Am J Physiol Heart Circ Physiol 282: H1787–H1792, 2002.
First published January 17, 2002; 10.1152/ajpheart.00607.2001.

Sildenafil-nitric oxide donor combination promotes ventricular tachyarrhythmias in the swine right ventricle

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Received 12 July 2001; accepted in final form 14 January 2002

Swissa, Moshe, Toshihiko Ohara, Moon-Hyoung Lee, Sanjay Kaul, Prediman K. Shah, Hideki Hayashi, Peng-Sheng Chen, and Hrayr S. Karagueuzian. Sildenafil-nitric oxide donor combination promotes ventricular tachyarrhythmias in the swine right ventricle. *Am J Physiol Heart Circ Physiol* 282: H1787–H1792, 2002. First published January 17, 2002; 10.1152/ajpheart.00607.2001.—We tested the hypothesis that sildenafil, singly or in combination with nitric oxide (NO) donors, promotes ventricular tachycardia (VT) and ventricular fibrillation (VF). Vulnerability to VT/VF was tested by rapid pacing in eight isolated normal swine right ventricles (RV). The endocardial activation was optically mapped, and the dynamic action potential duration (APD) restitution curves were constructed with metal microelectrodes. At baseline, no VT/VF could be induced. Sildenafil (0.2 μg/ml) or NO donor singly or in combination did not alter VT/VF vulnerability. However, when 2 μg/ml sildenafil was combined with NO donors, the incidence of VT and VF rose significantly ($P < 0.01$). VT with a single periodic wavefront was induced in five of eight RVs, and VF with multiple wavefronts was induced in all eight RVs. The sildenafil-NO donor pro-VT/VF combination significantly increased the maximum slope of the APD restitution curve and the amplitude of the APD alternans. The pro-VT/VF effects of sildenafil were reversible after drug-free Tyrode solution perfusion. We conclude that a sildenafil (2 μg/ml) and NO donor combination increases VT/VF vulnerability in the normal RV by a mechanism compatible with the restitution hypothesis.

ventricular fibrillation; electrical stimulation

SILDENAFIL (Viagra) is a popular drug that is used to manage erectile dysfunction (9). Shah (18) first reported two patients with extensive prior myocardial infarction in whom ventricular tachycardia (VT) was temporally associated with sildenafil use. From April 1998 through May 1999, a total of 1,473 major adverse events associated with siledenafil use were reported to the US Food and Drug Administration Internet site. These adverse events included 522 deaths, 517 myocardial infarctions, 255 sudden deaths or arrhythmias, 119 cardiovascular accidents, and 271 cases of syncope or hypotension (2, 5a). The cause of death was myocardial infarction in 200 patients, sudden death or arrhythmia in 94 patients, and unknown in 187 patients. Some of the patients who died suddenly, presumably by VT/ventricular fibrillation (VF), were on concomitant nitrate therapy. The American College of Cardiology/American Heart Association consensus suggested that neither sildenafil nor nitrates should be used within 24 h of the other in any dose and by any route of administration (5) because of doubling (synergism) of maximal blood pressure reduction (24). At present, however, there are no studies that have evaluated the direct proarrhythmic potential of siledenafil and its interaction with nitrates [i.e., nitric oxide (NO) donors] independent of hemodynamic compromise. In this study, we tested the hypothesis that sildenafil, individually or in combination with NO donors, increases ventricular vulnerability to VT/VF in the isolated normal swine right ventricle (RV) during heart rate acceleration and constant coronary perfusion.

MATERIALS AND METHODS

Tissue Preparation

All experiments were done in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Eight farm pigs (25–35 kg body wt) of either sex were anesthetized with 20 mg/kg iv thiopental sodium. The RV was isolated and perfused through the right coronary artery at a constant flow rate of 30 ml/min with oxygenated Tyrode solution as previously described (11).

