

From: THE METABOLISM, STRUCTURE, AND FUNCTION  
OF PLANT LIPIDS  
Edited by Paul K. Stumpf, J. Brian Mudd,  
and W. David Nes  
(Plenum Publishing Corporation, 1987)

#### LIPID METABOLISM IN POTATO LEAF DISKS:

#### EFFECT OF CALMODULIN ANTAGONISTS

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#### INTRODUCTION

Dibucaine (nupercaine), a local anesthetic, was recently shown to alter the lipid composition of barley-root membranes (1). When excised roots were placed in dibucaine there was an increase in the proportions of palmitic, stearic, and oleic acids and a decrease in the proportions of linoleic and linolenic acids. We have recently shown that the rate of autolytic degradation of phospholipids in potato leaf homogenates is inhibited by calmodulin antagonists (which include dibucaine) and stimulated by calmodulin (2). This study was undertaken in order to investigate the effect of dibucaine and other calmodulin antagonists on the polar lipid composition of potato leaves.

#### MATERIALS AND METHODS

Potato (Solanum tuberosum c.v. Kennebec) plants were grown in a greenhouse for 30-40 days. Twelve mature leaves (4-6 cm) were removed and 8 disks (9 mm in diameter) were cut from each leaf with a brass cork borer (#6). One disk from each leaf was floated (top-side up) in a petri dish containing 20 ml of various test solutions (pH 6.0). The petri dishes were placed in a vacuum chamber (640 mm Hg) for 2 minutes and then removed and incubated in the dark at 25°C. After the desired times disks were removed, placed in 1 ml of hot isopropanol and heated to 70°C for 10 min to inactivate the lipolytic enzymes. The lipids were extracted and separated by thin layer chromatography as previously described (1). Phosphatidylcholine and digalactosyl diacylglycerol were identified by comparison with known standards, removed from the TLC plates and analyzed quantitatively (3,4). Trifluoperazine sulfoxide was a generous gift from Smith, Kline, and French Laboratories. All other reagents were obtained from Sigma Chemical Co.

#### RESULTS

In the first experiment (Table 1) leaf disks were incubated in various test solutions for 18 hours. The rates of hydrolysis of phosphatidylcholine (PC) and digalactosyl diacylglycerol (DGDG) in dark controls were very low (about 4%). Each of the eight treatments increased the hydrolytic rates, however some caused much more pronounced increases. For each treatment the

Table 1. Hydrolysis of phosphatidylcholine (PC) and digalactosyl diacylglycerol (DGDG) in potato leaf disks which were floated in various test solutions for 18 hours. Number in parentheses is the relative % hydrolysis with the control set equal to zero.

Treatment	% hydrolyzed per 18 hours <sup>a</sup>	
	PC	DGDG
Control (H <sub>2</sub> O)	3.9 (0) <sup>b</sup>	4.2 (0) <sup>c</sup>
2 mM dibucaine	75.6 (71.7)	62.9 (58.7)
50 μM chlorpromazine	32.4 (28.5)	30.5 (26.3)
50 μM w <sub>7</sub>	21.5 (17.6)	33.4 (29.2)
50 μM trifluoperazine	43.7 (39.8)	32.8 (28.6)
50 μM trifluoperazine sulfoxide	7.1 (3.2)	11.5 (7.3)
10 mM CaCl <sub>2</sub>	11.7 (7.8)	10.5 (6.3)
50 μM calcium ionophore, A 23187	10.9 (7.0)	15.3 (11.1)
1 mM indomethacin	42.2 (38.3)	40.7 (36.5)

<sup>a</sup>The values reported are the means of three separate experiments

<sup>b</sup>The level of PC at 18 hours was 229 nmol/12 disks

<sup>c</sup>The level of DGDG at 18 hours was 288 nmol/12 disks

rates of hydrolysis of PC and DGDG were comparable, which probably indicates that they are both degraded by the same enzyme. The rates of hydrolysis of PC and DGDG were stimulated the most by the dibucaine treatment. The other three calmodulin antagonists (chlorpromazine, w<sub>7</sub>, and trifluoperazine) also caused significant rate increases (21.5-43.7%). The hydrolytic rates were much lower with trifluoperazine sulfoxide than trifluoperazine, which is suggestive of an authentic calmodulin interaction rather than a nonspecific effect of the drugs. The calcium ionophore and CaCl<sub>2</sub> each caused a small increase in the hydrolytic rates. However, indomethacin, a calcium antagonist, caused a large stimulation in the rates of hydrolysis, comparable to those observed with the calmodulin antagonists.

Because dibucaine caused the largest stimulation of hydrolysis in Table 1, a time-course study of its effects on potato leaf disks was conducted (Fig. 1). This study revealed that the rate of hydrolysis of PC was linear from 0 to 6 hours (about 13% of the PC was hydrolyzed in 6 hours) and suggested that the rates of hydrolysis observed in Table 1 were probably also linear for 0 to 18 hours.

#### DISCUSSION

In this study we demonstrate that four calmodulin antagonists and one calcium antagonist stimulate the rate of breakdown of PC and DGDG in potato leaf disks. These results are very different from our *in vitro* studies with potato leaf homogenates (1) where calmodulin antagonists inhibited the rate of autolytic PC breakdown. In another related study fluphenazine (also a calmodulin antagonist) when applied to senescing pea leaves was shown to delay membrane deterioration as measured by several criteria (5). In the study of barley roots (1), dibucaine was shown to increase the proportions of saturated fatty acids. It is conceivable that such effects were caused by the selective stimulation of an acyl hydrolase which is specific for the esters of polyunsaturated fatty acids. Very little is known about the properties of the lipolytic enzymes in pea leaves and barley roots, but it is likely that they are very different than those that we have studied in

potato leaves (2). The different effects caused by the drugs used in these four studies could easily be explained by different sensitivities of the lipolytic enzymes of each type of tissue to the drugs.

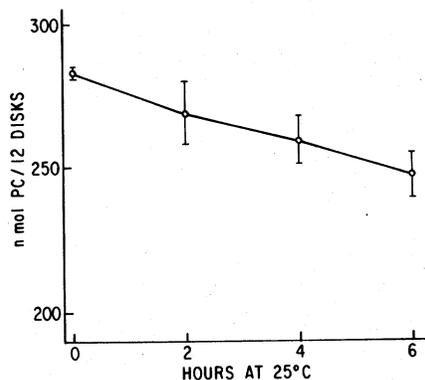


Figure 1. Time-course study of the rate of hydrolysis of phosphatidylcholine (PC) in potato leaf disks floated in 2 mM dibucaine. Data points are the means of three experiments  $\pm$  S.D.

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