Myocyte function in hypertrophy and heart failure

- Sian Harding
- BSc Module 1
- 27/10/11

Natural history of heart failure

Myocardial infarction
Valve disease
Genetic defect
Alcohol/drugs
Infection/sepsis

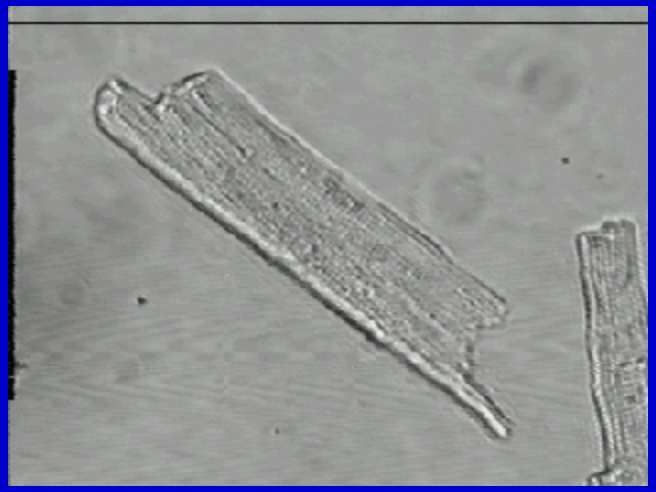
Damage

Hypertrophy
Dilatation
Volume loading
Sympathetic stimulation

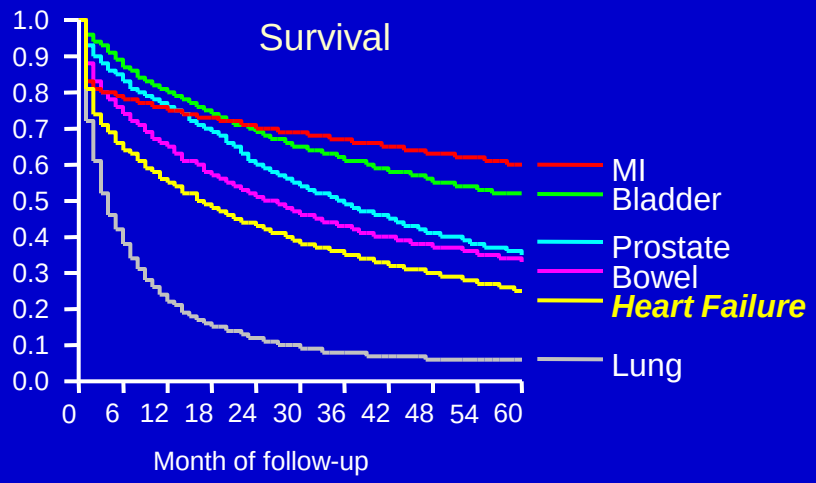
Apparent recovery/ compensation

Decompensation/ Heart failure

Death



Dying muscle cell (myocyte)



Heart failure

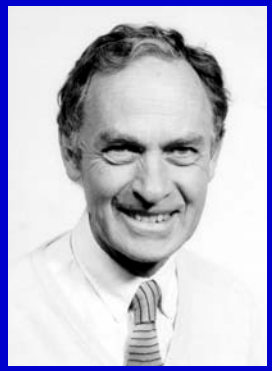
Definition …syndrome… which arises when the heart is unable to maintain an appropriate blood pressure without support

P.Harris, Br Heart J (1987) 58:190-203

Evolutionary origin

The body response is an ancient evolutionary reflex similar to the response to exercise and haemorrhage

P. Harris Cardiovasc. Res (1983) 17



Lascaux cave painting – man attacked by bison. *MIchael Holford*

What happens to the remaining myocytes in the failing heart?

Myocardial infarction
Valve disease
Genetic defect
Alcohol/drugs
Infection/sepsis

Damage

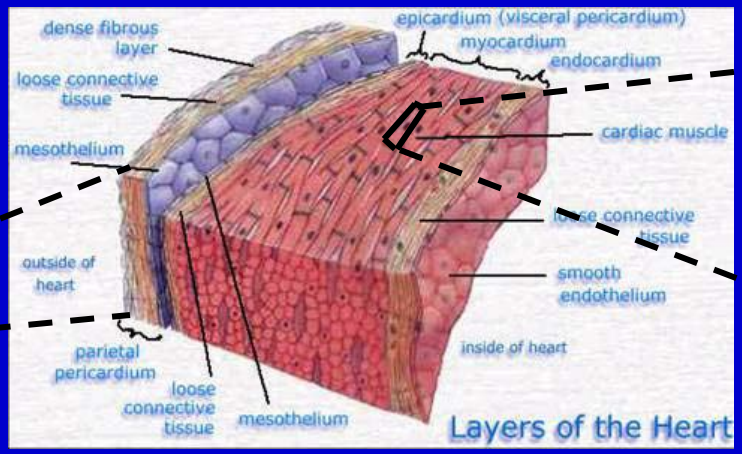
Hypertrophy
Dilatation
Volume loading
Sympathetic stimulation

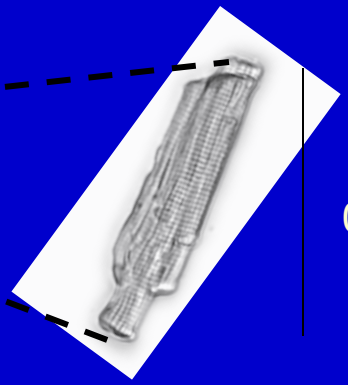
Apparent recovery/
compensation

Decompensation/
Heart failure

Death

Isolation of myocytes from the ventricle of the heart





0.1mm

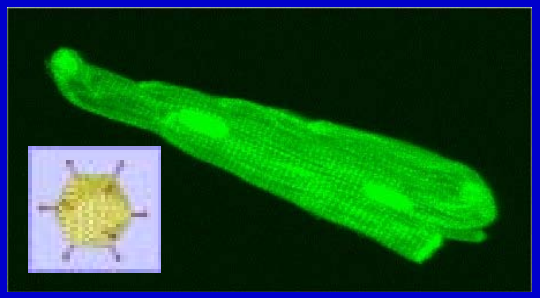
Myocyte

Experimental preparation: Isolated, superfused ventricular myocyte

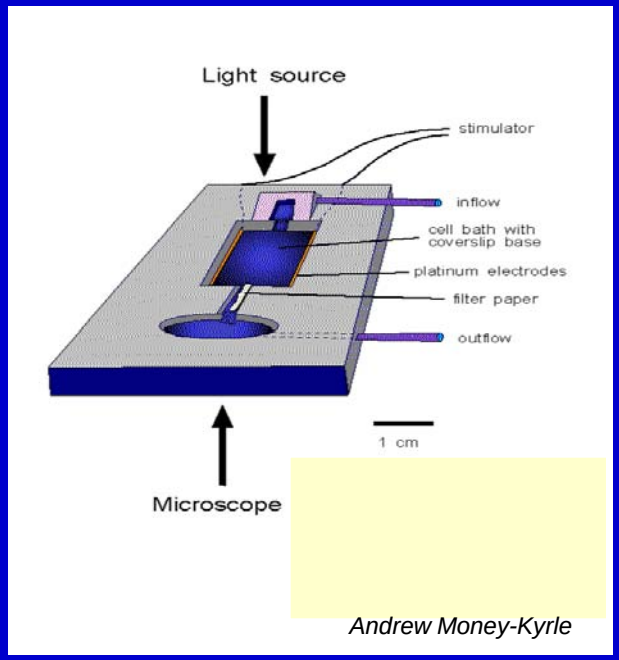
Advantages

- Retains contractile function
- Can be obtained from small myocardial samples
- Viability can be established
- Absolute measure of contractility
- No endogenous modulators
- Hypoxia unlikely

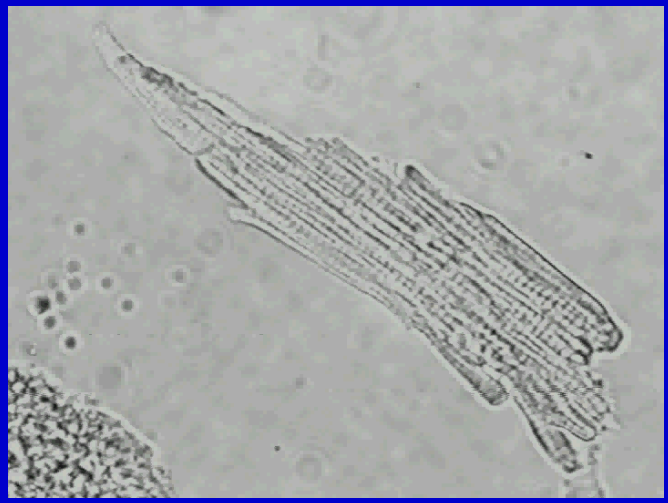
- Extends range of useful methods
  - Electrophysiology
  - Calcium transient measurements
- Access to agents with poor diffusion
- **Gene transfer**



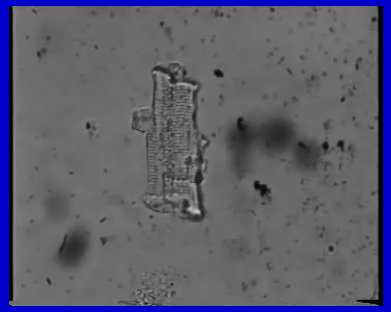
Transfection with Green Fluorescent protein using adenovirus

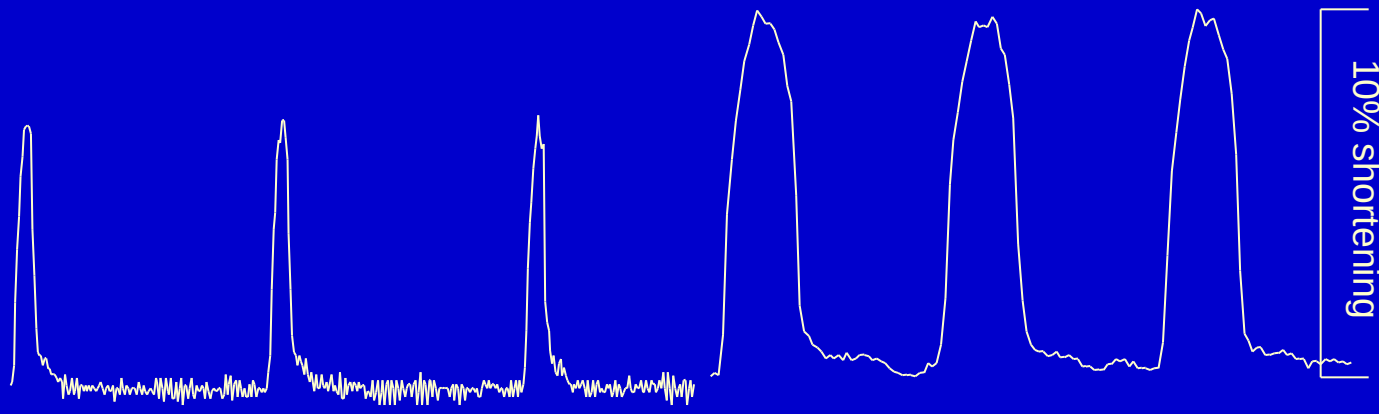


Andrew Money-Kyrle



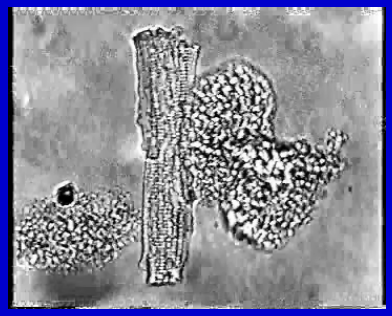
Ventricular myocytes from failing and non-failing human heart

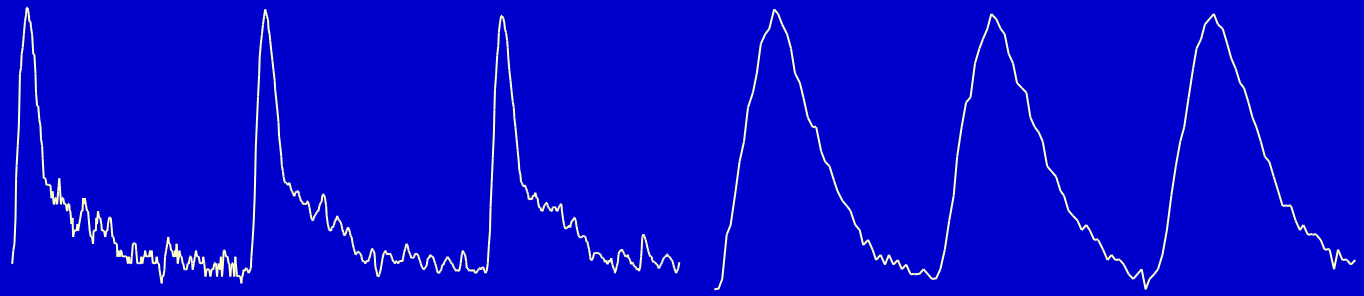




Non-failing, 0.2 Hz

Non-failing, 1 Hz





Failing, 0.2 Hz

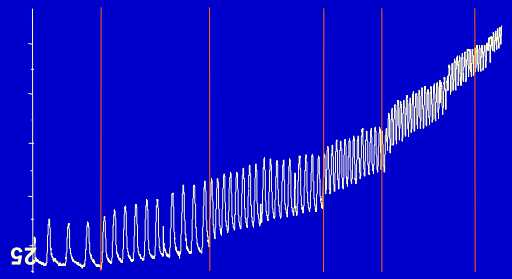
Failing, 1 Hz

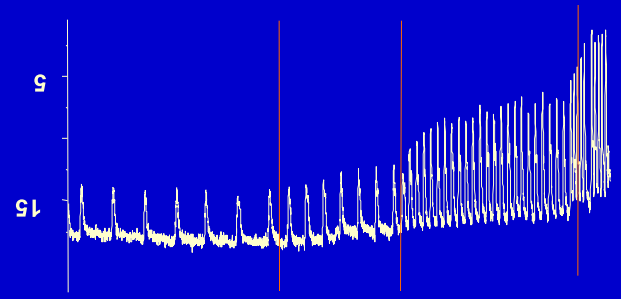
Loss of response to increased stimulation frequency in ventricular myocytes from failing and non-failing human heart

