0026-895X/87/050500-06$02.00/0

MOLECULAR PHARMACOLOGY, 31:500-505

Effect of Pseudopterosin A on Cell Division, Cell Cycle Progression, DNA, and Protein Synthesis in Cultured Sea Urchin Embryos

WILLIAMS S. ETTOUATI and ROBERT S. JACOBS

Marine Science Institute and Department of Biological Sciences, University of California, Santa Barbara, California 93106

Received November 3, 1986; Accepted February 26, 1987

SUMMARY

Pseudopterosin A is a diterpene riboside isolated from *Pseudopterogorgia bipinata*, a soft coral of the order Gorgonacea. Pseudopterosin A inhibits the first cleavage in fertilized sea urchin eggs (*Strongylocentrotus purpuratus, Strongylocentrotus franciscanus*, and *Lytechinus pictus*) with an IC_{50} of 25 μM. In time of addition studies, Pseudopterosin A (4×10^{-5} M) progressively blocked the first cleavage when added within the first 50 min post fertilization; when added after 50 min the first division occurred normally. Cell cycle studies show that Pseudopterosin A had to be in continuous contact with the sea urchin eggs during the initial 45 min post fertilization to significantly inhibit the first cleavage. Shorter drug contact time progressively reduced the degree of inhibition, suggesting that inhibition of cytokinesis by Pseudopterosin A was correlated with mitosis. Pseudopterosin A (4×10^{-5} M) inhibited the uptake and incorporation of [^{3}H]thymidine during the S_2 phase. This concentration of Pseudopterosin A also inhibits protein synthesis as measured by the uptake and incorporation of [^{3}H]phenylalanine. In this case the inhibition started before the S_1 phase. Cytological examination revealed that sea urchin embryos did not progress beyond early prophase. Notably, the nuclear envelope remained intact and chromatin was condensed into chromosomes in the arrested embryos. These synchronously dividing embryos did not show any abnormalities such as lysis, swelling, or morphological changes different from control embryos.

We have been investigating the site and mechanism of action of certain unique marine natural products in fertilized sea urchin eggs (1). The goal is to develop compounds that may have a unique mechanism of action that could serve as useful probes to investigate the complex sequence of events occurring during the first cycle of division in the sea urchin embryo. This led to investigation of Elatone, a marine natural product from the alga *Laurentia elata* (2, 3), Stylpoldione from the brown alga *Stypopodium zonale* (4, 5), and Manoalide from the sponge *Luffariella variabilis* (6). More recently, two new classes of compounds have been identified: the Pseudopterolides and the Pseudopterosins. These compounds were isolated from soft corals. We have found that the Pseudopterolides, extracted from *Pseudopterogorgia* (7), produce multiple nuclei. The Pseudopterosins, an entirely new class of diterpenoid glycosides extracted from *Pseudopterogorgia elisabethae* (8), are reported here. By utilizing the inherent synchrony of an invertebrate embryo cell culture system (9), we have examined how Pseudopterosin A affects phases of the cell cycle and the initiation and completion of certain mitosis-dependent processes. These unique glycosides have also been found to be significantly more potent than indomethacin in blocking phorbol myristate acetate-induced topical inflammation when applied to the skin of mice. Pseudopterosin A inhibits the stretch-reflex response in mice induced by intraperitoneal injection of phenyl-quinone (8). Our initial work with this marine natural product showed it to be a potent inhibitor of cleavage in the fertilized sea urchin egg that was qualitatively different from previously described marine natural products (10).

Materials and Methods

Cell cleavage study. For all assays, fresh sea water was collected and filtered through Whatman No. 1 filter paper. Sea urchins, either *Strongylocentrotus purpuratus*, *Strongylocentrotus franciscanus*, or *Lytechinus pictus*, were induced to spawn by injection of 0.5 M KCl through the soft tissue of the oral surface into the coelomic cavity (1). Incubation temperatures for the three species were 15, 16, and 18.5°, respectively. Under these conditions mitosis lasts 1 hr. At a time interval corresponding to the first division in the control samples, the percentage of Pseudopterosin A-treated embryos completing division was determined microscopically. Results were expressed as the percentage inhibition of egg cleavage, relative to controls. The effect of

This research was sponsored in part by the National Oceanic and Atmospheric Administration, National Sea Grant College Program, Department of Commerce, under Grant NA80AA-D-120, through the California State Resources Agency, Project No. R/MP-21. The U.S. Government is authorized to reproduce this paper and distribute it for governmental purposes.

ABBREVIATIONS: TCA, trichloroacetic acid; DMSO, dimethyl sulfoxide.

Pseudopterosin A at different stages of the cell cycle was determined by adding Pseudopterosin A at 10-min intervals after fertilization to aliquots of sea urchin embryos and observing the percentage inhibition of first cleavage.

Reversibility of Pseudopterosin A was determined by adding the drug 3 min post fertilization, and, at specific times, 1-ml aliquots of the 1% (v/v) suspension of eggs in sea water were washed three times with 100 ml of filtered sea water, resuspended at 1% (v/v) in sea water, and allowed to complete the 2-hr incubation period. Results were then expressed as percentage inhibition of egg cleavage, relative to controls.

