Research Paper

Inhibition by sildenafil of contractility of isolated non-pregnant human myometrium

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ABSTRACT

Objective: To investigate the ability of sildenafil to inhibit the contractility of isolated non pregnant human myometrium. **Materials and Methods:** The inhibitory effect of three concentrations (3, 10, and 30 μM) of sildenafil on 55 mM KCl-induced contractility of isolated non-pregnant human myometrium was studied. The ability of the guanylyl cyclase inhibitor ODQ (10 μM), the adenylyl cyclase inhibitor MDL-12,330A (10 μM), the non-specific potassium channel blocker TEA (2 mM), and the calcium-sensitive potassium (BKCa) channel blocker iberiotoxin (100 nM) to reverse the inhibition of 10 μM sildenafil on KCl-induced myometrial contractility was also studied. **Results:** Sildenafil produced a concentration-dependent inhibition of KCl-induced myometrial contractility that was statistically significant at all three concentrations of sildenafil used. The inhibition by 10 μM sildenafil of KCl-induced myometrial contractility was not reversed by the concurrent administration of ODQ or MDL-12,330A. The inhibition of 10 μM sildenafil of myometrial contractility was partially reversed by concurrent administration of TEA and totally and significantly reversed by the concurrent administration of iberiotoxin. **Conclusions:** These results suggest that sildenafil inhibits the contractility of isolated non-pregnant human myometrium. The results suggest that sildenafil does so by opening BKCa channels.

Key words: Contractility, myometrium, non-pregnant, sildenafil

INTRODUCTION

Sildenafil is a smooth muscle relaxant which has been approved by the US Food and Drug Administration for the treatment of erectile dysfunction,[1] and pulmonary arterial hypertension.[2] In the former condition it acts by relaxing the corpus cavernosum and in the latter by relaxing the pulmonary artery. It is well established that sildenafil inhibits phosphodiesterase-5 (PDE5), the enzyme that catalyzes the metabolism of the second messenger cyclic GMP

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(cGMP).[3,4] This action leads to increased cellular levels of cGMP. Sildenafil has been shown to relax various isolated smooth muscles such as human vas deferens,[5] rabbit corpus cavernosum,[6] human seminal vesicle,[7] porcine retinal artery,[8] rat duodenum,[9] and human detrusor.[10] Sildenafil has also been shown to relax isolated pregnant human myometrium in two studies.[11,12] In the first of these two studies,[11] it was found that sildenafil caused relaxation of the myometrium in a dose-dependent manner which was not reversed by the guanylyl cyclase inhibitor methylene blue or the non-specific potassium channel blocker tetraethyl ammonium (TEA) at 5 and 10 mM concentrations. TEA at a concentration of 20 mM reversed the relaxant effect of sildenafil. These findings suggested to the authors that potassium channels are likely to mediate the relaxant effect of sildenafil on the myometrium. To our knowledge, the inhibitory effect of sildenafil on isolated non-pregnant human myometrium has not been studied. Since there are marked anatomical, physiological, biochemical, and pharmacological differences between the pregnant and non-pregnant human myometrium, this study was designed to test the hypothesis that sildenafil can inhibit the contractility of the isolated non-pregnant human myometrium. Further, if sildenafil was found to do so, it was planned to investigate the possible mechanisms involved in the inhibitory effect of sildenafil on myometrial contractility.

MATERIALS AND METHODS

The study was conducted in two phases. During the first phase, conducted using 10 myometrial strips obtained from 10 patients who underwent hysterectomy, the effects of the vehicle dimethyl sulfoxide (DMSO) and three concentrations of sildenafil (3, 10, and 30 μM) on KCl-induced myometrial contractility were studied. This was followed by studying the ability of the reversal agents1H-[1,2,4] oxadiazol[4,3-a] quinoxalin-1-one (ODQ, a guanylyl cyclase inhibitor), MDL-12,330A (an adenylyl cyclase inhibitor), and TEA to reverse the inhibitory effect of 10 μM sildenafil on KCl-induced myometrial contractility.

Since the results of the first phase of the study suggested that potassium channels are involved in the inhibitory effect of sildenafil on myometrial contractility, we conducted a second study phase to investigate the ability of the calcium-sensitive potassium (BKCa) channel blocker iberiotoxin to reverse the inhibitory effect of sildenafil. During the second phase of the study, conducted on 10 additional strips obtained from 10 additional patients who underwent hysterectomy, the ability of iberiotoxin to reverse the inhibitory effect of 10 μM sildenafil on KCl-induced myometrial contractility was studied.

Selection of patients

The patients were selected from the Department of Obstetrics and Gynecology, Christian Medical College, Vellore, India. Inclusion criteria were patients 20 to 50 years of age undergoing hysterectomy for benign conditions like polyps, fibroids, and pelvic organ prolapse. Exclusion criteria were postmenopausal patients and those with malignant conditions. Written informed consent was obtained from each patient included in the study. The study was approved by the Institutional Review Board (IRB minute number 7370 dated 8.12.2010).

