Sildenafil (Viagra) induces powerful cardioprotective effect via opening of mitochondrial K\textsubscript{ATP} channels in rabbits

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Ockaili, Ramzi, Fadi Salloum, John Hawkins, and Rakesh C. Kukreja. Sildenafil (Viagra) induces powerful cardioprotective effect via opening of mitochondrial K\textsubscript{ATP} channels in rabbits. \textit{Am J Physiol Heart Circ Physiol} 283: H1263–H1269, 2002; 10.1152/ajpheart.00324.2002.—Sildenafil citrate (Viagra) is the pharmacological agent used to treat erectile dysfunction in men. Because this drug has a vasodilatory effect, we hypothesized that such an action may induce a preconditioning-like cardioprotective effect via opening of mitochondrial ATP-sensitive K (K\textsubscript{ATP}) channels. Rabbits were treated with sildenafil citrate (0.7 mg/kg iv) either 30 min (acute phase) or 24 h (delayed phase) before 30 min of ischemia and 3 h of reperfusion. Mitochondrial K\textsubscript{ATP} channel blocker 5-hydroxydecanoate (5-HD, 5 mg/kg iv) was given 10 min before ischemia-reperfusion. Infarct size was measured by tetrazolium staining. Sildenafil caused reduction in arterial blood pressure within 2 min of treatment, which returned to nearly baseline levels 3 min later. The infarct size (% risk area, means ± SE) reduced from 33.8 ± 1.7 in control rabbits to 10.8 ± 0.9 during the acute phase (68% reduction, \(P < 0.05\)) and 19.9 ± 2.0 during the delayed phase (41% reduction, \(P < 0.05\)). 5-HD abolished protection with an increase in infarct size to 35.6 ± 0.4% and 36.8 ± 1.6% during the acute and delayed phase, respectively (\(P < 0.05\)). Similar acute and delayed cardioprotective effects were observed when sildenafil was administered orally. Systemic hemodynamics also decreased after oral administration of the drug. However, these changes were mild and occurred slowly. For the first time, we demonstrate that sildenafil induces both acute and delayed protection against ischemia-reperfusion injury in vivo, which are mediated by opening of mitochondrial K\textsubscript{ATP} channels.

ATP-sensitive potassium channel; ischemia-reperfusion; phosphodiesterase-5

SILDENAFIL CITRATE (Viagra) is the first oral agent approved for treatment of erectile dysfunction in men (5, 11). It is a selective inhibitor of phosphodiesterase-5 (PDE-5), an enzyme that catalyzes the breakdown of a potent smooth muscle relaxing agent cGMP. Sildenafil has been shown to enhance nitric oxide (NO)-driven cGMP accumulation in the corpus cavernosum of rabbits without affecting cAMP formation. In the absence of NO drive, sildenafil had no functional effect on the human and rabbit isolated corpus cavernosum but potentiated the relaxant effects of NO on these tissues (20). Also, it has been shown that sildenafil causes mild to moderate decreases in systolic and diastolic pressure because of the inhibition of PDE-5 in smooth muscles in the vascular bed (12). In the present studies, we hypothesized that such a mild vasodilatory effect of sildenafil in the vasculature could potentially release agents such as adenosine, bradykinin, or NO, which may trigger a preconditioning-like effect in the heart. Because opening of the mitochondrial ATP-sensitive K (mitoK\textsubscript{ATP}) channel mediates the cardioprotective effect of preconditioning induced by adenosine (1, 2, 4, 6) or sublethal ischemia (3), we further hypothesized that these channels could potentially be involved in the cardioprotective effect of sildenafil. Accordingly, the goals of the present study were the following: 1) to show that the sildenafil induces both acute and delayed protection against ischemia-reperfusion injury in vivo, and 2) to demonstrate whether the protective effect of this drug is blocked by 5-hydroxydecanoate (5-HD), a selective blocker of the mitoK\textsubscript{ATP} channel (14). By using our in situ rabbit model of myocardial infarction, for the first time, we demonstrate that sildenafil induce both acute and delayed cardioprotective effects, which are dependent on the opening of mitoK\textsubscript{ATP} channel.

MATERIALS AND METHODS

Animals. Male New Zealand White rabbits (2.8–3.3 kg) were used for the studies. The care and use of the animals...
were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of Virginia Commonwealth University and the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (DHHS Publication No. (NIH) 80-23; Office of Science and Health Reports, Bethesda, MD 20205).

