16. M. P. Kamps and B. M. Sefton, *Anal. Biochem.* **176**, 22 (1989).
17. G. Munoz and S. H. Marshall, *ibid.* **190**, 233 (1990).
18. T. R. Hurley and B. M. Sefton, *Oncogene* **4**, 265 (1989).
19. Phosphorylated polypeptides were localized in the unfixed gel by brief autoradiography. The corresponding zones were cut out, homogenized, and eluted by overnight incubation at 37°C with 10 volumes of a solution containing 0.1 M NH_4HCO_3, 0.1% SDS, 1 mM PMSF, and 5 mM IAA. After addition of BSA (10 μg), the supernatants were lyophilized, and SDS was extracted with methanol. Purified polypeptides were dissolved in 40 μl of 0.1 M tris-HCl (pH 6.8) and incubated for 1 hour at 37°C with V8 protease (0.5 μg) (Boehringer Mannheim). The samples were mixed with an equal volume of sample buffer and analyzed by SDS-PAGE and autoradiography. Lanes 1 and 2, 56-kD polypeptides before V8 treatment; lanes 3 and 4, after V8 treatment. Lanes 1 and 3, 56-kD polypeptide isolated by MAb to CD59 (MEM-43); lanes 2 and 4, 56-kD polypeptide isolated by MAb to CD4 (MEM-16).
20. I. Štefanová, V. Hořejší, I. J. Ansotegui, W. Knapp, H. Stockinger, unpublished data.
21. We thank I. Hilgert and O. Majdic for providing MAbs, D. J. Anstee for MAb to CD55 BRIC128, A. Bernard for MAb to CD29 K20, P. Dráber for MAb to Thy-1 1aG4, B. Sefton for rabbit antiserum to $p56^{lck}$, E. M. Shevach for MAb to Ly-6 D7, and R. Vilella for MAb to CD55 143-30. Supported by the Austrian Research Council, a fellowship from the Sandoz Research Institute Vienna, Austria (I.Š.), and the Swedish Institute and the Swedish Cancer Society (I.J.A.).

28 May 1991; accepted 26 August 1991

Activity-Dependent Synaptic Competition in Vitro: Heterosynaptic Suppression of Developing Synapses

YI-JIUAN LO AND MU-MING POO*

The development and stability of synaptic connections in the nervous system are influenced by the pattern of electrical activity and the competitive interaction between the adjacent nerve terminals. To investigate this influence, a culture system of nerve and muscle cells has been developed in which a single embryonic muscle cell is coinnervated by two spinal neurons. The effect of electrical activity on the synaptic efficacy was examined after repetitive electrical stimulation was applied to one or both neurons. Brief tetanic stimulation of one neuron resulted in immediate functional suppression of the synapse made by the unstimulated neuron innervating the same muscle cell. This heterosynaptic suppression was largely absent when the tetanic stimulation was applied concurrently to both neurons. This result demonstrates that activity-dependent synaptic competition can be studied in vitro at a cellular level.

THE EFFICACY OF SYNAPTIC TRANSmission is susceptible to activity-dependent modulation, a process that underlies much of the plasticity in synaptic function (*1*). In developing nervous systems the pattern of electrical activity also exerts a critical influence on the stabilization and elimination of nerve connections (*2*). In neonatal animals each skeletal muscle fiber is innervated by several axons; all but one are eliminated as the animal matures (*3*). The process of synapse elimination is markedly affected by the activity of the motor nerves (*4*). However, little is known about the cellular mechanisms underlying the activity dependence of synaptic elimination and the nature of competitive interactions among coinnervating nerve terminals. We have designed a cell culture system to study the synaptic physiology associated with the activity-dependent competition between developing neurons.

Cultures of embryonic spinal neurons and myotomal muscle cells were prepared from 1-day-old *Xenopus* embryos (*5*). Functional synaptic contacts between the nerve and muscle cells are established within 24 hours after cell plating (*6*). Experiments were carried out on mononucleated spherical myocytes, which were innervated by two nearby cocultured spinal neurons (see Fig. 1A). The use of the spherical myocyte (average diameter, 35 μm) ensured close proximity of the nerve terminals on the myocyte surface. The synaptic efficacy was examined by whole-cell voltage-clamp recording (*7*) of evoked synaptic currents (ESCs) in the innervated myocyte.

