provide 31.6 kWh of energy storage, which will provide a vehicle range of 415 km (264 miles). For the same battery volume, the Ovonic NiMH battery will increase the range per charge to 480 km (300 miles). The environmental impact of eventual disposal of the Ovonic NiMH battery has also been studied (*20*). Knoll and colleagues concluded that, according to existing Environmental Protection Agency regulations, batteries that use this technology can be safely disposed of in landfills. It has also been shown (*21*) that with existing technology, Ovonic batteries can be recycled into metallurgical additives for cast iron, stainless steel, or new Ovonic NiMH battery electrodes. The commercial viability of each of these technologically feasible recycling programs will depend on process economics.

Future developments of Ovonic NiMH batteries will include improvements through the continued optimization of the MH materials and electrodes as well as improvements to the positive electrode and cell design (*2*). For example, some of the ongoing research at OBC focuses on application of the company's synthetic materials techniques to the development of an improved positive electrode with enhanced storage capacity through the use of engineered valence control. The chemical reaction that occurs during the charge of a conventional $Ni(OH)_2$ electrode involves transfer of one electron per Ni atom. We are developing materials that use the exchange of up to two electrons per atom. In addition, MH alloys with twice the storage capacity of first-generation materials have been measured in the laboratory, and cell designs in which lightweight substrates, current collection components, and containers are used are now being developed. Because the overall energy density of the battery is determined by the entire system, these combined approaches are targeted at the fabrication of batteries with both an energy storage density of 150 Wh kg^{-1} and the characteristics shown in Fig. 7.

Conclusion

In the development of the Ovonic NiMH battery, we have used aspects of physics, chemistry, metallurgy, and materials science. In particular, materials concepts (*10–13*) were focused on structural and compositional disorder to develop an NiMH battery with the characteristics necessary for practical EV use in the near, middle, and distant future.

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RESEARCH ARTICLES

Regulation of Gene Expression in Hippocampal Neurons by Distinct Calcium Signaling Pathways

Hilmar Bading,* David D. Ginty, Michael E. Greenberg†

Calcium ions (Ca^{2+}) act as an intracellular second messenger and can enter neurons through various ion channels. Influx of Ca^{2+} through distinct types of Ca^{2+} channels may differentially activate biochemical processes. *N*-Methyl-D-aspartate (NMDA) receptors and L-type Ca^{2+} channels, two major sites of Ca^{2+} entry into hippocampal neurons, were found to transmit signals to the nucleus and regulated gene transcription through two distinct Ca^{2+} signaling pathways. Activation of the multifunctional Ca^{2+}-calmodulin–dependent protein kinase (CaM kinase) was evoked by stimulation of either NMDA receptors or L-type Ca^{2+} channels; however, activation of CaM kinase appeared to be critical only for propagating the L-type Ca^{2+} channel signal to the nucleus. Also, the NMDA receptor and L-type Ca^{2+} channel pathways activated transcription by means of different cis-acting regulatory elements in the c-*fos* promoter. These results indicate that Ca^{2+}, depending on its mode of entry into neurons, can activate two distinct signaling pathways. Differential signal processing may provide a mechanism by which Ca^{2+} controls diverse cellular functions.

In neurons, transient changes in the concentration of intracellular calcium ($[Ca^{2+}]_i$) can trigger various processes including neurotransmitter release, modulation of synaptic transmission, excitotoxic cell death, and alterations in gene expression (*1*, *2*). The concentration of intracellular Ca^{2+} can be increased by Ca^{2+} influx across the plasma membrane and by release of Ca^{2+} from internal stores (*3*). To control Ca^{2+} entry, neurons have multiple types of Ca^{2+}-permeable ion channels (*4*). The segregation of Ca^{2+} channels into distinct subcellular regions of neurons may serve to generate highly localized Ca^{2+} signals (*5*). Consequently, the mode of Ca^{2+} entry into neu-

rons might govern the intracellular transmission of Ca^{2+} signals and thereby elicit specific cellular responses to elevated $[Ca^{2+}]_i$ (5).

Among the Ca^{2+}-regulated processes critical for the long-term structural and functional modification of neurons are changes in gene expression (2). Activation of either of two types of Ca^{2+} channels—the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor and the voltage-gated L-type Ca^{2+} channel—induces expression of the c-*fos* gene and other immediate early genes (IEGs) (6). IEGs are rapidly and transiently transcribed in many cell types in response to a variety of extracellular signals (2, 7). Several IEGs, including c-*fos*, encode transcription factors and control secondary programs of gene expression that may ultimately determine the phenotypic response of the cell (2, 7).

It is unclear whether Ca^{2+} entry through different types of Ca^{2+} channels regulates transcription by a common mechanism or whether particular types of Ca^{2+} channels are linked to distinct signaling pathways. We used primary cultures of hippocampal neurons to investigate Ca^{2+} signaling pathways that couple Ca^{2+} flux through either NMDA receptors or L-type Ca^{2+} channels to transcriptional induction of the c-*fos* gene (8).

