Clinical Science Immediate Publication. Published on 9 Nov 2006 as manuscript CS20060193

Growth hormone decreases phase II ventricular tachyarrhythmias during acute myocardial infarction in rats

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Key words: myocardial infarction, growth hormone, action potential, ventricular arrhythmias

Short title: growth hormone and ventricular arrhythmias

D.G.T. and A.C.M. were supported by a grant (PENED-01ED511) from the national General Council for Research and Technology.

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Abstract

Growth hormone administration during acute myocardial infarction (MI) ameliorates subsequent left ventricular (LV) dysfunction. In the present study, we examined the effects of such treatment on arrhythmogenesis. Fifty three Wistar rats (218±17g) were randomized into two intraperitoneal injections of either growth hormone (2IU/kg) (n=26) or normal saline (n=27), given 24 hours and 30 minutes, respectively, prior to MI, generated by left coronary artery ligation. A single-lead electrocardiogram was recorded for 24 hours post-MI, using an implanted telemetry system. Episodes of ventricular tachycardia (VT) and ventricular fibrillation (VF) during the first (phase I) and the following (phase II) hours of MI were analysed. Monophasic action potential was recorded from the lateral LV epicardium at baseline and 24 hours post-MI and signal duration at 90% of repolarization (APD90) was measured. Infarct size was calculated 24 hours post-MI.

Infarct size and phase I VT+VF did not differ significantly between groups, but phase II hourly duration of VT+VF episodes was 82.8±116.6s/hour in the control group and 18.3±41.2s/hour in the growth hormone group (p=0.0027), resulting in a lower arrhythmic (p=0.016) and total (p=0.0018) mortality in treated animals. Compared to baseline, APD90 prolonged significantly 24 hours post-MI in the control group, displaying an increased beat-to-beat variation, but remained unchanged in the growth hormone group. We conclude that growth hormone decreases phase II ventricular tachyarrhythmias during MI in the rat. This finding may have implications in cardiac repair strategies.

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Introduction

Myocardial infarction (MI) and its consequences remain a leading cause of morbidity and mortality worldwide and call for new treatment strategies. Growth hormone (GH) and its mediator, insulin-like growth factor-1 (IGF-1), have specific cardiovascular effects and are currently being evaluated as a promising new therapy in this regard [1]. These effects consist of enhancement of neovascularization [2], reduction of apoptosis [3,4], and mainly of an augmentation of actin and myosin synthesis, leading to increased myocyte cell volume [4-6]. Furthermore, animal [7] and human [8] studies indicate that activation of the GH/IGF-1 axis occurs during the first hours after MI, exerts cardioprotective effects on ischaemic myocardium and participates in the repair of the infarcted area [7,8]. Thus, the concept of administration of GH/IGF-1 during acute MI was recently advocated, aiming at prevention of left ventricular (LV) dysfunction [9,10].

However, little is known on the effects of GH/IGF-1 on ventricular arrhythmias during acute MI. This period is characterized by increased arrhythmogenesis, occurring in two peaks, i.e. during the first (phase I) and the following 10-12 hours (phase II) after coronary occlusion [11]. We hypothesized that the cardioprotective effects of early GH administration may decrease ventricular tachyarrhythmias during acute MI. We tested this hypothesis in the rat model, which exhibits a high frequency of ventricular tachyarrhythmias, with a time course corresponding to that seen in humans [12,13]. Thus, the present study aimed to examine the effects of growth hormone treatment primarily on the incidence of ventricular tachyarrhythmias and, secondarily, on infarct size.

Methods

The study was conducted in 63 female Wistar rats, aged 20±1 weeks. This sample size gives a satisfactory power of approximately 80% to detect a (meaningful) 50% reduction in ventricular tachyarrhythmias. The animals received appropriate care and the investigation conforms to the guiding principals of the Declaration of Helsinki. All rats were housed in a climate-controlled environment, with a 12:12-hour light-dark cycle and were given water and standard rat chow ad libitum.

Implantation of telemetry transmitter

The animals were intubated, mechanically ventilated using a rodent ventilator (model 7025, Ugo Basile, Comerio, VA, Italy) and anaesthetised with isoflurane. A continuous electrocardiogram (ECG) telemetry transmitter (Dataquest, Data Sciences International, Transoma Medical, Arden Hills, MN, USA) was implanted in the abdominal cavity, using a previously described method [12]. The rats were then housed in individual cages, placed on a receiver continuously capturing the signal, independently of animal activity. The ECG signal was displayed in real-time with the use of a computer program (A.R.T. 2.2, Dataquest, Data Sciences International, Transoma Medical, Arden Hills, MN, USA) and stored for analysis.

