Kidney International, Vol. 64 (2003), pp. 1356–1364

Amitriptyline eliminates calculi through urinary tract smooth muscle relaxation

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Amitriptyline eliminates calculi through urinary tract smooth muscle relaxation.

Background. We investigated the effects of amitriptyline in the urinary tract smooth muscle and urolithiasis.

Methods. Cats presenting with obstructive acute renal failure (ARF) received amitriptyline, and renal function and survival rates were analyzed. Isometric contractions and membrane potentials of rat, pig, or human isolated urinary tract smooth muscle were recorded in the presence or absence of amitriptyline.

Results. Twenty cats with obstructive ARF caused by urethral plugs received amitriptyline. In all cases, plugs were completely eliminated, and renal function returned to normal, with a 100% survival rate in the follow-up. Amitriptyline produced potent relaxations in rat urethral strips, accompanied by significant reductions in urethral ring membrane potential. This effect was prevented by pretreatment of urethral rings with 4-aminopyridine (4-AP), a voltage-dependent potassium channel blocker. Amitriptyline abolished in a reversible manner acetylcholine-, bradykinin-, and KCl-induced contractions in rat isolated bladder, and this effect was also prevented by 4-AP. Of interest, spontaneous and KCl-induced contractions of pig and human isolated ureter were also blocked by amitriptyline.

Conclusion. Our results indicate that amitriptyline is an effective and potent relaxant of urinary tract smooth muscle and this effect is mediated by opening of voltage dependent–potassium channels. We suggest that amitriptyline administration may help to promote elimination of urinary calculi.

Urolithiasis clinical presentations range from painful self-limited crises to urinary flow obstruction with consequent hydronephrosis and renal failure [1, 2]. Despite the high prevalence and costs involved, pharmacologic management of the disease has not evolved in the last years [3–5]. Hydration, analgesic, and antispastic drugs have been used during crises in order to relief symptoms and eliminate the calculi. However, a considerable number of patients experience pain for a long period of time, and invasive modalities of treatment are sometimes necessary to solve the problem [6–9]. The discovery of drugs effective in treating calculus-induced urinary flow obstruction would certainly decrease costs, hospitalization time, and complication rates secondary to extracorporeal shock wave lithotripsy and surgery.

Anecdotal reports show that the tricyclic antidepressive amitriptyline is able, in some cases, to induce the extrusion of urinary calculi of considerable dimensions [10]. Furthermore, preliminary data from our and other laboratories suggest that amitriptyline could increase the elimination urinary calculi in an animal model of recurrent cystitis (Achar et al, unpublished data) [11]. Thus, we investigated the role of amitriptyline in obstructive acute renal failure (ARF) induced by urethral plugs in a model of cat urolithiasis. As exciting results were produced, we then analyzed possible mechanisms of action of amitriptyline in the urinary tract of different animal species.

In the present study, we demonstrated for the first time that amitriptyline was able to completely reverse established obstructive ARF in cats, and this effect was mediated by urinary tract smooth muscle relaxation triggered by voltage-dependent potassium channel opening. These results suggest that amitriptyline might have a beneficial role in the treatment of urolithiasis manifestations, helping to promote calculus elimination and pain relief.

Key words: amitriptyline, urinary tract smooth muscle, voltage-dependent potassium channel, relaxation, obstructive acute renal failure, urolithiasis.

Received for publication March 11, 2003
and in revised form May 7, 2003
Accepted for publication May 30, 2003

METHODS

In vivo studies

Male cats presenting at a veterinarian hospital with advanced obstructive ARF received amitriptyline (Prodome, 1 mg/kg orally). Animals were treated for 30 days,

an arbitrary period of time set in order to prevent commonly observed recurrence of obstruction. Calculus elimination, renal function, and survival rates were evaluated. Urinalysis, plug composition analysis, serum creatinine, and blood urea nitrogen (BUN) measurement were performed by the use of commercially available kits (LABTEST Diagnostics, Vista Alegre, Minas Gerais, Brazil).

In vitro studies

Electrophysiologic studies (EPS). Rat urethral rings were everted, placed in a 2 mL perfusion chamber, and superfused at a rate of 3 mL min^{-1} with Krebs solution (pH 7.4, 37°C, aerated with 5% CO_2, 95% O_2). Micropipettes (borosilicate glass capillaries, 1B120F-6, World Precision Instruments, Sarasota, FL, USA) were made by means of a horizontal puller (Narishige model PN3, Tokyo, Japan) and filled with 2 mol/L KCl (tip resistance, 20 to 40 MΩ and tip potential <6 mV). The microelectrodes were mounted in silver/silver chloride half-cells on a micromanipulator (Leitz, Leica, Wetzlar, Germany) and connected to an electrometer (Intra 767, World Precision Instruments). The urethral rings were initially equilibrated for 1 hour. After this period of time, urethral impalements were carried out. The electrical signals were continuously monitored on an oscilloscope (54645A, Hewlett Packard, Palo Alto, CA, USA) and recorded in a potentiometric chart recorder (2210, LKB-Produkter AB, Bromma, Sweden). The successful implantation of the electrode was evidenced by a sharp drop in voltage upon entry into a cell, minimal change (<10%) in microelectrode resistance, and a stable potential (±3 mV) for at least 1 minute after impalement, and a sharp return to zero upon exit from the cell. Membrane potential measurements were obtained in Krebs solution before and after use of 1 pmol/L amitriptyline, in the absence and in the presence of one of the following drugs: 1 mmol/L glybenclamide, 1 mmol/L apamin, 1 mmol/L charybdotoxin, 1mmol/L chlotrimazole, and 1 mmol/L 4-aminopyridine (4-AP). Amitriptyline and potassium channels blockers were added to the preparations 10 and 20 minutes before the impalements, respectively.