Treatment of hippocampal neurons with glutamate (10 μM) caused a rapid increase in c-*fos* transcription as determined by nuclear run-on (9) and Northern (RNA) blot analyses (Fig. 1). The glutamate-induced increase in expression of c-*fos* mRNA was completely blocked by the selective NMDA receptor antagonist D(−)-2-amino-5-phosphonovalerate (APV), but not by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), which specifically blocks the non-NMDA type of glutamate receptor (Fig. 1A). When both glutamate receptor subtypes were blocked with sodium kynurenate (1 mM) and $MgCl_2$ (11.3 mM), glutamate treatment also failed to induce expression of c-*fos* mRNA (Fig. 1A). Chelation of extracellular Ca^{2+} by the addition of EGTA to the medium prevented stimulation of c-*fos* expression by glutamate, suggesting that a transmembrane Ca^{2+} flux caused the transcriptional response (Fig. 1A). Treatment of cultures with nifedipine, an inhibitor of L-type Ca^{2+} channels, had only a small inhibitory effect on induction of c-*fos* by glutamate (Fig. 1A). Taken together, these findings demonstrate that in hippocampal neurons glutamate stimulates transcription of c-*fos* by activating Ca^{2+} flux through the NMDA type of glutamate receptor. Stimulation of the non-NMDA type of glutamate receptor or activation of L-type Ca^{2+} channels appears not to be required for induction of c-*fos* expression by glutamate.

To initiate entry of Ca^{2+} through L-type Ca^{2+} channels, we exposed hippocampal cultures to increased concentrations of extracellular KCl, which causes membrane depolarization. This treatment resulted in induction of c-*fos* expression (Fig. 1B). Immunocytochemical analysis with antibodies that recognize the c-*fos* protein demonstrated strong nuclear staining in neurons

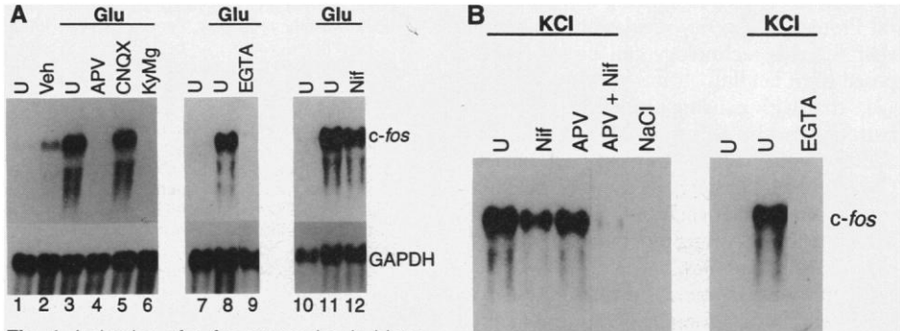

Fig. 1. Induction of c-*fos* expression in hippocampal neurons. (**A**) Northern blot analyses of total RNA from untreated neurons (U, lanes 1, 7, and 10) or neurons treated with H_2O (Veh, lane 2), glutamate (Glu, lanes 3, 8, and 11), glutamate in the presence of APV (100 μM) (lane 4), CNQX (40 μM) (lane 5), sodium kynurenate (1 mM) and $MgCl_2$ (11.3 mM) (KyMg, lane 6), 1 mM EGTA (lane 9), or nifedipine (5 μM) (Nif, lane 12). (**B**) Northern blot analyses of total RNA from untreated neurons (lane 6), neurons treated with NaCl solution (lane 5), KCl solution (lanes 1 and 7), or neurons treated with KCl solution in the presence of APV (100 μM) (lane 3), 1 mM EGTA (lane 8), nifedipine (5 μM) (lane 2), or nifedipine (5 μM) and APV (100 μM) (lane 4). Neurons were treated with APV, CNQX, sodium kynurenate and $MgCl_2$, and nifedipine for 10 minutes before stimulation. Neurons were treated with EGTA for 5 minutes before stimulation. All Northern blots were stripped and reprobed for constitutively expressed glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA to control for RNA loading.