Cytological studies. Two-ml aliquots of cells were removed at various times after fertilization, fixed in ethanol/glacial acetic acid (3:1) for 24 hr, and resuspended in 45% (v/v) acetic acid for 24 hr to produce maximum swelling and clearing of the cytoplasm. Then they were stained in 1% orcein in 75% acetic acid for 24 hr before examination by phase or Nomarski microscopy (× 100–400) (11).

[^{3}H]Thymidine uptake rate as a function of time after fertilization. Uptake of [^{3}H]thymidine was measured using the method of Vacquier and Brandiff (12). Pseudopterosin A (4×10^{-5} M) or vehicle control was added 5 min post fertilization. Duplicate 2-ml aliquots were removed at 15-min intervals after fertilization and transferred to tubes containing [^{3}H]thymidine (specific activity 20 Ci/mmol) in sea water. The stock solution was diluted using unlabeled thymidine in sea water to yield a final activity of 15 μCi/mmol at a final concentration of 1×10^{-5} M. After a 5-min "pulse," eggs were sedimented, washed repeatedly with ice-cold sea water containing 1 mg/ml of unlabeled thymidine, and then killed by the addition of 5 ml of 10% TCA. Aliquots of the TCA-soluble extract were then counted in the liquid scintillant Ecosint (National Diagnostics) on an LKB 1219 liquid scintillation counter.

Cumulative incorporation of [^{3}H]thymidine into DNA. Incorporation of [^{3}H]thymidine into TCA-insoluble material was measured by the method of Hinegardner *et al.* (13), as modified by White and Jacobs (3). Aliquots of a 1% (v/v) slurry of fertilized *L. pictus* embryos were added to beakers containing [^{3}H]thymidine (specific activity 6.7 Ci/mmol, New England Nuclear Corp., Boston, MA) to yield a final activity of 3 μCi/ml and a final concentration of 4.5×10^{-7} M. Pseudopterosin A (4×10^{-5} M) or vehicle control was added approximately 3–5 min post fertilization. Embryos were gently stirred throughout the incubation period. Duplicate 2-ml samples were removed at 10-min intervals and precipitated in 4 volumes of ice-cold 10% TCA containing a thousand-fold excess of nonradioactive thymidine. The TCA-insoluble fraction was washed three times by centrifugation, resuspended in cold sea water with 5% TCA, and collected on GF/C glass fiber filters using a Millipore filtration manifold. The filters were then washed with 30 ml of ice-cold 5% TCA, air dried, and solubilized in Protosol (New England Nuclear) for 7 hr prior to the addition of 10 ml of Ecosint. Samples were allowed to sit overnight for dissipation of any chemiluminescence; they were then counted on an LKB 1219 liquid scintillation counter.

[^{3}H]Phenylalanine uptake rate as a function of time after fertilization. The experimental protocol used for determining the rate of uptake of [^{3}H]phenylalanine was essentially the same as the method described by White and Jacobs (4).

Cumulative incorporation of [^{3}H]phenylalanine into proteins. Aliquots of a 1% (v/v) slurry of fertilized *L. pictus* embryos were added to beakers containing [^{3}H]phenylalanine (specific activity 130 Ci/mmol, Amersham), yielding a final activity of 0.5 μCi/ml. Pseudopterosin A (4×10^{-5} M) or vehicle control was added approximately 3–5 min post fertilization. The procedure followed was essentially identical with that described previously for thymidine incorporation.

Chemicals. Pseudopterosin A was supplied by Dr. William Fenical (Scripps Institution of Oceanography, La Jolla, CA). Purified Pseudopterosin A (as ivory crystals) was stored at −70°. Pseudopterosin A solutions were freshly prepared using DMSO as the solvent. All other chemicals were supplied by Sigma Chemical Co. except where otherwise noted.

Results

Effects of Pseudopterosin A on sea urchin embryo cleavage. As shown in Fig. 1, Pseudopterosin A produced a concentration-dependent irreversible inhibition of the first cleavage in *S. purpuratus* sea urchin embryos. The embryo underwent highly synchronous divisions when incubated at 16° in either sea water or a 0.5% (v/v) solution of DMSO in sea water. The IC_{50} for the inhibition of cleavage was approximately 2.5×10^{-5} M. Observations made during the treatment periods showed no lysis, swelling, or morphological changes. An identical dose response curve was obtained using *S. Franciscanus* and *L. pictus* embryos (data not shown), indicating that the inhibitory activity of Pseudopterosin A was not restricted to a particular species of echinoderm embryo. Pseudopterosin A (50×10^{-5} M) produced 100% inhibition of the first mitosis but showed cytotoxicity.

Cell cycle study with Pseudopterosin A. Pseudopterosin A was added to sea urchin embryos at different times in the cell cycle. Fig. 2 shows that the degree of inhibition of egg cleavage declined progressively when Pseudopterosin A was added 10 min post fertilization. This observation suggests that events prior to the mitosis are critical to the mode of action of Pseudopterosin A. Eggs exposed to Pseudopterosin A later in the cell cycle (50 min after fertilization) completed the first cleavage but were arrested in the two-cell stage. When the drug was added any time after the end of the S_1 phase or before prophase, cytokinesis occurred normally.

