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Sildenafil inhibits agonist-evoked rat uterine contractility: influence of guanylyl cyclase inhibition

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Abstract

Sildenafil shows a potent relaxant effect on corpus cavernosum smooth muscles by prolonging cyclic guanosine monophosphate (cGMP) actions. We investigated whether this inhibitory effect of sildenafil was also displayed on the uterine musculature. Isolated uteri of non-pregnant rats were used to measure the possible sildenafil-induced inhibition of contractions evoked by various oxytocic agents, viz., prostaglandin E_2, oxytocin and acetylcholine. The relation of these effects to sildenafil action on cGMP was also examined, using methylene blue as a guanylyl cyclase inhibitor. Sildenafil (30 and 100 nM) was found to shift to the right the non-cumulative concentration–response curves of the test agonists in a concentration-dependent manner. The shift was accompanied by a reduction in the maximal response of the tissue to all uterine stimulants selected. Sildenafil also elicited a marked concentration-dependent increase in EC_{25} of prostaglandin E_2, oxytocin and acetylcholine, as compared to their respective control values. Preincubation of the uterine strip with methylene blue (10 μM) reduced the inhibitory effects of sildenafil on oxytocin- and acetylcholine-evoked contractions, at submaximal concentrations of each agonist. The results suggest that sildenafil inhibits the uterotonic potentials of various oxytocic agents and that this effect could be probably related to the drug's action on cGMP.

Keywords: Sildenafil; Prostaglandin E_2; Oxytocin; Acetylcholine; Guanylyl cyclase; Methylene blue; Uterus; (Rat)

1. Introduction

Uterine quiescence is of utmost need in many obstetric problems (Lopez Bernal and TambyRaja, 2000) such as in spontaneous preterm labor which occurs with a high incidence, reaching 5–10% of all pregnancies and accounting for 75% of neonatal mortality and morbidity, including long-term handicap (Challis, 2000). Increased uterine contractility and distension are major symptoms in such obstetric problems (Hagberg and Wennerholm, 2000). These symptoms result from various underlying causes, including enhanced prostaglandins production with induction of oxytocic receptors (Saji et al., 2000). Although many of these problems are often impossible to prevent, early tocolytic therapy can help (Hagberg and Wennerholm, 2000) since during labor the smooth muscle transits from a state of relaxation to one of contraction, due to the release of oxytocic agents (Grammatopoulos and Hillhouse, 1999). The cyclic guanosine monophosphate (cGMP) system may help to maintain pregnancy, as increased cellular cGMP prevents uterine contraction during exposure to stimulating agents (Izumi and Garfield, 1995). Accordingly, agents capable of enhancing the cGMP level could be beneficial for reducing uterine susceptibility to endogenously released oxytocic substances, including prostaglandins, oxytocin and acetylcholine. The cGMP level is determined by a balance between activation of guanylyl cyclase, which catalyses cGMP formation from guanosine triphosphate (GTP), and cyclic nucleotide phosphodiesterase-5, which drives the breakdown of nitric oxide-stimulated cGMP (Corbin and Francis, 1999). Sildenafil (Viagra™)—which has been used as medication in male impotence (Boolell et al., 1996) and has attracted widespread attention—is a potent reversible inhibitor of the cGMP-specific phosphodiesterase, phosphodiesterase-5 (Boolell et al., 1996). Phosphodiesterase-5 is an important modulator of smooth muscle relaxation (Cartledge and Eardley, 1999). Consequently, sildenafil has been proved to increase smooth muscle relaxation in corpus cavernosum arterioles (Boolell et al., 1996) and thus could be a good candidate to test for

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its ability to produce similar inhibitory effects on the uterine musculature to produce quiescence and reduce contraction evoked by oxytocic agents.

The objective of the present investigation was to examine the effects of sildenafil on rat uterine contractions induced by various oxytocic agents, namely prostaglandin E_2, oxytocin and acetylcholine. Concentration–response curves for the test agonists were made in the absence and presence of sildenafil (30 or 100 nM). The influence of inhibition of cGMP synthesis by methylene blue, a potent guanylyl cyclase inhibitor, on the effects of sildenafil was also studied in order to relate the observed effects of the drug on agonist-evoked uterine contractions to its action on the cGMP system. Hence, the effects of sildenafil on chosen submaximal concentrations of oxytocin and acetylcholine were studied in uterine strips with or without pretreatment with methylene blue.

