Central \(\beta\)-Adrenergic Mechanisms May Modulate Ischemic Ventricular Fibrillation in Pigs

Gerald W. Parker, Lloyd H. Michael, Craig J. Hartley, James E. Skinner, and Mark L. Entman

A central noradrenergic process may permit expression of the stress-related increase in cardiac vulnerability to ventricular fibrillation (VF). Thus, the effect of central \(\beta\)-adrenergic receptor blockade with L-propranolol (0.01 and 0.05 mg/kg) on ischemia-induced VF vulnerability was evaluated in the psychologically stressed pig model and compared with Ringer’s solution and D-propranolol (0.05 mg/kg). The ischemia of a maximum 15-minute left anterior descending coronary artery occlusion was used since we previously determined that pigs surviving 15 minutes usually do not fibrillate. Time to the onset of VF was analyzed by time-to-event analysis and ranged from 0.75 to 13.8 minutes in vulnerable pigs. Intracerebroventricular administration of L-propranolol (0.05 mg/kg) prolonged the time to VF compared with Ringer’s solution and D-propranolol (\(p<0.05\)). The high dose of L-propranolol also reduced the incidence of VF (7/15 fibrillated) compared with Ringer’s solution (12/12 fibrillated) and D-propranolol (6/7 fibrillated). The lower dose of L-propranolol was without effect on VF vulnerability (7/9 fibrillated). The plasma concentration resulting from central administration of 0.05 mg/kg L-propranolol was found to be 9.05±3.25 ng/ml, which is significantly below therapeutic antiarrhythmic blood levels. We conclude that the reduced vulnerability to ischemia-induced VF after intracerebroventricular administration of propranolol is due to alteration of a central \(\beta\)-adrenergic receptor–mediated phenomenon as opposed to an effect on the heart directly or to nonspecific membrane stabilization. (Circulation Research 1990;66:259–270)

Various psychological risk factors have been related epidemiologically to cardiac rhythm disturbances in the presence and absence of coronary artery disease.1–6 These rhythm disturbances include ventricular tachycardia and ventricular fibrillation (VF), the underlying mechanisms of sudden cardiac death. Although some of the psychological stressors are clearly related to acute phasic stress and imminent emotional distress accompanied by hemodynamic alterations consistent with autonomic imbalance, many are related to more tonic psychosocial factors that are often imperceptible.1,5,7 Because the response may be temporally distant from the provocative event, causality of central nervous system–mediated arrhythmias associated with tonic psychosocial stress in humans is often difficult to discern.

Our laboratory has developed a unique pig model of sudden cardiac death in which operationally defined tonic psychological stress is associated with the development of ischemia-induced VF. Specifically, lack of behavioral adaptation to the novel laboratory environment is associated with the development of VF in most cases within 15 minutes after occlusion of the left anterior descending (LAD) coronary artery. Reduction of psychological stress by behavioral adaptation to the laboratory (learned adaptation), however, significantly reduces the vulnerability of the pig heart to ischemia-induced fibrillation.8 Furthermore, cryogenic blockade of a specific frontocortical brain stem pathway is also effective in preventing VF.9 Despite the increased vulnerability observed in the laboratory-unadapted pig, there were no overt signs of stress nor differences in basal heart rate in the unadapted compared with

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the adapted state. Together, these studies indicate that specific cortical influences on the brain stem and autonomic nervous system modulate myocardial vulnerability to ischemia-induced VF evoked by tonic psychological stress associated with the novel laboratory environment.

The cerebral neurochemical mechanisms mediating the increased myocardial vulnerability to psychological stress are unknown. Several neurophysiological studies have suggested a central catecholaminergic synaptic mechanism associated with the cerebral response to environmental stimuli. Central catecholaminergic mechanisms are associated with responses to new, noxious, or meaningful stimuli that invoke event-related slow potentials. The event-related slow potentials are characterized by slow membrane potential shifts caused by inactivation of a specific potassium current and are modulated by norepinephrine release and subsequent stimulation of cyclic AMP formation. Additionally, other experiments have suggested a relation between β-adrenergic receptor stimulation and synaptic plasticity involved in neurophysiological models of learning. Because the concept of environmental stress implies cognition of meaningful stimuli, we hypothesized that central noradrenergic β-receptor-mediated mechanisms contribute to the cerebral cortex’s influence on autonomic regulation of the heart, which in turn mediates increased sensitivity to ischemia-induced VF.

The purpose of the present study was to evaluate myocardial vulnerability to VF in the laboratory-unadapted psychologically stressed pig after central β-adrenergic receptor blockade. We demonstrate that administration of l-propranolol into the lateral cerebral ventricles exerts a specific potent effect on vulnerability to ischemic VF.

