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Roscovitine, a specific inhibitor of cyclin-dependent protein kinases, reversibly inhibits chromatin condensation during *in vitro* maturation of porcine oocytes

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Summary

In the present study the effects of roscovitine on the *in vitro* nuclear maturation of porcine oocytes were investigated. Roscovitine, a specific inhibitor of cyclin-dependent protein kinases, prevented chromatin condensation in a concentration-dependent manner. This inhibition was reversible and was accompanied by non-activation of $p34^{cdc2}$/histone H1 kinase. It also decreased enzyme activity of MAP kinase, suggesting a correlation between histone H1 kinase activation and the onset of chromatin condensation. The addition of roscovitine (50 µM) to extracts of metaphase II oocytes revealed that the MAP kinase activity was not directly affected by roscovitine, which indicates a possible link between histone H1 and MAP kinase. Chromatin condensation occurred between 20 and 28 h of culture of cumulus-oocyte complexes (COCs) in inhibitor-free medium (germinal vesicle stage I, GV1: 74.6% and 13.7%, respectively). Nearly the same proportion of chromatin condensation was detected in COCs incubated initially in inhibitor-free medium for 20–28 h and subsequently in roscovitine-supplemented medium (50 µM) for a further 2–10 h (GV I: 76.2% and 18.8%, respectively). This observation indicates that roscovitine prevents chromatin condensation even after an initial inhibitor-free cultivation for 20 h. Extending this initial incubation period to ≥ 22 h led to an activation of histone H1 and MAP kinase and increasing proportions of oocytes exhibiting chromatin condensation in the presence of roscovitine. It is concluded that histone H1 kinase is involved in the induction of chromatin condensation during *in vitro* maturation of porcine oocytes.

Key words: Chromatin condensation, $p34^{cdc2}$ kinase, Porcine oocytes, Roscovitine

Introduction

Mammalian oocytes are arrested at the diplotene stage of meiotic prophase. After the removal of fully grown oocytes with surrounding cumulus cells from their follicles and cultivation in suitable media, the oocytes resume meiosis (Pincus & Enzmann, 1935). Nuclear maturation is characterised by chromatin condensation, disappearance of the nucleolus and disassembly of the nuclear membrane, leading to germinal vesicle breakdown (GVBD; Motlik & Fulka, 1976). These events are accompanied by specific changes in protein phosphorylation that are controlled by enzymes which are intracellularly modified within specific signal transduction pathways.

One important enzyme is maturation promoting factor (MPF) (Masui & Markert, 1971), a member of the cyclin-dependent protein kinases. This serine threonine kinase consists of the catalytic subunit $p34^{cdc2}$, a homologue of the yeast cdc2 protein kinase, and the regulatory subunit cyclin B (Gautier *et al.*, 1988). During the growth phase of oocytes, the two subunits form the inactive pre-MPF phosphorylated at Thr 161, Thr 14 and Tyr 15 of $p34^{cdc2}$. This phosphorylation is partly catalysed by the wee 1 kinase (Mitra & Schultz, 1996). With the onset of nuclear maturation, the dimer is activated by specific dephosphorylation of the Tyr 15 residue catalysed by the cdc25 phosphatase (Norbury & Nurse, 1992).

MPF can be determined by its histone H1 kinase activity (Arion *et al.*, 1988). In porcine and bovine

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oocytes it increases at about 20 h and 6 h after onset of maturation, respectively, reaching a first maximum during metaphase I. Following a decrease during the anaphase I transition, the activity of the enzyme shows a second maximum at the metaphase II stage (Christmann *et al.*, 1994; Fissore *et al.*, 1996). Lamins are phosphorylated by $p34^{cdc2}$ and are involved in nuclear membrane disassembly (Peter *et al.*, 1992). There are conflicting data about the role of $p34^{cdc2}$ in chromatin condensation. According to Kubelka *et al.* (1995), chromatin condensation in porcine oocytes occurs independently of MPF activation. In bovine oocytes, Tatemoto & Terada (1998) suggest that $p34^{cdc2}$ exerts a profound influence on chromatin condensation. On the other hand Kubelka *et al.* (2000) concluded from their studies on bovine oocytes that chromatin condensation takes place in the absence of MPF activation.

