cloned in the Eco RI site of Bluescript SK and sequenced as described (*13*). Only one sequence change was identified (see text) and this change at codon 175 was found in two independent clones.
16. B. Vogelstein *et al.*, *Science* **244**, 207 (1989).
17. P. Hinds, C. Finlay, A. J. Levine, *J. Virol.* **63**, 739 (1989).
18. C. A. Finlay *et al.*, *Mol. Cell. Biol.* **8**, 531 (1988).
19. D. Eliyahu *et al.*, *Oncogene* **3**, 313 (1988).
20. S. H. Friend *et al.*, *Nature* **323**, 643 (1986); W.-H. Lee *et al.*, *Science* **235**, 1394 (1987); Y.-K.T. Fung *et al.*, *ibid.* **236**, 1657 (1987).
21. D. P. Lane and L. V. Crawford, *Nature* **278**, 261 (1979); D. I. H. Linzer and A. J. Levine, *Cell* **17**, 43 (1979).
22. J. A. DeCaprio *et al.*, *Cell* **54**, 275 (1988).
23. M. R. Green, *ibid.* **56**, 1 (1989).
24. P. Sarnow, Y. S. Ho, J. Williams, A. J. Levine, *ibid.* **28**, 387 (1982); P. Whyte, H. E. Ruley, E. Harlow, *Nature* **334**, 124 (1988).
25. G. G. Hicks and M. Mowat, *J. Virol.* **62**, 4752 (1988).
26. M. Mowat *et al.*, *Nature* **314**, 633 (1985); D. G. Munroe, B. Rovinski, A. Bernstein, S. Benchimol, *Oncogene* **2**, 621 (1988).
27. D. Wolf and V. Rotter, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 790 (1985); H. Masuda, C. Miller, H. P. Koeffler, H. Battifora, M. J. Cline, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 7716 (1987).
28. H.-W. Sturzbecher, C. Adison, J. R. Jenkins, *Mol. Cell. Biol.* **8**, 3740 (1988).
29. D. Eliyahu, A. Raz, P. Gruss, D. Givol, M. Oren, *Nature* **312**, 646 (1984); L. F. Parada, H. Land, R. A. Weinberg, D. Wolf, V. Rotter, *ibid.*, p. 649.
30. B. Vogelstein and D. Gillespie, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 615 (1979).
31. The authors thank M. Litt, S. Orkin, L. Leinwand, D. Givol, and J. Stein for providing probes; D. Schwartz for help with pulsed-field gel electrophoresis; D. and M. Brattain for providing cells lines; and T. Gwiazda for preparing the manuscript. Supported by the Clayton Fund, the McAshan Fund, the Howard Hughes Medical Institute, (training grants GM07309 and GM07184), and the National Institutes of Health (grants HD20619, CA42857, CA28854, CA47527, and CA35494).

6 February 1989; accepted 15 March 1989

Expression of a Cloned Rat Brain Potassium Channel in *Xenopus* Oocytes

MACDONALD J. CHRISTIE, JOHN P. ADELMAN, JAMES DOUGLASS, R. ALAN NORTH

Potassium channels are ubiquitous membrane proteins with essential roles in nervous tissue, but little is known about the relation between their function and their molecular structure. A complementary DNA library was made from rat hippocampus, and a complementary DNA clone (RBK-1) was isolated. The predicted sequence of the 495–amino acid protein is homologous to potassium channel proteins encoded by the *Shaker* locus of *Drosophila* and differs by only three amino acids from the expected product of a mouse clone MBK-1. Messenger RNA transcribed from RBK-1 in vitro directed the expression of potassium channels when it was injected into *Xenopus* oocytes. The potassium current through the expressed channels resembles both the transient (or A) and the delayed rectifier currents reported in mammalian neurons and is sensitive to both 4-aminopyridine and tetraethylammonium.

POTASSIUM CHANNELS ARE MEMbrane proteins that are selectively permeable to K^+ ions (*1*). Some kinds of K^+ channels are opened by depolarization; outward K^+ currents limit the duration of single action potentials (delayed rectifier) or set the pattern of bursts of action potentials (transient or A current). Other K^+ channels are opened or closed by second messengers to mediate the actions of synaptic transmitters (*1, 2*). We have determined some functional properties of a mammalian K^+ channel of known primary structure by expression in *Xenopus* oocytes of a cDNA clone isolated from rat brain.

