min with argon. Silane (300 μl) was added in a beaker near to the surfaces and left overnight to coat the cover glass. Immediately before use, the silanized surfaces were rinsed in deionized water and allowed to dry. We used λ DNA cI857 Sam7 (250 ng/μl) and bacterial chromosomal *E. coli* DNA (strain GN48 in 1% low-melting agarose plugs). The *E. coli* DNA concentration was determined by optical density to be 3.6 ng/μl. Both DNAs were stained in a 5:1 or 10:1 ratio [base pairs per dye (YOYO-1) molecule; Molecular Probes (Eugene, OR)]. Immediately before use, stained DNA was diluted in a 50 mM MES buffer (pH 5.5) to a final concentration of 0.2 pM. Typically, 10 μl of that solution was loaded onto a silanized cover slip. An unsilanized, but well-rinsed, cover slip then was floated gently on top and allowed to incubate at least 15 min at ambient temperature and humidity before examination on the microscope. The binding of DNA on silanized surfaces was dependent on the pH: the number of bound molecules in 50 mM MES buffer (pH = 5.5) is ≈10^4 times that in 50 mM tris buffer (pH = 8.0). Video images were taken on an inverted fluorescence microscope equipped with a 60×, 1.4 numerical aperture objective, 0.9 to 2.25× video zoom, 100-W xenon source, optimized fluorescence filter set, and an intensified charge-coupled device camera. Video signals were averaged (typically 16 frames) and contrast-enhanced electronically by a commercial box and stored on tape. Images were digitized on a personal computer and analyzed with custom-written software.

7. J. B. Brzoska, N. Shahidzadeh, F. Rondelez, *Nature* **360**, 719 (1992).
8. M. Yanagida, Y. Hiraoka, I. Katsura, *Cold Spring Harbor Symp. Quant. Biol.* **47**, 177 (1983); M. Yanagida *et al.*, *Applications of Fluorescence in the Biomedical Sciences* (Liss, New York, 1986), pp. 321–345.
9. The probability of anchoring at both ends increases with incubation time.
10. This fragment (1 million base pairs) of DNA is covalently bound to the silanized surface at only one of its 5′ ends. All other methods of covalent (chemical) attachment of oligonucleotides to a surface that we know of lead to multiple parasitic bindings [see V. Lund, R. Schmid, D. Rickwood, E. Hornes, *Nucleic Acids Res.* **16**, 10861 (1988)].
11. This force is typically of the order of an electron volt per angstrom [see J. N. Israelachvili, *Intermolecular and Surface Forces* (Academic Press, San Diego, CA, 1985), pp. 24–28].
12. D. Bensimon, A. Simon, V. Croquette, A. Bensimon, in preparation.
13. For a molecule of length L and velocity v, the shear force is $F \approx \eta L v$, where η is the viscosity (10^{-2} poise for water). Taking a lower estimate for the rupture force to be ≈ 10^{-9} N, we find that shear velocities of a few hundred micrometers per second are enough to break an *E. coli* chromosomal DNA fragment.
14. C. Bustamante, D. Keller, G. Yang, *Curr. Opin. Struct. Biol.* **3**, 363 (1993).
15. Stained λ DNA (7×10^6 molecules) was loaded onto a silanized surface and observed as described in Fig. 1. The molecules were then imaged with a scanning force microscope, in immersion oil with a standard pyramidal tip, V-shaped cantilever (100 μm long, 0.6 μm thick) with a spring constant k of 0.21 N/m. The piezoelectric tube has a 75-μm scanning range. The image shown is a part of a 4 μm by 4 μm scan (512 by 512 pixels). It was taken in topographic mode, at a 5-Hz line frequency.
16. From the full width at half maximum of the profile, one estimates the lateral resolution to be of the order of 30 nm, 10 times better resolved than with standard optical microscopy. Furthermore, we have verified that the DNA was fully linearized without knots by screening the length of a molecule by SFM.
17. R. E. Dickerson, H. R. Drew, B. N. Conner, M. L. Kopka, P. E. Pjura, *Cold Spring Harbor Symp. Quant. Biol.* **47**, 13 (1983).
18. R. K. Saiki *et al.*, *Science* **230**, 1350 (1985); M. A. Innis *et al.*, Eds., *PCR Protocols* (Academic Press, San Diego, CA, 1990).
19. J. A. Langdale and A. D. B. Malcolm, *Gene* **36**, 201 (1985).
20. J.-L. Guesdon, *J. Immunol. Methods* **150**, 33 (1992).
21. The right cohesive end of λ DNA was 3′ end-labeled with Dig-dUTP by terminal transferase and ligated to λ DNA. Silanized surfaces were treated with 60 μl of Protein A (1 mg/ml) in water for 2 hours at room temperature, rinsed three times in deionized water, and then incubated with 60 μl of anti-Dig solution (0.1 mg/ml) for 2 hours at room temperature. Surfaces were then rinsed three times in deionized water. Then 4 μl of YOYO-1–stained molecules (1.6 pM in 10 mM tris, pH = 8) were deposited on these prepared surfaces, covered with a rinsed unsilanized cover slip, and left to incubate in a humid atmosphere at room temperature for 1 hour, then left to dry.
22. P. Lichter *et al.*, *Science* **247**, 64 (1990).
23. I. Parra and B. Windle, *Nature Genet.* **5**, 17 (1993).
24. Furthermore, the possible detection of a single protein or antibody through coupling to a DNA "flag" and the subsequent combing of the bound DNA could be used to improve the sensitivity of protein detection, which is usually done by immunological assays (enzyme-linked immunosorbent assay) with a sensitivity of the order of 10^{-17} mol [see H. Labrousse *et al.*, *J. Immunol. Methods* **48**, 133 (1982)].
25. We thank J. P. Changeux, A. Ullmann, F. Rougeon, M. Hofnung, and M. Yaniv for scientific discussion and support. A.B. is a Human Frontier Science Postdoctoral Fellow. A.S. is a U.S. National Science Foundation Postdoctoral Fellow. Laboratoire de Physique Statistique and Laboratoire de Physique de la Matière Condensée are laboratories associated with the Centre National de la Recherche Scientifique and the Universités Paris 6 and Paris 7.