Reversibility of Pseudopterosin A. The action of Pseudopterosin A appeared to be relatively selective. That is, the effects were time dependent and cell cycle dependent. As shown in Fig. 3, Pseudopterosin A must be in contact with the sea urchin embryo for at least the first 50 min post fertilization to inhibit the first cleavage. Pseudopterosin A effects were reversible by washing during the first 50 min of exposure. After this time, the effects could not be reversed by repeated washing.

Cytological examination of Pseudopterosin A in arrested sea urchin embryos. Microscopic examination of the embryos revealed that Pseudopterosin A blocked mitosis. Fig. $4A_1$ shows the control cell during early prophase, 60 min post

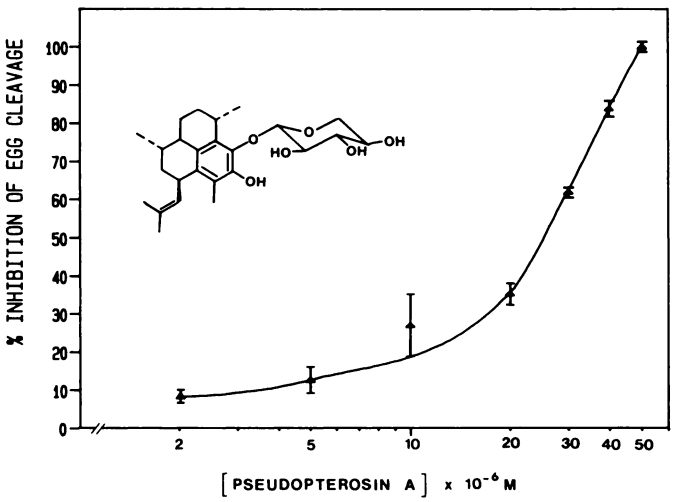

Fig. 1. Log dose response curve for inhibition of *S. purpuratus* embryo cleavage. Results from six representative experiments are shown. Pseudopterosin A was added 5 min after fertilization. The *error bars* represent standard error.

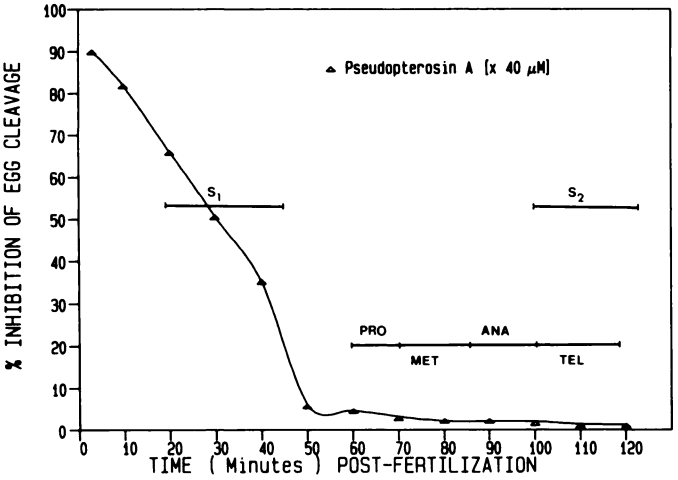

Fig. 2. The effect of Pseudopterosin A at different stages of the cell cycle in *S. purpuratus* sea urchin embryos. The fertilized sea urchin eggs were incubated with Pseudopterosin A at different times after fertilization. S_1 and S_2 are the first and second periods of DNA synthesis, respectively. *PRO*, *MET*, *ANA*, and *TEL* are prophase, metaphase, anaphase, and telophase of mitosis, respectively.

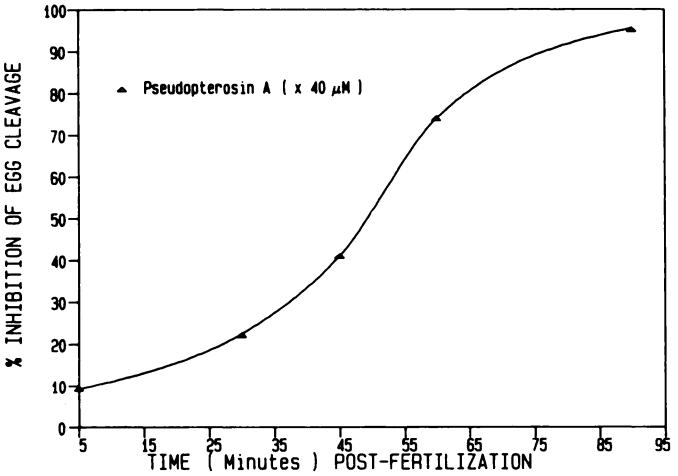

Fig. 3. Reversibility of Pseudopterosin A during the first division cycle of *S. purpuratus*. The drug was added 3 min post fertilization, then, after a certain period of time, a 1-ml sample of 1% (v/v) suspension of eggs in sea water was washed three times with 100 ml of filtered sea water.

fertilization, during which the fibrous chromosomes have begun to condense and become discernable as individual structures. During this phase the nuclear membrane begins to break down. Fig. $4B_1$ shows the cell at 80 min post fertilization. Metaphase has begun in the control embryos. As anaphase begins (Fig. $4C_1$), the centromers separate and the chromosomes begin to move toward the poles of the spindle. Fig. $4D_1$ shows an embryo in two-cell stage with nuclei in interphase. The cleavage membrane is visible, and the chromosomes are still condensed. Fig. 4, A_2–D_2, depicts the same time interval in cells exposed to 4×10^{-5} M Pseudopterosin A. The structural changes from 60 min to 120 min indicate that the cells did not progress beyond prophase. The chromatin was condensed and the nuclear membrane remained intact in these arrested embryos (Fig. 4, A_2–D_2).