A cDNA library made from rat hippocampus polyadenylated RNA was screened with two radiolabeled oligonucleotide probes (*3*). The sequences of the probes were based on conservation of amino acid sequences between the *Shaker* A (*4–6*) and MBK-1 (*7*) predicted proteins. *Shaker* is a family of *Drosophila* mutants that expresses abnormal transient or A-type K^+ channels; at least four distinct proteins encoded by the *Shaker* locus form K^+ channels when expressed in *Xenopus* oocytes. MBK-1 is a clone isolated from a mouse brain cDNA library having a homologous nucleotide sequence, but MBK-1 has not been shown to direct the formation of functional channels. One of the rat brain clones (RBK-1) that gave positive hybridization with both probes was purified, and the nucleotide sequence of the 1.7-kb cDNA insert was determined (Fig. 1). The sequence contains one long open-reading frame that encodes a protein of 495 amino acids (molecular mass, 54.6 kD). The translation product predicted from RBK-1 is 69% homologous to the 453 residues that constitute the core common to the different proteins that could be formed from transcripts of *Shaker* cDNA. Significantly greater homology is found within the predicted membrane-spanning regions H1 to H6, and in the arginine-rich amphipathic helix (S4), which may represent the channel voltage sensor. The putative translation products of RBK-1 and MBK-1 differ by only three of their 495 amino acids, and there are no differences in the H1 to H6 or S4 regions; the sequence is identical to the expected product of a cDNA clone (RCK-2) isolated from rat cortex (*8*).

The RBK-1 sequence was subcloned into a plasmid expression vector (*3*), and the mRNA transcripts synthesized in vitro were injected into *Xenopus* oocytes (*9*). Voltage-clamp recordings made 24 to 96 hours later showed that the oocytes that had been injected produced large outward currents in response to depolarizing voltage commands. The currents were dependent on the amount of mRNA injected (*10*) and were not seen in uninjected cells. Some of the properties of the current are shown in Fig. 2; from a holding potential of −80 mV the current was first observed with depolarizations to −50 mV and was maximally activated at 0 or +10 mV.

The current resulted from the movement of K^+ ions, as shown by measurement of its reversal potential (*11*) in different K^+ ion concentrations (Fig. 3). The reversal potential (E_{rev}) was linearly related to the extracellular potassium concentration ($[K]_o$) by $E_{rev} = m\log([K]_o/[K]_i)$ (the Nernst equation) where $[K]_i$, the intracellular potassium concentration, was assumed to be 110 m*M*. The slope of the relation (m) was 55 ± 2 mV per decade (SEM, n = 5 oocytes; theoretical value is RT/F = 58), providing convincing evidence that the channels expressed in the oocyte membrane are highly selective for K^+ ions.

We also determined whether the properties of the expressed channels were similar to those of the known classes of K^+ channels in mammalian neurons. The current developed (activated) within a few milliseconds of the applied depolarization (*12*), and the voltage dependence of activation of the K^+ conductance was well fit by a Boltzmann function centered at −30 mV (Fig. 2C). When the depolarizing pulse was terminated, the current subsided within a few milliseconds (*12*). Steady-state inactivation was studied by changing the holding potential for 5 s before applying the depolarizing command to +10 mV; the midpoint of the inactivation curve was at about −30 mV (n = 3). The current inactivated only partially during a maintained depolarization of up to 10 s in duration. The inactivation had a fast component (time constant, 50 to 100 ms) and a much slower component (time constant, 5 to 10 s), neither of which was strongly

Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

voltage-dependent (*12*). These properties are most similar to the transient or A current described in many vertebrate neurons (*1, 2, 13*); such a current is present in both rat (*14*) and guinea pig (*15*) hippocampal neurons. The best estimates of the kinetics come from ensemble analysis of single-channel A currents because these are uncontaminated with other currents (*16*); such recordings from guinea pig dorsal root ganglion cells indicate that inactivation occurs (at 0 mV) according to two exponential functions with time constants of 100 ms and 4 s, close to those found for the oocyte current.

The K^+ conductance produced by injection of RBK-1 mRNA was characterized with channel blockers. 4-Aminopyridine (4-AP) reduced the amplitude of both the fast and slowly inactivating components of the K^+ current; the concentration giving 50% reduction (EC_{50}) was 160 ± 30 μ*M* (SE, *n* = 4) (Fig. 4). The blockade was fully reversible on washing when the concentration was <300 μ*M*. The effect of 4-AP was use-dependent and voltage-dependent, being greater at −40 than at 0 mV and developing progressively during the first test pulse. 4-AP appeared to block equally well the reversed current in the inward direction. Barium and tetraethylammonium (TEA) also reduced the current, with respective EC_{50}'s of 2.0 ± 0.5 m*M* (*n* = 5) and 0.8 ± 0.2 m*M* (*n* = 6); the effect of both these blockers was greater at −40 than at 0 mV. The effectiveness of 4-AP provides further identification of the expressed current as a transient or A current, but the sensitivity to TEA is surprisingly high. The A current in rat or guinea pig hippocampal cells is unaffected by TEA at 3 or 25 m*M*, even though other K^+ conductances are blocked (*13–15*).

