Spread of Synaptic Depression Mediated by Presynaptic Cytoplasmic Signaling

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Postsynaptic activity may modulate presynaptic functions by transsynaptic retrograde signals. At developing neuromuscular synapses in *Xenopus* nerve-muscle cultures, a brief increase in the cytosolic calcium ion (Ca^{2+}) concentration in postsynaptic myocytes induced persistent depression of presynaptic transmitter secretion. This depression spread to distant synapses formed by the same neuron. Clearance of extracellular fluid did not prevent the spread of depression, and depression could not be induced by increasing the Ca^{2+} concentration in a nearby myocyte not in contact with the presynaptic neuron. Thus, the spread of depression is mediated by signaling in the presynaptic cytoplasm, rather than by a retrograde factor in the extracellular space.

Activity-dependent modulation of synaptic connections is crucial to the normal development and functioning of the nervous system (*1*). Persistent changes in the efficacy of synaptic transmission, known as long-term potentiation (LTP) or long-term depression (LTD), can be induced by a brief period of synaptic activity at various central and peripheral synapses (*2*). These forms of activity-dependent synaptic modulation not only occur at the active synapses, but may also spread to other synapses (*3*, *4*). Such spread of synaptic modulation affects the specificity of activity-induced changes and has important consequences for signal processing in the nervous system. In principle, the spread of synaptic modulation may be confined within the cytoplasm of the pre- or postsynaptic neuron, thereby affecting only synapses associated with that cell. On the other hand, membrane-permeant diffusible factors produced by the active synapse may spread through the extracellular space to affect a less restricted set of nearby synapses. For example, induction of LTP at one synapse in the CA1 region of the hippocampus results in potentiation of nearby synapses, and membrane-permeant diffusible retrograde factors appear to be responsible (*4*). Here, however, we found that long-term depression induced at one developing neuromuscular synapse resulted in depression at distant synapses made by the same presynaptic neuron. This spread appeared to be mediated by membrane-impermeant signals within the presynaptic cytoplasm.

Persistent depression of developing neuromuscular synapses can be induced by repetitive stimulation of an adjacent neuron co-innervating the same postsynaptic muscle cell, a form of heterosynaptic depression that may underlie activity-dependent synaptic competition (*5*, *6*). Similar synaptic depression can also be induced by direct postsynaptic activation of acetylcholine (ACh) receptors through iontophoretic application of pulses of ACh (*7*). Both heterosynaptic and ACh-induced depression require influx of Ca^{2+} into the postsynaptic muscle cell (*5*–*7*). Moreover, with the use of photolabile caged Ca^{2+} chelators, we recently showed that an increase in the postsynaptic Ca^{2+} concentration in the region of the synaptic contact is sufficient to induce persistent synaptic depression (*8*). We have now examined whether synaptic depression spreads from one synapse to another made by the same presynaptic neuron in *Xenopus* nerve-muscle cultures (*9*), and, if so, whether this spread is mediated through extra- or intracellular pathways.

Myocytes loaded with nitr-5–AM (*10*) were manipulated into contact with the neurite of a preexisting nerve-muscle pair

