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ABSTRACT

Neutral endopeptidase inhibition (NEP-I) and angiotensin converting enzyme inhibition (ACE-I) act synergistically to produce acute beneficial hemodynamic effects in models of heart failure. Blockade of the formation of angiotensin II (Ang II) acting together with potentiation of the natriuretic peptides, bradykinin and other vasoactive peptides may mediate the interaction of dual enzyme inhibition. In this study, the potential roles of Ang II repression and bradykinin potentiation were evaluated in conscious cardiomyopathic hamsters with compensated heart failure. The Ang II AT_1 receptor antagonist, SR 47436 (BMS-186295), was administered at 30 μmol/kg, i.v. followed by i.v. infusion at 1 μmol/kg/min in combination with NEP-I (SQ-28603 at 30 μmol/kg i.v.). Cardiac preload (left ventricular end diastolic pressure) and afterload (left ventricular systolic pressure) decreased significantly more after the combination of Ang II blockade and NEP-I than after either treatment alone. This indicated that repression of Ang II contributes importantly to the NEP-I/ACE-I interaction. Bradykinin B_2 receptor antagonism by Hoe 140 at 100 μg/kg, i.v. significantly blunted the decrease in left ventricular end diastolic pressure but not the decrease in left ventricular systolic pressure after dual NEP-I/ACE-I (SQ-28603 and enalaprilat each at 30 μmol/kg, i.v.). This suggests that bradykinin potentiation contributes to the preload-reducing, but not the afterload-reducing, acute effects of NEP-I/ACE-I. Hence, both Ang II repression and bradykinin potentiation are factors contributing to the synergistic hemodynamic effects of combined NEP-I and ACE-I in hamsters with heart failure. The bradykinin-mediated enhanced effect of combined NEP-I/ACE-I to reduce cardiac preload could improve the beneficial effects of ACE-I in the treatment of heart failure.

ACE-I blocks the formation of Ang II and hence attenuates its vasoconstrictor, antinatriuretic and growth enhancement properties. NEP-I prevents the enzymatic inactivation of ANP and therefore protects or potentiates its vasodilatory, natriuretic and antiproliferative actions. Studies have shown that concurrent administration of NEP-I and ACE-I in models of hypertension and heart failure result in an interaction that leads to cardiovascular effects greater than those caused by either treatment given singly. For instance, the antihypertensive effect of ACE-I in conscious spontaneously hypertensive rats was enhanced by coadministration of selective inhibitors of NEP (Seymour *et al.*, 1991; Pham *et al.*, 1993). In dogs with pacing-induced heart failure, NEP-I potentiated the vasodilatory effects of ACE-I (Seymour *et al.* 1993); and in a similar model, subchronic treatment with ACE-I potentiated the renal hemodynamic and excretory responses to NEP-I (Margulies *et al.*, 1991). In cardiomyopathic hamsters with heart failure, the combination of the ACE inhibitor, enalaprilat, and the selective NEP inhibitor, SQ-28603, produced decreases in cardiac preload and afterload, whereas each treatment alone had minimal effects (Trippodo *et al.*, 1993). These studies support the concept that the coadministration of ACE-I and NEP-I leads to synergistic effects and could have use in the treatment of hypertension and heart failure.

Possible mechanisms by which NEP-I and ACE-I interact to produce enhanced cardiovascular effects have been previously discussed (Seymour *et al.*, 1993, Trippodo *et al.*, 1993). Attenuation of the formation of Ang II by ACE-I might unmask the vasodilatory effects of ANP and other natriuretic peptides such as BNP and CNP. This may be particularly relevant in heart failure where the biological actions of ANP are blunted, perhaps partly because of the counteracting effects of Ang II (Raya *et al.*, 1989; Margulies *et al.*, 1991). Potentiation of the vasodilatory effects of bradykinin by both ACE-I and NEP-I might also contribute to the synergism. Although bradykinin can be potentiated by ACE-I alone, greater enhancement of the activity of this peptide may come

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ABBREVIATIONS: ACE-I, angiotensin converting enzyme inhibition; Ang II, angiotensin II; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CNP, C-type natriuretic peptide; HR, heart rate; LVEDP, left ventricular end diastolic pressure; LVSP, left ventricular systolic pressure; MAP, mean arterial pressure; NEP-I, neutral endopeptidase inhibition.

