Original Article

Avanafil Inhibits the Contractility of the Isolated Caprine Detrusor Muscle

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Abstract

Context: Avanafil is a smooth muscle relaxant that is clinically used to treat erectile dysfunction. It is an inhibitor of phosphodiesterase-5 (PDE5), the enzyme that catalyzes the metabolism of cyclic guanosine monophosphate (cGMP). The inhibitory effect of avanafil on isolated detrusor muscle contractility has not been studied. **Aims:** This study investigated the inhibitory effect of avanafil on isolated caprine (goat) detrusor muscle contractility and the possible mechanisms involved. **Settings and Design:** 80 mM potassium chloride (KCl)-induced contractility of the isolated goat detrusor was studied using a physiograph. **Materials and Methods:** Ten caprine detrusor strips were made to contract using 80 mM KCl before and after addition of three concentrations (10, 30, and 60 μM) of avanafil. Three reversal agents, ODQ, a guanylyl cyclase inhibitor; glibenclamide, an adenosine triphosphate (ATP)-sensitive potassium channel blocker; and iberiotoxin, a calcium-sensitive potassium (BKCa) channel blocker, were investigated for their ability to reverse the inhibitory effect of 30 μM avanafil on KCl-induced detrusor contractility. **Statistical Analysis Used:** The nonparametric statistical test, Kruskal–Wallis test, was used for the analysis of the data. **Results:** Avanafil caused a statistically significant inhibition of detrusor contractility at 30 and 60 μM concentrations. The inhibitory effect of 30 μM avanafil on detrusor contractility was significantly reversed by the addition of ODQ, glibenclamide, and iberiotoxin. **Conclusions:** Avanafil inhibits the contractility of the isolated detrusor by inhibiting PDE5, leading to raised cellular levels of cGMP. The raised levels of cGMP could have inhibited detrusor contractility by activating cGMP-dependent protein kinase, by opening ATP-sensitive potassium channels, and by opening BKCa. Avanafil could be evaluated for treating clinical conditions requiring relaxation of the detrusor like overactive bladder.

Keywords: *Avanafil, contractility, detrusor, urinary bladder*

Introduction

Avanafil is a smooth muscle relaxant that has been approved by the United States Food and Drug Administration for the treatment of erectile dysfunction (ED), where it acts by relaxing the corpus cavernosum.[1,2] Avanafil is a relatively new potent and selective phosphodiesterase-5 (PDE5) inhibitor.[3] Other drugs belonging to the family of PDE5 inhibitors include sildenafil, tadalafil, and vardenafil.[3] PDE5 is the enzyme involved in the catabolism of the second messenger cyclic guanosine monophosphate (cGMP).[4] Inhibition of PDE5 leads to raised levels of cGMP in the cell. cGMP is known to mediate the smooth muscle relaxant effect of avanafil. Avanafil has previously been shown to inhibit the contractility of the isolated rat, human, and rabbit corpus cavernosum. Sakamoto *et al*.[5] showed that avanafil has a potent relaxant effect on the isolated rabbit corpus cavernosum with an EC_{30} of 2.1 nM. Gur *et al*.[6] studied the inhibitory effect of avanafil on the contractility of the rat and human corpus cavernosum both *in vitro* and *in vivo*. The authors found that avanafil promotes relaxation of the corpus cavernosum by acting via the nitric oxide-cGMP pathway.

Recently, we showed that sildenafil[7] and tadalafil[8] inhibit the potassium chloride (KCl)-induced contractility of the isolated nonpregnant human myometrium. To date, the inhibitory effect of avanafil on the contractility of isolated detrusor muscle has not been studied. In this paper, we report that avanafil inhibits the KCl-induced contractility of the isolated caprine (goat) detrusor. It was felt that if avanafil is found to inhibit the contractility of the isolated detrusor, it may be useful in

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the treatment of clinical conditions requiring relaxation of the detrusor muscle, like overactive bladder (OAB).

Materials and Methods

Ten caprine urinary bladder samples were obtained from a local butcher shop from 10 mature goats of either sex. Goat bladder was chosen for this study because of easy availability.[9] The urinary bladder samples were transported to the pharmacology laboratory in physiological salt solution (PSS). The composition of the PSS was in mM: Nacl: 111.5; KCl: 4.6; $MgSO_4$: 1.16; NaH_2PO_4: 1.16; $CaCl_2$: 2.5; $NaHCO_3$: 21.9; and glucose: 11.1. In the laboratory, strips of detrusor measuring 10 mm × 3 mm were cut from the bladder samples as done previously in our laboratory.[9,10] The strips were observed under a hand lens to note the orientation of the muscle fibers as described previously.[9] The urothelium was removed by gentle scraping of the strips. The strips were mounted in an organ bath containing adequately oxygenated PSS maintained at a temperature of 37°C. A resting tension of 10 mN (about 2.5 g) was applied to the suspended strip. The study was approved by the Institutional Review Board and Animal Ethics Committee (IRB Min. No. 11220, dated March 5, 2018).

