around the grafted HUVECs. The core of graft was relatively a vascular and various sizes of vessels were observed in the graft. Revealing that grafted HUVECs newly formed miscovessels in the brain, which radiated out from the graft and fused with host vessels. Intravenous injection of Evans Blue prior to sacrificing the animals resulted in no extravasation of dye indicating that the blood-brain-barrier had formed in grafted HUVECs. Glial fibrillary immunohistochemistry demonstrated host astrocytes extending to the glial foot on grafted endothelial cells. This study demonstrated that ectopic origin endothelial cells from ectopic origin are able to form a blood-brain-barrier and that HUVECs are one of the candidate cell lines for *ex vivo* gene therapy in the for central nervous systems.

19. Morphological Changes of Immunopositive Cells of AMPA Glutamate Receptor Subunits within Intrastriatal Grafts in a Rat Model of Parkinsons Disease

Kazunari Todaka, Yasushi Ishida, Itsumi Kuwahara
Department of Psychiatry, Miyazaki Medical College, 5200 Kihara, Kiyotake-cho, Miyazaki-gun, Miyazaki 889-1692, Japan

To elucidate the morphological changes of immunopositive cells of α-amino-3-hydroxy-5-methyl-4-isoxazolepro-

pionate (AMPA) receptor subunits within intrastriatal "developing" grafts of fetal ventral mesencephalon (VM) in a rat model of Parkinson's disease, immunohistochemistry was performed 1, 4 and 12 weeks following transplantation. Unilateral lesions of mesostriatal dopamine pathway were created stereotaxically by injecting 6-hydroxydopamine (6-OHDA) into the left medial forebrain bundle of female Wistar rats (120–130 g). Three weeks after the formation of the 6-OHDA lesions, neural transplantation was performed in the 6-OHDA-lesioned rats which showed methamphetamine-induced rotation (at least 7 turns/min for 60 min) ipsilateral to the lesion. Fetal VM tissue was obtained from Day 15 rat embryos. For the immunohistochemical study, grafted animals were allocated into 3 groups: 1 week post grafting, the group 4 weeks post grafting, and 12 weeks post grafting. Immunohistochemistry was performed with the ABC method to detect AMPA glutamate receptor subunits (GluR1, GluR2/3 and GluR4) or tyrosine hydroxylase (TH) immunopositive cells. In addition, we performed a double-labeling immunofluorescence study to elucidate the similarity of the cellular distributions of TH and each AMPA receptor. One week after transplantation, TH-positive cells were detected without any immunoreactivity of the AMPA receptor subunits in the grafts. Four weeks after transplantation, TH-positive cells, distributed homogeneously in the grafts, seemed to be multipolar and larger compared to those at 1 week post grafting. At this stage, we could observe positive cells of AMPA receptors distributing homogeneously in the grafts. Twelve weeks after transplantation, the number of GluR1-positive cells decreased as compared with that at four weeks post grafting whereas TH-positive cells appeared to be more matured in shape and size. On the other hand, the cellular distributions of GluR2/3- and GluR4-positive cells showed no change as compared with those at 4 weeks post grafting. The results suggest that AMPA receptors, especially GluR1, may function in developing dopaminergic neurons in grafts.

20. Electrophysiological Properties of Spinal Cord Axons Remyelinated by Various Cell Transplantation Conditions

T. Imaizumi[1], J.D. Kocsis[2], K.L. Lankford[2], K. Hashi[1]
[1]Department of Neurosurgery, Sapporo Medical University School of Medicine, Japan, [2]Department of Neurology, Yale University School of Medicine, Neuroscience Research Center, USA

Transplantation of Schwann cells (SCs) induced remyelination of demyelinated rat dorsal column axons and improved conduction. To investigate how effective various transplant conditions may be to improve the electrophysiological function, we compared SCs of different ages and culture with acutely dissociated and SCs from different spicies (rats and pigs). Moreover, we studied the effect of cyclosporin. Dorsal columns (DCs) of adult rats were demyelinated at T11 by X-ray irradiation and ethidium bromide, and transplanted with SCs (3X104). Three weeks later, the spinal cord was removed, pinned in a recording chamber and field potentials (FPs) were recorded, to investigate the conduction properties of the specimen. Following transplantation of SCs, histological examination revealed SC-like patterns of remyelination of demyelinated axons. All transplant conditions improved in their electrophysiological properties compared to control demyelinated axons. Cell culture and cyclosporin treatment following SC transplantation showed less improvement than freshly dissociated SCs. The age (young adult 2.5 month old, and aged, 18 month old) of SCs did not significantly influence the conduction improvement. Xenotransplantation of cultured pig SCs (transgenic for hCD59, which is a human compliment inhibitor and might suppress hyper acute rejection if transplanted to humans) resulted in an improvement of conduction, but was less than that of the allografted cells. This reduction in electrophysiological function may result from immunosuppression and differences in culture.

21. Genetically Engineered Cell Line Suitable for Encapsulated Transplant in the Central Nervous System

Youichi Saitoh[1], Yutaka Eguchi[2], Yasushi Hagihara[1], Yoshihide Tsujimoto[2], Toshiki Yoshimine[1]
Departments of [1]Neurosurgery and [2]Medical Genetics, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

In a previous study, the authors demonstrated that when Neuro2A, a xenogeneic cells for rats, which carries the POMC (preopiomelanocortin) gene coding the precursor of ACTH and β-endorphin in a polymer capsule, was transplanted in the rat cerebrospinal fluid (CSF) space, it secreted β-endorphin and reduced the rat's pain. In addition, Neuro2A cells bearing the POMC gene linked with the Tet-On system, whose promoter is inducible by administration of tetracycline derivatives, secreted ACTH and β-endorphin according to the amount of tetracycline administered. However, those Neuro2A cells unexpectedly secreted low amounts of β-endorphin, despite the fact that Neuro2A cells transfected with POMC gene under SV40 promoter secreted equal amounts of ACTH and β-endorphin. It may be that the Tet-On system itself specifically interferes with the processing of POMC specifically or large molecular precursors in general. In the present study, the pUHD10-3-NL-1-β-endorphin and pUHD172-1n ln were co-transfected to BHK21, which does not have the POMC converting enzyme (BHK-Tet-End). NL-1 is a new cDNA that encodes a peptidase of the same family as Neprilysin. BHK-Tet-End secreted β-endorphin depending on the amount of tetracyclin administered. This system can be applicable to any cell line and that cell line can secrete interested neural peptides which can be controlled by the amount of tetracycline, and may be used for clinical *ex vivo* gene therapy.

22. Expression of Receptor Tyrosine Kinase RYK in Developing Rat Central Nervous System

Kazuyo Kamitori[1], Mitsuru Machide[1], Noriko Osumi[2], Shinichi Kohsaka[1]
[1]Department of Neurochemistry, [2]Department of Biochemistry and Cellular Biology, National Institute of Neuroscience, 4-1-1 Ogawa-higashi, Kodaira, Tokyo, 187-8502, Japan

RYK is a widely expressed receptor protein tyrosine kinase, which has been isolated from mice and humans, but it still remains to be an orphan receptor without any overt function. There are some similarities between RYK and *Drosophila* Lio/Drl, which is involved in learning and memory, and in axonal pathfinding. RYK and Lio/Drl share unique intracellular tyrosine kinase domains with common amino acid substitutions in the subdomains involved in the ATP binding. They have relatively small extracellular domains with two leucine-rich motifs, which are generally known to participate in protein interactions and in cell adhesions.