Failing

Non-failing

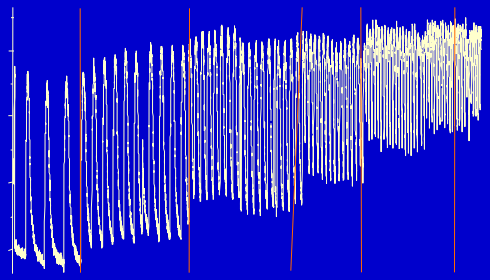
Contraction

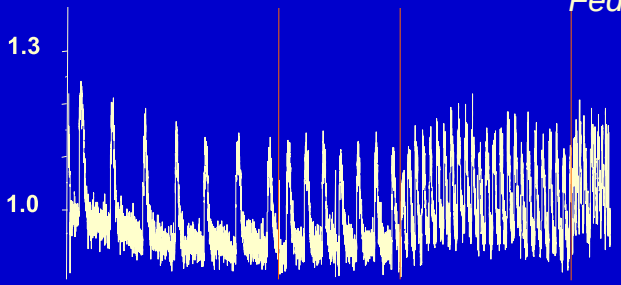




Federica del Monte

calcium

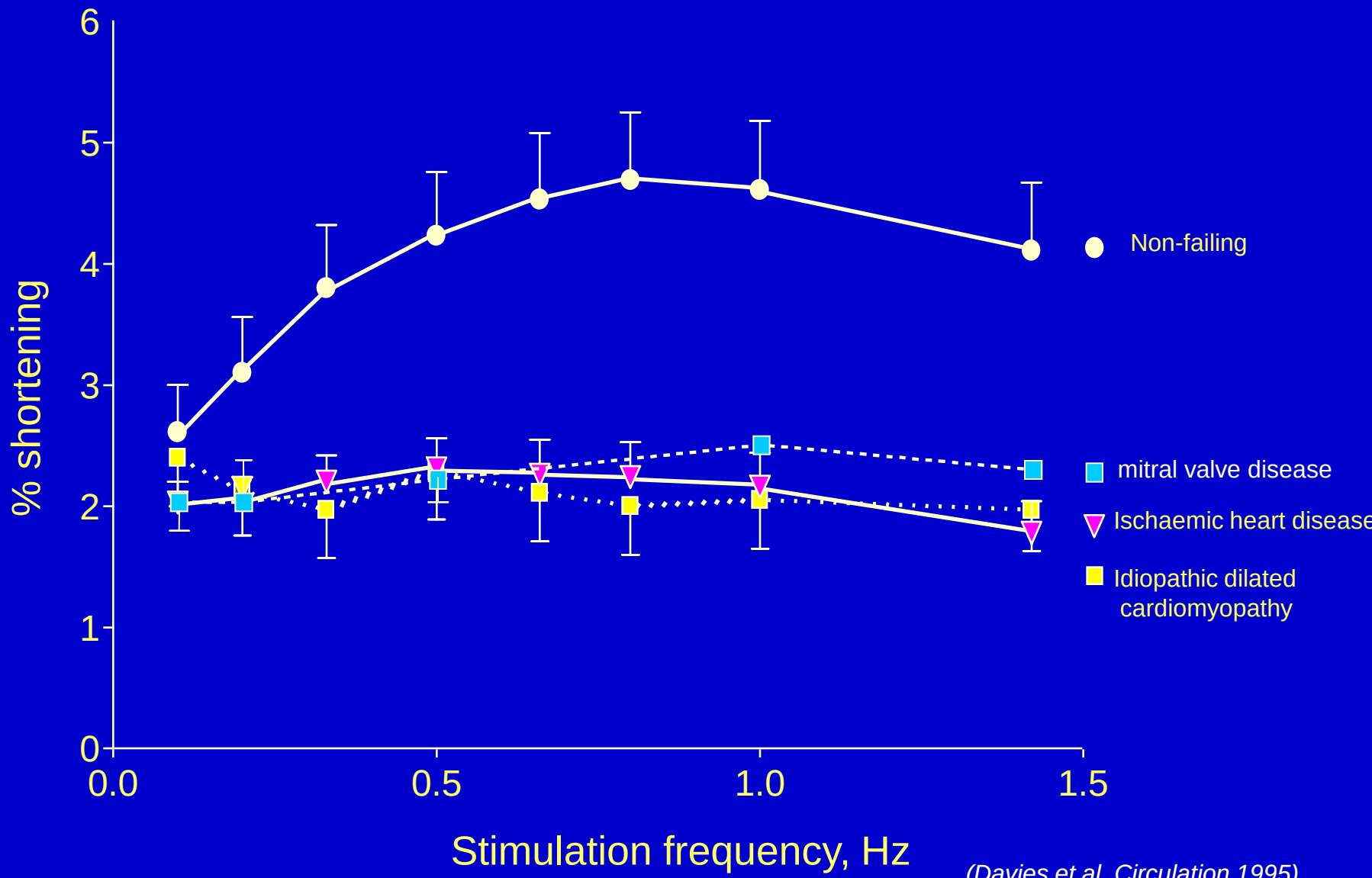




Frequency

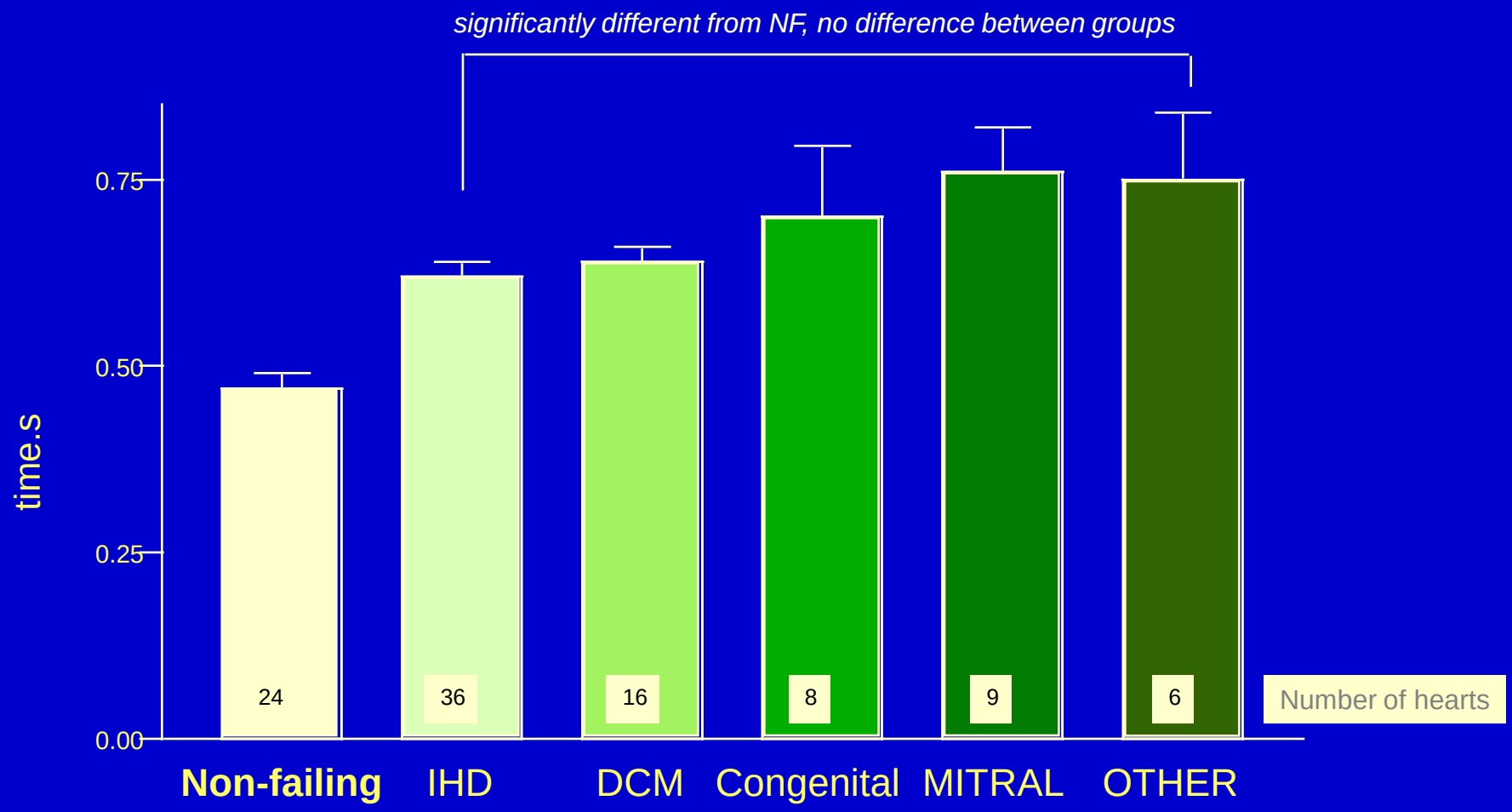
Ken MacLeod

Depressed frequency response in myocytes from failing human ventricle

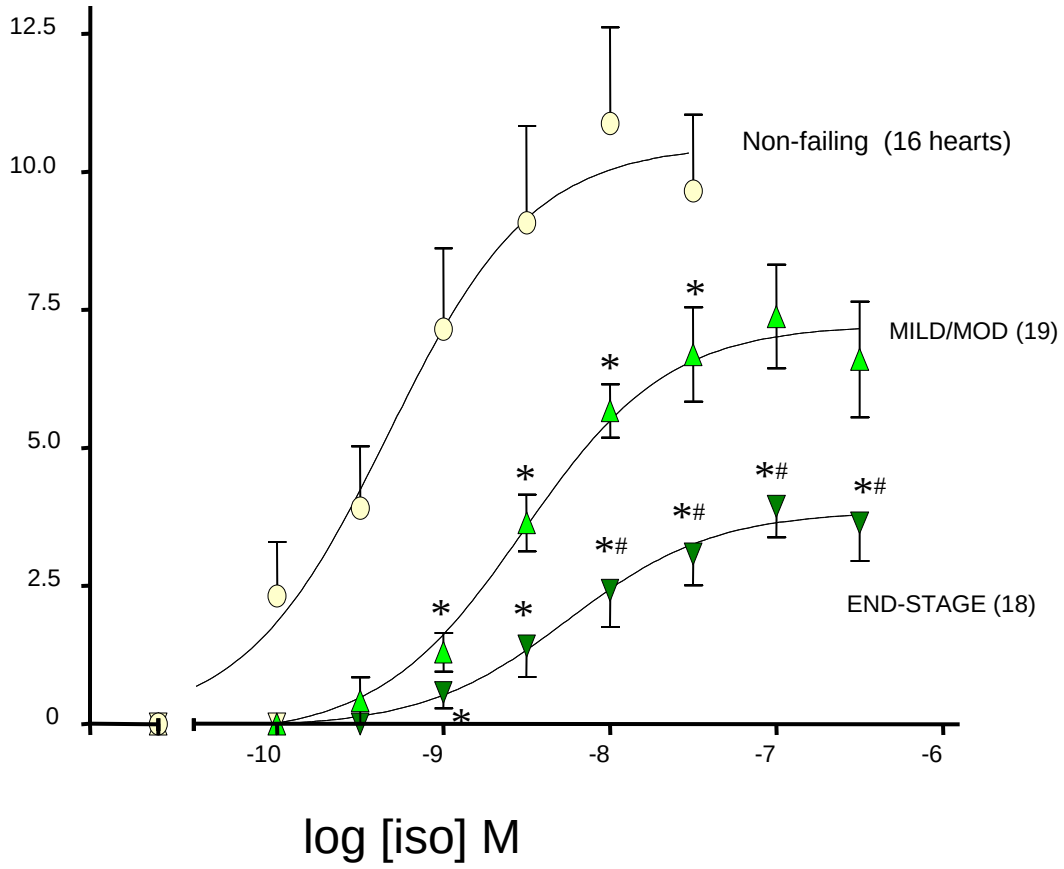


(Davies et al, Circulation 1995)

Slow relaxation is a common feature of myocytes from failing human heart

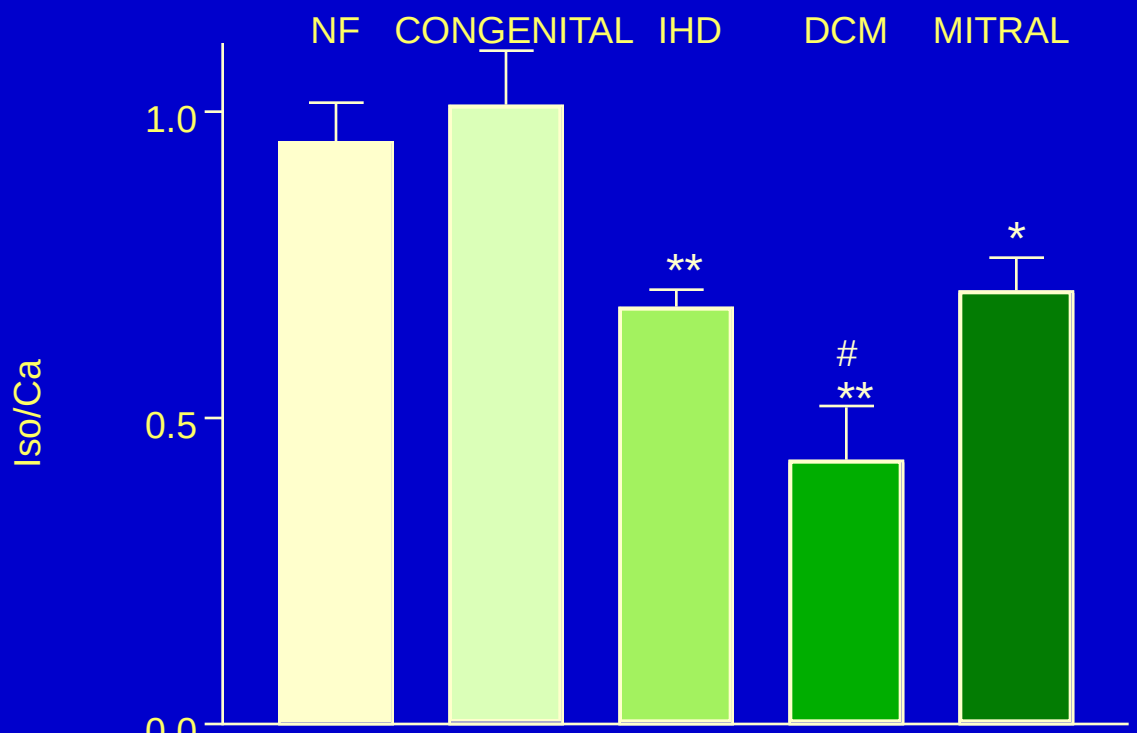


Beat duration



Updated from Harding, JMCC 1992 24:549

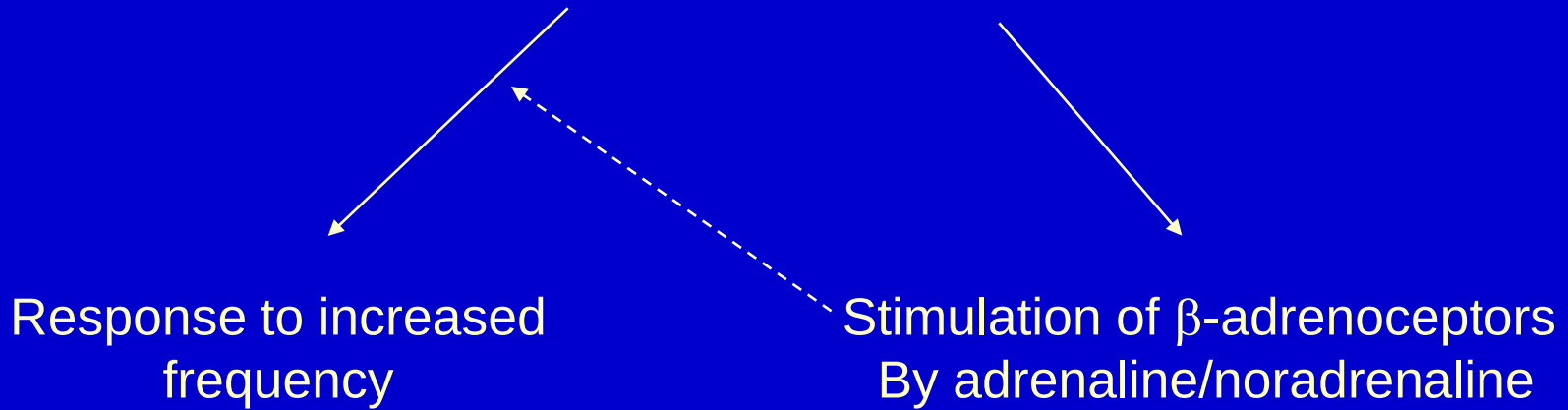
Maximum response to isoprenaline in human left ventricular myocytes



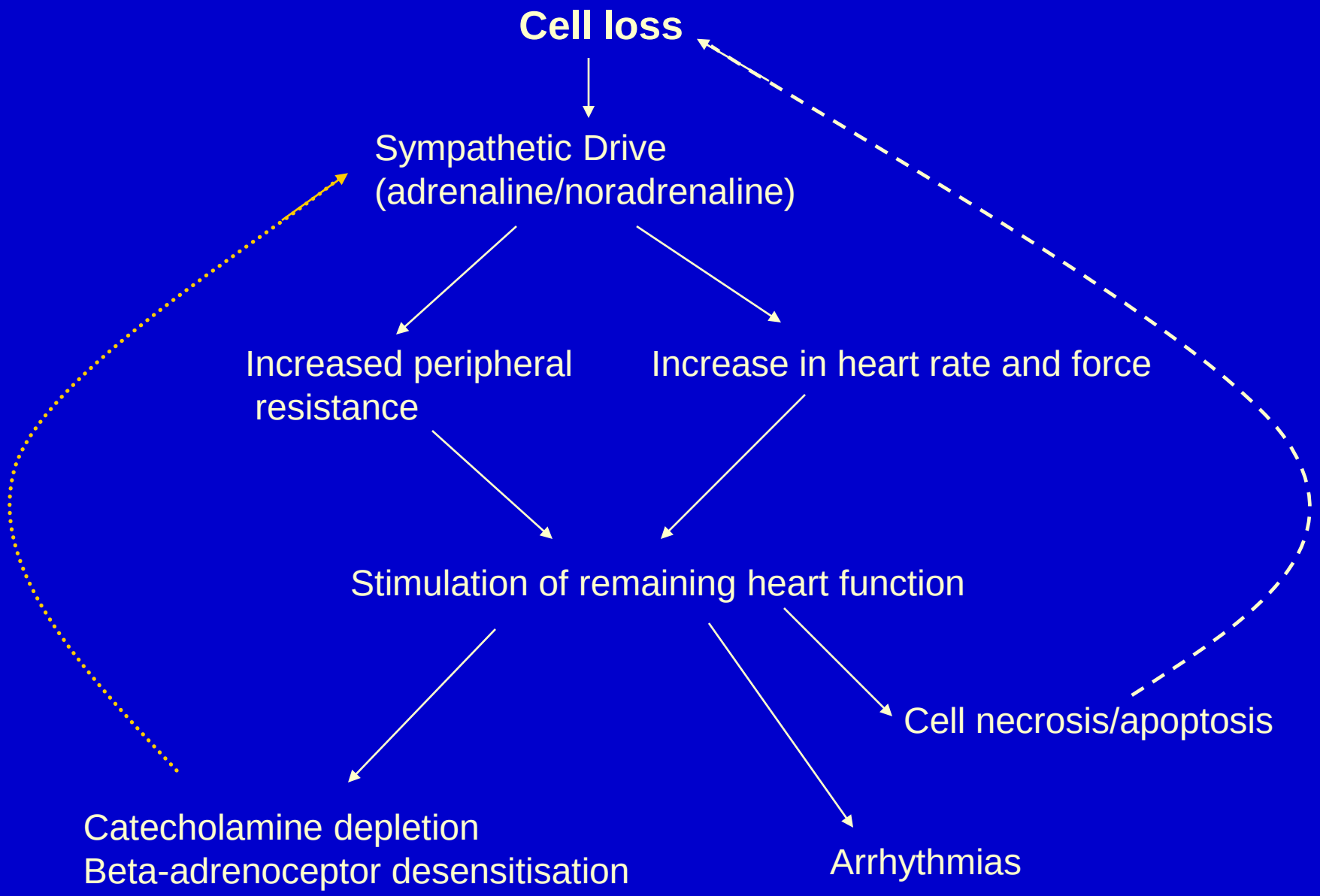
	NF	CONGENITAL	IHD	DCM	MITRAL
Number of hearts	16	5	28	6	8
Age of subjects	48±4	22±2	51±1	42±7	62±2
Average NYHA class	–	3.2	3.7	3.8	3

* P<0.01;**P<0.001 vs NF
# P<0.01 vs IHD, MITRAL

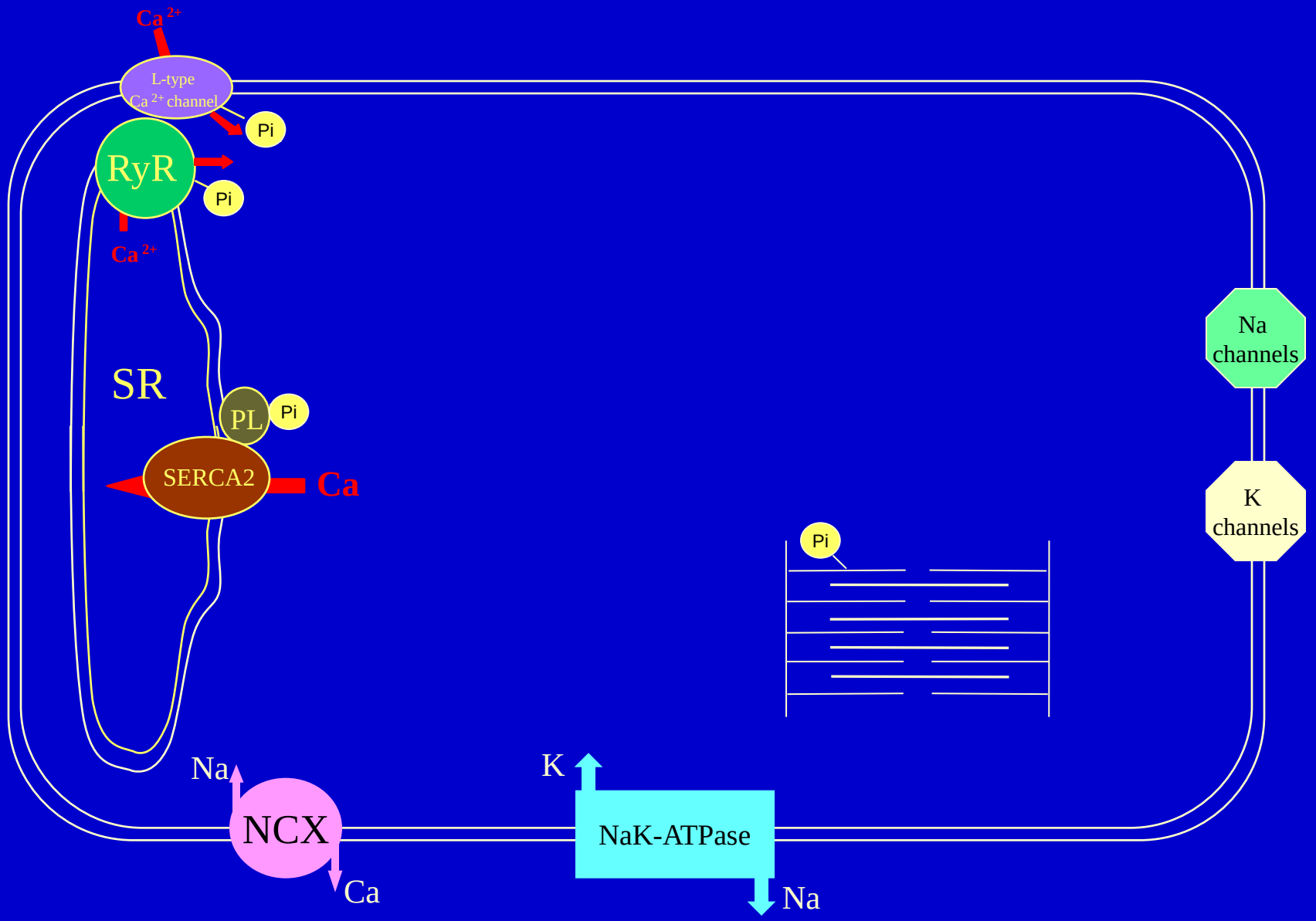
Mechanisms to increase contractile force