Effect of Pseudopterosin A on [^{3}H]thymidine uptake. The effects of Pseudopterosin A on [^{3}H]dThd uptake rates were examined throughout the first cell cycle in *L. pictus* sea urchin embryos. Accurate determination of the extent of macromolecular synthesis using the labeled precursor technique generally requires that the entry of the radiolabeled precursor molecule into the cell be unimpeded. Fig. 5A shows the inhibition of thymidine uptake by Pseudopterosin A. In control embryos, the rate of thymidine uptake increased rapidly as a function of time after fertilization, as was observed by Vacquier and Brandiff (12). This rate gradually became maximal and stable at approximately 100–120 min after fertilization. Pseudopterosin A-treated eggs showed a decreased rate of uptake throughout the cell cycle. The rate of [^{3}H]dThd uptake did not increase after 60 min incubation.

Effect of Pseudopterosin A on cumulative [^{3}H]thymidine incorporation. To examine the possibility that DNA replication is altered, we studied the effects of Pseudopterosin A on [^{3}H]Thd incorporation into DNA throughout the first division cycle (Fig. 5B). Two periods of incorporation were observed. The S_1 period, occurring 30–50 min after fertilization, was initiated and completed well before the beginning of mitosis (M phase-independent S_1 period). The S_2 period occurs during mitosis 100–120 min post fertilization. Pseudopterosin A did not markedly depress incorporation during the S_1 period, but inhibited the incorporation of thymidine into DNA during mitosis (50 min post fertilization to 120 min). The degree of thymidine incorporation into DNA was significantly limited relative to control incorporation with this concentration of Pseudopterosin A (4×10^{-5} M).

Effect of Pseudopterosin A on [^{3}H]phenylalanine uptake rates. In *L. pictus* embryos, the rate of uptake of [^{3}H]phenylalanine is shown (Fig. 6A). The increased rate of amino acid uptake we observed during the first cell cycle in sea urchin embryos was in excellent agreement with results reported by Raff *et al.* (14). As can be seen, Pseudopterosin A markedly depressed the rate of uptake throughout the cell cycle. At 120 min, the rate of uptake was only 15% of the control.

Effect of Pseudopterosin A on cumulative [^{3}H]phenylalanine incorporation. The reduced incorporation of [^{3}H]phenylalanine into protein, observed in Fig. 6B, might be accounted for by a Pseudopterosin A-mediated inhibition of amino acid uptake. The [^{3}H]phenylalanine incorporation increased rapidly after fertilization in the control embryos. In our studies, this pattern of amino acid incorporation appears to be quite similar to that reported by Fry and Gross (15). As can be seen (Fig. 6B), in metaphase the incorporation curve shows a 10% depression at 60 min. This depression is believed to be the result of metaphase cells synthesizing proteins at 25% of the rate of interphase cells (15). Throughout the entire cell cycle, embryos incubated in the continuous presence of 4×10^{-5} M Pseudopterosin A incorporated the amino acid at 5% of the rate seen in the control. Because of the marked reduction in [^{3}H]phenylalanine uptake observed (Fig. 6A) in Pseudopterosin A pulsed preparations, the lack of incorporation seen during continuous Pseudopterosin A exposure (Fig. 6B) is probably the result of depressed amino acid transport.

Discussion

Pseudopterosin A, a diterpene riboside isolated from the soft coral *Pseudopterogorgia elisabethae*, represents a new chemical class of cell division inhibitors. In preliminary studies we found that Pseudopterosin A produced an irreversible, concentration-dependent inhibition of the sea urchin embryo cleavage (10).

