Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 850–854, February 1982
Developmental Biology

Cyclic AMP-mediated control of meiosis: Effects of progesterone, cholera toxin, and membrane-active drugs in *Xenopus laevis* oocytes

(adenylate cyclase/cell division/insulin/methylase inhibitor/phosphatidylinositol)

SABINE SCHORDERET-SLATKINE*, MICHEL SCHORDERET†, AND ETIENNE-EMILE BAULIEU‡§

*Département de Gynécologie et d'obstétrique, Hôpital Cantonal, Genève, Switzerland; †Département de Pharmacologie, Ecole de Médecine, Genève, Switzerland; and ‡U 33 Institut National de la Santé et de la Recherche Médicale, Laboratoire des Hormones, 94270 Bicêtre, France

Communicated by Elwood V. Jensen, October 2, 1981

ABSTRACT **Progesterone depressed rapidly (50% at 1 min) and persistently cyclic AMP (cAMP) concentration that had been elevated by cholera toxin in *Xenopus laevis* oocytes. cAMP remained below 1 pmol per oocyte (mean basal level) for ≈1 hr and thereafter rose to ≈120% of control values, while germinal vesicle (nucleus) breakdown did not occur. In the absence of cholera toxin, progesterone treatment for 6 hr maintained cAMP concentration below the basal level (but not lower than 80%), and germinal vesicle breakdown occurred. Experiments in the presence of phosphodiesterase inhibitors suggested that progesterone modulates adenylate cyclase activity. The maturation promoting factor, which is formed after 3–5 hr of progesterone treatment and provokes germinal vesicle breakdown after its injection into untreated oocytes, also decreased cAMP concentration, an observation that may explain its "autoamplification." Nonsteroidal inducers of meiosis reinitiation (e.g., propranolol, methoxyverapamil, mersalyl) diminished the cholera toxin-mediated accumulation of cAMP, in contrast to compounds devoid of meiotic-inducing capacity and antagonists to progesterone action, such as gammexane (an inositol analogue) and 5′-deoxy-S-(2-methylpropyl)-5′-thioadenosine (a methylase inhibitor), that increased the nucleotide level. The fine control, suggested by the effects of small changes in cAMP levels, gives evidence of great sensitivity to a critical determinant governing meiotic cell division.**

A large body of data suggests the involvement of membrane sites for progesterone and other steroidal and nonsteroidal inducers of *in vitro* meiosis reinitiation in *Xenopus laevis* (1–3). Membrane interactions are followed by intracellular events, such as Ca^{2+} movements, nucleus-independent protein synthesis, protein phosphorylation/dephosphorylation, and the formation of a cytoplasmic "maturation-promoting factor" (MPF). MPF itself can induce meiotic cell division (maturation) in ≈2–3 hr, when it is transferred into recipient oocytes that have not been exposed to progesterone (4, 5).

In addition, a regulatory role for cyclic AMP (cAMP) known to be implicated in the control of cell division processes (6, 7), has been suggested on the basis of two series of experiments. Purified subunits of cAMP-dependent protein kinase, isolated from rabbit muscle, have been injected into recipient oocytes (8). The catalytic subunit inhibited the progesterone-induced process, whereas the regulatory subunit directly induced cell division (as did the inhibitor of the catalytic subunit). Secondly, progesterone-induced reinitiation of meiosis was inhibited by cholera toxin. This observation led to experiments which suggested that cAMP is involved in the control of progesterone-induced meiosis (9, 10). This, together with the results of MPF injected at various times into the oocytes (10), indicated a regulatory role for cAMP in MPF formation and amplification—i.e., during the 3- to 5-hr period of progesterone exposure. Measurements of cAMP in progesterone-treated oocytes of *Xenopus laevis* have been ambiguous. They showed either no changes (11, 12) or an early and short decrease for 10 min (13), or no initial change but a later and transitory decrease taking place between hours 3 and 5 of exposure to progesterone (14).

By using cholera toxin as a magnifying tool, we show that progesterone can decrease cAMP levels and presumably adenylate cyclase activity in *Xenopus laevis* oocytes almost immediately and with a persistent effect. Furthermore, MPF itself is able to decrease cAMP levels. The effects of various pharmacological agents known to mimic progesterone (1, 15–17) and those of agents devoid of meiotic-inducing capacity and antagonists to progesterone action were also investigated. It appears that, in order to allow germinal vesicle breakdown (GVBD) to occur, cAMP should remain below control levels from the beginning of exposure to progesterone or other active agents for up to 3–5 hr. Under such conditions, enough active MPF is formed, which in turn can also depress the level of cAMP, providing a possible mechanism for the process of autoamplification.

