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Voiding Dysfunction

Characteristics of Spontaneous Activity in the Bladder Trigone

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Abstract

Background: During bladder filling, the trigone contracts help keep the ureteral orifices open and the bladder neck shut. The trigone generates spontaneous activity as well as responding to neuromuscular transmitters, but the relationship between these phenomena are unclear.

Objectives: To characterise the cellular mechanisms that regulate and modify spontaneous activity in trigone smooth muscle.

Design, setting, and participants: Muscle strips from the superficial trigone of male guinea-pigs were used for tension experiments and immunofluorescent studies.

Measurements: In isolated trigonal cells, intracellular Ca^{2+} was measured by epifluorescence microscopy using the fluorescent Ca^{2+} indicator Fura-2.

Results and limitations: Spontaneous intracellular Ca^{2+} transients and contractions were observed in trigonal single cells and strips and were significantly higher compared to the bladder dome. Ca-free superfusate and verapamil terminated spontaneity. T-type Ca^{2+} channel block with $NiCl_2$ depressed slightly Ca^{2+} transients but not spontaneous contractions. Neither the BK_{Ca} channel blocker iberiotoxin nor the SK_{Ca} channel blocker apamin had any effect on single cell activity. By contrast, the Cl^- channel blocker niflumic acid attenuated significantly both Ca^{2+} transients and muscle contractions. Agonist stimulation (carbachol, phenylephrine) up-regulated activity. Gap junction labelling (Cx43) was approximately 5 times denser in the trigone than in detrusor smooth muscle. The gap junction blocker 18-ß-glycyrrhetinic acid modulated spontaneous contractions in the trigone but not in the bladder dome.

Conclusions: Trigone myocytes employ membrane L-type-Ca^{2+} channels and Cl^- channels to generate spontaneous activity. Intercellular electrical coupling ensures its propagation and, thus, sustains contraction of the whole trigone.

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1. Introduction

The trigone has recently attracted urologists' attention again, initiated by a discussion of whether it should be spared in intradetrusor botulinum toxin injections to treat bladder overactivity [1,2]. It is strategically located between the ureteric orifices and bladder outlet; however, little is known about its function during the micturition cycle. The superficial trigone develops, with the ureter, from an outgrowth of the mesonephric duct and provides, as a transverse-orientated interureteric muscle, competent vesico-ureteric anchoring [3,4]; it represents an area of dual parasympathetic-muscarinic and sympathetic-adrenergic innervation. There is evidence that micturition is initiated by relaxation of the trigone, which then funnels urine into the proximal urethra; relaxation may be NO/cGMP-mediated through nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase–positive nerves [5,6].

However, information about trigone behaviour during filling is sparse, and spontaneous activity might play a crucial role. Various smooth muscles in the lower urinary tract show spontaneous contractile activity during the filling phase. In the bladder dome, this behaviour may help the organ maintain a state of minimal surface area; in the urethra, it contributes significantly to closure pressure [7]. Although spontaneous activity has been intensively investigated in both dome and urethra, studies in the trigone are lacking. Two papers, which primarily investigated agonist effects on detrusor muscle, noted pronounced spontaneous activity of the trigone. Contractile activity was present in 71% and 89% of trigone strips from pigs and humans, respectively, compared to 20% from the dome [8]. Microelectrode recordings showed rhythmic variation of membrane potential in the dome; but in the trigone, there were superimposed additional bursts of spikes [9]. This study also described up-regulation of trigone activity by agonist stimulation.

We set out to corroborate previous reports of spontaneous activity in the trigone, to elucidate its origin and physiological characteristics, and to explore possible routes of modulation at an intra- and intercellular level. Data were compared to those from similar detrusor preparations of the bladder dome.

