Adrenergic signalling in the heart

- Steven Marston
- 17/10/2011

- CVBsc Module 1

Coordinated response of the heart to exercise

	Rest	Hard exercise
Oxygen consumption (litres/min)	0.25	3.0
Cardiac output (litres/min)	4.8	21.6
Heart rate (beats/min)	60.0	180.0
Stroke volume (ml)	80.0	120.0
End-diastolic volume (ml)	120.0	140.0
Residual volume (end-systolic)	40.0	20.0
Ejection fraction	0.67	0.86
Cycle time (s)	1.0	0.33
Duration of systole (s)	0.35	0.2
Duration of diastole (s)	0.65	0.13

(After Rerych, S. K., Scholz, P. M., Newman, G. E. *et al.* (1978) *Annals of Surgery*, **187**, 449–458)

Coordinated response of the heart to exercise

	Rest	Hard exercise	
Oxygen consumption (litres/min)	0.25	3.0	
Cardiac output (litres/min)	4.8	21.6	5x
Heart rate (beats/min)	60.0	180.0	3x
Stroke volume (ml)	80.0	120.0	1.5x
End-diastolic volume (ml)	120.0	140.0	
Residual volume (end-systolic)	40.0	20.0	
Ejection fraction	0.67	0.86	
Cycle time (s)	1.0	0.33	1/3
Duration of systole (s)	0.35	0.2	
Duration of diastole (s)	0.65	0.13	1/5

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HOW DOES THIS WORK?

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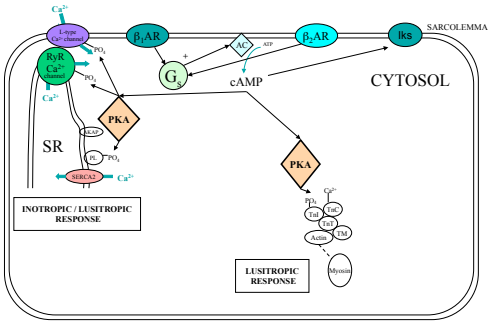
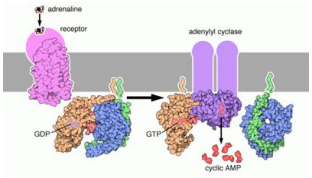
HOW DOES THIS WORK?

- Noradrenaline is released from sympathetic nerve terminals and circulating noradrenaline is increased 20-fold
- Noradrenaline binds to and activates β1-receptors
- This activates G_s protein leading to increased cytosolic cAMP and activation of protein kinase A

Phosphorylation of intracellular targets in ventricular muscle via cAMP-dependent protein kinase (PKA)



Adrenaline receptor acts through G_s to activate adenyl cyclase

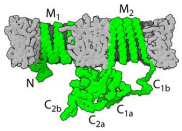


β1-AR binds G_s

When β1-AR receptor is activated GDP is exchanged for GTP in G_s

$G_{s\alpha}$ subunit dissociates and can bind to adenyl cyclase to activate it.

Adenylate cyclase catalyzes the conversion of ATP to 3',5'-cyclic AMP (cAMP) and pyrophosphate.



Adenylyl cyclase is a transmembrane protein. It passes through the plasma membrane twelve times.

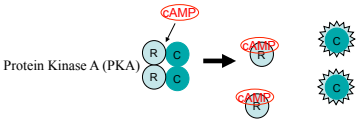
The important parts for its function are located in the cytoplasm and can be subdivided into the N-terminus, C1a, C1b, C2a and C2b.

The C1 region exists between transmembrane helices six and seven and the C2 region follows transmembrane helix 12.

The C1a and C2a domains form a catalytic dimer where ATP binds and is converted to cAMP.

cAMP activates PKA

- Each PKA is a holoenzyme that consists of two regulatory and two catalytic subunits. Under low levels of cAMP, the holoenzyme remains intact and is catalytically inactive.
- When the concentration of cAMP rises, cAMP binds to the two binding sites on the regulatory subunits, which leads to the release of the catalytic subunits.
- The free catalytic subunits can then catalyse the transfer of ATP terminal phosphates to protein substrates at serine, or threonine residues. This phosphorylation usually results in a change in activity of the substrate.



