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Possible role of Na^+-K^+-ATPase in the regulation of human corpus cavernosum smooth muscle contractility by nitric oxide

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1 This study was designed to determine the role of sodium-potassium adenosine triphosphatase (Na^+-K^+-ATPase) in the regulation of human corpus cavernosum smooth muscle contractility by nitric oxide (NO). In addition, we determined if the modulation of Na^+-K^+-ATPase activity by NO is dependent on the increases in intracellular cyclic GMP concentration.

2 The effect of NO donors, sodium-nitroprusside (SNP) and S-nitroso-glutathione (S-NO-Glu), and a permeable cyclic GMP analogue, 8-bromo-cyclic GMP, on Na^+-K^+-ATPase activity (measured as ouabain-sensitive ^{86}Rb-uptake) was studied in human cultured corpus cavernosum smooth muscle cells (HCCSMC). In addition, the effect of the cyclic GMP lowering agent, methylene blue, on NO-induced increase in Na^+-K^+-ATPase activity was studied.

3 SNP (1 μM) caused time-dependent increases in ouabain-sensitive Rb-uptake (33-72%) over 2-20 min in HCCSMC. The stimulation of ouabain-sensitive Rb-uptake by SNP was concentration-dependent (30 and 102% with 0.1 and 1 μM SNP, respectively). Similarly, significant increases in ouabain-sensitive Rb-uptake were obtained with 1 and 10 μM S-NO-Glu. In contrast, incubation of HCCSMC with 8-bromo-cyclic GMP (100 μM) did not increase ouabain-sensitive Rb-uptake.

4 S-NO-Glu induced-increase in intracellular cyclic GMP synthesis, but not the increase in ouabain-sensitive Rb-uptake, was completely inhibited by methylene blue in HCCSMC.

5 The Na^+-K^+-ATPase inhibitor, ouabain, caused a concentration-dependent increase in tension (0.5 to 2 fold) in tissues contracted with 15 mM KCl. SNP and S-NO-Glu caused a concentration-dependent relaxation (concentration required to cause half maximal relaxation (ED_{50}) = 0.04 and 0.2 μM, respectively) of HCC strips contracted with 15 mM K^+. Ouabain (0.1 to 10 μM) inhibited the response to SNP and S-NO-Glu by shifting the concentration-response curves to the right and preventing full smooth muscle relaxation.

6 These results indicate that the activity of Na^+-K^+-ATPase modulates the contractility of HCC smooth muscle, and that NO stimulates Na^+-K^+-ATPase activity in HCCSMC independently of its ability to increase the intracellular cyclic GMP concentration. They also suggest that stimulation of Na^+-K^+-ATPase activity plays an important role in NO-induced relaxation of HCC smooth muscle

Keywords: Ouabain; sodium nitroprusside; S-nitroso-glutathione; methylene blue; cyclic GMP; ^{86}Rb-uptake; penile erection

Introduction

In corpus cavernosum, nitric oxide (NO) released from the endothelium lining the lacunar spaces and autonomic dilator nerves has been shown to cause the relaxation of human trabecular smooth muscle (Kim *et al.*, 1991; Azadzoi *et al.*, 1992; Rajfer *et al.*, 1992). NO is thought to cause the relaxation by activating soluble guanylate cyclase resulting in accumulation of intracellular guanosine 3':5'-cyclic monophosphate (cyclic GMP) (Kim *et al.*, 1991). In vascular smooth muscle, cyclic GMP has been shown to lower the intracellular Ca^{2+} concentration by stimulating Ca^{2+}-ATPase activity, or indirectly by opening K^+ channels with subsequent hyperpolarization and closure of voltage-dependent Ca^{2+} channels (Lincoln & Cornwell, 1993). These are proposed as the underlying mechanism(s) in cyclic GMP/NO-induced relaxation of vascular smooth muscle.