ESCs elicited by the presynaptic neurons were first measured by low-frequency test stimuli. A brief episode of tetanic stimulation was then applied to one neuron, and the synaptic efficacy of both neurons was compared afterward with test stimuli (Fig. 1B). The amplitudes of the ESCs elicited by neurons 1 and 2 averaged 3.4 ± 0.3 (±SEM; $n = 7$) and 1.6 ± 0.4 nA (±SEM; $n = 8$), respectively, at the onset of the experiment. A train of 80 impulses were then elicited in neuron 2 over a period of 40 s. After the stimulus, the synaptic efficacy of neuron 2 increased to an average of 2.6 nA, but that of the unstimulated neuron 1 decreased to 1.7 nA. Over the next 30 min, the ESCs of neuron 1 remained at a suppressed level, while those of neuron 2 remained elevated. Figure 1C depicts the similar change in ESC amplitudes in three such stimulation experiments.

We have performed 13 experiments involving preferential tetanic stimulation. The number of nerve impulses initiated during the tetanus varied between 50 to 100 (at 2 to 5 Hz) (Fig. 2A). In all eight cases where the initial synaptic efficacy was higher at the stimulated synapse (S), significant suppression of the unstimulated synapse (US) was found for all eight synapses within the first 10 min after the tetanic stimulation (rank sum test, $P < 0.05$). In contrast, only one out of eight stimulated synapses showed significant reduction. Significant suppression of unstimulated synapse was also found in three out of five cases where the stimulated synapses had lower initial efficacy. None of the stimulated synapses showed any suppression, and three showed significant potentiation. With the exception of three cases where the suppressed synapse showed substantial recovery of the initial synaptic response with time, persistent suppression was observed for as long as the recording could be made (up to 1 hour; Fig. 2B). The origin of the variability in the extent of suppression among different synapses is unknown. It may be related to the physical proximity of the coinnervating neurites on the muscle surface, which cannot be determined with the phase-contrast optics used here.

In the second series of experiments, the same tetanic stimulation was applied concurrently to both neurons innervating the same myocyte, and the ESCs elicited by each neuron before and after the tetanus were compared. From a total of seven pairs of synapses that were tetanized synchronously (100 pulses, 2 Hz), only three out of 14 synapses showed significant reduction of mean ESC amplitude after the tetani ($P < 0.05$, rank sum test) (Fig. 2A). This result indicates that heterosynaptic suppression is largely absent after synchronous tetanic stimulation. The importance of the synchrony in pre- and postsynaptic activities for protecting the synapse from suppression is illustrated by the experiments in Fig. 3. Simultaneous stimulation (1 + 2) of both neurons produced little or no effect on the ESCs elicited by either neuron, but the same train of stimuli delivered to the neurons asynchronously (1 then 2, with a 100-ms delay between the stimuli applied to the two neurons) resulted in immediate suppression of one or both neurons. Asynchronous tetani often led to asymmetric suppression of the two neurons, as shown in all three cases illustrated. In cases (B) and (C), substantial recovery was observed within 10 min after initial suppression. We did not observe any correlation between the asymmetry in suppression and the sequence of

Department of Biological Sciences, Columbia University, New York, NY 10027.

*To whom correspondence should be addressed.

