0026-895X/96/020360-05$3.00/0

MOLECULAR PHARMACOLOGY, 49:360–364 (1996).

Stimulation of Ca^{2+}-Dependent Membrane Currents in *Xenopus* Oocytes by Microinjection of Pyrimidine Nucleotide-Glucose Conjugates

HAK YONG KIM, DAVID THOMAS, and MICHAEL R. HANLEY

Department of Biological Chemistry, School of Medicine, University of California, Davis, California 95616-8635

Received September 22, 1995; Accepted October 31, 1995

SUMMARY

Microinjection, but not extracellular application, of cytidine-5′-diphosphate-D-glucose (CDPG) has been shown to elicit Ca^{2+}-dependent currents in *Xenopus laevis* oocytes. These responses were comparable to those of inositol-1,4,5-trisphosphate ($InsP_3$) in being both rapid and dose dependent. For example, maximal amplitudes of CDPG-induced current were similar (~365 ± 75 nA at 1 μM CDPG) to those of $InsP_3$. The CDPG currents were insensitive to removal of extracellular Ca^{2+}, indicating the dependence on Ca^{2+} release from intracellular Ca^{2+} stores but not on Ca^{2+} entry through plasma membrane. CDPG-induced currents were reduced or abolished by pretreatment with thapsigargin, by injection of the Ca^{2+} chelator 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid, or by extracellular perfusion of the Cl^- channel blocker niflumic acid but were insensitive to injection of the $InsP_3$ antagonist heparin. These results suggest that CDPG induces Ca^{2+} discharge from intracellular Ca^{2+} stores via a mechanism distinct from that of $InsP_3$ in *Xenopus* oocytes. Another pyrimidine nucleotide-glucose derivative, uridine-5′-diphosphate-α-D-glucose, also induced Ca^{2+}-dependent currents, but the activity was lower than that of CDPG (maximal amplitude, 272 ± 62 nA). Other nucleotide-glucose compounds (adenosine-5′-diphosphate-D-glucose, guanosine-5′-diphosphate-D-glucose, and thymidine-5′-diphosphate-D-glucose) had no current responses when injected into oocytes. After injection of CDPG, CDPG-induced Ca^{2+} release appeared to couple to a Ca^{2+} entry pathway similar to that coupled to $InsP_3$. These results indicate that pyrimidine nucleotide-glucose conjugates may provide novel pharmacological tools for the study of Ca^{2+} signaling in oocytes.

Ca^{2+} signaling is crucial in the regulation of cell responses in a wide variety of cells. Multiple mechanisms contribute to regulation of cytosolic Ca^{2+} levels. Notably, $InsP_3$ is recognized as the key second messenger regulating Ca^{2+} discharge from intracellular stores (1). In addition to $InsP_3$, however, other endogenous or exogenous compounds can stimulate Ca^{2+} mobilization from stores. For example, cADP-ribose can activate the ryanodine receptor to release stored Ca^{2+} (2). Similarly, sphingosine-1-phosphate has recently been shown to elevate intracellular Ca^{2+}, albeit by an unknown mechanism (3, 4). In this regard, a sphingolipid-gated Ca^{2+} channel has been demonstrated in microsomes isolated from a rat basophilic leukemia cell (5) and expressed in *Xenopus* oocytes from mRNA derived from a human umbilical vein endothelial cell (6).

This work was supported in part by grants from the National Institutes of Health and the Council for Tobacco Research (M.R.H.). D.T. was supported by a National Institutes of Health Training Grant in Molecular and Cellular Biology.

In *Xenopus* oocytes, as in other cells, receptor-activated Ca^{2+} release proceeds through activation of the $InsP_3$ receptor on the endoplasmic reticulum (7, 8). Although sphingosine-1-phosphate has been shown to increase intracellular ionized calcium by acting on a specific surface receptor, possibly the lysophosphatidic acid receptor, in *Xenopus* oocytes (9), these cells do not respond to intracellular microinjection of either cADP-ribose (10) or sphingosine-1-phosphate (9). Thus, *Xenopus* oocytes appear to be somewhat simpler in the pharmacological sensitivity of their Ca^{2+} stores.

Recently, we reported the usefulness of the *Xenopus* oocyte as a bioassay for detection and purification of novel cellular factors influencing cytosolic Ca^{2+} elevation (11, 12). In the course of fractionating Jurkat lymphocyte extracts, we obtained evidence for new factors, distinct from inositol phosphates, capable of stimulating Ca^{2+}-dependent membrane currents by intracellular Ca^{2+} discharge (12). Preliminary chemical characterization of such partially purified extracts indicated the presence of pyrimidine nucleotide derivatives.

