Confocal Ca^{2+} imaging of mouse sinoatrial node

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Recent studies have demonstrated that intracellular Ca^{2+} plays an important role in cardiac pacemaking (Ju & Allen, 2001). However, the mammalian sinoatrial node (SAN) is a heterogeneous structure and studies on the centre leading pacemaker region suggest that central pacemaker cells do not require the sarcoplasmic reticulum (SR) calcium release for spontaneous activity (Lancaster *et al.*, 2004). In order to study the Ca^{2+} dependent pacemaker mechanisms in different regions of mammalian preparation, we developed a new technique to image Ca^{2+} from intact mouse sino-atrial preparations. Mice (7 -10 weeks) were deeply anaesthetized. The right atrium was opened under a dissecting microscope to expose the crista terminalis, the intercaval area and the interatrial septum as described by Verheijck *et al.* (2001). The preparation was pinned into a sylgard block with a 3×5mm open window that allowed microscopic imaging. The SAN preparation was loaded with the fluorescent Ca^{2+} indicator Fluo-4 AM (10 μM) by incubation in Tyrode solution at 4°C for 5 h. The SAN area was recognized by its anatomic landmarks. 10 mM 2,3–butanedione monoxime (BDM) was used to reduce the motion artifact. The loading of dye into the central region normally was weaker than for peripheral regions for reasons that are unclear. It is known that Na^{+} channels are required for peripheral but not for the central pacemaker activity. Therefore 100 μM lidocaine was added to the perfusate to block any pacemaker type activity from the periphery of the SAN. Spontaneous Ca^{2+} signals from the central SAN were imaged using a confocal microscope (LSM410) in XY and XT modes. We found that the SAN Ca^{2+} signal was modulated by the SR Ca^{2+} release channel modulator caffeine and by ATP. These results show that it is possible to record Ca^{2+} signals from the centre of leading pacemaker region in mouse heart.

Ju, Y.K. & Allen D.G. (2001) *Clinical and Experimental Pharmacology and Physiology* **28**, 703-8.
Lancaster, M.K., Jones, S.A., Harrison, S.M. & Boyett, M.R. (2004) *Journal of Physiology* **556**, 481-494.
Verheijck, E.E., van Kempen, M.J.A., Veereschild, M., Lurvink, J., Jongsma, H.J. & Bouman, L.N. (2001) *Cardiovascular Research* **52**, 40-50.

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