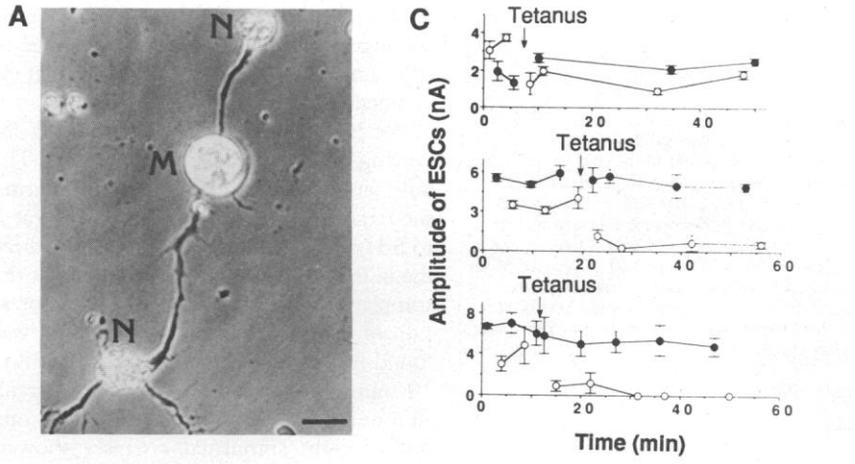

Fig. 1. Heterosynaptic suppression of synaptic efficacy. (**A**) Phase contrast micrograph of a typical 1-day-old *Xenopus* nerve-muscle culture. A spherical myotomal myocyte (M) was innervated by two cocultured spinal neurons (N). Bar, 20 μm. (**B**) The membrane current (continuous trace) of a doubly innervated myocyte [holding potential (V_h) = −70 mV, filtered at 150 Hz]. Neurons 1 and 2 were stimulated alternatively at low frequency (0.05 Hz) to induce ESCs (small triangles). Events not marked are currents that resulted from spontaneous ACh secretion from either neuron (*6*). High-frequency stimulation (2 Hz, 80 pulses) was applied to neuron 2 at the time marked by the thick bar. Samples of ESCs are shown by the traces below at higher time resolution (filtered at 2.5 kHz). Scales: slow trace, 2 nA and 40 s; fast trace, 1 nA and 10 ms. (**C**) Results from three experiments in which the stimulated synapse (filled circles) showed either lower (upper graph) or higher (two lower graphs) efficacy than the unstimulated synapse (open circles) before the tetanus. The upper case depicts the same experiment as in (B). The tetanus applied was 80, 100, and 50 pulses at 2, 2, and 5 Hz, respectively. Error bars represent SEM; n = 3 to 6.