ABBREVIATIONS: $InsP_3$, inositol-1,4,5-trisphosphate; CDPG, cytidine-5′-diphosphate-D-glucose; UDPG, uridine-5′-diphosphate-α-D-glucose; ADPG, adenosine-5′-diphosphate-D-glucose; GDPG, guanosine-5′-diphosphate-D-glucose; TDPG, thymidine-5′-diphosphate-D-glucose; BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; LPA, 1-oleoyl-L-α-lysophosphatidic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Accordingly, we evaluated several nucleotide-glucose conjugates for current-inducing activity on microinjection into oocytes. The results suggest that the microbial products CDPG and UDPG, but not other related conjugates, induce Ca^{2+}-dependent membrane currents on microinjection into oocytes but are inactive when applied extracellularly. In this report, we describe the characterization of these responses.

Experimental Procedures

Materials. Thapsigargin was purchased from LC Services (Woburn, MA); BAPTA was from Molecular Probes (Eugene, OR); and niflumic acid, heparin, and CDP-, UDP-, TDP-, ADP-, and GDP-glucose were from Sigma Chemical Co. (St. Louis, MO). Thapsigargin and niflumic acid were dissolved in DMSO, BAPTA was dissolved in water, and all nucleotide-glucose compounds were dissolved in 10 mM HEPES, pH 7.0. All were stored as stock solutions at −20°.

***Xenopus* oocytes.** *Xenopus* oocytes were obtained by ovarectomy as described previously (11–13). Follicular cells were removed from oocytes through treatment with collagenase (2 mg/ml for 2 hr at room temperature) followed by rolling of oocytes on plastic Petri dishes. Defolliculated oocytes were maintained in modified L-15 medium (diluted 1:1 with 30 mM HEPES, pH 7.4, containing 0.25% chicken ovalbumin, 1 mM L-glutamine, and 50 μg/ml gentamycin).

Data acquisition and current analysis. Two-electrode whole-cell voltage-clamp experiments with *Xenopus* oocytes were performed as described previously (11–13). Briefly, stage V and VI oocytes were impaled with two microelectrodes with resistances of 1 MΩ and back-filled with 3 M KCl. Oocytes were voltage-clamped at −60 mV in OR2 medium (82 mM NaCl, 2 mM $CaCl_2$, 2.5 mM KCl, 1 mM $MgCl_2$, 1 mM Na_2HPO_4, and 5 mM HEPES, pH 7.4) with the use of a TEV-200 clamp amplifier (Dagan, MN). For experiments requiring removal of extracellular Ca^{2+}, oocytes were perfused with Ca^{2+}-free OR2 medium (82 mM NaCl, 2.5 mM KCl, 5 mM $MgCl_2$, 0.1 mM EGTA, 1 mM Na_2HPO_4, and 5 mM HEPES, pH 7.4). We have found, in agreement with others (14, 15), that oocyte current responses can be unstable in Ca^{2+}-free buffers but can be stabilized by the addition of a high level of Mg^{2+} (5 mM). Currents were low-pass filtered at 50 kHz using the internal four-pole Bessel filter and digitized at sampling rates of 100 msec (10 Hz) or 500 msec (2 Hz) with the use of the TL-1 analog/digital converter (Axon Instruments, Foster City, CA) and the current analysis program SCAN (Dagan, Minneapolis, MN).

To obtain current-voltage relations, voltage ramps were run on the activated currents. The ramp I-V data was collected with the use of pCLAMP software (version 5.5) to sample current responses to potential changes at a rate of 4 kHz. Current output from the clamp amplifier was low-pass filtered at 50–100 kHz and digitized using the TL-1 interface board (Axon Instruments) at 12-bit resolution. The ramp protocol consisted of repeated episodes (every 5 sec) of a −100 mV to +60 mV ramp (2-sec duration ramp at a depolarization rate of 0.5 mV/msec) with an interepisode holding potential of −60 mV.

