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Potent Antifibrillatory Effects of Intrapericardial Nitroglycerin in the Ischemic Porcine Heart

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OBJECTIVES We investigated the antiarrhythmic effects of intrapericardial nitroglycerin (NTG) during acute myocardial ischemia in the porcine heart.

BACKGROUND Nitroglycerin is a nitric oxide donor that exerts potent effects on the cardiovascular system. Intrapericardial administration allows investigation of pharmacologic actions on cardiac tissue in an in vivo system while minimizing the confounding influences of systemic effects.

METHODS In 29 closed-chest pigs, myocardial ischemia was induced by intraluminal balloon occlusion of the left anterior descending coronary artery. Arrhythmia incidence was monitored during 5-min balloon inflations performed without drug and at 15, 45, 75, and 105 min after NTG (4,000 μg bolus) administered by percutaneous transatrial access into the pericardial space. Electrocardiograms were monitored for ischemia-induced T-wave alternans (TWA), a marker of electrical instability. The antiadrenergic potential of NTG was investigated by examining the drug's suppression of dobutamine-induced increase in myocardial contractility.

RESULTS Control coronary artery occlusion provoked ventricular fibrillation (VF) in all animals. Intrapericardial NTG suppressed VF at 45 min in all six pigs ($p < 0.05$) and reduced TWA across a parallel time course (from 459.1 ± 144.4 μV before drug to 42.22 ± 13.96 μV at 45 min, $p = 0.047$). The antifibrillatory effect occurred as early as 15 min and persisted for up to 75 min. Augmentation of maximum of the first time derivative of left ventricular pressure by dobutamine was blunted by intrapericardial NTG (from 3,999 ± 196 mm Hg/s before NTG to 3,543 ± 220 mm Hg/s at 15 min, $p = 0.012$).

CONCLUSIONS Intrapericardial NTG exerts a robust antifibrillatory action. Potential mechanisms include reduction in electrical instability and blunting of adrenergic effects. (J Am Coll Cardiol 2003;41:1831–7) © 2003 by the American College of Cardiology Foundation

Interest is increasing in the direct delivery of agents into the pericardial space for local treatment of cardiovascular disorders to achieve maximum therapeutic effects and to minimize side effects of systemic administration (1–6). The intrapericardial approach to local cardiac drug delivery possesses several intrinsic advantages: 1) delivery into a low-turnover reservoir, which maximizes contact with tissue and minimizes loss of agent into circulation; 2) access to coronary vessels and to the sympathetic and parasympathetic efferent fibers, both of which have significant segments of epicardial exposure, particularly at the base of the heart (7,8); 3) perfusion of atrial and ventricular epicardial tissue to affect ionic currents; 4) reduced exposure to degradative enzymes, notably those contained in erythrocytes; and 5) avoidance of systemic effects. Safe, rapid, reliable access without thoracotomy to the normal pericardial space has been demonstrated (4,9).

The well-established vascular effects of nitric oxide (NO) donors are augmented when administered intrapericardially. The NO donor sodium nitroprusside more effectively protected against platelet aggregation in stenosed and injured coronary arteries when administered intrapericardially than intravenously (3). Intrapericardial nitroglycerin (NTG) produced persistent coronary vasodilation without systemic hypotension or reflex elevations in heart rate (HR) (5). The vasodilatory effect, measured by intravascular ultrasound, was more pronounced and enduring (3 to 15 min) than an equal intracoronary dose (200 μg bolus). Baek et al. (6) demonstrated a prolonged vasodilatory effect and positive remodeling by the NO donor diazeniumdiolated bovine serum albumin, which has 22-h intrapericardial residence time, and suggested a clinical application in protecting against restenosis after angioplasty.

Nitroglycerin is capable of acting at multiple levels including the coronary vasculature, the cardiac autonomic nerve supply, and the myocytes themselves, stemming from its production of the highly permeable gas NO (10–12). The antiarrhythmic efficacy of intravenous NTG has been established both experimentally (13–16) and clinically (17–20). However, the hypotensive effect of systemic administration of NTG can decrease antiarrhythmic efficacy (16).