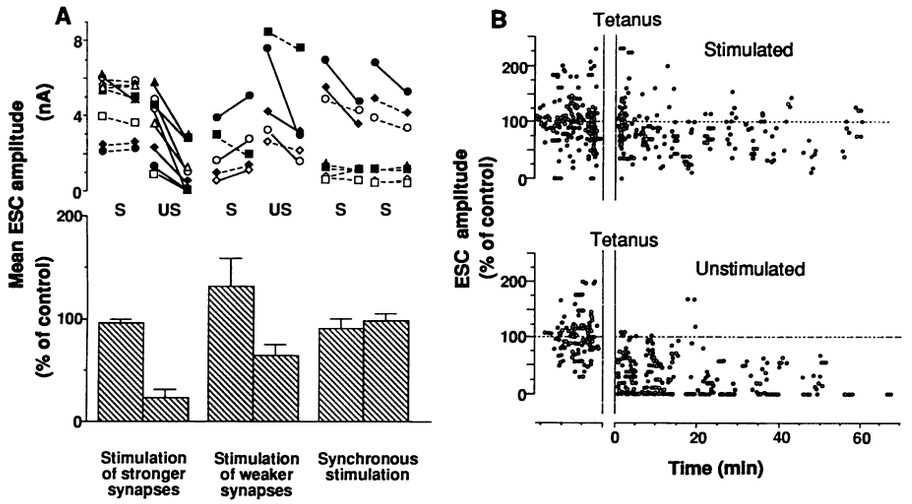

Fig. 2. Changes in the mean amplitude of ESCs before and after tetanic stimulation was applied to one or both coinnervating neurons. (**A**) Summary of results from 13 experiments on preferential stimulation and seven experiments on synchronous stimulation. Each point is the mean amplitude of ESCs elicited by test pulses during a period of 5 to 10 min before (left point of a connected pair) or immediately after (right point of the connected pair) the tetanic stimulation. Data from the stimulated (S) and unstimulated synapses (US) are shown. Solid lines indicate significant difference after stimulation (rank sum test, $P < 0.05$); dotted lines represent no significant difference. The histograms below depict the average value (± SEM) of the change in mean ESC amplitude, normalized for each synapse as the percentage of the pretetanized value, for the data directly above each histogram. Significant difference between the stimulated and unstimulated synapses was found for the two groups of preferential stimulation experiments ($P < 0.01$ and 0.02, two-tailed t test), and no significant difference was found for synapses that were tetanized synchronously ($P > 0.2$). Tetanic stimulation was a train of pulses at 2 to 5 Hz, applied for 10 to 50 s (total number of pulses between 50 to 100). No consistent difference was found among the results we obtained by using different tetanus parameters within this range. (**B**) The time course of heterosynaptic suppression. For all 13 preferential stimulation experiments, the amplitude of ESCs recorded from each synapse was normalized against the mean amplitude of the last six ESCs recorded before the tetanus. The slight downward trend of the ESCs at stimulated synapses may be attributed to either the synaptic depression from the test stimuli or the wash-out effect of the whole-cell recording.

tetanic pulses applied to the two neurons. Instead, the more severely suppressed synapses exhibited lower average synaptic responses before the tetani (A and B) or more rapid depression during the tetani (C), suggesting that effective heterosynaptic suppression requires not only presynaptic activity but also efficacious postsynaptic activation.

Polyneuronal innervation of skeletal muscle fibers before synapse elimination is normally restricted to the same endplate (*8*). In mature mammalian muscle fibers, coexisting nerve terminals from different motoneurons are physically separated (*9*). Synaptic competition thus appears to operate effectively only among nearby synapses. In the present study we tested this idea by using spindle-shaped myocytes that received innervation from two neurons at distinctly separate sites on their surfaces. We found complete absence of heterosynaptic suppression in all three cases examined. Although the precise configuration of the nerve terminal cannot be resolved in these experiments, the separation of nerve terminals (at the initial site of muscle contact) (Fig. 4) was in the range of 50 to 75 μm. This result suggests that physical proximity may be a crucial factor for heterosynaptic suppression.

The heterosynaptic suppression induced by preferential tetanic stimulation could result from either direct interaction between the coinnervating nerve terminals or retrograde modulation mediated through postsynaptic muscle cells. The synaptic responses we observed in the myocyte were monosynaptic in nature. The synaptic delay of the stimulated and the suppressed synapses was 1.64 ± 0.15 and 1.54 ± 0.14 ms (mean ± SEM; six synapses each), respec-