MATERIALS AND METHODS

Meiosis Reinitiation in *Xenopus laevis* Oocytes. Oocytes (stages V and VI) were isolated, exposed to progesterone or other agents, and studied as described (5). They were preincubated with cholera toxin (0.1 nM, Schwarz/Mann) for periods indicated in specific experiments. Progesterone or other agents were applied for either 15 min or 6 hr, two periods of time representative of rapid or prolonged effects, respectively. Injections of MPF were performed as described (5).

Measurements of cAMP. These were determined as before (10) and expressed as percentage of basal level ±SEM; 100% refers to the basal cAMP level. The SEM of control (untreated) oocytes was always <4% and is not indicated on the graphs. Because the basal level varies between oocytes taken out from different females (0.7–1.5 pmol per cell), data from several experiments were pooled by first normalizing the results within each experiment and expressing each point as percentage of basal level in oocytes from that particular female. Points are mean values ±SEM from three or more experiments.

RESULTS

Effect of Progesterone on cAMP Levels. Progesterone at 10 μM led to a rapid and sustained decrease in cAMP to ≈90% of the control level. This small decrease was statistically signif-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: MPF, maturation-promoting factor; cAMP, cyclic AMP; IBMX, 3-isobutyl-1-methyl-xanthine; GVBD, germinal vesicle breakdown; SIBA, 5′-deoxy, 5′-S-isobutyl-5′-thioadenosine.

§ To whom reprint requests should be sent.

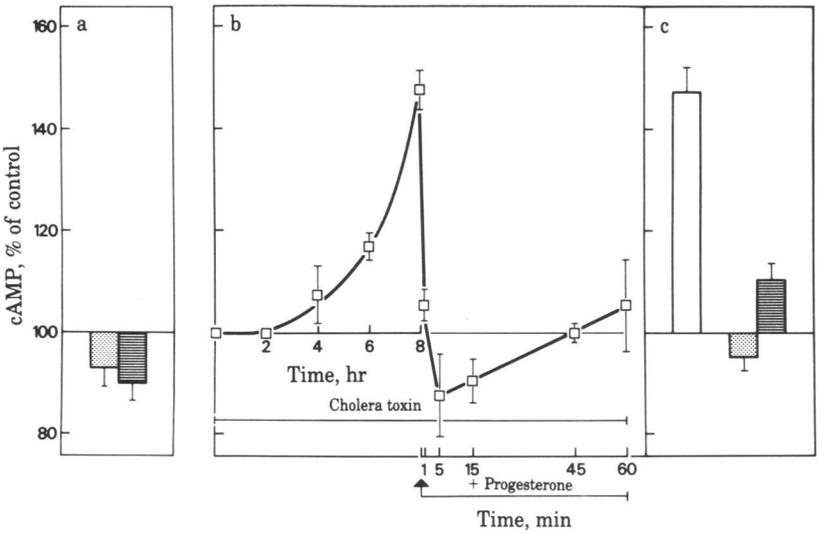

FIG. 1. Effect of progesterone on cAMP levels in oocytes treated with hormone alone or hormone plus cholera toxin. (*a*) cAMP level after 15-min (▨) and 6-hr (▤) treatment with 10 μM progesterone. Each value is the mean percentage (± SEM) of three pools of 10 oocytes versus the cAMP content of three control oocyte pools (100%). After homogenization of oocytes, 40 μl of supernatant (in duplicate) was used for cAMP measurement. (*b*) Time course of cAMP decrease during the first hr of exposure to progesterone of cholera toxin-treated oocytes. *Xenopus laevis* oocytes were incubated with cholera toxin (0.1 nM) for 9 hr. They were exposed to progesterone (10 μM) for various periods of incubation (1–60 min) during the last hr of cholera toxin treatment. Each value is obtained as in *a*. (*c*) Comparison between a 15-min and a 6-hr treatment with progesterone of cholera toxin-treated oocytes. Cholera toxin (0.1 nM) was added at time 0 and was present throughout the entire 8-hr incubation period (□). Progesterone (10 μM) was added either 15 min (▨) or 6 hr (▤) before the end of the 8-hr cholera toxin treatment. Results are expressed as in *a*.

icant, and the values shown in Fig. 1*a* were obtained after exposure to the steroid for 15 min and 6 hr in 20 experiments.

Reversal of Cholera Toxin-Induced Accumulation of cAMP by Progesterone. The oocytes were exposed to cholera toxin (0.1 nM). When progesterone (10 μM) was added after a delay of 2 hr or more, GVBD did not occur (9). However, as seen in Fig. 1*b*, progesterone induced a remarkably rapid decrease in the cellular cAMP normally produced in response to cholera toxin (10). After 1 min of exposure to progesterone, cAMP levels returned nearly to control values. Between 15 and 60 min of continuous progesterone treatment, cAMP levels ranged between 80 and 100% of those of control oocytes. Fig. 1*c* summarizes 27 experiments performed with nine different females. Progesterone applied for 15 min decreased the cAMP concentration below the level measured in control oocytes. After 6 hr, the cAMP concentration was ≈110–120%. Under these experimental conditions, cAMP was not extruded from the oocytes, as determined previously by measuring the nucleotide in the external medium (10).