Drug Perfusion

Sildenafil citrate (Pfizer) in tablet form (50 mg) was dissolved in 200 ml of Tyrode solution just before the experiment to form a stock solution of 250 μg/ml. The stock solution was then filtered, and a clear solution was obtained. Two different concentrations of sildenafil, 0.2 and 2 μg/ml, were used. These concentrations of sildenafil encompass plasma sildenafil levels reported in humans (0.13–1.5 μg/ml) after oral ingestion of 25- to 200-mg Viagra tablets (3, 10). We used 50 μM sodium nitroprusside ($n = 7$) or 10 μM nitroglycerine ($n = 4$) as NO donors (1, 17). Nitroglycerine was used in four

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isolated RVs after washout of the nitroprusside to determine if the NO donor species influences the proarrhythmic potential of sildenafil in the same RV.

Pacing and Optical Mapping

The isolated RV was paced with a bipolar electrode with twice diastolic threshold current and 5-ms pulse width. The nature of the global RV rhythm (VT or VF) was determined by recording a pseudoelectrocardiogram (ECG) via two electrodes placed on both sides of the RV (11). Endocardial "action potentials" were recorded with a pure iridium metal electrode as we have previously described (15). Testing of VT/VF vulnerability and construction of dynamic action potential duration (APD) restitution curves were made by progressively shorter pacing cycle lengths (CL) from 400 to loss of 1:1 capture using twice diastolic current threshold and 5-ms pulse duration (4, 12). Pacing began at 400-ms CL and then progressively decreased during the same pacing train without pauses between the different pacing CLs. When 1:1 capture was lost, the pacing train was considered complete. No further aggressive pacing protocols (i.e., higher current strengths, burst pacing) (11) were used in this study. A pair of defibrillating coil electrodes (CPI) was placed in the tissue bath 1 cm away from either side of the isolated RV and was connected to an HVS-02 external defibrillator (Ventritex; Sunnyvale, CA). The optical mapping used in the present study has been previously described in detail (13, 21). Briefly, the isolated RVs were stained for 20 min with the voltage-sensitive dye di-4-ANEPPS (Molecular Probes; Eugene, OR) and excited using a solid state laser at a wavelength of 532 nm. Fluorescent and scattered light was collected using an image-intensified charge-coupled device camera (Dalsa; Ontario, Canada). The data were acquired simultaneously from 128 × 128 sites over a 3 × 3-cm area with a temporal resolution of 2.3 ms. About 1.5- to 2-cm-wide edges of tissue remained outside the mapped region. The fluorescent signals were baseline subtracted and inverted. A moving median filter was applied, after which the signals were normalized to the average of the maximal five data points so that the range of the maximum signal amplitude was the same for each pixel. The signals of each pixel were then spatially averaged with the signals of eight neighboring pixels to reduce noise. Each pixel was then assigned a shade of red, representing the fully depolarized state, yellow, representing the repolarizing state, and black, representing the fully repolarized state. The boundaries of the depolarizing regions were delineated by red, and the repolarizing regions were delineated by green lines.

Study Protocol

After baseline testing of VT/VF inducibility and construction of dynamic APD restitution curves, we tested the effects of drug or drug combinations. In one protocol, the two NO donors were perfused singly; in a second protocol, the higher concentration of sildenafil (2 μg/ml) was perfused alone. In the remaining two protocols, the two NO donors were combined with the lower (0.2 μg/ml) and higher (2 μg/ml) concentrations of sildenafil. The order of drug perfusion was randomized, and a washout period of 20 min was allowed between the two different drug perfusion protocols. After each drug perfusion, the sequence of rapid pacing was repeated. The endocardial RV effective refractory period (ERP) was determined by the extrastimulus method using twice diastolic current threshold and 5-ms duration during regular pacing at a CL of 300 ms.

Data Analysis

APD restitution curve slopes were determined using exponential fits to the data. Student's *t*-test, χ^2-test, or ANOVA with Dunn's (Bonferroni) correction was used for multiple comparisons (6, 22). Values of $P < 0.05$ were considered significant. All data are presented as means ± SD.

