How and When Do Synapses Change Their Strength?

S02-01

LTP AND LTD BETWEEN PAIRS OF SINGLE HIPPOCAMPAL NEURONS.

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Long-term potentiation (LTP) and long-term depression (LTD) of excitatory synaptic transmission have been investigated at synapses formed by pairs of monosynaptically connected pyramidal cells in rat hippocampal slice cultures. Potentiation lasting >3 minutes was never induced when several 50 Hz tetani were elicited in single presynaptic neurons. In contrast, potentiation lasting >15 minutes was induced at CA3-CA1 and CA3-CA3 synapses when single low frequency presynaptic action potentials were synchronously paired with postsynaptic depolarization (20-80 x). We conclude that associative/cooperative interactions of multiple inputs are required for LTP induction. LTP could not be induced at all unitary synapses, even when formed on a single postsynaptic cell, indicating that not all synapses are equally plastic. The absence of plasticity at some contacts was not due to prior saturation at a maximally potentiated level. In contrast to LTP, both 3 Hz tetanization of a single presynaptic neuron (3 min.), and asynchronous pairings between pre- and postsynaptic activity (800 ms delay, 100 x), depressed previously potentiated synapses. The reversible induction of saturating LTP at synapses formed by two neurons demonstrates that the same synaptic contacts undergo potentiation and depression.

S02-02

LONG-TERM POTENTIATION (LTP) OF NMDA AND AMPA RECEPTOR-MEDIATED SIGNALS IN CA1 PYRAMIDAL CELLS.

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It has been proposed that CA1 LTP is expressed by uncovering clusters of postsynaptic AMPA receptors (AMPARs), explaining why quantal content increases, and why NMDAR signals undergo little change. Under some conditions, however, NMDAR signals can increase. I have re-examined this in guinea pig hippocampal slices, by eliciting LTP in one of two pathways, and subsequently isolating the NMDAR signal. By comparing the NMDAR signal in the conditioned pathway to that in the control pathway, a small potentiation was consistently observed. This was roughly 40% of the potentiation of the AMPAR signal when elicited by tetanic stimulation, but only 20% of the AMPAR potentiation when elicited by pairing low-frequency stimulation with postsynaptic depolarization. It was accompanied by an increase in the statistic $1/CV^2$, implying an increase in quantal content. One possible explanation why the potentiation and the change in $1/CV^2$ are smaller for the NMDAR signal than for the AMPAR component is that much of the NMDAR signal is generated by spill-over of glutamate released from terminals which do not make synapses with the postsynaptic cells where LTP induction takes place. AMPARs would not sense this glutamate release because of their lower affinity for the transmitter. If this model is correct, it could invalidate the 'latent AMPAR cluster' argument for a postsynaptic site of expression of LTP.

S02-03

ROLE OF THE NEURAL CELL ADHESION MOLECULE NCAM IN USE-DEPENDENT SYNAPTIC PLASTICITY. A. Artola, C.G. Becker, R. Gerardy-Schahn, T. Becker, H. Welzl, M. Schachner.

NCAM, a cell surface glycoprotein on neurons and glial cells, mediates cell-cell and cell-substrate interactions. Many processes during embryonic development are correlated with the regulated expression of NCAM. It has recently been shown that NCAM is also involved in long-term potentiation (LTP) and in learning and memory. One possible mechanism is that it regulates the extent of adhesivity between pre- and post-synaptic membranes. In principle this can be achieved through transcriptional control of the type and amount of NCAM expressed on cell membrane and/or posttranslational modifications by the addition of long chains of alpha-2-8-linked polysialic acid (PSA). PSA can modulate the efficacy of both NCAM-mediated interactions as well as those regulated by other cell ligands on cell surface and extracellular matrix. Specific removal of PSA with endoneuraminidase NE significantly impairs spatial learning of rats in the Morris water maze and abolishes LTP in CA1 of hippocampal slices. Our findings suggest that cell adhesion molecule-mediated interactions play a critical role in synaptic plasticity and learning and memory. We are further testing this hypothesis in NCAM deficient mice (Cremer et al., *Nature* 1994, **367**: 455)

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S02-04

STRUCTURAL PLASTICITY AND MECHANISMS OF LTP IN CA1 HIPPOCAMPAL ORGANOTYPIC CULTURES.

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Among the different mechanisms proposed to contribute to LTP are modifications of the structure of synapses. In support of this are results that implicate the adhesion molecules F1 and NCAM in LTP (Lüthi et al., 1994). By using Endo-N, a neuroaminidase that cleaves the polysialic portion of NCAM, we have obtained further evidence that induction of LTP and LTD requires the expression at the cell surface of the embryonic form of NCAM and that this expression is controlled by neuronal activity in a calcium-dependent manner. Evidence for structural modifications was also observed in a morphological analysis. Activated synapses were detected using a precipitation method that revealed, at the electron microscopy level, the accumulation of calcium in postsynaptic spines. Comparison of stimulated and control synapses 30 min after induction of LTP showed a major increase in the proportion of spines with perforated postsynaptic densities. These results are consistent with the interpretation that induction of synaptic plasticity may be associated with structural changes of synapses (work supported by FNRS).