In this present study we doned a rat RYK gene and characterized its expression pattern in the central nervous system. Developmental analysis by Northern blot revealed two peaks of RYK mRNA; on embryonic days 17 to 18 (E17~18) in the whole brain, and during postnatal weeks 1 to 4 (P1W~P4W) in the cerebrum. By *in situ* hybridization, RYK-expressing cells were observed throughout the central nervous system, mainly in the ventricular zone on E11 and E13. On E18 and E20, the remarkably high level of RYK mRNA was detected in the ventricular zone as well as in the cortical plate of the forebrain. These two regions overlapped the immunoreactive areas of nestin and MAP2, a neural stem cell marker and a mature neural marker, respectively. In the postnatal brain, RYK was predominantly expressed in neurons in the cerebral cortex, hippocampus, and cerebellum. Double-labeling of RYK with marker proteins confirmed expression of RYK in neural stem cells and neurons. These observations suggest that RYK plays contributory roles in the differentiation and maturation of neuronal cells in the central nervous system.

23. Isolation and Culture of Neural Precursor Cells from Embryonic Human Brain

Jun Takahashi[1], Hiroki Toda1, Kei Tashiro[2], Tasuku Honjo[3], Nobuo Hashimoto[1]
[1]Department of Neurosurgery, [2]Center for Molecular Biology and Genetics, [3]Medical Chemistry, Faculty of Medicine, Kyoto University, Kyoto 606-8507, Japan

Stem cells that can give rise to neurons, astrocytes, and oligodendrocytes have been found in the developing and adult central nervous system (CNS) of rodents. In the clinical application of neural stem cells for the patients with neuro-degenerative disease or brain injury, it is essential that these cells exist within the human brain. In this presentation, we show that the developing human CNS embodies neural precursor cells which are similar to their the counterpart in the adult rat hippocampus.

Permission to use human CNS tissue was obtained by the ethical committee of the Kyoto University Graduate School of Medicine. Whole brain was obtained after 11 weeks of gestation from a legally aborted fetus, and mechanically triturated. Cells were plated on fibronectin-coated dishes, and maintained in DMEM/F12 serum-free medium containing N2 supplement (Gibco), 20 ng/ml of bFGF, and 10 ng/ml of EGF. Phase-bright cells, similar to rat neural stem cells, proliferated and were cultured over 1 year. These cells can be frozen for preservation, and can also be introduced foreign transgene using a retroviral vector.

For differentiation experiments, the culturing medium was replaced by N2 medium containing retinoic acid (0.5 μM) and 0.5 % FBS. After 1 week, the medium was again replaced by N2 medium containing BDNF (20 ng/ml), NT-3 (40 ng/ml), and 0.5 % FBS. After another 2 weeks, cells were fixed with paraformaldehyde and used for immunofluorescence study. The study revealed 30 to 40 % of the cells were MAP2ab or GFAP-positive, and some cells were galactocerebroside-positive, which indicates that the cells obtained from the developing human brain could give rise to neurons, astrocytes, and oligodendrocytes. Another study revealed the existence of GABA-positive cells and glutamate-positive cells, which suggested that some cells were GABAergic or glutamatergic neurons.

These cells represent a renewable source of neurons and glia and may significantly facilitate research on human neurogenesis and the development of clinical neural transplantation.

24. Hippocampal Adult Neurogenesis and Neuronal Circuit Formation: An Analysis with Special Reference to Polysialic Acid-Neural Cell Adhesion Molecule

Tatsunori Seki, Yasumasa Arai
Department of Anatomy and Medical Education, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo, Tokyo 113-8421, Japan

It is generally thought that neurons are generated during the embryonic and early postnatal periods, but not in the adult. However, as neurogenesis continues in the dentate granule layer of the adult hippocampal formation, this would imply that new neuronal circuits are being added to the adult hippocampus by adult neurogenesis. Since these newly generated granule cells specifically express polysialic acid (PSA-)neural cell adhesion molecule (NCAM), it is possible to examine the new neuronal circuit formed by these cells using PSA immunohistochemisty. The present observation by confocal laser scanning microscopy and immunoelectron microscopy demonstrates contact between developing dendritic processes and radial glial processes, and also shows the glial processes enveloping dendritic processes. Further

more the number of PSA-expressing granule cells and radial glia cells show a parallel age-dependent decrease during aging. One possible function of the association of the two elements is that such a spatial and temporal relationship contributes to dendritic development of the newly generated granule cells. In the adult hippocampus distinct types of mossy fiber boutons exhibit different PSA expression patterns. No PSA immunoreactivity was detected in large mature mossy fiber boutons. On the other hand, immunoreactivity was found on the free surface of the immature type of mossy fiber boutons, namely irregularly-shaped boutons and medium-sized boutons containing fewer clear synaptic vesicles, mitochondria and synapses, suggesting that the synaptogenesis of the mossy fiber boutons persist in the adult hippocampus. As for PSA function, it was revealed that the PSA expression of mossy fibers is required for synaptic arrangement. Recently neurogenesis and PSA expression in the adult hippocampus are reported to be affected by neuronal activity and hormones, suggesting that they are closely associated with hippocampal plasticity.

25. Effects of Basic Fibroblast Growth Factor and Epidermal Growth Factor on Neural Stem Cells Sorted by Light Scattering

Banri Yamanoha and Takane Nakayama
Institute of Life Science, Soka University Hachioji, Tokyo 192-8577, Japan

The fate of neural stem cell in developmental brain progress depends intensively in differentiation by the primary culture system. The stem cell is differentiated terminally to neuron and glia cells, however, the regulation of the these processes remains largely unknown. Recent progress in the understanding of stem cell biology would suggest that there are heterogeneous precursors in the stem cell progeny and that these can be classified as belonging to the multipotential, bipotential and unipotential types of precursor. Furthermore, epigeneic factors that regulate the development of the brain might control the proliferation and differentiation of the stem/precursors. We have established a method for separation of neural precursor cells from the forebrain of embryonic day 12 embryos by flow cytometry using light scattering. The cells were assessed in serum-free medium, DMEM/F12 supplemented with insulin, transferrin and heparin (DF/TIH). When the sorted cells are plated into DF/TIH containing FGF-2 at 5×103 cell/cm^2, they form neurospheres and increased in size, whereas EGF response cells were not generated. Furthermore, the FGF-generated spheres are defined as nestin-immunoreactive cells, and differentiated to neurons without FGF-2. It has been suggested that FGF and EGF response precursor cells might generate each other at different stage in the development of brain. In order to define above address this, stem cells in embryonic day 15 and 18 were isolated by flow cytometer using the light scattering method. These cells formed spheres from which we could assess the response of the growth factors. The spheres from E15 were generated with FGF-2 and EGF, but the FGF-2 response spheres exceeded the number of EGF spheres by a factor of 10. Conversely, EGF response spheres were greater in number in E18 than in E15. The spheres are analyzed by immunocytochemistry using αNestin, αMAP and αGFAP. In a double antigen analysis, all of generated spheres expressed the antigen of nestin, a part of cell population to which belonged the FGF-2 response sphere expressed the antigen of MAP.