2. Materials and methods

2.1. Animals

Virgin female Wistar albino rats aged 4–5 months (120–150 g each) were used. They were purchased from the National Research Center, Giza, Egypt. The animals received a standard pellet diet and were allowed free access to water.

2.2. Source and preparation of uterine tissue

Contractile studies were performed on full-thickness uterine tissue obtained from non-pregnant rats. The animals received estradiol in olive oil, injected subcutaneously in the back of the neck at a dose of 0.15 mg/kg, 24 h before experimentation. The hormonal state was checked by examining the vaginal smears, and the animals were in the estrous phase. The animals were killed by cervical dislocation and the uterine horns were removed. The horns were then opened by sharp dissection and each horn strip was mounted into a thermostatically controlled organ bath of 20 ml capacity containing De Jalon solution consisting of the following (mM): NaCl 154, KCl 5.63, glucose 2.77, $NaHCO_3$ 5.95 and $CaCl_2$ 0.27. The uterine tissue was attached to an isotonic transducer (T3, Bioscience, UK) connected to a strain gauge coupler (FC117, Bioscience, UK) placed in a physiogragh (Washington 400MD2C, Bioscience, UK). The uterine strips were maintained at 30 °C, aerated with carbogen (95% O_2, 5% CO_2), under a 0.5 g load and equilibrated for 30 min.

2.3. Effects of sildenafil on agonist concentration–response relationships

This experiment was performed to study the effects of sildenafil on uterine contractions induced by certain agents that all share a property of being endogenously released oxytocic substances. Non-cumulative concentration–response curves to prostaglandin E_2, oxytocin and acetylcholine were made in a manner which allowed each tissue strip to serve as its own control. Each agonist was kept in contact with the preparation for 30 s, followed by washing twice during a 6-min period.

To study the effects of sildenafil, the uterine strips were then incubated with sildenafil (30 or 100 nM) for 15 min (Frith and Gibson, 2000; Vemulapalli and Kurowski, 2000), after which the non-cumulative concentration–response curves to prostaglandin E_2, oxytocin and acetylcholine were repeated. Each uterine tissue was used for only one concentration of sildenafil because the effect of sildenafil was long lasting and persisted for up to one hour even after several washings with fresh De Jalon solution.

The responses were quantified by measuring the peak height of uterine contraction and the results are given as percentages of the maximal control agonist response.

2.4. Effects of guanylyl cyclase inhibition on sildenafil activity

This experiment was carried out to study whether the observed inhibitory effects of sildenafil on agonist-evoked uterine contractility were related to the drug action on

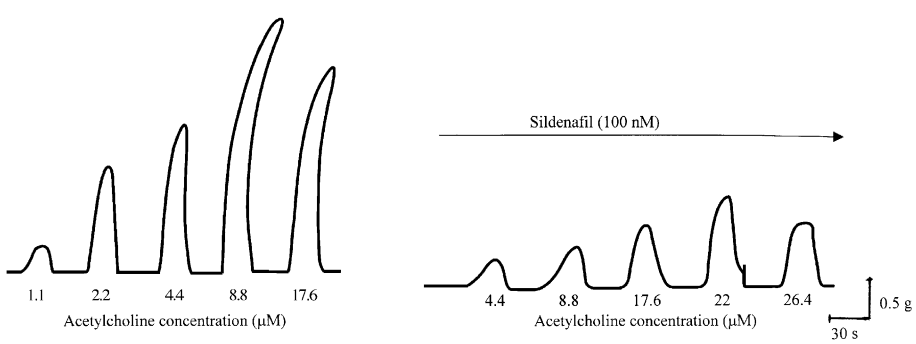

Fig. 1. Effects of different concentrations of acetylcholine on uterine tissue of non-pregnant rats in the absence and presence of 100 nM sildenafil. The contraction tracings are representative of results from experiments summarized in Fig. 4B.

cGMP. Hence, the effects of sildenafil were tested in the absence and presence of a guanylyl cyclase inhibitor viz., methylene blue, which inhibits cGMP production.