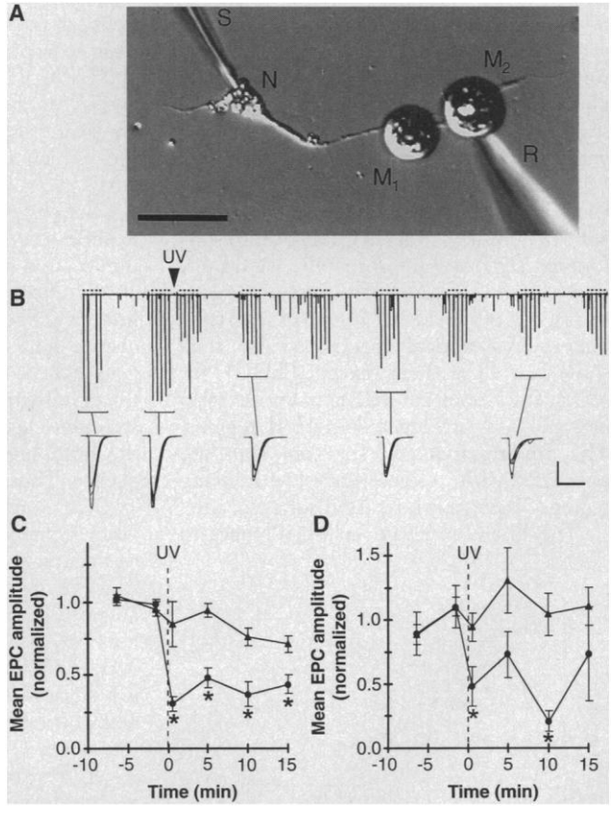

Fig. 1. Presynaptic spread of synaptic depression. (**A**) Differential interference contrast (DIC) image of a 1-day-old *Xenopus* culture in which a spherical myocyte (M_1) was manipulated into contact with the neurite of a spinal neuron (N) at a distance of ~40 μm from a preexisting synapse between the neuron and a cocultured myocyte (M_2). The manipulated myocyte (M_1) had been preloaded with nitr-5 by incubation for 1 hour in a separate culture dish with a solution containing nitr-5–AM (20 μM) and then exposed for 10 s to saline containing 20 mM K^+. R and S, whole-cell recording and extracellular stimulating pipettes, respectively. Bar, 50 μm. (**B**) An example of data collected for the synapse arrangement shown in (A). The continuous trace represents the membrane current recorded from the myocyte with preexisting innervation (M_2) under voltage-clamp conditions (holding potential, −70 mV, filtered at 1 kHz). EPCs were evoked at regular intervals at the times marked by the small dots. Photolysis of nitr-5 in myocyte M_1 was induced at the time indicated by the arrowhead with a 2-s pulse of a focused UV beam with a spot 20 μm in diameter. EPC traces are shown below at a higher time resolution (filtered at 2.5 kHz) for the recording periods indicated by the brackets. Scales: slow traces, 1.5 nA, 2 min; fast traces, 2 nA, 30 ms. (**C**) Synaptic depression induced in the innervated myocyte (M_2) after UV exposure of the nitr-5–loaded myocyte (M_1). Experiments similar to that shown in (B) are summarized. The mean amplitude of five or six EPCs recorded in the myocyte M_2 at 5-min intervals was normalized to the mean value for the same synapse during the 5-min control period before UV photolysis. Data are means ± SEM from 16 experiments in which myocyte M_1 was loaded with nitr-5 (●) and five experiments in which M_1 was not loaded with nitr-5 (▲). *$P < 0.05$ versus corresponding control value [single-factor analysis of variance (ANOVA)]. (**D**) Synaptic depression induced at the manipulated synapse by photolysis. Experimental protocol and analysis were similar to those in (B) and (C), with the exception that the whole-cell recording was from the nitr-5–loaded myocyte (M_1), according to the nystatin perforated patch technique to prevent washout of nitr-5 (*19*). The M_1 myocyte was either loaded (●) ($n = 13$) or not loaded (▲) ($n = 17$) with nitr-5. The asterisk indicates $P < 0.05$ versus corresponding control value (single-factor ANOVA).

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(Fig. 1A). When the contact was made, at a distance of ~50 μm (*11*) from the preexisting synaptic site, exposure of the nitr-5–loaded myocyte to ultraviolet (UV) light for 2 s (*12*) induced a marked synaptic depression of the preexisting synapse, as shown by a reduction in the mean amplitude of impulse-evoked excitatory postsynaptic currents (EPCs) recorded in the innervated myocyte (*13*) (Fig. 1). Five minutes after photolysis, the mean amplitude of EPCs (including failures) was reduced to 48 ± 5% (mean ± SEM, n = 16) of the pre-photolysis control value (Fig. 1, B and C). This decrease in mean EPC amplitude was predominantly attributable to a reduced mean amplitude of successfully evoked synaptic currents (54 ± 5% of the control value) and to a slight increase in the failure rate in eliciting evoked responses (from 0.63% before to 8.75% after UV exposure). In control experiments, myocytes not loaded with nitr-5 were similarly manipulated into contact with the presynaptic neuron. Exposure of these myocytes to UV did not induce significant depression (Fig. 1C). The difference between these control cells and nitr-5–loaded cells was still significant 25 to 30 min after UV illumination (67 ± 3% versus 33 ± 7%; $P < 0.05$). Consistent with previous observations (*14*), functional synaptic transmission was established soon after the nitr-5–loaded myocyte was manipulated into contact with the neurite. Although amplitude fluctuation of EPCs at the manipulated synapse was larger than that at preexisting synapses, a reduction in the mean EPC amplitude was also evident after UV illumination of the manipulated myocyte for 2 s (Fig. 1D). Thus, the manipulated myocyte rapidly established a synapse with the neurite, and an increase in postsynaptic Ca^{2+} concentration could induce depression of this synapse and an adjacent synapse made by the same presynaptic neuron. The decrease in the average evoked response was not accompanied by changes in the onset, rise, or decay times of the EPCs, or in the properties of the miniature excitatory postsynaptic currents (MEPCs) (Fig. 2, A and B). The amplitude distribution of MEPCs also was not affected by the induction of depression (Fig. 2C). Thus, the depression was attributable to a decrease in presynaptic transmitter release triggered by each action potential, rather than to a change in postsynaptic transmitter sensitivity or in the reliability of the recording method.

