Endothelin-1-induced activation of rat renal pelvic contractions depends on cyclooxygenase-1 and Rho kinase

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Grisk O, Packebusch M, Steinbach AC, Schlüter T, Kopp UC, Rettig R. Endothelin-1-induced activation of rat renal pelvic contractions depends on cyclooxygenase-1 and Rho kinase. Am J Physiol Regul Integr Comp Physiol 299: R1602–R1609, 2010. First published September 22, 2010; doi:10.1152/ajpregu.00452.2010.—Upper urinary tract peristalsis is generated in the proximal renal pelvis that connects to the renal parenchyma at the pelvis-kidney junction. It may be exposed to the high renal endothelin-1 (ET-1) concentrations. Dietary NaCl restriction increases renal pelvic ET<sub>A</sub> receptor expression. We investigated the contribution of ET<sub>A</sub> and ET<sub>B</sub> receptors to ET-1-stimulated rat renal pelvic contractions and whether the sensitivity of renal pelvic contractile activity to ET-1 stimulation increases with dietary NaCl restriction. We tested whether ET-1-induced contractile activity depends on cyclooxygenase (COX)-1 or -2 and to what extent spontaneous as well as agonist-induced peristalsis depends on Rho kinases (ROCK). Contractions of isolated renal pelvses were investigated by myography. ET-1 concentration-dependently increased pelvic contractile activity up to 400% of basal activity. ET<sub>A</sub> but not ET<sub>B</sub> receptor blockade inhibited ET-1-induced pelvic contractions. Basal and ET-1-stimulated contractions were similar in renal pelvses from rats on a high-NaCl diet or on a NaCl-deficient diet. COX-1 inhibition reduced spontaneous and almost completely blocked the ET-1-induced pelvic contractions. ROCK inhibition reduced spontaneous and ET-1 stimulated pelvic contractile activity by 90%. RT-PCR revealed that both ROCK isoenzymes are present in the renal pelvic wall. Western blot analyses did not show increased phosphorylation of ROCK substrates myosin phosphatase target subunit 1, ezrin, radixin, and moesin in ET-1-treated isolated renal pelvses. ET-1 is a powerful ET<sub>A</sub> receptor-dependent activator of renal pelvic contractions. COX-1 and ROCK activity are required for the ET-1 effects on pelvic contractions, which are not significantly affected by dietary NaCl intake.

smooth muscle; urinary tract

THE RENAL PELVIS collects urine after it passes the distal collecting duct openings of the renal papilla. Its smooth muscle layer generates peristaltic waves that move the urine via the ureter into the bladder. The uppermost parts of the renal pelvis are connected with the renal parenchyma at the pelvis-kidney junction (4). The peristalsis of the upper urinary tract that includes the renal pelvis and the ureter originates in the proximal renal pelvis and depends on myogenic electrical activity, which is maintained in isolated pelvic preparations and in renal transplants (6, 14, 15, 17). Renal pelvic contractions can be modulated by substances that reach the upper renal pelvis via endocrine and paracrine routes or as transmitters of the renal nerves (5, 12, 16).

Endothelin-1 (ET-1) is well known to elicit vascular smooth muscle contraction. ET-1 is present in the kidney with the highest concentrations in the renal medulla (7, 24), where it stimulates natriuresis (9, 25). Importantly, ET-1 is also present in the renal pelvic wall (11, 21), and the renal pelvis is endowed with ET<sub>A</sub> and ET<sub>B</sub> receptors, the ET<sub>A</sub> receptors being present on smooth muscle cells and ET<sub>B</sub> receptors on sensory nerve fibers (13). We have shown that ET-1 stimulates rhythmic contractions of isolated rat renal pelvses and that this effect is sensitive to ET<sub>A</sub> receptor blockade (13). The role of ET<sub>B</sub> receptors for the modulation of rat renal pelvic contractions is currently unclear. ET-1 activates phospholipases C and A<sub>2</sub> (28), resulting in arachidonic acid release from membrane phospholipids. Arachidonic acid is converted to prostaglandins by cyclooxygenase (COX)-1 and -2. The peristalsis of the upper urinary tract can be stimulated by COX-1 or COX-2 products depending on the species and experimental conditions (2, 3, 16). Currently, it is unknown whether COX activity is required for the ET-1-induced activation of renal pelvic contractions. We investigated whether ET-1 effects on renal pelvic contractions can be modulated by ET<sub>B</sub> receptor blockade and tested the hypothesis that COX activity is required for ET-1 to activate pelvic motility. To discriminate between isoenzymes, we used COX-1- and COX-2-specific inhibitors.