We investigated whether local delivery of nitroglycerin into the intact pericardial sac to minimize potential systemic effects could protect against myocardial ischemia-induced arrhythmias. T-wave alternans (TWA) was quantified to assess the agent's effects on ischemia-induced cardiac electrical instability. We also evaluated the agent's capacity to protect against dobutamine-induced increase in contractility as a measure of its potential antiadrenergic activity.

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Abbreviations and Acronyms

cGMP	= cyclic guanine monophosphate
ECG	= electrocardiogram
HR	= heart rate
LAD	= left anterior descending coronary artery
LV	= left ventricle/ventricular
LV dP/dt max	= maximum of the first time derivative of left ventricular pressure
NO	= nitric oxide
NTG	= nitroglycerin
SBP	= systolic blood pressure
TWA	= T-wave alternans
VF	= ventricular fibrillation
VPB	= ventricular premature beat

METHODS

Experimental preparation. This study was conducted according to the National Institutes of Health standards and protocols approved by the institution and conformed to the "Position of the American Heart Association on Research Animal Use." Yorkshire farm pigs (n = 29, either gender, 25 to 35 kg) were pre-anesthetized with telazol (4.7 mg/kg, intramuscularly) and xylazine (2.2 mg/kg, intramuscularly) and anesthetized with alpha-chloralose (bolus, 100 mg/kg, intravenously, followed by continuous infusion, 40 mg/kg/h, intravenously). Arterial PO_2, PCO_2, and pH were maintained in physiologic range with the use of a constant volume-cycled respirator (Harvard Apparatus, Holliston, Massachusetts) and supplemental oxygen through endotracheal intubation by tracheostomy. Femoral artery and vein were cannulated bilaterally with 8F introducer sheaths using standard protocol. Blood pressure was continuously monitored from a femoral arterial sheath, and intravenous fluids were administered through a femoral vein. Standard precordial 12-lead electrocardiograms (ECG) were recorded with a PRUCKA Cardiolab workstation (GE Medical Systems, Milwaukee, Wisconsin) and analyzed on the MARS workstation (GE Medical Systems). Unipolar electrograms were recorded from a monopolar intracoronary lead placed just beyond the angioplasty balloon positioned downstream of the first diagonal branch of the left anterior descending coronary artery (LAD) and from a left ventricular (LV) lead recorded with reference to Wilson's central terminal.

Percutaneous transatrial pericardial access. Percutaneous transatrial pericardial access was performed (4,9). The stiff end of a standard angioplasty guidewire (0.014-inch Wizdom guidewire, Cordis Corp., Hialeah, Florida) was placed within the lumen of a soft infusion catheter (0.038-inch SOS straight tip, open-ended angiographic guidewire, Bard Interventional Products, Billerica, Massachusetts), advanced until 1 to 2 mm protruded through the end of the infusion catheter, and locked with a stopcock in position with reference to the infusion catheter. This assembly was then advanced into an 8F multipurpose guide catheter (MP2, Boston Scientific, Natick, Massachusetts) previously positioned in the right atrial appendage via a femoral vein under fluoroscopic guidance. Puncture of the right atrial appendage was made with the guidewire tip, and the infusion catheter and guidewire were advanced as a unit into the pericardial space. Conformation of the infusion catheter to the exterior curvature of the heart on fluoroscopy verified its location within the pericardial space. The guidewire was then removed, and the infusion catheter left in place for drug delivery. The absence of trauma from the atrial puncture was verified by <1% hematocrit of pericardial fluid aspirated with the infusion catheter.

