P057

Pharmacological evidence for the existence of four separate β-adrenoceptors in chick embryo ventricular cardiac myocytes

NS Freestone, C Read, H Al-Hakem, KP Patel, CLS Sam. *Kingston University, Pharmacy, UK*

Introduction: There is conflicting evidence concerning the existence of various β-adrenoceptor sub-types in cardiac tissue. The presence of a low affinity version of the β1-adrenoceptor has been shown in mammalian heart cells (Freestone *et al*, 1999). This third stimulatory cardiac β-adrenoceptor has been confused with the inhibitory β3-adrenoceptor in cardiac tissue. Work is continuing to separate out the contributions of these different β-adrenoceptor sub-types to cardiac function. The aim of the present study was to pharmacologically isolate the contributions of the four cardiac β-adrenoceptors to the physiology of cardiac cells.

Method: Spontaneously beating cultures of ventricular myocytes were obtained as previously described from seven day old chick embryos (Rabkin, Freestone and Quamme, 1994). Specific combinations of β-agonists and antagonists were used to stimulate each of the four β-adrenoceptor sub-types individually and the beating rate responses of the cells monitored by video microscopy.

Results: The addition of 100nM isoprenaline (β1/β2 agonist) led to an increase in the mean spontaneous contraction rate from 56 ± 2.81 bpm to 73 ± 4.09 bpm (n=16, p = 0.0002). The subsequent introduction of 200nM propranolol (β1/β2 antagonist) reduced the mean spontaneous contraction rate by ≈34% (from 73 ± 4.09 bpm to 48 ± 3.82 bpm, n=16, p = 0.0003). A further reduction in the mean spontaneous contraction rate was observed with the sequential addition of a β3 agonist, 600nM BRL37344, (from 48 ± 3.82 bpm to 40 ± 1.84 bpm, n=16, p = 0.0108). Conversely, the addition of 1μM CGP12177 ($\beta1_L$-adrenoceptor agonist) resulted in an increase in the mean spontaneous contraction rate; however, the increase was 25% lower than the increase observed with the addition of 100nM isoprenaline. The addition of 1μM CGP12177 increased the mean spontaneous contraction rate by ≈27% (from 40 ± 1.84 bpm to 55 ± 4.30 bpm, n=16, p = 0.0021). BRL37344 (600 nm) when added alone reduced the contraction rate from 92.5 ± 12 bpm to 79.3 ± 13 bpm (p = 0.007; n=12).

Blockade of β2-adrenoceptors with ICI118551 revealed stimulation ($p < 0.0001$) of the contraction rate by noradrenaline (from 80 ± 0.55 bpm to 125 ± 0.16 bpm; n= 12) working through high affinity β1-adrenoceptors ($\beta1_H$). Blockade of β1-adrenoceptors with CGP20712 revealed stimulation ($p < 0.0001$) of the contraction rate by adrenaline (from 75 ± 0.53 bpm to 91 ± 0.07 bpm; n= 12) working through β2-adrenoceptors.

Discussion: This preliminary pharmacological evidence suggests that the combined β1/β2 agonist, ISO and $\beta1_{(H)}$, β2 and $\beta1_{(L)}$ agonists (adrenaline, noradrenaline and CGP12177 respectively) mediate positive chronotropic responses whilst agonists binding to the β3 adrenoceptor (eg. BRL37344) mediate negative chronotropic responses in chick embryo ventricular myocytes. Thus the presence of four distinct β-adrenoceptor populations mediating different contractile effects has been identified and characterised in one cardiac cell preparation for the first time.