Tissue preparation

After removal of the uterus from the patient, parts of the uterus showing gross pathological changes were noted and excluded. A 2 × 2 cm tissue specimen was resected from normal appearing areas of the lateral wall of the uterus. The specimen was then transported in physiological salt solution (PSS) to the pharmacology laboratory within 1 hour. The uterine specimens were examined under a magnifying glass to look for orientation of muscle fibers. Pathological changes like fibroids were also looked for and excluded. The serosa and endometrium were removed from the specimens. Myometrial strips measuring 10 × 3 × 3 mm were then made and mounted in a 20 ml organ bath containing PSS maintained at a temperature of 37°C and adequately aerated with oxygen. 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. A resting tension of 25 milliNewtons (mN; about 2.5 g) was applied to the mounted strip.

Drugs

KCl (Qualigens, Mumbai, India) was dissolved in double-distilled water to make a concentration of 168 mg/ml. KCl was prepared fresh before each experiment. Sildenafil (Sigma Aldrich, St Louis, MO, USA) was dissolved in dimethyl sulphoxide (DMSO), (Sigma Aldrich, St Louis, MO, USA) to give a concentration of 5 mg/ml. ODQ (Sigma Aldrich, St Louis, MO, USA) was dissolved in DMSO to give a concentration of 5 mg/ml. MDL-12,330A (Sigma, St Louis, MO, USA) in pure powder form was dissolved in DMSO to give a working concentration of 5 mg/ml. TEA (Qualigens, Mumbai, India) was dissolved in double-distilled water to give a concentration of 50 mg/ml. Iberiotoxin (Santa Cruz Biotechnology, Dallas, TX, USA) was dissolved in double-distilled water to give a 4 μM stock solution.

Experimental procedure

Experiments involving effect of sildenafil on KCl-induced contractility of the myometrium

After an equilibration period of 90 minutes, the tension was readjusted to 25 mN. The response of the myometrium to the administration of 55 mM KCl was then studied followed by the response after the administration of 55 mM KCl and 0.25 ml of the solvent used to dissolve sildenafil, DMSO. This was the maximum volume of DMSO used in the study. 55 mM KCl is the standard concentration of KCl used in our laboratory for stimulating the contraction of the isolated myometrium.[13,14]

We have found this concentration of KCl to be suitable because it is effective and can be washed out easily following muscular contraction. Then 55 mM KCl was administered again. After washing the bath, 55 mM KCl was added again concurrently with the test drug, sildenafil. Three concentrations of sildenafil were used: 3, 10, and 30 μM. The incubation time that was used in the study after administration of the drugs (DMSO, sildenafil, and reversal agents) to the bath was 10 minutes. During each tracing, after drug administration, a contact time of 90 seconds was given, after which the tissue was washed till the baseline was reached. A rest period of 10 minutes was used between drug treatments.

Experiments involving reversal of inhibitory effect of sildenafil on contractility of the myometrium

In order to elucidate the mechanism of inhibition of KCl-induced myometrial contractility by sildenafil, the following experimental procedures were performed: KCl was administered alone and then with 10 μM sildenafil and ODQ (10 μM). After washing out the organ bath solution and a rest period, KCl was added alone again, and then with 10 μM sildenafil and MDL-12,330A (10 μM). After washing out the organ bath solution and a rest period, KCl was added alone and then with 10 μM sildenafil and TEA (2 mM).

In the second phase of the study, the ability of the reversal agent iberiotoxin to reverse the inhibitory effect of 10 μM sildenafil on KCl-induced contractility was studied. During this phase of the study, for each mounted myometrial strip, the contractile response to 55 mM KCl was obtained followed by that to KCl in the presence of 10 μM sildenafil. After adequate washes and reaching the baseline, the response to 55 mM KCl and then KCl in the presence of 100 nM iberiotoxin and 10 μM sildenafil was obtained. The concentrations of reversal agents used in this study are those that have been used in previous studies.[11,15-17]

Statistical analysis

Contractility was quantified by the maximum height of contraction and the area under the contractile curve (AUCC), a method which has been standardized in our laboratory.[13,14] These parameters were determined by scanning the tracings after each experiment and analysis with the software Image Tool (University of Texas Health Sciences Center at San Antanio, TX, USA). This was done by comparing statistically the values of these parameters of the control data (after the administration of KCl alone) and the values of the test data (after the administration of the test drug (s) with KCl). The nonparametric test, Wilcoxon signed rank test, was used for all statistical analyses of the data.