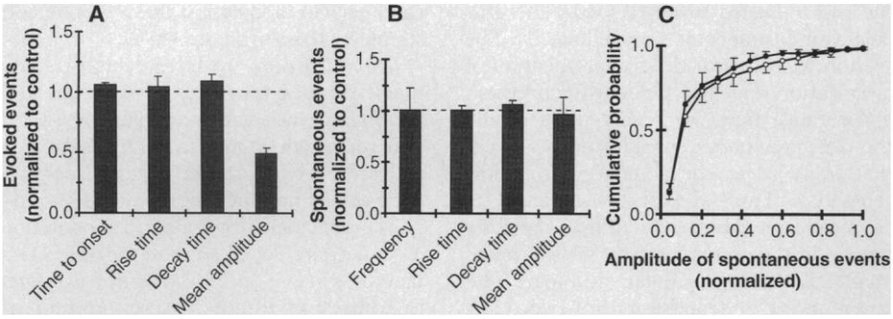

Fig. 2. Effects of depression on the properties of synaptic currents. (**A** and **B**) Various properties of EPCs (A) and MEPCs (B) after induction of synaptic depression at an adjacent synapse by UV photolysis of nitr-5 in a loaded postsynaptic myocyte, as described in Fig. 1, B and C. The mean value for synaptic currents at each individual synapse was normalized to that observed at the same synapse before UV photolysis, and the histograms represent the normalized values (means ± SEM) for all synapses (n = 16). (**C**) Composite graphs of MEPC amplitude distribution from experiments described in Fig. 1C. The cumulative probability refers to the fraction of total events observed in the innervated myocyte with amplitudes smaller than a given amplitude, during a 5-min period before (○) and after (●) UV exposure of the nitr-5–loaded myocyte. Data are means ± SEM (n = 6). There is no statistically significant difference between the two sets of data (Kolmogorov-Smirnov test, $P > 0.1$).

