Effect of BAY y 5959 on myocardial function and metabolism in normal and failing hearts

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Todaka, Koji, Jie Wang, Geng-Hua Yi, Anguo Gu, Shu-Ming Zhu, Hui Zhang, and Daniel Burkhoff. Effect of BAY y 5959 on myocardial function and metabolism in normal and failing hearts. *Am. J. Physiol.* 274 (*Heart Circ. Physiol.* 43): H1560–H1568, 1998.—BAY y 5959 is a dihydropyridine derivative with positive inotropic actions mediated by a direct increase in intracellular calcium. We characterized the direct myocardial actions of this new agent in hearts isolated from seven normal dogs and from five dogs with repeated coronary microembolization-induced heart failure. Inotropic actions of BAY y 5959 were accompanied by little effect on duration of contraction and by prolongation of the monophasic action potential (MAP); in contrast, isoproterenol decreased contraction and MAP durations. Whereas inotropic responsiveness to isoproterenol was blunted in embolized hearts, these actions of BAY y 5959 were relatively preserved in the heart failure state. Isoproterenol increased heart rate, whereas BAY y 5959 had little effect. Changes in coronary vascular resistance also decreased similarly for isoproterenol and BAY y 5959. Finally, for comparable inotropy, increases in myocardial oxygen consumption were similar for isoproterenol and for BAY y 5959. In summary, preserved inotropic responsiveness and lack of positive chronotropic actions are two clinically favorable features of this type of inotropic agents compared with a typical β-adrenergic agonist.

heart failure; inotropic agents; calcium; oxygen consumption; heart rate; coronary vascular resistance; action potential

TRADITIONAL INOTROPIC agents used in the treatment of congestive heart failure (CHF), such as catecholamines and phosphodiesterase inhibitors, enhance myocardial contractile force via adenosine 3′,5′-cyclic monophosphate (cAMP)-dependent pathways, which are downregulated in CHF (2, 7). Accordingly, inotropic responsiveness is frequently depressed in CHF patients, and tolerance may develop during longer term infusions. In addition, these agents increase heart rate and may enhance ventricular ectopy, both of which are undesirable side effects. The existence of a class of dihydropyridine compound derivatives that exert positive inotropic actions by enhancing systolic transsarcolemmal calcium flux through L-type calcium channels has been recognized for a long time; the most prominent of these is BAY K 8644 (21). However, lack of myocardial specificity of that compound greatly limited its potential clinical utility, mainly because of its vasoconstricting actions (10, 12).

More recently, BAY y 5959, a dihydropyridine derivative with cardioselective calcium-channel agonistic activity, has been discovered (1, 11). BAY y 5959 binds with high affinity to the cardiac dihydropyridine receptor component of the calcium channel and prolongs the single-channel open time (1). Preliminary studies of this compound in various models have shown its inotropic actions to be preserved in heart failure (20, 24), that it has bradycardic actions (with associated action potential prolongation) (6, 17), and that it may exert a relative oxygen-saving effect compared to inotropism with β-agonists (4, 5, 20). These features have renewed interest in this class of compounds as a potential new therapy for heart failure (16, 17). However, interpretation of results of studies in intact, awake dogs are complicated by direct or indirect effects of drug infusion on baroreflexes and potentially on vascular properties (preload and afterload), and the degree to which the identified characteristics reflect direct vs. indirect myocardial effects on the heart, while of primary importance, is not certain.

In view of the potential clinical application of this class of inotropic agents for treating heart failure, the purpose of this study was to test in normal and failing hearts the direct myocardial actions of BAY y 5959 on ventricular contractile state, coronary vascular resistance, myocardial oxygen consumption (MVO_2), heart rate, and action potential duration. To provide a relevant framework of interpreting the findings, we made comparisons to the effects of isoproterenol. Potential confounding effects of autonomic reflexes and changes in afterload and preload were obviated because the physiological conditions of an intact heart and blood perfusion were retained by performing these studies in isolated cross-perfused canine hearts. Normal hearts and hearts rendered myopathic by repeated coronary microembolization were studied. The constellation of myocardial actions of BAY y 5959 identified in this study supports the notion that this class of agents offers certain advantages over traditional inotropic agents in the treatment of heart failure.

METHODS

Isolated heart preparation. Hearts of seven normal dogs (22.2 ± 2.0 kg) and of five dogs with repeated coronary microembolization heart failure (25.3 ± 4.1 kg, methods described below) were studied using a standard isolated heart preparation. Details of this preparation have been provided previously (3). Briefly, the heart from the dog of interest was excised and metabolically supported by blood provided from a second support dog. The femoral arteries and veins of the support dog were cannulated and connected to a perfusion circuit consisting of a peristaltic pump, a heater, a blood filter, and an air trap. The pressure in the aortic root of the isolated heart, which is the perfusion pressure for coronary flow, was measured. Coronary blood flow was adjusted at the beginning of the protocol to provide a perfusion pressure of ~100 mmHg

and was kept constant throughout the experiment. Blood traveled through the coronary vasculature of the isolated heart and returned to the support dog by gravity. Coronary flow was collected through a wide-bore cannula placed in the right atrium and right ventricle and was measured by an in-line ultrasonic flowmeter (Transonic Systems model T108, Ithaca, NY). The difference between arterial and venous oxygen content (a-vO_2) was measured on-line by a commercially available spectrophotometer (AVOX Systems, San Antonio, TX). Oxygen consumption of the whole heart ($M\dot{V}O_2$) was determined by multiplying coronary flow by a-vO_2.

A water-filled balloon was placed within the left ventricle (LV) via the mitral valve. The volume of the balloon, and therefore of the LV, was controlled by a piston pump servosystem. A micromanometer (model SPC-360, Millar Instruments, Houston, TX) placed within the balloon was used to measure LV pressure. The heart was paced from the LV apex at constant rate (154 ± 11 beats/min) and was constrained to contract isovolumically. Blood temperature was kept at ~37°C by a heat exchanger.

Monophasic action potentials (MAPs) were recorded from the anterior or lateral surface of the LV by a commercially available MAP electrode (8). MAP amplitudes are expressed in relative terms.