Materials and Methods

Experimental Animal

Chronically instrumented, conscious Yorkshire-cross pigs (20–25 kg) were used for the study. The pig was selected for several reasons. First, since an individual’s response to psychological stressors is a factor contributing to the etiology of sudden cardiac death, it was critical to select an animal model in which vulnerability is similarly modulated by a behavioral response to environmental stimuli. The pig represents an appropriate animal model since operationally defined psychological stressors are known to increase the vulnerability of the pig heart to arrhythmogenesis. Second, since behavior is a variable in this study, which implies that interpretive responses to sensory stimuli need to be controlled, it was necessary to obtain experimental animals having a uniform psychosocial background and thus uniform behavioral response to the laboratory and personnel. Pigs were obtained from a single agricultural source located near our facility. The pigs were raised under identical conditions, transported to our lab in the same vehicle, and housed in the same environment during boarding and while in our laboratory. In addition, it was often possible to obtain littermates to serve as additional controls. A similar constancy of experimental animals is not easily obtained from other commonly used large animal models. These two factors, a constant psychosocial background and vulnerability to psychosocial stressors, made the pig an ideal model for the study.

Surgical Preparation

Pigs underwent aseptic brain cannula implantation to facilitate drug infusion as well as left lateral thoracotomy for implanting instrumentation. Anesthesia was induced with 15 mg/kg i.m. ketamine and 10 mg/kg i.v. methohexital. After tracheal intubation, anesthesia was maintained with methoxyflurane and oxygen. The pigs were ventilated with a tidal volume of 10–20 ml/kg and a respiratory rate of 10–15 breaths/min by use of an oxygen-flushed anesthetic machine and ventilator.

Two 25-gauge stainless steel spinal needles cut to a length of 50 mm were implanted into the lateral cerebral ventricles of the brain. One needle was inserted into the left ventricle, and the second needle was placed in the right ventricle. The needles were implanted under stereotaxic control at a location 22.5 mm rostral to bregma. Each cannula was inserted to depth of 21.5 mm so that the tip was positioned in the anterior horn of the left and right lateral cerebral ventricle. The needles were permanently secured to the cranium by a cranial acrylic crown attached to the skull with stainless steel screws. Cannula placement in the cerebral ventricles was confirmed at autopsy by observing fluorescein dye in the cerebrospinal fluid, which had been injected into the cannula before death. Placement was also confirmed histologically.

Thoracotomy in the fourth left intercostal space exposed the pericardium, which was then opened. The internal mammary artery was cannulated with polyethylene tubing (PE-100) to facilitate arterial blood gas analysis during surgery. One group of pigs was instrumented for measurement of various hemodynamic indexes; a second group assigned to the myocardial vulnerability protocol was instrumented further with a hydraulically activated ligature-occluding device capable of effecting reversible myocardial ischemia at a later time in the conscious state. The occluder was positioned on the proximal LAD coronary artery 1 cm distal to its bifurcation with the circumflex coronary artery. A pulsed Doppler blood flow velocity probe was placed distal to the occluder. Doppler ultrasonic wall-thickening probes for evaluating regional myocardial function were sutured to the epicardial surface in the region to be rendered ischemic (anterior wall) as well as in the nonischemic region (posterior wall) of the left ventricle. A high fidelity pressure transducer (model P7, Konigsberg) was positioned in the chamber of the left ventricle via a stab incision in the apex in those pigs assigned to the hemodynamic protocol. Stainless steel wire electrodes were sutured through the skin on all four extremities for monitoring standard lead
electrocardiograms. The distal end of the lead wires, catheter, and occluder were exteriorized caudal to the scapula and secured to the animal with a waterproof bandage. A chest tube was placed in the thoracic cavity before closing the thoracotomy and was removed postoperatively on evacuation of the pneumothorax. The animals were allowed 7–10 days postoperative recovery before beginning experimental manipulations. The animals were afebrile, eating well, and gaining weight by this time.

**Behavioral Protocol**

The behavioral response to experimental handling of the pig is known to be a critical factor influencing myocardial vulnerability to ischemia-induced VF. Therefore, the psychological state of the animal was experimentally controlled by providing consistent care in handling of the animals. The pigs were transported from their home cage in the vivarium to the laboratory on a portable cart. Minimal restraint was effected by taping the legs together in an extended position. In the laboratory, the pigs were placed in right lateral recumbency in a specially designed study chamber. This exposure to the unfamiliar laboratory, in effect, constitutes a set of novel stimuli that is associated with increased vulnerability to ischemia-induced arrhythmias. Behavioral adaptation, however, occurs after 6–8 daily experiences in the laboratory, and VF no longer occurs during identical ischemic insults.8 Thus, evaluation of VF latency after central administration of propranolol in this study was conducted in the laboratory-unadapted state, when pigs are known to manifest VF within 15 minutes after LAD coronary artery occlusion.

