J Anesth (2005) 19:45–53
DOI 10.1007/s00540-004-0268-y

Journal of
Anesthesia

Positive inotropic effect of nicorandil in adult rats in situ

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Abstract
Purpose. Although the cardiac protective and negative inotropic effects of nicorandil via opening the sarcolemmal or mitochondrial K_{ATP} channel and NO-like action are well known, positive inotropic effects of nicorandil in normal hearts have not been reported. The aim of the current study was to investigate how nicorandil affects left ventricular (LV) function of in situ adult rat hearts through the entire cardiovascular system.
Methods. We performed simultaneous and continuous measurements of LV pressure (P) by a catheter-tip micromanometer and LV volume (V) using a conductance catheter. We obtained steady-state LV P-V loops and intermittent curvilinear LV end-systolic pressure–volume relations (ESPVRs), LV end-systolic pressure (ESP_{mLVV}), and systolic pressure–volume area (PVA_{mLVV}) at midrange LVV (mLVV). We evaluated the effects of nicorandil on LV function by these mechanical indexes sensitively reflecting changes in LV contractility and work capability, preload (end-diastolic volume, EDV), and afterload (effective arterial elastance, Ea).
Results. Nicorandil (10 and 20 $\mu g \cdot kg^{-1} \cdot min^{-1}$) (blood concentration: 0.53 ± 0.14 and 1.48 ± 0.21 μg/ml) largely shifted ESPVR upward and thus significantly increased $ESP_{0.06}$, $PVA_{0.06}$, stroke volume due to increase in EDV, and ejection fraction without significant changes in Ea and heart rate. In contrast, an NO donor, nitroglycerin ($1 \mu g \cdot kg^{-1} \cdot min^{-1}$), significantly decreased Ea but did not change $ESP_{0.06}$, $PVA_{0.06}$, and EDV. Furthermore, either a nonselective K_{ATP} channel blocker, glybenclamide, or a mitochondrial K_{ATP} channel blocker, 5-hydroxydecanoate, abolished these nicorandil-induced positive inotropic actions.
Conclusion. These results suggest that nicorandil exhibited positive inotropic actions on LV function of in situ hearts in adult rats with resultant increased preload (EDV).

Key words Cardiac function · End-systolic pressure–volume relationship · Inotropic agent · K_{ATP} channels · Systolic pressure–volume area (PVA)

Address correspondence to: M. Takaki
Received: April 12, 2004 / Accepted: July 29, 2004

Introduction

The effect of nicorandil on different parts of the vascular system might be mediated by two different modes of actions: the large coronary arteries are dilated mainly through nitric oxide (NO)-like action, whereas coronary resistance vessels are dilated mainly through its K_{ATP} channel opening action [1]. Furthermore, nicorandil has been found to dilate veins, conductive coronary arteries, and small resistance arteries [1,2], suggesting the possibility that nicorandil decreases both preload and afterload and increases coronary blood flow. Either actions of nicorandil predominate at different concentrations: the NO-like actions are dominant at lower concentrations than those that exert the K_{ATP} channel opening actions in conscious dogs [3,4]. It was actually reported that at high concentrations nicorandil exerted negative inotropic effects on human left ventricular papillary muscles predominantly by opening the K_{ATP} channels [5]. In acute ischemic and congestive heart failure canine models, nicorandil was considered to improve cardiac contractility due to the reduction of both preload and afterload mediated by K_{ATP} channel opening actions with NO-like actions [6,7]. No positive inotropic effects of nicorandil on the normal heart model, however, have been reported.

We previously reported that end-systolic pressure–volume relations (ESPVRs) of the excised cross-circulated [8–10] and in situ ejecting rat hearts [11,12] are upward convex curves even within the physiological range, as in mouse hearts [13]. Furthermore, we have shown that systolic pressure–volume area (PVA, a total mechanical energy per beat) obtained from the curvilinear ESPVR is linearly related with myocardial oxygen consumption per beat in rat hearts [8–10] as in the canine heart [14]. This finding provided us with the possibility for evaluating left ventricular contractility and work capability of rat hearts using the ESPVR-PVA framework [11,12,15].

The purpose of the present study was to investigate how intravenously infused nicorandil affects the left ventricular function of in situ adult rat hearts. We hypothesized that nicorandil does not affect left ventricular function and work capability in adult rat hearts. To test this hypothesis, we obtained simultaneously continuous left ventricular pressure–volume data, described the pressure–volume loop and left ventricular ESPVR, and evaluated nicorandil-induced changes in ESPVR, ESP, and PVA at midrange left ventricular volume (ESP_{mLVV} and PVA_{mLVV}) and hemodynamic changes by preload (end-diastolic volume, EDV) and afterload (effective arterial elastance, Ea) [16,17].

Materials and methods

Left ventricular volumetric conductance catheter system

We used the previously developed type of a miniaturized 3 Fr conductance catheter and a conductance catheter signal processing apparatus (S-I Medico-tech, Osaka, Japan) for the rat [18]. The conductance catheter is equipped with six electrodes, of which the four inner electrodes (3 mm apart) sense three segmental conductance signals (G_1, G_2, and G_3).