A second important group of enzymes involved in the resumption of meiosis are members of the MAP kinase family, i.e. the 42 kDa and 44 kDa variants. These enzymes are activated within different signal transduction pathways by specific extracellular signals (growth factors, neurotransmitters). This is the reason why MAP kinases are also called extracellular signal-regulated kinases (ERKs, p42-ERK2, p44-ERK1). The intracellular signal transduction pathways consist of specific enzymes (i.e. c-*ras*, c-*raf*, c-*mos*) activating the MAP kinase kinases, or MEK, which subsequently phosphorylate and activate the MAP kinases (Pelech & Sanghera, 1992).

MAP kinase activity (determined using the substrate myelin basic protein, MBP) increases in bovine oocytes after 8 h at nearly the same time as H1 kinase (Kulbelka *et al.*, 2000), whereas in the pig, the activity increases before MPF activation at about 16 h (Motlik *et al.*, 1998; Wehrend & Meinecke, 2001). The activation of the enzyme by phosphorylation is accompanied by a mobility shift on SDS-PAGE. This shift occurs in cattle at 8 h (Kulbelka *et al.*, 2000) and in pigs at 16–20 h (Motlik *et al.*, 1998) after onset of oocyte culture. Subsequent to activation, MAP kinases are translocated into the nucleus of porcine oocytes (Inoue *et al.*, 1998) where they seem to influence chromatin condensation, GVBD and metaphase II arrest in both bovine and porcine oocytes (Inoue *et al.*, 1995, 1998; Motlik *et al.*, 1998).

The aim of the present study was to examine the influence of histone H1 kinase on the onset of chromatin condensation during culture of porcine cumulus–oocyte complexes (COCs) by reducing the enzyme activity with roscovitine, a highly specific inhibitor of cyclin-dependent protein kinases. This purine derivative inhibits $p34^{cdc2}$ by competitively reducing the binding of ATP to its binding site. Screening experiments with the MAP kinase variants ERK1 and ERK2 demonstrated 20-fold (ERK2) to 50-fold (ERK1) higher IC_{50} values, whereas other important enzymes (i.e. cAMP-dependent protein kinase, cdc25 phosphatase, DNA topoisomerase I/II) are hardly inhibited by roscovitine (De Azevedo *et al.*, 1997; Meijer *et al.*, 1997).

Materials and methods

In vitro maturation (IVM)

Ovaries were collected from prepubertal gilts at a local slaughterhouse and transported to the laboratory in 0.9% saline at 30–33 °C. Follicles with a diameter of 3–6 mm were flushed with 0.9% saline containing 1% (v/v) heat-inactivated fetal calf serum (FCS Sigma, Deisenhofen, Germany) using a 20 gauge needle connected to a 20 ml syringe. Oocytes with an evenly granulated cytoplasm and surrounded by a homogeneous cumulus cell layer were washed three times in Dulbecco's phosphate-buffered saline (PBS, Sigma) containing 10% (v/v) FCS. Twenty COCs were placed in 0.5 ml IVM medium (TCM 199, Sigma) with 20 µg/ml insulin (Sigma), 50 µg/ml gentamicin (Sigma), 20% (v/v) FCS supplemented with 20 IU/ml eCG (Intergonan, Intervet, Tönisvorst, Germany) in a 4-well multidish (Nunc, Roskilde, Denmark). COCs were matured at 39 °C, 5% CO_2 in a humidified atmosphere (95%) for the appropriate time depending on the experimental conditions.

Determination of nuclear maturation status

After the end of the IVM period, COCs were treated with 0.25% (w/v) hyaluronidase (bovine testes, Sigma), and cumulus cells were removed with a fine-bore Pasteur pipette. Oocytes were fixed in ethanol/acetic acid (3:1) for at least 24 h and stained in 2% (w/v) aceto/orcein solution. Nuclear maturation was determined under a phase-contrast microscope according to Motlik & Fulka (1976).

Determination of histone H1 and MBP kinase activity

At the end of cultivation COCs were denuded as described above. Ten oocytes were placed in 4 µl PBS and stored at –80 °C until activity determination.

Kinase activities of MAPK and MPF were determined concomitantly using histone H1 (Sigma) to assess MPF (Arion *et al.*, 1988) and MBP (Sigma) to evaluate MAPK activity (Sanghera *et al.*, 1990), with modifications according to Fissore *et al.* (1996).

