Volume 316, number 2, 170–174 FEBS 12041 January 1993


# GTPγS and phorbol ester act synergistically to stimulate both $Ca^{2+}$-independent secretion and phospholipase D activity in permeabilized human platelets

## Inhibition by BAPTA and analogues

Jens R. Coorssen and Richard J. Haslam

*Department of Pathology, McMaster University, Hamilton, Ont., L8N 3Z5, Canada*

Received 5 November 12992

We have tested the hypothesis that phospholipase D (PLD) is the effector of the unidentified G protein ($G_E$) mediating $Ca^{2+}$-independent exocytosis in platelets. Although GTPγS, and to a lesser extent phorbol 12-myristate 13-acetate (PMA), caused some secretion of 5-HT from electropermeabilized human platelets in the effective absence of $Ca^{2+}$ (pCa > 9), these stimuli had much more potent synergistic effects when added together. In all cases, secretion of 5-HT was closely correlated to the stimulus-induced formation of [$^3$H]phosphatidic acid ([$^3$H]PA) from [$^3$H]arachidonate-labelled phospholipids. Addition of ethanol inhibited both secretion and [$^3$H]PA formation and led to the accumulation of [$^3$H]phosphatidylethanol ([$^3$H]PEt), indicating that [$^3$H]PA was formed largely by activation of PLD. BAPTA and analogues caused dose-dependent inhibitions of both GTPγS-induced secretion and PLD activity in the permeabilized platelets. This action of BAPTA did not appear to be mediated by chelation of $Ca^{2+}$ or by direct inhibition of protein kinase C (PKC). The results suggest that PLD is the target of $G_E$ in platelets and that BAPTA can block PLD activation.

GTPγS; Phosphatidic acid; Phospholipase D; BAPTA; Secretion; Permeabilized platelet

## 1. INTRODUCTION

A previous study from this laboratory [1] showed that GTPγS can induce the $Ca^{2+}$-independent secretion of both dense and α-granule constituents from electropermeabilized human platelets without causing stimulation of phosphoinositide-specific phospholipase C. This action of GTPγS was dependent on PKC activity [2] and was potentiated by phorbol ester (PMA) [1,2]. Similar studies with other permeabilized cells, particularly neutrophils [3], HL-60 cells [4] and RINm5F cells [5], in which $Ca^{2+}$-independent secretion was observed, and with mast cells in which $Ca^{2+}$ was required [6,7], have led Gomperts and colleagues to propose that an unidentified G protein, designated $G_E$, may mediate GTPγS-induced exocytosis (reviewed in [8]). Since treatment of rabbit platelets with PMA enhances the ability of GTPγS to stimulate PLD in membrane preparations [9], we have suggested that PLD may be the effector of $G_E$ in platelets [1]. In the present study, we have investigated this possibility and have demonstrated a close correlation between the $Ca^{2+}$-independent release of a dense granule constituent (5-HT) and activation of PLD in permeabilized human platelets incubated with GTPγS and/or PMA.

There is evidence that the $Ca^{2+}$-chelator BAPTA [10] can inhibit GTPγS-induced secretion from a number of cell types by a mechanism that may be unrelated to its ability to bind $Ca^{2+}$ ions [11]. Thus in electropermeabilized platelets, the secretion of both dense granule and lysosomal constituents in response to GTPγS was abolished by 20 mM BAPTA but not by 20 mM EGTA, although the equilibrium affinities of these compounds for $Ca^{2+}$ were similar under the conditions of these experiments [12]. In permeabilized neutrophils, much less GTPγS-induced secretion of β-glucuronidase was seen in the presence of BAPTA than of EGTA [3]. Finally, dibromo-BAPTA (4 mM) inhibited GTPγS-induced exocytosis from mast cells, despite the presence of $Ca^{2+}$ [13]. Since these actions of BAPTA have not been adequately explained, we investigated the effects of BAPTA and analogues on both the $Ca^{2+}$-independent secretion of 5-HT from permeabilized platelets and the associated activation of PLD. The results provide further evidence of a role for PLD in secretion.

