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Selective Inhibition of Cytokinesis in Sea Urchin Embryos by the Marine Natural Product Pseudopterolide

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SUMMARY

Low concentrations (1–10 μM) of the marine natural product pseudopterolide inhibited cytokinesis and induced formation of multinucleate cells in fertilized *Strongylocentrotus purpuratus* embryos. As determined by immunofluorescence microscopy using fluorescent stains for actin filaments, microtubules, and chromosomes, pseudopterolide inhibited cytokinesis selectively by disrupting the contractile ring, whereas spindle microtubule organization and mitotic chromosome segregation to opposite spindle poles were unimpaired. At somewhat higher concentrations (16–20 μM), pseudopterolide induced formation of microtubule spiral asters, which are believed to be caused by rotation of the cytoplasm relative to the cell cortex. The effects of pseudopterolide on cytokinesis were cell-cycle dependent. The actions of pseudopterolide in fertilized sea urchin embryos were strikingly similar to the actions of another marine natural product, stypoldione, a structurally unrelated orthoquinone that reacts covalently with the sulfhydryl groups of glutathione, β-mercaptoethanol, cysteine, and a number of proteins [*Mol. Pharmacol.* 35:635–642 (1989)]. In the present study, pseudopterolide was also found to react with sulfhydryl groups of glutathione, β-mercaptoethanol, and cysteine. The results indicate that the cellular target for pseudopterolide, like the target for stypoldione, may be an especially sensitive sulfhydryl-containing protein involved in the formation or function of the contractile ring.

Pseudopterolide, an unusual irregular diterpenoid (Fig. 1), is a marine natural product isolated from the soft coral *Pseudopterogorgia acerosa* (1). Early studies on the biological activity of pseudopterolide revealed that the compound had anti-inflammatory activity (2). The compound was also found to inhibit cell proliferation in sea urchin embryos and in cultured Chinese hamster ovary cells. Incubation of fertilized sea urchin eggs with pseudopterolide resulted in the formation of multiple nuclei, apparently as a consequence of repetitive normal mitotic divisions in the absence of cytokinesis. This finding led to the suggestion (2) that pseudopterolide might inhibit cell division selectively by disrupting the formation or normal function of the contractile ring, an actin- and myosin-containing structure that is necessary for cytokinesis (3–5).

In the present study, we used immunofluorescence microscopy, with fluorescent stains for actin filaments, microtubules, and chromatin, to investigate the action of pseudopterolide on mitosis and cytokinesis in fertilized *Strongylocentrotus purpuratus* embryos. We also investigated the reversibility of the compound and the time dependence for its onset of action. The results indicate that low concentrations of pseudopterolide (approximately 10 μM and below) inhibit cytokinesis selectively, whereas mitosis and nuclear division are unimpaired. Mitosis occurs normally, but the contractile ring does not form and cell cleavage does not occur. The selective inhibition of cytokinesis by pseudopterolide in sea urchin embryos resembles the action of the cytochalasins (3) and the marine natural product stypoldione (6). Pseudopterolide possesses a unique chemical structure that is distinct from the cytochalasins and from stypoldione. However, we found that pseudopterolide, like stypoldione (7), can react with sulfhydryl residues. We hypothesize that the selective action of pseudopterolide may be caused by an interaction of the compound with a highly sensitive sulfhydryl-containing cell component that is critical to contractile ring formation and cytokinesis.

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Materials and Methods

Sea urchin gametes and embryos, pseudopterolide incubations, and effects on cell cleavage. *S. purpuratus* gametes were

ABBREVIATIONS: CFA, calcium-free artificial; DAPI, 4′,6-diamidino-2-phenylindole; DTNB, 5,5′-dithiobis(2-nitrobenzenoic acid); EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; PABA, *p*-aminobenzoic acid; PBS, phosphate-buffered saline; PIPES, piperazine-*N,N′*-bis(2-ethanesulfonic acid).

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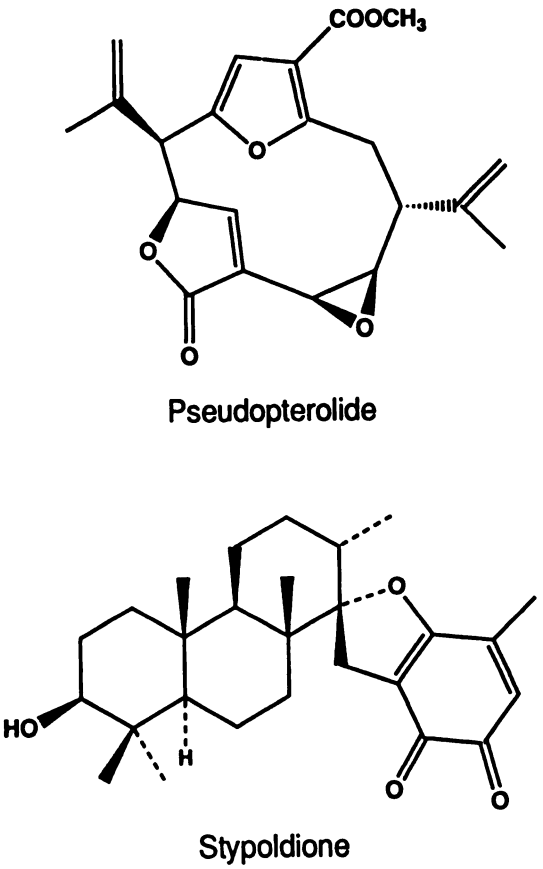

Fig. 1. Structures of pseudopterolide and stypoldione.

obtained by intracoelomic injection of 0.5 M KCl and were cultured in sea water, as previously described (6, 8). All experiments with sea urchin embryos were carried out at 15.5°. Briefly, eggs were shed into sea water, filtered three times through 150-μm Nitex mesh (Tetko, Inc., Elmsford, NY) to remove jelly coats, and concentrated by allowing the eggs to settle to the bottom of a beaker and aspirating the excess sea water. Eggs were resuspended in fresh filtered sea water at a concentration of approximately 1% (v/v). Sperm was collected directly from the aboral surface of male sea urchins and diluted into sea water (one drop of concentrated sperm/10 ml of sea water).

