Ventricular Arrhythmias and Local Electrograms After Chronic Regional Denervation of the Ischemic Area in the Pig Heart

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Cardiac denervation has been proved to reduce the incidence of coronary occlusion arrhythmias in dogs, but the effect of limiting the extent of sympathectomy to the ischemic area, particularly in hearts with sparse coronary collateral circulation, as in the human heart, needs further investigation. Ventricular arrhythmias and changes in epicardial direct current electrograms induced during acute left anterior descending coronary artery occlusion were recorded in 14 pigs subjected to regional denervation of the ischemic area 2 weeks before; these were compared with findings in 14 sham-operated control pigs. Regional denervation was induced by pericoronary application of phenol above the occlusion site and it was confirmed by the loss of myocardial catecholamine histofluorescence.

During 35 min of ischemia, significant differences in occurrence of ventricular premature beats, ventricular tachycardia, ST segment elevation, TQ segment depression and epicardial activation delays were observed between the two groups of experiments, with lower values of each variable in the denervated hearts. Ventricular fibrillation occurred 32 times in 11 control pigs and only 15 times in eight denervated hearts. In contrast, programmed ventricular extrastimuli delivered during 35 to 50 min of ischemia induced 39 fibrillatory episodes in 13 denervated hearts and only 14 episodes in seven control pigs.

Thus, denervation limited to the ischemic area in hearts with a human-like coronary artery pattern was associated with a decrease in the magnitude of early ischemic arrhythmias and electrocardiographic alterations, but the procedure was unable to prevent early induction of ventricular fibrillation.

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Deprivation of cardiac sympathetic nerve tone is known to exert antiarrhythmic effects early after coronary artery occlusion in the dog heart (1–6). Canine models limiting the extent of myocardial sympathectomy to the ischemic region, thus preserving the innervation of large normal myocardial areas, have been developed either by encircling the occluded myocardial region with phenol applied topically to the epicardium (7) or by infusing 6-hydroxydopamine into the coronary artery before its ligation (8). Although these studies support the hypothesis that regional denervation of the ischemic area is still able to produce antifibrillatory effects without any hemodynamic deterioration (7,8), further investigations are needed to evaluate the potential clinical applicability of the procedure, particularly in heart models with a coronary artery pattern comparable with that of humans.

The porcine heart, like the human heart, has a poorly developed coronary collateral circulation (9). In this model, regional denervation of the ischemic area was feasible by applying phenol around the coronary artery segment before occlusion; preliminary data (10) collected 60 min after phenol treatment suggested that, even in pigs, regional denervation of the ischemic myocardium exerted significant electrophysiologic effects, but no information was available regarding its possible antiarrhythmic consequences.

Therefore, this study attempts to analyze the potential antiarrhythmic effect of long-term (14 days) myocardial denervation limited to the ischemic area in the in situ pig heart and, additionally, the influence of sympathectomy on the evolving changes in the epicardial direct current electrograms. The 14 day model was chosen to ensure full sympathetic effects because at this time local catecholamine stores are severely depleted (1–3).

Methods

Twenty-eight pigs weighing 15 to 20 kg underwent two surgical procedures. The first operation, a left lateral intercostal thoracotomy was performed under general anesthesia.
with an intravenous injection of metomidate (4 mg/kg body weight), a short-acting nonbarbiturate hypnotic (Hypnodil, Janssen Pharmaceutical), followed by sodium thiopental (30 mg/kg). The pericardium was opened and the proximal segment of the left anterior descending coronary artery was isolated above the first diagonal branch. In 14 pigs phenol was topically applied around the isolated arterial segment to produce transmural regional myocardial sympathetic denervation distal to the application (10), whereas in the remaining 14 pigs the artery was dissected but phenol was not applied (sham-operated controls). The pigs were allowed to recover, and 14 days later a midsternotomy was performed under intravenous anesthesia with metomidate (4 mg/kg) followed by alpha-chloralose (100 mg/kg). The pericardium was re-opened and a pericardial cradle was performed to suspend the heart.

The left anterior descending coronary artery was also dissected below the first diagonal branch and a fine Prolene 5/0 snare was placed to occlude the artery and to produce a zone of acute ischemia within the previously denervated myocardium. During all interventions the pigs were mechanically ventilated with a Bird respirator at 45% oxygen concentration to maintain normal blood gases. To ensure a comparable heart rate among all pigs, a demand Medtronic pacemaker was used to pace the right atrium at 95 to 110 beats/min. Sodium penicillin and analgesics were administered after the first intervention. Episodes of spontaneous ventricular fibrillation were terminated whenever they occurred by internal direct current countershocks of 15 W/s. The pigs were handled in accordance with the Position of the American Heart Association on Research Animal Use.