Surgical procedure: infarction protocol. The rabbits were anesthetized with an intramuscular injection of ketamine HCl (35 mg/kg) and xylazine (5 mg/kg). Subsequent doses of ketamine-xylazine (10 and 2 mg/kg, respectively) were administered during the experiment as needed to maintain surgical anesthesia. Atropine was administered along with the anesthetic to keep the heart rate elevated especially during the surgery protocol. The body temperature was monitored and maintained at 38°C throughout the experimental protocol. The neck was opened with a ventral midline incision and a tracheotomy was performed followed by intubation. The animal was then mechanically ventilated on a positive-pressure ventilator using compressed room air at 30–35 cycles/min with a tidal volume of ~15 ml. Ventilator setting and PO2 were adjusted as needed to maintain the arterial blood gas parameters within the physiological range. The blood gases and pH were measured 12 times for all the groups during the infarction protocol. The arterial blood gases and pH values ranged between 7.20 and 7.50 with PCO2 maintained between 20 and 50 mmHg and the HCO3 level ranging between 15.0 and 28.0 mg/dL. The PO2 ranged between 60 and 150 mmHg with the O2 saturation constantly kept above 90%. The jugular vein was cannulated with a polyethylene (PE) catheter for continuous infusion of 0.9% saline solution. The carotid artery likewise was dissected and cannulated with a PE catheter for blood sampling and continuous arterial pressure monitoring. Electrocardiographic leads were attached to subcutaneous electrodes to monitor either limb lead II or lead III.

After stabilization of the hemodynamics, a left thoracotomy was performed through the fourth intercostal space, and the pericardium was opened to expose the heart. A 5-0 silk suture with an atraumatic needle was then passed around the left anterior descending (LAD) artery midway between the atroventricular groove and the apex. The ends of the tie were then threaded through a small vinyl tube to form a snare. To induce infarction, the LAD artery was occluded for 30 min by pulling the snare and then fixing it in place by clamping the vinyl tube with a hemostat. A bolus of heparin sodium (500 IU) was given immediately before coronary occlusion for prophylaxis against thrombus formation around the snare. Myocardial ischemia was confirmed visually in situ by regional cyanosis, ST elevation and depression, or T wave inversion on the electrocardiogram, hypokinetic and dyskinetic movement of the myocardium, and relative hypotension. After 30 min of ischemia, the snare was released, and the heart was allowed to reperfuse for 180 min. This was readily confirmed by hyperemia over the surface of the previously ischemic-cyanogetic segment. The thoracic cavity was covered with the saline-soaked gauze to prevent the heart from drying.

Measurement of infarct size. After completion of the ischemia-reperfusion protocol, 500 units of heparin were injected, and the heart was removed quickly and mounted on a Langendorff apparatus. The coronary arteries were perfused with 0.9% saline containing 2.5 mM CaCl2. After the blood was washed out, the ligation around the coronary artery was retightened, and ~2 ml of 10% Evan’s blue dye were injected as a bolus into the aorta until most of the heart turned blue. The hearts were then perfused with saline to wash out the excess Evans blue, removed from the Langendorff apparatus, frozen, and cut into four to six transverse slices from apex to base of equal thickness (~1 mm). The slices were then incubated in 1% 2,3,5-triphenyltetrazolium chloride (TTC) solution in isotonic pH 7.4 phosphate buffer at 37°C for 20 min. TTC reacts with NADH in the presence of dehydrogenase enzymes causing the viable cells to stain with a deep red color. Red-stained viable tissue was easily distinguished from the infarcted gray or white unstained necrotic tissue. The slices were subsequently fixed in 10% formalin solution. The area at risk was determined by negative staining with Evan’s blue. The areas of infarcted tissue, the risk zone, and the whole left ventricle were measured by computer morphometry using a Bioquant imaging software (BIO98). Infarct size was expressed as a percentage of the ischemic risk area.

Measurement of hemodynamics. Hemodynamic measurements included heart rate, mean arterial pressure, and systemic and diastolic blood pressure. Rate-pressure product was calculated as the product of heart rate and peak arterial pressure.