Reversal of Cholera Toxin-Induced Accumulation of cAMP by Progesterone in the Presence of Phosphodiesterase Inhibitors. Papaverine (0.1 mM), 3-isobutyl-1-methyl-xanthine (IBMX; 1 mM), and theophylline (10 mM) led to 2- to 3-fold increases in the cholera toxin-induced accumulation of cAMP after 15 min or 6 hr of continuous treatment of oocytes (Fig. 2). The effects of phosphodiesterase inhibitors were dose dependent (not shown). Progesterone again was active because a decrease in cAMP accumulation was observed after 15 min or 6 hr of simultaneous steroid treatment (Fig. 2).

Effects of Nonsteroidal Inducers of Meiosis on Cholera Toxin-Mediated Accumulation of cAMP: Comparison with Noninducing and Progesterone Antagonist Substances. A parallel study with various meiosis inducers (1, 15–17) provided evidence that these were also able to decrease the level of cAMP in oocytes (Fig. 3). Mersalyl (17), propranolol, and D600 decreased the cAMP elevation induced by cholera toxin, their inhibitory effects being more pronounced after 6 hr of continuous treatment. $ZnSO_4$ had never before been tested but is known to decrease adenylate cyclase activity in one or more other systems (18). Insulin (16) led to an initial decrease, followed by a potentiation of the cAMP accumulation induced by cholera toxin. Detailed studies on insulin action will be reported elsewhere. The decreasing effect on cAMP of mersalyl and of other drugs was dose dependent (data not shown) and could be correlated with their dose dependent capacity to achieve maturation (1, 15). However, the dose relationship could not be stud-

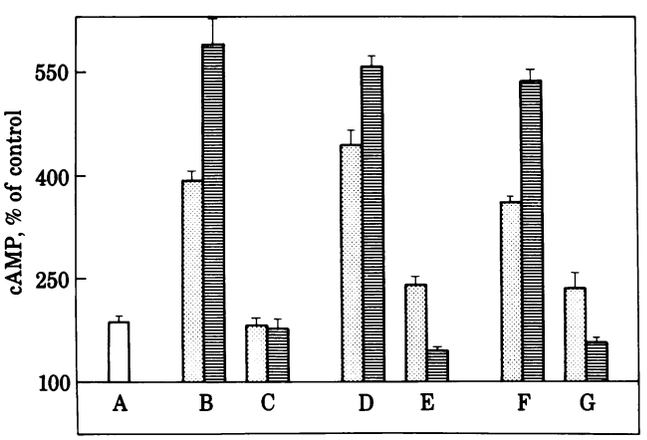

FIG. 2. Reversal of cholera toxin-induced accumulation of cAMP by progesterone: effects of inhibitors of phosphodiesterase. Cholera toxin (0.1 nM) was added at time 0 and was present in all samples throughout the entire 8-hr incubation period (□). Each phosphodiesterase inhibitor [papaverine (0.1 mM), IBMX (1 mM), or theophylline (10 mM)] was added alone or with progesterone (10 μM) either 15 min (□) or 6 hr (▤) before the end of the 8-hr cholera toxin treatment: *A*, control (cholera toxin alone); *B* and *C*, papaverine without (*B*) and with (*C*) progesterone; *D* and *E*, IBMX without (*D*) and with (*E*) progesterone; *F* and *G*, theophylline without (*F*) and with (*G*) progesterone. Mean percentages (± SEM) versus control values (100%) are given as in Fig. 1*a*.