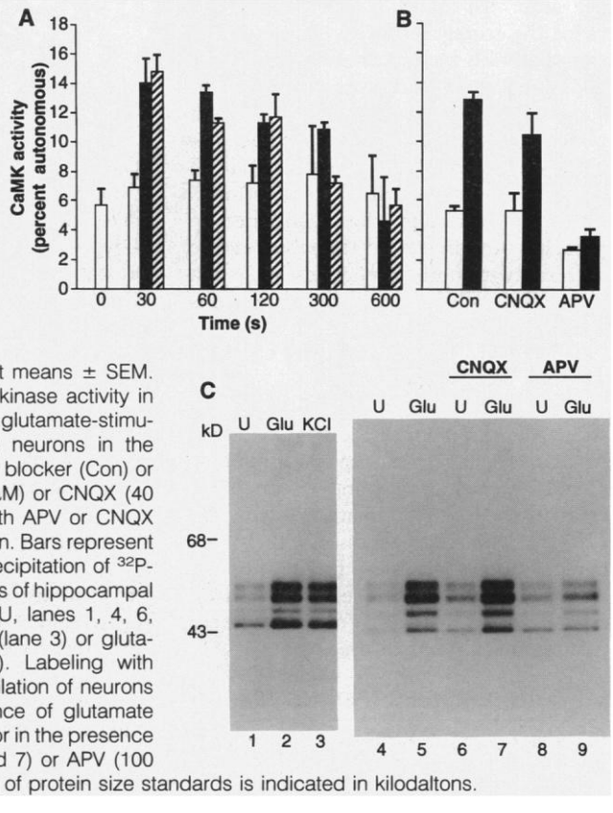

Fig. 2. Activation of CaM kinase in hippocampal neurons after stimulation of NMDA receptors or L-type Ca^{2+} channels. Percent autonomous activity is defined as the ratio of CaM kinase activity in the absence of Ca^{2+} relative to its activity in the presence of Ca^{2+} (13) (**A**) Time course of CaM kinase activity in neurons treated with H_2O (control) (open bars), glutamate (black bars), and KCl (hatched bars). Bars represent means ± SEM. CaMK, CaM kinase. (**B**) CaM kinase activity in unstimulated (open bars) and glutamate-stimulated (1 minute) (black bars) neurons in the absence of glutamate receptor blocker (Con) or in the presence of APV (100 μM) or CNQX (40 μM). Neurons were treated with APV or CNQX for 10 minutes before stimulation. Bars represent means ± SEM. (**C**) Immunoprecipitation of ^{32}P-labeled CaM kinase from lysates of hippocampal neurons that were untreated (U, lanes 1, 4, 6, and 8) or stimulated with KCl (lane 3) or glutamate (lanes 2, 5, 7, and 9). Labeling with [^{32}P]orthophosphate and stimulation of neurons was done either in the absence of glutamate receptor blocker (lanes 1 to 5) or in the presence of CNQX (40 μM) (lanes 6 and 7) or APV (100 μM) (lanes 8 and 9). Migration of protein size standards is indicated in kilodaltons.

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but not glial cells in hippocampal cultures that had been treated with KCl or glutamate (9). Induction of c-*fos* expression by KCl was effectively inhibited by nifedipine and, to a lesser extent, by APV (Fig. 1B). Induction of c-*fos* expression was completely blocked after chelation of extracellular Ca^{2+} with EGTA (Fig. 2B). This indicates that KCl treatment induces c-*fos* transcription primarily by stimulating Ca^{2+} influx through L-type Ca^{2+} channels but also in part by activating Ca^{2+} influx through NMDA receptors. As a means of activating c-*fos* expression by Ca^{2+} flux through L-type Ca^{2+} channels only, hippocampal neurons were treated with KCl in the presence of APV to inhibit Ca^{2+} influx through the NMDA receptor. Under these conditions, the increase in c-*fos* expression was completely blocked by nifedipine (Fig. 1B).

A well-characterized mediator of Ca^{2+} signaling events is the multifunctional Ca^{2+}-calmodulin–dependent protein kinase (CaM kinase) (*10*). In the pheochromocytoma cell line PC12, Ca^{2+} influx through voltage-sensitive Ca^{2+} channels induces c-*fos* transcription through a signaling pathway that may include activation of CaM kinase (*11*). However, the role of this kinase in the regulation of transcription in the hippocampus is unclear. KCl-induced membrane depolarization leads to an increase in CaM kinase activity in extracts prepared from hippocampal slices (*12*). To investigate whether exposure of cells to glutamate leads to stimulation of CaM kinase activity, we treated hippocampal neurons with glutamate or KCl and monitored the effects on CaM kinase activity. CaM kinase activity in hippocampal neurons was assessed by two independent methods: (i) Activity of CaM kinase in whole cell extracts was assayed in vitro by phosphorylation of a synthetic peptide substrate (*13*); and (ii) hippocampal neurons were labeled in vivo with [^{32}P]orthophosphate, the four subunits of CaM kinase were immunoprecipitated, and the extent of autophosphorylation was measured (*14*). Autophosphorylation of CaM kinase accompanies its activation (*15*).

Glutamate treatment or KCl-induced membrane depolarization of hippocampal neurons caused a rapid and transient induction of CaM kinase activity (Fig. 2). CaM kinase activity reached a maximum within 30 to 60 seconds after treatment with KCl or glutamate, and returned to the basal rate after 10 minutes (Fig. 2A). The effect of glutamate on CaM kinase activity was completely blocked by APV, whereas CNQX had no effect (Fig. 2, B and C). These results demonstrate that NMDA receptors mediate stimulation of CaM kinase activity by glutamate. In contrast, treatment of hippocampal neurons with KCl led to increased CaM kinase activity even in the presence of APV. This NMDA receptor–independent activation of CaM kinase activity appeared to require Ca^{2+} flux through L-type Ca^{2+} channels because it was blocked by nifedipine (*16*).

