S11-37

INBRED STRAIN DERIVED MURINE EMBRYONIC STEM CELL LINES

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Most of the germ-line competent ES cell lines used so far in gene targeting experiments have been derived from blastocysts of sublines of the mouse strain 129/Sv. Recently, ES cell lines that have been derived from the C57BL/6 inbred mouse strain were successfully used to generate knock-out mice. In addition, we have isolated several male ES cell lines from each the BALB/c, DBA/2 and C3H inbred strains. One of the BALB/c ES cell lines has been used to generate IL-4 deficient BALB/c mice. Of 4 male DBA/2 ES cell lines, two were transmitted through the germ-line and one is currently being used in gene targeting experiments. The C3H ES cell lines are currently being analyzed. With the availability of these ES cell lines it is now possible to induce a mutation on the genetic inbred background of choice and to analyze the induced mutation in different genetic backgrounds without laborious breeding.

Synaptic Release

S12-01

MOLECULAR AND FUNCTIONAL DIVERSITY OF INDIVIDUAL NERVE TERMINALS OF ISOLATED CORTICAL NEURONS.

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Learning is associated with changes in the strength of connections between neurons which can occur independently in individual synapses of the same cell. A possible presynaptic component of these adaptive changes is suggested by the differential expression, in vivo, of nerve terminal proteins (NTPs) involved at various stages of membrane fusion and transmitter exocytosis. Here we show that single cortical neurons cultured in isolation are capable of differential expression of NTPs at different synapses. Using the dye FM1-43 in mixed neuronal cultures, we also show that the intensity of uptake and release of the dye following K^+ stimulation at each synapse correlate with the levels of synaptophysin, synapsin I and SV2 but not of synapsin II. These data demostrate that differential expression of NTPs is predictive of synaptic strength. They also show that physiological variations of the levels of expression of synaptophysin, synapsin I and SV2 correlate with synaptic efficacy.

S12-02

SNARE Protein-Protein Interactions in Adrenal Chromaffin Cells

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Specificity of interaction between secretory vesicles and the plasma membrane in neurotransmission is thought to be determined by the interaction of the vesicle- and target membrane-associated SNARE proteins synaptobrevin, and SNAP-25 and syntaxin, respectively. *In vitro*, recombinant SNARE proteins form stable trimeric complexes that dissociate upon addition of SNAPs and NSF in the presence of MgATP.

We have investigated the role of t-SNARE heterodimer formation *in vivo* by using permeabilized chromaffin cells as a model system. We could show that syntaxin 1A, and peptides containing the full carboxyterminal heptad repeat region, bind with high affinity to endogenous SNAP-25. Synaptobrevin of the chromaffin granules does not bind to endogenous syntaxin or SNAP-25, but to complexes of syntaxin and SNAP-25. In the plasma membrane of chromaffin cells, only a small proportion of SNAP-25 is found in a complex with syntaxin, probably because the majority of syntaxin is associated with mSec1. We suggest that docking of chromaffin granules induces dissociation of mSec1 from syntaxin, to allow its association with SNAP-25. This t-SNARE heterodimer formation can be blocked by heptad repeat-containing peptides of syntaxin, that themselves are able to form a complex with plasma membrane SNAP-25 and synaptobrevin of the chromaffin granules. These peptides, by mimicking plasma membrane-anchored syntaxin, inhibit secretion of noradrenaline in permeabilized chromaffin cells. We thus conclude that dimerization of the two endogenous t-SNAREs is an essential step preceding docking and/or fusion of chromaffin granules.

S12-03

The SNARE complex and synaptic vesicle docking: an open ended question.

