

indicative of physiological control. But the SV channel is also strongly inwardly-rectifying, and effects of Ca^{2+} have been observed only at negative membrane potential (V_m). Because, in the steady state, tonoplast V_m is sustained positive by the electrogenic H^+ pumps^{3,4}, the physiological role of Ca^{2+} in activating the SV channel has, hitherto, been doubtful.

The equilibrium potential for Ca^{2+} across the tonoplast is greater than -200 mV^{5,6}. Opening of Ca^{2+} channels during a transient rise in $InsP_3$ (generated, for example, by hormones⁷, light⁸ or elicitors) can therefore be expected to induce a negative swing of V_m . Because cytosolic free Ca^{2+} will rise simultaneously, the net effect will be a massive increase in SV channel activity.

These considerations clearly indicate a central role for the SV channel in regulating solute fluxes across the tonoplast of cells whose volume habitually changes significantly. In the specific case of stomata, where guard-cell volume reduction is known to be preceded by a rise in

cytosolic free Ca^{2+} (ref. 9), coordinated release of ions can be achieved both at the tonoplast and (via Ca^{2+} gating of other ion channels¹⁰) at the plasma membrane.

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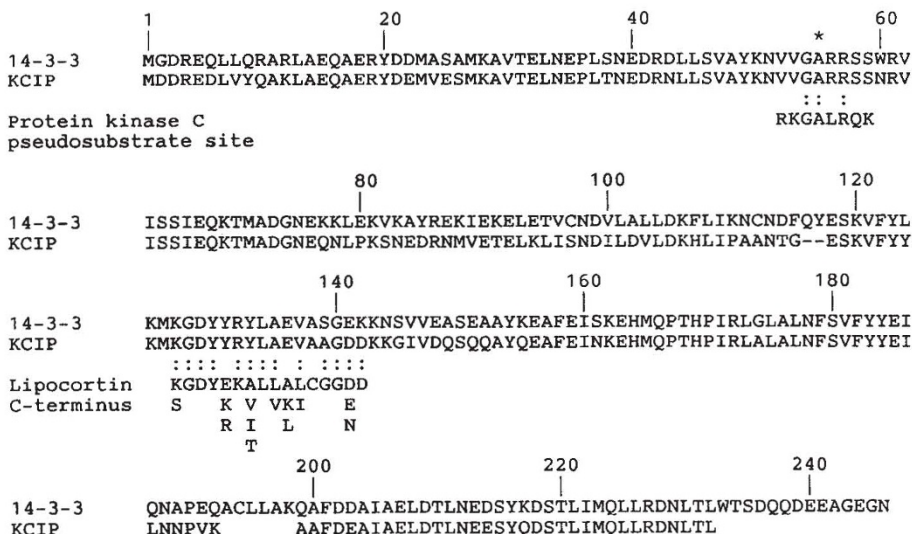
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Kinase and neurotransmitters

SIR—We have recently isolated¹ acidic proteins of relative molecular mass 29,000–33,000 from sheep brain that are potent inhibitors of Ca^{2+} -phospholipid-dependent protein kinase C. A search of the EMBL database reveals that peptides representing more than 90 per cent of the kinase C inhibitor isoforms are very similar to bovine brain 14-3-3 protein² (see figure). No physiological function had been attributed to this protein

until Yamauchi *et al.*³ showed that it is identical to an activator protein of tyrosine and tryptophan hydroxylases, the rate-limiting enzymes involved in catecholamine and serotonin biosynthesis respectively, processes essential for the synthesis of dopamine and other neurotransmitters.

Examination of the amino-acid sequences in the figure suggests a possible mechanism for kinase C inhibition.



Alignment of bovine brain 14-3-3 (η chain²) with peptides from kinase C inhibitor protein (KCIP) in single-letter code. Three main isoforms of the kinase inhibitor have been partially sequenced and although only one set of representative peptides is shown, the overall identity is approximately the same, 75 per cent. The additional alignment at positions 52–59 is the pseudosubstrate domain of α , β and γ forms of protein kinase C^{4,5}. The 'pseudosubstrate' alanine residue is marked with an asterisk. Residues occurring in at least two out of ten lipocortins⁷ are also indicated. A good alignment could not be obtained for some kinase C inhibitor peptides with distinctly different sequence, in the region between residues 191 and the C terminus of 14-3-3.

Residues 54–57 in the 14-3-3 protein isoform and all the kinase C inhibitor forms we have sequenced are reminiscent of part of the 'pseudosubstrate' domain of protein kinase C^{4,5}. The pseudosubstrate hypothesis has been proposed to account for the inhibitory sequences in the regulatory domains of a wide range of second messenger-dependent protein kinases^{4,5}. These sequences contain all the elements necessary for a recognition site for the particular kinase but the phosphorylatable serine or threonine is replaced by another residue (most commonly alanine). Competitive inhibition by this domain is relieved by the conformational change induced by binding of the second messenger. Note that the serines at positions 58 and 59 are potential substrate sites for kinase C whereas cyclic AMP-dependent and Ca^{2+} -calmodulin kinase II could phosphorylate Ser 59.

Although the kinase inhibitor and 14-3-3 are not calcium-binding proteins^{1,6} and there is no evidence of similarity with any putative calcium binding domains, one region (residues 127–142) is very similar to the conserved carboxy terminus of the family of calcium- and lipid-binding proteins, variously called lipocortins, endonexins and calpactins⁷.

The proteins 14-3-3 and kinase C inhibitor exist as at least seven distinct gene products, but it is not known whether different isoforms are responsible for the physiological roles of this novel multigene family.

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Scientific Correspondence

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