Eggs were fertilized by adding diluted sperm to the diluted egg suspensions (1 ml of sperm suspension/30 ml of diluted egg suspension). Pseudopterolide was dissolved in a vehicle consisting of 5% ethanol and 95% propylene glycol. Pseudopterolide at appropriate concentrations or vehicle control (5% ethanol/95% propylene glycol) was added to yield a final ratio of vehicle to sea water of 1:200 (v/v) and the desired final concentration of compound. Embryos to be examined by immunofluorescence microscopy were obtained as described above, except that final egg suspensions were prepared in CFA sea water containing 10 mM PABA, to remove fertilization envelopes. The embryos were washed twice with PABA-containing CFA sea water to remove excess sperm, resuspended at a ratio of 1 to 100 (v/v) in CFA sea water without PABA, and passed six times through 150-μm Nitex mesh filters to remove the fertilization membranes (6).

To determine the period during the cell cycle that was most sensitive to the action of pseudopterolide and after various periods of time, pseudopterolide was added and incubation was continued. To examine the reversibility of the compound, pseudopterolide was added to embryo suspensions within 1 min of fertilization. Exposure to the compound was terminated at desired times by sedimenting and resuspending the embryos three times in fresh sea water. Quantitation of division was performed by counting the number of divided and nondivided embryos after the control embryos had progressed to the end of first cleavage (approximately 120 min after fertilization).

Immunofluorescence microscopy. Fixation and staining of embryos for immunofluorescence microscopy were performed according to the method of Hollenbeck and Cande (9), with the following modifications (6). Embryos were gently pipetted onto air-dried polylysine-coated (1 mg/ml) coverslips and allowed to adhere for 2 min. Coverslips were drained and immediately placed into small weigh boats containing 2 ml of PIPES extraction buffer (0.4% Triton X-100 in 50 mM PIPES, 10 mM EGTA, 6 mM $MgSO_4$, pH 6.8). After 4.5–5.5 min, coverslips were fixed in 1% (w/v) paraformaldehyde, 1% (w/v) glutaraldehyde, in PIPES extraction buffer, for 10 min. Coverslips were washed three times with a solution of PBS, (137 mM NaCl, 2.7 mM KCl, 8 mM sodium phosphate, 1.5 mM potassium phosphate, pH 7.5), followed by two 5-min washes with a 4 mg/ml solution of sodium borohydride in PBS, to decrease aldehyde fluorescence. Coverslips were then washed three times with PBS, and 25 μl of mouse monoclonal anti-α-tubulin, diluted 1/100 in PBS, were added (6). Coverslips were incubated at 37° for 60 min and washed three more times with PBS. A 25-μl volume of a secondary staining solution was then added, which consisted of fluoroscein isothiocyanate conjugated to an anti-mouse IgG (1/25 in PBS) to stain the microtubules, rhodamine-conjugated phalloidin (1/20 in PBS) (Molecular Probes) to stain the actin filaments, and DAPI (10 μg/ml) to stain the chromatin and chromosomes. Coverslips were then incubated for an additional 60 min at 37° and washed three times with PBS. Coverslips were mounted onto slides with a solution of 0.1 M sodium borate, pH 8.0, and sealed with clear nail polish. Slides were stored at 4° in the dark until used. The fixed and stained embryos were viewed with a Zeiss Photomicroscope III, using Plan-Neofluor 25× and 40× objectives. Embryos were photographed using Kodak technical Pan film at 200 or 800 ASA film speed.

DTNB sulfhydryl assay. Different concentrations of stypoldione or pseudopterolide (50 μl in 100% ethanol) were added to solutions of β-mercaptoethanol, cysteine, or glutathione in sea water and were incubated for 15 min at room temperature (final concentration of sulfhydryl compounds, 20 μM; total reaction volume, 1 ml). DTNB (10 μl in 100% ethanol; final concentration, 200 μM) was then added, and the absorbance at 412 nm was determined after 30 min of incubation.

Results

Inhibition of cell division by pseudopterolide. *S. purpuratus* embryos develop relatively synchronously after fertilization, when incubated in sea water at 15.5°. The first mitosis typically occurs between 70 and 90 min after fertilization, and cleavage of the cell to form two blastomeres (cytokinesis) begins in anaphase and is usually complete at approximately 110 min. The second mitotic division ensues rapidly after termination of the first division, normally beginning approximately 130–140 min after fertilization and lasting approximately 15 min. Cytokinesis giving rise to four blastomers begins during anaphase and is complete approximately 150 min after fertilization.

Pseudopterolide inhibited the first division of sea urchin embryos in a concentration-dependent manner, with 50% inhibition (IC_{50}) occurring at a pseudopterolide concentration of approximately 10 μM (Fig. 2). Division was blocked completely at slightly greater than 40 μM pseudopterolide. Similar to results previously described with this compound in sea urchin eggs and cultured Chinese hamster ovary cells (2), at the pseudopterolide concentration that inhibited cleavage by 50% (10 μM) nuclear division appeared to occur in the absence of cytoplasmic division in blocked embryos. No morphological evidence for the formation of cleavage furrows could be detected, by light microscopy using Nomarski optics, in cells that failed to cleave (Fig. 3).