Monophasic action potential recordings

Epicardial monophasic action potential (MAP) signals were recorded as a marker of myocardial ischaemia [14,15]. The method used in our laboratory for MAP recordings has been described previously [16]. In brief, a MAP probe (model 200, EP Technologies, Sunnyvale, CA, USA) was placed on the lateral LV ventricular wall and was secured by hand, exerting mild, constant pressure against the epicardium, to eliminate electrical artifacts. The signal was amplified with the use of a preamplifier (model 300, EP Technologies, Sunnyvale, CA, USA) and filtered at 50 Hz (for elimination of power line interference) using a digital notch filter. The signal was further filtered, using a band pass filter, allowing a signal range between 0.05 Hz and 500 Hz. Two-minute recordings were stored into a personal computer, equipped with an analog-to-digital converter (BNC 2110, National Instruments Corporation, Dallas, TX, USA). The software utilized in this study, developed and validated in our Institution [17], permits recording and off-line analysis of MAP signals. Fifty sinus beats per recording were analysed and action potential duration at 90% of repolarization (APD90) was measured at baseline and 24 hours after MI. Arrhythmic signals and signals displaying electrical artifacts were excluded. The standard deviation of APD90 was calculated for each recording, as a measure of beat-to-beat variation, which correlates with myocardial ischaemia [14,15].

Drug administration and generation of MI

Rats were randomized, in an 1:1 fashion, into two intraperitoneal injections of either 2IU/kg GH (S-8648, Sigma-Aldrich Corporation, St Louis, MO, USA) (n=26) or normal saline (n=27), given 24 hours and 30 minutes prior to MI generation. The dosage used corresponds to that previously found to exert cardioprotection [18] and pre-treatment

was considered to have additional beneficial actions, through systemic and local GH/IGF-1 axis stimulation [19].

MI was generated as previously described [20], by an operator blinded to treatment assignment. The left coronary artery was ligated using a 6-0 suture, placed between the pulmonary artery cone and the left atrial appendage. Following these anatomical landmarks ensures generation of similar infarct size in all experiments [20]. A six-lead ECG was obtained and ST-segment elevation was considered a proof of induced MI. ECG recording was continued for 24 hours or until spontaneous death and no resuscitation attempts were allowed at any time during the study. Twenty-four hours after MI the survivors were re-anaesthetised, the site of previous left thoracotomy was reopened and MAP recordings were repeated at the same sites. The rats were then sacrificed using a lethal dose of potassium chloride and the heart was harvested for measurement of infarct size. The study protocol is depicted in figure 1.

Infarct size

Infarct size was measured using previously described methods [21]. The heart was excised, frozen (in -20ºC for 1 hour), hand-cut in five 2 mm slices, incubated (in triphenyltetrazolium chloride for 15 min at 37ºC) and fixed (in 10% formalin for 20 min). The slices were scanned with the use of a high resolution scanner (Scanjet 4570c/5500c, Hewlett-Packard, Palo Alto, CA, USA), the areas of infarcted and non-infarcted myocardium were measured from both sides of each slice and averaged. Planimetry was performed using a previously validated software program (Image Tool, University of Texas, USA). The measured areas were then multiplied times the slice thickness to determine the volumes of infarcted and non-infarcted myocardium for each slice. These values were summed and infarct size (expressed as a percentage) was defined as the ratio between the infarcted divided by the total LV volume.

Heart rate

We analysed continuous 5-min ECG recordings, from which non-sinus beats were excluded. The mean value of these RR intervals was used to determine heart rate, calculated at baseline, at the 5^{th}, 30^{th} and 60^{th} minute post-MI and hourly thereafter.

