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COMMENTARY

The PI-PLC inhibitor U-73122 is a potent inhibitor of the SERCA pump in smooth muscle

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In this issue MacMillan and McCarron in 2010 demonstrated that the phospholipase C (PLC) inhibitor U-73122 can potently inhibit Ca^{2+} release from isolated smooth muscle cells independent of its effect on PLC. Their data suggest that the PLC inhibitor can block the sarcoplasmic/endoplasmic reticulum calcium ATPase pump in smooth muscle and cast doubt on the reliability of U-73122 as the main pharmacological tool to assess the role of the phosphotidyl inositol-PLC pathway in cellular signalling.
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Abbreviations: IP_3, inositol trisphosphate; PLC, phospholipase C; PI-PLC, phosphotidyl inositol-phospholipase C; SERCA, sarcoplasmic/endoplasmic reticulum calcium ATPase

The initial discovery of the N-aminosteroid homologue of N-ethylmaleimide, U-73122, as an inhibitor of phospholipase C (PLC) by Bleasdale *et al*. (1990) and Smith *et al*. (1990), provided a tool to help assess the contribution of PLC to cellular signalling pathways in a variety of cell types. The data presented in their early papers demonstrated that U-73122 could reduce thrombin-induced inositol trisphosphate (IP_3) production in platelets and polymorphonuclear neutrophils. This inhibitory effect of U-73122 appeared to be dependent on the presence of a pyrroledione group, as replacement of this with pyrrolidinedione (to form U-73343) abolished the inhibitory effects of the molecule on IP_3 synthesis and Ca^{2+} release.

In the 20 years since the discovery of this molecule, approximately 2000 papers have been published, where the effects of U-73122 have been attributed to its inhibitory effects on PLC in a variety of cell types including smooth muscle (Ellershaw *et al*., 2002), interstitial cells of Cajal (Kim *et al*., 2003; Johnston *et al*., 2005) and pancreatic acinar cells (Yule and Williams, 1992). In the current issue, MacMillan and McCarron (2010) suggest that U-73122 interferes with Ca^{2+} handling in smooth muscle independent of an effect on PLC. Their data call into question the reliability of this molecule when used alone as a tool to investigate the contribution of PLC to cellular signalling.

In their study MacMillan and McCarron (2010) attempted to establish the contribution of IP_3 receptors to the propagation of agonist-evoked Ca^{2+} waves in guinea-pig, freshly dispersed colonic smooth muscle cells. They found that U-73122 abolished the excitatory effects of exogenous carbachol, consistent with the idea that IP_3 synthesis was inhibited via blockade of PLC. To test that these effects were attributable to an effect on PLC, the authors examined the effects of U-71322 on Ca^{2+} transients, which do not involve PLC activation, by either photo releasing caged IP_3 or by evoking Ca^{2+} release from ryanodine receptors using caffeine. Surprisingly, U-73122 abolished Ca^{2+} oscillations induced by both protocols, strongly suggesting that U-71322 can inhibit Ca^{2+} oscillations by a mechanism that does not involve PLC. The authors noted that the effects of U-71322 (such as reduced amplitude and rate of decay of the caffeine or IP_3-evoked Ca^{2+} transients, as well as elevated basal Ca^{2+} levels) were remarkably similar to the effect of the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) pump inhibitor CPA. Therefore, this study suggests that U-73122 (but not its 'inactive' analogue, U-73343) is a potent inhibitor of the SERCA pump in smooth muscle.

These observations may help to explain one of the discrepancies in the original study (Bleasdale *et al*., 1990), which showed that U-71322 was able to inhibit agonist responses at concentrations lower than that needed to inhibit IP_3

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production. For example, Ca^{2+} transients evoked by the thromboxane receptor agonist U-46619 were abolished by 2 μM U-73122 whereas IP_3 production was only inhibited by 50% in the presence of 10 μM U-73122.

Several other studies have suggested that U-73122 has effects unrelated to the inhibition of PLC including the depletion of intracellular stores in PC12 cells (Clementi *et al*., 1992), potentiation of IP_3-mediated Ca^{2+} release and direct stimulation of cation channels in excised patches from murine pancreatic acinar cells (Mogami *et al*., 1997). Taken together, the results of MacMillan and McCarron (2010) suggest that great care should be taken in the interpretation of experiments that use U-73122 as the primary pharmacological tool to assess the contribution of the phosphotidyl inositol-PLC pathway in cells.

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