Isometric contraction recordings

Adult male Wistar rats (250 to 300 g) were sacrificed and urethra and bladder were removed. Adult pigs were anesthetized and middle ureteral segments were obtained. Human normal ureteral rings were obtained from healthy kidney donors. Institutional Ethic's Committee approval and patient's informed consent for the use of the ureter segments were obtained. Tissue specimens were excised and immediately placed in a Krebs solution. Organ samples were carefully freed from fat and connective tissue. Isolated organ preparations (urethral and bladder strips, and ureteral rings) were mounted for isometric recording in 5 mL chambers containing Krebs solution (supplemented with 1 mmol/L $BaCl_2$ when analyzing pig ureteral rings) maintained at 37°C and bubbled with a gaseous mixture of O_2 (95%) and CO_2 (5%). Preparations were submitted to optimal tensions (rat urethra and bladder, and human ureter, 1 g; and pig ureter, 4 g) and equilibrated for 1 hour. Tension was recorded through SCANS 60.106A Acquisition System and processed by the software Proto-5 (Letica, Barcelona, Spain). Concentration-response [1 nmol/L to 1 mmol/L acetylcholine (ACh)] or single (for 80 mmol/L KCl or 1 μmol/L bradykinin) curves were carried out in rat bladders in the absence or presence of amitriptyline (100 nmol/L to 10 μmol/L). Human and pig spontaneous ureteral contractions were recorded also in the absence or presence of increasing concentrations of amitriptyline. The effect of KCl-induced contractions was also addressed in those preparations. In order to investigate the role of voltage-dependent potassium channels, experiments were repeated in the presence of 4-AP or vehicle.

Statistical analysis

All data are expressed as means ± SEM. Comparisons between groups were analyzed with the Student *t* test (for two groups) or one-way analysis of variance (ANOVA), followed by the Newman-Keuls test when indicated (for three or more conditions). $P < 0.05$ was considered statistically significant. For EPS, when more than one impalement was performed on the same preparation, measurements were averaged and considered as one.

RESULTS

In this study, 20 male cats presenting with obstructive ARF received amitriptyline for 30 days. Blood and urinary alterations observed demonstrate the severity of the disease in those animals (Fig. 1 and Table 1). Due to the poor outcome usually observed in these cases [12, 13] and to our initial positive data, experiments were carried out without the inclusion of a placebo-controlled group. Calculus elimination was observed and urinary flow was restored in all animals. A survival rate of 100% and, as can be seen in Figure 1, complete reversal of renal dysfunction was observed in all cases following amitriptyline administration. These effects were achieved in a period of time no longer than 72 hours. No major amitriptyline-related adverse effect was detected. Slight and transient somnolence was seen in more debilitated animals. Restoration of renal function coincided with disappearance of such side effect.

In order to identify possible mechanisms of action for amitriptyline, rat urethral strips were mounted in organ baths for isometric contraction recordings. At this point, we changed species as in vitro studies with cat tissues were considered unethical by our local committee. Amitriptyline produced potent reductions in resting, non-

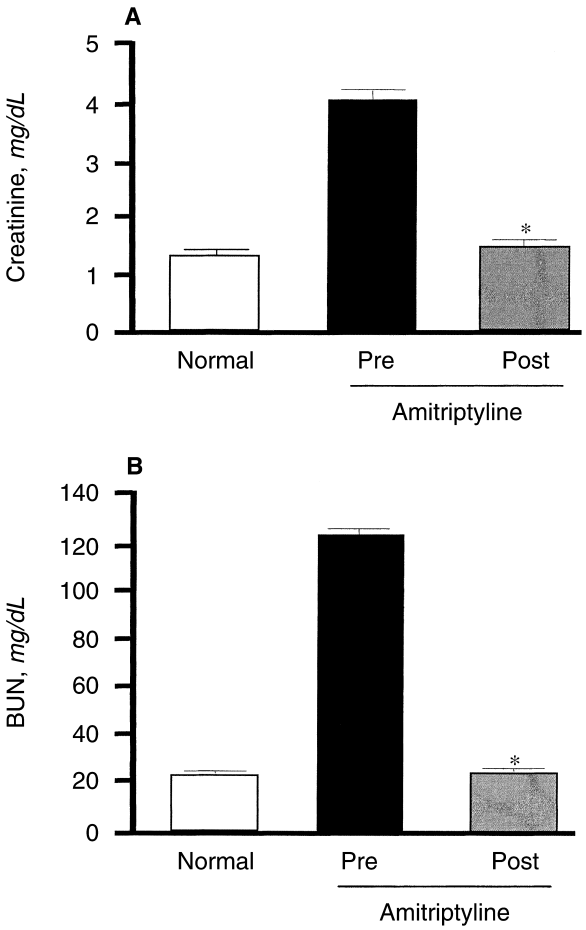

Fig. 1. Creatinine (*A*) and blood urea nitrogen (BUN) (*B*) levels in cats presenting with acute renal failure (ARF) secondary to urolithiasis before and after treatment with amitriptyline (1 mg/kg of body weight/day, 30 days). $*P < 0.05$; $N = 20$. Analysis was also performed in five healthy untreated cats and values obtained are presented for comparison.