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Release of Adenosine by Activation of NMDA Receptors in the Hippocampus

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Adenosine is present in the mammalian brain in large amounts and has potent effects on neuronal activity, but its role in neural signaling is poorly understood. The glutamate receptor agonist *N*-methyl-D-aspartate (NMDA) caused a presynaptic depression of excitatory synaptic transmission in the CA1 region of guinea pig hippocampal slices. This depression was blocked by an adenosine A1 receptor antagonist, which suggests that activation of the NMDA subtype of glutamate receptor raises the concentration of extracellular adenosine, which acts on presynaptic inhibitory A1 receptors. Strong tetanic stimulation caused a heterosynaptic inhibition that was blocked by both NMDA and A1 receptor antagonists. Enkephalin, which selectively inhibits interneurons, antagonized the heterosynaptic inhibition. These findings suggest that synaptically released glutamate activates NMDA receptors, which in turn releases adenosine, at least in part from interneurons, that acts at a distance to inhibit presynaptically the release of glutamate from excitatory synapses. Thus, interneurons may mediate a widespread purinergic presynaptic inhibition.

Adenosine is a purine that is present in large amounts in the mammalian brain (*1*). When applied to brain slices, adenosine exerts a powerful presynaptic inhibition of excitatory synaptic transmission, which is mediated by A1 receptors (*2*). Furthermore, the concentration of extracellular adenosine is increased during hypoxia (*3*), synaptic stimulation (*4*), and the application of glutamate (*5*, *6*). The NMDA subtype of glutamate receptor is necessary for glutamate-evoked elevation of the concentration of adenosine, and this effect is Ca^{2+}-dependent (*6*). However, the source of this adenosine and the physiological mechanisms that control its concentration in the extracellular space are not known. We used here the hippocampal slice preparation in conjunction with electrophysiological techniques to address these issues.

We used standard techniques for preparing guinea pig hippocampal slices (*7*). Application of NMDA to the bath caused a depression in excitatory synaptic transmission, as described (*8*). Although the depression of field potentials is partly due to the direct depolarization of the postsynaptic cells, a number of observations indicate that this cannot fully account for the depression. First, the depression was of similar magnitude when the postsynaptic depolarization at the soma was prevented with whole-cell voltage clamping (Fig. 1). Second, the inhibition lasted longer than the inward current induced directly by NMDA (Fig. 1B; compare top and bottom graphs). Third, the inhibition was associated with a clear increase in paired-pulse facilitation (PPF) of excitatory transmission (Fig. 1, A and C) (227 ± 36% of the control, n = 11), a presynaptic phenomenon that is very sensitive to changes in transmitter release. Fourth, after the application of NMDA there was a marked, transient reduction of the frequency of spontaneous excitatory postsynaptic currents (EPSCs) (n = 5) (Fig. 1D). During this reduction in frequency, there was no significant change

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in the distribution of amplitudes of spontaneous EPSCs in any of the five experiments ($P > 0.05$, Kolmogorov-Smirnov test) (Fig. 1E).