Recently, NO has also been shown to stimulate Na^+-K^+-adenosine triphosphatase (Na^+-K^+-ATPase) activity in blood vessels (Gupta *et al.*, 1992; 1994). However, this effect of NO is reported to be independent of its ability to increase intracellular cyclic GMP levels (Gupta *et al.*, 1994). Na^+-K^+-ATPase is thought to play a critical role in the maintenance of vascular tone (see reviews by Blaustein, 1993; Kaplan, 1985), since inhibition of its activity has been shown to cause vasoconstriction, and to attenuate relaxation caused by endothelium-dependent and -independent vasodilators (DeMey & Vanhoutte, 1980; Foley 1984; Rappaport *et al.*, 1985a,b). In the present study, we investigated whether NO stimulates Na^+-K^+-ATPase activity in human corpus cavernosum smooth muscle, and if increases in cyclic GMP concentration play a role in NO-induced stimulation of Na^+-K^+-ATPase activity. The effect of NO-donors sodium nitroprusside (SNP) and S-nitroso-glutathione (S-NO-Glu), and a permeable cyclic GMP analog, 8-bromo-cyclic GMP, on ouabain-sensitive ^{86}Rb-uptake (a measure of Na^+-K^+-ATPase activity) were studied in human culture corpus cavernosum smooth muscle cells (HCCSMC). In addition, we studied the effect of ouabain (inhibitor of Na^+-K^+-ATPase activity) on SNP and S-NO-Glu-induced relaxation of isolated strips of human corpus cavernosum smooth muscle.

Methods

Tissue procurement

Human corporal biopsies were obtained from impotent men at the time of penile prosthesis implantation. Protocols for the procurement of corporal biopsies were approved by the local Institutional Review Board for Human Studies. All tissue donors issued informed consent. Tissues were placed in ice-

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cold physiological salt solution at the time of removal and transported to the laboratory for immediate organ chamber studies or cell culture.

Measurement of Na^+-K^+-ATPase activity in human cultured corpus cavernosum smooth muscle cells

Human corpus cavernosum smooth muscle cell (HCCSMC) cultures were prepared as described earlier (Moreland *et al.*, 1995). Na^+-K^+-ATPase activity was determined by measuring the ouabain-sensitive ^{86}Rb-uptake as described in cultured vascular smooth muscle cells (Brock *et al.*, 1982). In brief, confluent HCCSMC (passage 2–3) were made quiescent in Dulbecco's Modified Eagle Medium (DMEM) containing 0.4% foetal calf serum for 24 h. Cultures were then washed three times and incubated for 30 min at 37°C with physiological salt solutions (PSS, pH 7.4) containing in mM: NaCl 118.3, KCl 4.5, $MgCl_2$ 0.6, NaH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 5.5 and HEPES 20. The incubation media was then aspirated and 1 ml of PSS containing 2 μCi ml^{-1} $^{86}RbCl$ was added to the cells. After different time intervals (0–20 min), medium was removed and cells were washed with ice-cold 0.1 M $MgCl_2$ (1 ml × 5) to remove radioisotope from the extracellular compartment. Following this, cells were extracted with 1 ml of 6% trichloroacetic acid and the intracellular $^{86}Rb^+$ content was determined by gamma counting. To determine Na^+-K^+-ATPase activity, cells were exposed to ouabain (100 μM) 10 min before the addition of $^{86}RbCl$. Ouabain caused maximum inhibition of ^{86}Rb-uptake (approximately 50%) under these conditions. Approximately 15 and 40% inhibition of ^{86}Rb-uptake was also observed with 1 and 10 μM ouabain, respectively (data not shown). The ouabain-sensitive ^{86}Rb-uptake, which is known to be a measure of Na^+-K^+-ATPase activity (Akera *et al.*, 1981), was calculated as the difference in the rates of ^{86}Rb-uptake in the presence and absence of ouabain. The specific activity of $^{86}RbCl$ was determined on the basis of extracellular K^+ concentration of 4.5 mM and ^{86}Rb-uptake was calculated as nmol min^{-1}per 10^6 cells. To determine whether ouabain-induced inhibition of ^{86}R-uptake was a result of depolarization of the cell membrane, we studied the effect of a depolarizing solution (PSS containing 15 mM KCl) on ouabain-sensitive Rb uptake by cultured HCCSMC. Ouabain (100 μM) inhibited ^{86}Rb-uptake to the same extent in control (48%) and 15 mM KCl-depolarized cells (44%) suggesting a lack of effect of depolarization on ouabain-sensitive ^{86}Rb-uptake.

