Hemodynamic Effects of a Calcium Channel Promoter, BAY y 5959, are Preserved after Chronic Administration in Ischemic Heart Failure in Conscious Dogs[1]

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ABSTRACT

BAY y 5959 is a dihydropyridine derivative that binds to L-type calcium channels in a voltage-dependent manner and promotes calcium entry into the cell during the plateau of the action potential by influencing mean open time. Because myofilament responsiveness to calcium is preserved in congestive heart failure (CHF), the inotropic responsiveness to this compound should be preserved in CHF, and tolerance should not develop despite long-term treatment. To test these hypotheses, CHF was induced in 14 chronically instrumented dogs by daily (30 ± 5 days) intracoronary microsphere injections. The effects of BAY y 5959 (2-h i.v. infusions of 3 μg/kg/min and 10 μg/kg/min) were determined before heart failure, after heart failure was established and then 2 h after the end of a 5-day continuous BAY y 5959 intra-atrial infusion. Before CHF, the positive inotropic effect of BAY y 5959 at a dose of 10 μg/kg/min [left ventricular dP/dt (LV$dP/dt$) increased from 2955 ± 132 mmHg to 4897 ± 426 mmHg, P < .05] was associated with bradycardia (HR decreased from 92 ± 4 to 78 ± 6 b/min, P <.05), slight increases in mean arterial pressure (it increased from 100 ± 2 mmHg to 113 ± 5 mmHg, P <.05) and did not alter left ventricular end-diastolic pressure. In CHF, BAY y 5959 continued to induce dose-dependent increases in left ventricular systolic pressure, LV$dP/dt$ and mean arterial pressure, as well as causing bradycardia and a significant decrease in left ventricular end-diastolic pressure. After a 5-day infusion of BAY y 5959, base-line LV$dP/dt$ and left ventricular end-diastolic pressure improved. The responses of LV$dP/dt$ and mean arterial pressure to BAY y 5959 were similar to those of the control state. The sustained responses in CHF and after long-term infusion suggest that BAY y 5959 may be an effective and potent inotropic agent for treatment of CHF that does not lead to tolerance to its positive inotropic effects.

The use of inotropic agents is an important form of therapy for many patients with CHF. Currently used inotropic agents generally fall into one of two classes: β-agonists or phosphodiesterase inhibitors. Although they are efficacious in many settings, their use is sometimes limited by afterload-reducing effects, by decreased effectiveness in heart failure, by the development of tolerance and by potential proarrhythmic effects such as sinus tachycardia and ventricular ectopy.

BAY y 5959 is a dihydropyridine derivative that binds to L-type calcium channels in a voltage-dependent manner and promotes calcium entry into the cell during the plateau of the action potential by influencing mean open time (Bechem *et al.*, 1997). In contrast to previous calcium promoters, BAY y 5959 has been shown to be relatively myocardial-specific, lacking significant vasoconstrictor properties that are present in previous compounds in this class of drugs, such as BAY k 8644 (Bechem *et al.*, 1997; Huetter *et al.*, 1994). A recent study has demonstrated that in normal conscious dogs, the positive inotropic effects of BAY y 5959 are comparable to those of two other traditional inotropic agents, dobutamine and milrinone (Sato *et al.*, 1997). It remains to be determined whether the positive inotropic effect of BAY y 5959 exists in the heart failure state and whether tolerance, defined as a reduced inotropic response after prolonged exposure, develops with the continuous use of BAY y 5959. Because myofilament responsiveness to calcium is preserved in the heart failure state (Hajjar and Gwathmey, 1992), it is hypothesized that the inotropic responsiveness to this compound should be similar to that observed in the normal heart and that tolerance should not develop after long-term treatment.

ABBREVIATIONS: LA, left atrium; LAP, left atrial pressure; LV, left ventricle; LV$dP/dt_{max}$, peak left ventricular dP/dt; CHF, congestive heart failure; MAP, mean arterial pressure; LVEDP, left ventricular end-diastolic pressure; LVSP, left ventricular systolic pressure; LAD, left anterior descending artery; LCX, left circumflex coronary artery; BAY y 5959, (−)-(R)-isopropyl-amino-5-cyano-1, 4 dihydro-6-methyl-4-(3-phenyl-quinoline-5-yl)-pyridine-3-carboxylate.

Accordingly, this study was designed to determine whether inotropic responsiveness to BAY y 5959 is preserved in an animal model of heart failure and whether tolerance develops after a 5-day continuous infusion.