Exposure of injected oocytes to a Ca^{2+}-free and high Mg^{2+} (11 instead of 1 m*M*) solution had no effect on the peak current; a positive shift in the activation voltage by 7 mV occurred in this solution, similar to the effect of manganese observed in CA3 hippocampal neurons (*15*). In five further experiments, EGTA was pressure-injected into the oocyte (estimated final concentration, 1 to 2.5 m*M*), with no effect on the K^+ current.

```
                                                                          -34
                                                                    5'----TCCTGGCCTCCTACCCCTGCACCCTGCATCCATC   -1

ATG ACG GTG ATG TCA GGG GAG AAT GCA GAC GAG GCT TCG GCC GCT CCA GGT CAC CCC CAG GAT GGC AGC TAC CCA AGG CAG GCG GAC CAC   90
Met Thr Val Met Ser Gly Glu Asn Ala Asp Glu Ala Ser Ala Ala Pro Gly His Pro Gln Asp Gly Ser Tyr Pro Arg Gln Ala Asp His   30

                                            *   *   *
GAC GAC CAC GAA TGC TGC GAG CGC GTG GTG ATC AAC ATC TCC GGG CTG CGC TTC GAG ACG CAG CTC AAG ACT CTG GCC CAG TTC CCC AAC  180
Asp Asp His Glu Cys Cys Glu Arg Val Val Ile Asn Ile Ser Gly Leu Arg Phe Glu Thr Gln Leu Lys Thr Leu Ala Gln Phe Pro Asn   60

ACG CTG CTG GGC AAC CCG AAG AAA CGC ATG CGC TAC TTT GAC CCT CTG AGG AAT GAG TAC TTC TTT GAC CGC AAC CGG CCC AGC TTC GAT  270
Thr Leu Leu Gly Asn Pro Lys Lys Arg Met Arg Tyr Phe Asp Pro Leu Arg Asn Glu Tyr Phe Phe Asp Arg Asn Arg Pro Ser Phe Asp   90

GCC ATC CTT TAT TAC TAC CAG TCG GGG GGG CGC CTG CGC AGG CCG GTC AAC GTG CCC CTG GAC ATG TTC TCC GAG GAG ATT AAA TTT TAC  360
Ala Ile Leu Tyr Tyr Tyr Gln Ser Gly Gly Arg Leu Arg Arg Pro Val Asn Val Pro Leu Asp Met Phe Ser Glu Glu Ile Lys Phe Tyr  120

GAG TTG GGC GAG GAG GCC ATG GAG AAG TTC CGG GAA GAT GAG GGC TTC ATC AAG GAA GAG GAG CGC CCC CTA CCC GAG AAG GAG TAC CAG  450
Glu Leu Gly Glu Glu Ala Met Glu Lys Phe Arg Glu Asp Glu Gly Phe Ile Lys Glu Glu Glu Arg Pro Leu Pro Glu Lys Glu Tyr Gln  150

CGC CAG GTG TGG CTG CTC TTT GAG TAT CCG GAG AGC TCA GGA CCT GCA CGG GTT ATT GCC ATT GTA TCC GTC ATG GTC ATC CTC ATC TCC  540
Arg Gln Val Trp Leu Leu Phe Glu Tyr Pro Glu Ser Ser Gly Pro Ala Arg[Val Ile Ala Ile Val Ser Val Met Val Ile Leu Ile Ser  180
                                                                                     H1
                                                                                                  *   *   *
ATA GTC ATC TTT TGC CTG GAG ACT CTC CCT GAG CTG AAG GAT GAC AAG GAC TTC ACG GGC ACC ATT CAC CGC ATC GAT AAC ACC ACA GTC  630
Ile Val Ile Phe Cys Leu]Glu Thr Leu Pro Glu Leu Lys Asp Asp Lys Asp Phe Thr Gly Thr Ile His Arg Ile Asp Asn Thr Thr Val  210

ATC TAC ACT TCT AAC ATC TTC ACA GAC CCT TTC TTC ATT GTG GAA ACC TTG TGT ATC ATC TGG TTC TCT TTT GAG CTG GTG GTG CGC TTC  720
Ile Tyr Thr Ser Asn Ile Phe Thr Asp Pro[Phe Phe Ile Val Glu Thr Leu Cys Ile Ile Trp Phe Ser Phe Glu Leu Val Val Arg Phe  240
                                                                          H2