Cyclic GMP measurements

HCCSMC were incubated in PSS essentially as described above, except that the phosphodiesterase inhibitor zaprinast (30 μM) was added during the final 30 min of incubation. S-NO-Glu (10 μM) was added to the medium for the final 5 min of incubation and, methylene blue (30 μM) for the last 15 min of the incubation. At the end of the incubation, PSS was aspirated and 1 ml of 6% trichloroacetic acid (4°C) was added to the cells. The cells were scraped and collected in an Eppendorf tube, centrifuged at 2500 *g* and supernatant was extracted with water-saturated diethylether. The upper ether layer was discarded and the lower aqueous layer evaporated to dryness in a Speedvac. Dried extracts were reconstituted in 0.5 M sodium phosphate buffer, acetylated and cyclic GMP determined by radioimmunoassay. The recovery of added cyclic GMP in control assays was approximately 90%.

Organ chamber studies

Strips of human corpus cavernosum (~3 × 3 × 7 mm) were mounted to force transducers (Grass instruments FT03, Quincy, MA, U.S.A.) in 25 ml organ baths (37°C) containing PSS of the following composition (in mM): NaCl 118.3, KCl 4.7, $MgSO_4$ 0.6, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25%, CaEDTA 0.026 and glucose 11.1 and aerated with 5% CO_2/95% air to maintain a pH of 7.4. Optimal isometric tension for contraction was attained as described previously (Saenz de Tejada *et al.*, 1989). The tissues were contracted with 15 mM K^+ for 15 min, at which time a stable contraction was typically attained. Ouabain (0.1 μM–10 μM) was then added to the organ baths. Tissues were also treated with 1 μM phentolamine to block contractions caused by noradrenaline released from adrenergic nerve terminals in the corpus cavernosum. After a 20 min incubation with ouabain, tissues were relaxed with the NO donors, SNP and S-NO-Glu. A dose-response was obtained by cumulative additions of drugs to the chambers in half log increments starting at 1 nM and continuing until the maximal relaxation was achieved with each treatment. At the end of the experiment, 100 μM papaverine was administered to determine full relaxation. Data were calculated as percentage relaxation (means ± s.e.) from 15 mM K^+ contraction for each SNP or S-NO-Glu concentration and presented as % change in tone.

Materials

Ouabain, sodium nitroprusside, zaprinast and papaverine hydrochloride were obtained from Sigma Chemical (St. Louis, MO, U.S.A.), and ^{86}rubidium chloride was from New England Nuclear Research Products (Boston, MA, U.S.A.). Radioimmunoassay kit for cyclic GMP was purchased from Biotechnology Institute (Stoughton, MA, U.S.A.). S-nitrosoglutathione was a gift from NitroMed (Cambridge, MA, U.S.A.). All other chemicals were of reagent grade and obtained from common commercial sources. Ouabain for organ chamber studies was dissolved in dimethyl sulphoxide (DMSO, final concentration = 0.02%), which did not affect the tone of HCCSM strips or their ability to relax after treatment with NO donors (data not shown). All other drugs were dissolved in twice distilled water. Six well clusters for cell culture were obtained from Costar (Cambridge, MA, U.S.A.) and foetal bovine serum from Bocell Laboratories (Rancho Dominguez, CA, U.S.A.). Other cell culture supplies were from GIBCO (Grand Island, NY, U.S.A.).

Results

Time- and concentration-dependent stimulation of ouabain-sensitive Rb uptake by sodium nitroprusside and S-nitroso-glutathione

As shown in Figure 1, basal rate of total ^{86}Rb-uptake in HCCSMC was linear over 20 min. Ouabain-sensitive Rb-uptake, which represented approximately 50% of the total Rb-uptake, was also linear over this period. Addition of sodium nitroprusside (SNP; 1 μM) for 2 to 20 min along with $^{86}RbCl$ to the incubation media caused significant increases (33 to 72%) in ouabain-sensitive Rb-uptake by HCCSMC (Figure 2). As shown in Figure 3, the effect of SNP on ouabain-sensitive Rb-uptake was also concentration-dependent. During 5 min incubations, significant increases in ouabain-sensitive Rb uptake over control were observed at SNP concentrations of 0.01 to 1 μM (Figure 3). Significant increases (~50%) in ouabain-sensitive Rb-uptake were observed with another NO donor S-nitoso-glutathione (S-NO-Glu; 1 and 10 μM; 11.03 ± 1.4 and 11.21 ± 1.2 nmol per 10^6 cells vs. control = 7.6 ± 1.2 nmol per 10^6 cells; $n = 5$). Neither SNP nor S-NO-Glu, caused a significant change in the ouabain-insensitive Rb-uptake (data not shown).

