that both are able to exert their CN inhibitory action. However, their different cellular localization in the neuron suggests that each isoform plays distinct roles in neural functions.

P3-36

Immunocytochemical study of apelin in the rat brain

[1]Norio Iijima, [1]Masaki Tanaka, [2]Yasuhiko Ibata

[1]Dept. Anatomy and Neurobiology, Kyoto Prefectural University of Medicine, [2]Kyoto Prefectural University of Medicine

The apelin is an endogenous ligand for the seven-transmembran receptor, APJ, which was originally isolated from human genomic DNA by PCR and dominantly distributed in the lung. It was also reported that apelin and APJ were expressed in the rat brain. In this study, we investigated the areas in the rat central nervous system throughout the brain and spinal cord, in which apelin could play some functional roles. Apelin-like immunoreactivities (apelin–LI) @were detected in the neuronal perikarya distributed in the zona incerta in the thalamus. Nerve processes with apelin–LI were detected within the hypothalamus, for example in the paraventricular nucleus, the medial preoptic area, and the supraoptic nucleus. The inner layer of median eminence contained many fibers with apelin–LI. These observations suggested that apelin might affect the neuroendcrine systems via the hypothalamus or be transported directly to the posterior pituitary. The fibers with apelin–LI were also detected around the catecholaminergic neurons within the nucleus of solitary tract and the locus ceruleus. In addition to these areas, apelin–LI were located in the septal nucleus in the diencephalon, hippocampus, and cerebral cortex. From these localization of apelin–LI, it was speculated that apelin could play various functional roles in the rat brain in addition to neuroendocrine function.

P3-37

GABA and glycine receptor immunoreactivity in rat trigeminal ganglion

Hana Hayasaki, Kentaro Maemura and Masahito Watanabe

Department of Anatomy, Osaka Medical College

We examined mRNA expression for gamma-aminobutyrate A receptor ($GABA_A$) subunits ($f¿1$–6, $fÀ1$–3, $fÁ3$, $fÂ$) and glycine (GLY) receptor subunit ($f¿1/2$) in rat trigeminal ganglion (TG) by reverse transcription polymerase chain reaction (RT-PCR). In addition, we demonstrated that the localization of these subunit proteins by immunohistochemistry. Methods: Ten week-old male Wistar rats were used in the following experiments. For RT-PCR, cDNA was synthesized from total RNA extracted from TG, whole brain and spinal cord. For immunohistochemical study, cryosections of TG were prepared and the expression of each receptor subunit protein was examined by indirect immunofluorescent technique, and the observation was carried out with confocal laser scanning microscope. Results: All mRNAs for GABA and glycine receptor subunits studied were expressed in rat TG. Neuronal cell bodies were immunopositive for $GABA_A$ receptor $f¿1$, 2, 3, 5, 6, $fÀ1$–3, $fÁ3$, and $fÂ$ subunits. The immunoreactivity for $GABA_A$ receptor $f¿1$, 3, $fÀ1$–3, $fÁ3$, and $fÂ$ subunits was dependent on the cell size. Nerve fibers were only positive for $GABA_A$ receptor $f¿3$ subunit. The nerve cells did not show GLY immunoreactivity. Satellite cells were immunopositive for $GABA_A$ receptor $f¿2$, 3, 5, 6, and $fÂ$ subunits. In addition, satellite cells showed strongly positive immunoreactivity for GLY receptor $f¿1/2$ subunit. Conclusion: These results indicate that different combinations of subunits may confer particular pharmacological and physiological properties on TG. Furthermore, co-expression of GLY receptors with GABA receptors in satellite cells strongly suggests that they have certain physiological function in the sensory ganglion.

P3-38

Immunoelectron microscopic analyses of the neuronal elements in the suprachiasmatic nucleus of the Japanese quail

K. Hirunagi, E. Seto*, K. Fukuta*, N. Kansaku**

The Nagoya University Museum, *Graduate School of Bioagricultural Sciences, Nagoya University, **Laboratory of Animal Genetics and Breeding, Azabu University

The identification of the suprachiasmatic nucleus (SCN) in birds has been marked by intense controversy. Some studies suggest that the SCN is located in similar place in birds and mammals (mSCN), while others argue that the avian SCN is located in the lateral hypothalamus (vSCN). Yoshimura et al. (2001) have demonstrated that clock genes are expressed in the mSCN but not in the vSCN in Japanese quail. To confirm neuroanatomical characteristics of the mSCN, we examined immunoelectron microscopic analyses of neuronal elements in the mSCN of the Japanese quail using antibodies of vasoactive intestinal polypeptide (VIP), VIP receptor (VIPR) and an opsin. Adult Japanese quails were used. Free-floating Vibratome sections were processed for immunostaining according to a LSAB method (DAKO). After immunostaining, they were flat embedded in Araldite on glass slides. A small region containing mSCN was cut out of the total section under a dissection microscope and re-embedded for ultrathin sectioning. Medial SCN of the Japanese quail consists of small oval neurons ($10\times6 f\hat{E}m$). Immunoreactive (ir) neuronal elements are mainly found in the neuropil of the mSCN. About 60–80% of these elements are distributed within $3 f\hat{E}m$ from the cell membrane of soma. Although VIP ir cell body is considered as an immunohistochemical maker of mammalian SCN, we find no VIP ir cell body in this study. VIP ir elements are classified in two groups. About 40% of VIP ir neuronal elements are a synapse type. Another is a varicose type, which shows no synaptic specialization in the section. VIPR immunoreactivity is found as a fuzzy material on the membrane of the process, which seems to correspond to a synaptic junction. Opsin ir elements contain immunoreactive small granules. This neuronal element is probably a varicose of neuronal fiber. In several avian species, VIP and opsin co-exist in the deep brain photoreceptor. The presence of opsin ir terminals suggests that opsin ir deep brain photoreceptors of the lateral septum innervate the mSCN of the Japanese quail.

P3-39

Myosin V and II isoforms in presynaptic terminals of superior cervical ganglion neurons in culture

Yoshiko Takagishi, Sumiko Mochida*, Noriko Murakami**, Sugiko Futaki***, Kanako Itoh*, Yoshiharu Murata

RIEM, Nagoya University, Nagoya, Japan; *Tokyo Medical University, Tokyo, Japan; **New York State Institute for BRDD, New York, USA; ***Sekiguchi Biomatrix Signaling Project, ERATO, Aichi, Japan

Among the extended superfamily of myosin motor proteins, myosin V and non-muscle myosin II are abundant in neurons. To explore the potential presynaptic functions of these myosins, we used immunoconfocal microscopy to study the distributions of myosin V (a & b) and II (A & B) isoforms in cultured superior cervical ganglion neurons (SCGNs) forming axo-somatic synapses. Further, we expressed green fluorescent protein (GFP) fusion proteins in SCGNs to determine whether myosin Va and IIB are targeted to the presynaptic terminal. [Methods] SCGNs were taken from Wistar rats on postnatal day 7. The cells were cultured for 5–6 weeks and then were stained with isoform-specific antibodies for myosin Va, Vb, IIA and IIB. An anti-Bassoon antibody was also used to identify presynaptic terminals. GFP-myosin Va and IIB constructs were injected into the nucleus of SCGNs. The immunofluorescence cells and GFP-expressing constructs were imaged by confocal microscopy. [Results] Myosin Va was distributed throughout the soma and both axonal and dendritic processes of SCGNs. However, there was no spatial overlap between myosin Va and Bassoon at the presynaptic terminals surrounding