Calcineurin Enhances MEF2 DNA Binding Activity in Calcium-dependent Survival of Cerebellar Granule Neurons*

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Myocyte enhancer factor 2 (MEF2) has been shown recently to be necessary for mediating activity-dependent neuronal survival. In this study, we show that calcium signals regulate MEF2 activity through a serine/threonine phosphatase calcineurin. In cultured primary cerebellar granule neurons, the electrophoretic mobility of MEF2A protein was sensitive to the level of extracellular potassium chloride (KCl) and depolarizing concentrations of KCl led to hypophosphorylation of the protein. The specific inhibitors of calcineurin cyclosporin A (CsA) and FK506 could overcome KCl-dependent MEF2A hypophosphorylation. The effects of CsA and FK506 were KCl specific as they had little effect on MEF2A phosphorylation when granule neurons were cultured in the presence of full media. Hyperphosphorylation of MEF2A led to the loss of its DNA binding activity as determined by DNA mobility shift assay. Consistent with this, CsA/FK506 also inhibited MEF2-dependent reporter gene expression. These findings demonstrate that regulation of MEF2A by calcium signals requires the action of protein phosphatase calcineurin. By maintaining MEF2A in a hypophosphorylated state, calcineurin enhances the DNA binding activity of MEF2A and therefore maximizes its transactivation capability. The identification of MEF2 as a novel target of calcineurin may provide in part a biochemical explanation for the therapeutic and toxic effects of immunosuppressants CsA and FK506.

Calcium signaling is fundamental to many neuronal functions including the survival of developing neurons both in vivo as well as in vitro (1). A classic and important in vitro model that mimics the in vivo events of the trophic action of neuronal activity is potassium chloride (KCl)-dependent survival of primary cerebellar granule cells (2, 3). There, the elevated levels of extracellular potassium promote survival by opening L-type voltage-sensitive calcium channels leading to an influx of calcium into cells.

The molecular mechanisms by which calcium entry promotes neuronal survival have just begun to be defined. Several protein kinase-mediated signal transduction pathways are activated by calcium influx through L-type voltage-sensitive calcium channels (1). These include the classical protein kinase C isoforms, the calcium-dependent adenylate cyclases, members of the calcium-calmodulin dependent kinase family, and the components of Ras-MAPK signaling pathway. However, evidence for the involvement of these protein kinases in calcium-dependent neuronal survival has been limited. Recently, calcium influx has been shown to activate the phosphatidylinositol-3′OH kinase/Akt pathway and p38 MAPK signaling pathway providing a mechanism by which calcium promotes neuronal survival (4, 5).

In addition to the activation of protein kinases, calcium signals have also been shown to increase the activity of phosphatase calcineurin (also known as PP2B) (6–8). Calcineurin is a calcium and calmodulin-dependent serine/threonine phosphatase involved in many cellular processes including neuronal excitability (7) and the prevention of neurotoxicity (8). Although many of its identified substrates to date are structural proteins, one of the best studied calcineurin targets is nuclear factor of activated T cell (NFAT), a transcription factor that when dephosphorylated by calcineurin, translocates into the nucleus to activate gene expression (9–11). Interestingly, recent studies by several groups suggest that calcineurin may also regulate gene expression by acting on another important transcription regulator myocyte enhancer factor 2 (MEF2). It has been shown that putative MEF2-binding sites within promoters of Epstein-Barr virus lytic gene BZLF1, nur77 orphan steroid receptor, and some slow fiber-specific genes can confer calcium inducibility and are calcineurin sensitive (12–14). Despite its DNA binding sites having been implicated in mediating calcium/calcineurin-dependent gene transcription, MEF2 itself has not been shown to be the target of or regulated by calcineurin. Furthermore, the molecular mechanisms by which calcineurin regulates MEF2-dependent gene transcription remain undefined.