Results

The effects of nucleotide-glucose conjugates on *Xenopus* oocyte currents were tested with voltage-clamp experiments. Extracellular application had no effect. On the other hand, microinjection of CDPG (1 μM final concentration) induced a rapid, biphasic current response (Fig. 1A; peak current, 365 ± 75 nA) that was similar, but not identical, to that elicited by $InsP_3$ microinjection (Fig. 1B). In particular, the initial current spike to CDPG was much shorter in duration than that elicited by $InsP_3$ (~1 sec versus 5 sec). In Ca^{2+}-free medium, CDPG-induced currents were unchanged (peak current, 331 ± 4 nA; Fig. 1A) from those observed in normal

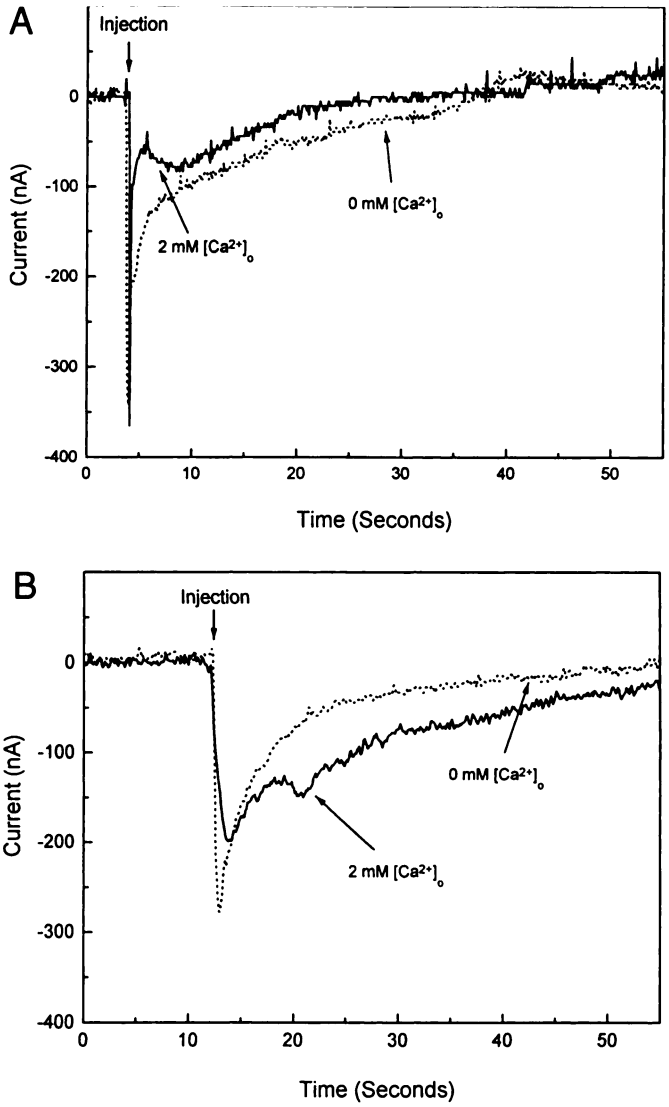


Fig. 1. Microinjection of (A) CDPG (1 μM) or (B) $InsP_3$ (1 μM) induces membrane currents in *Xenopus* oocytes in the presence (*solid lines*) or absence (*dotted lines*) of extracellular Ca^{2+}. These results are representative of at least six independent experiments in each case.

medium. These results suggest that the membrane currents elicited by CDPG are not dependent on Ca^{2+} entry but might involve Ca^{2+} discharge from intracellular Ca^{2+} stores.

To determine the dose-potency of CDPG, varying concentrations were injected, and peak current responses were measured. Fig. 2 illustrates that CDPG exhibited a bell-shaped dose-response curve with a threshold of <50 nM and maximal activity at 1 μM. At the highest concentration tested (10 μM), CDPG-induced currents were substantially decreased from the maximum level.

To establish the nature of the membrane currents activated by CDPG, we examined initially the I-V relationship (Fig. 3). The reversal potential for the CDPG-activated current (~−35 mV) indicated that the current may be carried in large part, but not exclusively, by Cl^-. This conclusion was further supported by the observation that the CDPG-activated current was substantially reduced (12% of control), but not abolished, by perfusion of 1 mM niflumic acid, an inhibitor of oocyte Cl^- channels (Table 1). For comparison, the rever-