Control responses to submaximal concentrations (about 50% of maximal responses) of oxytocin (3 nM) and acetylcholine (4 μM) were first obtained, and the tissues were then exposed twice to either agonist before proceeding with the evaluation of the drug effect. The uterine strips were then pretreated with methylene blue (10 μM) (Kuenzli et al., 1996) for 15 min, followed by another 15-min incubation with sildenafil (30 or 100 nM) in the presence of methylene blue. The tissues were then re-challenged with the same submaximal concentrations of either agonist, added twice. Using other uterine strips, similar experiments were performed in the same manner except that the tissues were incubated with sildenafil without pretreatment with methylene blue. The data are expressed as percentages of the control agonist response. The influence of methylene blue on the sildenafil effect, on agonist-induced contractions, was evaluated by comparing the drug's effect in absence and presence of methylene blue.

2.5. Data analysis

The agonist concentration–response curves obtained in the absence and presence of sildenafil were analyzed by regression line analysis, then the effective concentrations

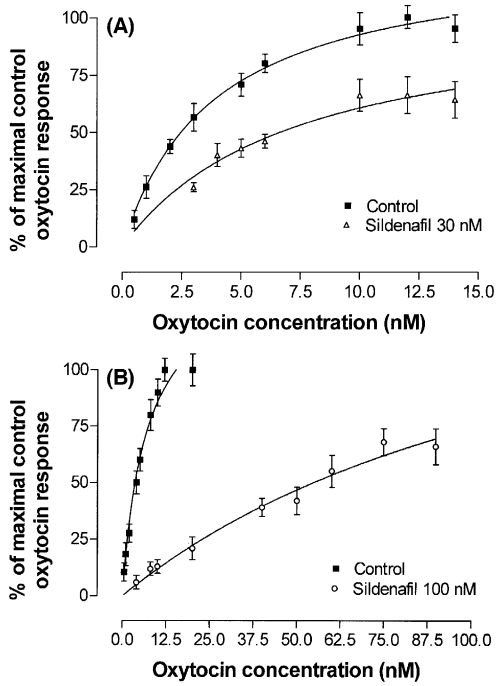

Fig. 3. Concentration–response relationship for oxytocin in uterine tissue of non-pregnant rats in the absence (control) and presence of sildenafil 30 nM (A) and 100 nM (B). EC_{25} was calculated to assess the sildenafil-induced inhibition of contraction evoked by oxytocin. Each point represents the mean of four experiments with mean S.D. shown by a vertical bar.

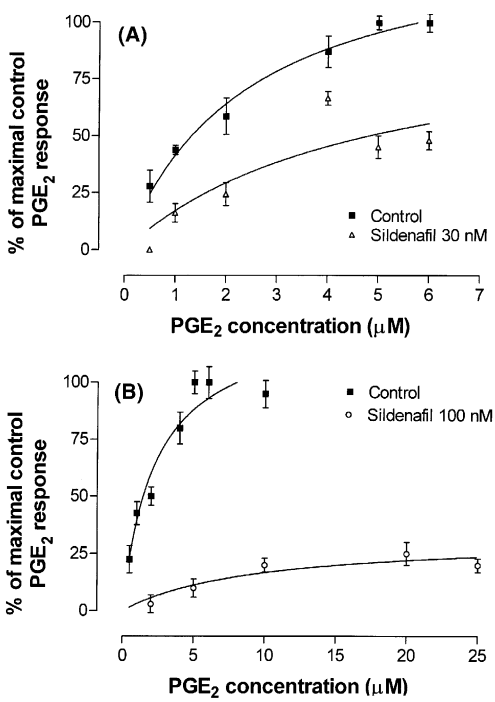

Fig. 2. Concentration–response relationship for prostaglandin E_2 in uterine tissue of non-pregnant rats in the absence (control) and presence of sildenafil 30 nM (A) and 100 nM (B). EC_{25} was calculated to assess the sildenafil-induced inhibition of contraction evoked by prostaglandin E_2. Each point represents the mean of four experiments with mean S.D. shown by a vertical bar.