Our findings suggest that a brief application of NMDA results in a presynaptic inhibition of excitatory synapses that outlasts the direct postsynaptic action and suggest that the inhibition may be mediated by a substance released by NMDA receptor activation. We first considered nitric oxide (NO), because NMDA can release NO (9) and NO can inhibit excitatory synapses (*10*). However, the inhibitor of NO synthase *N*-ω-nitro-L-arginine (200 μM) failed to alter the inhibitory action of NMDA ($n = 4$). Another possible candidate for mediating this inhibitory action is γ-aminobutyric acid (GABA) acting on presynaptic $GABA_B$ receptors, but the selective $GABA_B$ receptor antagonist CGP 35348 (500 μM) had no effect on the NMDA-induced depression ($n = 3$). Because NMDA can increase the concentration of adenosine (6) and adenosine inhibits excitatory synapses by acting on presynaptic A1 receptors (2), we tested the effect of the adenosine A1 receptor antagonist 8-cyclopentyltheophylline (CPT) on NMDA-induced inhibition. This specific antagonist considerably enhanced baseline synaptic transmission as a result of its blockade of the tonic presynaptic inhibitory action of ambient adenosine (*11*). In the presence of CPT, the NMDA-induced inhibition was markedly reduced ($n = 9$) (Fig. 1F) (*12*).