MEF2 belongs to a family of MADS (MCM1, agamous, deficiens, and serum response factor) box transcription factors and plays a critical role in muscle gene expression (15). Four members of mammalian MEF2s have been identified known as MEF2A to D. They bind to DNA as homo- and heterodimers through the consensus MEF2 binding sequence C(T/T)A(A/T)TA/G to regulate gene expression (16). In this study, a mechanism by which calcium signals regulate the function of MEF2A in cultured rat cerebellar granule neurons has been characterized. This signaling pathway involves calcineurin. Specifically, blocking intracellular calcium signals reduced MEF2-dependent reporter gene activity. Both KCl withdrawal and the inhibition of calcineurin by CsA and FK506 led to the hyperphosphorylation of MEF2A protein. The hyperphosphorylation of MEF2A inhibited its DNA binding activity. Consistent with

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Membrane Depolarization-dependent MEF2 Reporter Gene Activation in Cultured Primary Cerebellar Granule Neurons—To address the activation of MEF2-dependent gene expression by neuronal activity, we measured the levels of expression from a CAT reporter gene that contains two MEF2 DNA-binding sites within its 5'-regulatory region. Previous studies including our own have shown that MEF2 protein is expressed by cerebellar granule neurons in vitro and in vivo (21–24). Our recent work has demonstrated that expression from the MEF2 reporter gene is activated in granule neurons cultured in the presence of KCl and the enhanced expression requires MEF2 DNA-binding sites. However, it was not clear whether withdrawal of KCl could lead to the inhibition of MEF2 reporter gene expression. To test this, MEF2-driven CAT reporter gene activity was measured in the absence of KCl following transfection. Prolonged removal of trophic factor KCl results in neuronal apoptosis at a rate of about 30% of cell death within 24 h following KCl withdrawal (17). To exclude the possibility that the change of MEF2-dependent CAT activity following KCl withdrawal was due to cell death, CAT activity was measured 10 to 12 h after KCl removal, a time when most cells were still morphologically healthy and no obvious cell death could be detected. As expected, robust MEF2 DNA-binding site-dependent CAT activity could be detected when cells were cultured in the presence of KCl (25 mM) (Fig. 1) and this activity depended on calcium influx through L-type voltage-sensitive calcium channels. Removal of KCl (5 mM) resulted in a significant decrease in the level of CAT expression (Fig. 1). Dose-response analysis revealed that the degree of decline in MEF2-driven CAT activity paralleled the levels of extracellular KCl as less than optimal levels of KCl (10 mM) led to an intermediate change of CAT expression. These results suggested that calcium signals are critical for activation of MEF2-dependent gene regulation.

Analysis of MEF2A Protein in the Presence and Absence of KCl—To study the mechanisms by which the calcium signals regulate MEF2-driven reporter gene expression, we analyzed the expression of MEF2 protein in the presence and absence of KCl. Although the levels of MEF2 protein expressed by granule neurons within 6 h following KCl withdrawal did not change (data not shown), MEF2A protein prepared from KCl-deprived granule cells showed a significant alteration in its mobility when analyzed by Western blot. There were at least two major species of MEF2A protein detectable in KCl-treated granule cell extracts (Fig. 2A). KCl withdrawal, however, resulted in the disappearance of the faster migrating species with a corresponding increase in the level of the slower migrating species of MEF2A (Fig. 2A). The change of the ratio of the faster and slower migrating MEF2A species suggested that there is a

![Fig. 1. Effects of KCl withdrawal on MEF2-dependent CAT reporter gene expression in cultured primary granule neurons. Primary granule neurons cultured for 6 days in vitro were transfected with one MEF2 CAT reporter plasmid (pTKMEF2 × 2CAT) along with a construct encoding β-Gal (pRSVβ-Gal). 2 h after transfection, cells were washed three times with media without serum and placed in media containing different concentrations of KCl (+KCl, 25 mM; −KCl, 5 mM; or −KCl, 10 mM) for another 10 h before harvest for CAT and β-Gal assay. + indicates transfection with a construct containing 2 wild type MEF2 DNA-binding sites in pTKMEF2 × 2CAT reporter and mt with mutated MEF2 DNA-binding sites in the reporter. CAT activity in samples transfected with mt MEF2 × 2CAT reporter was treated as one and relative fold of CAT activity is presented.](image-url)
KCl-dependent change in the post-translational modification of MEF2A.