To determine whether stimulation of CaM kinase is critical for activation of c-*fos* by Ca^{2+} entry through either NMDA receptors or L-type Ca^{2+} channels, we used two compounds—calmidazolium, an inhibitor of calmodulin (*17*), and KN-62, a CaM kinase inhibitor (*18*)—both of which antagonized stimulation of CaM kinase activity (*19*) and CaM kinase autophosphorylation (Fig. 3D) in hippocampal neurons. Calmidazolium and KN-62 inhibited KCl-induced expression of c-*fos* mRNA in a dose-dependent manner (Fig. 3). In contrast, induction of c-*fos* expression by glutamate was largely unaffected by these agents (Fig. 3). These results suggest that Ca^{2+} signals evoked by activation of NMDA receptors and L-type Ca^{2+} channels are propagated to the nucleus through two pharmacologically distinguishable pathways. These experiments further suggest that CaM kinase has a role in the L-type Ca^{2+} channel signaling pathway but not in the NMDA receptor pathway. However, a limitation to the interpretation of these experiments is that the inhibitors calmidazolium and KN-62 might interfere with the function of molecules other than calmodulin or CaM kinase. Calmidazolium can affect Ca^{2+} influx into PC12 cells and snail neurons, and KN-62 reduces Ca^{2+} channel activity in the pancreatic β-cell line HIT-T15 (*20*). Nevertheless, the observation that calmidazolium and KN-62 inhibit CaM kinase activation without substantially affecting induction of c-*fos* expression after treatment with glutamate raised the possibility that the NMDA receptor signals to the nucleus by a pathway that is distinct from the L-type Ca^{2+} channel pathway.

The existence of distinct pathways that couple Ca^{2+} entry to gene expression was further assessed by examining the cis-acting regulatory sequences in the c-*fos* promoter that mediate increases in transcription after activation of NMDA receptors or L-type Ca^{2+} channels. For these experiments it was necessary to develop a method for transiently introducing DNA into hippocampal neurons in primary culture (*21*). Hippocampal neurons were transfected with the human c-*fos* gene containing various portions of the promoter region. The