**Experimental Protocol**

**Hemodynamic alterations as a function of laboratory adaptation.** Pigs were transported to the laboratory as described above on a daily basis for 8 days to assess hemodynamics as a function of laboratory adaptation. This was done to more fully evaluate hemodynamic status in this paradigm of tonic psychosocial stress in the pig. The Konigsberg pressure transducer, previously calibrated in vitro, measured left ventricular systolic and end-diastolic pressures. The first derivative of left ventricular pressure (dP/dt) was obtained by electronic differentiation. A triangular wave signal with known rate of change was used to provide direct calibration of the differentiator amplifier. A cardiographomtriged by the electrocardiogram provided instantaneous and continuous record of heart rate. LAD coronary artery blood flow velocity and regional myocardial wall thickening (left ventricular systolic thickening fraction) were recorded by their respective ultrasonic Doppler probes.16–18 The initial onset of the upstroke of left ventricular systolic pressure and peak negative dP/dt were used for timing the beginning and end of systole necessary for calculating systolic thickening fraction. To minimize recent phasic stress-induced hemodynamic alterations associated with moving the pig from the vivarium to the laboratory, data were not collected for 30 minutes after arrival in the laboratory and attachment to the physiograph preamplifier patient cables. Heart rate, left ventricular systolic and end-diastolic pressure, maximum rate of left ventricular pressure development, left ventricular systolic wall thickening, and LAD coronary artery blood flow were continuously recorded on an eight-channel recorder (Gould, Cleveland, Ohio). The above indexes were measured three times at 10-minute intervals while the pig was in a quiescent state. Once it was determined that significant differences did not exist among the three measurements (repeated analysis of variance, p=NS), the mean was used to represent baseline hemodynamic status on a given day in the laboratory.

**Effect of centrally administered propranolol on hemodynamics.** These same pigs were then used to evaluate the effect of centrally administered L-propranolol on the chronotropic and inotropic state of the heart. Once these baseline recordings had been obtained, either 0.01 mg/kg L-propranolol or Ringer’s solution (vehicle control) was slowly infused into the lateral cerebral ventricles via the brain cannula. The volume administered was held constant (250–300 μl) among the pigs and was given by slow infusion over a 10-minute period. The presentation of drug treatment was alternated with respect to laboratory day exposure so that Ringer’s solution was given on lab days 1, 3, 5, and 7 and L-propranolol was given on lab days 2, 4, 6, and 8. The data were initially analyzed for propranolol-induced changes with respect to the number of days of accrued laboratory adaptation. The number of daily experiences in the laboratory, however, was found not to be a factor in the observed hemodynamic alterations. Thus, the values from all the days within each drug treatment were pooled. A separate set of pigs was similarly instrumented and studied with a higher dose of L-propranolol (0.05 mg/kg i.c.v.) and the dextroretrota-

draphic combination of propranolol, D-propranolol (0.05 mg/kg i.c.v.).

**VF latency determinations.** Initial handling of the pigs and data collection were performed in an identical manner as described above. Once baseline hemodynamic indexes were recorded, pigs were given either 0.01 or 0.05 mg/kg L-propranolol, 0.05 mg/kg D-propranolol, or Ringer’s solution (vehicle control) infused slowly into the lateral cerebral ventricles over a 10-minute period. LAD coronary artery occlusion was effected 10 minutes after completion of the drug infusion. Completeness of coronary occlusion was verified by the LAD blood flow velocity signal, electrocardiographically, and by observing paradoxical left ventricular systolic wall thinning in the ischemic region.19 The time to the development of VF (VF latency) of a maximum 15-minute coronary artery occlusion was determined. A maximum 15-minute occlusion was selected because earlier studies demonstrated that VF will occur within 14 minutes after complete occlusion of the LAD coronary artery in unadapted pigs.8 Furthermore, adapted pigs not sus-
ceptible to VF remained so for at least 24 hours. Thus a 15-minute occlusion provided sensitivity for detecting antifibrillatory efficacy while minimizing the occurrence of occlusion/reperfusion–associated myocardial damage. If VF occurred, the occlusion was released, and the animal was electroconverted (200–250 W·sec extrathoracic shock) while unconscious. Reperfusion was also instituted if fibrillation did not occur after 15 minutes of ischemia. Some pigs were additionally used in a within-subject control protocol. These pigs were used on a subsequent occlusion trial only if recovery from occlusion/reperfusion–induced regional myocardial dysfunction could be prospectively documented by the ischemic zone wall-thickening data and if animals were free of electrocardiographic abnormalities. Gross, histochemical (triphenyl tetrazolium chloride stain), and histological examinations of the hearts were also performed to confirm the prospective characterization of these pigs. In the within-subject experimental design, presentation of drug treatment was randomized with respect to laboratory day exposure or occlusion trial.

Pharmacokinetics of centrally administered propranolol. Cerebrospinal fluid and plasma levels of propranolol were determined at 5 and 10 minutes after intracerebral ventricular administration of 0.05 mg/kg L-propranolol in a separate group of pigs. The samples were obtained under anesthesia (ketamine, sodium methohexital, and methoxyflurane) to facilitate cerebrospinal fluid collection at a site distant from drug infusion. Otherwise, infusion of L-propranolol into the right and left lateral cerebral ventricles was performed in an identical manner as previously described for VF latency determinations and hemodynamic evaluation. Cerebrospinal fluid samples were obtained at the level of the cisterna magna with a 20-gauge, 4-inch spinal needle. Blood was simultaneously collected from a femoral venous catheter. Intravenous infusion of 0.05 mg/kg L-propranolol via a lateral ear vein was also performed in three pigs to allow comparison of cerebroventricular versus intravenous administration. Plasma and cerebrospinal fluid samples were stored at −70°C until analyzed. Propranolol concentrations were determined by high-performance liquid chromatography.

