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# INWARD CA2+ CURRENT IN HUMAN PROSTATE SMOOTH MUSCLE – EVIDENCE OF L- AND T-TYPE CHANNELS

## Aims of Study

Benign prostatic hyperplasia (BPH) is considered to be a stromal disease that subsequently affects epithelial growth. Growth factors and suppressors activate downstream intracellular pathways, but the linkage to cellular proliferation is unclear. In many cells raised intracellular $Ca^{2+}$ is evoked by growth factors [1] and $Ca^{2+}$ channel activity correlates with cell proliferation. Two $Ca^{2+}$ channels in particular have been implicated, L-type and T-type, that differ in several characteristics, most significantly the range of activation potentials. T-type channels activate at more negative values [2]. T-type channels are prominent during cell growth in development, and activity is modulated by growth hormone or in conditions leading to cellular hypertrophy and proliferation [3], but are less evident in differentiated cells. $Ca^{2+}$ currents have not been systematically characterised in human prostate smooth muscle, the aim was to carry out an analysis as a prelude to investigate the cellular functions of growth factors.

## Methods

Prostate samples were obtained, with Ethical Committee approval, from patients undergoing either TURP or radical prostatectomy and stored in Ca-free HEPES-Tyrode's solution. Isolated cells were prepared by digestion in this solution, with collagenase (1mg/ml), hyaluronidase (0.5mg/ml) and antitrypsin (0.45mg/ml). In general it was easier to isolate cells from prostatectomy samples as retrieved tissue was less damaged. Experiments were carried out in $HCO_3^-$-buffered Tyrode's at 37°C. Whole-cell recordings used patch-electrodes, whole-cell capacitance was measured on membrane rupture to scale membrane currents to unit capacitance. $Cs^+$-based electrode solutions, to block outward currents, were used to record inward currents, $K^+$-based solutions to record resting potentials. Data are mean±s.d, differences between data sets ($p<0.05$) were tested using Student's *t*-tests.

## Results

Most cells elicited electrical responses, data from TURP chips or prostatectomy specimens were generally pooled as the objective was to characterise the different ionic currents. The resting potential immediately after membrane rupture was -63±11mV (n=8). Action potentials were difficult to elicit by current injection using K-filled electrodes, but could with Cs-filled electrodes. with a maximum upstroke velocity of about $0.8V.s^{-1}$. Inward current was reversibly abolished by removal of superfusate Ca. Peak inward current was at 0mV and was significantly less in cells from TURP chips compared to prostatectomy samples (1.9±0.27 *vs* 3.3±0.40 $pA.pF^{-1}$, n=14 *vs* 24). Cell size was similar in the two groups, 72±6 *vs* 71±5 pF.

The effect of 30µM verapamil and 20µM $NiCl_2$ were tested with three protocols. 1.Depolarisation from -100mV to –10mV; current partially blocked by verapamil, remainder blocked by $NiCl_2$. 2.Depolarisation from –40mV to –10mV; current totally blocked by verapamil. 3.Depolarisation from –100mV to –30mV; current totally blocked by $NiCl_2$. This was interpreted as there being an L-type current, blocked by verapamiil and activated above –40mV and a T-type current activated at more negative potentials and blocked by $NiCl_2$. Separate experiments showed that 30µM verapamil and 20µM $NiCl_2$ were maximal concentrations to achieve these actions. The voltage-dependence of activation was determined by eliciting currents with depolarising steps up to +40mV from: a holding potential of –100mV (activate T and L-type); a holding potential of –40mV (activate L-type), T-type current was the difference current between these protocols. For the L-type current half-maximum activation was at –7.2±1.0mV and for the T-type current at –36.1±2.0mV. The protocol permitted determination of the magnitude of the two current components. Total peak inward current at 0mV was 2.13±0.57 $pA.pF^{-1}$ (n=15). The L-type current was the larger component. At their respective maximum voltages L-type current density was 1.56±0.47 $pA.pF^{-1}$ (+10mV) and T-type current was 0.83±0.16 $pA.pF^{-1}$ (-20mV).

## Conclusions

This study is the first to investigate systematically inward current in human prostate smooth muscle. Mean peak net inward current is about 60% of that in detrusor [2] and can support an action potential. Inward current was separated into two components, using channel blockers and different holding potentials, of which about two-thirds is L-type current. Of interest is the $Ni^{2+}$-dependency of the T-type component as there are several isoforms of the *alpha*-subunit, which forms the channel pore. One of their differentiating characteristics is the $Ni^{2+}$-dependency, the *alpha*1G subtype has a low affinity for $Ni^{2+}$ compared to the *alpha*1H subtype [4]. In detrusor the low affinity (200µM $NiCl_2$) subtype is present [2] but in prostate the low [$NiCl_2$ ] (20µM) used for block suggests the *alpha*1H subtype. Thus any manipulation of prostate channel activity could have selective effects over detrusor. T-type $Ca^{2+}$ channels confer a greater ability to generate oscillatory activity in excitable cells and thus offer an interesting drug target to control mechanical function.

## References


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