The frequency and amplitude of renal pelvic contractions increase with increasing urine flow (4, 16). The pattern of pelvic peristalsis changes with the rate of urine production and has been suggested to be part of the urine concentrating mechanisms (4). High dietary NaCl intake is associated with high diuresis, and very low NaCl intake is associated with low urine excretion rates (7), due to different water intakes and different needs to eliminate or retain NaCl. We have previously shown that sodium restriction leads to increased renal pelvic ET<sub>A</sub> receptor expression in Sprague-Dawley rats (13). Therefore, we tested the hypothesis that renal pelvic contractions are more dependent on ET-1 in Sprague-Dawley rats subjected to dietary NaCl restriction than in rats kept on high NaCl intake.

Rho kinases (ROCK) importantly contribute to the regulation of smooth muscle function in many organs by activation of the contractile apparatus, increasing its Ca<sup>2+</sup> sensitivity, and by reorganization of the cytoskeleton (22, 32). ROCK inhibition decreases agonist-induced renal vasoconstriction to varying degrees depending on the agonist (1, 26). The role of ROCK activity for spontaneous as well as ET-1-induced pelvic motility is currently unknown. We tested the hypothesis that ROCK inhibition decreases spontaneous and ET-1-induced

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renal pelvic contractions. We found that ROCK inhibition potently inhibited spontaneous and ET-1-induced pelvic contractions, suggesting that ROCK activity is of major importance for rhythmic pelvic force development. To investigate whether the potency of ROCK inhibition to reduce agonist-induced pelvic contractions also occurs in response to other stimuli than ET-1, we tested whether ROCK inhibition also reduces adrenoceptor agonist-stimulated peristaltic activity. Two different rat strains (Sprague-Dawley and F344) were used to examine whether the effects produced by ET-1 in Sprague-Dawley rats could be reproduced in another rat strain.

METHODS

Animals

Experiments were performed in male Sprague-Dawley rats (Charles River, Sulzfeld, Germany) and in male F344 rats (Harlan Winkelmann, Borchen, Germany) in accordance with the American Physiological Society’s “Guiding Principles for the Care and Use of Animals” and the German Animal Protection Act. Permission was obtained from the Committee on Animal Welfare at the Ministry for Food Safety, Agriculture, and Fishery of the state Mecklenburg-Vorpommern, Germany.

Animals were kept in our animal facility at 22°C, and a relative air humidity of 60% with lights on from 6:00 AM to 6:00 PM and free access to standard chow containing 0.6% NaCl and fresh tap water. For experiments that were performed to test the effects of NaCl intake on renal pelvic contractile activity, animals were either fed a diet containing 1.8% NaCl together with isotonic saline as drinking fluid (high salt intake) or given a NaCl-deficient diet (catalog no. 960232; ICN Biomedicals, Eschwege, Germany) (13) together with tap water for 2 wk before the experiments.

Isolated Renal Pelvic Preparation and myography

Three-month-old rats were deeply anesthetized with pentobarbital sodium (60 mg/kg). Both kidneys were excised and immediately placed into ice-cold physiological salt solution (PSS). Dissection was performed with the aid of a stereomicroscope and fine dissection instruments in ice-cold PSS in petri dishes coated with silicone gel. The renal pelvises were fixed in the silicone gel with a thin injection needle placed through the pyeloureteric junction. Adhering epithelial and vascular tissue was removed.

Renal pelvises where mounted in a model 410A two-channel wire myograph (Danish Myotechnology, Aarhus, Denmark) following an approach similar to what we have described for small blood vessels (26). The organ bath was filled with bicarbonate-buffered PSS (37°C, pH 7.4) and gassed with carbogen (26). The preparations were stretched until spontaneous rhythmic contractions could be clearly observed. This was obtained at distending forces between 0.15 and 0.2 mN (Fig. 1). The amplitude and frequency of spontaneous rhythmic contractions were 0.2–0.4 mN and 10–15 min

experimental blocks, there were no significant differences in baseline contraction frequency and force between groups.

