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A novel mechanism for the genesis of arrhythmias? The role of the low affinity β1-adrenergic receptor and CGP12177 in spontaneous calcium release in rat atrial myocytes.

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Introduction: A number of β-adrenoceptor blocking drugs cause cardiostimulation at high concentrations. Some of these non-conventional partial agonists, eg. pindolol, have been contra-indicated in the treatment of ischemic heart disease because of their sympathomimetic effects (Podrid and Lown, 1982). Freestone *et al* (1999) have shown that the non-conventional partial agonist, CGP12177, structurally similar to pindolol, is 40 times more potent than isoprenaline (ISO) in causing arrhythmias in mouse ventricular myocytes. The pro-arrhythmic effect of CGP12177 occurs despite increasing intracellular calcium levels by only 30% of the amount resulting from ISO administration. Furthermore, in ferret ventricle it has been shown that CGP12177 causes an increase in the plateau phase of the action potential whilst shortening the overall action potential duration more potently than noradrenaline acting on conventional β1-adrenoceptors (Lowe *et al*, 1998). These effects are insensitive to propranolol but are blocked with moderate potency by bupranolol. This has led to the designation of a new receptor – the β1L-adrenoceptor (low affinity) as distinct from the classical β1-adrenoceptor now called the β1H adrenoceptor (high affinity).

Aim: In this study we have used CGP12177 in quiescent rat atrial cells to investigate the effect of this pro-arrhythmic agent on intracellular calcium release using laser scanning confocal microscopy.

Methods: Atrial cells were isolated from male WKY rats (200-250g) by a method developed in our laboratories (Freestone *et al*, 2000) and were loaded with the calcium fluorescent dye, Fluo 4-AM (5μM). Images of calcium events within quiescent cells were obtained in whole cell and line scanning mode of the Zeiss LSM510 Meta confocal microscope. Cells were perfused with drugs in physiological buffer as previously described (Aptel *et al*, 2002). Images were obtained approximately every 3ms. Cells were perfused with propranolol (200nM) alone, ISO alone (100nM) and CGP12177 (1μM) in the presence of propranolol (200nM) and the frequency of calcium events recorded.

Results: When perfused with propranolol there were 0.05 ± 0.03 s^{-1} whole cell calcium waves, 0.4 ± 0.1 s^{-1} large but localised calcium release events (wavelets) and 41 ± 6.7 s^{-1} calcium sparks observed (n =12). Administration of CGP12177 in the presence of propranolol (200nM) significantly increased the incidence of wavelets to, for example, 0.86 ± 0.17 s^{-1} at 1μM CGP12177 ($p < 0.005$; n=12). ISO administration resulted in an increase ($p < 0.01$) in spark frequency from 26.7 ± 4.5 s^{-1} to 38.7 ± 7.9 s^{-1} from basal (n = 5) but did not increase the frequency of whole cell calcium waves or wavelets. In those cells which did not exhibit waves or wavelets, CGP12177 increased the incidence ($p < 0.01$) of calcium sparks from 42 ± 5.3 s^{-1} to 62 ± 6.1 s^{-1} (n = 6).

Discussion: As shown previously for mouse ventricular myocytes, (Freestone *et al*, 1999) CGP12177 is associated with more potent arrhythmogenic effects in cardiac cells than ISO. Additionally, as previously shown by Aptel *et al* (2002), for human atrial myocytes, subcellular waves much larger than a calcium spark are sometimes evident in atrial cells.