The oocytes were lysed by repeated freezing and thawing in extraction buffer (final concentrations: 20 mM MOPS (pH 7.2; Merck, Darmstadt Germany), 10 mM *p*-nitrophenylphosphate (pNPP, Sigma), 20 mM β-glycerophosphate (β-GPP, Sigma), 0.1 mM

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sodium orthovanadate (Sigma), 20 mM sodium fluoride (Merck), 1 mM dithiothereitol (DTT, Merck), 5 mM EGTA (Sigma), 0.1 mM EDTA (Sigma), 20 µg/ml aprotinin (Boehringer, Mannheim, Germany), 20 µg/ml leupeptin (Boehringer) and 1 mM benzamidine (Sigma)). Afterwards the lysates were incubated in the kinase assay buffer (final concentrations: 50 mM MOPS (pH 7.2), 10 mM pNPP, 20 mM β-GPP, 0.5 mM sodium orthovanadate, 1 mM DTT, 5 mM EGTA, 0.1 mM EDTA, 20 µg/ml aprotinin, 20 µg/ml leupeptin, 1 mM benzamidine, 10 mM magnesium chloride (Merck), 0.3 mM ATP (Sigma), 5 µM PKA inhibitor (rabbit sequence; Sigma), 0.67 mg/ml MBP, 1 mg/ml histone H1 and 0.1 mCi/ml γ^{32}P-ATP (Amersham-Pharmacia, Freiburg, Germany)) for 30 min at 30 °C. The reaction was terminated by adding sample buffer at final concentrations of 0.11 mM Tris-HCl (pH 6.8), 11.3% glycerol (Aldrich, Deisenhofen, Germany), 0.28% SDS (Sigma), 0.11 mg/ml bromphenol blue (Sigma) and 3.1% mercaptoethanol (Sigma) and by boiling the samples for 3 min (Lämmli, 1970).

Histone H1 and MBP were separated on a 15% SDS polyacrylamide gel (Lämmli, 1970), stained with 0.1% coomassie brilliant blue R 250 (Serva, Heidelberg, Germany) and dried. Phosphorylation of the substrates was visualised by autoradiography (Biomax MS, Kodak, Rochester, USA). Radioactivity in the individual histone H1 and MBP bands was measured by a liquid scintillation analyser (model 1600 TR, Canberra-Packard, Dreieich, Germany). Mean values of the blank samples, which contained all the components required for the reaction but without the addition of oocytes, were subtracted from each value to obtain histone H1 and MAP kinase activities.

Experimental design

In the first experiment COCs were cultivated for 48 h in IVM medium supplemented with different concentrations of roscovitine (25.0, 37.5 and 50.0 µM; Calbiochem-Novabiochem, Schwalbach, Germany). Control COCs were incubated for the same period in IVM medium plus 0.5% (v/v) DMSO (solvent of roscovitine; Merck). At the end of culture, nuclear maturation status and histone H1 and MAP kinase activities were determined.

In order to demonstrate the specificity of the roscovitine inhibition of MPF, the activities of the kinases were determined in oocytes immediately after recovery from the follicles (0 h) or following culture in IVM medium for 48 h. In these experiments roscovitine (50 µM) or the solvent of roscovitine (0.2% v/v DMSO) was added to the kinase assay buffer.

In the second experiment COCs were matured in IVM medium for 20, 22, 24, 26 or 28 h and either fixed and stained for nuclear stage analysis or subsequently transferred to IVM medium supplemented with 50 µM roscovitine for an additional 10, 8, 6, 4 or 2 h. At the end of the culturing process (30 h), nuclear maturation status and histone H1 and MAP kinase activities were determined.

To examine possible effects of DMSO on the kinase activities and the nuclear maturation process, COCs were incubated in IVM medium supplemented with 0.5% (v/v) DMSO for 30 h. At the end of culture, oocytes were analysed for nuclear status and kinase activities.

In a third experiment, the reversibility of the roscovitine inhibition was determined. COCs were cultivated for 30 h in IVM medium supplemented with 50 µM roscovitine and subsequently transferred to inhibitor-free medium for various periods of time. Nuclear maturation stages and kinase activities were analysed after termination of the cultivation.

Statistical analysis

All experiments were repeated at least four times. The results were statistically analysed (SAS) using the *t*-test. A p value of 0.05 or less was considered to be significant. The relationship between GVI stages of treated and non-treated oocytes were measured using Pearson's product–moment correlation coefficient.

