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Nitric oxide synthase activity in the human urogenital tract

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Abstract Nitric oxide (NO) has been suggested as a non-adrenergic non-cholinergic neurotransmitter in the urogenital tract and has previously been shown to have a smooth muscle relaxing effect in the urogenital organs both in various animals and in humans. It has been shown that NO is a mediator of the erection and the dilatation of the bladder neck and urethra. The aim of the study was to analyse nitric oxide synthase (NOS) activity in the human urogenital tract. NOS activity was measured by the conversion of L-[U-^{14}C] arginine to L-[U-^{14}C] citrulline. In the upper urinary tract there was Ca^{2+}-dependent NOS activity in the renal pelvis, but no significant NOS activity could be found in the ureter. In the lower urinary tract we found high Ca^{2+}-dependent NOS activity in the urethra, intermediate activity in the bladder neck and comparatively low activity in the detrusor muscle. In the male genital tract the testis and epididymis had no significant NOS activity. The vas deferens, prostate, seminal vesicle and corpus cavernosum were found to have high levels of Ca^{2+}-dependent NOS activity. Ca^{2+}-independent NOS activity was not obtained in the urogenital tract. Our results correspond well with previous functional studies indicating NO to be an important nerve-induced mediator of erection and in the micturition reflex, but also suggest that NO may be involved in several other functions in the human urogenital tract.

Key word Human · Male genital tract · Nitric oxide Nitric oxide synthase · Urinary tract

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Nitric oxide (NO) has been suggested to be a non-adrenergic non-cholinergic neurotransmitter in the lower urinary tract in several species including sheep [8, 22], rabbit [1, 2], pig [16, 17] and man [2, 14]. NO has also been implicated as the nerve-induced mediator of erection in rat [3], rabbit [10] and man [4, 9]. It has also been suggested that nerve-induced release of NO mediates the relaxation of the bladder neck and urethra during the micturition reflex in humans [7, 14]. NO synthase (NOS) has been shown to be located within nerve fibres in human corpus cavernosum and urethra [4, 14], and it has also been suggested that nerve stimulation can induce the release of NO in both human corpus cavernosum and urethra [14]. NO is synthesised from the amino acid L-arginine by NOS with simultaneous formation of L-citrulline [15]. Several different NOSs have been suggested and three different isoforms, nerve NOS (n-NOS), endothelial NOS (e-NOS) and inducible NOS (i-NOS), have been cloned [13]. n-NOS and e-NOS are Ca^{2+} dependent whereas i-NOS is Ca^{2+}-independent and is activated as a host defence mechanism [13]. The aim of our study was to investigate the localisation of NOS in the human upper and lower urinary tract and in the male genital tract in humans.

Materials and methods

Specimens from human detrusor, bladder neck, prostatic urethra, proximal ureter, renal pelvis, testis, epididymis, vas deferens and prostate were obtained from patients subjected to radical surgery for cancer. Human corpus cavernosum was obtained from transsexual males undergoing reassignment surgery. The study was approved by the local ethics committee. The tissue samples were immediately frozen in liquid nitrogen and stored at −70 °C until studied for NOS activity. NOS activity was studied by a citrulline formation assay where the conversion of L-(U-^{14}C) arginine to L-[U-^{14}C] citrulline was measured [13]. Tissues were homogenised in ice-cold homogenisation buffer containing 320 mM sucrose, 10 mM hydroxyethylpiperazine ethanesulphonic acid (HEPES), 0.1 mM ethylene glycol tetra-acetic acid (EGTA), 1 mM DL-dithiothreitol, 10 µg/ml trypsin inhibitor, 10 µg/ml leupeptin, phenylmethylsulpho-

