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Relaxant action of sildenafil citrate (Viagra) on human myometrium of pregnancy

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KEY WORDS
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Objective: The purpose of this study was to investigate the effect of the cyclic guanosine monophosphate phosphodiesterase 5-specific inhibitor, sildenafil citrate, on the contractions of isolated pregnant human myometrium.
Study design: Myometrial samples were obtained from women who underwent elective cesarean delivery. Myometrial contractions that were recorded in response to sildenafil in the absence and presence of the potassium channel blocker, tetraethylammonium or the guanylate cyclase inhibitor, methylene blue (10 μmol/L) were studied. One-way analysis of variance with post-hoc analysis was used to test differences among groups.
Results: Sildenafil caused relaxation of myometrium in a concentration-dependent manner. The $\log_{10}$ EC_{50} value for this relaxation in the presence of 20 mmol/L tetraethylammonium was significantly different ($P<.01$) than values that were obtained with sildenafil alone or sildenafil in the presence of either methylene blue or 5 and 10 mmol/L tetraethylammonium.
Conclusion: Myometrial relaxation that is evoked by the direct application of sildenafil occurs independently of cyclic guanosine monophosphate. Potassium channels appear to be the likely candidates in mediating this response.

The electrical quiescence of the gravid uterus during most of gestation facilitates fetal growth and maturation. A variety of signaling pathways and molecules that act to hold uterine contractility in abeyance throughout pregnancy until term have been studied. These include the nitric-oxide–guanosine 3′5-cyclic monophosphate-protein kinase G (NO-cGMP-PKG) cascade[1,2] and ion channels.[3] The human myometrium expresses a plethora of ion channels, the most abundant of which appears to be a large-conductance calcium-activated potassium channel (BK_{Ca}) that is pivotal in the control of uterine excitability, principally as a result of its direct association with intracellular calcium levels.[3,4] In isolated pregnant human myometrial cells, a further 3 distinct voltage-gated whole-cell conductances (I_{K1}, I_{K2}, and

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I_{KA}), have been described that exhibit differential sensitivity to the potassium channel blockers 4-aminopyridine and tetraethylammonium ions.[5] It has been suggested that these currents may play a role in determining cellular membrane potential and contribute to myometrial quiescence.

Preterm delivery remains the single leading cause of death and morbidity of neonates. Approximately 50% of all preterm deliveries are due to preterm labor.[6] It is clear that once initiated, preterm labor is difficult to arrest fully, although transient suppression by pharmacologic means is possible. β_2-mimetics remain the most popular choice in the treatment of preterm labor by tocolysis, despite little evidence to support improvement in key perinatal outcomes with this group of agents.

Although our knowledge regarding the mechanisms that underlie normal physiologic labor has advanced considerably, the emergence of new candidate drugs that lack the side-effect profiles of commonly used tocolytic agents to treat preterm labor has been slow. Within the last few years, sildenafil citrate (Viagra) has been used successfully for the treatment of penile erectile dysfunction.[7,8] Several alternative potential therapeutic applications of sildenafil have come to light. For example, successful pregnancies in previously failed attempts of in vitro fertilization have been attributed to the improved vasodilation that is caused by sildenafil citrate.[9,10] In addition, there may be a role for sildenafil in the treatment of female sexual dysfunction.[11] Sildenafil citrate promotes smooth muscle relaxation by preventing the degradation of the second messenger cGMP by phosphodiesterase, PDE5.[12,13] Moreover, BK_{Ca} channels have been implicated directly or indirectly in the actions of sildenafil,[14,15] NO,[16-18] and cGMP.[19] The relaxant action of cGMP in smooth muscle, through its downstream effector, the enzyme protein kinase G, results in reduced intracellular Ca^{2+} levels and a reduced sensitivity of the contractile elements to Ca^{2+}. In light of the interaction between sildenafil citrate, the NO-cGMP pathway and BK_{Ca} channels, the aim of the present study was first to elucidate whether sildenafil citrate is able to modulate uterine contractility and second to establish a role, if any, for the involvement of cGMP and/or potassium channels in the action of sildenafil in the term pregnant human myometrium.