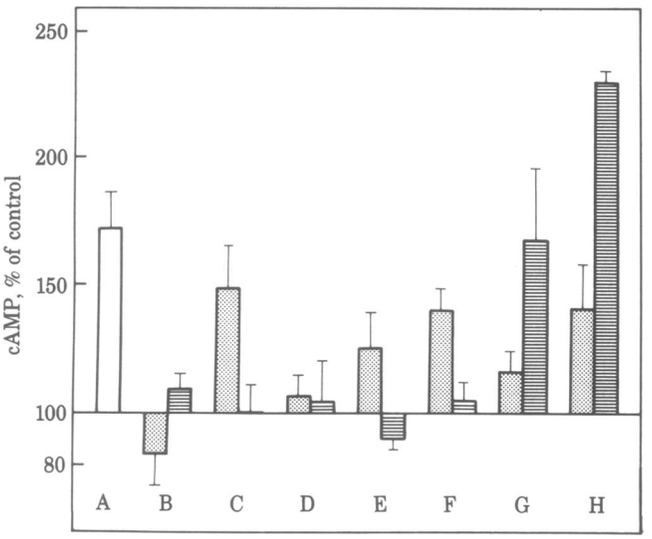

FIG. 3. Effects of progesterone and agonists on cholera toxin-induced accumulation of cAMP. Cholera toxin (0.1 nM) was added at time 0 and was present in all samples throughout the entire 8-hr incubation period (□). Progesterone (10 μM), mersalyl (0.1 mM and 1 mM), propranolol (1 mM), D-600 (1 mM), $ZnSO_4$ (0.1 mM), or insulin (7 μM) were added either 15 min (▨) or 6 hr (▤) before the end of the 8-hr cholera toxin treatment: *A*, control (cholera toxin alone); *B*, progesterone; *C* and *D*, mersalyl at 0.1 mM and 1 mM, respectively; *E*, propranolol; *F*, D-600; *G*, $ZnSO_4$; *H*, insulin. Mean percentages (± SEM) versus control values (100%) are given as in Fig. 1*a*.

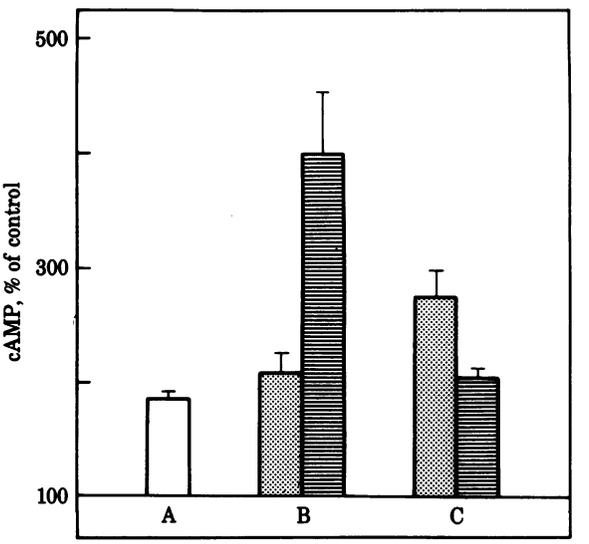

FIG. 4. Effects of SIBA and gammexane (antagonists of the reinitiation of meiosis by progesterone) on cholera toxin-induced accumulation of cAMP. Cholera toxin (0.1 nM) was added at time 0 and was present in all samples throughout the entire 8-hr incubation period (□). SIBA (1 mM) and gammexane (1 mM) were added either 15 min (▨) or 6 hr (▤) before the end of the 8-hr cholera toxin treatment: *A*, control (cholera toxin alone); *B*, with SIBA; *C*, with gammexane. Mean percentages (± SEM) versus control values (100%) are given as in Fig. 1*a*.

ied on the same oocytes because GVBD follow-up must be performed in the absence of cholera toxin, whereas kinetic studies of cAMP concentrations are achieved on cholera toxin treated oocytes.

Gammexane, an analogue of inositol that inhibits competitively the metabolism of inositol-containing phospholipids (19), and 5′-deoxy-5′-S-2-isobutyl-5′-thioadenosine (SIBA), a methylase competitive inhibitor (20), do not provoke meiosis and antagonize progesterone-induced meiotic maturation (21, 22).

