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Modulation of smooth muscle activity by nitric oxide in the human upper urinary tract

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Abstract The aim of the study was to ascertain whether nitric oxide (NO) might regulate motility in the human upper urinary tract. Smooth muscle activity in the human renal pelvis and proximal ureter was studied *in vitro* in organ baths, and nitric oxide synthase (NOS) activity was studied by measurement of citrulline formation. NO, glyceryl trinitrate (GTN) and sodium nitroprusside (SNP) significantly reduced the frequency of spontaneous rhythmic contractions in renal pelvis and proximal ureter. Exogenously applied NO elicited relaxations in pre-contracted renal pelvis. Calcium-dependent NOS activity was significant in the renal pelvis but undetectable in the ureter. Also, NOS activity was absent in hydronephrotic renal pelvis. NO, SNP and GTN inhibited smooth muscle activity in the human upper urinary tract. NOS activity was obtained in normal renal pelvis but not in hydronephrotic renal pelvis. Regulation of urinary tract NO concentrations might offer a strategy for treatment of renal colic and disturbances in upper urinary tract motility.

Key words Ureter · Renal pelvis · Nitric oxide · Upper urinary tract motility · Nitric oxide synthase activity

Nitric oxide (NO) has been shown to be a mediator of non-adrenergic, non-cholinergic nerve-induced smooth muscle relaxation [2, 6, 10]. NO synthase (NOS) exists in several isoforms that enzymatically convert L-arginine to NO and L-citrulline in equal amounts. Ca^{2+}-dependent isoforms, e-NOS and n-NOS, are present in vascular endothelial cells and in neurons, respectively, and a Ca^{2+}-independent inducible enzyme, i-NOS, is present in, for example, macrophages and neutrophils as part of the host defence system [8]. Recently, nitric oxide synthase (NOS)-immunoreactive neurons have been demonstrated in the ureter and renal pelvis in rats [11]. The physiological function of these neurons is, however, unknown. In the rabbit urethra, non-adrenergic, non-cholinergic nerve-mediated relaxation is caused by NO [1]. The presence of NOS and the localisation of NOS activity in the human lower urinary tract was recently demonstrated [5, 9]. Little is known about NOS activity or the effect of NO on smooth muscle activity in the upper urinary tract. The aim of the present study was to investigate the effects of NO and of NO donors such as glyceryl trinitrate (GTN) and sodium nitroprusside (SNP) in the human upper urinary tract and to establish the distribution of NOS activity in this region.

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Methods

Specimens of human renal pelvis and proximal ureter adjacent to the pelviureteral junction were obtained from patients undergoing nephrectomy for renal cell carcinoma or patients undergoing pyeloplasty for hydronephrosis. The study was approved by the local ethics committee.

Organ bath experiments

Smooth muscle preparations were prepared on ice and were dissected free from surrounding fat and connective tissue. The ureter segment was cut into 10- to 12-mm long spiral strips. The renal pelvis was cut into strips of the same size. The preparations were suspended vertically in 6-ml organ baths containing Tyrode's solution (concentration in mM: Na^+ 161; K^+ 2.8; Ca^{2+} 1.8; Mg^{2+} 0.5; Cl^- 144; HCO_3^- 24; $H_2PO_4^-$ 0.4; glucose 5.6) kept at 37 °C and continuously aerated with 5% CO_2 in O_2. Motor activity was

recorded isotonically by Harvard smooth muscle transducers, and tracings were made on a BBC 120 recorder. During a period of 90–120 min the preparations were left to stabilise. Most of the smooth muscle preparations presented spontaneous rhythmic contractions when stabilised. In some of the preparations, initially without contractile activity, spontaneous rhythmic contractions could easily be triggered by application of histamine (10^{-7} M–10^{-5} M). Approximately one quarter of the renal pelvis preparations did not respond with rhythmic contractions but presented tonic contraction after application of histamine (10^{-7} M). Frequency of rhythmic contractions was defined as the average number of contractions per minute during 5 min immediately before and after drug application, respectively. In the pre-contracted preparations relaxation reaching the level of the baseline was defined as 100% relaxation. The baseline was defined as the degree of contraction immediately before application of histamine.

