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EFFECT OF A METHYL-TRANSFERASE INHIBITOR, 5'-DEOXY-5'-S-ISOBUTYL-THIOADENOSINE (SIBA) ON cAMP LEVEL AND PROGESTERONE INDUCED MEIOSIS REINITIATION IN XENOPUS LAEVIS OOCYTES

Sabine Schorderet-Slatkine[1], Joëlle Finidori-Lepicard[2], Jacques Hanoune[3], and Etienne-Emile Baulieu[2].

[1]Département de Gynécologie et d'Obstétrique, Hôpital Cantonal, Genève, Suisse. [2]U 33 Inserm, Lab. Hormones, 94270 Bicêtre, France. [3]U 99 Inserm, Hôpital Henri Mondor, 94000 Créteil, France.

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SUMMARY

The reinitiation of meiosis induced by 1 µM progesterone in Xenopus laevis oocytes is inhibited in a dose-dependent manner by 5'-deoxy-5'-S-isobutyl-thioadenosine. This methyl-transferase inhibitor increases cAMP levels in oocytes, whether in presence or in absence of the hormone and/or isobutyl-methyl-xanthine, a phosphodiesterase inhibitor ; cAMP also is further increased in oocytes prealably treated by cholera toxin. Incubation of oocytes with 5'-deoxy-5'-S-isobutyl-thioadenosine stimulates the activity of membrane-bound adenylate cyclase. These results suggest that the effect of the drug on progesterone-induced meiosis is attributable, at least in part, to its action on membrane-bound adenylate cyclase activity.

INTRODUCTION

Progesterone is the physiological hormone resuming meiosis of Xenopus laevis oocytes, and it acts at the membrane level (review in 1). We have recently observed in experiments with intact cells and cell-free preparations that progesterone can inhibit membrane-bound adenylate cyclase activity (2), a mechanism responsible for the decrease of intracellular cAMP level involved in the meiotic cell division (3-6).

ABBREVIATIONS

SIBA : 5'-deoxy-5'-S-isobutyl-thioadenosine ; IBMX : Isobutyl-methyl-xanthine ; GVBD : Germinal vesicle breakdown ; MTA : 5'-methyl-thioadenosine ; SAH : 5'-adenosyl-homocysteine ; SIBU : 5'-deoxy-5'-S-isobutyl-uridine.

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It has been shown by Axelrod and colleagues (7), that in a series of biological systems, the enzymatic methylation of membrane phospholipids might be implicated in the transmission of biological signals. Since we have obtained evidence for "specific steroid sites" in Xenopus laevis oocyte plasma membrane that are operationally linked to adenylate cyclase activity and reinitiation of meiosis (1, 2), and also since we have already observed that gammexane, an inositol analogue interferring with inositol-containing phospholipid metabolism, was able to inhibit progesterone induced meiotic reinitiation (8), we decided to investigate whether Axelrod's concept could be applied to the progesterone-oocyte system. We now report that a competitive inhibitor of methyl-transferase, 5'-deoxy-5'-S-isobutyl-thioadenosine (SIBA) (9), inhibits progesterone induced meiosis in Xenopus laevis oocytes. SIBA by itself is shown to increase cAMP accumulation in presence or in absence of progesterone, and it also does it in presence of isobutyl-methyl-xanthine (IBMX), a phosphodiesterase inhibitor and/or in cells prealably treated by cholera toxin. Then we propose that the enhancement of cAMP level by SIBA is attributable at least in part, to an increased adenylate cyclase activity, and we demonstrate that membrane-bound adenylate cyclase is actually involved.

MATERIALS AND METHODS

Xenopus laevis oocytes (stage V or VI) were collected as described before, after collagenase treatment (10). Experiments were performed at room temperature in a modified Barth's medium (11), and the induction of maturation was achieved by continuous exposure of cells to progesterone 1 µM. Maturation was scored by the outward demonstration of a white spot at the animal pole representative of germinal vesicle breakdown (GVBD). SIBA was mostly used on the basis of its competitive antagonism to methyl-transferase activity (9). Other analogues were used such as 5'-methyl-thioadenosine (MTA), 5-adenosyl-homocysteine (SAH), 5'-deoxy-5'-S-isobutyl-uridine (SIBU) and sinefungin (9). These compounds were generously provided by Pr. E. Lederer and Dr. M. Robert-Gero (Institut des Substances Naturelles de Gif-sur-Yvette). SIBA and analogues were directly added to the incubation medium (pH 7.4), in such a way that oocytes could be exposed to hormone at the same time.

Levels of cAMP were measured on 10 oocytes batches, in triplicate. After transfer into a glass homogenizer containing 200 µl of buffer and heating at 100°C for 10 min, they were homogenized and centrifuged at 4°C. cAMP determinations in duplicate were made on 40 µl of supernatant by radioimmunoassay (4).