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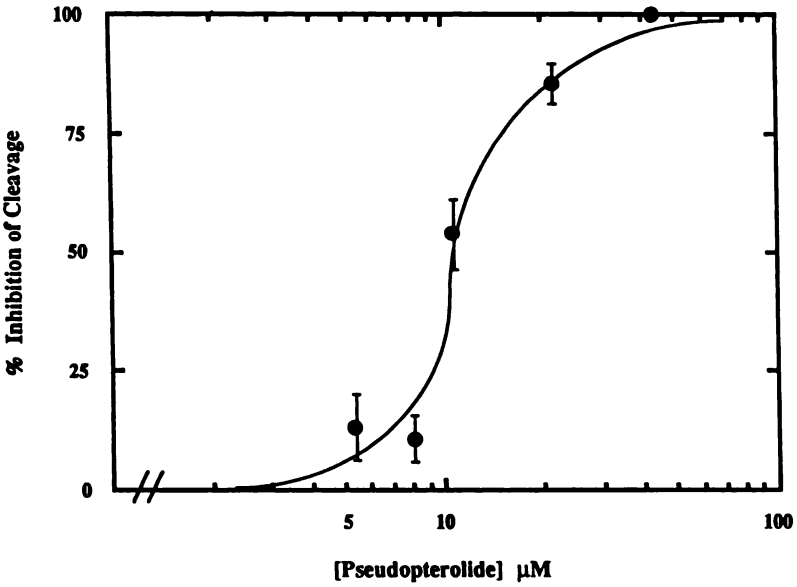

Fig. 2. Concentration dependence of inhibition of cell division by pseudopterolide. Indicated concentrations of pseudopterolide were added to *S. purpuratus* embryos 30 min after fertilization. The number of embryos that had undergone cleavage to the two-cell stage were scored at 120 min of incubation, and the percentage of inhibition of cleavage was calculated. *Error bars*, standard error.

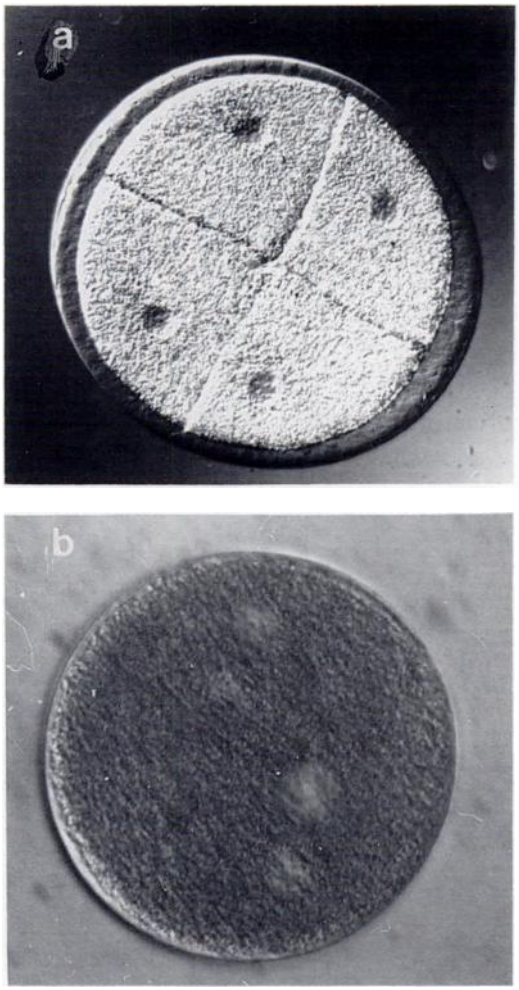

Fig. 3. Effects of 10 μM pseudopterolide on cytokinesis and cleavage furrow formation. Pseudopterolide or solvent control was added to egg suspensions 30 min after fertilization. Embryos were viewed with Nomarski optics at approximately 160 min after fertilization. a, Untreated control embryo at the four-cell stage. b, Embryo treated with pseudopterolide. Magnification, 800×.

Selective inhibition of cytokinesis at low concentrations of pseudopterolide. Direct evidence that low concentrations of pseudopterolide inhibited cytokinesis without detectably affecting mitotic spindle organization or function was obtained by immunofluorescence light microscopy. A monoclonal antibody to tubulin was used to visualize mitotic spindle microtubules, rhodamine-conjugated phalloidin was used to visualize actin filaments, and DAPI was used to visualize chromatin and chromosomes (see Materials and Methods). It was clear that both the first and second mitoses occurred normally but that the contractile rings required for formation of the cleavage furrows, to yield the two-blastomere and four-blastomere stages, did not form. A typical example of such a blocked embryo is shown in Fig. 4, at 155 min after fertilization, when control embryos are normally progressing from the two-cell to the four-cell stage.

By immunofluorescence microscopy, untreated embryos at this stage of development were characterized by a prominent microtubule-free space between the two-blastomere products of the first division, two sets of mitotic spindles, and condensed and organized chromosomes. Shown in Fig. 4a is an untreated embryo in late anaphase of the second division, stained with a monoclonal antibody against tubulin. The embryo contained two clearly distinct blastomeres that were separated by a prominent microtubule-free space (Fig. 4a, *arrow*), indicating the location of the plasma membranes separating the two cells. Each blastomere contained a normal spindle in late anaphase. Each half-spindle remained attached to its opposite half-spindle by a thin microtubule bridge. A single, relatively compact, mass of chromatin was located approximately centrally in each half-spindle (Fig. 4b). Staining with rhodamine-phalloidin revealed the presence of a distinct contractile ring (Fig. 4c, *arrow*) that lay perpendicular to a more diffusely staining band of apparently organized actin filaments, which may be aligned microvilli in the cleavage furrow from the preceding cytoplasmic division (5).