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Neurotransmitter release occurs via Ca^{2+} triggered fusion of synaptic vesicles (SV) with the presynaptic plasmamembrane (PM). Vesicles that appear stably docked at the presynaptic active zone are thought to constitute the readily releasable pool that are activated by calcium. *In vitro*, the SV protein synaptobrevin associates with the PM proteins syntaxin and synaptosomal associated protein of 25 kDa (SNAP-25). In turn these three proteins (SNAREs) act as receptors for the soluble NSF attachment protein (SNAP) that allows the subsequent binding of the ATPase N-ethylmaleimide sensitive fusion protein (NSF). NSF hydrolyses ATP to disrupt the macromolecular complex. The association of the SNAREs and their subsequent activation by NSF has been postulated to function in both the docking and fusion of synaptic vesicles (Söllner et al. 1993, Cell 75. 409). This hypothesis appears at odds with observations that tetanus toxin, which inhibits neurotransmitter release by cleaving synaptobrevin, does not decrease the number of docked vesicles in treated terminals (Hunt et al., 1994 Neuron 12. 1269). We have investigated this conflict by analysing the effect of clostridial neurotoxins on distinct intermediates of synaptic vesicle fusion that can be isolated in vitro. We have shown SNAPs and NSF bind to, and upon ATP hydrolysis, disrupt an SDS resistant complex which forms when the three SNAREs associate with cachother (Hayashi et al., 1994 EMBO J 13 5051). Our observations highlight the physiological relevance of this intermediate. Furthermore, tetanus toxin treatment compromises the stability of the SDS resistant complex without perturbing its interactions with SNAP and NSF, suggesting the intrinsic stability of this intermediate is essential in producing fully functional NSF activated intermediates. Interestingly both fragments of tetanus toxin cleaved synaptobrevin are found in these destabilized complexes leaving open the possibility that the cleaved protein could participate in a SNARE dependent mechanism of synaptic vesicle docking.

S12-04

ACTIONS OF CLOSTRIDIAL NEUROTOXINS ON SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS

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The functional role of SNARE proteins in the mammalian CNS has been investigated by means of clostridial neurotoxins. Pre-treatment of rat hippocampal slice cultures with botulinum neurotoxin (BoNT) type F (40ng/ml for 48-72 h), which cleaves both isoforms of the SNARE protein synaptobrevin, strongly reduced (on average by 10 times) the frequency, but not the amplitude, of miniature excitatory postsynaptic currents (mEPSCs) as well as the amplitude of EPSCs evoked with stimulation in the dentate gyrus, recorded from whole-cell voltage-clamped CA3 pyramidal cells. Application of three secretogogues, α-latrotoxin (ltx), the Ca^{2+} ionophore ionomycin, or the protein kinase C (PKC) stimulator phorbol ester to BoNT/F-treated cultures failed to produce the increase in mEPSC frequency observed in control cultures. We conclude that synaptobrevin plays an essential role in: 1) the spontaneous fusion of synaptic vesicles with the presynaptic membrane, 2) fusion in response to Ca^{2+} influx mediated by either action potentials or ionomycin, 3) fusion that has been rendered independent of Ca^{2+} influx by either PKC activation or ltx application.

S12-05

THE CELLULAR PRION PROTEIN IS NOT ASSOCIATED WITH GABA(A) RECEPTORS VIA A BIOCHEMICALLY STABLE LINK

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The so-called prion diseases are most likely caused by the conformational conversion of the cellular prion protein PrP(C) into an abnormal, pathological form. The function of PrP(C) is not known. From electrophysiological measurements in prion-less mice it has been proposed that PrP(C) may contribute to the structural integrity of central synapses containing gamma-aminobutyric acid type A (GABA(A)) receptors. We tried to demonstrate such a possible interaction of PrP(C) and GABA(A) receptors by copurification of these proteins from calf brain membranes. Preparations of PrP(C) and GABA(A) receptors were analysed for the presence of GABA(A) receptors or PrP(C), respectively. No evidence for PrP(C) - GABA(A) receptor complexes could be obtained in our experiments, although the protein purification schemes used should favour the preservation of intermolecular linkages. We conclude that the role of PrP(C) might be different from that of providing GABA(A) receptors a linkage to cytoskeletal structures at postsynaptic membranes.