S02-05

AGRIN INDUCES ACHR SUBUNIT GENE EXPRESSION IN CULTURED MUSCLE CELLS.

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Acetylcholine receptor (AChR) gene expression by synaptic myonuclei is regulated by neural factors attached to synaptic basal lamina (BL). One factor in synaptic BL is agrin which causes AChR to aggregate at the synapse. A non-aggregating isoform of agrin is expressed in muscle. We have examined recombinant neural and muscle chick agrin isoforms for involvement in AChR ε-subunit gene expression in cultured rat myotubes. Unlike soluble fragments of chick agrin, agrin attached to subtrates increased ε-subunit mRNA levels. The effect was similar in magnitude to that of ARIA, another BL factor, and it was observed for both neural and muscle agrins. Co-transfection of an ε-subunit gene promoter fragment linked to a luciferase reporter gene with plasmids encoding isoforms of agrin increased luciferase activity in Sol8 muscle cells. C2C12 cells stably transfected to secrete agrin and injected into muscle in vivo increased ε-subunit mRNA. These data are consistent with a role of agrin in neural regulation of AChR expression at the neuromuscular junction. They further suggest a potential role for muscle derived agrin in AChR expression.

S02-06

INVESTIGATION OF POINT MUTATION-INDUCED RECTIFICATION IN RECOMBINANT $GABA_A$ RECEPTORS

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Point mutation of homologous charged amino acid residues in the putative M1-M2 cytosolic loop of $\alpha3\beta2\gamma2$ $GABA_A$ receptors induces outward rectification in the GABA-evoked current mediated by these receptors. When neutral or positively charged residues of one or more subunits in this domain are substituted by negatively charged residues, the amount of steady state rectification increases according to the number of charges involved (Backus *et al.* (1993), *Neuroreport*, **5**,285-288). The aim of the present study was to investigate the mechanism of this rectification. Using the cDNAs described by Backus *et al.*, we expressed functional $GABA_A$ receptors in *Xenopus* oocytes and used a two-electrode voltage clamp to compare the current-voltage (I-V) characteristics of the wild type (WT) receptor and those with the point mutation in the γ (Lys259Glu) subunit alone and in the α (Arg273Glu) and γ (Lys259Glu) subunits together. In contrast to the outwardly rectifying steady state I-V relationship, voltage jump experiments revealed a linear instantaneous I-V relationship for both the WT and mutated receptors over the voltage range -100 mV to 40 mV. This indicated that the mutations in the M1-M2 cytosolic loop modified the channel kinetics, rather than introduce a voltage-dependence of the single channel conductance. Our finding suggests that sensing of the transmembrane field by the charged residue at the γ subunit position 259 and homologous sites of the α and β subunits might play a role in determining the voltage-dependence of $GABA_A$ receptor channel kinetics.

S02-07

THE AVIAN BRAIN $GABA_A$ RECEPTOR γ4 SUBUNIT: EXPRESSION AND FUNCTIONAL PHARMACOLOGY.

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The γ subunit of heteropentameric $GABA_A$ receptors contributes to a high-affinity benzodiazepine binding site and is therefore an important determinant of $GABA_A$ receptor pharmacology. In this study we characterize the recently cloned γ4 subunit (Harvey et al., 1993, FEBS Lett., 331,211-216), which in avian species replaces the mammalian γ3 subunit. We co-expressed γ4 subunits with rat α3 and β2 subunits and demonstrated functional expression of GABA-activated receptor channels in transfected HEK-293 cells. Expression was confirmed by nuclear injection in *Xenopus* oocytes which were then used to determine the pharmacology of the recombinant α3β2γ4 receptor. The evoked current for GABA was potentiated by sodium pentobarbital, suppressed by picrotoxin and blocked by Zn^{2+}, in a dose-dependent manner. The benzodiazepine full agonists flunitrazepam and triazolam strongly potentiated the GABA-evoked current, whereas the partial agonists bretazenil and abecarnil enhanced the response less strongly and the β-carboline agonists DMCM and β-CCM had inhibitory effects. However, the inverse agonist Ro15-4513 enhanced the GABA response and the positive agonist zolpidem was inactive at α3β2γ4 receptors. Our results show that the avian γ4 subunit confers on $GABA_A$ receptors a novel pharmacology which differs from that imparted by mammalian γ subunits.