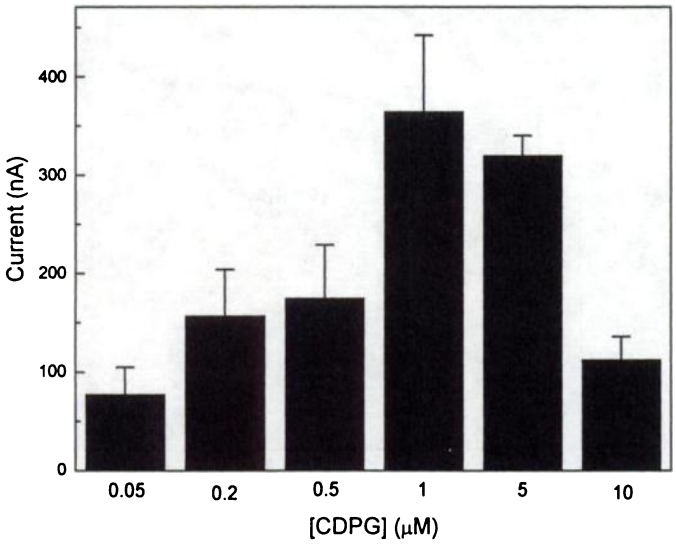


Fig. 2. Concentration dependence of maximal currents stimulated by different doses of injected CDPG. Values are average ± standard deviation of six or more determinations.

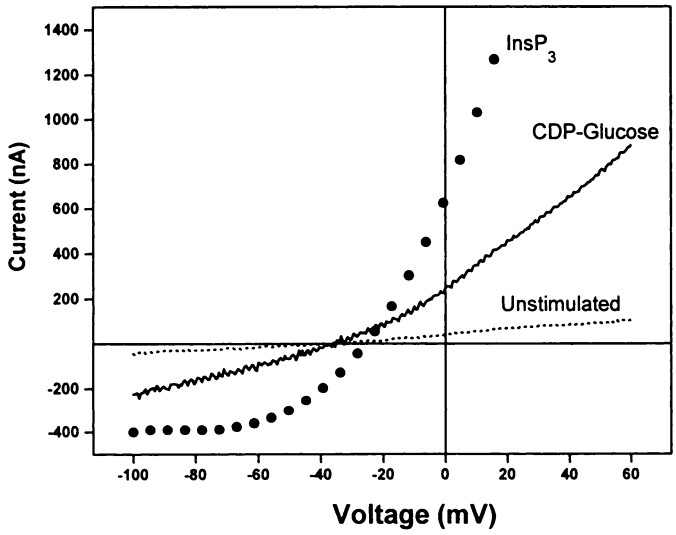


Fig. 3. I-V relationship of CDPG-evoked and $InsP_3$-evoked currents in *Xenopus* oocytes. For experimental details, see Experimental Procedures. The reversal potentials were −35 mV for CDPG and −25 mV for $InsP_3$. Results are representative of four independent experiments.

sal potential of current elicited by $InsP_3$ injection was ~ −25 mV, and the current was completely abolished by niflumic acid. These results underscored fundamental differences between CDPG- and $InsP_3$-induced current responses. Indeed, the shapes of the I-V curves for CDPG- and $InsP_3$-induced currents were quite distinct, particularly at negative potentials (> −60 mV), where current evoked by CDPG responded in a more linear fashion to voltage changes.

We have characterized the dependence of CDPG-induced currents on Ca^{2+} discharge by using several pharmacological reagents. Pretreatment with thapsigargin, an inhibitor of SERCA-type Ca^{2+} ATPases (16) that depletes Ca^{2+} stores in oocytes (17), completely blocked the activity of CDPG (Table 1). This suggested an absolute requirement for intact Ca^{2+} stores for CDPG responses. Injection of BAPTA (1 mM final concentration) eradicated CDPG-induced currents (Table 1). These results indicate that CDPG-induced currents are Ca^{2+} dependent, arise from Ca^{2+} discharge from intracellular stores, and are carried principally by Cl^-.

To evaluate whether these CDPG-stimulated currents were arising from activation of the $InsP_3$ receptor, oocytes were pretreated by microinjection of heparin (500 μg/ml). Under these conditions, all $InsP_3$-induced currents were abolished. However, heparin pretreatment had little or no effect on CDPG-induced currents (Table 1). This result suggests that CDPG does not elicit its action through the $InsP_3$ receptor.

To determine the structural specificity of CDPG-induced currents, a variety of related nucleotides and nucleotide-glucose conjugates were tested. Only a pyrimidine-glucose conjugate, UDPG, exhibited activity, whereas another pyrimidine nucleotide-glucose conjugate, TDPG, and two purine nucleotide-glucose conjugates, ADPG and GDPG, were inactive (Table 2). UDPG appeared to be less active than CDPG in that it gave lower peak current responses at matched doses.