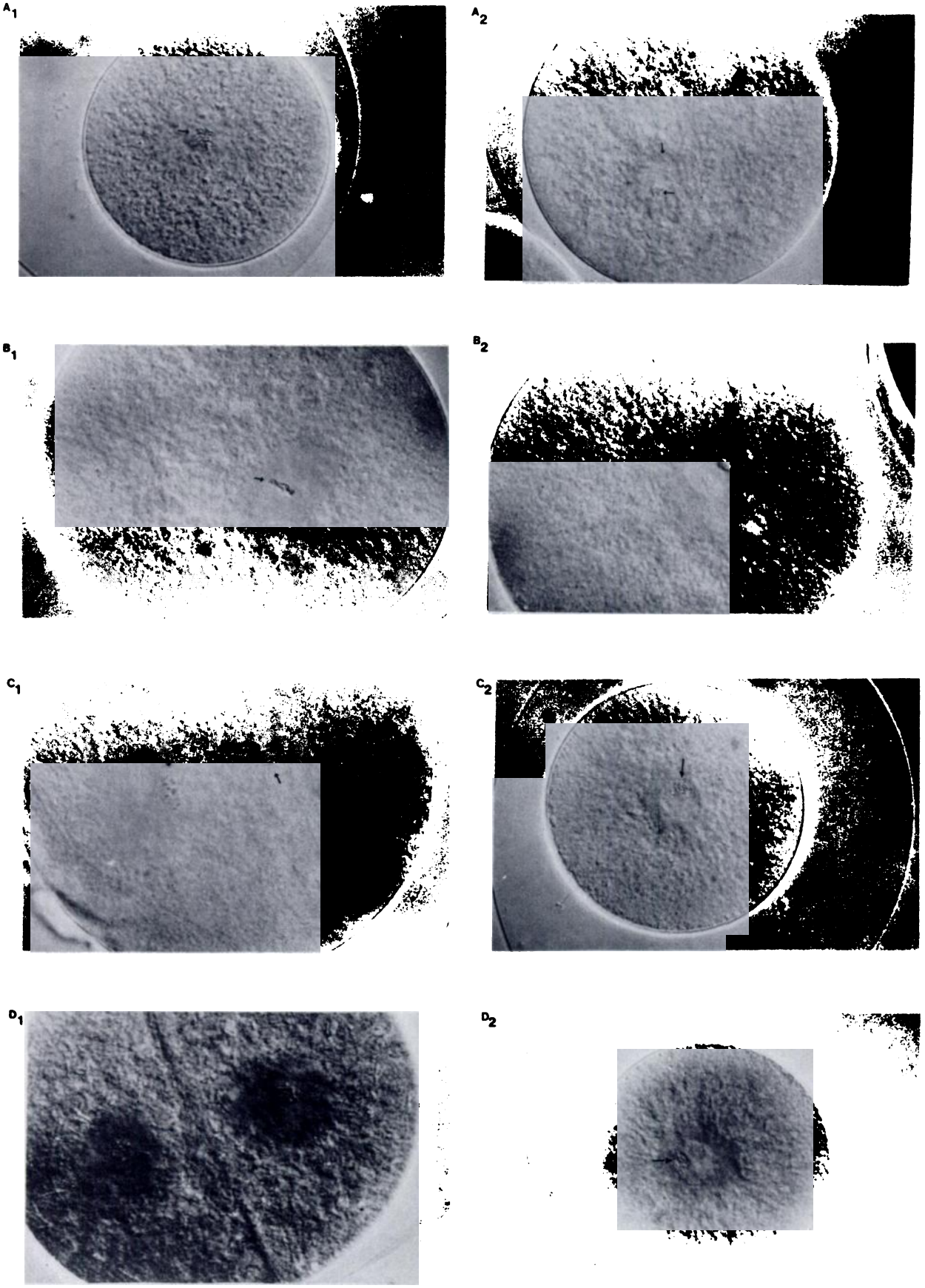

Fig. 4. *S. purpuratus* embryos following incubation in Pseudopterosin A or DMSO solvent control. A_1. Control embryo fixed at 60 min after fertilization to show normal early prophase. A_2. Embryo incubated in the continuous presence of 4×10^{-5} M Pseudopterosin A; the chromatin is condensed but the chromosomes are not visible. B_1. Control embryo fixed at 80 min metaphase; the chromosomes are aligned in a plane at the cell's equator, halfway between the poles of the spindle. B_2 was treated the same as A_2. The cells are still in an early prophase stage. The nuclear membrane is still visible. C_1. Control embryo fixed at 90 min late anaphase; the centromeres separate and the new chromosomes move toward the poles of the spindle. C_2. In the continuous presence of 4×10^{-5} M Pseudopterosin A, the embryo shows an early prophase nucleus. D_1. Control embryo fixed at 120 min shows an embryo in two-cell stage with nuclei in interphase. D_2. The treated cells show the same nucleus as an early prophase with chromosomal condensation.