After clear rhythmic contractions had been obtained, renal pelvic preparations were allowed to equilibrate for 20 min. Each block of experiments was performed in a strictly randomized order, including the appropriate vehicle controls for each set of experiments. Agonist responses with and without the addition of enzyme inhibitors were obtained as cumulative concentration-response curves. The time interval between individual ET-1 concentration steps was 5 min. Only one ET-1 concentration-response curve could be obtained per preparation due to the long-lasting effects of the agent. Pilot studies showed that adrenoceptor-agonist-induced effects on pelvic peristalsis occurred more rapidly than ET-1-induced effects. Furthermore, unlike ET-1-induced effects, adrenoceptor agonist-induced effects were fully reversible after the bathing solution was changed. An exception was the α2-adrenoceptor agonist guanabenz, which also elicited prolonged effects. Therefore, the adrenoceptor agonists norepinephrine, phenylephrine, and isoproterenol were administered in random order to the renal pelvises, whereas guanabenz was always given as the last substance. After the individual concentration-response curves had been obtained, the bathing solution was changed three times and 20 min elapsed until the next concentration-response curve was obtained. The time interval between individual concentrations was 2 min for adrenoceptor agonists. The concentrations of adrenoceptor agonists were in a range known to preferentially activate the desired adrenoceptor type (19, 20, 29, 34).
inhibition on adrenoceptor-dependent stimulation of pelvic peristalsis was performed in individual pelvises for each adrenoceptor agonist tested.

The receptor blockers and enzyme inhibitors or their vehicles were administered 15 min before ET-1 or adrenoceptor agonist administration. All drugs used in myograph studies were obtained from Sigma (Deisenhofen, Germany). DMSO was added to the stock solutions of the ETA receptor blocker BQ123, the ETB receptor blocker BQ788, the COX-1 inhibitor valeryl salicylic acid (VSA), and the COX-2 inhibitor NS-398, which were further diluted in isotonic saline. Final DMSO concentrations were ≤0.05% in the organ bath. All other drugs, including the Rho kinase inhibitor Y-27632, were dissolved in isotonic saline.

mRNA Detection

RNA isolation from isolated renal pelvises and real-time RT-PCR followed the same procedures as described previously (13). Design of synthetic oligonucleotide primers for gene expression analyses of Rho kinase genes were performed on the basis of published sequences. The following primers were designed to span an intron to avoid amplification of genomic DNA: Rock1 (GenBank accession no. NM_031098) forward primer, 5'-CTG GAT GGA TTG GAT GCT TT-3' and reverse primer, 5'-GCA TTA ACC TTC CTG GTG GA-3'; and Rock2 (GenBank accession no. NM_013022) forward primer, 5'-AGA TCA GTG CAG CCG CTA TT-3', and reverse primer, 5'-ACC ACG CCT GAC AGG TTC TT-3'. The relative content of mRNA was determined using the geometric mean of the contents of β-actin and Ywhaz (tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activator protein, zeta polyepetide) as endogenous references for normalization.

ROCK Activation and Western Blot Analysis

To examine whether the agonists activated ROCK, we incubated isolated renal pelvises in PSS at 37°C for 20 min with 10−5 M ET-1, 10−5 M norepinephrine, or no agonist (26). The phosphorylation of ROCK substrates was determined using Western blot analysis. Aortic rings obtained from the same animals served as positive controls (26). After incubation, the pelvises were shock-frozen in aceton on dry ice for 10 min and fixed in a dry ice-acetone slurry containing 10% trichloroacetic acid (TCA) and 10 mM dithiothreitol (DTT) for 1 h. To remove TCA and DTT, we washed the tissue four times with acetone for 10 min and fixed in a dry ice-acetone slurry containing 10% acetone at −70°C.

Before Western blot analyses were performed, pelvises were put in 300 μl of lysis buffer (pH 7.4) containing 150 mM NaCl, 50 mM Tris-HCl, 10 mM EDTA, 0.1% Tween 20, 0.1% β-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, and 10 μg/ml protease inhibitor cocktail (Sigma-Aldrich, Munich, Germany). Pelvises were frozen in liquid nitrogen and ground (mortar). Thereafter, the homogenate was treated with supersonic waves twice for 10 s at 4°C and stored at −70°C until Western blot analysis. Protein concentration was determined using the Biuret reaction (Carl Roth, Karlsruhe, Germany).

