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FLUORESCENT PHORBOL ESTER BINDING TO THE RECEPTOR PROTEIN KINASE C IN-VITRO AND IN-VIVO

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Fluorescent phorbol esters from the fruits of the plant Sapium indicum have been isolated,characterised and were collectively called the Sapintoxins. Only Sapintoxins A,B and D were found to be pro-inflammatory causing erythema of mouse ear and aggregation of platelets(Edwards et al 1983). These are the only naturallyocourring fluorescent phorbol esters known to date, although semi-synthetic esters have been produced (Tran et al 1984).
The study of phorbol ester binding has been limited to the use of radiolabelled esters, usually at the C-20 hydroxyl group. This group is prone to exchange and in vivo labelling of the tissues has required the use of sectioning followed by autoradiography for long periods of time(Worley et al). The identification of the phorbol ester receptor as protein kinase C has led to the use of kinase activity to indirectly study phorbol ester binding (Castagna et al). This receptor protein has been used in our laboratories to screen a number of phorbol and daphnane esters (Ellis et al) including these fluorescent esters(Figure 1). In vivo binding of these phorbol esters was studied by using fluoresence microscopy with the fibroblast cell line Swiss 3T3 and the pituitary cell line GH_3. In common with the in vitro data, fluorescence was observed at the cell membranes and some in the cytosol,but no nuclear fluorescence. The labelling of the cells by the inactive phorbol esters Sapintoxin C and α-Sapinine was increased in the presence of TPA. This effect may be due to a change in membrane fluidity caused by using 1μM TPA. At the same concentration however,there was no change in fluorescence of the cells labelled with the more active esters. This is a similar response to that found by Tran et al, when cells were labelled with the semi-synthetic ester, Dansyl-TPA.
Figure 1 tables the affinity of these fluorescent phorbol esters for protein kinase C by listing the Ka for activation of the kinase,as well as the Ki for phorbol dibutyrate binding. This data compares well with the erythema and platelet aggregation data of Edwards et al, showing that structural differences of the phorbol esters alter biological activity. It would appear that this trend is also followed in cell binding of these phorbol esters, with the esters most active in the in vitro tests causing the highest cellular labelling. These fluorescent esters are a useful probe to the action of phorbol esters in vivo and could offer an alternative to the traditional radiochemical assays for receptor binding.

FIGURE 1 Biological activity of Sapintoxins; (* induce erythema)

Compound	Ka PKC activation	Ki PDB binding	GH_3/ 3T3 binding
Sap A *	76 nM	22 nM	+++
Sap B *	15600 nM	750 nM	++
Sap C	>100 μM	>10 μM	+
Sap D *	28 nM	1.5nM	+++
α-Sap	>100 μM	>10 μM	+

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