fects of combined NEP-I and ACE-I in cardiomyopathic hamsters with heart failure. These factors were studied not only because of the known effects of the inhibitors on their metabolism, but also because potent specific inhibitors of the Ang II AT_1 receptor and the bradykinin B_2 receptor were available. Ang II AT_1 receptors mediate virtually all of the known biological actions of Ang II (Timmermans *et al.*, 1993). Bradykinin B_2 receptors are likely responsible for many of the cardiovascular and renal effects of bradykinin (Regoli *et al.*, 1990). In this study we used the Ang II AT_1 receptor antagonist, SR 47436 (BMS-186295) (Cazaubon *et al.*, 1993) and the bradykinin B_2 receptor antagonist, Hoe 140 (Wirth *et al.*, 1991). The results from the use of these specific probes indicate that both the repression of Ang II and the potentiation of bradykinin contribute to the acute synergistic effects of NEP-I and ACE-I to reduce cardiac preload in cardiomyopathic hamsters with heart failure.

Methods

All procedures involving animals were in accordance with the Bristol-Myers Squibb Pharmaceutical Research Institute Animal Care and Use Committee.

Animal preparation

Cardiomyopathic male hamsters of the BIO TO-2 strain (Sole, 1986) were obtained from Bio Breeders, Inc., Fitchburg, MA. The animals were housed two to five per cage in a light and dark cycle of 14 h and 10 h, respectively, for a minimum of 2 wk before study. The animals were maintained on Purina 5001 pelleted Chow (Purina, Richmond, IN) and tap water *ad libitum*; they were studied at approximately 260 days of age in a stage of nonedematous, compensated heart failure (Fox *et al.*, 1993; Trippodo *et al.*, 1993; Panchal and Trippodo, 1993). All experiments were conducted in conscious, unrestrained, cardiomyopathic hamsters 3 h after placement of catheters. At the end of each experiment, the animals were euthanized with sodium pentobarbital (100 mg/kg, i.v.).

The hamsters were briefly anesthetized with methohexital sodium (70 mg/kg, i.p., Brevital, Eli Lilly & Co., Indianapolis, IN). Polyethylene catheters (PE10 connected to PE50) were filled with isotonic saline (0.9% NaCl) containing heparin (4 IU/ml). The PE10 ends of the catheters were used for cannulation. The right jugular vein was cannulated and supplemental doses of methohexital (1 mg/kg, i.v.) were administered as needed during surgery. The right carotid artery was cannulated for the measurement of MAP; in some experiments the catheter was advanced into the left ventricle for the measurement of LVSP and LVEDP. The free ends of the catheters were passed under the skin and exteriorized at the back of the neck near the scapula. The animals were allowed to recover unrestrained for 3 h before initiating measurements. The arterial or ventricular catheter was connected to a pressure transducer (Model P23XL, Gould Electronics, Valley View, OH) for measurement of MAP or LVEDP and LVSP. Cardiovascular pressures and HR were recorded

ducted in conscious cardiomyopathic hamsters to determine a dose regimen of SR 47436 (BMS-186295) that would nearly completely block the pressor response to Ang II for at least 2 h. The pressor responses to two challenges of Ang II (100 ng/kg, i.v. dissolved in 0.9% NaCl, 1 ml/kg) were determined. This dose of Ang II produced more than a 30% increase in MAP. Based on the preliminary experiments, SR 47436 (BMS-186295) was administered to five cardiomyopathic hamsters at 30 μmol/kg, i.v. followed by continuous i.v. infusion at 1 μmol/kg/min. Challenges of Ang II were then repeated at 10-min to 30-min intervals up to 150 min after the bolus injection of SR 47436 (BMS-186295).

Bradykinin depressor response. Because i.v. bolus injections of bradykinin caused respiratory distress in the cardiomyopathic hamsters, depressor responses to intraarterial administration of this peptide were studied. There were no changes in the behavior of the animals to suggest pain or distress after the administration of bradykinin, i.a. In eight conscious cardiomyopathic hamsters the depressor response to 0.9% NaCl (1 ml/kg, i.a.) was determined. This was followed by two challenges of bradykinin (10 μg/kg, i.a., dissolved in 1 ml/kg 0.9% NaCl). This dose of bradykinin and saline produced a depressor response that was in the mid to upper range of the bradykinin dose-response relationship for depressor effects. Based on preliminary experiments, Hoe 140 was administered at 100 μg/kg, i.v. (dissolved in 0.9% NaCl, 1 ml/kg). Challenges of bradykinin were then repeated at 5-min to 30-min intervals up to 180 min. A second injection of 0.9% NaCl was administered at the end of the experiment.