The conductance catheter method of measuring LVV has been described in detail by Baan et al. [19,20]. Briefly, instantaneous intraventricular conductance volume $V(t)$ [$= V_1(t) + V_2(t) + V_3(t)$] is obtained from the measured conductance $G(t)$ [$=G_1(t) + G_2(t) + G_3(t)$] by the following equations:

$$V(t) = (1/\alpha) \times \rho \times L^2 \times G(t) - Vc \quad (1)$$

$$Vc = (1/\alpha) \times \rho \times L^2 \times Gp \quad (2)$$

where α is a dimensionless empirical constant for the $V(t) - G(t)$ relationship. This value was reasonably assumed to be 1.0 [15,18,20]. L (cm) is the distance between two adjacent sensing electrodes; ρ (Ω·cm) is the specific resistivity of blood; Gp ($S = 1/\Omega$) is the parallel conductance (i.e., conductance formed by tissues surrounding left ventricular cavity); and Vc (ml) is constant offset volume.

Surgical preparation

The investigation conformed with the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan.

Retired breeder male Wistar rats (400–530 g body weight; 10–20 weeks, adult rats) were anesthetized with pentobarbital sodium (50 mg/kg i.p.). Immediately before data sampling, an additional pentobarbital sodium (10 mg/kg i.p.) was administered to obtain a similar anesthetic level in individual adult rats. The trachea was intubated, and each rat was ventilated by a respirator (SN-480-7; Shinano, Tokyo, Japan) with room air ($F_{I_{O_2}} = 0.21$) to maintain P_{O_2}, P_{CO_2}, and pH within their normal range by measurements twice during the predrug infusion period. The chest was opened via a midline sternotomy, and the pericardium was dissected to expose the heart. A conductance catheter (custommade by Inter Medical, Tokyo, Japan; see below) was introduced into the left ventricle through an apical stab with a pursestring suture. The positioning of this catheter is critical to obtain reliable LVV data. We always confirmed that all segmental conductance volume changes were synchronous throughout each experiment. A 2.5 Fr catheter-tip micromanometer (Millar Instruments, Houston, TX, USA) was also inserted through the apex into the left ventricle. A polyethylene tube (3 Fr) was cannulated into the external jugular vein for intravenous injection or continuous infusion of the drug. A string occluder was placed loosely around the ascending aorta. The respirator was stopped during data acquisition to avoid respiratory fluctuation of cardiac signals.

Experimental protocols

Experiments were performed in 42 rat hearts. At first, specific resistivity of sampled blood was measured in a specially designed small cuvette (volume content, 0.2 ml) that was connected to the processing apparatus. When cardiac hemodynamics was stable, a series of left ventricular pressure–volume loops was obtained during increasing afterload by a gradual ascending aortic occlusion to evaluate left ventricular ESPVR. Left ventricular pressure and the three individual segmental conductance volume signals were digitized in 12-bit accuracy at a sampling frequency of 500 Hz for later analyses. The aortic occlusion was performed for 1–2 s until EDV slightly increased. The duration of occlusion was limited not to evoke any arrhythmia.

The sequence of experimental interventions is shown in Fig. 1. After collecting the baseline pressure–volume data, we continuously infused drugs (infusion rate: 4 ml·kg^{-1}·h^{-1}) via the polyethylene tube cannulated in the external jugular vein with a syringe pump. The animals were divided into six groups (each, $n = 7$), as follows: vehicle subgroup, physiological saline infused; N10 subgroup, nicorandil (10 μg·kg^{-1}·min^{-1}) infused; N20 subgroup, nicorandil (20 μg·kg^{-1}·min^{-1}) infused; TNG subgroup, nitroglycerin (1 μg·kg^{-1}·min^{-1}) infused; Glyben + N20 subgroup, after pretreatment with glybenclamide (10 mg/kg i.v.), nicorandil (20 μg·kg^{-1}·min^{-1}) infused; and 5-HD + N20 subgroup, after pretreatment with 5-hydroxydecanoate (5 mg/kg i.v.), nicorandil (20 μg·kg^{-1}·min^{-1}) infused.

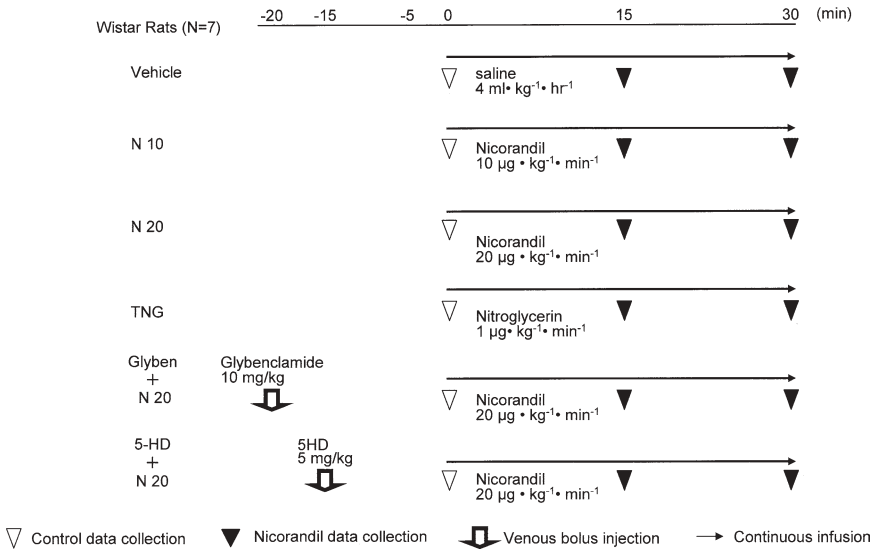

Fig. 1. Protocols for analysis of physiological function. Each group is composed of seven rats. *N*, nicorandil; *TNG*, nitroglycerin; *5-HD*, 5-hydroxydecanoate; ▽, control data collection; ▼, nicorandil data collection; ⇩, venous bolus injection; → continuous infusion of nicorandil