Results

Experiment 1

After cultivation in IVM medium supplemented with 25.0, 37.5 or 50.0 µM roscovitine for 48 h, 39.7 ± 22.4% (n = 124), 77.9 ± 12.9% (n = 128) and 85.4 ± 8.9% (n = 223) of the COCs, respectively, exhibited a nucleus without any signs of chromatin condensation (GV I). In contrast, only 3.4 ± 2.6% (n = 130) of the COCs incubated in IVM medium supplemented with 0.5% (v/v) DMSO for 48 h remained in the GV I stage. This observation demonstrates that roscovitine prevented chromatin condensation in the majority of the oocytes, whereas the use of DMSO did not affect the nuclear maturation process.

Maturation of COCs in IVM medium supplemented with 50 µM roscovitine for 48 h led to a significant decrease ($p < 0.001$) in histone H1 and MAP kinase activities in comparison with COCs treated with DMSO (Table 1).

It has to be emphasised that roscovitine treatment for 48 h led to a significant ($p < 0.001$) increase in MAP kinase activities in comparison with oocytes analysed immediately after being recovered from the follicles, whereas histone H1 kinase activities remained unchanged (Table 1). This indicates that roscovitine

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Table 1 Histone H1 kinase (H1k) and MAP kinase (MAPK) activities of oocytes analysed immediately after recovery from follicles (0 h) or following culture in medium supplemented with DMSO (0.5%, v/v) and roscovitine (50 µM), respectively

	0 h	48 h DMSO	48 h Roscovitine
H1k	102.3 ± 43.0[a]	460.4 ± 105.9[b]	110.9 ± 47.2[a]
MAPK	580.5 ± 80.7[a]	2114.0 ± 277.8[b]	815.7 ± 136.2[c]

Values are enzyme activities in cpm per 10 oocytes (mean ± SD) of four replicates.
[a,b,c] Means with different superscripts in a line differ significantly ($p < 0.05$).

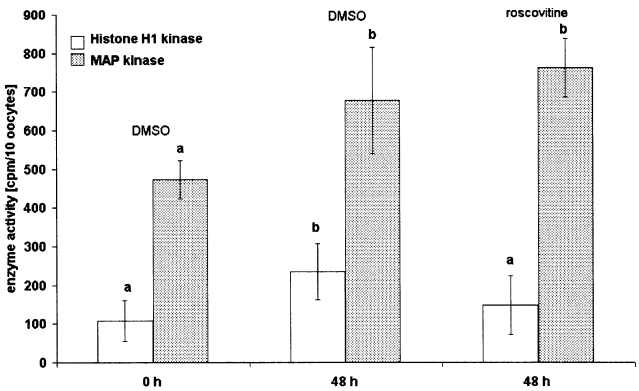

Figure 1 Histone H1 and MAP kinase activities of oocytes analysed immediately after recovery from follicles (0 h) or following culture in inhibitor-free medium for 48 h. Denuded oocytes were subjected to histone H1/MAP kinase assay in the presence of 0.3% (v/v) DMSO or 50 µM roscovitine. Each column represents the mean (± SD) of four replicates of 10 oocytes, respectively. [a,b] Means with different superscripts differ significantly ($p < 0.05$).

completely blocked histone H1 kinase activities but prevented MAP kinase activation only partially. To exclude the possibility that this inhibition of MAP kinase activity was caused by a direct effect of roscovitine, histone H1 and MAP kinase activities of oocytes were determined by supplementing the kinase assay with roscovitine or DMSO either immediately after recovery from the follicles or after cultivation in IVM medium for 48 h. The results demonstrate that the presence of roscovitine during the assay procedure led to a significant ($p < 0.05$) decrease in histone H1 kinase activities in comparison with DMSO, whereas MAP kinase activities remained unaffected (Fig. 1). These observations argue in favour of an indirect inhibition of MAP kinase activities by roscovitine.

Experiment 2

When COCs were incubated in inhibitor-free medium for 20, 22, 24, 26 and 28 h the following percentages remained in the GV I stage: 74.6 ± 6.1% ($n = 182$), 69.7 ± 7% ($n = 189$), 4.49 ± 7.4% ($n = 192$), 22.3 ± 9.1% ($n = 316$) and 13.7 ± 5.6% ($n = 172$), respectively.

These percentages of oocytes exhibiting a GV I stage corresponded with the proportion of oocytes in GV I following an initial culture in IVM medium for 20, 22, 24, 26 and 28 h, respectively, and subsequently a second incubation period in roscovitine-supplemented medium for 10, 8, 6, 4 and 2 h, respectively (Table 2).