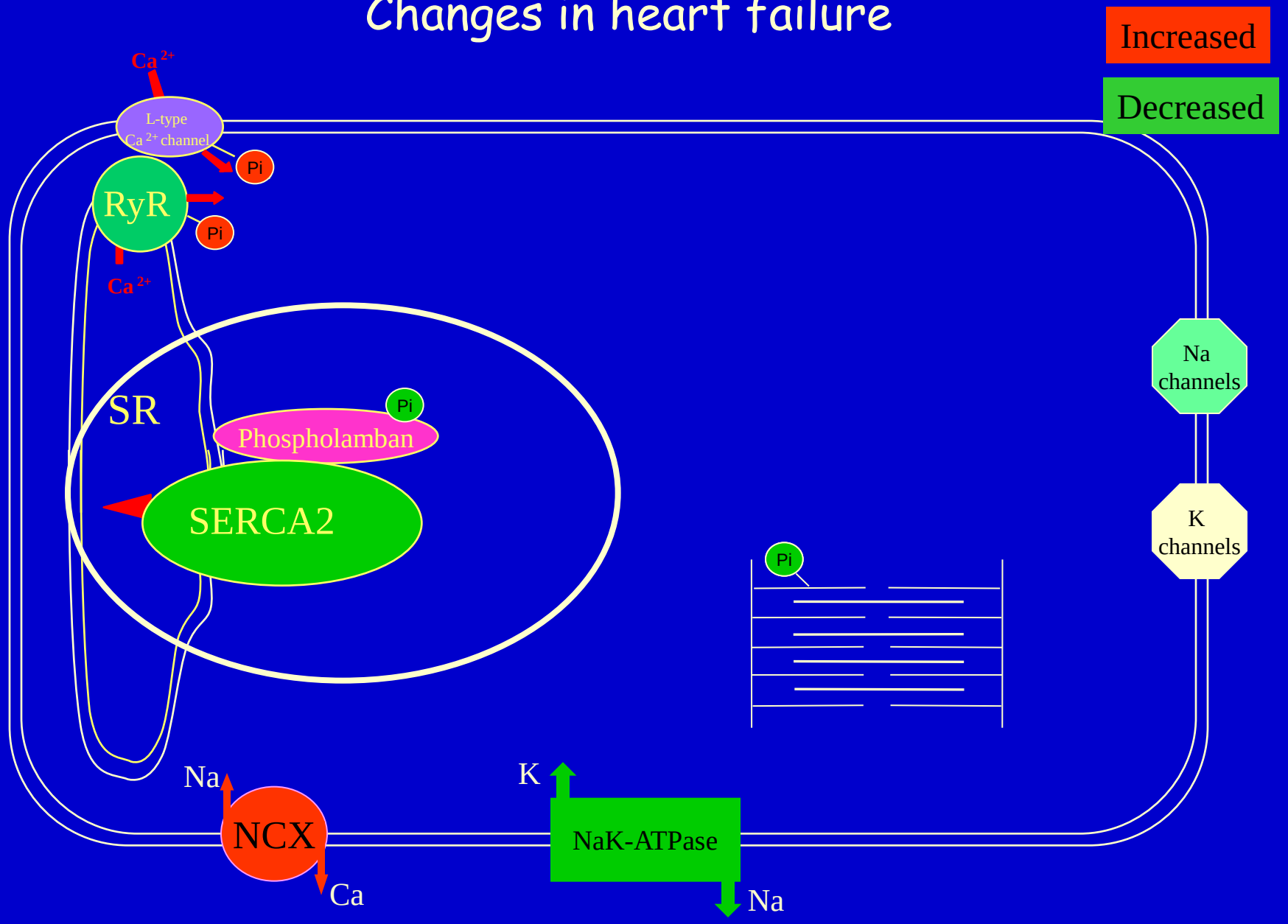
Both lost in the failing heart


Cell loss
Sympathetic Drive
(adrenaline/noradrenaline)
Increased peripheral resistance
Increase in heart rate and force
Stimulation of remaining heart function
Cell necrosis/apoptosis
Catecholamine depletion
Beta-adrenoceptor desensitisation
Arrhythmias

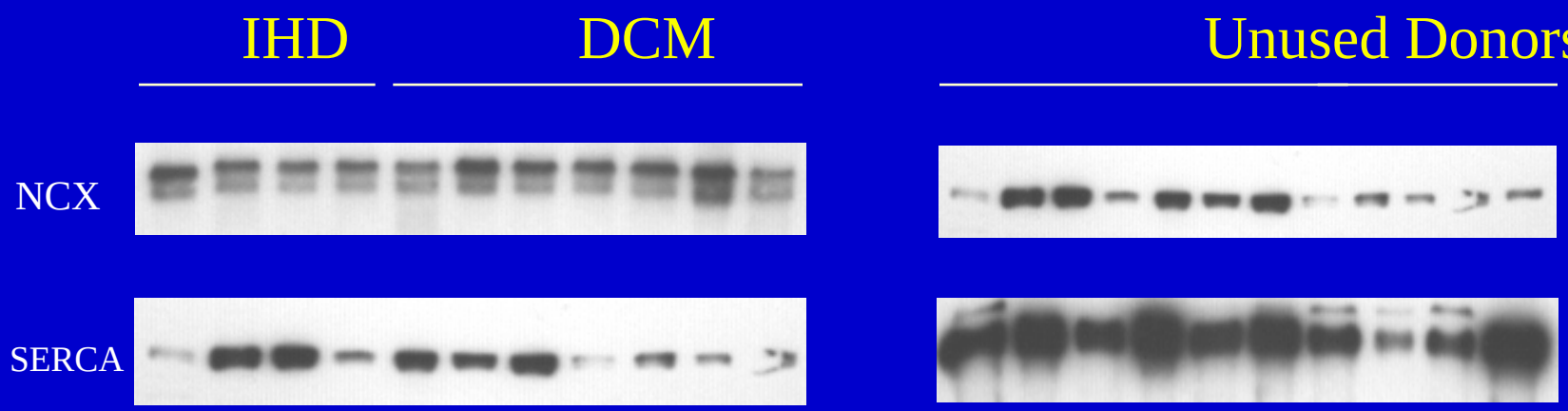
Calcium movements in excitation-contraction coupling

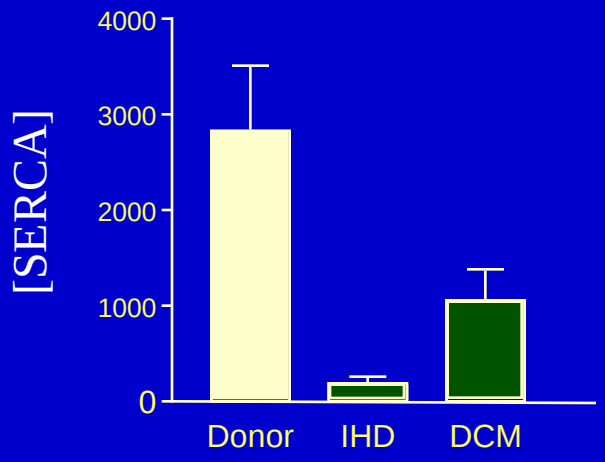


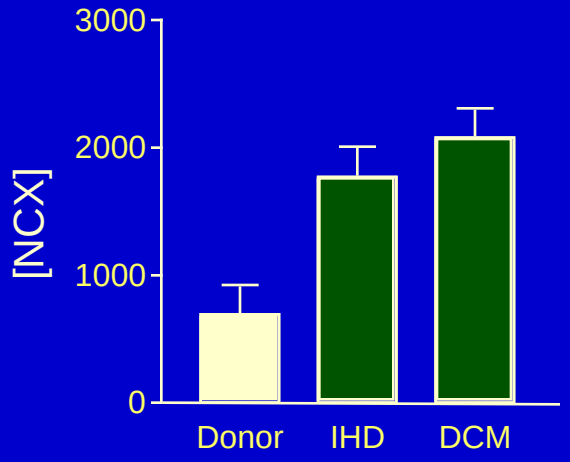
Calcium movements in excitation-contraction coupling
Changes in heart failure



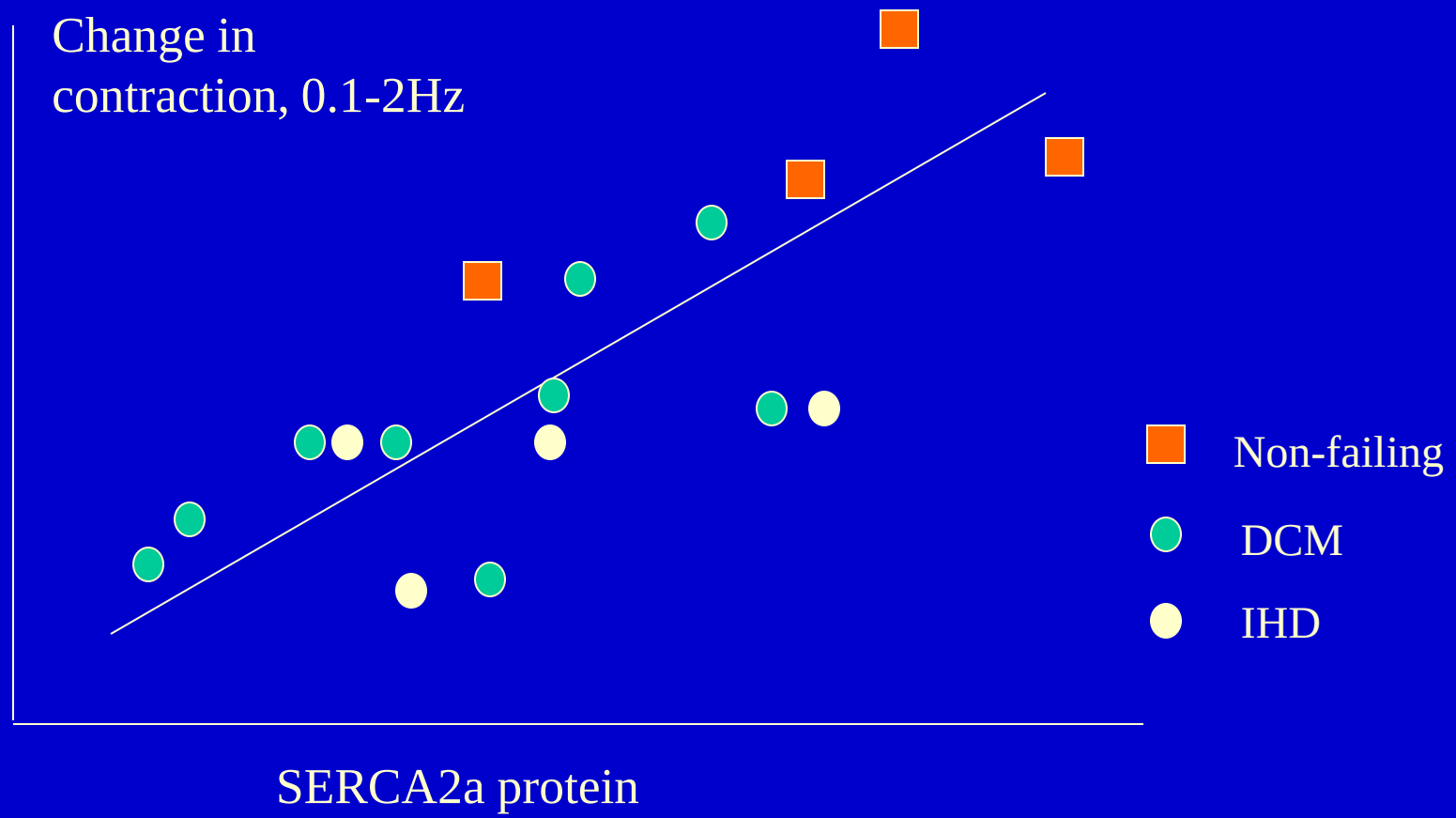
SERCA and NCX Expression





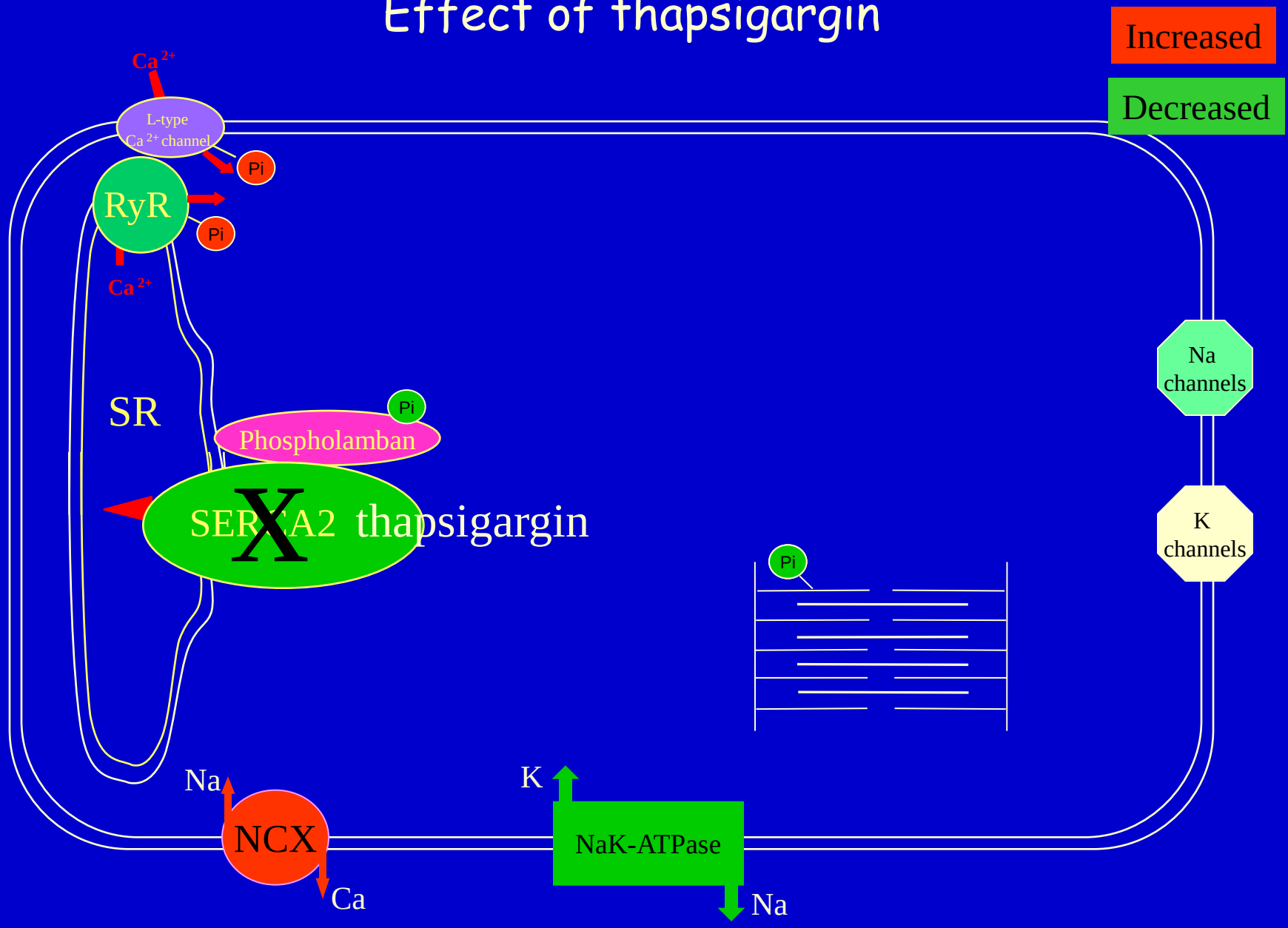


SERCA2a protein levels correlate with frequency response

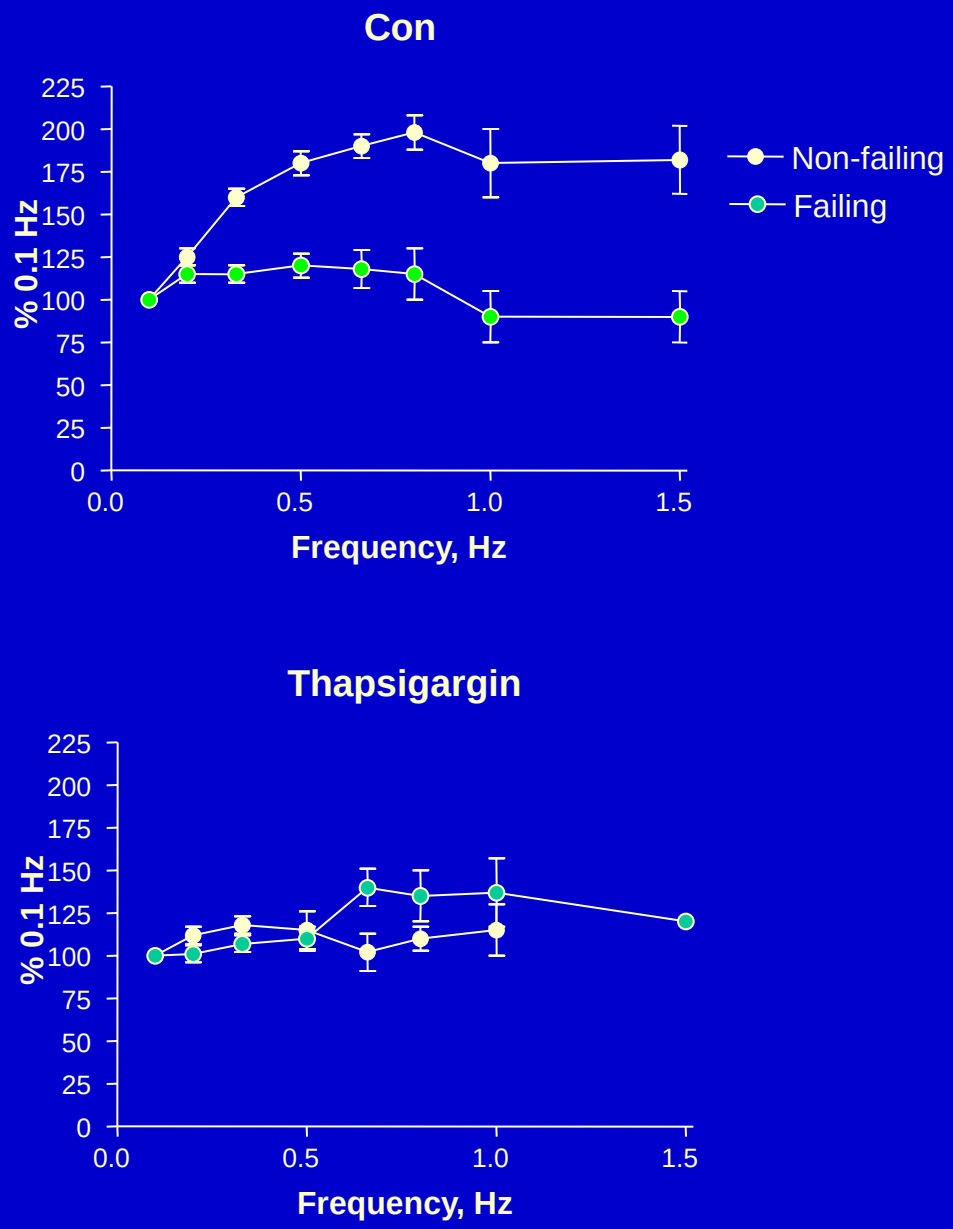


Hasenfuss et al, Circ Res 1994, 75:434-442

Calcium movements in excitation-contraction coupling
Effect of thapsigargin



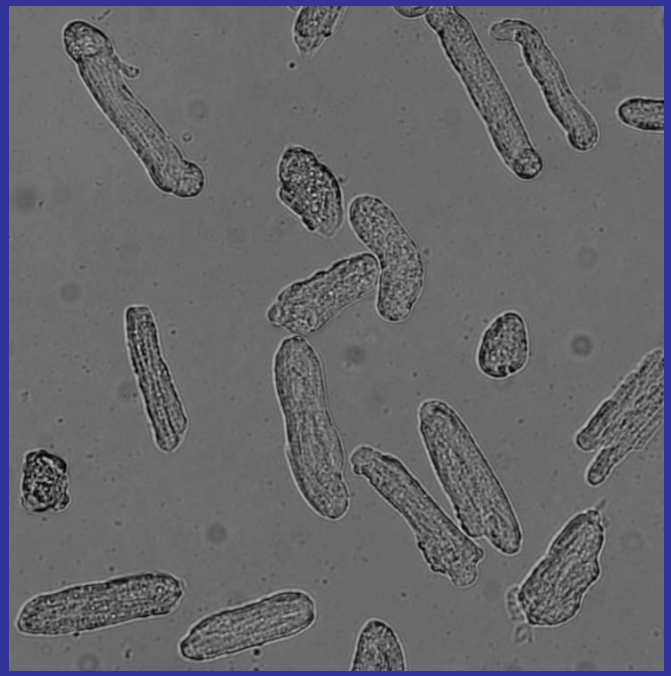
Thapsigargin mimics effect of failure on frequency response

