44. INVOLVEMENT OF A VOLTAGE-DEPENDENT CALCIUM CHANNEL IN SIGNAL TRANSDUCTION IN THE 2-CELL EMBRYO

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Platelet-activating factor (PAF) is an autocrine trophic factor for the preimplantation embryo that induces an increased $[Ca^{2+}]_i$ in the 2-cell embryo. The $[Ca^{2+}]_i$ transient had an absolute requirement for influx of external calcium. The transients were inhibited by blockers of L-type calcium channel blockers but not by a variety of non-L-type channel blockers. This study used whole-cell patch clamp methodology to assess whether the early mouse embryo expressed a functional calcium channel with the properties of an L-type channel. Standard whole-cell patch clamp techniques were used to study Ca^{2+} currents in two-cell embryos. Membrane potential was held at -60mV and depolarizing voltage pulses of 1 s duration were applied between -20 and +80 mV at intervals of 5 s. Currents were low-pass filtered, sampled and digitized at 0.2 kHz. Ba^{2+} was used as the charge carrier. The currents at each voltage-step were recorded before and after treatment of embryos with different kinds of L-type Ca^{2+} channel blockers: diltiazem (75 μM), nifedipine (80 μM) and verapamil (80 μM). Inward currents were measured as the difference between the whole cell currents before and after the addition of a drug or control to the bath solution (NaCl 55 mM, KCl 4.69 mM, $MgCl_2$ 0.2 mM, Na_2EDTA 0.11 mM, glucose 5 mM, $CaCl_2$ 2.04 mM (equivalent to 1.94 mM free-Ca^{2+}), Hepes 20.4 mM, $BaCl_2$ 50 mM (equivalent to 49.99 mM free-Ba^{2+}), adjusted to pH 7.4, 300 mosM/kg. Using diltiazem, a current of 0.23 ± 0.03 nA (mean ± SEM) was detected and was maximal at a voltage of 36.94 ± 2.59 mV. A similar current was evident when either nifedipine or verapamil were used. Prior treatment of embryos with exogenous PAF resulted in a significant ($P < 0.05$) reduction in the proportion of embryos expressing the current and the size of the current compared with those pretreated with rPAF acetylhydrolase. The results show that 2-cell embryos possess a depolarisation-activated membrane channel, with the properties of an L-type calcium channel. The desensitisation of channel activity by prior PAF challenges suggest that the current was activated during PAF-induced calcium signaling.

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