To establish that the mobility change of MEF2A resulting from KCl withdrawal was indeed due to variable degrees in phosphorylation, protein extracts from granule neurons deprived of KCl were treated with calf intestinal alkaline phosphatase (AP). Treatment of KCl-deprived protein samples with AP reversed the mobility of MEF2A from slow to fast migration and resulted in the appearance of a MEF2A species migrating even faster than the lower MFEA2A band found in KCl-treated neurons (Fig. 2B). These findings confirmed the KCl-dependent hypo- and hyperphosphorylation of MEF2A in granule cells and suggested that calcium signals maintain MEF2A protein in a hypophosphorylated state through the activation of a protein phosphatase.

Analysis of the Calcium-dependent Phosphatase Involved in MEF2A Dephosphorylation—To characterize the phosphatase involved in dephosphorylation of MEF2A in response to membrane depolarization in granule neurons, we studied the effects of calcineurin-specific inhibitors on MEF2A mobility by Western blot. Recent studies have identified several calcium responsive promoters and mapped the calcium-responsive and cyclosporin A (CsA)-sensitive elements to the putative MEF2 DNA-binding site (9, 12, 14). But it was not clear from those studies whether MEF2 was a target of this signaling pathway. To address this question, we tested the effect of blocking calcineurin on MEF2A phosphorylation. Treatment of granule neurons cultured in the presence of KCl with either CsA or FK506 led to a change in the level of MEF2A phosphorylation as indicated by the disappearance of the faster migrating band of MEF2A (Fig. 3A, B and C). CsA at a concentration as low as 0.01 μM effectively reversed the effect of KCl and led to increased MEF2A phosphorylation. The effect of CsA or FK506 on MEF2A phosphorylation was relatively specific to KCl as the mobility of MEF2A prepared from granule cells cultured under full media conditions (serum + KCl) was not significantly altered by CsA or FK506 (Fig. 3C). However, serum alone failed to maintain MEF2A in a hypophosphorylated state even in the absence of calcineurin inhibitors (Fig. 3D). The specific effects of calcineurin inhibitors were further corroborated by the failure of okadaic acid, an inhibitor to serine/threonine phosphatases PP1 and PP2A, to alter the migration of MEF2A (Fig. 3E).

These results suggested that calcineurin was the major phosphatase to dephosphorylate MEF2A in response to calcium signals in granule neurons and was required in mediating calcium-dependent MEF2A hypophosphorylation.

Kinetic Analysis of MEF2A Hyperphosphorylation in Response to KCl Withdrawal or the Addition of CsA—KCl withdrawal has been shown to trigger granule cell death, and it has been suggested that granule neurons irreversibly commit themselves to the apoptotic pathway about 2 h following KCl withdrawal (25). To correlate MEF2A dephosphorylation with cell death, the kinetics of MEF2A hyperphosphorylation after KCl removal or the addition of CsA were analyzed. The mobility change of MEF2A was detectable as early as 15 to 30 min following the addition of CsA to cells where an alteration in MEF2A migration was first detected at about 30 to 60 min following the addition of CsA (Fig. 4A). A similar but somewhat delayed pattern was seen with CsA where an alteration in MEF2A migration was first detectable at 30 to 60 min following the addition of CsA (Fig. 4B). In general, withdrawal of KCl was more effective in altering the pattern of MEF2A migration than CsA. The inclusion of CsA in addition to KCl withdrawal did not result in any further up-shift in MEF2A migration or shift in the kinetics to an earlier time point (Fig. 4C). These findings suggested that the change in MEF2A phosphorylation preceded the final commitment of granule neurons to the apoptotic pathway following KCl withdrawal and therefore were consistent with the finding that MEF2 is required for calcium-promoted neuronal survival.12