NO solution was prepared by pure NO gas (water solubility 7 ml/100 ml at standard temperature and pressure) bubbling through ultrafiltrated water deoxygenated with helium. This gave a stock solution of 3×10^{-3} M [13].

NOS activity

NOS activity was measured by the conversion of L-[U^{-14}C] arginine to L-[U^{-14}C] citrulline.

For the citrulline formation assay, tissues were homogenised in ice-cold homogenisation buffer containing 320 mM sucrose, 10 mM HEPES, 0.1 mM EGTA, 1 mM DL-dithiothreitol, 10 μg ml^{-1} trypsin inhibitor, 10 μg ml^{-1} leupeptin, 100 μg ml^{-1} phenylmethylsulfonyl fluoride and 2 μg ml^{-1} aprotinin (adjusted to pH 7.2 at 20 °C with 1 M HCl). The homogenate was centrifuged at 10000 *g* for 30 min at 4 °C, and the soluble fraction used for the measurement of NOS activity. The tissue extract was added to tubes prewarmed to 37 °C, containing 100 μl of a 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, UK; 150000 dpm), and 1.2 mM $MgCl_2$. 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$^{\omega}$-monomethyl-L-arginine (2 mM each), to determine the level of the Ca^{2+}-dependent and the Ca^{2+}-independent NOS activity, respectively. The reaction was terminated by removal of substrate and dilution by the addition of 1.5 ml of 1:1 (v/v) H_2O/Dowex AF 50W-X8, pH 7.5. Five millilitres of H_2O was added to the incubation mix, and 2 ml of the 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 of tissue (wet weight) per minute.

Drugs

The drugs used were obtained from the following sources: GTN (Schwartz Pharma, Monheim, Germany); SNP (Hoffmann-La Roche, Basel, Switzerland); NO gas, 100% (AGA, Lidingö, Sweden); histamine chloride, aprotinin, L-arginine, L-citrulline, Dowex AF 50W-X8, DL-dithiothreitol, NADPH, N$^{\omega}$-nitro-L-arginine, trypsin inhibitor, phenylmethylsulfonyl fluoride and L-valine (Sigma, USA); L-[U^{-14}C] arginine, 150000 dpm (Amersham, UK), leupeptin (Peninsula Laboratories, Belmont, Calif., USA); N$^{\omega}$-monomethyl-L-arginine (Wellcome Research Laboratories, UK).

Statistics

Experimental data were expressed as mean values ± SEM. Statistical significance was tested according to Student's *t*-test for paired or unpaired observations.

Results

Spontaneous rhythmic contractions were seen in the human renal pelvis and ureter preparations. In some preparations rhythmic contractions did not occur until histamine (10^{-7}–10^{-5} M) was applied. A stable basal tone was seen in the preparations after 120 min stabilisation time. The frequency of spontaneous contractions in the renal pelvis was 2.0 ± 0.2 min^{-1} ($n = 10$), and in the proximal ureter it was 2.4 ± 0.3 min^{-1} ($n = 4$).

NO, GTN and SNP significantly and dose-dependently reduced the frequency of spontaneous rhythmic contractions in the human renal pelvis (Figs. 1, 2) and proximal ureter (Figs. 1, 3). NO and the NO donors were more effective in reducing the frequency of spontaneous contractions in the renal pelvis than in the proximal ureter ($P < 0.05$). There was a small reduction in amplitude of rhythmic contractions in the proximal ureter upon application of the NO donors(GTN 6 ± 2% and SNP 15 ± 4%, $P < 0.05$; NO 2 ± 2%, NS). In the renal pelvis no significant reduction in the amplitude of spontaneous contractions was seen. In 25% of the renal pelvis preparations, application of histamine (10^{-7} M) did not elicit rhythmic contractions, but induced a sustained contraction (Fig. 4). In these preparations exogenous NO (10^{-7} M–10^{-5} M) caused dose-dependent relaxations ($n = 6$, $P < 0.01$) (Figs. 4, 5). NO, GTN or SNP did not affect the basal tone in the preparations which were not pre-contracted. The NOS inhibitor N$^{\omega}$-nitro-L-arginine (10^{-4} M) did not affect frequency or amplitude of spontaneous rhythmic contractions in the renal pelvis or the proximal ureter.

