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EDITORIAL

Protein kinase A-independent responses to β-adrenoceptor agonists

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Since the discovery of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) and the elucidation of its role as an intracellular 'second messenger', understanding of the function of this molecule has progressed to a stage where the cyclic AMP pathway is frequently cited as the archetype of signal transduction mechanisms. The action of agonists at G_s-coupled receptors – such as β-adrenoceptors and VPAC receptors for vasoactive intestinal peptide (VIP) and pituitary adenylyl cyclase-activating peptide (PACAP) – can be understood as a simple cascade involving activation of adenylyl cyclase, elevation of cytoplasmic cyclic AMP levels and activation of cyclic AMP-dependent protein kinase (PKA), leading to phosphorylation of target proteins (Foreman, 1996).

This simple schema has, however, required some rethinking in recent years as a result of new discoveries. Mammalian adenylyl cyclase has been shown to exist as five subfamilies, accounting for at least eight isoenzymes. These isoenzymes differ in such characteristics as their sensitization by βγ G-protein subunits (Thomas & Hoffman, 1996) and P-site mediated inhibition by adenine nucleoside derivatives (Johnson *et al*., 1997), and also contribute differentially to changes in tissue responsiveness to G_s-coupled receptor agonists in disease (Guenifi *et al*., 2000), under inflammatory conditions (Billington *et al*., 1999) or following prolonged receptor activation (Eckhardt *et al*., 2000). Such heterogeneity allows for radically varying patterns of cyclic AMP response to receptor agonists in different cells and even in the same cell at different stages of development or under different physiological or pathological conditions.

PKA also exhibits heterogeneity. PKA holoenzymes are formed from two catalytic (C) subunits and two regulatory (R) subunits, the R subunits being liberated upon binding of two molecules each of cyclic AMP. Free C subunits are catalytically active and responsible for cyclic AMP-dependent phosphorylation events. To date, three C subunits (Cα, Cβ and Cγ) and four R subunits (RIα, RIβ, RIIα and RIIβ) have been identified, with PKAI and PKAII holoenzymes being formed from assembly of RI or RII subunits, respectively, with C subunits that exhibit restricted tissue distribution (Taskén *et al*., 1995). RI and RII subunits are also expressed differentially by tissues: for example, the PKAII holoenzyme accounts for >90% of the PKA activity in guinea-pig lung (Giembycz & Diamond, 1990). Selective engagement of PKAI by epidermal growth factor receptor complexes has been demonstrated (Tortora *et al*., 1997), suggesting the possibility that cross-talk between intracellular signalling pathways might be specific to individual isoforms of the signalling enzymes.

The activation of PKA in smooth muscle cells by elevated cytoplasmic cyclic AMP results in relaxation as a result of phosphorylation of effector proteins including myosin light chain kinase and ion channels that regulate cytosolic Ca^{2+} concentrations (Anderson, 1995); actions of cyclic AMP in many other cells have been explained by the rapid PKA-dependent phosphorylation of effector proteins (Dent & Giembycz, 1995). However, two alternative mechanisms of action of cyclic AMP have been demonstrated within the scope of cyclic nucleotide-regulated protein kinase activity. First, cyclic AMP may mimic the related purine nucleoside, guanosine 3′:5′-cyclic monophosphate (cyclic GMP) to activate cyclic GMP-dependent protein kinase (PKG), and this has been suggested to be an important pathway for actions of cyclic AMP in smooth muscle cells (Jiang *et al*., 1992; Torphy *et al*., 1982; White *et al*., 2000). Secondly, PKA phosphorylates the Ser^{133} residue of a cytoplasmic protein – cyclic AMP responsive element binding protein (CREB) – leading to nuclear binding of activated CREB to cyclic AMP-responsive elements (CRE) in the promoter regions of cyclic AMP-responsive genes and changing the expression of gene products (Shaywitz & Greenberg, 1999). In this way, changes to the phenotype of cells can be induced by cyclic AMP, in addition to the more acute responses mediated by PKA.

In this issue of the journal, Lucia Spicuzza and colleagues report the inhibition of acetylcholine (ACh)-induced contraction of guinea-pig tracheal smooth muscle by a β-adrenoceptor agonist, isoprenaline, through a mechanism independent of PKA (Spicuzza *et al*., 2001). While other agents associated with intracellular cyclic AMP or cyclic GMP elevation – VIP, a phosphodiesterase inhibitor, a non-selective PKA activator or a PKG activator – displayed inhibition of ACh spasmogenesis that was blocked by treatment of tissues with a PKAII inhibitor, the inhibition due to isoprenaline was unaffected by inhibitors of PKAI, PKAII or PKG. This finding supports previous demonstrations of PKA-independent actions of β-adrenoceptor agonists in other smooth muscle systems that contrast with the PKA-dependent actions of β-agonists in cardiac muscle and brain (Spicuzza *et al*., 2001).

The mechanism by which isoprenaline acts in these smooth muscle systems remains to be defined. Cyclic AMP-independent actions of $G_{s\alpha}$ have been described in airway smooth muscle, including the direct activation of the high conductance Ca^{2+}-sensitive K^+ channel (BK_{Ca}) (Kume *et al*., 1994), but these occur in parallel with cyclic AMP-dependent events and would be expected to be observed for all G_s-

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coupled receptors, including VPAC receptors as well as β-adrenoceptors. Elsewhere, cyclic AMP has been shown to induce thyroid cell proliferation through a combination of PKA-dependent activation of mammalian target of rapamycin (mTOR)/70-kDa ribosomal protein S6 kinase ($p70^{S6k}$) and PKA-independent activation of phosphatidylinositol 3-kinase (PI-3K)/Rac1 protein kinase (PKB/Akt) (Cass *et al*., 1999). These PKA-dependent and independent actions occur in parallel and would be expected to be observed for all agents acting through cyclic AMP elevation. The existence of a PKA-independent mechanism mediating *exclusively* the action of a β-adrenoceptor agonist in airway smooth muscle is an extraordinary finding and one that demands further investigation.

As described by Spicuzza *et al*., attempts to confirm the finding by the use of alternative inhibitors of PKA enzymes were unsuccessful (Spicuzza *et al*., 2001). The inhibitory cyclic nucleotide analogues used in this study are all competitive inhibitors at the cyclic AMP-binding site of PKA R subunits and their effectiveness would be reduced in the presence of very high intracellular cyclic AMP concentrations that might result from β-adrenoceptor activation by isoprenaline. For this reason, the demonstration that an inhibitor acting at the ATP-binding site of the C subunit also failed to suppress the actions of isoprenaline would be very valuable in supporting the thesis that these actions are independent of PKA. Currently available drugs have proved unsuitable for these experiments in β-agonist-treated airway smooth muscle cells and alternative approaches, including the transfection of endogenous inhibitors of PKA, will need to be used (Spicuzza *et al*., 2001). In the event that a purely PKA-independent anti-spasmogenic action of isoprenaline is confirmed, a rigorous effort to define its mechanism will, no doubt, follow.

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