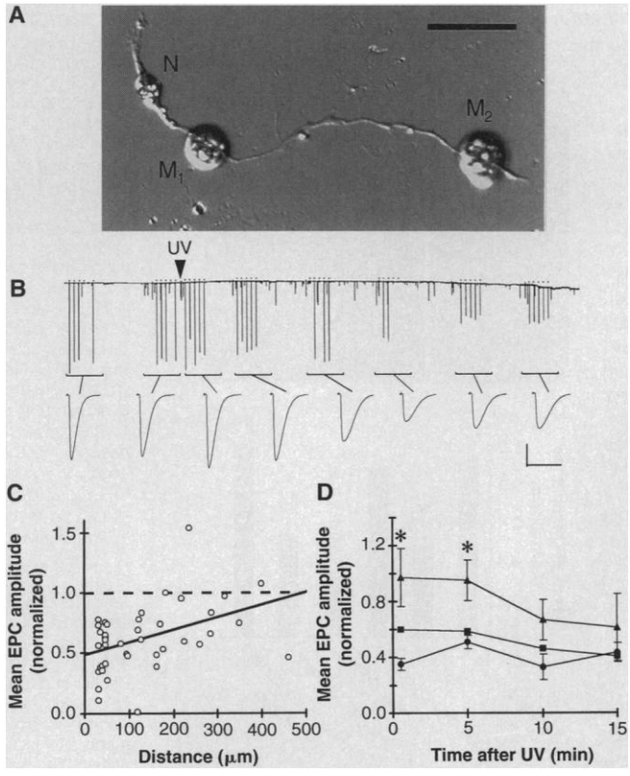

Fig. 3. Distance dependence of the presynaptic spread of depression. (**A**) A DIC image of a 1-day-old *Xenopus* culture in which a nitr-5–loaded myocyte (M_1) was manipulated into contact with the neurite at a site distant from the preexisting synapse on another myocyte (M_2). Bar, 50 μm. (**B**) Experiment similar to that shown in Fig. 1B, with the exception that the nitr-5–loaded myocyte was positioned 400 μm from the innervated myocyte. Scales as in Fig. 1B. (**C**) Summary of the distance dependence of the spread of depression. The nitr-5–loaded myocytes (M_1) were positioned at various distances (30 to 460 μm) from the innervated myocyte (M_2). Each data point represents the mean EPC amplitude recorded from one M_2 myocyte 5 min after a 2-s exposure of myocyte M_1 to UV light, normalized to the control EPC amplitude obtained from the same synapse before nitr-5 photolysis. The solid line represents the best linear fit of the data, with a correlation coefficient (r) of 0.42. (**D**) Delay in onset of depression at distant synaptic sites. Changes in the mean EPC amplitude in myocyte M_2 after photolysis at M_1 were averaged for experiments in which M_1-M_2 distances were <50 μm (●), between 100 and 150 μm (■), and >200 μm (▲). Data were from the same set of experiments as that shown in (C). Data are means ± SEM. The asterisk indicates $P < 0.05$ versus corresponding value for <50 μm group (single-factor ANOVA).

The spread of synaptic depression along the presynaptic neuron was examined by positioning the nitr-5–loaded myocyte at various distances from the preexisting synapse (Fig. 3A). Significant depression was apparent even when the myocyte was positioned at a remote location. An example of depression at a synapse 400 μm from the contact site is shown in Fig. 3B. Although the extent of synaptic depression was similar to that observed in nearby synapses 15 min after photolysis, the onset of depression showed a delay, with no apparent depression during the first 5 min after photolysis. The extent of depression at 5 min after photolysis showed an apparent dependence on the distance between the myocytes, which ranged from 30 to 460 μm in 41 separate experiments (*15*) (Fig. 3C). The

data collected from these experiments were also grouped for three different ranges of distances. Statistically significant differences were detected between values of the close (<50 μm) and far (>200 μm) groups during the first 5 min, but not 10 min after photolysis (Fig. 3D). These results suggest that there is an increased delay of onset of depression for synapses at greater distances, although similar depression may later be attained.

Upon photolysis, it is possible that the myocyte secretes a membrane-permeant substance that diffuses extracellularly to adjacent and distant synapses and induces presynaptic depression. We therefore exposed to UV light a nitr-5–loaded myocyte positioned at a distance of 50 to 70 μm from the preexisting synapse as above, but this time the myocyte was not placed in contact with the neurite (Fig. 4A). No significant synaptic depression was observed, as compared with controls (Fig. 4C). Thus, either the production or the delivery of the retrograde signal, or both, depends on physical contact with or extremely close proximity to the presynaptic neuron. In addition, we were able to position a nitr-5–loaded myocyte on the neurite of an adjacent, noninnervating neuron, and found that no depression was induced in the preexisting synapse after UV photolysis, consistent with the idea that retrograde signaling from the UV-exposed myocyte to the presynaptic neurite requires physical contact and is not mediated by diffusible factors.

To examine further whether long-range extracellular signaling contributes to the spread of depression to distant synaptic sites, we introduced a suction pipette to remove the extracellular fluid surrounding the nitr-5–loaded myocyte (*16*). The efficiency of clearance of extracellular fluid by suction was confirmed by local injection of dye solution (Fig. 4B). Under this condition of clearance flow, we observed essentially the same presynaptic spread of depression as that observed in the absence of the flow (Fig. 4C). Thus, the observed spread of depression must be mediated by a signaling process in the presynaptic cytoplasm that is unaffected by extracellular clearance. The rate of spread of depression can be accounted for by passive diffusion of a cytoplasmic signaling molecule, although a role for active processes cannot be excluded (*17*).

Previous studies on heterosynaptic and ACh-induced synaptic depression have shown that suprathreshold stimulation of the presynaptic neuron in synchrony with postsynaptic activation fully prevents the induction of synaptic depression (*5–8*). We examined whether presynaptic spread of depression can also be prevented by presynaptic activation coincident with UV exposure. A nitr-5–loaded myocyte was positioned in contact with the presynaptic neuron at a distance of ≤50 μm from the preexisting synapse. Presynaptic firing of action potentials at a frequency of 2 Hz concurrent with the 2-s UV exposure of the myocyte prevented the synaptic depression (Fig. 4C). This result confirms that synaptic depression monitored in the present study was similar to the Hebbian modulation previously demonstrated at these synapses (*5–8*).

