ATP modulates intracellular Ca^{2+} and firing rate through a $P2Y_1$ purinoceptor in cane toad pacemaker cells

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Recent studies on cardiac pacemaker cells have demonstrated that interventions which affect intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) also influence firing rate (Zhou & Lipsius, 1993; Ju & Allen, 1998; Bogdanov *et al.*, 2001). To investigate the involvement of $[Ca^{2+}]_i$ in modulation of heart rate by ATP, we examined the effect of extracellular ATP (10-100 μM) on $[Ca^{2+}]_i$ and spontaneous firing rate in single pacemaker cells isolated from the sinus venosus of cane toads*. In spontaneously firing cells, ATP initially increased peak $[Ca^{2+}]_i$, diastolic $[Ca^{2+}]_i$, and the firing rate. These early effects were followed by a late phase in which the peak $[Ca^{2+}]_i$, diastolic $[Ca^{2+}]_i$ and the firing rate all declined. Previous studies suggested that positive phase was mediated by P2 purinoceptors, activated by ATP, while the negative phase was mediated by P1 purinoceptors, activated by adenosine (Burnstock & Meghji, 1981). To define the subtype of purinoceptors involved we used αβ-methylene ATP, adenosine, and UTP (respectively $P2X_{1,3}$, P1 and $P2Y_{2,4,6}$ selective agonists). However, we found that these agonists caused no significant change in $[Ca^{2+}]_i$ and had little or no effect on firing rate. In contrast the $P2Y_1$ selective agonist 2-MesADP (1 μM) mimicked the biphasic effects of ATP and these effects were inhibited by the non-selective purinoceptor antagonist suramin and by the $P2Y_1$ selective antagonist MSR 2179.

Immunohistochemistry using an anti-$P2Y_1$ antibody demonstrated that $P2Y_1$ receptors were present on the cell surface. To establish the specificity of the antibody we performed Western blotting analysis on the protein extracts from toad tissues including sinus venosus and aorta as well as rat aorta as positive control. The immunoreaction with the $P2Y_1$ antibody resulted in a major band of apparent molecular weight of approximately 57 kDa in all three samples. Thus the $P2Y_1$ antibody recognized a similar molecular weight protein in both amphibian and mammalian tissues as reported by others (Moore *et al.*, 2000).

To investigate the nature of the biphasic response we studied the effect of ATP on Ca^{2+} store content. We found that the effects of ATP were related to the sarcoplasmic reticulum (SR) Ca^{2+} store. After depletion of the SR Ca^{2+} store with caffeine or ryanodine, ATP no longer had any effect on $[Ca^{2+}]_i$ or firing rate. Furthermore, the SR Ca^{2+} store content was decreased during the late phase of 2-MesADP application. The effect of ATP was coupled to phospholipase C (PLC) activity because the PLC inhibitor U-73122 eliminated the effect of ATP.

Our study shows that in toad pacemaker cells, the biphasic effects of ATP on pacemaker activity are mainly through $P2Y_1$ purinoceptors, which are able to modulate Ca^{2+} release from the SR Ca^{2+} store. We propose that inositol 1,4,5-triphosphate generated by PLC facilitates SR Ca^{2+} release causing the early increase in peak $[Ca^{2+}]_i$. The increased Ca^{2+} release partially depletes the SR Ca^{2+} store accounting for the subsequent decline in peak $[Ca^{2+}]_i$.

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