*Correspondence address:* R.J. Haslam, Department of Pathology, McMaster University, 1200 Main Street West, Hamilton, Ont., L8N 3Z5 Canada. Fax: (1) (416) 577–0198.

*Abbreviations:* PLD, phospholipase D; GTPγS, guanosine 5′-[γ-thio]triphosphate; PMA, phorbol 12-myristate 13-acetate; PA, phosphatidic acid; PEt, phosphatidyl-ethanol; BAPTA, 1,2-bis(*o*-aminophenoxy)ethane-*N,N,N′*,N′-tetraacetic acid; PKC, protein kinase C


*Published by Elsevier Science Publishers B.V*

## 2. EXPERIMENTAL

### 2.1. *Materials*

[5,6,8,9,11,12,14,15-$^3$H]Arachidonic acid (100 Ci/mmol) and [$\gamma$-$^{32}$P]ATP (3000 Ci/mmol) were obtained from Du Pont, Mississauga, ON, Canada, and [*side-chain*-2-$^{14}$C]5-HT (55 mCi/mmol) from Amersham, Oakville, ON, Canada. GTP$\gamma$S was from Boehringer Mannheim, Laval, PQ, Canada, and PMA and BAPTA from Sigma, St. Louis, MO, USA. BAPTA was also obtained from Fluka, Ronkonkoma, NY, USA. 5,5'-Dibromo-BAPTA was from Molecular Probes, Eugene, OR, USA and 5,5'-dinitro-BAPTA was a generous gift from Dr. M.P. Charlton, Department of Physiology, University of Toronto, Toronto, ON, Canada. Other materials were from sources listed previously [1,14].

### 2.2. *Labelling and electropermeabilization of platelets*

Human platelets, isolated as described previously [14], were resuspended at $5 \times 10^9$/ml in citrated plasma, pH 6.5, and incubated at 37°C for 1.5 h with [$^3$H]arachidonate (5 $\mu$Ci/ml). [$^{14}$C]5-HT was added in three equal amounts during the last 30 min (to a final concentration of 0.25 $\mu$Ci/ml). The platelets were then washed in $Ca^{2+}$-free Tyrode's solution containing 5 mM PIPES (pH 6.5) and 0.35% (w/v) bovine serum albumin and, after addition of 5 mM EGTA, were permeabilized by high-voltage electric discharges [14]. The permeabilized platelets were cooled to 4°C, freed from low-$M_r$ solutes by gel filtration [14] and eluted in a medium (pH 7.4) containing 3.9 mM $MgCl_2$ and the potassium salts of glutamic acid (160 mM), HEPES (20 mM), EDTA (2.5 mM) and EGTA (2.5 mM). ATP (final concentration 5 mM) was added to the eluate, which was adjusted to contain $5 \times 10^8$ platelets/ml. This suspension was stored at 0°C until used (30–60 min).

### 2.3. *Incubations*

All samples of permeabilized platelet suspension were first equilibrated for 15 min at 0°C with an additional 10 mM EGTA (to give a pCa > 9 even after release of platelet $Ca^{2+}$ [1]), with sufficient $MgCl_2$ to give 5.0 mM $Mg^{2+}_{free}$ and with any other additions. Concentrations of added $MgCl_2$ were calculated using binding constants given elsewhere [10,15]. After equilibration, samples (final vol. 0.4 ml containing $1.6 \times 10^8$ platelets) were incubated for 10 min at 25°C before measurement of [$^{14}$C]5-HT secretion in 0.05 ml portions [14] and extraction of lipids from the remainder [16]. [$^3$H]PA and [$^3$H]PEt were then isolated by t.l.c. [17] and counted for $^3$H as in [9]. Basal values for extracellular [$^{14}$C]5-HT and for $^3$H-labelled lipids were obtained from permeabilized platelet suspension stored at 0°C and subtracted from values obtained after incubations. These corrections were very small for [$^{14}$C]5-HT and [$^3$H]PEt, but significant [$^3$H]PA was present prior to incubations. To normalize results from different experiments, $^3$H-labelled lipids were expressed as percentages of total platelet $^3$H. In some experiments, PKC activation was measured in small samples of permeabilized platelet suspension (0.1 ml) containing [$\gamma$-$^{32}$P]ATP (16 $\mu$Ci) by determining the phosphorylation of its major substrate, pleckstrin [1,14].