Effect of methylene blue on S-NO-Glu-induced increases in cyclic GMP concentration and ouabain-sensitive Rb-uptake in human cultured corpus cavernosum smooth muscle cells

We next studied the effect of the guanylate cyclase inhibitor/cyclic GMP lowering agent, methylene blue, on S-NO-Glu-

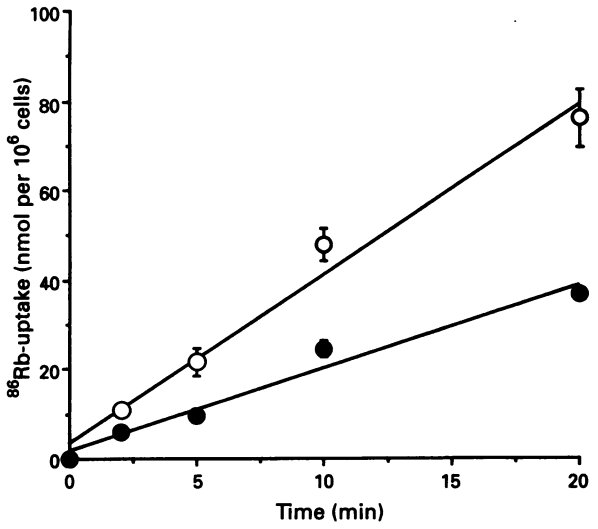

Figure 1 Time course of total (○) and ouabain-sensitive (●) $^{86}Rb^+$-uptake by human cultured corpus cavernosum smooth muscle cells. Quiescent cells (passage 2–3) were incubated for 30 min in PSS containing 20 mM HEPES. $^{86}Rb^+$ was added for indicated periods at the end of incubation as described in Methods. Cells were exposed to ouabain (100 μM) 10 min before the addition of $^{86}Rb^+$. Results are mean ± s.e. of 3 to 5 experiments.

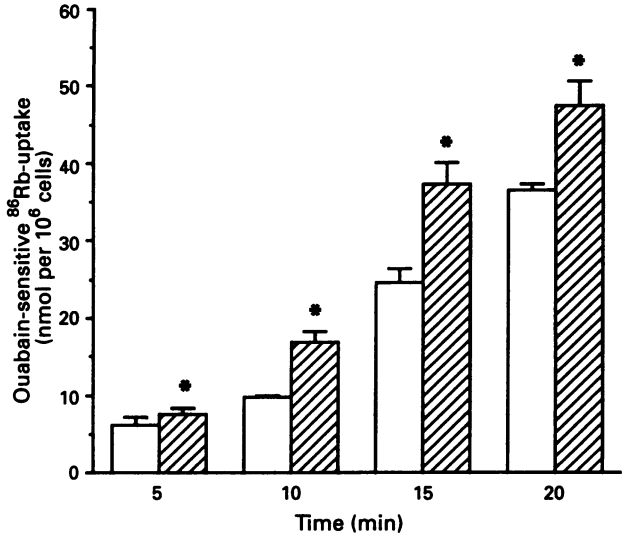

Figure 2 Time-dependent stimulation of ouabain-sensitive $^{86}Rb^+$-uptake by SNP in human cultured corpus cavernosum smooth muscle cells. Quiescent cells were incubated as described in Figure 1 and in Methods. SNP (1 μM, ▨) was added to the cells along with $^{86}Rb^+$ for 5 min after a 30 min preincubation. Data are mean ± s.e. of 3 to 5 experiments. *Indicates values significantly different from the respective controls (open columns) as determined by paired t test.

induced increases in cyclic GMP concentration and ouabain-sensitive Rb-uptake in human cultured corpus cavernosum smooth muscle cells. A 15 min pretreatment of cells with 30 μM methylene blue prevented the S-NO-Glu-induced increase in cyclic GMP of HCCSMC; however, it had no effect on intracellular cyclic GMP concentration in unstimulated cells (Figure 4a). In contrast, the ability of S-NO-Glu (10 μM) to increase ouabain-sensitive Rb-uptake was not reduced significantly in the presence of methylene blue (Figure 4b).