Repeated coronary microembolization heart failure model. The details of the procedures used to induce heart failure using repeated coronary microembolization have been provided elsewhere (13, 14, 18). Five mongrel dogs were anesthetized (1–2% inhaled isoflurane) and underwent sterile surgery for chronic instrumentation via a left thoracotomy. A solid-state pressure transducer (Konigsberg model P6.5, Konigsberg Instrument, Pasadena, CA) was inserted into the LV through the apex. Fluid-filled catheters were inserted into the left atrium and the aorta. Another thin [Tygon catheter, diameter 1.8–2.3 (ID 1.0–1.3) mm, Cardiovascular Instruments, Boston, MA], fluid-filled catheter was introduced into the proximal portion of the left circumflex coronary artery for injections of microspheres over the ensuing weeks. After at least 10 days recovery, baseline hemodynamics were recorded while the dogs were awake and resting comfortably on a laboratory table. Approximately 25,000–50,000 glass microspheres (diameter ~90 μm) suspended in saline were injected daily for ~30 days (average total no. of microspheres 1,050,000 ± 209,000) until measurements of peak rate of rise of LV pressure (dP/dt_{max}), LV end-diastolic pressure (LVEDP), and resting heart rate were consistent with a state of heart failure (detailed below). The animals were then observed for between 7 and 10 days; at the end of this observation period, hemodynamic measurements were repeated with the dogs lying quietly on their side to ensure persistence of the heart failure state. The results, summarized in Table 1, show that there was a significant decrease in dP/dt_{max} and a significant elevation in LVEDP ($P < 0.05$, Wilcoxon signed-rank sum test); the changes in these parameters suggest that the animals were in a state of moderate heart failure. The hearts of these animals were studied in isolation as described above and comprised the CHF group.

Table 1. *Hemodynamic measurements obtained before and after completion of microembolization regime*

	Baseline	Embolized
HR, beats/min	92 ± 17	101 ± 15
AoP, mmHg	101 ± 6	92 ± 6
LVEDP, mmHg	6.6 ± 2.1	16.2 ± 1.9*
dP/dt_{max}, mmHg/s	3,070 ± 394	2,175 ± 422*

Values are means ± SE. Postembolization (embolized) values were obtained 7–10 days after stopping embolization. HR, heart rate; AoP, mean aortic pressure; LVEDP, left ventricular (LV) end-diastolic pressure; dP/dt_{max}, peak rate of rise of LV pressure. *$P < 0.031$ by Wilcoxon signed-rank sum test.

Isolated heart protocols and data analysis. Inotropic, lusitropic, metabolic, and electrophysiological effects of BAY y 5959 were assessed in normal and failing hearts. After the surgical preparation was completed, hearts were allowed to stabilize for ~30 min before the protocol was started. Ventricular volume was adjusted to provide an end-diastolic pressure (EDP) of ~5 mmHg. Hemodynamic recordings (LV pressure, coronary blood flow, a-vO_2) and MAP recordings were made at baseline during stepwise increases in BAY y 5959 infusion (titrated to increase contractile state by ~75%). BAY y 5959-liposome was prepared by diluting a standardized preparation of 25 mg BAY y 5959, sucrose, egg lecithin, and ascorbic acid with a standardized reconstitution medium containing glycerol and sodium caprylate in water; this preparation was then diluted in saline to final concentrations ranging between 10 and 40 μg/ml (23.6 and 94.2 μmol/l), depending on heart sensitivity to BAY y 5959, and was infused directly into the arterial perfusion line ~1.5 m from the heart, which allowed ample time for uniform mixing in blood before reaching the heart. At the highest infusion rate studied, hemodynamic measurements were made at three different volumes (spanning EDPs between 0 and 15 mmHg) to construct end-systolic pressure-volume relationships (ESPVR) and to assess the relationship between workload and $M\dot{V}O_2$. The results obtained with BAY y 5959 were compared to those obtained with isoproterenol titrated to create similar degrees of inotropism. Because of the relatively long half-life of BAY y 5959, the order of drug infusion could not be randomized. Therefore, some hearts received only BAY y 5959, and some hearts received isoproterenol followed by BAY y 5959. Selected results from the two groups were analyzed separately to test whether isoproterenol pretreatment modified the effects of BAY y 5959. In all, the BAY y 5959 group consisted of seven normal hearts (5 of which were pretreated with isoproterenol) and five CHF hearts (all of which were pretreated with isoproterenol), and the isoproterenol group consisted of five normal and five CHF dogs.

Ventricular contractile state was assessed by isovolumic peak developed pressure (peak minus minimum isovolumic LV pressure) at a fixed volume. Left ventricular relaxation was assessed by pressure half-time ($t_{½}$), which was defined as the time for LV pressure to fall to 50% of its value at the point of peak rate of decline in LV pressure ($-dP/dt_{max}$). The duration of contraction was defined as the width of the isovolumic pressure curve at a pressure level equal to 10% of the peak developed pressure; this parameter was called D_{10}.

To assess the metabolic cost of inotropism, we determined the relationship between $M\dot{V}O_2$ and total mechanical work, indexed by the pressure-volume area (PVA): $M\dot{V}O_2 = A \times PVA + B$. PVA was defined in the usual manner as the area on the pressure-volume diagram contained within the triangular region bounded by the linear ESPVR, the end-diastolic pressure-volume relationship, and the vertical line corresponding to the volume at which the isovolumic contraction occurred (23). The $M\dot{V}O_2$ intercept of this relation (i.e., *B*; the unloaded $M\dot{V}O_2$) has been shown to vary directly with contractile state, whereas the slope, *A*, is relatively independent of contractile state (23). Because changes in contractile state are generally brought about by changes in intracellular calcium, changes in *B* have been hypothesized to reflect altered energy demands for calcium cycling (23).

Inotropic, lusitropic, and metabolic effects were related to plasma concentrations of BAY y 5959, which were estimated

from the rate of drug infusion, the measured coronary blood flow (CBF), and a calibration curve. The calibration curve was derived from 22 freshly frozen plasma samples spanning a wide range of concentrations (0–800 nmol/l), which were analyzed by high-performance liquid chromatography. Samples were transported overnight on dry ice from our laboratory to Bayer (West Haven, CT), where the assay was performed. The formula determined from this procedure was as follows: $[\text{BAY y 5959}]_{plasma}$ (in nmol/l) = 0.97[infusion rate (in nmol/min)/CBF (in l/min)], where 0.97 is the empirically determined scaling factor.

Average dose-response curves were computed for each group of hearts by averaging drug concentrations at the same level of inotropism after fitting each response to a sigmoidal curve [Boltzmann equation: $y = (A_1 - A_2)/[1 + e^{(x - x_0)/d}] + A_2$, where A_1, A_2, x_0, and d are fit parameters].

Statistical analysis. Data are presented as means ± SD. Wilcoxon's signed-rank sum test was used for determination of differences in in vivo hemodynamic parameters between baseline and CHF states for animals that underwent repeated coronary embolizations. Multiple linear regression was used to test for statistical significance of differences in trends such as effects of drug concentration on hemodynamic parameters. $P < 0.05$ was regarded significant.