## 3. RESULTS

### 3.1. *Relationship between secretion and [$^3$H]PA formation*

In the absence of additions, incubation of permeabilized platelets at a pCa > 9 did not lead to [$^{14}$C]5-HT secretion, though some [$^3$H]PA was formed (Fig. 1). However, equilibration of the platelets with 100 $\mu$M GTP$\gamma$S, or even with 100 nM PMA, caused significant secretion of [$^{14}$C]5-HT at pCa > 9 and enhanced [$^3$H]PA formation. Moreover, when added together, these stimuli induced a much more marked secretion that was on average 2.2-fold greater than the sum of their individual effects (Fig. 1A). In these experiments, the effect of

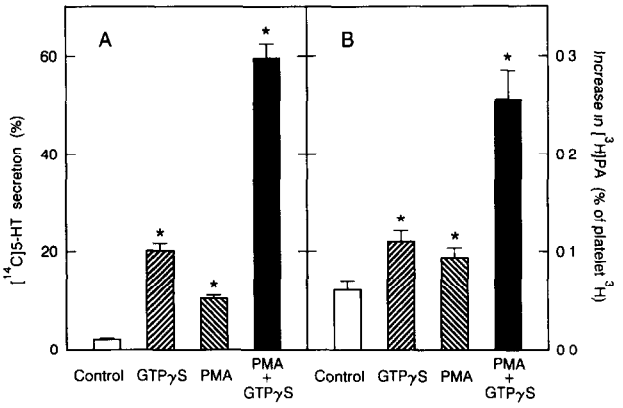


Fig. 1. Effects of GTP$\gamma$S and PMA on $Ca^{2+}$-independent 5-HT secretion and PA formation in permeabilized platelets. Samples of labelled permeabilized platelets were incubated with no stimulus (control), 100 $\mu$M GTP$\gamma$S, 100 nM PMA or 100 $\mu$M GTP$\gamma$S and 100 nM PMA, as indicated. Secretion of [$^{14}$C]5-HT (A) and the increases in [$^3$H]PA (B) were determined. To normalize results from different experiments, [$^3$H]PA was expressed as a percentage of the total $^3$H in the permeabilized platelets; basal [$^3$H]PA (0.071 ± 0.004% of platelet $^3$H) was subtracted. Values shown are means ± S.E. from all experiments ($n$ = 17); the significance of changes was evaluated by two-sided paired $t$-tests (*$P < 0.001$).

PMA alone was weaker and the synergism between GTP$\gamma$S and PMA stronger than in a previous study at pCa > 9 [1] in which the [$Mg^{2+}_{free}$] was 2.7 mM, rather than 5.0 mM. Measurement of [$^3$H]PA accumulation in the same experimental samples (Fig. 1B) showed that the stimulus-induced formation of this phospholipid closely paralleled secretion. In particular, the increases in [$^3$H]PA caused by simultaneous addition of GTP$\gamma$S and PMA amounted to 2.4-fold the sum of the increases they induced individually.

### 3.2. *Role of PLD activity in [$^3$H]PA formation*

Since phosphoinositide-specific phospholipase C activity was not detected in permeabilized platelets incubated at pCa > 9 [1], the role of PLD in [$^3$H]PA formation was evaluated. The decreases in the amounts of [$^3$H]PA formed on addition of ethanol and the associated accumulation of [$^3$H]PEt by transphosphatidylation were used as measures of PLD activity [17]. Addition of 400 mM ethanol markedly inhibited the accumulation of [$^3$H]PA caused by GTP$\gamma$S alone or by GTP$\gamma$S and PMA (Fig. 2A); in three separate experiments, these inhibitions amounted to 64 ± 7% and 74 ± 7%, respectively (mean values ± S.E.). These actions of ethanol were associated with the formation of amounts of [$^3$H]PEt comparable to or larger than the decreases in [$^3$H]PA (Fig. 2B). When present together, GTP$\gamma$S and PMA had markedly supra-additive effects on [$^3$H]PEt formation (Fig. 2B), showing that these stimuli acted synergistically to increase PLD activity. Although ethanol had little effect on [$^3$H]PA accumulation in platelets incubated with PMA alone, [$^3$H]PEt formation was al-