TTC GCC TGC CCC AGC AAG ACA GAC TTC TTT AAG AAC ATC ATG AAC TTC ATC GAC ATT GTG GCC ATC ATC CCT TAT TTC ATT ACC CTG GGC  810
Phe Ala]Cys Pro Ser Lys Thr Asp Phe Phe Lys Asn[Ile Met Asn Phe Ile Asp Ile Val Ala Ile Ile Pro Tyr Phe Ile Thr Leu Gly  270
                                                                         H3

ACA GAG ATA GCT GAG CAG GAG GGG AAT CAG AAG GGC GAG CAG GCC ACT TCC CTG GCC ATC CTC AGG GTC ATC CGC TTG GTA AGG GTG TTC  900
Thr Glu Ile Ala]Glu Gln Glu Gly Asn Gln Lys Gly Glu Gln Ala Thr Ser[Leu Ala Ile Leu Arg Val Ile Arg Leu Val Arg Val Phe  300
                                                                                                    S4-like

AGA ATC TTC AAA CTC TCC CGC CAC TCC AAG GGC CTT CAG ATC CTG GGC CAG ACC CTC AAA GCT AGT ATG AGG GAG TTA GGG CTG CTC ATC  990
Arg Ile Phe Lys Leu Ser Arg His Ser Lys Gly Leu]Gln Ile Leu Gly Gln Thr Leu Lys Ala Ser Met Arg Glu[Leu Gly Leu Leu Ile  330

TTT TTC CTC TTC ATT GGC GTC ATA CTG TTT TCT AGT GCA GTG TAC TTT GCG GAG GCG GAA GAA GCT GAG TCG CAC TTC TCC AGT ATC CCC 1080
Phe Phe Leu Phe Ile Gly Val Ile Leu Phe Ser Ser Ala Val Tyr Phe Ala]Glu Ala Glu Glu Ala Glu Ser His Phe Ser Ser Ile Pro  360
                    H4

GAT GCT TTC TGG TGG GCG GTG GTG TCC ATG ACC ACT GTG GGA TAC GGT GAC ATG TAC CCT GTG ACA ATT GGA GGC AAG ATC GTG GGC TCC 1170
Asp[Ala Phe Trp Trp Ala Val Val Ser Met Thr Thr Val Gly Tyr Gly Asp Met Tyr Pro Val Thr Ile]Gly Gly Lys[Ile Val Gly Ser  390
                                          H5

TTG TGT GCC ATC GCT GGT GTG CTG ACA ATT GCC CTG CCC GTA CCT GTC ATT GTG TCC AAT TTC AAC TAT TTC TAC CAC CGA GAA ACT GAG 1260
Leu Cys Ala Ile Ala Gly Val Leu Thr Ile Ala Leu Pro Val Pro Val Ile Val]Ser Asn Phe Asn Tyr Phe Tyr His Arg Glu Thr Glu  420
                        H6
                                                                                        ___________
GGG GAA GAG CAG GCT CAG TTG CTC CAT GTT AGT TCT CCT AAC TTA GCC TCT GAC AGT GAC CTC AGC CGC CGC AGC TCC TCT ACT ATC AGC 1350
Gly Glu Glu Gln Ala Gln Leu Leu His Val Ser Ser Pro Asn Leu Ala Ser Asp Ser Asp Leu Ser Arg Arg Ser Ser Ser Thr Ile Ser  450

                                        *   *   *                                                 *   *   *
AAG TCT GAG TAC ATG GAG ATC GAA GAG GAC ATG AAC AAT AGC ATA GCC CAC TAC AGG CAG GCT AAT ATC AGA ACT GGT AAC TGC ACC GCA 1440
Lys Ser Glu Tyr Met Glu Ile Glu Glu Asp Met Asn Asn Ser Ile Ala His Tyr Arg Gln Ala Asn Ile Arg Thr Gly Asn Cys Thr Ala  480

                        *   *   *
ACT GAT CAA AAC TGC GTT AAT AAG AGC AAG CTC CTG ACC GAT GTT TAA AAAAAGCACCAGGCAAGCAATCAAAAGCCCCCAAACAAAACCCTTGGCGACTCCT 1543
Thr Asp Gln Asn Cys Val Asn Lys Ser Lys Leu Leu Thr Asp Val stop

GTCCCACTCTGTAGATACTTTACTAAAACCGTAGTCTTTGAATGCTTTATTTAACTGGCAATGCACTGTTGCATTGTGAATTTGGGGGGTGGGCAAACCTGAAGCTTTCAAGATCACATT 1662

TAAAAAACAAAACCAACCAAACAAGCAAAAAGAAAAAAAAAAACCCAACAAAAAAAAAAA----3'
                                                              1721
```