Table 1. Findings from urinalysis[a] in 20 adult male cats presenting at a veterinarian hospital with obstructive acute renal failure (ARF)

Abnormality	Number
Hematuria	18 (90%)
Bacteriuria	14 (70%)
Leukocytes	10 (50%)
Cylindruria	6 (30%)
Struvite crystals[b]	12 (60%)
Calcium oxalate crystals[b]	8 (40%)
Amorphous urate crystals[b]	4 (20%)

[a] Urine samples obtained through cystocentesis
[b] Similar crystal distribution was observed from plug content analysis

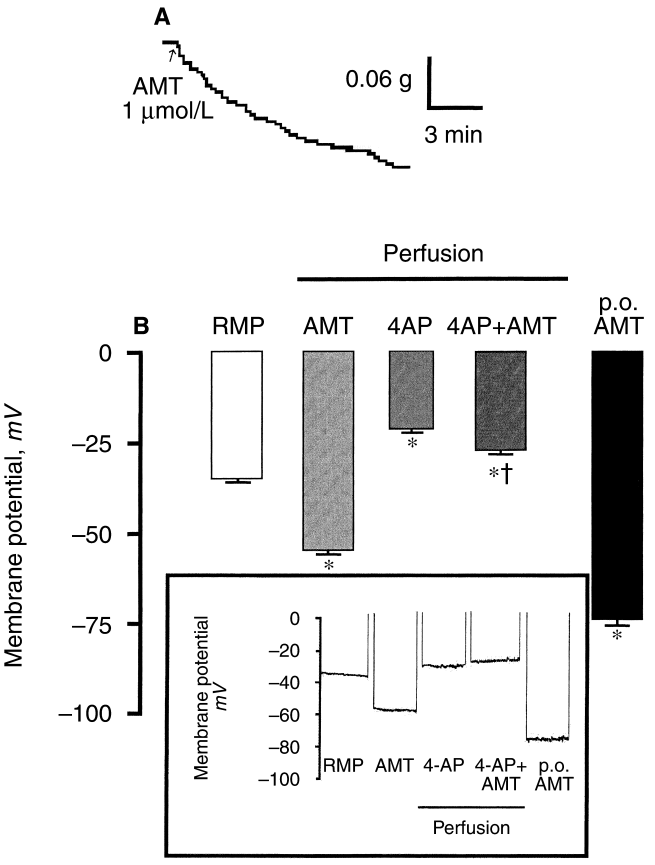

Fig. 2. Effects of amitriptyline (AMT) on rat isolated urethra. (*A*) Representative tracing showing the effect of 1 µmol/L amitriptyline on basal rat urethral strip smooth muscle tone. Similar findings were obtained in seven other experiments. (*B*) Membrane potential was measured in everted urethral rings under the following conditions: resting membrane potential (RMP), after perfusion with 10 pmol/L amitriptyline in the absence or presence of 4-aminopyridine (4-AP) (1 mmol/L), and after oral administration of amitriptyline (gavage, 1mg/kg/day, 5 days). Each sample represents a different animal ($N = 6$ to 9). Data are expressed as means ± SEM. $*P < 0.001$ vs. RMP. $\dagger P < 0.001$ vs. amitriptyline perfusion. Inset: Typical tracings representing the findings depicted in (B).

stimulated basal tone of rat urethra, with maximal relaxant responses at micromolar levels (Fig. 2A). Due to problems during dissection of urethral tissue from adjacent structures (including penis bone), smooth muscle layers, and consequently contractile apparatus integrity, are frequently compromised, making urethral isometric recordings poorly reproducible. Thus, to corroborate and extend our findings, EPS were also carried out with urethral rings. Short-term perfusion with 1 pmol/L amitriptyline produced marked reductions in resting membrane potentials (Fig. 2B). It is noteworthy that ex vivo experiments performed with urethras removed from animals that received oral amitriptyline (1 mg/kg) for 5 days demonstrated an even more intense hyperpolarization (Fig. 2B). It has been described that amitriptyline can block monovalent cation channels in different preparations [14–17]. Thus, we repeated the experiments with amitriptyline in the presence of distinct potassium channel blockers. Perfused 4-AP (1 mmol/L), an inhibitor of voltage-dependent potassium channels [18], abolished amitriptyline-induced hyperpolarization in rat urethral rings (Fig. 2B), while the blocker alone had a slight but significant depolarizing effect on resting membrane potentials. On the other hand, no significant modification

