Abstract  We examined the contribution of K+ channels to the relaxation responses induced by different redox forms of nitric oxide (NO+, NO– and NO•) in comparison with those evoked by electrical field stimulation (EFS) of nitrergic nerves in the sheep urethra. K+ channel blockers with different selectivity profile were used. Sodium nitroprusside (SNP) and different S-nitrosothiols were used as NO+ donors, Angeli’s salt as an NO– donor and nitroglycerin (GTN) was chosen as a representative compound known to require metabolic activation in the target tissue. Pure NO gas was used to prepare NO• solutions. Relaxation evoked by EFS of nitrergic nerves or by exogenous NO• was not inhibited by any of the K+ channel blockers, but was enhanced by 4-aminopyridine [inhibitor of voltage-dependent K+ (Kv) channels]. This suggests that, whereas K+ channel activation and hyperpolarization of the postsynaptic membrane do not contribute to relaxation, prejunctural modulation of the nitrergic neurotransmission by Kv channels may be relevant. Relaxation induced by NO+ or NO– donors was not affected by K+ channel blockade with the following exceptions: glybenclamide, a blocker of ATP-sensitive K+ channels (KATP), enhanced responses to SNP and Angeli’s salt, 4-aminopyridine inhibited relaxation evoked by Angeli’s salt and GTN, and charybdotoxin, a blocker of large-conductance, Ca2+-activated K+ channels (BKCa), inhibited those induced by the S-nitrosothiol S-nitrosoglutathione. These results do not suggest the existence of a general mechanism of action on K+ channels for compounds releasing either NO+ or NO– in the ovine urethra. In contrast, ODQ only moderately inhibited relaxations to NO+. In addition, the NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (carboxy-PTIO) effectively inhibited responses to NO• whilst not affecting those to EFS or NO–, suggesting a close similarity between the nitrergic transmitter and nitroxyl ion.

We conclude that nitrergic relaxation induced either by the endogenous transmitter or by exogenous NO donors in the ovine urethra is not mediated by postsynaptic alterations in the K+ conductance; only a prejunctural modulation through Kv channels seems to be significant. In addition, the production and/or release of alternative redox forms of NO, such as NO–, may be involved in neurotransmission processes in the urethra.

Keywords Nitric oxide · Nitrergic relaxation · Nitroxyl anion · Nitrosionium cation · NO donors · S-nitrosothiols · Potassium channels · Urethra

Introduction  Electrical field stimulation (EFS) of the sheep urethra induces non-adrenergic non-cholinergic (NANC) relaxation (García-Pascual et al. 1991) that is mediated by increases in cGMP levels (García-Pascual and Triguero 1994) and blocked by nitric oxide (NO) synthesis inhibitors (García-Pascual et al. 1991; Triguero et al. 2000). In addition, the presence of constitutive NO synthase (NOS) activity in nerve fibres in the smooth muscle layer of the urethra has been shown (Triguero et al. 1993; García-Pascual et al. 1996). These observations suggest strongly the involvement of the L-arginine-NO pathway in the urethral NANC
relaxation that mediates the decrease in resistance accompanying micturition. However, whether NO itself or a NO-containing compound is the ultimate transmitter mediating nitrergic relaxation in the urethra remains controversial (García-Pascual and Triguero 1994; García-Pascual et al. 2000). In this sense, several relatively stable NO adducts, specially S-nitrosothiols, have been investigated as possible nitrergic carriers (Kerr et al. 1992; García-Pascual et al. 1999), but definitive data supporting such a role are still lacking. Furthermore, the fact that the NO redox form NO• itself does not react with thiol groups under physiological conditions, whereas alternative redox activated states of NO, such as the nitrosionium cation (NO+) derived from endogenous S-nitrosothiols (Arnelle and Stamler 1995) or the nitroxy anion (NO•−), formed directly by NO synthase (Schmidt et al. 1996), are more likely to nitrosylate thiol groups directly, favours the view that alternative redox forms of NO might act as nitrergic mediators. Recently, it has been suggested that the NO•− donated by Angeli’s salt is a better candidate than NO• to serve as the nitrergic transmitter in the rat anococcygeus muscle (Li et al. 1999).

Membrane K+ channels are one of the best known proteins of biological significance, the function of which could be modified by redox interactions with NO adducts. K+ channels at nerve terminals may modulate neurotransmitter release by affecting electrical events of the cell membrane (Stjärne et al. 1991). In addition, opening K+ channels in smooth muscle cell membranes increases K+ efflux, hence resulting in membrane hyperpolarization, closure of voltage-dependent Ca2+ channels and decreases in the cytosolic Ca2+ concentration, thus leading to relaxation of the smooth muscle cells (Nelson and Quayle 1995). Activation of K+ channels via cGMP-dependent or independent mechanisms may be involved in the relaxations induced by nitrergic nerve stimulation, NO and NO donors (Bolotina et al. 1994; Jiang et al. 1998). However, this role of K+ channels does not seem to be common to all tissues and species and, where present, differences related to the K+ channel types involved also exist.