Lack of effect of permeable cyclic GMP analogue on ouabain-sensitive Rb-uptake

To determine further if NO-induced increases in ouabain-sensitive Rb-uptake were mediated by increased intracellular

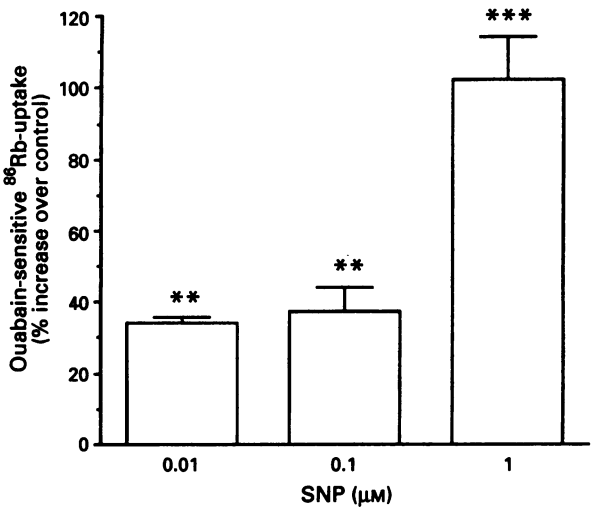

Figure 3 Concentration-dependent stimulation of ouabain-sensitive $^{86}Rb^+$-uptake by SNP in cultured human corpus cavernosum smooth muscle cells. Quiescent cells were incubated as described in Methods and treated with SNP (0.01 to 1 μM) along with $^{86}Rb^+$ for the final 5 min. Ouabain-sensitive ^{86}Rb-uptake by control group was 10.05 ± 1.44 nmol per 10^6 cells. Data are mean ± s.e. of 5 experiments and are represented as percentage increase over control (100%). $^{**}P < 0.01$; $^{***}P < 0.001$ indicate values significantly different from control. Statistical evaluation of the data were performed by ANOVA followed by Student-Newman-Keuls multiple comparisons test.

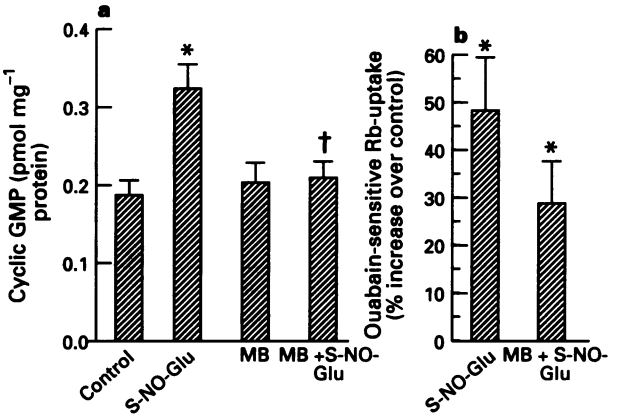

Figure 4 Effect of methylene blue (MB) on S-nitroso-glutathione (S-NO-Glu) on intracellular cyclic GMP concentration (a) and ouabain-sensitive $^{86}Rb^+$-uptake (b) in human cultured corpus cavernosum smooth muscle cells. S-NO-Glu (10 μM) and methylene blue (30 μM) were added to the incubation media for the final 5 and 15 min of incubation, respectively. Data are mean ± s.e. of 7 experiments. *, †$P < 0.05$ indicate values significantly different from control and S-NO-Glu, respectively, as determined by ANOVA followed by Student-Newman-Keuls multiple comparisons test.

cyclic GMP concentrations, we studied the effect of a permeable cyclic GMP analogue, 8-bromo-cyclic GMP, on ouabain-sensitive Rb-uptake in HCCSMC. A 10 min incubation of HCCSMC with 8-bromo-cyclic GMP (100 μM), which completely relaxed precontracted human isolated corpus cavernosum smooth muscle (data not shown), did not cause a significant increase in ouabain-sensitive Rb-uptake (12.4 ± 0.7 nmol 5 min^{-1} per 10^6 cells, $n = 5$) compared to control (9.7 ± 1.1 nmol 5 min^{-1} per 10^6 cells, $n = 5$; $P = 0.135$ by paired t test).