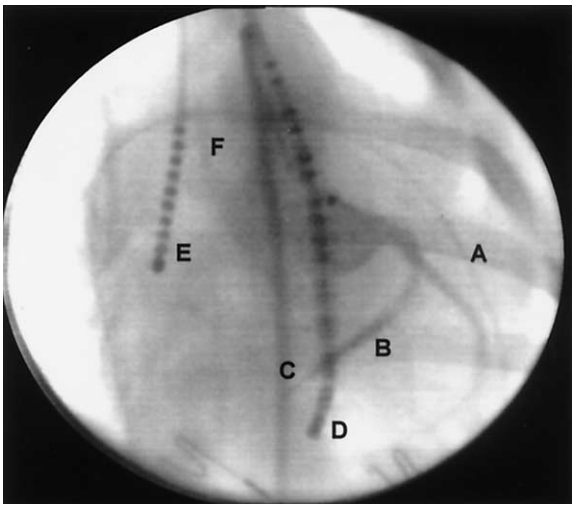


Figure 1. Fluoroscopic image of experimental setup for electrophysiology studies. Infusion catheter **(A)** in the pericardial space conforms to the contour of the heart. Angioplasty balloon **(B)** completely occludes the left anterior descending coronary artery as demonstrated by dye injection into the left main coronary artery. Intracoronary guidewire **(C)** monitors the intracoronary electrocardiogram (ECG). Electrocatheter **(D)** positioned in the left ventricular (LV) ischemic zone records the LV ECG. Pacing catheter **(E)** is in the right atrium, positioned via the right internal jugular vein; 8F multipurpose guide catheter **(F)** in the right atrial appendage is used for introducing the pericardial infusion catheter.

Myocardial ischemia induction. Myocardial ischemia was induced in a closed-chest preparation by intraluminal occlusion of the LAD between the first and second diagonal branches with standard percutaneous transluminal coronary angioplasty techniques and equipment (Fig. 1). Under fluoroscopic guidance, the left main coronary artery was cannulated with an 8F Judkins right guide catheter (JR4 with side holes, Boston Scientific). A 0.014-inch angioplasty guidewire (0.014-inch Wizdom guidewire, Cordis) was threaded through the LAD past the second diagonal branch. An angioplasty balloon, 2.5- to 3.5-mm in diameter and 10- to 20-mm long, was passed over the guidewire to position the proximal end just beyond the first diagonal branch and was inflated to occlude the vessel completely, as verified with angiography. Occurrence of reperfusion arrhythmias was avoided by slow-release of the balloon. Between occlusions, the balloon was pulled back into the guide catheter to allow blood flow to resume and to minimize endothelial trauma. Heparin (5,000 U bolus, intravenously, followed by 1,000 U/h) was administered to

prevent thrombosis. This closed-chest model of intracoronary artery occlusion yielded a high incidence of ventricular fibrillation (VF) and proved to be reliable, reproducible, and highly suitable for studying the effects of drugs on ischemia-induced arrhythmias.

Experimental design. Effects of intrapericardial NTG on severe ventricular arrhythmias were studied (n = 6). Six 5-min LAD coronary artery occlusions were performed 30 min apart (preconditioning, control, 15 min post-drug, 45 min post-drug, 75 min post-drug, and 105 min post-drug). Nitroglycerin (4,000 μg from a 5 mg/ml stock solution diluted with normal saline) was injected intrapericardially (10-ml bolus) at 15 min after control occlusion. Preconditioning occlusion results were discarded because of established variability. Severity of occlusion-induced arrhythmias was graded as VF, ventricular tachycardia consisting of $\geq$4 consecutive ventricular premature beats (VPB) lasting $<$15 s, and isolated VPBs. ST-segment deviation was monitored from precordial lead V_3. Saline (10 ml bolus), rather than NTG, was administered intrapericardially (n = 5) to verify the reproducibility of the experimental model and to provide control data for the vehicle. Whenever VF ensued, the balloon was deflated, retracted, and the heart was defibrillated.

The effect of intrapericardial NTG (4,000 μg bolus) on TWA was investigated (n = 5) during heart-rate pacing at 120 beats/min. Coronary occlusions and intrapericardial access were performed by the same protocol. Additional instrumentation included: 1) multipolar, steerable 7F electrocatheter (Bard Electrophysiology, Lowell, Massachusetts) inserted into the LV retrogradely from the aorta to obtain unipolar electrograms from the endocardial surface in the ischemic zone; 2) LAD coronary guidewire to record unipolar electrograms from the epicardium in the ischemic zone; and 3) 6F quadripolar electrocatheter (Bard Electrophysiology) positioned in the right atrium with the two most distal poles used for pacing at 120 beats/min. Heart rate was maintained constant during TWA measurement to rule out this variable. Arrhythmia grade was not analyzed in this group due to the ectopy attributable to the LV catheter.