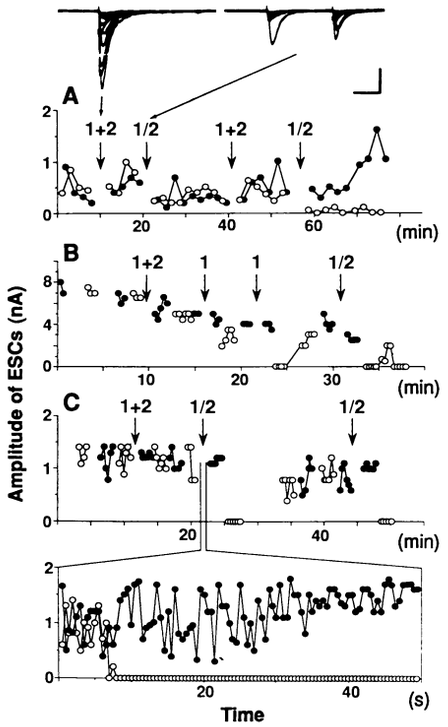

Fig. 3. Effects of synchronous and asynchronous stimulation on ESCs in three separate experiments (A, B, and C). Recordings were similar to those in Fig. 1B, except that the same tetanic stimulation (2 Hz, 50 s) was applied to both neurons innervating the same myocyte, either synchronously (1 + 2) or asynchronously (1/2, 100-ms interval). Insets of oscilloscopic traces above depict samples of ESCs, recorded in the myocyte during the synchronous (left) and asynchronous (right) tetanic stimulation, respectively. Scales: 1 nA and 40 ms. Open and closed symbols refer to data from cells 1 and 2, respectively. In (A) each data point represents average value of three measurements of ESCs over 1 min. In (B) and (C), individual measurements are shown. (**A**) Repeated synchronous and asynchronous stimulation. (**B**) Synchronous stimulation, then the same tetanus applied selectively to neuron 1, followed by asynchronous stimulation of both neurons. (**C**) Repeated asynchronous stimulations after synchronous stimulation. (Inset) The amplitude of the ESCs for both neurons during the period of tetanic stimulation. The suppressed neuron 2 showed rapid depression during the tetanus. Tetanus-induced synaptic depression recovered quickly after the tetani; thus, it cannot account for the persistent posttetanic suppression observed.

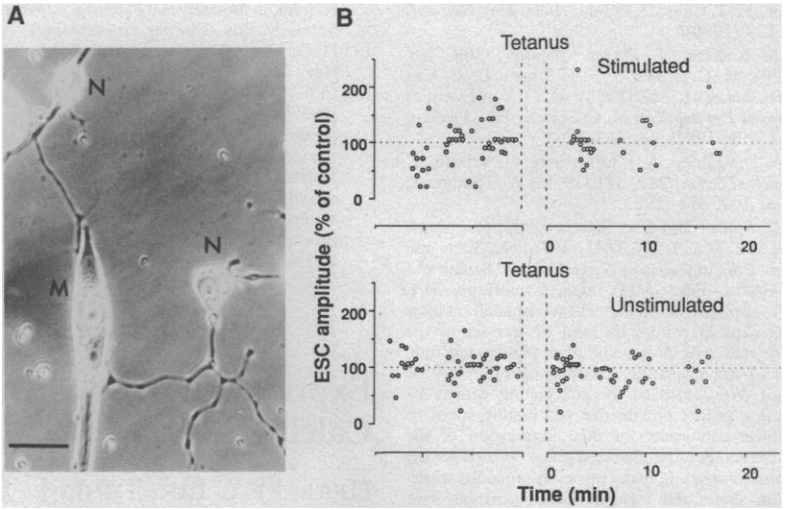

Fig. 4. Absence of heterosynaptic suppression between distant nerve terminals. (**A**) Phase-contrast micrograph of a spindle-shaped myocyte (M), which was innervated by two spinal neurons (N) at its two ends. Bar, 20 μm. (**B**) Data from three preferential stimulation experiments, with myocytes similar to that in (A) and a protocol as in Fig. 1B. The amplitudes of ESCs elicited by the stimulated and unstimulated neurons were normalized as for Fig. 2B.

tively. These were not significantly different from the synaptic delay observed in singly innervated myocytes (1.62 ± 0.30 ms, n = 6) ($P > 0.5$, t test). Moreover, when one of the neurons was damaged at the end of the experiment and its synaptic response disappeared, responses could still be elicited by the other neuron, suggesting that the connections to the myocyte were monosynaptic. Whole-cell recordings at the neuronal soma (five cases) detected no synaptic response when impulses were initiated in the other neuron. Nevertheless, we cannot exclude the possibility that evoked secretion of acetylcholine (ACh) from the nerve terminal is modulated by local electrical or humoral interactions among presynaptic neuritic processes. Alternatively, postsynaptic activation may have modified the ACh receptor topography (*10*) or induced the release of retrograde regulatory factors, both of which could alter synaptic efficacy. Since these studies were carried out under voltage-clamp conditions, postsynaptic depolarization is not required for the induction of heterosynaptic suppression. However, other events associated with activation of postsynaptic ACh receptors may be responsible for the suppression.