RESULTS

Vulnerability to VF

Table 1 shows the incidence of VT/VF in all eight isolated RVs. At baseline, VF could be induced twice in only one of eight isolated RVs (Table 1). In this RV, VF could also be induced when it was perfused with a low concentration of sildenafil and NO donor (Table 1). In the remaining seven RVs, repeated trials (at least 5 trials in each RV for a total 44 trials) failed to induce VF at baseline. Similarly, when the lower (0.2 μg/ml) or higher (2 μg/ml) sildenafil concentration was perfused individually, no VF could be induced. Similarly, no VF could be induced when the two NO donors were perfused individually (Table 1). Because the effects of the two NO donors were similar, the results were pooled. When, however, 2 μg/ml sildenafil was combined with either of the two NO donors, VF was induced in all eight RVs ($P < 0.01$ for all comparisons; Table 1). Induction of VF was reasonably reproducible in all eight RVs (39 induced VF episodes of 75 trials,

Table 1. *Effects of sildenafil and NOD, individually and in combination, on the incidence of VT/VF during rapid pacing*

	Control	NOD	LCS + NOD	HCS	HCS + NOD	Washout
Number of pig RVs	8	7	8	7	8	7
VT induction	0/8	1/7	1/8	0/7	5/8*	0/7
VF induction	1/8	0/7	1/8	0/7	8/8*	1/7
Number of trials	44	40	40	36	97	35
Number of VT episodes	0/42	1/40	1/39	0/36	22/58*	0/33
% VT episodes	0	2.5	2.5	0	38	0
Number of VF episodes	2/44	0/39	1/39	0/36	39/75*	2/35
% VF episodes	5	0	2.5	0	52	6
Number of VT/VF episodes	2/44	1/40	2/40	0/36	61/97*	2/35
% VT/VF episodes	6	2.5	5	0	63	6

Values are means ± SE. VT, ventricular tachycardia; VF, ventricular fibrillation; NOD, nitric oxide donors [sodium nitroprusside (50 μM) or nitroglycerine (10 μM)]; LCS and HCS, low (0.2 μg/ml) and high (2 μg/ml) concentrations of sildenafil, respectively; RV, right ventricle. *$P < 0.01$ for all comparisons.

52%; Table 1). The mean VF CL was 99.34 ± 10 ms (range 80–113 ms). In 17 episodes, the onset of VF was preceded by three to five beats of periodic activations (VT), and, in 16 episodes, rapid pacing degenerated to VF immediately without a prior transient period of VT. The pro-VF of the 2 μg/ml sildenafil-NO donor combination was reversible upon 20 min of a drug-free washout period (Table 1).

Vulnerability to VT

At baseline, VT could not be induced in any of the eight isolated RVs. NO donor singly or combined with the low concentration of sildenafil could induce VT in only one of eight isolated RVs (Table 1). The high concentration of sildenafil also failed to induce VT (Table 1). However, when the higher concentration of sildenafil was combined with NO donor, VT was induced in five of eight RVs (22 induced VT episodes of 58 trials, 38%, $P < 0.01$ for all comparisons; Table 1). VT had a mean CL of 169 ± 30 ms (range 110–230 ms) and monomorphic ECG characteristics. The VT could last for >10 min, often requiring cardioversion for termination. The period of the VT was either slower ($n = 12$) or faster ($n = 5$) than the pacing CL that induced the VT. The mean pacing CL that induced VT was 182 ± 39 ms (range 110–250 ms). Three episodes of VT with a mean CL of 157 ± 12 ms were initiated during regular pacing at a CL of 400 ms within 5 min of high sildenafil concentration-NO donor perfusion. The pro-VT effects of the 2 μg/ml sildenafil-NO donor combination were reversible within 20 min of drug-free perfusion in all five RVs (Table 1).

Activation Pattern During VT/VF

The mapped VF episodes ($n = 22$) were characterized by the presence of three to four independent wavefronts propagating in different directions, consistent with our previous reports (26). In contrast, however, mapped VT episodes ($n = 15$) showed regular activation patterns ($n = 13$) that were initiated either from the border, outside the mapped region ($n = 12$), or the center ($n = 1$) of the mapped tissue. Figure 1*A* illustrates an episode of VT initiated from the center of the tissue by a periodic discharge mechanism. In one episode, the VT was sustained by a single counterclockwise-rotating spiral with a period of 130 ms (Fig. 1*B*).