The effects of NTG on intracoronary dobutamine-induced increases in myocardial contractility were investigated to determine whether or not the agent's antifibrillatory effect involved post-receptor antiadrenergic action (n = 5). This inotropic sympathomimetic agent was chosen because of its relatively high selectivity for $beta_1$-adrenergic receptors, with only mild stimulatory influence on $beta_2$- or $alpha_1$-vascular receptors (21,22). Three 250-μg bolus doses of dobutamine were injected into the left main coronary artery at 30-min intervals. The first two injections provided control information. At 15 min before the third injection, NTG (4,000 μg bolus) was instilled into the pericardial space. Changes in HR, systolic blood pressure (SBP), and LV contractility, as measured by the maximum of the first time derivative of left ventricular pressure (LV dP/dt max), were recorded via a 7F pigtail catheter (Cordis Corp.) in the LV.

The hemodynamic effects of NTG administered via the intravenous or intrapericardial routes were compared (n = 8). Nitroglycerin (4,000 μg bolus) was injected in separate interventions into the pericardial space and into a femoral vein. Changes in HR, SBP, and LV dP/dt max associated with the two routes of administration were compared.

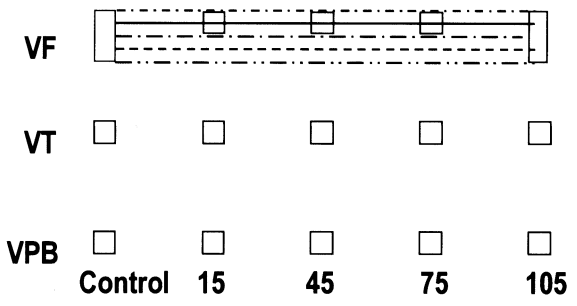
TWA and ST-segment analysis. Precordial lead V_3 (which recorded the highest TWA values among the surface leads), intracoronary, and LV intracavitary ECGs were analyzed at resting baseline and during occlusion for TWA magnitude, a robust marker of propensity to ischemia-induced lethal arrhythmias (23–25) by the modified moving average beat method (25). According to this technique, a stream of beats was divided into odd and even bins, and the morphology of the beats in each bin was averaged over a few beats to create a moving average complex; TWA was computed every 15 s as the maximum difference in amplitude between the odd-beat and the even-beat average complexes from the J-point to the end of the T-wave.

ST-segment deviation in the same leads and time points was calculated as the difference between isoelectric and the J-point and J-point-plus-60-ms levels for beats averaged for 15 s. ST-segment change was determined as the maximum difference in ST-segment deviations.

Statistics. Arrhythmia grade and VPB incidence were analyzed using Friedman's two-way analysis of variance by ranks with Dunn's post-hoc test. All other data are reported as mean $\pm$ SEM. Values for ST segments, TWA, HR, SBP, and LV dP/dt max were analyzed by analysis of variance with correction for repeated measures. A p value of $<$0.05 was considered statistically significant.

RESULTS

Intrapericardial NTG suppresses ventricular arrhythmias. Intrapericardial NTG consistently suppressed ischemia-induced VF. Control occlusions in all six animals resulted in VF, on average within 4 min after the start of occlusion (Fig. 2, top panel). At 45 min after intrapericardial NTG, arrhythmia grade was significantly reduced ($p < 0.05$) with five of six pigs displaying only VPBs (Fig. 2, bottom panel). This reduction to VPBs occurred in three of six pigs as early as 15 min after intrapericardial NTG administration and generally dissipated by 75 min. Intrapericardial NTG did not influence the degree of ischemia-induced ST-segment deviation (lead V_3, control: 2.90 $\pm$ 0.57 mm; 15 min post-NTG: 3.18 $\pm$ 0.87 mm; 45 min post-NTG: 3.52 $\pm$ 1.11 mm; 75 min post-NTG: 3.12 $\pm$ 0.74; 105 min post-NTG: 2.00 $\pm$ 0.98; p = NS). Intrapericardial NTG did not influence the magnitude of ischemia-induced hypotension and tachycardia, which averaged $<$10 mm Hg and $<$10 beats/min, respectively, and did not differ among occlusions.