Materials and Methods

Surgical preparation. Eighteen mongrel dogs (24–32 kg) of either sex were chronically instrumented for hemodynamic measurements and repeated microembolization as described previously (Knecht *et al.*, 1997). Four animals died unexpectedly soon after coronary embolization, so this study is based on results from the 14 chronically instrumented dogs that survived the embolization procedure. Briefly, animals were anesthetized (inhaled isoflurane 1–2%) and mechanically ventilated. A thoracotomy was performed in the left fifth intercostal space under sterile conditions. Tygon catheters (inside diameter 0.04–0.05 in., outside diameter 0.07–0.09 in., Cardiovascular Instr. Corp., Boston, MA) were placed in the descending thoracic aorta and the LA. A solid-state pressure gauge (P6.5, Konigsberg Instruments, Pasadena, CA) was placed in the apex of the LV, and a Tygon catheter was also inserted into the LV for calibration of the solid-state pressure gauge during experimental measurements. A custom-made silicon catheter was implanted in the proximal portion of the dominant coronary artery. Of the 14 dogs reported in this study, the LAD was dominant in six dogs, and the LCX was dominant in six dogs. Two dogs had catheters in both LAD and LCX. The catheters and wires were run s.c. and externalized through the back of the dog, the chest was closed in layers and a chest tube was inserted to reduce the pneumothorax. Antibiotics were given after surgery as necessary. Dogs were allowed to recover fully from surgery and were trained to lie quietly on a laboratory table before experiments.

Hemodynamic recordings. Hemodynamic measurements were obtained with common recording techniques in all animals. Briefly, arterial and LA pressures were measured by attaching the previously implanted catheters to P23ID strain-gauge transducers (Statham Instruments, Inc, Oxnard, CA). LV systolic pressure was measured with the previously implanted solid-state pressure gauge, which had been calibrated *in vitro* against an electronic signal of known size and was cross-calibrated *in vivo* with measurements of pressure from the LV and LA catheters. All the pressure transducers were calibrated *in vitro* against a mercury manometer with atmospheric pressure as zero and cross-calibrated *in vivo* with pressure recorded from the implanted aortic, LA and LV catheters. Mean values of aortic pressure and atrial pressure were all determined on-line using 3-Hz averaging filters (DA26, Medtron Engineering, Olivenhain, CA). Data were recorded on an 8-channel thermal writing chart recorder (30-V8808-10, Gould Electronics, East Rutherford, NJ), and periods of interest were digitized [Gateway 2000, 486 computer equipped with a National Instruments (Austin, TX) analog-to-digital conversion system] for off-line analysis. Drift in the pressure gauges, amplifiers and chart recorder was eliminated by frequent calibration during experiments.

Experimental design and protocol. On the day of each series of measurements, a 19-gauge i.v. catheter was inserted in a peripheral vein of a hind leg for drug infusions. LVSP, LVEDP, MAP, LAP and HR were measured after the dogs were quiet and accustomed to the laboratory. The LV pressure signal was differentiated to assess LV$dP/dt_{max}$. In order to assess *beta*-adrenergic receptor-mediated cardiac inotropic responsiveness, a bolus injection of isoproterenol (0.5 μg/kg) was administered, and changes in LVSP, LV$dP/dt_{max}$, MAP and HR were measured. After performing the base-line hemodynamic measurements and an isoproterenol challenge, a placebo infusion [placebo: 20 ml of reconstituted diluent solution without BAY y 5959, consisting of 1,2 propylene glycol (7.75 g), polyethylene glycol 400 (2.5 g), water (10.48 g), ascorbic acid (0.02 g), sodium ascorbate (0.026 g) and sodium hydroxide solution 2 N (0.05 g)] was

administered for 2 h, and the above measurements were repeated. Subsequently, BAY y 5959 was infused at a rate of 3 μg/kg/min for 2 h; measurements were repeated at the end of that interval and again after 2 h at an infusion rate of 10 μg/kg/min. Each dog underwent this protocol before the induction of heart failure (as detailed below). Approximately 3 days after the establishment of a stable chronic heart failure state, a second similar series of hemodynamic measurements was performed. Finally, to test whether a long-term infusion of BAY y 5959 resulted in the development of tolerance, BAY y 5959 was infused continuously for 5 days at the rate of 3 μg/kg/min using a portable infusion pump (Model 5400, SIMS Deltec, Inc, St. Paul, MN) through the previously implanted LA catheter. In order to provide a placebo group of animals to compare the degree of heart failure, 3 animals underwent a 5-day infusion of placebo at the equivalent of 3 μg/kg/min. On the sixth day, the infusion pump was turned off for 2 h and the animals were rechallenged with the drug using the same protocol outlined above. In order to assess tolerance to BAY y 5959, we allowed a 2-h hiatus between turning off the infusion pump and acute administration of BAY y 5959. This was done because the half life of BAY y 5959 is approximately 3 h (Investigator's Brochure, Miles Inc., West Haven, CT), in order to avoid complete washout of the drug and yet to obtain stable base-line hemodynamics. Placebo-infusion animals did not undergo the tolerance challenge.