Fig. 1. Nucleotide and predicted translation product sequence of RBK-1 cDNA. Hydrophobic, potential transmembrane domains H1 to H6 and the putative voltage-sensor (S4-like) region are boxed. Asterisks, possible sites for N-linked glycosylation. Solid bar, a possible A kinase phosphorylation site. Nucleotides 538–574 and 951–987 are the oligonucleotide sequences used as probes.

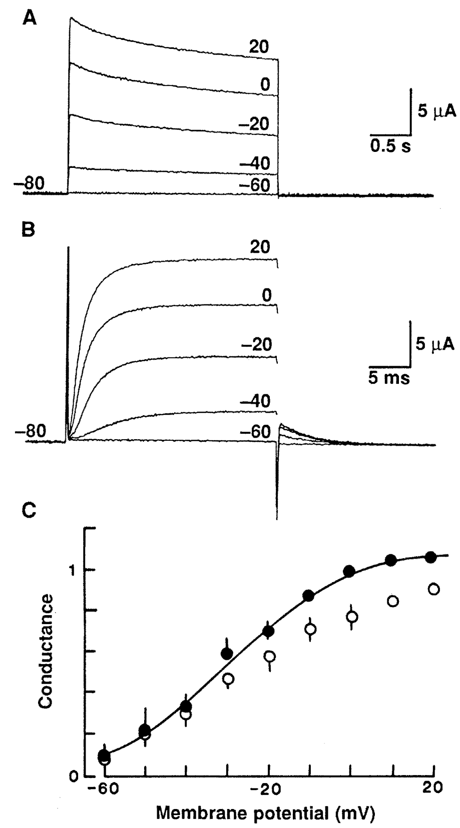

Fig. 2. Oocytes injected with RBK-1 mRNA show transient outward current when depolarized. (**A** and **B**) Currents evoked by depolarizing voltage-command pulses from a holding potential of −80 mV (records are single sweeps, leak not subtracted). The depolarizing step was 2.5 s in duration and was to the potential (in millivolts) indicated beside each trace. (B) is the rising phase of the current at faster recording speed. (**C**) Potassium conductance as a function of membrane potential. Filled circles were derived from the peak currents in (A). The values are chord conductances for which a driving force from −102 mV is assumed. The conductances ranged greatly from cell to cell (42.5 to 196 μS at 0 mV), perhaps because of variability in the number of channels expressed; the values have been normalized to unit conductance at 0 mV. The line is best least-squares fit of the conductance *G* to the function $G = G_{max}/[1 + \exp(V_{0.5} - V)/k]$ where *V* is the potential, $V_{0.5}$ (midpoint for activation) = −30.5 ± 2.7 mV, and *k* (slope factor) = 13.6 ± 1.8 mV. Open circles are the conductances of the same cells, measured at the end of the depolarizing command (duration, 1 s). Vertical lines indicate SEM, where this exceeds the size of the symbol; results from four or five cells are shown at each point, except for +10 and +20 mV where two cells were studied (these points not used for fitting).

Finally, the current was not blocked by apamin (3 μ*M*, *n* = 3) or quinine (100 μ*M*, *n* = 3), both of which block certain types of Ca^{2+}-activated K^+ currents, indicating that the expressed K^+ channels are not Ca^{2+}-activated.

Comparison with predicted *Shaker* proteins (*4–6*) demonstrates that RBK-1 represents the conserved central core region of *Shaker*; the divergent regions found at the amino and carboxyl termini of the *Shaker* products are missing from RBK-1 [and MBK-1 (*7*)]. A putative A-type K^+ channel was purified from rat brain (*17*) by using a dendrotoxin affinity column, and the major component had an apparent molecular mass of 76 kD on SDS-polyacrylamide gel. RBK-1 encodes a protein of 54.6 kD, but glycosylation at some or all of the five potential N-linked sites (Asn-X-Thr/Ser) (Fig. 1) might increase the molecular mass to a value near that reported for the dendrotoxin receptor.

The RBK-1 K^+ channel amino acid composition resembles one of the four internally homologous domains of the voltage-sensitive Na^+ channel (*18*), as first pointed out for the *Shaker* product and MBK-1 (*4, 6*). Thus, RBK-1 may encode a single subunit of a normally heteropolymeric channel or homopolymeric channel. Our experiments do not allow one to distinguish these possibilities, although the strong similarities between the currents expressed from RBK-1 and those observed in mammalian neurons might be expected from homopolymerization. Timpe *et al.* (*19*) found no evidence for heteropolymerization by injecting oocytes with ShA mRNA mixed with ShB or ShC mRNAs. The differences between the conductance expressed from RBK-1 and that present in differentiated mammalian cells (for example, the high sensitivity to both 4-AP and TEA) might result from posttranslational modifications or the influence of other associated proteins (*20*).

Note added in proof: Charybdotoxin also reversibly blocked the K^+ current; the EC_{50} was 5.5 ± 0.4 n*M* (*n* = 3).