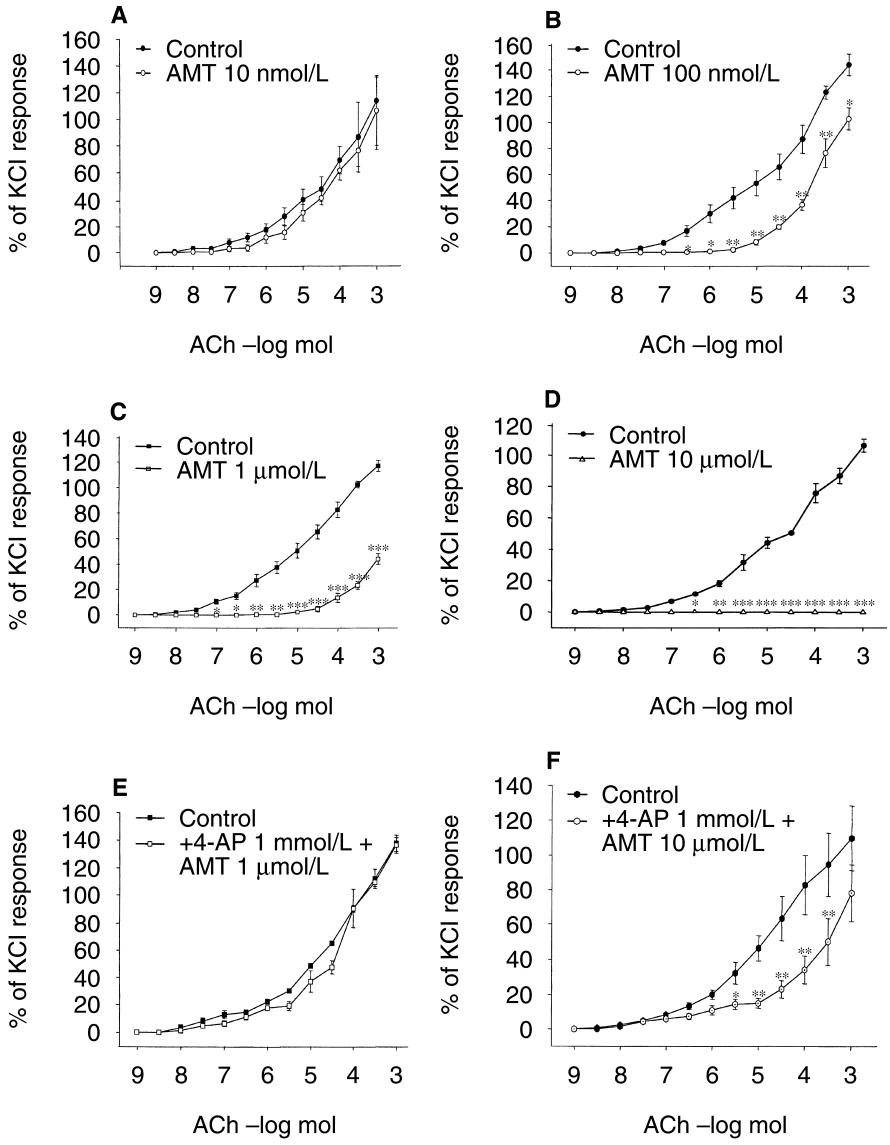

Fig. 3. Effect of amitriptyline (AMT) on acetylchlonine (ACh)-induced contractions on rat bladder strips. ACh (1 nmol/L to 1 mmol/L) cumulative curves were performed in the absence or in the presence of AMT 10 nmol/L (*A*), 100 nmol/L (*B*), 1 μmol/L (*C*), and 10 μmol/L (*D*). Effect of 1 μmol/L and 10 μmol/L amitriptyline in the presence of 4-aminopyridine (4-AP) (1 mol/L) (*E* and *F*). Data are expressed as means ± SEM of the percentage of 80 mmol/L KCl-elicited contractions (N = 6 to 8). $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

of amitriptyline effect was detected following perfusion of rat urethras with high- (3 mmol/L chlotrimazole or 3 mmol/L charybdotoxin) or low- (3 mmol/L apamin) conductance Ca^{2+}-activated potassium channel blockers, or with an adenosine triphosphate (ATP)-sensitive (3 mmol/L glybenclamide) potassium channel antagonist (see [19] for review) (data not shown), suggesting that amitriptyline acts on a specific potassium channel subtype.

