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Ovarian hormones control the changing expression of claudins and occludin in rat uterine epithelial cells during early pregnancy

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Summary

Regulation of the uterine luminal environment is important for successful attachment and implantation of the blastocyst. The contents and volume of luminal fluid are regulated in part by the tight junctions. Using immunofluorescence microscopy, protein and RNA analysis, the cellular distributions of tight junction components claudins and occludin were observed during early pregnancy and under various hormonal regimens. Results indicate that occludin and claudin-4 distribution changed during early pregnancy and in response to ovarian hormones. At the time of implantation and in response to progesterone administration to ovariectomised rats, occludin and claudin-4 showed increased immunolabelling in luminal epithelium. Interestingly, occludin protein detection in uterine luminal epithelial cells at the time of implantation was statistically significantly decreased at the time of implantation compared to day 1 of pregnancy. This suggests that a cytoplasmic pool of occludin is present at day 1 of pregnancy and is redistributed to the tight junctions at the time of implantation. The presence of occludin and claudin-4 in the tight junctions at the time of implantation and in response to progesterone suggests that the paracellular pathway is impermeable to water and Na^+ at this time, and that the transport of such substances takes place via the transcellular pathway.

Introduction

Tight junctions (TJs), with adherens junctions and desmosomes, form the junctional complex at the apical region of the lateral plasma membrane

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in epithelial cells (Farquhar and Palade, 1963). The morphology of TJs visualised using freeze fracture reveals an interconnecting network of continuous intramembranous protein strands (TJ strands). Each TJ strand connects with a corresponding TJ strand on the adjacent cell, forming paired TJ strands, seen as the points of fusion in ultrathin sections (Staehelin, 1973). It is now also known that TJs of different levels of permeability show ion and size selectivity (Gumbiner, 1993). The permeability and the selectivity of TJs in a particular cell type are determined by the composition and ratio of transmembrane proteins in the interconnecting strands of the TJ network (Claude and Goodenough, 1973; Tsukita et al., 2001). These proteins include claudins, a large family of transmembrane proteins (Furuse et al., 1998; Morita et al., 1999), and occludin, a transmembrane protein with several splice variants (Furuse et al., 1993; Ghassemifar et al., 2002; Muresan et al., 2000).

In uterine epithelial cells (UECs), TJs form a key component of the major change for uterine receptivity, called the plasma membrane transformation (Murphy, 1993, 2000; Murphy and Shaw, 1994). In particular, freeze-fracture studies of rat UECs during early pregnancy (Murphy et al., 1982) and under different regimes of ovarian hormones (Murphy et al., 1981) show that TJ in the luminal epithelium (LE) are under hormonal control. Oestrogen stimulation results in a TJ network of strands that are parallel. Progesterone causes the TJ network to extend further down the lateral plasma membrane and the TJ strands are geometrically more complex and more interconnected, reflecting an impermeable TJ network, which is like that seen at the time of implantation. The function of TJs in this epithelium thus appears to be to tightly control the volume and composition of the luminal fluid at the time that the blastocyst is developing and attaching (Murphy et al., 1982).

There have been preliminary studies on some of the major TJ proteins in UECs. Claudin-1, -3, -5 and -7, occludin and ZO-1 have been studied in rat uterine LE during the oestrous cycle (Mendoza-Rodriguez et al., 2005). These proteins were most abundant in TJs during pro-oestrus and oestrus when oestrogen and progesterone levels peak. ZO-1, claudin-1 and occludin have also been studied in uterine LE during early pregnancy (Orchard and Murphy, 2002). Occludin is not present on days 1 and 3 of pregnancy; however, on days 6 and 7, occludin is localised to the TJ region of LE cells. This was the first evidence that TJ molecules are influenced by the progesterone-dominated environment of early pregnancy. Beyond these three TJ proteins, the molecular composition of LE TJs during early pregnancy has not been elucidated. Moreover, the molecular composition of glandular epithelium (GE) TJs and their role in controlling the paracellular pathway in uterine glands have not been studied.

To thus understand how TJ molecules, and the claudins in particular, might control the composition of the luminal environment in which the blastocyst implants, we here study occludin and claudin-1 to claudin-4 in LE and GE in the pregnant rat uterus with immunohistochemical and molecular techniques and we also determine the influence of ovarian hormones on the expression of these TJ proteins during early pregnancy and the period of uterine receptivity.

Materials and methods

The study was approved by the University of Sydney Animal Ethics Committee as protocols K03/3-2002/3/33444 and K03/3-2005/3/3980.

Pregnant animals

Twenty adult, virgin female Wistar rats were used and housed in plastic cages under a 12-h light/dark cycle. The animals had free access to tap water and rat food cubes, and room temperature was maintained at 21 °C. Vaginal smearing was used to confirm normal cycling and to detect the pro-oestrus stage of the oestrous cycle (Long and Evans, 1922). Pregnant rats were obtained by mating pro-oestrus females with fertile males overnight. The presence of sperm in a vaginal smear the following morning confirmed successful mating and was designated day 1 of pregnancy. Five animals for each day of pregnancy were anaesthetised on the morning of days 1, 3, 6 or 7 of pregnancy with an intraperitoneal injection of sodium pentobarbitone (Nembutal; Boehringer, Ingelheim, Germany). Both horns of the uterus were excised and cut into 5-mm-long pieces. For immunofluorescence microscopy, pieces of tissue were immediately coated in OCT embedding compound (Tissue Tek, USA), frozen in super-cooled isopentane (BDH, Poole, UK), and stored in liquid nitrogen. Tissue randomly assigned for polymerase chain reaction (PCR) or protein analysis was snap frozen and stored in liquid nitrogen until use.

Ovariectomised animals

Twenty virgin adult female Wistar rats were used to study the effects of ovarian hormones, with five