Effect of ouabain on trabecular smooth muscle contractility and relaxation to NO donors

We next studied the role of Na^+-K^+-ATPase in trabecular smooth muscle contractility and in NO-induced relaxation in human corpus cavernosum smooth muscle strips contracted with 15 mM K^+ as described in Methods. Addition of the Na^+-K^+-ATPase inhibitor, ouabain, to the organ chambers caused a concentration-dependent increase in tension in tissues contracted with 15 mM K^+ (Table 1). The NO donor, SNP, caused a concentration-dependent relaxation of trabecular smooth muscle. In the absence of ouabain, relaxation of K^+ contracted tissues was first observed with 1 nM SNP; the half maximal relaxation occurred at approximately 40 nM, and maximal relaxation at 10 μM SNP. As shown in Figure 5 and in Table 1, ouabain inhibited the ability of SNP to initiate, as well as to cause a complete relaxation of precontracted trabecular smooth muscle in a concentration-dependent manner. The concentration of SNP required to cause half maximal relaxation in the presence of different ouabain concentrations was increased by approximately 2 to 25 fold, respectively (Table 1). S-NO-Glu also caused concentration-dependent relaxation (ED_{50} = 0.1 μM; full relaxation at 50 μM) of human trabecular smooth muscle precontracted with 15 mM K^+. Ouabain also shifted the dose-response for S-NO-Glu-induced relaxation to the right and inhibited its ability to cause a full relaxation (Table 1 and Figure 6).

Discussion

The results of the present study indicate that the NO donors, SNP and S-NO-Glu, cause a concentration-and time-dependent increase in Na^+-K^+-ATPase activity in human cultured corpus cavernosum smooth muscle cells. Taken together with the findings that ouabain causes contraction of corpus cavernosum smooth muscle and inhibits the ability of SNP and S-No-Glu to cause relaxation, these results suggest that stimulation of Na^+-K^+-ATPase activity plays a significant role in NO-induced relaxation of human corpus cavernosum smooth muscle. Consistent with these findings, NO has recently been reported to stimulate Na^+-K^+-ATPase activity in the rabbit aorta (Gupta *et al.*, 1992; 1994).

Our results also suggest that the stimulation of Na^+-K^+-ATPase activity by NO is independent of its ability to increase intracellular cyclic GMP concentration. This conclusion is based on the following observations: (i) 8-bromo-cyclic GMP, which caused full relaxation of corpus cavernosum smooth muscle *in vitro* (data not shown), did not increase ouabain-sensitive Rb-uptake in HCCSMC, and (ii) the cyclic GMP lowering agent, methylene blue, did not significantly inhibit S-NO-Glu-induced increase in ouabain-sensitive uptake, despite preventing the increase in intracellular cyclic GMP synthesis. Further, a lack of effect of cyclic GMP analogues on Na^+-K^+-

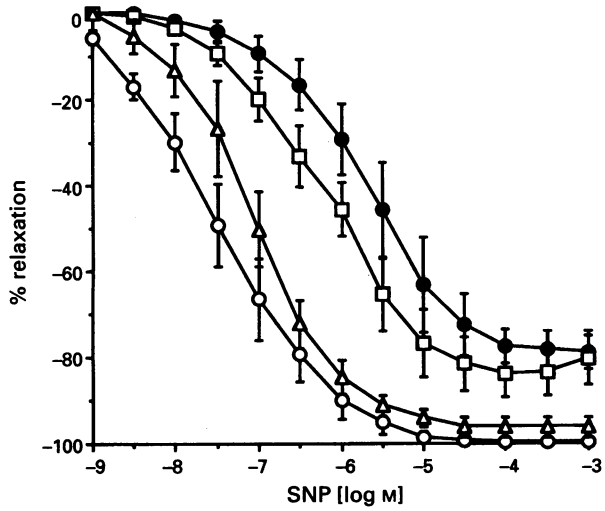

Figure 5 SNP-induced relaxation of human corpus cavernosum smooth muscle strips in the absence (○) and presence of (0.1, 1 and 10 μM ouabain). Results are mean ± s.e. of 4 experiments. Ouabain (1 and 10 μM) significantly inhibited the ability of SNP (50 nM – 1 mM) to relax the smooth muscle ($P < 0.05$, paired t test).