Since we needed confirmation of these results in functional assays and we had to verify the effects of amitriptyline in upper parts of the urinary tract, another series of experiments was performed employing rat bladder and pig ureteral strips. Concentration-dependent curves to ACh were carried out in rat bladder strips mounted for isometric tension recordings and results were expressed as percentage of maximal response to 80 mmol/L KCl (Fig. 3 A to D). The experiments were performed in the absence or presence of increasing concentrations of amitriptyline (0.01 to 10 μmol/L). Amitriptyline presented a significant, noncompetitive inhibitory effect on ACh-induced contractions ($IC_{50} = 4.0 \times 10^{-7}$ mol/L). At 10 μmol/L, amitriptyline abolished the contractile effect of ACh. Several mechanisms of action have been described for amitriptyline, one of them being a competitive blockade of muscarinic receptors. Thus, in order to rule out a selective anticholinergic action of amitriptyline, receptor-dependent (bradykinin) or -independent (80 mmol/L KCl) contractions were generated in rat bladder strips, and amitriptyline effect was once more addressed. Bradykinin (1 μmol/L) elicited phasic contractions in rat bladder, which were progressively inhibited by amitriptyline in a concentration range very similar to that necessary to block ACh-triggered responses

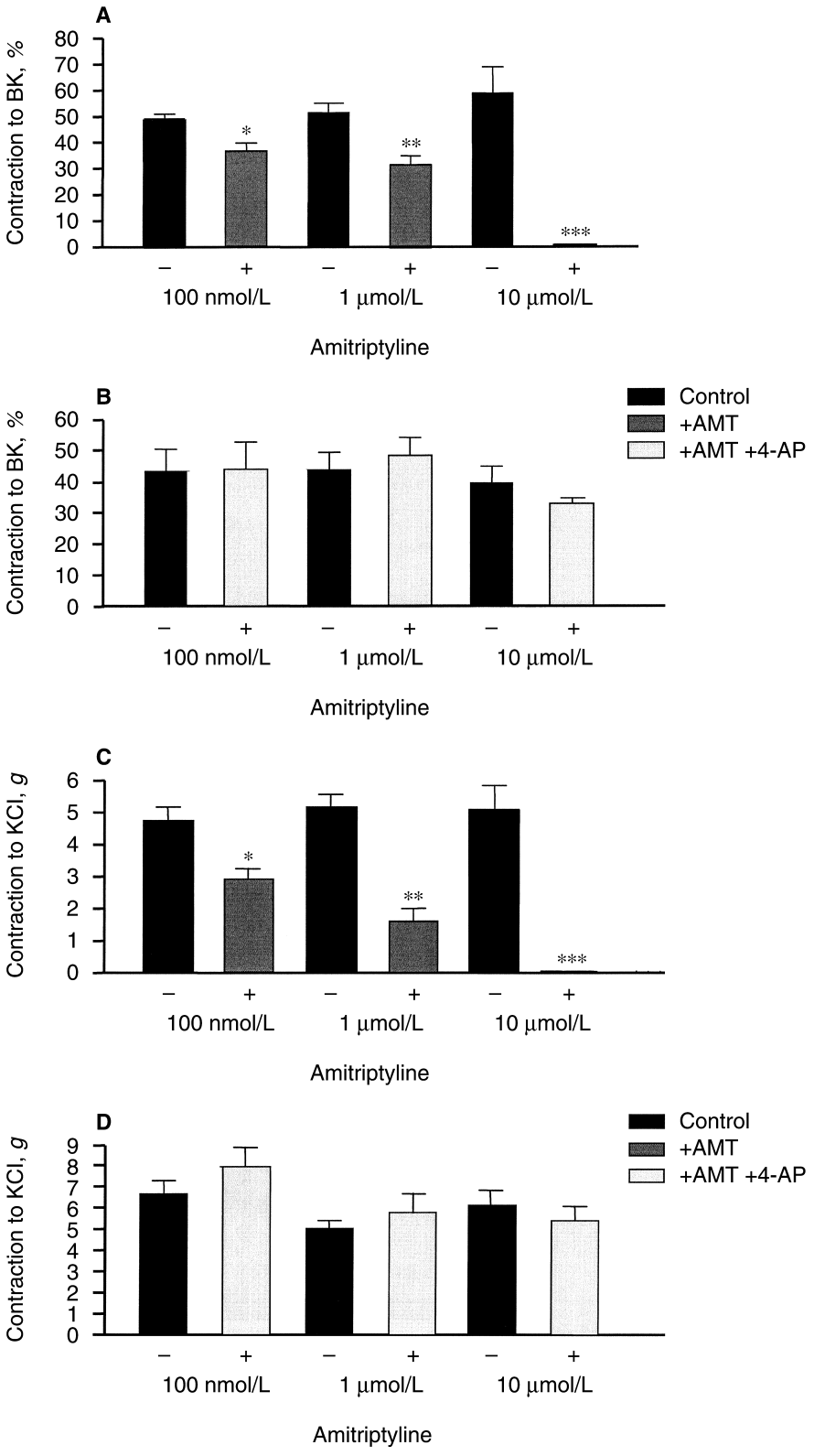

Fig. 4. Effect of amitriptyline (AMT) on bradykinin (BK)- or KCl-induced contractions in rat bladder strips. Single curves to bradykinin 1 μmol/L (*A* and *B*) or 80 mmol/L KCl (C and *D*) were built in the absence or in the presence of increasing concentrations of amitriptyline. The action of 4-aminopyridine (4-AP) on amitriptyline inhibitory effect is demonstrated in (B and D). KCl data are expressed in grams of contraction and bradykinin contractions expressed as percentage of KCl response. Data represent means ± SEM ($N = 7$). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

