

Effects of R 56 865, a Preventer of Cellular Calcium Overload, on Left Ventricular Diastolic Properties During Pacing-Induced Ischemia in Dogs

G. Vandeplassche, C. Hermans, L. Wouters, and M. Borgers

Department of Cardiovascular Pharmacology, Janssen Research Foundation, Beerse, Belgium

Summary: The effects of R 56 865, a compound with unusual calcium antagonistic properties, on altered left ventricular (LV) diastolic properties were studied during pacing-induced ischemia in dogs with coronary stenosis. Severe coronary artery stenosis was produced on both the left anterior descending (LAD) and circumflex (Cx) coronary arteries in anesthetized β -blocked dogs. The right atrium was paced at 200 beats/min for 3 min. In the post-pacing period and before drug intervention, the most characteristic observation was a significant increase in LV end-diastolic pressure (LVEDP) and in the time constant of (τ) LV pressure decrease; after return of LV hemodynamics to baseline values (15 min), R 56 865 (0.16

mg $\cdot$ kg⁻¹) or solvent (control) was injected and four subsequent pacing runs were performed at 30-min intervals. In the postpacing period of these four subsequent runs, there was a steep increase in LVEDP and τ -values while HR returned more slowly to baseline values. After R 56 865 infusion, the increase in LVEDP and τ -values was significantly lower than in the pretreatment run and all LVEDP values were significantly lower than in the control group. We conclude that R 56 865 effectively attenuates ischemia-induced changes in LV diastolic stiffness. **Key Words:** R 56 865—Calcium overload—Ventricular relaxation.

In several studies, a profound impairment of left ventricular (LV) relaxation and LV diastolic compliance has been observed in patients during an episode of exercise-induced angina pectoris (1-4). A similar increase in LV end-diastolic pressure (LVEDP) has been observed during pacing-induced tachycardia in dogs with critical coronary stenosis (5,6).

The cardiac pathophysiology underlying these observations has been the subject of considerable debate. Impairment of LV diastolic compliance has been ascribed to a sustained intracellular calcium availability resulting in a persistent contractile element interaction in the ischemic myocardium throughout diastole (7). The increase in LVEDP has been explained by an impairment of intracellular calcium homeostasis; however, if this is true, the increase in LVEDP is not mediated by increased calcium influx through the slow channels because pretreatment with verapamil does not prevent it (7).

The compound R 56 865 (N-[1-[4-(4-fluorophenoxy)butyl]-4-piperidinyl]-N-methyl-2-benzothiazolamine) (Janssen Research Foundation, Belgium) has been shown in various species to protect against ouabain-induced cardiac toxicity (8-11) and against functional defects elicited by ischemia or hypoxia in isolated heart preparations (12-14). We evaluated whether R 56 865 influences changes in LV diastolic properties elicited by pacing-induced ischemia in dogs with coronary stenoses.

MATERIALS AND METHODS

Experimental procedure

The experiments were performed on 20 mongrel dogs of either sex ranging in body weight from 15 to 25 kg. After premedication with piritramide (5 mg $\cdot$ kg⁻¹) and anesthesia with nembutal (15 mg $\cdot$ kg⁻¹), the animals were intubated and ventilated (50% O₂, 50% N₂O) using a Siemens Servo-ventilator (type 900). The ECG was de-

Received September 10, 1990; revision accepted December 15, 1990.

Address correspondence and reprint requests to Dr. G.

Vandeplassche at Janssen Research Foundation, Department of Cardiovascular Pharmacology, Turnhoutseweg, 30, B-2340 Beerse, Belgium.

rived from limb leads (standard lead II). LV pressure (LVP) and aortic blood pressure (AoP) were measured by retrograde catheterization through the femoral arteries with catheter-tip manometers 7F (Honeywell). Zero-line calibration (LVP) was achieved by simultaneously recording the pressure signal through the lumen of the catheter with an external pressure transducer (Gould 231D) positioned at midchest level and equating both zero lines on the recorder.