Material and methods

In vitro contractility

Human myometrial samples were obtained from women who underwent elective cesarean delivery at term gestation (39-42 weeks), all of whom gave written informed consent. Ethical approval for this study was obtained from Southern Derbyshire Ethics Committee. Muscle biopsy specimens, which were procured from the upper midline of the lower uterine segment, were collected in Krebs-Henseleit solution (118 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L $CaCl_2$, 1.2 mmol/L KH_2PO_4, 25 mmol/L $NaHCO_3$, 11 mmol/L glucose, pH 7.4) and transported to the laboratory. After removal of decidual and serosal tissue, the biopsy specimen was cut with the muscle fibers that run longitudinally, to yield myometrial strips (approximately, $2 \times 2 \times 10$ mm). Tissue strips were mounted individually in a 4-chamber organ bath (Power Lab; A/D Instruments, Oxfordshire, UK) and attached to isometric force transducers, and 2*g* tension was applied. The chambers contained 10 mL of oxygenated (95% oxygen/5% carbon dioxide mix) Krebs-Henseleit solution and were maintained at 37°C for the duration of the experiment. Samples were stimulated with 0.5 nmol/L oxytocin, and the contractions were allowed to develop. After a maximal equilibration period of 2 hours, contractions were recorded in response to cumulative additions of sildenafil citrate in the concentration range of 10 nmol/L to 1 mmol/L every 30 minutes. For antagonism studies, after the equilibration period, myometrial strips were preincubated with either tetraethylammonium (TEA; 5, 10, or 20 mmol/L) or methylene blue (10 μmol) for 30 minutes before the cumulative application of sildenafil citrate. The potassium channel blocker TEA at low concentrations (<2 mmol/L) selectively blocks BK_{Ca} channels. Both the guanylate cyclase inhibitor (methylene blue) and TEA were made in Krebs-Henseleit solution as concentrated stock solutions and diluted as appropriate. Sildenafil citrate was dissolved directly in dimethyl sulfoxide (DMSO) to obtain a 1-mol/L stock solution and diluted to the appropriate concentration in Krebs-Henseleit solution. The maximum DMSO content did not exceed 0.2%. The effects of vehicle (DMSO) were tested by the preparation of test solutions with Krebs-Henseleit in place of sildenafil. The sildenafil citrate was provided by Pfizer (Sandwich, UK). All other chemicals were obtained from Sigma (Poole, Dorset, UK).

Data and statistical analysis

Contractile activity, which was assessed by the integration of the mechanical responses over a 25- minute period in the presence of sildenafil citrate, were compared with those that were obtained with or without TEA or methylene blue with Chart software (version 4.01; A/D Instruments). Results are expressed as percent relaxation relative to the control period and plotted against the sildenafil concentration. Concentration-effect curves were fitted to a 1-site model with variable slope with Prism software (version 3.0; GraphPad Software, Inc, San Diego, Calif) according to the following 4-parameter logis-