The role of electrical activity in synaptic competition has been a subject of many recent studies (*11*). Altering the amount of electrical activity in one of the coinnervating axons in neonatal rats or in adult animals after nerve regeneration has been reported to confer a competitive advantage (*12*) or disadvantage (*13*) on motoneurons during the elimination of polyneuronal innervation. Our results are consistent with the view that electrical activity confers a competitive advantage. The effect of heterosynaptic suppression accumulates with repeated tetanic stimulation of the same synapse (Fig. 3B). It remains to be determined whether long-term functional suppression may eventually lead to permanent structural alteration, such as the retraction of the nerve terminal. At multiply innervated adult rat and *Xenopus* skeletal muscle fibers, there is also physiological evidence for heterosynaptic interactions between nerve terminals coinnervating the same muscle fiber, although only short-term effects have been reported (*14*).

In 1949, Hebb (*15*) postulated that the strength of a synapse may be enhanced if the activity at the synapse repeatedly takes part in exciting the postsynaptic cell. This idea may be extended to suggest that a synapse is strengthened or stabilized by its own activity, but suppressed or eliminated by asynchronous activity from other synapses on the same postsynaptic cell. Our results are consistent with this generalized interpretation of Hebb's postulate (*16*). In the development of ocular dominance columns in the visual cortex, a key factor in synaptic competition may reside in the relative amount and the coincidence of the electrical activity in the competing pathways (*17*). Our observation that synchronous stimulation of the coinnervating neurons protects them from synaptic suppression demonstrates in vitro that the lack of synchrony between the pre- and postsynaptic activity leads to synaptic suppression.

In conclusion, we have developed an in vitro system for studying activity-dependent synaptic competition at a cellular level. The simplicity of the culture environment may prove to be useful in elucidating the cellular mechanisms underlying activity-dependent changes in both pre- and postsynaptic cells. The rapid appearance and the persistence of heterosynaptic suppression underscore the delicate and plastic nature of early synaptic connections.

REFERENCES AND NOTES

1. R. S. Zucker, *Annu. Rev. Neurosci.* **12**, 13 (1989); T. H. Brown, E. W. Kairiss, C. L. Keenan, *ibid.* **13**, 475 (1990); D. V. Madison, R. C. Malenka, R. A. Nicoll, *ibid.* **14**, 379 (1991).
2. D. Purves and J. Lichtman, *Principles of Neural Development* (Sinuar, Sunderland, MA, 1985); C. Shatz, *Neuron* **5**, 745 (1990); M. Constantine-