Change of MEF2A DNA Binding Activity in Response to KCl Withdrawal or the Inhibition of Calcineurin by CsA/ FK506—To study the biochemical consequences of the change
in MEF2A dephosphorylation by calcineurin, we assessed MEF2A DNA binding activity by electrophoretic mobility shift assay. Previous studies of nur77 promoter in T cell activation have shown that the DNA binding activity of MEF2 is constitutive and insensitive to calcium signals, and only the transactivation activity of MEF2 is calcium signal-dependent (14). To our surprise, we found that MEF2A DNA binding activity in granule neurons was calcium-dependent and could be modulated by calcineurin activity. KCl withdrawal or addition of CsA/FK50 resulted in diminished DNA binding by MEF2A (Fig. 5, A and B). The decline in MEF2A DNA binding activity closely correlated with the change in its pattern of migration, as the decrease in MEF2A DNA binding activity followed a time course consistent with the occurrence and the level of hyperphosphorylated MEF2A species. However, CsA did not significantly affect the DNA binding activity of MEF2A when granule neurons were cultured in full media (Fig. 5C), consistent with our previous finding that CsA did not result in MEF2A hyperphosphorylation in the presence of full media. These findings suggested that one direct consequence of calcineurin-dependent MEF2A hypophosphorylation in response to calcium influx into granule neurons is the enhancement of the DNA binding ability by MEF2A. The hyperphosphorylation of MEF2A resulting from a decrease in calcineurin activity leads to an inhibition of MEF2A DNA binding. These observations provide a new mechanism by which calcium signals regulate MEF2 activity.

### The Expression of MEF2 CAT Reporter Gene Is CsA/FK506 Sensitive—
The decline of MEF2A DNA binding activity resulting from the inhibition of calcineurin suggested that calcineurin was involved in the activation of MEF2A-dependent genes. To test this, we analyzed the activity of MEF2-driven CAT reporter gene following the inhibition of calcineurin by CsA or FK506. Calcium-dependent MEF2 CAT gene expression was found to be CsA/FK506 sensitive. Both CsA and FK506 significantly reduced the expression of calcium-dependent and MEF2-mediated CAT reporter gene (Fig. 6) suggesting that the activity of calcineurin is required to promote MEF2-dependent gene expression.

### DISCUSSION

Calcium signals are critical in mediating many cellular responses including neuronal survival. In this report, we have defined a mechanism whereby calcium signals regulate the activity of MEF2A protein in cultured primary cerebellar granule neurons. Calcium-dependent and calmodulin-regulated calcineurin has been identified as the major phosphatase that regulates MEF2A activity in response to membrane depolarization. The mobility of MEF2A on a SDS-polyacrylamide gel electrophoresis is both calcium and calcineurin sensitive. Blocking calcium influx by either lowering the levels of extracellular concentrations of KCl or the inhibition of calcineurin with CsA or FK506 results in the hyperphosphorylation of MEF2A. Hyperphosphorylation of MEF2A significantly reduces its ability to bind to DNA and therefore inhibits its transactivation activity. By maintaining MEF2A protein in a hypophosphorylated state, calcineurin enhances MEF2-dependent gene expression.

Previous studies have mapped the calcineurin-dependent induction of the Nur77 promoter to the putative MEF2 DNA-binding site (14). However, the mechanism by which MEF2 factor mediates the calcineurin-sensitive response in T cells was not clear. Although the transactivation activity of MEF2 requires calcium signals, its DNA binding activity seems to be constitutive and insensitive to calcium signals (14). Our studies show that in cultured cerebellar granule neurons, MEF2A DNA binding activity is calcium sensitive and modulated by calcineurin. The reason for this difference is not clear. It is possible that MEF2 proteins may be regulated differently in response to calcium signals in different cell types. The balancing effect of other phosphatases or kinases may be different in those cells. Finally, different isoforms of MEF2 could be regulated differently. As the antibodies used in the nur77 promoter study do not distinguish between different MEF2 isoforms, it is
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The finding that MEF2A is a downstream target of calcineurin in response to calcium influx raises the question of whether calcineurin dephosphorylates MEF2A directly or indirectly. Given that no other phosphatase cascades have been shown to be the direct substrate of calcineurin in granule neurons, the most simple explanation for this finding is that MEF2A protein is a direct substrate of calcineurin. MEF2 is a nuclear protein. Although it has been shown recently that MEF2 can translocate from nucleus to cytoplasm in myocyte in response to TGF-β (26), it does not seem to do so in response to the withdrawal of KCl. On the other hand, calcineurin resides primarily in the cytoplasm. Calcineurin can translocate into the nucleus together with its substrate such as NFAT but has not been shown to do so by itself (6, 27, 28). NFAT is expressed in granule cells and can be found in the nucleus of cells treated with KCl (data not shown). Therefore, it is possible that calcineurin molecules transported into the cell nucleus with NFAT in response to calcium signals may dephosphorylate MEF2A directly in the nucleus.