26. Differentiation of Transplanted Adult Hippocampus-Derived Neural Stem Cells in Adult Rat Retina with Mechanical Injury

Akihiro Nishida[1,2], Masayo Takahashi[1], Hidenobu Tanihara[3], Akira Mizoguchi[2], Chizuka Ide[2], Yoshihito Honda[1]
[1]Department of Ophthalmology and Visual Sciences, [2]Department of Anatomy and Neurobiology, Kyoto University Graduate School of Medicine, [3]Tenri Yorozu Hospital, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto, Japan

We previously reported that adult rat hippocampus-derived neural stem cells (AHSCs) migrated into injured adult rat retina after being transplanted into the intravitreal space. This study is to investigate the potentials of the AHSCs to differentiate into neuronal or glial lineages in adult rat retinas with mechanical injury.

Clonal AHSCs carried marker genes encoding cytoplasmic β-galactosidase (β-gal) were used for this study. Mechanical injury was made in the adult rat retina with a hooked 30-gauge needle under direct observation surgical microscope. The cell suspension (containing 90,000 AHSCs) was slowly injected into the vitreous space. The incorporation and differentiation of the grafted cells were investigated by immunohistochemical studies using antibodies to β-gal, nestin, MAP2ab, MAP5, GFAP, HPC-1 and calbindin.

After the creation of mechanical injury, at the first, second and fourth post-treatment week the migration of the grafted AHSCs were found in host retina in 10 %, 50 % and 40 % of the experimental eyes, respectively. The cells migrated into the wide area surrounding the wound, where GFAP expression was upregulated in Müller cells. In the non-injured group, grafted cells were not found inside the host retinas. Immunohistochemical studies showed that, at the first week, the majority of the grafted AHSCs expressed nestin, a marker for immature status, but not any other markers. On the other hand, at the fourth week after transplantation, the grafted cells showed immunoreactivity for MAP2ab (10 %), MAP5 (14 %) and GFAP (14 %), suggesting the progress in the processes of differentiation into neuronal and glial lineages after retinal transplantation. However, the grafted cells showed no immunoreactivity for HPC-1, a marker of amacrine cells, and calbindin, a marker of horizontal and some amacrine cells.

Our results revealed that the incorporation and subsequent differentiation into neuronal and glial lineages of the

grafted AHSCs can be achieved even in adult rat retinas after mechanical injury.

27. Transplantation of Human Neural Stem Cells Repairs the Injured Central Nervous System in the Adult Rat

Osamu Honmou, Takaaki Kato, Yukinori Akiyama, Kazuo Hashii
Department of Neurosurgery, Sapporo Medical University, Sapporo 060-8543, Japan

A central issue in cell transplantation therapy for the damaged central nervous system is to determine which type of cell is most effective in eliciting functional recovery. Although it is known that transplantation of neural stem cells can lead to functional improvement in animal models, less is known about results of transplantation of neural stem cells derived from humans. We report here that transplantation of neural stem cells derived from adult human brain and human fetals tissue in the demyelinated and the traumatic models of adult rats leads to functional improvement. The dorsal columns of the adult rat spinal cord were demyelinated by X-irradiation and intraspinal injections of ethidium bromide. Clonal cell suspensions of human neural stem cells were injected into the demyelinated lesion site. Lesions were examined histologically 3 weeks after transplantation. Light and electron microscopic examination revealed the demyelinated axons were remyelinated.

The frontal cortexs of the adult rats were partially removed. Clonal cell suspensions of human neural stem cells were injected into the injured lesion. Lesions were examined histologically 2 weeks after transplantation. Light microscopic examination revealed the neural tissue reconstruction. These results demonstrate that transplantation of neural stem cells derived from both adult and fetal human brains results in appropriate changes in neural reconstruction in the adult mammalian CNS.

28. Transplantation of Clonal Neural Stem Cells Derived from Adult Human Brain Elicits Functional Repair of Demyelinated Spinal Cod Axons in the Adult Rat

Yukinori Akiyama, Osamu Honmou, Takaaki Kato, Kazuo Hashi
Department of Neurosurgery, Sapporo Medical University, Sapporo 060-8543 Japan

Clonal neural stem cells derived from adult human brain tissue were transplanted into the demyelinated rat spinal cord to determine if myelin formation could occur and if conduction could be improved. The dorsal columns of the adult rat spinal cord were demyelinated by X-irradiation and intraspinal injections of ethidium bromide. Cell suspensions of clonal neural stem cells derived from adult human brain were injected into the glial-free lesion site. Light and electron microscopic examination of the dorsal columns revealed cells engaging and myelinating axons in a manner highly reminiscent of Schwann cells in the demyelinated regions 21 days after transplantation. In addition, an in situ hybridization study with COT-1 DNA probe indicated that the new myelin was formed by the human cells. The dorsal columns were removed and maintained in an *in vitro* recording chamber; conduction properties were studied using field potential recording techniques. The demyelinated axons exhibited inhibitation of and blocking of conduction, and a reduction in their ability to follow high frequency stimulation. Axons remyelinated by transplantation of clonal neural stem cells derived from adult human brain exhibited restoration of conduction through the lesion, with re-establishment of normal conduction velocities. The axons remyelinated following transplantation showed enhanced impulse recovery to paired pulse stimulation and greater frequency-following capability as compared to demyelinated axons. These results demonstrate the functional repair of demyelinated axons in the adult CNS by transplantation of clonal neural stem cells derived from adult human brain.

29. Hibernation and Grafting of Long-Term Cultured Sphere of Neuronal Progenitor Cells in the Ischemic Rat Brain

Takeshi Kondoh[1], Hideyuki Akiyama[1], Takehiko Ueyama[2], Naoya Saitoh[2], Norihiko Tamaki[1]
[1]Department of Neurosurgery, Kobe University School of Medicine, [2]Division of Molecular Pharmachology, The Biosignal Center, Kobe University, Kusunoki-cho 7-5-1, Chuou-ku, Kobe 650-0017, Japan

Neuronal progenitor cells have been widly studied with the purpose of regeneration of injured central nervous system. For the grafting of these cells, not pure single cell suspension of stem cells but spheres composed with progenitor cells have been reported to demonstrate improved survival after grafting. In this study, rat neuronal stem cells were obtained from E-14 rat subventriclur zone followed by free floating culture in EGF-containing medium as previously reported by Weiss et al. After four passage in four weeks, single stem cells were left to grow without dissociation for two months by changing the medium weekly. The spheres became 500–800 μm in diameter and then preserved in Hibernation Medium E (Gibco BRL) for a week at 4 °C. Those sphere were labeled by PHK26 right before the grafting. Two different type of cerebral ischemia has been prepared in host adult rats. One is middle cerebral artery (MCA) occulusion by phtochemical method and the other is endothelin injection (ET-1; 0.05 ng/animal) into unilateral striatum. One week following transplantation of hibernated neuroprogenitor.

