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# PRODUCTION AND REGULATION OF NITRIC OXIDE IN THE RAT BLADDER

## Aims of Study

In many smooth muscle systems, the nitric oxide (NO) signaling pathway plays an important role in modulating smooth muscle function. Furthermore, NO synthase (NOS) activity is regulated by protein-protein interactions in caveolae, specialized plasmalemmal microdomains involved in the integration and modulation of various signal transduction processes. In the urinary bladder however, the extent to which NO effects normal bladder function and its role in detrusor dysfunction remain unresolved. Moreover, neither the identification of the relevant caveolin isoform nor its co-localization with NOS have been described in the urinary bladder. The purpose of this study was to demonstrate the location of NO production, determine the functional role of NO and identify potential mechanisms regulating the production of NO.

## Methods

To detect NO production in rat bladder sections, tissue was incubated in an NO indicator dye (DAF-2DA) at 37E for 1 hour. Changes in integrated fluorescence intensity were measured before and after carbachol or electrical field stimulation (EFS) using a multiphoton/confocal imaging system. To determine the functional effects of NO, bladder strips were placed in organ baths between platinum electrodes and equilibrated for 60 minutes. The contractile responses to EFS and carbachol were determined before and after exposure to a nitric oxide synthase inhibitor (L-NAME) or an NO donor (sodium nitroprusside). To determined the distribution of NOS and caveolin, bladders were processed for immunohistochemistry. Tissue sections (10 Φm) were fixed in acetone washed in PBS and incubated overnight with primary antibodies (eNOS, nNOS, caveolin-1, caveolin-3). After washing, sections were incubated with fluorescent-labeled secondary antibodies or processed using the ABC method.

## Results

Extensive fluorescence was detected in the urothelium, smooth muscle bundles, and localized in neuronal fibers. Basal NO production in smooth muscle and urothelial layers was markedly increased by carbachol and EFS. EFS induced contractions were significantly increased in the presence of L-NAME and attenuated after administration of SNP. The carbachol dose response curve was potentiated in the presence of L-NAME and SNP caused an immediate relaxation of carbachol induced contractions. Exposure to L-NAME resulted in a dose dependent increase in amplitude of spontaneous activity. Extensive immunostaining of caveolin-1 was detected in SM bundles and urothelium, consistent with prominent staining of plasmalemma. Immunoreactivity for eNOS and nNOS was also detected. Colocalization of caveolin-1 with eNOS was identified in the bladder, particularly in the urothelium.

## Conclusions

These findings indicate that NO production is responsive to physiologic stimuli, and neurally mediated or receptor activated smooth muscle contraction is modulated by an inhibitory effect of NO. Furthermore, colocalization of caveolin and NOS suggests that production of NO may be regulated by targeting of NOS to caveolae and its interaction with caveolin, analogous to NOS regulatory mechanisms described in the cardiovasculature.