Equal amounts of pelvic protein (500 μg) were electrophoretically separated on 10% sodium dodecyl sulfate polyacrylamide gel with 5% stacking gel and transferred to nitrocellulose. Membranes were blocked with nonfat dry milk and incubated with polyclonal rabbit antibody against the cytoskeletal proteins ezrin, radixin, and moesin (ERM; 1:1,000) and with polyclonal rabbit antibody against the myosin phosphatase target subunit 1 (MYPT1; 1:1,000). Alternatively, membranes were incubated with polyclonal rabbit anti- phospho-ERM (ezrin, Thr567; radixin, Thr564; moesin, Thr558) antibody (1:1,000; Chemicon, Temecula, CA) and with polyclonal rabbit anti-phospho-MYPT1 (Thr696) antibody (1:1,000, Millipore, Billerica, MA). This was followed by incubation with a peroxidase-labeled secondary antibody (goat anti-rabbit, 1:5,000–1:10,000; Chemicon). Immunoreactive bands were detected using an enhanced chemiluminescence kit (ECL Plus; Amersham Pharmacia Biotech, Little Chalfont, UK). β-Actin (monoclonal mouse anti-actin antibody, 1:10,000, Chemicon) was used as loading control.

Statistics

All data are means ± SE. Comparisons of multiple group means were performed using one-way or two-way ANOVA with or without repeated measurements, as appropriate, followed by post hoc testing with the Student-Newman-Keuls test using SigmaStat statistical software (SPSS, Chicago, IL). Differences were taken as significant at $P < 0.05$.

RESULTS

**Myograph Experiments**

**Experiment 1.** ET-1 stimulated rhythmic contractions in isolated renal pelvises in a concentration-dependent manner in Sprague-Dawley and F344 rats with similar potency (Fig. 2A). Changes in mean force and frequency of contractions paralleled each other. Mean frequency of pelvic contractions increased from 9.2 ± 1.0 min−1 under baseline conditions to 18.6 ± 1.5 min−1 at maximum ET-1 concentrations in Sprague-Dawley rats ($P < 0.001$; not shown). ETB, but not ETA receptor blockade significantly reduced the stimulatory effect of ET-1 on pelvic contractions (Fig. 2B), whereas it had no significant effect on spontaneous peristaltic activity. COX-1 inhibition slightly reduced spontaneous contractile activity in isolated Sprague-Dawley rat renal pelvises. Contractile activity fell in VSA-treated pelvises and was significantly lower than respective control levels during the first dosing step of ET-1 (1 nM) due to reductions in frequency and force. ET-1 at 1 nM had no significant effect on pelvic contractions in vehicle- and NS-398-treated preparations. VSA almost completely abolished the ET-1-induced rise in contractile activity. COX-2 inhibition was without effect (Fig. 2C). Similar results were obtained in renal pelvises from F344 rats (data not shown).

**Experiment 2.** Baseline contractile activities in isolated renal pelvises from Sprague-Dawley rats kept on either a NaCl-deficient (n = 7) or high-NaCl diet (n = 6) were 0.76 ± 0.21 and 0.92 ± 0.21 mN/min, respectively (no significant difference). Distending forces under control conditions were similar between the two groups. ET-1-induced similar concentration-dependent rises in contractile activity of renal pelvises from both groups (Fig. 3).

**Experiment 3.** The nonselective ROCK inhibitor Y-27632 reduced spontaneous renal pelvic contractile activity to 10% of baseline activity within 10 min after it had been administerd (Fig. 4A). Frequency of pelvic contractions fell from 13.2 ± 1.0 min−1 under control conditions to 8.5 ± 1.4 min−1 by 10 min after administration of Y-27632 ($P < 0.05$). During ROCK inhibition, contractile activity remained significantly reduced even when ET-1 was administered in increasing doses (Fig. 4B).