Statistical Analysis
The time to the onset of VF subsequent to LAD coronary artery occlusion (VF latency) was analyzed by Kaplan-Meier time-to-event or survival-curve analysis followed by Gehan’s test of significance. Hemodynamic indexes within each treatment group and for laboratory adaptation were analyzed by repeated-measure analysis of variance. When an overall treatment effect was detected, between-group analysis of either percent change or absolute change from baseline was used to isolate specific drug effects at individual time points (analysis of variance followed by the Bonferroni multiple-comparison t test). The latter was preceded by evaluating the homogeneity of baseline values between the four treatment groups (analysis of variance). Data are expressed as mean±SD.

Results
VF Latency Determination
The latency to the onset of VF subsequent to complete occlusion of the LAD coronary artery in the psychologically unadapted pig was determined after intracerebroventricular administration of 0.05 and 0.01 mg/kg L-propranolol, 0.05 mg/kg D-propranolol, and Ringer’s solution. Survival curve analysis revealed that L-propranolol at a dose of 0.05 mg/kg was an effective pharmacological intervention in prolonging the time to the development of VF as well as reducing the incidence of VF after acute occlusion of the LAD coronary artery (Figure 1). Ischemia-induced VF occurred in all 12 occlusion trials in the Ringer’s solution treatment group with VF latencies ranging from 0.75 to 12.5 minutes. L-Propranolol (0.05 mg/kg i.c.v.), however, prolonged the time to VF compared with Ringer’s solution (p<0.01) and D-propranolol (p<0.05). The incidence of ischemia-induced VF was also reduced to 47% (7/15 fibrillated) compared with Ringer’s solution (12/12 fibrillated) and D-propranolol (6/7 fibrillated). VF latencies in the vulnerable pigs ranged from 1.5 to 13.8 minutes. There were no significant differences between the lower dose L-propranolol (7/9 fibrillated) and D-propranolol (6/7 fibrillated) treatment groups compared with the Ringer’s group.

A within-subject experimental design was also used to minimize between-subject variability. L-Propranolol (0.05 mg/kg i.c.v.) prolonged the time to the onset of VF compared with Ringer’s solution and D-propranolol in all cases (Figure 2). VF latencies after randomized presentation of L-propranolol and Ringer’s solution were 12.72±1.42 and 6.18±1.42 minutes, respectively (p=0.016; one-tail paired t test). A similar randomized presentation of L-propranolol and D-propranolol resulted in VF latencies of 9.75±3.21 and 7.29±2.43 minutes (p=0.0432). In these nine pigs in which experimental presentation of drug treatment was randomized with respect to laboratory day exposure, only four (44%) developed VF subsequent to LAD coronary artery occlusion after central administration of 0.05 mg/kg L-propranolol. Ischemia-induced VF occurred in all nine of those same pigs after central administration of either D-propranolol or Ringer’s solution.

Hemodynamic Effect of Centrally Administered Propranolol
Hemodynamics were also evaluated at 5, 10, 15, 20, and 30 minutes after intracerebroventricular administration of 0.05 and 0.01 mg/kg L-propranolol, 0.05 mg/kg D-propranolol, and Ringer’s solution (vehicle control) in a separate group of pigs. Data for each treatment are summarized in Table 1 and Figure 3 and indicate the following: 1) Baseline heart rate, left ventricular systolic pressure, maximum left ventricu-
lar dP/dt and dP/dt per developed pressure, and left ventricular systolic wall thickening were similar between the four treatment groups ($p=NS$ by analysis of variance). 2) L-Propranolol (0.05 and 0.01 mg/kg) induced a moderate decrease in heart rate, maximum left ventricular dP/dt, and maximum left ventricular dP/dt per developed pressure ($p<0.0005$ by repeated-measure analysis of variance). The peak heart rate decrease after 0.05 mg/kg i.c.v. L-propranolol was 27 beats/min and after 0.01 mg/kg i.c.v. L-propranolol was 13 beats/min. The maximum decrease in left ventricular dP/dt after 0.05 and 0.01 mg/kg i.c.v. L-propranolol was 19% and 16%, respectively. Maximum left ventricular dP/dt per developed left ventricular systolic pressure achieved a maximum decline from baseline of 16% and 13%. The above hemodynamic effects were observed as early as 5 minutes after completion of drug infusion, but peak responses were not achieved until 15–25 minutes in individual animals. Between-group analysis of either absolute change or percent change from baseline at individual time points revealed a dose-dependent L-propranolol effect on heart rate but not on maximum left ventricular dP/dt or dP/dt per developed pressure (Figure 3). 3) L-Propranolol (0.05 mg/kg) was associated with a slight reduction in systolic wall thickening after various dosages.

**Figure 1.** Graph showing latency to the onset of ventricular fibrillation (VF) subsequent to complete occlusion of the left anterior descending coronary artery in the psychologically stressed pig. Pigs were given either Ringer’s solution (vehicle control), 0.05 mg/kg D-propranolol (d-P on figure), or 0.05 mg/kg L-propranolol (l-P on figure) infused slowly into the lateral intracerebral ventricles 10 minutes before coronary artery occlusion. VF latencies for the treatment groups were analyzed by the Kaplan-Meier survival curve analysis in which estimated probabilities for not developing VF (ordinate) are plotted against time (abscissa). VF occurred in all 12 occlusion trials in the Ringer’s treatment group and in six out of seven occlusion trials in D-propranolol-treated pigs. VF latencies ranged from 0.75 to 12.5 minutes. L-Propranolol (0.05 mg/kg), however, prolonged the time to VF compared with both Ringer’s solution ($p<0.01$) and D-propranolol ($p<0.05$). The incidence of VF was reduced to 47% (7/15 fibrillated) in the 0.05 mg/kg L-propranolol-treated pigs. The lower dose of L-propranolol and D-propranolol were not significantly different from Ringer’s solution. *$p<0.05$ compared with Ringer’s solution or D-propranolol.

