stimuli was not significantly different between TRN and SED hearts ($P > 0.05$ unpaired t-test n = 9-10).
We suggest that despite the occurrence of both cardiac hypertrophy and electrical remodelling in response to voluntary exercise, these changes do not pre-dispose the exercise trained heart to arrhythmias. Our findings are consistent with the overall beneficial effect of mild exercise on the heart.

Maron BJ (2005). Heart **91**, 1380-1382.

McCrossan ZA *et al*. (2004). Cardiovasc Res **63**, 283-292.

Natali AJ *et al*. (2002). J Physiol **541**, 863-875.

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PC25

**Carbon monoxide inhibits human cardiac L-type $Ca^{2+}$ channels**

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Conditions of stress such as myocardial infarction stimulate up-regulation of heme oxygenase-1 (HO-1) to provide cardioprotection, but its mechanism of action is unknown (Clarke et al., 2003). One of the products of HO-1 catabolism is carbon monoxide (CO). We have investigated the ability of CO to act as a modulator of L-type $Ca^{2+}$ channels, using whole-cell patch clamp recordings from HEK 293 cells stably expressing the human $\alpha_{1C}$ $Ca^{2+}$ channel subunit as previously described (Scragg et al., 2005).
CO, applied via the CO donor molecule CORM-2 (1-70μM; Williams et al., 2004), caused reversible, voltage-independent $Ca^{2+}$ channel inhibition of up to ca. 40%, whereas its inactive form (iCORM) was without significant effect. CO-mediated inhibition was independent of protein kinase G activation since effects were unaltered in cells pretreated with PET-cGMPS (100nM). Two distinct NO donors, SIN-1 (10μM) and GSNO (2mM) failed to mimic the inhibitory actions of CO and did not significantly alter $Ca^{2+}$ currents. The actions of CO were prevented by the antioxidant MnTMPyP (100μM). Inhibition of NADPH oxidase (apocyanin; 30μM, or diphenyleneiodonium; 3μM), or xanthine oxidase (allopurinol, 1μM) did not affect the inhibitory actions of CO. Instead, inhibitors of complex III (but not complex I) of the mitochondrial electron transport chain, namely antimycin A (3μM) and stigmatellin (1μM) and a mitochondrially-targeted antioxidant (Mito Q; 250nM), fully prevented the effects of CO.
Our data indicate that the cardioprotective effects of HO-1 activity may be attributable to an inhibitory action of CO on cardiac L-type $Ca^{2+}$ channels. Inhibition arises from the ability of CO to promote generation of reactive oxygen species from complex III of mitochondria.

Clark JE et al. (2003) Circ Res 93, e2-e8.

Scragg JL et al. (2005) FASEB J 19, 150-152

Williams SE et al. (2004) Science 306, 2093-2097

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PC26

**SAPHIR: a Guyton-based extensible, modular 'core model' of blood pressure regulation for the Physiome**

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We present current progress on the SAPHIR project, an open-source, modular modeling environment of cardiovascular and respiratory physiology using state-of-the-art multi-scale simulation methods. Our model initially targets blood pressure and body fluid homeostasis. We present re-implementations of two legacy models that treated overall regulation of blood pressure (Guyton et al. 1972), and fluid regulation (Ikeda et al. 1979).
The basic "core model" includes lumped-parameter input-output descriptions of relevant organs as modules, i.e., heart, vasculature, intra- and extracellular spaces, lungs, kidneys, and muscles. This core model can be modified/extended by customizing existing modules or by replacing one or several of the core modules by more detailed, mechanistic models at finer resolution. As modeling/simulation environments, Berkeley-Madonna was used for Ikeda's model (JF, PB); Fortran (SRT), Matlab/Simulink (PH, FG) and the M2SL C++ software library (Rennes laboratory, AH & VL), were used for implementation of the original Guyton models. M2SL will be the basic solver package for the multi-module modeling environment.
The resulting modular modeling environment is compact enough to run on a personal computer and yet accomodate detailed mechanistic submodules.
This open-source, modular approach allows for (i) selected extensions/refinements of the model (e.g. addition of a pancreas module and regulation of blood glucose) and (ii) assessment of system-level consequences of local perturbations (e.g. models of functional consequences of genetic polymorphisms). One important goal is to keep the model compact enough to ensure fast execution time (in view of eventual use in clinical settings), yet to allow detailed sub-modules (to maintain system-integrated feedback loops).
This approach will eventually provide a platform for patient-specific exploration of therapeutic scenarios in the context of the IUPS Physiome.

Guyton AC, Coleman TG & Granger HJ (1972). Annu Rev Physiol 34, 13-46.

Ikeda N, Marumo F, Shirataka M & Sato T (1979). Annals of Biomedical Engineering 7, 135-166.