Induction of heart failure. Heart failure was induced by daily injection of glass microspheres (Spheriglass, 90 μm in mean diameter) through the previously implanted catheter in the dominant coronary artery (Knecht *et al.*, 1997). In brief, the microspheres were continuously agitated in a saline suspension (25,000 microspheres/ml, 50,000 microspheres/day) and injected daily until LVEDP was approximately 15 mmHg and HR was approximately 120 beats/min. Further details concerning the establishment of CHF are provided under "Results."

Statistical analysis. All results are expressed as means $\pm$ S.E. Changes in resting hemodynamic parameters between pre- and post-heart failure values were compared using one-way analysis of variance (ANOVA). Base-line changes in hemodynamics due to an intervention were compared to changes in the same intervention after the establishment of heart failure using a two-way ANOVA. A Duncan's multiple range test was used for multiple comparisons. Statistical significance was determined at P < .05.

This study was approved by the Institutional Animal Care and Use Committee, College of Physicians & Surgeons of Columbia University, and animals were cared for in accordance with the Guiding Principles for the Use and Care of Laboratory Animals (N.I.H. Publication No 82-23, 185).

Results

Daily coronary microembolization leads to CHF. After 30 $\pm$ 5 days of embolization with a total of 1,310,714 $\pm$ 261,171 microspheres, moderate heart failure developed. The base-line hemodynamic profile of the animals before and 3 days after the final embolization is summarized in figure 1. LV$dP/dt_{max}$ was reduced by approximately 25%, and there was a resting tachycardia. LVEDP increased significantly from 6 $\pm$ 0.5 to 17 $\pm$ 0.7 mmHg. LVSP and MAP were slightly decreased. These effects persisted after the 5-day infusion of the placebo agent in three control animals, as can be seen in table 1; this demonstrates that the daily coronary microembolization model leads to persistent CHF.

Effects of chronic infusion of BAY y 5959. Hemodynamic alterations were measured 2 h after the beginning and at the end of the 5-day infusion on three of the dogs (with infusion pump kept turned on). The results, summarized in table 2, showed that LVSP, LV$dP/dt_{max}$, LVEDP and MAP
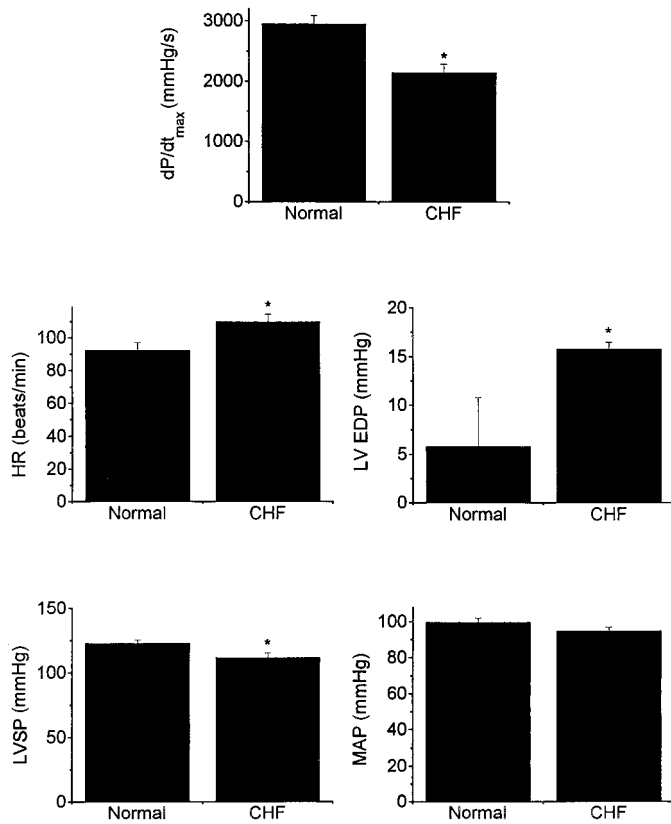
Fig. 1. Chronic heart failure (CHF) was evidenced by significant decreases in LV$dP/dt_{max}$ and LVSP and by significant increases in LVEDP and HR. *P < .05 from normal.

