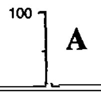
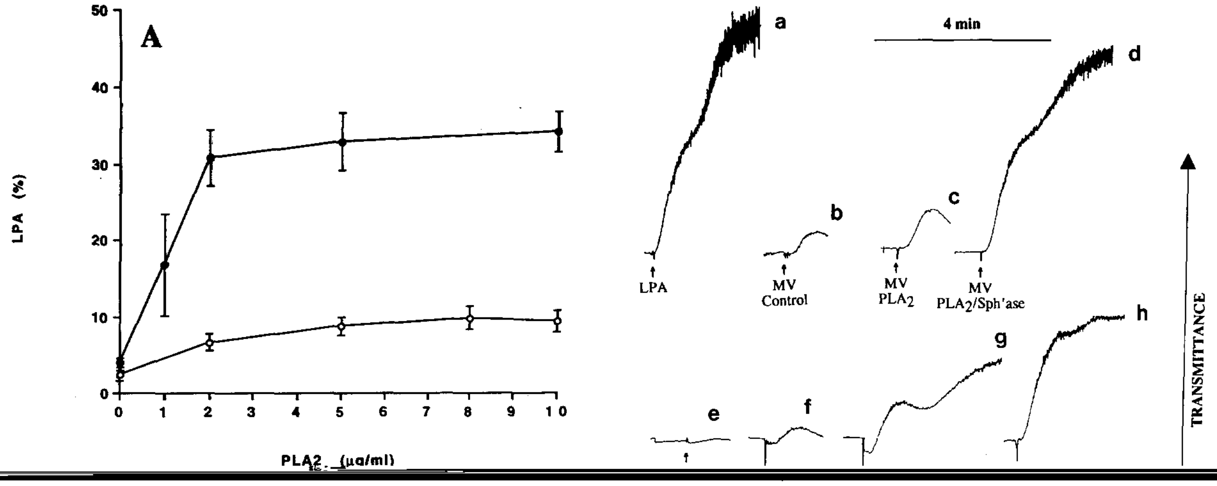


which almost 80% of sphingomyelin (SPH) was hydrolyzed, $sPLA_2$ could degrade 50% of PC, 25% of PE, and 12% of PS present in microvesicles (Figure 2A). Hy-


A
LPA (%)
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
9
10
PLA2 (µg/ml)
a
b
c
d
4 min
LPA
MV
Control
MV
PLA$_2$
MV
PLA$_2$/Sph'ase
e
f
g
h
TRANSMITTANCE

brain SPH, PLA_2 from pig pancreas, sphingomyelinase and α-toxin from S. aureus, lipopolysaccharide from E. coli K235, 1-oleoyl-LPA, and bovine serum albumin, essentially fatty acid free, were obtained from Sigma. $[^{32}P]$o-phosphate was from Amersham. Silica gel 60 plates (0.25 mm thick) were obtained from Merck. N-acetyl-D-sphingosine (C2-ceramide) was synthesized by acetylation of D-sphingosine with acetic anhydride as described (Gaver and Sweeley, 1966).

$MgCl_2$, 5 mM glucose, 10 mM HEPES (pH 7.4). Erythrocytes were suspended in buffer A at 5×10^6 cells/ml. $CaCl_2$ (1 mM, final concentration) was then added, followed by 2 μM A23187, and incubation was performed at 37°C for 45 min under magnetic stirring. Intact cells were eliminated by centrifugation at 1,500 × g for 15 min, and the supernatant was centrifuged at 13,000 × g for 20 min, allowing it to obtain a microvesicle pellet. In preliminary experiments, a relationship was established between the hemoglobin level of the vesicles lysed pensions of human platelets (Simon et al., 1982). Protein was determined according to Bradford (1976) using bovine serum albumin as a standard. Polyacrylamide gel electrophoresis in the presence of so-

sensitive sub-micro phosphorus determination. Anal. Chim. Acta *24*, 203–204.

Bradford, M. M. (1976). A rapid and sensitive method for the quantita-

Kolesnick, R., and Golde, D. W. (1994). The sphingomyelin pathway

in tumor necrosis factor and interleukin-1 signaling. Cell 77, 325–328.

Chow, E. P., Tizard, R., and Pepinsky, R. B. (1989). Structure and

and Sarda, L. (1992). Separation and characterization of the precursor

and activated forms of porcine and human pancreatic colipase by

Schroit, A. J., and Zwaal, R. F. A. (1991). Transbilayer movement of