Nitric oxide acts through different signaling pathways in maturation of cumulus cell-enclosed mouse oocytes

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ABSTRACT

Background: Nitric oxide (NO) have a dual action in mouse oocyte meiotic maturation which depends on its concentration, but the mechanisms by which it influences oocyte maturation has not been exactly clarified. In this study different signaling mechanisms which exist for in vitro maturation of meiosis was examined in cumulus cell-enclosed oocytes (CEOs) after injection of pregnant mare's serum gonadotropin (PMSG) to immature female mice.

Methods: The CEOs were cultured in spontaneous maturation and hypoxanthine (HX) arrested model.

Results: Sodium nitroprusside (SNP, an NO donor, 10mM) delayed germinal vesicle breakdown (GVBD) significantly during the first 5 hrs of incubation and inhibited the formation of first polar body (PB1) at the end of 24 hrs of incubation. SNP (10^{-5}M) stimulated the meiotic maturation of oocytes significantly by overcoming the inhibition of HX. Sildenafil (a cGMP stimulator, 100 nM), had a significant inhibitory effects on both spontaneous meiotic maturation and HX-arrested meiotic maturation. Forskolin (an adenylate cyclase stimulator, 6μM) and SNP (10mM) had the same effects on GVBD. Forskolin reversed the SNP (10^{-5}M) stimulated meiotic maturation.

Conclusion: These results suggest that differences in pathways are present between SNP-inhibited spontaneous meiotic maturation and SNP-stimulated meiotic maturation in mouse oocytes

Keywords: Nitric oxide; Sildenafil; Oocytes maturation; Signaling pathway

INTRODUCTION

The NO-generating system has been demonstrated in the reproductive tract of several mammalian species, and plays a role in a variety of reproductive function such as steroidogenesis, pregnancy, folliculogenesis, and tissue remodeling (1,2,3).

In several reports it has been demonstrated that the Nw-nitro-L-arginine methyl ester (L-NAME, an NO inhibitor) significantly suppressed the resumption of meiosis which was reversed by addition of sodium nitroprusside (SNP) to the culture (4, 5, 6, 7, 8, 9). Also it has been demonstrated that aminoguanidine (AG, an NO inhibitor) decreased cGMP production in preovulatory follicles addition of an NO donor (SNAP) blocked this suppression (9). These results indicated that NO produced a high concentration of cGMP. The cGMP has an important role in maintaining the meiotic arrest of oocytes. It is well established that NO actions on endothelial cells, smooth muscle cells and acrosome reaction are mediated via soluble guanylate cyclase (sGC) and cGMP (10, 11, 12) but the mechanisms of NO on oocyte maturation have not been determined. NO with a variety of biological functions does not act just by changing cGMP level, it can also mediates its effect through inhibition of adenylyl cyclase (AC), alteration of phosphodiesterase (PDE), activation of calcium-dependent potassium channels and G-proteins (13, 14).

The aim of this study was to determine the most common pathway of NO action during meiotic maturation of oocyte in mouse. The results may be important in understanding of the mechanisms of regulation in reproduction of mammals.

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MATERIAL AND METHODS

M199 and bovine serum albumin (Sankyo Kagaku, Tokyo, Japan), Leibovitz's L15 medium (Invitrogen, Grand Island, NY), Waymouth's MB752/1 medium (Sankyo Kagaku, Tokyo, Japan), sodium pyruvate (Nacalai Tesque, Kyoto, Japan), L-glutamine, eCG, penicillin, streptomycin (Meiji Seika, Tokyo, Japan) , the sildenafil (Pfizer), hypoxanthine (HX), sodium nitroprusside (SNP), Nw-nitro-L-arginine methyl ester (LNAME), forskolin and PMSG (Sigma Chemicals Co).

Sodium nitroprusside (SNP), Nw-nitro-L-arginine methyl ester (L-NAME) were directly dissolved in culture media and used immediately.

Isolation, culture and examination of oocytes were performed according to the previously described method (15).

Statistical analysis

All data are presented as the mean ± S.E.M. Each experiment was repeated at least four times. Significant differences between oocytes in different concentrations of chemicals were determined for various parameters using an independent t- test.

RESULTS

In this study, SNP induced GV-arrested CEOs in comparison with control during the first 5 hrs ($P < 0.05$) (Fig. 1).

Sildenafil (100 nM) inhibited spontaneous meiotic resumption in both CEOs (30.00±2.5 versus 100%, $P < 0.05$) and DOs (4.00±3.00 versus 96.06 ±1.20%) ($P<0.05$) (Fig. 2). This effect of Sildenafil was significantly different from SNP, which only inhibited the formation of PB1 in CEOs (not in Dos) at the end of 24 hrs culture (Fig3). Similarly, Sildenafil also inhibited significantly HX-arrested oocyte maturation in CEOs (GVBD: 19.78 ± 1.56% versus 27.39±2.22%; PB1: 3.51±2.03% versus 5.37±2.03%) (Fig4).