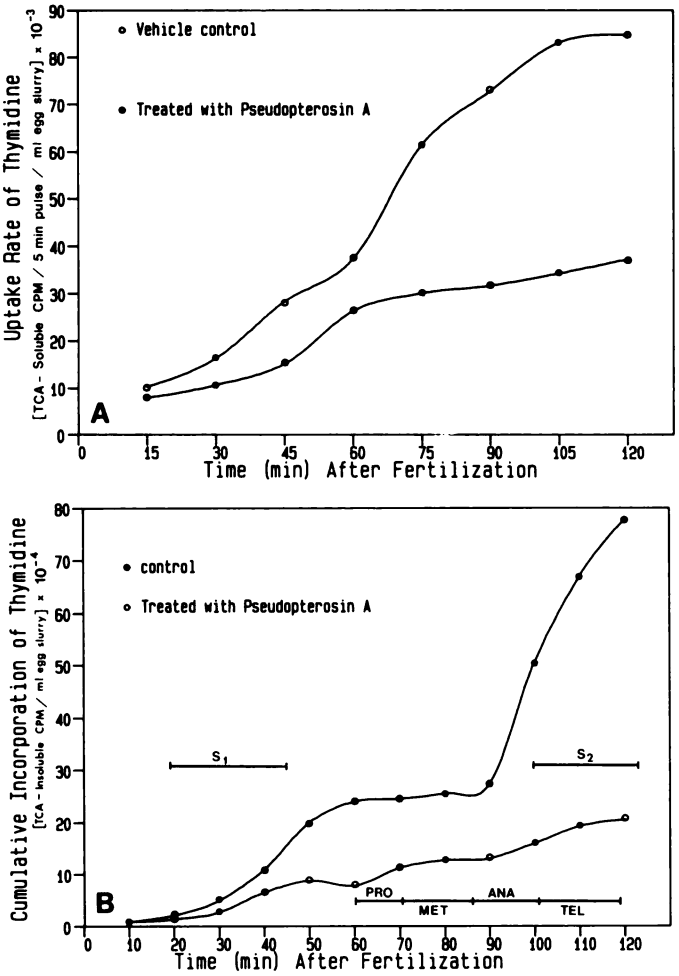

Fig. 5. A. [^{3}H]Thd uptake into fertilized eggs during the first division cycle. Pseudopterosin A (4×10^{-5} M) or DMSO (as control) was added 5 min after fertilization. At 15-min intervals after fertilization, duplicate egg aliquots were "pulsed" with [^{3}H]Thd for 5 min, washed three times by centrifugation, and the unincorporated TCA-soluble [^{3}H]Thd was measured. B. Effect of Pseudopterosin A on cumulative [^{3}H]thymidine incorporation during the first cell cycle in *L. pictus* sea urchin. Pseudopterosin A (4×10^{-5} M) or vehicle control was added 3 min after fertilization. [^{3}H]Thymidine was added immediately after fertilization. S_1 and S_2 are the first and second periods of DNA synthesis, respectively. *PRO, MET, ANA,* and *TEL* are prophase, metaphase, anaphase, and telophase of mitosis, respectively.

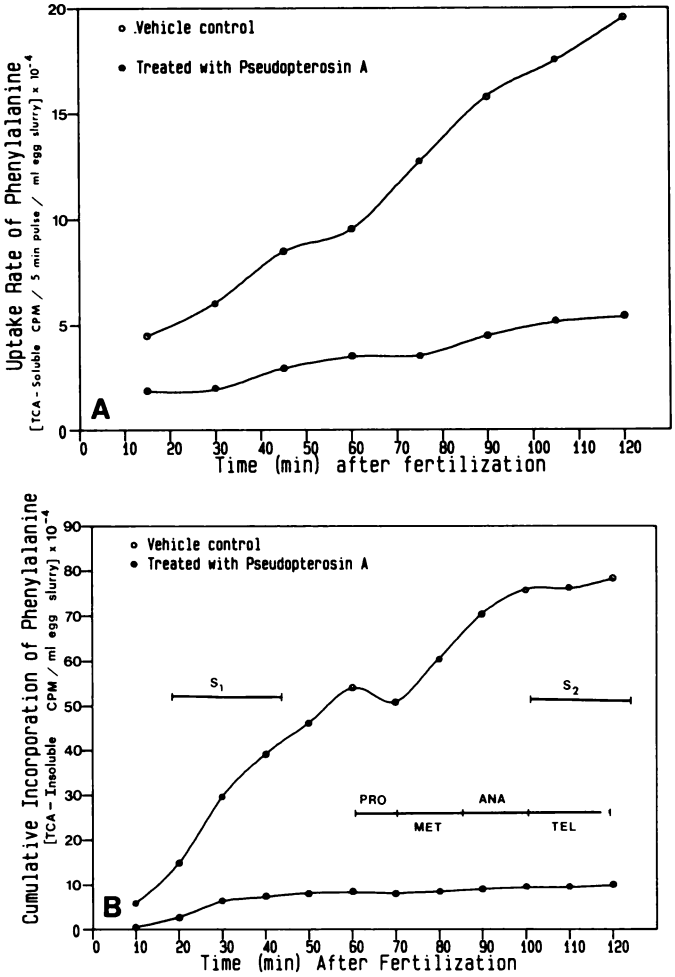

Fig. 6. A. Effect of Pseudopterosin A on [^{3}H]phenylalanine uptake rate during the first division cycle of *L. pictus*. Uptake rate is defined as the amount of [^{3}H]phenylalanine accumulated into the TCA-soluble pool by the end of the 5-min pulse. B. Effect of Pseudopterosin A on [^{3}H] phenylalanine incorporation during the first division cycle of *L. pictus*. Incorporation rate is defined as the amount of [^{3}H]phenylalanine accumulated into TCA-soluble material by the end of the pulse. S_1 and S_2 are the first and second periods of DNA synthesis, respectively. *PRO, MET, ANA,* and *TEL* are prophase, metaphase, anaphase, and telophase of mitosis, respectively.