In conclusion, we have demonstrated a rapid and long-range cytoplasmic spread of presynaptic modulation induced by a localized retrograde signal from a postsynaptic cell (*18*). The existence of such a presynaptic spread has direct consequences for the spatial specificity of synaptic modulation. The activity-dependent retrograde effect may be synapse specific only for a limited time, because distant synapses are soon affected. The resulting interaction between distant synapses may play an important role in signal processing and plasticity in the nervous system.

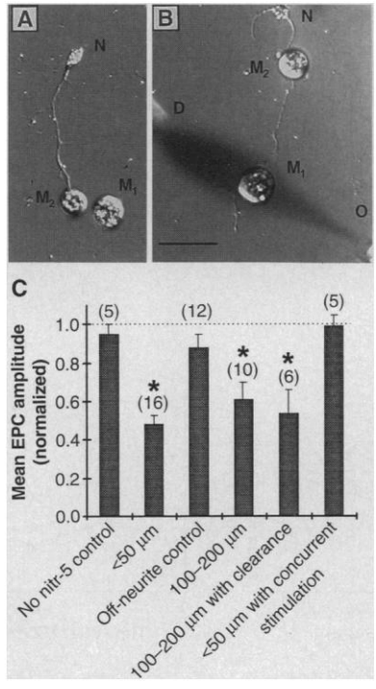

Fig. 4. Spread of synaptic depression under various experimental conditions. (**A**) A DIC image of the synapse arrangement in a control experiment, in which the nitr-5–loaded myocyte (M_1) was positioned at a site 50 μm from the preexisting synapse but not in contact with the presynaptic neuron or postsynaptic myocyte (M_2). (**B**) A DIC image of a culture illustrating the arrangement for extracellular clearance flow. A rapid suction flow was introduced across the nitr-5–loaded myocyte (M_1) in order to wash away potential extracellular factors secreted by the UV-illuminated myocyte. Ringer's solution containing trypan blue was injected from a second, smaller pipette to reveal the flow pattern. O, suction pipette. D, dye inlet pipette. Bar, 50 μm. (**C**) Mean EPC amplitudes recorded from the postsynaptic myocyte of a preexisting synapse (normalized to the control value) 5 min after UV illumination of a manipulated myocyte. (No nitr-5 control) The manipulated myocytes exposed to UV light were not loaded with nitr-5–AM. (<50 μm) Standard experiments with the nitr-5–loaded myocyte positioned within 50 μm of the recorded myocyte and on the neurite of the presynaptic neuron. (Off-neurite control) Nitr-5–loaded myocytes were positioned at a distance of ~70 μm from the preexisting synapse and not in contact with the neurite, as shown in (A). (100 to 200 μm) The distance between the nitr-5–loaded myocyte (positioned on the neurite) and the recorded myocyte was between 100 and 200 μm. (100 to 200 μm with clearance) The same as (100 to 200 μm), with the exception that a suction flow of culture medium was applied across the nitr-5–loaded myocyte, as shown in (B). (<50 μm with concurrent stimulation) The same as (<50 μm), with the exception that the presynaptic neuron was stimulated to fire action potentials at a frequency of 2 Hz for 2 s during the period of UV exposure of M_1. Data are means ± SEM for the number of experiments indicated in parentheses. Asterisk indicates $P < 0.05$ versus no nitr-5 control (single-factor ANOVA).

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9. *Xenopus* nerve-muscle cultures were prepared as described [N. C. Spitzer and J. C. Lamborghini, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1641 (1976); N. Tabti and M.-m. Poo, in *Culturing Nerve Cells*, G. Banker and K. Goslin, Eds. (MIT Press, Cambridge, MA, 1991), pp. 137–154]. The cells were plated on clean glass cover slips and were used for experiments after incubation for 24 hours at room temperature (20° to 22°C). The culture medium consisted of 50% (v/v) Leibovitz's medium (L-15; Gibco), 1% fetal bovine serum (Gibco), and 49% Ringer's solution [115 mM NaCl, 2 mM $CaCl_2$, 2.5 mM KCl, and 10 mM Hepes (pH 7.3)].
10. Isolated myocytes were loaded with the caged Ca^{2+} chelator nitr-5 by incubation of nerve-muscle cultures for 60 min in culture medium containing 20 μM nitr-5–AM {acetoxymethyl ester of 1-(2-amino-5-