Table I. Inhibition by SIBA of the progesterone (1 µM)-dependent release of meiosis in the oocytes of Xenopus laevis expressed as percentage of germinal vesicle breakdown (GVBD). SIBA and progesterone were added together in the Barth medium and GVBD scored after 18 h.

SIBA (M)	% GVBD
-	100
10^{-5}	100
5.10^{-5}	72
10^{-4}	50
5.10^{-4}	21
10^{-3}	0

Adenylate cyclase activity was determined in a plasma membrane containing fraction and in cytosol obtained after subcellular fractionnation (2). Oocytes were homogenized in 10 vol bicarbonate 1 mM, EDTA 3 mM ; the crude homogenate was centrifuged 1,000x15 gxmin ; the supernatant was centrifuged 10,000x20 gxmin, the pellet obtained (P-10,000 fraction) was enriched in plasma membrane. The supernatant was finally centrifuged 165,000x90 gxmin to prepare the cytosol fraction. The enzyme activity was measured as previously described (12). Assays were performed on fractions containing 20-50 µg of protein, for 30 min at 30°C.

RESULTS

As indicated in Table I, SIBA was an inhibitor of progesterone induced maturation of Xenopus laevis oocytes. The 50 % efficient dose was 0.1 mM. These results were obtained by continuous exposure of oocytes to both progesterone and SIBA in the incubation medium. Injection of cells with 100 nl of SIBA 2 mM followed by progesterone incubation did not prevent GVBD. Incubation of oocytes with progesterone and SIBA analogues showed that MTA was also an inhibitor of meiosis reinitiation, although less active than SIBA (ED $\sim$ 0.5 mM), and that SIBU, SAH and sinefungin were not active at the tested concentrations (up to 1 mM) (data not shown). It was interesting to observe that after extensive washing following exposure to SIBA for several hours, oocytes thereafter exposed to progesterone recovered completely their ability to mature under progesterone action.

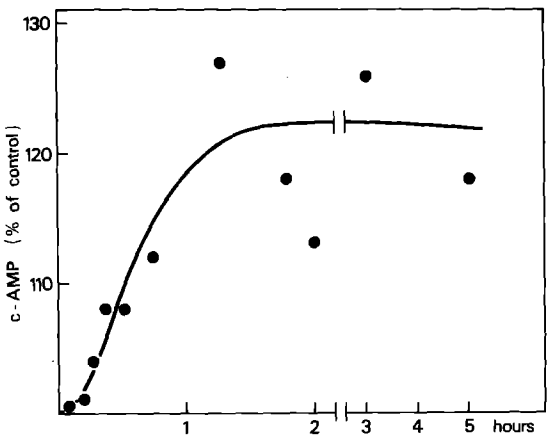
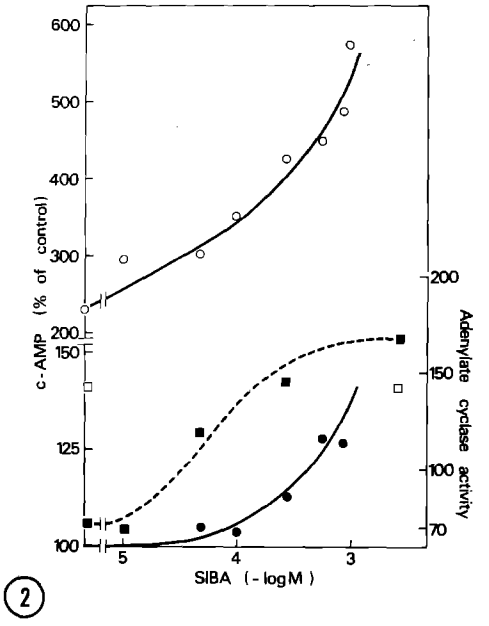


Figure 1. Time-course of cAMP accumulation in Xenopus laevis oocytes after exposure to SIBA 1 mM. The data points were obtained during 5 experiments and refer to the corresponding control values ; each was obtained from 3 groups of 10 oocytes.

Since it has been observed that cAMP level is decreased by progesterone when meiosis occurs and that an increase of cAMP level above control values antagonizes progesterone action (4), we investigated the effect of SIBA on cAMP level. By itself, the drug increased cAMP level inside oocytes, significantly already after 15 min of SIBA 1 mM exposure, and the increase (10-30 %) was maintained for at least 3 h (Fig. 1). Dose-dependency between 10 µM and 1 mM SIBA was observed whatever in presence or not of cholera toxin. In untreated oocytes, incubation with SIBA 1 mM increased cAMP levels by 30-50 % above control level ; in oocytes preincubated 4 h with SIBA, the addition of 1 mM SIBA induced an enhancement of 100-150 % above the value observed in oocytes incubated with cholera toxin alone (Fig. 2).

