

DIFFERENTIAL ROLES OF NEURONAL- AND UROTHELIUM-DERIVED NO IN THE MODULATION OF THE SPONTANEOUS ACTIVITY OF MOUSE DETRUSOR SMOOTH MUSCLE

Hypothesis / aims of study

Nitric oxide (NO) is known to be released from nerves and urothelium in urinary bladder, but its role has not been well established. c-Kit antigen expressing interstitial cells (ICs) in the bladder are considered to be targets of NO, and consequently may modulate the contractility of the detrusor. Examining the relationship between NO and ICs, and their function in the bladder, may help us to understand the pathophysiology of bladder dysfunction. Therefore, we aim to localize the immunoreactivity of neuronal nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS) and c-Kit in the mouse bladder, and investigate the function of NO and ICs in the spontaneous activity of urothelium-intact and -removed detrusor preparations from nNOS knockout (nNOS^{-/-}) and sibling control (nNOS^{+/+}) mice.

Study design, materials and methods

nNOS, eNOS and c-Kit antibodies were applied to immunostain whole mount bladder preparations from both nNOS^{+/+} and nNOS^{-/-} mice. To measure electrical activity of bladder smooth muscle, detrusor strips were isolated from mouse bladders. Changes in membrane potential of smooth muscle cells (SMCs) were recorded using sharp electrode intracellular recording. To image Ca²⁺ dynamics, tissue strips were exposed to 10 μ M Oregon Green 488 BAPTA-1 AM for 70 min, and then image series were acquired with a Leica SP2 upright confocal microscope. Simultaneous intracellular recording and confocal imaging was also performed to determine the relationship between spontaneous electrical activity and concurrent Ca²⁺ dynamics.

Results

nNOS-immunopositive neurons and nerve fibers were seen in the detrusor layer of nNOS^{+/+} mouse bladder. However, these nNOS-immunopositive structures were absent in nNOS^{-/-} bladders. Both nNOS and eNOS expressing cells were present in the basal urothelial layer of nNOS^{+/+} and nNOS^{-/-} bladders; an area which is very close to the suburothelial c-Kit positive ICs. c-Kit immunopositive spindle-shape ICs and mast cells were identified in the suburothelial and detrusor layer of mouse bladder. In spontaneously active SMCs, global increases in intracellular Ca²⁺ concentration (whole cell flashes) were coincident with spontaneous action potentials (sAPs). sAPs and whole cell flashes occurred at a lower frequency in nNOS^{-/-} SMCs (sAPs: mean 2.1 min⁻¹ vs. 4.3 min⁻¹, number of animals (*n*) = 13 for nNOS^{-/-}, *n* = 19 for nNOS^{+/+}, *P* < 0.05; whole cell flashes: median 0 min⁻¹ vs. 1.1 min⁻¹, *n* = 7, *P* < 0.0001). The frequency of sAPs in nNOS^{-/-} preparations was enhanced through the addition of a nitric oxide donor, diethylamine NONOate sodium salt (NONOate; 100 μ M) (9.4 min⁻¹ vs. 1.9 min⁻¹, *n* = 10, *P* < 0.05). Additionally, NONOate increased the frequency of sAPs when the cGMP pathway was blocked by 1H - [1, 2, 4] oxadiazolo [4, 3-a] quinoxalin-1-one (10 μ M) (8.6 min⁻¹ vs. 3.2 min⁻¹, *n* = 5, *P* < 0.05). An eNOS inhibitor, N^G-mono-methyl-L-arginine (10 μ M) and removal of the urothelium both significantly increased the frequency of sAPs in the nNOS^{-/-} preparations (control: 1.3 min⁻¹ vs. 2.3 min⁻¹, *n* = 4, *P* < 0.05; urothelium-removed: 3.8 min⁻¹, *n* = 13 vs. urothelium-intact: 2.1 min⁻¹, *n* = 6, *P* < 0.05). A c-kit inhibitor, imatinib mesylate (10 μ M) inhibited the frequency of sAPs in nNOS^{+/+} preparations only when the urothelium was removed (control: 4.3 \pm 0.7 min⁻¹ vs. imatinib mesylate: 2.7 \pm 0.8 min⁻¹; *n* = 4, *P* < 0.05).

Interpretation of results

The fact that the frequency of spontaneous activity of mouse detrusor is low when the nNOS gene has been disrupted, whilst exogenous NO enhances the frequency of sAPs even when the cGMP pathway has been blocked, suggests that neuronal NO may enhance the generation of sAPs without having to activate the cGMP cascade. The c-Kit positive ICs present in the suburothelial layer immediately below the basal NOS containing urothelial cells may, however, be targets of urothelium-derived NO. The fact that the c-kit inhibitor, imatinib mesylate, suppresses the frequency of sAPs only in the absence of the urothelium suggests that the function of c-Kit positive ICs may be suppressed by an inhibitor released from the urothelium. Additionally the augmentation of the frequency of sAPs generated in nNOS^{-/-} SMCs when eNOS is inhibited or the urothelium is removed, supports the proposition that urothelium-derived NO plays an inhibitory role in the spontaneous activity of detrusor, probably by suppressing the activity of suburothelial ICs.

Concluding message

This study has for the first time demonstrated c-Kit immunopositive ICs in the mouse bladder. The results suggest that neuronal-derived NO facilitates the generation of spontaneous activity via a cGMP-independent pathway, whereas urothelium-derived NO inhibits spontaneous activity of the mouse detrusor probably by suppressing the function of suburothelial ICs. The overall degree of excitation of the bladder will thus be a balance between these two effects of NO. Upsetting the balance may cause bladder dysfunction.

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Is this a clinical trial?	No
What were the subjects in the study?	ANIMAL
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	Yes
Name of ethics committee	UK Animals (Scientific Procedures) Act 1986 and European Communities Council Directive 86/09/EEC