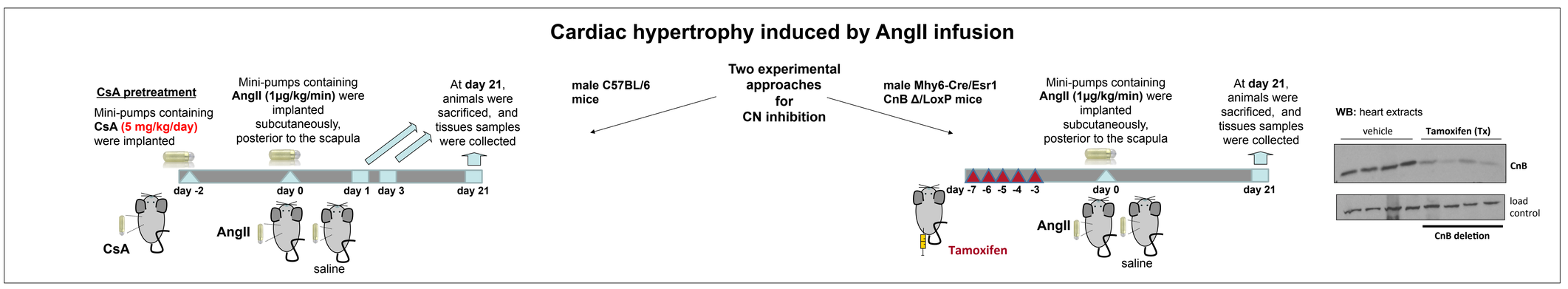
Role of Calcineurin in the development of cardiac hypertrophy

Sara Martínez-Martínez[1], Noelia Lozano Vidal[1], Mª Dolores López Maderuelo[1],Ana Vanesa López Alonso[2], Rut Alberca Rodríguez[1], Luis Jesús Jiménez-Borreguero[2] and Juan Miguel Redondo[1]

Departamentos de [1]Biología Vascular e Inflamación y [2]Epidemiología, Aterotrombosis e Imagen, Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC). Madrid, España.

Previous studies have shown that the Calcineurin (CN)/NFAT signalling pathway is an established important regulator of cardiac hypertrophy. However, most studies are based on gain- and loss-of-function initiated at or before birth, and there are no detailed studies about the role of this pathway in the progression of pathological cardiac remodelling during adulthood.
To investigate the role of CN in the progression of disease we have analyzed the effect of cyclosporin A (CsA), a pharmacological CN inhibitor, on angiotensin II (AngII)-induced hypertension and cardiac hypertrophy in mice. Our results show that although CsA treatment does not alter the AngII-induced hypertension, it blocks the increase of cardiac mass, ventricular wall thickness and cardiomyocyte size when hypertrophy is established (21 days after AngII infusion). Unexpectedly, AngII infusion induces hypertrophy as early as three days and this effect is also inhibited by CsA. Interestingly, CsA impairs cardiac function and has no effect on AngII-induced fibrosis. A complementary analysis based on the inducible genetic deletion of CN in heart just before the induction of hypertrophy indicates that CN deficiency results in a clear reduction of hypertrophy accompanied by a blockade of AngII-induced fibrosis. Furthermore, CN deficient mice, but not CsA-treated mice, do not develop systolic dysfunction.