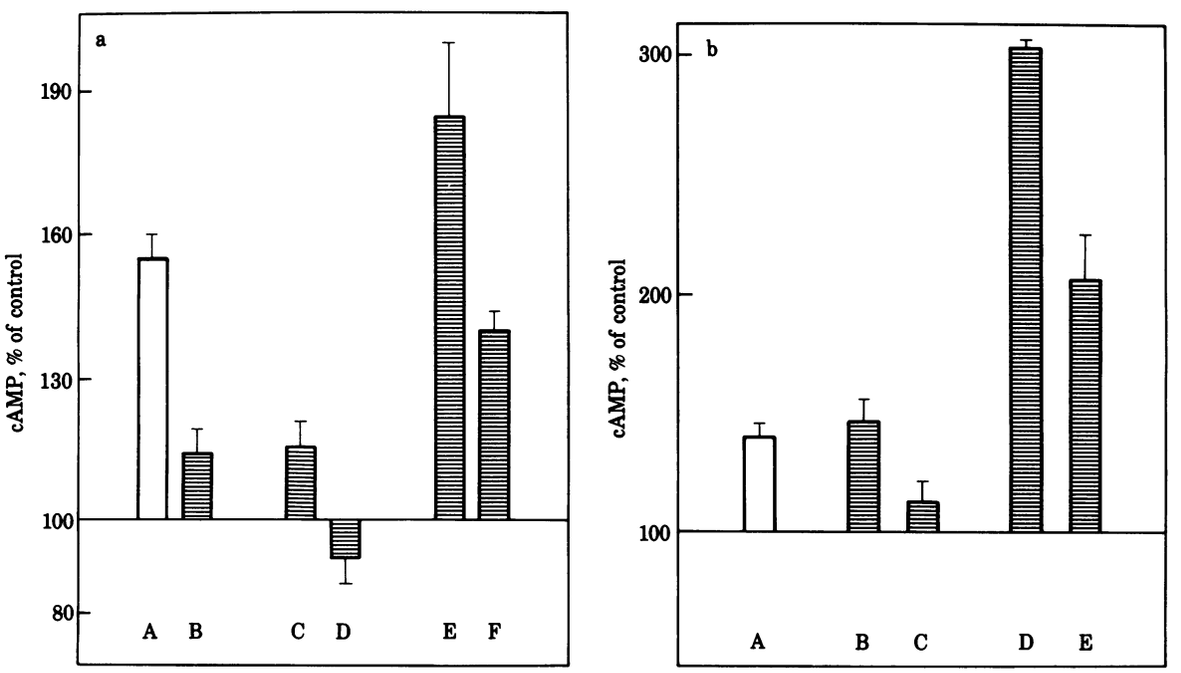

FIG. 5. Effect of injected MPF on cAMP concentration in oocytes exposed to cholera toxin in the absence or presence of progesterone or IBMX. Cholera toxin (0.1 nM) was added at time 0 and was present in all samples throughout the entire 8-hr incubation period (□). MPF was obtained from mature oocytes as described in Fig. 4 and injected 6 hr after the beginning of cholera toxin treatment. (*a*) Progesterone (10 μM) and insulin were applied 2 hr after the beginning of the cholera toxin treatment: *A*, control (cholera toxin alone); *B*, MPF (50 nl); *C* and *D*, progesterone without (*C*) and with (*D*) MPF (50 nl); *E* and *F*, insulin without (*E*) and with (*F*) MPF (50 nl). (*b*) IBMX was added at 6 hr: *A*, control (cholera toxin alone); *B*, MPF (10 nl); *C*, MPF (100 nl); *D* and *E*, IBMX, without (*D*) and with (*E*) MPF (100 nl). At the end of the 8-hr incubation, cAMP was measured. Mean percentages (± SEM) versus control values (100%) are given as in Fig. 1*a*.

Both increased the cAMP level in oocytes (Fig. 4).

MPF Decreases the cAMP Levels in Oocytes. In cholera toxin-treated oocytes, cAMP accumulation was greatly decreased 2 hr after injection of MPF (Fig. 5). This inhibitory effect of MPF was obtained only when sufficient amounts of active cytoplasm were injected because 10 nl was not enough, but 50 or 100 nl was active. Kinetic studies indicated that the decrease in cAMP did not occur during the first hr (not shown), in contrast with the immediate effects of progesterone (Fig. 1). Furthermore, the decrease in cAMP by MPF also was observed in the presence of IBMX (Fig. 5*b*).

When both progesterone exposure and MPF injection were performed, the level of cAMP in cholera toxin-exposed oocytes was lower than the basal level (Fig. 5*a*). This finding strikingly correlates with the 100% GVBD observed under these circumstances. cAMP levels were also determined 2 hr after the injection of MPF (30–60 nl of cytoplasm) into untreated oocytes. Values lower than those of controls were observed and were comparable to those of oocytes continuously exposed to progesterone (not shown).

DISCUSSION

A possible role for cAMP in progesterone-induced meiosis of *Xenopus laevis* oocytes has been postulated (8, 10), and in this work we substantiate this possibility, making use of cholera toxin and phosphodiesterase inhibitors to magnify cAMP changes. The results led to a designation of adenylate cyclase as a target of progesterone action. Moreover, we amplify the concept in showing a remarkable correlation between the effect of a variety of compounds upon cAMP level and their efficiency as meiosis inducers.

Under the combined influence of progesterone plus cholera toxin cAMP was found to be lower than after cholera toxin alone, even though it remained higher than after progesterone alone (10), correlating with the antagonistic effect of cholera toxin. The effect of progesterone to decrease cAMP, whether in untreated or cholera toxin treated oocytes, suggests the possibility that the steroid inhibits the activity of adenylate cyclase, in accordance with early experiments showing a decrease in cAMP formation (10, 23). The rapidity and the persistency of the cAMP decrease by progesterone were striking. Experiments performed in the presence of three phosphodiesterase inhibitors likely excluded an important effect at the level of the phosphodiesterase that are very active in *Xenopus laevis* oocytes (24, 25).