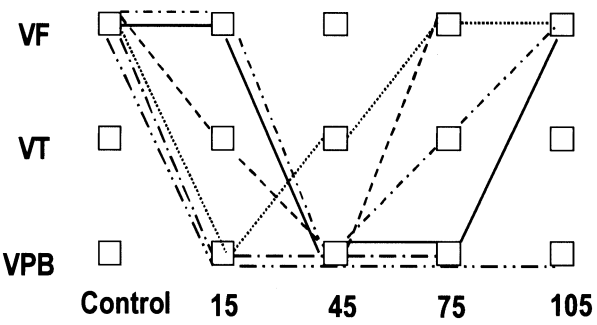


Figure 2. Suppression of ischemia-induced arrhythmias with intrapericardial nitroglycerin. **(Top)** Coronary artery occlusion consistently provoked severe arrhythmias after intrapericardial saline rather than nitroglycerin administration (n = 5). **(Bottom)** Arrhythmia severity was reduced 45 min after intrapericardial nitroglycerin (4,000 μg bolus) compared with control ($p < 0.05$). This reduction in arrhythmia grade appeared as early as 15 min and lasted up to 75 min in four of six animals. Each **line type** represents an individual experiment. VF = ventricular fibrillation; VPB = ventricular premature beats; VT = ventricular tachycardia (<10-s duration).

Intrapericardial NTG reduces TWA. Intrapericardial NTG decreased ischemia-induced TWA magnitude in a parallel time course with its suppression of ischemia-induced ventricular arrhythmias (n = 5). Nitroglycerin suppressed TWA during the occlusion at 45 min after NTG (intracoronary lead, control: 459.1 ± 144.4 μV before drug to 42.22 ± 13.96 μV; p = 0.047), but TWA recovered by 75 min post-NTG (276.6 ± 233.7 μV; p = 0.606) compared with control (Figs. 3 and 4). In the other leads, the reduction in TWA did not reach significance (LV lead: control, 112.42 ± 76.38 vs. NTG, 27.18 ± 12.35 μV; p = 0.289, and precordial lead V_3: control, 162.04 ± 120.1 vs. NTG, 45.48 ± 30.89 μV; p = 0.293). In this group, ST-segment deviations did also not vary among the control and post-drug coronary occlusions (control, 5.16 ± 1.35 mm; 15 min post-NTG, 4.50 ± 1.04 mm; 45 min post-NTG, 4.48 ± 0.78 mm; 75 min post-NTG, 4.58 ± 0.53 mm; p = NS).

Intrapericardial NTG attenuates adrenergic stimulation. Intrapericardial NTG significantly blunted the intracoronary dobutamine-induced augmentation of LV dP/dt max (n = 5). When intracoronary dobutamine was given at 15 min after intrapericardial NTG, an antiadrenergic effect

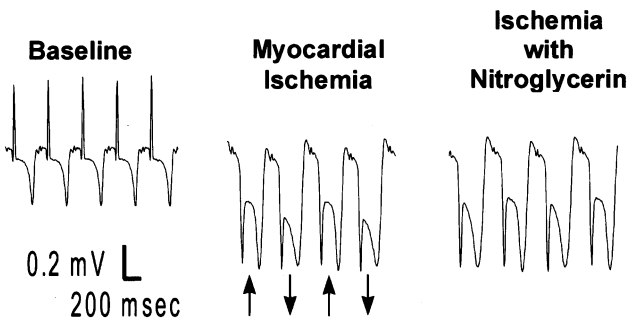


Figure 3. Ischemia-induced T-wave alternans (TWA) in intracoronary electrocardiogram tracings in a representative experiment; TWA was not evident at baseline, before occlusion of the left anterior descending coronary artery **(left).** Marked TWA (105.26 μV) appeared just before ventricular fibrillation onset during occlusion without drug **(middle)** but was minimized (42.10 μV) during the occlusion at 45 min after intrapericardial nitroglycerin (4,000 μg bolus) **(right)**.