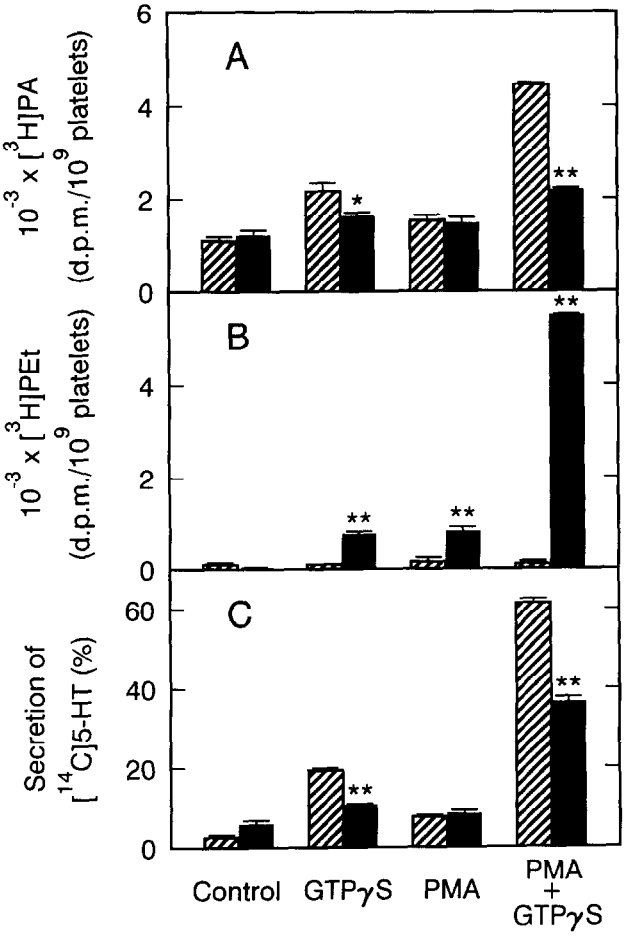


Fig. 2. Effects of ethanol on $Ca^{2+}$-independent PA and PEt formation and on the associated 5-HT secretion. Samples of labelled permeabilized platelets were incubated with no stimulus (control), 100 $\mu$M GTPγS, 100 nM PMA or 100 $\mu$M GTPγS and 100 nM PMA, in each case in the absence (▨ and presence (■) of 400 mM ethanol. The formation of both [$^3$H]PA (A) and [$^3$H]PEt (B) and the secretion of [$^{14}$C]5-HT (C) were determined. Values are means ± S.E. from triplicate incubations in a single experiment; significant effects of ethanol (two-sided unpaired *t*-test) are shown: $*P < 0.05$; $**P < 0.01$

ways observed. However, in control incubations with ethanol, little or no [$^3$H]PEt was detected and ethanol tended to increase rather than decrease [$^3$H]PA accumulation, though this was rarely significant in individual experiments (Fig. 2A). These results indicate that stimulus-induced [$^3$H]PA formation is largely attributable to PLD activity, whereas in control incubations, [$^3$H]PA appears to be formed by other mechanisms.

Ethanol (400 mM) markedly inhibited [$^{14}$C]5-HT secretion induced by GTPγS or by GTPγS and PMA (Fig. 2C); these inhibitions amounted to 74 ± 3% and 47 ± 3%, respectively (means ± S.E.; 3 experiments). However, ethanol had no significant effect on the weak secretion caused by PMA alone (Fig. 2C). This observation may, in part, be explained by a small increase in [$^{14}$C]5-HT secretion caused by ethanol in control samples. As a whole, these results demonstrate a correlation between the effects of ethanol on the accumulation of [$^3$H]PA attributable to PLD activation and its effect on [$^{14}$C]5-HT secretion. The residual secretion observed in the presence of ethanol correlated with both the residual stimulus-induced [$^3$H]PA accumulation and with [$^3$H]PEt formation.