**Figure 2.** Bar chart showing ventricular fibrillation (VF) latency in the psychologically stressed pig subsequent to complete occlusion of the left anterior descending coronary artery (within-subject experimental design). Pigs were given either 0.05 mg/kg L-propranolol (l-prop on figure), 0.05 mg/kg D-propranolol (d-prop on figure), or Ringer’s solution infused slowly into the lateral intracerebral ventricles 10 minutes before coronary artery occlusion. Each bar represents the VF latency of an individual pig. Successive experiments in the same pig are connected by a common baseline. Drug treatments were randomized with respect to laboratory day exposure and paired within each animal. In these nine pigs, only four (44%) given L-propranolol developed VF subsequent to left anterior descending coronary artery occlusion. Ischemia-induced VF occurred in all nine of these same pigs when given either D-propranolol or Ringer’s solution.
Thickening (p<0.05), but statistical significance was only marginal. Furthermore, analysis of percent change from baseline between the four treatment groups at individual time points did not reveal a significant between-group effect. 4) Left ventricular systolic and end-diastolic pressures were not significantly affected by any of the four treatment regimens (p=NS). 5) D-Propranolol and Ringer’s solution after intracerebral infusion were not associated with significant alterations in the measured hemodynamic indexes.

**Heart Rate Response to LAD Coronary Artery Occlusion**

Baseline heart rate in the quiescent state was similar in the L-propranolol (0.05 mg/kg and 0.01 mg/kg), D-propranolol, and Ringer’s solution treatment groups (Table 2; p=NS by analysis of variance). Although heart rate can fluctuate markedly after LAD coronary artery occlusion in the conscious pig, the general trend evoked by ischemia consisted of a biphasic heart rate response in most pigs. Heart rate increased in the early phase of ischemia (0–4 minutes) in all cases and in all treatment groups; this increase was followed by a later decline in those individual pigs with VF latencies greater than 4 minutes. The highest heart rate (peak) reached during the first 4 minutes of ischemia was lower in the 0.05 mg/kg L-propranolol group compared with the Ringer’s group (Table 2; p<0.05). However, there was no difference in peak heart rate in the 0.05 mg/kg L-propranolol compared with both the D-propranolol and low-dose L-propranolol treatment groups, despite differences in VF vulnerability (Table 2; p=NS). Likewise, the heart rate measured at the last period of sinus rhythm before VF (or at 15 minutes) was also moderately depressed in pigs receiving 0.05 mg/kg L-propranolol compared with pigs receiving Ringer’s solution (p<0.05; Table 2) but not compared with the other two groups (p=NS; Table 2). Since heart rate may be a determinant of the