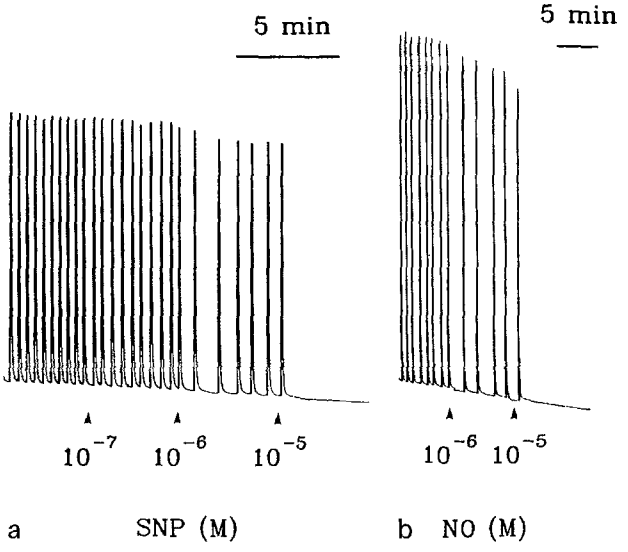

Fig. 1 Isotonic recording of spontaneous rhythmic contractions in the isolated human **a** proximal ureter and **b** renal pelvis. Reduction of frequency after application of nitric oxide (*NO*) and sodium nitroprusside (*SNP*), respectively

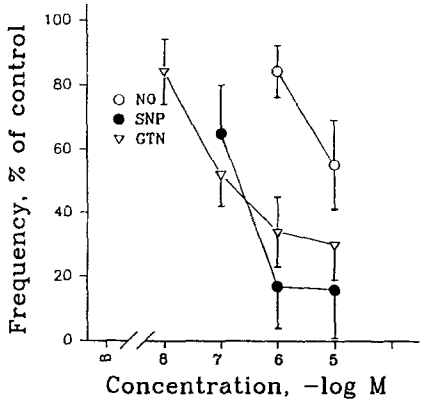

Fig. 2 Dose-dependent inhibitory effects of nitric oxide (*NO*), sodium nitroprusside (*SNP*) and glyceryl trinitrate (*GTN*) on frequency of contractions in the isolated human renal pelvis

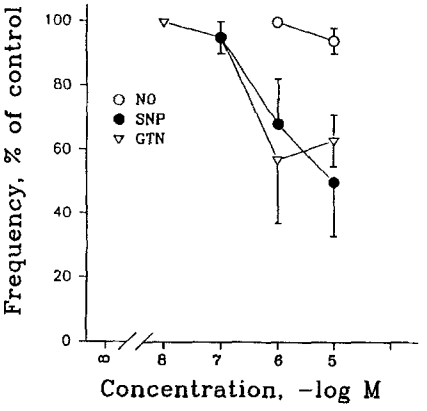

Fig. 3 Dose-dependent inhibitory effects of nitric oxide (*NO*), sodium nitroprusside (*SNP*) and glyceryl trinitrate (*GTN*) on frequency of contractions in the isolated human proximal ureter

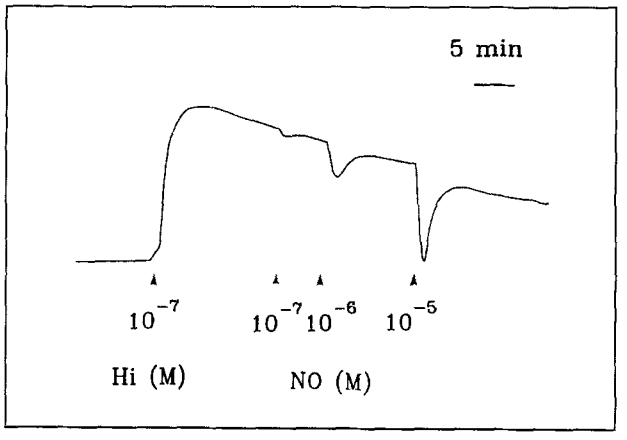

Fig. 4 Isotonic recording of relaxations induced by exogenous nitric oxide (*NO*; 10^{-7}–10^{-5} M) in the isolated human renal pelvis. The tissue was pre-contracted with histamine (*Hi*; 10^{-8}–10^{-7} M)