nyl fluoride 100 μg/ml and 2 μg/ml aprotinin (adjusted to pH 7.2 at 20 °C with 1 M HCl). The homogenate was centrifuged at 10 000 *g* for 30 min at 4 °C and the soluble fraction used for the measurement of NO synthase activity. The tissue extract was added to tubes prewarmed to 37 °C, containing 100 μl buffer consisting of 50 mM potassium phosphate, pH 7.2, 50 mM L-valine, 100 μM NADPH, 1 mM L-citrulline, 20 μM L-arginine and L-[U-^{14}C] arginine (Amersham, 150 000 dpm), 1.2 mM $MgCl_2$ and 0.24 mM $CaCl_2$. Duplicate incubations for 10 min at 37 °C were performed for each sample in the presence or absence of either EGTA (2 mM) or EGTA plus N^{ω}-monomethyl-L-arginine (2 mM each), to determine the level of the Ca^{2+}-dependent and Ca^{2+}-independent NOS activity, respectively. The reaction was terminated by removal of substrate and dilution by addition of 1.5 ml 1:1 (v/v) H_2O/Dowex AF 50W-X8, pH 7.5. Five millilitres H_2O was added to the incubation mix, and 2 ml supernatant was removed and examined for the presence of L-[U-^{14}C] citrulline by liquid scintillation counting. The level of citrulline is expressed as picomoles per gram tissue (wet weight) per minute. Drugs manufacturers were as follows: aprotinin, L-arginine, L-citrulline, Dowex AF 50W-X8, DL-dithiothreitol, NADPH, trypsin inhibitor, phenylmethylsulphonly fluoride and L-valine (Sigma, USA); L-[U-^{14}C] arginine (150 000 dpm, Amersham, UK); leupeptin (Peninsula Labs., USA); N^{ω}-monomethyl-L-arginine (Wellcome Research Laboratories, UK).

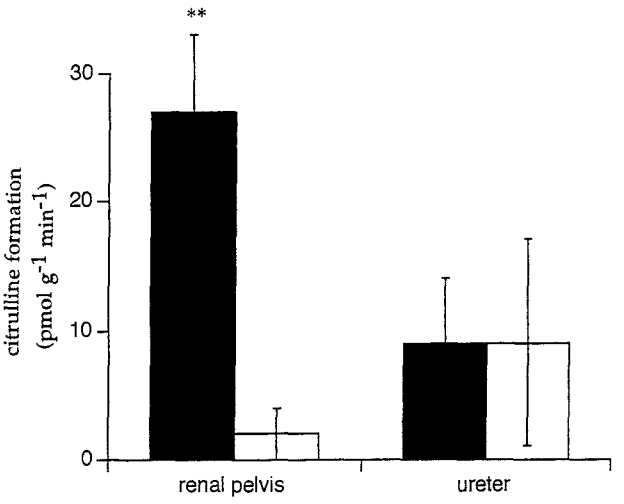

Fig. 1 Calcium-dependent (*filled bars*) and calcium-independent (*blank bars*) NO synthase (NOS) activity in the human upper urinary tract as measured by citrulline formation (pmol/min per gram). There was a significant Ca^{2+}-dependent NOS activity in the renal pelvis ($n=6$) but not in the ureter ($n=5$), and no significant Ca^{2+}-independent NOS activity could be found. Statistics are according to Student's *t*-test for paired data. ** $P<0.01$. Means ± SEM

Results

Human upper urinary tract

In the human renal pelvis Ca^{2+}-dependent NOS activity was found ($n=6$, $P<0.01$), whereas there was no significant Ca^{2+}-independent NOS activity (Fig. 1). In the proximal ureter ($n=5$) no significant Ca^{2+}-dependent or Ca^{2+}-independent NOS activity was obtained (Fig. 1).

Human lower urinary tract

In the human detrusor ($n=11$, $P<0.01$), bladder neck ($n=17$, $P<0.001$) and proximal urethra ($n=7$, $P<0.05$), Ca^{2+}-dependent NOS activity was evident (Fig. 2). The NOS activity in the urethra ($P<0.05$) and the bladder neck ($P<0.05$) was significantly higher than in the detrusor smooth muscle (Fig. 2). There was no Ca^{2+}-independent NOS activity in the human lower urinary tract under these clinical conditions (Fig. 2).

Human male genital tract

In the testis ($n=4$) and epididymis ($n=5$) no significant Ca^{2+}-dependent or Ca^{2+}-independent NOS activity could be found (Fig. 3). In the vas deferens ($n=10$, $P<0.01$), seminal vesicle ($n=9$, $P<0.001$) and prostate ($n=11$, $P<0.001$) Ca^{2+}-dependent NOS activity was obtained but not Ca^{2+}-independent activity was evident (Fig. 3). In the corpus cavernosum there was Ca^{2+}-dependent NOS activity ($n=2$), but no Ca^{2+}-independent activity was found (Fig. 3).