A femoral vein was cannulated for injection (room temperature) of the solvent cyclodextrine 10% or for injection of the compound R 56 865 ($0.16 \text{ mg} \cdot \text{kg}^{-1}$). Pacing electrodes were connected to a catheter which was advanced through the left jugular vein until the electrodes were placed into the right atrium. A left thoracotomy was performed through the fifth intercostal space, and the pericardium was opened. The proximal circumflex coronary artery (Cx) and left anterior descending coronary artery (LAD) were freed from adipose tissue.

Propranolol ($0.5 \text{ mg} \cdot \text{kg}^{-1}$ intravenously, i.v.) was injected at this stage of the experiment to prevent ventricular fibrillation (VF) (15). Electromagnetic flow probes (Skalar) for flow measurement and lexan clips to reduce antegrade coronary blood flow to 50% of baseline value were placed on LAD and Cx arteries. Continuous registration of the signals was performed on an eight-channel ink-jet recorder (Mingograph).

The pressure values and ECG signals were fed by transducer amplifiers into a digital minicomputer (PDP 11 23) connected to a dual diskette drive and displayed on a MacIntosh. The following variables were calculated on line: heart rate (HR) (beats/min), systolic and diastolic aortic blood pressure (AoPs and AoPd) (mm Hg), LVP (mm Hg) and its first derivative ($\text{LVdP}/dt_{\text{max}}$ and $\text{LVdP}/dt_{\text{min}}$) (mm Hg $\cdot \text{s}^{-1}$), LVEDP (mm Hg), and rate pressure product (RPP). The time constant of LVP decrease (τ) (ms) was measured as described previously (16).

Experimental protocol

Pretreatment pacing run. The left atrium was paced at a rate of 1.5 times resting HR for 3 min, and hemodynamics were recorded 15, 30, and 46 s and 1, 2, 3, 4, and 5 min after the end of the pacing period. Dogs entering ventricular failure after the pretreatment pacing-run showed a substantial reduction in both AoP and LVdP/dt values that was not comparable to results obtained from the other dogs. Therefore, the former dogs were excluded from the analysis. Most probably, a critical degree of stenosis or the presence of a too-extensive myocardial ischemia or both were responsible for this phenomenon. A reduction in AoPs $>20\%$ was considered a criterion to exclude dogs from analysis. Four dogs developed LV failure and 3 developed VF; these 7 dogs were excluded from analysis. The remaining dogs were randomly allocated to R 56 865 or its solvent (control).

Pacing runs subsequent to pharmacologic intervention. Dogs received i.v. injection of R 56 865 ($0.16 \text{ mg} \cdot \text{kg}^{-1}$; $n = 6$) or its solvent (β -hydroxypropylcyclodextrine; $n = 7$) 15 min after the first pretreatment pacing run. Thereafter, four subsequent pacing runs were performed at 30 min intervals, and the influence of R 56 865 on resting and postpacing hemodynamics was analyzed. The effects of treatment on LV hemodynamics were followed ~ 105 min after injection with subsequent pacing runs every 30 min (i.e., 15, 45, 75, and 105 min postinjection).

Statistical analysis

The values of the first run of seven animals in the control and six in the R 56 865 group were taken to evaluate the changes induced by pacing tachycardia. The statistical significance of the changes from the resting values at each recorded time interval was determined by Wilcoxon's test.

The effects of treatment were analyzed by computing, for each subsequent run, the average change during the first minute. This was done by computing for each run the time-average by integration with the linear trapezoidal rule. Within-group comparisons were made by comparing the average change of each run with the change obtained for the pretreatment run using Wilcoxon's test. The differences between the individual average change and those obtained from the pretreatment runs were computed. Group comparison was made for each posttreatment run. The differences obtained for the control group were compared with those of the experiments with R 56 865 by Mann-Whitney U test. Results were expressed as percentages of the pretreatment run. In all statistical tests, two-tailed probabilities <0.05 were considered statistically significant.