Embryos incubated with 10 μM pseudopterolide underwent normal mitotic division but did not undergo cytokinesis. Shown in Fig. 4d is an embryo in mid-anaphase of the second division, stained with the tubulin antibody. The embryo was a single

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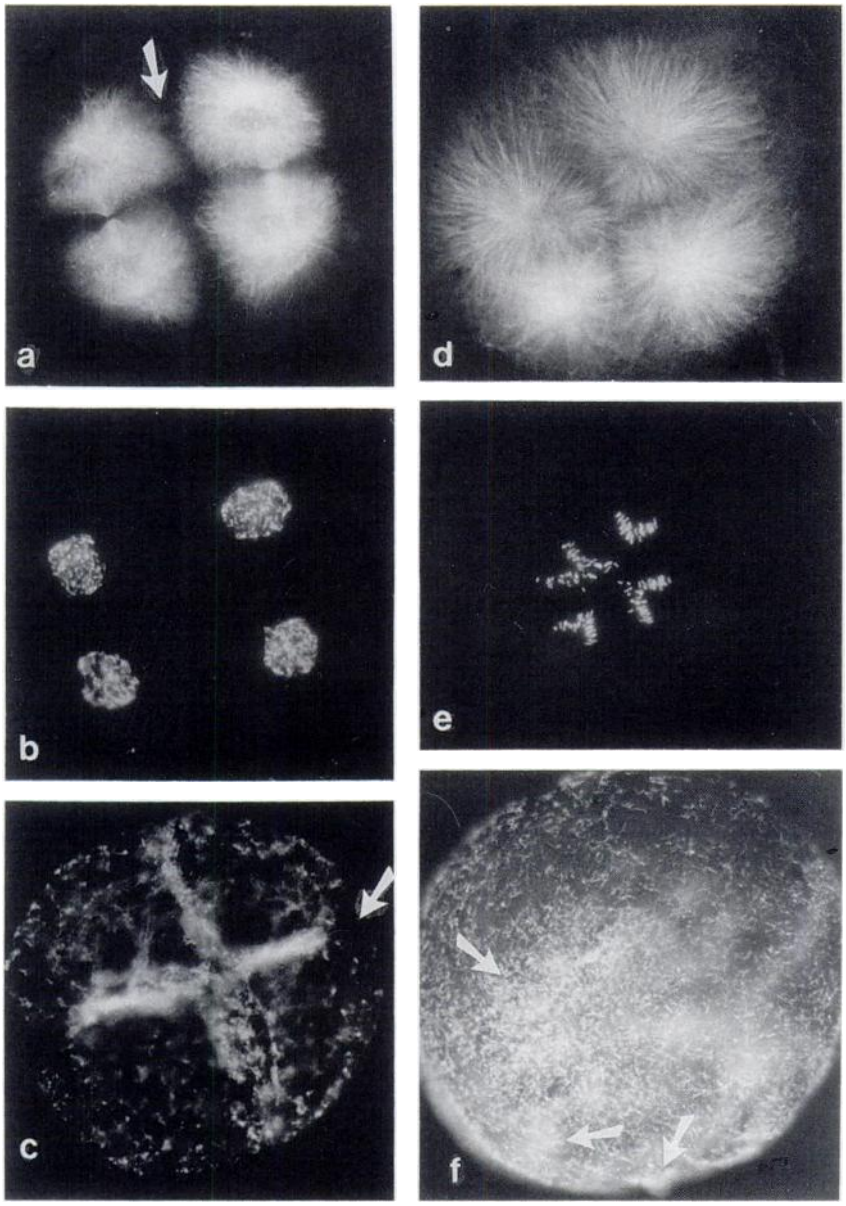

Fig. 4. Effects of 10 μM pseudopterolide on the contractile ring. Embryos were extracted and fixed 155 min (untreated controls) or 158 min (pseudopterolide-treated) after fertilization and were stained with anti-tubulin (a and d), DAPI (b and e), or rhodamine-phalloidin (c and f). Pseudopterolide or solvent control was added to egg suspensions 30 min after fertilization. a and b, Untreated control embryo, showing two late anaphase spindles with the chromosomes grouped near the poles. In a, a prominent microtubule-free space (*arrow*) indicates the location of the plasma membranes separating the two cells. c, Phalloidin staining of the same embryo. The contractile ring of the second cell cleavage (*arrow*) lay perpendicular to a more diffusely staining region of organized actin that may be microvilli in the cleavage furrow. d and e, Embryo incubated with 10 μM pseudopterolide. Cytokinesis had not taken place in this embryo, but two spindles and four separate sets of chromosomes are clearly visible, indicating that the embryo was in mid-anaphase of the second mitosis. f, Staining with rhodamine-phalloidin, revealing the absence of a contractile ring or cleavage furrows. The actin formed small fibrous aggregates that coalesced, in several regions near the surface of the embryo, into irregular dense patches (*arrows*).

cell. The two spindles were normal, except that there were no membranes to separate one blastomere from the other, so that the microtubules of all of the half-spindles were overlapping. Each half-spindle contained a compact mass of chromosomes (Fig. 4e). There was no sign of a cleavage furrow, and staining with rhodamine-phalloidin revealed a complete absence of contractile rings (Fig. 4f). Instead, the actin appeared to be organized into small fibrous aggregates that were distributed near the surface of the embryo, which in many cells appeared to coalesce into irregular dense patches (Fig. 4f, *arrows*). Similar results were obtained at the time of first division (data not shown).

Early block of division and induction of "microtubule spirals" by high concentrations of pseudopterolide. The selective ability of pseudopterolide to inhibit cytokinesis was lost at concentrations of pseudopterolide somewhat higher than the IC_{50}. For example, many blocked embryos incubated with 20 μM pseudopterolide (>IC_{80}) did not undergo cytokinesis or mitosis but, instead, became blocked shortly after pronuclear migration and apparently before pronuclear fusion. A typical embryo blocked at such an early stage is shown in Fig. 5. Interestingly, many microtubules were present in such cells, but they were distributed abnormally, in the form of "microtubule spirals" (Fig. 5a) (6, 10, 11), which are believed to be caused by rotation of the cytoplasm relative to the cell cortex (11). Similar microtubule spirals have been observed at the same stage of development when normal sea urchin embryos were exposed to elevated temperatures (11) or when embryos were incubated with the marine natural product stypoldione (6) (see below). DAPI staining of the embryo incubated with 20 μM pseudopterolide allowed clear visualization of the two distinct pronuclei, with the sperm DNA still highly compact and brightly stained and the maternal DNA diffuse and less brightly stained (Fig. 5b). A similar pattern was observed in most of the embryos incubated with 20 μM pseudopterolide.