The depletion of intracellular Ca^{2+} stores by discharge has been shown to induce Ca^{2+} influx through voltage-insensitive channels on the plasma membrane (18). This Ca^{2+} entry pathway has been called "capacitative calcium entry" (18), and oocytes exhibit such a pathway (17). To determine whether CDPG-induced Ca^{2+} discharge was coupled to a Ca^{2+} entry pathway, after injection of CDPG, external Ca^{2+} was removed and subsequently replaced with a high Ca^{2+} medium (10 mM extracellular ionized calcium). Removal of extracellular Ca^{2+} after initial current responses to CDPG

TABLE 1

Effects of various drug treatments on CDP-glucose-induced Ca^{2+}-dependent currents in *Xenopus* oocytes

Treatment	Peak current	% of Control	*n*
	nA		
Control	365 ± 75	100	12
TG[a]	0	0	3
BAPTA injection[b]	0	0	5
Heparin injection[c]	272 ± 62	80	4
Niflumic acid[d]	40 ± 16	12	4

[a] TG (1 μM) applied extracellularly in Ca^{2+}-free OR2 (2 hr).
[b] BAPTA injection (1mM, 15 min).
[c] Heparin injection (500μg/ml, 1 hr).
[d] 1 mM applied extracellularly by perfusion (1 min) before injection.

TABLE 2

Structural requirements for eliciting current responses of related nucleotides and nucleotide-glucose conjugates

All compounds were injected to give a final estimated intracellular concentration of 1 μM. For experiments requiring removal of extracellular calcium, oocytes were perfused with a Ca^{2+}-free OR2 medium as described in Experimental Procedures. None of the following nucleotide diphosphates or triphosphates were active on microinjection: CDP, CTP, UDP, UTP, GDP, GTP, ADP, or ATP. Glucose and inositol were also inactive. Values are mean ± standard deviation of six or more independent determinations.

Nucleotide-glucose conjugate	Currents	
	2 mM $[Ca^{2+}]_o$	0 mM $[Ca^{2+}]_o$
	nA	
CDPG	365 ± 75	331 ± 4
UDPG	252 ± 26	248 ± 28
ADPG	0	0
GDPG	0	0
TTPG	0	0

abolished currents (Fig. 4A). However, current decayed with a slow time course that was identical in the presence or absence of extracellular Ca^{2+} (see Fig. 1A). Subsequent replacement of Ca^{2+}-free medium with high Ca^{2+} medium (10 mM) activated sustained currents (84 nA). These results suggest that CDPG induced both initial Ca^{2+} mobilization from intracellular Ca^{2+} stores and later Ca^{2+} entry. For comparison, the same sequence of events is shown after injection of $InsP_3$ (Fig. 4B).

Xenopus oocytes have endogenous receptors to LPA that activate the phosphatidylinositol signaling pathway to produce Ca^{2+}-dependent currents through $InsP_3$ (13, 19). We examined the interaction between CDPG and endogenous $InsP_3$-induced currents through the ordered addition of extracellular LPA before or after CDPG injection. LPA-induced currents were 10-fold higher than the maximum current elicited by CDPG, indicating that Ca^{2+} release by CDPG was substantially less than that of LPA. After stimulation of currents by LPA, subsequent injection of a maximal dose of CDPG (1 μM) had no effect (data not shown). However, after injection of a maximal dose of CDPG, stimulation by LPA elicited normal peak currents (data not shown). This result suggests that microinjected CDPG mobilizes Ca^{2+} from a limited store that is, nevertheless, sensitive to endogenous $InsP_3$.