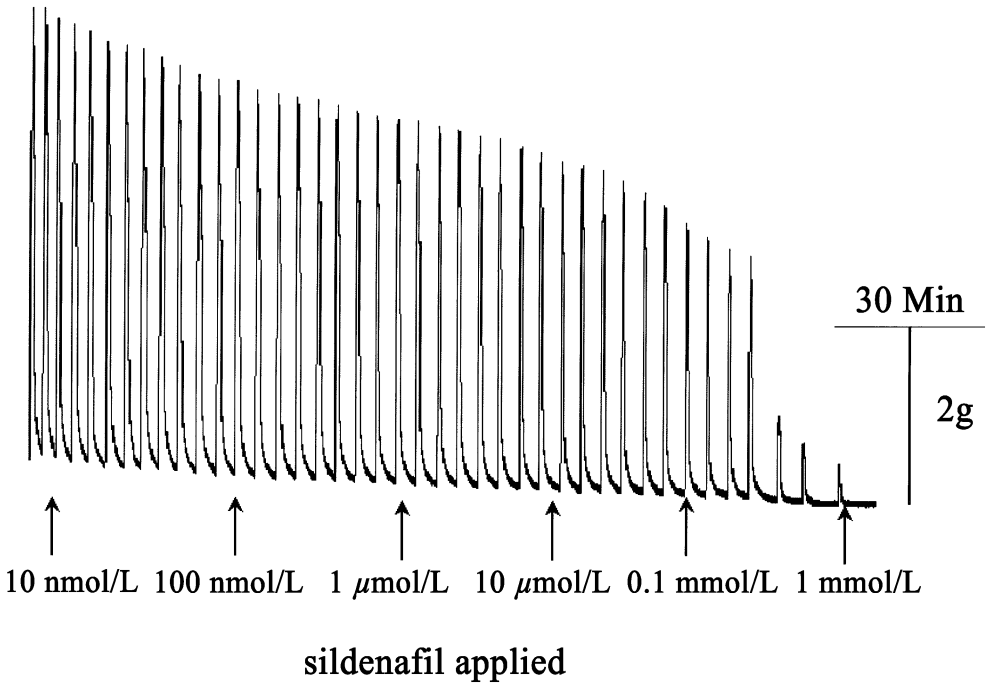

Figure 1 Oxytocin-induced contractions that were recorded from term pregnant human myometrium. Tissue strips were placed under 2*g* tension, and sildenafil citrate was applied cumulatively. A representative concentration-dependent relaxation to sildenafil is observed.

tic equation: $Y = Y_{max}/(1+10\hat{}[(\log_{10}EC_{50} - X)^{*}H])$, where Y is the percentage of myometrial relaxation, Y_{max} is the maximal relaxation achieved, $\log_{10}EC_{50}$ is the log of the sildenafil concentration that produces 50% myometrial relaxation, X is the sildenafil concentration, and H the Hill slope. The EC_{50} values for sildenafil in the presence and absence of blockers were then obtained from the best fit of the concentration-response curve to the pooled data. Results are expressed as mean ± SEM of the number of observations. Differences among groups were compared with the use of 1-way analysis of variance and post-hoc Bonferroni correction with Prism software. A probability value of $<.05$ was considered statistically significant.

Results

Sildenafil citrate produced relaxation of human myometrial strips in a concentration-dependent manner (Figure 1) compared with the activity in matched control strips (ie, oxytocin alone; n = 12 strips). This was observed as a reduction in contraction amplitude, although the frequency of contractions appeared to be unchanged. Maximal relaxation was achieved at the highest concentration of sildenafil that was applied (1 mmol/L; Fig 1), but full tissue relaxation was not always observed. The effect of vehicle that was applied at the corresponding percent concentrations with Krebs-Henseleit solution instead of sildenafil demonstrated that the relaxation was due to sildenafil alone and not DMSO (n = 11; Figure 2). Methylene blue (10 μmol/L) had no effect on myometrial contractility in the absence of sildenafil (n = 4 strips), nor did preincubation with 10 μmol/L methylene blue (n = 6 strips)

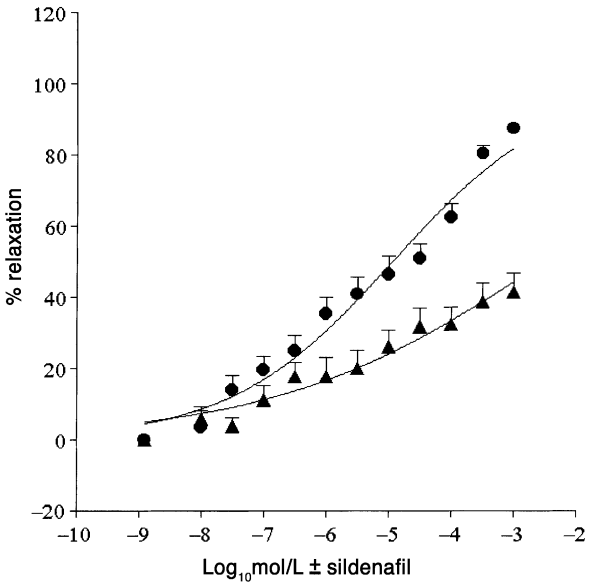

Figure 2 Graphic representation of the effects of DMSO alone compared with sildenafil that was prepared with DMSO as vehicle on oxytocin-induced myometrial contractions. The *closed circles* represent DMSO + sildenafil; the *closed triangles* represent DMSO + Krebs-Henseleit solution (for sildenafil).