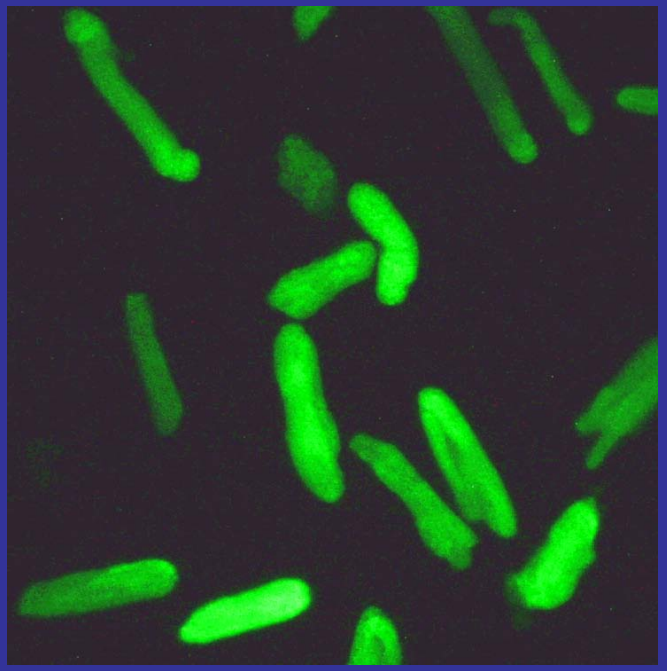


Increasing SERCA2a activity experimentally

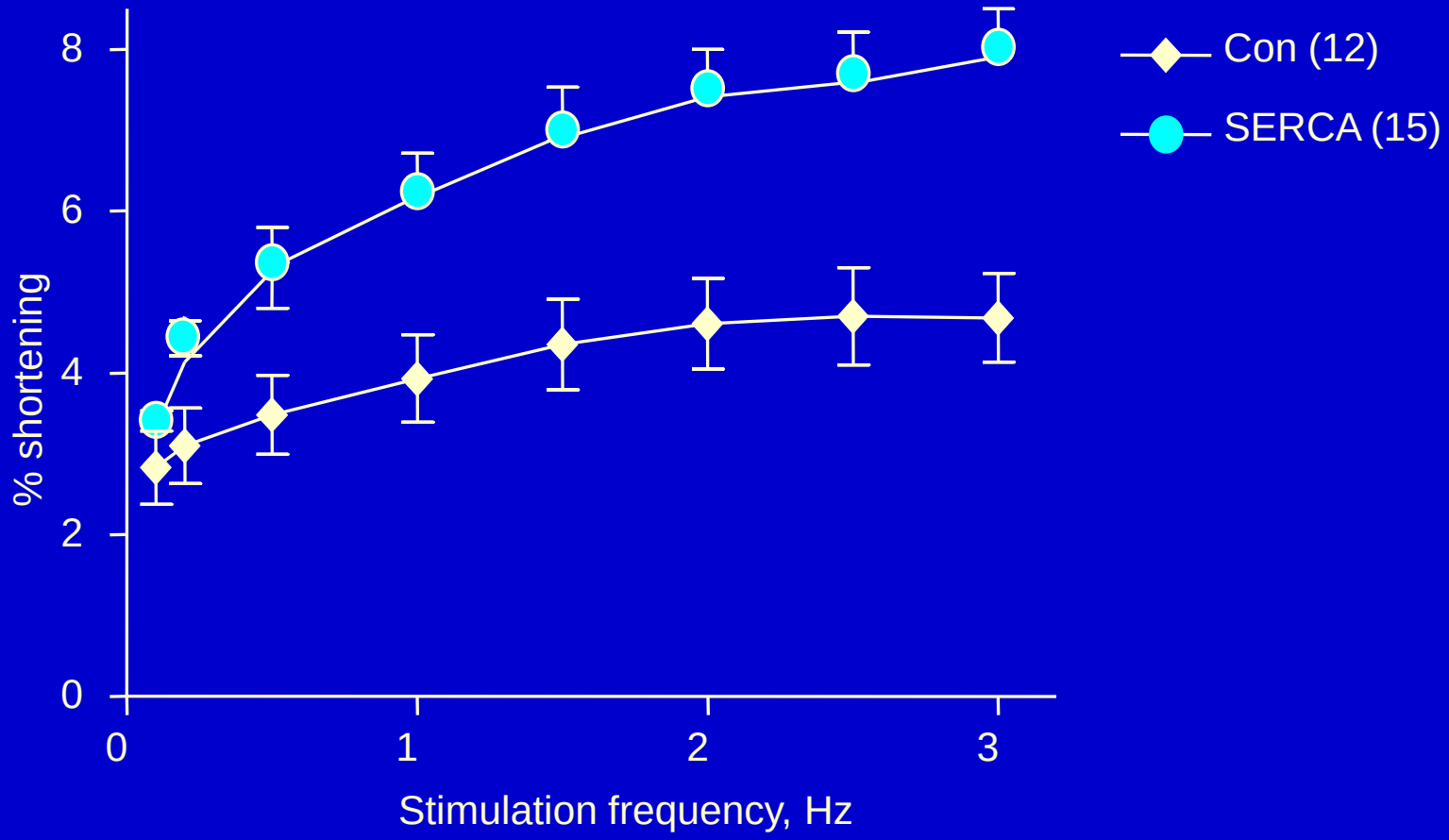
- A replication-deficient adenovirus carrying both the SERCA2a cDNA and GFP under the control of separate CMV promoters and a structurally similar adenovirus carrying the reporter gene GFP were used
- Ventricular myocytes were isolated from the left ventricles of the human hearts
- Myocytes were infected with either Ad.GFP or Ad.SERCA2a

Adult myocytes after 48 h in culture with adenovirus with GFP

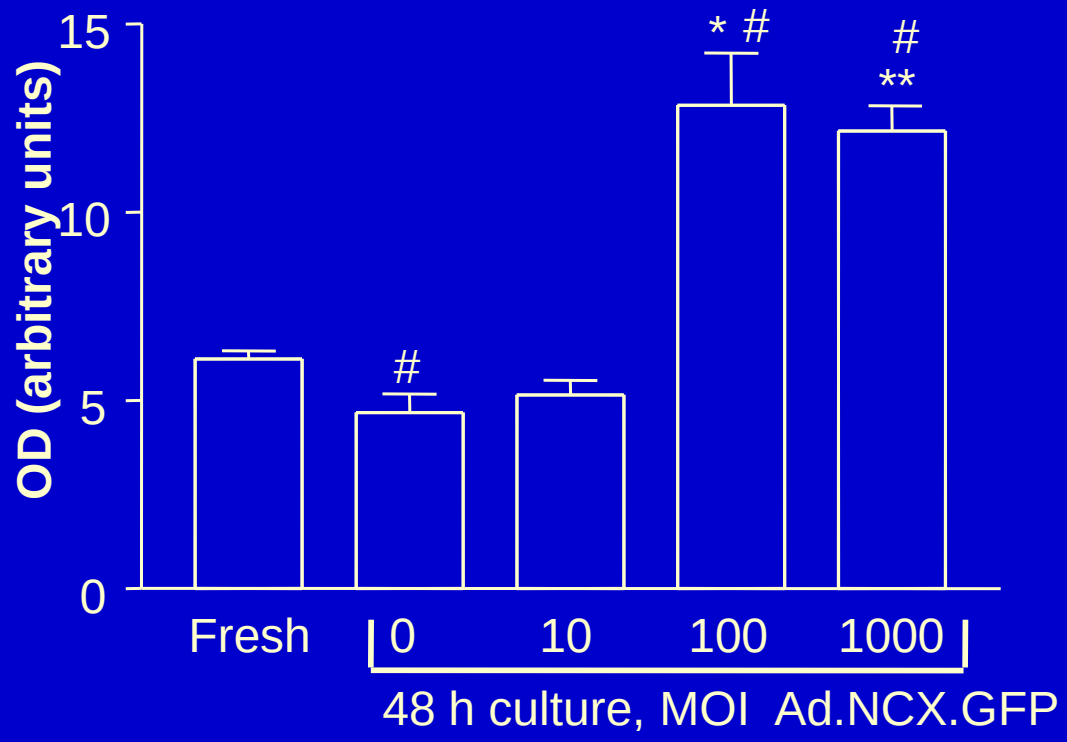


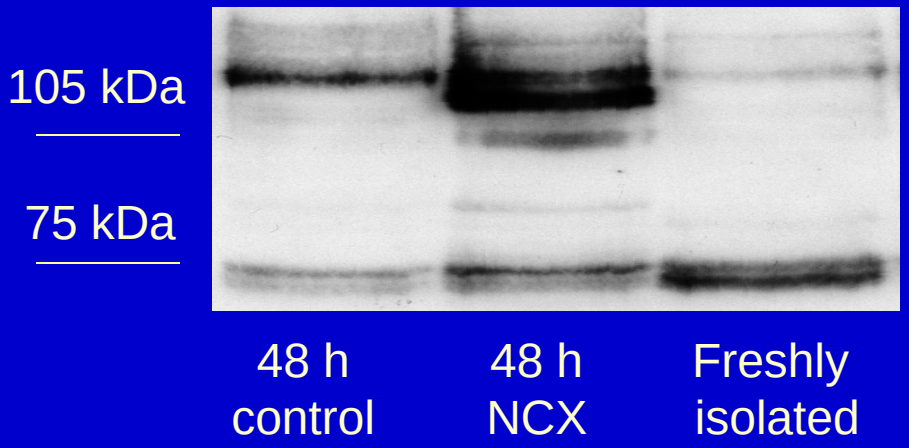


Overexpression of SERCA2a restores frequency response

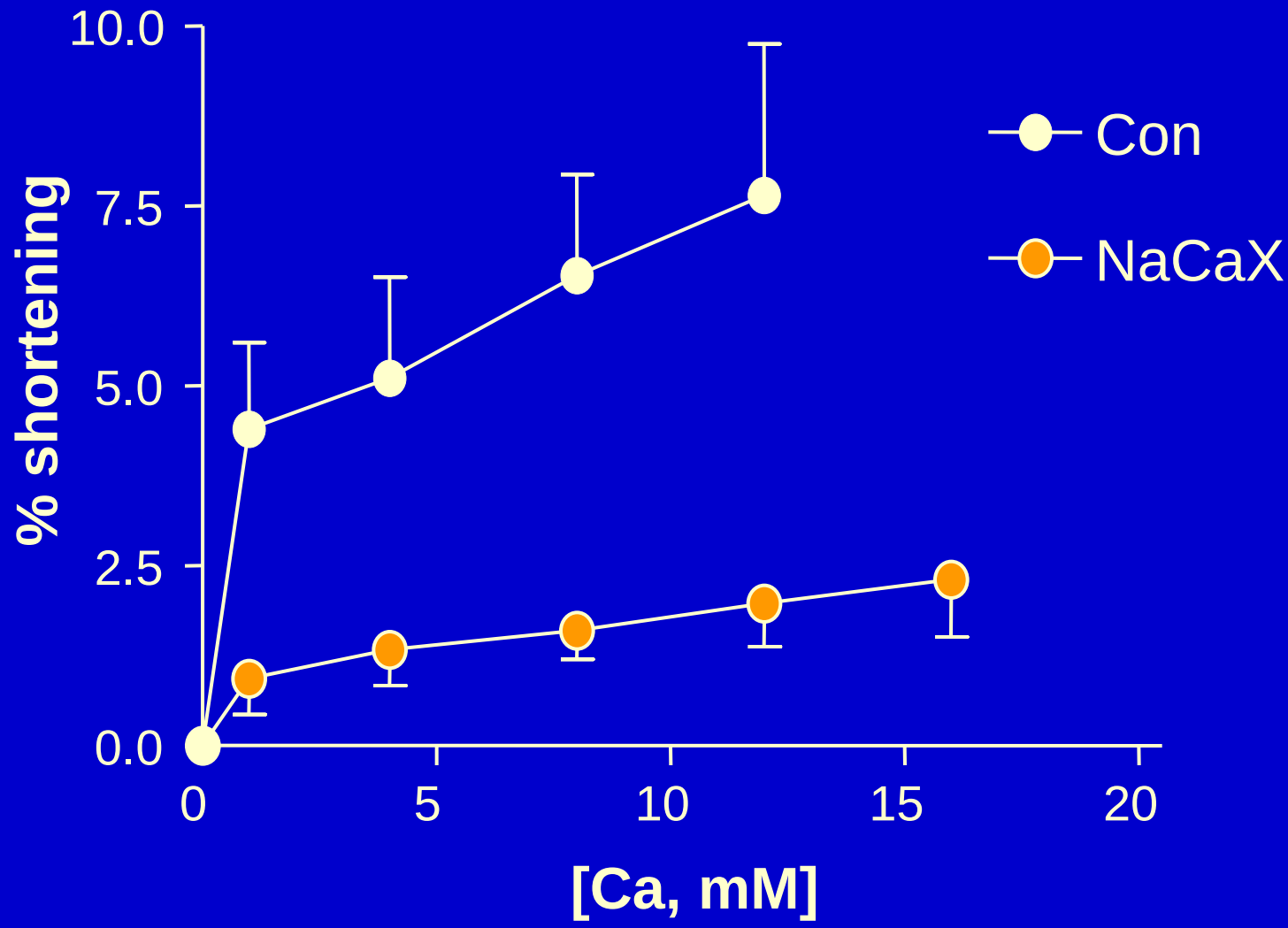


Protein levels of the Na/Ca exchanger after adenoviral overexpression

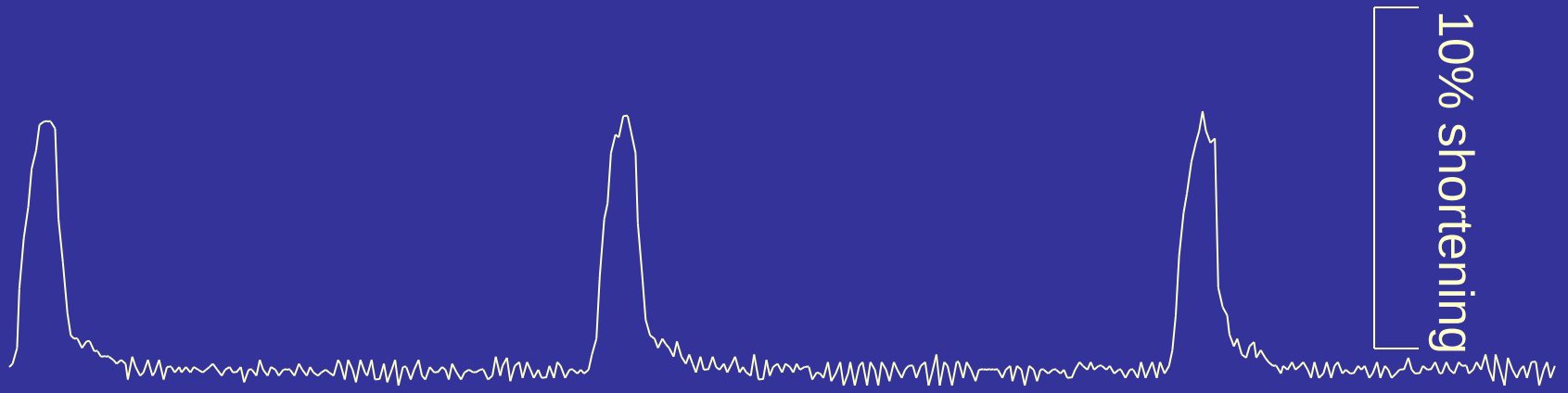




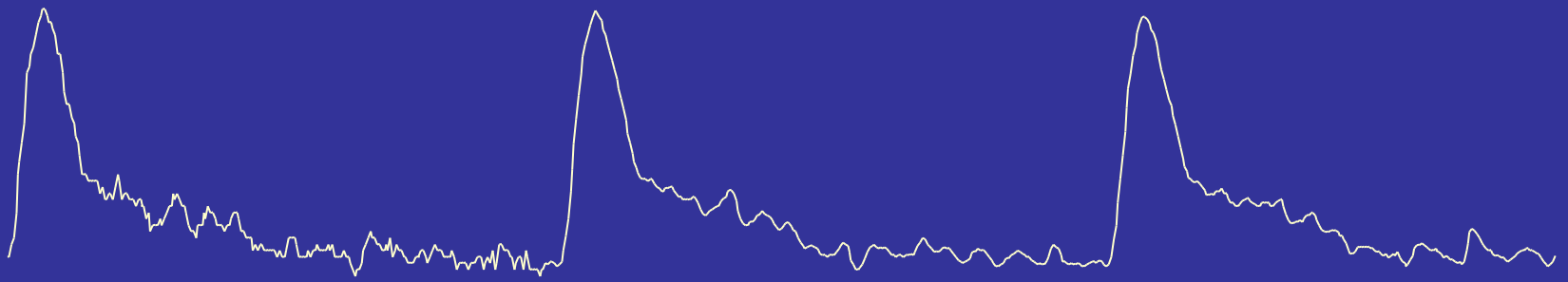
Ca concentration-response curves, n=6 preparations



Slowed relaxation in cells from failing heart



Non-failing, 0.2 Hz



Failing, 0.2 Hz

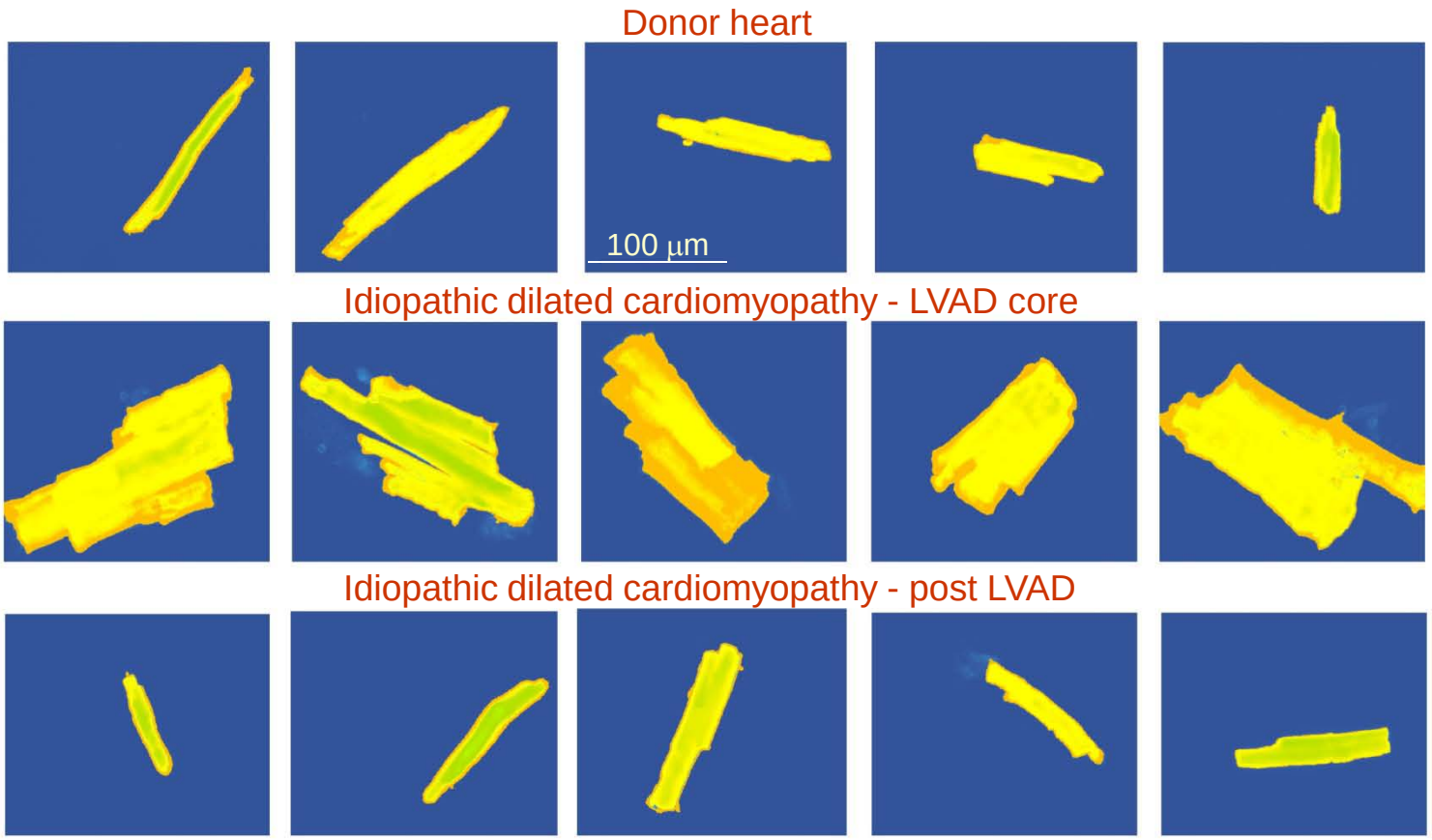
Myocyte size does not predict function

Slowing is related to hypertrophy, but not to increased cell size

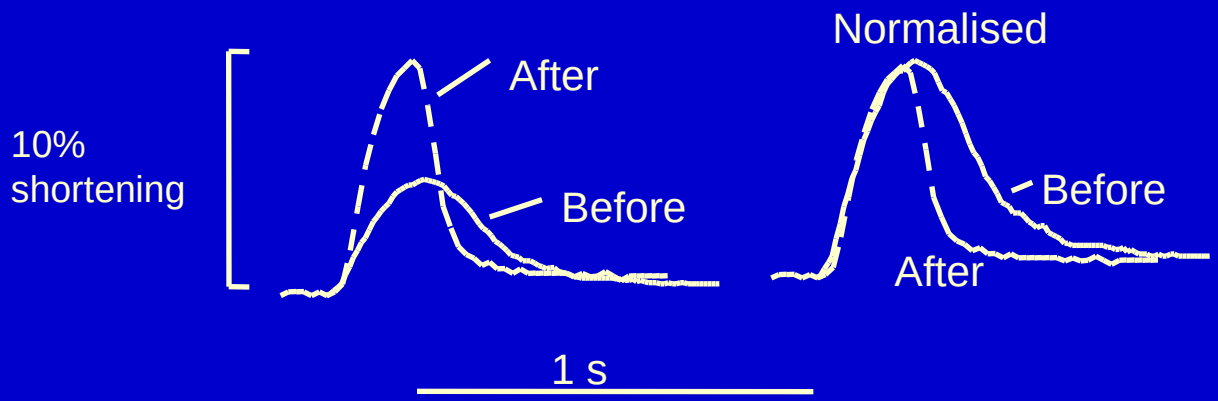
DEL MONTE, F., O'GARA, P., POOLE-WILSON, P.A., YACOUB, M.H., and HARDING, S.E. (1995) Cell geometry and contractile abnormalities of myocytes from failing human left ventricle. Cardiovasc.Res. ***30****, 281-290.*

