Stimulation of matrix-metalloproteinase-1 and tissue inhibitor of metalloproteinase-1 gene expression in rats by the preovulatory prolactin peak

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Abstract

Since structural luteolysis involves deterioration of tissue, the gene expression of matrix-metalloproteinase-1 (MMP-1) and the respective tissue inhibitor of this metalloprotease (TIMP-1) were measured at various times on the day of pro-oestrus and in animals in which the preovulatory prolactin surge was blocked for the duration of 3 cycles by bromocriptine. An additional group of prolactin-blocked rats received a prolactin replacement injection on the afternoon of pro-oestrus. In spontaneously pro-oestrous rats, MMP-1 and TIMP-1 gene expression increased significantly (P < 0.01) prior to the occurrence of the preovulatory LH surge but simultaneously with the onset of the preovulatory prolactin surge. When prolactin release was blocked by bromocriptine for 3 cycles, no such changes were observed during the afternoon of pro-oestrus. However, an intraperitoneal injection of bovine prolactin at the time when the preovulatory prolactin surge occurs normally increased MMP-1 and TIMP-1 gene expression (P < 0.01). These results indicate that MMP-1 and TIMP-1 gene expression are stimulated by the preovulatory prolactin surge.

Previous work has shown that the preovulatory LH surge activates the enzymatic cascade which leads to increased collagenase activity.

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Introduction

The preovulatory luteinizing hormone (LH) peak in most species studied hitherto causes activation of the proteolytic enzymes matrix-metalloproteinase-1 and -2 (MMP-1 and MMP-2) (1–4). This activation can be prevented by indomethacin indicating that LH acts via prostaglandins (5). The two metalloproteases cleave collagen within the follicle thereby softening the follicular tissue which will finally result in the release of the oocytes.

In most species the regression of corpora lutea results in reduced progesterone secretion and in a simultaneously occurring destruction of luteal tissue, i.e. in functional and structural luteolysis (6, 7). In rats, however, these two processes are temporally separated by several days (8). As a result of the preovulatory LH and prolactin surge the luteinized and later the ruptured follicles secrete large amounts of progesterone the secretion of which ceases when no mating stimulus is applied to the animal (9, 10). If mating does not occur, the corpora lutea are then structurally preserved until the next pro-oestrous day, when they regress as a result of the preovulatory prolactin surge. Thus, in older corpora lutea prolactin is a luteolytic signal (8).

Recently we demonstrated that porcine luteal cells express and release increasing amounts of MMP-1 and MMP-2 as they undergo luteolysis (11). Furthermore, it is well documented that the process of ovulation also implicates increased LH-stimulated release of follicular collagenases (5, 12, 13). Since in rats the preovulatory LH surge causes ovulation and the preovulatory prolactin surge causes luteal regression of the corpora lutea formed during the previous ovulation, the effects of the preovulatory prolactin surge and the expression of collagenase genes was investigated. Recently Quinn et al. (14) published the cDNA sequence for rat MMP-1 which allows synthesis of specific primers for the reverse transcription polymerase chain reaction (RT-PCR). In addition to matrix-metalloproteases, these tissues also synthesize and secrete tissue inhibitors of metalloproteases (TIMP). Thus, the MMP-1 is inhibited by TIMP-1 (15, 16). The TIMP-1 has also been cloned (17) and the sequence in cattle, human, mouse, and rabbit is so similar that we tried the primer nucleotide sequence which was successfully used for demonstrating human TIMP-1 gene expression (18).

The preovulatory prolactin surge can very effectively be blocked by dopaminergic compounds such as the lysergic acid derivative 2-Br-α-ergocryptine (bromocriptine) (19).
and such blockade was shown to prevent structural luteolysis of old corpora lutea (8). In the present experiments we made use of this experimental paradigm and inhibited pituitary prolactin release by 2-Br-α-ergocryptine over a period of three oestrous cycles and studied MMP-1 and TIMP-1 gene expression in prolactin-deprived animals. Furthermore, we investigated the effects of prolactin replacement by injecting bovine prolactin into rats in which prolactin was blocked over 3 oestrous cycles. This latter experimental design was shown to result in structural luteolysis of the accumulated older corpora lutea (8). In the ovaries of prolactin-blocked and the appropriate control animals as well as in normally cyclic animals, we studied the expression of MMP-1 and TIMP-1 genes utilizing a semiquantitative RT-PCR.