S02-08

LASER-FLASH PHOTOLYSIS OF CAGED GLUTAMATE

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Flash photolysis of "caged" compounds provides a means to rapidly change the concentration of biologically active products. We used *CNB-L-Glutamate* ($t_{1/2} \approx 21\mu s$, quantum yield ≈ 0.14) to investigate the kinetics of non-NMDA glutamate receptors in cultured hippocampal neurons. Membrane currents were recorded in the whole-cell mode of the patch-clamp technique in the presence of bicuculline, strychnine and TTX. A Nd:YAG laser (flash duration ≈ 7 ns at 355 nm) was used to epi-illuminate a ≈ 250 µm field. At a holding potential of -60 mV the currents induced by a flash showed a rapid activation ($t_{1/2}$ < 7 ms) followed by a slower inactivation, but the amplitude was much smaller than expected (< 100 pA). Surprisingly, rapid superfusion ($t_{1/2} \approx 200$ ms) of the cells with 0.5 mM CNB-L-Glutamate led to a slow inward current, which desensitized partially after several hundred ms. Similar currents but with ≈ 2 fold larger amplitude were elicited with 0.5 mM free glutamate. Both effects were reversibly blocked by CNQX (10 µM). These findings suggest that CNB-L-Glutamate, as sold, may not be biologically inert. Instead, the caged compound may exhibit an intrinsic action on AMPA receptors or may have become contaminated with glutamate. In both cases the photolytic dynamic range of CNB-L-Glutamate (without further purification) would be reduced.

Nitric Oxide

S03-01

Inducible nitric oxide synthase deficient mice

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To directly define the physiological role of inducible nitric oxide synthase (iNOS) we constructed a strain of mice deficient in iNOS. These mice are viable, fertile and without evidence of histopathological abnormalities. However, in contrast to wild-type and heterozygous mice, which are highly resistant to the protozoa parasite Leishmania major infection, mutant mice are uniformly susceptible. The infected mutant mice developed a significantly stronger Th1 type of immune response than the wild-type or heterozygous mice. The mutant mice showed reduced non-specific inflammatory response to carrageenin and were resistant to lipopolysaccharide-induced mortality.

S03-03

Nitric oxide and the Cardiovascular System

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The circulation is controlled by the central nervous system, hormones and local vascular mechanism. The endothelium is in a strategic anatomical position in the blood vessel wall between the blood (and platelets and monocytes) and vascular smooth muscle. Endothelial cells are stimulated by mechanical and hormonal signals and it release mediators modulating contraction and proliferation of vascular smooth muscle, platelet function, coagulation and monocyte adhesion. Nitric oxide (NO) and prostacyclin (PGI_2) and a putative hyperpolarizing factor (EDHF) mediate relaxation. NO inhibits smooth muscle proliferation and (with PGI_2) platelet function. Bradykinin induced NO production is regulated by angiotensin converting enzyme on the endothelium; this enzyme converts angiotensin I into angiotensin II, and inactivates bradykinin. Endothelin-1, thromboxane A_2 (TXA_2) and prostaglandin H_2 (PGI_2) are endothelium-derived contracting factors. In contrast to TXA_2 and PGH_2 which activate platelets, endothelin-1 has no platelet effects, but proliferative properties in vascular smooth muscle. Under physiological conditions, the endothelium plays a protective role as NO prevents adhesion of circulating blood cells, keeps the vasculature in a vasodilated state and inhibits vascular smooth muscle proliferation. In disease states, decreased NO release contributes to enhanced vasoconstriction, adhesion of platelets and monocytes and proliferation of vascular smooth muscle, all events which are involved in cardiovascular disease.

S03-02

THERAPEUTIC STRATEGIES FOR THE INHIBITION OF INDUCIBLE NITRIC OXIDE SYNTHASE

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In recent years, NO, a gas previously considered a potentially toxic chemical, has become established as a diffusible universal messenger mediating cell-cell communication throughout the body. In mammals, NO is a recognized mediator of blood vessel relaxation that helps to maintain blood pressure. In the central nervous system NO acts as a non-conventional neurotransmitter and participates in the establishment of long-term plasticity required for memory formation. In addition, NO is responsible for some parts of the host response to sepsis and inflammation and contributes to certain disease states. A number of strategies have emerged with regard to a pharmacological control of pathological NO overproductions. I will discuss these novel therapeutic approaches that may provide new means for clinical medicine.

S03-04

The Role of Nitric Oxide (NO) in Apoptosis

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Nitric oxide (NO) is recognized as a ubiquitous messenger throughout physiology and pathophysiology. Cyclic GMP-independent signaling pathways comprise cytostasis and/or cytotoxicity related to NO. Activation of the cytokine/lipopolysaccharide inducible NO-synthase (iNOS) causes nitrite accumulation in the cell supernatant and apoptotic cell death of macrophages (RAW 264.7 cells) or ß-cells (RINm5F cells). Apoptosis is characterized by morphological (chromatin condensation) and biochemical criteria (DNA fragmentation), while iNOS-inhibitors (N^G-monomethyl-L-arginine) are used to trace back NO-generation to cell death. Prior to DNA laddering we observed the accumulation of the tumor suppressor p53. Stabilization of p53 was correlated to the extend of DNA damage, initiated by a chemically heterogeneous group of NO-releasing compounds. As potential protective modulators of apoptosis we focused on Bcl-2 overexpression and superoxide production. Regulation of NO-production, activation or inhibition of specific intracellular signaling pathways modulate the cellular response to a potentially cytotoxic molecule like nitric oxide.