KCl (Qualigens, Mumbai, India) was dissolved in double-distilled water to obtain a concentration of 149 mg/ml. KCl aliquot was prepared fresh every day. Avanafil (Santa Cruz Biotechnology, Dallas, TX, USA) was dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St Louis, MO, USA) to give a 5 mg/ml stock solution. ODQ (Sigma-Aldrich) was dissolved in DMSO to give a 4.45 mM stock solution. Glibenclamide (Sigma-Aldrich) was dissolved in DMSO to give a 10 μM stock solution. Iberiotoxin (Santa Cruz Biotechnology) was dissolved in double-distilled water to give a 4 μM stock solution.

Following an equilibration period of 45 min, the tension was readjusted to 25 mN. The response of the detrusor strips to the administration of 80 mM KCl was then studied followed by the response to 80 mM KCl after the administration of 225 μl of the solvent used to dissolve avanafil, DMSO. This was the maximum volume of DMSO used as a vehicle and administered into the organ bath during the study. 80 mM KCl is the standard concentration of KCl used in our laboratory for stimulating the contraction of the isolated detrusor.[10] Then, 80 mM KCl was administered again. After washing the bath, 80 mM KCl was added again after allowing 5 min of contact time with the test drug, avanafil, at a concentration of 10 μM. During each tracing, after drug administration, a contact time of 90 s was given, after which the tissue was washed till the baseline was attained. This procedure was then repeated with two higher concentrations of avanafil (30 and 60 μM).

In order to elucidate the possible mechanism of the inhibitory effect of avanafil on the isolated detrusor, the following procedure was performed: the detrusor strip was first made to contract with 80 mM KCl. After washing out the KCl and a rest period, the reversal agent (ODQ/glibenclamide/iberiotoxin) was incubated with 30 μM avanafil in the organ bath for 5 min after which 80 mM KCl was added and the detrusor contraction was obtained. The reversal effect of each of the three reversal agents on the inhibitory effect of 30 μM avanafil on 80 mM KCl-induced detrusor contractility was studied.

Contractility was computed by measuring the maximum height of contraction and the area under the contractile curve (AUCC) of the tracings, a method which we have standardized in our laboratory.[7-10] These parameters were calculated by scanning the tracings after each experiment and analysis using the software Image Tool (University of Texas Health Sciences Center at San Antonio, TX, USA). The percentage inhibition of the height of contraction and the AUCC following the administration of the test drug(s), that is, avanafil or avanafil with the reversal agent, in comparison with the prior administration of KCl, was calculated. The nonparametric statistical test, Kruskal–Wallis test, was used for statistical analysis of the percentage inhibition of the test drug(s), that is, avanafil with or without a reversal agent, on the 80 mM KCl-induced contractility of the detrusor strips.

Results

The results of the effect of DMSO and the three concentrations (10, 30, and 60 μM) of avanafil used in the study on KCl-induced contractility of isolated detrusor strips are shown in Table 1. As shown, DMSO did not significantly inhibit KCl-induced detrusor contractility ($P > 0.05$). The lowest concentration of avanafil (10 μM) also did not significantly inhibit the detrusor ($P > 0.05$), whereas the 30 and 60 μM concentrations of avanafil caused a significant inhibitory effect of KCl-induced detrusor contractility ($P < 0.05$). A representative tracing of the concentration-dependent inhibitory effects of avanafil on KCl-induced detrusor contractility is given in Figure 1.

The results of the effects of the three reversal agents used in the study, ODQ, glibenclamide, and iberiotoxin, on 30 μM avanafil-induced detrusor relaxation are shown in Table 2. All three reversal agents reversed the inhibitory effect of avanafil on detrusor contractility since in their presence the percentage inhibition of detrusor contractility decreased and became statistically nonsignificant ($P > 0.05$). Representative tracings of the effect of avanafil on KCl-induced detrusor contractility and the effect of the reversal agent iberiotoxin on 30 μM avanafil inhibition of KCl-induced detrusor contractility are shown in Figure 2.