In preparations from renal pelvis and proximal ureter obtained from patients ($n = 10$) with hydronephrosis due to obstruction of the pelviureteral junction,

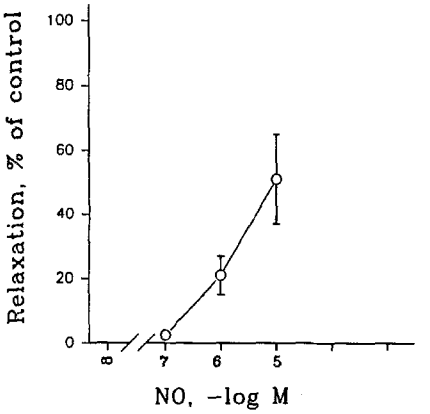

Fig. 5 Dose-dependent relaxation by exogenous nitric oxide (*NO*) in isolated human renal pelvis, pre-contracted with histamine (10^{-7} M)

NO, GTN and SNP dose-dependently reduced the frequency of spontaneous contractions, as in the non-obstructed tissues (not shown).

NOS activity, as measured by citrulline formation, was found in the human upper urinary tract. There was significant calcium-dependent NOS activity in the renal pelvis (22 ± 6 pmol g^{-1} min^{-1}, $P < 0.01$), but no calcium-independent activity. No significant activity was seen in the proximal ureter. Neither calcium-dependent nor calcium-independent activity was seen in the hydronephrotic renal pelvis.

Discussion

Results from the present study provide evidence that NO and the NO donors, GTN and SNP, are inhibitors of tonic and rhythmic smooth muscle activity in the human upper urinary tract. Ca^{2+}-dependent NOS activity was found in the human renal pelvis which suggests a possible role for endogenous NO as a modulator of smooth muscle activity. It has been shown that the frequency of peristaltic action is limited by the renal pelvic pacemaker [3]. The pacemaker has been suggested to be of myogenic origin [7]. In most tissues, NO induces smooth muscle relaxation by activating guanylate cyclase and increasing cGMP levels in smooth muscle cells [12]. Since NO can induce hyperpolarisation in intestinal smooth muscle [14], it is possible that a similar action by NO in the upper urinary tract could result in a decreased frequency of rhythmic contractions as well as smooth muscle relaxation. Thus both an effect on pacemaker frequency and a general smooth muscle-relaxing effect might be at hand. A decrease in renal pelvic frequency is reflected by an increase in diameter of the renal pelvis, as determined by ultrasound scanning, in healthy asymptomatic subjects [4]. Thus, the effect of NO and NO

donors might have clinical implications, because an agent that decreases peristaltic frequency and induces relaxation in smooth muscle could reduce intrapelvic pressure and facilitate stone passage.

In our experiments the NOS inhibitor N^{ω}-nitro-L-arginine (10^{-4} M) did not affect spontaneous smooth muscle activity in the isolated proximal ureter or the renal pelvis, indicating that under the present experimental conditions the endogenous production of NO was not sufficient to modulate smooth muscle activity. The NOS activity is probably located within neurons, since NOS-staining neurons are present in the wall of the renal pelvis in rats [11]. It is conceivable that the ischaemia during the nephrectomy might have resulted in decreased viability of the nerves. In support, the preparations responded only poorly to electric nerve stimulation (unpublished observations). However, since significant Ca^{2+}-dependent NOS activity was found in the renal pelvis, further studies are required to elucidate the physiological role of endogenous NO in the human upper urinary tract.

In hydronephrotic renal pelvis no NOS activity was observed. However the effects of NO and NO donors resembled those in the normal renal pelvis. It is unclear whether the absence of NOS activity is involved in the mechanism causing pyeloureteral obstruction or whether it is a result of the hydronephrosis. In the intestine, pyloric stenosis and Hirschsprung's disease have been suggested to be due to a deficiency of NOS neurons [15, 16].

In conclusion, exogenous NO, SNP and GTN inhibit rhythmic and tonic smooth muscle contractions in the human upper urinary tract. Endogenous NO might be a modulator of smooth muscle activity in the human upper urinary tract, possibly by reducing peristaltic rate or by causing smooth muscle relaxation in the renal pelvis. Regulation of urinary tract NO concentrations might offer a strategy for treatment of renal colic and disturbances in upper urinary tract motility.

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