Nicorandil (Chugai, Tokyo, Japan), 5-hydroxydecanoate (Wako, Osaka, Japan), and nitroglycerin (Nippon Kayaku, Tokyo, Japan) were dissolved in physiological saline. Glybenclamide (Sigma, St. Louis, MO, USA) powder was dissolved in dimethyl sulfoxide (DMSO, final concentration, 0.3%). The data collection was performed 15 and 30 min (when the blood concentration of nicorandil reached almost maximum) after onset of infusion. At the end of the experiment, rat blood was sampled and stored at −80°C (7 adult rats). Nicorandil blood concentration was measured by high pressure liquid chromatography by Panafirm Laboratories (Kumamoto, Japan). Left ventricular pressure (LVP) was monitored by a pen recorder (Recti-Horiz-8K; NEC San-ei, Tokyo, Japan) throughout the experiment.

In the final part of each experiment, *Gp* and *Vc* were measured by injecting hypertonic saline (10% NaCl solution, 0.020–0.025 ml) into the pulmonary artery to transiently change the resistivity of the blood in the left ventricle, and we calculated the *Vc* value [15,18,20]. The calculated *Vc* value obtained by Eq. 2 was subtracted from the measured left ventricular conductance volume to obtain the left ventricular absolute blood volume, absolute LVV.

At the end of each experiment, injecting a lethal dose of pentobarbital sodium killed all 42 rats. The left ventricle including the interventricular septum was excised and weighed after both the atria and right ventricular free wall were trimmed off. The left ventricular weights measured (0.713 ± 0.048 g; range, 0.625–0.808 g) were used to normalize LVV by 1 g left ventricular mass in individual hearts.

Data analysis

A curvilinear ESPVR of in situ rat left ventricle is obtained by drawing an upper enveloping curve on a series of pressure–volume loops in a similar manner to our previous method [11,15] (Fig. 2). The left ventricular end-systolic pressure–volume data on the left upper shoulder of all the pressure–volume loops were plotted and fitted by the method of least squares using the following equation (Eq. 3) [11,15]:

$$\mathrm{LVP} = A\left\{1-\exp\left[-B\left(\mathrm{LVV}-V_0\right)\right]\right\} \tag{3}$$

where A, B, and V_0 are fitting parameters. We obtained the best-fit ESPVR curve in each of the 42 hearts.

Previous articles [11,15] validated this assumption; i.e., the end-systolic pressure–volume point obtained by an isovolumic one-beat clamp was located on or near the ESPVR curve of ejecting contractions. PVA of an isovolumic contraction represents the maximal capability of external mechanical work of the left ventricle at a given preload [21]. PVA as a function of LVV was obtained by integrating Eq. 3 from the extrapolated V_0 along the volume axis. The following Eq. 4 is the obtained function [11,15]:

$$\mathrm{PVA} = A\Big/\left(\mathrm{LVV}-V_0\right) - A\left\{1-\exp\left[-B\left(\mathrm{LVV}-V_0\right)\right]\right\}\Big/B \tag{4}$$

where LVV ranged between V_0 and about 0.25 ml/g.

In the present study, we chose 0.06 ml/g (=mLVV in the present study) as an appropriate common LVV, because the mean value of [V_0 + (maximum ESV − minimum ESV) on the ESPVR × 1/2] [11,12,15] was approximately 0.061 ± 0.011 ml/g (see Fig. 2). Mean V_0 value was 0.015 ± 0.009 ml/g.

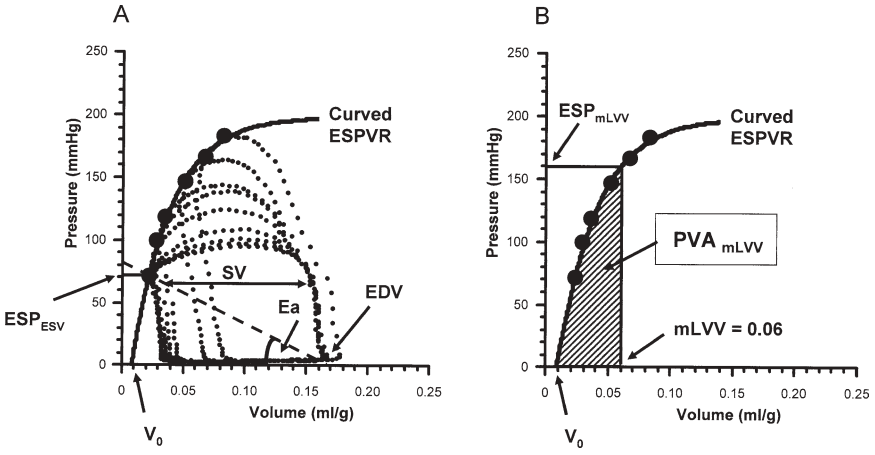

Fig. 2. A Representative left ventricular (*LV*) pressure (*P*)–volume (*V*) loops in ejecting rat hearts in situ during an aortic occlusion. *Solid circles*, end-systolic P-V data on the enveloping curve. *ESPVR*, end-systolic pressure–volume relation; *Ea*, effective arterial elastance; ESP_{ESV} (ESP at end-systolic volume)/*SV* (stroke volume); *EDV*, end-diastolic volume. **B** A representative curved ESPVR; *mLVV*, midrange LVV; ESP_{mLVV}, ESP at mLVV (=0.06 ml/g); PVA_{mLVV}, systolic pressure–volume area at mLVV (=0.06 ml/g). Volume axes indicate normalized absolute LV volume for 1 g LV mass in **A** and **B**. V_0, volume–axis intercept

ESP at ESV (ESP_{ESV}) was specifically defined to differentiate from ESP_{mLVV}. Ea is defined as the ESP_{ESV}/stroke volume (SV) ratio of the left ventricle under stable hemodynamics [16,17], and thus Ea depends on SV. SV was obtained by left ventricular (EDV − ESV). Ejection fraction (EF) was obtained by SV/left ventricular (EDV − V_0) (Fig. 2).