RESULTS

Ten patients who fulfilled the selection criteria were enrolled into the first phase of the study. Their ages ranged from 35 to 50 years with a mean age of 43 years. The clinical diagnoses of the patients were: Fibroids, five patients; endometrial polyp, one patient; pelvic organ prolapse, two patients; adenomyosis, one patient; and simple hyperplasia, one patient. Ten patients who fulfilled the selection criteria were enrolled into the second phase of the study. Their ages ranged from 36 to 49 years with a mean age of 42 years. The clinical diagnoses of these patients were: Fibroids, 8 patients; endometriosis, 1 patient; and adenomyosis, 1 patient. In order to ensure, as much as possible, that all myometrial specimens used in the study had similar physiological conditions, all the specimens were obtained from patients fulfilling the following criteria: Non-pregnant, non-lactating, pre-menopausal patients 20 to 50 years old with non-malignant conditions.

The results of the effect of DMSO and sildenafil on KCl-induced contractility of isolated non-pregnant human myometrium are shown in Table 1. As shown, DMSO did not significantly inhibit KCl-induced myometrial contractility, whereas sildenafil produced a concentration-dependent inhibitory effect of KCl-induced myometrial contractility that was statistically significant at all three concentrations of sildenafil used. The results of the effects of the reversal agents on sildenafil-induced myometrial relaxation are shown in Tables 2 and 3. ODQ and MDL-12,330 A were not found to reverse the inhibitory effect of sildenafil on KCl-induced myometrial contractility. TEA partially reversed the inhibitory effect of sildenafil on myometrial contractility. Iberiotoxin completely and significantly reversed the inhibitory effect of sildenafil on myometrial contractility. Sample tracings of the effect of sildenafil on KCl-induced myometrial contractility and the effects of the reversal agents on 10 μM sildenafil's inhibition of KCl-induced myometrial contractility are shown in Figures 1 and 2.

DISCUSSION

This study has shown for the first time that sildenafil inhibits the contractility of isolated non-pregnant human myometrium [Table 1; Figures 1 and 2]. Although these findings cannot be directly extrapolated to the pregnant human myometrium, they support those of previous studies that have shown that sildenafil inhibits the contractility of isolated pregnant human myometrium.[11,12] In the first phase of this study, it was found that the guanylyl cyclase inhibitor ODQ did not reverse the inhibitory effect of 10 μM sildenafil on KCl-induced myometrial contractility [Table 2]. The adenylyl cyclase inhibitor MDL-12,330A also acted like ODQ in this regard, and did not reverse the inhibition of myometrial contractility due to sildenafil [Table 2]. In this context, sildenafil is a specific inhibitor of PDE5, a PDE isozyme that specifically catalyzes the metabolism of cGMP, without catalyzing the metabolism of cAMP.[18] *In vitro* studies conducted previously have shown that PDE5 is present in the human myometrium.[19,20]

Table 1: Percent inhibition of DMSO and sildenafil of KCl-induced contractility of isolated non-pregnant human myometrium (*n*=10 for each drug administration)

Drug Administration	% Inhibition of height		% Inhibition of AUCC	
	Mean (SEM)	*P*	Mean (SEM)	*P*
55 mM KCl+DMSO (0.25 ml)	–0.75 (1.34)	0.912	–0.29 (4.35)	0.845
55 mM KCl+3 μM sildenafil	19.72 (4.94)	0.001	25.91 (5.63)	0.000
55 mM KCl+10 μM sildenafil	36.39 (3.41)	0.000	48.51 (5.33)	0.000
55 mM KCl+30 μM sildenafil	57.55 (6.74)	0.000	74.16 (3.68)	0.000

Values of percent inhibition are compared with those following prior administration of KCl only AUCC=Area under contractile curve, DMSO=Dimethyl sulfoxide, SEM=Standard error of mean

Table 2: Effects of reversal agents on 10 μM sildenafil's inhibition of KCl-induced contractility of isolated non-pregnant human myometrium (*n*=10 for each drug administration)

Drug administration	Mean (SEM)	
	% Inhibition of height	% Inhibition of AUCC
10 μM ODQ+10 μM sildenafil+55 mM KCl	54.97 (6.33)	54.32(9.36)
10 μM MDL-12,330A+10 μM sildenafil+55 mM KCl	58.44 (5.29)	67.34 (5.06)
2 mM TEA+10 μM sildenafil+55 mM KCl	17.70 (2.31)	16.38 (4.12)

Values of percent inhibition are compared with those following prior administration of KCl only AUCC=Area under contractile curve, SEM=Standard error of mean, TEA=Tetraethyl ammonium. See table 1 for effect of 10 μM sildenafil on KCl-induced myometrial contractity in the absence of reversal agents

Table 3: Effect of iberiotoxin on 10 μM sildenafil's inhibition of KCl-induced contractility of isolated non-pregnant human myometrium (*n*=10 for each drug administration)

