

## PAF INDUCED CHANGES IN INTRACELLULAR $Ca^{2+}$ AND MEMBRANE POTENTIAL IN THE 2-CELL MOUSE EMBRYO

*Y. Li<sup>1,2</sup>, M. L. Day<sup>2</sup>, C. O'Neill<sup>1,2</sup>*

<sup>1</sup>Human Reproduction Unit, Royal North Shore Hospital, University of Sydney, NSW, Australia;

<sup>2</sup>Physiology, University of Sydney, NSW, Australia

Platelet-activating factor (PAF) is an autocrine survival factor for the preimplantation embryo. PAF induces a transient increase in intracellular  $Ca^{2+}$  ( $[Ca^{2+}]_i$ ) in 2-cell embryos that is caused by the interdependent influx of external calcium and release of calcium from internal stores. A membrane current with L-type calcium channel properties is activated during PAF-induced calcium signalling. Since the L-type channel in many cell types is primarily voltage-gated we were interested to learn whether this was also the case in the 2-cell embryo. The present study investigated the relationship between the PAF-induced  $Ca^{2+}$  transient and changes in membrane potential ( $E_m$ ) in the 2-cell embryo.

The perforated whole-cell patch-clamp technique was used to detect changes in  $E_m$  and standard calcium imaging techniques were used to measure changes in  $[Ca^{2+}]_i$  in 2-cell embryos from QS mice. Embryos were first loaded with Fluo-3 and then pretreated with PAF:acetylhydrolase to degrade the embryo derived PAF before patch clamping. Whole-cell perforated patch-clamping was performed by inclusion of 240mg/ml Nystatin in the pipette solution. Changes in  $E_m$  and  $[Ca^{2+}]_i$  were recorded simultaneously after treatment of the embryo with PAF.

In 2-cell embryos PAF induced a change in  $E_m$ , consisting of an initial small depolarisation of  $2.4 \pm 0.2$  mV ( $42 \pm 4$  sec after addition of PAF) followed by one or more transient hyperpolarisations of  $-8 \pm 1$  mV ( $100 \pm 9$  sec after addition of PAF). Transient increases in  $[Ca^{2+}]_i$  paralleled the membrane hyperpolarisations and were initiated at  $84 \pm 8$  sec after addition of PAF. These responses to PAF were seen in 58% of 2-cell embryos ( $n = 52$ ). It is not yet clear whether these changes in  $E_m$  account for the activation of calcium influx through the L-type channel. The results show for the first time that the 2-cell embryo is an electrically active organism.