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FUNCTIONAL IMAGING OF THE INTERSTITIAL CELLS OF THE BLADDER USING CALCIUM IMAGING

Aims of Study

Interstitial cells have been identified in the urological tract in different species. In the bladder they form two distinctive networks, one in the suburothelium and one in between the smooth muscle cells of the detrusor.
The interstitial cells of the bladder (ICB) are involved in NO signalling and are immunoreactive for the vanilloid receptor VR 1 and for connexin 43, a gap junction protein. They are also stained by c-kit labelling, used in the gastro-intestinal tract as a marker for interstitial cells of Cajal.
Immunohistochemical data suggest that these interstitial cells might play a role in the pathophysiology of neurogenic bladder disease.
Isolated guinea pig ICB show a spontaneous rise in intracellular calcium concentration and respond to cholinergic stimulation.
The analogy of these cells to the interstitial cells of Cajal in the gut could suggest a similar physiological role for the ICB: they could generate an automatic pacemaker signal or act as intermediaries in the transmission of nerve signals to smooth muscle cells.
Fluorescent dyes recording changes in intracellular calcium are a valuable tool to study viable ICB's, comparable to previously described methods for gastrointestinal interstitial cells of Cajal.
The aim of this study is to develop an animal model to examine vital networks of interstitial bladder cells using calcium imaging techniques.

Methods

We use freshly isolated bladder tissue from 6 female Whistar rats. The bladder is opened longitudinally; the suburothelial network of interstitial cells is exposed by careful microdissection of the urothelium in 3 animals. A more profound dissection is carried out in 3 other animals to investigate the detrusal network of ICB's.
The dissected tissue is loaded with a fluorescent dye, Fluo-4 acetoxymethyl (Molecular Probes Inc) 9 µM for 30 minutes then washed in Krebs' solution for one hour. Confocal laser scanning microscopy is used to study fluorescence emission from the calcium-sensitive dyes, with a 488nm excitation line on a Bio-Rad MRC 1024 confocal microscope at a rate of 3 frames per second.
Measurements are analysed with standard software for Windows (Scion Image and Microsoft Excell). Intracellular calcium oscillations are expressed at a rate of F/F_0 of the fluorescence generated by an event (F) against the baseline (F_0)
Subsequently immunohistochemistry is performed on 4 µm frozen sections with primary antibodies directed against C-kit to confirm the presence of the ICB networks.

Results

According to the level of microdissection, we are able to examine the suburothelial or the detrusal network of interstitial cells of the bladder.
Laser scanning microscopy reveals a network of fluorescent cells, morphologically identical to previously described interstitial cells of the bladder.
Spontaneous activity in interstitial cells located in between the detrusor smooth muscle cells has been observed as a doubling of the fluorescence rate, lasting 20 seconds before returning to baseline.
Immunohistochemistry allowed us to identify the examined cells as being c-kit positive cells.

Conclusions

Functional imaging of the interstitial cells of the bladder using calcium imaging is a feasible way of visualising viable networks of interstitial cells. We will use this technique to study the

physiology of the ICB and to examine their possible pathophysiological role in detrusor overactivity.
More experiments are needed to characterise the spontaneous activity of the interstitial cells located in between the detrusor smooth muscle cells.