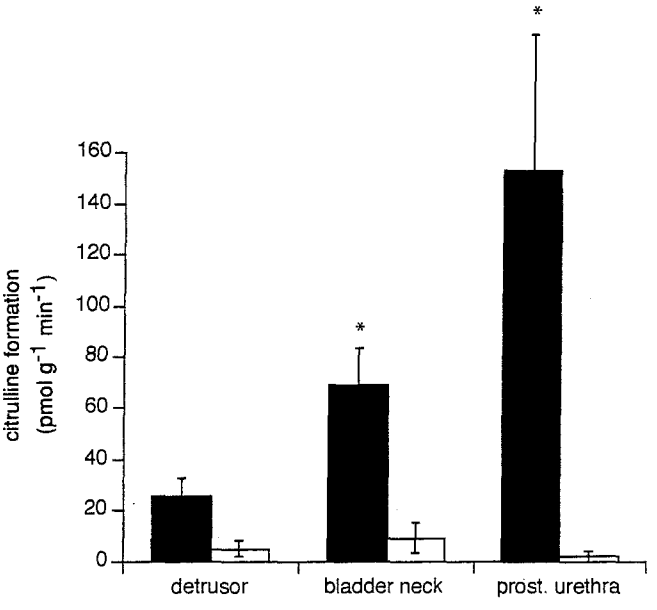

Fig. 2 Calcium-dependent (*filled bars*) and calcium-independent (*blank bars*) NO synthase (NOS) activity in the human lower urinary tract as measured by citrulline formation (pmol/min per gram). Both the urethra ($n=7$) and the bladder neck ($n=17$) showed significantly higher NOS activity than the detrusor muscle ($n=11$), denoted by *. Statistic are according to Student's *t*-test for unpaired data, * $P<0.05$. Means ± SEM

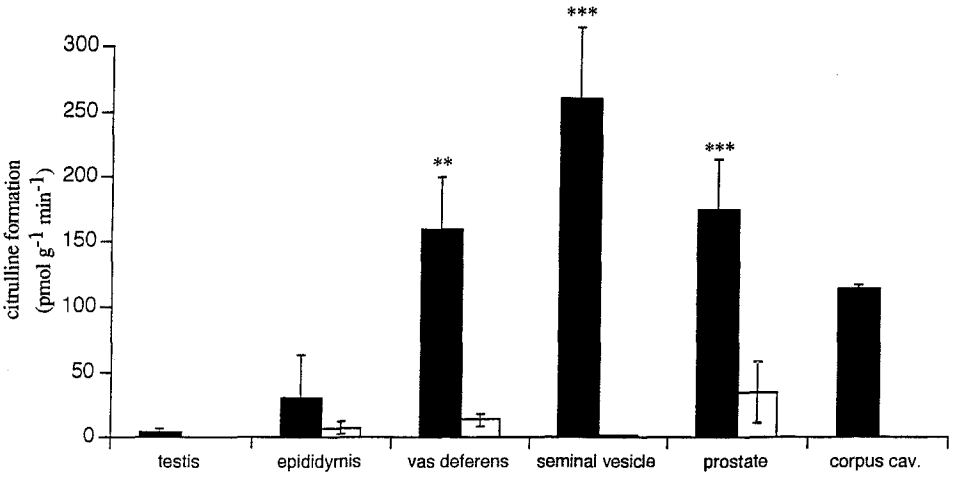

Fig. 3 Calcium-dependent (*filled bars*) and calcium-independent (*blank bars*) NO synthase (NOS) activity in the male genital tract as measured by citrulline formation (pmol/min per gram). Significant calcium-dependent NOS activity was found in the vas deferens ($n=10$), seminal vesicle ($n=9$), prostate ($n=11$) and corpus cavernosum ($n=2$) but not in the testis ($n=4$) or epididymis ($n=5$). No significant calcium-independent NOS activity was found in the male reproductive organs. Statistics are according to Student's *t*-test for paired data. ** $P<0.01$, *** $P<0.001$. Means ± SEM