Propranolol, mersalyl, D-600, tetracaine, insulin, and $ZnSO_4$ which can reinitiate meiosis, have all been found to lower cAMP levels in *Xenopus laevis* oocytes, as does progesterone, the physiological inducer. These agents have been shown to inhibit the activity of adenylate cyclase in a variety of other systems (26–31). Of particular interest is insulin, which induces meiosis but in a somewhat delayed manner as compared to progesterone (16)—a peculiarity that may be explained by a biphasic effect on cAMP to be reported elsewhere.

Gammexane inhibits progesterone action on meiosis by interference at an early (membrane?) step (21) and it increased cAMP level in cholera toxin-treated oocytes. SIBA also antagonizes progesterone action (22), and we found that it also increased cAMP level; this result may be related to a process of methylation of phospholipids occurring in oocyte membrane as in other systems in response to hormones or mediators (32).

The logical follow-up of the present results consisted in the identification and measurement of adenylate cyclase activity in *Xenopus laevis* oocytes. We have recently observed (see *Note Added in Proof*) that ≈20–30% of adenylate cyclase activity found in oocyte homogenates are present in a plasma membrane-containing fraction, whereas 60% are soluble. Only the membrane-associated adenylate cyclase activity decreased when exposed to progesterone, in very good agreement with the small diminution of cAMP level in hormone-treated oocytes. In cell-free experiments with membrane-associated cyclase, we were able to demonstrate the same steroid specificity on enzyme activity as that observed for meiosis in intact cells (1). Experiments are in progress in order to study the molecular mechanism of hormone action on cyclase.

Whatever is the mechanism of progesterone action of cAMP metabolism, the effect of a rather limited decrease of its concentration remains intriguing. The effect of progesterone on cAMP values has even been difficult to assess rigorously in the absence of cholera toxin. However, we have never found cAMP values above control levels during the first hours of meiosis reinitiation, and in Fig. 6 we represent the small decrease taking place under the influence of progesterone alone (dotted zone) that, if persistent, is sufficient to allow MPF to be formed and GVBD to occur. We calculated that our results are compatible with the meiotic effect of the injection of regulatory subunit of protein kinase (8). The binding of cAMP by the amount of catalytic subunit reported to induce 50% GVBD (8) would correspond to a diminution of ≈0.1–0.2 pmol of free cAMP per oocyte—that is ≈10–20% of the intracellular cAMP. Moreover the sustained decrease in cAMP that we found, again pointed to an

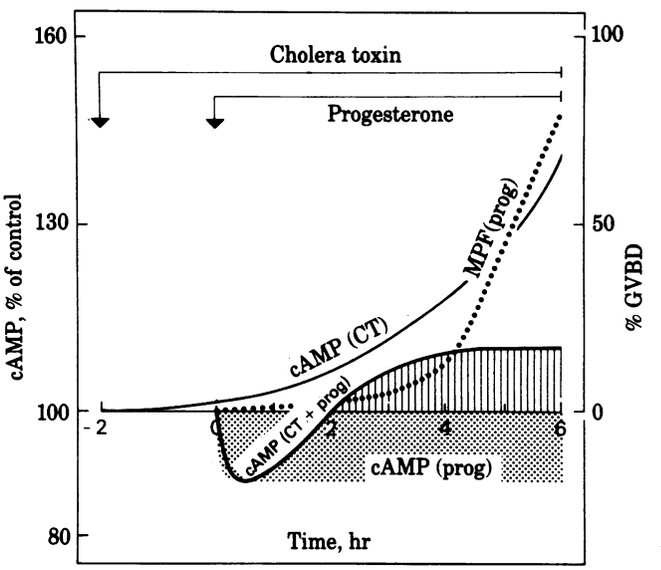

FIG. 6. Scheme of the time course of cAMP changes provoked by progesterone or cholera toxin, or both. Comparison with the progesterone-induced formation of MPF. In most cases, GVBD begins 6 hr after the start of incubation of *Xenopus laevis* oocytes with progesterone (10 μM) and has occurred in all cells by 8 hr. MPF increases 2–3 hr before GVBD, between hours 3 and 4 and hours 5 and 6 of progesterone incubation (3). The beginning of the curve (•••••) of MPF formation under progesterone (prog) treatment is naturally very imprecise because the rate of increase is slow, and MPF can only be tested by transfer of a limited amount of cytoplasm into recipient oocytes. The dotted zone represents the cAMP values observed during incubation of maturing oocytes in progesterone, starting at time 0; all values are below the 100% control values of untreated oocytes. The cholera toxin (CT) solid line represents cAMP accumulation under the influence of cholera toxin (0.1 nM) incubated since hour −2. The progesterone + cholera toxin curve represents cAMP accumulation when oocytes, incubated from hour −2 in cholera toxin, are also exposed to progesterone from time 0 and, therefore, do not mature. After an early decrease of cAMP, values above the 100% control level are reached, and these increased concentrations of cAMP preclude the formation of MPF necessary for provoking GVBD.