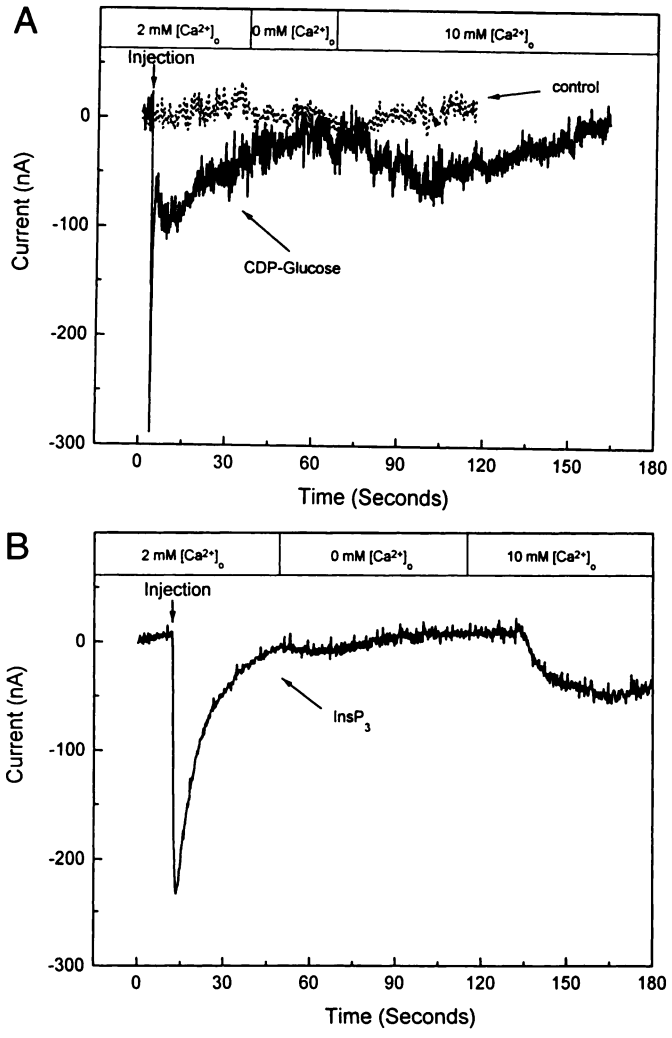


Fig. 4. CDPG and $InsP_3$ activate sustained Ca^{2+} entry as assessed by current responses. After injection of (A) CDPG (1 μM) or (B) $InsP_3$ (1 μM), external Ca^{2+} was removed and subsequently replaced with 10 mM extracellular ionized calcium. *Bars on top*, extracellular Ca^{2+} concentrations. The resulting time courses of currents are shown. In control oocytes, there were no injections. These data are representative of four independent experiments.

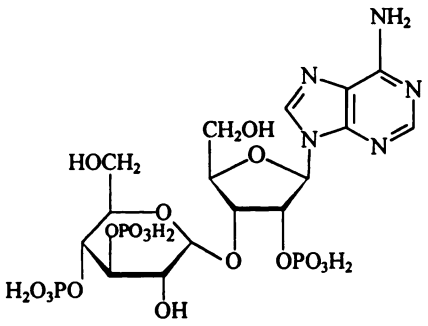
Discussion

Preliminary chemical analyses of partially purified Ca^{2+} discharge factors from Jurkat lymphocytes (11, 12) indicated the presence of pyrimidine-sugar derivatives.[1] Thus, we evaluated several known microbial products that are nucleotide-glucose adducts for intracellular Ca^{2+} discharge activity in oocytes. A CDP pyrophosphodiester conjugate with glucose (i.e., CDPG) was shown to rapidly induce Ca^{2+}-dependent membrane currents in oocytes. The activation of these currents appeared to require intact Ca^{2+} stores, suggesting that the currents were activated by Ca^{2+} discharge. Significantly, this mechanism of Ca^{2+} release appeared by several criteria to be distinct from that of $InsP_3$, such as insensitivity to heparin, a different I-V pattern for induced currents, and time course of action. Although CDPG-induced currents were Ca^{2+}- dependent, the shift in the observed reversal potential from that of $InsP_3$-induced currents (e.g., −35 mV versus −25 mV, respectively) suggests recruitment of a novel current contribution. Although the origin of this shift is unclear, it may be due to a variable K^+ current that has been variably observed with receptor (20) or $InsP_3$ stimulation (21). Thus, CDPG appears to be a novel pharmacological probe of Ca^{2+}-discharge mechanisms.

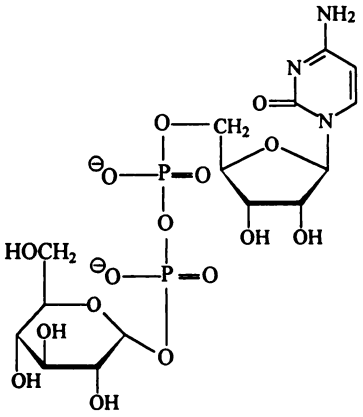
The site sensitive to CDPG appears to be quite specific in its structural requirements in that the closely related pyrimidine-glucose conjugate UDPG was active, whereas the methyluracil variant TDPG was inactive. Neither nucleotide diphosphates, triphosphates nor glucose was active. Thus, the site mediating the Ca^{2+}-discharge actions of CDPG is responsive to very specific pyrimidine nucleotide-sugar molecular species. Intriguingly, novel microbial products that potently activate the $InsP_3$ receptor, the adenophostins, also belong to the chemical category of nucleotide-glucose conjugates (Fig. 5; see Refs. 22 and 23). In the case of the adenophostins, the conjugates are a purine with a different mode of chemical linkage to a phosphorylated glucose moiety. However, the overall similarities of structures and activities between adenophostin and CDPG raise the important possibility that specific nucleotide-sugar conjugates may comprise a novel class of pharmacological probes for sites involved in Ca^{2+} discharge.