The proportions of untreated and roscovitine-treated oocytes in GV I were correlated ($r = 0.994$). These observations support the idea that oocytes remaining in the GV I stage after an initial culture in IVM medium did not start to condense their chromatin during the second incubation period in roscovitine-supplemented medium.

In comparison with oocytes analysed immediately after recovery from the follicles (0 h), histone H1 and MAP kinase activities increased significantly ($p < 0.001$) in oocytes cultivated in IVM medium for 22, 24 and 26 h and then transferred to roscovitine-supplemented medium for an additional 8, 6 and 4 h, respectively (Fig. 2). This indicates that in the course of the inhibitor-free cultivation period both kinases were activated. Thus, it is important to note, that in comparison with the control oocytes incubated in IVM medium supplemented with 0.5% (v/v) DMSO for 30 h, the histone H1 kinase activities of oocytes cultivated for 22 h in IVM medium and subsequently for further 8 h in roscovitine-supplemented medium exhibited significantly lower ($p < 0.001$) histone H1 kinase levels. That means that roscovitine prevented complete activation of the histone H1 kinase despite the fact that about one-third of the oocytes had already begun to condense their chromatin (Table 2).

Furthermore, in comparison with control oocytes significantly ($p < 0.01$) lower MAP kinase activities were detected in oocytes cultivated for 22 and 24 h in IVM medium and subsequently in roscovitine-supplemented medium for a further 8 and 6 h, respectively (Fig. 2). This also demonstrates that roscovitine exerted an inhibitory effect on the MAP kinase activities, too.

Experiment 3

When COCs were cultivated in roscovitine-supplemented IVM medium for 30 h, 86.2 ± 9.2% ($n = 223$) remained at the GV I stage. Following transfer into inhibitor-free IVM medium 85.0 ± 13.8% ($n = 186$) of the oocytes reached metaphase II after a culture period of 48 h (Table 3), which demonstrates the reversibility of roscovitine's action. It should be noted that after 24 h of culture in inhibitor-free IVM medium 58.4 ± 15.5% of the oocytes remained in the GV I stage (Table 3). In contrast, only 44.9 ± 7.4% ($p < 0.05$) of the oocytes were in GV I stage after culture for 24 h in IVM medium

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Table 2 Nuclear maturation status of COCs cultivated first in inhibitor-free medium and subsequently in roscovitine (50 µM)-supplemented medium

First culture: inhibitor-free (h)	Second culture: roscovitine (h)	*n*	Oocytes in GVI stage (%)	Oocytes in metaphase I stage (%)
20	10	143	76.2 ± 0.5^{a}	17.0 ± 3.8^{a}
22	8	226	64.3 ± 8.6^{b}	20.8 ± 7.1^{a}
24	6	202	45.1 ± 8.6^{c}	42.4 ± 16.6^{b}
26	4	182	24.2 ± 4.5^{d}	64.2 ± 6.0^{c}
28	2	210	18.8 ± 3.9^{e}	69.8 ± 7.4^{c}
Control (30 h DMSO)	–	223	2.5 ± 2.8^{f}	92.3 ± 7.0^{d}

Values are the percentages (mean ± SD) of oocytes in GV I and metaphase I stage from at least four replicates.
^{a,b,c,d,e,f} Means with different superscripts in a column differ significantly ($p < 0.05$).

Table 3 Nuclear maturation status of oocytes cultivated first in IVM medium supplemented with roscovitine (50 µM) for 30 h and subsequently in inhibitor-free medium

First culture: roscovitine (h)	Second culture: inhibitor-free (h)	*n*	Oocytes in GV I stage (%)	Oocytes in D, M1, AI, T1 stages (%)	Oocytes in MII stage (%)
30	0	223	$86.2^{a} \pm 9.1$	$2.3^{a} \pm 5.1$	-
30	18	145	$71.3^{b} \pm 15.3^{b}$	$16.8^{b} \pm 9.4$	$4.1^{a} \pm 4.9$
30	24	132	$58.4^{c} \pm 15.5$	$19.6^{b} \pm 11.9$	$17.5^{b} \pm 7.3$
30	30	172	$16.3^{d} \pm 9.1$	$45.7^{c} \pm 11.1$	$32.9^{c} \pm 17.6$
30	42	154	$6.6^{d,e} \pm 6.6$	$34.1^{d} \pm 16.5$	$59.2^{d} \pm 16.6$
30	48	186	$1.5^{e} \pm 2.3$	$13.5^{a,b} \pm 12.1$	$85.0^{e} \pm 13.8$

Values are the percentages (mean ± SD) of oocytes in GV I, diakinesis, metaphase I, anaphase I, telophase I (D, M1, A1, T1) and metaphase II (MII) stage from at least six replicates.
^{a,b,c,d,e} Means with different superscripts in a column differ significantly ($p < 0.05$).