RESULTS

Postpacing hemodynamics before drug administration

In 13 of 20 animals, a significant increase occurred in HR and τ -value up to 1 min and in LVEDP up to 5 min after the first pretreatment pacing run was stopped. In these animals, LV function remained preserved, with changes in AoP and $\text{LVdP}/dt_{\text{max}}$ and $\text{LVdP}/dt_{\text{min}} <10$ and $<200 \text{ mm Hg} \cdot \text{s}^{-1}$, respectively (Table 1). Changes in AoP $<10 \text{ mm Hg}$ and in $\text{LVdP}/dt_{\text{max}}$ and $\text{LVdP}/dt_{\text{min}}$ values $<200 \text{ mm Hg} \cdot \text{s}^{-1}$ were considered of no physiologic importance, despite statistical significance. The hemodynamic variables reflecting pacing-induced changes in the presence of a reduced coronary perfusion were considered to be HR, τ -value, and especially LVEDP.

Influence of R 56 865 on resting hemodynamics

Intravenous administration of R 56 865 ($0.16 \text{ mg} \cdot \text{kg}^{-1}$) or its solvent, given 15 min after the first pacing run before initiation of the subsequent runs induced no significant changes in hemodynamic measurements (Table 2).

Influence of R 56 865 on postpacing hemodynamic measurements

After administration of R 56 865 ($n = 6$), a significantly slighter increase in LVEDP was obtained for all four subsequent runs as compared with the control group. Furthermore, although in the control group the values of the increase in LVEDP tended to be higher than in the pretreatment period, they were significantly lower for the treated animals. In the control group ($n = 7$), subsequent pacing runs resulted in a steeper increase in LVEDP than in the pretreatment run (Fig. 1).

TABLE 1. Effect of pacing-induced ischemia and of R 56 865 in dogs with severe stenosis of LAD and Cx coronary arteries on LV hemodynamics

Parameter	HR	AoPs	AoPd	LVEDP	LVdP dt_{max}	LVdP dt_{min}	τ Value
Pacing-induced ischemia							
Control	92 $\pm$ 11	100 $\pm$ 6	77 $\pm$ 6	5.5 $\pm$ 0.3	1,667 $\pm$ 127	1,652 $\pm$ 129	43 $\pm$ 3.3
Postpacing							
15 s	118 $\pm$ 10*	105 $\pm$ 6*	82 $\pm$ 4*	13.0 $\pm$ 1.6*	1,543 $\pm$ 192	1,371 $\pm$ 174*	68 $\pm$ 9*
30 s	113 $\pm$ 10*	108 $\pm$ 7*	87 $\pm$ 7*	10.0 $\pm$ 0.6*	1,624 $\pm$ 178	1,515 $\pm$ 176	61 $\pm$ 10*
45 s	113 $\pm$ 10*	107 $\pm$ 7*	86 $\pm$ 7*	9.2 $\pm$ 0.4*	1,719 $\pm$ 208	1,627 $\pm$ 172	53 $\pm$ 7*
60 s	112 $\pm$ 10*	106 $\pm$ 6*	85 $\pm$ 7*	8.6 $\pm$ 0.6*	1,798 $\pm$ 239	1,705 $\pm$ 182	48 $\pm$ 6*
2 min	111 $\pm$ 11*	106 $\pm$ 7*	85 $\pm$ 7*	7.5 $\pm$ 0.7*	1,851 $\pm$ 224	1,790 $\pm$ 177*	44 $\pm$ 4
3 min	109 $\pm$ 11*	106 $\pm$ 7*	84 $\pm$ 8*	6.8 $\pm$ 0.6*	1,874 $\pm$ 226	1,844 $\pm$ 179*	42 $\pm$ 4
4 min	107 $\pm$ 10*	105 $\pm$ 7	82 $\pm$ 8	6.3 $\pm$ 0.5*	1,841 $\pm$ 198	1,832 $\pm$ 173*	40 $\pm$ 3
5 min	105 $\pm$ 10	104 $\pm$ 7	81 $\pm$ 7	6.0 $\pm$ 0.5*	1,826 $\pm$ 186	1,497 $\pm$ 298*	40 $\pm$ 3
R56 865							
Before	88 $\pm$ 17	102 $\pm$ 11	79 $\pm$ 13	6.1 $\pm$ 1.4	1,634 $\pm$ 381	1,538 $\pm$ 275	47 $\pm$ 9
After							
5 min	93 $\pm$ 16	100 $\pm$ 13	77 $\pm$ 15	6.1 $\pm$ 1.5	1,625 $\pm$ 435	1,530 $\pm$ 376	47 $\pm$ 9
15 min	83 $\pm$ 12	95 $\pm$ 11	74 $\pm$ 13	5.5 $\pm$ 1.3	1,508 $\pm$ 345	1,409 $\pm$ 256	46 $\pm$ 7