### 3.3. *Inhibition of 5-HT secretion and PLD activation by BAPTA and analogues*

High (mM) concentrations of BAPTA have been reported to inhibit $Ca^{2+}$-independent secretion from permeabilized cells, including platelets [11,12]. The results in Table I confirm this and show that BAPTA also inhibited the increases in [$^3$H]PA caused by GTPγS and/or PMA. To study the relationship between the effects of BAPTA on secretion and PLD activity, [$^3$H]PEt formation was measured in the presence of 200 mM ethanol, which inhibited the secretion of [$^{14}$C]5-HT induced by GTPγS alone and by GTPγS with PMA by only 34 ± 4% and 15 ± 2%, respectively (mean values ± S.E., 5 experiments). Under these conditions, the $IC_{50}$ for inhibition of the GTPγS-induced secretion of [$^{14}$C]5-HT

Table I

Effects of BAPTA on $Ca^{2+}$-independent 5-HT secretion, PA formation and pleckstrin phosphorylation in permeabilized platelets

| Additions | Secretion of [$^{14}$C]5-HT (8%) | | $10^{-3}$ x Increase in [$^3$H]PA (d.p.m./$10^9$ platelets) | | $^{32}$P in pleckstrin (pmol/$10^9$ platelets) | |
|---|---|---|---|---|---|---|
| | − BAPTA | + BAPTA | − BAPTA | + BAPTA | − BAPTA | + BAPTA |
| None | 3 ± 1 | 4 ± 0 | 3.3 ± 0.4 | 2.9 ± 0.4 | 101 ± 8 | 73 ± 6 |
| GTPγS | 26 ± 1 | 7 ± 1** | 5.7 ± 0.3 | 3.6 ± 0.1** | 219 ± 8 | 111 ± 2** |
| PMA | 12 ± 1 | 8 ± 1* | 5.2 ± 0.5 | 3.8 ± 0.1* | 329 ± 3 | 320 ± 25 |
| GTPγS + PMA | 51 ± 3 | 25 ± 1** | 12.3 ± 0.8 | 7.6 ± 0.5* | 319 ± 9 | 326 ± 7 |

Samples of labelled permeabilized platelets were incubated with no stimulus, 100 $\mu$M GTPγS, 100 nM PMA or 100 $\mu$M GTPγS and 100 nM PMA, in each case in the absence and presence of 10 mM BAPTA. The secretion of [$^{14}$C]5-HT and formation of [$^3$H]PA were determined from triplicate incubations in the same experiment. [$\gamma$-$^{32}$P]ATP was added to additional duplicate samples for measurement of pleckstrin phosphorylation. Values are means ± S.E.; the significance of the effects of BAPTA was evaluated by two-sided unpaired *t*-tests ($*P < 0.05$; $**P < 0.01$).

