DISTRIBUTION OF COMPONENTS OF THE SNARE COMPLEX IN RELATION TO TRANSMITTER RELEASE SITES AT THE FROG NEUROMUSCULAR JUNCTION.

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Neurotransmitter release from nerve terminals involves targeting, docking and calcium-dependent fusion of synaptic vesicles at the presynaptic membrane. According to the SNARe hypothesis, the vesicle protein VAMP and the plasma membrane proteins synaptin and SNAP25 form a trimeric complex. Association of the N-ethylmaleimide sensitive fusion protein (NSF) and soluble NSF attachment proteins (SNAP) with the SNARE (SNAP receptor) complex should promote fusion. At the frog neuromuscular junction, neurotransmitter release sites are regularly spaced at 1 μm intervals along the nerve terminal, directly facing presynaptic folds which contain a high density of acetylcholine receptors. Immunostaining and laser confocal scanning microscopy were used to compare the distribution of presynaptic proteins implicated in exocytosis with that of fluorescent α-bungarotoxin. Synaptin, SNAP-25 and calcium channels were located almost exclusively at release sites. Synaptobrevin (VAMP) was distributed more widely in the cytoplasm of the nerve terminal, presumably corresponding of microvesicles associated with each active zone. N-ethylmaleimide sensitive fusion protein (NSF) and soluble NSF attachment proteins (eSNAP) displayed a diffuse distribution throughout the nerve terminal, cytoplasm and also co-localized in distinct concentrated zones adjacent to the presynaptic membrane. Thus, our observations are consistent with the hypothesis that SNARE complexes mediate vesicle docking at active zones. They also suggest a role for NSF and eSNAP in vesicle retrieval by endocytosis.

ELECTRON MICROSCOPIC LOCALIZATION OF INTERFERON-γ (IFNγ) IN MOUSE LIVER

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In addition to its cellular receptor, IFNγ displays a high affinity for heparin sulfate. intravascular injected (+)-IFNγ has been demonstrated (H. Lortat-Jacob et al.) to be concentrated specifically in the liver. The macro-autoradiography confirmed this localization. The emulsion-semin-thin sections showed signal in the Disse space and were associated with the hepatocyte membrane. This preferential tunicular localization of the IFNγ led us to localize the IFNγ in the mouse liver by electron microscopic methods. The basal level of IFNγ was determined in the normal mouse liver to visualize the physiological state, and its variations either after intraperitoneal injection of IFNγ or after endogenous stimulation of its synthesis, via the LPS stimulation. The mice were sacrificed, the liver was quickly taken and fixed, either in 2% paraformaldehyde/0.05% glutaraldehyde for the cryo-ultramicroscopy study, or in paraformaldehyde/lactate phosphate/0.05% glutaraldehyde followed by 0.1% OsO4 and embedding in Epon white. Indirect immunogold technique was used to localize IFNγ after incubation of ultrathin sections with anti-IFNγ antibodies (1:100/1:200). The sections were counterstained with uranyl acetate and lead citrate, and observed under EM120 Philips E.M. at 60 kV. The immunospecificity of the labeling was controlled using IFNγ preabsorbed antibodies.

A positive signal was observed in normal liver, in Disse space, on hepatocyte membrane, inside the hepatocyte cytoplasmin coated vesicles, mitochondria or in the nucleus. No signal was detected in the bilary canaliculi. The same IFNγ localization was observed with a greatly enhanced gold labeling after injection or neosynthesis of IFNγ. These results show the presence of physiological IFNγ in mouse liver and an increased gold signal in correlation with the presence of IFNγ after exogenous injection or endogenous synthesis.

ACTIVITY-INDUCED INTERNALIZATION AND RAPID DEGRADATION OF SODIUM CHANNELS IN CULTURED FETAL NEURONS.

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A regulatory mechanism for neuronal excitability consists in controlling sodium channel density at the plasma membrane. In cultured fetal neurons, about 20% of sodium channels are of the voltage-dependent type, and the remainder are high voltage-activated (HVA) and toxin-sensitive sodium channels. These results show the presence of physiological IFNγ in mouse liver and an increased gold signal in correlation with the presence of IFNγ after exogenous injection or endogenous synthesis.

We have investigated by Confocal Microscopy the effects of the normal and truncated receptors of the GHR on the endocytosis of the GH endocytosis and its nuclear translocation associated with the GHR. The GHR belongs to the cytokine receptor superfamily, which are characterized by i) a single membrane spanning domain, ii) about 20% homology and conserved pairs of cystein residues, iii) the absence of consensus signal for tyrosine kinase activity. Rat GH receptor cDNA was stably transfected in CHO-K1 cells. The complete rat cDNA coding for amino acids (aa) 1-638 was expressed in CHO-638. A stop codon was inserted at aa 295, which formed the extracellular membrane spanning domain without the intracytoplasmic domain (CHO294). Cells were plated in 6 well plates, containing glass coverslips. At the subconfluent state, cells were incubated in medium without serum overnight. It was then removed and replaced by fresh medium without serum containing 1 μg/ml human GH for 1, 5, 15, 30, 60 min. After stimulation, cells were fixed in 2% paraformaldehyde in 0.1 M phosphate buffer and processed for immunofluorescence. Internalized GH and GHR were visualized using either specific antibody against GH (Dako, France) or antihuman GHR antibodies (Novum, Sweden). Secondary antibodies were used in combination with rhodamin-green* anti-mouse antibodies (Novum, Sweden).

INTRACELLULAR TRAFFIC OF GROWTH HORMONE (GH) AND ITS RECEPTOR (GHR) IN CHO CELLS TRANSFECTED WITH GHR cDNA: A Confocal Microscopy Study

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