Modification of Ventricular Fibrillation Latency Following Coronary Artery Occlusion in the Conscious Pig

The Effects of Psychological Stress and Beta-adrenergic Blockade

By James E. Skinner, Ph.D., J. T. Lie, M.D., and M. L. Entman, M.D.

SUMMARY
Abrupt occlusion of the left anterior descending coronary artery was performed on 45 unanesthetized farm pigs in order to evaluate the relative effects on the latency to ventricular fibrillation (VFL) of 1) adaptation of the animals to the laboratory, and 2) beta-receptor blockade by propranolol. Compared to control values, VFL was greatly lengthened (i.e., VF delayed or prevented) by adaptation (P < 0.01), was shortened by large (2 mg/kg) doses of racemic propranolol in unadapted animals (P < 0.02), and was again increased, while under the influence of this drug, by adaptation (P < 0.05). Neither a lower (0.2 mg/kg) dose of racemic propranolol, 2 mg/kg of dextropropranolol, nor ventricular pacing to a higher heart rate had an effect on VFL. The results suggest that reduced psychological stress was very effective in retarding or preventing the onset of VF, that low doses of propranolol were ineffective, and that higher doses were deleterious in unadapted animals.

Additional Indexing Words:
Myocardial infarction
Sudden death
Propranolol
Dextropropranolol
Adaptation

A RECENT REPORT by Rahe et al.1 has revealed that an increase on a scale of life-change, including events such as death of a spouse, divorce, loss of a job predicts persons who will develop myocardial infarction or coronary death with as much accuracy as the physical risk factors of high blood pressure and high serum cholesterol. Their study implies that the life-change variable may precipitate the onset of cardiac illness and death, and suggests that if the persons had not been exposed to the psychological stress, they might not have developed an infarct nor have died at the time that they did. Three independent normative studies of nontraumatic deaths of persons living in modern urban settings have established consistently that 13–14% of all these deaths are sudden and are unexplained following autopsy.3–4 These results suggest that ventricular fibrillation may be initiated by factors other than diffuse and extensive coronary artery disease.

Several recent observations in the conscious animal have confirmed that psychological factors have an important influence on the mechanisms underlying ventricular fibrillation (VF) initiation. Schaper5 has reported that laboratory-raised beagles show a lower mortality rate following gradual coronary occlusion than do mongrels. Gregg6 has stated that trained dogs accustomed to the laboratory do not manifest VF following acute coronary occlusion. Lown et al.7 have demonstrated that an increase in stress produced by electric shock will lower the threshold for electrically elicited arrhythmias and fibrillation.

Beta-adrenergic blocking agents are widely used in patients because of their antiarrhythmic properties.8 Khan et al.9 have reported recently that a racemic mixture of propranolol in moderate doses (0.1 mg/kg) produces a decreased mortality in conscious dogs following acute occlusion of the left circumflex coronary artery. Mortality was reduced from 72% (18/27 animals) to 24% (6/25 animals) as a consequence of a decrease in the incidence of VF within the first two hours after occlusion. An injection of dextropropranolol (0.07 mg/kg), calculated to produce an "equi-active membrane effect" to that produced by the racemic mixture, had no effect in decreasing mor-
tality. The authors concluded that the protective effect of the racemic dose was due to beta-adrenergic blockade by the levoisomer and was not the result of membrane stabilization by the dextroisomer.

The present study was undertaken to evaluate the relative effects in the conscious pig of psychological stress and beta-adrenergic blockade on the ventricular-fibrillation latency (VFL) following acute coronary occlusion. Three groups of animals were prepared: group A received various degrees of stress that was evoked by introducing the animals into an unfamiliar laboratory environment and was varied by adapting the animals differentially to the laboratory and experimenters; group B received varying dosages of propranolol, with the psychological stress held constant; and group C experienced both reduced psychological stress and significant beta blockade (see table 1).

Methods

Coronary Occlusion

Young Hampshire farm pigs (40–60 lb) from the Huntsville prison facility were chosen as the experimental animals. Unlike mongrel dogs whose reaction to the experimental situation may be aggression or docility, depending upon whether they were pets or strays, these pigs show a uniform behavioral reactivity pattern to the laboratory and personnel. Also, pigs have a coronary circulation that resembles that of man more closely than that of the common carnivores.5–10

A coronary occluder was developed because the widely used balloon-cuff type11,12 was found to be impractical. Pigs have intramural coronary arteries buried 4–6 mm below the epicardial surface. In preliminary experiments in our chronic pig preparations, it was found that the intramural arteries, when surrounded by a bulky device such as a balloon-cuff occluder, tended to bow up out of their channels and thus cause unintentional interference with coronary flow. The device which we developed is illustrated in figure 1 and consists of a hydraulically operated piston inside a double-action set of chambers, made by connecting two polyethylene tuberculin syringes together with melted polyethylene. The piston moves a wire inside a small polyethylene tube, which will tighten and release a non-stretchable ligature placed around the artery.