Cardiovascular effects

In this series of experiments, baseline measurements of LVEDP, LVSP and HR were determined in groups of conscious cardiomyopathic hamsters. Compounds or vehicles were administered at 1 ml/kg, i.v., unless indicated otherwise, and measurements were repeated at 5-min to 30-min intervals up to 90 min after administration of the last agent.

SR 47436 (BMS-186295), SQ-28603 and the combination of these agents. SR 47436 (BMS-186295) was administered at 30 μmol/kg, i.v. (0.3 ml) followed by a continuous i.v. infusion at 1 μmol/kg/min (0.01 ml/min). SR 47436 (BMS-186295) was prepared in 0.028 M KOH and diluted to a final concentration of 0.017 M KOH. KOH solution (0.017 M) was administered i.v. to the vehicle group at 0.3 ml followed by a continuous infusion at 0.01 ml/min. SQ-28603 was dissolved in 0.84% $NaHCO_3$ and administered at 30 μmol/kg, i.v. This dose of SQ-28603 was previously shown to result in a doubling of plasma ANP concentration within 90 min in this model (Trippodo, *et al.*, 1993). The vehicle for SQ-28603 was previously shown to have only minimal cardiovascular effects in cardiomyopathic hamsters (Trippodo *et al.*, 1993); similar minimal effects were observed in this study (see below). One group of cardiomyopathic hamsters received the combination of SR 47436 (BMS-186295) and SQ-28603. In this group, SR 47436 (BMS-186295) was administered according to the same dosage regimen described above; 30 min after the bolus injection of SR 47436 (BMS-186295), SQ-28603 was administered at 30 μmol/kg, i.v.

30 min later SQ-28603. The vehicles and doses of the compounds were the same as indicated above.

Statistical analyses

Differences in age, body weight and baseline values among groups were evaluated by analysis of variance. Differences in changes from baseline among groups were evaluated by analysis of covariance with repeated measures and contrasts. The baseline value for each variable was used as the covariate. The level of significance was taken at $P < .05$. All data are expressed as means ± S.E.M.

Compounds

Ang II and bradykinin were purchased from Sigma Chemical Co. (St. Louis, MO); Hoe 140 was purchased from Peninsula Laboratories (Belmont, CA); enalaprilat was supplied by Merck Sharp & Dohme Research Laboratories (West Point, PA); SQ-28603 was synthesized by Bristol-Myers Squibb Pharmaceutical Research Institute (Princeton, NJ) and SR 47436 (BMS-186295) was synthesized by Sanofi Recherche (Montpellier, France).

Results

Inhibition of pressor and depressor responses

Ang II pressor response. The pressor responses to duplicate injections of Ang II were 29 ± 2 mm Hg and 31 ± 3 mm Hg (fig. 1). After the administration of SR 47436 (BMS-186295), the pressor responses to Ang II at intervals up to 150 min were less than 6 mm Hg. These results indicate that nearly complete inhibition of the pressor response to

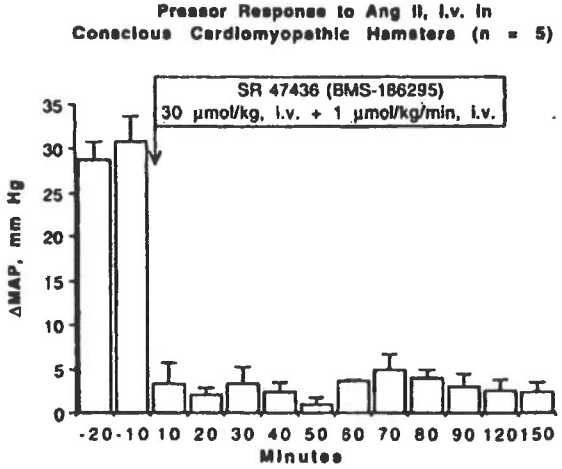

Fig. 1. Changes in MAP in response to Ang II, 100 ng/kg, i.v. before and after the administration of SR 47436 (BMS-186295) in conscious cardiomyopathic hamsters (age 263 ± 5 days; b.wt. 124 ± 6 g).

Hg for intervals up to 180 min. These results indicate tha after the administration of Hoe 140, the depressor response to i.a. injections of bradykinin in 0.9% NaCl was attributabl mostly to the vehicle effect and that the depressor effect bradykinin alone was nearly completely abolished for th duration of study.