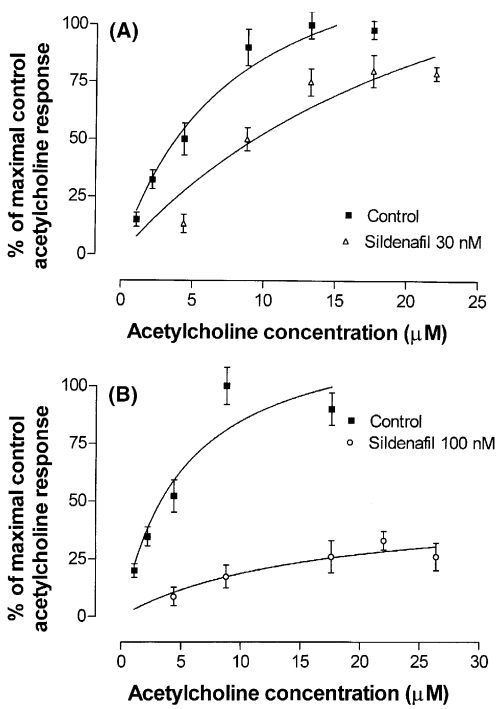

Fig. 4. Concentration–response relationship for acetylcholine in uterine tissue of non-pregnant rats in the absence (control) and presence of sildenafil 30 nM (A) and 100 nM (B). EC_{25} was calculated to assess the sildenafil-induced inhibition of contraction evoked by acetylcholine. Each point represents the mean of four experiments with mean S.D. shown by a vertical bar.

25 (EC_{25}) were statistically evaluated by Student's *t*-test for paired data. The influence of methylene blue on sildenafil effects was determined by Student's *t*-test for unpaired data. A probability level of less than 0.05 was accepted as being significant. The results were given as means of four experiments ± one standard deviation of the mean. GraphPad Software InStat (version 2) was used to carry out the statistical analysis.

2.6. Drugs and chemicals

The following drugs and chemicals were used: sildenafil (Pfizer, USA), prostaglandin E_2, acetylcholine bromide and methylene blue (Sigma, USA) and oxytocin (Sandoz, Switzerland). All other chemicals were of analytical grade.

3. Results

3.1. Effects of sildenafil on agonist concentration–response relationships

Addition of uterine stimulants namely, prostaglandin E_2, oxytocin and acetylcholine elicited immediate tissue contractions followed by a spontaneous fall and return to baseline even without washout of the agonists.

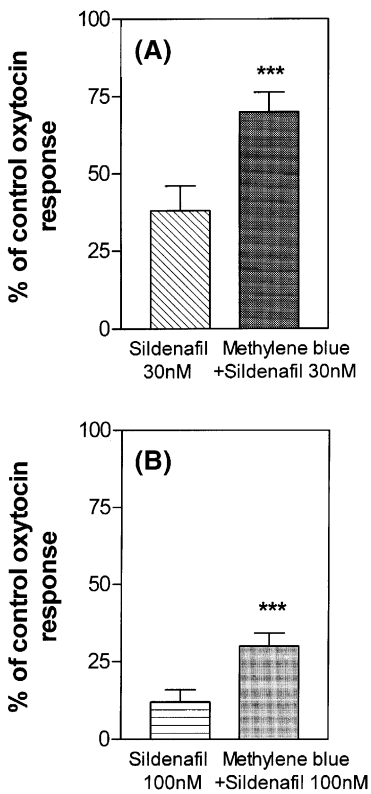

Fig. 5. Effects of sildenafil 30 nM (A) and 100 nM (B) on oxytocin (3 nM)-evoked contractions in uterine tissue of non-pregnant rats with or without preincubation with methylene blue (10 μM). Each column represents the mean of four experiments with mean S.D. shown by a vertical bar. *** Significantly different from sildenafil group at $P < 0.001$.