HR, heart rate; AoPs and AoPd, systolic and diastolic aortic pressure; LVEDP, left ventricular end-diastolic pressure; LV dP dt_{max} and dt_{min} , maximum and minimum of the first derivative of LV pressure; τ -value, time constant of LV pressure decrease.

Values are mean $\pm$ SEM.

* $p < 0.05$, postpacing values versus control values.

Similar pharmacologic effects on subsequent pacing runs were noted for HR (Fig. 2). After solvent administration, HR decreased less markedly than in the pretreatment run during the first minute postpacing. This difference remained significant after the third pacing period. In contrast, HR returned faster to baseline values within 45 min after R 56 865; this effect was significantly different from that noted in the control group as well as from the value in the pretreatment run.

In the control group (solvent-only), τ -value increased during the first minute postpacing to a value higher than the increase in the pretreatment pacing

run. In contrast, after administration of R 56 865, the pacing-induced increase in τ -value was significantly lower than that in the pretreatment pacing run (Fig. 3). Despite substantially lower absolute values for increase in τ in the first minute postpacing in R 56 865-treated dogs, statistical significance for a difference after solvent treatment was not reached owing to the high variability in both groups.

DISCUSSION

In this study, pacing-induced ischemia in dogs with critical coronary stenosis was used as a model

