Wnt peptides control mammalian cancer cell membrane potential

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Abstract

The Wnt ligands are a family of 19 secreted polypeptides that play important functional roles during development and in disease. Wnt binding to cell membrane receptors leads to intracellular Ca^{2+} release ($[Ca^{2+}]_i$)and nuclear translocation of ß-catenin, a transcription factor co-activator [1]. To investigate early events in this signalling pathway we have used medium throughput techniques (Qpatch) as well as single cell patch recording with simultaneous $[Ca^{2+}]_i$ imaging to show that an early step in the Wnt signal cascade is the control of membrane potential.

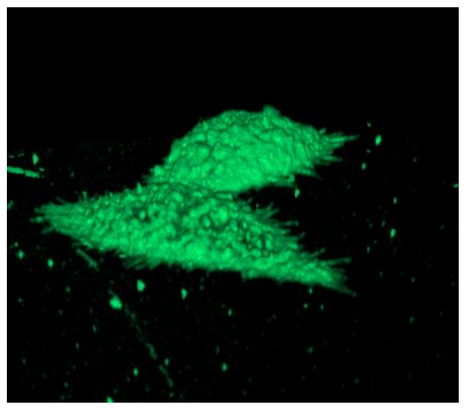
Using PC3 prostate cancer cell lines, local application of Wnts (5A, 9B or 10B) via a puff pipette of produced an small initial depolarisation, followed by a hyperpolarization of the cells of up to 30 mV. Medium-throughput measurements, using controlled microfluidics, also consistently showed an early (<3s) current response at Wnt concentrations of 1.9 nM.

In Ca^{2+} imaging experiments, previously [1], a bolus delivering higher sustained concentrations to the bath produced a larger but delayed (100-200s) $[Ca^{2+}]_i$ rise. We observe a large cationic current reversing around +10mV associated with this rise in Ca^{2+}. The phenomena are simply explained by store operated calcium release. The net effect ensures Wnt activation leads to Ca^{2+} entry, possibly by a voltage-independent non-selective pathway and a release of $[Ca^{2+}]_i$.

We suggest that Wnt control of membrane potential is a signal amplifying mechanism for low nanomolar levels of Wnt, and further suggests that modulation of this critical cell signal transduction pathway could be critical for gene transcription and cellular function. A majority of Wnts do this.

Introduction:

PC3 cells are an epithelial prostate tumour cell line. A range of Wnts trigger a delayed Ca^{2+} rise [1] in these and other cancer cell lines. Recorded in patch clamp the cells have low membrane potential [2] but exhibit an outward current activated apparently at (non-physiological) positive potentials [3]. The main candidate channel carrying this K^+ current is the BK(Ca) α subunit although a KCNN4 channel is also reported [4]. Without baseline subtraction a small 'leak' current is sometimes apparent in recordings. All of these currents are modulated by Wnts as described below



1. Methods

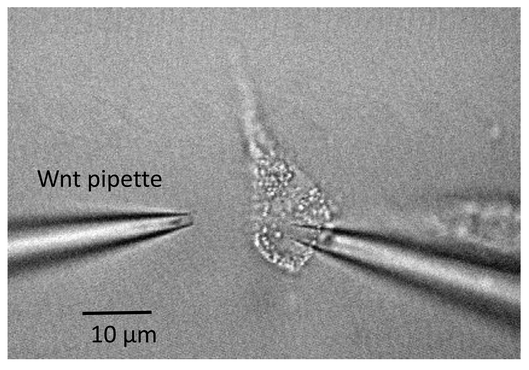
Cell culture: PC3 cells (passages 25-37) were cultured in RPMI 1640 supplemented with 5mM L-glutamine and Fetal Bovine Serum [1] for 48-72 hours.

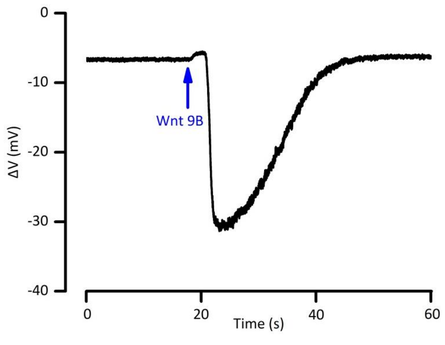
Single cell patch clamp: Cells were recorded using an Axon 200B amplifier in a bath continuously perfused with extracellular solutions at 37°C. Cells were imaged using an upright Zeiss LSM510 confocal microscope (Zeiss). The extracellular solutions contained (in mM) NaCl, 145; KCl, 4; $CaCl_2$, 2; $MgCl_2$,1; HEPES, 10, adjusted to pH 7.4. The intracellular pipette solution was chosen to minimise a Cl^- leak and contained (in mM): K-gluconate, 140; $MgCl_2$, 1; Na_2ATP, 4; EGTA, 1; HEPES, 10 to pH 7.2.