Arrhythmia analysis

The acquired ECG tracings were displayed and analysed off-line independently by two of the authors (D.A.E., G.G.B.), blinded to treatment assignment. We report VT and VF duration, according to the guidelines provided by the Lambeth Conventions for

determination of experimental arrhythmias [22]. VT was defined as 4 or more consecutive ventricular ectopic beats and VF was defined as a signal in which individual QRS deflections could not easily be distinguished from one another. Even with these guidelines, separating VF from VT was often difficult, and this has been the experience of others [12]. Therefore, in this study, we report VT and VF collectively. The duration of each VT or VF episode was measured using the time-scale provided by the recording software. For each rat, the sum of VT+VF duration was divided by the actual survival time (i.e. the time at risk for experiencing a tachyarrhythmia) and is reported as hourly duration, expressed as seconds per hour. Since different mechanisms have been suggested to account for VT/VF occurring during the first and the following hours after coronary occlusion [11], hourly VT+VF duration is also reported separately for phase I (during the first hour after MI generation) and for phase II (from the 61st minute to the end of the recording or to spontaneous death). Tachyarrhythmic death was defined as ventricular asystole, preceded by sustained VF and bradyarrhythmic death as ventricular asystole, preceded by bradycardia (<200beats per minute) associated with complete heart block.

Statistical analysis

All values are given as mean ± one standard deviation, unless otherwise specified. Mortality rates were compared using Yates' corrected chi square. Differences in continuous variables were compared using Student's t-test, or the analysis of variance for repeated measures, followed by Tukey's multiple comparisons test, as appropriate. Arrhythmia frequencies were not normally distributed and were compared using the Mann-Whitney U-test. Statistical significance was defined at an alpha level of 0.05.

Results

We studied 63 rats weighing 218±16g. Of these, 10 (5 (16.1%) had received GH and 5 (15.6%) normal saline) died during the surgical procedure and were excluded. Fifty three animals were included in the study, of which 26 (220±23g) received GH and 27 (216±9g) normal saline.

Mortality

There were 10 tachyarrhythmic deaths, 1 (3.8%) in the GH group (during phase I) and 9 (33.3%) in the control group (4 during phase I and 5 during phase II). Arrhythmic mortality was significantly (p=0.016) lower in the GH group, compared to controls. No bradyarrhythmic deaths occurred in GH-treated rats, but there were 3 (11.1%) in the control group (1 during phase I and 2 during phase II); this difference between groups was not statistically significant (p=0.24). Overall mortality was significantly lower in the GH group, compared to controls (p=0.0018).

Infarct Size

Infarct size was calculated for the 40 survivors, 15 in the control group and 25 in the GH group. Mean infarct size was 40.1±7.7% in controls and 35.4±8.2% in treated rats (p=0.08).

Heart rate and ventricular tachyarrhythmias

As shown in figure 2, there were no significant differences in heart rate between groups during the entire observational period (F=1.31, p=0.17). During this period, total VT+VF duration was significantly (two-sided exact p=0.0029) longer in the control group (n=27, 129.2±219.4s/hour), compared to the GH group (n=26, 27.6±56.5s/hour). Phase I duration of VT plus VF episodes was 89.5±215.2s/hour in the control group (n=27) and 41.7±69.9s/hour in the GH group (n=26); this difference did not reach statistical significance (two-sided exact p=0.83). In contrast, phase II hourly duration of VT+VF episodes was 82.8±116.6s/hour in the control group (n=22) and 18.3±41.2s/hour for the GH group (n=25), (two sided exact p=0.0027). Hourly VT/VF duration is shown in figure 3, the distribution of VT/VF over time is shown in figure 4 and a representative example from each group is shown in figure 5. The mean episode duration in the GH group was 3.9±4.3s, that was significantly (p=0.01) shorter compared to the 8.2±7.3s mean duration in the control group.

MAP recordings

There was a significant variance in LV APD90 (F=92.5, p<0.001), that was due to a significant increase in LV APD90 24 hours post-MI in the control group (n=10), compared to baseline. In contrast, no significant changes in LV APD90 were observed in the GH group (n=16). Compared to baseline, beat-to-beat variation of LV APD90 24 hours post-MI increased significantly in the control group, but remained unchanged in the GH-treated group (table 1, figure 6).

Discussion

Early post-MI treatment with GH reduces infarct size [9,18], produces a hypertrophic response to the injured myocardium [4-6,9,23,24] and enhances cardiac repair [3,5,6,9,18,24]. These actions preserve LV size and function, prevent heart failure and increase survival [3-6,9]. We hypothesized that these favourable effects may reduce arrhythmogenesis. We used a double dosing regimen, consisting of (a) pre-treatment with GH, aiming at stimulation of IGF-1 production [19] and (b) GH administration immediately prior to MI generation [18]. We report a reduction in phase II ventricular tachyarrhythmias in treated rats, resulting in decreased arrhythmic and total mortality.

