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Short communication

Progesterone receptor isoform A inhibits isoform B-mediated transactivation in human amnion

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Abstract

Human amnion cells were transfected with progesterone receptor A and/or B, and the progesterone-dependent reporter construct, mouse mammary tumor virus promoter (MMTV), linked to a luciferase gene. In progesterone receptor B-expressing amnion that had been cultured before the onset of labour, treatment with progesterone resulted in an eightfold increase of the reporter activity, whereas in laboured cells, no such increase was seen. In contrast, progesterone receptor A was a weak activator of transcription in laboured and non-laboured amniocytes. When the isoforms A and B of the progesterone receptor were co-transfected, progesterone receptor A exhibited a marked inhibitory effect on progesterone receptor B-mediated transcription. These results show that progesterone receptors A and B function differentially, and progesterone receptor A is a transdominant repressor of progesterone receptor B-mediated transcription in human term amnion. © 2001 Published by Elsevier Science B.V.

Keywords: Progesterone receptor; Progesterone withdrawal; (Human); Amnion; Transfection

1. Introduction

Progesterone is the main "pro-pregnancy" hormone responsible for uterine quiescence during gestation. However, by the end of pregnancy and before the onset of labour, there is an increased expression of "pro-labour" factors, which are normally down-regulated by progesterone during gestation (Keelan et al., 1997). Since there is no detectable fall in maternal progesterone serum levels towards the end of pregnancy, a 'functional' progesterone withdrawal must be considered.

The human progesterone receptor exists as two isoforms, progesterone receptor A (94 kDa) and progesterone receptor B (116 kDa). Progesterone receptor A is an N-terminal truncated form of progesterone receptor B (Kastner et al., 1990; Tora et al., 1988). Both isoforms can regulate the transcription of progesterone-dependent genes upon hormone stimulation. In general, progesterone receptor B is an activator of transcription, whereas the A form exhibits differential effects. It can be an activator, similar to progesterone receptor B, but can also be inactive (Kastner et al., 1990; Meyer et al., 1992), or a repressor of the transcriptional activity of progesterone receptor B (Vegeto et al., 1993; McDonnell et al., 1994).

The aim of current study was to determine the role of progesterone receptor A in mediating the transcriptional effect of progesterone in human gestational tissue, amnion. Since we found that the A receptor was an ineffective activator of transcription as compared with isoform B, we went on to investigate whether progesterone receptor A can repress the transcriptional function of progesterone receptor B, and therefore, might account, at least in part, for the 'functional' progesterone withdrawal before the onset of labour.

2. Material and methods

2.1. Cell culture

For amnion cell culture, fetal membranes were collected from elective caesarian sections at term (non-laboured) or after spontaneous delivery (laboured). The amnion layer

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was peeled from the chorion and washed twice in phosphate-buffered normal saline. The amnion was cut into strips and incubated at room temperature in 0.5 mM EDTA for 15 min. This step was followed by two washes with phosphate-buffered normal saline, and the amnion digested in dispase grade II at 37 °C for 40 min. The digested strips were then placed in 100 ml Dulbecco's modified Eagle's medium with 10% fetal bovine serum, and the epithelial cells were separated from the membranes by vigorously shaking for 3 min. The membranes were discharged and the media containing the cells were centrifuged at 1800 rpm for 10 min. The pellet was re-suspended in 100–175 ml of Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 10 U/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine. Cells were grown in 24-well tissue culture plates at 37 °C and 5% CO_2-humidified atmosphere until they were 70–90% confluent. Before use, cells were washed free from serum.

