Calcineurin inhibition and cardiac hypertrophy: A matter of balance

Leslie A. Leinwand*

Department of Molecular, Cellular, and Developmental Biology, Porter Addition, Room A3B40, University of Colorado, Boulder, CO 80309-0347

The heart is remarkably plastic, changing its size, shape, and function in response to a wide variety of stimuli. Beyond birth, by far the greatest cardiac growth occurs by cellular hypertrophy, rather than hyperplasia. When normal cardiac size has been reached, additional changes in size can occur in response to stimuli such as hypertension, defective genes, or exercise, to name a few. Although some of this hypertrophy is adaptive, some of it is maladaptive and can ultimately result in cardiac failure, the largest health care burden in the United States. A great deal of attention has been paid to the molecular pathways of hypertrophy, and it is clear that they are complex, having many intersections. A series of observations has pointed toward calcineurin as a key mediator of cardiac hypertrophy, and two articles in this issue of PNAS have further underscored this assertion (1, 2). Calcineurin is a serine–threonine phosphatase that is activated by Ca^{+2}-calmodulin. Calcineurin dephosphorylates nuclear factor of activated T cells (NFAT), which then translocates to the nucleus where it acts combinatorially with other transcription factors to activate downstream targets. A report by Molkentin *et al*. in 1998 demonstrated that an activated calcineurin could provoke massive cardiac hypertrophy and that the immunosuppressive agents, cyclosporin A (CsA) and FK506, could block this process (3). Further, expression of a downstream target of calcineurin, activated NFAT3, had the same effect and could be prevented by CsA and FK506 (3). These observations provoked heated arguments about the appropriateness of both calcineurin as a therapeutic target and immunosuppressants as drugs to prevent/treat cardiac hypertrophy.

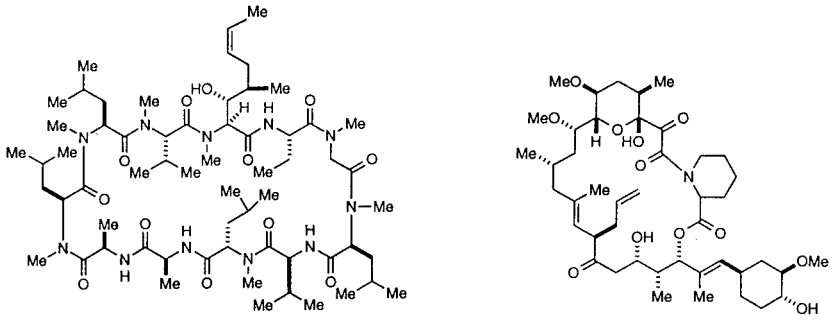

Fig. 1. Chemical immunosuppressive agents cyclosporin A (CsA) and FK506 may be replaced by protein inhibitors in the battle to inhibit calcineurin and cardiac hypertrophy.

Why Such Controversy?

Calcineurin has long been a therapeutic target during postsurgical attempts to prevent rejection of transplanted organs. The immunosuppressant drugs, CsA and FK506 (Fig. 1), form complexes with cyclophilin and FKBP12, respectively, which then complex with the catalytic subunit of calcineurin. This approach of testing the effect of pharmacologic inhibition of calcineurin activity might have seemed straightforward, but the seemingly conflicting results obtained from such trials (see below) have divided workers into camps, arguing about the promise and pitfalls of calcineurin inhibition to treat or prevent cardiac hypertrophy. The lure is obvious: drugs already exist and have been used for a considerable time. However, calcineurin is a ubiquitous protein, undoubtedly involved in many different pathways in multiple organ systems. CsA is known to cause nephrotoxicity, which may preclude its use as a prophylactic measure for hypertrophy (4). It has also been shown that, at least in some cases, patients undergoing treatment with CsA develop hypertension and hypertrophy (5). Finally, CsA and FK506 have targets that are independent of calcineurin (6, 7). For these reasons, it has been argued that prophylactic administration of CsA or FK506 to prevent hypertrophy would be ill-advised.