Statistics

All data were expressed as mean ± SD (standard deviation).

Statistical comparisons were performed by using the repeated-measure analysis of variance (ANOVA) for the hemodynamics. When multiple comparisons were made, one-way ANOVA and Dunnet post hoc test [exceptionally, Fisher protected least squares difference (PLSD) post hoc test for comparison of EDV among N20, Glyben + N20, and 5HD + N20] were used to determine the level of statistical significance. In all statistical tests, P values < 0.05 were considered statistically significant.

Results

Blood concentration of nicorandil

Blood concentration of nicorandil infused at the rate of 10 μg·kg^{-1}·min^{-1} was 525 ± 143 ng/ml after 30 min and 553 ± 170 ng/ml after 60 min. There were no significant differences between concentration after 30 min and that after 60 min. Thus, blood concentration of nicorandil infused at 20 μg·kg^{-1}·min^{-1} was measured after 30 min (when the blood concentration of nicorandil reached almost maximum) to be 1477 ± 208 ng/ml. This concentration of nicorandil is lower than that which exerts negative inotropic effects [5].

Curvilinear baseline ESPVR

Figure 3 shows representative data for each baseline series of left ventricular pressure–volume loops of in situ rat hearts while changing afterload by gradual aortic occlusion. We drew enveloping curves (ESPVR) on the series of pressure–volume loops. We obtained similar results in the other 39 rat hearts.

Comparison of effects of nicorandil infusion with those of vehicle infusion

Nicorandil infusion at 20 μg·kg^{-1}·min^{-1} (N20) markedly shifted ESPVR upward from the baseline ESPVR and increased left ventricular EDV (Fig. 3B). To investigate whether the positive inotropic effect of nicorandil is caused by an increase in circulation blood volume, we compared the effect of N20 with that of vehicle (saline) infusion (vehicle). In Fig. 3A,B, N20 shifted ESPVR upward associated with increased EDV at 30 min infusion, but the vehicle did not change ESPVR at 30 min infusion in each rat. We calculated $PVA_{0.06}$ values from each ESPVR. N20 increased $PVA_{0.06}$ in this heart from 3.9 to 5.6 mmHg·ml·beat^{-1}·g^{-1} (45% increase from the baseline) at 30 min. N10 and N20 significantly ($P < 0.05$) increased averaged $PVA_{0.06}$ from that in each baseline and vehicle at 30 min (Fig. 4A).

N10 and N20 decreased averaged Ea from each baseline at 30 min, but there were no significant differences in averaged Ea among vehicle, N10, and N20 (Fig. 4B). N10 and N20 significantly increased averaged EDV from each baseline at 30 min (Fig. 4C). N20 increased left ventricular EDV maximally from the baseline at 30 min (0.208 ± 0.027 ml/g vs 0.183 ± 0.024 ml/g), but there were no significant differences in left ventricular EDV between N10 and N20. N10 and N20 significantly increased EF from each baseline and vehicle at 30 min (Fig. 4D). N10 and N20 increased mean $ESP_{0.06}$ and SV