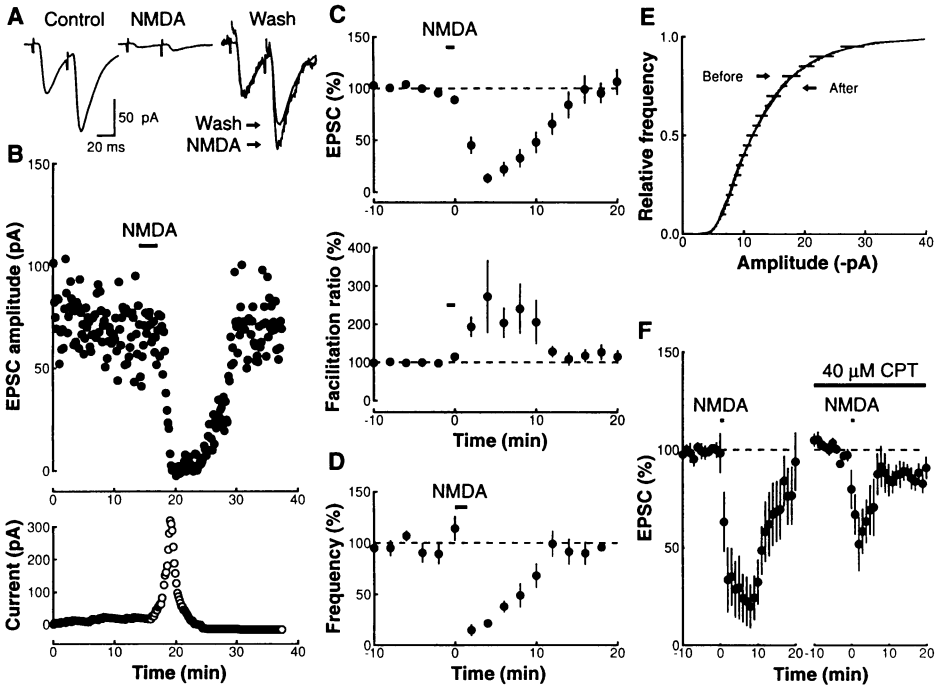

Fig. 1. NMDA induces a presynaptic depression of CA1 excitatory synaptic transmission, which is blocked by an adenosine A1 receptor antagonist. (**A**) Sample traces of whole-cell recording from a CA1 pyramidal cell (holding potential = −80 mV). Paired-pulse facilitation was elicited by stimulating the input twice with a 40-ms interval. Bath application of 10 μM NMDA for 3 min induced a robust depression of excitatory transmission, which completely recovered after 15 to 20 min of washing. The depression was accompanied by an increase in the facilitation ratio (compare the EPSC observed during the depression scaled to the EPSC after washout). (**B**) (Top) Time course of the NMDA-induced depression of the EPSC [same cell as in (A)]. (Bottom) Application of NMDA also directly induced an inward current in the postsynaptic neuron. (**C**) Average of all the experiments where 10 μM NMDA was applied in the bath for 1 to 3 min to induce depression ($n = 11$). (Top) NMDA caused a decrease in the EPSC amplitude. Complete recovery was obtained after a 20-min washout. (Bottom) The corresponding modification of the presynaptic activity, documented by the increase in the facilitation ratio (227 ± 36% after 3 min, $n = 11$). (**D**) The frequency of spontaneous EPSCs is transiently reduced after the application of NMDA. The control frequency, which averaged 1.63 ± 0.15 Hz, was normalized to 100% for each experiment and then averaged ($n = 5$). (**E**) A plot of the cumulative frequency distribution of spontaneous EPSC amplitudes before (broken line) and after the application of NMDA (solid line) for the experiments illustrated in (D). (The broken and dotted lines are superimposed.) Data from the five individual experiments were pooled, and the average distributions are shown (mean ± SEM). (**F**) The NMDA-induced depression of synaptic transmission was partially inhibited by CPT, an adenosine A1 receptor antagonist. A summary of all the whole-cell experiments ($n = 9$) is as follows: after a stable base line was recorded, NMDA was applied in the bath to induce the control depression. After complete recovery, 40 μM CPT was perfused. The blockade of the presynaptic inhibitory A1 receptors increased the basal synaptic transmission about twofold. After 20 to 30 min of CPT treatment, the inhibitory effects of NMDA were reduced.

This effect was not due to an action of CPT on NMDA receptors because responses evoked by NMDA iontophoresis were unaffected by CPT (*13*). Because the action of CPT was very slowly reversible, we did not examine routinely the washout of this effect.

Synaptic release of glutamate by strong tetanic stimulation also caused an inhibition like that seen with exogenous agonist application. In these experiments two independent, but overlapping, pathways were stimulated. Tetanic stimulation of one pathway resulted in a heterosynaptic inhibition of the other pathway, which could be seen with either whole-cell recording (Fig. 2, A and B) or field potential recording (Fig. 2, C through F, and Fig. 3). The duration of this inhibition varied from 5 to 15 min, depending on the strength of the stimulus in the tetanized pathway. Both the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) ($n = 11$) (Fig. 2A) and NMDA ($n = 12$) (Fig. 2B) components of the EPSC recorded with whole-cell pipettes exhibited a heterosynaptic inhibition of similar magnitude (*14–16*). The PPF, recorded with whole-cell pipettes, was increased to 215 ± 48% ($n = 10$) during this inhibition, which suggests a presynaptic mechanism (*15*). Although the $GABA_B$ receptor antagonist CGP 35348 blocks a much shorter lasting (~1 s) form of heterosynaptic depression (*17*), it had no effect on this phenomenon ($n = 6$). Heterosynaptic inhibition was blocked by the NMDA receptor antagonist D-2-amino-5-phosphonovaleric acid (APV) (25 to 50 μM) (*16*), as shown in Fig. 2C for a single experiment and in Fig. 2D, a summary of all experiments ($n = 9$). The inhibition was also antagonized by the A1 receptor antagonist CPT. An example is shown in Fig. 2E, and a summary of all experiments is shown in Fig. 2F ($n = 9$) (*18*). As is the case in the experiments with NMDA application, CPT caused an increase in synaptic responses, and therefore the stimulus strength was decreased to evoke control-sized responses before the effect of CPT on heterosynaptic inhibition was examined (*19*). Although the effect of APV was reversed within 20 min of washing, the effect of the A1 receptor antagonist only partially reversed after an hour wash.

These results suggest that synaptically released glutamate activates NMDA receptors, which increases the concentration of extracellular adenosine, resulting in the inhibition of glutamate release from excitatory synapses. What cellular compartment is responsible for this NMDA receptor–dependent elevation in extracellular adenosine concentration? Potential sources include glial cells, synaptic terminals, interneurons, and pyramidal cells. Although it is not possible with the available evidence to

unequivocally identify the source, there are a number of findings relevant to this issue. Although an indirect action of glutamate on glia cells cannot be excluded, a direct action seems unlikely as NMDA receptors are rarely seen on this cell type (*20*). Synaptic terminals, either excitatory or inhibitory, also appear to be an unlikely major source because an NMDA receptor–dependent rise in adenosine concentration is not observed in synaptosomal preparations (*21*).