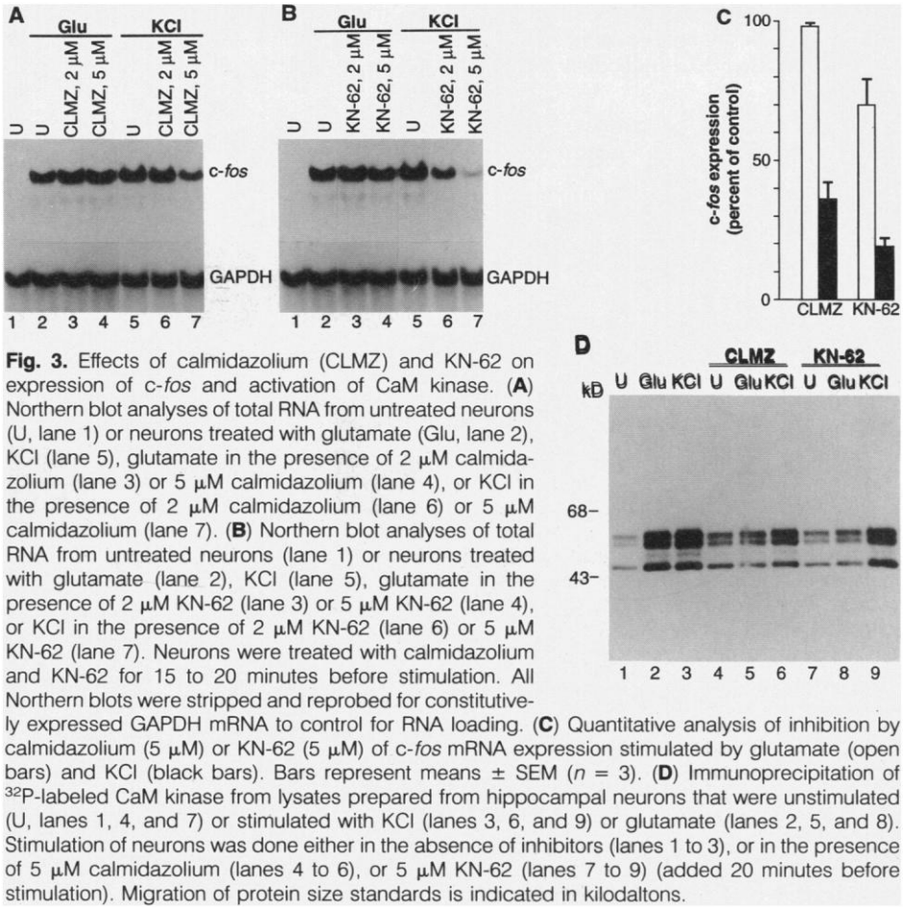

Fig. 3. Effects of calmidazolium (CLMZ) and KN-62 on expression of c-*fos* and activation of CaM kinase. (**A**) Northern blot analyses of total RNA from untreated neurons (U, lane 1) or neurons treated with glutamate (Glu, lane 2), KCl (lane 5), glutamate in the presence of 2 μM calmidazolium (lane 3) or 5 μM calmidazolium (lane 4), or KCl in the presence of 2 μM calmidazolium (lane 6) or 5 μM calmidazolium (lane 7). (**B**) Northern blot analyses of total RNA from untreated neurons (lane 1) or neurons treated with glutamate (lane 2), KCl (lane 5), glutamate in the presence of 2 μM KN-62 (lane 3) or 5 μM KN-62 (lane 4), or KCl in the presence of 2 μM KN-62 (lane 6) or 5 μM KN-62 (lane 7). Neurons were treated with calmidazolium and KN-62 for 15 to 20 minutes before stimulation. All Northern blots were stripped and reprobed for constitutively expressed GAPDH mRNA to control for RNA loading. (**C**) Quantitative analysis of inhibition by calmidazolium (5 μM) or KN-62 (5 μM) of c-*fos* mRNA expression stimulated by glutamate (open bars) and KCl (black bars). Bars represent means ± SEM (n = 3). (**D**) Immunoprecipitation of ^{32}P-labeled CaM kinase from lysates prepared from hippocampal neurons that were unstimulated (U, lanes 1, 4, and 7) or stimulated with KCl (lanes 3, 6, and 9) or glutamate (lanes 2, 5, and 8). Stimulation of neurons was done either in the absence of inhibitors (lanes 1 to 3), or in the presence of 5 μM calmidazolium (lanes 4 to 6), or 5 μM KN-62 (lanes 7 to 9) (added 20 minutes before stimulation). Migration of protein size standards is indicated in kilodaltons.

human α-globin gene, driven by the simian virus 40 early promoter, was transfected together with human c-*fos* as a control for transfection efficiency. Expression of the transfected genes and the endogenous rat c-*fos* gene was measured by a ribonuclease protection assay (*22*). In control experiments, hippocampal cultures were transfected with a plasmid containing the bacterial *lacZ* gene under the regulation of the Rous sarcoma virus long terminal repeat. Detection of the transfected *lacZ* gene product in an enzyme reaction with X-gal as a substrate demonstrated that virtually all of the transfected cells are neurons (*9*).

Treatment of hippocampal neurons with glutamate or KCl increased the amount of c-*fos* mRNA transcribed from the endogenous rat gene (c-fos^R) and from the transfected human gene pF4 (c-fos^H), which carries 750 bp of c-*fos* upstream regulatory sequence (Fig. 4A). These experiments demonstrate that it is possible to efficiently transfer plasmid DNA into primary hippocampal neurons and that expression of the transfected human c-*fos* gene recapitulates the regulation of the endogenous rat c-*fos* gene.

A 5′ deletion to nucleotide −222 relative to the transcriptional start site at +1 (plasmid pF222) reduced the ability of glutamate to induce transcription of the transfected c-*fos* gene (Fig. 4B). Thus, DNA sequences upstream of nucleotide −222 function in the glutamate response. The plasmid pF222 was still partially responsive to glutamate; however, this appeared to be almost completely a consequence of glutamate-induced membrane depolarization and subsequent activation of L-type Ca^{2+} channels because it was blocked by nifedipine (Fig. 4B). In contrast to the effect of glutamate, KCl treatment was effective at stimulating synthesis of c-*fos* mRNA from plasmid pF222 (Fig. 4B) (*23*). This effect of KCl was inhibited by nifedipine but not by APV, demonstrating that the transcriptional response was the result of activation of L-type Ca^{2+} channels (Fig. 4B). Taken together, these results indicate that NMDA receptors and L-type Ca^{2+} channels, the principal ion channels mediating induction of c-*fos* expression by glutamate and KCl treatment, respectively, require different c-*fos* promoter elements to activate transcription. Whereas sequences that lie between nucleotides −750 and −222 relative to the transcriptional initiation site in the c-*fos* promoter are important for the NMDA receptor pathway, induction of c-*fos* expression in hippocampal neurons by L-type Ca^{2+} channel activation can occur independently of DNA sequences 5′ of nucleotide −222.