Controversy has been ignited by inconsistent results in attempts to treat various induced and genetic models with these drugs (see Table 1). In an attempt to validate calcineurin as a potential drug target and to further understand the pathways of cardiac hypertrophy, genetic inhibition of calcineurin activity has now been achieved. In this issue of PNAS, two reports show convincingly that three different endogenous protein inhibitors of calcineurin can block several forms of cardiac hypertrophy (1, 2). Many of those skeptical about calcineurin's central role and its potential therapeutic target may now have a harder time making their arguments. Although the authors do not claim that calcineurin will play a role in all of hypertrophy, it is now more clear than ever that its role is a significant one.

The role of calcineurin in cardiac hypertrophy was initially posited on the basis of the finding that GATA4 and MEF2, cardiac-enriched transcription factors, were important in activating cardiac genes during hypertrophy and that NFAT could bind to GATA4 and to MEF2 (14, 15). Because NFAT was a known target of calcineurin's action, the logical next step was to test the effect of activating calcineurin and NFAT in the heart. The key report that spawned most of the heated discussions and further tests of the inhibition of calcineurin was that mentioned above, in which CsA and FK506, both in clinical use to prevent organ rejection, prevented calcineurin-induced hypertrophy (3). Subsequently, reports have described the ability and inability of these drugs to prevent a variety of models of

See companion articles on pages 3322 and 3328.

*E-mail: leinwand@stripe.colorado.edu.

hypertrophy. Some of these are summarized in Table 1, and the results are confusing. For example, inconsistent results have been achieved in ostensibly similar models of pressure overload-induced hypertrophy in which one of three outcomes was observed: hypertrophy was completely prevented, was unaffected, or it was attenuated, and the animals died as a result of CsA treatment. The effect of calcineurin inhibition by CsA on genetic models has been tested and, whereas the hypertrophy resulting from mutations in some sarcomeric proteins can be prevented by CsA, it does not prevent the hypertrophy resulting from cardiac expression of an activated retinoic acid receptor (9). The end result of these reports has been to leave the community of scientists who are interested in therapeutics with an unsatisfying notion about the potential for inhibition of calcineurin and cardiac hypertrophy.

Nonpharmacologic Inhibition of Calcineurin

Despite the controversy surrounding immunosuppressant therapy for cardiac hypertrophy, or perhaps because of it, new lines of investigation using additional approaches have now underscored the importance of calcineurin in cardiac hypertrophy *in vivo*. Two groups have now used genetic means to inhibit calcineurin activity and have demonstrated quite convincingly that three different protein inhibitors of calcineurin inhibit three different models of cardiac hypertrophy (1, 2). The activity of calcineurin can be modulated not only by the immunophilins that are the targets of CsA and FK506 but also by at least three proteins: AKAP79, cabin-1/cain, and MCIP1 (16, 17). AKAP79 binds calcineurin in conjunction with protein kinases A and C. Cabin-1/cain binds both calcineurin and the transcription factor, MEF2, and its overexpression leads to calcineurin and MEF2 inhibition and can prevent hypertrophic responses in cardiac myocytes (16). Both AKAP79 and cabin-1/cain are ubiquitously expressed proteins, but neither is expressed at high levels in the heart, suggesting that they are unlikely to regulate cardiac calcineurin activity. However, the observation that cabin-1/cain can directly negatively regulate MEF2 suggests that it *could* have effects in the heart, if expressed. Another class of protein inhibitors of calcineurin is termed MCIPs (myocyte enriched calcineurin interacting proteins). MCIPs are highly expressed in striated muscle and are unrelated to any of the above-mentioned inhibitors of calcineurin. Because of their enrichment in striated muscle, they would seem to be attractive as potential selective inhibitors of calcineurin. Of the two family members, MCIP1, and not MCIP2, is regulated by calcineurin activity, providing a backup means of protecting the cell from the deleterious consequences of unrestrained calcineurin activity (17, 18).