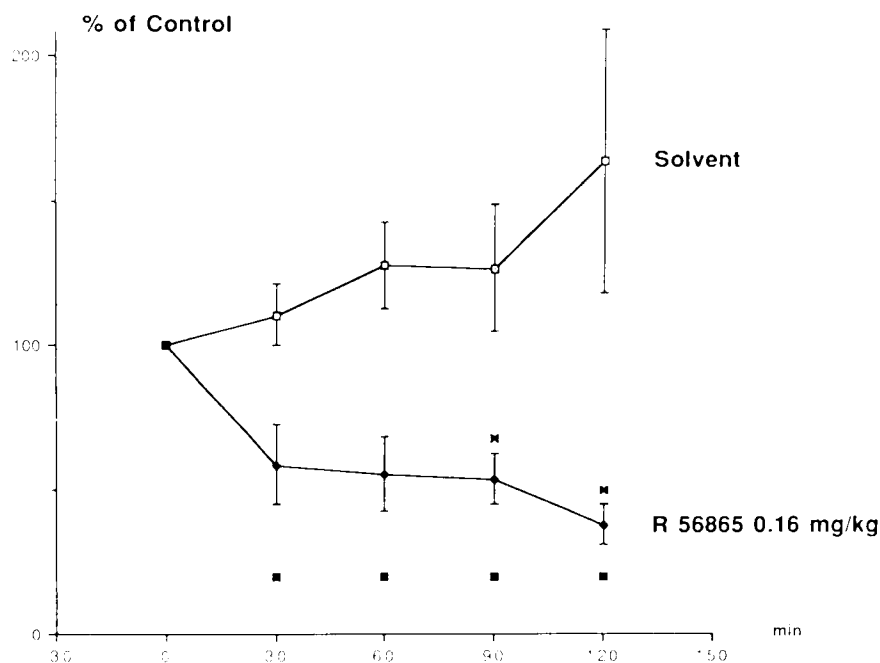
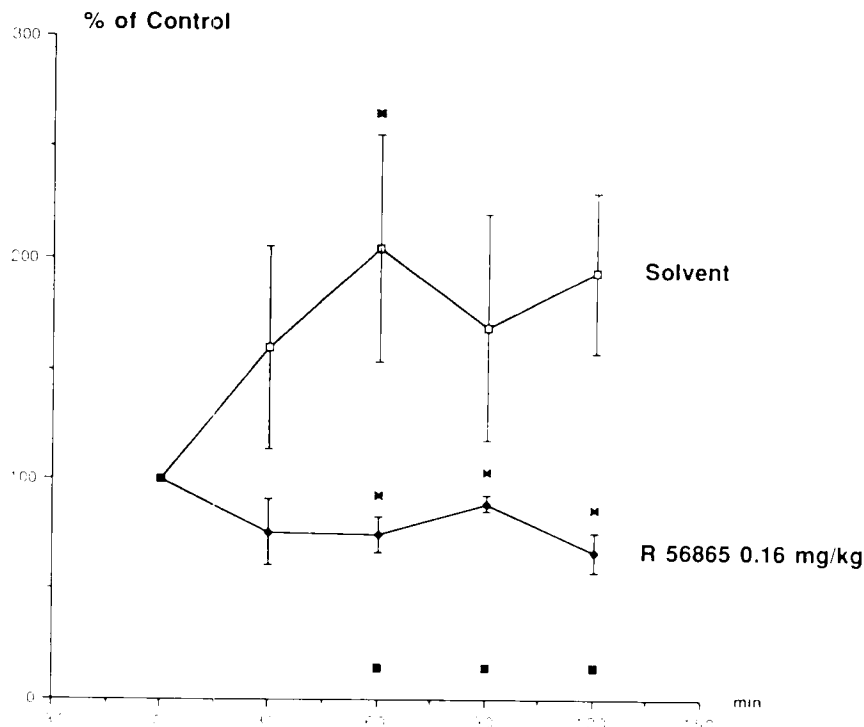


FIG. 1. Effect of four subsequent pacing runs on left ventricular end-diastolic (LVEDP) in solvent- and R 56 865-treated groups. The changes occurring during the first minute in the control pacing run are set at 100%; the changes occurring in subsequent pacing runs (i.e., after solvent or compound administration) are expressed as percentage of change in comparison with the changes noted in the control run. * $p < 0.05$, R 56 865 versus pretreatment value; $p < 0.05$, R 56 865 versus control group (solid squares).

FIG. 2. Effect of four subsequent pacing runs on heart rate (HR) in solvent- and R 56 865-treated groups. The changes occurring during the first minute in the control pacing run are set at 100%; changes occurring in subsequent pacing runs (i.e., after solvent or compound administered) are expressed as percentage of change in comparison with the changes noted in the control run. * $p < 0.05$; R 56 865 versus pretreatment value; $p < 0.05$; R 56 865 versus control group (solid squares).



to study pharmacologic effects on impaired ventricular relaxation. Such an ischemia elicited by energy demand induces a steep increase in LVEDP and in the time constant of LV pressure decrease (τ -value), indicative of impaired diastolic ventricular function. These findings are in agreement with results obtained in other studies (7,17–19). A similar

increase in LVEDP induced by atrial pacing (1) or by exercise (20) has been observed in patients with angina pectoris. An impairment of LV isovolumic relaxation during pacing-induced ischemia was also observed by McLaurin and colleagues (21).

