F-PM-1 THE FORMATION OF CHOLINERGIC SYNAPSES BETWEEN SYMPATHETIC NEURONS IN CELL CULTURE. P.H. O'Lague*, K. Obata*, P. Claude*, D.D. Potter*, and E.J. Furshpan*, Department of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115.

The ability of sympathetic neurons to form synapses in cell-culture has been examined using electrophysiological and electronmicroscopical techniques. Superior cervical ganglia of newborn rats were mechanically dissociated into individual cells and small clusters. This cell suspension contained mostly principal neurons and a smaller number of ganglionic non-neuronal cells. Put into culture at low density, the neurons sent out processes which formed a complex network. In media containing bicarbonate (26mM) the few non-neuronal cells proliferated and eventually formed a cellular carpet in which the neurons and their processes became embedded. In bicarbonate-free media the growth of the non-neuronal cells was suppressed and the neuronal network developed in near isolation. In either medium the resting and action potentials recorded from the neurons were similar to those reported for intact ganglia. In the presence of the carpet of non-neuronal cells, but only rarely in absence, the neurons formed synapses with each other de novo. The synapses were all excitatory, but were of two types. One, which was relatively rare, was shown to operate by electrical transmission. The other, and predominant, type had several characteristics of chemical transmission and was shown in pharmacological experiments to be cholinergic. The presence of excitatory, cholinergic synapses between these sympathetic neurons was unexpected.

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F-PM-2 MEMBRANE STRUCTURE AT FRESH-FROZEN FROG NEUROMUSCULAR JUNCTIONS. J.E. Heuser* and T.S. Reese*, LNNS, NINDS, NIH, Bethesda, Maryland 20014.

Cutaneous pectoris muscles were prepared for freeze-fracturing by freezing without preliminary exposure to fixatives or cryoprotectants in order to avoid artifacts caused by these agents. Nerve terminals were recognized after fresh-freezing by the characteristic double rows of large intramembranous particles which delineate "active zones" (J. Neurocytol. 3, 1974). However, fresh-frozen nerve terminals differed in certain respects from fixed terminals. (1) Deformations of the presynaptic membrane were lacking at active zones, even after electrical stimulation which produces many deformations in aldehyde-fixed terminals. Since these deformations are thought to be sites of synaptic vesicle discharge, their unnatural abundance in fixed terminals illustrates that aldehydes profoundly slow the time-course of synaptic vesicle exocytosis. (2) Deformations of the presynaptic membrane outside of the active zones were numerous in fresh-frozen terminals recovering from stimulation. Many of the deformations contained clusters of 4-10 unusually large intramembranous particles. In suitable cross-fractures, these particles appeared to be continuous with fine cytoplasmic filaments, which may be the frozen counterparts of the cytoplasmic coats that distinguish endocytotic pits in thin sections. Possibly, the large particles are intramembranous anchoring-points for these cytoplasmic filaments. (3) In aldehyde-fixed tissues, nearly all of the intramembranous particles clustered in the postsynaptic membrane beneath the nerve terminal adhere to the cytoplasmic half of the fractured muscle membrane but in fresh-frozen tissue nearly half of these particles adhere, instead, to the external half of the postsynaptic membrane. Aldehyde fixation may anchor these particles to the dense cytoplasmic material which underlies the postsynaptic membrane.

F-PM-3 MOLECULAR BASIS FOR POSTSYNAPTIC CONDUCTANCE CHANGES. C.F. Stevens, Department of Physiology and Biophysics, University of Washington, Seattle, Washington 98195.

We have been investigating the effects of acetylcholine and other agonists at the frog neuromuscular junction in an effort to understand the molecular events underlying the post-synaptic conductance change. Acetylcholine is applied to the post-junctional membrane by nerve-evoked release, and acetylcholine as well as other agonists are iontophoretically applied to endplates visualized with Nomarski differential interference contrast optics. Local agonist concentrations are measured with an ion-selective microelectrode, and post-junctional conductance changes evoked by the agonists are investigated with the two microelectrode voltage clamp technique. By studying the time course and amplitude of endplate currents and miniature endplate currents, and the spectrum of agonist-induced conductance fluctuations, we have developed a specific picture of post-junctional mechanisms: according to our view, one or two agonist molecules bind rapidly to a post-junctional receptor and produce a conformational change which opens an associated channel. This channel remains open, on the average, between about .8 and 5 ms and has an average conductance, when open, of between about 15 and 30 picomhos, depending upon which agonist caused the underlying conformational change. A dipole moment change, which we have measured, also accompanies the channel opening conformation transition.

F-PM-4 ACETYLCHOLINE RECEPTORS IN NORMAL AND DENERVATED MUSCLE. J.P. Brockes* and Z.W. Hall*, Department of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115

Most acetylcholine (ACh) receptors in adult mammalian skeletal muscle occur at the neuromuscular junction. Denervation causes a large increase in the number of receptors outside the junction. We have separately purified ACh receptors from normal rat diaphragm (junctional receptors) and the receptors that appear in extrajunctional regions of the diaphragm after denervation (extrajunctional receptors) and have compared their properties. The reaction of the two purified receptors with I^{125}-α-bungarotoxin was studied by kinetic methods and found to be similar, although not identical. The toxin-receptor complexes of the two receptors were indistinguishable by gel filtration and by zone sedimentation in a sucrose gradient, and showed identical precipitation curves with rabbit antiserum to the eel ACh receptor. Both toxin-receptor complexes bind concanavalin A and are therefore probably glycoproteins. Low concentrations of d-tubocurarine were about 10-fold more effective in decreasing the rate of toxin binding to junctional than to extrajunctional receptors. The two toxin-receptor complexes could be separated by isoelectric focusing. Thus junctional and extrajunctional receptors are similar but distinct molecules. We have also investigated whether synthesis of receptors occurs after denervation. Denervated muscles were incubated with ^{35}S-methionine and the ACh receptor was purified. Radioactivity was associated with material whose isoelectric point and sedimentation behavior was the same as the receptor and which could be precipitated by an antiserum to the eel ACh receptor. We conclude that synthesis of the ACh receptor occurs after denervation. We thank Dr. J. Patrick for the antiserum. Supported by the Muscular Dystrophy Associations of America and NIH grants NS09646 and NS30984.