In the articles by Rothermel *et al.* and De Windt *et al.* (1, 2), genetic approaches were taken to inhibit calcineurin activity, but each study has unique aspects as well. Both used transgenic overexpression of protein inhibitors of calcineurin, and the use of the α-cardiac myosin heavy chain promoter provided cardiac-specific expression of the transgene. The inhibitors used were either truncated forms of AKAP79 and cabin-1/cain (2) or MCIP1 (1). The three inhibitors were tested for their effect on several distinct models of cardiac hypertrophy. The first was the well known hypertrophic response of the heart to β-adrenergic stimulation, and the three protein inhibitors were found to be effective in inhibiting hypertrophy. Although wild-type animals showed ≈22% increase in heart/body weight in response to isoproterenol, MCIP1 expression limited hypertrophic growth to an ≈8% increase. Cabin-1/cain expression reduced hypertrophic growth from a 20% increase to ≈10%. The effect of cabin-1/cain activity expression was also tested on aortic constriction of the expressing transgenic mice and also used in an acute administration of the protein inhibitors via adenovirus mediated gene transfer to the rat heart, which had been subjected to aortic constriction. Both were found to be effective. An added measure of efficacy in the paper by DeWindt *et al.* (2) was that the authors measured calcineurin activity and showed that activity was depressed by the peptide inhibitors. However, as they note, there are reservations with the calcineurin activity assay. The authors of the MCIP1 paper tested the efficacy of MCIP1 expression on the genetic model of hypertrophy and failure resulting from cardiac expression of activated calcineurin. Once again, the effect was striking in that it was similar to the effects of treatment with CsA or FK506.

Table 1. Effects of calcineurin inhibition on cardiac hypertrophy

Model	Species	Outcome	Ref.
Renovascular hypertension (2 kidney, 1 clip)	Mice	Prevention	8
Tropomodulin overexpression	Mice (transgenic)	Prevention	9
Myosin light chain-2	Mice (transgenic)	Prevention	9
Fetal β tropomyosin	Mice (transgenic)	Prevention	9
Activated calcineurin	Mice (transgenic)	Prevention	3
Activated NFAT3	Mice (transgenic)	Prevention	3
Pressure overload (abdominal aorta)	Rat	Prevention	10
Pressure overload (aortic banding)	Mice	Attenuated LVH, heart failure	11
Retinoic acid receptor	Mice (transgenic)	No prevention of hypertrophy	9
Pressure overload (aortic banding)	Mice	No prevention of hypertrophy	12
Pressure overload (aortic banding)	Rat	No prevention of hypertrophy	13
Pressure overload (aortic banding)	Rat	Attenuated LVH, death	9

LVH, left ventricular hypertrophy.

Is All Hypertrophy Bad?

Of added import was the test of the effect of MCIP1 on exercise-induced hypertrophy. Interest in this experiment stems from the little that is known about the overlap of physiologic and pathologic hypertrophic pathways. It is well appreciated that exercise conditioning results in cardiac enlargement that is beneficial, whereas hypertrophy that results from a pathologic stimulus such as pressure overload can ultimately be deleterious. MCIP1 expression was shown to be effective in inhibiting exercise-induced hypertrophy. Herein lies the rub. From a basic science perspective, it is very useful to know that the calcineurin pathway is common, at least at some level, to both forms of hypertrophy. However, these observations raise the issue of the potential deleterious effects of inhibiting hypertrophy. Both groups reported some deleterious consequences of calcineurin inhibition in their founder populations. For example, transgenic lines expressing high levels of the cabin-1/cain or AKAP79 peptides showed thin ventricular walls, suggesting an inhibition of normal developmental hypertrophy, and mice expressing MCIP1 had a 5–10% decrease in cardiac mass (1, 2). This potential hazard could presumably be overcome by appropriate timing and dosage. But these observations underscore the complexities of these proteins as therapeutic modalities. Consistent with these concerns are the observations in two animal models of pressure

overload in which prevention of the hypertrophic response resulted in death, presumably because the initial hypertrophy is a necessary compensatory mechanism (9, 11).