**Experiment 4.** To test whether the strong inhibitory effect of Y-27632 on agonist-stimulated renal pelvic contractions was confined to ET-1, we successively applied several adrenoceptor agonists (Fig. 5). Norepinephrine caused concentration-dependent rises in contractile activity up to a maximum of 200% of control values in both Sprague-Dawley and F344 rats (Fig. 5, A and B, and Supplemental Fig. 2). The administration of selective adrenoceptor agonists revealed that pelvic peristaltic...
sis could be activated by either α1- or α2-receptor-dependent mechanisms. However, the α2-adrenoceptor agonist guanabenz stimulated contractile activity more potently than norepinephrine or the α1-adrenoceptor agonist phenylephrine. The β-adrenoceptor agonist isoproterenol (0.01–10 μM in 7 half-logarithmic concentration steps) had no significant effect on renal pelvic contractile activity except for the highest concentration used (10 μM), where a slight stimulation was observed that may have been due to nonselective actions of the substance (not shown). ROCK inhibition potently blocked α-adrenoceptor-induced contractions (Fig. 5C).

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**Fig. 2.** *A*: endothelin-1 (E-1) increased renal pelvic contractile activity in a concentration-dependent manner in Sprague-Dawley and F344 rats. Pelvic contractile activity represents the product of contraction frequency and mean force during the recording intervals and is given relative to control values. B: ET<sub>α</sub> receptor blockade with BQ123 inhibited E-1-induced increases in pelvic contractile activity. ET<sub>β</sub> receptor blockade with BQ788 had no significant effect on ET-1-induced pelvic contractions. *P < 0.05, BQ123 vs. vehicle. C: the cyclooxygenase-1 (COX-1) inhibitor valeryl salicylic acid (VSA; i, COX inhibitor) reduced pelvic contractions and almost completely blocked the ET-1-induced rise in contractile activity. The COX-2 inhibitor NS-389 was without significant effect. ‡P < 0.05 vs. control. *P < 0.05, VSA vs. vehicle.

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**Fig. 3.** E-1 caused similar increases in contractile activity in isolated renal pelvises from Sprague-Dawley rats exposed to NaCl restriction or high-NaCl intake for 2 wk.

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**Fig. 4.** *A*: Rho kinase (ROCK) inhibition strongly reduced spontaneous contractions in Sprague-Dawley rat renal pelvises (representative original tracing). B: ROCK inhibition with Y-27632 (i) reduced spontaneous contractions and virtually abolished the ET-1-induced rise in pelvic contractile activity. ‡P < 0.05 vs. control. *P < 0.05 vs. vehicle.
Real-Time PCR and Rho Kinase Activation

Real-time PCR showed that mRNA of both ROCK isoforms was expressed in pelvic tissue (Fig. 6A). To test whether stimulation of isolated renal pelvises with ET-1 or norepinephrine caused ROCK activation, we investigated phosphorylation of the ROCK substrates ERM using the Western blot technique. No significant increases in ERM phosphorylation were detected in isolated renal pelvises, whereas a clear rise in ERM phosphorylation occurred in aortic rings from the same animals (Fig. 6B). Similar results were obtained for MYPT1 (not shown).

DISCUSSION

ET-1 is involved in the regulation of renal vascular and epithelial function (9, 28). Although the presence of ET-1 and ET receptors in the rat renal pelvic wall has been shown previously (11, 13, 21), the actions of ET-1 on rat renal pelvic peristalsis have not been systematically investigated. In the present study we have shown that exogenous ET-1 potently increases renal pelvic contractile activity via ETA receptor-dependent mechanisms in two strains of rats, confirming our previous results (13). ETB receptor blockade did not significantly affect ET-1-induced contractions, indicating that ETB receptor activation does not contribute to the ET-1 effects on contractile activity in isolated rat renal pelvises. ETA or ETB receptor blockade had no significant effect on spontaneous contractions, suggesting that endogenous ET-1 formation within the
renal pelvic wall does not significantly contribute to spontaneous pelvic contractions under in vitro conditions. Our findings correspond well with an early study (18) on endothelin actions on mammalian smooth muscle preparations where ET<sub>A</sub> receptor-dependent contractions could be elicited in human renal pelvic strips. In this study (18), the effective ET-1 concentration range was similar to the present data, but no indication was made as to whether the pelvic strips had maintained rhythmic contractions.