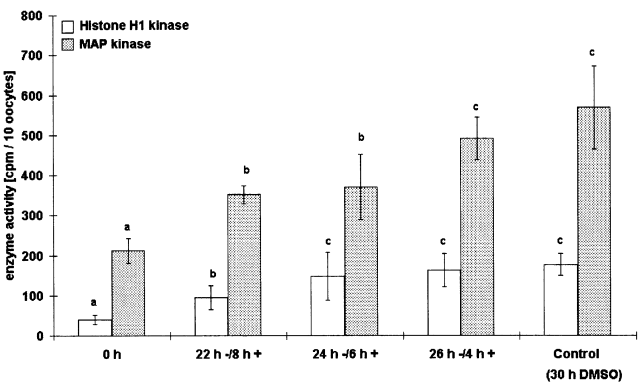

Figure 2 Histone H1 and MAP kinase activities of oocytes cultured first in inhibitor-free medium (0 h, 22 h–, 24 h–, 26 h–) and subsequently in roscovitine (50 µM) supplemented medium (8 h+, 6 h+, 4 h+). Control oocytes were incubated in inhibitor-free medium supplemented with 0.5% (v/v) DMSO for 30 h. Each column represents the mean (± SD) of four replicates of 10 oocytes, respectively. ^{a,b,c} Means with different superscripts differ significantly ($p < 0.05$).

without previous roscovitine inhibition (see experiment 2). This indicates that the onset of nuclear maturation is delayed following withdrawal of roscovitine.

Enzyme activities during the reversibility reaction demonstrated that oocytes cultivated for 30 h in roscovitine-supplemented IVM medium showed a significant increase ($p < 0.001$) in MAP kinase but not in histone H1 kinase activities in comparison with oocytes analysed immediately after recovery from the follicles (0 h). Transfer into inhibitor-free culture medium and further incubation for 18 h resulted in a significant increase ($p < 0.001$) in the histone H1 kinase activities. A further significant rise ($p < 0.001$) in the activities of both kinases was seen after 30 h of inhibitor-free cultivation, whereas only the MAP kinase activities increased significantly ($p < 0.001$) during the following 18 h of incubation (Fig. 3). Changes in histone H1 and MAP kinase activities during the inhibitor-free cultivation after 30 h of roscovitine treatment showed that the reversibility of nuclear maturation was accompanied by the reversibility of the

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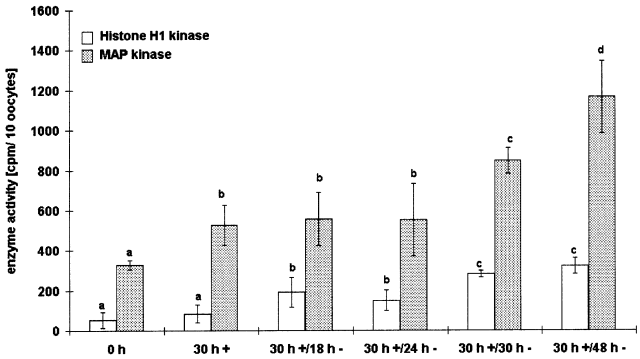

Figure 3 Histone H1 and MAP kinase activities of oocytes cultured first in roscovitine (50 μM)-supplemented medium for 30 h (30 h+) and subsequently in inhibitor-free medium (18 h–, 24 h–, 30 h–, 48 h–). Each column represents the mean (± SD) of three replicates of 10 oocytes, respectively. ^{a,b,c,d} Means with different superscripts differ significantly ($p < 0.05$).

enzyme activities. Such a temporal course of the kinase activities following roscovitine inhibition indicates that MPF is involved in the initiation of chromatin condensation.