APD Restitution and Refractoriness

NO donors singly and in combination with either the low or high sildenafil concentration significantly ($P <$

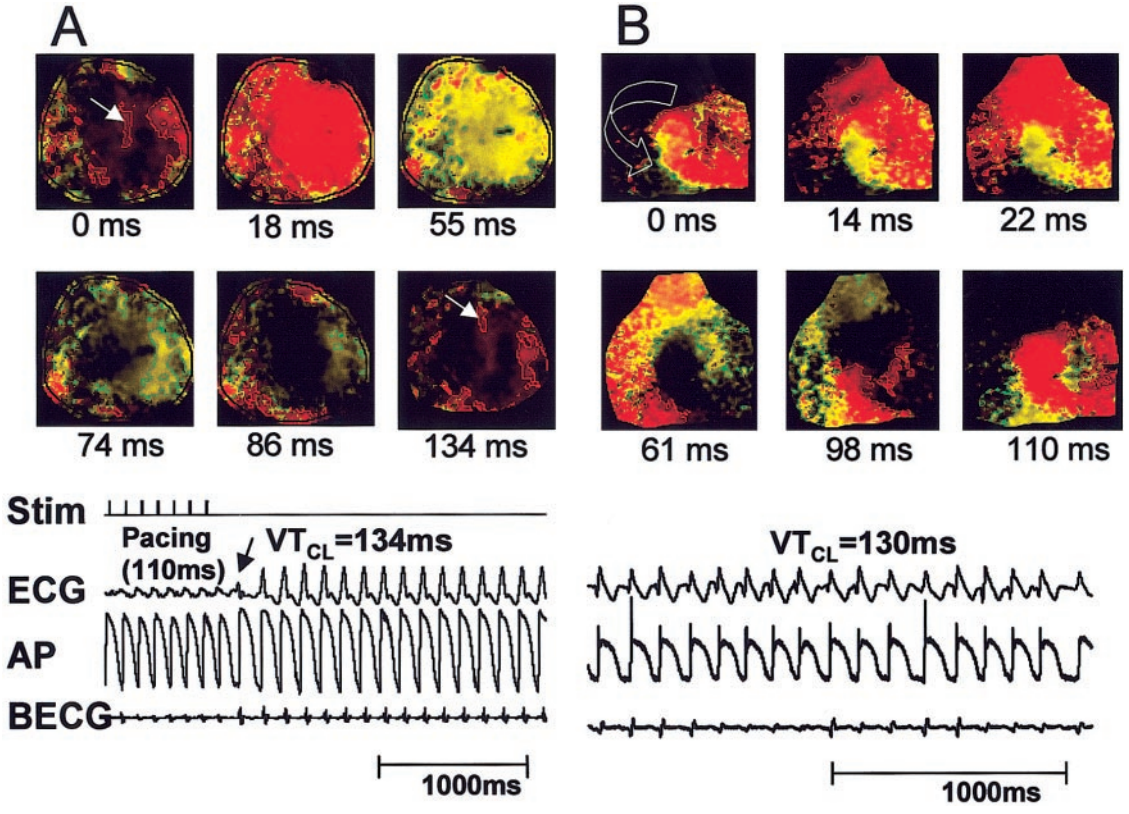

Fig. 1. Optical snapshots during ventricular tachycardia (VT) induced during perfusion of a 2,000 ng/ml sildenafil and 50 μM sodium nitroprusside combination in an isolated perfused swine right ventricle. The most depolarized region is red and the most repolarized is black. The red and green lines depict wavefront and waveback, respectively. The number under each frame is the time (in ms), starting from zero (chosen arbitrarily). *A*: note the presence of a focal activation that gives rise to a large concentric activation. *B*: episode of induced VT sustained by a large counterclockwise (arrow) reentrant wavefront. Stim, stimulus artifact; CL, cycle length; ECG, pseudoelectrocardiogram; AP, action potential; BECG, bipolar electrogram.

Table 2. *Effects of sildenafil and NOD, individually and in combination, on the ERP, APD restitution curve, and APD_{90}*

	Control (n = 8)	NOD (n = 7)	LCS + NOD (n = 8)	HCS (n = 7)	HCS + NOD (n = 8)	Washout (n = 7)
ERP, ms	202 ± 9*	178 ± 12	185 ± 17	198 ± 18*	190 ± 22	202 ± 10*
APD restitution slope	1.3 ± 0.1	2.1 ± 0.2†	1.9 ± 0.2†	1.6 ± 0.3	1.9 ± 0.4†	1.2 ± 0.1
DI with slope > 1, ms	48 ± 15	73 ± 10†	70 ± 13	66 ± 14	65 ± 10	44 ± 11
APD_{90}, ms						
PCL = 400 ms	243 ± 7	225 ± 5	228 ± 11	241 ± 10	227 ± 10†	245 ± 11
PCL = 240 ms	171 ± 8	147 ± 12	159 ± 7	186 ± 8	179 ± 10‡	174 ± 6