TABLE 1

Hemodynamic measurements after a 5-day continuous placebo infusion on three animals with heart failure

	Control	Heart Failure	After 5-Day Placebo Infusion
LVSP (mmHg)	121 ± 6	105 ± 1.3*	110 ± 1.7*
LVEDP (mmHg)	7 ± 1.2	21 ± 1.8*	19 ± 3.7*
LV$dP/dt$ (mmHg/s)	3042 ± 127	2215 ± 145*	2419 ± 311*
MAP (mmHg)	122 ± 4.6	84 ± 2.4*	83 ± 4.7*
HR (beats/min)	90 ± 5	98 ± 11	104 ± 15.6

Values are mean ± S.E. *P < .05 from control.

TABLE 2

Hemodynamic measurements at the beginning and the end of a 5-day continuous infusion of BAY y 5959 on three animals with heart failure

	Base line	Beginning	End
LVSP (mmHg)	105 ± 6.5	135 ± 14*	139 ± 9.8*
LVEDP (mmHg)	18 ± 4	10 ± 1.8*	11 ± 2.5*
LV$dP/dt$ (mmHg/s)	1880 ± 74	2780 ± 166*	2789 ± 394*
MAP (mmHg)	91 ± 5.3	103 ± 8.8*	115 ± 4.7*
HR (beats/min)	110 ± 7.2	78 ± 3.5*	100 ± 10.6

Values are mean ± S.E. *P < .05 from base line.

were consistently improved by the 5-day continuous infusion of BAY y 5959. These changes were comparable to the changes induced by acute administration of BAY y 5959 at the same dose (3 μg/kg/min).

No visible deleterious effects were observed during or immediately after the infusion of BAY y 5959. Despite the report of excitation and convulsions in chronically instrumented dogs at high doses (30 μg/kg/min) of a previous compound (Investigator's Brochure, Miles Inc., West Howen, CT), no such effects were observed during this study.

Hemodynamic effects of BAY y 5959 are preserved in the failing heart and after 5 days of continuous infusion. Before the induction of heart failure, BAY y 5959 induced dose-dependent changes in LV hemodynamics. After establishment of heart failure (evidenced by a marked decrease in dP/dt_{max} and a rise in LVEDP), BAY y 5959 retained these dose-dependent hemodynamic effects (fig. 2). These included dose-dependent increases in peak LV pressure, dP/dt_{max} and MAP, as well as a dose-dependent decrease in resting HR. Whereas BAY y 5959 had no significant effect on LVEDP in the control (non-heart failure) state, drug infusion significantly decreased this parameter in the heart failure state.

After the 5-day continuous infusion of BAY y 5959, baseline dP/dt_{max} slightly improved, compared with the original heart failure state, despite the 2-h drug withdrawal, and the peak dP/dt_{max} response to the 10 μg/kg/min infusion of BAY y 5959 was similar to that of the control state (fig. 3). Changes in MAP after infusion of BAY y 5959 were similar to those changes observed for LV$dP/dt_{max}$, as shown in figure 3E.

Base-line LVEDP decreased slightly after the 5-day infusion; although there was a trend for LVEDP to decrease further in response to drug infusion, this was not statistically significant. The bradycardia effect was not present, however, after the continuous infusion of BAY y 5959. Rather, there was a resting tachycardia that was slightly, but not significantly, faster than that observed in the original heart failure state (fig. 3D).

Comparison with hemodynamic effects of isoproterenol. As shown in the top panel of figure 4, hemodynamic responses to isoproterenol are blunted in the heart failure state. These included blunted response of LVSP, LV$dP/dt_{max}$ and HR; however, the hypotensive effect of isoproterenol was not altered. In contrast, as shown in the bottom panel of figure 4, the hemodynamic effects of BAY y 5959 were preserved in the heart failure state. Another important distinction between these agents was that blood pressure increased (not decreased) and HR decreased (not increased) with BAY y 5959.

Discussion

The possibility of the development of a dihydropyridine derivative with calcium-promoting (instead of calcium-blocking) actions for the treatment of heart failure has been discussed for many years. Theoretically, as these agents directly increase the concentration of intracellular calcium, they result in significant positive inotropic effects independent of the cAMP-related mechanisms that are significantly impaired in heart failure. Because it is generally believed that myofilament responsiveness to calcium is well maintained in the heart failure state (Hajjar and Gwathmey, 1992), this class of compounds would be expected to exert a preserved positive inotropic effect on the failing heart.