2. Methods

2.1. *Tissue preparation and tension experiments*

Tissue was obtained from the bladders of 70 male guinea-pigs (400–499 g, Dunkin-Hartley), killed by Schedule-1 cervical dislocation in accordance with the UK Animals (Scientific Procedures) Act, 1986. The bladder dome was resected cranially to the orifices, the base longitudinally opened on the ventral site, and the trigonal area exposed. The superficial layer was distinguished from underlying detrusor by its paler, whitish appearance. After removing the mucosa by blunt dissection, a thin strip (average: 6.4 mg) between the ureteral orifices was cut; only one strip per bladder was prepared. Strips were also dissected from the dome. Preparations (28 trigone, 21 dome) were mounted in a horizontal superfusion trough between a fixed hook and an isometric force transducer and superfused with a 24 mM-$NaHCO_3$/5%CO_2-buffered Tyrode's solution (5–10 ml min^{-1}, 37 °C, pH 7.4). All chemicals throughout were from Sigma unless specified in the text. Transducer output was recorded through a bridge amplifier and displayed on a moving-paper chart recorder.

2.2. *Cell isolation, measurement of intracellular calcium ($[Ca^{2+}]_i$)*

Cells were prepared from muscle strips (37 animals) as might be used for tension measurements by dissociation with a collagenase-based enzyme mixture [10]. Cells were loaded with the fluorescent Ca^{2+} indicator Fura-2 (Molecular Probes) at 37 °C for 9 min and stored at 4 °C for later use. An aliquot of cell suspension was placed in a glass-bottomed chamber maintained at 37 °C, mounted on the stage of an inverted microscope. After cells had settled to the chamber floor, it was superfused with Tyrode's at 1.5 ml min^{-1}. Cells were illuminated alternately at 340:380 nm (32 Hz) and fluorescent light collected between 410 and 510 nm with a photomultiplier tube. The ratio of fluorescence on excitation at 340 or 380 nm is a function of $[Ca^{2+}]_i$: Calibration of the signal has been detailed elsewhere [10].

2.3. *Connexin immunofluorescence*

Five de-urothelialised strips were placed immediately in optimal cutting temperature (OCT) embedding medium and snap-frozen in liquid N_2. Triplicate sections (10 μm) for each specimen were cut on a cryostat and mounted on 3-aminopropyltriethoxysilane (APES)-coated slides. Sections were post-fixed in methanol (−20 °C, 5 min), blocked in 1% bovine serum albumin (BSA) for 45 min and incubated with primary antibodies against connexin43 and connexin45 (2 h, room temperature: Cx43, mouse mAB, Chemicon 1:1000; Cx45; gift from Professor N Severs, Imperial College London). Binding was visualised using a Cy3-conjugated secondary antibody (goat, anti-mouse, Chemicon, 1:1000), and nuclei counterstained with 4′,6-diamidino-2-phenylindole (DAPI; Invitrogen, 1:50,000) in the same step (45 min, room temperature [RT]). Three representative images per section were taken using a confocal microscope (Zeiss LSM 510 Meta, x40) at fixed pinhole and detector gain settings. The number of punctate connexin fluorescent particles was counted in each image using the ImageJ freeware program (rsb.info.-nih.gov/ij/; constant threshold, particles 2-infinity).

2.4. *Experimental protocols and data analysis*

Experimental variables obtained during interventions were compared to the mean of pre- and postintervention values (controls) and expressed either as absolute values or percentage of control. Control experiments, using only the vehicle for test agents, were always carried out and exerted no significant effects. Values are mean ± SD, n = number of cells or muscle strips. Differences between means of data sets were examined by paired or unpaired student t tests and those between two incidences by Fisher's exact test. The null hypothesis was rejected at $p < 0.05$.

3. Results

3.1. *Characteristics of spontaneous activity*

Spontaneous activity was recorded in trigonal single cells and strips as Ca^{2+} transients and contractions, respectively. Myocytes were about half the size of those from the bladder dome (Fig. 1A). The resting $[Ca^{2+}]_i$ was 45.5 ± 21.1 nM (n = 107 cells), and cells always showed either discrete Ca^{2+} transients (Fig. 1B) or, less often, more sustained and fused

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