Cell-cycle sensitivity and reversibility of pseudopterolide. To investigate the period during the cell cycle of the developing embryo that was most sensitive to pseudopterolide,

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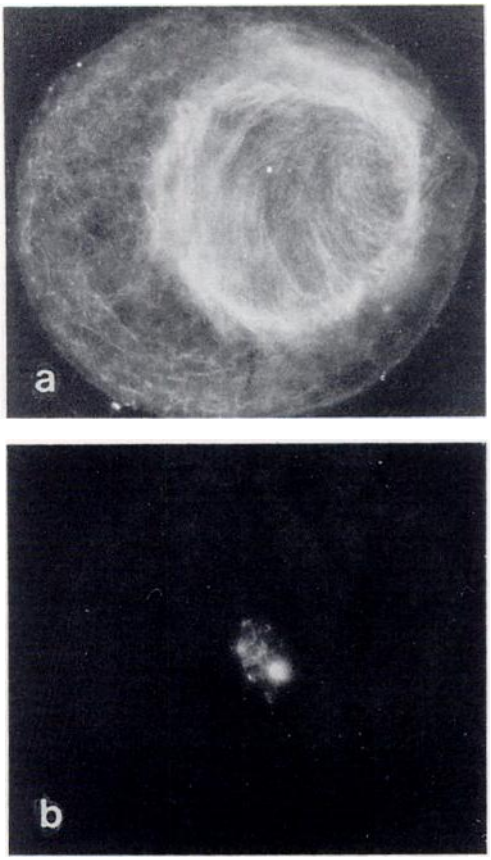

Fig. 5. Induction of microtubule spiral asters by 20 μM pseudopterolide. Pseudopterolide was added 30 min after fertilization; embryos were extracted and fixed 165 min after fertilization and were stained with antitubulin antibody (a) or DAPI (b).

embryos were fertilized and pseudopterolide was added at different times after fertilization. Incubation was then continued until control embryos had completed first cleavage. A concentration of 16.2 μM pseudopterolide was used, which inhibits division by approximately 75% when present beginning immediately after fertilization. Pseudopterolide inhibited division by the maximal amount at this concentration when it was added as late as 45 min after fertilization (Fig. 6). Thus, the action of pseudopterolide on cytokinesis does not seem to be due to effects on early events that occur during the first 45 min of development. When pseudopterolide was added 60 min after fertilization, approximately 10–20 min before the first mitosis, the compound still induced approximately 50% of the inhibition of cytokinesis that it induced when added immediately after fertilization. Even when added 90 min after fertilization, when a large proportion of embryos had completed the first division, the compound still induced approximately 20% of the inhibition it induced when added immediately after fertilization. Thus, the period of greatest sensitivity to pseudopterolide for inhibition of cytokinesis appears to be just before or at the time of mitosis and cytokinesis.

We examined how readily the action of pseudopterolide on cytokinesis could be reversed by incubating embryos with the compound, beginning immediately after fertilization, and removing the compound, by sedimenting and resuspending the embryos in fresh sea water, at different times during development. As shown in Fig. 7, exposure of embryos for brief periods of time shortly after fertilization induced substantial inhibition of cleavage. For example, exposure to the drug for 30 min beginning immediately after fertilization resulted in approximately 70% inhibition. Thus, the compound appears to be able to exert its action rapidly, and the action is not readily reversed after removal of the compound from the medium.

Inhibition of contractile ring formation by stypoldione. The selective inhibition of cytokinesis by pseudopterolide and the ability of the compound to induce formation of microtubule spirals were remarkably similar to the actions of the orthoquinone stypoldione (Fig. 1) in fertilized sea urchin embryos (6). Low concentrations of stypoldione selectively inhibited cytokinesis, while mitosis continued normally (50% inhibition of cytokinesis occurred at 2.5 μM stypoldione), whereas somewhat higher concentrations of stypoldione (20–80 μM) inhibited cell progression shortly after fertilization and induced formation of microtubule spirals (6). In the previous study on stypoldione, we did not determine whether stypoldione inhibited formation of the contractile ring.

In the present work, we incubated fertilized embryos with 5 μM stypoldione and triply stained the blocked cells at the time of mitotic division, to determine whether the contractile ring was present. Shown in Fig. 8 is an embryo fixed 165 min after fertilization. The embryo was a single cell containing two mid-anaphase mitotic spindles, each with two distinct compact masses of chromosomes (Fig. 8, a and b). There was no remnant of a first contractile ring or any sign of the first cleavage furrow normally found in control embryos (see Figs. 3a and 4c), and only a vague indication of the possible presence of organized actin filaments was present in the putative division plane for the first cytoplasmic cleavage. Similar absence of contractile rings occurred in embryos in late anaphase of the first mitotic division (data not shown).

Sulfhydryl reactivity of pseudopterolide. The similarities in the biological actions of pseudopterolide and stypoldione prompted us to determine whether pseudopterolide, like stypoldione, could react with sulfhydryl groups. A colorimetric assay using Ellman's reagent (DTNB) (12) was used to examine the reactivity of pseudopterolide with sulfhydryl groups of β-mercaptoethanol, cysteine, and glutathione, sulfhydryl compounds previously found to react with stypoldione (7). Different concentrations of pseudopterolide or stypoldione were first incubated with the sulfhydryl compounds, using a standardized set of conditions (see Materials and Methods), and then the remaining free sulfhydryl concentration was determined colorimetrically (Fig. 9). Pseudopterolide was less reactive with sulfhydryl compounds than stypoldione, but it is clear that pseudopterolide could react with all three of the sulfhydryl compounds tested.