RESULTS

Effects of BAY y 5959 on LV pressure and MAPs. Representative MAP and isovolumic LV pressure recordings from a heart isolated from an animal that underwent repeated coronary embolizations in response to increasing doses of isoproterenol are shown in Fig. 1. As is well known, isoproterenol shortened the MAP duration (Fig. 1*A*) while enhancing contractile force dose dependently (Fig. 1*B*). In addition, a decrease in the total duration of contraction is also evident. At the highest dose of isoproterenol studied, peak developed pressure increased by 65 ± 19% in normal hearts and 52 ± 9% in failing hearts, and this was associated with an average 9.3 ± 2.5 and 10.1 ± 2.6% decrease in D_{10} (index of overall duration of contraction), respectively. BAY y 5959 also created a significant inotropic response but, in contrast to isoproterenol, lengthened the MAP (Fig. 2, *A* and *B*; data from same heart as presented in Fig. 1). In contrast to isoproterenol, contraction duration was little affected by BAY y 5959. With a comparable average of 61 ± 17 and 57 ± 10% increase in developed pressure in normal and failing hearts, D_{10} was decreased by only 7.0 ± 2.5 and 6.6 ± 1.7%, respectively ($P < 0.01$ vs. normal group by multiple linear regression).

Average contractile responses to both agents in normal and CHF groups are summarized in Fig. 3. As reported and well recognized previously, the response of failing hearts to isoproterenol is depressed markedly as evidenced by the statistically significant rightward shift of the dose-response curve of the CHF group (Fig. 3*A*, $*P = 0.007$, normal vs. CHF). In contrast, the dose-response curve of the CHF group to BAY y 5959 was shifted by a smaller, statistically nonsignificant ($P = 0.413$) degree relative to that of the normal group (Fig. 3*B*). Figure 3*B*, *inset*, shows that isoproterenol pretreatment did not affect the effectiveness of BAY y 5959 in any discernable way.

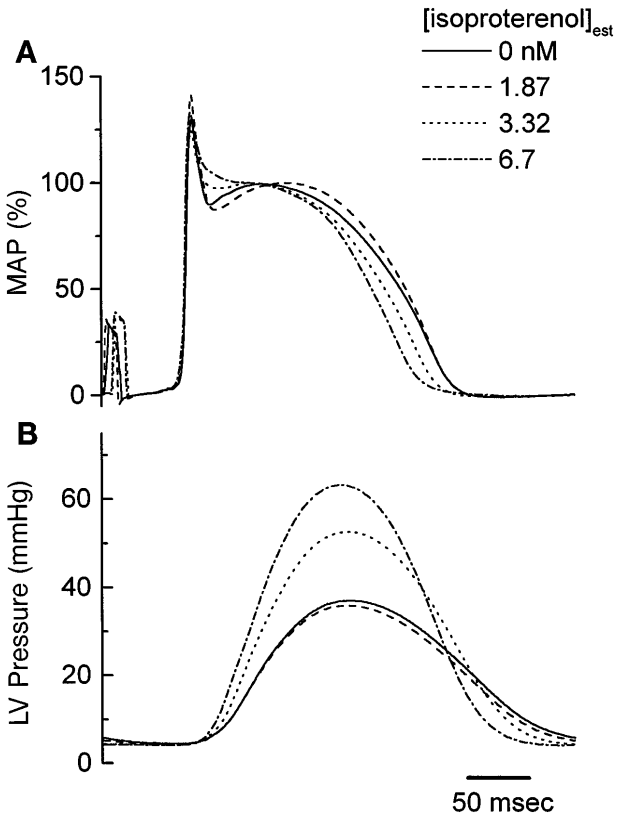

Fig. 1. Effects of isoproterenol on monophasic action potential (MAP, *A*) and on isovolumic left ventricular (LV) pressure waves (*B*) in a representative heart isolated from a dog with moderate heart failure due to repeated coronary microembolization. As is well known, inotropism with this β-agonist is associated with decreases in action potential duration and decreases in duration of contraction. Estimated blood concentrations of isoproterenol ($[\text{isoproterenol}]_{est}$) were 0, 1.87, 3.32, and 6.7 nM.

The MAP duration (MAPD) was indexed by $MAPD_{90}$, the duration at 90% repolarization. Average results for both isoproterenol and BAY y 5959 are summarized in Fig. 4; to make the comparison easier, we normalized $MAPD_{90}$ to its baseline value and plotted it against normalized developed pressure, which indexed the degree of inotropism. The baseline values are as follows: $MAPD_{90}$ was 200 ± 25 ms in normal hearts and 211 ± 8 ms in CHF hearts (P = NS by unpaired t-test); developed pressure was 68 ± 15 mmHg in normal hearts and 40 ± 14 mmHg in CHF hearts ($P < 0.01$ by unpaired t-test). As shown in the representative case, isoproterenol decreased $MAPD_{90}$, whereas it was prolonged by BAY y 5959 in both normal and CHF hearts for comparable degrees of inotropism. Multiple-regression analysis showed that the differences in these relations between isoproterenol and BAY y 5959 were statistically significant in both normal and CHF hearts ($P < 0.001$).

Effects on relaxation. Although BAY y 5959 enhances peak intracellular calcium, we found that the rate of relaxation was increased by this agent. For comparable degrees of inotropy, $t_{½}$ was decreased by both agents to roughly the same degree ($P = 0.83$ by multiple linear regression analysis, Fig. 5). Baseline values of $t_{½}$ were

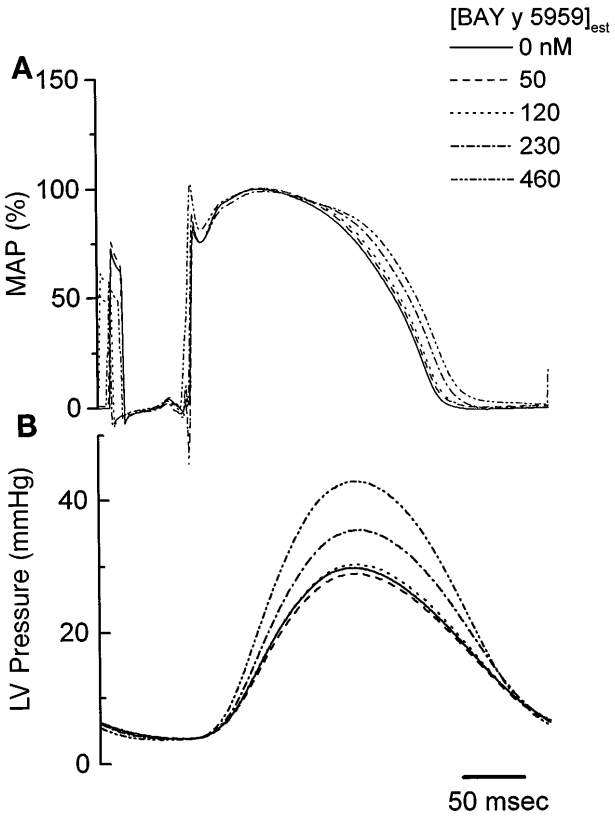

Fig. 2. Effects of BAY y 5959 on MAP (*A*) and on isovolumic LV pressure waves (*B*) in representative heart isolated from a dog with moderate heart failure due to repeated coronary microembolization (same heart as in Fig. 1). Inotropism with this myocardial-specific calcium agonist was associated with increased action potential duration and no significant change in overall duration of contraction. Estimated blood concentrations of BAY y 5959 ($[BAY\ y\ 5959]_{est}$) were 0, 50, 120, 230, and 460 nM.