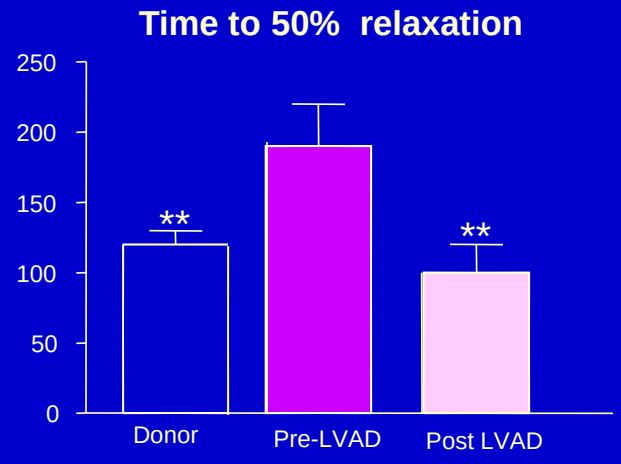
NAQVI, R.U., DEL MONTE, F., O'GARA, P., HARDING, S.E., and MACLEOD, K.T. (1994) Characteristics of myocytes isolated from the hearts of renovascular hypertensive guinea-pigs. Am.J.Physiol. 266, H1886-H1895

Human left ventricular myocytes



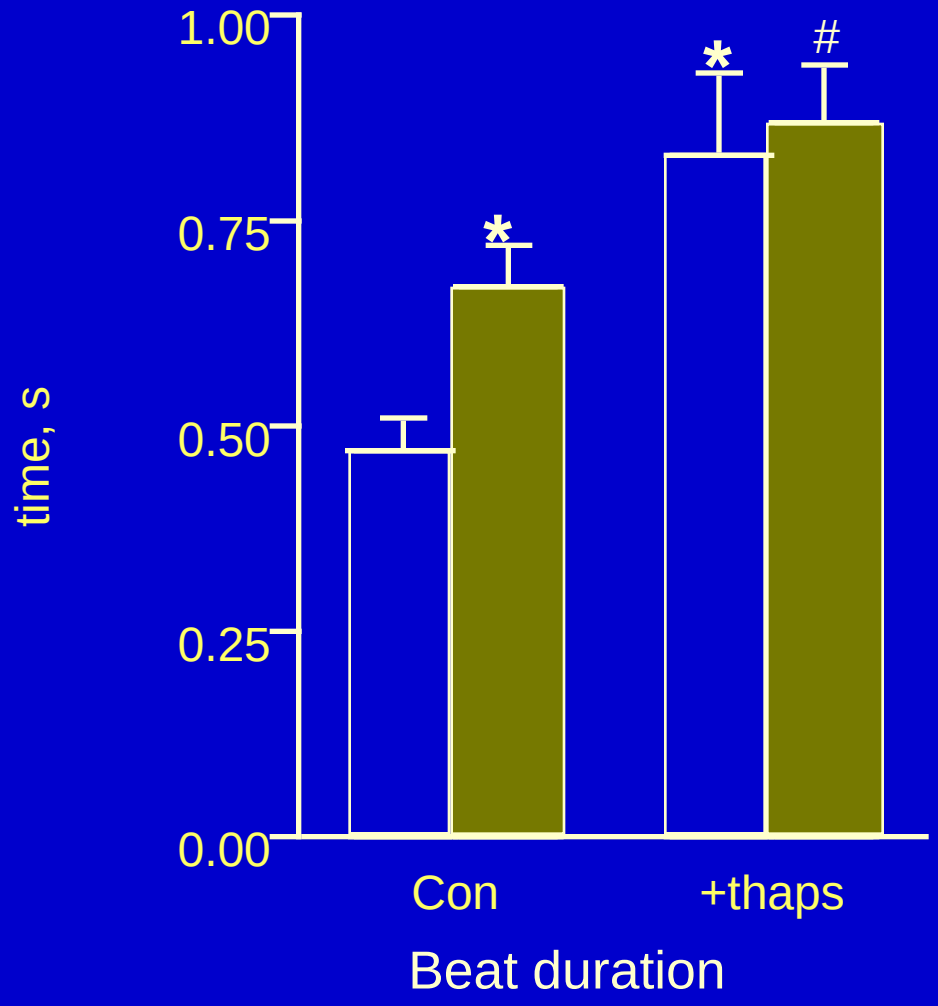
Recovery of contractile function of human left ventricular myocytes after 8 months on LVAD





Terracciano et al, Circulation 2004: Correlation between recovery and SR Ca stores

SERCA2a inhibition by thapsigargin abolishes the difference between relaxation in myocytes from failing and non-failing human hearts



NF (10)

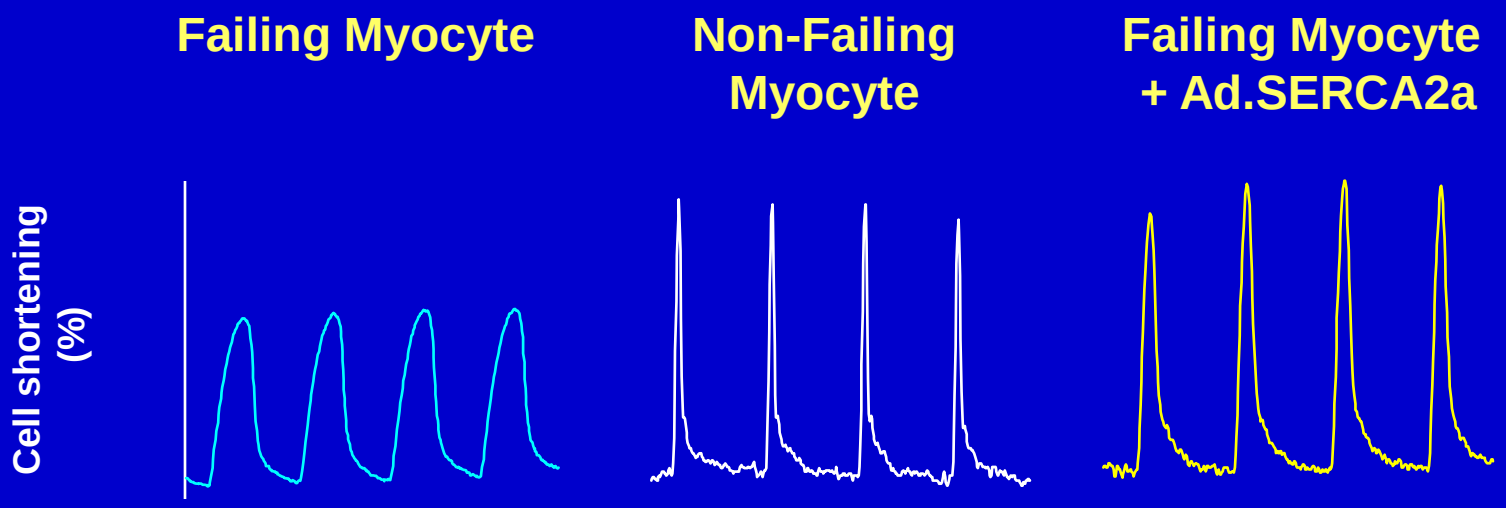
F (18)

* significantly different from NF control

significantly different from F control

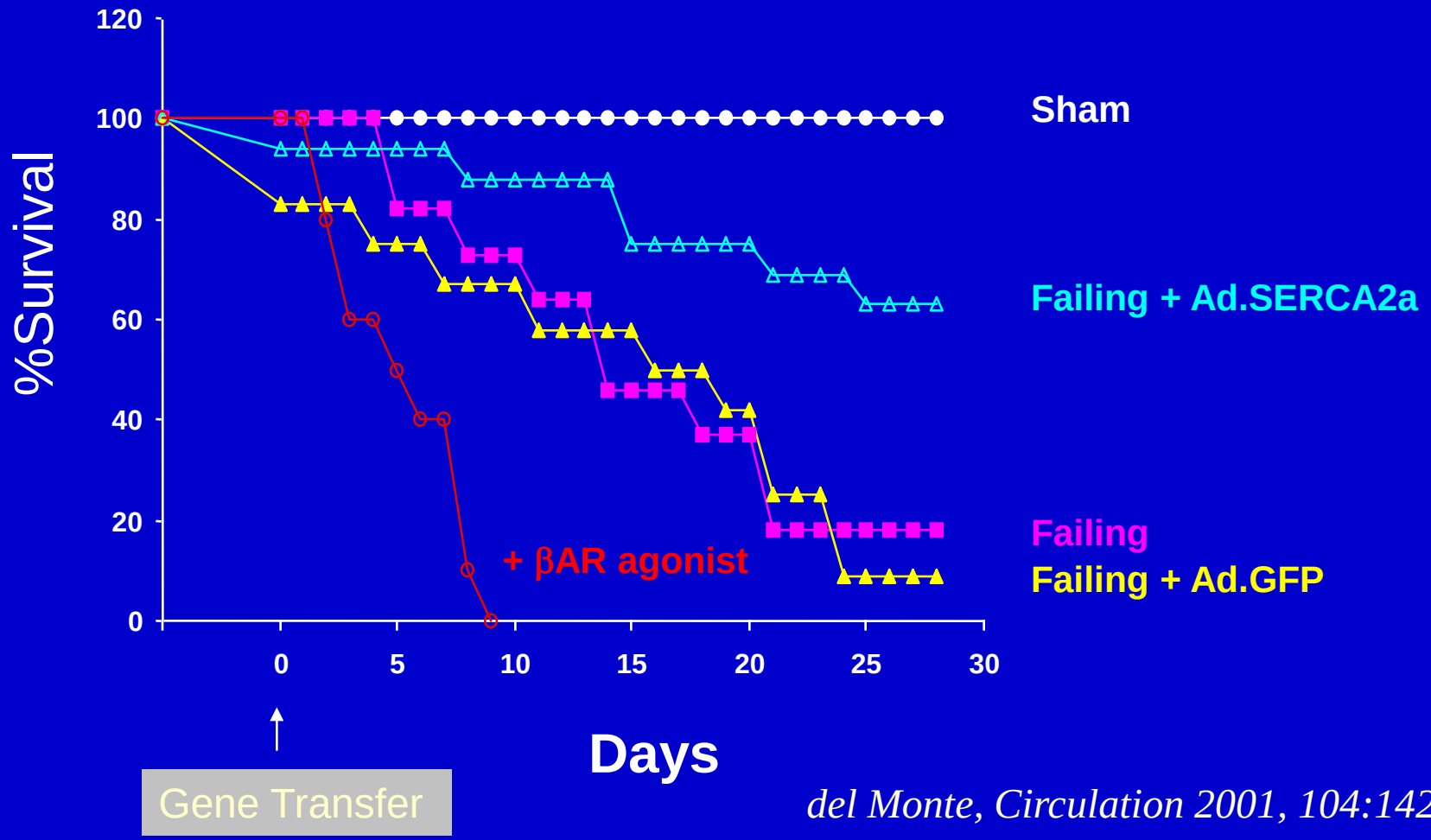
(Davia et al, Cardiovasc.Res 1997 33:88)

Overexpression of SERCA2a speeds contraction and relaxation in human cells



Del Monte et al Circulation 1999

Effect of SERCA2a Gene Transfer on Survival in Rats with Pressure-Overload Hypertrophy in Transition to Heart Failure



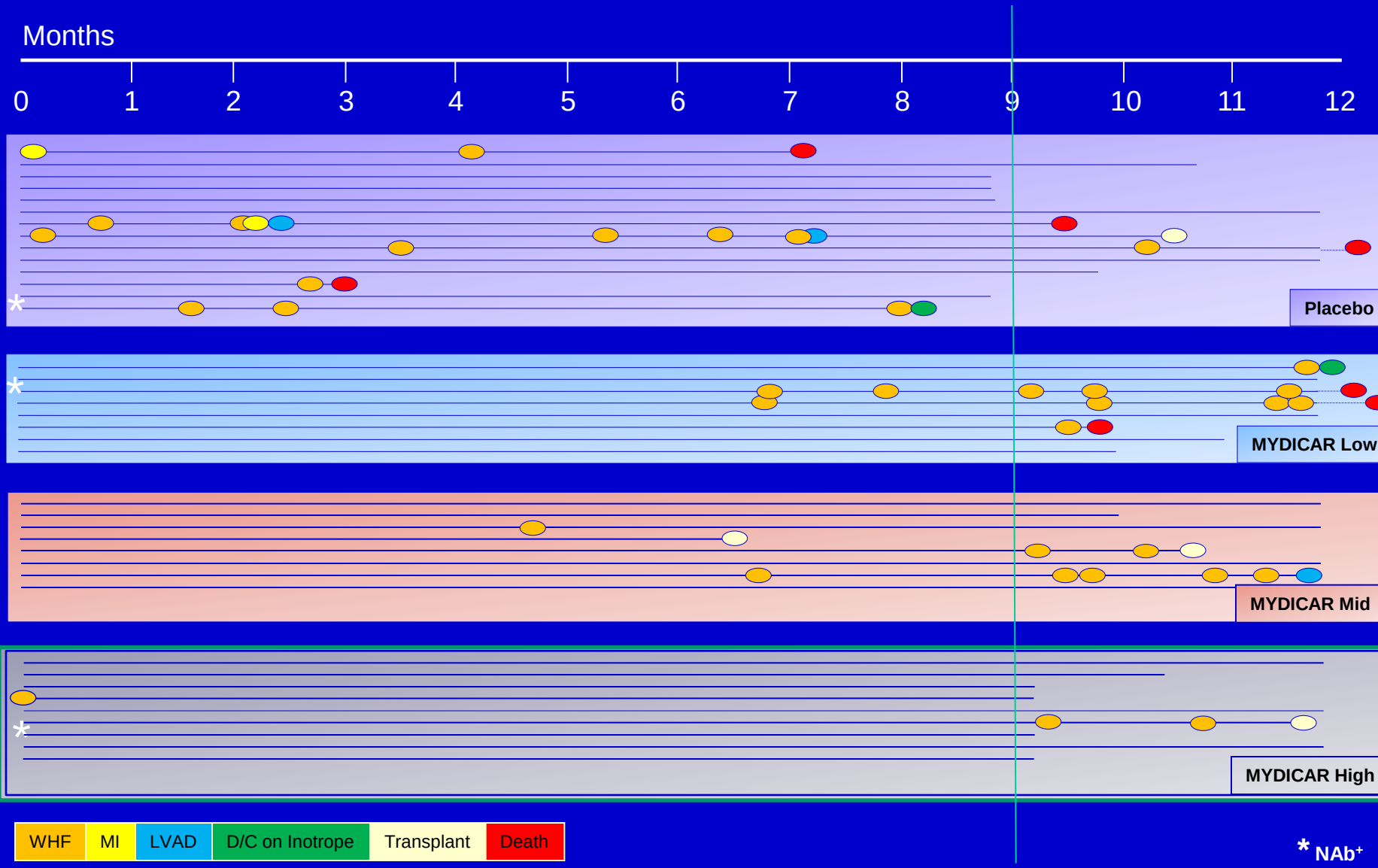
del Monte, Circulation 2001, 104:1424

SERCA2a as a possible gene therapy target

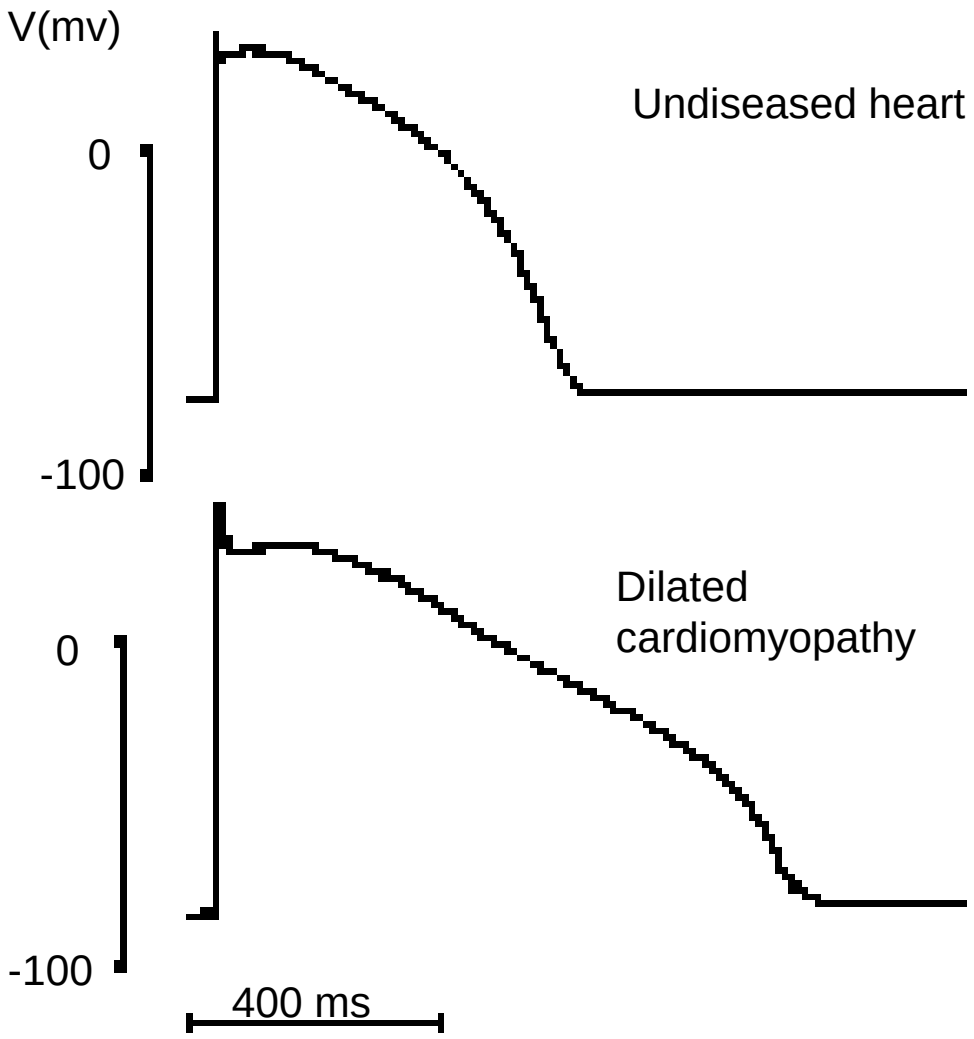
- Either SERCA2a overexpression or depletion of phospholamban are effective in increasing contraction and speeding relaxation in mouse/rat/rabbit/human myocytes
- No increase in mortality in PLB-KO or rats overexpressing SERCA2a
- No evidence for induction of arrhythmia: suppression of β-adrenoceptor-mediated aftercontractions.
- Rescue of some, but not all, mouse heart failure models by PLB-KO cross
- Rescue of heart failure in rat/hamster/pig by gene transfer by PLB-KO