Discussion

This study has shown for the first time that avanafil inhibits the contractility of the isolated caprine detrusor

Table 1: Inhibitory effects of dimethyl sulfoxide and avanafil on potassium chloride-induced contractility of isolated caprine detrusor (*n*=10 for each drug administration)

Drug administration	Mean (SEM), *P*	
	Percentage inhibition of height	Percentage inhibition of AUCC
80 mM KCl + DMSO (225 µl)	−4.7 (4.67), 0.45	3.7 (1.08), 0.496
80 mM KCl + 10 µM avanafil	19.4 (6.72), 0.29	23.7 (1.53), 0.29
80 mM KCl + 30 µM avanafil	38.5 (5.55), 0.01	47.9 (1.36), 0.005
80 mM KCl + 60 µM avanafil	38.7 (3.74), 0.019	40.7 (1.04), 0.049

Values of percentage inhibition were obtained by comparing values after the test drug with values due to prior administration of KCl only. AUCC: Area under contractile curve; DMSO: Dimethyl sulfoxide; SEM: Standard error of mean; KCl: Potassium chloride

Table 2: Reversal of inhibition of potassium chloride-induced detrusor contractility by avanafil using reversal agents (*n*=10 for each drug administration)

Drug administration	Mean (SEM), *P*	
	Percentage inhibition of height	Percentage inhibition of AUCC
80 mM KCl + 30 µM avanafil + 10 µM ODQ	14.0 (6.68), 0.427	10.6 (3.00), 0.326
80 mM KCl + 30 µM avanafil + 10 µM glibenclamide	5.0 (7.08), 0.705	−1.13 (1.81)*, 0.705
80 mM KCl + 30 µM avanafil + 100 nM iberioto	−15.1 (6.88)*, 0.45	−9.1 (1.65)*, 0.705

*Mean values are negative due to the total reversal of the inhibitory effect of avanafil on KCl-induced myometrial contractility by glibenclamide and iberiotoxin. The inhibitory effect of 30 µM avanafil on detrusor contractility in the absence of a reversal agent is given in Table 1. Values of percentage inhibition were obtained by comparing values after the test drugs with values due to prior administration of KCl only. AUCC: Area under contractile curve; SEM: Standard error of mean; KCl: Potassium chloride

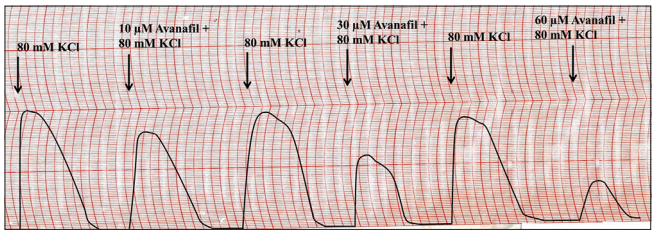

Figure 1: Representative trace from the study showing the inhibitory effects of 3 concentrations of avanafil (10, 30, and 60 µM) on 80 mM potassium chloride-induced contractility of the isolated goat detrusor

muscle. Avanafil at 30 and 60 µM concentrations significantly inhibited the KCl-induced contractility of the detrusor [Table 1]. Avanafil is known to be a smooth muscle relaxant, which is the basis for its use in ED. The concentrations of avanafil used in this study are comparable to the concentrations of sildenafil and tadalafil that we used in two earlier studies, which investigated the inhibitory effects of sildenafil and tadalafil on the isolated human myometrium.[7,8]

The findings of this study throw light on the possible mechanisms of action of avanafil in relaxing the isolated detrusor: the inhibitory effect of avanafil was reversed by the three reversal agents, ODQ, glibenclamide, and iberiotoxin [Table 2 and Figure 2]. ODQ is a guanylyl cyclase inhibitor and hence reduces cellular levels of cGMP supporting the known fact that avanafil relaxes smooth muscle by inhibiting PDE5 and hence increasing cellular levels of cGMP. It was also found in this study that the adenosine triphosphate (ATP)-sensitive potassium channel blocker glibenclamide and the calcium-sensitive potassium (BKCa) channel blocker iberiotoxin reversed the inhibitory effect