<table>
<thead>
<tr>
<th>Table 1. Hemodynamic Response After Intracerebroventricular Administration of L-Propranolol, D-Propranolol, and Ringer’s Solution</th>
<th>After drug administration</th>
<th>n</th>
<th>Baseline</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>20 min</th>
<th>30 min</th>
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<tr>
<td>Heart rate (beats/min)</td>
<td>14</td>
<td>142.6±10.6</td>
<td>123.2±7.9</td>
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<td>16</td>
<td>130.1±19.6</td>
<td>124.3±23.5</td>
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<td>Ringer’s solution</td>
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<td>LVSP (mm Hg)</td>
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<td>D-Propranolol (0.05 mg/kg)</td>
<td>5</td>
<td>2,916±250</td>
<td>2,972±215</td>
<td>2,962±222</td>
<td>3,070±251</td>
<td>2,961±209</td>
<td>2,921±199</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Ringer’s solution</td>
<td>14</td>
<td>3,479±553</td>
<td>3,392±354</td>
<td>3,346±542</td>
<td>3,423±459</td>
<td>3,426±440</td>
<td>3,571±580</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>LV+dP/dt/P</td>
<td>9</td>
<td>33.3±3.7</td>
<td>29.6±2.9</td>
<td>29.8±4.1</td>
<td>28.9±3.7</td>
<td>28.1±3.7</td>
<td>28.0±3.2</td>
<td>&lt;0.0005</td>
<td></td>
</tr>
<tr>
<td>L-Propranolol (0.05 mg/kg)</td>
<td>16</td>
<td>36.8±4.4</td>
<td>33.4±2.1</td>
<td>33.1±2.4</td>
<td>31.9±4.6</td>
<td>31.7±3.9</td>
<td>32.0±4.5</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>D-Propranolol (0.05 mg/kg)</td>
<td>5</td>
<td>30.9±2.3</td>
<td>31.0±2.5</td>
<td>31.1±2.4</td>
<td>32.2±3.3</td>
<td>31.4±2.5</td>
<td>30.2±3.2</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Ringer’s solution</td>
<td>14</td>
<td>36.9±3.9</td>
<td>36.7±3.8</td>
<td>35.8±4.1</td>
<td>36.4±3.2</td>
<td>36.5±2.9</td>
<td>37.2±4.3</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>LV systolic T (%)</td>
<td>7</td>
<td>28.6±9.4</td>
<td>26.1±6.6</td>
<td>25.9±7.0</td>
<td>27.3±7.5</td>
<td>27.0±7.7</td>
<td>27.0±7.7</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>L-Propranolol (0.05 mg/kg)</td>
<td>13</td>
<td>26.7±9.9</td>
<td>25.8±9.1</td>
<td>25.5±9.8</td>
<td>25.9±9.7</td>
<td>25.8±10.1</td>
<td>25.7±10.5</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>D-Propranolol (0.05 mg/kg)</td>
<td>5</td>
<td>33.1±8.2</td>
<td>32.3±7.7</td>
<td>32.4±7.7</td>
<td>32.5±8.4</td>
<td>31.7±7.8</td>
<td>31.5±7.5</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Ringer’s solution</td>
<td>11</td>
<td>29.1±8.9</td>
<td>29.8±9.9</td>
<td>28.6±8.5</td>
<td>29.4±9.1</td>
<td>29.0±9.3</td>
<td>29.0±9.4</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD. Values of p are by repeated-measure analysis of variance. LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; LV+dP/dt, maximum left ventricular dP/dt; LV+dP/dt/P, LV+dP/dt per developed left ventricular pressure; LV systolic T, left ventricular systolic wall thickening fraction; NS, not significant.
FIGURE 3. Graphs showing heart rate and maximum left ventricular dP/dt (MAX LV dP/dt) after cerebroventricular administration of 0.05 mg/kg L-propranolol (l-prop on figure), 0.01 mg/kg L-propranolol, 0.05 mg/kg D-propranolol (dprop on figure), and Ringer’s solution (vehicle control). Heart rate is expressed as absolute change from baseline in beats/min; MAX LV dP/dt is expressed as percent change from baseline. Values represent mean±SEM. *p<0.01 compared with Ringer’s solution. **p<0.01 compared with the lower dose of L-propranolol.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Baseline HR (beats/min)</th>
<th>HR after drug administration (beats/min)</th>
<th>HR after LAD occlusion (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>L-Propranolol (0.05 mg/kg)</td>
<td>15</td>
<td>143±15</td>
<td>140±14</td>
<td>140±14</td>
</tr>
<tr>
<td>L-Propranolol (0.01 mg/kg)</td>
<td>7</td>
<td>145±15</td>
<td>145±14</td>
<td>141±14</td>
</tr>
<tr>
<td>d-Propranolol (0.05 mg/kg)</td>
<td>7</td>
<td>136±14</td>
<td>134±18</td>
<td>132±14</td>
</tr>
<tr>
<td>Ringer’s solution</td>
<td>12</td>
<td>137±20</td>
<td>137±19</td>
<td>132±20</td>
</tr>
</tbody>
</table>

Values are mean±SD. HR, heart rate; LAD, left anterior descending coronary artery; phase 1, peak HR during early phase of ischemia (0–4 minutes); phase 2, stable HR within 1 minute of the onset of ventricular fibrillation or reperfusion. Three baseline measurements were made before drug infusion. Resting HR is given for 1, 5, and 10 minutes after drug infusion and before ischemia. F values are by one-way analysis of variance.

*p<0.01 compared with Ringer’s solution by t test with Bonferroni correction.

†p=NS for the four treatment groups.

‡p<0.01 for the four treatment groups.
propensity to develop VF, the relation between peak heart rate and VF latency from all the pigs was analyzed. A correlation between VF latency and peak heart rate in the first 4 minutes of ischemia was not observed ($r = -0.296, p > 0.05$; Figure 4, top panel). A relatively weak correlation, however, was observed between VF latency and heart rate nearest the onset of VF ($r = -0.566, p < 0.05$; Figure 4, bottom panel). Although heart rate may be one mechanism contributing to the differences observed in VF vulnerability, the data suggest that other factors are involved.

**Hemodynamics as a Function of Laboratory Adaptation**

Heart rate, left ventricular systolic and end-diastolic pressure, maximum rate of left ventricular pressure development, left ventricular systolic wall thickening, and coronary artery blood flow were evaluated on a daily basis for 8 days (Figure 5). The initial transport of the pig from the vivarium to the laboratory is accompanied by acute hemodynamic alterations. This acute phasic stress variable was minimized by allowing 30 minutes to elapse before beginning data collection. Significant differences were not observed (repeated-measurement analysis of variance) in the above hemodynamic indexes during psychological adaptation to the unfamiliar environment. The lack of overt hemodynamic alterations in the unadapted compared with the adapted pig occurred despite the known increased myocardial vulnerability to ischemia-induced VF associated with initial exposure to a novel laboratory environment.8

**Propranolol Pharmacokinetics**

Cerebrospinal fluid and blood plasma analysis of L-propranolol levels after intracerebral administration of 0.05 mg/kg propranolol revealed concentrations of 1,201–5,991 ng/ml propranolol in the cerebrospinal fluid as opposed to 4.9–13.7 ng/ml propranolol in the plasma 10 minutes after completion of drug infusion (Table 3). Intravenous administration of the same dose of L-propranolol, on the other hand, produced cerebrospinal fluid levels of only 1.2–1.4 ng/ml, while 6.7–62.5 and 2–15.4 ng/ml were detected in the plasma at 5 and 10 minutes, respectively (Table 4).