The mechanism of the abnormalities in diastolic function causing an increase in LVEDP during an

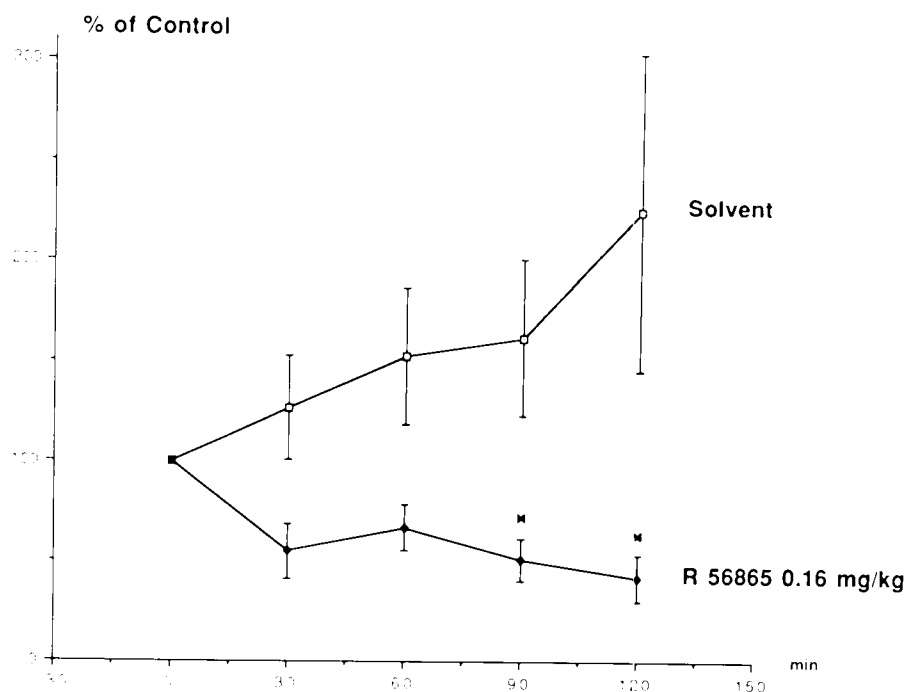


FIG. 3. Effect of four subsequent pacing runs on the time constant of left ventricular pressure decrease (τ -value) in solvent- and R 56 865-treated groups. The changes occurring during the first minute in the control pacing run are set at 100%; changes occurring in subsequent pacing runs (i.e., after solvent or compound administration) are expressed as percentage of change in comparison with the changes noted in the control run. * $p < 0.05$; R 56 865 versus pretreatment value.

anginal attack remains controversial. In contrast to ischemia elicited by coronary artery occlusion, ischemia induced by increasing myocardial energy demand in the presence of coronary stenosis in open-chest dogs results in an increase in myocardial muscle stiffness (19). Thus, the changes in the physical properties of the myocardium induced by high-energy demand ischemia appear to be directly related to an impairment of the active relaxation process, affecting primarily the myocardial cell. High-energy demand ischemia may impair cellular metabolism so that calcium handling is altered, which can cause a persistent interaction of contractile proteins throughout diastole and thus increased active diastolic stiffness. It has been reported that verapamil cannot prevent, whereas caffeine increases, this effect of pacing-induced tachycardia (7). The possibility that such a mechanism is indeed involved in the decay of ventricular relaxation capacity induced by ischemia is supported by several observations. Paulus and co-workers (7) showed that a reversible form of hypoxic cardiac muscle contracture may explain the decreased ventricular distensibility. The cause of the increase in myocardial stiffness after cardiac pacing, contributing to the observed diastolic abnormalities of pacing ischemia, could be similar to the stiffness observed during hypoxic cardiac muscle contracture, i.e., persistence of cross-bridges because of impaired intracellular calcium sequestration and because of the formation of rigor bonds (22). Verapamil, blocking calcium influx through the L-type slow channels, did not prevent such ischemic relaxation. In contrast, caffeine, blocking Ca^{2+} reuptake by the sarcoplasmic reticulum, increased such a loss of ventricular distensibility elicited by pacing-induced tachycardia (7). The response to caffeine and verapamil was similar in both hypoxic contracture and pacing-induced ischemia, indicating that intracellular calcium redistribution plays a central role under both conditions. Therefore, both hypoxic contracture and the increased LVEDP under ischemic conditions could be mediated by impaired extracellular calcium homeostasis rather than by an increased calcium influx through the slow calcium channels in the myocardial cells.