**Chronic regional myocardial denervation.** Phenol (carbolic acid, 88%) topically applied to the proximal segment of the left anterior descending coronary artery was expected to produce transmural sympathetic denervation of the distal anteroseptal region supplied by the treated artery (10). This chemical agent produced necrosis of the efferent sympathetic nerve fibers en route to the myocardium at their pericoronary course. To evaluate the extent of possible structural damage of the arteries treated with phenol on a long-term basis, histopathologic sections of these arterial segments were processed for hematoxylin-eosin or Verhoeff staining and examined by light microscopy. The presence of sympathetic denervation was assessed in all cases by catecholamine histofluorescent reaction in myocardial samples.

**Catecholamine histofluorescence.** The glyoxylic acid reaction for biogenic amines (11) was used to evoke histofluorescence in adrenergic nerve fibers. The fluorescence reaction was separately evaluated by two observers and was averaged from four consecutive slices studied in each biopsy. The biopsy sections were immediately frozen with liquid nitrogen, stored at −40°C and cut in a −40°C cryostat at sections of 20 to 30 µm. The slices were left for 15 s in a solution containing glyoxylic acid monohydride (Sigma), 2 g; glucose, 5.4 g; sodium phosphate 5.5 g; distilled water, 100 ml; 10 N sodium hydroxide sufficient to titrate the solution to pH 7.4 and water to complete a final volume of 150 ml. The preparations were dried of excess fluid and were incubated for 10 min at 80°C. Mineral oil covered the preparations, and a second incubation at 80°C for 190 s was carried out to remove autofluorescent air bubbles. The preparations were then analyzed in a fluorescence microscope Leitz with a K 490 filter. Myocardial biopsies for histofluorescence analysis were obtained at 50 min of ischemia. Longer ischemic periods may significantly reduce the fluorescent reaction (10,12).

**Epidermal direct current extracellular potentials.** A rubber membrane containing three rows of three cotton wick electrodes separated by an interelectrode distance of 5 mm was gently sutured to the anterior surface of the heart so that it covered the center of the ischemic area. The electrodes were made with nonpolarizable materials and consisted of thin polyethylene tubes containing a cotton thread filled with isotonic saline solution (13). The potential differences between the recording epicardial sites and the relative zero potential obtained with a reference cotton wick electrode sutured to the mediastinal fat were measured with a high impedance direct current buffer amplifier. The electrograms were obtained with an Elema Mingograf 82 ink jet recorder 25 to 100 mm/s paper speed and a signal amplitude of 1 mV/mm. Recordings from all electrodes were made in the control state and every 5 min during 50 min of coronary occlusion. Continuous recordings from electrocardiographic (ECG) leads and from one epicardial electrode were also obtained to analyze ventricular arrhythmias.

**Arrhythmia induction and measurement of refractory period.** Programmed electrical stimulation with one and two extrastimuli at the ischemic left ventricular myocardium or at the normal right ventricular outflow tract was performed to measure the effective refractory period of the ventricles and to compare possible differences in the capacity to induce ventricular arrhythmias between denervated and control hearts. Square wave pulses of 2 ms duration at twice the diastolic threshold were delivered with a Medtronic 5325 programmable electrical stimulator through epicardial bipolar electrodes. After 8 beats at a constant cycle length of 600 ms, one extrastimulus was introduced late in diastole and progressively rendered more premature at interval steps of 10 to 20 ms until it failed to excite the ventricle (refractory period). Episodes of ventricular fibrillation or ventricular tachycardia were terminated by internal direct countershock of 15 W/s. If fibrillation was not induced, a second programmed extrastimulus was coupled to the former premature beat, which was fixed 30 ms beyond the refractory period. The stimulation protocol was randomly initiated at the left or right ventricle and was completed between 35 and 50 min of ischemia because at this time the spontaneous arrhythmias tended to vanish. A more complete stimulation
protocol (three or more extrastimuli) would have prolonged the ischemic period and thus reduced the histofluorescent reaction (10). In that case, denervation induced by phenol alone could not be assessed.

Data analysis. The TQ segment shift was measured at the flat part of the PQ segment, the ST segment elevation at 120 to 150 ms from the beginning of the Q wave and the local activation time from the Q wave to the midpoint of the intrinsic deflection (13). These variables were measured in all nine electrodes of the epicardial rubber membrane, but only the mean value was considered. Data from each group of 14 pigs were expressed as the mean value ± 1 SD. Ventricular premature beats and episodes of ventricular tachycardia (>7 consecutive ventricular ectopic beats) were tabulated as the total number of events occurring every minute during 35 min of occlusion in each group of experiments.