Peristaltic activity of the renal pelvis originates from rhythmic electrical activity in atypical smooth muscle cells and interstitial cells of the proximal renal pelvis that spreads via gap junctions to typical smooth muscle cells (15–17). Recently, it was shown that hyperpolarization-activated cation-3 (HCN3) channels are critical for the maintenance of rhythmic contractions of the mouse upper urinary tract (6). Since ET-1 caused a concentration-dependent parallel rise in contraction frequency and force, our data suggest that ET-1 activates the electrical activity of renal pelvic pacemaker cells in addition to the contractile apparatus of pelvic smooth muscle cells.

Prostaglandins modulate upper urinary tract peristalsis, and the effects depend on the species and type of prostaglandin (16). Also, depending on the species, stimulatory effects of prostaglandins on upper urinary tract peristalsis may be due to COX-1 or COX-2 activity (2, 3, 16). Nonselective COX inhibition decreased electrically stimulated contractions in human ureters (2) and spontaneous contractile activity in guinea pig and rat renal pelvises (3). Selective COX-1 inhibition stimulated contractions in rat renal pelvises, whereas selective COX-2 inhibition had no significant effect (3). Furthermore, COX-2 inhibition did not significantly affect electrically induced contractions in human isolated ureters but decreased electrically stimulated pig ureter contractions (2). Because of the pronounced prostaglandin effects on spontaneous as well as stimulated upper urinary tract peristalsis and because ET-1 receptors can activate phospholipase A<sub>2</sub> (28), we investigated the effect of selective COX inhibitors on ET-1-induced contractions. The COX-1 inhibitor VSA caused a significant reduction in spontaneous pelvic contractile activity at 5 μM, which has been reported to stimulate renal pelvic contractions in Wistar rats (3). Since we obtained similar findings in two rat strains, it is unlikely that this discrepancy can be attributed to the rat strain used. Since indomethacin decreased contractile activity in rat renal pelvics and COX-2 inhibition with NS-389 was without effect (3), which is in line with the present data, it is more likely that selective COX-1 inhibition inhibits rather than stimulates pelvic contractions. Of note, in the present study the COX-1 inhibitor VSA almost completely abolished the ET-1-induced rise in peristaltic activity. Together, our data indicate that ET-1 actions on pelvic motility critically depend on COX-1 activity.

Our finding that COX-1 inhibition effectively abolished the ET-1-induced rise in peristaltic activity suggests that COX-1 products may have a permissive effect on ET-1 actions. Alternatively or additionally, ET-1 may stimulate COX-1-dependent prostaglandin synthesis, which in turn may mediate the ET-1-induced effects on pelvic peristalsis. In fact, there is evidence for a dual role of COX-1 products in ET-1 signaling in the renal pelvis. Thus COX inhibition resulted in decreases in action potential frequency, amplitude, and duration in electrically driven cells but not in pacemaker cells of the guinea pig renal pelvis (16). This suggests that the blockade of ET-1-induced pelvic peristalsis in response to COX-1 inhibition in rats is due in part to the absence of a permissive effect of renal pelvic prostaglandin formation on agonist-induced peristalsis. On the other hand, ET-1 increases the renal formation and release of prostaglandins, including PGF<sub>2α</sub> and PGE<sub>2</sub>, via ET<sub>A</sub> and ET<sub>B</sub> receptor-dependent mechanisms (33). Both prostaglandins stimulate pelvic contractions (16). Furthermore, isolated renal pelvices release PGE<sub>2</sub> in response to protein kinase C activation (10), which can be activated by ET<sub>A</sub> receptors (28). Thus, in addition to their permissive role for the effects of ET-1 on the renal pelvis, COX-1 products may be important mediators of ET-1-induced peristalsis.

The present data suggest that ET-1 is a modulator of upper urinary tract motility. Immunohistochemistry has shown that ET<sub>A</sub> receptor expression is confined to smooth muscle in the pelvic wall (13). With decreasing urine production rates, the pelvic peristalsis is reduced (4, 16) and may become more dependent on paracrine stimulators of smooth muscle activity that may also alter the pattern of peristaltic activity. Our data on ET-1-induced contractions in pelvices from rats on high-NaCl or NaCl-deficient diets do not support this hypothesis, because there was no significant difference between the two groups in response to ET-1 stimulation. The 1.4-fold elevation in renal pelvic ET<sub>A</sub> receptor expression in NaCl-restricted rats reported previously (13) may not be sufficient to cause a significant difference in the sensitivity of pelvic contraction to pharmacological ET-1 stimulation.