Cardiovascular effects

SR 47436 (BMS-186295), SQ-28603 and the combina tion of these agents. Age, body weight and baseline value were similar ($P > .05$) among the four groups of cardiomy pathic hamsters in this series of experiments. These value (means ± S.E.M.) were as follows in the vehicle (n = 10 SQ-28603 (n = 6), BMS-186295 (n = 9) and BMS-186295 SQ-28603 (n = 7) groups, respectively: age (days), 256 ± 261 ± 4, 258 ± 1, 261 ± 2; b.wt. (g), 116 ± 2, 118 ± 3, 117 2 and 117 ± 3; LVEDP (mm Hg), 19 ± 2, 18 ± 3, 17 ± 2 an 21 ± 2; LVSP (mm Hg), 111 ± 3, 117 ± 5, 112 ± 2 and 10 ± 3; HR (beats/min), 350 ± 10, 378 ± 12, 338 ± 16 and 36 ± 6. The changes in LVEDP and LVSP after the administr tion of SR 47436 (BMS-186295) were not significantly diffe ent from those in the vehicle (0.017 M KOH) group (fig. 3 After the administration of SQ-28603 alone, LVEDP d creased only slightly (by <5 mm Hg), although the decrea at the 30-min time point was significantly greater than th change in the vehicle group. LVSP decreased by approx mately 10 mm Hg after the administration of SQ-2860 alone; the decreases at two time points were significant

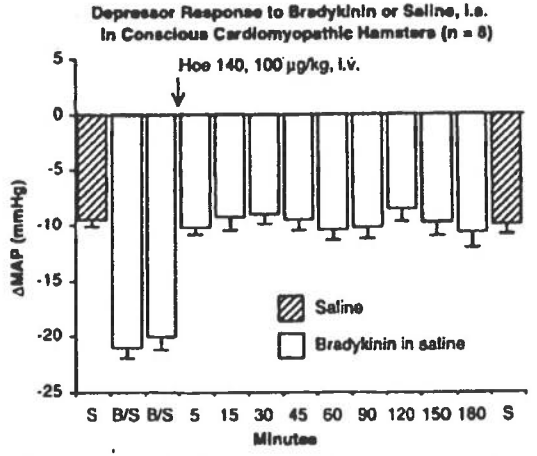

Fig. 2. Changes in MAP in response to bradykinin, 10 µg/kg, i.a. saline (0.9% NaCl), 1 ml/kg, i.a. before and after the administration Hoe 140 in conscious cardiomyopathic hamsters (age 259 ± 2 da b.wt. 112 ± 2 g).

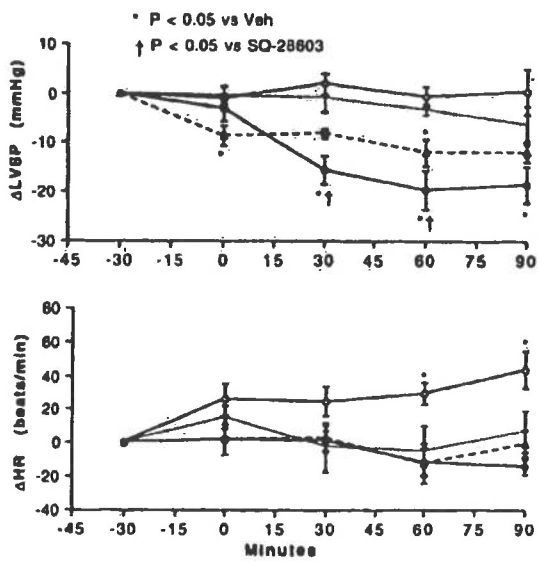

Fig. 3. Cardiovascular changes in conscious cardiomyopathic hamsters after the administration of vehicle (0.017 M KOH), SQ-28603, SR 47436 (BMS-186295) or the combination of SQ-28603 and SR 47436 (BMS-186295). The group receiving the combination treatment is designated as 295 + 603. SQ-28603 was administered as an i.v. bolus at 30 μmol/kg. SR 47436 (BMS-186295) was administered at 30 μmol/kg, i.v. followed by a continuous i.v. infusion at 1 μmol/kg/min. In the combination treatment group, SQ-28603 was administered 30 min after the bolus injection and start of infusion of SR 47436 (BMS-186295).

greater than the changes in the vehicle group. The combination of SQ-28603 and SR 47436 (BMS-186295) produced decreases in LVEDP and LVSP that were significantly greater than the vehicle effects and the changes due to the administration of these compounds alone. For instance, at 90 min after the administration of the combination treatment, LVEDP was decreased by 11 ± 3 mm Hg from a baseline of 21 ± 2 mm Hg; LVSP was decreased by 18 ± 4 mm Hg from a baseline of 107 ± 3 mm Hg. HR changes were minimal in all groups, although significant increases relative to the vehicle group were observed at two time points after the administration of SR 47436 (BMS-186295).