To date, the only drug of this class generally available for evaluation has been BAY k 8644. This agent prolongs the mean open time of the L-type calcium channel in a voltage-independent manner (Schramm *et al.*, 1983; Brown *et al.*, 1986; Hess *et al.*, 1986). Under this circumstance, the pro-
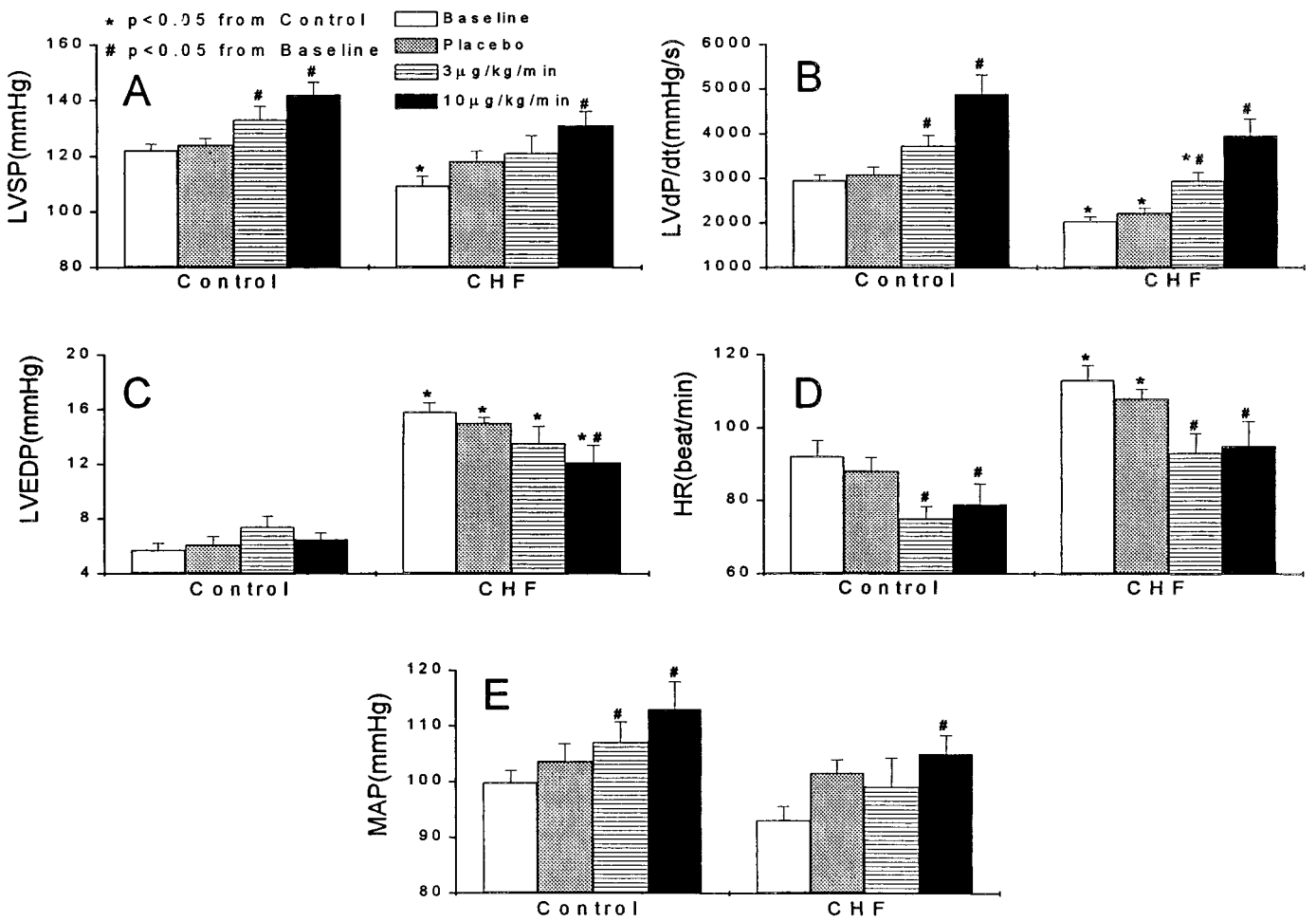
Fig. 2. BAY y 5959 caused dose-dependent increases in LVSP, LV*dP/dt*, MAP and bradycardia responses in control state ($n = 11$). The hemodynamic effects of BAY y 5959 retained after establishment of CHF ($n = 9$). In addition, the elevated LVEDP was significantly reduced after administration of BAY y 5959 in the CHF state. Placebo had no effects on hemodynamics in any circumstance (control, $n = 9$; CHF, $n = 4$).

found positive inotropic response is accompanied by a marked vasoconstrictor response (Rump *et al.*, 1992). Experimental data also showed that when applied to isolated rabbit hearts, BAY k 8644 increased myocardial infarct size (Rump *et al.*, 1993). In the same experimental preparation, BAY k 8644 did not increase myocardial contractility because of a concomitant marked reduction of coronary blood flow that counteracted the direct myocardial effects (Rump *et al.*, 1993). In normal conscious dogs, administration of BAY k 8644 produces significant increases in MAP, LVSP, HR and systemic vascular resistance but causes no change in cardiac output, stroke volume or left ventricular percent area shortening. Increased LV*dP/dt*$_{max}$ and rate of shortening could be shown only after autonomic nervous system blockade (Pagel *et al.*, 1994). Thus BAY k 8644 lacks myocardial specificity (Ishii *et al.*, 1986), and the potentially clinically beneficial positive inotropic effects were undermined by its vasoconstrictor properties.