In this regard, it is interesting to note that CDPG reliably gave smaller current responses that were abolished by pretreatment with LPA. On the other hand, CDPG pretreatment did not alter current responses to LPA. This suggests that a limited compartment within the $InsP_3$-sensitive stores responds to CDPG, and it may imply a functional heterogeneity within the receptor-regulated Ca^{2+} stores in oocytes. Intriguingly, although both $InsP_3$ and CDPG current responses were sensitive to the depth of the injection micropipette, CDPG appears to act only when injected very near the plasmale-

[1] H. Y. Kim, D. Thomas, and M. R. Hanley, unpublished observations.



ADENOPHOSTIN A



CDP-GLUCOSE

Fig. 5. Structures of adenophostin A and CDP-glucose. The stereoisomer configuration was as described previously (22, 23).

mma (data not shown), whereas $InsP_3$ responses could be detected regardless of depth (24).

Although these observations, in the strictest sense, indicate a pharmacological activity of nucleotide-sugar conjugates in Ca^{2+} homeostasis, it is interesting to note that the concept of a physiological interaction between such metabolites and Ca^{2+} control processes has been suggested before (25). Specifically, in irreversible hepatocyte injury, enhanced Ca^{2+} entry into hepatocytes has been proposed as a common pathway in cell death (26, 27). The candidate mediator of injury-induced Ca^{2+} influx in hepatocytes was, in fact, UDP-glucose (25), which is normally the endogenous glycogen biosynthesis precursor. This correlation might imply an unsuspected, and possibly widespread, role for UDP-glucose in regulating Ca^{2+} signals. However, the physiological role of pyrimidine-nucleotide conjugates in regulated Ca^{2+} discharge remains speculative.

Acknowledgments

We thank Dr. Rich Nuccitelli (Section of Molecular and Cellular Biology, University of California at Davis) for use of the *Xenopus laevis* colony.