Study protocol. All animals were subjected to an infarction protocol consisting of 30 min of sustained ischemia by occlusion of the coronary artery followed by 180 min of reperfusion. The effect of sildenafil was studied in the absence or presence of 5-HD in two phases; i.e., the acute and delayed phases. In the acute phase, myocardial infarction protocol was carried out 30 min after treatment with sildenafil. In the delayed phase, the ischemia-reperfusion protocol was carried out 24 h later. The rabbits were randomly assigned into one of the following groups. In group I (saline control, n = 10), rabbits received 0.9% saline. In group II (sildenafil, acute phase, n = 6), Viagra tablets were crushed and 0.7 mg/kg sildenafil was dissolved in 3 ml saline. This preparation was given as an intravenous bolus (approximating on a mg/kg basis), the clinical dose of 50 mg administered to a 70-kg patient as described by Przyklenk and Kloner (15). The animals were subjected to ischemia-reperfusion 30 min later. Group III (sildenafil, delayed phase, n = 6) animals were treated with sildenafil as in group II and subjected to ischemia-reperfusion 24 h later. Group IV (5-HD, n = 7) consisted of control rabbits treated with 5-HD (5 mg/kg iv) 10 min before sustained ischemia and reperfusion. In group V (sildenafil + 5-HD, acute phase, n = 6), sildenafil-treated rabbits as in group II were given 5-HD (5 mg/kg iv) 10 min before ischemia and reperfusion. In group VI (sildenafil + 5-HD, delayed phase, n = 6), the sildenafil-treated rabbits as in group III were given 5-HD (5 mg/kg iv) 10 min before ischemia and reperfusion.

In addition, a subset of three groups of animals (n = 5–6 per group) were given sildenafil citrate orally (1.4 mg/kg) or saline (control) to determine the early and delayed cardioprotective effect of the drug through this route. Because there is 40% bioavailability of sildenafil citrate after oral administration (http://www.pfi zer.com/hml/pi/s/viagrapfi .pdf), we used double the dose of the intravenous route; i.e., 1.4 mg/kg, which is equivalent to clinical dose of 100 mg for a 70-kg patient.

Statistics. All measurements of infarct size and risk areas are expressed as group means ± SE. Changes in hemodynamics and infarct size variables were analyzed by a two-way repeated-measures ANOVA to determine the main effect of time, group, and time-by-group interaction. If the global tests showed major interactions, post hoc contrasts between different time points within the same group or between different groups were performed using t-test. Statistical differences were considered significant if the P value was <0.05.
RESULTS

Hemodynamics. Intravenous administration of sildenafil citrate (0.7 mg/kg) caused a rapid decrease in hemodynamics as indicated by the 24.5%, 47.3%, and 38.8% decline in systolic, diastolic, and mean arterial pressures, respectively, within 2 min (Fig. 1A). The systemic hemodynamics returned to nearly baseline levels by 5 min after treatment with sildenafil. No significant changes in heart rate were observed following treatment with sildenafil (not shown). The effect of orally administered sildenafil citrate on systemic hemodynamics was milder and slower compared with the intravenous dose of the drug. The orally administered sildenafil caused an −9.2%, 12.5%, and 10.3% decrease in systolic, diastolic, and mean arterial pressure, respectively, after 30 min of treatment with the drug (Fig. 1B). This hypotensive response remained significantly depressed even at 60 min after oral administration of the drug. No changes in heart rate were observed. Also, no significant changes in systemic hemodynamics were observed in the control animals given saline orally (data not shown).

The heart rate, mean arterial pressure, and rate-pressure product during baseline, preischemia, 30 min of ischemia, and 180 min of reperfusion periods are shown in Tables 1 and 2. The hemodynamics remained reasonably stable, although they gradually decreased in all the groups during the experimental protocol. Except at the indicated time points, the mean values were not significantly different between the groups at any time point.

Infarct size. The infarct size (% of risk area) reduced from 33.8 ± 1.7 to 10.8 ± 0.9 during the acute phase (68% reduction, means ± SE, P < 0.05) and 19.9 ± 2.0 during the delayed phase (41% reduction) in the sildenafil-treated rabbits (Fig. 2A). A similar reduction in infarct size was observed acutely (after 60 min) and 24 h later when sildenafil was administered orally (Fig. 2B). The infarct-limiting effect of sildenafil was abolished in animals treated with 5-HD as shown by the significant increase in infarct size to 35.6 ± 0.4 during the acute phase and 36.8 ± 1.6 in the delayed phase (P < 0.05 vs. groups II and III treated with sildenafil, Fig. 2A). Control animals treated with 5-HD had an infarct size of 33.5 ± 1.9, which was not different from an infarct size of 33.8 ± 1.7 in the saline controls (P > 0.05). A similar trend in the changes in infarct size was observed when expressed as a percentage of the left ventricle (not shown). Similarly, the risk areas expressed as a percentage of the left ventricle were not statistically significantly different between the groups. These data suggest that changes in the infarct size observed among various groups were not related to the percentage of the risk area of the left ventricle, which was occluded by our technique. Representative sections of the heart treated with sildenafil citrate intravenously clearly demonstrated a significantly larger area of viable tissue (brick red color) compared with the saline-treated control and sildenafil + 5-HD-treated animals, which had much larger gray and white areas in the risk zone (Fig. 3).