Drug administration	% Inhibition of height		% Inhibition of AUCC	
	Mean (SEM)	*P*	Mean (SEM)	*P*
55 mM KCl+10 μM sildenafil	21.17 (5.61)	0.006	40.58 (4.99)	0.002
55 mM KCl+100 nM iberiotoxin+10 μM sildenafil	–6.17(10.23)	0.23*	–5.44 (10.41)	0.922*

Values of percent inhibition are compared with those following prior administration of KCl only AUCC=Area under contractile curve *The mean values of percent inhibition are negative since the mean values after addition of iberiotoxin, sildenafil and KCl exceeded those after addition of KCl only

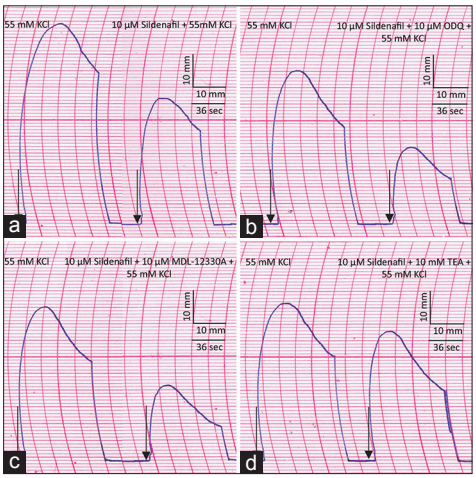

Figure 1: Sample traces from the first study phase: (a) contractile effect of 55 mM KCl before (left side) and after (right side) addition of 10 μM sildenafil. (b) Contractile effect of 55 mM KCl before (left side) and after (right side) administration of 10 μM ODQ and 10 μM sildenafil. (c) Contractile effect of 55 mM KCl before (left side) and after (right side) administration of 10 μM MDL-12,330A and 10 μM sildenafil. (D) Contractile effect of 55 mM KCl before (left side) and after (right side) addition of 2 mM tetraethyl ammonium (TEA) and 10 μM sildenafil

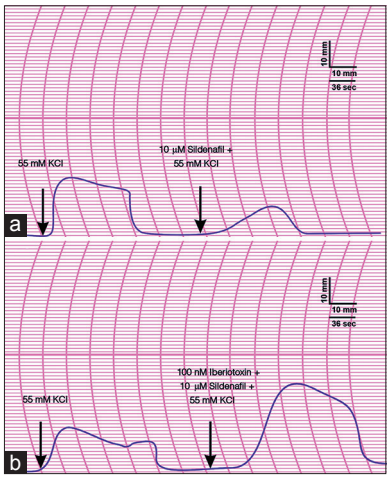

Figure 2: Sample traces from the second study phase: (a) Contractile effect of 55 mM KCl before (left side) and after (right side) addition of 10 μM sildenafil. (b) Contractile effect of 55 mM KCl before (left side) and after (right side) addition of 100 nM iberiotoxin and 10 μM sildenafil

The non-specific potassium channel blocker TEA partially reversed the inhibitory effect of sildenafil on KCl-induced myometrial contractility during the first phase of this study [Table 2]. This suggests that the opening of cell membrane-bound potassium channels was involved in the mechanism of action of sildenafil. During the second phase of this study, the specific BKCa channel blocker

iberiotoxin[21] completely and significantly [Table 3] reversed the inhibitory effect of sildenafil on myometrial contractility, suggesting that the type of potassium channel opened by the administration of sildenafil was the BKCa channel. Iberiotoxin has been shown to reverse the relaxant effect of sildenafil previously in the human detrusor,[10] the rabbit ductus arteriosus,[22] and penile resistance arteries.[23] There is experimental evidence from previous studies on isolated smooth muscles that sildenafil can directly open potassium channels without the need for raised cellular levels of cGMP.[24,25] In the first phase of our study, the guanylyl cyclase inhibitor ODQ did not reverse the inhibitory effect of sildenafil, suggesting that cGMP was not involved in the inhibitory effect of sildenafil on the myometrium in our study [Table 2]. BKCa channels are abundant in the human myometrium and are known to play a major role in myometrial contractility.[26,27] There is also evidence that the myometrium is different from other tissues in that BKCa channels in the myometrium can function independently of cGMP.[28] Alternatively, since cGMP can regulate BKCa channels,[29,30] it is possible that the raised cellular levels of cGMP due to inhibition of PDE5 by sildenafil could have opened the BKCa channels.

CONCLUSION

In conclusion, this study has shown that suitably low concentrations of sildenafil inhibit the contractility of the isolated non-pregnant human myometrium. The results suggest that the likely mechanism involved is the opening of BKCa channels. The results suggest that sildenafil has the potential to be used in clinical conditions like preterm labor requiring inhibition of myometrial contractility.

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Conflicts of interest

There are no conflicts of interest.

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