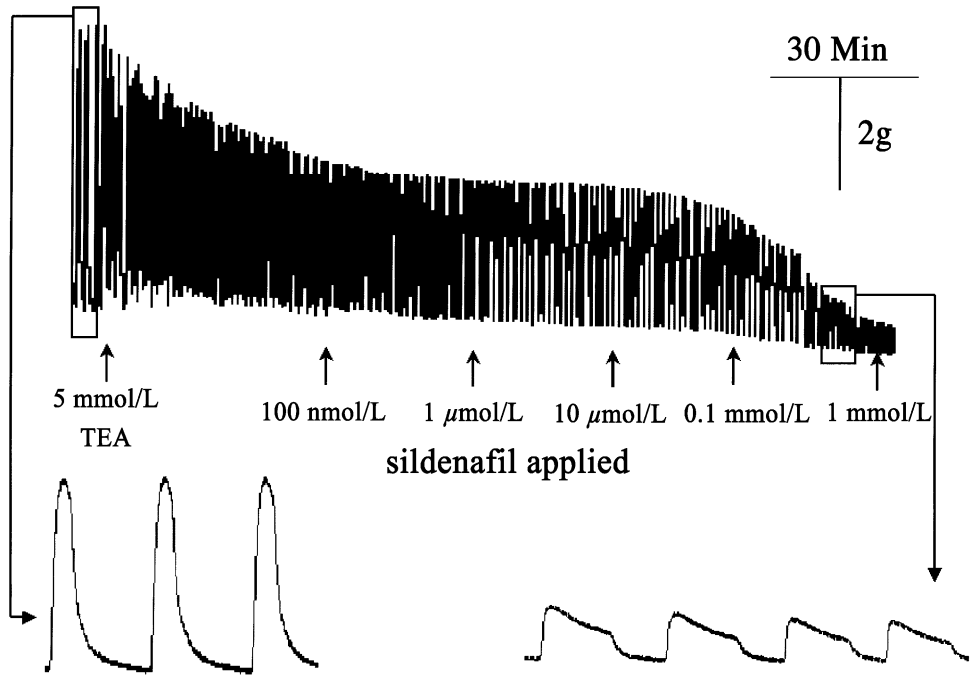

Figure 3 In the presence of 5 mmol/L TEA, myometrial contractions were spike-like and reduced in amplitude compared with the inset, which illustrates representative oxytocin-induced activity. The application of sildenafil resulted in a broadening of the contractions, which had a delayed return to the baseline.

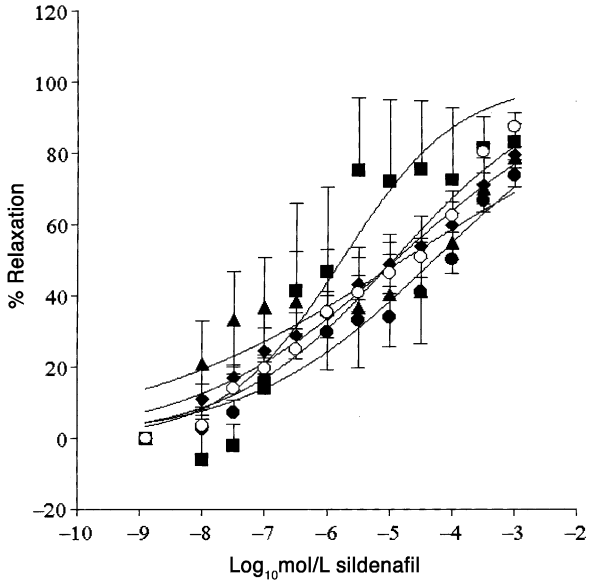

Figure 4 Concentration-response curves were generated in response to sildenafil alone (*open circles*), sildenafil + 10 μmol/L methylene blue (*closed diamonds*), and sildenafil + 5 mmol/L TEA (*closed octagons*), and sildenafil + 20 mmol/L TEA (*closed squares*). Data were best-fit to a 1-site receptor model. The *closed triangles* represent sildenafil + 10 mmol/L TEA.

