INHIBITION OF THE PROGESTERONE-DEPENDENT INDUCTION OF MEIOSIS BY GAMMEXANE IN XENOPUS LAEVIS OOCYTES

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Summary

Progesterone induces in vitro maturational events in Xenopus laevis oocytes. We report that gammexane (γ -chlorocyclohexane), an inositol analog, strongly inhibits progesterone action, providing a new tool for studying the effect of steroid on meiosis. The compound does not block the overall protein synthesis on oocytes. The antagonist effect is reversible and might take place during early stage of progesterone action since it does not abolish the activity of progesterone activated cytoplasm on maturation. It has been suggested that progesterone is effective at the surface level in Xenopus oocytes, and it is postulated that the inhibitor acts on the plasma membrane, possibly by affecting phosphatidyl-inositol metabolism and/or turnover.

Introduction

Xenopus laevis oocytes in the first meiotic prophase progress synchronously to the second meiotic metaphase, when submitted to progesterone exposure in vitro (1). They remain in the second meiotic metaphase until fertilization or activation. Microinjection experiments have indicated that progesterone may act first upon the oocyte surface (1, 2), suggesting a site of action of the steroid at the membrane level. Recent evidence in favour of this proposal was provided

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by experiments using La^{3+} (3) and propranolol-like drugs (4), which were able to mimick the action of progesterone. Moreover, an extensive range of amphiphilic cationic drugs, such as neuroleptics, tricyclic antidepressants, anorexiants, local anaesthetics and antiarrhythmic agents were also found to be potent non steroidal inducers of maturational events in Xenopus laevis oocytes (5). These results reinforce the suggestion that progesterone may act first at the membrane level (6), possibly by interacting with membrane phospholipids and by promoting thereby well defined Ca^{2+} displacement (3). We now report that gammexane (γ-chloro-cyclo-hexane or lindane), a widely used insecticide which exerts probably some of its effects as an inositol antagonist (7) interfering with phosphatidylinositol metabolism (8, 9), is an inhibitor of progesterone-induced maturation in Xenopus laevis oocytes.

Materials and Methods

Ripe oocytes of Xenopus laevis females were collected as described elsewhere (10) and submitted to collagenase treatment in order to obtain full-grown oocytes free of follicular cells (11). The experiments were performed at room temperature in a modified Barth's medium (12). The induction of maturation was routinely achieved by continuous exposure of oocytes to progesterone. Maturation was scored by the outward demonstration of a white spot at the animal pole representing germinal vesicle breakdown (GVBD). Gammexane 0.02 - 1 mM was used as antagonist. It was dissolved in dimethylformamide and added to the Barth medium, pH 7.4, 60 min to 1 min before the exposure of oocytes to various concentrations of progesterone 0.01 - 20 μM.

Proteins were studied by a double labelling technique (13, 14), using radioactive leucine obtained from Amersham : L-(4,5-^{3}H) leucine 58 Ci/mmol and L-(U-^{14}C) leucine 324 mCi/mmol. Appropriate amounts of non radioactive amino acid were added to obtain identical concentrations of ^{3}H and ^{14}C-labelled aminoacids and a $^{3}H/^{14}C$ desintegration per minute (dpm) ratio of about 10.

Twenty-five oocytes were exposed to progesterone (1 μM) in the presence of gammexane (1 mM) and twenty-five oocytes were exposed to progesterone alone (1 μM), until the appearance of GVBD in the latter group of oocytes. Both groups of oocytes were injected with ^{3}H leucine and incubated for 2 hr. Control oocytes for progesterone plus gammexane