Discussion

Selective inhibition of cytokinesis by pseudopterolide. By immunofluorescence microscopy using fluorescent phalloidin, a toxin that binds selectively to filamentous actin, we found that low concentrations of the marine natural product pseudopterolide (1–10 μM) inhibited formation of the contractile ring and prevented cell cleavage during the first and second mitotic divisions of sea urchin embryos. Using a fluorescent tubulin antibody to stain microtubules and fluorescent DAPI to stain chromatin, we found that mitotic spindles formed and appeared to function normally. Microtubule organization in spindles of embryos incubated with pseudopterolide was indis-

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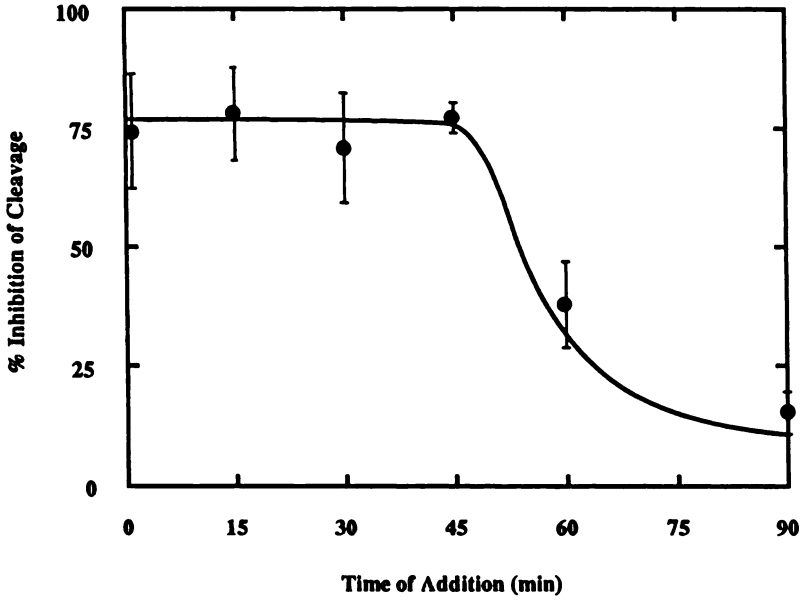

Fig. 6. Cell-cycle sensitivity to pseudopterolide. Pseudopterolide (16.2 μM) was added to embryo suspensions at the times indicated after fertilization, and incubation was continued until control embryos had completed first cleavage. *Error bars*, standard error.

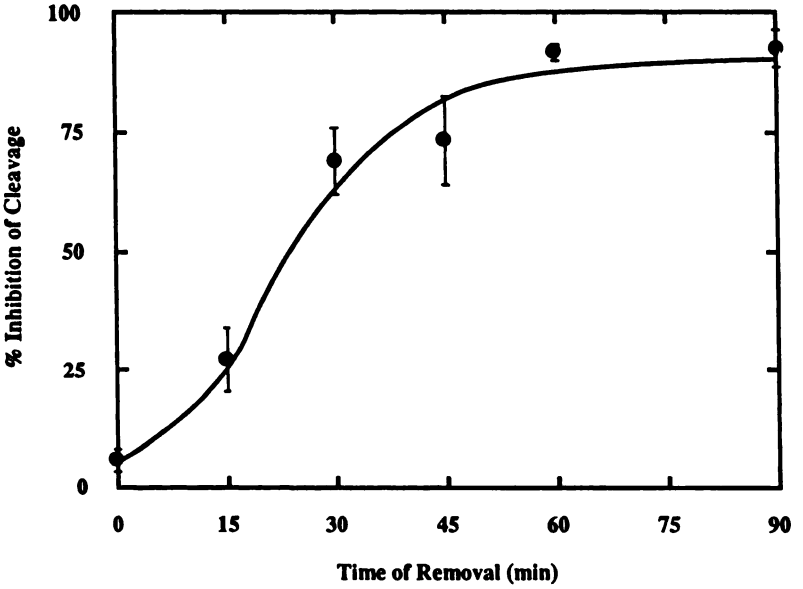

Fig. 7. Reversibility of pseudopterolide. Pseudopterolide (16.2 μM) was added to embryo suspensions immediately after fertilization and was removed at the indicated times. *Error bars*, standard error.

tinguishable from that of control spindles, and chromosomes appeared to segregate properly to their opposite spindle poles. Thus, pseudopterolide uncoupled mitosis from cytokinesis at the lowest effective concentrations, apparently by inhibiting formation of the contractile ring.

By adding pseudopterolide to fertilized egg suspensions at different times before the normal completion of first cleavage, we found that the compound inhibited division when added as little as 20–30 min before the onset of mitosis. The data indicate that pseudopterolide might inhibit cytokinesis by acting on a cell component or cell process whose function is critical during the period of mitosis and cytoplasmic division. The fact that pseudopterolide could inhibit division even when present in the incubation medium for short periods of time beginning shortly after fertilization indicates that the compound may bind very tightly or irreversibly to its cellular target and that the target is present early in the cell cycle.

Induction of microtubule spirals. At somewhat higher concentrations than required to inhibit cell division by 50% (e.g., 20 μM), pseudopterolide inhibited progression of egg cells through the cell cycle shortly after sperm entry and induced formation of spiral microtubule asters. Spiral microtubule asters were thought initially to be part of the normal sequence of events occurring during the first cell cycle in *S. purpuratus* embryos (10). However, spiral asters do not appear to occur normally (6, 9, 11) but, rather, form by rotation of the cytoplasm relative to the cell cortex upon incubation of embryos at elevated temperatures (11) or incubation with certain compounds, such as stypoldione (6) or pseudopterolide. The mechanism responsible for induction of spiral asters by pseudopterolide and stypoldione is unknown but may be due to a common action of the two compounds on actin filaments that function in anchoring the cell cortex to the internal cytoplasm (see below).