of intrapericardial NTG was registered in decreased LV dP/dt max (from 3,999 ± 196 mm Hg/s to 3,543 ± 220 mm Hg/s, p = 0.012), but this effect, although suggestive, did not achieve statistical significance at 45 min (3,694 ± 312 mm Hg/s, n = NS) and was no longer evident at 75 min (4,048 ± 708 mm Hg/s, p = NS) after NTG (Fig. 5). Intrapericardial NTG did not blunt the intracoronary dobutamine-induced rise in HR (from 141 ± 10 beats/min to 137 ± 15 beats/min at 15 min, p = 0.62, to 153 ± 16 beats/min at 45 min, to 152 ± 18 beats/min at 75 min).

HR and hemodynamic response to intravenous versus intrapericardial NTG administration. Intravenous NTG (4,000 μg bolus) resulted in a transient reduction in systolic arterial blood pressure and LV contractility (dP/dt max) (n = 6, Fig. 6). By comparison, intrapericardial NTG (4,000-μg bolus) produced a slightly delayed, more moderate, and more persistent decrease in SBP and with a slight reduction in LV contractility (dP/dt max). In both groups,

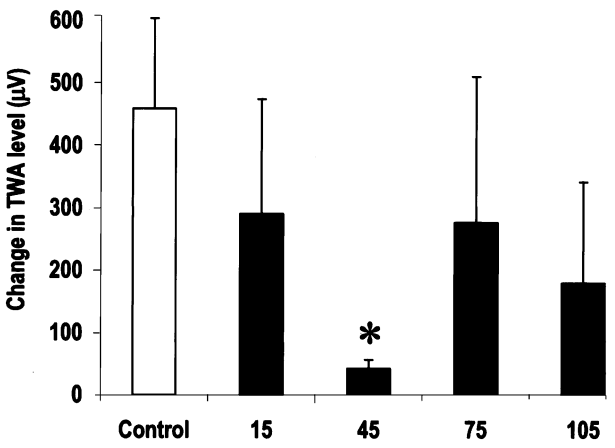


Figure 4. Intrapericardial nitroglycerin significantly attenuated the magnitude of ischemia-induced T-wave alternans (TWA) in the intracoronary lead at 45 min after delivery (*$p < 0.05$), in parallel with the agent's effect on arrhythmias; TWA recovered to pre-drug levels by 75 min after intrapericardial nitroglycerin.

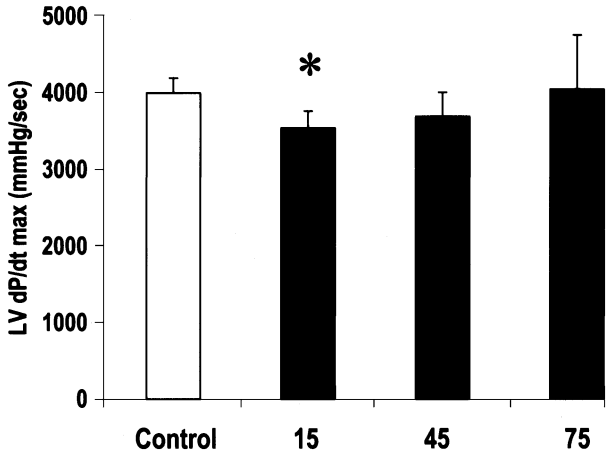


Figure 5. Effect of intrapericardial nitroglycerin on the rise in contractility in response to intracoronary bolus injections of dobutamine. At 15 min after intrapericardial nitroglycerin, the rise in contractility induced by intracoronary dobutamine was significantly blunted (*$p < 0.05$). Thereafter, nitroglycerin's effect did not achieve statistical significance. LV dP/dt max = maximum of the first time derivative of left ventricular pressure.

there was a transient rise in HR with maximum increase in the range of 10 beats/min.