effect of the cyclic nucleotide at the level of MPF formation and amplification (10). We propose then that progesterone initially provokes MPF formation, and later on that MPF by itself decreases cAMP by a not-yet-known mechanism that may account for its "autocatalytic amplification" demonstrated previously in recipient oocytes (33, 34). Therefore, both progesterone and MPF would contribute to the persistent low cAMP level. Conversely, as also schematized in Fig. 6, when oocytes are exposed to progesterone after cholera toxin, GVBD does not occur, and the cAMP increase (0.1–0.2 pmol per cell) blocks the formation and amplification of MPF.

All experiments performed with progesterone and those with a variety of agonists and antagonists point to a remarkable correlation between reinitiation of meiosis in *Xenopus laevis* oocytes and the decrease of cAMP. That a ≈10% decrease (mean value) is necessary and enough is difficult to understand because the basal cAMP concentration in oocytes varies much according to each female (a series of experiments should be always conducted with oocytes from a single animal). The driving force for maintaining the ≈1 μM cAMP concentration in untreated oocyte is unknown. It is clear that this level by itself is not the critical determinant allowing or not allowing meiosis to resume. For instance, the possible consequences of the decrease of cAMP on protein phosphorylation should be evaluated. Among other possible determinants of meiosis, Ca^{2+} ions have been much studied, and their release from membrane binding sites has been evoked as an important correlate of meiosis reinitiation (1, 35, 36). Indeed, data showing directly Ca^{2+} release have been published (35, 36). Ca^{2+} changes could be implicated in several features of cAMP metabolism (37) and coupled changes of Ca^{2+} and cAMP may be obligatorily involved. Other effects of Ca^{2+} changes also could occur [for instance in the control of ATPase activity (38)].

In any case, the concept implicating cAMP in the control of meiotic cell division seems applicable to other species. In *Rana pipiens* oocytes, a decrease in cAMP is provoked by progesterone (39, 40), and an inhibition of progesterone-induced meiosis by dibutyryl cAMP has been reported. In mammalian oocytes in culture, it has been shown that increased cAMP levels can maintain mouse oocytes in the prophase of the first meiotic division (41, 42). Further work comparing the amphibian and the mammalian systems is warranted because the MPFs of their respective oocytes display similar properties (43). Moreover, some analogies between MPF and mitotic factors from amphibian (44) and mammalian (45) cells have been shown. Therefore, the present results may be interesting in the general consideration of control of cell division, possibly initiated at the cell surface.

Note Added in Proof. Data on adenylate cyclase in *Xenopus laevis* oocytes are now published (46). Results obtained by Sadler and Maller (47) are also confirmatory.

We acknowledge the excellent technical help of Gisèle Gilliéron-Allenbach and contributions of Anne Atger, Françoise Boussac, Jean-Claude Lambert, and Adèle Wolfson to the manuscript. This work was supported by Inserm and the Fond National Suisse (contracts 3.210.77 and 3.327.78).