References

1. Berridge, M. J. Inositol trisphosphate and calcium signalling. *Nature (Lond.)* **361:**315–325 (1993).
2. Lee, H. C., T. F. Walseth, G. T. Bratt, R. N. Hayes, and D. L. Clapper. Structural determination of a cyclic metabolite of NAD^+ with intracellular Ca^{2+}-mobilizing activity. *J. Biol. Chem.* **264:**1608–1615 (1989).
3. Ghosh, T. K., J. Bian, and D. L. Gill. Intracellular calcium release mediated by sphingosine derivatives generated in cells. *Science (Washington D. C.)* **248:**1653–1656 (1990).
4. Mattie, M., G. Brooker, and S. Spiegel. Sphingosine-1-phosphate, a putative second messenger, mobilizes calcium from internal stores via an inositol trisphosphate-independent pathway. *J. Biol. Chem.* **269:**3181–3188 (1994).
5. Kindman, L. A., S. Kim, T. V. McDonald, and P. Gardner. Characterization of a novel intracellular sphingolipid-gated Ca^{2+} permeable channel from rat basophilic leukemia cells. *J. Biol. Chem.* **269:**13088–13091 (1994).
6. Kim, S., V. Lakhani, D. J. Costa, A. I. Sharara, J. G. Fitz, L.-W. Huang, K. G. Peters, and L. A. Kindman. Sphingolipid-gated Ca^{2+} release from intracellular stores of endothelial cells in mediated by a novel Ca^{2+}-permeable channel. *J. Biol. Chem.* **270:**5266–5269 (1995).
7. Han, J. K., and R. Nuccitelli. Inositol 1,4,5-trisphosphate-induced calcium release in the organelle layers of the stratified, intact egg of *Xenopus laevis*. *J. Cell Biol.* **110:**1103–1110 (1990).
8. DeLisle, S., and M. J. Welsh. Inositol trisphosphate is required for the propagation of calcium waves in *Xenopus* oocytes. *J. Biol. Chem.* **267:**7963–7966 (1992).
9. Durieux, M. E., S. J. Carlisle, M. N. Salafranca, and K. R. Lynch. Response to sphingosine-1-phosphate in *X. laevis* oocytes: similarities with lysophosphatidic acid signaling. *Am. J. Physiol.* **264:**C1360–C1364 (1993).
10. Parys, J. B., S. W. Sernett, S. DeLisle, P. M. Snyder, M. J. Welsh, and K. P. Campbell. Isolation, characterization, and localization of the inositol 1:4,5-trisphosphate receptor protein in *Xenopus laevis* oocytes. *J. Biol. Chem.* **267:**18776–18782 (1992).
11. Thomas, D., and M. R. Hanley. Evaluation of calcium influx factors from stimulated Jurkat T-lymphocytes by microinjection into *Xenopus* oocytes. *J. Biol. Chem.* **270:**6429–6432 (1995).
12. Kim, H. Y., D. Thomas, and M. R. Hanley. Chromatographic resolution of an intracellular calcium influx factor from thapsigargin-activated Jurkat cells: evidence for multiple activities influencing calcium elevation in *Xenopus* oocytes. *J. Biol. Chem.* **270:**9706–9708 (1995).
13. Ferguson, J. E., and M. R. Hanley. Phosphatidic acid and lysophosphatidic acid stimulate receptor-regulated membrane currents in the *Xenopus laevis* oocyte. *Arch. Biochem. Biophys.* **297:**388–392 (1992).
14. Parekh, A. B., M. Foguet, H. Lübbert, and W. Stühmer. Ca^{2+} oscillations and Ca^{2+} influx in *Xenopus* oocytes expressing a novel 5-hydroxytryptamine receptor. *J. Physiol.* **469:**653–671 (1993).
15. Parker, I., and Y. Yao. Relation between intracellular Ca^{2+} signals and Ca^{2+}-activated Cl^- current in *Xenopus* oocytes. *Cell Calcium* **15:**276–288 (1994).
16. Thomas, D., and M. R. Hanley. Pharmacological tools for perturbing intracellular calcium storage. *Methods Cell Biol.* **40:**65–89 (1994).
17. Petersen, C. C. H., and M. J. Berridge. The regulation of capacitative calcium entry by calcium and protein kinase C in *Xenopus* oocytes. *J. Biol. Chem.* **269:**32246–32253 (1994).
18. Putney, J. W., Jr. Capacitative calcium entry revisited. *Cell Calcium* **11:**611–624 (1990).
19. Durieux, M. E., M. N. Salafranca, K. R. Lynch, and J. R. Moorman. Lysophosphatidic acid induces a pertussis toxin-sensitive Ca^{2+}-activated Cl^- current in *Xenopus laevis* oocytes. *Am. J. Physiol.* **263:**C896–C900 (1992).
20. Parekh, A. B., H. Terlau, and W. Stühmer. Depletion of $InsP_3$ stores activates a Ca^{2+} and K^+ current by means of a phosphatase and a diffusible messenger. *Nature (Lond.)* **364:**814–818 (1993).
21. Ferguson, J. E., J. K. Han, J. P. Kao, and R. Nuccitelli. The effects of inositol trisphosphates and inositol tetrakisphosphate on Ca^{2+} release and Cl^- current pattern in the *Xenopus laevis* oocyte. *Exp. Cell Res.* **192:**352–365 (1991).
22. Takahashi, M., T. Kagasaki, T. Hosoya, and S. Takahashi. Adenophostins A and B: potent agonists of inositol-1,4,5-trisphosphate receptor produced by *Penicillium brevicompactum*. *J. Antibiot.* **46:**1643–1647 (1993).
23. Takahashi, M., K. Tanzawa, and S. Takahashi. Adenophostins, newly discovered metabolites of *Penicillium brevicompactum*, act as potent agonists of the inositol 1,4,5-trisphosphate receptor. *J. Biol. Chem.* **269:**369–372 (1994).
24. Oron, Y., and N. Dascal. Regulation of intracellular calcium activity in *Xenopus* oocytes. *Methods Enzymol.* **207:**381–390 (1992).
25. Schiessel, C., C. Forsthove, and D. Keppler. ^{45}Calcium uptake during the transition from reversible to irreversible liver injury induced by D-galactosamine in vivo. *Hepatology* **4:**855–861 (1984).
26. Schanne, F. A. X., A. B. Kane, E. E. Young, and J. L. Farber. Calcium dependence of toxic cell death: a final common pathway. *Science (Washington D. C.)* **206:**700–702 (1979).
27. Kane, A. B., E. E. Young, F. A. X. Schanne, and J. L. Farber. Calcium dependence of phalloidin-induced liver cell death. *Proc. Natl. Acad. Sci. USA* **77:**1177–1180 (1980).

Send reprint requests to: Dr. Michael R. Hanley, Department of Biological Chemistry, School of Medicine, University of California, Davis, CA 95616-8635.