Utilizing the fact that fertilized eggs progress synchronously through the cell cycle, we observed that the ability of Pseudopterosin A to inhibit the first cell division is rapidly reduced as eggs progress into mitosis. At 50 min post fertilization there was less than 10% inhibition of egg cleavage for the first cycle, whereas the second cycle was inhibited (data not shown). These results suggest that events prior to the beginning of the M phase are critical to the mode of action of Pseudopterosin A. Pseudopterosin A will be active only if it is in contact with the embryo for the first 55 min post fertilization; that is, it must be in contact with the cell prior to the initiation of prophase.

The cytological examination supported the kinetic studies and revealed that sea urchin embryos did not progress beyond early prophase when incubated in the presence of Pseudopterosin A (4×10^{-5} M). Chromatin was condensed into chromosomes but the nuclear membranes remained intact in Pseudopterosin A-arrested embryos, indicating that Pseudopterosin A exerts its inhibiting action prior to prophase. With respect to events occurring during mitosis, Hinegardner *et al.* (13) demonstrated that, with the exception of the first DNA replication period of the first cell cycle (the S_1 period), DNA synthesis (S_2) occurs simultaneously with telophase of mitosis during early development of the *S. purpuratus* embryo. The S_2 DNA replication period shares (with all subsequent replication periods) the common feature of partially overlapping mitosis. In contrast, the S_1 period occurs only during the first cell cycle which precedes mitosis, and is considered M phase independent.

The embryos treated with Pseudopterosin A (4×10^{-5} M) showed that, during the S_1 phase, [^{3}H]dThd incorporation was approximately 90% of the controls at 40 min post fertilization. During mitosis, the [^{3}H]dThd incorporation level remained relatively constant and the increase typical of S_2 was abolished (35% of control). With respect to [^{3}H]dThd uptake in sea urchin preparations, our data suggest that the S_2 blockade of incorporation could be the result of blockade of the increase in

thymidine uptake during mitosis. Vacquier and Brandiff (12) showed that the thymidine uptake rate increased rapidly after fertilization, and Nishioka and Magagna (16) showed that thymidine uptake was strictly dependent upon the presence of extracellular sodium. Pseudopterosin A produced 60% inhibition of uptake during the S_2 replicative period; it had less effect on [^{3}H]dThd uptake during S_1 than during S_2. This suggests that Pseudopterosin A may have a more marked effect on thymidine uptake during S_2. By using embryos preloaded with [^{3}H]dThd, we have demonstrated that the S_2 phase DNA synthesis is inhibited by Pseudopterosin A. This observation suggests that the ability of Pseudopterosin A to inhibit DNA synthesis might be a consequence of pharmacological effects that are independent of mitosis and not directly dependent on DNA synthesis. If mitosis could not be initiated, then the S_2 phase similarly would not be initiated. The uptake itself does not appear to be a physiological requirement, since sea urchin eggs are fully capable of developing in sea water completely lacking external metabolites (16).

Examination of the rates of amino acid uptake and incorporation into protein revealed that these processes were also inhibited by Pseudopterosin A. Wagenaar and Mazia (17) have shown that protein synthesis inhibitors prevent the breakdown of the nuclear membrane. Emetine blocked [^{3}H]thymidine incorporation completely during S_2 in echinoderm embryos, while producing no inhibition of incorporation during S_1. In contrast to Pseudopterosin A, these drugs inhibit the chromosomal condensation. The sites of action could be multiple, involving membrane transport and systems that are not cell cycle dependent as well as molecular processes that are mitosis associated or dependent. Pseudopterosin A might inhibit cleavage by inhibiting translation. At 200 μM (46 μg/ml), Pseudopterosin A had no effect on microtubule assembly *in vitro*.[1] The results presented demonstrate that Pseudopterosin A is active early in the cell cycle. The main difference between Pseudopterosin A and Emetine is: Emetine had no effect on thymidine incorporation, until almost the end of mitosis (18). Conversely, Pseudopterosin A had an effect on thymidine incorporation 30 min after fertilization. Emetine and Pseudopterosin A showed a rapid and complete inhibition of amino acid incorporation into proteins.

Gerace *et al.* (19, 20) have shown that the lamina polypeptides are involved in the structural organization of the nuclear envelope. Depolymerization of the lamina induces the breakdown of the nuclear envelope (21), and phosphorylation/dephosphorylation mediates the structural dynamics of the lamina during cell division (22, 23). Because Pseudopterosin A-treated embryos are arrested with the nuclear membrane intact, it would be of interest to determine if Pseudopterosin A has any influence on the rate of phosphorylation of the nuclear lamina during mitosis. This compound is also a potent anti-inflammatory agent that may act by indirectly interfering with eicosanoid production in PMA-treated preparations (8). Although phosphorylation/dephosphorylation reactions driven by protein kinase C have recently been implicated in the inflammatory process (24), we have no evidence to claim that the inhibition of mitosis is linked to the anti-inflammatory effects of Pseudopterosin A. This hypothesis will be explored.

[1] L. Wilson, personal communication.

Acknowledgments

The authors are indebted to Ms. Michelle Lee for technical assistance during the incorporation studies. We wish to thank Elise Clason for demonstration of the sea urchin assay. The authors are grateful to Dr. William Fenical (Scripps Institution of Oceanography) for his generous supplies of purified Pseudopterosin A.