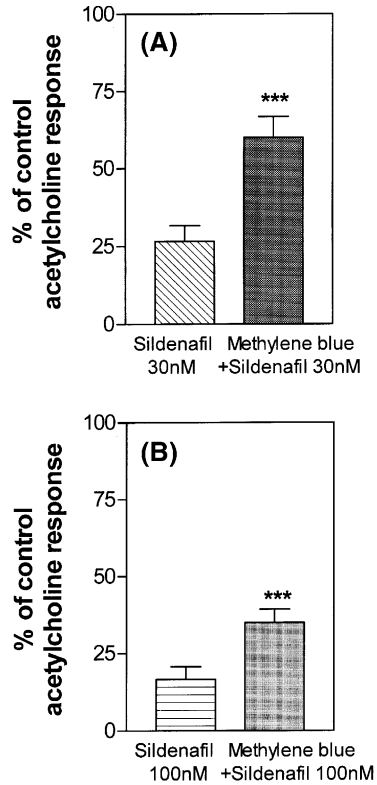

Fig. 6. Effects of sildenafil 30 nM (A) and 100 nM (B) on acetylcholine (4 μM)-evoked contractions in uterine tissue of non-pregnant rats with or without preincubation with methylene blue (10 μM). Each column represents the mean of four experiments with mean S.D. shown by a vertical bar. *** Significantly different from sildenafil group at $P < 0.001$.

Incubation of uterine tissues with sildenafil, 30 or 100 nM, did not produce any reduction in the baseline, but produced a shift to the right of the concentration–response curves for the different stimulants. Fig. 1 shows representative responses of uterine tissue to different concentrations of acetylcholine in the absence and presence of sildenafil. The EC_{25} of the test agonists were significantly increased by sildenafil, in a concentration-dependent manner. Sildenafil, 30 nM, produced an increase in the EC_{25} of prostaglandin E_2 from 0.45 ± 0.2 μM (mean ± S.D.) to 1.35 ± 0.4 μM (Fig. 2A), while that of oxytocin was from 1.43 ± 0.5 to 2.41 ± 0.3 nM (Fig. 3A), and the EC_{25} of acetylcholine was from 1.60 ± 0.3 to 4.7 ± 0.3 μM (Fig. 4A). Sildenafil at 100 nM provoked a more pronounced elevation of the EC_{25} values for all test agonists; the EC_{25} of prostaglandin E_2 was increased from 0.56 ± 0.3 to 18.41 ± 5.1 μM (Fig. 2B), that of oxytocin from 1.80 ± 0.5 to 24.5 ± 5.2 nM (Fig. 3B) and that of acetylcholine from 1.45 ± 0.6 to 16.18 ± 5.1 μM (Fig. 4B). The shift was accompanied by a reduction in the maximal responses of the tissue to the test oxytocic agents.

3.2. Effects of guanylyl cyclase inhibition on sildenafil activity

Preincubation of the uterine tissue with methylene blue at 10 μM reduced the inhibitory effects of sildenafil (30 and 100 nM) on oxytocin-(Fig. 5) and acetylcholine-(Fig. 6) evoked uterine contractions. In the presence of methylene blue and sildenafil the agonist-induced contractility was increased about onefold, as compared to the effects of sildenafil alone.

4. Discussion

The introduction of sildenafil, in the middle of the 1990s, has tremendously changed the treatment of male sexual dysfunction due to its effects on smooth muscle. There have been limited investigations of sildenafil effects in women, and it has recently been prescribed for patients with antidepressant-induced sexual dysfunction (Nurnberg et al., 1999). In a preliminary report, the use of vaginal sildenafil has been demonstrated to improve uterine artery blood flow in patients undergoing *in vitro* fertilization (Sher and Fisch, 2000). The present study focused on the possible role of sildenafil in uterine reactivity towards oxytocic agents.

The results of this investigation make it not unreasonable to assume that sildenafil produces uterine quiescence as it blunted the contractility elicited by uterotonic agents in the isolated uterus of non-pregnant rats. Incubation of the uterine strips with sildenafil, at the concentrations selected viz., 30 and 100 nM, produced a right shift of the concentration–response curves for prostaglandin E_2, oxytocin and acetylcholine, in a concentration-related manner. Sildenafil also reduced the maximal responses of the tissue to different stimulant agonists, concomittantly with a concentration-dependent increase in the EC_{25} of these agonists as compared to the control values.