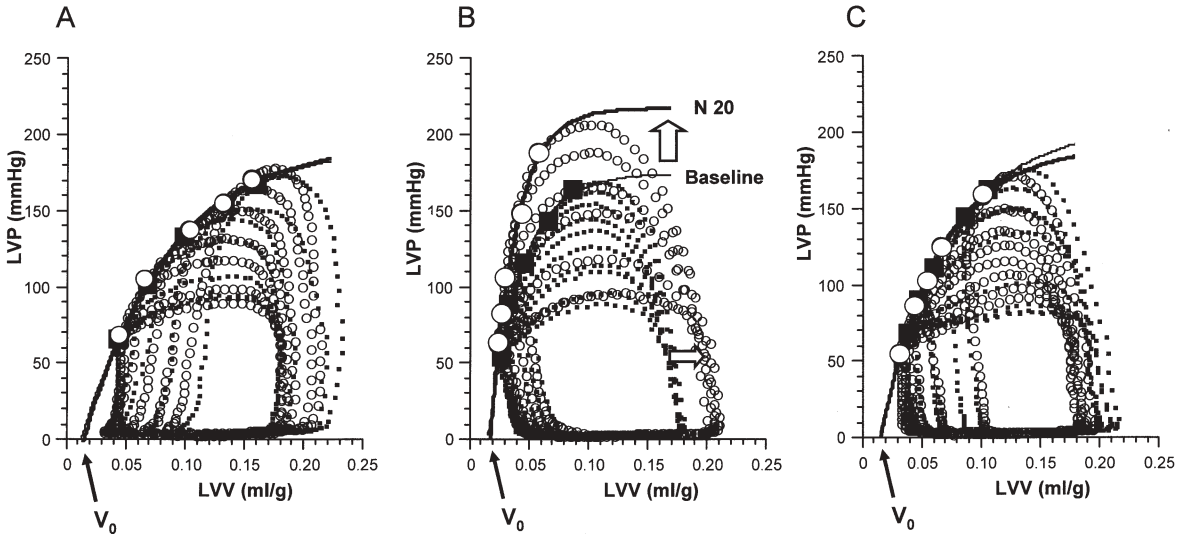

Fig. 3. Comparisons of left ventricular (LV) pressure–volume (P-V) loops and ESPVRs (end-systolic pressure–volume relations) in each heart between preinfusion (*solid squares*) and at 30 min infusions of vehicle (saline 4 ml·kg^{-1}·h^{-1}) (**A**), nicorandil 20 μg·kg^{-1}·min^{-1} (N20) (**B**) and nitroglycerin 1 μg·kg^{-1}·min^{-1} (TNG) (**C**) (*open circles*). **A** Vehicle did not change baseline ESPVR. Vehicle ESPVR overlapped with baseline ESPVR. **B** N20 ESPVR shifted upward from baseline ESPVR. **C** TNG ESPVR overlapped with baseline ESPVR. V_0 (volume-axis intercept) was not changed during each experiment

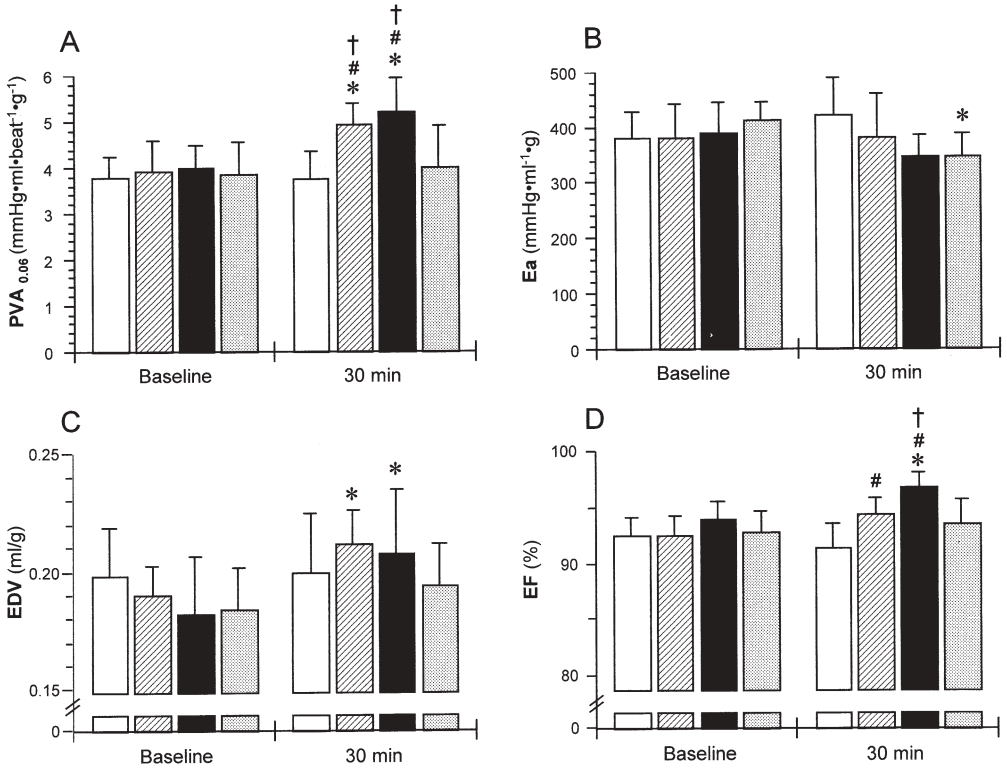

Fig. 4. Comparison of left ventricular (LV) systolic pressure–volume area ($PVA_{0.06}$) at a midrange LV volume (mLVV) of 0.06 ml/g (**A**), Ea (effective arterial elastance) (**B**), end-diastolic volume (EDV) (**C**), and ejection fraction [EF = SV/(EDV − V_0)] (**D**) among vehicle (*open columns*), nicorandil 10 μg·kg^{-1}·min^{-1} (N10) (*striped columns*), nicorandil 20 μg·kg^{-1}·min^{-1} (N20) (*solid columns*), and nitroglycerin 1 μg·kg^{-1}·min^{-1} (TNG)-infused rat hearts (*gray columns*). *Significantly different vs baseline (Dunnet); #significantly different vs vehicle (Dunnet); †significantly different vs TNG (Dunnet)

Table 1. Effects of nicorandil and nitroglycerin on left ventricular mechanoenergetic indexes