Effects of noradrenaline

- The heart rate increases
  chronotropy
- Ventricular pressure rises quicker and higher arterial pressure is produced
  inotropy
- The duration of systole grows briefer and relaxation is faster.
  lusitropy

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Chronotropy

Adrenaline acts on pacemaker potential

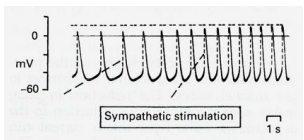

Figure 4.7 Effect of sympathetic stimulation on the pacemaker potential of the frog heart during the boxed interval. The dashed gradients highlight the increased slope of the pacemaker potential. The upper double-dashed line draws attention to the increased size of the action potentials, which is due to the enhancement of the inward calcium current by catecholamines. Note also the relatively slow onset of tachycardia. (From Hutter, O. F. and Trautwein, W. (1956) *Journal of General Physiology*, **39**, 715–733, by permission)

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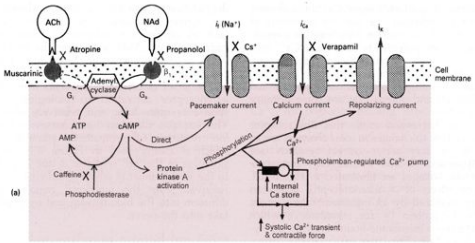
Chronotropy

cAMP induced by adrenaline increases inward Na^+ current

Activated PKA increases inward Ca^{2+} current and outward K^+ current



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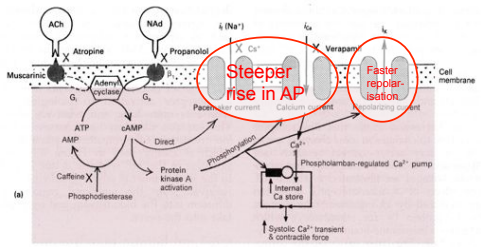

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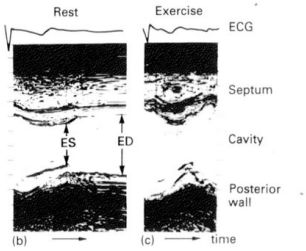
Inotropy

End diastolic dimension *increased* 2%

End systolic dimension *decreased* 5%

Stroke volume *increased* 24%

M-mode echocardiography



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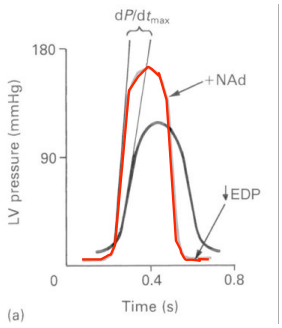
Inotropy

dP/dtmax *increased*

Peak pressure *increased*

Contraction duration *decreased*

Faster relaxation (lusitopy)



Effects of noradrenaline

- The heart rate increases
  - chronotropy
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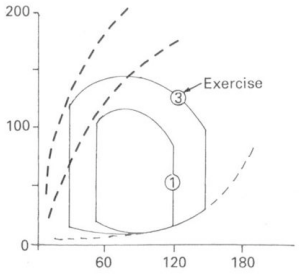
Inotropy