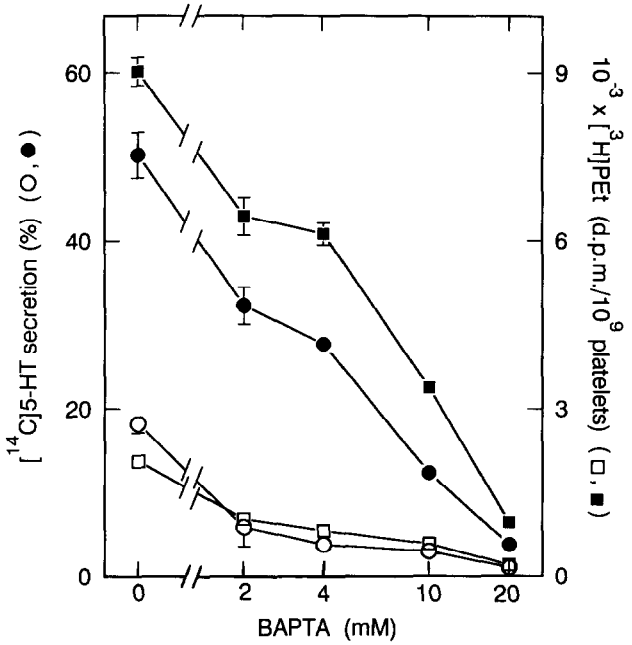


Fig. 3. Inhibition of $Ca^{2+}$-independent 5-HT secretion and PEt formation by BAPTA. Samples of labelled permeabilized platelets were incubated with the indicated concentrations of BAPTA and either 100 $\mu$M GTP$\gamma$S (○, □) or 100 $\mu$M GTP$\gamma$S and 100 nM PMA (●, ■), in each case in the presence of 200 mM ethanol. Secretion of [$^{14}$C]5-HT (○, ●) and the formation of [$^3$H]PEt (□, ■) were determined. Values are means ± S.E. from triplicate incubations in the same experiment.

by BAPTA was less than 2 mM, whereas the $IC_{50}$ for inhibition of the synergistic effect of GTP$\gamma$S and PMA was about 5 mM (Fig. 3). The results also show that BAPTA caused parallel dose-dependent inhibitions of PLD activation ([$^3$H]PEt formation). In five separate experiments, 10 mM BAPTA inhibited GTP$\gamma$S-induced secretion and [$^3$H]PEt formation by 71 ± 3% and 64 ± 5%, respectively, whereas the corresponding effects of GTP$\gamma$S with PMA were inhibited by 66 ± 3% and 52 ± 3%, respectively (mean values ± S.E.). Dibromo-BAPTA was considerably more effective than BAPTA as an inhibitor of both the $Ca^{2+}$-independent [$^{14}$C]5-HT secretion and [$^3$H]PEt formation induced by GTP$\gamma$S with PMA; 1 mM dibromo-BAPTA and 4 mM BAPTA were roughly equipotent (Table II). Dinitro-BAPTA had effects on secretion and PLD activity intermediate between those of BAPTA and dibromo-BAPTA (not shown).

To investigate the mechanism of action of BAPTA, the phosphorylation of pleckstrin during $Ca^{2+}$-independent secretion from permeabilized platelets was also studied (Table I). BAPTA (10 mM) did not affect the phosphorylation of this protein induced by PMA added with or without GTP$\gamma$S, indicating that BAPTA did not directly inhibit PKC. However, BAPTA did inhibit the phosphorylation of pleckstrin caused by GTP$\gamma$S alone, suggesting that PA formed by PLD, or a PA metabolite, may activate PKC.

## 4. DISCUSSION

The results provide three independent lines of evidence consistent with the hypothesis [1] that the target of $G_E$ in the platelet is PLD. First, GTP$\gamma$S and PMA acted synergistically in permeabilized platelets to stimulate both $Ca^{2+}$-independent secretion of 5-HT and PLD activity, whether the latter was measured as the accumulation of [$^3$H]PA in the absence of ethanol or of [$^3$H]PEt in the presence of ethanol. This synergistic activation of PLD is in accord with a previous report in which rabbit platelet membranes were studied [9], as well as with recent work on permeabilized HL-60 cells [18]. Second, ethanol inhibited the GTP$\gamma$S-induced secretion of 5-HT from permeabilized platelets and caused comparable decreases in [$^3$H]PA accumulation, which were attributable to the formation of [$^3$H]PEt by PLD-catalysed transphosphatidylation. Similar effects of ethanol on stimulus-induced PA accumulation and secretion in intact mast cells [19] and differentiated HL-60 cells [20] have been cited as evidence of a role for PLD in these cells. Third, BAPTA and analogues caused parallel concentration-dependent inhibitions of GTP$\gamma$S-induced secretion and PLD activity in permeabilized platelets, despite the presence of sufficient EGTA to give a pCa > 9. This unexpected result not only greatly strengthens the correlation between secretion and PLD activity, but can also explain previously reported anomalous inhibitory effects of BAPTA and analogues on secretion from permeabilized platelets [12] and other cells [3,11,13]. It is of interest that dibromo-BAPTA, which has an equilibrium affinity for $Ca^{2+}$ about tenfold lower than that of BAPTA [10,21], was about fourfold more potent than BAPTA as an inhibi-

