Calcineurin and skeletal muscle growth

To the editor — Calcineurin is recognized as a crucial, though not the sole, signalling intermediate in the regulation of skeletal muscle fibre hypertrophy in mice and rats[1,2,3]. Recently, Bodine and colleagues[4] refuted a function for calcineurin in growth because, unlike us (Fig 1b,c in ref. 1), they were unable to prevent hypertrophy of overloaded muscle fibres with the calcineurin inhibitors, cyclosporin A (CsA) or FK506.

Their failure to block fibre hypertrophy may be because they injected a lower dose of CsA, administered both inhibitors only once daily, initiated drug treatments 1 day later, and used different drug vehicles compared to our regime[1,2]. They show average calcineurin activities to be 20% lower after CsA treatment and unchanged by CsA in overloaded muscles[4], compared to our observation of 65% inhibition with a higher dose of CsA (Fig. 1a)[3]. Clear dose-dependent effects of CsA on calcineurin activity and CsA blood levels exist (Fig. 1b). Furthermore, their report of increased muscle mass after 1 week of overload-FK506 treatment may be caused by inflammation, a well-documented characteristic of the overload model[5–7], emphasizing that wet mass is not a reliable measure of muscle hypertrophy[1]. Indeed, muscle protein content is only higher at 15 days and decreased protein concentrations recovered by 30 days of overload[6,7]. Finally, the authors attribute our CsA drug effects to general toxicity. As stated[1], animal growth and daily locomotor activity levels were unaffected by CsA treatment (Fig. 1c,d). Given these arguments, and our recent demonstration of an extensive calcineurin-mediated dephosphorylation of NFAT and MEF2 during overload[3], it is premature to rule out a function for calcineurin in skeletal muscle growth.

Shannon E. Dunn, Alain R. Simard, Renée A. Prud'homme and Robin N. Michel*

Neuromuscular Research Laboratory, Laurentian University, Sudbury, Ontario, P3E 2C6, Canada

**e-mail: RNMICHEL@nickel.laurentian.ca*

Reply — Regarding our recent papers showing that the Akt pathway is necessary and sufficient for skeletal muscle hypertrophy *in vitro* and *in vivo*[4,10], Dunn *et al.* focus on our skepticism concerning the function of the calcineurin pathway in these processes.

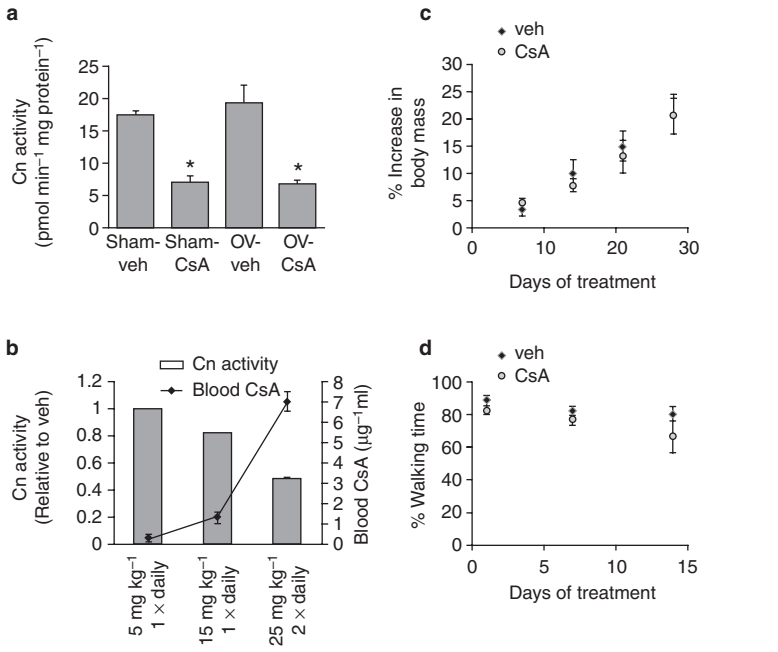

Figure 1 **Effects of cylcosporin A. a**, Mean ±SE total calcineurin (Cn) activity in plantaris lysates ($n = 3$ per group) from 5 d Sham or overloaded (OV) mice treated with vehicle (veh) or CsA (25 mg kg^{-1}, twice daily). Asterisk, different from Sham-veh. **b**, Plantaris mean ±SE relative to Cn activity (left) and blood CsA level (right) from rats treated for 5 d at a CsA dosage of 5 mg kg^{-1} once daily (reported to be ineffective in preventing fibre growth during muscle regeneration[8]), 15 mg kg^{-1} once daily[4] and 25 mg kg^{-1} twice daily[1]. **c**,**d**, Mean ±SE percent change in animal body weight (**c**) and percent walking time during an open field test[9] (**d**) after veh or CsA treatment ($n = 6$–9 group^{-1} time point^{-1}).

After noting their comments, we remain skeptical based on the following points:

First, we reported that there is no significant increase in calcineurin activity during muscle hypertrophy, and Dunn *et al.*'s data is in agreement with this finding (compare Fig. 1b from Bodine *et al.*[4] with bar 1 and bar 3 in Fig. 1a of their response letter); this lack of calcineurin activation contrasts to the marked activation of the Akt pathway[4,10]. It is difficult to claim that calcineurin is a key inducer of hypertrophy if its activity does not change during this process.

