An N-Acetyl-Aspartyl-Glutamate (NAAG)-like Peptide in the Rat Hypothalamic Magnocellular (MC) Nuclei.

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Among several acidic oligopeptides known to be present in the brain, NAAG received greatest attention as a potential excitatory neurotransmitter. Several laboratories produced antibodies against NAAG conjugated to carrier protein at its carboxyl terminus with carbodiimide. In contrast to these earlier studies, we have attempted to raise C-terminally directed antibodies that would recognize NAAG without the risk of reacting with other N-acetylated peptides known to be present in brain. For this purpose, we immunized rabbits with the synthetic model peptide Gly-Asp-Glu (GAG) coupled to hemocyanin with glutaraldehyde. By enzyme immunoassay, GAG, NAAG, and the tetrapeptide Val-GAG reacted equally well with the resulting antiserum (EC50 values in the nM range); Asp-Glu and Gly-Glu were a thousand-fold less reactive and Glu showed practically no cross-reactivity. By immunocytochemistry, the GAG antiserum stained neuronal structures known to contain NAAG (e.g., in the olfactory bulb and spinal cord); in addition, it stained a population of MC neurons and their processes in the hypothalamic paraventricular and supraoptic nuclei and the posterior pituitary. Staining was effectively blocked by NAAG and GAG but was unaffected by oxytocin and vasopressin. These results suggest that hypothalamic MC neurons contain NAAG or a similar oligopeptide with a C-terminal -Asp-Glu sequence. Such a peptide may be secreted in the posterior pituitary, or may act as an excitatory neurotransmitter within the MC nuclei (through local synaptic contacts) or at distant brain regions that receive projections from the hypothalamic MC system.

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Electron microscopic cytochemistry of sulfated and neutral carbohydrates in the endothelial cells lining the capillaries of the rat salivary glands.

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In an attempt to study the cytophysiological activities of endothelial cells lining the capillaries of the rat salivary glands, the cellular sulfated and neutral carbohydrates were visualized by means of electron microscopic cytochemical methods. Parotid, submandibular and sublingual glands from adult male rats of Fischer strain were perfusion-fixed with 0.1M cacodylate buffered 1% glutaraldehyde(GA)-2% paraformaldehyde(PFA)(pH.7.4). Then, the glandular tissue pieces were dissected out from the donor animals, immersion-fixed in similarly buffered 2.5%GA-2%PFA for 2 hours at room temperature. Some of these fixed tissue pieces were sectioned with a microslicer (D.S.K) and reacted for high iron diamine(HID) reagent under standard staining conditions, whereas others were not subjected to such a staining. All the tissue pieces were then ethanol-dehydrated and embedded in Quetol 812, according to routine techniques. Ultrathin sections were prepared from the resin embedded tissue blocks and mounted on nylon grids. Sections from the HID reacted tissues were stained with thiocarbohydrazide-silver proteinate-physical development(TCH-SP-PD), whereas those from the intact tissues were reacted with periodic acid(PA)-TCH-SP-PD.

In the endothelial cells lining the capillaries of the three salivary glands, different ultrastructures exhibited positive HID-TCH-SP-PD and PA-TCH-SP-PD reactions; these ultrastructures were the surface coat of the plasma membrane, pinocytotic vesicles, lysosomes, membranes of Golgi complexes etc. Of particular note was the finding that the positive HID reactions of the surface coat of the luminal plasma membrane were continuous in the parotid gland, whereas those in the other two glands tended to be more or less discontinuous or stippled. Such variations in the reactions of the surface coat could represent certain aspects of varying cytophysiological activities of endothelial cells with different salivary glands.

An Efficient Enzyme Immunoassay (EIA) for the Characterization of Antisera to Glutamate (Glu) and Aspartate (Asp) Based on Covalent Coupling of the Amino Acids to Polystyrene Microplates. L.H. Abdullah*, P. Ordronneau and P. Petrusz. Univ. of North Carolina, Chapel Hill, NC.

Antisera raised in rabbits to Glu and Asp conjugated to hemocyanin with glutaraldehyde (GA) were first characterized by immunocytochemistry and immunoblot analysis (Hepler et al., J. Histochem. Cytochem., 36:13-22). The antisera were specific for their respective haptens and recognized, in addition to free Glu and Asp, the non-endogenous excitatory amino acid (EAA) receptor ligands quisqualate and kainate (Van Eyck et al., J. Histochem. Cytochem. 37:927, 1989). In order to study the conformational requirements for ligand recognition by these and other antisera, we developed a rapid and efficient EIA method, using covalent coupling of the amino acids to microtiter plates with GA. This ensures stable attachment as well as uniform and optimal orientation of the amino acids. Microplates were first activated with GA at pH 4.5 and Glu or Asp (0.1 M) were then added at pH 8.0. The plates were treated with 0.1 M ethanolamine to block unreacted aldehyde groups. Non-specific binding was blocked by 3% normal goat serum containining 0.05% Tween-20. The plates were then incubated with optimal dilutions of anti-Glu or anti-Asp sera (for four antisera, these ranged from 1:20,000 to 1:400,000) combined with increasing concentrations of the ligands to be tested. The bound antibodies were detected by peroxidase-conjugated goat anti-rabbit IgG and subsequent incubation with tetramethylbenzidine substrate. This assay proved very useful for rapid initial screening of antisera, and for efficient testing of antiserum cross-reactivities with a large number of relevant amino acids, dipeptides, and EAA receptor agonists and antagonists.

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Production of Basement Membrane Components in vitro—— A Recombinant Experiment using Cultured Chick Embryonic Skin as revealed by Immunocytochemical Methods —— Y. Akimoto, A. Obinata*, H. Endo* and H. Hirano. Dept Anat, Kyorin Univ Sch Med, Mitaka, Tokyo 181, and *Dept Physiol Chem, Fac Pharm Sci, Teikyo Univ, Sagamiko, Kanagawa 199-01, Japan.

Production of extracellular matrix components such as laminin(LM), type IV-collagen(CL), fibronectin(FN) and tenascin(TN) during the formation of the basement membrane has been analyzed immunocytochemically using cultured epidermis-dermis recombinant skin. The epidermis and dermis of the tarsometatarsal skin from 13-day-old chick embryos were separated each other by the treatment with EDTA and Dispase. The basal lamina was removed both in the epidermis and dermis. The separated epidermis was overlayed onto the separated dermis, i.e., recombined, then cultured for 1-7 days in a chemically defined medium (BGJb) on the Millipore filter. Cryosectioned semi- and ultra-thin sections were made, stained with rhodamin- or colloidal gold-labeled antibodies against LM, CL, FN or TN, and examined by light and transmission electron microscopy.

In the normal embryonic skin at 13-20 days in ovo, all of the four components were found localizing in the basement membrane. Furthermore, FN and TN were distributed in the extracellular matrix of the dermis. In the epidermis-dermis recombinant skin, LM and FN appeared at the epidermal-dermal junction 1-2 days after recombination. Positive sites were observed as patches locating immediately beneath the epidermis. CL and TN appeared 2-3 days after recombination. The staining pattern became continuous and linear in profile 4-7 days after recombination. The present results indicate that LM and FN appear first among the four, then, CL and TN are added successively during the course of the basement membrane formation in vitro.

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