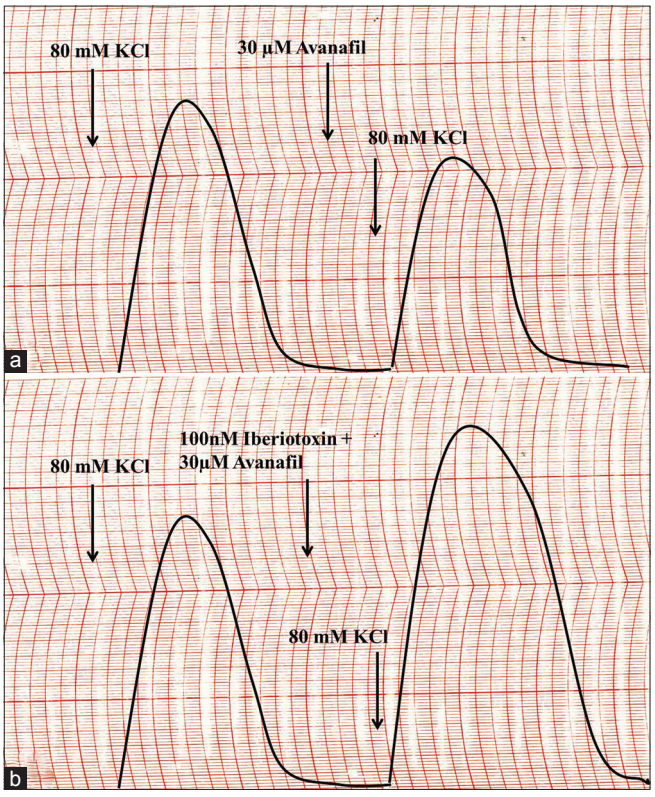

Figure 2: Representative traces from the study: (a) Contractile effect of 80 mM potassium chloride before (left side) and after (right side) addition of 30 µM avanafil. (b) Contractile effect of 80 mM potassium chloride before (left side) and after (right side) addition of 100 nM iberiotoxin and 30 µM avanafil

of avanafil on detrusor contractility [Table 2]. This suggests that in this study, avanafil also inhibited detrusor contractility by causing the opening of ATP-sensitive potassium channels

and BKCa. This could have been due to the increased cellular levels of cGMP due to inhibition of PDE5 by avanafil. These data are supported by the fact that cGMP is known to regulate the gating of ATP-sensitive potassium channels[11] and BKCa.[12] Moreover, it is known that both ATP-sensitive potassium channels and BKCa play a crucial role in detrusor contraction and relaxation and detrusor function and dysfunction.[13]

There have been only a few studies that have investigated the inhibition by PDE5 inhibitors of the contractility of the isolated detrusor. To the best of our knowledge, the current study is the only one on the inhibition of the contractility of the isolated detrusor by avanafil. It is of interest that Oger *et al*.[14] found that sildenafil's inhibition of carbachol-induced isolated human detrusor contractility was reversed by all three reversal agents used in the current study, namely, ODQ, glibenclamide, and iberiotoxin. These authors found sildenafil's inhibitory effect even at a concentration of 3 μM, unlike in the current study where avanafil was found to inhibit detrusor contractility at 30 and 60 μM, but not at 10 μM [Table 1]. Filippi *et al*.[15] found that 100 nM vardenafil significantly increased sodium nitroprusside-induced relaxation of the isolated human detrusor made to contract with carbachol. These findings support the data that regarding PDE5 inhibitors, the order of potency *in vitro* is vardenafil >sildenafil >avanafil.[4,16]

As mentioned in the introduction, Sakamoto *et al*.[5] found that avanafil is active in the isolated rabbit corpus cavernosum at low concentrations (in the nM range). However, we found that in the detrusor avanafil is active only at higher concentrations (in the μM range; [Table 1]). The reason for this difference in the action of avanafil between the corpus cavernosum and the detrusor could be that there are higher concentrations of cGMP, the substrate for PDE5, in the corpus cavernosum muscle cells than in the detrusor muscle cells.[17] Hence, lower concentrations of PDE5 inhibitors such as avanafil are likely to be required for acting in the corpus cavernosum than in the detrusor.[4,17]

Disorders of the lower urinary tract like OAB are common chronic medical conditions that have a great impact on the quality of life of affected patients.[18] OAB is characterized by urinary urgency, usually accompanied by frequency and nocturia. Anticholinergics are presently the first-line therapy for OAB, since they lower intravesical pressure, increase capacity, and reduce the frequency of detrusor contractions.[19] However, although they have initial good response rates, they can later have decreasing efficacy.[20] Moreover, they are known to produce adverse effects such as dry mouth, blurred vision, xerostomia, and constipation, which can limit their use.[19] Hence, it is felt that newer alternatives are needed.[20] One such class of new drugs that could be useful for treating OAB is PDE5 inhibitors.[21,22] Avanafil can be administered orally to patients and has a good pharmacokinetic profile.[2,23] The safety profile of avanafil is also good with no major adverse effects reported to date.[2,23] Hence, in the light of the above data, avanafil is a potential new candidate for the treatment of clinical conditions like OAB that require relaxation of the detrusor muscle. Indeed, there is preliminary clinical evidence that PDE5 inhibitors are useful in the treatment of OAB. For example, Amano *et al*.[24] administered 5 mg of tadalafil once a day to patients with benign prostatic hyperplasia. After 12 months of treatment, the authors found significant improvement in OAB symptom score, residual urine volume score, and international prostate symptom score compared to initial scores.

Conclusions

This study has shown for the first time that at suitably low concentrations, the PDE5 inhibitor avanafil inhibits the contractility of the isolated detrusor. The results of the study suggest that avanafil inhibits detrusor contractility by raising cellular levels of cGMP, leading to activation of cGMP-dependent protein kinase, and by opening ATP-sensitive potassium channels and BKCa. Avanafil could be further evaluated for the treatment of clinical conditions requiring relaxation of the detrusor like OAB.

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Conflicts of interest

There are no conflicts of interest.

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