τ Value increased during pacing-induced ischemia anesthetized dogs with coronary stenosis. This further slowing of isovolumic pressure decay could be a manifestation of a rather load-insensitive relaxation of the ventricular cardiac muscle in response to ischemia (23). Thus, incomplete relaxation or partial inactivation results in persistent interaction of contractile elements throughout the diastole. As such, it imposes an active stiffness on the passive elasticity of the ventricular myocardium.

R 56 865 i.v. prevents the ischemia-induced increase in LV diastolic stiffness. These results corroborate the earlier documented protective effects

of R 56 865 against the cardiac dysfunction induced by ouabain or ischemia and hypoxia in vitro and in vivo (8–10,12,13).

Whether the protective effects of R 56 865 observed during ischemia and its protection against ouabain-induced toxicity are mediated through the same mechanism is not clear. R 56 865 prevents contractions elicited by perturbations of ion channels in isolated cardiomyocytes (14), probably by limiting intracellular calcium overload or the toxic consequences thereof (10); this was demonstrated recently in in vitro experiments using isolated rat and rabbit cardiomyocytes (14). After exposure of the cells to pathologic stimuli (veratridine, singlet oxygen, lysophosphatidylcholine, or ouabain) irreversible hypercontracture was observed as a consequence of intracellular calcium overload. Tetrodotoxin (inhibitor of fast sodium channel) and reduced extracellular sodium, but not nifedipine, protected against these pathologic stimuli, which suggested that intracellular calcium overload induced by these stimuli was mediated by dysfunction of the fast sodium channel, resulting in sodium overload followed by calcium overload owing to sodium/calcium exchange. R 56 865 dose-dependently prevented hypercontracture induced by pathologic stimuli, suggesting that R 56 865 may interfere with this pathologic sodium channel dysfunction in a way different from that of class I antiarrhythmic drugs. A possible explanation for the effects we observed may be that R 56 865 finally results in preservation of homeostasis for ions such as calcium in the cell. By such a mechanism, the compound then may prevent sustained intracellular calcium availability and interrupt the persistent interaction of contractile element in diastole. Such a pharmacologic effect may then attenuate impairment of diastolic-ventricular relaxation induced by myocardial ischemia.

Acknowledgment: We thank Professor Dr. I. Robertson and Dr. F. De Clerck for critical comments, I. Gevers for typing the manuscript, and L. Leijssen and G. Jacobs for preparing the photographs.

REFERENCES

1. Dwyer EM Jr. Left ventricular pressure volume alterations and regional disorders of contraction during myocardial ischemia induced by atrial pacing. *Circulation* 1970;42:1111–22.
2. Barry WH, Brooker JZ, Alderman EL, Harrison DC. Changes in diastolic stiffness and tone of the left ventricle during angina pectoris. *Circulation* 1974;49:255–63.
3. Mann T, Goldberg S, Mudge GH, Grossmann W. Factors contributing to altered left ventricular diastolic properties during angina pectoris. *Circulation* 1979;59:14–20.
4. Bourdillon PD, Lorell BH, Mirsky I, Paulus WJ, Wynne J, Grossman W. Increased regional myocardial stiffness of the left ventricle during pacing-induced angina in men. *Circulation* 1983;27:316–23.
5. Serizawa T, Mirsky I, Carabello BA, Grossman W. Diastolic