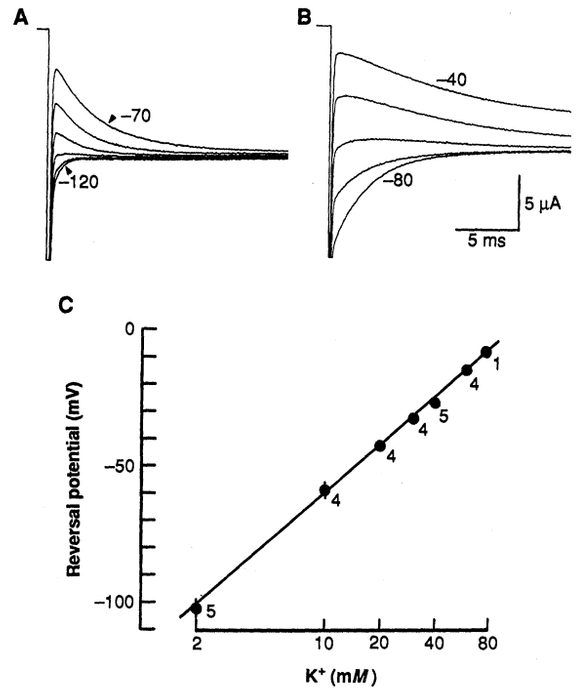

Fig. 3. The expressed current is a K^+ current. (**A** and **B**) Tail currents at different membrane potentials. The cell was depolarized (to 0 mV) for 10 ms from a holding potential of −70 mV and then stepped to various test potentials. In (A) (2 m*M* K^+) the test potentials were −70 through −120 mV in 10-mV increments. In (B) (10 m*M* K^+) they were −40 through −80 mV in 10-mV increments. (**C**) The reversal potential of the current as a function of the K^+ concentration. Points are mean values for the number of cells indicated; vertical bars are SEM, where this exceeds the size of the symbol. The line is the theoretical line for an intracellular K^+ concentration of 110 m*M* (*20*).

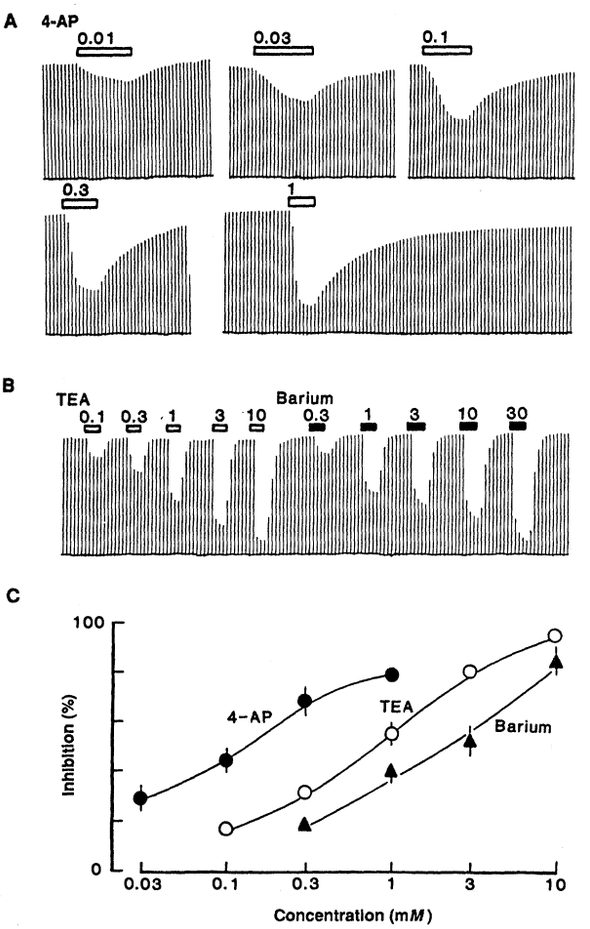

Fig. 4. The expressed current is blocked by 4-AP, TEA, and barium. (**A** and **B**) The membrane currents evoked at intervals of 30 s by a depolarizing command pulse of 0.5 s duration to −10 mV. The current amplitude without added drugs was 1.9 μA (leakage of 0.12 μA was not subtracted, but records were filtered at 15 Hz to eliminate capacitative transients). The bars above the recordings indicate the periods during which the superfusing solution contained 4-AP, TEA, or barium at the millimolar concentrations indicated. All recordings were from the same oocyte; in (A) the trace has been interrupted to remove the segments during which the effect of 4-AP washed out. (**C**) The effects of the blocking drugs as a function of concentration for a number of oocytes (4-AP, *n* = 4; TEA, *n* = 6; and barium, *n* = 5).

REFERENCES AND NOTES

1. B. Rudy *et al.*, *Neuroscience* **25**, 729 (1988); R. Latorre and C. Miller, *J. Membr. Biol.* **71**, 11 (1983).
2. B. Hille, *Ionic Channels in Excitable Membranes* (Sinauer, Sunderland, MA, 1984).
3. Total RNA was extracted from rat hippocampus by the method of Chirgwin, and the polyadenylated fraction was purifed by means of oligo(dT) cellulose chromatography [J. M. Chirgwin A. E. Przybyla, R. J. MacDonald, W. J. Rutter, *Biochemistry* **18**, 5294 (1979)]. Complementary DNA was prepared by the method of U. Gubler and B. J. Hoffman [*Gene* **25**, 263 (1983)] and cloned into λgt10 as described [J. P. Adelman, C. T. Bond, J. Douglass, E. Herbert, *Science* **235**, 1514 (1987)]. Oligonucleotide probes (Fig. 1) were synthesized on an Applied Biosystems 380A DNA synthesizer with β-cyanoethylphosphoramidite chemistry. Probes were radiolabeled at their 5′ termini and used to screen 200,000 hippocampus cDNA clones. One of 14 clones that showed positive hybridization to both probes was purified, the cDNA insert was cloned into M13 vector, and the nucleotide sequence was determined by the method of Sanger [F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977)].
4. B. L. Tempel, D. M. Papazian, T. L. Schwarz, Y. N. Jan, L. Y. Jan, *Science* **237**, 770 (1987); D. M. Papazian, T. L. Schwarz, B. L. Tempel, Y. N. Jan, L. Y. Jan, *ibid.*, p. 749.
5. T. L. Schwarz, B. L. Tempel, D. M. Papazian, Y. N. Jan, L. Y. Jan, *Nature* **331**, 137 (1988); L. C. Timpe *et al.*, *ibid.*, p. 143.
6. O. Pongs *et al.*, *EMBO J.* **7**, 1087 (1988).