inhibit the relaxation that was produced by sildenafil. In control experiments, the examination of the effects of TEA alone on oxytocin-induced contractions, when 5 mmol/L TEA (n = 6 strips) or 10 mmol/L TEA (n = 6 strips) was introduced to the tissue baths separately, showed an immediate decrease in contraction amplitude that was accompanied by an increased frequency of contractions that was noted with contractions that appeared spike-like (5 mmol/L TEA, Figure 3). These effects remained unchanged for the duration of the experiment (data not shown). This concentration of TEA is likely to have caused virtually complete blockade of BK_{Ca} channels.[4] In the presence of TEA, sildenafil application resulted in the prolongation of contractions and a reduction in amplitude (Figure 3). In 10 mmol/L and 20 mmol/L TEA, the broadening of the contractions increased still further. With 20 mmol/L TEA, although contractions were erratic and of varying amplitude, increasing concentrations of sildenafil appeared to stabilize tissue contractility. The complete inhibition of contractile activity was observed at lower sildenafil concentrations in high TEA concentrations. Complete concentration-response curves in the presence of TEA and methylene blue are shown in Figure 4. The mean $\log_{10}EC_{50}$ value for sildenafil-induced relaxation in 20 mmol/L TEA (−5.77) was significantly different ($P < .01$) compared with sildenafil alone (−4.94), sildenafil plus methylene blue (−4.91), sildenafil plus 5 mmol/L TEA (−4.29), and sildenafil plus 10 mmol/L TEA (−4.79; Table).

Comment

The main outcome of this study is that the direct application of sildenafil citrate relaxes term pregnant, nonla-

Table Summary of mean (± SEM) $log_{10}EC_{50}$ values for sildenafil-mediated myometrial relaxation in the absence and presence of pharmacological blockers

	Sildenafil	Sildenafil/TEA (5 mmol/L)	Sildenafil/TEA (10 mmol/L)	Sildenafil/TEA (20 mmol/L)	Sildenafil/methylene blue (10 μmol/L)
$Log_{10}EC_{50}$	−4.94 ± 0.08	−4.29 ± 0.29	−4.79 ± 0.38	−5.77 ± 0.25*	−4.91 ± 0.27
N	12	6	6	5	6

N, Number of samples.
* $P < .01$, when compared with sildenafil alone, 5 and 10 mmol/L TEA, and methylene blue groups.

boring human myometrium in an in vitro model of contractility. The millimolar concentrations that are required for sildenafil's maximal relaxant action indicates possible nonspecific effects on cell membranes or transport mechanisms. However, the nonspecific potassium channel blocker TEA at 20 mmol/L TEA resulted in an overall leftward shift in the concentration-response curve, which significantly altered the $log_{10}EC_{50}$ value of the sildenafil response. This finding indicates that channels other than myometrial BK_{Ca} channels contribute to this effect because the latter are more sensitive to blockade by lower extracellular TEA concentrations. We suggest that the blockade of BK_{Ca} channels by TEA actually unmasks TEA-insensitive currents that may be the target of sildenafil action. Three populations of voltage-gated potassium currents have been reported in pregnant human myometrium, but 70% of the current that is carried by both I_{K1} and I_{K2} is sensitive to 10 mmol/L TEA.[5] Although sildenafil may be causing relaxation, in part by selectively activating the remaining I_{K1} or I_{K2} current components, their sensitivity to TEA suggests that their contribution is likely to be meager. Although no calcium channel blockers were tested, blockade of underlying calcium channels could underlie the sildenafil effect. Further experiments will be designed to investigate the effects of sildenafil on spontaneous contractility because the potassium channel contribution to the former is likely to differ from that evoked by oxytocin.