This leaves either interneurons or pyramidal cells as the likely source. Previous studies have shown that opioid receptors are selectively localized on interneurons in the CA1 region (*22*) and that activation of these receptors hyperpolarizes interneurons (*23*) and also inhibits transmitter release from their terminals (*24*). We therefore used the opioid peptide enkephalin to suppress selectively interneuronal function. Enkephalin partially inhibited the heterosynaptic inhibition, and this effect was completely reversible. An example of a typical experiment is shown in Fig. 3A, and a summary of all

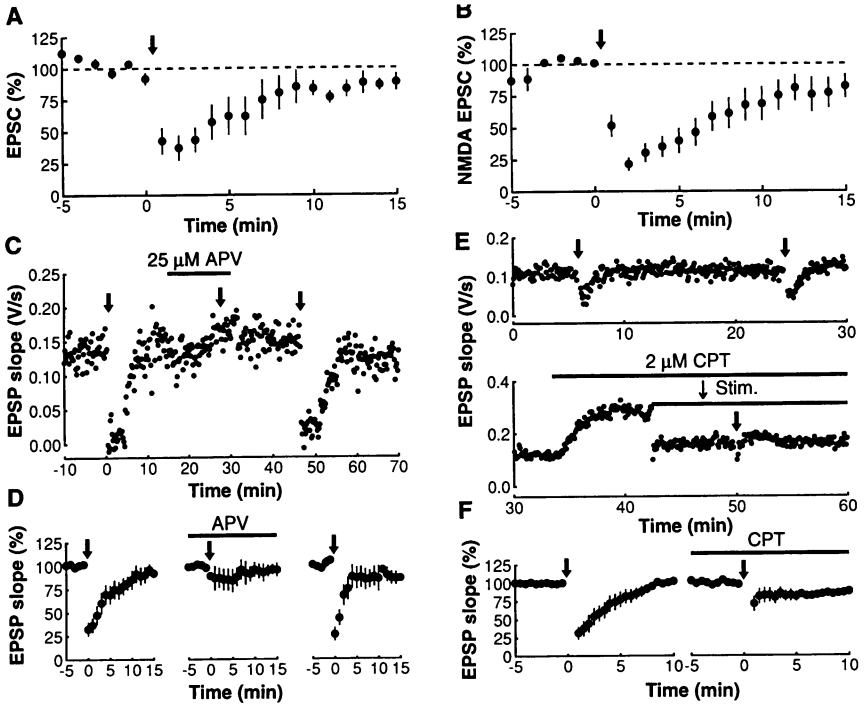

Fig. 2. High-frequency synaptic stimulation evokes a heterosynaptic depression of excitatory synaptic transmission. (**A**) Two independent pathways were stimulated with two sets of bipolar stimulating electrodes, and the EPSCs of one pathway were recorded at a holding potential of −80 mV with whole-cell pipettes (test pathway). In the other pathway, a 100-Hz, 1-s tetanus (arrows in this and subsequent panels) was applied to induce heterosynaptic depression of the EPSCs in the test pathway. (**B**) A similar heterosynaptic inhibition was observed on the pharmacologically isolated NMDA component of the EPSC (*14*). (**C**) When the slice was superfused with 25 μM D-APV, the heterosynaptic depression, recorded extracellularly, was completely suppressed and the effect was totally reversible. (**D**) Summary graph of nine experiments similar to that shown in (C) in which the effect of D-APV (25 to 50 μM) on the heterosynaptic depression was examined. The left panel shows the average of the control depression in nine slices. As shown in the middle graph, bath-applied D-APV suppressed this depression, although it recovered after about 30 min of washout (right panel). (**E**) The adenosine A1 receptor antagonist CPT blocks a tetanus-induced depression. Field EPSPs were recorded, and after a stable base line was obtained, a strong tetanus was repeatedly applied in another independent pathway, resulting in a depression of the test pathway. CPT (2 μM) was then applied to the bath, which caused an enhancement of EPSPs because of blockade of tonic activation of presynaptic A1 receptors. The tetanus-induced depression was considerably reduced in the presence of CPT. In this experiment, the stimulus strength (Stim.) of the tetanized pathway was also adjusted. (**F**) Summary graph of this series of experiments. On the left is shown the average of control tetanus-induced depression (n = 9). In the presence of CPT, the depression was significantly inhibited (right).