- myocardial stiffness in gradually developing left ventricular hypertrophy in dog. *Am J Physiol* 1982;242:H633-7.
6. Sasayama S, Takahashi M, Nakamura M, et al. Effect of diltiazem on pacing-induced ischemia in conscious dogs with coronary stenosis: improvement of post-pacing deterioration of ischemic myocardial function. *Am J Cardiol* 1981;48:460-7.
 7. Paulus JP, Serizawa T, Grossman W. Altered left ventricular diastolic properties during pacing-induced ischemia in dogs with coronary stenosis. *Circ Res* 1982;50:218-27.
 8. Vollmer B, Meuter CH, Stockbroeckx R, Janssen PAJ. R 56865 prevents electrical and mechanical signs of ouabain intoxication in guinea pig papillary muscle. *Eur J Pharmacol* 1987;142:137-40.
 9. Schneider J, Beck E, Scheufer L. Protective effects of R 56865 on cardiac glucoside intoxication. *J Mol Cell Cardiol* 1987;19(suppl III):S84.
 10. Schneider J, Beck E, Borgers M. Dose-dependent protection by R 56865 against digitalis toxicity in the guinea pig heart-lung preparation. *Naunyn-Schmiedeberg Arch Pharmacol* 1988;337:R65.
 11. Finet M, Bravo G, Godfraind T. The protective action of R 56865 against ouabain-induced intoxication in rat heart isolated atria and ventricles. *Eur J Pharmacol* 1989;164:555-63.
 12. Garner JA, Bernier M, Hearse DJ. R 56865 abolishes reperfusion-induced arrhythmias in vivo without altering hemodynamics. *Circulation* 1989;11:501.
 13. Garner JA, Bernier M, Hearse DJ. R 56865, a potent new anti-arrhythmic agent, effective during ischemia and reperfusion in the rat heart. *J Cardiovasc Pharmacol* 1990;16:468-79.
 14. Borgers M, Ver Donck L. R 56865, a new cardioprotective principle based on prevention of ion channel pathology. *J Mol Cell Cardiol* 1990;22(suppl 1):47.
 15. Reynolds RD, Calzadilla SV, Lee RJ. Spontaneous heart rate, propranolol and ischemia induced ventricular fibrillation in the dog. *Cardiovasc Res* 1978;12:653-8.
 16. Thompson DS, Waldron CB, Jull SM, et al. Analysis of left ventricular pressure during isovolumic relaxation in coronary artery disease. *Circulation* 1982;65:690-7.
 17. Grossman W, Mann JT. Evidence for impaired left ventricular relaxation during acute ischemia in man. *Eur J Cardiol* 1978;7(suppl):239-49.
 18. Mann T, Goldberg S, Mudge GH, Grossman W. Factors contributing to altered left ventricular diastolic properties during angina pectoris. *Circulation* 1979;59:14-20.
 19. Bourdillon PD, Paulus WJ, Serizawa T, Grossman W. Effects of verapamil on regional myocardial diastolic function in pacing-induced ischemia in dogs. *Am J Physiol* 1986;251(Heart Circ Physiol 20):H834-40.
 20. Flessas AP, Connely GP, Handa S, et al. Effects of isometric exercise on the end-diastolic pressure, volumes and function of the left ventricle in men. *Circulation* 1976;53:837-47.
 21. McLaurin LP, Rolett EL, Grossman W. Impaired left ventricular relaxation during pacing-induced ischemia. *Am J Cardiol* 1973;32:751-7.
 22. Lewis MJ, Housmans PR, Claes VA, Brutsaert DL, Henderson AH. Myocardial stiffness during hypoxic and reoxygenation contracture. *Cardiovasc Res* 1980;14:339-44.
 23. Chuck LHS, Goethals MA, Parmley WW, Brutsaert DL. Load-insensitive relaxation caused by hypoxia in mammalian cardiac muscle. *Circ Res* 1981;48:797-804.