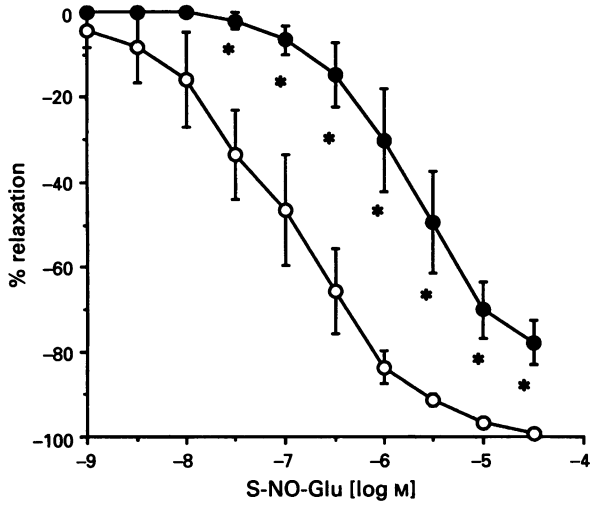

Figure 6 Effect of ouabain on S-NO-Glu-induced relaxation of human corpus cavernosum smooth muscle strips. Ouabain (10 μM, ●) was added to organ chambers after a stable contraction was achieved with 15 mM K^+. Results are mean ± s.e. of 4 experiments. Ouabain significantly inhibited the ability of S-NO-Glu (50 nM – 0.5 mM) to relax the smooth muscle (*$P < 0.05$, paired t test).

Table 1 Ouabain-induced contractions, and inhibition of SNP and S-NO-Glu-induced relaxation in human corpus cavernosum smooth muscle strips

	Contraction		*Relaxation*		
	(% of	*SNP*		*S-NO-Glu*	
Ouabain (μM)	*15 mM KCl contraction)*	*EC_{50}* (μM)	*Maximum* (%)	*EC_{50}* (μM)	*Maximum* (%)
0.0	100	0.04	100	0.11	100
0.1	103 ± 3	0.07	94.8 ± 2.6	–	–
1.0	158 ± 15*	0.75	87.2 ± 7.6*	–	–
10.0	296 ± 3.85*	1.00	79.4 ± 5.6*	5.0*	77.8 ± 5.2*

Ouabain was added after obtaining a steady state contraction with 15 mM KCl. SNP and S-NO-Glu dose-responses were obtained by cumulative addition following contractions with ouabain. The half maximal concentration (ED_{50}) of SNP and S-NO-Glu which caused 50% relaxation of precontracted strips were derived from Figures 5 and 6, respectively. Results are mean ± s.e. of 4 experiments each performed in tissues obtained from different patients.

*Values significantly different from control (no ouabain), $P < 0.05$ by ANOVA followed by Student-Newman-Keuls multiple comparisons test.

ATPase activity and stimulation of Na^+-K^+-ATPase activity independent of intracellular cyclic GMP accumulation by NO in aortic smooth muscle has been reported (Gupta *et al.*, 1994). Thus, in addition to guanylate cyclase, Na^+-K^+-ATPase represents a target for NO action in corpus cavernosum smooth muscle.

A number of effects of NO have recently been reported to be mediated by cyclic GMP-independent mechanisms. For example, NO causes a direct activation of calcium-sensitive K^+-channels in aortic smooth muscle (Bolotina *et al.*, 1994). SNP inhibits glucose-induced insulin release by activating ATP-sensitive K^+-channels presumably via a cyclic GMP-independent pathway, since cyclic GMP itself potentiates insulin-release without affecting ionic movements (Antoine *et al.*, 1993). NO, but not permeable cyclic GMP analogues, has been reported to ADP-ribosylate and inhibit the activity of glyceraldehyde-3-phosphate dehydrogenase (Brune & Lapetina, 1989; Molina y Vedia *et al.*, 1992), and to reduce intracellular calcium in fibroblasts (Garg & Hassid, 1991). In addition, NO regulates NMDA receptors via S-nitrosylation of free thiol groups (Lipton *et al.*, 1993). Whether NO increases the activity of Na^+-K^+-ATPase by one of these mechanisms remains to be determined.