CUPID: Time to Multiple Clinical Events

As of May 21, 2010



Prolonged action potential is a hallmark of the failing myocyte



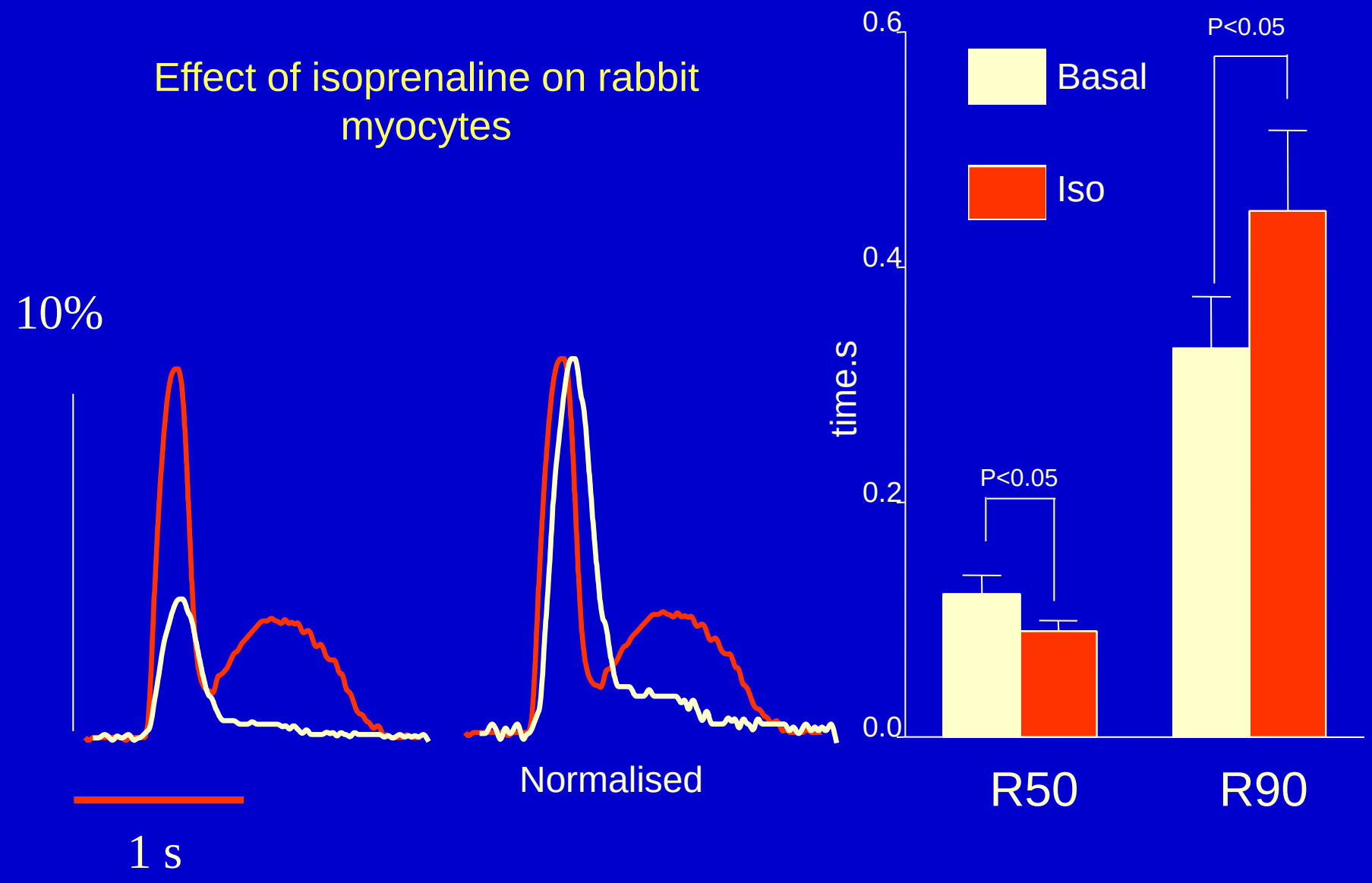
*(Beuckelmann et.al. Circulation Research **73**:379, 1993)*

Ca and K currents in human ventricular myocytes

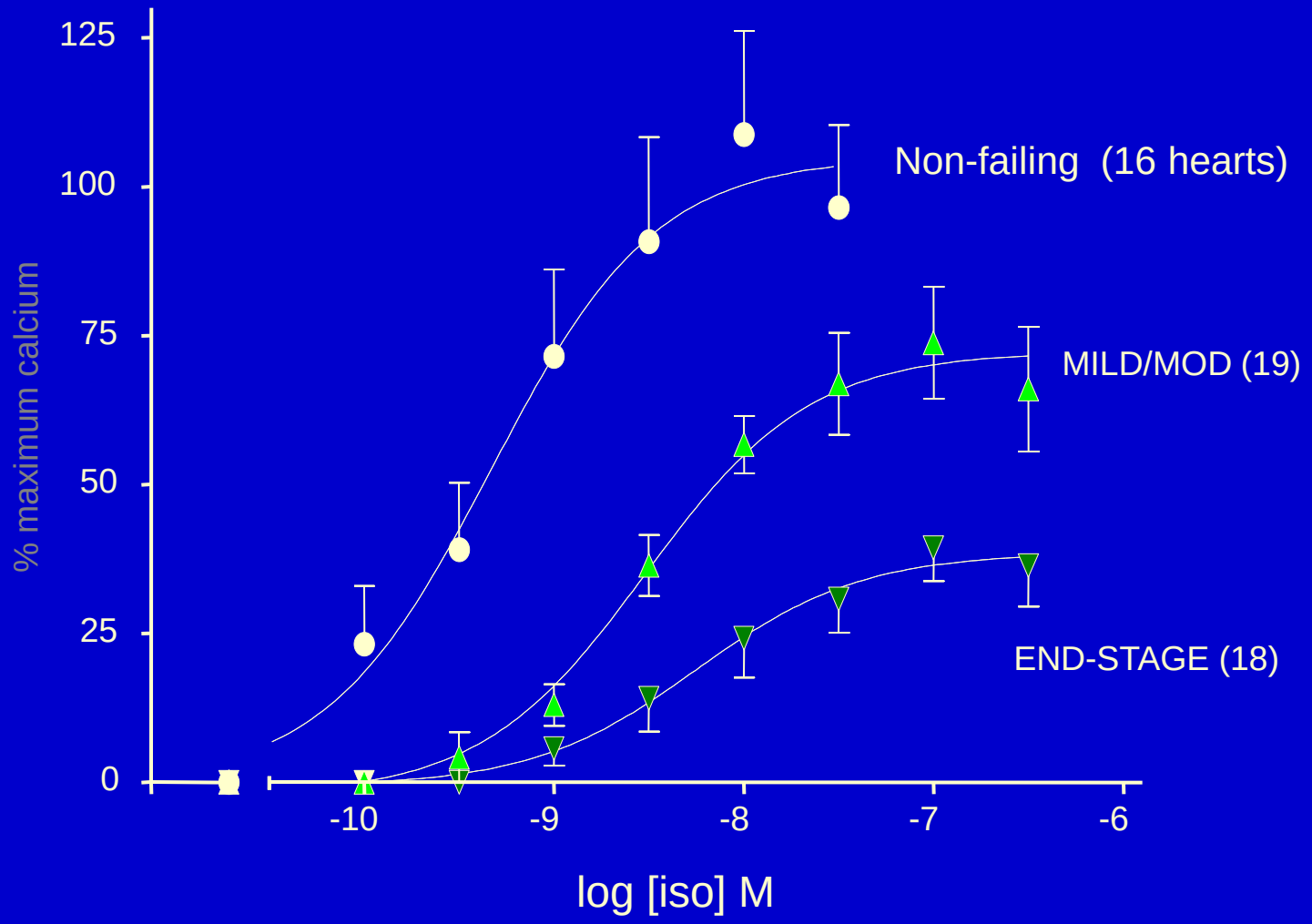
- $I_{CA\text{-}L}$ unchanged (Beuckelmann 1992, Mewes 1994)
- $I_{KS/R}$ small or absent (Beuckelmann 1993)
- I_{K1} decreased in HF (Beuckelmann 1993)
- I_{TO} epi>endo, gradient decreased in HF (Nabauer 1996)
- I_f unchanged (Hoppe, 1998)

  - NB NaK ATPase decreased – reduced hyperpolarisation and raised intracellular Na

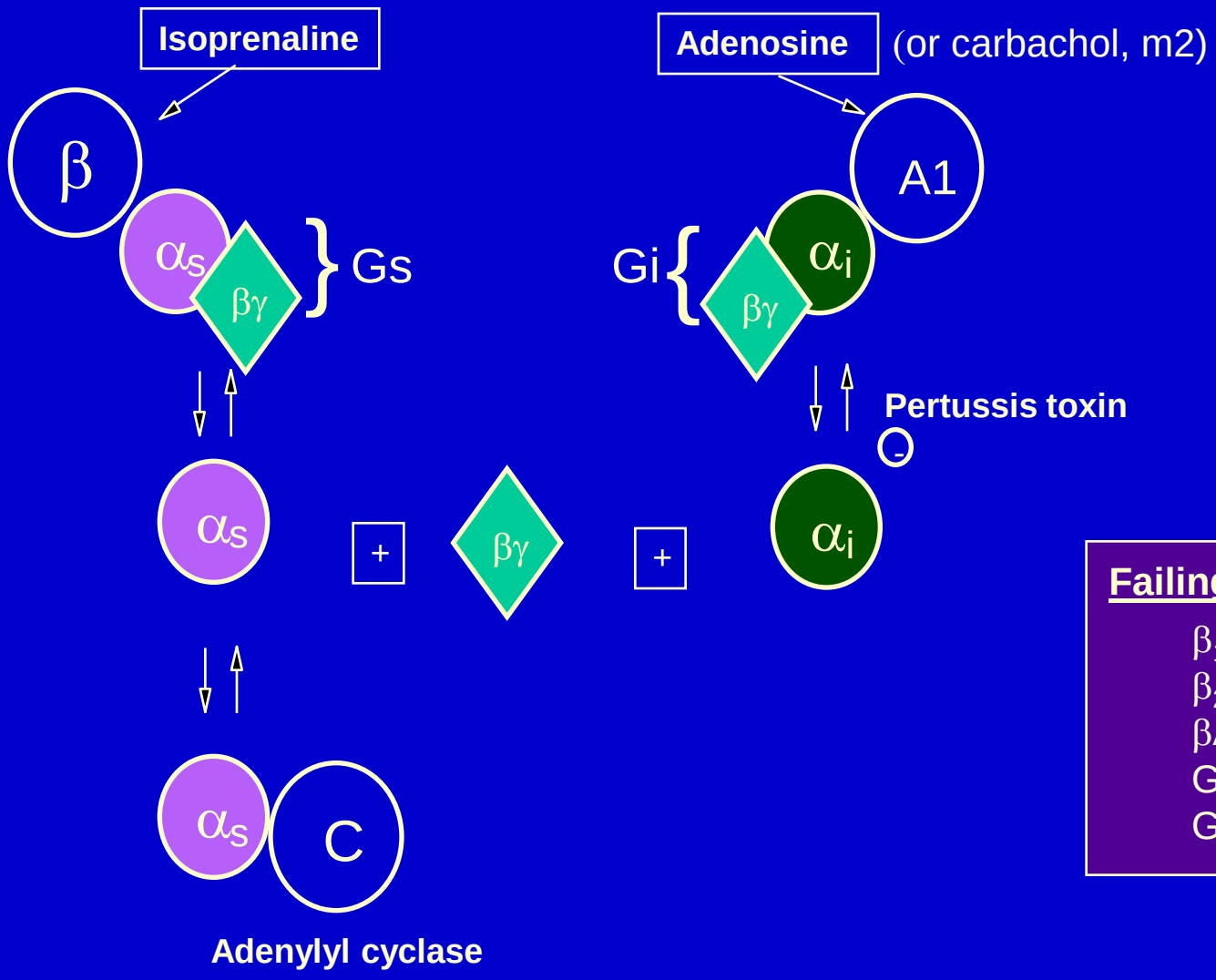
Effect of isoprenaline on rabbit myocytes