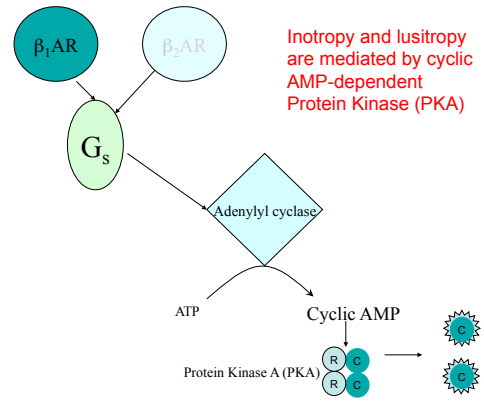
Peak pressure *increased*

End-diastolic volume *increased*

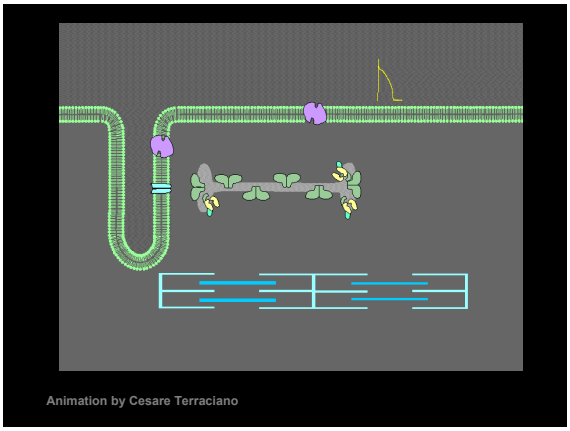
End systolic volume *decreased*

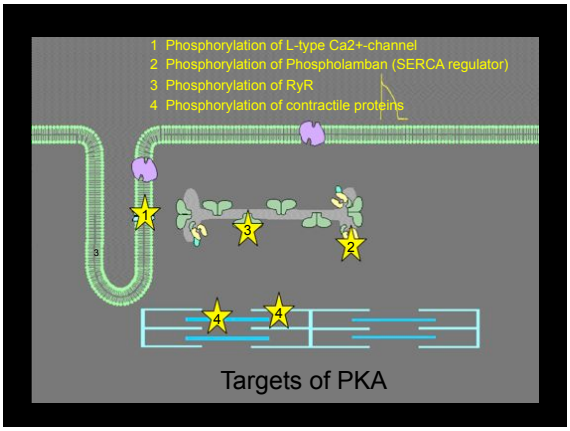
Stroke volume and work per cycle *increased*



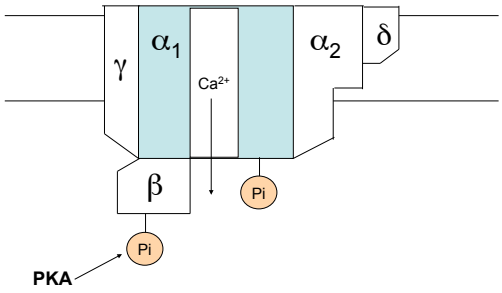


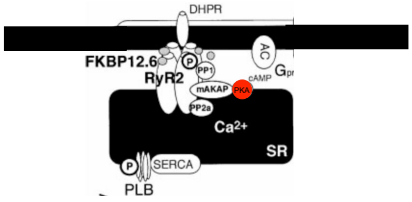
Inotropy and lusitropy are mediated by cyclic AMP-dependent Protein Kinase (PKA)





Phosphorylation of sarcolemmal L-type calcium channel by PKA increases open probability, contributes to pacemaker depolarisation and prolongs plateau of ventricular AP



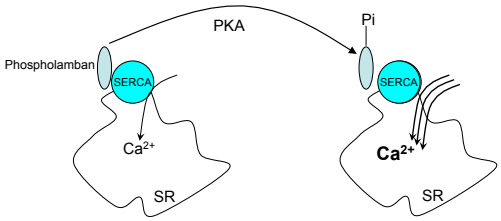


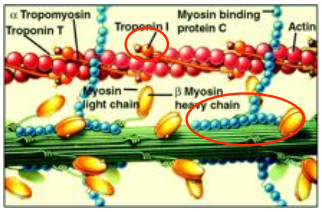
PKA is found in a complex with RyR, mAKAP , phosphatase and FKBP12.6

Phosphorylation of RyR2 with exogenous PKA "may activate RyR2 in the same dynamic way that PKA modifies cardiac contractile proteins"

Valvidia et al 1995 Science 267: 1997-2000

Phospholamban has a tonic inhibitory effect on the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a). Phosphorylation by PKA relieves this inhibition, allowing greater Ca^{2+} uptake into the SR. This speeds relaxation, and enhances the subsequent contraction especially at high frequencies

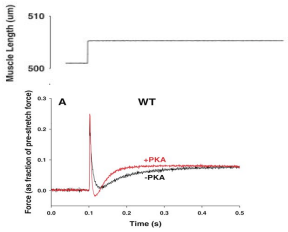




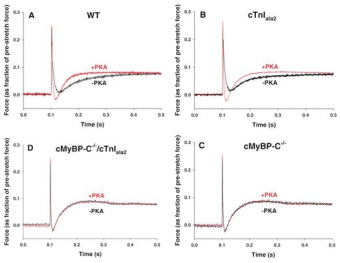
PKA phosphorylates MyBP-C in the thick filaments and Troponin I in the thin filaments