Our observation that the guanylate cyclase inhibitor, methylene blue, failed to inhibit the sildenafil-induced relaxation argues against a role for cGMP in mediating this effect. This finding is at odds with the reported blockade by methylene blue of the inhibition by sildenafil of oxytocin and acetylcholine-contractions in rat myometrium.[20] The latter observation is strongly suggestive of a role for cGMP in mediating the relaxation because of sildenafil but may also be explained by the longer incubation times with sildenafil before the addition of test agents. The present study differs in that only direct effects of sildenafil were examined.

Control of smooth muscle excitability occurs by second-messenger–mediated pathways that involve cyclic adenosine monophosphate and cGMP. Because sildenafil's potent clinical application in the treatment of impotence is mediated through the NO-cGMP cascade,[12] this pathway is the probable target of sildenafil action in other organ systems. The existence of a NO-cGMP-PKG pathway is well-documented in pregnant human[2,21] and rat[22] myometrium. It has been postulated that the NO-cGMP pathway signaling pathway underlies the uterine quiescence that predominates during pregnancy and that down-regulation of this pathway at term promotes myometrial contractility.[2,22] However considerable controversy exists with respect to the effects of cGMP in relaxing pregnant myometrium.[23,24] Specifically, the apparent role of cGMP in bringing about myometrial quiescence is refuted by several lines of evidence that demonstrate that pregnancy is associated with reduced levels of PKG compared with the nonpregnant state,[23,25] decreased PKG enzyme activity,[23] refractoriness to nonhydrolyzable analogs of cGMP, and cGMP-specific phosphodiesterase inhibitors.[25] Clearly, the role of cGMP in myometrium requires clarification. Interestingly, Zhou et al,[19] on the basis of electrophysiologic data, have proposed that membrane-bound PKG promotes BK_{Ca} channel activity in the pregnant nonlaboring human myometrium and that this mechanism may underlie the maintenance of gestational quiescence.

Although cGMP is unlikely to play a role in the actions of sildenafil that are reported herein, there is emerging evidence that sildenafil is able to act through cGMP-dependent and independent mechanisms that involve ion channels. For example, vasodilation of fetal rabbit ductus arteriosus occurs through the sildenafil-induced cGMP-mediated activation of BK_{Ca} channels.[26] Direct effects of sildenafil at prejunctional BK_{Ca} channels have been reported in human vas deferens.[14] Concern has also been expressed that sildenafil, albeit at much higher concentrations than those used therapeutically, prolongs the repolarization of the cardiac action potential by acting on the rapid component of the delayed rectifier (HERG) current with potentially serious cardiovascular implications.[27] The prolongation of the downstroke of myometrial contractions by sildenafil in the presence of TEA, as observed in our study, is consistent with the latter blocking potassium currents that would tend to repolarize the cell.

From the present study, we suggest that TEA-resistant potassium channels are the likely candidates in the generation of the sildenafil-evoked relaxation. The observation that higher concentrations (10 and 20 mmol/L) of TEA altered the waveform of the sildenafil response, thereby unmasking potential contributions from voltage-gated potassium conductances by TEA, suggests novel mechanisms of sildenafil action. Because sildenafil's therapeutic application is based directly on the NO-cGMP pathway, the fact that this pathway is apparently down-regulated at term gestation in the human myometrium implies that sildenafil is probably not acting through this route. Moreover, it is not known whether preterm labor is itself a premature manifestation of the normal signaling axes that are operative during labor. If so, it might be expected that a down-regulation of this pathway may have taken place already in preterm labor and therefore may be of little therapeutic benefit as a target of sildenafil action.

The present study demonstrates that sildenafil citrate, at relatively high concentrations, is able to relax pregnant human myometrium. This raises the possibility that sildenafil or related molecules may have future potential tocolytic application. The vaginal application of sildenafil appears to be effective and certainly would limit concern regarding the systemic effects on the maternofetal unit.[9] In view of our findings, a detailed understanding of the mechanisms that are involved in the sildenafil-mediated relaxation of pregnant human myometrium would establish whether they have tocolytic potential.

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