**Discussion**

Ventricular tachycardia degenerating into VF is the most consistently observed mechanism of sudden cardiac death.4,24 Although myocardial ischemia and infarction are known contributing factors in predisposing to VF, not all persons with ischemic heart
disease or infarction die unexpectedly. Furthermore, acute pathological lesions of the coronary arteries and myocardium are not observed or are inadequate to account for the fatal event in 10–15% of the cases.25 Thus, although ischemia is a major factor triggering VF, other factors in addition to diffuse coronary artery disease may mediate increased vulnerability in susceptible individuals.

This study provides further evidence in an animal model that central nervous system input is one such

TABLE 3. Plasma and Cerebrospinal Fluid Propranolol Concentrations After Intracerebroventricular Administration of 0.05 mg/kg L-Propranolol in the Pig

<table>
<thead>
<tr>
<th>Pig</th>
<th>Plasma (ng/ml)</th>
<th>Cerebrospinal fluid (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
<td>10 min</td>
</tr>
<tr>
<td>41</td>
<td>6.4</td>
<td>4.9</td>
</tr>
<tr>
<td>41A</td>
<td>16.7</td>
<td>13.7</td>
</tr>
<tr>
<td>44</td>
<td>12.7</td>
<td>8.4</td>
</tr>
<tr>
<td>45</td>
<td>7.5</td>
<td>6.1</td>
</tr>
<tr>
<td>49</td>
<td>16.8</td>
<td>10.9</td>
</tr>
<tr>
<td>50</td>
<td>11.2</td>
<td>10.3</td>
</tr>
</tbody>
</table>
intravenous and intracerebral propranolol. This further suggests that a plasma concentration in the 10 ng/ml range would not be associated with an antifibrillatory effect due to myocardial receptor antagonism since previously used intravenous doses of 0.2 mg/kg and 2 mg/kg racemic propranolol (4–40 times the dose used in the present study), which would be associated with even higher plasma levels, were not effective in preventing VF in this same pig model.8 Moreover, propranolol was specifically evaluated for potential antifibrillatory efficacy in the pig, and a plasma level of 600 ng/ml was without effect in reducing the occurrence of VF after LAD coronary occlusion.29

Baseline hemodynamic and cardiac function before occlusion is also not expected to influence or be predictive of increased myocardial vulnerability to VF in this model since no significant changes were observed in the measured indexes of cardiac inotropy and chronotropy during an 8-day laboratory adaptation protocol. This observation extends a previous finding that resting heart rates were similar in laboratory-unadapted compared with laboratory-adapted pigs despite their known difference in vulnerability to ischemia-induced VF.10 The lack of hemodynamic alterations and overt signs of stress associated with exposure to a novel environment confirm the utility of this paradigm in modeling the influence of the often imperceptible tonic psychosocial stress associated with life-change events on myocardial vulnerability in man.1,5,7 It also points out the need to recognize psychological variables, whether in animals or man, when evaluating antiarrhythmic efficacy.

Decreased vulnerability to ischemia-induced VF after central administration of propranolol, however, is associated with moderate hemodynamic alterations. Both doses of L-propranolol resulted in a moderate, but a significant, decrease in heart rate and maximum left ventricular dP/dt but no effect on left ventricular systolic pressure and end-diastolic pressure. There were no significant hemodynamic alterations after central administration of D-propranolol and Ringer’s solution.

After induction of ischemia, peak heart rate in the first 4 minutes of ischemia and the stable heart rate nearest the onset of VF were moderately depressed in the high-dose L-propranolol–treated pigs compared with pigs receiving Ringer’s solution. However, both peak heart rate and heart rate nearest the onset of fibrillation were not different in pigs receiving 0.05 mg/kg L-propranolol, 0.01 mg/kg L-propranolol, and D-propranolol despite differences in VF vulnerability. Furthermore, there was no correlation between VF latency and peak heart rate in the early phase of ischemia and only a relatively weak correlation near the onset of VF. The hemodynamic and heart rate data, therefore, do not identify a specific physiological parameter that predicts antifibrillatory action. Studies in other species have also reported a negative chronotropic effect as well as a decrease in mean arterial blood pressure after administration of pro-

---

**Table 4. Plasma and Cerebrospinal Fluid Propranolol Concentrations After Intravenous Administration of 0.05 mg/kg L-Propranolol in the Pig**