Of the contractile apparatus

Stretch-activation

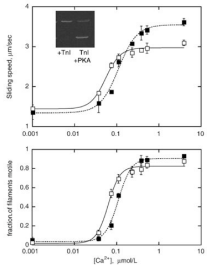


Cardiac myofibrils are stretched at 50% Ca^{2+}-activation and held. There is a delayed tension response which plays a role in synchronising contraction in the following systole. The rate of this is increased by PKA phosphorylation of MyBP-C



The effects of PKA phosphorylation on stretch activation depend on MyBP-C and are independent of troponin I since they the same in a non-phosphorylatable TnI mutant and are absent in a MyBP-C null mutant.

PKA phosphorylation of troponin I decreases Ca^{2+}-sensitivity of contraction



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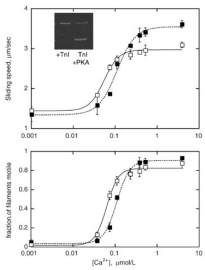


How can this *increase* contractility?

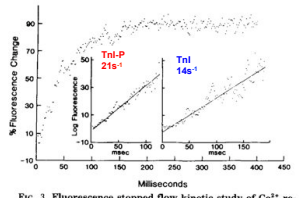
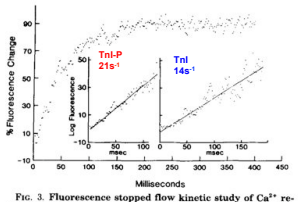


FIG. 3. **Fluorescence stopped flow kinetic study of Ca^{2+} removal from phosphorylated and nonphosphorylated cardiac Tn_{IA}.** A solution of 2 μM Tn_{IA}, 2 mM EGTA, 2.1 mM CaCl (pCa 4), 3 mM $MgCl_2$, 10 mM MOPS, 90 mM KCl, pH 7.01, was mixed in equal molar ratio with a solution of 12 mM EGTA, 3 mM $MgCl_2$, 10 mM MOPS, 90 mM KCl, pH 7.01. The solutions were mixed in 1:1 ratio and gave a 15% fluorescence increase statically and kinetically. *Inset A*, semilog plot of the fluorescence increase in Tn_{IA} reconstituted with phosphorylated TnI. *Inset B*, same plot with Tn_{IA} reconstituted with nonphosphorylated (control) TnI. Each *trace* represents signal averaged data of five stopped flow traces. Linear regression analysis of data in *inset A* and *B* (*solid line*) gave rate constants of 21 s^{-1} and 14.5 s^{-1} with $r = 0.9$ and 0.8, respectively.

The decrease in Ca^{2+}-sensitivity is due to faster dissociation of Ca^{2+} from troponin C



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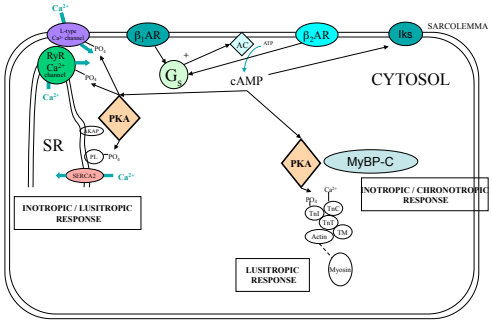
Faster dissociation of Ca^{2+} allows faster relaxation- Lusitropy and is essential for the inotropic response in whole heart

- the primary effect of PKA phosphorylation of cTnI is reduced Ca^{2+}-sensitivity of force, whereas phosphorylation of cMyBP-C accelerates the kinetics of force development.
- These results predict that PKA phosphorylation of myofibrillar proteins in living myocardium contributes to both the accelerated relaxation in diastole and increased rates of force development in systole.