Interaction of pseudopterolide with sulfhydryl-containing compounds. The mechanism by which pseudopter-

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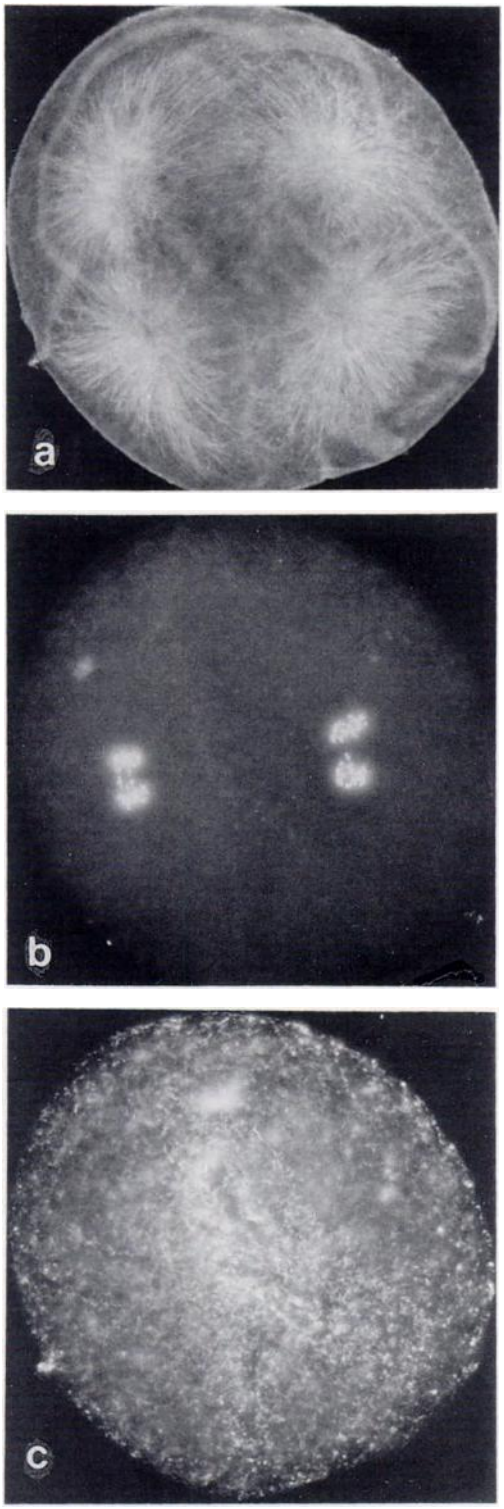

Fig. 8. Inhibition by stypoldione of contractile ring formation. Stypoldione (5 μM) was added to embryos 30 min after fertilization. Embryos were extracted and fixed 165 min after fertilization and were stained with antitubulin (a), DAPI (b), or rhodamine-phalloidin (c). The embryo shown is a single cell containing two mid-anaphase spindles (a), each with two distinct compact masses of chromosomes (b). There were no cleavage furrows, and only vague indication of the possible presence of organized actin filaments can be seen in the putative division plane for the first cytoplasmic cleavage (c).

olide selectively inhibits cytokinesis is unknown and is currently under investigation. Also, the chemical mechanism for the reactivity of pseudopterolide with sulfhydryl groups is under investigation.[1] One possibility is that pseudopterolide inhibits cytokinesis by reacting with sulfhydryl groups of one or more biological molecules. Such a hypothesis is strengthened because of the similarities in the biological actions of pseudopterolide and stypoldione. In the present study, we found that pseudopterolide reacted with glutathione, β-mercaptoethanol, and cysteine, compounds previously found to react with stypoldione. However, the reactivity of pseudopterolide with sulfhydryl groups was weaker than that of stypoldione under the conditions used and, in addition, relatively low reactivity was observed at 10 μM pseudopterolide (Fig. 9), the concentration that half-maximally inhibited cytokinesis (Fig. 2). Nevertheless, the possibility that pseudopterolide inhibits cytokinesis through its sulfhydryl reactivity remains attractive, because pseudopterolide inhibits cytokinesis approximately 4-fold less potently than stypoldione and because, due to its lipid solubility, the reactivity of pseudopterolide *in vitro* may not reflect the true situation in cells.

Possible disruption of actin filament structure or function by a selective interaction of pseudopterolide with a sensitive sulfhydryl-containing molecule. It is surprising that compounds that react with sulfhydryl residues appear to inhibit cytokinesis and contractile ring formation selectively. Pseudopterolide, like stypoldione, is a highly lipophilic molecule and, if the two compounds act through sulfhydryl reactivity, one reason for the apparent selectively of the two compounds may be related to their hydrophobicity. Both compounds might accumulate preferentially in hydrophobic cell compartments such as the plasma membrane or internal membrane-containing organelles. In addition, potential cytoplasmic receptors for the compounds might be protected by the high concentrations of glutathione present in the cytoplasm (1–5 mM) (13–15). Thus, cytoplasmic receptors for pseudopterolide and for stypoldione may be protected by a combination of the lipid-partitioning of the compounds and the high concentrations of glutathione in the cytoplasm. The fact that pseudopterolide and stypoldione both induce formation of microtubule spiral asters is also consistent with this idea, because such action of the compounds might be exerted in the cortex of the cell, adjacent to the plasma membrane.

A possible target for pseudopterolide is the contractile ring. Both filamentous actin and myosin are found in this structure, which forms adjacent to the plasma membrane at the cleavage furrow during cytokinesis and is essential for furrowing and cytoplasmic division (3–5). Compounds that react with sulfhydryl groups can inhibit the ATPase activity of myosin and the binding of myosin to actin (16, 17). Further, injection into cells of *N*-ethylmaleimide-modified heavy meromyosin (18) or antibodies against myosin (19) block cytokinesis selectively, producing multinucleate cells. Thus, due to its location near the plasma membrane, its importance in cell cleavage, and the importance of sulfhydryl groups in its function, a reasonable possibility is that the receptor for pseudopterolide and for stypoldione resides in the contractile ring itself or that it may be a sulfhydryl-containing molecule involved in anchoring the

[1] S. Sharma and B. Lipshutz, unpublished observations.