31.3 ± 5.5 ms for the normal group and 29.8 ± 4.6 ms for the CHF group ($P = 0.57$ by unpaired *t*-test).

Effects on heart rate. To test and compare the chronotropic actions of BAY y 5959 and isoproterenol, we interrupted the pacing for several minutes to examine the native heart rate at each drug infusion rate. Results, summarized in Fig. 6, show that positive chronotropic actions of isoproterenol were identified, with heart rate increasing in relation to the degree of inotropism achieved. However, heart rate did not increase with BAY y 5959 infusion (P = NS by multiple linear regression) despite the same degree of inotropism as achieved with isoproterenol. Statistical analysis showed that this difference in heart rate responses was statistically significant in both normal and CHF hearts ($P < 0.001$). Baseline values of heart rate were 108 ± 21 beats/min in normal hearts and 92 ± 17 beats/min for CHF hearts ($P = 0.07$ by unpaired *t*-test).

Effects on coronary resistance. To test whether BAY y 5959 affects smooth muscle tone in the heart, we examined its effect on coronary vascular resistance. As summarized in Fig. 7, coronary resistance decreased with increasing degrees of inotropism caused by BAY y 5959. Furthermore, the changes in resistance in both normal and CHF hearts were similar to those observed after isoproterenol infusion for comparable degrees of inotropism. Multiple linear regression revealed no difference in these effects between isoproterenol and BAY y 5959. Baseline values of coronary resistance were 1.12 ± 0.39 $mmHg \cdot min \cdot ml^{-1}$ for normal hearts and 1.35 ± 0.50 $mmHg \cdot min \cdot ml^{-1}$ for CHF hearts ($P = 0.25$ by unpaired *t*-test). Although metabolic coronary autoregulation-mediated dilation in response to enhanced contractility may have masked possible vasoactive properties of BAY y 5959, any such effect would be unlikely in view of the fact that our result showed no difference in the relationship between resistance and inotropic state between the two agents in either normal or heart failure states.

Effects on oxygen consumption. Representative results regarding the ESPVR and the MVO_2-PVA relationship from a failing heart are shown in Fig. 8. BAY y 5959 injection to a blood concentration of ~700 nM increased the slope of the ESPVR with little effect on the volume-axis intercept (Fig. 8*A*). This increase in contractility was accompanied by an upward shifting of the MVO_2-PVA relationship as seen in Fig. 8*B*. These

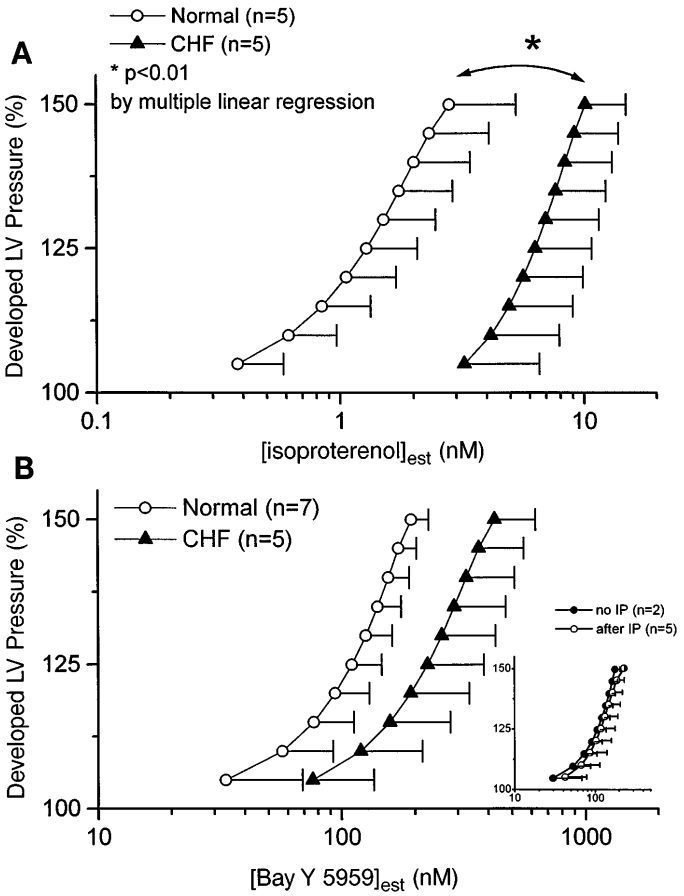

Fig. 3. Average (±SD) dose-response curves of normal and congestive heart failure (CHF) animals to intracoronary infusions of isoproterenol (*A*) and BAY y 5959 (*B*). Whereas isoproterenol responsiveness was markedly blunted (normal vs. CHF, $*P = 0.007$ by multiple-regression analysis), responsiveness to BAY y 5959 was not significantly different between the 2 groups (normal vs. CHF, $P = 0.413$ by multiple-regression analysis). *Inset*: pretreatment of normal hearts with isoproterenol (IP) did not influence subsequent dose-response curve to BAY y 5959.