Plasmid pF222 may be responsive to L-type Ca^{2+} channel stimulation because it contains a DNA element that has been implicated in activation of c-*fos* transcription after stimulation of voltage-sensitive Ca^{2+} channels in PC12 cells (*22*). This element (TGACGTTT), termed the Ca^{2+} response element (CaRE), is located at nucleotide −60 relative to the site of transcriptional initiation in the c-*fos* promoter and resembles the cyclic adenosine monophosphate response element (CRE) (TGACGTCA). The c-*fos* CaRE and the CRE appear to be functionally indistinguishable in their ability to mediate Ca^{2+}-regulated gene expression in PC12 cells (*22*). We investigated the role of the CaRE and CRE in the induction of c-*fos* expression by L-type Ca^{2+} channel activation in hippocampal neurons. A minimal c-*fos* promoter construct containing 42 nucleotides of upstream regulatory sequences (plasmid pAF42) showed essentially no response to stimulation with either KCl or glutamate (Fig. 4C). Insertion of one copy of the CaRE or CRE 5′ of nucleotide −42 (plasmids pAF42CaRE and pAF42CRE, respectively), conferred sensitivity to KCl and, to a lesser extent, to glutamate (Fig. 4C). Similar to the results obtained with plasmid pF222 (Fig. 4B), the glutamate response appeared to be largely a consequence of stimulation of L-type Ca^{2+} channels because it was inhibited by nifedipine (Fig. 4C). Quantitative analysis of these results revealed that the amounts of c-*fos* mRNA transcribed from plasmids pAF42CRE and pAF42CaRE after KCl-induced activation of L-type Ca^{2+} channels were 5.4 and 1.7 times that in untreated cells, respectively. In contrast, the amount of c-*fos* mRNA transcribed from plasmids pAF42CRE and pAF42CaRE increased only slightly (in this experiment, 1.3-fold) or slightly decreased, respectively, after activation of the NMDA receptor pathway with glutamate in the presence of nifedipine. Similar results were obtained in other experiments. We cannot rule out the possibility that the CRE or CaRE has a limited capacity to confer a transcriptional response to stimulation of NMDA receptors, but we could not detect such a response with our ribonuclease protection assay. Nevertheless these results do demonstrate that in hippocampal neurons a single copy of the CRE or CaRE mediates stimulation of c-*fos* expression after Ca^{2+} flux through L-type Ca^{2+} channels but is less potent in conferring a transcriptional response of c-*fos* after NMDA receptor activation.

Plasmid pF222 may be relatively unresponsive to NMDA receptor stimulation because it lacks the cis-acting serum response element (SRE) located at nucleotide −300 relative to the site of transcriptional initiation in the c-*fos* promoter. Because the SRE is necessary for induction of transcription of c-*fos* by a variety of agents including growth factors (*24*), we examined the possibility that the SRE might also control transcription of c-*fos* in response to glutamate. Insertion of one copy of the SRE

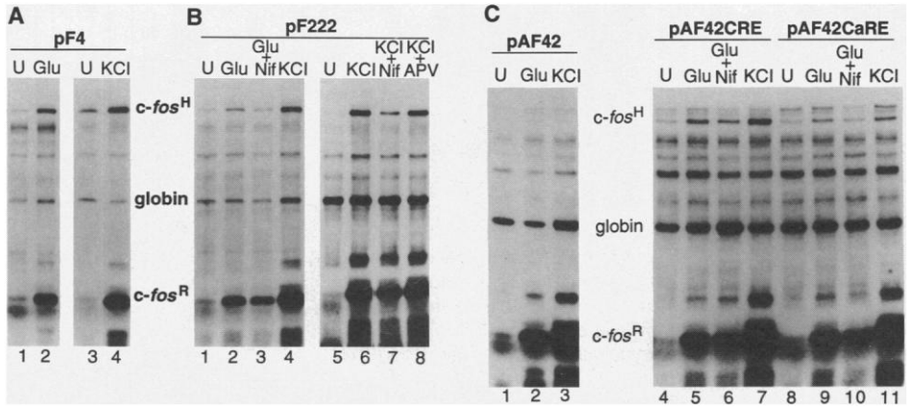

Fig. 4. Analyses of cis-acting regulatory elements that mediate induction of c-*fos* expression upon treatment of hippocampal neurons with glutamate or KCl. RNase protection analyses were used to measure expression of the endogenous rat c-*fos* gene (c-fos^R), the transfected human α-globin gene, and human c-*fos* gene (c-fos^H) after transfection of the indicated plasmids. (**A**) Plasmid pF4 (human genomic c-*fos* gene containing 750 bp of upstream regulatory sequence). RNA was isolated from unstimulated neurons (U, lanes 1 and 3) or neurons stimulated with glutamate (Glu, lane 2) or KCl (lane 4). (**B**) Plasmid pF222 (human genomic c-*fos* gene containing 222 bp of upstream regulatory sequence). RNA was isolated from unstimulated neurons (lanes 1 and 5) or neurons stimulated with glutamate (lane 2), glutamate and 5 μM nifedipine (lane 3), KCl (lanes 4 and 6), KCl and 5 μM nifedipine (lane 7), or KCl and 100 μM APV (lane 8). (**C**) Plasmids pAF42 (human genomic c-*fos* gene containing 42 bp of upstream regulatory sequence) (lanes 1 to 3), pAF42CRE (lanes 4 to 7), and pAF42CaRE (lanes 8 to 11). RNA was isolated from unstimulated neurons (lanes 1, 4, and 8) or neurons stimulated with glutamate (lanes 2, 5, and 9), glutamate and 5 μM nifedipine (lanes 6 and 10), or KCl (lanes 3, 7, and 11). Neurons were treated with nifedipine or APV for 10 minutes before stimulation. Nif, nifedipine.