1. Baulieu, E. E., Godeau, F., Schorderet, M. & Schorderet-Slatkine, S. (1978) *Nature (London)* **275**, 593–598.
2. Brachet, J. (1974) *Ann. Biol.* **13**, 403–434.
3. Masui, Y. & Clarke, H. J. (1979) *Int. Rev. Cytol.* **57**, 185–282.
4. Masui, Y. & Markert, C. L. (1971) *J. Exp. Zool.* **177**, 129–146.
5. Schorderet-Slatkine, S. & Drury, K. C. (1973) *Cell Differ.* **2**, 247–254.
6. Bourne, H. R., Coffino, P., Melmon, K. L., Tomkins, G. M. & Weinstein, Y. (1975) *Adv. Cyclic Nucleotide Res.* **5**, 771–786.
7. Pastan, I. H., Johnson, G. S. & Anderson, W. B. (1975) *Annu. Rev. Biochem.* **44**, 491–522.
8. Maller, J. L. & Krebs, E. G. (1977) *J. Biol. Chem.* **252**, 1712–1718.
9. Godeau, F., Boquet, P., Schorderet, M., Schorderet-Slatkine, S. & Baulieu, E. E. (1978) *C. R. Hebd. Seances Acad. Sci. Paris* **286**, 685–688.
10. Schorderet-Slatkine, S., Schorderet, M., Boquet, P., Godeau, F. & Baulieu, E. E. (1978) *Cell* **15**, 1269–1275.
11. O'Connor, C. M. & Smith, L. D. (1976) *Dev. Biol.* **52**, 318–322.
12. Pays-De Schutter, A., Kram, R., Hubert, E. & Brachet, J. (1975) *Exp. Cell Res.* **96**, 7–14.
13. Maller, J. L., Butcher, F. R. & Krebs, E. G. (1979) *J. Biol. Chem.* **254**, 579–582.
14. Bravo, R., Otero, C., Allende, C. C. & Allende, J. E. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1242–1246.
15. Schorderet-Slatkine, S., Schorderet, M. & Baulieu, E. E. (1977) *Differentiation* **9**, 67–76.
16. El Etr, M., Schorderet-Slatkine, S. & Baulieu, E. E. (1979) *Science* **205**, 1397–1399.
17. Brachet, J., Baltus, E., Pays-De Schutter, A., Hanock-Quertier, J., Hubert, E. & Steinert, G. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1574–1578.
18. Malmquist, J., Israelsson, B. & Ljungqvist, U. (1979) *IRCS Med. Sci.* **7**, 233.
19. Michell, R. H. (1975) *Biochim. Biophys. Acta* **415**, 81–96.
20. Enouf, J., Lawrence, F., Tempete, C., Robert, C., Robert-Gero, M. & Lederer, E. (1979) *Cancer Res.* **39**, 4497–4502.
21. Schorderet-Slatkine, S., Schorderet, M. & Baulieu, E. E. (1977) *Biochem. Biophys. Res. Commun.* **77**, 496–502.
22. Schorderet-Slatkine, S., Finidori-Lepicard, J., Hanoune, J. & Baulieu, E. E. (1981) *Biochem. Biophys. Res. Commun.* **100**, 544–550.
23. Mulner, O., Huchon, D., Thibier, C. & Ozon, R. (1979) *Biochim. Biophys. Acta* **582**, 179–184.
24. Allende, C. C., Bravo, R. & Allende, J. E. (1977) *J. Biol. Chem.* **252**, 4662–4666.
25. Mulner, O., Cartaud, A. & Ozon, R. (1980) *Differentiation* **16**, 31–39.
26. Davies, T. F., McLachlan, S. M., Povey, P. M., Smith, B. R. & Hall, R. (1977) *Endocrinology* **100**, 974–979.
27. Hancock, A. A. & Hess, M. E. (1979) *Biochem. Pharmacol.* **28**, 2601–2606.
28. Hepp, K. D., Rinninger, J., Langley, J. & Renner, R. (1978) *FEBS Lett.* **91**, 325–328.
29. Lambert, B. & Jacquemin, C. (1979) *FEBS Lett.* **105**, 19–22.
30. Mavier, P. & Hanoune, J. (1975) *Eur. J. Biochem.* **59**, 593–599.
31. Nathanson, J. A., Freedman, R. & Hoffer, B. J. (1976) *Nature (London)* **261**, 330–332.
32. Hirata, F. & Axelrod, J. (1980) *Science* **209**, 1082–1090.
33. Reynhout, J. K. & Smith, L. D. (1974) *Dev. Biol.* **38**, 394–400.
34. Drury, K. C. & Schorderet-Slatkine, S. (1975) *Cell* **4**, 269–274.
35. Moreau, M., Vilain, J. P. & Guerrier, P. (1980) *Dev. Biol.* **78**, 201–214.
36. Wasserman, W. J., Piato, L. H., O'Connor, C. M. & Smith, L. D. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1534–1536.
37. Berridge, M. J. (1975) *Adv. Cyclic Nucleotide Res.* **6**, 1–98.
38. Warren, G. B., Houslay, M. D. & Metclafe, J. C. (1975) *Nature (London)* **255**, 684–687.
39. Morrill, G. A., Schatz, F., Kostellow, A. B. & Poupko, J. M. (1977) *Differentiation* **8**, 97–104.
40. Speaker, M. G. & Butcher, F. R. (1977) *Nature (London)* **267**, 848–849.
41. Cho, W. K., Stern, S. & Biggers, J. D. (1974) *J. Exp. Zool.* **187**, 383–386.
42. Stern, S. & Wassarman, P. M. (1973) *J. Cell Biol.* **59**, 335a.
43. Balakier, H. & Czolowska, R. (1977) *Exp. Cell Res.* **110**, 466–469.
44. Wasserman, W. J. & Smith, L. D. (1978) *J. Cell Biol.* **78**, R15–22.
45. Sunkara, P. S., Wright, D. A. & Rao, P. N. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2799–2802.
46. Finidori-Lepicard, J., Schorderet-Slatkine, S., Hanoune, J. & Baulieu, E. E. (1981) *Nature (London)* **272**, 255–257.
47. Sadler, S. & Maller, J. L. (1981) *J. Biol. Chem.* **256**, 6368–6373.