Combination of enalaprilat and SQ-28603 and the effects of Hoe 140 on this combination. Age, body weight and baseline values were similar ($P > .05$) among the four groups of cardiomyopathic hamsters in this series of experiments. These values (means ± S.E.M.) were as follows in the vehicle ($n = 9$), Hoe 140 ($n = 6$), enalaprilat + SQ-28603 ($n = 6$), Hoe 140 + enalaprilat + SQ-28603 ($n = 10$) groups, respectively: age (days), 259 ± 2, 262 ± 2, 260 ± 1 and 258 ± prilat and SQ-28603 resulted in a significantly smaller decrease (approximately 5 mm Hg) in LVEDP as compared with the combination of enalaprilat and SQ-28603, but did not blunt the changes in LVSP. The changes in HR in all groups were generally small, although significant decreases relative to the vehicle or the enalaprilat plus SQ-28603 groups were observed at some time points in the animals receiving Hoe 140 plus the combination of enalaprilat and SQ-28603.

Effects of Hoe 140 on the combination of SR 47436 (BMS-186295) and SQ-28603. Age (258 ± 1 days), b.wt.

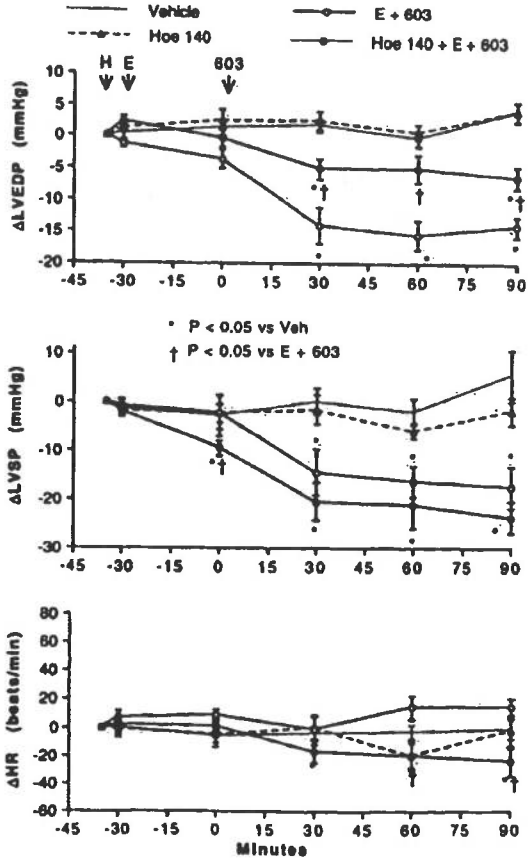

Fig. 4. Cardiovascular changes in conscious cardiomyopathic hamsters after the administration of vehicle (0.84% $NaHCO_3$), Hoe 140 (H, 100 μmol/kg, i.v.), the combination of enalaprilat (E, 30 μmol/kg, i.v.) and SQ-28603 (603, 30 μmol/kg, i.v.), or the combination of Hoe 140 plus enalaprilat and SQ-28603 (same doses as above). Arrows indicate times of administration of compounds.

SQ-28603. Because different vehicles had to be used in the two series of experiments using the ACE inhibitor, enalaprilat or the Ang II antagonist, SR 47436 (BMS-186295), and because slightly different vehicle effects were observed, direct comparison of groups from these two series of experiments would not be valid. Therefore, to eliminate the "vehicle effects" in such a comparison, the average change observed at