Second, we reported that pharmacologic inhibition of the calcineurin pathway with either cyclosporin or FK506 does not block skeletal muscle hypertrophy, and that expression of constitutively active forms of calcineurin does not cause hypertrophy[4,10]. Several other groups similarly reported that pharmacologic or genetic blockade of calcineurin does not block skeletal muscle hypertrophy[8,11], and that transgenic overexpression of constitutively active calcineurin does not cause hypertrophy[2,12] — of note, the latter paper is from the Dunn *et al.* group. The other pharmacologic studies[8,11] are noteworthy in that they reported inhibition of other calcineurin-mediated processes in skeletal muscle without any effect on hypertrophy. Similarly, we completely blocked calcineurin-mediated NFAT phosphorylation in cultured myotubes, and still

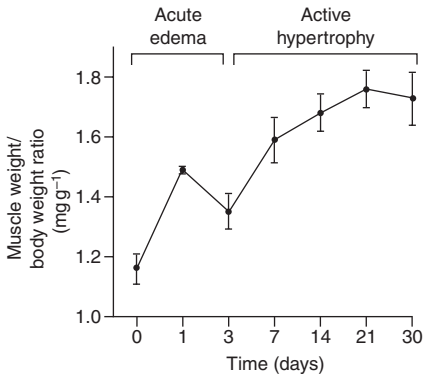

Figure 2 **Plantaris muscle hypertrophy is induced by chronic overload.** Rat plantaris wet weight relative to animal body weight from 0–30 days of compensatory hypertrophy. Values are means ± S.E., $n = 5$–8 animals per group. A transient acute edema is seen only at day-1, followed by a steady increase in muscle weight caused by hypertrophy, which seems to plateau between 2 and 4 weeks. An agent that blocked the hypertrophy response would be expected to exert its effects by day-14, whereas an agent that only decreased muscle weight between day 14 and day 30 would seemingly be affecting a post-hypertrophy process, and could result from long-term toxicity, known to result from chronic treatment with CsA (ref. 9) or the vehicle used, Cremophor EL[14,15].

did not see an inhibition of hypertrophy[10].

Third, perhaps the hardest point to reconcile with Dunn *et al.*'s comments is their own published finding[1] that pharmacologic blockade of calcineurin did not significantly inhibit skeletal muscle hypertrophy during the first 2 weeks after functional overload — inhibition was only evident at 4 weeks[1]. As a major increase in muscle mass is achieved in these models by two weeks[13] (also see Fig. 2), and is not accounted for by inflammatory edema, but by increases in fibre size and muscle protein[13], and as this increase cannot be blunted by calcineurin blockade, the data cited from both our studies and that of Dunn *et al.* demonstrate that the hypertrophy induced by two weeks of functional overload does not require the calcineurin pathway. The late effects of high doses of calcineurin blockers would either be consistent with a second form of late hypertrophy, which is indeed calcineurin-dependent, or with a non-specific toxicity caused by the agents used, as has been reported[14,15].

In contrast to the difficulty in definitively demonstrating a function for the calcineurin pathway in overload-induced skeletal muscle hypertrophy, the case for the Akt pathway seems quite strong[4,10]. However, future studies might still demonstrate a role for calcineurin under some other settings of skeletal muscle hypertrophy, just as calcineurin has been implicated in certain settings of cardiac hypertrophy, but not in others[16].

George D. Yancopoulos and David J. Glass

Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591-6707, USA

email: gdy@regeneron.com or david.glass@regeneron.com

1. Dunn, S. E., Burns, J. L. & Michel, R. N. *J. Biol. Chem.* **274,** 21908–21912 (1999).
2. Dunn, S. E., Chin, E. R. & Michel, R. N. *J. Cell Biol.* **151,** 663–672 (2000).
3. Dunn, S. E. Simard, A. R., Bassel-Duby, R., Williams, R. S. & Michel, R. N. *J. Biol. Chem.* **276,** 45243–45254 (2001).
4. Bodine, S. C. *et al. Nature Cell Biol.* **3,** 1014–1019 (2001).
5. Armstrong, R. B., Marum, P., Tullson, P. & Saubert, C. W. *J. Appl. Physiol.* **46,** 835–842 (1979).
6. Noble, E. G., Tang, G. Q. & Taylor, P. B. *Can. J. Physiol. Pharmacol.* **62,** 1178–1182 (1984).
7. Ianuzzo, C. D. & Chen, V. *J. Appl. Physiol.* **46,** 738–742 (1979).
8. Serrano, A. L. *et al. Proc. Natl Acad. Sci. USA* **98,** 13108–13113 (2001).
9. Moll, J. *et al. Nature* **413,** 302–307 (2001).
10. Rommel, C. *et al. Nature Cell Biol.* **3,** 1009–1013 (2001).
11. Musaro, A. *et al. Nature Genet.* **27,** 195–200 (2001).
12. Naya, F. J. *et al. J. Biol. Chem.***275,** 4545–4548 (2000).
13. Adams, G. R. & Haddad, F. *J. Appl. Physiol.* **81,** 2509–2516 (1996).
14. Sanchez, H. *et al. J. Mol. Cell Cardiol.* **32,** 323–331 (2000).
15. Gelderblom, H., Verweij, J., Nooter, K. & Sparreboom, A. *Eur. J. Cancer* **27,** 1590–1598 (2001).
16. Zhang, W. *et al. Circ. Res* **84,** 722–728 (1999).