DISCUSSION

Sildenafil citrate (Viagra) is currently the only approved oral drug for treatment of erectile dysfunction in men. However, little is known about other beneficial effects of this drug. We report here our novel observation about the preconditioning-like effect of sildenafil in the adult rabbit heart. Our results show that intravenous administration of sildenafil induces an acute (early) and delayed cardioprotective effect as indicated by a significant reduction in the infarct size compared with the saline-treated controls. Because the drug is taken orally by patients, we further showed that feeding the rabbits with sildenafil citrate reduced infarct size acutely (after 1 h) as well as 24 h later, which was comparable to the infarct size reduction obtained by intravenous administration of the drug. The selective blocker of mitoKATP channels 5-HD, when administered before the ischemia-reperfusion protocol, abolished both the early as well as delayed cardioprotection induced by sildenafil citrate. Intravenous administration of sildenafil citrate caused a severe transient decrease in the systemic hemodynamics (diastolic, systolic, and mean arterial blood pressure) within 2 min after treatment, which returned to nearly baseline levels by 5 min after treatment with sildenafil (not shown). The effect of orally administered sildenafil citrate on systemic hemodynamics was milder and slower compared with the intravenous dose of the drug. The orally administered sildenafil caused an −9.2%, 12.5%, and 10.3% decrease in systolic, diastolic, and mean arterial pressure, respectively, after 30 min of treatment with the drug (Fig. 1B). This hypotensive response remained significantly depressed even at 60 min after oral administration of the drug. No changes in heart rate were observed. Also, no significant changes in systemic hemodynamics were observed in the control animals given saline orally (data not shown).

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levels after 3 min. Although a significant decrease in systemic hemodynamics was also observed after oral administration of the drug, these changes were mild and occurred slowly. The hemodynamics remained largely unchanged among the groups during ischemia-reperfusion protocol. To our knowledge, this is the first study demonstrating 1) the direct cardioprotective effect of sildenafil in vivo, and 2) the involvement of the mitoK\textsubscript{ATP} channel in mediating this protection in the ischemic heart.

Sildenafil is a potent selective inhibitor of PDE-5 in vascular smooth muscle cells, which is known to enhance erectile function in men (20). Sexual stimulation results in the release of NO from nerves and endothelial cells in the corpus cavernosum of the penis that stimulates guanylate cyclase with subsequent formation of cGMP. Accumulation of cGMP leads to smooth muscle cell relaxation in the arteries, arterioles, and sinusoids in the corpus cavernosum, which allow this erectile tissue to fill with blood and causing an erection.
Men with erectile dysfunction may be unable to produce adequate amounts of cGMP because it may be broken down by PDE-5, which is found in high levels in the genitalia. Sildenafil inhibits PDE-5 allowing an increase in cGMP and improving vasodilation. Besides genitalia, PDE-5 is also found in other vascular and visceral smooth muscles (21). As a result, the administration of sildenafil causes vasodilation and decrease in the blood pressure. We hypothesized that such a vasodilatory action of sildenafil could potentially release endogenous mediators of preconditioning such as adenosine, bradykinin, or NO. One or more of these mediators may trigger signaling cascade leading to opening of the mitoKATP channel resulting in acute and delayed cardioprotective effects. Indeed our results show a very impressive acute cardioprotective effect that is comparable or even better than ischemic preconditioning (28) and pharmacological preconditioning induced by activation of adenosine receptors, monophosphoryl lipid A, or bradykinin (9, 13, 17, 26, 27). The cardioprotective effect after 24 h was less pronounced during the delayed phase; i.e., a 41% reduction of infarct size compared with 67% in the acute phase, implying that there may be gradual waning of the sustained protective effect. Alternatively, it is possible that the sildenafil-induced protection is biphasic, with the acute and delayed phase protection controlled by separate mechanisms.

Fig. 3. Representative sections of the heart demonstrating reduction of postischemic infarct size 30 min following treatment with sildenafil and blockade of the protective effect with 5-HD. At the end of the experimental protocol, as described in MATERIALS AND METHODS, the hearts were perfused with Evans blue to demarcate the risk area. Each heart was then sliced into 4–5 sections and stained with 2,3,5-triphenyltetrazolium chloride followed by fixation in formalin. Blue areas represent normal perfused tissue. Viable areas are stained brick red, whereas infarcted are gray or white. Note that significant viable area in the sections of the heart treated with sildenafil compared with saline control or sildenafil + 5-HD-treated rabbit. Similar pattern was observed in the sections of the heart from delayed phase groups (not shown).