DISCUSSION

Intrapericardial NTG potently suppresses ischemia-induced VF in parallel with TWA, a measure of cardiac electrical instability. These effects may be mediated through the agent's antiadrenergic action, which was implicated by its blunting the myocardial inotropic response to intracoronary dobutamine. The present electrophysiologic and antiarrhythmic results are consistent with nitroglycerin's well-established action as a NO donor.

Antifibrillatory action of NTG. Previous studies performed in a canine model indicated that intravenous NTG reduced the incidence of ischemia- and reperfusion-induced VF, increased ventricular electrical stability, reduced ischemic injury, and raised the VF threshold of nonischemic myocardium, especially when hypotensive effects and reflex sympathetic activation were prevented (13–16). Intravenous pretreatment with the NO precursor L-arginine protected against both stunning and reperfusion-induced VF in canines, probably by lessening endothelial injury (17). Clinically, intravenous NTG reduced the incidence of sustained repetitive ventricular response during electrophysiologic testing (18) and the number of ventricular ectopic beats in patients during acute myocardial infarction (19) and exercise testing (20).

Whether or not intrapericardial NTG reduces the extent of ischemic burden and the potential contribution of this factor to its antiarrhythmic action were unclear. Nitroglycerin's antiarrhythmic actions occurred in the absence of a significant reduction in ischemia-induced ST-segment levels in pigs, a species known to have poor collateral circulation (26), which minimizes the effects of vasodilator drugs on extent of ischemia. However, because ST-segment level

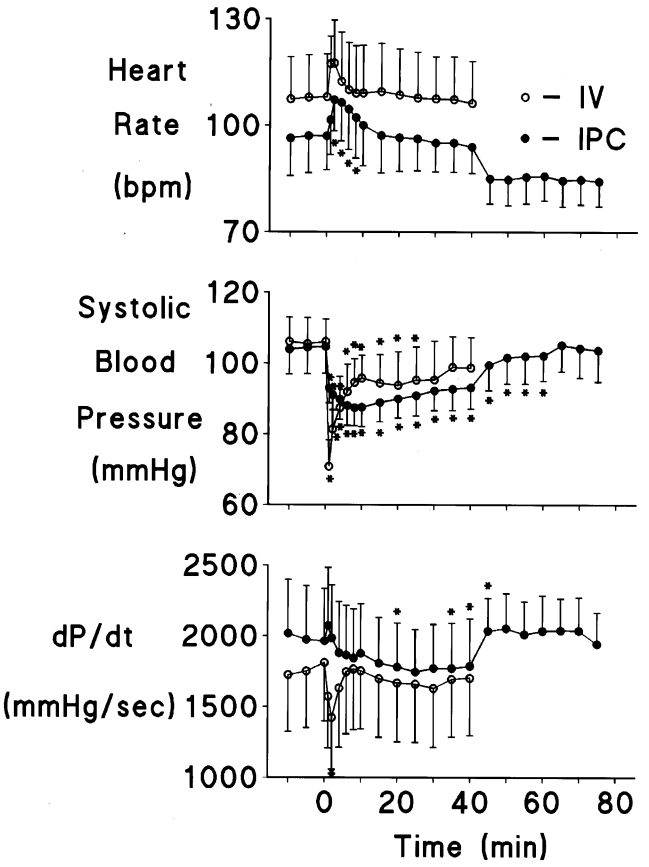


Figure 6. Heart rate and hemodynamic response to intravenous (IV) **(open circles)** as compared with intrapericardial (IPC) **(filled circles)** nitroglycerin administration. The period of IV monitoring was 40 min, corresponding to a time when the effect of IV nitroglycerin would be expected to be completely dissipated, and, in fact, the values had all returned to baseline at that point. The effects of IPC nitroglycerin were monitored for 75 min, the anticipated period of action and potential antiarrhythmic effects. *p = difference from pre-drug baseline, <0.05. The results were obtained in a total of eight animals, four of which received both IV and IPC nitroglycerin, and the remaining four received the drug through either the IV or IPC route. Randomizing the protocol was intended to reduce possible sequence-related effects.

does not provide a direct measure of myocardial blood flow and ischemia (27,28), definitive demonstration will require further experiments with assessment of regional myocardial blood flow and metabolism.