30. Stimulation in Hippocampal Pyramidal Neuron Evokes Increases in HCNP Precursor Protein Messenger RNA in CA1 Field of Rat Hippocampal Slice

Tamaki Iwase[1,2], Noriyuki Matsukawa[1], Shigehisa Mitake[1], Hitoo Nishino[2], Takayuki Yamamoto[3], Kosei Ojika[1]

[1]Second Department of Internal Medicine and [2]Second Department of Physiology, Nagoya City University Medical School, [3]Choju Medical Institute, Fukushimura Hospital, Japan

Hippocampal cholinergic neurostimulating peptide (HCNP) is an undecapeptide isolated from the hippocampus of young rats. HCNP enhances the cholinergic development of the rat medial septal nucleus *in vitro*. The HCNP precursor protein is a 21 kDa protein that binds Mg-ATP, and is associated with the opioid-binding protein. The highest expression of the HCNP precursor protein messenger RNA (HCNP mRNA) is in hippocampal pyramidal neurons. The N-methyl-D-aspartate (NMDA) receptor specifically mediates release of HCNP from hippocampal neurons *in vitro*. The neurotrophic factors such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) modulates synaptic activity and their expression is modulated by neuronal activity. Hippocampal long-term potentiation (LTP) has been studied as a model for the neuronal basis of learning and memory. In this presentation, we show the effects of neural activity on expression of the HCNP mRNA. We selected the model of long-term potentiation in rat hippocampal slices, and used non-radioisotopic *in situ* hybridization. We stimulated the Shaffer collateral-CA1 pyramidal neuron synapse in adult rat hippocampal slice with extracellular recording techniques. One slice received only test stimuli delivered at 0.1 Hz, at an intensity that evoked a 1 mV population spike; the other slice received tetanization consisting of 2 trains of stimuli (1 s long at 100 Hz with an interstimulus interval of 20 s) as well as test stimuli. *In situ* hybridization revealed both stimuli increased expression of the HCNP mRNA in CA1 of the rat hippocampal slice. Although the release of HCNP from hippocampal neurons is specifically mediated by the NMDA receptor, this result indicates that some other mechanisms may regulate the HCNP mRNA level.

31. Abnormal Expression of Glial/Neuronal Proteins in the Mouse Brain after Intraventricular Administration of Basic Fibroblast Growth Factor (bFGF) In Utero

Makoto Ohmiya, Atsumi Nitta, Hiroshi Nomoto, Megumi Ito, Shoei Furukawa
Laboratory of Molecular Biology, Gifu Pharmaceutical University, Mitahora-higashi, Gifu 502-8585, Japan

Neural stem cells proliferate, move to their destined positions and differentiate during sequential processes of constructing the central nervous system. These processes are thought to involve actions of neurotrophic factors, although exact details of the mechanisms remain unknown. In this study, we investigated effects of basic fibroblast growth factor (bFGF) on neurogenesis following intraventricular administration of bFGF (0.1–0.4 mg) 14 days old embryos. Hydrocephalus-like head morphology appeared in bFGF-treated animals after several postnatal days, in which a markedly enlarged ventricular space and thin structure of the cerebral cortex were apparent. In bFGF-treated animals, cells expressing MAP2 and/or GFAP existed in the ventricular zone of irrespective of the postnatal age, and Hu protein and/or nestin were expressed even in differentiated neuronal cells located in the cortical plate/cerebral cortex. Furthermore, astrocyte-like cells possessed immunoreactivity of tyrosine hydroxylase in the whole cerebral cortex in bFGF-treated animals. These observations demonstrated that exogenously added bFGF threw the expression of neuronal and glial proteins into disorder during neurogenesis and caused abnormal brain structure resembling hydrocephalus. bFGF is thought to play essential roles for normal development of the cerebral cortex.

32. CNTF and GDNF Promote Survival of Axotomized Beta Ganglion Cells in the Cat Retina

Masami Watanabe
Department of Physiology, Institute for Developmental Research, Kasugai 480-0392, Japan

Transection of the axons of retinal ganglion cells (RGCs) at the level of the optic nerve (ON) results in their eventual death. We have previously shown that axotomized RGCs could not only survive but also regenerate their axons following transplantation of peripheral nerve segments. However, the total numbers of regenerated RGCs were lower than 5 %. To obtain greater numbers of surviving cells, neurotrophic factors were injected into the vitreous of adult cats. RGCs were labeled with DiI injected into the both lateral geniculate nuclei 14 days prior to transecting the ON. After transection of the ON, BDNF (1 μg), NT-4/5 (1 μg), GDNF (1 μg), CNTF (1 μg) was injected into the vitreous. After 14 days survival, the eye was enucleated and the retina was dissected. Proportions of cell types were determined with systematic injections of Lucifer Yellow. The densities of RGCs were measured in the area centralis of the right normal retina and left ON transected retina. The average cell density was reduced to 18.5 % in untreated retinas with ON transection, while the average densities were 28.5 % (BDNF), 21.8 % (NT-4/5), 36.8 % (GDNF) and 35.7 % (CNTF) in the retinas with neurotrophic factor injections. These values were, however, statistically insignificant. Relative numbers of surviving beta cells in the untreated retina were 12.1 % compared with the normal retina, and 39.6 % and 46.5 % cells in the retinas with CNTF or GDNF injections, respectively. Surviving alpha cells were 67 % to 71 % of those in the normal retina irrespective of neurotrophic factor injections. The results suggest that CNTF and GDNF are effective for the survival of axotomized beta cells, and their receptors may be expressed on the surface of beta cells.

33. Influence on Gene Expression of Neurotrophic Factors Produced in Astrocyte Stimulated by ACTH1-24

Minoru Kokubo[1], Kiyofumi Asai[2], Masayuki Morikawa[2], Yoko Kawai[2], Yoshiro Wada[1], Taiji Kato[2]

[1]Department of Pediatrics, Nagoya City University Medical School, [2]Department of Bioregulation Research, Nagoya City University Medical School, Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan

We examined the effects of ACTH1-24, which is used in the therapy of West's syndrome, on astrocytes. We stimulated rat astrocytes with CRF, ACTH1-24, Dexamethazone (Dex) *in vitro*, and examined changes of NGF, BDNF, NT-3 and CNTF mRNA, by reverse transcription polymerase chain reaction (RT-PCR). Down-regulation of CNTF mRNA was observed when stimulated with ACTH1-24. Northern blot analysis revealed the down-regulation was observed within 4 h after stimulation over 10 nM of ACTH1-24. It is reported that ACTH1-24 induces cAMP. It is generally accepted that cAMP exerts its cellular effects mostly via the activator of cAMP-dependent protein kinase (PKA). However, treatment of cultured astrocytes with H-89, a selective inhibitor of PKA, did not reverse ACTH1-24-induced suppression of CNTF mRNA. Up-regulation of NT-3 mRNA was also found when stimulated with 1 mM Dex. The possibility thus exists that the blood-brain barrier (BBB) is not complete in the immature and damaged brain, which might lead to West's syndrome. The administration of ACTH1-24 may affect astrocytes directly and exert changes of neurotrophic factors. ACTH1-24 stimulates secretion of glucocorticoid in the adrenal grands. It has been reported that glucocorticoids induce neurotrophic factors, and thus glucocorticoids induced by ACTH1-24 may also exert changes of neurotrophic factors.