To our knowledge, there is no information regarding the role of K+ channels in the relaxation of sheep urethral smooth muscle produced either by the endogenous nitrergic transmitter or by exogenous NO and NO donors. Thus, the aim of the present study was to compare the effects of different K+ channel blockers on relaxation induced by different types of nitrergic stimulation: EFS of nitroglycerine, which inhibits voltage-gated (Kv) channels (Robertson and Nelson 1994); charybdotoxin, which inhibits large-conductance, Ca2+−activated (BKCa) channels (Nelson and Quayle 1995); apamin, which interacts selectively with small-conductance Ca2+−activated (SKCa) channels, and glybenclamide, which blocks ATP-sensitive (KATP) channels (Kolb 1990). In addition, we extended our study to establish whether NO•− donated by Angeli’s salt could account for the relaxant activity of the nitrergic transmitter in the sheep urethra.

### Methods

**Drugs.** Pure NO gas was obtained from L’Air Liquide (Madrid, Spain). (±)Noradrenaline bitartrate (NA), atropine sulphate, guanethidine sulphate, EDTA sodium salt, EGTA, sodium nitrite, l-cysteine, N-acetyl-l-cysteine, glutathione, SNP, 8-bromoguanosine-3′,5′-cyclic monophosphate (8-Br-cGMP), glybenclamide and apamin were obtained from Sigma (St. Louis, Mo., USA). GTN solution (1% in ethanol:propylene glycol:water, 1:1:1.33) and zaprinast were from Merck (Darmstadt, Germany) and Calbiochem (La Jolla, Calif., USA), respectively. SNAP, 4-aminoypyridine, H[1,2,4]-oxadiazole-[4,3-a]-quinoxalin-1-one (ODQ), 4H-8-bromo-1,2,4-oxadiazoloxazin-4,3-diazobenz(b,1,4) oxazin-1-one (NS 2028) and 2-(4-carboxyphenyl)-4,4,5-tetramethyl imidazole-1-oxyl 3-oxide (carboxy-PTIO) were purchased from Alexis (Läufelfingen, Switzerland). Hydroxylamine hydrochloride, sodium ethoxide and isopropyl nitrate, for synthesis of Angeli’s salt, were obtained from Sigma-Aldrich (Steinheim, Germany). NA was dissolved in distilled water with 0.1 mM ascorbic acid, glybenclamide, zaprinast, NS2028 and ODQ in dimethylsulphoxide and charybdotoxin in bovine serum albumin (0.1%). All other drugs were dissolved in distilled water. Solutions were stored at −20°C and working dilutions were made daily.

**Preparation of NO•−, S-nitrosothiols and Angeli’s salt solutions.** A saturated, aqueous solution of NO•− was prepared daily from pure NO gas as previously described (García-Pascual et al. 1999). NO concentrations, in both the saturated and the working dilutions, were determined before use by means of a NO electrode (ISO-NO, WPI, Herts., UK). GSNO was synthesized as described previously (Hart 1985). Solutions of SNC and SNAC were prepared by addition of sodium nitrite (100 mM) to the same amount of a solution containing 250 mM HCl, 1 mM EDTA and 100 mM L-cysteine (for SNC) or N-acetyl-l-cysteine (for SNAC). Solutions of SNAP and GSNO were freshly prepared in distilled water. S-nitrosothiol concentrations were determined spectrophotometrically (model UV-1601 UV-visible spectrophotometer; Shimadzu, Tokyo, Japan) assuming molar absorption coefficients (εmax) of 16.6 M−1 cm−1 for SNC, (εmax) 17.8 M−1 cm−1 for SNAP, (εmax) 16 M−1 cm−1 for GSNO and (εmax) 614 M−2 cm−2 for SNAP. Working dilutions were prepared in de-oxygenated distilled water immediately before use, kept on ice and maintained in the dark. Angeli’s salt (trioxodinitrate, Na2 N2 O3) was synthesized following the method described by Hughes and Cammack (1999). The purity of the product was tested spectrophotometrically and was in agreement with the data reported by Hughes and Cammack (1999). Working solutions were made in NaOH (0.1 mM), kept on ice and protected from light.

**Tissue preparation.** Lower urinary tracts from female lambs (2–3 months) were collected at the abattoir shortly after sacrifice and transported to the laboratory in cold Krebs‘ solution (in mM): NaCl 119, KCl 4.6, CaCl2 1.5, MgCl2 1.2, NaHCO3 15, KH2PO4 1.2, EDTA 0.01 and glucose 11. From each urinary tract, the urethra was removed, opened longitudinally and pinned in a Sylgard Petri dish filled with Krebs‘ solution. The mucosa, most of the submucosa, fat and connective tissue were removed by sharp dissection under stereomicroscope. Then, transverse strips (approximately 1×1×5 mm) were cut for recording mechanical activity.

**Recording of mechanical activity.** Urethral strips were transferred to 5-ml organ baths containing Krebs‘ solution warmed to 37°C