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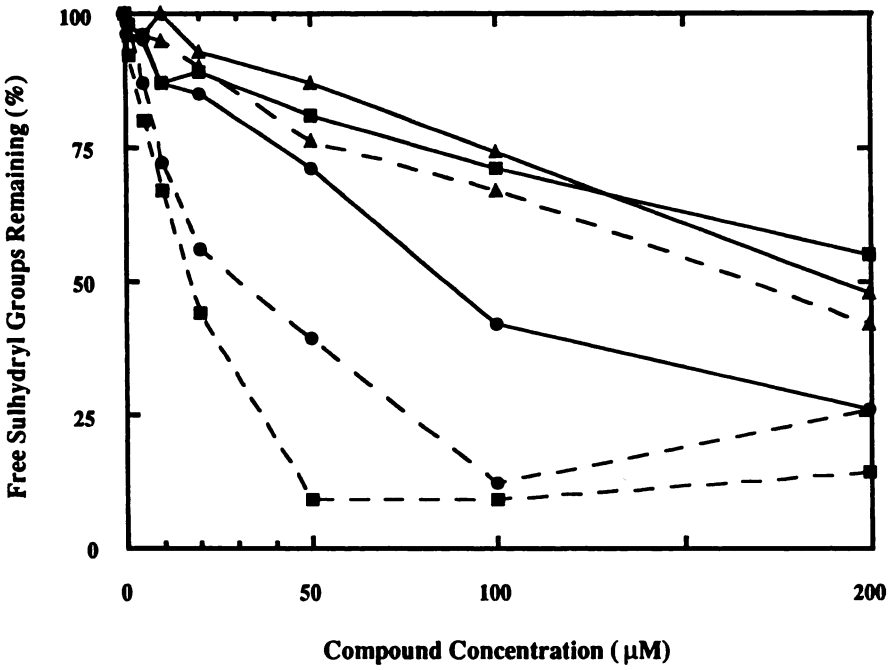

Fig. 9. Sulfhydryl reactivities of pseudopterolide and stypoldione. β-Mercaptoethanol, cysteine, or glutathione (20 μM) was incubated with the indicated concentrations of pseudopterolide (——) or stypoldione (– – –) for 15 min at room temperature. The remaining free sulfhydryl concentration was then determined colorimetrically (see Materials and Methods). ●, β-Mercaptoethanol; ▲, glutathione; ■, cysteine.

contractile ring to the cytoplasmic surface of the plasma membrane.

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References

1. Bandurraga, M. M., W. Fenical, S. F. Donovan, and J. Clardy. Pseudopterolide, an irregular diterpenoid with unusual cytotoxic properties from the caribbean sea whip, *Pseuodpterogorgia acerosa*. *J. Am. Chem. Soc.* **104**:6463–6465 (1982).
2. 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).
3. Schroeder, T. E. The contractile ring. I. Fine structure of dividing mammalian (HeLa) cells and the effects of cytochalasin B. *Z. Zellforsch. Mikrosk. Anat.* **109**:431–449 (1970).
4. Schroeder, T. E. The contractile ring and furrowing in dividing cells. *Ann. N. Y. Acad. Sci.* **582**:78–87 (1990).
5. Otto, J. J., and T. E. Schroeder. Association of actin and myosin in the contractile ring. *Ann. N. Y. Acad. Sci.* **582**:179–184 (1990).
6. O'Brien, E. T., D. J. Asai, R. S. Jacobs, and L. Wilson. Selective inhibition of cytokinesis in sea urchin embryos by low concentrations of stypoldione, a marine natural product that reacts with sulfhydryl groups. *Mol. Pharmacol.* **35**:635–642 (1989).
7. O'Brien, E. T., D. J. Asai, A. Groweiss, B. H. Lipshutz, W. Fenical, R. S. Jacobs, and L. Wilson. Mechanism of action of the marine natural product stypoldione: evidence for reaction with sulfhydryl groups. *J. Med. Chem.* **29**:1851–1855 (1986).
8. Jacobs, R. S., and L. Wilson. Fertilized sea urchin eggs as a model for detecting cell division inhibitors. *Drugs Pharm. Sci.* **27**:481–493 (1986).
9. Hollenbeck, P. J., and W. Z. Cande. Microtubule distribution and reorganization in the first cell cycle of fertilized eggs of *Lytechinus pictus*. *Eur. J. Cell Biol.* **37**:140–148 (1985).
10. Harris, P., M. Osborn, and K. Weber. A spiral array of microtubules in the fertilized sea urchin egg cortex examined by indirect immunofluorescence microscopy and electron microscopy. *Exp. Cell Res.* **126**:227–236 (1980).
11. Schroeder, T. E., and D. E. Battaglia. "Spiral asters" and cytoplasmic rotation in sea urchin eggs: induction in *Strongylocentrotus purpuratus* eggs by elevated temperature. *J. Cell. Biol.* **100**:1056–1062 (1985).
12. Ellman, G. L. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* **82**:70–77 (1959).
13. Rapkine, L. Chemical processes of cellular division. *C. R. Hebd. Seances Acad. Sci.* **191**:871–874 (1930).
14. Ii, I., and H. Sakai. Glutathione reductase in the sea urchin egg. *Biochim. Biophys. Acta* **350**:151–161 (1974).
15. Fahey, R. C., S. D. Mikolajczyk, G. P. Meier, D. Epel, and E. J. Carroll, Jr. The glutathione thiol-disulfide status in the sea urchin egg during fertilization and the first cell division cycle. *Biochim. Biophys. Acta* **81**:336–345 (1976).
16. Sekine, T., and W. W. Keilley. The enzymatic properties of *N*-ethylmaleimide modified myosin. *Biochim. Biophys. Acta* **81**:336–345 (1964).
17. Silverman, R., E. Eisenberg, and W. W. Kielley. Interaction of SH_1-blocked HMM with actin and ATP. *Nature New Biol.* **240**:207–208 (1972).
18. Meeusen, R. L., J. Bennett, and W. Z. Cande. Effect of microinjected *N*-ethylmaleimide modified heavy meromyosin on cell division in amphibian eggs. *J. Cell Biol.* **86**:858–865 (1980).
19. Mabuchi, I., and M. Okuno. The effects of myosin antibody on the division of starfish blastomeres. *J. Cell Biol.* **74**:251–263 (1970).

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