Because sequential measurements were made on the same heart to define the time course of the ECG changes, the differences between denervated and control hearts were statistically evaluated by analysis of variance (ANOVA) for repeated measurements, with time as the within-subjects factor and denervation as the between-subjects factor. Differences in refractory periods from baseline to 35 to 50 min of ischemia (one ischemic measurement) in the same pig were evaluated by the paired Student’s t test, whereas group differences between the baseline or ischemic values were analyzed by the unpaired t test. A p value <0.05 was considered significant. Differences in the incidence of ventricular fibrillation in both groups were assessed by the chi-square test.

Results

Histology. Histologic examination of the coronary arteries 2 weeks after application of phenol revealed a slight perivasculare nonspecific infiltrative reaction with a preserved arterial wall structure. Myocardial biopsy samples obtained from the region supplied by the phenol-treated arterial segment showed no histofluorescent reaction, whereas tissue samples taken from the myocardium irrigated by the nontreated circumflex coronary artery revealed a normal network of adrenergic nerve fibers. Coronary dissection alone did not result in denervation, as denoted by the presence of normal histofluorescent fibers in the myocardium distal to the dissection level.

Ventricular arrhythmias (Fig. 1 to 3). A well defined biphasic pattern of arrhythmias was detected in all 14 control pigs during 35 min of coronary occlusion (Fig. 1 and 2). Single ventricular premature beats and couplets had a first peak activity between 2 and 10 min and a second increase during 20 to 30 min of occlusion. Both phases were separated by a period virtually free of arrhythmias. The 14 denervated hearts showed, with respect to control pig hearts a signifi-
virtual abolition of the second phase. Ventricular tachycardia was observed in 7 of the 14 control pigs, whereas only one episode was detected in the 14 denervated hearts (Fig. 2). Ventricular fibrillation developed 32 times in 11 control pigs; in contrast, there were 15 episodes in 8 denervated hearts (Fig. 3). However, these differences were not statistically significant.

Electrical stimulation (Fig. 4). Programmed ventricular stimulation performed between 35 and 50 min of coronary occlusion induced 14 episodes of ventricular fibrillation in seven control hearts (5 times from the right ventricular pacing site and 9 times from the apex of the ischemic left ventricle) and 39 episodes in 13 denervated pig hearts (19 times from the right and 20 times from the left pacing sites). Although these differences are not statistically significant, they are in contrast with the lower incidence of spontaneous arrhythmias observed during the first 35 min of ischemia in the denervated group of experiments. Ventricular tachycardia was similarly inducible in both groups of pigs.

The preocclusion refractory period of the left ventricle tended to be longer in phenol-treated hearts than in control hearts, although this difference was not significant (327 ± 39 versus 307 ± 42 ms). Between 35 and 50 min of ischemia there was a significant shortening of the effective refractory period in all cases (from 307 ± 42 to 220 ± 43 ms [p < 0.05] in control hearts and from 327 ± 39 to 234 ± 49 ms [p < 0.05] in denervated hearts).

Epicardial direct current electrograms (Fig. 5 to 8). During 35 min of coronary occlusion, denervation of the ischemic area induced with respect to control hearts a significant difference in the magnitude of the ST segment elevation (ANOVA, F = 8.3, p < 0.004) and TQ segment depression (ANOVA, F = 6.5, p < 0.01). During all ischemic time intervals, the mean values of the TQ and ST segment displacement were lower in the denervated than in control pigs (Fig. 5). A transient recovery in ST segment elevation occurred between 15 and 25 min of ischemia in control hearts, whereas this recovery phase was less pronounced in the denervated group.

Prolongation of the local epicardial activation time appeared in all experiments after coronary occlusion, but the magnitude of this delay was significantly different between the two groups of experiments (ANOVA, F = 25.07, p < 0.0001). At each time interval the lower mean epicardial delays corresponded to the denervated hearts (Fig. 6). Like the ST segment changes, the local activation transiently recovered during 10 to 20 min of ischemia in control hearts, but it remained nearly unchanged in the denervated group (Fig. 6 and 7). At the end of the recovery period there was a progressive loss of intrinsic deflection amplitude leading to monophasic electrograms. Figures 7 and 8 illustrate the early occurrence of monophasic potentials in nondenervated pig hearts.
The major finding of this study was the demonstration that denervation limited to the ischemic area and to its surrounding normal myocardium in porcine hearts with a coronary artery occlusion followed different metabolic environments during the two phases of arrhythmias: phase Ia (10 to 15 min of ischemia) is characterized in part by a local increase in potassium ion (23, 24), hydrogen ion (19) and cyclic adenosine monophosphate (c-AMP) (25) levels, whereas during phase Ib (20 to 30 min of ischemia) c-AMP returns to baseline levels (25), and catecholamines and alpha-adrenoceptors increase in the ischemic area (26-30).