Electrophysiological changes during phase I include accumulation of extracellular K^+, enhancement of outward repolarizing K^+ currents and relative Na^+ channel inactivation [25]. These changes favour the flow of 'injury current' that triggers VF [25]. In our study, we observed a reduction in phase I VT/VF, albeit not statistically significant. We feel that this is due to the broad variation in the incidence of ventricular tachyarrhythmias during this period [12], resulting in wide confidence intervals. Nonetheless, a direct electrophysiologic action of GH on ventricular myocytes cannot be excluded and this view is supported by reports of a significant effect of GH/IGF-1 on calcium channels in atrial myocytes [26] and of rapid delayed rectifier potassium current in neonatal ventricular myocytes [27]. Furthermore, GH/IGF-1 may lead to an 'insulin-like' stimulation of Na^+/K^+ pump activity [28], resulting in T-wave alterations [29].

The most pronounced antiarrhythmic effect of GH treatment was observed in phase II ventricular tachyarrhythmias, the onset of which occurs after a quiescent period and coincides with the gradual transition of reversible into irreversible myocardial injury [11]. These tachyarrhythmias originate mostly from the border zone between ischaemic and normal myocardium [11]. Differences in the electrophysiologic milieu between these areas favour the development of re-entry and abnormal automaticity, regarded as likely prevalent mechanisms of phase II arrhythmogenesis [11]. Previous studies have indicated that the cardioprotective action of the GH/IGF-1 axis is primarily exerted at the border zone [2,7,24], reducing myocardial ischaemia and infarct size [9,18,30]. The mechanisms of GH/IGF-1 cytoprotection are not understood, but activation of the phosphatidylinositol kinase pathway is likely [28,30]. Our results indicate that these actions not only lack a proarrhythmic potential, but they lead to a decrease in phase II ventricular tachyarrhythmias.

We report a trend towards a decreased infarct size after GH treatment, which did not reach statistical significance. We feel that this does not contradict the previously reported [9,18,30] cardioprotective actions of GH/IGF-1 for two reasons. *First* and foremost, our study had an only 40% power to detect a 10% decrease in infarct size. *Second*, the lack of statistical significance may be secondary to the higher mortality observed in the control group; mortality correlates with infarct size [12] and, as per our protocol, deceased rats (with presumably larger infarcts) were not included in infarct size measurements. The reduction of both, tachyarrhythmic and bradyarrhythmic deaths, the latter indicative of pump failure [12], reinforces this assumption.

In our study, the preservation of MAP signals in treated rats confirms the antiischaemic-cardioprotective actions of GH/IGF-1. We report a significant prolongation of LV APD90 in the control group, coupled with increased beat-to-beat variation in MAP duration. Both these characteristics, indicative of peri-infarct ischaemia and increased arrhythmogenesis [13-15], were eliminated in GH-treated rats.

In addition to the above considerations, several other mechanisms may be operative and merit further study. *First*, acute GH administration may induce coronary vasodilation [31], resulting in increased oxygen supply to the border zone and, thereby, to decreased arrhythmogenesis [32]. *Second*, the previously reported favourable haemodynamic effects of GH administration [3,6,23], may reduce LV wall stress and decrease stretch-induced ventricular tachyarrhythmias [33]. *Third*, ischaemia leads to intracellular accumulation of catecholamines and to phase II tachyarrhythmias [11]. This process may be attenuated by GH, as evidenced by previous reports of a marked decrease in myocardial noradrenaline content after MI [34]. However, the lack of difference in heart rate between treated and control rats in our study, suggests that this mechanism may be operative only during long-term GH treatment.

Strengths and limitations of the study

The present work is the first to demonstrate reduced post-MI arrhythmogenesis after GH treatment. The miniature telemetry recording system used in our experiments permits the study of both, phase I and phase II tachyarrhythmias, without the confounding effects of anaesthesia [11,12]. Moreover, MAPs have been shown to reproduce the repolarization time course of transmembrane action potentials with high fidelity [14]. Thus, MAP recordings are suitable for studying the characteristics of local electrophysiology and ischaemia [14,15]. Despite these merits, four limitations may be

apparent. *First*, the dosing regimen used in our study did not permit the evaluation of the relative contribution of GH and IGF-1 on the antiarrhythmic effect. *Second*, our study did not include measurements of LV function or haemodynamics. Although this issue has been addressed in previous reports [4,6,31], further studies of post-MI GH treatment are necessary, incorporating data on both, LV function and arrhythmogenesis. *Third*, our study was confined to the rat model of permanent coronary occlusion and did not examine the possible antiarrhythmic effects of GH/IGF-1 axis in the presence of reperfusion. *Lastly*, the timing of growth hormone administration in our study may reduce the relevance of our findings to post-myocardial infarction treatments in humans.

