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 a transient increase in $[Ca^{2+}]_i$ in the 2-cell embryo. The $[Ca^{2+}]_i$ transient had an absolute requirement for influx of external calcium and was 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.

Pre-implantation mouse embryos were recovered after superovulation of female QS mice by intraperitoneal injections of equine chorionic gonadotrophin (10 i.u.) followed 48 hours later by human chorionic gonadotrophin (10 i.u.) and mating. Mice were killed by cervical dislocation and 2-cell embryos were flushed from the reproductive tract into Hepes-modified HTF medium containing 3 mg/ml BSA. The zona pellucida was removed by brief treatment with 0.5% pronase. Standard whole-cell patch-clamp techniques were used to study Ca^{2+} currents in two-cell embryos. The membrane potential was held at -60mV and depolarising voltage pulses of 1s duration were applied between -20 and +80 mV at intervals of 5 s. Currents were low-pass filtered, sampled and digitised 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, consisting of NaCl 55mM, KCl 4.69mM, $MgCl_2$ 0.2mM, Na_2EDTA 0.11mM, glucose 5mM, $CaCl_2$ 2.04mM (1.94 mM free Ca2+), Hepes 20.4mM, $BaCl_2$ 50mM (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 pre-treated 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 challenge suggests that the current was activated during PAF-induced calcium signalling.