(P16) Novel Blockers for Store-Operated Ca^{2+} Entry Channels: 2-Aminoethoxydiphenyl Borate (2-APB) and the Analogue Compounds

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Capacitative Ca^{2+} entry (CCE) is the mechanism to replenish the intracellular Ca^{2+} stores and essential to the intracellular Ca^{2+} signaling. CCE is mediated by Ca^{2+}-permeable channels in the plasma membrane that are generally referred to as "store-operated channels (SOCs)". We reported 2-aminoethoxydiphenyl borate (2-APB) is a membrane-permeable blocker for the IP3 receptors (J. Biochem. 122 498-505, 1997) and to inhibit CCE (Science 287 1647-1651, 2000), suggesting an essential role for IP_3 receptors in the induction of CCE. On the other hand, it was shown that CCE could be induced independently of the presence of IP3Rs, raising the possibility that 2-APB directly blocks SOC. In the present study, we first tested this possibility using the cell line deficient in IP3Rs and found that 2-APB effectively blocked CCE. Inhibitory effect of 2-APB for CCE was stronger than that for IP_3-induced Ca^{2+} release (IICR), with IC50 values for CCE and IICR being 5 and 20 μM, respectively (Receptors _ Channels 7 429-439, 2001). The above results show that 2APB has an inhibitory effect on SOC in addition to IP3R; however, more selective SOC blockers are desirable for the elucidation of physiological roles of SOC. To find such SOC-selective blockers, we synthesized 166 2-APB analogues using 2-APB as the leading compound and screened them in IP3R-deficient cells and CHO cells. As the result of the screening, we could find some 2-APB analogues that were highly inhibitory to SOC but were ineffective in inhibiting IICR. IC50 values of these compounds for CCE inhibition were 100-1000-fold lower than those for IICR inhibition. Using these compounds, it was demonstrated that CCE is necessary for the sustained Ca^{2+} oscillation induced by ATP in CHO cells.
In conclusion, in addition to the finding that 2-APB has dual inhibitory effects for CCE and IICR, we have discovered novel 2-APB analogues that selectively block SOC. These compounds should be a valuable tool to elucidate physiological roles of SOC.