Together with our studies on p38 MAPK-dependent MEF2 activation in neurons, data presented here show that two complementary biochemical mechanisms, phosphorylation and dephosphorylation, regulate the activity of MEF2 protein in a synchronized fashion in response to calcium signals. The findings that both p38 MAP kinase and phosphatase calcineurin activate MEF2 in the presence of calcium suggest that these enzymes function through different sites on MEF2. The p38 MAPK sites within MEF2A have been identified in the C-terminal of the protein where its transactivation domain resides (29, 30). The hypothesized sites for calcineurin on MEF2A proteins remain unknown. Given that calcineurin inhibitors affect MEF2A DNA binding activity, it is most likely that calcineurin sites are at the N-terminal region of the protein, which contains the highly conserved DNA binding and dimerization domains of MEF2 (30). It is known that the substrate specificity of calcineurin is not only due to a specific sequence but rather determined by both primary and higher order structural features (6). Therefore, it is not surprising that preliminary analysis of this region of MEF2 protein failed to identify any obvious calcineurin sites. Previous studies of MEF2 phosphorylation in COS cells have shown that the only known in vivo phosphorylation site within this region of MEF2 protein is a casein kinase II site (31). However, the casein kinase II site is an unlikely calcineurin target as phosphorylation of this site has been shown to enhance MEF2C DNA binding. These results suggest that the pattern and regulation of MEF2 phosphorylation in granule neurons in response to calcium signaling may be very different from that in COS cells.

Given that MEF2 is required for neuronal survival, blocking calcineurin activity with CsA or FK506 might be expected to induce neuronal death. However, our preliminary studies show that CaA and FK506, either alone or in combination, fail to block the calcium-promoted survival of granule neurons consistently. The reason for this is unclear. One possible explanation is that CsA and FK506 may not completely abolish calcineurin activity in vivo. Consistent with this, our own analysis demonstrates that in general, KCl withdrawal results in a higher degree of MEF2A upshifting and CaA or FK506, even at a higher dose, is less effective than CaA withdrawal in shifting MEF2A to a hyperphosphorylated form. This is in agreement with the finding that a much higher degree of residual MEF2A DNA binding activity could be detected in CaA-treated cells (Fig. 5, A and B). Therefore, it is possible that the residual MEF2A activity could account for the lack of cell death in the presence of CaA or FK506. Or alternatively, unidentified CaA-insensitive mechanisms might contribute to cell survival. Consistent with this hypothesis, CaA and FK506 were found to be much less effective in inhibiting MEF2A DNA binding and transactivation activities in the presence of full media. The mechanisms by which full media protect MEF2A from the inhibitory effects of calcineurin inhibitors are not known. It is conceivable that instead of targeting the phosphatase calcineurin, the survival signals of full media may inhibit the activity of inhibitory kinase(s) whose activities would normally become unopposed following KCl withdrawal or inhibition of calcineurin function when granule neurons are grown in the presence of CaA. It is interesting to note that this additional survival signals could only be activated by a combination of serum and KCl because serum alone seems to be unable to prevent MEF2A from being hyperphosphorylated even in the absence of calcineurin inhibitors.

The identification of calcineurin as the major MEF2A phosphatase may have broad implication not just limited to granule neurons and could provide insight into the therapeutic and toxic effects of CaA observed clinically. It is widely accepted that the immunosuppressive action of CsA is due to inhibition of NFAT, a transcription factor important for cytokine gene expression during immune response (32–34). MEF2 proteins are expressed by a variety of cell types in the immune system including macrophages, B cells, and T cells (data not shown) (29, 35). Their target genes in these immune cells have only just begun to be identified. For example, MEF2 proteins have been suggested to regulate the expression of immunoglobulin gene light chain or J chain in B cells (36, 37), nur77 in T cells (14), c-fos in macrophages during an inflammation response (29), and more importantly, the regulator of cytokine-inducible gene NF-κB (5). Therefore, inhibition of MEF2 may underlie part of the mechanisms by which CsA suppresses immune responses. The side effects of CaA and FK506, such as nephrotoxicity and neurotoxicity, could be partly due to the inhibition of MEF2 in those cell types where MEF2 factors have been shown to be expressed.
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REFERENCES