During 5 hrs of incubation, action of SNP was the same as the forskolin on GVBD (Fig.5).

Forskolin (6μm) a specific stimulator of AC, blocked the effect of SNP on meiotic maturation after 24 hrs incubation (GVBD: 29.99±3.98% versus 82.437±3.11%, $P < 0.001$; PB1: 19.99±3.34% versus 49.94±2.98%, $P < 0.01$). Forskolin had no effect on GVBD and PB1 in comparison with control group. (GVBD: 23.67±2.01% versus 25.05±1.03%; PB1: 18.45 ±2.1% versus 16.09±1.05%, $P > 0.05$, Fig.6).

DISCUSSION

This study shows that cAMP-elevating reagents in contrast to cGMP-elevating reagents could reverse the induced effects of SNP on the HX-arrested oocyte maturation. Therefore the stimulatory effects of NO on mouse oocyte meiotic resumption is based on the signal pathway of cAMP, while the inhibitory mechanism is through the cGMP pathway.

There are many data concerning the effects of NO on meiotic maturation of various mammalian species, however, the signaling mechanism by which NO exerts these effects have not been clearly elucidated (7,14-19).

It is well known that NO have many biological effects through activation of sGC and induction of cGMP synthesis in several somatic cell and reproduction systems (20-23).

For instance, the NO-induced acrosome reaction is mediated via the synthesis of cGMP and activation of PKG (24) which have important effects on sperm number, motility, and morphology (25). However, some experimental data also indicate that NO can induce its biological effects via non-cGMP-dependent pathway e.g. directly activates ion channel or inhibits AC activity (26). It has been reported that dual actions of NO, on inhibition and stimulation of CEOs, depends to its concentration (27).

In this study it was found that SNP effects were in CEOs not in DOs; whereas Sildenafil inhibited spontaneous oocyte maturation in CEOs and Dos, with greater potency on DOs. These controversies suggest there is another signaling pathway than cGMP which is involved in NO-mediated spontaneous oocyte maturation. This result is consistent some other reports (28) that showed iNOS-derived NO maintains the intra follicular cGMP level to inhibit oocyte meiotic maturation.

This study shows that 5 hrs exposures to SNP like using forskolin as a cAMP- elevating reagent prevents GVBD completely and therefore cAMP may also be involved in the NO-mediated spontaneous oocyte maturation.

The meiotic arrest of the oocytes is due to elevation of cAMP in medium (29, 36). In the present study, GVBD and PB1 in CEOs arrested by HX increased by using the SNP not by Sildenafil (cGMP). Which illustrates the stimulatory effects of NO is via the cGMP-independent pathway on HX-arrested model.

The NO physiological functions are via regulation of cAMP as SNP inhibited forskolin effects which indicate that the target of NO is enzyme (30, 31, 35). HX as a natural inhibitor of meiotic resumption acts with the same potency as PDE3A, the presence of PDE3A in rat and mouse oocytes

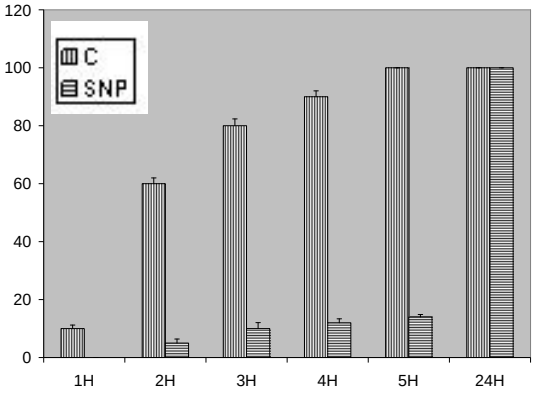

Figure 1. CEOs were cultured in maturation medium in the presence or absence of 1 mM SNP for 24 hrs and observed every hr during the first 5 hrs and again after 24 hrs. Oocytes were compared with control (C) group for GVBD at different times (P< 0001, vs. control).

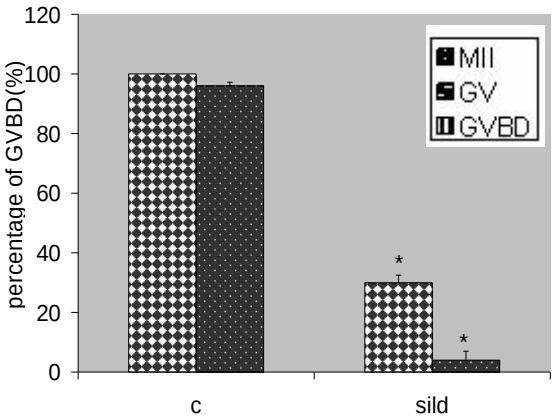

Figure 2. CEOs or DOs were cultured with Sildenafil (100nM) for 24 hrs (H). Bars indicate the percentage of oocytes at GVBD (*P < 0.05 vs. control).