7. B. L. Tempel, Y. N. Jan, L. Y. Jan, *Nature* **332**, 837 (1988).
8. A. Baumann, A. Grupe, A. Ackermann, O. Pongs, *EMBO J.* **7**, 2457 (1988).
9. Oocytes (Dumont stage V-VI) were harvested (*20*) from adult female *X. laevis* under anesthesia [0.35% MS-222 (3-aminobenzoic acid ethyl ester), Sigma]. Theca and follicular layers were removed by incubation for 3 hours in Ca^{2+}-free and 82.5 m*M* NaCl ND-96 solution. Denuded oocytes were injected 1 to 24 hours later with 0.5 to 50 ng of mRNA (in 50 nl). Oocytes were incubated at 18°C for up to 120 hours in ND-96, which is 96 m*M* NaCl, 2 m*M* KCl, 1.8 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 2.5 m*M* sodium pyruvate, 0.5 m*M* theophylline, 5 m*M* Hepes, and gentamycin (50 μg/ml).
10. Recordings of membrane current were made 24 to 96 hours after injection of mRNA. Oocytes were continuously superfused (3 ml/min) with ND-96 (pyruvate, theophylline, and gentamycin omitted) at 23° to 25°C and were voltage-clamped with two microelectrodes (resistance 0.1 to 1 megohm) by using standard techniques [A. S. Finkel and P. W. Gage, in *Voltage and Patch Clamping with Microelectrodes*, T. G. Smith, H. Lecar, S. J. Redman, P. W. Gage, Eds. (Williams and Wilkins, Baltimore, 1985), pp. 47–94]. Single sweep current traces were low-pass filtered at 3 or 10 kHz before recording and are presented without leak subtraction. Oocytes (89%) displayed large K^+ currents 48 to 96 hours after injection of 25 to 100 ng of RBK-1 mRNA (two RNA preparations injected into 45 oocytes from four *Xenopus* donors). The outward current evoked by stepping from −90 (or −70) to −30 mV was 4.3 ± 0.45 μA (peak) and 3.1 ± 0.33 μA after 1 s (n = 30). Leak currents during these steps did not exceed 0.2 μA. No rapidly developing outward currents were observed in uninjected or water-injected oocytes (n = 44). As reported [N. Dascal, *CRC Crit. Rev. Biochem.* **22**, 317 (1987)], slowly developing outward currents were occasionally observed in uninjected oocytes during steps to −20 or 0 mV, but these did not exceed 0.1 μA and were not affected by tetraethylammonium (1 m*M*) or 4-AP (3 m*M*). The peak amplitude of the outward current was strongly related to the amount of mRNA injected. After 48 to 60 hours, the current evoked by stepping from −90 to −30 mV was 0.08 ± 0.06 μA (2 out of 8 positive) for 500 pg of mRNA, 0.82 ± 0.18 μA (12 out of 14 positive) for 5 ng of mRNA and 1.81 ± 0.37 μA (7 out of 8 positive) for 50 ng of mRNA.
11. For K^+ concentrations of 2 to 20 m*M*, the reversal potential of the tail current was measured by stepping to 0 mV for 10 ms and then measuring the current amplitude immediately after stepping back to different potentials. For 40, 60, and 80 m*M* K^+, reversal of the current was observed directly. NaCl was reduced when KCl was increased in these experiments, so the good agreement with the Nernst equation implies that Na^+ was not significantly permeable. The current was also unaffected by substituting isethionate for 90% of the Cl^- ions (n = 3).
12. The time constant for activation was 2.7 ± 0.3 ms at −20 mV, and 6.0 ± 1.3 ms at −40 mV (mean ± SEM, n = 5); measurements were inaccurate at 0 and +20 mV because currents may have been contaminated by capacitative transients. At the end of the depolarizing step the current declined with a time constant of 5.0 ± 0.3 ms (2 m*M* K^+, at −70 mV, n = 4). The faster time constants of inactivation during a sustained depolarization were 68.3 ± 8.4 ms at −40 mV, 36.5 ± 1.0 ms at −10 mV, and 43.9 ± 13.0 ms at +10 mV (n = 4); the slower time constants were 11.0 ± 2.3 s at −40 mV, 5.8 ± 1.4 s at −10 mV, and 10.8 ± 3.9 s at +10 mV (n = 4). Recovery from inactivation reached 60% ± 10% (n = 4) within 1 s and was complete between 30 and 60 s.
13. J. A. Connor and C. F. Stevens, *J. Physiol. (London)* **213**, 21 (1971); M. A. Rogawski, *Trends Neurosci.* **8**, 214 (1985).
14. J. V. Halliwell, I. B. Othman, A. Pelchen-Matthews, J. O. Dolly, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 493 (1986); M. Segal and J. L. Barker, *J. Neurophysiol.* **51**, 1409 (1984); M. Segal, M. A. Rogawski, J. L. Barker, *J. Neurosci.* **4**, 604 (1984).
15. B. Gustaffson, M. Galvan, P. Grafe, H. A. Wigstrom, *Nature* **299**, 252 (1982).
16. H. Kasai, D. Kameyama, K. Yamaguchi, J. Fukuda, *Biophys. J.* **49**, 1243 (1986).
17. H. Rehm and M. Lazdunski, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4919 (1988).
18. M. Noda *et al.*, *Nature* **312**, 121 (1984).
19. L. C. Timpe, Y. N. Jan, L. Y. Jan, *Neuron* **1**, 659 (1988).
20. B. Rudy *et al.*, *ibid.*, p. 649.
21. Supported by Department of Health and Human Services grants DA03160, DA03161, DA04154, DK32979, and MH40416. We thank H. Lester for help with establishing the oocyte injection methods, C. Miller for providing charybdotoxin, and Y. Wu for technical help.