2.2. Transient transfection

A liposome-mediated transfection method (Tfx®-50; Promega, Southampton, UK) was used to introduce DNA into the cells, following the manufacturers instructions. To investigate the effect of progesterone receptor A and B overexpression on progesterone responsive genes, amnion cells were transiently transfected with 0.5 μg of progesterone receptor A, progesterone receptor B or an empty expression vector (pSG5), and 0.5 μg of the progesterone-sensitive reporter construct, mouse mammary tumour virus promoter (MMTV), linked to a luciferase gene (pMSG vector). To assess the repressive effect of the A isoform of the progesterone receptor on the B-mediated reporter expression, amniocytes were transfected with progesterone receptor B (0.5 μg/well) and increasing amounts of expression vector for progesterone receptor A (0.02, 0.05, 0.1 or 0.2 μg/well) and the MMTV–luciferase reporter. To ensure consistent transfection, a total of 1.5 μg/well DNA was added with the difference made up using empty vector. Cells transfected with an empty vector served as controls.

Forty-eight hours after transfection, the cells were treated with progesterone at a concentration of 10 nM, or its vehicle, for 24 h. After treatment, the medium was removed and the cells were incubated with 200 μl of reporter lysis buffer (Promega) for 15 min. Lysates were stored at −80 °C until analysis.

2.3. Luciferase assay

Samples were centrifuged at $13\,000 \times g$ for 1 min. Twenty microliters of the supernatant and 40 μl of the luciferase assay reagent (Promega) were mixed, and luciferase activity was measured in a luminometer (Turner Design TD20/20, Promega).

2.4. Drugs and solutions

Dulbecco's modified Eagle's medium, EDTA and phosphate-buffered normal saline were purchased from Sigma (Poole, UK). Penicillin/streptomycin, L-glutamine and dispase grade II were purchased from Gibco (Life Technologies, Paisley, UK). Fetal bovine serum was bought from Helena (BioSciences, Sunderland, UK). Progesterone (Sigma) was dissolved in ethanol and diluted in phosphate-buffered normal saline.

2.5. Data analysis

Results are shown as means ± S.E.M. Statistical evaluation was performed with one-way analysis of variance followed by Dunnett's test for multiple comparisons. Differences with probability values of $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Progesterase receptor A and B-mediated transcriptional activity

Fig. 1 shows the effect of progesterone on the transcriptional activity of non-laboured and laboured amnion cells transfected with the empty vector, or expression vectors for the progesterone receptors A or B (each 0.5 μg DNA/

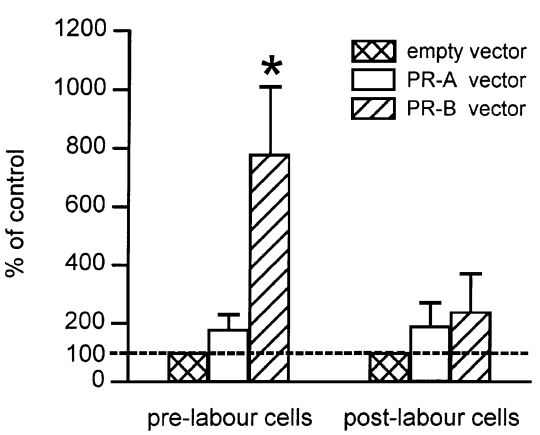

Fig. 1. Effects of progesterone receptor A and B transfection on responsiveness to progesterone in laboured and non-laboured cells. Cells were transfected with 0.5 μg DNA/well of expression vector for progesterone receptor A, B or an empty vector. Cells were treated with progesterone (10 nM), and the progesterone-dependent mouse mammary tumour virus promoter (MMTV)–luciferase construct was used as reporter of transcriptional activity. Responses are expressed relative to vector-transfected cells. Progesterone receptor B transfection resulted in a marked increase of reporter activity in response to progesterone in cells harvested prior to labour, but not in cells post-labour. Progesterone receptor A-transfected cells, laboured and non-laboured, were largely unresponsive to progesterone. Data are shown as means ± S.E.M., $n = 5–8$. $^{*}P < 0.05$ versus vector-transfected cells.

well). Non-laboured cells transfected with progesterone receptor B and treated with progesterone exhibited an eightfold-increased activity of the MMTV–luciferase reporter. Laboured cells transfected with progesterone receptor B showed only a small statistically insignificant increase in reporter activity after progesterone treatment. Transfection of progesterone receptor A into either non-laboured or laboured cells caused only a small statistically insignificant increase of reporter activity under progesterone stimulation, as compared with cells transfected with empty vector. Cells transfected with the empty vector instead of progesterone receptors B responded to progesterone with a small increase of reporter activity, which amounted to $135 \pm 32\%$ in laboured cells ($n = 5$, $P = 0.32$) and $150 \pm 23\%$ in non-laboured cells ($n = 8$; $P = 0.03$, one-sided) with respect to the vehicle of progesterone.