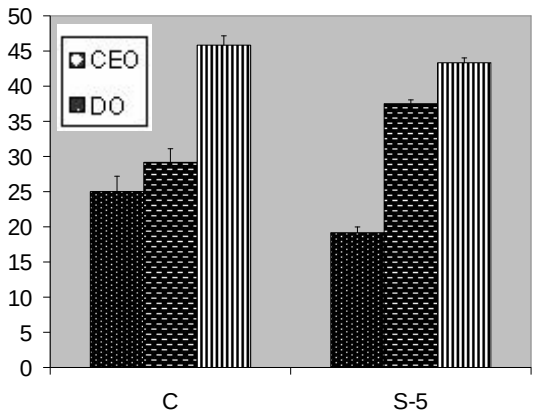

Figure 3. DOs were cultured in the HX-medium with the SNP (10^{-5})
(P > 0.05 vs. control).

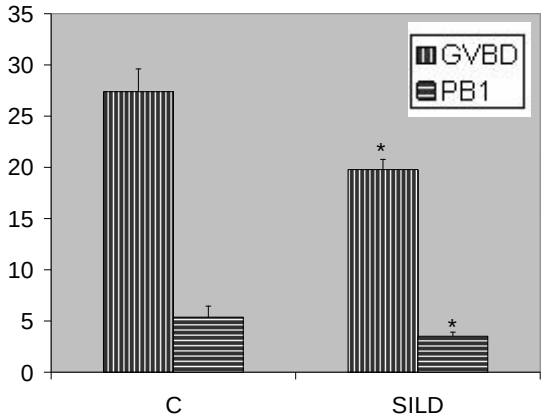

Figure 4. HX-arrested CEOs were cultured in the presence or absence of Sildenafil (100 mM) for 24 hrs (H) (*P < 0.05 vs. control).

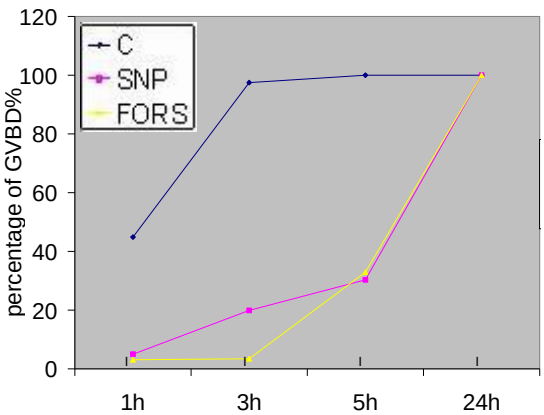

Figure 5. CEOs were cultured in maturation medium with SNP (10^{-5} mM), or forskolin (6 µM), for 5 hrs (H) (P < 0.05 vs. control).

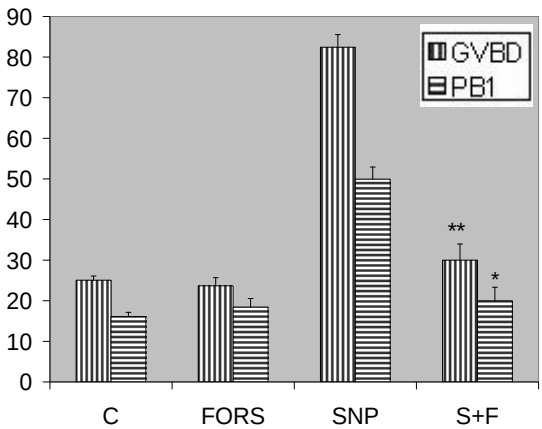

Figure 6. CEOs were cultured in HX-medium with forskolin (6 µM), SNP (10^{-5}), or SNP + forskolin for 24 hrs. (*P < 0.01, ** P < 0.001 vs. SNP).

have been proved by using specific PDE inhibitors (32, 33, 34).

As the AC stimulatory effects of forskolin on the spontaneous meiosis maturation could be reversed by SNP, the exogenous NO elicits meiotic resumption in mouse CEOs by the decrease in cAMP.

The only effects of NO in CEOs not in Dos meiotic maturation are consistent with the reports of Bu and Kazuo's (19, 16).

Now the question is whether NO up or down - regulates the PDE expression in changing the cAMP levels in oocytes.

In this study the distance between the cumulus cells and oocytes increased in the SNP-treated group in the other word at the end of the culture they easily strip off from oocytes with once pipetting. So the disruption of gap junctions (which are important for maintenance of metabolic coupling and facilitation of transfer of cAMP) might also be one of the possible mechanisms of NO effects on oocytes maturation (37, 38).

CONCLUSION

There is different signaling pathway in spontaneous and HX-arrested oocyte maturation. The key of the first meiotic division arrest is the concentration of cAMP and cGMP in a preovulatory follicle. Since NO is a well known factor that stimulates cGMP production, it may concluded that high concentration of NO arrest meiotic of oocytes and a decrease in NO generation results in resumption of meiosis.

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