34. Genomic Cloning of the Mouse HCNP (Hippocampal Cholinergic Neurostimulating Peptide) Precursor Protein Gene

H. Nakazawa[1,3], N. Matsukawa[1], H. Okada[2], T. Yamamoto[3], K. Ojika[1], R. Ueda[1]
[1]Second Department of Internal Medicine, Nagoya City University, [2]Department of Molecular Biology, Nagoya City University, Nagoya 467, [3]Choju Medical Institute Fukushimura Hospital, Toyohashi 441, Japan

Neurotrophic factors such as NGF and BDNF have been shown to play important roles in the development of the septo-hippocampal cholinergic system as well as in the mechanism of learning and memory formation. Hippocampal cholinergic neurostimulating peptide (HCNP), isolated from hippocampal tissue of young rats, enhances the *in vitro* synthesis of acetylcholine in medial septal nuclei. This novel undecapeptide is processed from its 21kDa precursor protein by specific cleaving enzyme. We have previously cloned rat, human and mouse cDNAs encoding the precursor proteins. *In situ* hybridization and/or immunohistochemical analyses of human, rat and mouse brain have revealed that the expression of HCNP gene and HCNP-related components were widely distributed in the target tissues of cholinergic neurons in the brain, especially in the hippocampus, and suggested that their abnormal expressions were involved in certain disease processes such as learning disorders in human as well as in animal models. In this study, we cloned the mouse genomic DNA using HCNP precursor cDNA as a probe and determined the entire nucleotide sequence of the 15kbp gene. In its promotor region, there were CAAT box and GC box consensus sequences, but no TATA box sequence was found between the CAAT box and the transcription initiation site. Several other enhancer binding sequences such as Sp-1, NF-κB, MyoD, GATA, MTF, IRF-1/2 were found in 5'-upstream region and introns of the gene.

35. Analysis for mRNA Levels of Tissue-Type Plasminogen Activator and Urokinase-Type Plasminogen Activator in Rat Brain after Middle Cerebral Artery Occlusion

Takeshi Ito, Katsunobu Takenaka, Tatsuya Kuroda, Shinji Noda, Noboru Sakai
Department of Neurosurgery, Gifu University School of Medicine, Gifu 500-8705, Japan

The roles of the tissue-type plasminogen activator (tPA) and the urokinase-type plasminogen activator (uPA) have been well reported in thrombolitic therapy. On the other hand, the functions of tPA and uPA are less understood in ischemic brain tissue. Therefore, changes in tPA and uPA mRNA in rat brain tissue after MCA occlusion and during the hypoxic stimulation in the neuronal cell line, PC 12 cells, were examined. Permanent middle cerebral artery (MCA) occlusion was induced by advancing a filament into the internal carotid artery in 36 adult male Sprague-Dawley rats. The ischemic cerebral MCA cortex, contralateral cortex and bilateral hippocampus specimens were collected at 0 (controls), 1, 3, 6, 12 and 24 hours after occlusion. Hypoxia was induced in PC 12 cells with a multigas incubator (set to 1 % O_2). Quantitative reverse transcription-polymerase chain reaction was performed to measure the alteration in mRNA levels. The mRNA levels of tPA and uPA were significantly increased after MCA occlusion in the ischemic cerebral cortex. The increases of tPA mRNA were time-dependent in insult and contralateral hippocampus. The increase of uPA mRNA was also seen in the hippocampus bilaterally. In PC 12 cells, the mRNA for tPA was significantly increased for 6–12 hours, while the mRNA for uPA was not detected at any time studied. Our results suggest that the activation of these proteases might involved in not only the insult but also in changes contralateral brain tissue.

36. Immunosuppressive Therapy with FK 506 as a Model of Traumatic Brain Injury

Masaki Shinoda[1], Kou Sakabe[3], Mitsunori Matsumae[2], Masayoshi Shibata[2], Atsushi Tsugu[2], Yoshihiko Katano[2], Ryuichi Tsugane[2]
[1]Department of Neurosurgery, Chigasaki Municipal Hospital, Chigasaki, Kanagawa, Japan, [2]Department of Neurosurgery,

Tokai University School of Medicine Isehara, Kanagawa, Japan, [3]Department of Morphology, Tokai University School of Medicine Isehara, Kanagawa, Japan

Immunological alterations following traumatic brain injury with or without an immunosuppressant (FK506) were examined. Young adult Sprague-Dawley rats were placed under general anesthesia, and an incisional injury to the right striatum was made with a knife (Futaba No. 19). Following the injury, an immunosuppressant (FK 506, Fujisawa Co. Ltd., 10 mg/kg) was administrated intraperitoneally; control animals received normal saline. One, 4, and 7 days, and 2 and 4 weeks after the operation, rats were sacrificed and perfused with 4 % paraformaldehyde. The brains were sectioned by paraffin fixation materials. Immunohistochemical examinations were performed after de-paraffin fixation. OX18 (major histocompatibility (MHC) class I), OX6 (MHC class II), ED1, and OX42 (complement receptor type 3 (CR3)) monoclonal antibodies were used as primary antibodies. The results showed a decrease of debris formation and glial proliferation in the FK 506-administered group. A lower amount of MHC class II IR-positive cells was recognized in day 4 to week 1 of the FK 506 treated group. We conclude that administration of an immunosuppressant (FK 506) decreases glial proliferation via the inhibition of the glial activating pathway. Immunosuppressive therapy has potential applications in clinical medicine, as a method of neuroprotection.

37. Molecular Mechanisms of the Neuroprotective Effect of bFGF on Neuronal Injury

Takakazu Kawamata[1], Tomoko Yamaguchi[2], Tomokatsu Hori[1]
Departments of [1]Neurosurgery and [2]Biochemistry, Neurological Institute, Tokyo Women's Medical University, 8-1 Kawada-Cho, Shinjuku-Ku, Tokyo 162-8666, Japan

Basic fibroblast growth factor (bFGF) is an 18-kDa neurotrophic polypeptide that promotes neural survival and neurite outgrowth. Although bFGF has recently been recognized as a promising molecule for the treatment of neuronal damage, including cerebral infarct volume reduction and enhancement of functional recovery, as we demonstrated previously, the precise molecular mechanisms by which bFGF exerts its neuroprotective effect have not yet been clarified. In the present study, introducing PC 12 cells as a model of neuronal cells, we examined the effect of bFGF on survival activity and investigated major the signaling transduction pathways by detecting Ras, PI3-kinase, and Bcl-2 with Western blot analysis to explore possible neuroprotective mechanisms of bFGF. bFGF at 1–100 ng/ml promoted cell viability dose-dependently up to 200 % following 24 hours incubation under control culture condition in PC 12 cells. Preincubation for 24 hours with 1–100 ng/ml of bFGF prevented cell death induced by A23187 up to 30–40 % in a dose-dependent manner. In Western blot analysis, bFGF activated Ras, PI3-kinase, and Bcl-2 in various degrees and at various time points. Ras was upregulated dominantly in the early stage up to 24 hours following bFGF stimulation returning to the level of baseline at 48 hours. On the other hand, PI3-kinase was activated in the later phase at 48 hours compared to 24 hours as a cellular response to bFGF. In the current study, however, Bcl-2, a major candidate molecule to suppress programmed cell death in various cell types, was upregulated less appreciably than Ras or PI3-kinase. Furthermore, neuroprotective effect of bFGF was inhibited prominently by Rapamycin (PI3-kinase inhibitor) or PD98059 (MEK inhibitor). The current findings indicate that several signaling transduction pathways including MAP kinase and PI3-kinase pathways are involved in neuroprotective mechanisms of bFGF over various time courses, which may partially share the molecular mechanism of NGF in cellular responses.

