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CELLULAR PROPERTIES OF HUMAN SUB-UROTHELIAL MYOFIBROBLASTS - THEIR ROLE IN BLADDER SENSATION

Aims of Study

The sensation of bladder fullness initiates the micturition reflex and alteration of its sensitivity may contribute to syndromes such as sensory urge and even bladder pain. Recently it has been shown that stretch of the urothelium releases ATP at the baso-lateral surface [1] and the purine is believed ultimately to excite sub-urothelial afferents. However, the intermediate steps in this process remain unclear. Recently a layer of sub-urothelial myofibroblasts have been identified that make intimate contacts with nerve endings [2] and express gap junction proteins [3] and raises the possibility that they form an electrical network of cells that act as an intermediary step in the sensation pathway. If so these cells should be electrically active and respond to ATP by generating excitatory responses. The cellular properties of these cells from human biopsy samples were therefore investigated to determine if they fulfilled these criteria.

Methods

Human bladder wall samples were obtained with Ethical Committee approval from patients undergoing either cystectomy or bladder augmentation. The urothelium was dissected from the underlying detrusor and digested at 37°C with a collagenase-based medium with constant stirring for 30 minutes. The tissue was partially disrupted into round urothelial cells and a layer of ovoid or spindle-shaped cells with or without dendrite-like structures. Experiments were performed at 37°C in a HCO_3^-/CO_2 buffered superfusate. Electrophysiological recordings were made with patch-type electrodes filled with a KCl/EGTA-based solution. Intracellular $[Ca^{2+}]$, $[Ca^{2+}]_i$ was measured with the fluorochrome Fura-2, excited at 340/380 nm (50Hz switching) and fluorescence intensity recorded between 410-480 nm. Data are presented as means±s.d.

Results

Cell capacitance was 27±16pF (n=53). Membrane potential was recorded in 16 cells and was on average –63±13mV. However the membrane potential showed either small fluctuation potentials or spikes that peaked around about -35mV. Membrane resistance was estimated to be 8.5-9.0x10^4 Ω.cm^2 from either the time constant of the resting potential change to small depolarising or hyperpolarizing currents or from the slope of the steady-state current-voltage relationship. Under voltage-clamp small, transient inward currents were followed by sustained outward currents on depolarisation from a holding potential of –100mV. Inward current was abolished in Ca-free superfusate, peaked at about –10 mV (25±10pA) and was smaller at 0 mV as the larger outward current overlaid the inward component. Outward current showed outward-going rectification with spontaneous transient components, exhibited a reversal potential at about –80mV and was greatly attenuated by 30mM tetraeythlammonium chloride. 100 µM ATP generated after a delay transient inward currents when the membrane was clamped at –60 mV, similar to the average resting membrane potential. The maximum peak inward current was 23±17pA (n=6). Separate experiments showed that ATP also elicited a transient increase of intracellular $[Ca^{2+}]$. 30µM ATP increased $[Ca^{2+}]$ from 90±60nM to a peak of 832±500nM (18 transients, 4 cells). ATP was also applied to several epithelial cells from the urothelium/sub-urothelium biopsy, but no Ca^{2+} transients were observed.

Conclusions

It is possible to isolate cells from the urothelial layer of the bladder identified as being distinct from epithelial cells: they were more spindle shaped, but were distinctive from smooth muscle cells by their small size and shape. Thus they are not contaminant smooth muscle cells from the underlying detrusor but represent a distinct urothelial/suburothelial cell population, previously identified as myofibroblasts. The cells had many characteristics of excitable cells: a resting membrane potential of about –60mV; regenerative spikes in many cells; inward current followed by outward current; and inward current generated on application of an

agonist. Thus these cells could form an electrical network that would distribute an electrical signal over a reasonable area upon focal depolarisation, which would be facilitated by the relatively high membrane resistance. The Ca-dependence and range of potentials over which inward current was generated imply that it is in part at least a Ca^{2+}-current. The characteristics of the outward current suggest that a significant fraction is a Ca^{2+}-dependent outward current, possibly through BK channels. These cells responded to ATP by inward current generation and a rise of intracellular [Ca^{2+}]. The inward current is consistent with the presence of P2X purinoceptors. These properties are consistent with the hypothesis that these cells form an intermediate stage in the modality of bladder sensation and could act as a variable gain integrating stage. Thus their further study is important to understand how bladder sensation may be modulated.

References

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