Effects of streptozotocin-induced type I diabetes mellitus on the sinoatrial-node of the rat heart

Yu Zhang[1], Yanwen Wang[1], Joseph Yanni[1], Mohammed Anwar Qureshi[2], Xue Cai[1], Natalie Gardiner[1], Frank Christopher Howarth[2], Halina Dobrzynski[1]

[1]Institute of Cardiovascular Sciences, University of Manchester; [2]Department of Physiology, UAE University

British Heart Foundation

The University of Manchester

Introduction

It has been estimated that 5 million people will suffer from diabetes in the UK by 2025.[1] Cardiovascular complications are common in type I diabetes mellitus (T1DM). There is increased risk of bradyarrhythmias, atrioventricular block and bundle branch block as a result of dysfunction of the cardiac conduction system (CCS).[2,3] The CCS is responsible for the generation and transmission of electrical activity in the heart (Figures 1 and 2) and consists of the sinoatrial node (SAN, the primary pacemaker), atrioventricular node (AVN), bundle of His (HIS), right and left bundle branches (RBB; LBB) and right and left Purkinje fibres (RPFs; LPFs). In the rat streptozotocin (STZ)-induced model of T1DM, *in vivo* ECG recordings have shown a significant ($P<0.05$) decrease in heart rate (HR) and prolongation of the QRS complex, evidence of dysfunction of the CCS (Figures 8-10).[4]

1: Cardiac conduction system

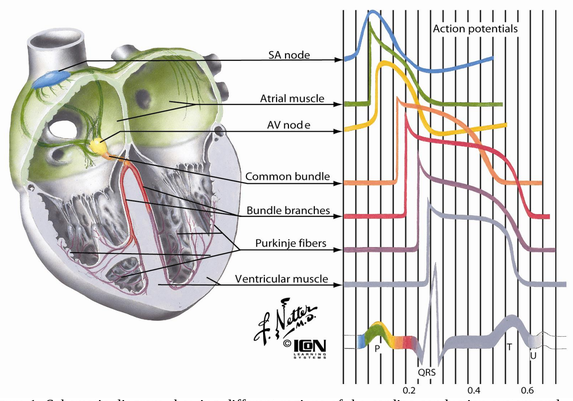


Figure 1: Schematic diagram showing different regions of the cardiac conduction system and the corresponding action potentials. AV, atrioventricular; SA, sinoatrial.

2: Membrane and Ca^{2+} clocks

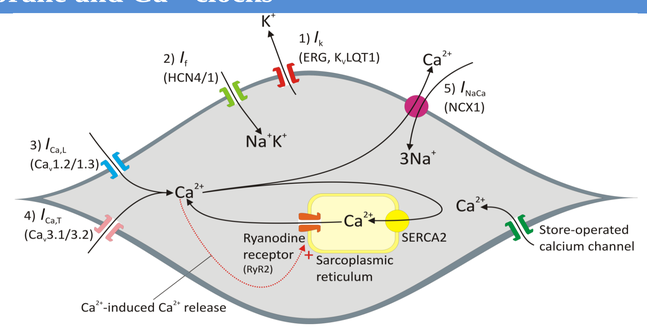
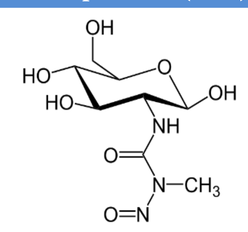


Figure 2: Schematic diagram of a SAN cell showing different ionic currents involved in the pacemaker potential and pacemaker activity. There is voltage-dependent decay of outward currents (I_K) and voltage-dependent activation of inward currents: I_f (funny current), $I_{Ca,L}$ (L-type Ca^{2+} current) and $I_{Ca,T}$ (T-type Ca^{2+} current). The sarcoplasmic reticulum is replenished with Ca^{2+} by SERCA2 (sarcoendoplasmic reticulum Ca^{2+} ATPase) and membrane store-operated Ca^{2+} channels (SOCC). Ca^{2+} is removed from nodal cells by the Na^+-Ca^{2+} exchanger in response to a spontaneous release of Ca^{2+} from the sarcoplasmic reticulum via the ryanodine receptor (RyR2). In removing Ca^{2+}, the Na^+-Ca^{2+} exchanger generates an inward current (I_{NaCa}). Adapted from Monfredi et al. (2010). [6]

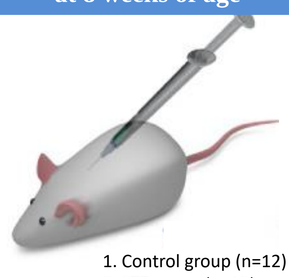
Methods and Results

Streptozocin (STZ)



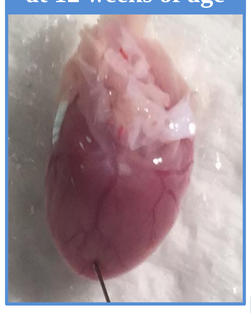
STZ is a naturally occurring chemical which is toxic to insulin producing β cells in mammalian pancreas