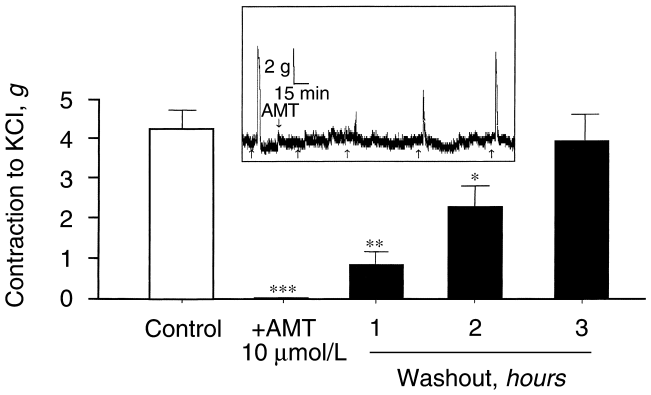

Fig. 5. Reversibility of amitriptyline (AMT) inhibitory effect on rat bladder strips. Eighty μmol/L KCl-induced contractions were recorded in the absence and in the presence of 10 μmol/L amitriptyline, and following 1, 2, and 3 hours of repetitive medium renewal without amitriptyline readministration. Data are expressed in grams of contraction and represent means ± SEM ($N = 5$). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Inset: Typical tracing demonstrating reversible action of amitriptyline. Upward and downward arrows indicate 80 mmol/L KCl and 10 μmol/L amitriptyline administration, respectively. Similar recordings were obtained in at least five experiments.

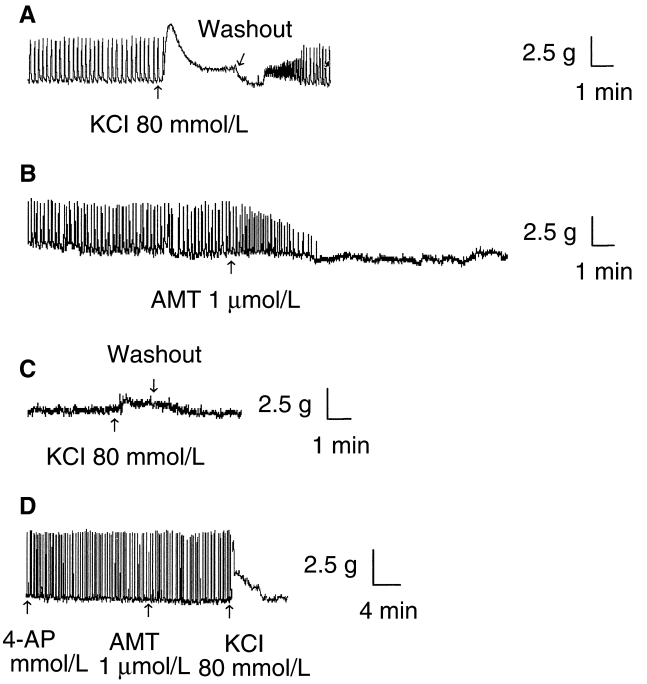

Fig. 6. Representative tracings of spontaneous and KCl-induced contractions of pig isolated ureteral rings in the absence (*A*) or in the presence (*B* and *C*) of 1 μmol/L amitriptyline. Amitriptyline inhibitory action was completely prevented by preadministration of 1 mmol/L 4-aminopyridine (4-AP) (*D*). Similar findings were obtained in at least five experiments.

(Fig. 4A). Surprisingly, even maximal contractions induced by KCl were markedly inhibited by 10 μmol/L amitriptyline (Fig. 4C). Given that amitriptyline appeared to be a rather nonspecific blocker of rat bladder contractions, we analyzed the reversibility of its inhibitory effect. Rat bladders exposed for 30 minutes to 10 μmol/L amitriptyline had their responses to KCl abolished (Fig. 5). Preparations were then washed in Krebs solution several times, and progressive recovery of KCl-induced response was demonstrated. After a washout period of 3 hours, amitriptyline effect was fully reversed, with recordings of KCl-elicited contractions similar to those obtained before amitriptyline administration (Fig. 5). Thus, this series of experiments ruled out a toxic, nonreversible effect of amitriptyline in urinary tract smooth muscle. To confirm that voltage-dependent potassium channels mediate amitriptyline inhibitory effect, ACh curves in the presence of amitriptyline were repeated in preparations preincubated with 4-AP (1 mmol/L) for 20 minutes. As can be verified in Figure 3 E and F, 4-AP, as seen in rat urethra, prevented amitriptyline from reducing the tonus of rat bladder strips. In a separate series of experiments, we ruled out a potentiating effect of 4-AP on ACh-elicited contractions in the absence of amitriptyline (data not shown), suggesting a specific effect of this potassium channel blocker on amitriptyline action in the urinary tract. The mechanism of action of amitriptyline in rat bladder strips seemed to be the same regardless the contractile agent used, as inhibition of bradykinin- and KCl-induced contractions by amitriptyline was also prevented by pretreatment of the preparations with 4-AP (Fig. 4 B and D).

