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DIURNAL VARIATION IN SYMPATHETIC CONTROL OF EXCITATION-CONTRACTION COUPLING: THE ROLE OF ÃŸ3 ADRENOCEPTORS AND NITRIC OXIDE

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We have previously shown a time-of-day variation in the response of systolic $[Ca^{2+}]_i$ to the non-specific ÃŸ-a drenergic (ÃŸ-ADR) agonist isoproterenol (ISO), linked to a variation in Nitric Oxide (NO) signalling [1]. This may reflect stimulation of ÃŸ3-ADRs, which induces a NO-dependent negative inotropic response [2]. As the action potential duration (APD) regulates systolic $[Ca^{2+}]_i$, and a time-of-day variation exists in the cardiac action potential [3], we set out to investigate the effect of β3-ADR stimulation and NO-signalling on the APD and systolic $[Ca^{2+}]_i$.

Ventricular myocytes were isolated by enzymatic digestion at time points corresponding to 3 hours into the male Wistar rats rest (ZT3) and active-period (ZT15). Measurement of systolic $[Ca^{2+}]_i$ was made in myocytes loaded with Fura-2 and APD using the whole-cell patch clamp technique.

A significant time-of-day variation was found in systolic $[Ca^{2+}]_i$ following stimulation with ISO (10nM), a non-specific ÃŸ-ADR agonist, which was higher in ZT3 (1040.0+116.9nM) compared to ZT15 myocytes (428.0+63.1nM) (n=3-5, S.E.M., 2-way ANOVA, $P<0.001$). The difference in systolic $[Ca^{2+}]_i$ during ISO stimulation was abolished following inhibition of NOS with L-NNA (500 μM) (2-way ANOVA, $P<0.001$). To determine whether this time-of-day variation in response to ISO can be explained by a variation in AP configuration in response to ÃŸ-ADR stimulation, APD at 30% (APD_{30}) and 50% (APD_{50}) were recorded. ISO stimulation increased APD_{30} and APD_{50} significantly more in ZT15 than ZT3 myocytes, with % increase in APD_{30} of 120.3+14.9% in ZT15 compared to 10.6+8.2% in ZT3 myocytes (n=3, S.E.M., students t-test, $P<0.001$), and APD_{50} of 95.9+13.2% in ZT15 compared to 11.6 +7.4% in ZT3 myocytes (n=3, S.EM., students t-test, $P<0.001$). We also investigated systolic $[Ca^{2+}]_i$ and APD in ZT3 and ZT15 myocytes in response to the specific ÃŸ3-ADR agonist BRL37344 (200nM), to determine if time-of-day variation in systolic $[Ca^{2+}]_i$ following ISO-stimulation could be explained by variation in ÃŸ3-ADR signalling. A significant

reduction in systolic $[Ca^{2+}]_i$ in ZT3 myocytes was found following BRL37344 stimulation, from 458.5+41.2nM to 361.2 +18.0nM (n=4, 2-way ANOVA, $P<0.001$) but no effect on ZT15 myocytes. BRL37344 also significantly reduced APD_{30}, (18.3+2.2ms to 14.4+1.6ms) (n=5, 2-way ANOVA, $P<0.001$), and APD_{50} (32.9+4.3ms to 26.5+3.1ms)(n=5, 2-way ANOVA, $P<0.001$) in ZT3 myocytes with no significant change in ZT15 myocytes.

Our data shows a reduction in systolic $[Ca^{2+}]_i$ in rest-period myocytes (ZT3) in response to ÃŸ3-ADR stimulation, which may reflect the reduction in APD. This suggests that the reduced response of systolic $[Ca^{2+}]_i$ to ISO-stimulation in active-period myocytes is not due to a strong negative inotropic action of ÃŸ3-ADR activation during the active period.