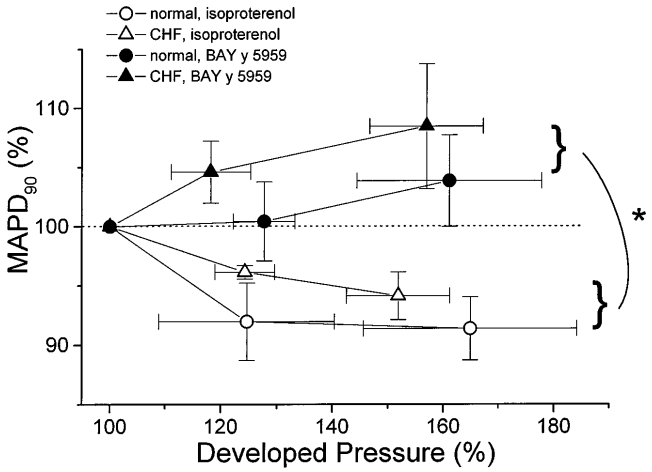

Fig. 4. Average changes in MAP duration (MAPD) [indexed by duration at 90% repolarization ($MAPD_{90}$)] plotted as a function of contractile state for BAY y 5959 and for isoproterenol. Contractile state was indexed by developed pressure normalized to its baseline value before drug infusion. In both normal and CHF hearts, $MAPD_{90}$ decreased in response to isoproterenol and increased in response to BAY y 5959 (*$P < 0.01$ by multiple linear regression for both groups of hearts).

characteristics were confirmed in other hearts examined as summarized in Fig. 9. Multiple linear regression analysis indicated that there was no significant influence of inotropism by either isoproterenol or BAY y 5959 on the MVo_2-PVA slope (Fig. 9*A*) in either normal or CHF hearts. In contrast, both agents significantly increased the intercept values, *B*, in both groups (Fig. 9*B*); furthermore, the increases were comparable for isoproterenol and BAY y 5959 for similar degrees of inotropism. Finally, the CHF group had a lower baseline intercept compared with normal, and this difference was statistically significant.

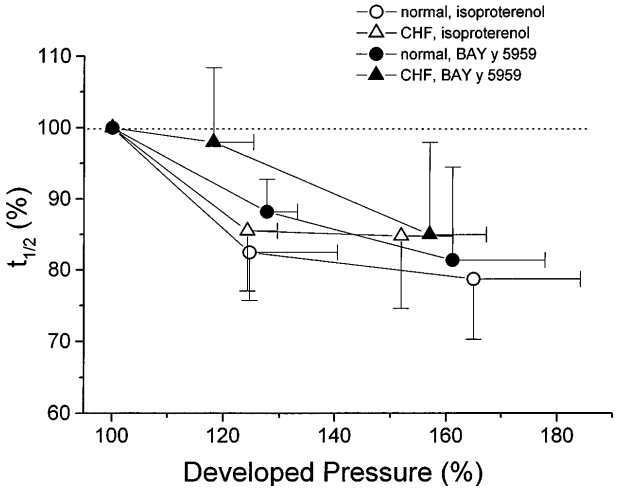

Fig. 5. Average changes in rate of relaxation [indexed by half-time of pressure fall ($t_{½}$)] plotted as a function of contractile state for BAY y 5959 and for isoproterenol. Contractile state was indexed by developed pressure normalized to its baseline value before drug infusion. In both normal and CHF hearts, $t_{½}$ decreased as developed pressure increased by either drug ($P < 0.01$ by multiple linear regression), but there were no differences in the slopes of any of these relations, indicating a comparable effect of these drugs on dynamic aspects of relaxation.

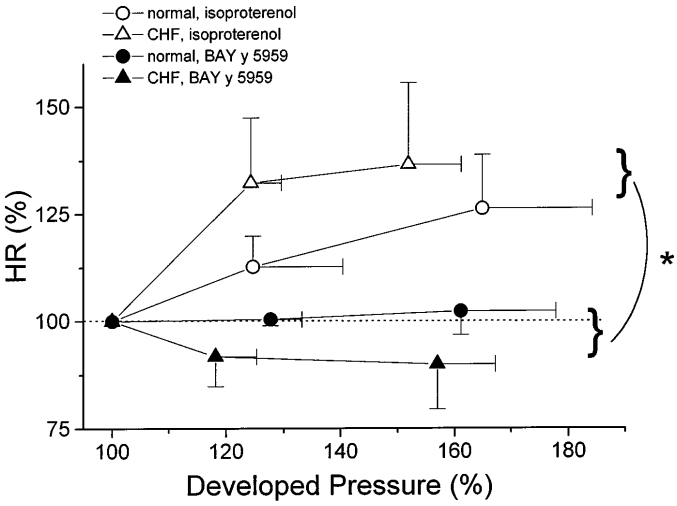

Fig. 6. Effect of BAY y 5959 and isoproterenol on native heart rate (HR) in both normal and CHF hearts as a function of contractile state. Both HR and developed pressure (the index of inotropic response) are normalized to their respective baseline values before drug infusion. Isoproterenol increased HR in both normal and CHF hearts (*$P < 0.01$ by multiple linear regression analysis for both groups). There was no statistically significant effect of BAY y 5959 on HR in either normal or CHF hearts, although a trend for an HR reduction in CHF hearts is noted.

DISCUSSION

The results of the present studies have provided new insights into the effects of BAY y 5959 on properties of the intact heart that have not been obtained from previous studies of hearts in situ. Most notably, it has been shown that changes in MVo_2 created with this agent are comparable to that observed when the inotropic state is increased comparably by a β-agonist. Mild bradycardic actions observed in our ex vivo preparation suggest that at least a component of its bradycardic

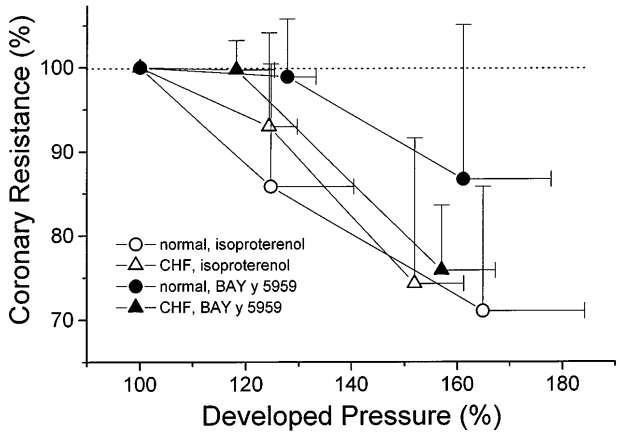

Fig. 7. Effects of BAY y 5959 and isoproterenol on coronary vascular resistance as a function of contractile state. Both coronary resistance and developed pressure (the index of inotropic response) are normalized to their respective baseline values before drug infusion. As shown, coronary vascular resistance varied similarly after both isoproterenol and BAY y 5959 with a vasodilatory response. Statistical analysis revealed that these responses were similar for the 2 drugs, suggesting that these effects are mediated by metabolic autoregulation and that BAY y 5959 did not interfere with this process.