[1-hydroxy-1-(2-nitro-4,5-methylenedioxyphenyl)-methyl]-phenoxy)-2-(2′-amino-5′-methylphenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; Calbiochem] [J. P. Y. Kao, A. T. Harootunian, R. Y. Tsien, *J. Biol. Chem.* **264**, 8179 (1989)]. On the assumption that a brief depolarization-induced increase in cytosolic Ca^{2+} loads nitr-5 with Ca^{2+}, the cells were then bathed in saline containing 20 mM K^+ for 10 s. Myocytes loaded with nitr-5–AM were transferred to the recording chamber containing untreated *Xenopus* nerve-muscle cultures. Cell manipulation was performed as previously described (*14*).

11. The distance refers to the center-to-center distance between the spherical myocytes. The diameter of the myocytes was 29 ± 7 μm (mean ± SEM, n = 50). Physical contact between the two myocytes was avoided because these cells undergo electrical coupling after contact in culture [M. Chow and M.-m. Poo, *J. Physiol.* (*London*) **346**, 181 (1984)].
12. Photolysis of nitr-5 in loaded myocytes was induced with a 2-s pulse of steady light from a 75-W xenon lamp. The light passed through a UG1 filter, a dichroic mirror (XFO 400; DCLP), a 40× water immersion objective, and a no. 1 cover glass before reaching the cell. The photolysis efficiency of the light source through the microscope was 14 and 42% per second for Ca^{2+}-free and Ca^{2+}-bound nitr-5, respectively [R. S. Zucker, *Cell Calcium* **14**, 87 (1993)] (*8*). Assuming that nitr-5 accumulated in cells to ~1 mM and was 40% loaded with Ca^{2+}, so that the free intracellular Ca^{2+} concentration remained at 100 nM, we calculated that a 2-s UV exposure increases the Ca^{2+} concentration to ~320 nM, with a half-time of decay of ~15 s and with extrusion slowed by the extra buffering provided by nitr-5 [L. Landò and R. S. Zucker, *J. Neurophysiol.* **72**, 825 (1989)]. We have observed a larger photolysis-induced increase in Ca^{2+} by direct Ca^{2+} imaging with the fluorescent dye fluo-3 in separate myocyte cultures and with a 10-s UV light exposure through the same optics.
13. Synaptic currents were recorded from innervated muscle cells by whole-cell recording methods [O. P. Hamill, A. Marty, E. Neher, B. Sakmann, F. J. Sigworth, *Pfluegers Arch.* **391**, 85 (1981); S. H. Young and M.-m. Poo, *Nature* **305**, 634 (1983)] with a patch-clamp amplifier (Axopatch 1D; Axon Instruments). The solution inside the recording pipette contained 150 mM KCl, 1 mM NaCl, 1 mM $MgCl_2$, and 10 mM Hepes (pH 7.2). Recordings were made at room temperature in Hepes-buffered Ringer's solution, to which B27 supplement [1:100 (v/v); Gibco] was added to provide antioxidants to help prevent UV-induced cell damage. Extracellular stimulation of the presynaptic neuron was achieved with a patch electrode at the cell body under loose-seal conditions. The recorded membrane current was filtered at 1 kHz and stored on videotape, for later playback on a storage oscilloscope (TDS320; Tektronix) or an oscillographic recorder (TA240; Gould) and for analysis by computer with the SCAN program (J. Dempster, University of Strathclyde, Glasgow, UK).
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15. The site of innervation on spherical myocytes is centrally located on the substrate-facing surface and not at the apparent perimeter of the myocyte (*6*). Therefore, distances between the two myocytes were obtained by measuring the distance between the centers of the two myocytes along the neurite. Two myocytes may be located on the same neurite without branches, on two different branches of the same neurite, or on two different neurites, regardless of their relative distances from the cell body.
16. Local perfusion of culture medium was achieved with a large suction micropipette (inner diameter, 10 to 20 μm) connected by means of fluid-filled tubing to a fluid reservoir. The suction or outflow pressure was adjusted by changing the level of the fluid reservoir relative to that of the culture dish. The flow pattern was tested before the experiment with medium containing trypan blue (Fig. 4B) and was monitored during the experiment by observing the movement of small cell debris in the culture.
17. The rate of diffusion along the neurite can be estimated by the equation $s^2 = 2Dt$, where s is the average distance of diffusion over time t, and D is the diffusion coefficient. For a globular protein of 45 kD, such as ovalbumin, the diffusion coefficient in these *Xenopus* neurites was 15.8 ± 2.1 $\mu m^2/s$ [S. Popov and M.-m. Poo, *J. Neurosci.* **12**, 77 (1992)]. Thus, the average time required for diffusion of a cytosolic protein of 45 kD over a distance of 150 to 200 μm is ~12 to 20 min. Simple diffusional signaling between the synapses could thus account for the delay in the onset of synaptic depression observed at distant sites. Active retrograde transport could achieve a rate of 30 to 180 μm/min [R. D. Allen, J. Metuzals, I. Tasaki, S. T. Brady, S. P. Gilbert, *Science* **218**, 1127 (1982)], allowing more rapid signaling than diffusion.
18. The cytosolic signal that mediates the spread of depression should be distinguished from the initial retrograde signal provided by the postsynaptic myocyte after the increase in cytosolic Ca^{2+}, the nature of which remains obscure. In principle, the latter could be a membrane-permeant diffusible signal that acts locally at the synapse. Alternatively, the signal may be mediated by membrane-bound surface molecules that act across the synaptic cleft. Evidence suggests that nitric oxide (NO) may mediate a presynaptic modulation of transmitter secretion induced by long-term repetitive postsynaptic depolarizations at *Xenopus* neuromuscular junctions [T. Wang, Z. Xie, B. Lu, *Nature* **374**, 262 (1995)]. Our attempts to demonstrate a role for NO in hetereosynaptic or ACh-induced synaptic depression, which may involve mechanisms distinct from those activated by long-term repetitive depolarizations, have so far been unsuccessful.
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Structures of an MHC Class II Molecule with Covalently Bound Single Peptides