Values are means ± SE; n = number of RVs. ERP, effective refractory period [during regular pacing at cycle length (PCL) = 300 ms]; APD, action potential duration; DI, diastolic interval; APD_{90}, APD at 90% repolarization. *$P < 0.05$ compared with NOD, †$P < 0.05$ compared with control and washout; ‡$P < 0.05$ compared with control, NOD, and LCS + NOD.

0.05) increased the maximum slope of the APD restitution curve compared with the control (Table 2). The high concentration of sildenafil alone had no significant effect on the maximum slope of the APD restitution (Table 2). The high concentration sildenafil-NO donor combination changed the APD compared with the control (baseline), which was CL dependent. While at 400-ms CL, the drug combination shortened the APD compared with the control; at a 240-ms pacing CL (i.e., the shortest CL with regular 1:1 capture), the APD prolonged significantly compared with the control (Table 2). The combination of the higher concentration of sildenafil and NO donor was the only drug regimen that simultaneously caused a significant increase in the maximum slope of the APD restitution curve (1.3 ± 0.1 vs. 1.9 ± 0.5, $P < 0.05$) and the APD (171 ± 8 vs. 179 ± 10 ms, $P < 0.05$; Table 2) compared with the control. No other drug or drug combination compared with the control had these simultaneous effects on the APD restitution and on the APD at the shortest captured pacing CL. Consistent with the restitution hypothesis, these two effects (increases in the slope of the APD restitution and APD) (7, 25) promote APD oscillations with increasing amplitude during rapid pacing. Figure 2 illustrates an example of increasing amplitude of the APD gradient with the high concentration of sildenafil and NO donor that precedes VF.

DISCUSSION

The ability of the sildenafil-NO donor combination to promote VT/VF in the isolated perfused normal swine RV constitutes the major finding of this study. An equally important finding was the lack of proarrhythmic effect when the same concentration of sildenafil was perfused singly or at lower concentration with or without a NO donor.

Mechanisms of VT/VF Induced by Sildenafil-NO Donor Combination

Automatic mechanisms. NO stimulates the formation of cGMP, and sildenafil inhibits its degradation by inhibiting phosphodiesterase type 5 (PDE5) activity, causing a net increase in intracellular cGMP concentrations (3, 20). Although a selective inhibitor of PDE5, sildenafil was shown to also inhibit PDE1, an important cardiac PDE (23). Recent work has shown that sildenafil may significantly increase both cGMP and cAMP levels in isolated human cardiac tissue through the inhibition of PDE1 (19). An increase in cellular cGMP has been shown to stimulate the pacemaker current, which may promote an "automatic" tachycardia (14). Whereas an automatic mechanism might cause a focal activity, an intramural reentry (scroll wave) that periodically activates an endocardial site