In conclusion, our findings indicate that early post-MI GH/IGF-1 axis activation reduces ventricular tachyarrhythmias in the rat. Although a variety of mechanisms may be operative, including a direct effect of GH on ion transport, a border zone cytoprotective effect may best explain our results. Our findings gain importance, in light of recent advances in cardiac repair strategies and their possible relation with arrhythmogenesis [35]. Future studies should explore the mechanisms of cytoprotective and antiarrhythmic effects of early GH/IGF-1 activation during MI with or without reperfusion.

Acknowledgements

This work was supported, in part, by the national General Council for Research and Technology (project: PENED-01ED511). We wish to thank Boston Scientific/Iatriki Efzin for offering the MAP probe and preamplifier. Anastasia Alevizatou, Apostolos Andronikou, Christos Dounis and Dimitris Oikonomidis are acknowledged for their assistance during the experiments and Eleni Goga for her invaluable help as a research coordinator.

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Table

Table 1. Action potential duration at 90% of repolarization (APD90) and beat-to-beat variation in APD90 in the two groups.

	Control (n=10)	Growth hormone (n=16)
APD90 baseline (ms)	92.38±10.28	93.12±5.27
APD90 24 hours (ms)	116.40±11.14 *	94.93±4.99
Beat-to-beat variation† baseline (ms)	3.90±1.19	4.12±1.70
Beat-to-beat variation† 24 hours (ms)	13.50±7.60 *	3.81±1.86

* $p<0.001$ compared to baseline

† expressed as the standard deviation of APD90 in 50 sinus beats

ms= milliseconds

Figure legends

Figure 1. Study protocol, including electrocardiographic (ECG) telemetry system implantation, growth hormone (GH) or normal saline (NS) injection and myocardial infarction (MI) generation. Monophasic action potentials (MAP) were recorded at baseline and 24 hours post myocardial infarction, prior to infarct size measurements.

Figure 2. Sinus heart rate in beats per minute (bpm) in the two groups. Note the increase in heart rate after myocardial infarction (MI), without significant differences between growth hormone (GH) treated animals and controls.

Figure 3. Mean hourly duration (±standard error of the mean) of ventricular tachycardia (VT) and ventricular fibrillation (VF) during the whole observation period and during phases I and II. Asterisk denotes a significant ($p<0.05$) difference between growth hormone (GH) treated animals and controls.

Figure 4. Distribution of ventricular tachycardia and ventricular fibrillation over time. Note the significantly shorter tachyarrhythmia duration in the growth hormone (GH) group, which is more prominent during phase II. Two quiescent periods of low arrhythmogenesis are evident (dashed lines), one between phases I and II and the second after the twelfth hour post myocardial infarction (MI). Error bars depict standard error of the mean.

Figure 5. Representative example of ventricular tachycardia, degenerating into ventricular fibrillation in a control rat (lower panel) and a short episode of ventricular fibrillation in a growth hormone (GH)-treated rat (upper panel).

Figure 6. Representative examples of monophasic action potential recordings at baseline (A) and 24 hours post-myocardial infarction (MI) in a growth hormone (GH)-treated rat (B) and a control rat (C).

Figures

Figure 1.