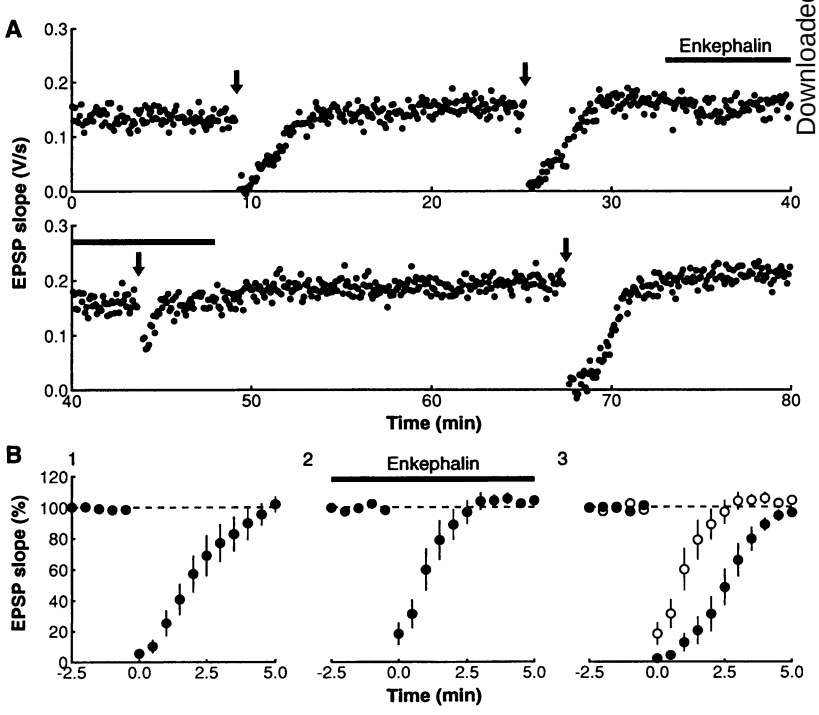

Fig. 3. Enkephalin, which selectively inhibits interneurons, reduces heterosynaptic inhibition of excitatory synaptic transmission. (**A**) Two independent pathways were stimulated with two sets of bipolar stimulating electrodes, and the extracellular field EPSPs of one pathway were recorded. Heterosynaptic depression was induced and recorded as described in Fig. 2. A tetanus (100 Hz for 1 s) was repeatedly given (arrows), and depressions of a similar magnitude were obtained with each tetanus. Met-enkephalin (10 μM) suppressed this depression, and the effect was completely reversible. (**B**) Summary graph of seven experiments in which the effect of enkephalin on the heterosynaptic depression was examined. Graph 1 shows the average of the control depression in seven slices. As shown in graph 2, extracellular application of enkephalin suppressed this depression in all cases, although an approximately 20-min washout of the drug resumed the depression completely (graph 3; closed circles). The depression in the presence of enkephalin (open circles) was superimposed on the graph of the depression after washout of enkephalin.

experiments is shown in Fig. 3B (n = 7). The metabolically stable analog D-alanine-D-leucine-enkephalinamide had a similar, but less rapidly reversible effect (n = 5). A similar reduction in the heterosynaptic inhibition of the NMDA receptor component of the EPSC was also observed: 2 min after the tetanus, the NMDA EPSC amplitude was 49 ± 18% of the control and in the presence of 20 μM enkephalin was 88 ± 20% of the control (n = 4). Enkephalin had no effect on the response to exogenously applied adenosine (n = 3), ruling out an effect on the uptake or action of adenosine. These results suggest that interneurons, which have synaptic NMDA receptors (*25*), are a source for a portion of the adenosine, although pyramidal cells could serve as an additional source.

The heterosynaptic inhibition described above could result either from the release of adenosine itself or, alternatively, from the release of the metabolic precursors of adenosine, adenosine triphosphate (ATP) or 5′–adenosine monophosphate (5′-AMP) (*1*). We favor a direct action of released adenosine, because the application of α,β-methylene ADP (500 μM), an inhibitor of ecto-5′-nucleotidase (*6*, *26*), which converts 5′-AMP to adenosine, had no effect on heterosynaptic inhibition (n = 8). Such a finding is in accord with neurochemical studies in which α,β-methylene ADP had little effect on the NMDA-induced release of adenosine (*6*).

In summary, we have presented here evidence that activation of NMDA receptors on hippocampal interneurons can release adenosine, which acts at a distance to inhibit the release of glutamate from excitatory synapses (Fig. 4). The actual mechanism by which NMDA receptors on interneurons or other cells cause the release of adenosine is unclear. Because the primary excitatory drive during a tetanus is provided by the non-NMDA receptors, the fact that NMDA receptor antagonists essentially abolish heterosynaptic inhibition raises the possibility that local Ca^{2+} entering through the synaptic NMDA receptors is responsible for the Ca^{2+}-dependent release of adenosine.