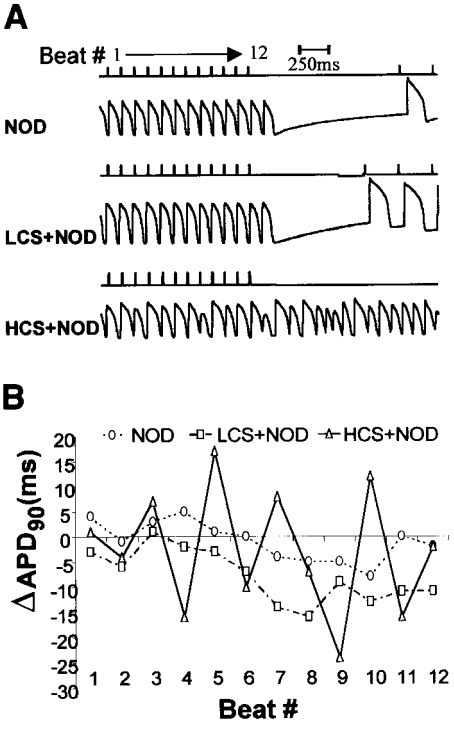

Fig. 2. Endocardial APs recorded with metal microelectrodes during perfusion with nitric oxide donor (NOD; 50 μM sodium nitroprusside) in combination with 0.2 μg/ml [low concentration sildenafil (LCS) + NOD] and 2 μg/ml [high concentration sildenafil (HCS) + NOD] sildenafil. *A*: the first 4 of 12 paced beats were paced at a CL of 120 ms, whereas the subsequent 8 beats were paced at a CL of 110 ms. Stimulus artifacts (stim) are above each recording. Note increasing AP duration (APD) and AP amplitude alternans with HCS + NOD that precede ventricular fibrillation. *B*: plot of the APD gradients on a beat-to-beat basis during pacing at 110 ms as shown in *A*. APD gradients between consecutive beats reach 42 ms with HCS + NOD, whereas it remains below 10 ms with the other drug regimens. (Note that the possible emergence of ectopic activity during pacing as a cause of increased APD gradient cannot be ruled out.)

might also give the appearance of a focal activity (27). A recent in vitro study on isolated cardiac myocyte showed that 30 μM sildenafil directly blocked the rapid outward K^+ current and cause a modest APD prolongation. Sildenafil's reversal of the NO donors' shortening of the ERP seen in the present study is consistent with the APD prolonging effect of sildenafil reported in isolated myocytes (8).

Reentrant mechanisms. Another potential mechanism for the high concentration sildenafil-NO donor combination to promote VT/VF may result from the ability of this combination to increase both the maximum slope of the APD restitution and the APD simultaneously during rapid pacing. No other drug or drug combination could manifest these two effects simultaneously. With increasing the pacing rate near the rate at which VT/VF could be induced, the amplitude of the APD gradient consecutive beats became progressively amplified, a property that is known to increase wavefront instability and promote wavebreak, leading to reentry (7, 25). The pro-VT/VF of the sildenafil-NO donor combination appears to be also compatible with the restitution hypothesis of wavefront stability. The sildenafil-NO donor combination may then trigger VT by an automatic or a reentrant mechanism and VF by wavebreak by a mechanism compatible with the restitution hypothesis. More work is needed to clarify this issue.

Limitations of the Study

It could be argued that our RV preparations may become unstable as a result of repeat fibrillation/defibrillation trials. However, the full reversibility of the proarrhythmic effects of the sildenafil-NO donor combination upon drug-free washout argues against this potential pitfall. Another limitation of our study could be that the effect of the sildenafil-NO donor combination was evaluated on normal and well-perfused ventricular myocardium. We do not know whether a similar response pattern also occurs in diseased and ischemic ventricular tissue. Finally, it must be emphasized that, in the present study, we did not use an aggressive pacing protocol (burst pacing and high currents of stimulation) to induce VF (12). The low yield of VF at baseline therefore could have resulted from the lack of an aggressive stimulation protocol rather than from the characteristics of the isolated RVs.

Clinical Implications

Sildenafil is contraindicated in patients with heart disease that necessitates the concomitant use of nitrates (NO donors). Our findings indicate that such a drug combination might increase ventricular vulnerability to VT/VF. Further studies using models of acute ischemia and chronic infarction will be needed to determine whether sildenafil alone is also arrhythmogenic because many sudden deaths in humans occur in the absence of nitrates (2).

We thank Avile McCullen and Meiling Yuan for technical assistance, Nina Wang for reading the manuscript, Elaine Lebowitz for secretarial assistance, and Dr. William J. Mandel for support.

This study was supported in part by a Fellowship Award from the Save A Heart Foundation, The American Physicians Fellowship for Medicine in Israel, The Israel Pacing Foundation (to M. Swissa), the Fukuda Memorial Foundation For Medical Technology (to T. Ojara), National Heart, Lung, and Blood Institute Specialized Center of Research Grant HL-52319, University of California Tobacco-Related Disease Research Program Grant 9RT-0041, American Heart Association National Center Grant-In-Aids 9750623N and 9956464N, the Cedars-Sinai Electrocardiographic Heartbeat Organization, the Grand Sweepstakes, and the Pauline and Harold Price Endowment.

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