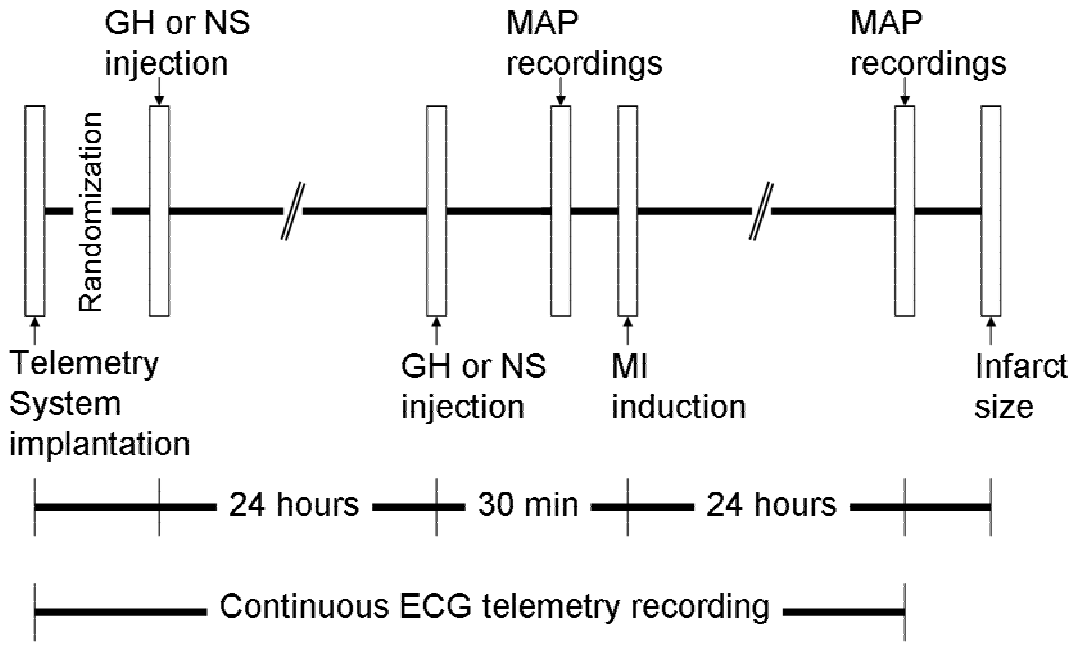


Figure 2.

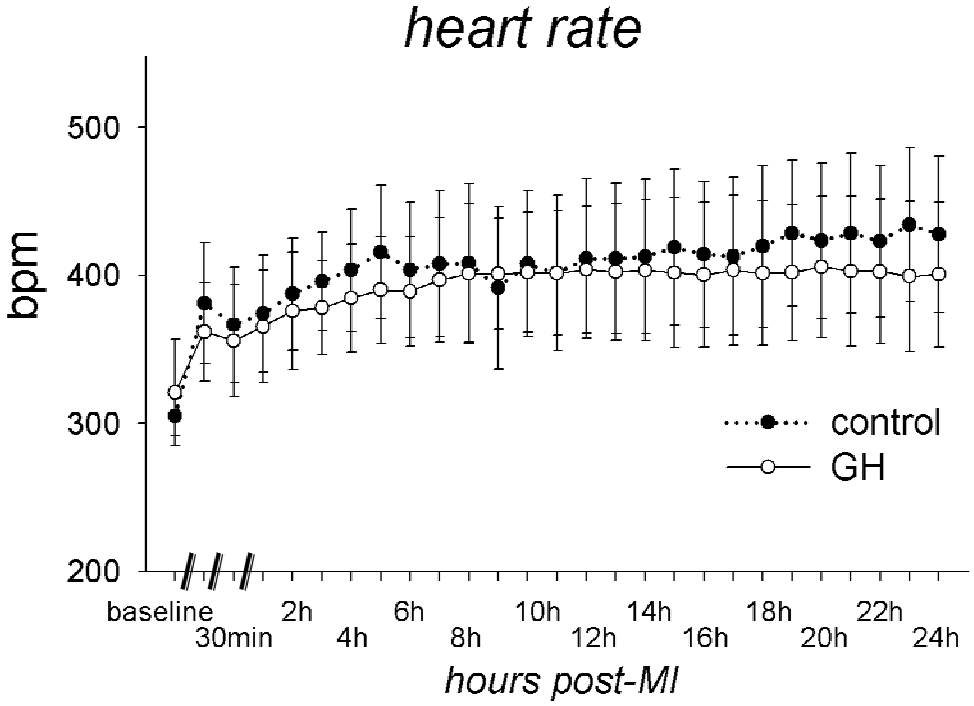


Figure 3.