<table>
<thead>
<tr>
<th>Pig</th>
<th>Plasma (ng/ml) 5 min</th>
<th>Plasma (ng/ml) 10 min</th>
<th>Cerebrospinal fluid (ng/ml) 5 min</th>
<th>Cerebrospinal fluid (ng/ml) 10 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>62.5</td>
<td>13.4</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>44</td>
<td>6.7</td>
<td>2.0</td>
<td>1.7</td>
<td>1.4</td>
</tr>
<tr>
<td>45</td>
<td>56.9</td>
<td>15.4</td>
<td>1.5</td>
<td>1.2</td>
</tr>
</tbody>
</table>

factor capable of influencing myocardial vulnerability. As has been shown previously, all pigs exposed to a novel laboratory environment develop VF within 14 minutes after the onset of ischemia. Pretreatment with direct intracerebral administration of L-propranolol, however, significantly prolonged the time to the onset of VF as well as reduced the incidence of ischemic VF from 100% to 47%. The dextrorotatory isomer that has less than 1% of the potency of the levotoratory isomer in antagonizing β-adrenergic receptors, however, failed to reduce the environmental stress-related vulnerability to ischemia-induced VF. Thus, the major finding of this study is that a central β-adrenergic receptor–mediated phenomenon appears to be involved in modulation of myocardial vulnerability to environmental stress-related arrhythmogenesis.

Since propranolol distribution into the blood compartment was expected to occur after intracerebroventricular administration, plasma and cerebrospinal fluid levels of the drug were determined to firmly establish the relative site of action (central vs. peripheral). Direct administration of 0.05 mg/kg L-propranolol into the lateral cerebral ventricles did result in measurable blood levels at 5 and 10 minutes after drug infusion. Plasma concentrations of propranolol that are associated with therapeutic effects are known to vary widely (20–1,000 ng/ml), particularly with reference to suppression of arrhythmical activity. Intravenous infusion of 10 mg racemic propranolol (1 mg/min) suppressed spontaneous ventricular ectopy in eight out of 12 patients, whereas, D-propranolol was without effect. Plasma levels associated with ectopy suppression following intravenous administration were in the range of 40–85 ng/ml.26 This level is comparable with plasma concentrations required to cause considerable cardiac β-adrenergic receptor antagonism as evidenced by a 40–80% inhibition of exercise-induced tachycardia.27 However, concentrations ranging from 100–800 ng/ml are sometimes necessary to control ventricular arrhythmias in humans.28 Thus, the measured plasma concentration of 9±3.2 ng/ml observed after intracerebroventricular injection should be below therapeutic antiarrhythmic blood levels. Cerebrospinal fluid levels, on the other hand, were on the order of 200–300-fold higher. Five minutes after the completion of drug infusion, plasma concentration for intravenous administration of L-propranolol was four times higher than for intracerebral administration; at 10 minutes, the plasma concentration was similar for...
pranolol into the cerebroventricular system. Reduction of sympathetic nervous system activity and a decrease in plasma norepinephrine concentration accompany the physiological response after cerebroventricular injection of pranolol. Thus, there is no doubt that centrally administered pranolol alters autonomic tone and stimulation of the heart by reducing (in all likelihood) sympathetic outflow.

Several studies have demonstrated a reduction in the incidence of ischemia-induced lethal ventricular arrhythmias after myocardial β-adrenergic blockade; however, there are conflicting reports. Peripheral myocardial β-adrenergic receptor antagonism achieved by the intravenous administration of β-blockers afforded protection against VF during coronary artery occlusion in the dog. Furthermore, 0.2 mg/kg i.v. propranolol significantly reduced the incidence of ischemia-induced VF with experimentally induced sympathetic hyperactivity in cats; the cardioselective β-blocker tolololomol (4 mg/kg i.v.), resulted in a 50% reduction in the repetitive extrasystole VF threshold in a psychologically stressed dog model. In contrast, peripheral administration of 0.2 and 2 mg/kg i.v. racemic propranolol in the psychologically stressed pig was ineffective in providing protection against acute ischemia-induced VF.

The present study demonstrates in the same pig model that central administration of 0.05 mg/kg l-propranolol provides a salutary antifibrillatory effect. The reason for the disparity of results in the pig compared with other models concerning cardiac β-adrenergic receptor blockade is only speculative. However, it is known that the pig is intrinsically sensitive to environmental stressors. It may be that vulnerability to the deleterious effects of environmental stress modulates the observed differences. If so, this vulnerability is likely shared by the pig and at least some patients.

The rationale for the present study stemmed from observations that various operationally defined stress or events (novel stimulus, unexpected cutaneous shock, and physical restraint) all appear to evoke a cortical noradrenergic β-receptor process of which the electrochemical correlates are known. These same stressor events also increase the susceptibility to lethal arrhythmias in pigs. The present study demonstrates that antagonism of cerebral β-adrenergic receptors reduces the susceptibility to environmental stress-related VF. The data suggesting that this is a centrally mediated β-adrenergic effect are 1) isomeric specificity of propranolol effect, 2) pharmacokinetic data demonstrating negligible peripheral propranolol levels with central administration, and 3) inability of peripheral (intravenous) administration to alter vulnerability to ischemic VF.

References


Key Words: propranolol • ventricular fibrillation • pigs • psychological stress
Central beta-adrenergic mechanisms may modulate ischemic ventricular fibrillation in pigs.

G W Parker, L H Michael, C J Hartley, J E Skinner and M L Entman

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