Characterisation of chloride currents in the mouse pre-implantation embryo

I. Pons-Meneghetti[1], M.L. Day[1], D.I. Cook[1] and M. Johnson[2], [1]Laboratory of Developmental Physiology, Department of Physiology, Anderson Stuart Building (F13), University of Sydney, NSW 2006, Australia and [2]Department of Anatomy, Dowlin St, Cambridge CB2 3DY, UK.

Pre-implantation embryonic development describes the process by which the embryo grows from the zygote (one-cell) to the blastocyst (64-254 cells) after which it implants into the placenta. Blastocysts are composed of two different cell types, the inner cell mass (ICM) and trophectoderm cells (TE). Chloride currents throughout these stages of embryonic development are not well characterised. A swelling activated Cl^- current was shown to be cell cycle dependent as well as developmentally regulated however this current was not examined in isotonic solutions (Kolajova *et al.*, 2001). In the blastocyst it is believed that Cl^- is transported by both paracellular (Manejwala *et al.*, 1998) and transcellular mechanisms (Brison & Leese, 1993) and that the expansion of the blastocoel cavity is largely reliant on Cl^- channels and a Cl^-/HCO_3^- exchanger (Zhao *et al.*, 1997). The cystic fibrosis transmembrane conductance regulator (CFTR) was recently shown to be present in human blastocysts and it may play a role in the process described above (Ben-Chetrit *et al.*, 2002). This study aimed to characterise Cl^- currents observed in isotonic conditions in the pre-implantation embryo by looking at the eight distinct members of the voltage gated chloride channel family (ClC) (1-7 and K) as well as CFTR.

The mRNA expression pattern of CFTR and ClC channels in the early mouse embryo was determined by RT-PCR. The channels observed in the pre-implantation embryo were ClC-2 to ClC-7, ClC-K and CFTR. Furthermore, ICM and TE cells were separated and RT-PCR of CFTR was carried out for each cell type. The results showed that CFTR mRNA is only present in TE cells. These data suggest that Cl^- channels may play an important roles in the pre-implantation embryo.

The whole-cell patch-clamp technique was used in order to characterise Cl^- currents in the mouse pre-implantation embryo. In the late four-cell stage two major currents were identified through the use of various Cl^- channel antagonists. These included a DIDS-sensitive (non-specific Cl^- channel blocker) and glibenclamide-sensitive (CFTR blocker) current. DIDS inhibited approximately 46% and glibenclamide 38% of the Cl^- current. When both drugs were added simultaneously, the Cl^- current was reduced by approximately 74% indicating that the DIDS and glibenclamide sensitive currents are individual currents. In the ICM glibenclamide had no effect on the Cl^- current whereas in a 3.5 day trophoblast cell line preliminary results indicate that there is a large glibenclamide sensitive current. These electrophysiological results are consistent with the CFTR mRNA expression pattern observed.

The exact role that Cl^- channels play in the pre-implantation embryo still remains to be identified. The results described above show that CFTR along with other ClC channels are present in the pre-implantation embryo at the mRNA level and that they are most likely to be responsible for the currents observed.

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