38. GABA-Induced Intrinsic Light-Scattering Changes Associated with Voltage-Sensitive Dye Signals in Embryonic Brainstem Slice Preparations: Coupling of Depolarization and Cell Shrinkage

Katsushige Sato, Yoko Momose-Sato, Kohtaro Kamino
Department of Physiology, Tokyo Medical and Dental University School of Medicine, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan

We have found new evidence for GABA-induced intrinsic optical changes associated with a voltage-sensitive dye signal in the early embryonic chick brainstem slices. The slices were prepared from 8-day old embryos, and they were stained with a voltage-sensitive dye (NK2761). Pressure ejection of GABA to one site within the preparation elicited optical changes. With 580 nm incident light, two components were identified in the GABA-induced optical change. The first component was wavelength dependent, while the second, slower change was independent of wavelength. Comparison with the known action spectrum of the dye indicates that the first component reflects a depolarization of the membrane, and that the second, slow component is a light-scattering change resulting from cell shrinkage coupled with the depolarization. Similar optical changes were also induced by glycine, although the amplitude of both the first and second signals was much smaller than for GABA. The optical changes induced by GABA persisted in the presence of picrotoxin and 2-hydroxysaclofen, suggesting that these optical responses include a novel GABA response, which has been termed $GABA_D$ in our previous reports.

39. Optical Mapping of Neural Network Activity in the Chick Spinal Cord Slice Preparations at an Intermediate Stage of Embryonic Development

Yoshiyasu Arai, Yoko Momose-Sato, Katsushige Sato, Hiraku Mochida, Kohtaro Kamino

Department of Physiology, Tokyo Medical and Dental University School of Medicine, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan

We have applied multiple-site optical recording of transmembrane potential changes to recording of neuronal pathway/network activity from embryonic chick spinal cord slice preparations. Spinal cord preparations were dissected from eight-day old chick embryos at Hamburger-Hamilton stage 33, and transverse slice preparations were prepared with the 13th cervical spinal nerve or with the 2nd or 5th lumbosacral spinal nerve intact. The slice preparations were stained with a voltage-sensitive merocyanine-rhodanine dye (NK2761). Transmembrane voltage-related optical (dye-absorbance) changes evoked by spinal nerve stimulation with positive square current pulses using a suction electrode were recorded simultaneously from 128/1020 loci in the preparation. Optical responses were detected from dorsal and ventral regions corresponding to the posterior (dorsal) and anterior (ventral) gray horns. The optical signals were composed of two components, fast spike-like and slow signals. In the dorsal region, the fast spike-like signal was identified as the presynaptic action potential in the sensory nerve, and the slow signal as the postsynaptic potential. In the ventral region, the fast spike-like signal reflects the antidromic action potential in motoneurons, and the slow signal is related to the postsynaptic potential evoked in the motoneuron. In preparations in which the ventral root was cut micro-surgically, the antidromic action potential-related optical signals were eliminated. The areas of maximal amplitude of the evoked signals in the dorsal and ventral regions were located near the dorsal root entry zone and the ventral root outlet zone, respectively. Quasi-concentric contour-line maps were obtained in the dorsal and ventral regions, suggesting the functional arrangement of the dorsal and ventral synaptic connections. Synaptic fatigue induced by repetitive stimuli in the ventral synapses was more rapid than in the dorsal synapses. In the ventral root-cut preparations, comparing the delay times between the ventral slow optical signals, we have been able to demonstrate that neural network-related synaptic connections are functionally generated in the embryonic spinal cord at Hamburger-Hamilton stage 33.

40. Transmission Electron Microscopic Study of the Perivascular Glial Structure in Cerebral White Matter and Grey Matter

Naoyuki Ono[1], Keiko Obara[1,2], Akihiro Kitabatake[1], Satoshi Kawamura[1], Mitsuru Suzuki[1]
[1]Department of Neuropsychiatry, [2]Second Department of Pathology, Iwate Medical University, 19-1 Uchimaru, Morioka 020-8505, Japan

We examined the perivascular glial cells of the hippocampal fimbria in adult rats by a transmission electron microscopy (TEM) and compared the results with observations of the perivascular glial cells of cerebral grey matter (rat temporal lobe cortex). Adult rats were perfusion-fixed under deep anesthesia, and horizontal ultra-thin sections of the fimbria and the cortex were observed by TEM. In the perivascular area of the fimbria, mono- or multi-layered cell rows composed of astrocytes, oligodendrocytes and microglia were found frequently with a low appearance of pericytes (2.3 %) compared to that of the temporal lobe cortex (5.5 %). Despite confirmation of the presence of three types of glial cells in the perivascular area of the cortex, these cells failed to form cell rows characteristic of the white matter and were observed discretely. Comparison of the vascular transverse sections by TEM between the fimbria and the cortex revealed the presence of astrocyte processes extensively covering the entire periphery of the basement membrane of the vascular outer wall in the former, and poor continuity of the neighbouring astrocyte process with the basement membrane of the vascular outer wall in the latter. The presence of astrocyte processes appearing to encapsulate the periphery of the vessel in the fimbria was confirmed by light microscopy and immune transmission electron microscopy. As such, there was a clear difference between the two sites in the perivascular cellular structure, particularly in the manner of contact with the astrocyte processes. The above findings demonstrate structural differences in glial cell arrangement in the perivascular tissue between the white and grey matter, suggesting that the presence of functional differences in the glial cell at different sites should be noted when interpreting the pathogenesis of white matter disease and the process of regeneration after cerebrovascular damage.

41. Cholinergic Denervation of the Brain Inhibits the Expression of Long-term Potentiation in Adult Rats but not in Young Rats

Tomoyuki Nishizaki[1], Tamotsu Nomura[1], Takeshi Kondoh[2], Norihiko Tamaki[2]
[1]Department of Physiology, [2]Department of Neurosurgery, Kobe University School of Medicine, Kusunoki-cho 7-5-1, Chuou-ku, Kobe 650-0017, Japan

Cholinergic systems have been found to be selectively denervated by 192 IgG-saporin, and in this study 192 IgG-saporin was injected into the lateral ventricle of the brain in two groups: a 2.0 mg dose in infant rats (2 weeks old, Group A) and a 4.2 mg dose in adult rats (8 weeks old, Group B). An immunohistochemical study demonstrated little ChAT activity in either the hippocampus or the septal nucleus of the brain in either group two weeks (acute) or 4 months (chronic) after injection. Perforant path long-term potentiation (LTP) was not induced in hippocampal slices in the acute stage, in Group B, but the LTP expression success rate was unaffected in brain slices from Group A. A similar effect was obtained in the chronic stage. The results of this study suggest that the cholinergic systems in the adult brain play a significant role in the expression of LTP. They also

suggest that the young brain has the ability to compensate for the damaged neuronal circuit.