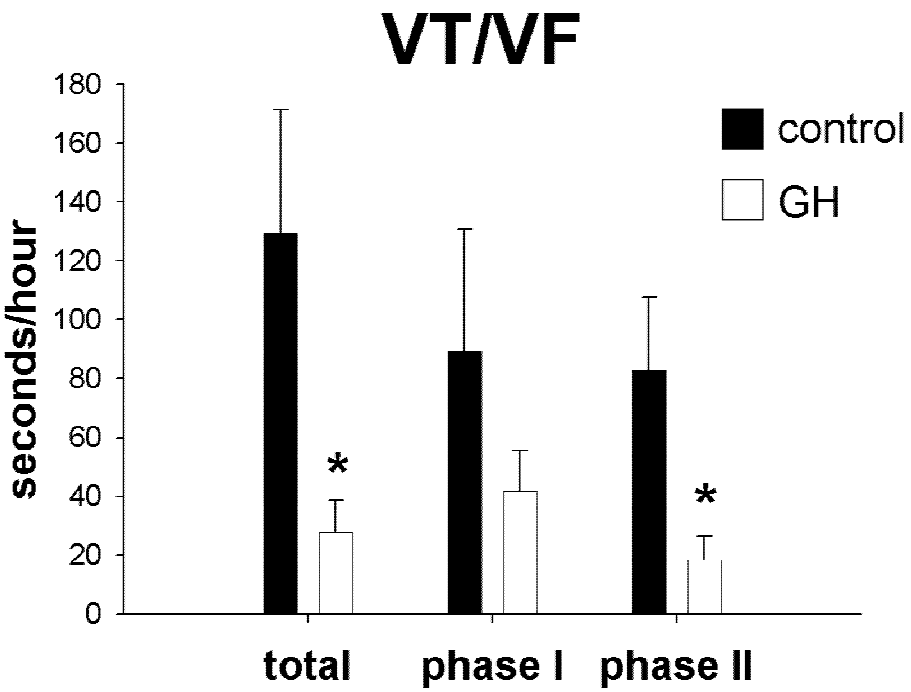


Figure 4.