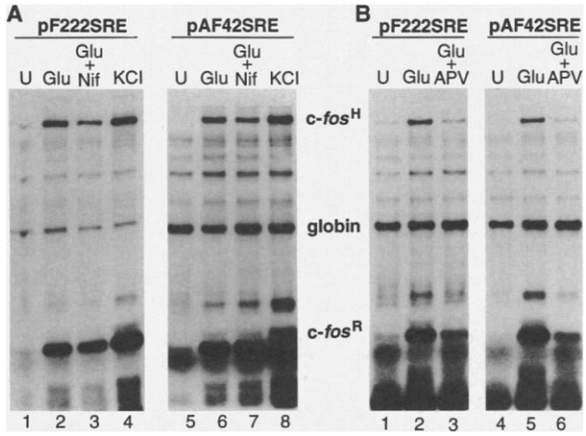

Fig. 5. Analyses of cis-acting regulatory elements required for induction of c-*fos* expression by NMDA receptor activation. Ribonuclease protection assays were done as described (Fig. 4). Hippocampal neurons were transfected with the indicated plasmids. (**A**) RNA was isolated from unstimulated neurons (lanes 1 and 5) or neurons stimulated with glutamate (Glu, lanes 2 and 6), glutamate and 5 μM nifedipine (lanes 3 and 7), or KCl (lanes 4 and 8). (**B**) RNA was isolated from unstimulated neurons (lanes 1 and 4) or neurons stimulated with glutamate (lanes 2 and 5) or glutamate and 100 μM APV (lanes 3 and 6). Neurons were treated with nifedipine and APV for 10 minutes before stimulation.

upstream of nucleotide −222 (plasmid pF222SRE) or upstream of nucleotide −42 (plasmid pAF42SRE) restored responsiveness to glutamate to these c-*fos* constructs (Fig. 5A). This transcriptional response appeared to be mediated by the NMDA receptor because it was blocked by APV (Fig. 5B). We conclude that in addition to its function as a growth factor response element, the SRE also functions as a Ca^{2+} response element that is a critical target of the NMDA receptor signaling pathway. Although the SRE was also capable of mediating a response to Ca^{2+} influx through the L-type Ca^{2+} channel after treatment of neurons with KCl (Fig. 5A), it is apparently not essential for this pathway because Ca^{2+} influx through L-type Ca^{2+} channels can stimulate expression of c-*fos* through the CaRE or CRE when the SRE is deleted.

Our finding that the SRE is important for induction of c-*fos* expression by the NMDA receptor pathway suggests that this pathway may be similar to signaling pathways activated by growth factor receptor tyrosine kinases. Although there is no evidence that NMDA receptors exhibit intrinsic tyrosine kinase activity, Ca^{2+} flux through NMDA receptors rapidly stimulates tyrosine and threonine phosphorylation of the mitogen-activated protein (MAP) kinase and activates its serine-threonine–specific phosphotransferase activity (*25*). MAP kinase and the MAP kinase–regulated ribosomal protein S6 kinase II ($pp90^{rsk}$) can phosphorylate transcription factors that interact with the SRE and control c-*fos* expression (*26*). This suggests a model in which Ca^{2+} entering through NMDA receptors leads to activation of MAP kinase, which, directly or indirectly, induces the phosphorylation of SRE-binding proteins and stimulates transcription of c-*fos*.

Our experiments have identified the SRE as an important regulatory element in the c-*fos* promoter that is targeted by the NMDA receptor pathway. However, it is conceivable that in the context of the endogenous gene other regulatory elements also participate in mediating NMDA receptor regulation of c-*fos* transcription. The finding that NMDA receptor activation leads to phosphorylation of the transcriptional regulatory site, serine-133, of the CRE- or CaRE-binding protein CREB (*27*) suggests that CREB and its DNA binding site may take part in the mechanism by which NMDA receptors control gene expression. Although our DNA transfection experiments demonstrate that a single copy of the CRE or CaRE can only confer a weak transcriptional response to the transfected gene upon NMDA receptor activation, the several CREs or CaREs located throughout the promoter of the endogenous c-*fos* gene (*28*) may augment the SRE-dependent transcriptional response to Ca^{2+} influx through NMDA receptors.