Imaging: 200 μM OGB5N (K_d =20 μM) was included in the pipette and excited at 488 nm. The Wnt timing and concentration was determined by including co-ejected Alexa647 dye. The dye had no effect on the cells, but allowed absolute calibration of the Wntsn (R&D Systems). Wnts were either applied by local application or as a 2 μl volume into the bath.

QPatch high-throughput recording: Cells were recorded in 16 or 48 well system (QPatch, Sophion). Gigaseals were obtained in >90% of the cells. The intracellular solution contained (in mM): $CaCl_2$, 5.3, $MgCl_2$, 1.7, EGTA, 10; HEPES, 10; KF, 20; KCl, 100 and Na_2ATP, 4; adjusted to pH 7.2 and osmolarity 295 mOsm.

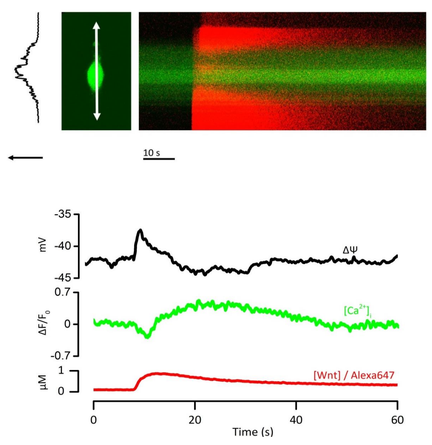
2. Results: Wnts hyperpolarize the membrane potential





Close application of Wnt9B, producing a depolarization-hyperpolarization response. Wnt5A responses comparable.

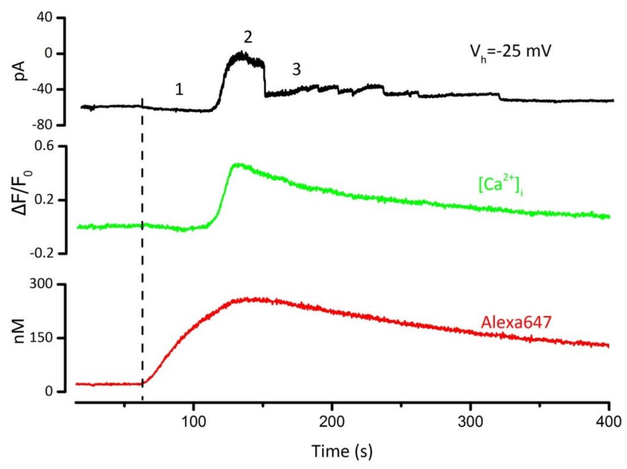
3. Results: Wnts cause rapid Ca^{2+} increase



Line scan
8 ms / line.
Green OGB5N
Red
Alexa47+Wnt9B

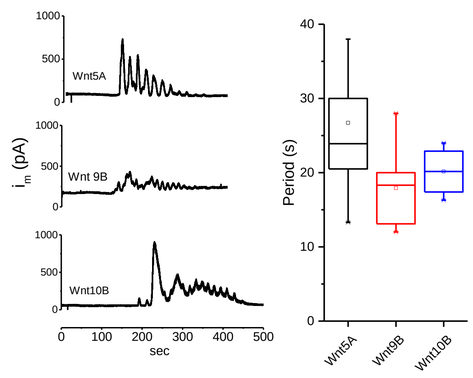
Puff application of Wnt9B. Initial deplolarization , <100 ms. Ca^{2+} rise matches 20 s latency hyperpolarization