In summary, the studies in this issue of PNAS show that nonpharmacologic manipulation of calcineurin activity can have profound effects on genetic, mechanical, and exercise-induced hypertrophy, as well as on normal developmental hypertrophy. These observations solidify calcineurin's central role in cardiac hypertrophy. Whether inhibitors of its activity will be effective therapeutic agents in clinical use remains to be seen.

1. Rothermel, B. A., McKinsey, T. A., Vega, R. B., Nicol, R. L., Memmen, P., Yang, J., Antos, C. L., Shelton, J. M., Bassel-Duby, R., Olson, E. N. & Williams, R. S. (2001) *Proc. Natl. Acad. Sci. USA* (2001) **98,** 3328–3333.
2. De Windt, L. J., Lim, H. W., Bueno, O. F., Liang, Q., Delling, U., Braz, J. C., Glascock, B. J., Kimball, T. F., del Monte, F., Hajjar, R. J. & Molkentin, J. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 3322–3327.
3. Molkentin, J., Lu, J., Antos, C., Markham, B., Richardson, J., Robbins, J., Grant, S. & Olson, E. (1998) *Cell* **93,** 215–228.
4. Aperia, A., Ibarra, F., Svensson, L., Klee, C. & Greengard, P. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 7394–7397.
5. Lipkin, G., Tucker, B., Giles, M. & Raine, A. (1993) *J. Hypertens.* **11,** 439–442.
6. duBell, W., Gaa, S., Lederer, W. & Rogers, T. (1998) *Am. J. Physiol.* **275,** 2041–2052.
7. Kaibori, M., Okumura, T., Ito, S., Oda, M., Inoue, T. & Kamiyama, Y. (1999) *Transplant Proc.* **31,** 804–805.
8. Murat, A., Pellieux, C., Brunner, H. & Pedrazzini, T. (2000) *J. Biol. Chem.* **275,** 40867–40873.
9. Sussman, M., Lim, H., Gude, N., Taigen, T., Olson, E., Robbins, J., Colbert, M., Gualberto, A., Wieczorek, D. & Molkentin, J. (1998) *Science* **281,** 1690–1693.
10. Shimoyama, M., Hayashi, D., Takimoto, E., Zou, Y., Oka, T., Uozumi, H., Kudoh, S., Shibasaki, F., Yazaki, Y., Nagai, R. & Komuro, I. (1999) *Circulation* **100,** 2440–2454.
11. Meguro, T., Hong, C., Asai, K., Takagi, G., McKinsey, T., Olson, E. & Vatner, S. (1999) *Circ. Res.* **84,** 735–740.
12. Ding, B., Price, R., Borg, T., Weinberg, E., Halloran, P. & Lorell, B. (1999) *Circ. Res.* **84,** 729–734.
13. Zhang, W., Kowal, R., Rusnak, F., Sikkink, R., Olson, E. & Victor, R. (1999) *Circ. Res.* **84,** 722–728.
14. Mesali, N., Nakamura, K., Zvaritch, E., Dickie, P., Dziak, E., Krause, K., Opas, M., MacLennan, D. & Michalak, M. (1999) *J. Cell. Biol.* **144,** 857–868.
15. Chin, E., Olson, E., Richardson, J., Yang, Q., Humphries, C., Shelton, J., Wu, H., Bassel-Duby, R. & Williams, R. (1998) *Genes. Dev.* **12,** 2499–2509.
16. Taigen, T., Windt, L., Lim, W. & Molkentin, J. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 1196–1201.
17. Rothermel, B., Vega, R., Yang, J., Wu, H., Bassel-Duby, R. & Williams, R. (2000) *J. Biol. Chem.* **275,** 8719–8725.
18. Yang, J., Rothermel, B., Vega, R., Frey, N., McKinsey, T., Olson, E., Bassel-Duby, R. & Williams, R. (2000) *Circ. Res.* **87,** 61–68.