3.2. Repressive function of progesterone receptor A on progesterone receptor B

To identify a possible repressive effect of progesterone receptor A on receptor isotype B function, cells were co-transfected with constant amounts of progesterone receptor B (0.5 μg DNA/well) and increasing amounts of A (0.02, 0.05, 0.2 or 0.5 μg DNA/well). The MMTV–luciferase construct was again used as reporter. As shown in Fig. 2, co-transfection of receptor A resulted in a dose-dependent inhibition of the effect of receptor B on reporter activity in the presence of progesterone. At the most effective dose of A (0.1 μg/well), the response to progesterone was reduced to 40% of that seen with progesterone receptor B alone. These experiments clearly demonstrate the repressive effect of progesterone receptor A on B-mediated transcriptional activity in human amnion cells.

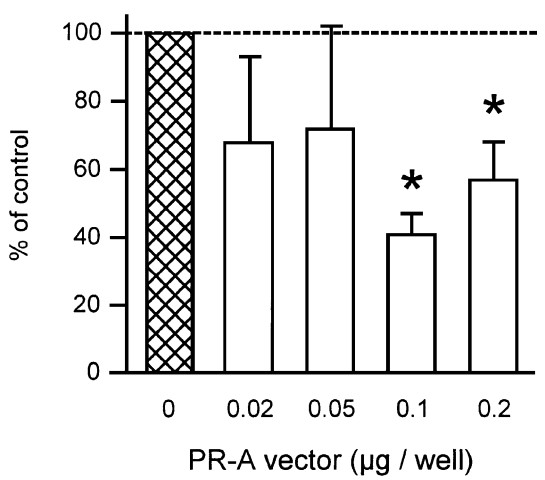

Fig. 2. Repressive effect of progesterone receptor A on B-mediated transcriptional activity. Non-laboured amnion cells were transfected with 0.5 μg DNA/well of expression vector of progesterone receptor B and increasing amounts (0, 0.02, 0.05, 0.1 or 0.2 μg DNA/well) of progesterone receptor A. Cells were treated with progesterone (10 nM), and the progesterone receptor-dependent mouse mammary tumour virus promoter (MMTV)–luciferase construct was used as reporter of transcriptional activity. Responses are expressed as percent of control (progesterone receptor B but with no A transfected). Progesterone receptor B-mediated transcriptional activity was inhibited by A-isoform co-expression in a dose-dependent fashion. Progesterone receptor A was most effective at the dose of 0.1 μg DNA/well. Data are shown as means ± S.E.M., $n = 5$. * $P < 0.05$ versus no PR-A transfection.

4. Discussion

The current study, for the first time, demonstrates that the progesterone receptor isoforms A and B act differentially on progesterone response elements in human gestational tissue. We have observed here that progesterone receptor B is a potent activator of transcription in term non-laboured amnion, but its effect is largely abolished in laboured amniocytes. In contrast, progesterone receptor A by itself appears to be a weak activator of progesterone-sensitive genes, but if co-expressed with progesterone receptor B, it is a potent repressor of hormone-induced transcriptional activity in human amnion. The latter effect was not simply due to an excess of progesterone receptor A but occurred at a ratio of progesterone receptor B to A of 5:1. Progesterone receptor A, therefore, appears to be a dominant repressor of progesterone receptor B function in amniocytes, which suggests that activation and repression by the A receptor are two independent effects. While in some other cell types, progesterone receptor A and B are of similar efficacy as transactivators (Kastner et al., 1990; Meyer et al., 1992), our observations confirm and extend previous findings by Vegeto et al. (1993), who reported that the A form of the progesterone receptor is an ineffective activator of transcription but a potent repressor of progesterone receptor B-mediated transcription in HeLa cells. Cell-specific transcriptional co-factors probably influence the interaction of the receptor with the general transcription apparatus, and are hence, the determinants of the cell-specific effects of the two progesterone receptor isoforms.