Table II

Comparison of the inhibitory effects of dibromo-BAPTA and BAPTA on $Ca^{2+}$-independent secretion and PLD activity in permeabilized platelets

| Inhibitor | Secretion of [$^{14}$C]5-HT (%) | $10^{-3}$ × Increase in [$^3$H]PEt (d.p.m./$10^9$ platelets) |
|---|---|---|
| None | 41 ± 2 | 9.6 ± 0.4 |
| Dibromo-BAPTA (1 mM) | 27 ± 1* | 7.9 ± 0.7 |
| Dibromo-BAPTA (4 mM) | 8 ± 1* | 3.2 ± 0.1* |
| BAPTA (4 mM) | 23 ± 1* | 7.4 ± 0.2* |

Samples of labelled permeabilized platelets were incubated with 100 $\mu$M GTP$\gamma$S and 100 nM PMA in the presence of 200 mM ethanol and the indicated concentrations of dibromo-BAPTA or BAPTA. The secretion of [$^{14}$C]5-HT and formation of [$^3$H]PEt were determined from triplicate incubations in the same experiment; values are means ± S.E. The significance of changes was evaluated by two-sided unpaired $t$-tests (*$P < 0.05$).

tor of secretion and PLD activity. Although BAPTA binds $Ca^{2+}$ more rapidly than does EGTA [10], which may account for the ability of the former to block neurotransmitter release [22], differences in the $Ca^{2+}$-binding kinetics of these chelating agents are unlikely to explain the present results. Thus, we have observed similar inhibitions of GTPγS-stimulated secretion and PLD activity by BAPTA in the presence of $Ca^{2+}$ buffered to a final pCa of 6 [2]. Moreover, dinitro-BAPTA, which has little or no $Ca^{2+}$-binding capacity [21], was slightly more potent than BAPTA as an inhibitor of secretion and PLD activity in our experiments. We conclude that BAPTA and analogues do not inhibit secretion by chelation of $Ca^{2+}$. In addition, BAPTA did not appear to exert a direct inhibitory effect on PKC in permeabilized platelets and may therefore act on either $G_E$ or PLD itself.

Although our results are most simply explained if activation of PLD by a hypothetical $G_E$ mediates the stimulation of secretion by GTPγS, more complex mechanisms involving multiple GTP-binding proteins and target enzymes cannot easily be excluded, particularly in intact cells. However, the absence of significant GTPγS-induced inositol phosphate or diacylglycerol formation in permeabilized platelets at pCa > 9 [1,2] indicates that phospholipase C activity has no role in secretion under the conditions of the present study. Moreover, although preliminary work shows that GTPγS and PMA act synergistically to stimulate phospholipase $A_2$ at pCa > 9, inhibition of this enzyme did not affect secretion from permeabilized platelets (Coorssen, Davidson and Haslam, in preparation). These considerations focus attention on the possibility that PA generated by PLD acts as a second messenger that mediates secretion from platelets. A similar role for PLD has also been suggested by others working with mast cells [19], HL-60 cells [20] and pancreatic islets [23]. However, in intact human platelets, PLD activity accounts for only 10–20% of the total PA that accumulates after stimulation by thrombin [2,24]; most of the PA is formed by the sequential actions of phospholipase C and diacylglycerol kinase. Thus, a major role for PLD in secretion from intact platelets is only likely if the PA generated by this enzyme differs in its fatty acid composition or, more plausibly, in its subcellular localization from that formed as a result of phospholipase C activity. Some support for this possibility is provided by our observation that the PA accumulating in control incubations of permeabilized platelets was formed by a PLD-independent mechanism and was not associated with secretion. There are multiple mechanisms through which PA generated by PLD might enhance secretion. These include activation of PKC, as suggested by the GTPγS-induced phosphorylation of pleckstrin [1], stimulation of other protein kinases [25], effects on factors that regulate low-$M_r$ GTP-binding proteins [26] and a direct membrane-fusogenic action [27].