5 December 1988; accepted 2 February 1989

Inescapable Versus Escapable Shock Modulates Long-Term Potentiation in the Rat Hippocampus

TRACEY J. SHORS, THOMAS B. SEIB, SEYMOUR LEVINE, RICHARD F. THOMPSON

A group of rats was trained to escape low-intensity shock in a shuttle-box test, while another group of yoked controls could not escape but was exposed to the same amount and regime of shock. After 1 week of training, long-term potentiation (LTP) was measured in vitro in hippocampal slices. Exposure to uncontrollable shock massively impaired LTP relative to exposure to the same amount and regime of controllable shock. These results provide evidence that controllability modulates plasticity at the cellular-neuronal level.

EXPOSURE TO INESCAPABLE SHOCK in laboratory animals has been linked to marked changes in endocrine activity and central nervous system neurochemistry (*1*), suppressed immunological function (*2*), increased gastric ulceration (*3*), reduced activity (*4*), weight loss (*5*), decreased aggression and lowered dominance status (*6*), and analgesia (*7*). Most of these effects can be ameliorated when the animal can control the aversive event; control is defined as the capacity to make an instrumental response to an aversive stimulus.

Of particular interest are the learning deficits observed after exposure to inescapable shock (*8*). These deficits cover a wide range of tasks (*9*) and are often transferred from one task to another (*10*). Similar regimes have a detrimental effect on LTP in the rat hippocampus (*11*). LTP is a form of neuronal plasticity characterized by an increase in synaptic response to a constant volley after brief tetanic stimulation of afferent fibers (*12*). Because of its relatively long time course, localization in the hippocampus (although not exclusively), and correlation with behavioral learning (*13–17*), LTP has been suggested as a component of associative memory formation (*18*).

Prior exposure to uncontrollable shock eliminated LTP in the in vitro hippocampal slice preparation (*11*). To determine whether this effect, like those described above, could be ameliorated by permitting the animal to exert control, we placed Long-Evans male rats (n = 12), weighing 200 to 250 g, and a second group of yoked controls (n = 12) in identical soundproof shuttle boxes. Boxes were linked to a scrambled-shock generator, and the rats were subjected to low-intensity shock (60 Hz, 1 mA) every minute for 30 min. Yoked controls could not escape, but experimental animals were able to escape by running through an archway (8 cm by 8 cm) and tripping a balance switch that shut off the current to the boxes of both groups simultaneously. After seven daily sessions of 30 shock presentations with an intertrial interval (ITI) of 60 s, the experimental group had mastered the behavior to the extent that the duration of each shock had dropped on average from 3.8 to 1.5 s and over 75% of the responses were less than 1.5 s (Fig. 1).

Immediately after the seventh morning of training, animals from both groups were killed and hippocampal slices (400 μm) were prepared (*19*). Twelve additional Long-Evans males were taken directly from their home cages and killed, and hippocampal slices were prepared. Trunk blood was collected from all rats for corticosterone radioimmunoassay (*20*). Recordings were performed "blind" by the experimenter.

Extracellular field potentials were recorded from the cell body layer of CA1 after pulsed stimulation of the Schaffer collateral branches of CA3 pyramidal cell axons. After a 10-min stability period, input-output functions were obtained. The potential before tetanus was set at one-half the maxi-

T. J. Shors, T. B. Seib, R. F. Thompson, Department of Psychology, University of Southern California, Los Angeles, CA 90089.
S. Levine, Department of Psychiatry, Stanford University, Stanford, CA 93405.