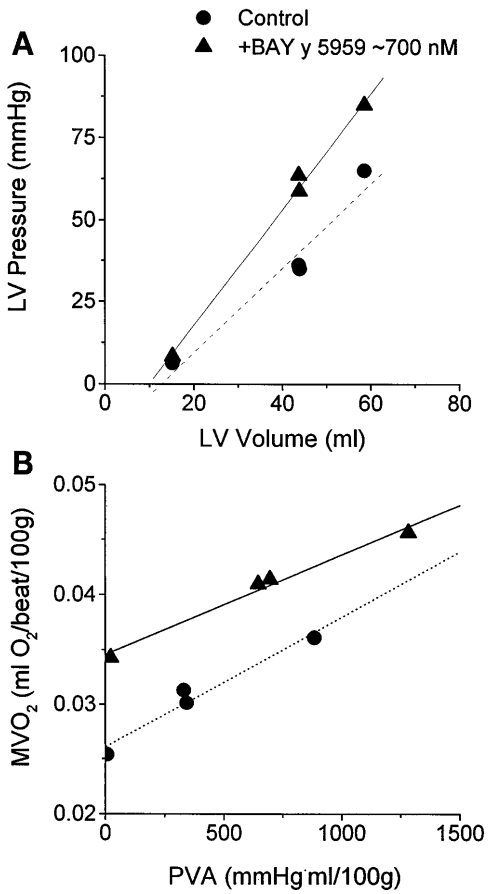

Fig. 8. Representative end-systolic pressure-volume relationship (ESPVR, *A*) and myocardial oxygen consumption ($M\dot{V}O_2$)-pressure-volume area (PVA) relationship (*B*) under control conditions and after infusion of BAY y 5959 to achieve blood concentration of ~700 nM in a CHF heart. Consistent with the positive inotropic action, the slope of the ESPVR increased with little increase in volume-axis intercept. Similar to reports with other inotropic agents, BAY y 5959 increased the $M\dot{V}O_2$ intercept with little effect on the slope of the relationship.

actions in situ may be mediated by direct myocardial effects independently of reflexes. Coronary flow measurements revealed that metabolic autoregulation of the coronary vascular bed in response to inotropic-mediated increased work was not affected by this calcium channel promoter. Finally, whereas action potential duration was increased, there was a mild shortening of the overall contraction duration and increase in the rate of relaxation under conditions in which heart rate is maintained constant.

Results of previous in vitro studies have shown that BAY y 5959 enhances L-type calcium channel conductance during electrical depolarization and prolongs the action potential (1). The inotropic actions of this agent have been attributed to changes in intracellular calcium and are independent of pathways involving cAMP. The results of the present study provide a detailed characterization of the direct myocardial physiological actions of this agent in intact blood-perfused canine hearts. Studies have been performed in both normal hearts and in hearts rendered myopathic by repeated coronary microembolization (13, 14). Hemodynamic measurements have shown that the degree of heart failure achieved with this model is more moderate than that achieved with other experimental models of heart failure (e.g., rapid pacing-induced heart failure). However, this model is relatively stable over long periods of time (13), and the method of achieving myocardial damage simulates the most common etiology of chronic heart failure, making it a good model for studying effects of cardiotonic agents.

We showed for the intact heart that BAY y 5959 has positive inotropic actions that are associated with an increase in the action potential duration and minor reduction in overall duration of contraction when heart rate is maintained constant by pacing. These effects contrast with the shortened action potential duration and larger decrease in contraction duration seen with β-agonists. Even though these effects of β-agonism on the dynamics of contraction are heart rate independent (and relate to phosphorylation of myofilament proteins and phospholamban), they are generally considered to be the means by which adequate diastolic duration can be maintained in the face of increased heart rate.

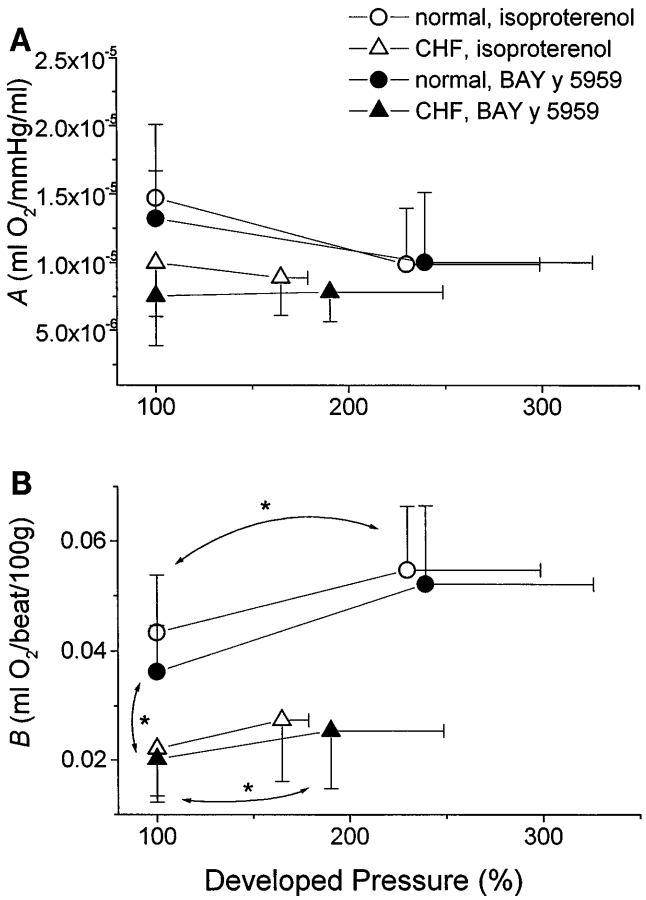

Fig. 9. Impact of BAY y 5959 and isoproterenol on slope *A* (*A*) and $M\dot{V}O_2$ intercept *B* (*B*) in normal and CHF hearts as a function of the degree of inotropism. As in previous studies, multiple linear regression analysis revealed no statistically significant effect on slope *A* of the relationship, but there was a consistent inotropic-related effect on intercept *B* of the relationship. Furthermore, changes in intercept *B* were similar for isoproterenol and for BAY y 5959. Finally, there was a statistically significant difference in baseline values of intercept *B* between normal and CHF hearts. $*P < 0.05$.

Because BAY y 5959 does not increase heart rate and does not impair relaxation (as may have been the case for an agent that enhances intracellular calcium), the lack of a contraction duration shortening effect should not result in a detrimental effect on overall hemodynamics. Indeed, preliminary studies in intact animals (both with and without heart failure) and patients have failed to reveal any hemodynamic consequences related to long durations of contraction or relaxation (4, 11, 17, 19, 24). The reason that relaxation is not impaired by BAY y 5959 has not been elucidated but is consistent with results obtained previously in isolated muscle strips for both BAY y 5959 and BAY K 8644 (9). It has been presumed that although peak calcium is enhanced by these agents, the rate of calcium sequestration by the sarcoplasmic reticulum is not influenced (positively or negatively), and thus the rate of relaxation is unaltered.