*Acknowledgements:* This work was supported by a grant from the Medical Research Council of Canada (MT-5626). J.R.C. held an M.R.C. Studentship. The authors thank Keith Davidson for development of the computer programme used to calculate $[Ca^{2+}_{free}]$ and $[Mg^{2+}_{free}]$ and Qun Du for carrying out experiments with dinitro-BAPTA.

## REFERENCES

[1] Coorssen, J.R., Davidson, M.M.L. and Haslam, R.J. (1990) Cell Regulation 1, 1027–1041.
[2] Haslam, R.J. and Coorssen J.R., in: Mechanisms of Platelet Activation and Control (K.S. Authi, S.P. Watson and V.V. Kakkar, Eds.), Adv. Exp. Med. Biol., Plenum, New York, 1993, in press.
[3] Barrowman, M.M., Cockcroft, S. and Gomperts, B.D. (1986) Nature 319, 504–507.
[4] Stutchfield, J. and Cockcroft, S. (1988) Biochem. J. 250, 375–382.
[5] Vallar, L., Biden, T.J. and Wollheim, C.B. (1987) J. Biol. Chem. 262, 5049–5056.
[6] Howell, T.W., Cockcroft, S. and Gomperts, B.D. (1987) J. Cell Biol. 105, 191–197.
[7] Cockcroft, S., Howell, T.W. and Gomperts, B.D. (1987) J. Cell Biol. 105, 2745–2750.
[8] Gomperts, B.D. (1990) Annu. Rev. Physiol. 52, 591–606.
[9] Van der Meulen, J. and Haslam, R.J. (1990) Biochem. J. 271, 693–700.
[10] Tsien, R.Y. (1980) Biochemistry 19, 2396–2404.
[11] Knight, D.E., von Grafenstein, H. and Athayde, C.M. (1989) Trends Neurosci. 12, 451–458.
[12] Athayde, C.M. and Scrutton, M.C. (1990) Eur. J. Biochem. 189, 647–655.
[13] Penner, R. and Neher, E. (1988) FEBS Lett. 226, 307–313.
[14] Haslam, R.J. and Davidson, M.M.L. (1984) Biochem. J. 222, 351–361.
[15] Fabiato, A. and Fabiato, F. (1979) J. Physiol. (Paris) 75, 463–505.
[16] Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911–917.
[17] Bocckino, S.B., Wilson, P.B. and Exton, J.H. (1987) FEBS Lett. 225, 201–204.
[18] Geny, B. and Cockcroft, S. (1992) Biochem. J. 284, 531–538.
[19] Gruchalla, R.S., Dinh, T.T. and Kennerly, D.A. (1990) J. Immunol. 144, 2334–2342.
[20] Xie, M., Jacobs, L.S. and Dubyak, G.R. (1991) J. Clin. Invest. 88, 45–54.
[21] Pethig, R., Kuhn, M., Payne, R., Adler, E., Chen, T.-H. and Jaffe, L.F. (1989) Cell Calcium 10, 491–498.
[22] Adler, E.M., Augustine, G.J., Duffy, S.N. and Charlton, M.P. (1991) J. Neurosci. 11, 1496–1507.
[23] Metz, S.A. and Dunlop, M. (1990) Biochem. J. 270, 427–435.
[24] Huang, R., Kucera, G.L. and Rittenhouse, S.E. (1991) J. Biol. Chem. 266, 1652–1655.
[25] Bocckino, S.B., Wilson, P.B. and Exton, J.H. (1991) Proc. Natl. Acad. Sci. USA 88, 6210–6213.
[26] Takai, Y., Kaibuchi, K., Kikuchi, A. and Kawata, M. (1992) Int. Rev. Cytol. 133, 187–230.
[27] Leventis, R., Gagne, J., Fuller, N., Rand, R.P. and Silvius, J.R. (1986) Biochemistry 25, 6978–6987.