Urinary calculi are distributed mostly along the ureter when they are detected by image methods in the setting of painful crises. With that in mind, we analyzed the effects of amitriptyline in pig mid-ureteral rings mounted for isometric tension recordings. Following the equilibration period, nonstimulated ureteral rings exhibit periodic clusters of strong phasic contractions (Fig. 6 A and B). This activity was not mediated by parasympathetic innervation, since it could not be blocked by 1 μmol/L atropine administration (data not shown). Amitriptyline incubation progressively inhibited the frequency and amplitude of these twitches, and pig ureter contractile activity was abolished in the presence of 1 μmol/L amitriptyline, a concentration ten times lower than that necessary to block rat bladder contractile activity (Fig. 6B). Progressive renewal of Krebs solution in the absence of amitriptyline rescued the normal phasic contraction pattern observed before amitriptyline administration (data not shown). As demonstrated in rat bladder, amitriptyline inhibitory action was not selective for receptor-mediated stimulation, as KCl-elicited contractions were also blocked by amitriptyline in pig ureteral rings (Fig. 6 A and C). In addition, amitriptyline negative effect on smooth muscle tonus was also prevented by preincubation of ureteral preparations with 4-AP (Fig. 6D). Finally, in order to demonstrate that amitriptyline has a

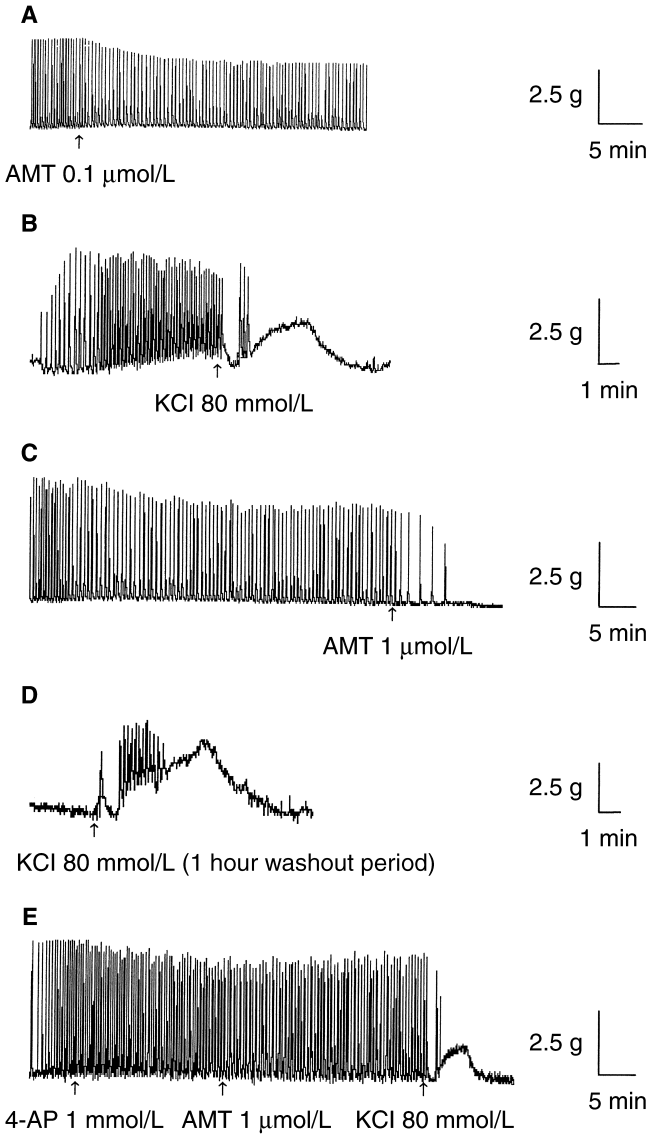

Fig. 7. Representative tracings of spontaneous and KCl-induced contractions on human isolated ureteral rings in the absence (*A* and *B*) or in the presence of 0.1 μmol/L and 1 μmol/L amitriptyline (*A* and *C*, respectively). Amitriptyline inhibitory action was completely reversed following medium renewal (*D*) and prevented by preadministration of 1 mmol/L 4-aminopyridine (4-AP) (*E*). Similar findings were obtained in at least four experiments.

potential use in clinical practice, we investigated the effect of this drug in human isolated ureteral rings obtained from kidney donors. As observed with pig ureter, the preparation, in most cases, exhibits spontaneous phasic contractions (Fig. 7). After a short period of time in presence of 0.1 μmol/L amitriptyline, this contractile activity was partially inhibited while 1 μmol/L amitriptyline completely abolished ureteral twitches (Fig. 7 A and C). In addition, even maximal tonic contractions elicited by 80 mmol/L KCl were completely prevented by preincubation of human ureter with 1 μmol/L amitriptyline (Fig. 7 B and C). Furthermore, as demonstrated in rat and pig preparations, amitriptyline inhibitory effect was reversed by Krebs solution renewal and prevented by preincubation of the preparations with 4-AP (Fig. 7 D and E).