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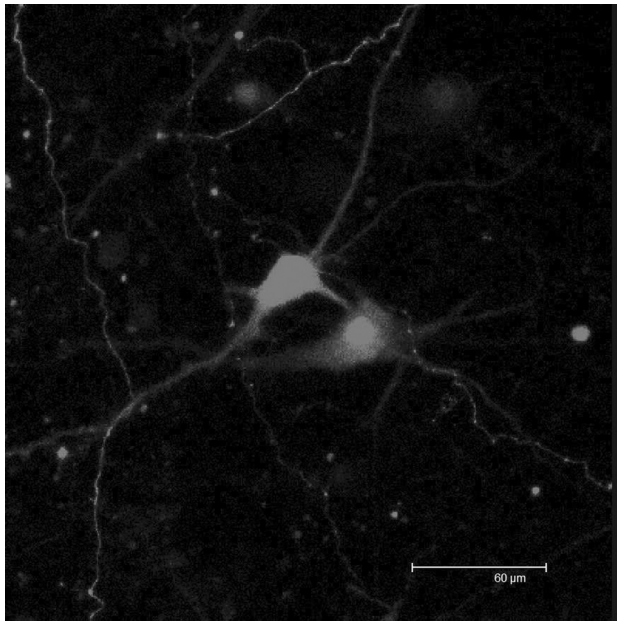
likely toxicity of these proteins was due to their incomplete folding in central neurones and/or formation of oligomers.
Here we report construction of an adenoviral vector to target expression of a newer member of the red protein family, DsRed2, to central NAergic neurones. DsRed2-fluorescent cells exhibit no signs of toxicity, show healthy morphology, and their electrophysiology is indistinguishable from similar neurones expressing EGFP. This opens a way for multi-colour fluorescent imaging in living brain tissue using cell-specific viral vectors.




NAergic neurones expressing DsRed2 in rat organotypic brainstem slice culture. Image width: 275 µm.

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PC33

**Structural requirements of human cardiac L-type $Ca^{2+}$ channels for inhibition by carbon monoxide**

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We have suggested that the cardioprotective effects of heme oxygenase-1 may be attributable to an inhibitory action of its product, carbon monoxide (CO) on cardiac L-type $Ca^{2+}$ channels. CO-mediated $Ca^{2+}$ channel inhibition arises from the ability of CO to promote generation of reactive oxygen species (ROS) from complex III of mitochondria (Dallas et al., 2008). Here, using mutagenesis in combination with whole-cell patch clamp recordings, we have probed the structural requirements of the $\alpha_{1C}$ subunit of the human L-type $Ca^{2+}$ channel, stably or transiently expressed in HEK 293 cells.

CO (applied using an established CO-releasing molecule, CORM-2 (Williams et al., 2004) at 30µM) exerts a strong inhibitory effect on the full-length splice variant (hHT) of the recombinant human cardiac L-type $Ca^{2+}$ channel $\alpha_{1C}$ subunit (53.2±2.8% inhibition, n=10 cells). This effect was fully prevented by pretreatment of cells with the reducing agent dithiothreitol (2mM). The rHT variant, lacking a cytoplasmic C-tail splice insert (Fearon et al., 2000), was insensitive to CO (n=10). Deletion mutagenesis studies demonstrated that a stretch of 34 amino acids (1785-1818) within the splice insert of hHT was essential for CO sensing. Given that CO inhibition of the channel arose via generation of ROS from mitochondria (Dallas et al., 2008), we investigated the potential involvement of each of the three cysteine residues (C1789, C1790 or C1810) in these effects of CO. Serine substitution of each of these residues fully prevented the effects of CO.
Our data suggest CO regulates $Ca^{2+}$ channel activity via redox modulation of one or more of three key cysteine residues in the C terminal tail of the channel. Whether or not these cysteines interact to regulate channel activity in the absence of CO remains to be determined.

Dallas ML et al. (2008). this meeting.

Fearon IM et al. (2000). Circ Res 87, 537-539.

Williams SE et al. (2004). Science 306, 2093-2097.

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PC34

**Discordant effects of $GABA_A$ receptor agonists in the retrotrapezoid nucleus on the central control of breathing in the rat**

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The retrotrapezoid nucleus (RTN), located close to the ventral medullary surface, contains central chemoreceptors that are activated by hypercapnia. In anesthetized animals, pharmacological inhibition of RTN causes apnoea, abolishes the response to increased $CO_2$ and blocks the peripheral chemoreflex (1). The physiological role of the RTN in non anesthetized animals is less clear. We have performed bilateral microinjections of $GABA_A$ receptor agonists (muscimol or isoguvacine) in the RTN of decerebrate artificially-perfused *in situ* rat preparations (2). Rats were decerebrated under deep halothane anaesthesia as assessed by an absence of reflex limb withdrawal to noxious pinching (as per ref. 2). Once decerebrate (i.e. insentient), halothane administration was discontinued making these preparations devoid of the undesirable effects of anaesthesia. The ventral medullary surface was surgically exposed and agonists were microinjected in the RTN under visual control while the phrenic nerve activity (PNA) was recorded simultaneously. Muscimol injections (1.75 mM, 30-60 nl, n=7) to the