42. Differential Expression of c-Fos and Zif268 in the Nigrostriatal System after Methamphetamine Administration in a Rat Model of Parkinson's Disease

Yasushi Ishida, Kazunari Todaka, Itsumi Kuwahara
Department of Psychiatry, Miyazaki Medical College, 5200 Kihara, Kiyotake-cho, Miyazaki-gun, Miyazaki 889-1692, Japan

Basal expression of the protein products of the inducible immediate early genes (IEGs) c-Fos and Zif268 was immunohistochemically investigated in four regions of the basal ganglia in rat brain. High basal levels of Zif268 and low levels of c-Fos were seen in the caudate-putamen (CPu). No basal levels of Zif268 were seen in three other regions: the globus pallidus (GP), entopeduncular nucleus, and substantia nigra pars reticulata (SNr). Low levels of c-Fos were seen in these three regions, the same as in the CPu. In a subsequent experiment, we examined methamphetamine-induced IEG protein expression in the basal ganglia of three groups of rats: normal control rats, rats with a unilateral 6-hydroxydopamine (6-OHDA) lesion in the nigrostriatal pathway, 6-OHDA rats, and rats with a unilateral 6-OHDA lesion followed by intrastriatal grafting with fetal ventral mesencephalon. In the 6-OHDA rats, methamphetamine (3 mg/kg, i.p.) induced c-Fos-like immunoreactivity (FLI) not only in the striatum on the intact side but in the substantia nigra pars reticulata (SNr) on the lesioned side. However, the hyperexpression of FLI in the SNr was not suppressed by intrastriatal grafts that completely suppressed the methamphetamine-induced rotation. These results indicate that opposite hemispheric asymmetries in FLI are induced by methamphetamine in the striatum and the SNr in the 6-OHDA rats. The decline in density of pallidal FLI ipsilateral to the lesion was restored by the intrastriatal grafts. The pattern of striatal expression of Zif268 following methamphetamine administration qualitatively reproduced that of c-Fos, while expression of Zif268 in other regions (GP, SNr) was unaffected by methamphetamine. Differential expression of these two IEGs may suggest gene-specific and region-specific functions of c-Fos and Zif268 in the basal ganglia.

43. Abnormal Formation of the Neural Circuit in the Forebrain of Nkx2.1 Knockout Mice

Kyoji Ohyama[1], Shioko Kimura[2], Masateru Katayama[1], and Koki Kawamura[1]
[1]Department of Anatomy, Keio University, School of Medicine, Japan, [2]Laboratory of Metabolism, National Cancer Institute, NIH, USA

Nkx2.1 is expressed in the ganglionic eminence (GE) and in hypothalamic neurons during the embryonic period in mice, and we performed immunohistochemical studies in *Nkx2.1* knockout mice to elucidate the functions of *Nkx2.1*. On embryo day 18 (E18), the number of neurons immunoreactive for calbindin D 28K, a calcium-binding protein, was greatly decreased in the amygdala and the piriform cortex. Bromodeoxyuridine labeling indicated that migration of early, but not later, generated neurons was impaired in the *Nkx2.1* knockout mice. Furthermore, the posterior branch of the anterior commissure, which connects the piriform cortex on both sides, did not cross the midline in the mutants. Instead it abnormally proceeded toward the diencephalic floor of the mutant, and unidentified nerve fibers were observed in the hypothalamus. Taken together, the results of this study suggest that the *Nkx2.1* gene is involved in the migration and differentiation of immature neurons in mice, and in the development of axon tracts, including the anterior commissure connecting the two sides of the paleocortex.

44. Adenovirus-Mediated Gene Transfer of Bcl-xl Rescues Cell Death in Primary Neuronal Culture

Norihiro Matsuoka[1], Hiroyuki Yukawa[1], Kazuhiro Ishii[2], Hirofumi Hamada[3], Nobuo Hashimoto[1], Shin-Ichi Miyatake[1]
[1]Department of Neurosurgery and Clinical Neuroscience, Faculty of Medicine, [2]Institute for Virus Research, Kyoto University, 54, Kawahara-cho, Shogoin, Sakyou-ku, Kyoto 606-8507, Japan, [3]Department of Molecular Biotherapy Research Chemotherapy Center, Cancer Institute, Tokyo, Japan

Apoptosis is the most common form of physiological cell death and plays an important role, especially in neuronal cell death during development. Bcl-xl, is a member of the Bcl-family that regulates and inhibits apoptosis, whereas other members, such as Bax, Bcl-xs, Bad, and Bak, do not. Bcl-2 is expressed in neuronal tissue during embryonic development but is down-regulated in the adult CNS. By contrast, Bcl-xl expression is retained in neurons of the adult CNS.

The effect of gene transfer of Bcl-xl in primary neuronal cells has never been elucidated, because these cells are too fragile for gene transfer techniques. Recombinant adenovirus vectors are known to be able to transfer genes of interest into a wide variety of cells, including neuronal cells, with high efficiency both *in vitro* and *in vivo*. In view of the duration of exposure to polypeptides, gene transfer and continuous expression may offer some advantage over direct injection of polypeptides. To investigate the possibility of gene therapy for the treatment of cerebral ischemic disease, we examined the effect of a replication-deficient recombinant adenovirus vector expressing human Bcl-xl on augmentation of the survival of primarily neuronal cells *in vitro*. Immunohistochemistry showed that the primary neuronal cells were successfully infected and that the Bcl-xl gene was transferred by this recombinant adenovirus with high efficiency. Bcl-xl gene transfer to the primarily cultured neurons prevented them from cell death cimpared to uninfected and control virus-infected neurons.

These findings suggest that the use of Bcl-xl-expressing recombinant adenovirus is a promising tool for *in vivo* gene therapy for ischemic cerebral disease or degenerative neuronal disease.

45. Effective Gene Transfection into the Entire Central Nervous System (Cns) of Non-human Primates

Yasushi Hagihara[1], Youichi Saitoh[1], Yasufumi Kaneda[2], Eiji Kohmura[1], Masaaki Taniguchi[1], Toshiki Yoshimine[1]
Departments of [1]Neurosurgery and [2]Gene Therapy Science, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka, Japan

Almost all of the clinical trials of gene therapy have been unsuccessful, because many gene delivery systems provide only limited transgene expression in humans, unlike in rodents. The difference between human and rodents must be responsible for this, and thus more practical conditions are required for evaluation of gene therapy strategy. We attempted *in vivo* gene transfection of the entire central nervous system (CNS) of non-human primates by using the hemagglutinating virus of Japan (HVJ)-AVE liposomes, a newly constructed anionic type liposome whose lipid composition is similar to that of HIV envelopes and coated by the fusogenic envelope proteins of inactivated HVJ. HVJ-AVE liposomes containing the lacZ gene (100 μg of lacZ gene diluted in 1 ml of balanced salt solution; BSS) was intrathecally injected through the cisterna magna of 3 Japanese macaques, and broad transgene expression was observed mainly in the neurons. LacZ gene expression was high in the hippocampus, brainstem, cerebellar vermis, and upper cervical cord (37.2 ± 1.9 %, 45.6 ± 18.7 %, 47.9 ± 17.8 %, and 42.3 ± 5.8 %, respectively), but significantly lower in the frontal, temporal, and occipital cortex (0.7 ± 1.6 %, 0 %, and 0 % respectively). "Empty" HVJ-AVE liposomes were also injected by the same procedure in one Japanese macaque, but no β-Gal-labeled cells were detected. An HVJ-AVE liposome-lacZ complex (100 μg of the lacZ gene diluted in 100 μl of BSS) was intrastriatally injected into 2 Japanese macaques, and dense focal transfection was observed around the injection sites. The transfection rate was 58.5 ± 7.1 % at the injection site, and it gradually declined distance. β-Gal-labeled cells were detected no more than 15 mm from the injection sites. We conclude that gene transfection of the CNS using HVJ-AVE liposomes will become a useful strategy for gene therapy clinically.