Pharmacological inhibition of CN: **CsA**

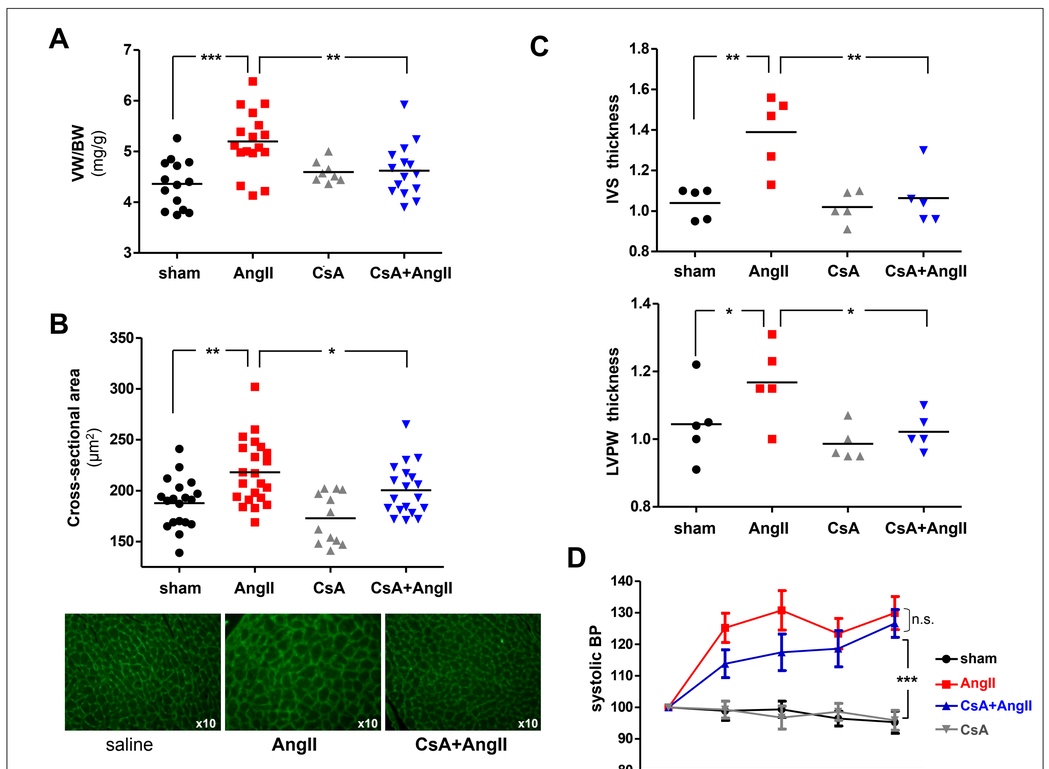


Fig.1 CsA inhibits the cardiac enlargement induced by AngII in a hypertension-independent manner.

A, CsA reduces the increase in cardiac mass mediated by AngII. After 21 days of AngII infusion, mice from 4 experimental groups (**sham**, control mice; **AngII,** mice treated with AngII for 21 days; **CsA,** mice treated with CsA for 21 days and **CsA+AngII**, AngII-treated mice that were also infused with CsA) were sacrificed and their ventricular weight was measured. Ventricular mass (mg) was normalized to body weight (g). **B, CsA diminishes cardiomyocyte size in AngII-treated mice.** Quantification of myocyte surface area from cardiac histological sections of mice stained with FITC-lectin (green) using ImageJ software (upper panel). Representative pictures are shown (lower panel). **C, CsA reduces the increase in thickness of interventricular septum (IVS) and left ventricular posterior wall (LVPW) mediated by AngII.** In vivo analysis by echocardiography was performed at day 21 of treatment. Wall thickness was measured in M-mode from cardiac longitudinal images and normalized to the thickness determined before the treatments. **D, CsA treatment has not a significant effect on AngII-induced hypertension.** Blood pressure (BP) of mice was monitorized during the longitudinal study. Systolic BP, measured at successive days after AngII minipump implantation, was normalized to systolic blood pressure of each mice before treatment (n=13 mean±SEM).

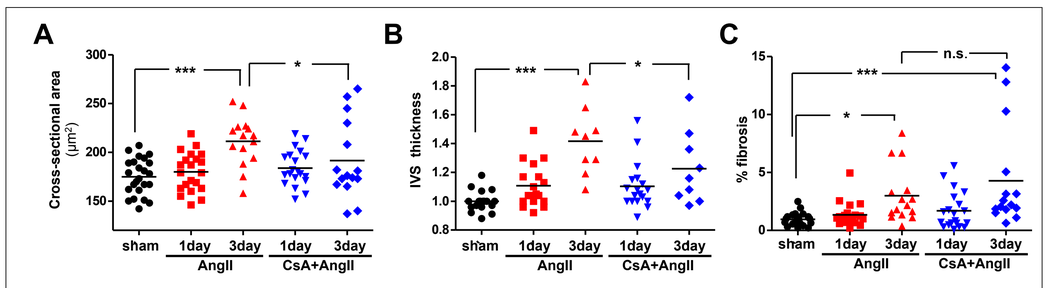
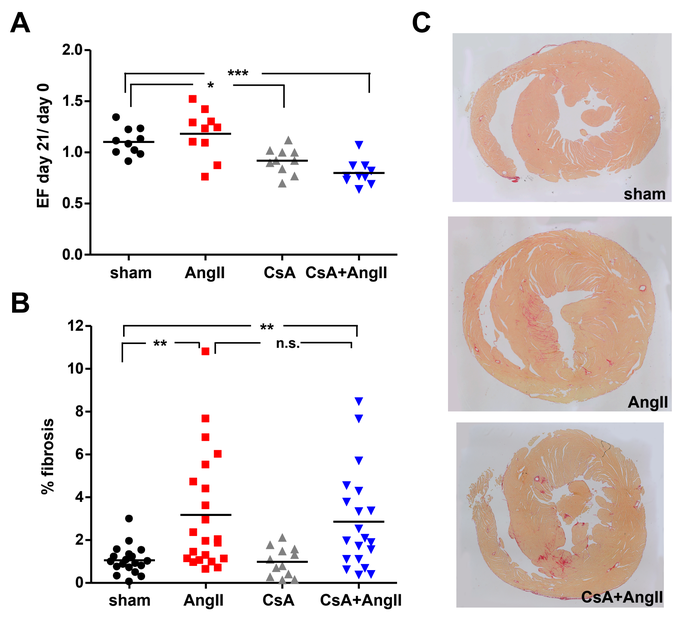


Fig.2 AngII induces a very early development of cardiac hypertrophy; CsA reduces cardiac mass but not fibrosis.