Materials and methods

Studies in pro-oestrous rats

Female Sprague Dawley rats weighing 250 g were housed under standardized conditions (lights on from 0700 to 1900 h, mean temperature 24°C, mean humidity 55%). The animals were allowed to adjust to these conditions for three weeks. Daily vaginal smears were then taken over a period of 3 oestrous cycles. At 0600 h in the morning of the following pro-oestrous day they were implanted with a jugular vein catheter under ether anaesthesia. Each animal was allowed to recover for at least 5 h. From a total of 40 pro-oestrous animals 4 blood samples were withdrawn at 15-min intervals at various times from 1400 h to 2300 h of the pro-oestrous day. Following withdrawal of the last blood sample the rats were decapitated, the ovaries removed, cleaned, weighed, and stored at −70°C until further processing within the following 48 h. LH and prolactin (PRL) in the serum of these animals were determined by radioimmunoassay (RIA) (20, 21). A serum pool obtained from ovariectomized rats was used to determine the characteristics of both the LH and PRL RIA. Intra- and interassay coefficients of variation were 7.5 and 11.5 (LH) and 5.2 and 10.5% (PRL) respectively. The sensitivity limit of the LH RIA was 0.2 ng/ml, the detection limit of the PRL RIA was 0.5 ng/ml.

The four hormone values prior to decapitation were used to form subgroups of rats according to the following criteria: LH concentrations still at basal values (LH subgroup I), LH concentrations increasing (LH subgroup II), and in a third group of animals serum LH concentrations decreasing (LH subgroup III). A second classification of the same animals according to their serum prolactin concentrations was slightly different: in a number of animals prolactin concentrations were still at basal values (prolactin subgroup I). In other animals prolactin concentrations increased (prolactin value subgroup II). In a third group prolactin levels were constantly increased (prolactin subgroup III) and in a fourth group prolactin levels decreased (prolactin subgroup IV).

Studies in prolactin-deprived pro-oestrous rats

A total of 27 regularly cyclic rats were divided into 2 subgroups. A total of 12 animals was treated with bromocriptine (Sandoz, Basel, Switzerland) over a period of 3 oestrous cycles. Each animal received an i.p. injection of 70 μg bromocriptine at 0700, 1500, and 2300 h on each day of 3 consecutive oestrous cycles. This treatment did not interfere with the regularity of the previously established regular oestrous cycles. On the third pro-oestrous day all animals received the 1500 h bromocriptine injection and 12 of the animals were decapitated 90 min later. Fifteen animals received in addition to the 1500 h bromocriptine injection 0.5 mg bovine prolactin administered simultaneously with bromocriptine. These animals were also decapitated 90 min later.

Individual frozen ovaries were homogenized with a dismembrator under permanent cooling with liquid nitrogen. The frozen tissue powder was dissolved in lysis buffer and the mRNA isolated utilizing oligo (dT)-coated dynabeads for affinity purification (Dynal, Oslo, Norway) according to the protocol of the manufacturer. The extracted mRNA was reversed transcribed into cDNA utilizing oligo (dT) primer. The cDNA was subjected to a PCR for either MMP-1 or TIMP-1 utilizing the primers described by Quinn et al. (14) and Carmichael et al. (18). The amplified PCR products were then separated by ethidium bromide-stained agarose gel electrophoresis which was directly subjected to densitometric scanning. PCR products were cloned and sequenced according to Sanger et al. (22) utilizing an ALF = DNA sequencer (Pharmacia, Freiburg, Germany) to prove the correct sequence.

Statistical treatment of data

Mean serum LH and prolactin concentrations in the different subgroups were subjected to analysis of variance followed by Bonferonni’s multiple t-test. Mean arbitrary units which resulted from the densitometric scanning of the PCR products were compared by a paired t-test with the respective other groups. A P value < 0.05 was considered to be significant.