Whereas inotropic responsiveness to isoproterenol was blunted in our model of moderate heart failure compared with normal hearts, the effectiveness of BAY y 5959 was preserved. Recent preliminary studies in conscious dogs have also shown preserved inotropic responsiveness in two different models of heart failure (rapid pacing and repeated coronary microembolization) (20, 24). Although it remains to be determined whether tolerance develops during long-term infusions [and results of preliminary studies suggest that it may not develop (24)], the finding of relatively preserved inotropic effectiveness in heart failure is a potentially important characteristic of this class of agents.

In the heart, myocardial specificity of BAY y 5959 was evidenced in the present study by a lack of vasoconstricting action on the coronary bed. Rather, coronary vasodilation occurred to a similar degree as was observed with isoproterenol for comparable degrees of inotropism. This suggests that the observed vasodilation could be related to metabolic autoregulation of the vascular bed in the face of increased workload rather than a direct vasodilatory effect of the drug on the vasculature (15). Because we did not examine effects on other vascular beds, we cannot address whether BAY y 5959 is truly myocardial specific or whether it may affect functioning of other organ systems.

In studies in which cardiac pacing was interrupted, it was shown that, whereas heart rate was increased dose dependently by isoproterenol, there was no significant effect (and in CHF animals a trend to decrease heart rate) in response to BAY y 5959. Bradycardic actions of BAY y 5959 have been noted after administration to conscious dogs, and it has been debated whether this reflects a direct myocardial effect or whether this is mediated by autonomic reflexes (19). Results of preliminary studies of in vitro cells suggest a direct bradycardic action (6). The observed tendency for a bradycardic action in our isolated CHF hearts indicates that direct, reflex-independent effects may be present (presumably effects on the sinus node) because reflexes are absent in this preparation and heart rate is controlled, as in situ, by the sinus node, which is intact with our methods of isolation. The fact that the bradycardic effects were much stronger in the conscious animals suggests that other factors may be involved, for example, as proposed, the baroreflexes. A drug with bradycardic or neutral effects on heart rate despite marked inotropic actions may have advantages for the treatment of heart failure over traditional agents with which $M\dot{V}O_2$ increases in proportion to heart rate. On the other hand, lack of a tachycardiac action may render changes in cardiac output less pronounced with BAY y 5959 than with a β-agonist for a given degree of inotropism; this is because a part of the increased cardiac output seen with β-agonists is due to the increase in heart rate and not to the inotropic effects.

As expected, there was an increase in $M\dot{V}O_2$ that accompanied BAY y 5959 positive inotropism. Furthermore, we demonstrated that the manner in which BAY y 5959 influenced the $M\dot{V}O_2$-PVA relationship was comparable to that observed with isoproterenol. Both agents increased the intercept of the relationship (which corresponds to an increase in unloaded oxygen consumption) in a dose-dependent manner. Results of recent preliminary studies in vivo and in vitro suggested that when BAY y 5959 and a β-agonist were administered at equipotent inotropic doses, the increase in oxygen consumption was less with BAY y 5959 than for a β-agonist, even when heart rate was controlled (4, 5, 20). This finding suggested a favorable direct effect of BAY y 5959 on myocardial energetics. The results of the present study, in which loading conditions are strictly controlled and in which inotropic actions can be precisely matched (much harder to achieve in situ), did not reveal any such effect. Whereas β-agonists may increase basal metabolism by elevating cAMP which, in turn, may influence several subcellular processes (which would not be the case for a calcium promoter), the sensitivity of the methods used in the present study could detect no energetic differences between these two types of agents. Differing results in these different preparations are not necessarily contradictory because $M\dot{V}O_2$ is very sensitively dependent on heart rate and contractility, as well as preload and afterload; subtle effects of these drugs on each of these factors could strongly influence the results. Therefore, although BAY y 5959 does not differ from β-agonist in the energy cost of inotropism, this does not necessarily predict an equally costly effect on myocardial energy metabolism in situ.

The conditions under which isolated canine heart studies are performed provide control over several important parameters in a setting that is free of autonomic reflexes. Consequently, it is relatively straightforward to determine the direct myocardial actions of a cardiotonic agent. However, several limitations must be acknowledged. First, the isolation and cross-perfusion depress resting contractile state so that the studies are performed with the heart slightly impaired compared with the in situ state. Second, the altered metabolic state of the support dog attributable

to the added stress of perfusing the isolated heart may alter blood levels of important cardioactive hormones compared with normal. Finally, questions related to drug effects in the intact circulation, obviously, cannot be determined. Two examples that have already been discussed above relate to the consequences of the lack of effects of BAY y 5959 on relaxation and to the effects of BAY y 5959 on cardiac output, which will be solely dependent on inotropic actions in the absence of increased heart rate. Naturally, many other factors not investigated in the present study need to be addressed in assessing the potential utility of this agent as a therapy for heart failure. For example, the implications of the action potential prolongation and effects on cardiac arrhythmias need to be defined. The effects of long-term infusions (with attention to the potential for developing tolerance) also need to be clarified. The rate of apoptosis, now considered to be an important factor in the progression of heart failure, may be influenced by a drug that increases intracellular calcium, especially in our embolization model, in which apoptosis has been demonstrated to occur (22). Finally, it will be important to elucidate the effects of BAY y 5959 on arterial and venous properties in various vascular beds.

In summary, lack of coronary vascular effects, preserved inotropic actions in heart failure, and lack of positive chronotropic effects are three aspects of the myocardial actions of BAY y 5959 that are potentially advantageous characteristics for heart failure therapy. Lack of influence on the duration of contraction and rate of relaxation have not been noted to influence systemic hemodynamics, although the in vivo consequences of these factors need further clarification, particularly in the heart failure state. Similarly, the implications of action potential prolongation (also noted previously in isolated muscle) also need to be studied (16). Finally, the direct metabolic cost of inotropism was demonstrated to be the same as for isoproterenol, a finding which was anticipated on the basis of current understanding of the determinants of $M\dot{V}O_2$. These findings suggest, in view of the unique mechanism of action, constellation of myocardial effects, and lack of effects on coronary vasculature, that further studies aimed at defining the potential clinical utility of this novel inotropic agent are warranted.