S12-06

SUBCELLULAR LOCALISATION AND ROLE OF SYNAPTOTAGMIN IN INSULIN-SECRETING PANCREATIC β-CELLS

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Various approaches have demonstrated the importance of the calcium-sensing and phospholipid-binding protein synaptotagmin in neurotransmitter release from small synaptic vesicles. We therefore investigated whether this protein may also be required for the secretion of insulin from Large Dense Core Vesicles (LDCV) in the endocrine pancreatic β-cell.

The synaptotagmin isoforms I, II and IV could be detected in the B-cell lines RINm5F, HIT-T15 and INS-1 by immunoblot. Subcellular fractionation of INS-1 cells demonstrated that synaptotagmin I comigrates with fractions enriched in insulin-containing secretory granules (LDCV) and in fractions containing synaptophysin, a marker of synaptic vesicle-like microvesicles in the B-cell. Immunohistochemical analysis by confocal microscopy demonstrated that also synaptotagmin II is found on both types of vesicles. In contrast, synaptotagmin IV is also found on structures different from the aforementioned vesicles. Primary rat β-cells express only synaptotagmin IV, but not I or II. To investigate the function of synaptotagmin II we used an affinity-purified antibody directed against its first calcium-binding domain. In streptolysin-O permeabilized HIT-T15 cells this antibody inhibits calcium-induced exocytosis by 60%, but not GTPγS-stimulated exocytosis. In conclusion, our findings suggest that synaptotagmin is also involved in calcium-stimulated exocytosis of endocrine hormones.

S12-07

CD SPECTRA OF THE LIGAND-BINDING DOMAIN OF THE NEURONAL ACETYLCHOLINE RECEPTOR α7 SUBUNIT

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The secondary structure of the domain of α7 containing the acetylcholine binding site has been studied using circular dichroism (CD). The peptide (residues 73-199), which is purified under denaturing conditions following expression in *E.coli*, has been renatured in the presence of n-octyl-β-D-glucopyranoside or n-octyl-oligo-oxyethylene. The results of the CD studies indicate that the peptide is refolded with a large proportion of β-sheet (70-72%) and minor amounts of α-helix (6-10%), β-turn (8%), and random coil (12-15%). However, the α-helix content increases slightly with addition of 40% trifluoroethanol. No structural changes were observed in the far CD region when the peptide was exposed to carbamylcholine or bromo-acetylcholine.

S12-08

Effects of postnatal NMDA receptor blockade on maturation of the rat primary somatosensory cortex

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$GABA_A$ receptor α-subunit variants are expressed in the developing rat primary somatosensory cortex (S1) in an area- and lamina-specific manner. Each of these subunits exhibits a distinct developmental profile reflecting the somatotopic organization of this area. Ablation of the thalamus at birth prevents the formation of barrels and dramatically alters the expression of the subunits α1–3 and α5, suggesting an activity-dependent mechanism underlying both phenomena. In this study, we have investigated the effects of postnatal NMDA receptor blockade on the development of S1 employing the non-competitive antagonist MK 801. Following a chronic blockade of cortical NMDA receptors by implantation of Elvax polymers releasing 10 µmol MK 801 locally into S1, no differences in the formation of barrels or in the immunohistochemical distribution of $GABA_A$ receptor subunits were detected. In contrast, in rats treated once at birth with a systemic injection of MK 801 (0.5 mg/kg), barrels did not form and the distribution of the α5-subunit was affected selectively: In layer IV of S1, the α5-subunit immunoreactivity was higher than in controls, similar to that seen after a thalamic lesion. However, although the expression of the α1- and α2-subunits reflected the malformation of the barrels, their laminar distribution was not altered. The results show that subcortical NMDA receptor-mediated activity is involved in the formation of barrels and in the laminar distribution of the $GABA_A$ receptor α5-subunit in the developing S1, whereas the expression of the α1-and α2-subunits is apparently not influenced by NMDA receptor blockade.