A, AngII infusion for 3 days induces a significant increase in myocyte size that is attenuated in CsA-treated mice. Quantification of cardiomyocyte area was performed as in Fig1.B. **B, IVS thickness is increased at day 3 of AngII infusion in a CsA sensitive fashion.** Mice were analysed by echocardiography at 1 day and 3 days after implanting AngII mini-pumps as in Fig.1C. **C, CsA treatment does not affect AngII-induced fibrosis at day 3.** Transversal sections of heart were stained with picrosirius red, and percentage of myocardium area staining for fibrosis was quantified by Image J software.

Fig.3 CsA impairs systolic cardiac function, and fails to reduce AngII-induced fibrosis.

A, Mice treated with CsA display a significant reduction in systolic cardiac function. Twenty-one days after AngII infusion, cardiac function of mice from four experimental groups was analysed by echocardiography. Percentage of ejection fraction was measured from M-mode tracings from each mice before treatment (day 0) and 21 days after AngII minipump implantation (day21). Changes in ejection fraction at day 21 are represented. **B and C, AngII-induced fibrosis when hypertrophy is established, is not reduced by CsA treatment.** Cardiac histological sections of mice sacrificed at day 21 were stained with picrosirius red to detect fibrosis. **B,** Quantification of percentage of myocardium area staining for fibrosis, and **C,** Representative images of picrosirius red staining (red indicates collagen deposition)



Genetic inhibition of CN: **Mhy6-Cre/Esr1 CnB Δ/LoxP mice**

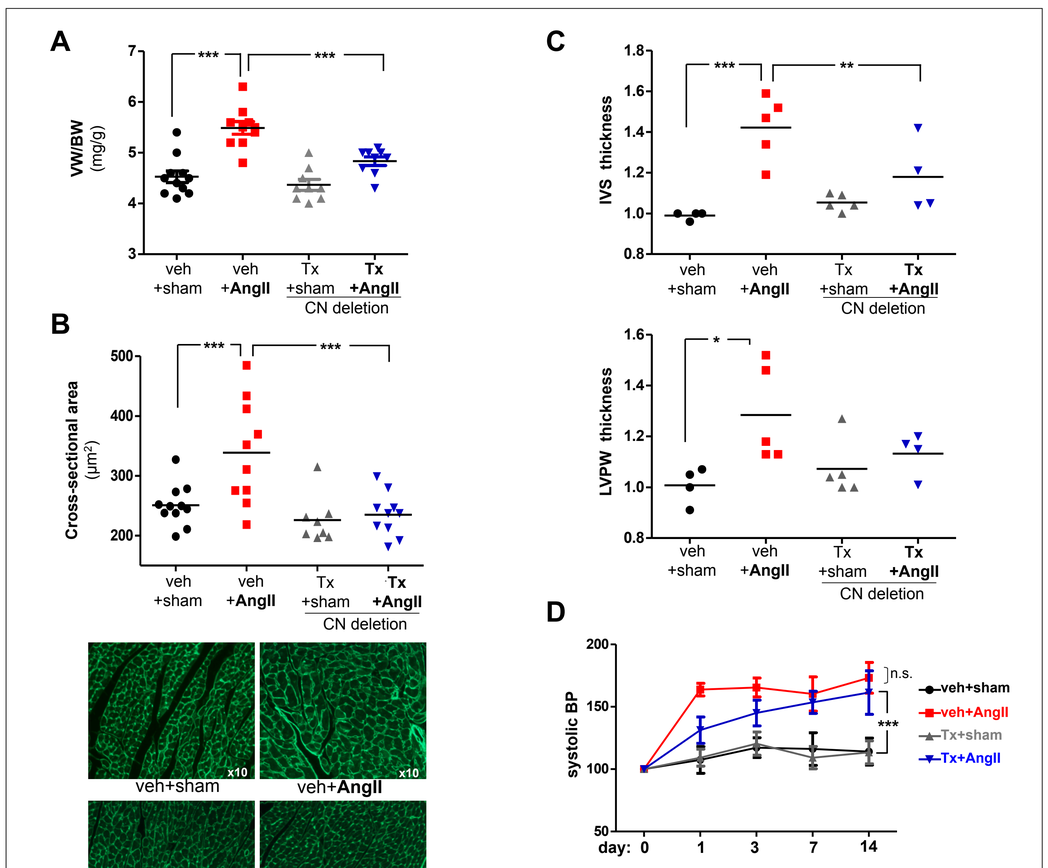


Fig.4 AngII-induced hypertrophy is inhibited in mice conditionally deficient in cardiac CN.