Paton, H. T. Cline, E. Debski, *Annu. Rev. Neurosci.* **13**, 129 (1990).
3. P. A. Redfern, *J. Physiol. (London)* **209**, 701 (1970); M. C. Brown, J. K. S. Jansen, D. C. Van Essen, *ibid.* **261**, 387 (1976); D. C. Van Essen, in *Neuronal Development*, N. C. Spitzer, Ed. (Plenum, New York, 1982), pp. 333–376.
4. R. A. D. O'Brien, A. J. C. Ostberg, G. Vrbova, *J. Physiol. (London)* **282**, 571 (1978); W. Thompson, *Nature* **302**, 614 (1983).
5. 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 glass cover slips in culture medium by transfer of the dissociated cells with a fine glass pipette and used after a 1-day incubation at room temperature (20° to 22°C). We controlled the cell plating density by selecting proper pipette size and plating speed to optimize conditions for dual innervation of the muscle cell. Because uncoated glass was used for the culture substratum, many myocytes remained spherical in shape and formed normal synapses with cocultured spinal neurons (*6*). Cultures were screened for dual innervation of the myocyte like that shown in Fig. 1A.
6. Z. Xie and M.-m. Poo, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7069 (1986); J. Evers, M. Laser, Y. Sun, Z. Xie, M.-m. Poo, *J. Neurosci.* **9**, 1523 (1989); J. Buchanan, Y. Sun, M.-m. Poo, *ibid.*, p. 1544.
7. O. P. Hamill *et al.*, *Pfluegers Arch.* **391**, 85 (1981); S. H. Young and M.-m. Poo, *Nature* **305**, 634 (1983); Y. Sun and M.-m. Poo, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2540 (1987). Recordings were made at room temperature in culture medium. The solution inside the recording pipette contained 150 mM KCl, 1 mM NaCl, 1 mM $MgCl_2$, and 10 mM Hepes buffer (pH 7.2). In all recordings, the membrane current was monitored by a patch-clamp amplifier (List EPC-7). The data were stored on a videotape recorder for later playback on a storage oscilloscope (Tektronix 5113) and for analysis by a microcomputer. Chart recording from an oscillographic recorder (Gould RS 3200) was used to monitor the sequence of events during the experiment but was not used for analysis of ESC amplitudes because of its slow response. We elicited the synaptic currents by stimulating presynaptic neurons extracellularly at the soma with heat-polished glass micropipettes. Two separate pipettes were used to stimulate the two coinnervating neurons. The pipettes were filled with Ringer's solution. The duration of these experiments was limited because these fragile embryonic neurons did not sustain prolonged contact by the stimulating electrode. Many of these developing synapses are also highly susceptible to synaptic depression under repetitive stimulation. The test stimuli were thus kept at a low frequency (0.05 Hz) and applied only for brief intervals intermittently. In control recordings of the evoked responses over long duration (up to 1 hour), we found that ESCs frequently exhibit a slight reduction in amplitude. We attribute this to the wash-out effect of the whole-cell recording.
8. M. C. Brown, J. K. S. Jansen, D. C. Van Essen, *J. Physiol. (London)* **261**, 387 (1976).
9. D. Kuffler, W. Thompson, J. K. S. Jansen, *Brain Res.* **138**, 353 (1977); T. Lømo, *Trends Neurosci.* **3**, 126 (1980).
10. M. M. Rich and J. W. Lichtman, *J. Neurosci.* **9**, 1781 (1989); M.-m. Poo and S. H. Young, *J. Neurobiol.* **21**, 157 (1989).
11. T. Magchielse and E. Meeter, *Dev. Brain Res.* **25**, 211 (1986); P. G. Nelson, C. Yu, R. Douglas Fields, E. A. Neale, *Science* **244**, 585 (1989); P. G. Nelson, R. Douglas Fields, C. Yu, E. A. Neale, *J. Neurobiol.* **21**, 138 (1990); A. A. Herrera and M. J. Werle, *ibid.*, p. 73; W. J. Betz, R. R. Ribchester, R. M. A. P. Ridge, *ibid.*, p. 21; D. C. Van Essen, H. Gordon, J. M. Soha, S. E. Fraser, *ibid.*, p. 223.
12. R. M. A. P. Ridge and W. J. Betz, *J. Neurosci.* **4**, 2614 (1984); R. R. Ribchester and T. Taxt, *J. Physiol. (London)* **344**, 89 (1983); *ibid.* **347**, 497 (1984).
13. E. M. Callaway, J. M. Soha, D. C. Van Essen, *Nature* **328**, 357 (1987).
14. W. J. Betz, M. Chua, R. M. A. P. Ridge, *J. Physiol. (London)* **418**, 25 (1989); R. Dunia and A. A. Herrera, in *Motoneuronal Plasticity*, A. Wernig and J. Carmody, Eds. (Elsevier, Amsterdam, in press).
15. D. O. Hebb, *The Organization of Behavior* (Wiley, New York, 1949), p. 62.
16. In G. Stent's interpretation of the Hebb postulate [*Proc. Natl. Acad. Sci. U.S.A.* **70**, 997 (1973)], postsynaptic depolarization is a necessary part of the cellular events that lead to synaptic elimination. In our study, heterosynaptic suppression was observed under voltage-clamp conditions, thus excluding the involvement of membrane depolarization. However, activation of ACh receptors could lead to postsynaptic changes, such as Ca^{2+} influx [E. R. Decker and J. A. Dani, *J. Neurosci.* **10**, 3413 (1990)], in the absence of membrane depolarization.
17. D. H. Hubel and T. N. Wiesel, *J. Neurophysiol.* **28**, 1041 (1965); M. P. Stryker and W. A. Harris, *J. Neurosci.* **6**, 2117 (1986).
18. We thank D. Sanes and W. Betz for helpful discussions. Supported by grants from National Institutes of Health (NS 22764), National Science Foundation (BNS 13306, BNS 12398), and Office of Naval Research (N00014-90-J1865).