STZ injection into rats at 8 weeks of age



1. Control group (n=12)
2. STZ group (n=12)

Collect hearts at 12 weeks of age



Techniques used

Functional experiments:

- *In vivo* ECG recordings
- *Ex vivo* ECG recordings
- Extracellular potential recordings
- Whole cell patch clamp recordings

Histology:

Masson's trichrome staining

Immunolabelling and Western blot (protein investigated):

- HCN4 (funny channel)
- Cx43 (gap junctional channel)
- RyR2 (Ca^{2+} handling protein)
- NF-M (sympathetic neuronal marker)
- Cav3 (cardiac cell membrane marker)

Table 1: Characteristics of rat T1DM model

	Control (n=12)	T1DM (n=12)
Body weight (g)	322.50	191.00**
Heart weight (g)	1.18	0.85**
Body/heart weight ratio	3.59	4.19**
Blood glucose (mg/dl)	101.91	533.33**

Data are mean ± SEM, **P<0.05.

Figure 3: *In vivo* heart rate

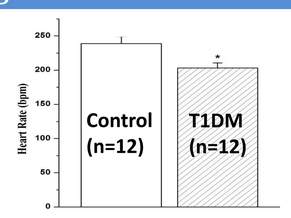


Figure 3: This figure shows that there is slower *in vivo* heart rate in T1DM rats. Data are mean ± SEM, *P<0.05. From Howarth et al., 2015.[4]

Figure 4: AV node action potential recording

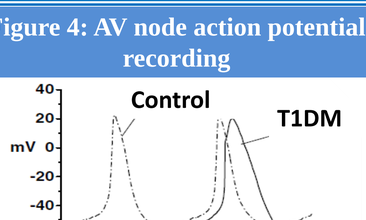


Figure 4: Action potential recordings from control and T1DM AV node myocytes. The T1DM AV node myocyte had a slower diastolic depolarisation and slower action potential upstroke.. From Yuill *et al.*, 2010.[5]

Table 2: *In vivo* ECG parameters

	Control	T1DM
QT (ms)	-0.60-0.58	8.25-2.82*
QRS (ms)	-0.13-0.18	1.57-0.29**
PQ (ms)	0.65-0.85	0.94-0.95

Table 2: This figure shows that the QT interval and QRS complex are prolonged in T1DM rats. Data are mean ± SEM, *P<0.05. From Howarth et al., 2015.[4]

Figure 5: Western Blot

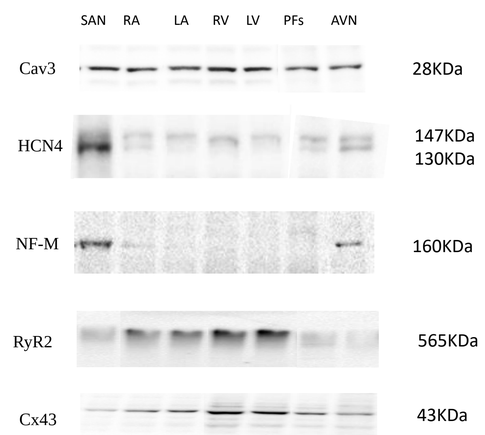


Figure 5: This figure show Western blot of SDS gel electrophoresis of homogenised tissue samples obtained from control rat hearts to test specificity of primary antibodies used in this study. As expected there was different levels of expression of proteins investigated in the different heart tissues. AVN, atrioventricular node; LA, left atrium; LV, left ventricle; PFs, Purkinje fibres; RA, right atrium; RV, right ventricle; SAN, sinoatrial node.