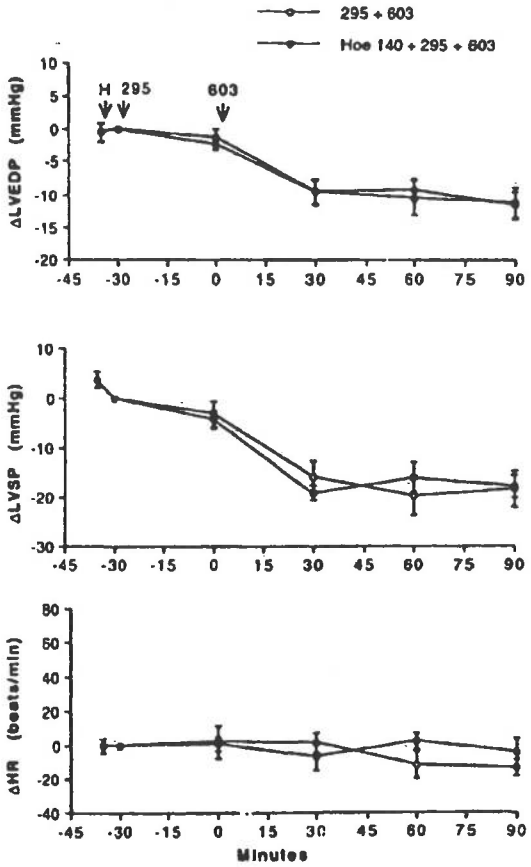

Fig. 5. Cardiovascular changes in conscious cardiomyopathic hamsters after the administration of the combination of SQ-28603 and SR 47436 (BMS-186295) or the combination of Hoe 140 plus SR 47436 (BMS-186295) and SQ-28603. The results from the combination of SQ-28603 and SR 47436 (BMS-186295) are the same as those shown in figure 3. SQ-28603 (603) was administered as an i.v. bolus at 30 μmol/kg. SR 47436 (BMS-186295) (295) was administered at 30 μmol/kg, i.v. followed by a continuous i.v. infusion at 1 μmol/kg/min. Hoe 140 (H) was administered as an i.v. bolus at 100 μg/kg. Arrows indicate times of administration of compounds.

with the animals receiving SR 47436 (BMS-186295) plus SQ-28603. A similar comparison between the groups receiving Hoe 140 with each of these combination treatments revealed no significant differences in the changes (minus vehicle effects) in LVEDP.

Discussion

The findings demonstrated that in hamsters with compensated heart failure, Ang II receptor blockade acted synergis-

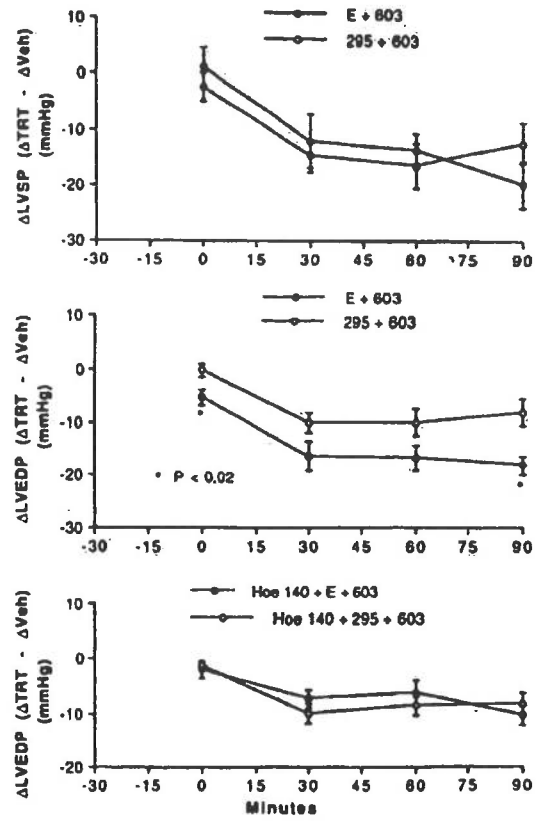

Fig. 6. Cardiovascular changes (minus vehicle effects) in conscious cardiomyopathic hamsters after the administration of the combination of enalaprilat (E, 30 μmol/kg, i.v.) and SQ-28603 (603, 30 μmol/kg, i.v.) or the combination of SQ-28603 (603, 30 μmol/kg, i.v.) and SR 47436 (BMS-186295) (295, 30 μmol/kg, i.v. + 1 μmol/kg/min, i.v.). The changes were determined from the results shown in figures 3 and 4. The bottom graph shows the changes (minus vehicle effects) in LVEDP in conscious cardiomyopathic hamsters receiving Hoe 140 in addition to the treatments indicated above; these changes were determined from the results in figures 4 and 5.

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