Results

Figure 1 details the LH and prolactin profile of 4 individual rats and their ovarian MMP-1 and TIMP-1 RT-PCR products. In animal 10 both LH and prolactin were still at basal levels and there were only faint PCR product bands detectable (lanes 2 and 3, Fig. 1). In animal 1 prolactin levels increased while LH levels were still at basal values: in this animal the MMP-1 gene expression was high and the TIMP-1 PCR product was
readily detectable (lanes 5 and 6). In animal 3 both hormones were increasing or high and the MMP-1 and TIMP-1 gene expression was also present (lanes 8 and 9) whereas gene expression of both products faded off (lanes 11 and 12) in animal 9 in which both hormones decreased. Mean levels of mRNAs encoding MMP-1 and TIMP-1 (Fig. 2) are low in animals with basal prolactin release (prolactin subgroup I), increased significantly ($P < 0.05$) with increased prolactin levels (prolactin subgroup II) to remain high when prolactin levels were at plateau values (prolactin subgroup III). The gene expression of both enzymes was reduced ($P < 0.05$) when prolactin levels decreased (prolactin subgroup IV). When the PCR product evaluation was performed according to basal, increasing or decreasing LH levels (LH subgroups I-III) MMP-1 and TIMP-1 gene expressions were highest ($P < 0.05$) prior to increased LH levels (Fig. 3). Since in all animals studied the increasing prolactin levels preceded the onset of the LH surge by 2 to 3 h, the time of basal LH levels coincided in most animals with the time of increasing prolactin levels, i.e. with subgroup II as shown in Fig. 2.

Three daily injections of bromocriptine for three cycles resulted in ovarian mass of $65 \pm 3$ mg and this was reduced to $56 \pm 1$ mg ($P < 0.05$) following a single injection of 0.5 mg bovine prolactin on the 3rd pro-oestrous day. It is evident that in the prolactin-treated animals MMP-1 gene expression (lanes 6–9) is higher than in the prolactin-blocked animals (lanes 1–4) (Fig. 4). Similar results can be visualised for the expression of the TIMP-1 gene (Fig. 5) which was barely detectable in the prolactin-blocked animals (lanes 6–9). The mean ovarian MMP-1 and TIMP-1 gene expression in the 15 pro-oestrous bromocriptine (3 cycles) and prolactin-treated animals was significantly ($P < 0.05$) higher than in pro-oestrous bromocriptine-treated animals (Fig. 6).

**Discussion**

There is ample evidence that activation of the enzymatic cascade which finally leads to activation of MMPs and TIMPs during ovulation is induced by LH (1–4). LH-induced collagenolysis in the follicle can be blocked...
by indomethacin, hence the effect of LH is mediated by a prostaglandin (5, 23, 24). Similarly, we demonstrated that in porcine luteal tissue luteolysis involves activation of MMPs and TIMPs (11). Often increased activity and release of substances is preceded by increased synthesis which involves activation of the appropriate gene. Indeed, increased MMP-1 and TIMP-1 gene expression were demonstrated prior to ovulation (25, 26), an

Figure 2 Ovarian MMP-1 and TIMP-1 gene expression in relation to prolactin levels. Subgroups were formed on the basis of basal, increasing, steadily elevated or decreasing serum PRL levels. Note that MMP-1 (open bars) and TIMP-1 (solid bars) gene expression increase significantly (* = P < 0.01) with increasing prolactin levels when compared with the group with basal prolactin levels (prolactin subgroup I, left hand graphs).

Figure 3 Ovarian MMP-1 (open bars) and TIMP-1 (solid bars) gene expression in pro-oestrous rats in relation to LH levels. Subgroups of animals were formed on the basis of basal, increasing or decreasing LH levels. Note that at the time when LH levels were not yet elevated gene expression of both enzymes is already statistically higher (* = P < 0.01) than during the time of increasing or decreasing LH levels.
observation confirmed in the present study. A simultaneous activation of MMP and TIMP systems may appear physiologically meaningless; however activation of MMP systems is always accompanied by activation of the appropriate TIMPs because only this guarantees rapid inactivation of otherwise dangerous proteolytic enzymes.