Reduction in TWA. The potent antifibrillatory effect of intrapericardial NTG was accompanied by a corresponding suppression of ischemia-induced TWA. Electrophysiologic mapping studies have determined that this alternating, beat-to-beat oscillation in T-wave morphology during myocardial ischemia is indicative of temporal and spatial unevenness of ventricular repolarization (29–31); TWA has also been demonstrated to be a measure of myocardial electrical instability that is correlated with the likelihood of life-threatening arrhythmias in diverse clinical conditions (32,33) and experimental settings (34). Intrapericardial NTG's suppression of TWA was maximum at 45 min post-drug, the period when NTG exerted its peak antiarrhythmic action. This effect was marked in

the intracoronary lead, which monitors the epicardial surface.

Attenuation of adrenergic influences. Fei et al. (35) demonstrated a pre-receptor, antiadrenergic effect of the NO precursor L-arginine using an open-chest canine model with a pericardial cradle. They reported that norepinephrine overflow from the coronary sinus induced by sympathetic nerve stimulation was diminished after bathing with L-arginine and postulated this mechanism for the agent's reduction of ischemia-induced ventricular arrhythmias. Our results demonstrate post-receptor antiadrenergic action, as intrapericardial NTG attenuated the inotropic response to intracoronary infusion of dobutamine, a sympathomimetic agent. This observation is consistent with previous findings indicating that NO significantly influences the myocardial contractile response to beta-adrenergic stimulation by dobutamine in normal dogs (36) and in humans with either LV dysfunction (37) or idiopathic dilated cardiomyopathy (38). The postulated basis for NO's negative inotropic responses during beta-adrenergic stimulation involves increased intracellular cyclic guanine monophosphate (cGMP), which inhibits the beta-adrenergic-stimulated increase in the L-type calcium channel current and reduces calcium affinity of the contractile apparatus (10) and has been shown to reduce cyclic adenosine 3′:5′-cyclic phosphate levels after direct beta-adrenergic stimulation (39), as well as the numerous downstream countervailing effects of cGMP in sympathetically stimulated myocardium. Nitroglycerin's suppression of TWA may also reflect blunting of adrenergic influences, which affect TWA magnitude (40). The basis for intrapericardial NTG's inability to blunt the HR increase provoked by intracoronary dobutamine is not known. A possible explanation is that the effects of NO on the sinus node pacemaker current (If) are quite variable and can include a stimulatory as well as an inhibitory response (41,42).

The functional half-life of intrapericardial NTG can be inferred from these data as 15 to 45 min, whereas intravenous NTG has a half-life of 3 to 5 min (43), with cardiovascular effects lasting 10 to 15 min. This prolongation in effect is most likely attributable to the absence of erythrocytes and their degradative enzymes from pericardial fluid, which had a hematocrit of <1%. The slow, gradual onset of the hemodynamic effects of intrapericardial NTG confirms the minimum leakage of nitroglycerin from the pericardial space.

Implications. Intrapericardial administration of NTG exerts a potent antifibrillatory effect. This action probably relates to the formation of NO, which is capable of blocking adrenergic profibrillatory influences and improving calcium handling during severe myocardial ischemia. From a broader perspective, these results underscore the potential for sustained action by cardioactive agents when delivered intrapericardially and highlight the potential utility of the NO pathway. Percutaneous delivery of these substances into the pericardial space could prove valuable both in elucidating fundamental modes of pharmacologic action and in leading to new therapeutic approaches to contain triggers of life-threatening arrhythmias.

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