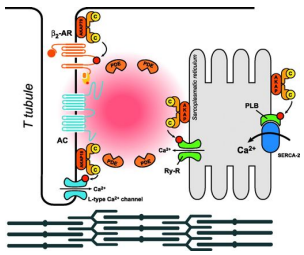
Phosphorylation of intracellular targets in ventricular muscle via cAMP-dependent protein kinase (PKA)



Regulation of cAMP- PKA system

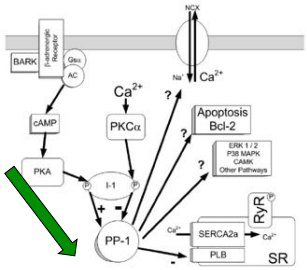
- BARK1 inactivates receptor and is activated by PKA phosphorylation (feedback mechanism)
- Phosphodiesterases degrade cAMP and create microdomains
- AKAPs anchor PKA to its target
- Phosphatases reverse PKA phosphorylation. Activity is controlled byPI-1, a substrate of PKA and PKC
- Muscarinic agonists antagonise adrenaline effects via phosphatase activation

TASKEN, K. et al. Physiol. Rev. 84: 137-167 2004;
doi:10.1152/physrev.00021.2003



Local gradients of cAMP and anchored pools of PKA mediate {beta}-adrenergic regulation of cardiac function. Signaling through the {beta}2-adrenergic receptor localized in microdomains of the sarcolemma formed by t tubules generates a local gradient of cAMP that is also shaped by phosphodiesterases. cAMP activates PKA localized via AKAPs in the vicinity of its substrates such as the {beta}2-adrenergic receptor ({beta}2-AR), the L-type Ca2+ channel, and the ryanodine receptor (RyR) and phospholamban (PLB).

The PP-1/ phosphatase inhibitor 1 regulation system



The β-adrenergic receptor couples with an inhibiting receptor kinase (BARK) and the stimulatory G-protein/adenylate cyclase complex (GSα-AC). Normal activation of this pathway increases cAMP synthesis and stimulates protein kinase A (PKA), which phosphorylates I-1 protein to act as an inhibitor of phosphatase PP-1.
PKA phosphorylates phos-pholamban (PLB) to enhance sarcoplasmic reticular (SR) calcium uptake, which is further augmented by the decline in PP-1 activity.

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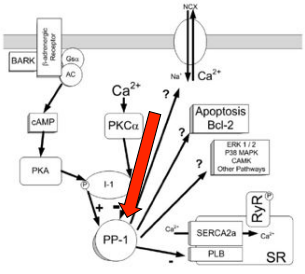
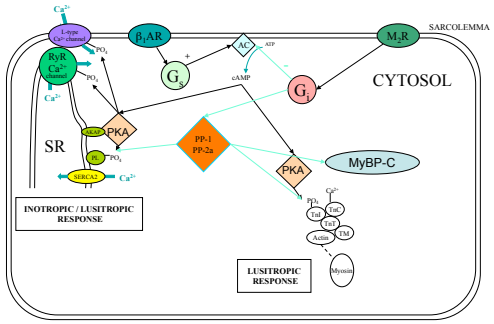
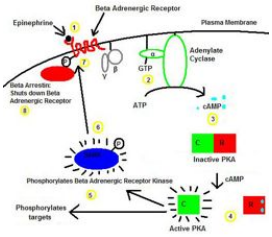


In settings in which PKCα is stimulated by calcium or various stress agonists, I-1 is phosphorylated at a different site, resulting in stimulation of PP-1 and reduced phosphorylation of PLB. The result is reduced calcium uptake by the sarcoplasmic reticulum and diminished contractile force.

Muscarinic stimulation can enhance dephosphorylation of PKA targets





BARK Activation

* (steps 1) Upon stimulation of the Beta adrenergic receptor by epinephrine, Gs will be activated.

* (steps 2 and 3) Gs alpha will then stimulate adenyl cyclase to make cAMP.

* (steps 3 through 6) cAMP will then activate cAMP dependent kinase (PKA), which among other proteins that it acts on, it will phosphorylate serine and threonine residues on BARK.

* (step 7) BARK, itself a serine/threonine kinase, will then phosphorylate serine and threonine resides on the B-adrenergic receptor itself.

* (step 8) This will facilitate Beta-arrestin's binding to the receptor. Additional stimulation by epinephrine will now be unable to activate Gs due to arrestin.

Therefore, BARK is a negative feedback enzyme which will prevent over stimulation of the B-adrenergic receptor.