Apart from its well defined relaxant effect on penile corpus cavernosum (Boolell et al., 1996), later studies offered evidence consistent with the accepted view that sildenafil produces enhancement of relaxation and inhibition of contractility of smooth muscle. Incubation with sildenafil (30–100 nM or 0.1–30 μM) augments the electrical field stimulation-induced relaxation of isolated rabbit clitoral corpus cavernosum (Vemulapalli and Kurowski, 2000) and inhibits electrically-evoked contractions in ring segments of human vas deferens (Medina et al., 2000b). It does not, however, affect the contractions induced by noradrenaline (Medina et al., 2000b). It also produces direct relaxation of carbachol-induced tone and increases the amplitude and duration of nitrergic relaxations in anococcygeus muscles in mice of either sex, when used at concentrations of 10–300 nM (Frith and Gibson, 2000). Inhibition of gastrointestinal motility (Bortolotti et al., 2001), reduction of contractile activity of esophageal musculature of patients with achalasia, and suppression of esophageal sphincter tone and residual pressure as well as contraction amplitude (Bortolotti et al., 2000) were also reported for sildenafil. Similarly, the drug (10^{-8}–3×10^{-5} M) causes concentration-dependent relaxation in the internal mammary arteries, radial arteries, and forearm veins, while it has a modest relaxant effect in the coronary arteries (Medina et al., 2000a). In addition, sildenafil amplifies the relaxation induced by the nitric oxide donor, sodium nitroprusside, in different blood vessels (Medina et al., 2000a,c) and in urogenital smooth muscles in male and female mice, while it does not affect the relaxant effects of papaverine (Frith and Gibson, 2000).

Data from the present study also revealed that the observed suppressive effects of sildenafil on uterotonic potentials of the test agonists might be related to enhancement of cGMP-mediated relaxation. It has been demonstrated that methylene blue inhibition of cGMP production by a block of guanylyl cyclase, which catalyzes the formation of cGMP from GTP (Kuenzli et al., 1996), led to suppression of sildenafil inhibitory effects on agonist-evoked uterine contractions.

It has been documented that the mechanism of action of sildenafil is via selective inhibition of phosphodiesterase-5 (Boolell et al., 1996; Wallis, 1999), an action that probably relates to the effect of sildenafil on the uterine musculature. Results of studies on rat uterus suggest that it is a nitric oxide-producing organ (Yallampalli et al., 1994). Nitric oxide drives cGMP formation through a nitric oxide-guanylyl cyclase relation pathway that exists in uterine smooth muscle, leading to a relaxant effect (Yallampalli et al., 1994). Termination of the smooth muscle relaxation is then accomplished by hydrolysis of the second messenger by phosphodiesterases (Andersson and Wagner, 1995). Phosphodiesterase-5, the target of sildenafil, is particularly abundant in smooth muscles enriched with other components of the cGMP signaling cascade (Saji et al., 2000) including the genitourinary tract. Therefore, agents that increase cellular cGMP might probably lead to a reduced smooth muscle tone and inhibited responsiveness to stimulant agonists. By blocking phosphodiesterase-5 that destroys nitric oxide-stimulated cGMP, sildenafil prevents the degradation of cGMP (Boolell et al., 1996), and thus through the addition of prostaglandin E_2, oxytocin and acetylcholine, the accumulated cellular cGMP attenuates uterine contractions, resulting in antagonism and suppression of the oxytocic effects of the test agonists. This assumption finds support from a previous report demonstrating that, during exposure to stimulating agents, including oxytocin and carbachol, the increased cellular cGMP prevents uterine contraction (Izumi and Garfield, 1995). On the other hand, at odds with the mode of action of sildenafil, results of a previous study showed that in human vas deferens, the inhibition induced by sildenafil was not modified by the guanylyl cyclase inhibitor, 1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxaline-1-one (1–30 μM), but

was abolished by the potassium channel blockers, tetraethylammonium, iberiotoxin and charybdotoxin (Medina et al., 2000b).

It could be concluded that sildenafil might prove effective for uterine maladies that involve increased susceptibility of smooth muscle to uterotonic agents, and that the mechanisms underlying this effect could involve the drug's action on the cGMP system. The present investigation might provide the groundwork for future studies to define both the effects of sildenafil in pregnant rat uterus and the use of sildenafil or other phosphodiesterase-specific inhibitors for modulation of uterine smooth muscle tone in female genital dysfunction.

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