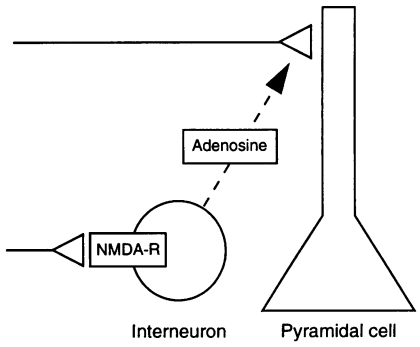

Fig. 4. Proposed mechanism for the synaptic control of extracellular adenosine in the CA1 region of the hippocampus. NMDA receptor (NMDA-R) activation by a strong tetanus of excitatory afferent fibers or by extracellular application of NMDA causes release of adenosine from the interneuron, presumably through a Ca^{2+}-dependent mechanism, which then gives rise to a widespread presynaptic inhibition of excitatory synaptic transmission by activating presynaptic adenosine A_1 receptors.

REFERENCES AND NOTES

1. J. W. Phillis and P. H. Wu, *Prog. Neurobiol.* **16**, 187 (1981); T. V. Dunwiddie, *Int. Rev. Neurobiol.* **27**, 63 (1985).
2. E. M. Silinsky, *J. Physiol. (London)* **346**, 243 (1984); T. V. Dunwiddie and H. L. Haas, *ibid.* **369**, 365 (1985); C. R. Lupica, W. R. Proctor, T. V. Dunwiddie, *J. Neurosci.* **12**, 3753 (1992); D. A. Prince and C. F. Stevens, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 8586 (1992); M. Scanziani, M. Capogna, B. H. Gähwiler, S. M. Thompson, *Neuron* **9**, 919 (1992); K. P. Scholz and R. J. Miller, *ibid.* **8**, 1139 (1992).
3. T. Zetterström *et al.*, *Neurosci. Lett.* **29**, 111 (1982).
4. Y. Sekino and I. Koyama, *ibid.* **148**, 109 (1992); L. M. Grover and T. J. Teyler, *ibid.* **154**, 39 (1993); J. B. Mitchell, C. R. Lupica, T. V. Dunwiddie, *J. Neurosci.* **13**, 3439 (1993).
5. I. Pull and H. McIlwain, *Biochem. J.* **136**, 893 (1973); K. Hoehn and T. D. White, *J. Neurochem.* **54**, 256 (1990).
6. C. G. Craig and T. D. White, *J. Neurochem.* **60**, 1073 (1993).
7. R. A. Nicoll and B. E. Alger, *J. Neurosci. Methods* **4**, 153 (1981). Hippocampal slices were prepared from 3- to 5-week-old male Hartley guinea pigs. The composition of the external recording solution was 119 mM NaCl, 2.5 mM KCl, 1.3 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.0 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, and 11 mM glucose. Picrotoxin (100 μM) was present in all experiments to block $GABA_A$ synaptic responses. A cut was routinely made to separate the CA3 region from the CA1 region. All experiments were carried out at room temperature (22° to 25°C). Either extracellular field potential recording or whole-cell voltage-clamp recording was used. Field potential recordings were made in the CA1 region with a glass pipette filled with 3 M NaCl. To evoke synaptic potentials, we delivered stimuli at 0.2 Hz through bipolar stainless steel electrodes placed in the stratum radiatum. Whole-cell recordings were made from CA1 pyramidal cells with "blind" whole-cell recording techniques [M. G. Blanton, J. J. LoTurco, A. R. Kriegstein, *J. Neurosci. Methods* **30**, 203 (1989); P. A. Coleman and R. F. Miller, *J. Neurophysiol.* **61**, 218 (1989)] with patch pipettes filled with an internal solution containing 122.5 mM Cs gluconate, 17.5 mM CsCl, 10 mM Hepes, 0.2 mM EGTA, 8 mM NaCl, 2 mM MgATP, and 0.3 mM Na_3 guanosine triphosphate (pH 7.2, 290 to 300 mosM). When filled with this solution, patch pipettes had resistances of 4 to 6 megohms. At least 30 min elapsed between break-in and the application of NMDA so that the potentiating effects of NMDA could be washed out [T. Manabe, P. Renner, R. A. Nicoll, *Nature* **355**, 50 (1992)]. Synaptic responses, which were recorded with an Axopatch 1D or an Axoclamp 1B, were filtered at 1 kHz, digitized at 2 to 5 kHz, and stored in a hard disk. In field potential recording, initial excitatory postsynaptic potential (EPSP) slopes were measured with a least squares method. In the whole-cell recording, EPSC amplitudes were measured by averaging a 5-ms window around the peak and subtracting the average value obtained during a 5-ms window immediately before the stimulus. The data are expressed as means ± SEM. Drugs used were methionine enkephalin, NMDA, adenosine, picrotoxin, *N*-ω-nitro-L-arginine, α,β-methylene ADP (Sigma), D-APV (Cambridge Research Biochemicals), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and CPT (Research Biochemicals).