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The high-resolution x-ray crystal structures of the murine major histocompatibility complex (MHC) class II molecule, I-E^k, occupied by either of two antigenic peptides were determined. They reveal the structural basis for the I-E^k peptide binding motif and suggest general principles for additional alleles. A buried cluster of acidic amino acids in the binding groove predicted to be conserved among all murine I-E and human DR MHC class II molecules suggests how pH may influence MHC binding or exchange of peptides. These structures also complement mutational studies on the importance of individual peptide residues to T cell receptor recognition.

Antigen presentation by MHC class II molecules involves the intracellular generation of antigen peptide fragments, their binding to MHC molecules, and the transport of the complexes to the cell surface for recognition by the αβ T cell receptor (TCR) of $CD4^+$ T cells (*1*, *2*). Although MHC molecules bind peptides promiscuously, there often is a characteristic peptide binding motif for each MHC allele controlled by a few of the peptide amino acids (*3*, *4*). We have produced a number of soluble murine I-E^k molecules, each of which is homogeneously occupied by a different peptide tethered by means of a flexible protein linker to the NH_2-terminus of the β chain (*5*–*7*). Several of these proteins crystallized readily. Two yielded crystals suitable for x-ray diffraction analysis (*8*): one was occupied by a peptide derived from murine hemoglobin [Hb(64–76)] (*9*) and a second with a peptide from murine heat shock protein 70 [Hsp(236–248)] (*10*). The structures of these complexes (Fig. 1) were determined at 2.3 Å and 2.7 Å resolution, respectively (*11*–*13*).

The peptides, well resolved in both structures, are bound in a groove between the α helices of the MHC α1 and β1 domains, with both the NH_2- and COOH-termini of the peptides protruding from the ends of the groove. The path of the flexible linkers from the NH_2-termini of the peptides to the COOH-terminus of the β1 domain avoids the upper surface of the molecules, which accounts for their lack of interference in TCR recognition (*5*, *14*). The two I-E^k structures are similar to each other and, with the exception of minor backbone variations, to that of two human DR peptide structures, DR1 complexed with an influenza hemagglutinin peptide [HA(306–318)] (*15*) and DR3 complexed with the invariant chain CLIP peptide (*16*).

Despite the fact that the two I-E^k–peptide complexes crystallized in different lattices, the asymmetric unit in both cases was the same dimer of αβ heterodimers. However, the geometry of this dimer is different from that of the dimer seen in the DR1 and

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