Role of myocardial denervation during ischemia. The major finding of this study was the demonstration that denervation limited to the ischemic area and to its surrounding normal myocardium in porcine hearts with a coronary artery distribution similar to that in humans was able to reduce the incidence of early occlusion arrhythmias, but failed to protect against early electrical induction of ventricular fibrillation. The beneficial antiarrhythmic effect was present during the two phases of arrhythmias and was characterized by a marked reduction in ventricular premature beats and ventricular tachycardia together with a relative protection against early spontaneous ventricular fibrillation. Although some dog heart experiments have reported a comparable greater effect of cardiac sympathectomy on ventricular ectopic beats rather than on ventricular fibrillation (15, 25, 31), others found predominant antifibrillatory effects (1-5, 18) and also unequal distribution of the antiarrhythmic action throughout the two phases of arrhythmias (4, 8, 18). Left stellectomy or atenolol treatment was more effective in phase Ib (4, 18), whereas chemical myocardial denervation predominantly affected phase Ia (8).

The antiarrhythmic effect of cardiac denervation is probably due to the induced depletion of local myocardial catecholamines (8, 31-33) because arrhythmias are prone to develop when partially depolarized fibers, like the ischemic cells, are brought into contact with catecholamines (21). The influence of postdenervation heart rate changes on the course of the arrhythmias is not a factor in our study because the experiments were done at fixed atrial paced rates.

Lack of protection against induced ventricular fibrillation. In contrast to the lower incidence of spontaneous ventricular fibrillation, programmed ventricular extrastimuli induced a greater number of fibrillatory episodes in denervated than in control hearts. This lack of protection may be due to the timing of our stimulation protocol (between 35 and 50 min of ischemia), a period when the denervated hearts, as compared with control hearts, had not yet reached a homogeneous distribution of monophasic potentials within the ischemic area. Inhomogeneities in the development of monophasic potentials may reflect local differences in the
degree of cellular inexcitability (34,35), a situation known to favor the genesis of arrhythmogenic pathways. A proarrhythmic effect induced by the electrical stimulation itself would affect both groups of experiments but, on the other hand, there is no evidence for an increase in local adrenergic activity during electrical induction of ventricular premature beats (25). Chronic denervation of the canine myocardium may render the heart more sensitive to circulating catecholamines (sympathetic supersensitivity) (36). Even though this phenomenon has not been reported in pigs, its presence in our denervated model should have produced a worsening of rather than a protection against spontaneous arrhythmias.

The ability to electrically induce fibrillatory episodes in the denervated heart suggests that the “substrate” for reentry is still present. Therefore, the antiarrhythmic effect of regional denervation does not seem to be related to a direct electrophysiologic action that prevents reentry. Instead, it would be likely due to the suppression of premature beats that can trigger the generation of reentrant circuits.

**Epicardial direct current electrograms.** During 15 to 25 min of ischemia, nondenervated control hearts exhibited a transient reduction in ST segment elevation concurrent with a momentary disappearance of arrhythmias after phase la and with a transient improvement in the epicardial activation delay. The transitory reduction in ST segment elevation observed in this study was not observed in previous pig heart experiments (10,34). This distinct ST segment course may be related to the anesthetic agent used because alpha-chloralose and barbiturates are known to exert different electrophysiologic actions on epicardial potentials (37). Residual effects on ST segment changes due to the previous thoracotomy are difficult to determine because the preocclusion epicardial electrograms had a normal ST segment contour in all cases.

The mechanism responsible for the transient amelioration of the electrocardiographic alterations and arrhythmias is poorly understood. The close temporal relation between the transient electrical improvement and the moment at which the extracellular potassium ion accumulation reached its plateau phase (23,24), provided support for the hypothesis that during this phase the membrane potential might partially recover and therefore restore, to some extent, the ischemic electrophysiologic derangements (10,34). However, isolated heart preparations have been able to demonstrate a transient electrical recovery even when the extracellular potassium ion was apparently held constant (38,39).

**Predenervation of the ischemic area lessened the magnitude of the ECG changes and nearly abolished the transient recovery in ST segment and local conduction delays.** Lessening of the transient recovery periods may be a direct effect of sympathectomy or an indirect consequence of the decreased magnitude in ST segment and epicardial delay changes. The results herein reported are in accordance with the acute denervation model in pigs (10) as well as with the retarded development of the histologic injury in denervated
dog hearts (40). Our 14 day model showed a more consistent effect on TQ segment depression than did the short-term experiments (10,41). Lessening of activation delays is in accordance with the effect of stellectomy (37,41) or with the worsening of conduction observed after stellate stimulation (42).

Methodologic considerations. The lack of histofluorescence was taken as indicative of denervation because catecholamine-depleted hearts fail to show fluorescent fibers (43), and 2 weeks after denervation the norepinephrine stores are severely depleted (1-3).

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References


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