In contrast to BAY k 6844, BAY y 5959 does not lead to vasoconstriction of either the peripheral or the coronary vascular beds (Huetter *et al.*, 1994; Sato *et al.*, 1997). Instead, coronary vasodilation occurs, as indicated by an increase in coronary blood flow and a decrease in coronary vascular resistance. The lack of peripheral effects suggests that this compound is relatively myocardial-specific, and this specific-

ity may be mediated by its unique electrophysiological properties, which render it ineffective on stable repolarized tissue such as smooth muscles (Bechem *et al.*, 1997).

Despite the expectation of a potential use of this class of drugs for the treatment of heart failure, the hemodynamic effects of BAY y 5959 in a clinically relevant experimental model of heart failure have not been determined. In our study, we used a canine model of multi-micro infarct-induced heart failure (Knecht *et al.*, 1997). Although the degree of heart failure achieved with embolization is moderate compared with rapid cardiac pacing-induced heart failure, we believe that this serves as a more stable and a more clinically relevant model, because it mimics the most common clinical etiology of CHF. Furthermore, as we have shown here and elsewhere (Knecht *et al.*, 1997), this model leads to a persistent heart failure state for at least 5 days after the cessation of microembolization. In our previous studies of BAY y 5959, we utilized an isolated blood-perfused failing-heart preparation to demonstrate that the hemodynamic effectiveness of BAY y 5959 was preserved despite the blunting of inotropic responsiveness to isoproterenol (Todaka *et al.*, 1998). In the present study, our data confirm that in the normal heart, BAY y 5959 is a potent inotropic agent and that this is accompanied by a mild increase in blood pressure and by a decrease in HR. In the heart failure state, the inotropic
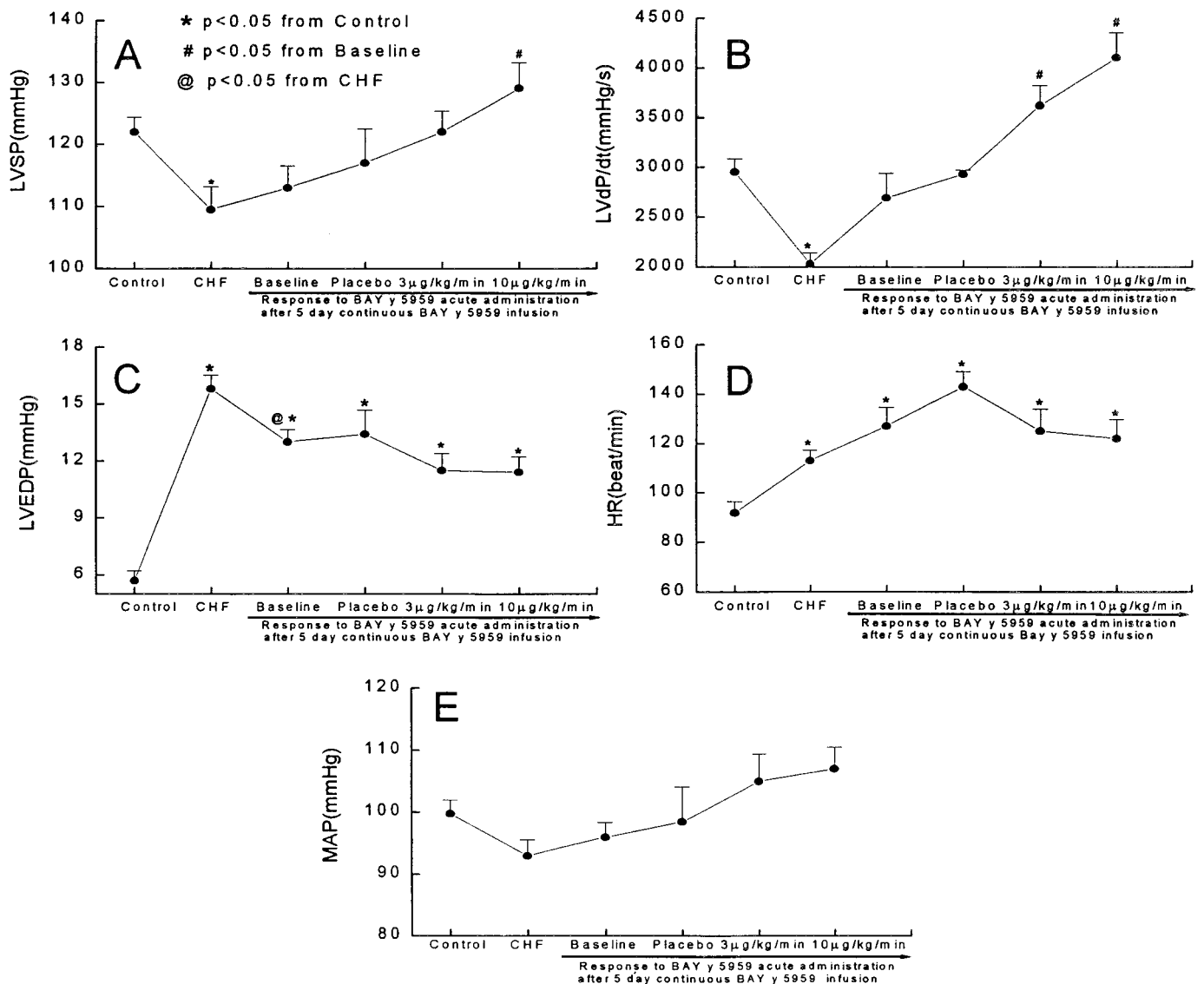
Fig. 3. Five-day continuous BAY y 5959 infusion was successfully performed in eight animals. In the CHF state, LVSP and LV*dP/dt* were significantly decreases, and LVEDP and HR were significantly increased from control values (Control). Five days of BAY y 5959 infusion slightly improved base-line LVSP (Baseline), LV*dP/dt*, LVEDP and MAP, but HR was further increased. Administration of BAY y 5959 (3 μg/kg/min and 10 μg/kg/min) was repeated 2 h after interruption of 5-day continuous BAY y 5959 infusion. The positive inotropic effect of BAY y 5959 was still preserved, although its bradycardia effect was not present.