46. Protective and Regenerative Effects of Intracerebral Grafting of Encapsulated GDNF-Producing Cells on Dopaminergic Neurons: Long-Term Evaluation Using an Animal Model of Parkinson's Disease

Isao Date, Tetsuro Shingo, Hideyuki Yoshida, Kenjiro Fujiwara, Kazuki Kobayashi, Takashi Ohmoto
Department of Neurological Surgery, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama 700-8558, Japan

Intracerebral grafting of encapsulated neurotrophic-factor-producing cells is a useful method of delivering neurotrophic factors into the brain. We established a cell line producing glial cell line-derived neurotrophic factor (GDNF) by gene transfer. The cells were encapsulated into a hollow fiber composed of a semipermeable membrane and grafted into the right striatum of rats. The host rats were intrastriatally injected with 6-hydroxydopamine on the right before or after grafting, and the long-term protective or regenerative effects of GDNF capsules on host dopaminergic neurons were evaluated. Neurochemical analysis revealed that the grafted capsules continued to secrete GDNF for 6 months, and histological analysis showed excellent survival of encapsulated GDNF-producing cells. Dopaminergic neurons in the substantia nigra and dopaminergic fibers in the striatum survived well, and apomorphine-induced rotational behavior of the host animals improved significantly. We conclude that encapsulated GDNF-producing-cell grafting has a long-term protective and regenerative effect on dopaminergic neurons in the animal models of Parkinson's disease.

47. Reconstruction in the Cerebral Infarcted Area and Functional Recovery Induced by Intracranial Transplantation of Central Nervous System Stem Cells with Mesenchymal Tissues in Ischemic Rats

Atsushi Fukunaga, Koichi Uchida, Takurou Hayashi, Motoyuki Yamada, Yoshiaki Kuroshima, Takeshi Kawase
Department of Neurosurgery, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

Intracranial transplantation of central nervous system stem cells with mesenchymal tissue into ischemic rats has been performed to evaluate how the grafted tissues can survive and the cognitive and motor function in rats can recover.

In our study, cerebral infarction was created in adult male Wistar rats by a modified Koizumi-Longa method. Reperfusion was performed an hour after middle cerebral artery occlusion. The rat mesencephalic neural plate at the early somite stage (embryo day 10.5) together with adjacent ventral mesenchymal tissues was microscopically dissected out and immediately implanted into the ischemic rat striatum. After implantation, the motor function was evaluated by the teadmill task and tail tests in the subsequent 5 weeks. One month after implantation, cognifive function was evaluated by the Morris water-maze method. Histological and immunohistochemical examination of the graft was performed with hematoxylin-eosin (HE), cresyl violet (CV), and dopamine- and adenosine 3',5'-monophosphate-regulated phosphoprotein 32 (DARPP-32) one and two months after implantation.

A huge non-tumoral mass was observed in the rat striatum on HE -stained amd CV-stained following intracranial transplantation sectims. DARPP-32-positive neuron-like cells were found in the graft suggesting that some dopaminergic nervous input into the grafted neuron-like cells. The water-maze study, the teadmill task , and tail tests showed recovery of cognitive and motor function in the grafted to the control levels. The difference in recovery of cognitive and motor function between the implanted group and the sham group was statistically significant. In conclusion, the results of this study suggested that CNS stem cells grafted with mesenchyme may survive and differentiate into mature CNS tissues within the adult ischemic rat brain, and therefore have the potential to be effective in restoring the cognitive and motor function of the rat model.

48. Transplantation of Neural Stem Cells into the Retina Injured by Transient Ischemia

Yasuo Kurimoto[1], Hiroto Shibuki[1], Yumi Kaneko[1], Masaki Ichikawa[1], Toru Kurokawa[1], Nagahisa Yoshimura[1], Masayo Takahashi[2]
[1]Department of Ophthalmology, Shinshu University School of Medicine, Matsumoto 390-8621, Japan, [2]Department of Ophthalmology and Visual Science, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan

Central nervous system stem cells are multipotent cells with the ability to 1) self-renew, 2) migrate, and 3) differentiate into neurons, astrocytes, and oligodendrocytes. They can be engrafted as integral members of the host cytoarchitecture throughout the central nervous system. Therefore, their use as graft material is promises to provide new strategies for the treatment of neurodegenerative diseases and neuro-injuries. When stem cells were transplanted into the eyes of infant rats, they were integrated into the host retina. However, no stem cells were integrated into the host retina when they were transplanted into the intact eyes of adult rats (Takahashi et al., *Mol. Cell Neuroscience* **12** (1998) 340). In the present study, we examined whether the grafted stem cells can be integrated into host retina injured by transient ischemia. Transient retinal ischemia was induced in adult rats by raising the intraocular pressure to 110 mmHg for 60 minutes in the ischemic group. Immediately after reperfusion, β-galactosidase-labeled stem cells derived from the hippocampus of adult rats (Palmer et al., *Mol Cell Neuroscience* **8** (1997) 389) were injected into the vitreous cavity of the treated eyes. The rats were sacrificed 1, 2, 3, or 8 weeks after treatment, and their eyes were processed for histochemical studies. The stem cells were also injected the eyes of non-ischemic control rats, which were sacrificed 3 weeks later. In the ischemic group, transplanted stem cells invaded into the retinal ganglion cell layer 1 week after transplantation, and the retinal inner nuclear layer 2–3 weeks after transplantation. They had become integrated into the various layers of the retina and showed neural-network-like morphology 8 weeks after transplantation. By contrast, no stem cells invaded the host retina in the control group. In conclusion, intravitreally injected neural stem cells can be integrated into host retina that has been subjected to ischemia-reperfusion injury. Such neuronal stem cells may be good candidates for reconstruction of the neural network of ischemia-injured retina.

49. Locomotor Performance Acquired in the Rat after Spinal Cord Replacement: Quantitative Assessment and Electromyographic Study

Takao Hase, Takeshi Nishio, Saburo Kawaguchi
Department of Integrative Brain Science, Kyoto University Graduate School of Medicine, Kyoto 606-8501, Japan

We previously reported quantitative measurement of neural connections across the graft (Asada et al., 1998) in an animal model of spinal cord repair in the neonatal period (Iwashita et al., 1994). The present study aimed at a quantitative assessment of locomotor function in the same experimental model. Spinal cord segments T10~12 were removed from neonatal rats, and embryonic spinal cord segments (ESC) or a peripheral nerve segment (PN) were grafted in the in place, or no grafting was performed. Locomotor performance was assessed by using the open field locomotor scale (Basso et. al., 1995) from day 0 to week 5, and comparisons were made with normal development. The scale ranged from a score 0 (complete paraplegia) to 21 (normal locomotion). At 4–5 weeks of age electromyography of all four limbs was performed simultaneously during locomotion to examine hind-forelimb coordination in greater detail. By three weeks after birth the normal rats acquired a mature locomotor pattern with a score of 21. Half of the animals grafted with ESC had a score of 18~20 and showed a locomotor pattern similar to that of the normal animals. A slight abnormality was seen in ankle joint movement in the open field study and the occasional occurrence of an odd step on electromyographic analysis, i.e., a sequence in which only the forelimbs were activated and the hindlimbs were silent. Locomotor performance in the remaining half of the animals grafted with ESC showed various grades of hind-forelimb coordination and scored 11~13. Increased occurrence of odd steps indicated poor hind-forelimb coordination. The rats grafted with PN showed weight-supported steps but lacked hind-forelimb coordination. The animals that were left ungrafted showed some hindlimb movement with maximal scores of 8 but never attained weight-supported steps. Electromyograms of the hindlimbs were almost silent. The grade of locomotor performance appears to depend on the quantity and quality of neural connections reestablished across the graft.