Since an increase of cAMP level may be due to an augmentation of adenylate cyclase activity or to a decrease of cAMP breakdown, adenylate cyclase activity was directly measured on the membrane-containing fraction of oocyte homogenate. Enzymatic activity, in absence (Fig. 2) or in presence of guanyl-5'-yl imidodiphosphate (data not shown), was found increased



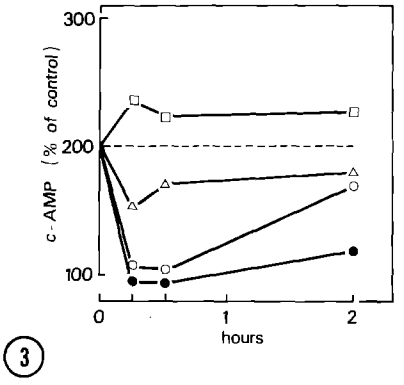


Figure 2. Concentration-dependance of cAMP accumulation and adenylate cyclase activity by SIBA. cAMP levels were measured as in Figure 1 and expressed as percent of control value. The lower curve (●) refers to untreated oocytes and the upper curve (○) to cholera toxin treated oocytes. Adenylate cyclase activity was expressed in pmol cAMP formed per mg protein in 20 min (2). The curve (■) refers to values obtained in P-10,000 fractions (membrane bound enzyme) from oocytes incubated with increasing concentrations of SIBA. Adenylate cyclase activity was also measured in the soluble fraction (□). Oocytes were incubated with SIBA for 90 min.

Figure 3. SIBA and IBMX inhibition of the decrease in cAMP level induced by progesterone in cholera toxin treated oocytes. Oocytes were incubated for 4 h with cholera toxin 10 pM and drugs added at time 0 : Progesterone 1 µM (●), progesterone 1 µM + SIBA 1 mM (○), progesterone 1 µM + IBMX 1 mM (△), progesterone 1 µM + SIBA 1 mM + IBMX 1 mM (□). The level of cAMP in cholera toxin alone treated oocytes is indicated by the dotted line.

after SIBA exposure of oocytes ($ED_{50} \sim 0.1$ mM), reinforcing the suggestion that the methyl-transferase inhibitor acts at the membrane level. Soluble adenylate cyclase activity was not modified by SIBA.

While progesterone itself maintains cAMP level in oocytes under the control value for several hours (5), the cyclic nucleotide remained above control level when SIBA was added at the same time as the hormone (~ 110 %) (data not shown). In cholera toxin treated oocytes, the results followed the same trend : it can be seen on Figure 3 that after 4 h of cholera toxin exposure establishing cAMP level at 200 % of control values, progesterone

lowered it very rapidly, but in presence of SIBA there was a reincrease already by 2 h. In the presence of IBMX, the progesterone effect was decreased, as expected, and when SIBA was added to progesterone and IBMX, the cAMP values stayed above the cholera toxin alone level. In none of these experimental conditions, of course, was meiotic maturation observed.

DISCUSSION

SIBA, a methyl-transferase inhibitor, blocks progesterone action on meiosis in Xenopus laevis oocytes. Since progesterone action is initiated at the membrane level and provokes a decrease of membrane bound adenylate cyclase activity that in turn decreases cAMP level in oocytes, we asked the question whether SIBA acts on membrane component(s) of these cells. It was demonstrated that SIBA increases cAMP level in oocytes, whether untreated or treated by cholera toxin and/or IBMX that establish higher levels of the cyclic nucleotide. Moreover, we found that the membrane-bound adenylate cyclase activity was stimulated by SIBA.

Whether or not SIBA acts by interferring with phospholipid methylation cannot be ascertained definitively by these experiments and will be only formally demonstrated by direct analysis of phospholipid metabolism. The washing experiment demonstrating the reversibility of SIBA effect on oocytes may be interpreted in different ways ; for instance progesterone could provoke changes in lipid methylation, and these changes be antagonized by the presence of SIBA ; alternatively progesterone would not modify lipid methylation, but the changes provoked by SIBA could preclude progesterone action while they would be easily reversible after washing. Indeed the mechanism(s) implicated in SIBA activities in oocytes remain unknown. However, even if SIBA may interfere with several cellular enzymes other than methyl-transferases, we favour the idea that membrane components are affected since injection of the drug into oocytes did not provoke any effect.

In conclusion, SIBA increases cAMP level in Xenopus laevis oocytes, and it has been recently reported that a parent compound (3-deaza-SIBA) has

the same effect in mouse lymphocytes (13). This effect is attributable at least in part to a change at the level of the membrane bound adenylate cyclase. Whatever the mechanism by which it increased activity, this antagonizes progesterone action, and the results strongly reinforce the proposition that cAMP metabolism is involved in the process of meiosis reinitiation that never occurs when cAMP level is above control values (5).

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