Inhibition of Na^+-K^+-ATPase activity with ouabain was coincident with the contraction of human corpus cavernosum smooth muscle strips. This suggests that Na^+-K^+-ATPase activity plays a role in maintenance of corpus cavernosum smooth muscle tone, similar to that reported in vascular smooth muscle (see reviews by Blaustein, 1977; 1993; DeMey & Vanhoutte, 1980; Foley 1984; Rappaport *et al.*, 1985a,b). In blood vessels, inhibition of Na-pump activity causes depolarization, resulting in an increase in intracellular calcium concentration, and vasoconstriction (Haddy *et al.*, 1980). The contraction of vascular smooth muscle caused by the inhibitors of Na^+-K^+-ATPase activity has also been proposed to be due to an increase in the intracellular Na^+ concentration. Such an increase in intracellular Na^+ would lead to increases in intracellular Ca^{2+}, probably via Na^+-Ca^{2+} exchange, and contraction (Blaustein, 1993, DeMay & Vanhoutte, 1980). Ouabain-induced contractions of trabecular smooth muscle were not due to the release of noradrenaline from adrenergic nerves (Tan & Powis, 1985) since experiments were performed in the presence of adrenoceptor antagonist, phentolamine.

The stimulation of Na^+-K^+-ATPase activity by NO would result in hyperpolarization of the corpus cavernosum smooth muscle cell membrane, with subsequent closure of voltage-sensitive Ca^{2+} channels followed by relaxation of corpus cavernosum smooth muscle. A number of studies in blood vessels support this hypothesis. For example, NO derived from endothelium and/or SNP has been shown to cause hyperpolarization and relaxation of arteries (Tare *et al.*, 1990; Rand & Garland, 1992) and ouabain blocks the hyperpolarization caused by acetylcholine in canine coronary smooth muscle (Feletou & Vanhoutte, 1988). Furthermore, inhibitors of Na^+-K^+-ATPase activity have been shown to inhibit relaxation induced both by endothelium-dependent (acetylcholine) and -independent (SNP) vasodilators (DeMey & Vanhoutte, 1980; Foley 1984; Rappaport *et al.*, 1985a,b). Alternatively, stimulation of Na^+-K^+-ATPase activity could also cause a decrease in calcium influx leading to attenuation of contraction (Kahn *et al.*, 1994).

These findings may have important implications in certain disease states which cause impotence in man. For example, NO synthesis and Na^+-K^+-ATPase activity have been shown to be inhibited in human subjects with diabetes and hypertension (Haddy *et al.*, 1980; Hilton, 1986; Panza *et al.*, 1990; Calver *et al.*, 1992; Elliott *et al.*, 1993), and in a number of experimental models of these diseases (Kjeldsen *et al.*, 1987; Gupta *et al.*, 1992; Tesfamariam *et al.*, 1993; Blaustein, 1993). Relaxation of corpus cavernosum smooth muscle is reported to be inhibited in impotent men with diabetes (Saenz de Tejada *et al.*, 1989), in diabetic rabbits (Azadzoi *et al.*, 1991b), and in rabbits treated with high cholesterol diets (Azadzoi *et al.*, 1991a). In addition, endogenous ouabain has recently been identified as an adrenal cortical hormone which may also be a paracrine hormone. An increase in the plasma concentration of endogenous ouabain has been reported in certain diseases such as essential hypertension (see review by Blaustein, 1993; Hilton, 1986). Such an increase in endogenous ouabain concentration would hypothetically result in inhibition of Na^+-K^+-ATPase activity, thus increasing the reactivity of trabecular smooth muscle to various contractile agonists (e.g. noradrenaline, endothelin) in addition to attenuation of NO-mediated relaxation, which may contribute to impotence. Whether an impairment of NO synthesis and Na^+-K^+-ATPase activity, and/or increased endogenous concentrations of ouabain are related to defects in endothelium-dependent and non-adrenergic non-cholinergic nerve-mediated relaxation of corpus cavernosum smooth muscle in these conditions is not known and is a subject of further investigation in our laboratory.

In summary, we have demonstrated that the activity of Na^+-K^+-ATPase modulates contractility of human corpus cavernosum smooth muscle. These data also suggest that NO stimulates Na^+-K^+-ATPase activity, an effect that does not involve increases in intracellular cyclic GMP levels, in corpus cavernosum smooth muscle. Thus, in addition to increasing the intracellular cyclic GMP concentration, stimulation of Na^+-K^+-ATPase activity is an important mechanism by which NO causes relaxation of corpus cavernosum smooth muscle.

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