Fig. 2. A: bar diagram showing infarct size (% risk area) after intravenous administration of sildenafil citrate. Saline control, animals receiving 0.9% saline. Sildenafil (acute phase), rabbits receiving sildenafil (0.7 mg/kg iv) 30 min before ischemia-reperfusion. Sildenafil (delayed phase), animals receiving sildenafil (0.7 mg/kg iv) 24 h before ischemia-reperfusion. 5-HD, control (saline-treated) rabbits received 5-hydroxydecanoate (5 mg/kg) 10 min before sustained ischemia and reperfusion. Sildenafil + 5-HD (acute phase), sildenafil-treated rabbits given 5-HD (5 mg/kg iv) 10 min before sustained ischemia and reperfusion. Sildenafil + 5-HD (delayed phase), rabbits treated with sildenafil 24 h before ischemia-reperfusion were given 5-HD. Results are means ± SE in 6–7 rabbits in each group. *P < 0.05 compared with control, sildenafil, sildenafil + 5-HD (acute and delayed), and 5-HD groups. B: reduction of infarct size (% of risk area) after oral administration of sildenafil citrate. Rabbits were given sildenafil (1.4 mg/kg) or equivalent volume of saline before ischemia-reperfusion protocol, which was carried out after 60 min (acute phase) and 24 h later (for delayed phase).
Another interesting observation in the present study is that both the acute and delayed cardioprotective effects were blocked by 5-HD, suggesting that the opening of mitoK\textsubscript{ATP} channels plays an important role in the infarct size reduction by sildenafil in our model. Several studies have now conclusively demonstrated that opening mitoK\textsubscript{ATP} channels plays an important role in ischemic as well as pharmacological preconditioning in the heart (7, 8, 18, 22). Mitochondria are known to play an essential role in cell survival by ATP synthesis and maintenance of Ca\textsuperscript{2+} homeostasis. Opening the mitoK\textsubscript{ATP} channel partially compensates the membrane potential, which enables additional protons to be pumped out to form a H\textsuperscript{+} electrochemical gradient for both ATP synthesis and Ca\textsuperscript{2+} transport (16). The acute protection induced by sildenafil may be mediated by the opening of the mitoK\textsubscript{ATP} channel either directly or through a variety of signaling pathways such as activation of protein kinase C and mitogen-activated protein kinases. The delayed phase could be through the signaling cascade leading to the synthesis of inducible NO synthase, generation of NO, and opening of the mitoK\textsubscript{ATP} channels as described previously (19, 23, 24, 29–31). Currently, we do not have the evidence in support of this notion, and further investigations are needed to determine the cellular and molecular mechanisms of cardioprotection by sildenafil.

In the present study, the sildenafil was given in normal rabbits with no sexual stimulation. It has been suggested that sexual activity is comparable to moderate exercise, particularly in men with coronary artery disease who may have been physically inactive to engage in sexual activity (10). Because exercise triggers a preconditioning-like effect in animal models (25), it is possible that men taking sildenafil before sexual intercourse could potentially have an additive cardioprotective effect of moderate exercise. On the other hand, concerns over the safe use of sildenafil in patients with ischemic heart disease have been raised. Whereas the overall safety of sildenafil use has been well established, the coadministration of long- and short-acting nitrate preparations has been associated with significant hypotension and adverse cardiovascular effects. In addition, caution has been recommended in prescribing sildenafil to patients with unstable ischemic coronary syndromes, severe left ventricular dysfunction, or patients who are on multiple-drug antihypertensive regimens (5, 12). Therefore, careful clinical studies are required to evaluate the preconditioning-like effect of sildenafil in patients with ischemic heart disease.

In conclusion, for the first time, we have demonstrated that intravenous or oral administration of sildenafil citrate induces significant cardioprotective effect against ischemia-reperfusion injury, the impact of which was powerful within 30 min and persisted to a slightly lesser degree 24 h after administration of the drug. The extent of protection observed with sildenafil was comparable to preconditioning induced by subthalamic ischemia and several other pharmacological agents. Furthermore, our results show that the cardioprotective effect of sildenafil was mediated by the opening of mitoK\textsubscript{ATP} channel, a proposed end effector of myocardial preconditioning (8, 22). Further investigations are needed to understand the molecular mechanism(s) of the sildenafil-induced cardioprotective effect, which would help in expanding the utility of this drug for other cardiovascular diseases in addition to the current use for treatment of erectile dysfunction in men.

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