responsiveness is preserved and the drug is associated with a significant reduction in LVEDP and HR, as noted in previous studies of normal dogs (Sato *et al.*, 1997) and in a recent study of patients with heart failure (Rousseau *et al.*, 1997). After a 5-day continuous infusion of BAY y 5959, there is no evidence of the development of tolerance with respect to the inotropic actions, although the bradycardic response diminishes.

The preservation of inotropic effectiveness in the failing heart, despite the documented deterioration of *beta* adrenergic responsiveness, and the continued effectiveness of long-term administration are important characteristics of an agent proposed for use in the treatment of heart failure. The continuous-infusion regimen not only enabled us to evaluate the development of tolerance but also provided further evidence of hemodynamic benefits resulting from BAY y 5959 treatment of heart failure. The measurements of hemody-

namics in the conscious state, although limited by the use of load- and HR-dependent indices of contractile function, made possible the assessment of heart failure and the hemodynamic responses to BAY y 5959 without the interference of the negative inotropy of sedation or anesthesia.

Because our experimental protocol requires survival for almost 10 weeks (3 weeks for surgical recovery, training and initial experiment, 4–5 weeks for embolization and 1 week for the drug infusion and terminal experiment) and because implantation of the aortic flow probe for calculation of systemic vascular resistance significantly jeopardizes the survival of the animal (there is a risk of rupture of the pulmonary artery or aorta), total peripheral resistance was not calculated. Therefore, the issue of whether BAY y 5959 has vasomotor effects was not directly addressed in our study. There is a moderate increase in MAP after administration of BAY y 5959 in both the normal and the heart failure state.
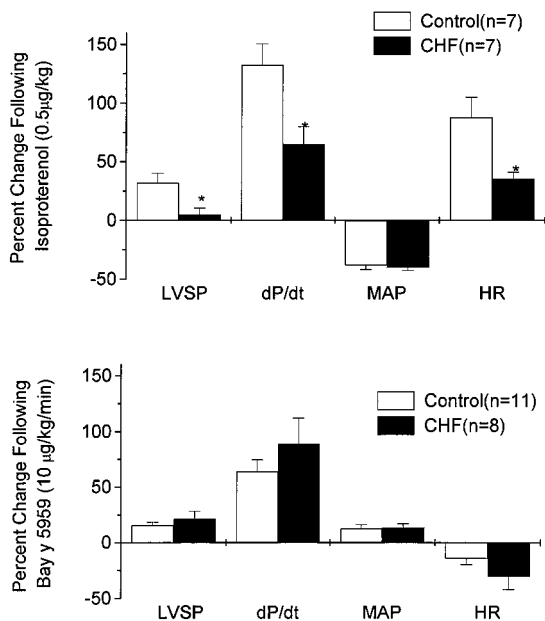
Fig. 4. Graphs show the impact of isoproterenol and BAY y 5959 on hemodynamics in both control and CHF states. The inotropic and chronotropic responses of failing hearts to isoproterenol were eliminated. However, the hemodynamic effects of BAY y 5959 were well maintained in CHF. It is important to note that the responses of MAP and HR to BAY y 5959, compared with isoproterenol, were increased (not decreased) and decreased (not increased), respectively. *$P < .05$ from control.