Rho kinase is an important mediator of vascular smooth muscle responses to vasoactive substances, including both fast vasoconstrictor responses and chronic remodeling (22, 26, 32). Its role for spontaneous and agonist-induced rhythmic contractions in the renal pelvis has not been investigated. Our data show that pelvic ROCK activity is important to maintain spontaneous contractions and for agonist-induced contractile activity. The contractile responses to ET-1 were greatly reduced albeit not completely abolished. The present study confirms that norepinephrine-induced activation of rat renal pelvic contractions is α-adrenoceptor dependent and extends these data (5) by showing that α<sub>2</sub>-adrenoceptor activation stimulates contractile activity more potently than α<sub>1</sub>-adrenoceptor activation. ROCK inhibition potently inhibited α-adrenoceptor-induced contractions. The data indicate that the strong ROCK dependence of agonist-induced pelvic contractile activity is not limited to ET<sub>A</sub> receptor activation. Also, α-adrenoceptor-activated renal pelvic contractions strongly depend on ROCK activity.

To test whether agonists activated ROCK in the renal pelvis and whether ROCK acted as a mediator of agonist-induced peristalsis in addition to a permissive effect on agonist-induced contractions, we measured the phosphorylation of known ROCK substrates in agonist-stimulated smooth muscle cells. Although we found clear increases in ERM and MYPT1 phosphorylation in response to ET-1 and norepinephrine in aortic rings, we failed to detect similar effects in renal pelvices from the same animals. These findings may suggest that ERM and MYPT1 are major ROCK substrates in tonically contracting aortic rings but not in phasically contracting renal pelvices. An alternative explanation may be that ERM and MYPT<sub>1</sub> phosphorylation and dephosphorylation oscillate rapidly in peristaltic organs with phosphorylation peaking at the height of
tion development. If so, the increased ERM and MYPT1 phosphorylation may have been undetected due to our methods not allowing for sufficiently high temporal resolution.

Endothelin receptor and adrenoceptor-dependent ROCK activation may occur not only via G_{12/13} proteins (22) but also via Ca^{2+}-dependent signaling (22, 23, 28). In addition, activation of Ca^{2+} signaling has been shown to also occur downstream of ROCK activation (27, 31). In the rat ureter, ROCK inhibition strongly attenuates electrical field stimulation-induced contractions (27). These effects are due in part to reduced dihydropyridine-sensitive inward Ca^{2+} currents and in part to reduced myosin light chain phosphorylation (27). Furthermore, ROCK inhibition has been shown to inhibit Ca^{2+} entry into rat penile artery smooth muscle cells via nonselective cation channels (31). Thus, in addition to regulating the contractile apparatus and cytoskeletal proteins, ROCK may also regulate L-type Ca^{2+} channels (27) and nonselective cation channels (31). ROCK-dependent modulation of Ca^{2+} signaling could contribute to changes in discharge frequency of pacemaker cells and in force development of typical smooth muscle cells in the renal pelvis.

Perspectives

In the present study we have shown that ET-1 potently stimulates renal pelvic contractions by activation of ET_{\alpha} receptors in a COX-1- and ROCK-dependent manner. Future research on pelvic function needs to address how ET-1 affects the generation of rhythmic pelvic activity with respect to signal transduction and the ionic currents involved. Furthermore, ROCK substrates that are critical for the generation of rhythmic electrical and mechanical activity of the pelvic wall need to be defined. The importance of agonists such as ET-1 for the regulation of upper urinary tract motility in vivo needs to be addressed to clarify the physiological relevance of the present pharmacological findings. The present findings may also be the basis to assess whether systemic administration of ROCK inhibitors has undesired side effects on upper urinary tract motility. In addition, they provide basic information relevant for the understanding of the pathophysiology of congenital and acquired defects in the upper urinary tract such as ureteropelvic junction (UPJ) obstruction, which has been found to be associated with reduced sympathetic innervation of the UPJ in humans (8) and increased pelvic ET-1 contents in rats (21). Importantly, increased ROCK activity contributes to maintained contractile force in the obstructed rat bladder (30), which may also occur in response to upper urinary tract obstructions.

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DISCLOSURES

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