8. G. L. Collingridge, S. J. Kehl, H. McLennan, *J. Physiol. (London)* **334**, 33 (1983); J. A. Kauer, R. C. Malenka, R. A. Nicoll, *Nature* **334**, 250 (1988).
9. S. J. East and J. Garthwaite, *Neurosci. Lett.* **123**, 17 (1991).
10. C. L. Boulton, A. J. Irving, G. L. Collingridge, J. Garthwaite, *J. Physiol. (London)* **467**, 181P (1993).
11. H. L. Haas and R. W. Greene, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **337**, 561 (1988). To adjust for this enhancement, we reduced the stimulus strength, although in CPT there was usually a gradual decay of the synaptic response, thus making stable baseline responses difficult to obtain.
12. The remaining inhibition recorded in CPT could result from a variety of mechanisms, such as incomplete blockade of A1 receptors or incomplete voltage clamping of the postsynaptic cell during the application of NMDA.
13. NMDA responses were recorded with an extracellular electrode placed in the stratum radiatum and evoked by brief (5-s) iontophoretic application of NMDA from a nearby electrode. The control response was 1.32 ± 0.10 mV, and after a 30-min application of 100 μM CPT the response was 1.28 ± 0.11 mV (n = 3). The noncompetitive NMDA receptor antagonist MK-801 (20 μM) reduced the response to 0.21 ± 0.04 mV.
14. To examine the NMDA component of the EPSC, we applied the AMPA receptor antagonist CNQX (20 μM) and held the cell at +40 mV to relieve the Mg^{2+} block of the NMDA receptor channel.
15. L. M. Grover and T. J. Teyler, *Synapse* **15**, 149 (1993).
16. Z. I. Bashir and G. L. Collingridge, *Eur. J. Neurosci.* **4**, 485 (1992).
17. J. S. Isaacson, J. M. Solis, R. A. Nicoll, *Neuron* **10**, 165 (1993).
18. Similar blockade of a shorter lasting heterosynaptic inhibition has been reported (*4*).
19. To compensate for the increased synaptic drive in CPT, we used two approaches. First, the stimulus strength in the tetanized pathway was reduced to evoke responses identical in size to those evoked before the application of CPT. Second, a low concentration of antagonist (0.2 μM CPT) largely blocked the ambient actions of adenosine. Higher concentrations, therefore, had little further effect on synaptic strength but markedly reduced the heterosynaptic inhibition without the need to change the stimulus strength. In 0.2 μM CPT, the average inhibition 3 min after the tetanus was 83 ± 18%, whereas that in 10 μM CPT was 40 ± 17% ($P < 0.02$; n = 7).
20. M. M. Usowicz, V. Gallo, S. G. Cull-Candy, *Nature* **339**, 380 (1989); D. J. A. Wyllie, A. Mathie, C. J. Symonds, S. G. Cull-Candy, *J. Physiol. (London)* **432**, 235 (1991); S. G. Cull-Candy and D. J. A. Wyllie, *Annu. Rev. N.Y. Acad. Sci.* **633**, 458 (1991).
21. K. Hoehn and T. D. White, *J. Neurochem.* **54**, 1716 (1990).
22. W. Zieglgänsberger, E. D. French, G. R. Siggins, F. E. Bloom, *Science* **205**, 415 (1979); R. A. Nicoll, B. E. Alger, C. E. Jahr, *Nature* **287**, 22 (1980).
23. D. V. Madison and R. A. Nicoll, *J. Physiol. (London)* **398**, 123 (1988).
24. G. A. Cohen, V. A. Doze, D. V. Madison, *Neuron* **9**, 325 (1992); M. Capogna, B. H. Gähwiler, S. M. Thompson, *J. Physiol. (London)* **470**, 539 (1993).
25. P. Sah, S. Hestrin, R. A. Nicoll, *J. Physiol. (London)* **430**, 605 (1990).
26. F. Pons, R. F. Bruns, J. W. Daly, *J. Neurochem.* **34**, 1319 (1980); R. A. Cunha, A. M. Sebastião, J. A. Ribeiro, *ibid.* **59**, 657 (1992).

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