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Changes of the suprachiasmatic nuclei (SCN) of rats by light after being raised under continuous darkness

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To identify the cells involved in the entrainment in rat suprachiasmatic nucleus (SCN), the distributions of AVP, VIP and c-fos were studied using immunofluorescence method. Male rats (Wistar) were raised under continuous darkness from their fetal period. At eight postnatal weeks, rats were lighted at 11:00 am for 30 minutes, or received light at 10:00 pm during the same period before sacrificed (group DL). Some rats were killed in the darkness without receiving light (group D). Rats raised under the conventional condition (14L-10D) and killed at corresponding times were used as controls. Then we made frozen sections after fixing the brains in 4% paraformaldehyde/PBS for 1day. The results were as follows; (1) As for the distribution of AVP, in the groups D and DL the sections were stained less intensely than the control rats. In D and DL, the sections of the rat SCNs obtained in the morning gave more intense signal than those in the night with the same results in controls. (2) As for that of VIP, the sections of the groups D and DL were stained less intensely than those of controls. But in the controls, the sections lighted in the morning gave a more intense signal than those in the night, while there was no difference in the intensity between the samples of the morning and the night in both of D and DL. (3) Regarding with the c-fos, either the sections from group D or controls did not show any signal. But in the group DL, both sections lighted in the morning and night gave positive signal in SCN and the adjacent surrounding region. (4) The double labeling revealed that most of fos-positive cells in SCN were VIP-neurons, and a small number of cells located in the peripheral region of SCN were positive both for fos and for tyrosine hydroxylase which is a marker for catecholamine neurons.

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The development early postnatally of the rat Pre-Pro-Somatostatin mRNA neurons in cerebral cortex

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The development early postnally of the Pre-Pro-Somatostatin mRNA neurons in the rat cerebral cortex was studied using digoxigenin Alkaline phosphase labelled antisense-RNA (Dig-Ap cRNA) probe. The following results were revealed: 1. SS mRNA neurons are observed in the piriform cortex cingalate cortex and neocortex. 2. SS mRNA neurons are preferentially localized in the deep layer of piriform cortex, the number and size of positive cells increase from P1 to P5, the positive cells are distributed in all layers of the cingulate cortex except the layer 1. 4. Neocortical SS mRNA neurons have a specific liminar distrbution, during in infragranular layer, besides the infragranular. These results indicated SS14, SS28(1 – 12) derived from the same Pre-Pro-Somatostatin may be involved the modulation of the rat brain development early neonatal period.

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Immunohistochemical Localization of Secretin, Cholecystokinin (CCK) and Somatostatin in The Rat Small Intestines after Acute CDDP Treatment

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CDDP, a platinum complex, is a antitumor drug and is well-studied renal tubular failure. However, little information about gut hormones such as secretin, CCK and somatostatin on small intestines is obtained. In the present study, acute effects of CDDP treatment on localization of gut hormones in the rat small intestines were examined by immunohistochemistry.

Forty male Sprague-Dawley rats were used for this experiment. Rats were injected intravenously with CDDP (3 mg/kg) in saline or were non-treated (control). After the rat euthanized at 1, 3, 5 and 10 days after CDDP treatment, the small intestines (duodenum, jejunum, ileum) were quickly removed and cut longitudinally. Three pieces of each organ were fixed at 4 °C in 10% formalin in 0.1M phosphate buffer (pH 7.2), embedded in paraffin, and cut at 5 μm. The Immunohistochemical detection performed using a 1:2000 dilution of rabbit anti-pig secretin, CCK and somatostatin, and Avidin-Biotin-Immuno-Peroxidase procedure, using biotinylated goat anti-rabbit IgG serum and avidin- biotin-peroxidase complexes. Control sections were processed in parallel and consisted of omission of primary antiserum with nonimmune animal (goat, rabbit and sheep) serum IgG. The total number of positive cells per a complete cross section was counted. Moreover, each positive cell was classified as being either crypt or villus associated. These results were statistically analyzed by 3-way ANOVA or Student's t-test.

In the duodenum, the numbers of secretin-positive cells and somatostatin-positive cells dramatically increased at 5 days after CDDP treatment. In the jejunum, the number of CCK-positive cells was increased at a day after CDDP treatment, and those of secretin-positive cells and CCK-positive cells were increased 5 days after CDDP treatment. In the ileum, the number of CCK-positive cells was increased at a day after CDDP treatment. Change in the number of secretin-positive cells may be caused by irregularity of gastric metabolism induced nephrotoxicity. Change in the numbers of CCK-positive cells may promote excretion of bile. Therefore, somatostatin may regulate secretin and CCK secretion. These results suggest that distribution of gut hormone-positive cells might be controlled under renal metabolism and excretion of bile in the CDDP-treated rat small intestines.

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Immunohistochemical Detection of Rab 3 Protein in the Pituitary Glands of the Rat Species and the GH-RH Transgenic Rat

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Rab proteins are low molecular weight GTP-binding proteins. Among these proteins, the Rab3 family is considered to be involved in the exocytosis of secretory granules in the cell of the central nervous system and anterior pituitary gland. In the present study, we investigated the localization of Rab3 protein in the pituitary glands of normal rats and the hyperplastic pituitary glands of GH-RH transgenic (Tg) rats, using the immunohistochemical methods.

Rab3 protein was expressed in the anterior, intermediate, posterior lobes of the normal rat pituitaries. Double staining for anterior pituitary hormones revealed expression of Rab3 in GH cells, but rare or occasional expression was observed in the other anterior pituitary hormone producing cells. Rab3 was strongly positive in the pituitaries of GH-RH Tg rats, which showed GH cell hyperplasia.

Rab3 was widely expressed in the anterior pituitary cells of the normal rats. In addition, Rab3 and GH were co-expressed in the same cells, suggesting that Rab3 may be involved in regulating exocytosis of secretory granules containing anterior pituitary hormones, especially GH.