In rats, ovulation is not only preceded by a preovulatory LH peak which has been shown to activate collagenase activity but also by a prolactin surge (27, 28). This preovulatory prolactin surge serves two functions: first, in addition to LH, prolactin is luteotrophic in that the LH-induced luteinization and the associated increased progesterone secretion is augmented (10); secondly, the preovulatory prolactin surge has a luteolytic effect on corpora lutea of the previous ovulation (8). The onset of the preovulatory prolactin surge precedes the onset of the LH surge usually by 2–4 h (24, 27, 28) and from the present experiments it appears that this early phase of the preovulatory prolactin surge is necessary to increase gene expression of collagenases, particularly of MMP-1 which was studied in the present experiments. Clearly MMP-1 gene expression was increased at times of increased prolactin concentrations when serum LH concentrations were still low.

In earlier experiments it was shown that long-lasting blockade of prolactin by dopaminergic drugs will result in accumulation of corpora lutea which, however, do not produce progesterone (8, 10). This accumulation of a functional corpus luteum resulted in increased ovarian weight (8), an effect confirmed in the present study.

The temporal coincidence of increasing preovulatory prolactin concentrations with increased MMP-1 gene expression is not necessarily causally linked. Therefore, we blocked prolactin release over 3 oestrous cycles with bromocriptine. As shown previously, such blockade of prolactin release will not disturb oestrous cyclicity and ovulation (10). Hence, there is enough LH inducible collagenolytic activity to induce ovulation. Blockade of prolactin release will disrupt structural luteolysis of corpora lutea of the previous ovulations (8, 10). Therefore the ovaries of prolactin-deprived animals enlarge due to the accumulation of old corpora lutea. When an injection of bovine prolactin was given at the time of the third pro-oestrus, this caused regression of the accumulated corpora lutea confirming previous observations (8). In the ovaries of bromocriptine-treated versus bromocriptine and prolactin-treated animals MMP-1 gene activity differed significantly in that the prolactin treatment caused increased MMP-1 gene expression.
expression within 90 min after injection of the hormone. This is evidence for the hypothesis that the preovulatory prolactin surge causes increased expression of the MMP-1 gene. Hence, it can be concluded that also under physiological conditions the preovulatory prolactin surge which always precedes the preovulatory LH surge by several hours, may induce increased synthesis of MMP-1.

The preovulatory stimulation of collagenases is always accompanied by increased synthesis and release of inhibitors of metalloproteases. In the present study synthesis of TIMP-1 as measured by TIMP-1 gene expression was also increased under the influence of the preovulatory prolactin surge as well as in prolactin-treated bromocriptine pretreated rats. In line with our results are the observations by Mann et al. (29), who demonstrated that granulosa cell-derived TIMP activity is high in LH-treated granulosa cell cultures and that TIMP gene expression is also increased in pregnant mare serum gonadotrophin (PMSG)-primed 'pro-oestrous' rats. The authors did not measure LH and prolactin concentrations in the PMSG/human chorionic gonadotrophin (hCG)-treated animals but it is well known that such treatment will induce preovulatory prolactin surges (24). Earlier it was demonstrated that granulosa cell-derived TIMP-1 is increased in preovulatory granulosa cell cultures (25) and that TIMP activity is also increased in pregnant mare serum gonadotrophin (PMSG)-primed 'pro-oestrous' rats. The authors did not measure LH and prolactin concentrations in the PMSG/ human chorionic gonadotrophin (hCG)-treated animals but it is well known that such treatment will induce preovulatory prolactin surges (24).

In summary, we demonstrated that in the rat ovaries MMP-1 and TIMP-1 gene expression increases prior to the onset of the preovulatory LH surge at a time when prolactin levels increase. In addition, blockade of the preovulatory prolactin surge by bromocriptine and injection of bovine prolactin at the time when the preovulatory prolactin surge would normally occur also induces increased MMP-1 and TIMP-1 gene expression. It is therefore concluded that increased synthesis of MMP-1 and TIMP-1 is, at least in part, induced by the preovulatory prolactin surge.

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