directly addressed in the present study, several possibilities were considered. First, because baroreflex control may be involved in BAY y 5959-induced bradycardia, it was reasoned that baroreflex resetting may take place (Fritsch *et al.*, 1989; Brooks *et al.*, 1993; Head, 1995). That is, as the baroreceptor is exposed to a new constant-pressure condition or chemical stimulus (if a chemical stimulus is able to alter the vascular tone of the carotid sinus or alter the central gain of the reflex) for a period of time, the working point and gain of the reflex will be reset. In the case of continuous BAY y 5959 infusion, the baroreceptor may have adapted to this agent itself as well as to its pressor effects, resulting in the reappearance of the tachycardia in CHF. If this is the case, whether this resetting occurred in the central or the peripheral components of the baroreflex system needs to be determined (Qian *et al.*, 1997; Heesch *et al.*, 1996). These data imply that some tolerance of the HR response to BAY y 5959 does develop after long-term use of the agent. Second, a less likely explanation is that tachyarrhythmias may be induced by the 5-day infusion of BAY y 5959. However, in a separate study in our laboratory, we did not observe a proarrhythmic effect of this agent. Specifically, ambulatory Holter monitoring was recorded in eight dogs before (65.1 ± 12 h) and during (77 ± 7 h) continuous BAY y 5959 infusion ($3\ \mu$g/kg/min); data analysis revealed that BAY y 5959 did not elicit proarrhythmic activity.

In summary, BAY y 5959, a novel dihydropyridine derivative, demonstrates a potent positive inotropic effect. Unlike traditional positive inotropic agents involving cAMP pathways, the positive inotropic response to BAY y 5959 is well preserved in the heart failure state that is accompanied by bradycardia, at least during 2-h infusions. There is no evidence of tolerance (except HR response) in terms of diminished inotropic response to BAY y 5959 after a 5-day period of continuous administration. The profile of hemodynamic actions of this agent offers several theoretical advantages over that of other inotropic agents (β-agonists and phosphodiesterase inhibitors) and, with a clinically acceptable side-effect profile, could provide a powerful addition to the armamentarium in the treatment of CHF.

This increase probably results from the increase in inotropy rather than *via* systemic vasoconstriction, in view of the fact that we and others have reported a lack of measurable vasoconstrictor effects of BAY y 5959 in the normal animal (Sato *et al.*, 1997; Todaka *et al.*, 1998), although these data have not been replicated in the heart failure state. We also did not examine the effect of BAY y 5959 on venous properties. The striking dose-dependent decrease in LVEDP due to this agent in the heart failure state may signal a possible effect of BAY y 5959 on venous properties. Alternatively, the decrease in LVEDP may simply result from the improvement of cardiac pump function. Nevertheless, the effect of BAY y 5959 on arterial and venous properties in various vascular beds in both the normal and the heart failure state need to be further elucidated.

It has been suggested that the BAY y 5959-induced bradycardia is mediated through autonomic reflexes because it is eliminated after ganglionic blockade (Uechi *et al.*, 1995). We, however, observed similar bradycardic effects in isolated failing hearts devoid of reflexes (Todaka *et al.*, 1998), a result that indicates a direct, reflex-independent effect on pacemaker function, on the cardiac conduction system or on myocytes. A study of *in vitro* myocytes also suggested direct negative chronotropic activity (Dembowsky *et al.*, 1996; Bechem *et al.*, 1997). In our study, we found an even more striking bradycardic response after the establishment of heart failure. To our surprise, however, whereas there was no evidence of the development of tolerance to the inotropic properties of BAY y 5959, base-line HR increased after the 5-day continuous infusion, and rechallenging the animal with BAY y 5959 had no effect on HR. Although the mechanisms of the resting tachycardia and the loss of bradycardia response to BAY y 5959 after continuous infusion were not

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