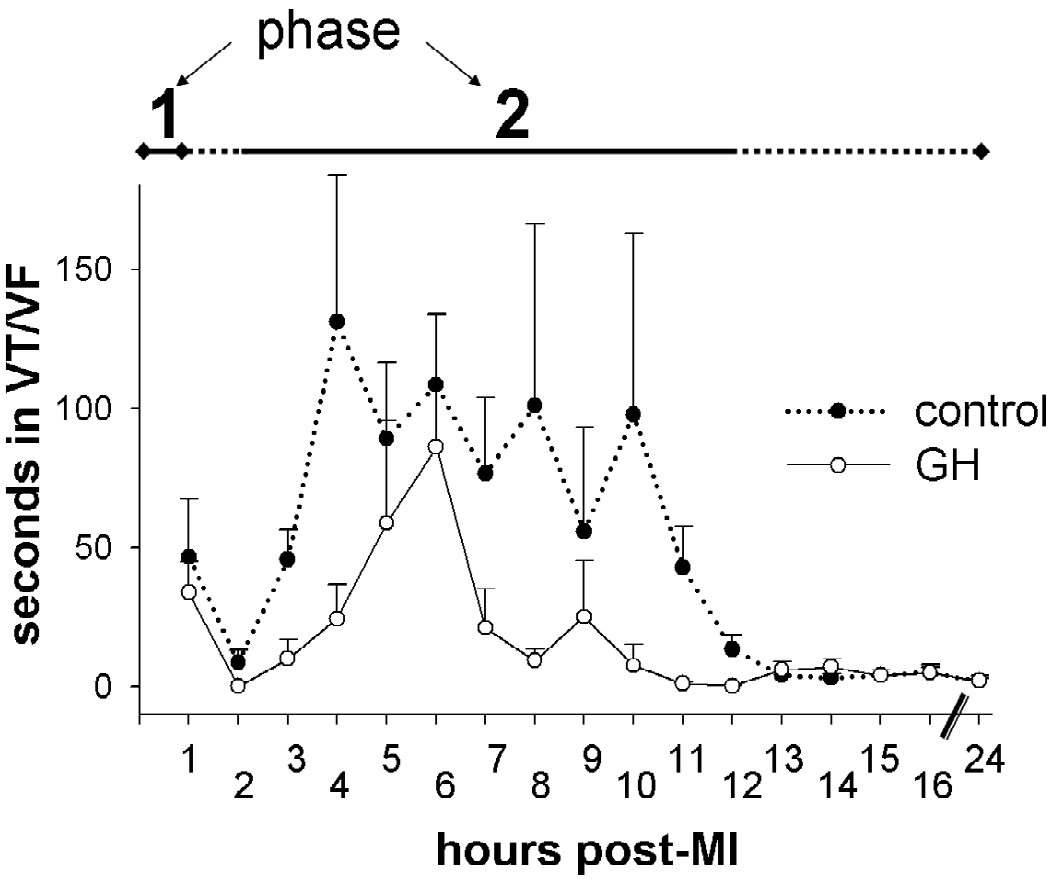
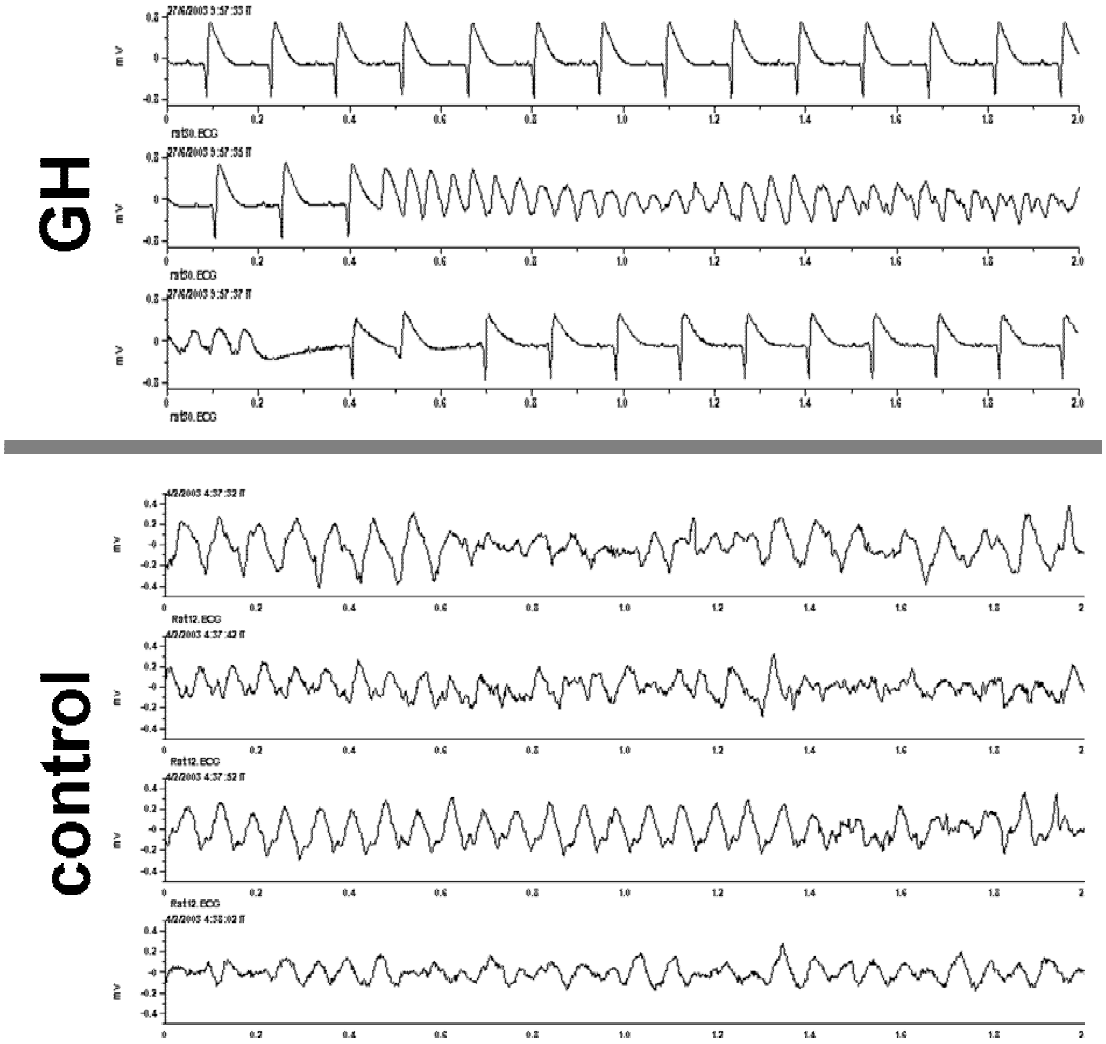


Figure 5.



Clinical Science Immediate Publication. Published on 9 Nov 2006 as manuscript CS20060193

Figure 6.