DISCUSSION

The discovery of drugs effective in treating urolithiasis would be a significant step in the management of a condition for which conservative approaches have a rather limited success rate. In fact, previous attempts to identify beneficial agents have produced negative or modest results, and drugs such as calcium channel blockers and nitrates, for instance, were tested in a controlled way [20, 21] but never included in common clinical practice. Our study is the first to demonstrate that amitriptyline, a drug used for many years for the treatment of depression and other conditions, facilitates the elimination of urinary calculi in felines. In the presence of urinary flow obstruction, these animals only rarely pass the calculi spontaneously, and obstructive ARF due to urolithiasis in cats is accompanied by a high mortality rate, even when invasive approaches are employed. In the present study, 20 animals with advanced obstructive ARF that received amitriptyline survived following passing a urethral plug. The calculi elimination occurred in a period of time no longer than 72 hours and renal function returned to normal in all of them. Due to ethical issues, we were not allowed to compare these results with those obtained from a control group. However, comparison with historical data [12, 13] demonstrated the marked beneficial effect of amitriptyline in such a condition.

Urinalysis and analysis of the constitution of eliminated plugs showed a varied composition, including struvite, calcium oxalate, and amorphous urate crystals. This finding and the rapid resolution of the obstruction induced by the drug suggest that amitriptyline mechanism of action probably would not involve a specific biochemical alteration in the urine. Thus, we hypothesized that amitriptyline could modify urinary tract smooth muscle tone, as demonstrated previously for other types of vascular and nonvascular smooth muscle preparations [22–24]. Our results in rat urethra confirmed that amitriptyline produces potent smooth muscle relaxations that appear to be mediated by opening of voltage-dependent potassium channels. The fact that only 4-AP among five potassium channel blockers was effective in inhibiting amitriptyline action points to a very specific mechanism of action for this drug. It is noteworthy that amitriptyline reduced urethral tone even in the absence of a precontraction, suggesting a mechanism independent of any particular ligand pathway. In fact, experiments with rat isolated bladder strips demonstrated that KCl-, ACh-, or bradykinin-elicited contractions were completely blocked by amitriptyline through the same mechanism. This is an in-

teresting finding since, during painful crises due to urolithiasis, several mediators are probably involved in urinary tract smooth muscle marked contractions that usually accompany the case. We speculate that amitriptyline would be effective in blocking smooth muscle tone increases induced by most if not all of the known and unknown contracting agents. In addition, these experiments were also useful to demonstrate that amitriptyline effect is fully reversible, ruling out a possible permanent toxic effect as a cause for smooth muscle relaxation. The fact that medium renewal for a period of time longer than 3 hours was necessary to completely wash out amitriptyline and to obtain complete recovery of rat bladder contractions points to a long-lasting effect of a single dose of the drug. This would be another advantageous characteristic for a drug to be used in the clinical setting of urolithiasis.

Ureteral stones are the main concern when treating patients with urolithiasis. Although we had convincing results in rat urethra and bladder, we proceeded to studies utilizing pig isolated ureteral rings to verify if amitriptyline effect would persist despite species and urinary tract region differences. Indeed, spontaneous and induced contractions were abolished by pretreatment of pig ureter with amitriptyline. As seen for rat bladder, this effect was not ligand-dependent, as atropine-resistant twitches, and KCl-induced contractions were equally inhibited by amitriptyline. Once again, the mechanism of action of amitriptyline was demonstrated to be dependent on opening of voltage-dependent 4-AP–sensitive potassium channels.

Since our main goal is to identify drugs potentially useful in the management of urolithiasis, we considered it appropriate to test amitriptyline in human isolated preparations. Ureteral rings from normal kidney donors mounted for isolated bath preparations presented a behavior very similar to that observed for pig ureter under basal conditions. The response of human tissue samples to amitriptyline was also comparable, and ureteral contractions were prevented by the administration of micromolar concentrations of the drug. As described for rat bladder and pig ureter, amitriptyline effect was also reversible. The potency of amitriptyline in human (as well as pig) ureteral rings was even higher than that observed in lower parts of the urinary tract, with spontaneous and KCl-elicited contractions being abolished at 1 μmol/L in contrast to 10 μmol/L amitriptyline necessary to produce the same effect in rat bladder. It should be emphasized that human ureteral contractions were quickly eliminated by the use of amitriptyline at a concentration within the range of steady-state plasma levels found in patients taking low to moderate (100 mg) doses of the drug for the treatment of depression [25–27].

CONCLUSION

We have identified for the first time a new desirable effect and mechanism of action for a well-known drug. Amitriptyline was found to produce potent, rapid, long-lasting, reversible, and extremely effective blockade of urinary smooth muscle contractions regardless the identity of the contracting agent. Due to the considerable number of patients treated with amitriptyline in the past decades, its pharmacokinetics and adverse effect profile are well recognized. Taken together, these elements suggest that amitriptyline is potentially a candidate to be used in the treatment of urolithiasis-induced painful crises. To address this hypothesis, a placebo-controlled clinical trial is currently underway in our institution to investigate the effects of short-term amitriptyline administration on calculus elimination and pain relief.

ACKNOWLEDGMENTS

This study has received financial support from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). We gratefully acknowledge the invaluable assistance of Dr. Luis F.P. de Figueiredo and Dr. José C.C.B. Silva in obtaining pig and human ureteral specimens, respectively. We also thank the technical assistance of Nelson Mora.

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