Figure 6

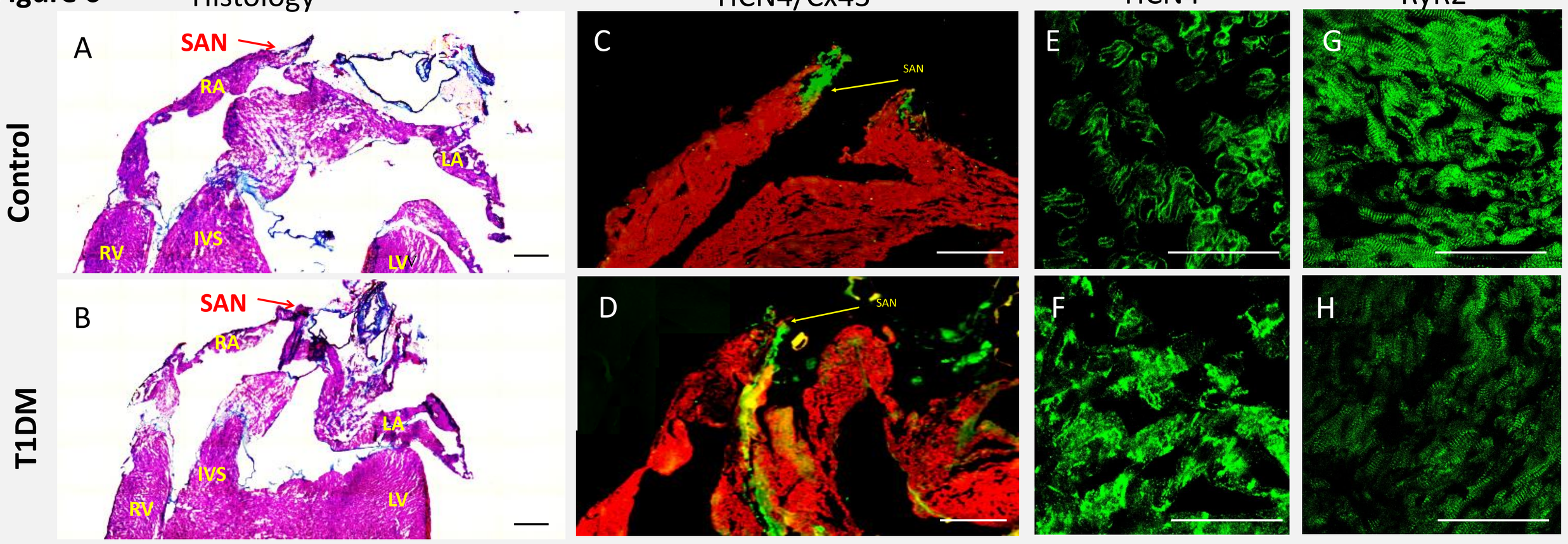


Figure 6: A and B show Masson's trichrome stained whole tissue sections from one control rat heart and one T1DM rat heart at the level of the SAN. C and D show low magnification confocal images of adjacent tissue sections immunolabelled for HCN4 and Cx43 at the level of the SAN. Cx43 signal is in red and HCN4 is in green. E-H show high magnification confocal images at the level of the SAN - HCN4 signal is in green (E and F) and RyR2 signal is in green (G and H). Scale bar = 50 µm.

Semi-quantification of immunolablelling

Figure 7: HCN4 expression was significantly increased in the SAN in T1DM hearts. RyR2 expression was significantly decreased in the SAN in T1DM hearts. Data are mean ± SEM; n=5 control hearts and n=5 T1DM hearts; *P<0.05.

Beating rate (ECG recording)

Figure 8: *Ex-vivo* beating rate was reduced by 17% in STZ rats compare to controls.

Effect of ryanodine on *ex-vivo* beating rate

Heart rate (bpm)	Heart rate with ryanodine (bpm)
252.2	160.0

Figure 9: Beating rate of *ex-vivo* SAN preparations was reduced by 36% on application of 2µM ryanodine. n=3 T1DM hearts. Application of ryanodine caused irregular beats/arrhythmias.

Effect of Cs^+ on *ex-vivo* beating rate

Heart rate (bpm)	Heart rate with Cs^+ (bpm)
252.2	170.6

Figure 10: Beating rate of *ex-vivo* SAN preparations was reduced by 32% on application of 2mM Cs^+. n=3 T1DM hearts.

Funny current

Figure 11: This figure shows that we were able to record funny current (I_f) for which HCN4 is responsible in isolated SAN cells form T1DM rats. Top, cell capacitance. Bottom, current relationship to I_f based on 33 cells from 3 T1DM rats.

Conclusion

1. Downregulation of RyR2 in the SAN could explain the slower heart rate of T1DM rats.
2. HCN4 increase in T1DM rats could be a compensatory mechanism.
3. Complex interplay between membrane currents and Ca^{2+}-clock signalling may increase risk of bradyarrhythmias.

Acknowledgement

We thank Professor Mark Boyett for his valuable comments.

References

1. www.diabetes.org.uk
2. Movahed MR, Hashemzadeh M, Jamal MM (2005). Diabetes mellitus is a strong, independent risk for atrial fibrillation and flutter in addition to other cardiovascular disease. *International Journal of Cardiology* **105**, 351-35.
3. Aksnes TA, Kjeldsen SE, Rostrup M, Störset O, Hua TA, Julius S (2008). Predictors of new-onset diabetes mellitus in hypertensive patients: the VALUE trial. *Journal of Human Hypertension* **22,** 520–527.
4. Howarth FC, Jacobson M, Shafiullah M, Adeghate E (2005). Long-term effects of streptozotocin-induced diabetes on the electrocardiogram, physical activity and body temperature in rats. *Experimental Physiology* **90**, 237-245.
5. Yuill KH, Tosh D, Hancox JC (2010). Streptozotocin-induced diabetes modulates action potentials and ion channel currents from the rat atrioventricular node. *Exp Physiol* **95**, 508-17.
6. Monfredi O[1], Dobrzynski H, Mondal T, Boyett MR, Morris GM (2010). The anatomy and physiology of the sinoatrial node--a contemporary review. *Pacing Clin Electrophysiology* **33**, 1392-406.