Discussion

In this study we have analysed the NOS activity in different parts of the human urogenital tract. In both upper and lower urinary tract as well as in the male genital tract we found evidence for NOS activity, which was found to be Ca^{2+}-dependent. Both nerve NOS (n-NOS) and endothelial NOS (e-NOS) are Ca^{2+}-dependent. There are several indications suggesting that the NOS activity in the urogenital tract is due to n-NOS activity. Thus both immunohistochemical and NADPH-diaphorase staining have shown the presence of n-NOS in neurons in the urogenital tract [3, 4, 17]. Furthermore, nerve-induced relaxations are attenuated by NOS inhibitors [1, 8, 10, 12] and nerve stimulation elicits the release of NO metabolites in the urogenital tract [14]. It has been shown that nerve-induced smooth muscle responses in the lower urinary tract are in good agreement with the localisation of NOS activity [7]. However, it is possible that some of the Ca^{2+}-dependent NOS activity was due to e-NOS activity. As expected, we were unable to demonstrate i-NOS activity in the human urogenital tract. Ca^{2+}-independent NOS activity is induced by various cytokines and toxins [15] and is not expected to be present in the urogenital tract under these clinical conditions.

In the upper urinary tract we found significant Ca^{2+}-dependent NOS activity in the renal pelvis. There are studies suggesting that neuropeptide Y and vasoactive intestinal peptide (VIP) can control the smooth muscle tone in the renal pelvis and the pyeloureteral junction [20]. Our findings suggest that NO might be of importance in regulating the tone in the renal pelvis, causing relaxation of the smooth muscle fibres, since it has been shown that NO induces relaxation in the renal pelvis and inhibits the spontaneous smooth muscle activity (Iversen et al., unpublished data). The innervation of the ureter is sparse, especially in the upper third [23], which is in good agreement with our results showing no significant NOS activity in the proximal ureter.

In the lower urinary tract NO has been suggested to play a major role as a nerve-induced mediator during the micturition reflex in a range of species [1, 2, 8, 16, 17, 22] including man [2, 7, 14]. Our results, showing high activity of Ca^{2+}-dependent NOS both in the human urethra and bladder neck, are in good agreement with previous studies [4, 7, 14].

In the male genital tract several studies have shown that NO is a mediator of erection in animals such as rat [3], rabbit [10] and humans [9, 12, 14]. NOS-containing nerves are present in the human corpus cavernosum [4, 14] and the NO metabolites NO_2^- and NO_3^- can be measured after nerve stimulation in erectile tissue [14]. Our finding of NOS activity in the human corpus cavernosum correlates well with previous studies [14, 18]. The Ca^{2+}-dependent NOS activities measured in the prostate, seminal vesicle and vas deferens were found to be high compared with the other urogenital tissues studied. However, the functional importance of NO production in these organs is unclear but since they are innervated from the same nerves as the bladder, urethra and penis [21] it is likely that this NOS activity is also of the n-NOS type. NOS-staining nerve fibres from the pelvic plexus have been described as penetrating the peripheral human prostate [4]. It is well known that there is α-adrenergic sympathetic innervation in the prostate [5, 6]. It is possible that the α-adrenergic innervation may be antagonised by an NANC-induced relaxation mediated by NO. It has been suggested that in the vas deferens of rodents the sympathetic noradrenergic nerves provides a general tone in the proximal part whereas purinergic nerves cause rapid contracts in the prostatic part [19]. NO may also have a role here as an antagonist. In the seminal vesicle the very high NOS activity could be of importance in relaxing the smooth muscle to enable the vesicle to harbor large amounts of seminal fluid and NO may also be involved in the ejaculation reflex.

Conclusion

Ca^{2+}-dependent NOS activity was found in various parts of the human urogenital tract. The results correlate well with previous studies indicating NO to be an important nerve-induced mediator in the micturition reflex and of erection. The data also suggested that NOS activity is involved in several other functions in the human urogenital tract since the ability to synthesis NO is present in several areas of the urogenital tract.

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