1. Ghosh, A., and Greenberg, M. E. (1995) Science 268, 239–247
2. Franklin, J. L., and Johnson, E. M., Jr. (1992) Trends Neurosci. 15, 501–508
3. Hack, N., Hidak, H., Wakefield, M. J., and Balazs, R. (1995) Neuroscience 57, 9–20
4. Miller, T. M., Tansey, M. G., Johnson, E. M., Jr., and Creedon, D. J. (1997) J. Biol. Chem. 272, 9647–9653
5. Zechn, D., Craig, R., Hanford, D. S., McDonough, P. M., Sabbadini, R. A., and Glembotski, C. C. (1998) J. Biol. Chem. 273, 8232–8239
6. Kies, C. B., Ren, H., and Wang, X. (1998) J. Biol. Chem. 273, 13367–13370
7. Miller, T. M., and Johnson, E. M., Jr. (1996) J. Neurosci. 16, 4696–4706
8. Dudek, H., Datta, S. R., Franke, T. F., Birnbaum, M. J., Yao, R., Cooper, G. M., Segal, R. A., Kaplan, D. R., and Greenberg, M. E. (1997) Science 275, 661–665
9. McDermott, J. C., Cardoso, M. C., Yu, Y. T., Andres, V., Leifer, D., Krause, D., Lipton, S. A., and Nadal-Ginard, B. (1995) Mol. Cell. Biol. 15, 2564–2577
10. Lyons, G. E., Micales, B. K., Schwarz, J., Martin, J. F., and Olson, E. N. (1995) J. Neurosci. 15, 5727–5738
11. Lin, X., Shah, S., and Bulleit, R. F. (1996) Brain Res. Mol. Brain Res. 42, 307–316
12. Leifer, D., Golden, J., and Kowall, N. W. (1994) Neuroscience 63, 1067–1079
13. Ikeshima, H., Imai, S., Shimoda, K., Hata, J., and Takano, T. (1995) Neurosci. Lett. 200, 117–120
14. Miller, T. M., and Johnson, E. M., Jr. (1996) J. Neurosci. 16, 7487–7495
15. De Angelis, L., Borch, S., Meltzchionna, R., Berghole, L., Bacarani-Contrera, M., Parise, F., Ferrari, S., and Assou, G. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 12353–12363
16. Shibasaki, F., Price, R. E., Milan, D., and McKeon, F. (1996) Nature 382, 370–373
17. Luo, C., Shaw, K. T., Raghavan, A., Arambarri, J., Garcia-Cazorla, F., Perrine, B. A., Hogan, P. G., and Rao, A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 899–9012
18. Han, J., Jiang, Y., Li, Z., Kravchenko, V. V., and Ulevitch, R. J. (1997) Nature 386, 286–289
19. Molkentin, J. D., Block, L. M., Martin, J. F., and Olson, E. N. (1996) Mol. Cell. Biol. 16, 2627–2636
20. Molkentin, J. D., Li, L., and Olson, E. N. (1996) J. Biol. Chem. 271, 17199–17204
21. Hoyt, T., Kiyono, T., Williamson, K., and Xu, X. (1995) Immunity 2, 461–472
22. Masuda, E. S., Naito, T., Tokushima, H., Campbell, D., Saito, F., Hannum, C., Ariu, K., and Ariu, N. (1995) Mol. Cell. Biol. 15, 2697–2706
23. Swanson, B. J., Jack, H. M., and Lyons, G. E. (1998) Mol. Immunol. 35, 445–458
24. Satyaraj, E., and Storb, U. (1998) J. Immunol. 161, 4795–4802
25. Raso, S., Karray, S., Guckstetter, E. R., and Keshland, M. E. (1998) J. Biol. Chem. 273, 26123–26129