4. Results: Three current components induced by Wnts



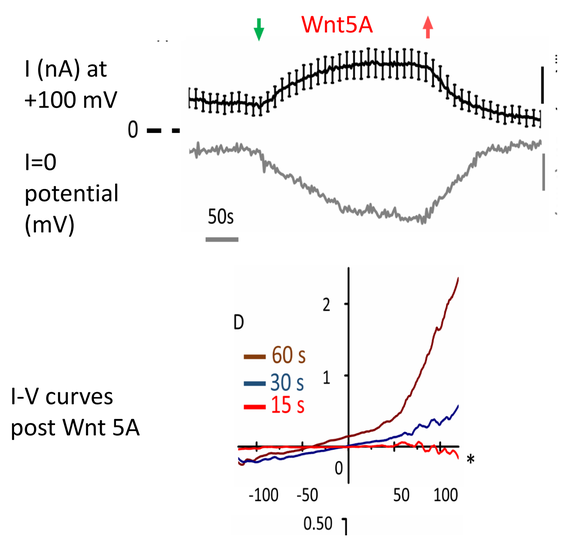
Bolus application of Wnt 9B, V_h = 0 mV. 1, 2, and 3 correspond to distinct temporal components of the currents. Note extended time scale.

5. Results: Wnt 5A, 9B & 10B currents oscillate



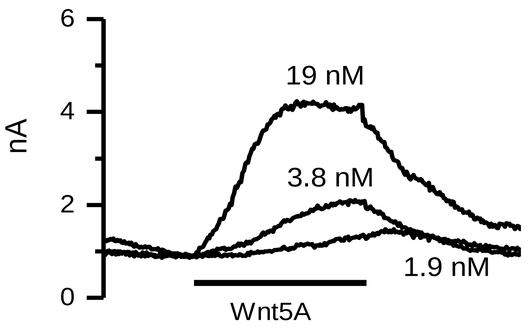
Bolus application of Wnts, V_h = 0 mV. Simultaneous oscillations in Ca_i implicate store release (see [1]).

6. Results: Medium throughput patch clamp systems monitor dynamics and sensitivity to Wnts



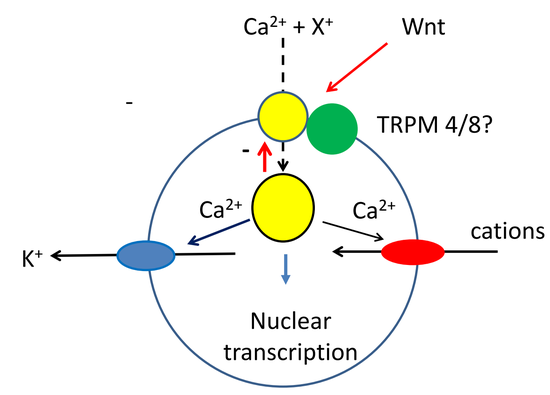
I-V curves post Wnt 5A

QPatch ramp I-Vs indicate responses to Wnt 5A, 9B and 10B Vh=-10 mV.



K(Ca) current sensitivity to Wnt 5A

A simple model



1. Wnt activates cation channel (t < 5s)
2. Ca influx activates K(Ca) (t~ 10s)
3. Ca influx induces SOCE and release (t~100s)
4. Ca rise lead to nuclear transcription

Conclusions:

1. Wnt ligands at nM concentrations hyperpolarize the cell membrane potential by activating an outward K current increasing Ca
2. Wnt ligands activate oscillatory membrane currents in several cancer cell lines, indicating store control and release.
3. At least two ionic conductances, controlled by $[Ca^{2++}]_I$, are involved: 1) a K(Ca) and 2) a cation conductance. The latter may be associated with the secretory role of the cell.

References:

[1] C. Thrasivoulou, M. Millar, A. Ahmed, Activation of intracellular calcium by multiple Wnt ligands and translocation of beta-catenin into the nucleus: a convergent model of Wnt/Ca2+ and Wnt/beta-catenin pathways. *J Biol Chem* **288**, 35651-35659 (2013).
[2] M. E. Laniado, S. P. Fraser, M. B. Djamgoz, Voltage-gated K(+) channel activity in human prostate cancer cell lines of markedly different metastatic potential: distinguishing characteristics of PC-3 and LNCaP cells. *Prostate* **46**, 262-274 (2001).
[3] J. Yan, R. W. Aldrich, LRRC26 auxiliary protein allows BK channel activation at resting voltage without calcium. *Nature* **466**, 513-516 (2010)
[4] Lallet-Daher H, Roudbaraki M, Bavencoffe A, Mariot P, Gackière F, Bidaux G, Urbain R, Gosset P, Delcourt P, Fleurisse L, Slomianny C, Dewailly E, Mauroy B, Bonnal JL, Skryma R, Prevarskaya N Intermediate-conductance Ca2+-activated K+ channels (IKCa1) regulate human prostate cancer cell proliferation through a close control of calcium entry. *Oncogene* **28**, 1792-1806 (2009).