Index	Treatment	Baseline	30 min
$ESP_{0.06}$ (mmHg)	Vehicle	150.2 ± 13.3	150.3 ± 17.2
	Nicorandil (10 μg·kg^{-1}·min^{-1})	150.0 ± 15.3	181.2 ± 11.3*
	Nicorandil (20 μg·kg^{-1}·min^{-1}):N20	152.9 ± 18.1	189.7 ± 20.7*
	Nitroglycerin (1 μg·kg^{-1}·min^{-1})	151.5 ± 32.6	154.7 ± 33.2
	Glybenclamide (10 mg/kg) + N20	141.6 ± 20.5	147.6 ± 24.7
	5-Hydroxydecanoate (5 mg/kg) + N20	152.4 ± 21.8	158.1 ± 26.6
ESP_{ESV} (mmHg)	Vehicle	61.7 ± 6.1	68.8 ± 9.0
	Nicorandil (10 μg·kg^{-1}·min^{-1})	62.8 ± 6.2	67.4 ± 11.6
	Nicorandil (20 μg·kg^{-1}·min^{-1}):N20	59.5 ± 11.2	59.3 ± 10.4
	Nitroglycerin (1 μg·kg^{-1}·min^{-1})	64.4 ± 4.1	56.0 ± 7.3
	Glybenclamide (10 mg/kg) + N20	66.5 ± 6.2	65.2 ± 9.9
	5-Hydroxydecanoate (5 mg/kg) + N20	65.0 ± 8.2	68.2 ± 7.2
Stroke volume (ml/g)	Vehicle	0.171 ± 0.02	0.173 ± 0.02
	Nicorandil (10 μg·kg^{-1}·min^{-1})	0.169 ± 0.01	0.185 ± 0.01
	Nicorandil (20 μg·kg^{-1}·min^{-1}):N20	0.160 ± 0.02	0.182 ± 0.02*
	Nitroglycerin (1 μg·kg^{-1}·min^{-1})	0.160 ± 0.01	0.166 ± 0.01
	Glybenclamide (10 mg/kg) + N20	0.154 ± 0.02	0.155 ± 0.02
	5-Hydroxydecanoate (5 mg/kg) + N20	0.154 ± 0.02	0.152 ± 0.02
Heart rate (beats/min)	Vehicle	356.6 ± 38.1	350.6 ± 47.1
	Nicorandil (10 μg·kg^{-1}·min^{-1})	354.0 ± 43.8	347.1 ± 47.3
	Nicorandil (20 μg·kg^{-1}·min^{-1}):N20	348.0 ± 33.8	352.3 ± 48.7
	Nitroglycerin (1 μg·kg^{-1}·min^{-1})	348.0 ± 45.7	348.9 ± 37.9
	Glybenclamide (10 mg/kg) + N20	334.3 ± 28.1	344.6 ± 41.5
	5-Hydroxydecanoate (5 mg/kg) + N20	352.3 ± 16.1	352.3 ± 17.6

Values are mean ± SD
All groups, $n = 7$
$ESP_{0.06}$, end-systolic pressure at a midrange left ventricular volume (mLVV = 0.06 ml/g); ESP_{ESV}, end-systolic pressure at end-systolic left ventricular volume; N, nicorandil
* $P < 0.05$ vs baseline (Dunnet)

from the baseline at 30 min, but did not change mean ESP_{ESV} and heart rate (Table 1).

Comparison of positive inotropic effects of nicorandil infusion with no effects of nitroglycerine (TNG) infusion

In contrast to positive inotropic effects of nicorandil, nitroglycerin infusion at the rate of 1 μg·kg^{-1}·min^{-1} (TNG) did not change pressure-volume loops and ESPVR from the baseline (see Fig. 3C). TNG significantly decreased only averaged Ea from the baseline, but did not change averaged $PVA_{0.06}$, EDV, and EF at all (Fig. 4). TNG also decreased averaged ESP_{ESV}, but not significantly (see Table 1).

Effects of K_{ATP} channel blockers on the positive inotropic effect of nicorandil infusion

Pretreatment with a nonspecific K_{ATP} channel blocker, glybenclamide (10 mg/kg i.v.) (Glyben), antagonized (Fig. 5B) both the N20-induced upward shift of ESPVR and increase in EDV (Fig. 5A). Pretreatment with a mitochondrial K_{ATP} channel blocker, 5-hydroxydecanoate (5 mg/kg i.v.) (5-HD), also abolished (Fig. 5C) both the N20-induced upward shift of ESPVR and increase in EDV (Fig. 5A). Either Glyben or 5-HD alone did not affect ESPVR and EDV at all. The N20-induced increases in averaged $PVA_{0.06}$, EDV, and EF were abolished by pretreatment with Glyben or 5-HD (Fig. 6A,C,D). Although there were significant differences in averaged Ea between N20 and pretreatments with Glyben and with 5-HD, each averaged Ea was not significantly different from each baseline (Fig. 6B). Thus, it seems likely that Ea was not affected by N20 or by pretreatments with Glyben or with 5-HD.

Discussion

The shape of ESPVR in the rat left ventricle was an upward convex curve. We cannot use Ees (maximal elastance) to evaluate left ventricular contractility, and instead we used ESP_{mLVV} and PVA_{mLVV} in rat hearts [11,12,15]. As shown in the previous study [15], when both V_0 values in the baseline and drug-infused hearts are not significantly different, the evaluation method for the left ventricular contractility by using ESP_{mLVV} is reasonable. It is PVA, however, that closely and linearly