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REFERENCES

1. **Bechem, M., S. Goldmann, R. Gross, S. Hallermann, S. Hebisch, J. Hutter, H. P. Rounding, M. Schramm, J. Stoltefuss, and A. Straub.** A new type of Ca-channel modulation by a novel class of 1,4-dihydropyridines. *Life Sci*. 60: 107–118, 1997.
2. **Bristow, M. R.** Changes in myocardial and vascular receptors in heart failure. *J. Am. Coll. Cardiol*. 22, *Suppl*. A: 61A–71A, 1993.
3. **Burkhoff, D., P. P. de Tombe, W. C. Hunter, and D. A. Kass.** Contractile strength and mechanical efficiency of left ventricle are enhanced by physiological afterload. *Am. J. Physiol*. 260 (*Heart Circ. Physiol*. 29): H569–H578, 1991.
4. **Dembowsky, K., M. Bechem, S. Goldmann, R. Gross, S. Hebisch, J. Hutter, P. Rounding, M. Schramm, J. Stoltefuss, and A. Straub.** The calcium promotor BAY y 5959 increases cardiac contractility without an oxygen wasting effect in vitro as well as in vivo (Abstract). *Circulation* 90: I-483, 1994.
5. **Dembowsky, K., U. Jahnel, S. Goldmann, R. Gross, J. Hutter, W. Schrock, J. Stoltefuss, and A. Straub.** The novel calcium-promotor BAY y 5959 increases cardiac economy in rat working heart and guinea pig papillary muscle (Abstract). *J. Heart Failure* 3: 40, 1996.
6. **Dembowsky, K., U. Jahnel, E. Lohrmann, J. Stoltefuss, and R. Gross.** Modulation of the beating frequency of cardiomyocytes in vitro by various calcium channel activators (Abstract). *Circulation* 94: I-556, 1996.
7. **Feldman, M. D., L. Copelas, J. K. Gwathmey, P. Phillips, S. E. Warren, F. J. Schoen, W. Grossman, and J. P. Morgan.** Deficient production of cyclic AMP: pharmacologic evidence of an important cause of contractile dysfunction in patients with end-stage heart failure. *Circulation* 75: 331–339, 1987.
8. **Franz, M. R., D. Burkhoff, H. Spurgeon, M. L. Weisfeldt, and E. G. Lakatta.** A new myocardial contact electrode accurately records membrane repolarization. *Eur. Heart J*. 7: 34–41, 1986.
9. **Gross, R., M. Bechem, M. Kayser, M. Schramm, R. Taniel, and G. Thomas.** Effects of the calcium agonistic dihydropyridine BAY K 8644 on the heart. In: *Cardiovascular Effects of Dihydropyridine-Type Calcium Antagonists and Agonists*, edited by A. Fleckenstein, C. van Breemen, R. Gross, and F. Hoffmeister. Heidelberg, Germany: Springer-Verlag, 1985, p. 218–232.
10. **Gross, R., M. Kayser, M. Schramm, R. Taniel, and G. Thomas.** Cardiovascular effects of the calcium-agonist dihydropyridine BAY k 8644 in conscious dogs. *Arch. Int. Pharmacodyn. Ther*. 277: 203–216, 1985.
11. **Hutter, J., M. Bechem, S. Goldmann, R. Gross, S. Hebisch, P. Rounding, M. Schramm, J. Stoltefuss, and A. Straub.** Haemodynamic action of the novel positive inotropic dihydropyridine BAY y 5959 in anaesthetized and conscious dogs (Abstract). *Eur. Heart J*. 15: 202, 1994.
12. **Ishii, K., Y. Sato, and N. Taira.** Similarity and dissimilarity of the vasoconstrictor effects of BAY k 8644 on coronary, femoral, mesenteric and renal circulations in dogs. *Br. J. Pharmacol*. 88: 369–377, 1986.
13. **Knecht, M., D. Burkhoff, G. H. Yi, S. Popilskis, and J. Wang.** Coronary endothelial dysfunction precedes heart failure and reduction of coronary reserve in awake dogs. *J. Mol. Cell. Cardiol*. 29: 217–227, 1997.
14. **Knecht, M., G. H. Yi, S. Popilskis, M. Packer, D. Burkhoff, and J. Wang.** Daily coronary microembolization in awake dogs leads to heart failure (Abstract). *J. Am. Coll. Cardiol*. 25: 222A, 1995.
15. **Mohrman, D. E., and E. O. Feigl.** Competition between sympathetic vasoconstriction and metabolic vasodilation in the canine coronary circulation. *Circ. Res*. 42: 79–86, 1978.
16. **Rousseau, M. F., W. Hayashida, C. van Eyll, S. Ahn, M. Bornemann, and H. Pouleur.** Calcium promotor BAY y 5959: the optimal way to deliver positive inotropism and class III antiarrhythmic effects? (Abstract) *Circulation* 90: I-217, 1994.
17. **Rousseau, M. F., P. E. Massart, C. van Eyll, J. Etienne, S. Ahn, L. Stoleru, M. Bornemann, and H. Pouleur.** Dissociation between the inotropic and systemic effects of the calcium promoter BAY y 5959 in patients with heart failure. *J. Am. Coll. Cardiol*. 27, *Suppl*. A: 280A, 1996.
18. **Sabbah, H. N., P. D. Stein, T. Kono, M. Gheorghiade, T. B. Levine, S. Jafri, E. T. Hawkins, and S. Goldstein.** A canine model of chronic heart failure produced by multiple sequential coronary microembolizations. *Am. J. Physiol*. 260 (*Heart Circ. Physiol*. 29): H1379–H1384, 1991.
19. **Sato, M., M. Uechi, K. Asai, T. A. Patrick, R. K. Kudej, and S. F. Vatner.** Effects of a novel inotropic agent, BAY y 5959, in

conscious dogs: comparison with dobutamine and milrinone. *Am. J. Physiol.* 272 (*Heart Circ. Physiol.* 41): H753–H759, 1997.

20. **Sato, N., M. Uechi, R. K. Kudej, K. Asai, D. E. Vatner, and S. F. Vatner.** Comparison of a novel calcium channel agonist and dobutamine in conscious dogs with heart failure (Abstract). *J. Am. Coll. Cardiol.* 27: 27A, 1996.
21. **Schramm, M., R. Towart, B. Lamp, and G. Thomas.** Modulation of calcium ion influx by the 1,4-dihydropyridines nifedipine and BAY k 8644. *J. Cardiovasc. Pharmacol.* 7: 493–496, 1985.
22. **Sharov, V. G., H. N. Sabbah, H. Shimoyama, A. V. Goussev, M. Lesch, and S. Goldstein.** Evidence of cardiocyte apoptosis in myocardium of dogs with chronic heart failure. *Am. J. Pathol.* 148: 141–149, 1996.
23. **Suga, H.** Ventricular energetics. *Physiol. Rev.* 70: 247–277, 1990.
24. **Wang, J., G. H. Yi, S. M. Zhu, H. Zhang, M. Packer, and D. Burkhoff.** Hemodynamic effects of a novel calcium channel promoter, BAY y 5959, are preserved in ischemic heart failure. *Circulation* 94, *Suppl.* I: I-557, 1996.