A, Mice with CN deficiency in heart do not show increased cardiac mass in response to AngII. After tamoxifen treatment (for CN deletion), AngII minipuumps were implanted in mice and after 21 days of AngII infusion mice were sacrificed. Ventricular weights from these mice were measured as Fig.1A. **B, Cardiomyocyte size is increased in response to AngII in CN-expressing mice but not in CN deficient mice.** Quantification of myocyte surface area from cardiac histological sections of mice was performed as Fig.1B, (upper panel). Representative pictures are show (lower panel). **C, CN deficient mice display reduction in thickness ventricular wall in response to AngII.** In vivo analysis by echocardiography was performed after 21 days of AngII infusion. IVS and LVPW thickness is represented as Fig.2C. **D, CN deficient mice display a normal hypertensive response to AngII infusion.** Blood pressure of mice was monitorized during experimental protocol, and systolic BP measurements are represented as Fig.1D.

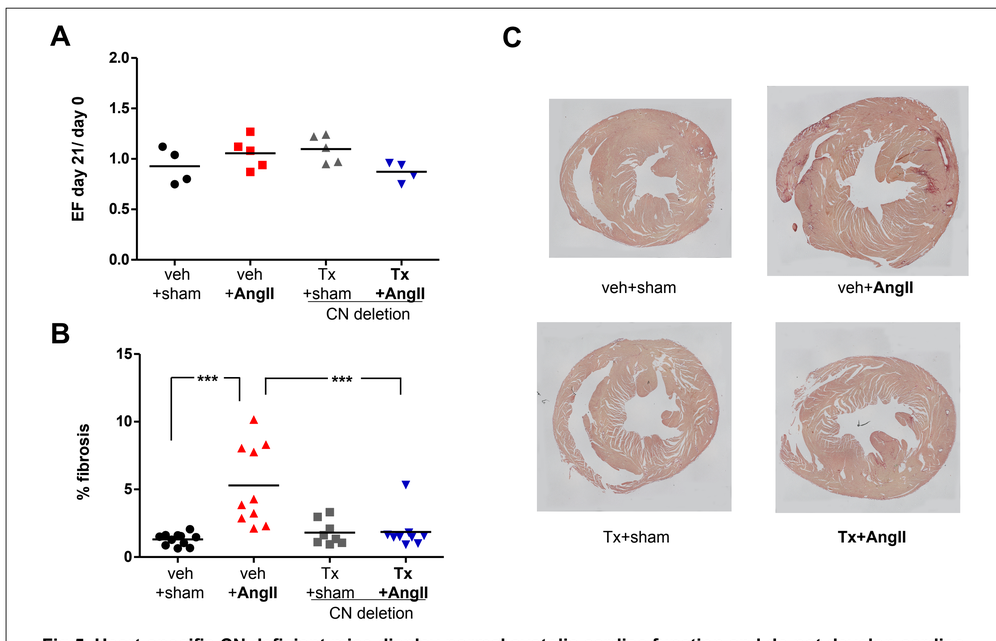


Fig.5 Heart-specific CN deficient mice display normal systolic cardiac function and do not develop cardiac fibrosis in response to AngII.

A, Cardiac CN deficiency does not induce systolic cardiac dysfunction. After 21 days of AngII infusion, cardiac function of mice from four experimental groups was analysed by echocardiography. Percentage of ejection fraction was measured from M-mode tracings from each mice before treatment (day 0) and 21 days after AngII minipump implantation (day21). The change of ejection fraction at study completion is represented. **B and C, AngII-induced fibrosis, when hypertrophy is established, is inhibited in CN deficient mice.** Cardiac histological sections of mice sacrificed at day 21 were stained with picrosirius red to detect fibrosis. **B,** quantification of percentage of myocardium area staining for fibrosis, and **C,** representative images.

Statistical analysis
Differences among groups were analysed by one-way ANOVA following by Newman-Keuls test. In blood pressure experiments, we analysed data by two-ANOVA following by Bonferroni test. *p<0.05 is considered significant. **p<0.01, ***p<0.001.

Conclusion

These results demonstrate that CN is essential for cardiac enlargement mediated by AngII, and suggest that the deleterious effect of CsA in cardiovascular diseases may be linked to the side effects of immunosuppressants unrelated to the inhibition of CN.