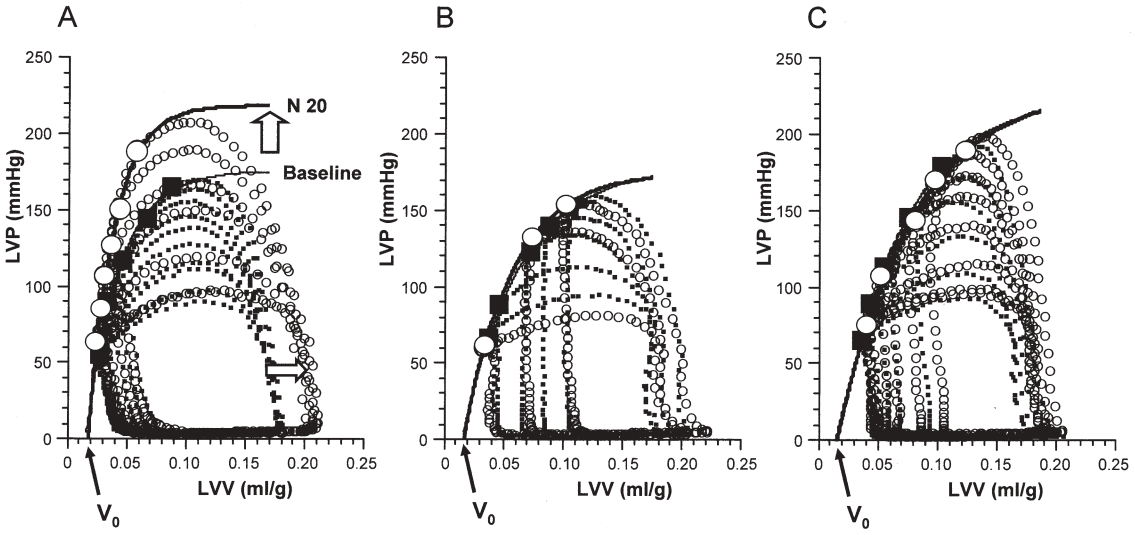

Fig. 5. Comparisons of left ventricular (LV) pressure–volume (P-V) loops and ESPVRs (end-systolic pressure–volume relations) among hearts at 30 min infusion with nicorandil 20 μg·kg^{-1}·min^{-1} (N20) (**A**), glybenclamide 10 mg/kg i.v. + nicorandil 20 μg·kg^{-1}·min^{-1} (Glyben + N20) (**B**), and 5-hydroxydecanoate 5 mg/kg i.v. + nicorandil 20 μg·kg^{-1}·min^{-1} (5-HD + N20) (**C**). Both glybenclamide and 5-hydroxydecanoate antagonized upward shifts of ESPVRs by N20. *Solid squares*, baseline ESPVRs; *open circles*, N20 ESPVR (**A**), Glyben + N20 ESPVR (**B**), and 5-HD + N20 ESPVR (**C**). V_0 (volume-axis intercept) was not changed during each experiment

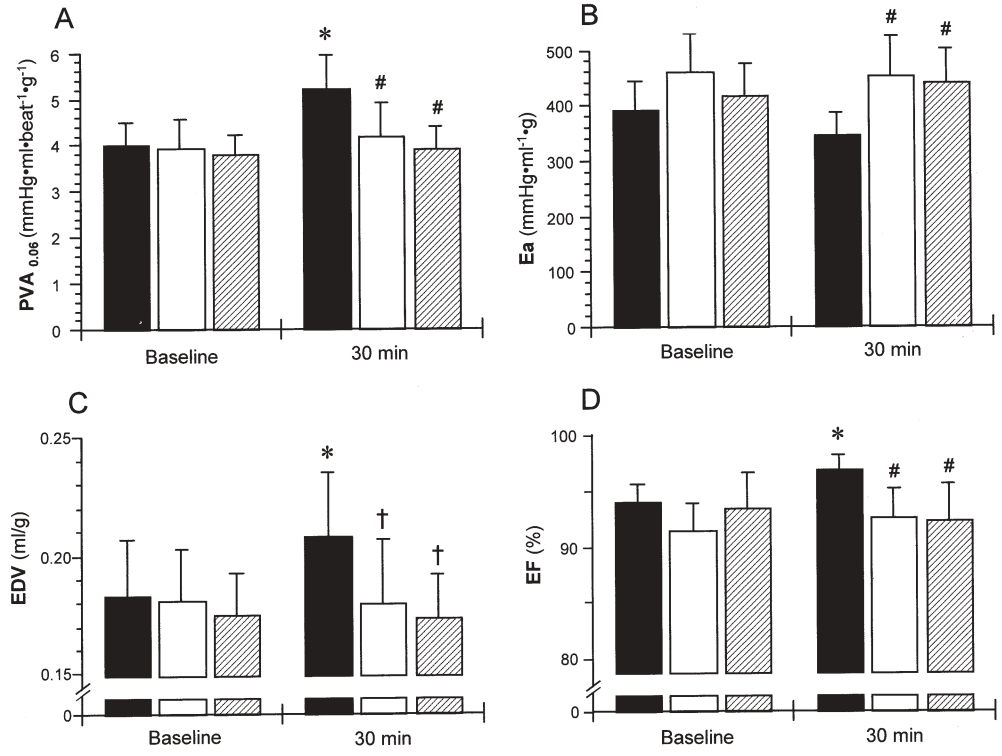

Fig. 6. Comparison of left ventricular (LV) systolic pressure–volume area ($PVA_{0.06}$) at a midrange LV volume (mLVV) of 0.06 ml/g (**A**), *Ea* (effective arterial elastance) (**B**), end-diastolic volume (EDV) (**C**), and ejection fraction [EF = SV/(EDV − V_0)] (**D**) among nicorandil 20 μg·kg^{-1}·min^{-1} (N20) (*solid columns*), glybenclamide 10 mg/kg i.v. + nicorandil 20 μg·kg^{-1}·min^{-1} (Glyben + N20) (*open columns*), and 5-hydroxydecanoate 5 mg/kg i.v. + nicorandil 20 μg·kg^{-1}·min^{-1} (5-HD + N20) (*striped columns*) infused rat hearts in controls and at 30 min N20 infusion. *Significantly different vs. baseline (Dunnet); # significantly different vs N20 (Dunnet); † significantly different vs N20 (Fisher PLSD)

correlates with cardiac oxygen consumption per beat under a variety of loading conditions in a stable contractility in the cross-circulated excised rat hearts, although a different type of model from the present one [8–10]. Therefore, the PVA can reasonably be used as a measure of a total mechanical energy (=work capability) as a function of preload of a ventricular contraction even under a nonlinear ESPVR in a rat left ventricle. We compared total mechanical energy with PVA at the same preload, mLVV.