Discussion

The present study demonstrates that roscovitine reversibly prevents the chromatin condensation of porcine oocytes during *in vitro* maturation and concomitantly leads to a significant decrease in histone H1 and MAP kinase activities. Since roscovitine added to oocyte extracts inhibits only the MPF and not the MAP kinase activity, it is suggested that the concurrent decrease in MAP kinase activity is caused by interactions between the MPF and MAP kinase transduction pathways (Peter *et al.*, 1992).

An influence of MPF (Ajiro *et al.*, 1996; Motokura & Arnold, 1993; Newport & Spann, 1987; Tatemoto & Terada, 1998) and MAP kinases (Shapiro *et al.*, 1999) on the condensation process during mitosis and meiosis was reported.

Since both kinase activities decreased in the present experiments a definite correlation of chromatin condensation initiation to one of the kinases could not be proved. However, the data suggest that histone H1 kinase plays a key role in this process because in roscovitine-treated oocytes at GV I stage, histone H1 kinase levels remained low whereas MAP kinase activities started to increase.

The assumption that MPF regulates chromatin condensation is supported by the observation that the percentages of GV I oocytes seen after maturing COCs first in inhibitor-free medium (20–28 h) correlated highly with the proportion of GV I oocytes resulting from cultivation of COCs in inhibitor-free medium (20–28 h). This demonstrates that roscovitine inhibited only those oocytes exhibiting uncondensed chromatin.

Regarding the histone H1 and MAP kinase activities it is suggested that the significant increase in the activities after 22 h of inhibitor-free cultivation was due to the increasing proportion of maturing oocytes (about 35%). This indicates again that the activation of MPF and MAP kinase occurred concomitantly with the initiation of chromatin condensation. The finding that roscovitine did not completely inhibit MPF activity during the further 8 h of cultivation after 22 h of culture in inhibitor-free medium can be explained by the mechanism of action of the drug. Roscovitine inhibits MPF by competitive interaction with the ATP binding site (De Azevedo *et al.*, 1997). Since the amount of activated histone H1 kinase molecules and of ATP in the oocytes increases during initiation of chromatin condensation, the inhibition of MPF by roscovitine might be less effective. This would also explain the finding that oocytes which have already started to resume meiosis in the course of the first culture period in inhibitor-free medium progressed to M I even in the presence of roscovitine. Luscher *et al*. (1997) have described the involvement of MPF in GVBD during mitosis and Motlik *et al*. (1998) have presented clear correlations between GVBD and MPF activation in porcine oocytes. Therefore it could be suggested that the initial increase in MPF activity occurring concomitantly with the onset of chromatin condensation is sufficient to induce all subsequent processes leading to GVBD and M I.

In contrast to the present results Kulbelka *et al.* (1995) assume that chromatin condensation of porcine oocytes occurs independently of histone H1 kinase activation because protein synthesis inhibition by cycloheximide induced chromatin condensation but decreased MPF activities. However, it is possible that the slight increase in histone H1 kinase activity detected after cycloheximide treatment (Kulbelka *et al.*, 1995) is sufficient for the initiation of chromatin condensation. With regard to the present results it should be stressed that the prevention of chromatin condensation in almost all oocytes only occurred when histone H1 kinase activities were inhibited by roscovitine at the same level as in oocytes analysed immediately after recovery from follicles. Furthermore, the delay in the onset of chromosome condensation after withdrawal of roscovitine supports the suggestion that MPF plays an important role during the chromatin condensation process.

The results of Tatemoto & Terada (1998) obtained with bovine oocytes support the present suggestion that histone H1 kinase is involved in the chromatin condensation process. These authors have demonstrated that chromatin condensation in the presence of cycloheximide is accompanied by an increase in his-

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tone H1 kinase activity. In contrast to these data Kulbelka *et al.* (2000) reported that the inhibition of nuclear maturation in bovine oocytes by butyrolactone I, a specific inhibitor of cyclin-dependent protein kinases, induced chromatin condensation but decreased MPF and MAP kinase activities. The authors also stated that chromatin condensation does not require activation of either histone H1 or MAP kinase. Although a part of these contradictory observations can be explained by the use of different inhibitors, further investigations of the role of histone H1 kinase in the chromatin condensation process are required.

In conclusion it can be stated that roscovitine reversibly inhibits chromatin condensation in porcine oocytes. Since this inhibition is accompanied by a significant decrease in histone H1 kinase activities, it is assumed that the kinase is causally involved in this initial process during resumption of meiosis.

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