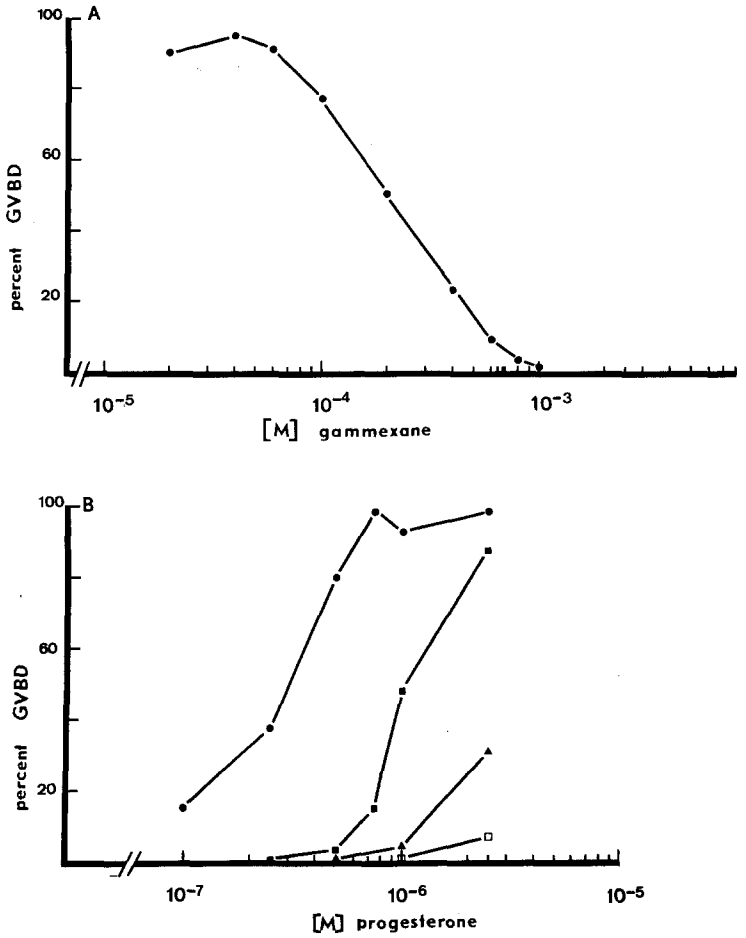

Figure 1 : Inhibition of the progesterone-dependent release of meiosis by gammexane in the oocytes of Xenopus laevis

The data represent the percentage of GVBD, of 100 oocytes incubated at room temperature in 10 ml Barth medium (pH 7.4). The oocytes are all taken from the same female for each type of experiments (A or B). Comparable results were obtained with oocytes taken from three other females. Maturation was scored after 18 hr continuous exposure to progesterone (in the absence or in the presence of gammexane).

A. Frequency of oocyte maturation induced by 1 µM progesterone, as a function of gammexane concentration. Gammexane was added 5 min before progesterone in the oocyte suspension.

B. Effects of progesterone on the frequency of oocyte maturation either in the absence (●) or in the presence of 0.1 mM (■), 0.2 mM (▲) or 0.5 mM (□) gammexane.

treated oocytes were incubated in Barth medium and received ^{14}C leucine.

Treated and control oocytes were pooled and homogenized together in 2 ml of the saline solution. The 105,000 g supernatant was obtained and protein analysis was performed using dialysis concentration and electrophoresis, as previously described (13). Sodium dodecyl sulfate (SDS 0.1%) polyacrylamide gel (6%) electrophoresis was performed at 2 mA per tube per 20 min and then at 5 mA per tube. Gels were re-electrophoresed in 7% acetic acid for 2 hr and immediately sliced. Soluene (0.5 ml) was added, and the suspension was left 3 hr at 50°C. Ten ml of a mixture of PPO (0.6g), POPOP (0.6g) and toluol (1 liter) were added to each vial.

Results

Gammexane is here described as an inhibitor of progesterone-induced maturation in Xenopus laevis oocytes (fig. 1). In oocytes continuously exposed to 1 μM progesterone (fig. 1A), the addition of gammexane in the incubation medium led to dose-dependent inhibitory effect. At 1 mM concentration, gammexane completely prevents the hormonal action, including the appearance of the Maturation Promoting Factor (MPF) as tested in recipient oocytes (11). Dose response curves with progesterone alone and with progesterone in the presence of 3 different concentrations of gammexane are shown on the section B of the figure 1.

To examine the possibility that the effects of gammexane are neither toxic nor irreversible, oocytes incubated during 18 hours in 5 mM gammexane alone were extensively washed and exposed to 10 μM progesterone. GVBD was observed in all oocytes. Further, activated cytoplasm removed from progesterone matured oocytes (10) was able to induce maturation when injected into oocytes prealably treated for 30 min with 5 mM gammexane and then continuously exposed to the same agent.