In contrast to the NMDA receptor pathway, the signal generated by Ca^{2+} flux through L-type Ca^{2+} channels may be propagated by CaM kinase and can efficiently stimulate transcription of c-*fos* through the CRE or CaRE even in the absence of the SRE. However, Ca^{2+} influx through L-type Ca^{2+} channels can also induce c-*fos* expression through the SRE. In PC12 cells, Ca^{2+} influx through voltage-sensitive Ca^{2+} channels can also activate c-*fos* expression through the SRE (*29*). This pathway may include activation of CaM kinase or MAP kinase–mediated phosphorylation of SRE-binding proteins, or both (*26, 29, 30*).

It is not known how Ca^{2+} entering hippocampal neurons through different types of channels can activate different signaling pathways. Ca^{2+} imaging studies and immunostaining analyses suggest that NMDA receptors and L-type Ca^{2+} channels may be localized to different subcellular regions of hippocampal neurons (*5*). The effects of Ca^{2+} influx may therefore depend on the availability of the particular mechanisms for signal processing at the site of Ca^{2+} entry. Alternatively, the various signaling pathways might respond to different concentration thresholds of Ca^{2+}; the difference in the response of the signaling pathways might reflect differences in the amount of Ca^{2+} that enters through NMDA receptors as compared to L-type Ca^{2+} channels. However, this possibility seems unlikely, because glutamate and KCl treatment lead to similar increases in $[Ca^{2+}]_i$ (*31*), and both stimuli cause a comparable induction of CaM kinase activity (Fig. 2). Of course, depending on the stimulus, Ca^{2+} concentrations might differ locally within the neuron.

We have demonstrated that changes in $[Ca^{2+}]_i$ influence gene expression in hippocampal neurons through at least two signaling mechanisms that are preferentially used depending on the mode of Ca^{2+} entry. Our finding that the differential activation of these Ca^{2+}-dependent signaling pathways can lead to activation of transcription through distinct DNA regulatory elements, suggests that these two pathways may induce distinct patterns of gene expression and may be a mechanism by which a single second messenger controls diverse cellular functions.

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volume of depolarization solution [10 mM Hepes (pH 7.4), 170 mM KCl, 1.3 mM $MgCl_2$, and 0.9 mM $CaCl_2$]. Control cultures were either untreated or treated with vehicle (water or dimethyl sulfoxide) or 0.41 volume of NaCl solution [10 mM Hepes (pH 7.4), 170 mM NaCl, 1.3 mM $MgCl_2$, and 0.9 mM $CaCl_2$]. To prevent excitotoxic cell death, sodium kynurenate (1 mM) and $MgCl_2$ (11.3 mM) (final concentrations) were added to the medium 10 minutes after stimulation. Total RNA was collected 50 minutes after stimulation and analyzed by Northern blot analysis.

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Chips off of Asteroid 4 Vesta: Evidence for the Parent Body of Basaltic Achondrite Meteorites

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For more than two decades, asteroid 4 Vesta has been debated as the source for the eucrite, diogenite, and howardite classes of basaltic achondrite meteorites. Its basaltic achondrite spectral properties are unlike those of other large main-belt asteroids. Telescopic measurements have revealed 20 small (diameters ≤10 kilometers) main-belt asteroids that have distinctive optical reflectance spectral features similar to those of Vesta and eucrite and diogenite meteorites. Twelve have orbits that are similar to Vesta's and were previously predicted to be dynamically associated with Vesta. Eight bridge the orbital space between Vesta and the 3:1 resonance, a proposed source region for meteorites. These asteroids are most probably multikilometer-sized fragments excavated from Vesta through one or more impacts. The sizes, ejection velocities of 500 meters per second, and proximity of these fragments to the 3:1 resonance establish Vesta as a dynamically viable source for eucrite, diogenite, and howardite meteorites.

About 6 percent of the meteorites falling to Earth have an igneous composition indicative of an origin in lava flows or basaltic intrusions on other planetary bodies. Although a few individual basaltic achondrite meteorites are now generally recognized to have been derived from the moon and Mars (*1*), there remains a debate over the parent body for most of these meteorites: the eucrites, diogenites, and howardites (*2*). Compositionally, the eucrites have the characteristics of basalt, the diogenites are plutonic, and the howardites are polymict breccias consisting of eucrite and diogenite fragments.

Asteroid 4 Vesta has been at the center of the debate over the parent body of the howardite-eucrite-diogenite (HED) meteorites. Vesta's optical spectrum, first measured by McCord *et al.* (*3*), contains a strong absorption band attributed to pyroxene centered near 9000 Å. On the basis of comparison with laboratory spectra of basaltic achondrites and Apollo 11 lunar samples, McCord *et al.* concluded that Vesta's surface is basaltic. Subsequent researchers (*4*) have interpreted that Vesta has a differentiated basaltic crust composed of a Mg-rich and Ca-poor pigeonite. Vesta's spectrum and apparent basaltic achondrite composition is unlike those of any other large main-belt asteroids, over 500 of which have been investigated (*5*). Drake (*6*) thus argued that Vesta must be the source for the basaltic achondrite meteorites. However, apparent dynamical difficulties in delivering fragments from Vesta to the Earth have

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