In the present study, nicorandil infusion at concentrations of about 0.5–1.5 μg/ml significantly increased left ventricular $PVA_{0.06}$, EDV, SV, and EF of in situ rat hearts, but did not increase Ea. The increase in $PVA_{0.06}$ indicates the enhancement of left ventricular contractility (positive inotropic actions) and work capability. The increased EDV seems likely to be caused by the increase in venous return from the peripheral capacitance vessels due to the positive inotropic effect of nicorandil.

The same volume of vehicle affected neither left ventricular function nor EDV, suggesting that the increase of EDV induced by nicorandil does not result from a simple increase of the total circulating blood volume. The increase in EDV induced by nicorandil is not likely due to venous contractions, because nicorandil dilates capacitance vessels, as does nitroglycerin, but does not contract capacitance vessels [6]. Neither elongation of relaxation time nor the enhancement of lusitropy in the left ventricle seems likely to increase EDV, because the left ventricular end-diastolic pressure–volume curve at the filling phase was not affected by nicorandil (our observation).

The present dose of nicorandil is five to ten times higher than a clinical dose. However, the effective dose of any drugs for rats is generally higher than that in other species. We could not simply compare the present effective dose in rats with the clinical dose. Consequently, we could not simply extrapolate the result of nicorandil in rats for human patients.

In the acute ischemic heart failure canine model, nicorandil at similar concentrations to the present ones increased cardiac output and SV mediated by the drug's K-channel opening activities and by the reduction of venous tone by its nitrate properties [6]. Furthermore, in the canine acute congestive heart failure model, nicorandil may exert beneficial hemodynamic effects mainly due to decrease in both afterload and preload rather than the coronary vasodilator effect [7]. The present result in the adult rat heart, however, revealed that nicorandil increased preload (EDV) without any effects on afterload (Ea and ESP_{ESV}). Taken together, the different mechanisms from those in these failing heart models would contribute to the present nicorandil-induced positive inotropic actions with resultant increased preload in normal adult rats. Basal cardiac hemodynamics may be different between the failing heart model and the present normal adult rat hearts.

Nitroglycerine also did not affect $PVA_{0.06}$, EDV, SV, and EF, although it significantly decreased Ea from the baseline. Glybenclamide or 5-hydroxydecanoate antagonized nicorandil-induced positive inotropic effects on the left ventricle of the in situ rat hearts. Therefore, positive inotropic actions of nicorandil observed in the adult rat hearts are unlikely to be caused by the indirect coronary vasodilator effect.

Mitochondrial K_{ATP} channel openers such as diazoxide and pinacidil depolarized the mitochondrial membrane and thus reduced Ca^{2+} influx through the potential-dependent mitochondrial uniporter [22,23]. Furthermore, these drugs also activate release of Ca^{2+} from mitochondria through the mitochondrial permeability transition pores [22,23]. It might be possible that the K_{ATP} channel opener nicorandil reduces Ca^{2+} influx into mitochondria by depolarization of the mitochondrial membrane and activates release of Ca^{2+} from mitochondria. The resultant increase of cytosolic calcium concentration in the myocardium may exert the positive inotropic action. In contrast, there is another report showing that mitochondrial K_{ATP} channel openers will depolarize by only 1–2 mV and have little direct effect on Ca^{2+} uptake [24]. According to this report, mitochondrial K_{ATP} channel openers may exert no changes in cytosolic calcium concentration and thus no positive inotropic actions. Furthermore, a recent paper suggests that diazoxide (a mitochondrial K_{ATP} channel opener) and 5-hydroxydecanoate (a mitochondrial K_{ATP} channel blocker) may act on cardiac mitochondria independently of mitochondrial K_{ATP} channels [25].

Taken together, the present result exhibiting antagonism by only one mitochondrial K_{ATP} channel blocker, 5-hydroxydecanoate against the novel positive inotropic effect of nicorandil, could not determine the underlying mechanisms. On the other hand, in young Wistar rat hearts where basal left ventricular mechanoenergetic indexes did not differ from those in the present adult rat hearts, we could not obtain any positive inotropic effects of nicorandil (our unpublished observations). Therefore, age-related changes in pharmacodynamics or metabolism may cause the different response to nicorandil between young and adult rat hearts.

Limitations of the present study

High-dose pentobarbital anesthesia had negative inotropic effects [26]. Therefore, the possibility that pentobarbital anesthesia had already depressed left ventricular contractility cannot be excluded. Although heart rate affected the linear ESPVR in canine hearts [27], in the present in situ rat hearts, the mean heart rate

was within 334–357 beats/min, and thus the curved left ventricular ESPVR was unchanged within this physiological heart rate range, as previously reported [11].

Conclusion

The present results indicate that nicorandil specifically enhances left ventricular contractility (novel positive inotropic actions) and work capability of in situ adult rat hearts associated with resultant increased EDV and without vasodilator actions. We believe that the present methods are highly sensitive to evaluate any changes in left ventricular function in in situ adult rats.

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