4 June 1991; accepted 9 September 1991

Expression Cloning of an Adenylate Cyclase–Coupled Calcitonin Receptor

HERBERT Y. LIN, TONJA L. HARRIS, MERILEE S. FLANNERY, ALEJANDRO ARUFFO, EUGENE H. KAJI, ALAN GORN, LEE F. KOLAKOWSKI, JR., HARVEY F. LODISH,* STEVEN R. GOLDRING

A calcitonin receptor complementary DNA (cDNA) was cloned by expression of a cDNA library from a porcine kidney epithelial cell line in COS cells. The 482–amino acid receptor has high affinity for salmon calcitonin (dissociation constant $K_d \approx 6$ nM) and is functionally coupled to increases in intracellular cyclic adenosine monophosphate (cAMP). The receptor shows no sequence similarity to other reported G protein–coupled receptors but is homologous to the parathyroid hormone–parathyroid hormone–related peptide (PTH-PTHrP) receptor, indicating that the receptors for these hormones, which regulate calcium homeostasis, represent a new family of G protein–coupled receptors.

CALCITONIN IS A 32–AMINO ACID hormone that lowers serum calcium concentrations by increasing renal calcium excretion and inhibiting osteoclast-mediated bone resorption (*1*). A major pathway for intracellular signaling by calcitonin is through increases in cytosolic cAMP (*2*). The calcitonin receptor is thought to couple to $G_{s\alpha}$, the heterotrimeric guanosine triphosphate (GTP)–binding protein that is sensitive to cholera toxin (*3*). The receptor can also couple to an additional signaling pathway via a pertussis toxin–sensitive G protein in isolated osteoclasts (*4*) and in LLC-PK_1 cells (*5*).

We cloned the calcitonin receptor by expression in COS cells using a previously reported strategy (*6*). A size-fractionated cDNA library was constructed (7) from LLC-PK_1 cells (*8*), a porcine kidney epithelial cell line that expresses $\sim 3 \times 10^5$ calcitonin receptors per cell with an apparent dissociation constant (K_d) of ~3 nM (Fig. 1A). Pools of mini-prep cDNA (*9*) containing 10^4 recombinants were transfected into COS cells and screened for binding to radioiodinated salmon calcitonin (SCT) by emulsion autoradiography (*10*). After screening 30 pools representing 3×10^5 clones, we identified two positive pools from which two positive clones with cDNA inserts 2.2 and 3.9 kilobases in length were isolated. The 2.2-kilobase clone (3J8-14-F1) was a truncated version of the 3.9-kilobase clone (2B5-0-1) but encoded the same open reading frame. We studied and characterized the properties of the 2.2-kilobase clone.

Radioiodinated SCT binds to LLC-PK_1 cells and to COS cells transfected with the cloned calcitonin receptor (CTR) cDNA (Fig. 1). Transfected COS cells expressed $\sim 2 \times 10^6$ receptors per cell (assuming 10% of the transfected cells expressed the receptor) with an apparent K_d of ~6 nM, similar to that expressed by LLC-PK_1 cells. Bovine PTH(1-34) [see (*11*)] did not compete for binding of radioiodinated SCT to the CTR transfectants.

The cloned receptor is functionally coupled to increase in intracellular cAMP (Fig.

H. Y. Lin, E. H. Kaji, H. F. Lodish, Whitehead Institute for Biomedical Research, Cambridge, MA 02142, and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.
T. L. Harris, M. S. Flannery, A. Aruffo, A. Gorn, Department of Medicine, Harvard Medical School and Medical Services (Arthritis Unit), Massachusetts General Hospital, Boston, MA 02114.
L. F. Kolakowski, Jr., Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.
S. R. Goldring, Department of Medicine, Harvard Medical School and Medical Services (Arthritis Unit), Massachusetts General Hospital, Boston, MA 02114, and New England Deaconess Hospital, Boston, MA 02215.

*To whom correspondence should be addressed.