References

1. Jacobs, R. S., and L. Wilson. Fertilized sea urchin eggs as a model for detecting cell division inhibitors, in *Modern Analysis of Antibiotics* (A. Aszalos, ed.). Marcel Dekker, Inc., New York, 481–493 (1986).
2. Jacobs, R. S., S. White, and L. Wilson. Selective compounds derived from marine organisms: effects on cell division in fertilized sea urchin eggs. *Fed. Proc.* **40**:26–29 (1981).
3. White, S. J., and R. S. Jacobs. Inhibition of cell division and of microtubule assembly by Elatone, a halogenated sesquiterpene. *Mol. Pharmacol.* **20**:614–620 (1981).
4. White, S. J., and R. S. Jacobs. Effect of stypoldione on cell cycle progression, DNA and protein synthesis, and cell division in cultured sea urchin embryos. *Mol. Pharmacol.* **24**:500–508 (1983).
5. O'Brien, E. T., R. S. Jacobs, and L. Wilson. Inhibition of bovine brain microtubule assembly *in vitro* by stypoldione. *Mol. Pharmacol.* **24**:493–499 (1983).
6. Glaser, K. B., and R. S. Jacobs. Molecular pharmacology of manoalide inactivation of bee venom phospholipase A_2. *Biochem. Pharmacol.* **53**:449–453 (1986).
7. Clason, E. L., and R. S. Jacobs. Inhibition of cell division and of the inflammatory process by the marine natural product pseudopterolide. *Fed. Proc.* **43**:3913 (1984).
8. Look, A. S., W. Fenical, R. S. Jacobs, and J. Clardy. The Pseudopterosins; a new class of antiinflammatory and analgesic natural products from the sea whip. *Proc. Natl. Acad. Sci. USA* **83**:6238–6240 (1986).
9. Mazia, D., and K. Dan. The isolation and biochemical characterisation of the mitotic apparatus of dividing cells. *Proc. Natl. Acad. Sci. USA* **38**:826–838 (1952).
10. Ettouati, W. S., and R. S. Jacobs. Inhibition of cell division in sea urchin eggs by the marine natural product Pseudopterosin A. *Fed. Proc.* **45**:580 (1986).
11. Mazia, D. Chromosome cycles turned on in unfertilized sea urchin eggs exposed to NH_4OH. *Proc. Natl. Acad. Sci. USA* **71**:690–693 (1974).
12. Vacquier, V. D., and B. Brandiff. DNA synthesis in unfertilized sea urchin eggs can be turned on and off by the addition and removal of procaine hypochloride. *Dev. Biol.* **47**:12–31 (1975).
13. Hinegardner, R. T., B. Rao, and D. E. Feldman. The DNA synthetic period during early development of the sea urchin egg. *Exp. Cell Res.* **36**:53–61 (1964).
14. Raff, R. A., J. W. Brandis, C. J. Huffman, A. L. Koch, and D. E. Leister. Protein synthesis as an early response to fertilization of the sea urchin egg: a model. *Dev. Biol.* **86**:265–271 (1981).
15. Fry, B. J., and P. R. Gross. Patterns and rates of protein synthesis in sea urchin embryos. *Dev. Biol.* **21**:105–124 (1970).
16. Nishioka, D., and L. S. Magagna. Increased uptake of thymidine in the activation of sea urchin eggs. *Exp. Cell Res.* **133**:363–372 (1981).
17. Wagenaar, E. B., and D. Mazia. The effect of emetine on first cleavage division in the sea urchin, *Strongylocentrotus purpuratus*, in *Cell Reproduction* (E. R. Dirksen, D. M. Prescott, and C. F. Fox, eds.). Academic Press, New York, 539–545 (1978).
18. Wagenaar, E. B. The timing of synthesis of proteins required for mitosis in the cell cycle of the sea urchin embryo. *Exp. Cell Res.* **144**:393–403 (1983).
19. Gerace, L., C. Comeau, and M. Benson. Organization and modulation of nuclear lamina structure. *J. Cell Sci. Suppl.* **1**:137–160 (1984).
20. Gerace, L., A. Blum, and G. Blobel. Immunocytochemical localization of the major polypeptides of the nuclear pore complex lamina fraction. Interphase and mitotic distribution. *J. Cell Biol.* **79**:546–566 (1978).
21. Gerace, L., and G. Blobel. The nuclear envelope lamina is reversibly depolymerized during mitosis. *Cell* **19**:277–287 (1980).
22. Ottaviano, Y., and L. Gerace. Phosphorylation of the nuclear lamina during interphase and mitosis. *J. Biol. Chem.* **260**:624–632 (1985).
23. Miake-Lye, R., and W. M. Kirschner. Induction of early mitotic events in a cell-free system. *Cell* **41**:165–175 (1985).
24. Touqui, L., B. Rothhut, A. M. Shaw, A. Fradin, and B. B. Vargaftig. Platelet activation—a role for a 40K anti-phospholipase A_2 protein indistinguishable from lipocortin. *Nature (Lond.)* **321**:177–180 (1986).

Send reprint requests to: Dr. Robert S. Jacobs, Department of Biological Sciences, University of California, Santa Barbara, CA 93106.