β-adrenoceptor desensitisation in ventricular myocytes from failing human heart

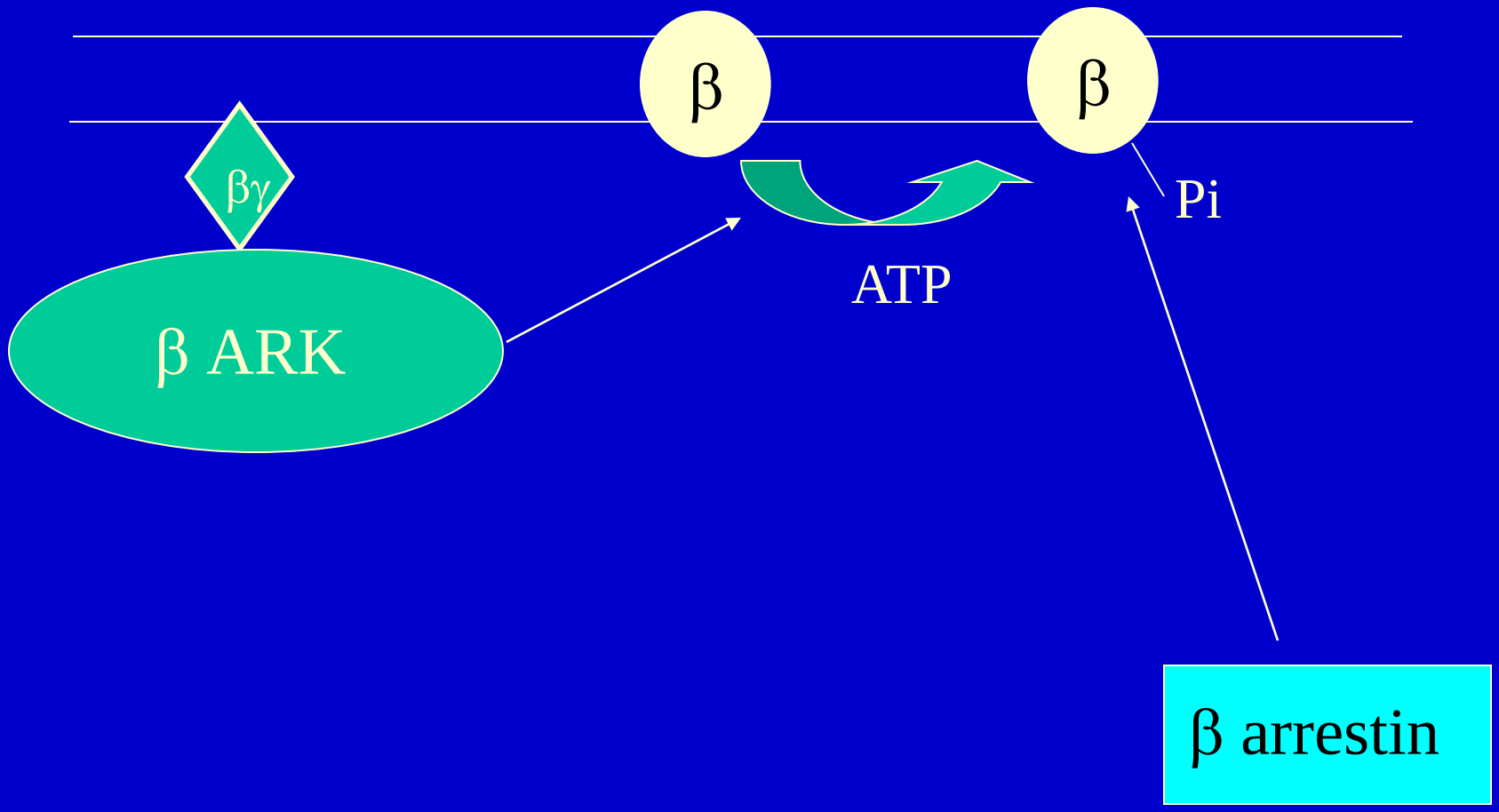


Updated from Harding, JMCC 1992 24:549

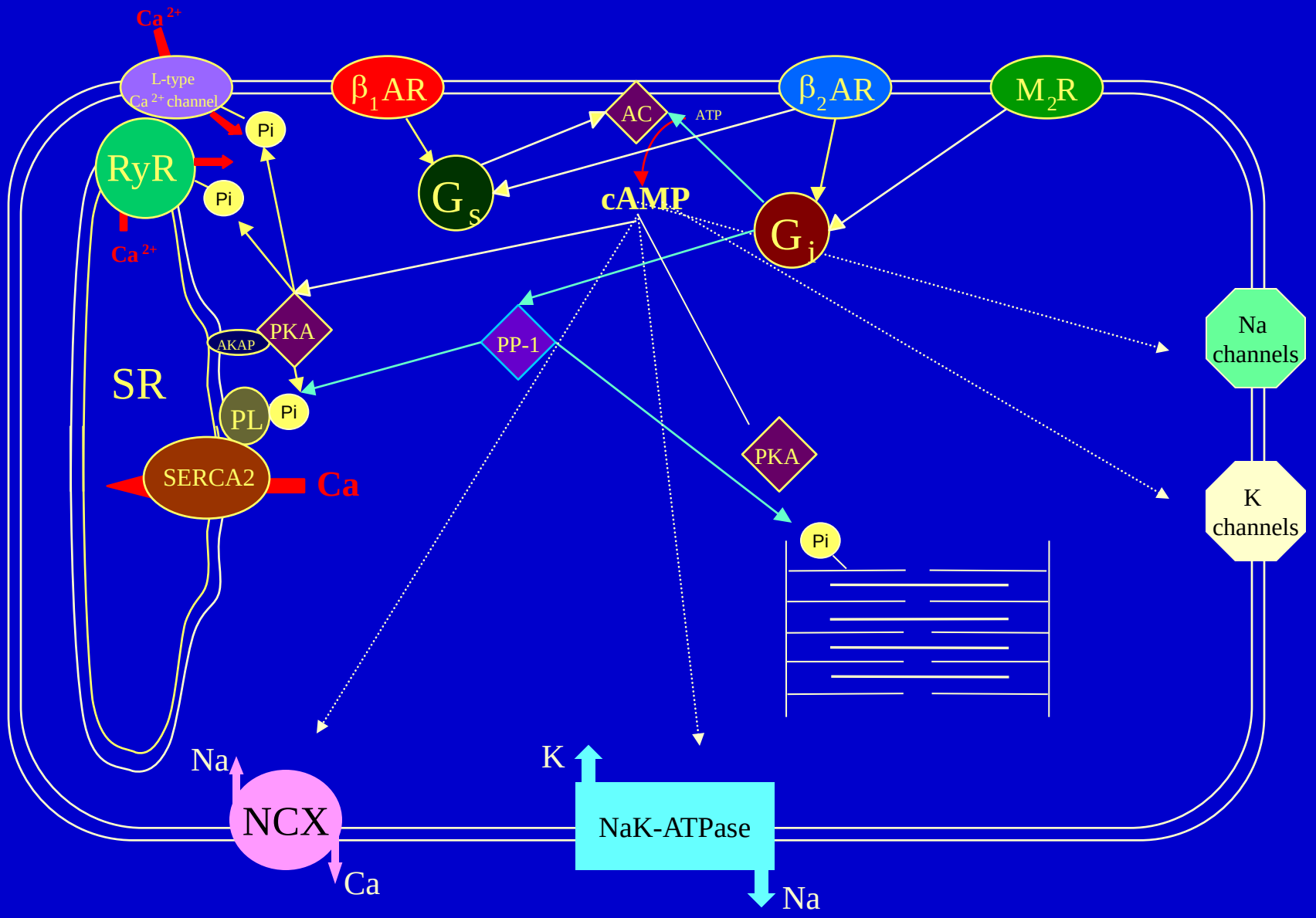


Failing human heart

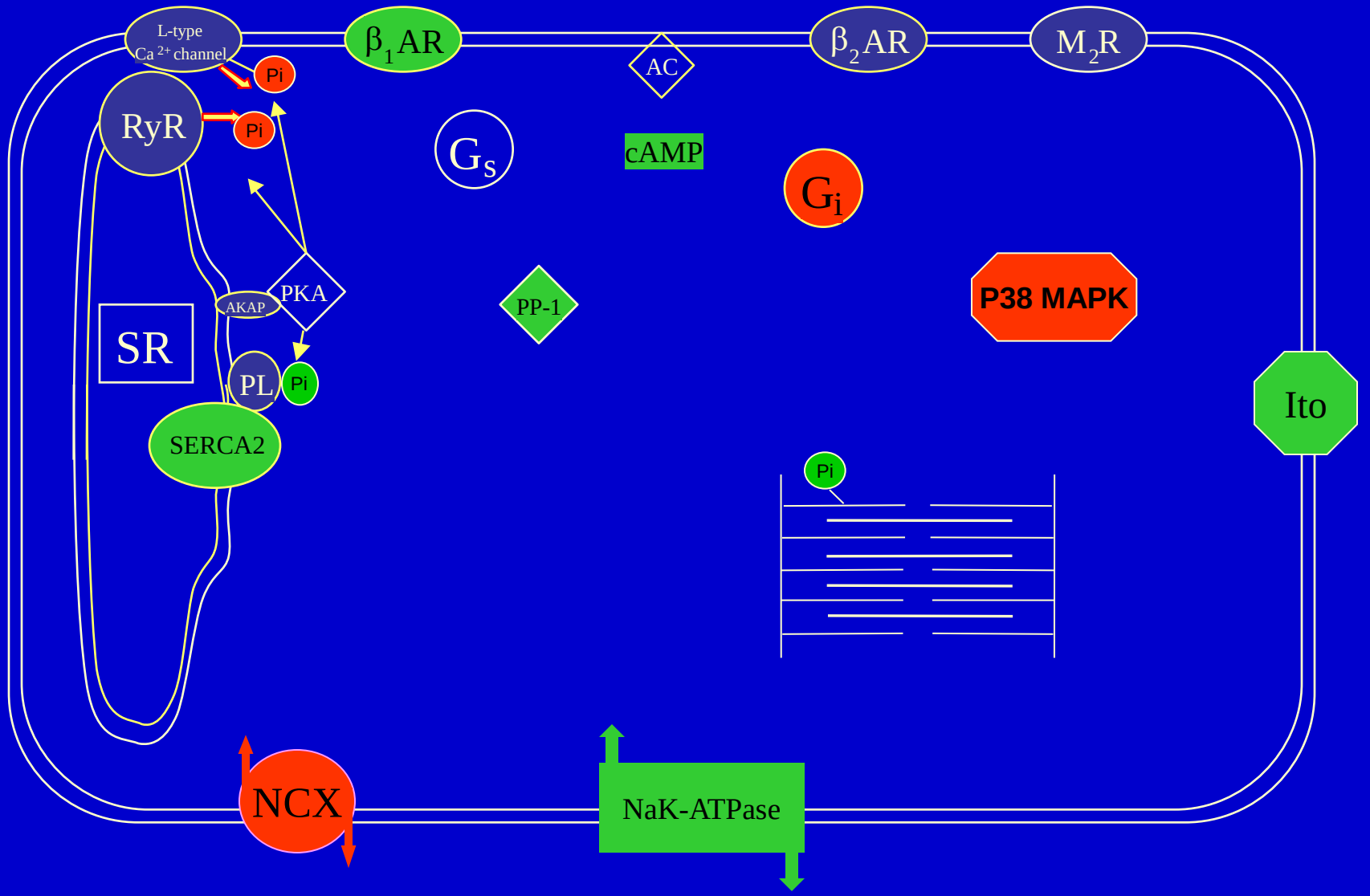
- β_1AR ↓
- β_2AR ↔
- βAR kinase ↑
- Gs ↔
- Gi ↑


β
β
βγ
Pi
ATP
β ARK
β arrestin

β-adrenoceptor control of calcium handling



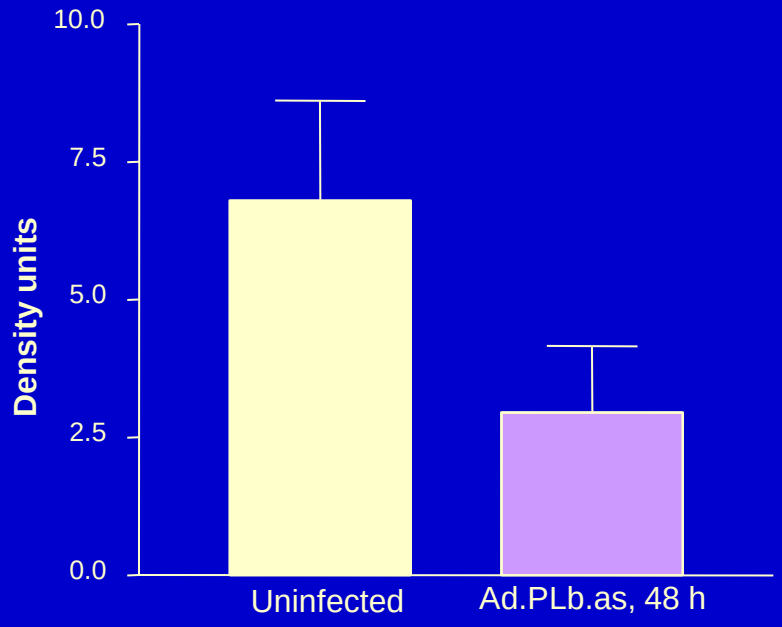
β-adrenoceptor control of calcium handling
Changes in heart failure

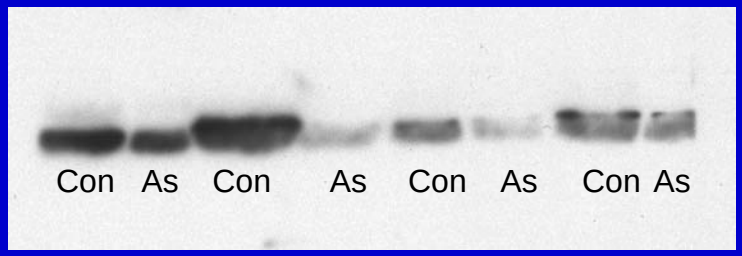


In failing human myocardium:-

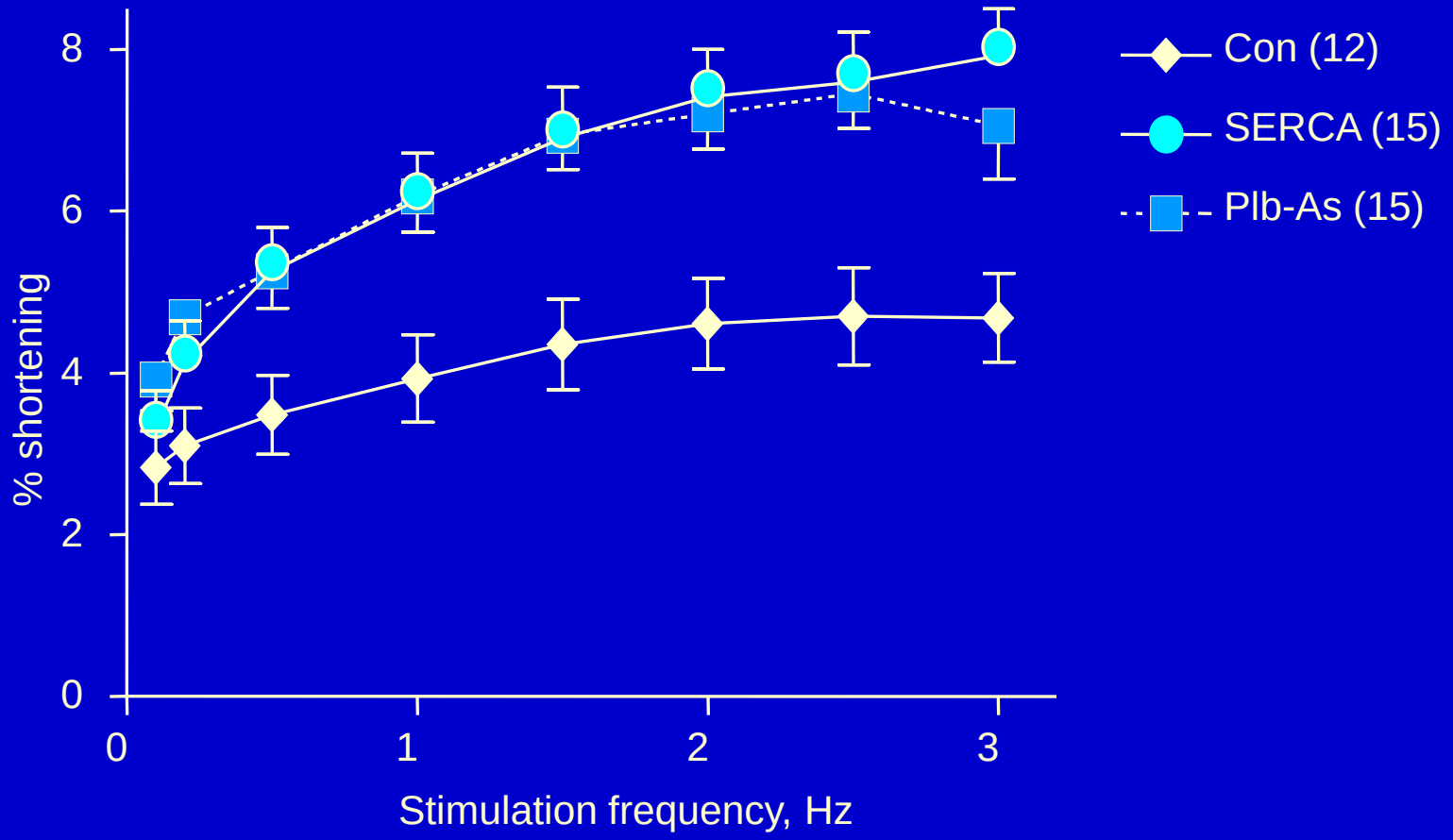
- SERCA2a activity is reduced more consistently than protein levels
- Cyclic AMP levels (basal or beta-adrenoceptor-stimulated) are reduced
- Back-phosphorylation experiments have shown reduced phospholamban phosphorylation
- Therefore, increased inhibitory effects of phospholamban may contribute to low SERCA2a activity

Down-regulation of phospholamban in rabbit myocytes 48 h after infection with Ad.Plb.As

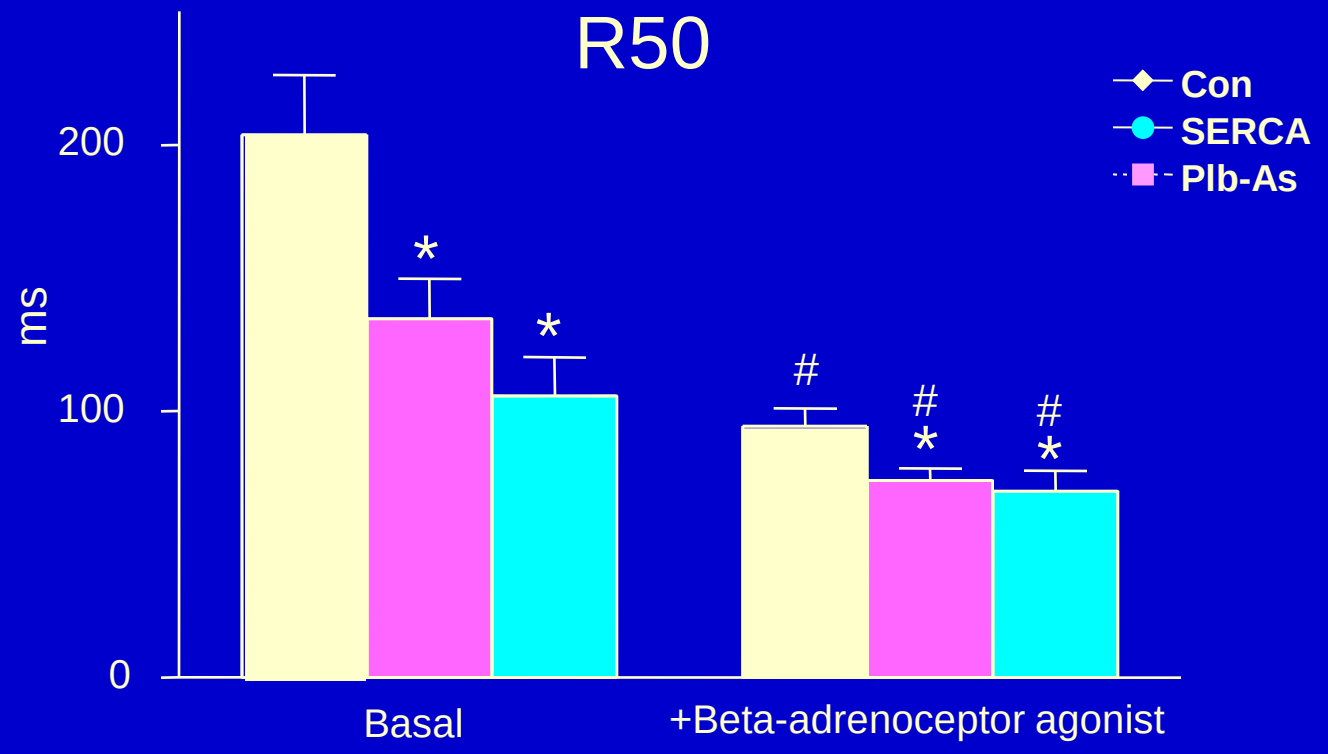




Effect of SERCA2a overexpression or PLB down-regulation on Contraction Amplitude vs frequency-response



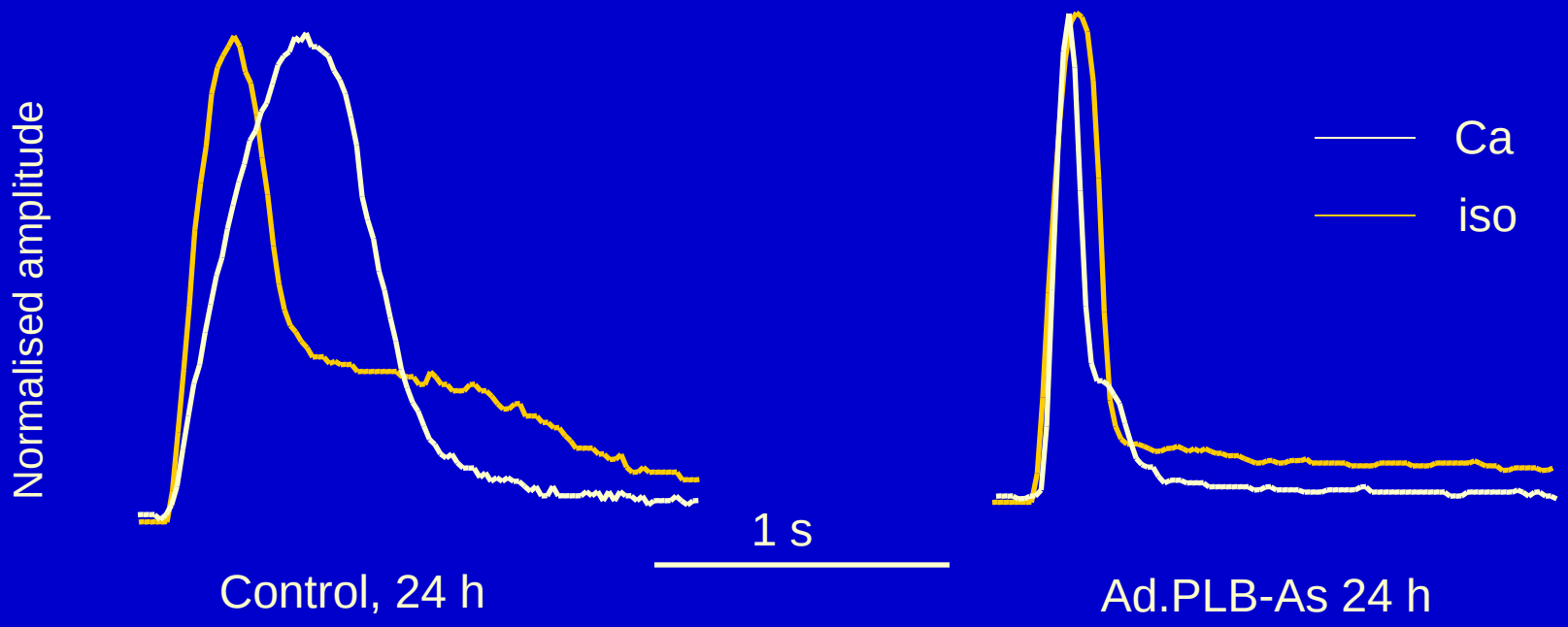
Acceleration of relaxation time following stimulation of SERCA2a activity