The mechanism of the repressive effect of the progesterone receptor A on the B isotype-mediated activity remains unclear. Vegeto et al. (1993) suggested that progesterone receptor A binds with a greater affinity, but in a non-productive manner, to a limited pool of cellular co-factors required for transcription which are common for both isoforms. McKenna et al. (1999) describe nuclear receptor activators and repressors, which enhance or attenuate transcriptional activation by nuclear receptors such as the progesterone receptor. Transcriptional regulation by nuclear receptors is a multistep process in which diverse factors have temporally and spatially distinct functions. The relative expression of co-activators and co-repressors determines the response to a ligand in the target cell. Progesterone receptor A, in a certain cell and promoter context, could strongly interact with a co-repressor, and

thereby, repress the transcriptional activity mediated by progesterone receptor B. Isoform A as well as isoform B contain two transcriptional activation functions (AF), AF-1 and AF-2 (Sartorius et al., 1994; Giangrande et al., 1997). A third activation site (AF-3) unique to progesterone receptor B located at the N-terminal end has also been proposed (Sartorius et al., 1994). On the other hand, both progesterone receptor isoforms possess a so-called inhibitory function (IF), which operates only in the context of A, but not B receptor (Hovland et al., 1998). Therefore, the lack of AF-3 could in part account for the reduced transcriptional activity of progesterone receptor A, while IF might be responsible for receptor A-mediated repression of progesterone receptor B activity.

A second significant finding of the current study is the unresponsiveness of laboured amnion cells to progesterone receptor B stimulation by progesterone. This observation suggests that in amnion cells, the "functional progesterone" withdrawal, as associated with labour, is mediated through inhibition of progesterone receptor function. The ability of the A isoform of the progesterone receptor to attenuate B-mediated transactivation is tempting to speculate that there may be an increase in uterine progesterone receptor A expression which acts to repress receptor B, and therefore, contributes to functional progesterone withdrawal. Demonstration of such an increase has been confounded by the lack of antibodies specific for human progesterone receptor A isoform, and it also needs to be considered that current approaches, such as polymerase chain reaction, Western blot and immunohistochemistry yield only semiquantitative data. Therefore, these assays might be reliable to detect major changes in progesterone receptor expression, while our and others' observations indicate that relatively small changes in the progesterone receptor A/B ratio are sufficient to suppress progesterone receptor B function rather than receptor A being simply absent or present (Wen et al., 1994; Brandon et al. 1993; McDonnell et al., 1994).

Recent studies suggest that progesterone receptors are present in human amnion, although less abundantly than in decidua and chorion, and remain constantly expressed around term (Mitchell and Chibbar, 1995). The amnion is a major source of prostaglandins, which have been linked to the initiation of labour. Post-labour, there is an increase in prostaglandin production in the amnion (Brennand et al., 1995). One mechanism that might limit prostaglandin release before the onset of labour is an increased expression of prostaglandin dehydrogenase. This enzyme, which inactivates prostaglandins, is overexpressed in fetal membranes (e.g. amnion) during pregnancy in a progesterone-dependent manner, but decreases shortly before labour (Challis et al., 1999). Therefore, consistent with our results, inhibition of receptor isoform B-mediated function by isoform A might result in a fall of prostaglandin dehydrogenase expression, causing an increase of prostaglandin tissue levels and subsequently initiating labour.

In summary, the present data suggest that in the amnion, it is progesterone receptor B which mediates the transcriptional effects of progesterone. In amniocytes cultured after labour, however, the transcriptional activity of progesterone receptor B is largely abolished. Since the prevailing action of progesterone receptor A in amnion is transrepression, inhibition of progesterone receptor B-mediated transcriptional activity by A might be a candidate mechanism responsible for the functional progesterone withdrawal associated with human labour.

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