It has been shown previously that during progesterone-induced maturation in vitro, several electrophoretically defined soluble protein peaks are selectively increased (13,14). Fig. 2 shows that the normalized pattern of

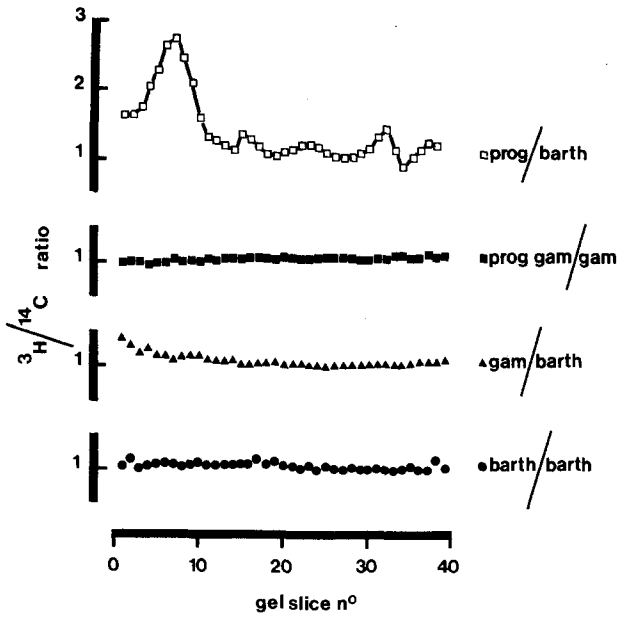

Figure 2 : Proteins during continuous treatment with hormone and gammexane.

Approximately 16 hr after exposure to 1 µM progesterone and 1 mM gammexane (when progesterone-treated oocytes show 100% GVBD), 25 oocytes are injected with ^3H leucine and incubated for 2 hr in modified Barth medium, and 25 oocytes treated with gammexane alone are injected with ^{14}C leucine and incubated for 2 hr (Prog. gam./gam.). Parallel experiment in the absence of gammexane is shown for comparison (Prog./Barth). Controls are obtained in the absence of progesterone and antagonist (Barth/Barth) or in the presence of gammexane alone (Gam./Barth). $^3H/^{14}C$ ratios have been normalized in order to make easier the comparison between various experiments (3,13,14).

synthesized proteins in oocytes exposed to gammexane and to progesterone did not show any peak of selective protein. However the antagonist effect of gammexane is not attributable to an inhibition of the overall protein synthesis machinery since the $^3H/^{14}C$ ratio of proteins obtained under the action of gammexane was not lower than in untreated oocytes.

Discussion

To explain the mechanism of the inhibitory effect of

gammexane on the progesterone induced GVBD, it is proposed that this inositol antagonist interacts in some way at the membrane level, possibly by interfering with phosphatidylinositol turnover and/or metabolism. It has been reported that gammexane inhibits phosphorus turnover in phosphatidylinositol of human lymphocytes cultures (8) and completely suppresses the acetylcholine-stimulated synthesis of phosphatidylinositol in brain slices (9).

Preceding experiments using amphiphilic cationic drugs as inducers of maturational events (5) have suggested that the mechanism of progesterone action in the induction of meiotic maturation may involve an initial step at the membrane level involving phospholipid disruption and defined Ca^{2+} displacement (3,5). These cationic drugs are able to concentrate into cellular membranes and to disturb the turnover of phospholipids with concomitant release of Ca^{2+} displaced from phosphatidic acid components (9). If the maturational events triggered by progesterone start first at the oocyte surface, the site of action of gammexane may involve also plasma membrane since the steps following hormone exposure, i.e. preferential synthesis of soluble proteins (14), appearance of the Maturation Promoting Factor MPF (11) and rupture of the germinal vesicle GVBD, are also inhibited (5). Nevertheless our results indicate that gammexane does not exert its inhibitory effects on progesterone-induced maturation by a direct effect on protein synthesis machinery since the overall $^{3}H/^{14}C$ ratio is not depressed. Indeed when MPF containing activated cytoplasm is injected into gammexane treated oocytes, selective protein synthesis and GVBD occur.

Whatever gammexane acts in interfering with phospholipids or by another mechanism, it may provide a new tool for exploring the membrane site of action of progesterone in the induction of meiotic maturation in Xenopus laevis oocytes.

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