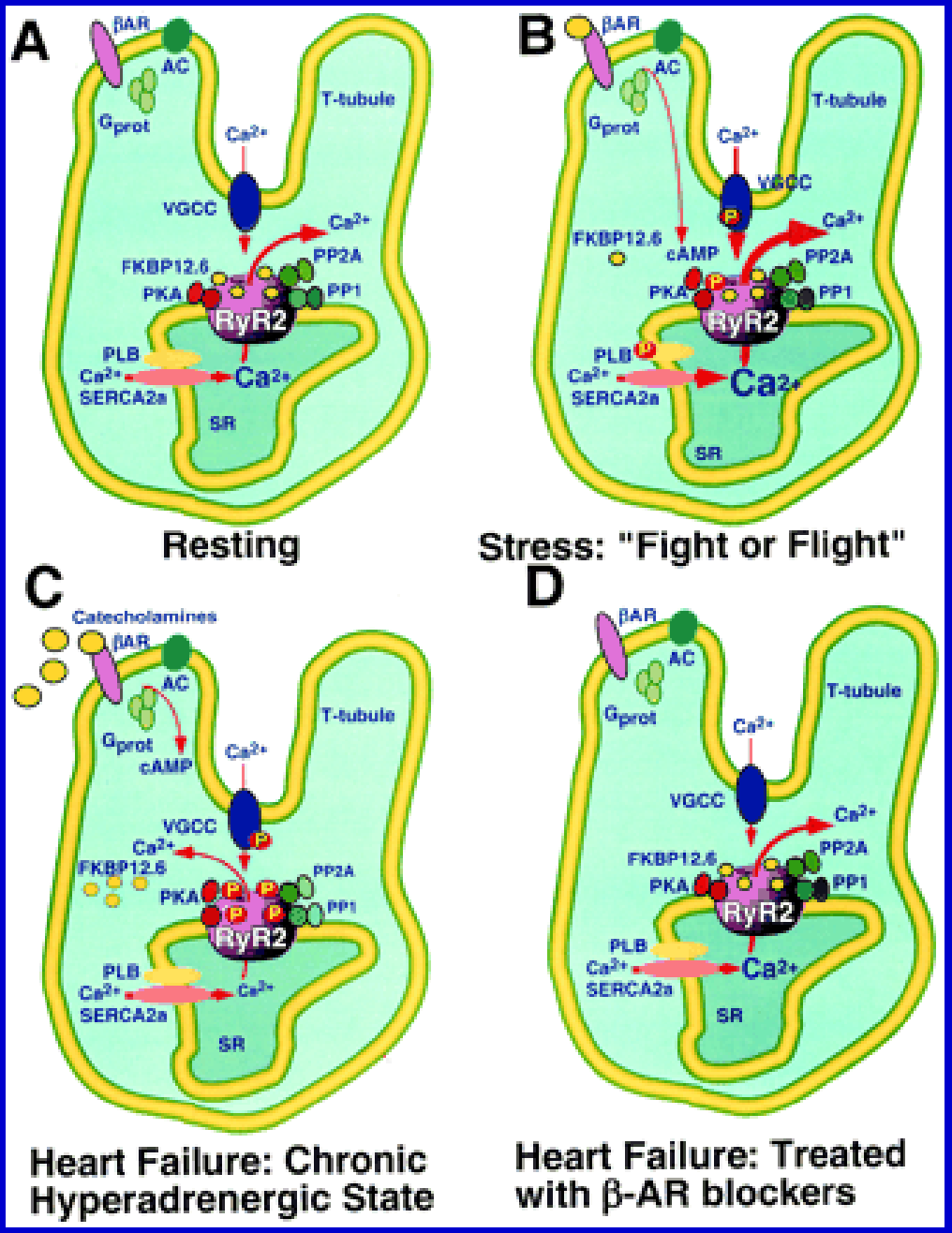


* sig diff from Con

sig diff from Basal

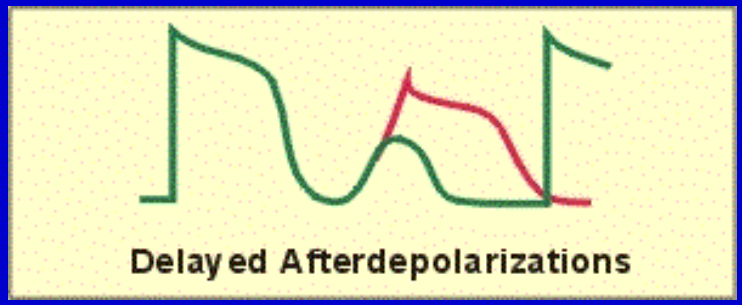
Human ventricular myocytes after down-regulation of phospholamban using PLB-antisense adenovirus



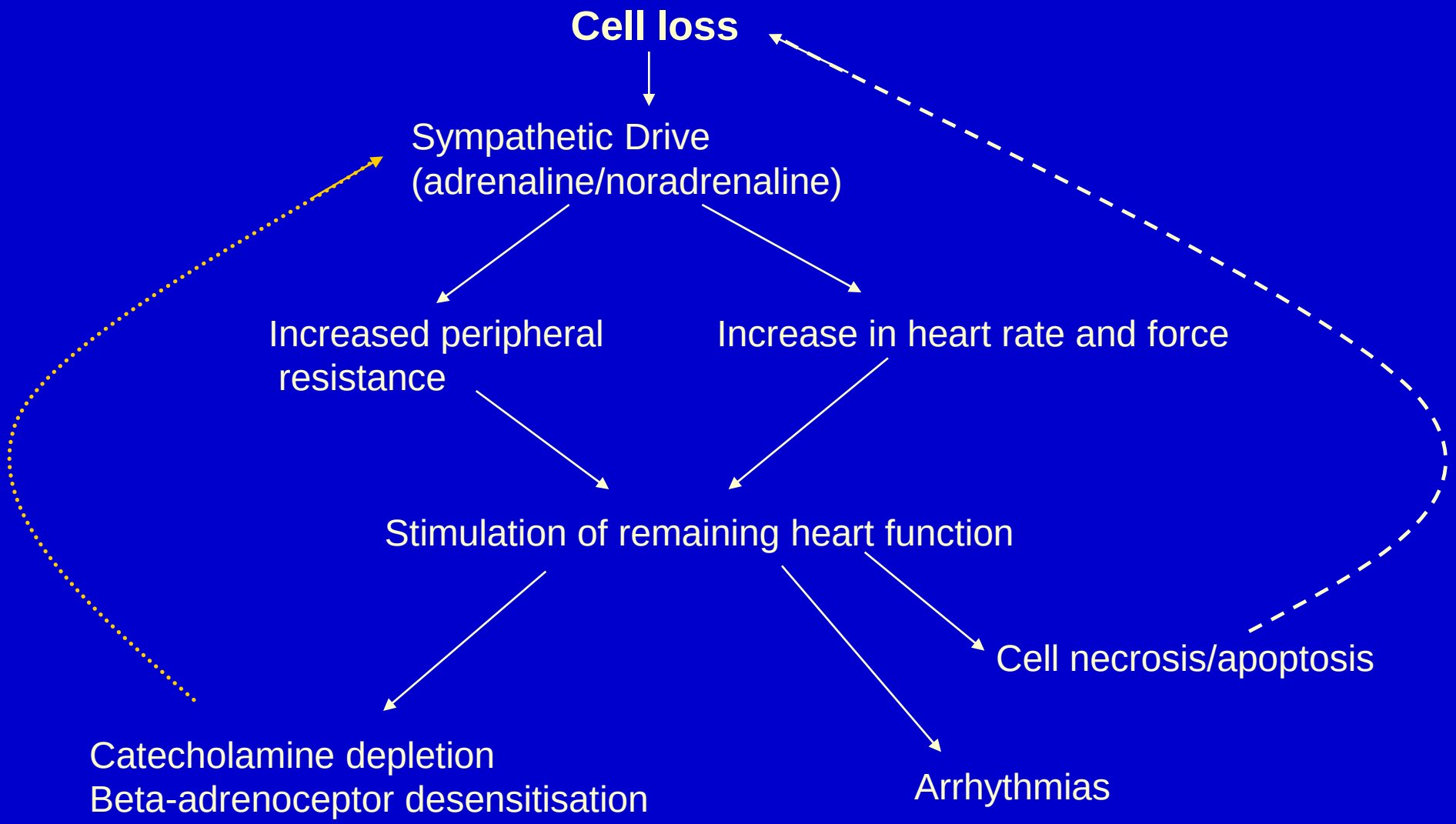


The cardiac SR Ca^{2+}-release channel in heart failure

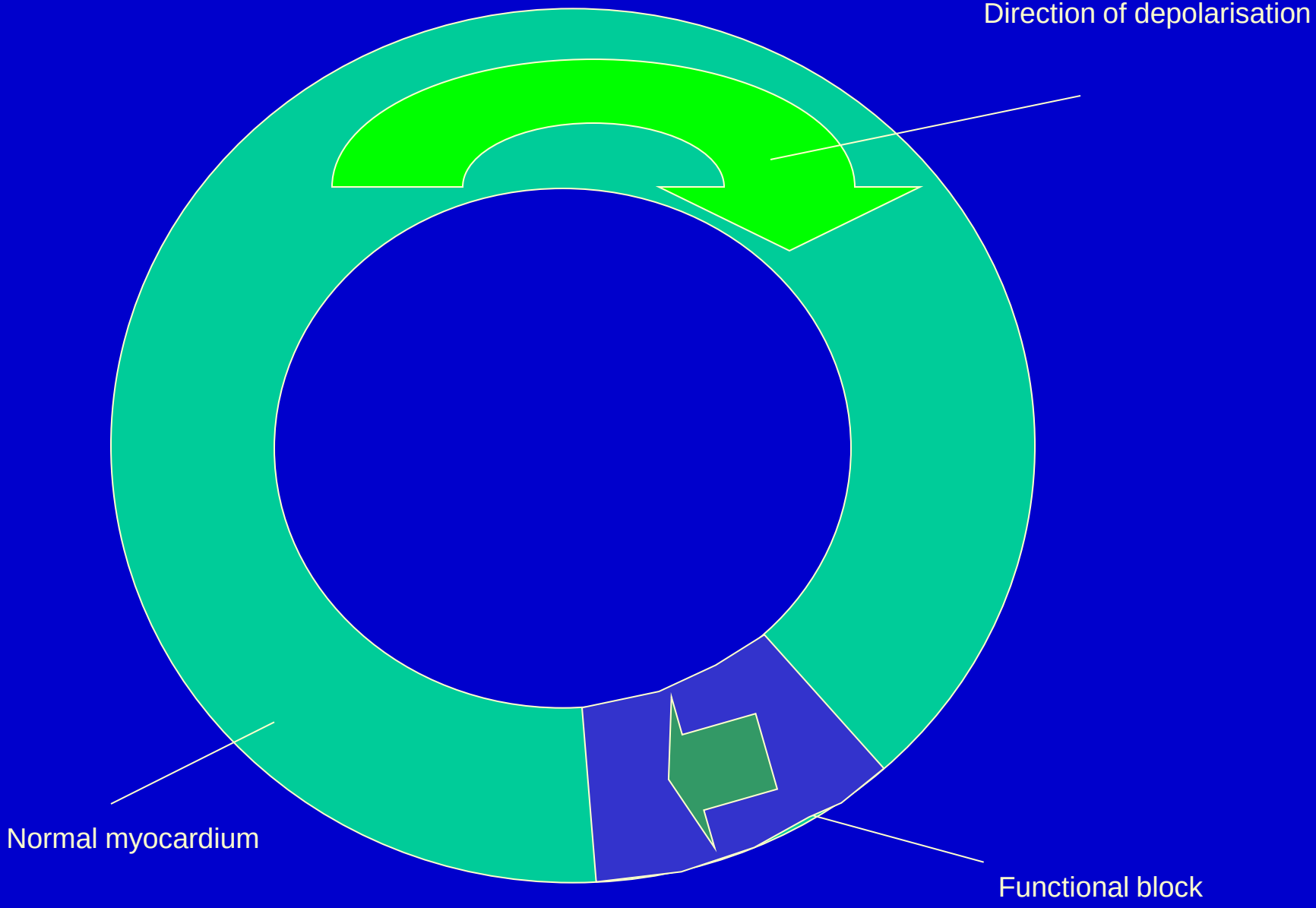
Marks et al Circulation 2002 105:272



Development of heart failure, role of sympathetic drive



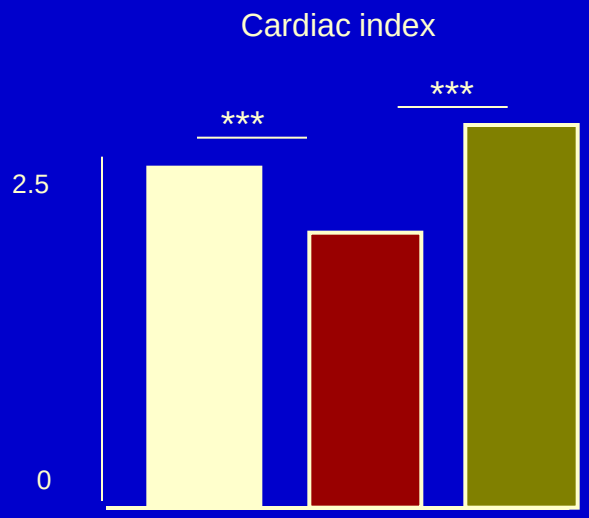
Re-entrant arrhythmias

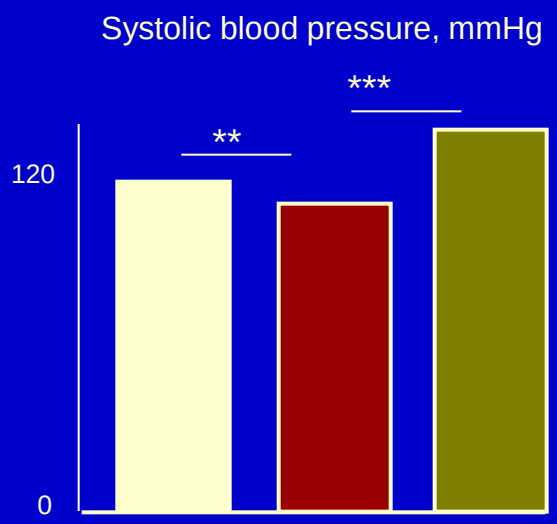


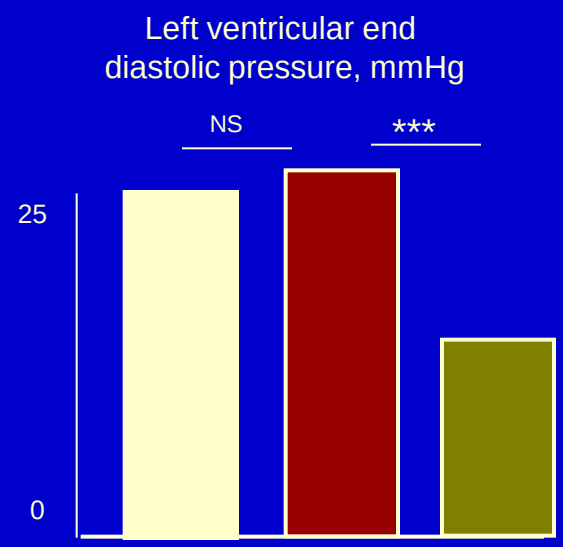
Failed trials with positive inotropes in heart failure

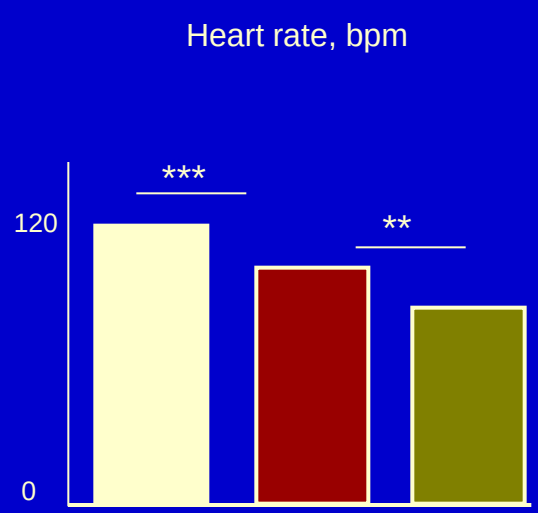
Trial	**Results**
PROMISE, milrinone 1991	Increased mortality, 28%
Xamoterol 1990	3-fold increase in mortality
PROFILE, flosequinan 1994	2-fold increase in mortality
Vesnarinone, 1993	Increased mortality

Improvement of heart failure by long-term β-blocker treatment









Before treatment

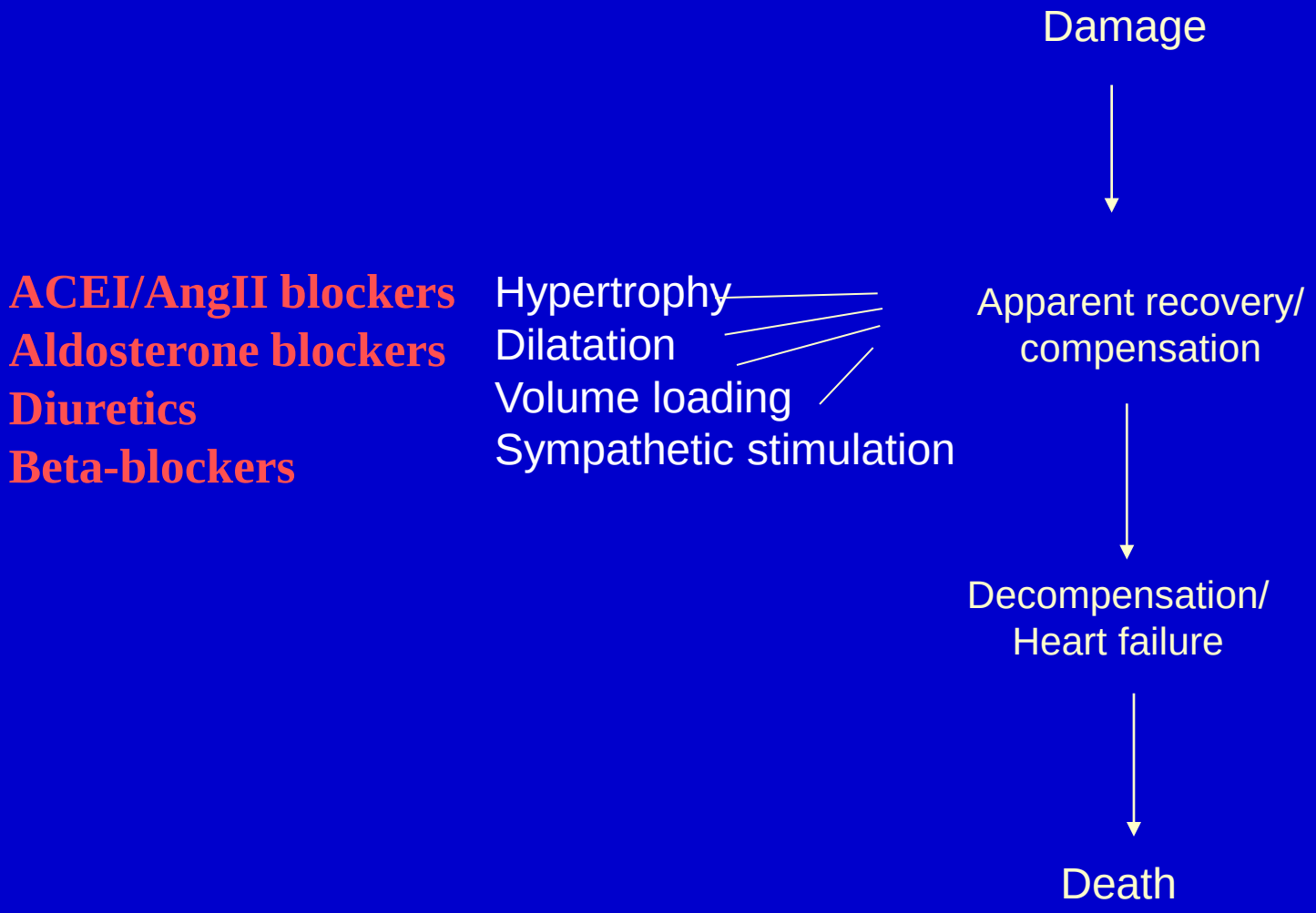
Metoprolol, acute, IV injection

Metoprolol, 2 months

Waagstein Circulation 1989

β-blocker trials

TRIAL	No. Pts	β-BLOCKER	Patients	RESULTS	
				All cause mortality	Clininal Outcomes
MDC	383	Metoprolo	NYHA II - III LVEF ≤ 40%	N.S.	Substantial ↓ in no. of patients requiring cardiac transplant (NS)
CIBIS I	641	Bisoprolol	NYHA III-IV LVEF ≤ 40%	N.S.	↑ Functional status and ↓ hospitalisation (p 0.01)
CIBIS II	2647	Bisoprolol	NYHA III-IV LVEF≤ 35%	↓ 32% ↓ 45% sudden death	Not yet analysed
US	1094	Carvedilol	NYHA II-IV LVEF≤ 35%	↓ 38% for combined death + admission	↓ 27% risk of hospitalisation for CVS disease
ANZ	415	Carvedilol	NYHA II-III LVEF≤ 45%	↓ 26% for combined death + admission	↑ LVEF (p<0.0001) ↓ LV cavity size (p<0.001)
Merit- HF	3991	Metoprolol	NYHA II-IV LVEF≤ 40%	↓ 35%	LVEF up 28% LV mass down
COPERNICUS	2289	Carvedilol	NYHA IV LVEF≤ 25%	↓ 35%	Not yet analysed


Damage
ACEI/AngII blockers
Aldosterone blockers
Diuretics
Beta-blockers
Hypertrophy
Dilatation
Volume loading
Sympathetic stimulation
Apparent recovery/
compensation
Decompensation/
Heart failure
Death

Alterations common to heart failure of various aetiologies

- Beta-adrenoceptor desensitisation
- SERCA2a activity decreased
- NaCa Exchanger increased
- RyR leaky
- Ito decreased
- Iks/r decreased
- NaK ATPase decreased
- Action potential duration increased