During surgery, the ligature was tied with the device in the occlusion position. This enabled the maximum tightness of the ligature to be preset and thereby prevent mechanical damage to the arterial wall when the device was later operated. The pericoronary nerves and fascia were separated carefully from the coronary artery so that they were not included within the ligature. The ligature was sewn through a few strands of the muscle underlying the dissected-free artery so that muscular action would aid in releasing the ligature when the device was set in the occlusion position. At the time of surgery, the effectiveness of the occlusion was determined by visual inspection of the collapse of the distal artery, cyanosis of the distal vessel, and marked ST-segment depression of the ECG. Later, in the closed-chest condition, the ECG criterion could determine the effectiveness of occlusion.

Five days after the pigs were received in the vivarium, each was operated under ether anesthesia, and an occluder was attached to its left anterior descending (LAD) coronary artery, just proximal to the origin of the main diagonal branch. This was an easily identifiable location and permitted consistent placement of the ligature from animal to animal. Stainless-steel wire electrodes were permanently sutured through the skin of the legs and the scalp for monitoring the standard lead ECG and the EEG. The day following surgery, the ECG was monitored for detection of any abnormalities. Each animal was allowed 7–10 days of postoperative recovery before experimentation was begun, except in the one case of the heart-rate control group, noted below.

A permanently implanted polyethylene catheter was inserted through the cut end of the mammary artery into the brachial artery for monitoring arterial blood pressure. The catheter was filled with heparin and sealed when not in use. All drugs were injected through a cannula in an ear vein 5 min prior to coronary occlusion. None of the pigs showed a significant behavioral reaction to the operation of the occluder.

If fibrillation occurred following coronary occlusion, the ligature was released and the arrhythmia converted with a 300–400 watt-sec external DC shock. The defibrillation shock was given after slow waves appeared in the EEG, indicating that the animal was unconscious or semiconscious. The slow EEG waves appeared within 30 sec following the onset of VF and disappeared within 10 sec following the return of the normal sinus rhythm. Most animals converted with only one shock, but sometimes a second shock was required, approximately 10 sec after the first. The ECG records of all animals returned to normal following disocclusion and defibrillation, and persistence of arrhythmias

Figure 1

Coronary-artery constrictor, constructed from two polyethylene tuberculin syringes. DO = disocclude syringe; I = weld joint, joined with melted polyethylene; L = ligature to be tied around artery; NC = polyethylene needle cover, welded to hydraulic chambers to protect wire; W = ooccluder syringe; PE 100 = polyethylene tubing (Adams Intramedic); T = metal T-joint for attaching W and PE 100 to heart muscle; W = anttempered 28-ga stainless-steel wire for movement of ligature; L = WF = wire fittings for attaching polyethylene tubing to needles (same wire as W); 19 ga = segment of 19-ga hypodermic needle; 20 ga = segment of 20-ga hypodermic needle.

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varied from 30 sec to 5 min following the return of the normal sinus rhythm. If the animal did not develop VF within 20 min following occlusion of the LAD coronary artery, the ligature was released, unless it was a terminal occlusion experiment. This 20-min interval is the maximum period of reversible myocardial ischemia that can be produced in the dog.13

Group Treatments

Table 1 shows the specific subgroup treatments for each of the three major groups. In group A, 17 native pigs were randomly assigned to four treatment subgroups. Each adaptation subgroup received different degrees of adaptation to the laboratory and experimenter. Subgroup 0 received no adaptation experiments; subgroups 4 and 8 received four or eight daily adaptation experiments prior to the first occlusion experiment. Each unit of adaptation consisted of taking the animal from the vivarium and placing it in the recording chamber with its feet tied together, attaching the recording wires and occluder tubes, and then leaving the animal undisturbed for 1 hr before returning it to its home cage. The adaptation experiments were exactly the same as the occlusion experiments, except the LAD coronary artery was not occluded, and therefore no defibrillation shocks were given.

Five additional animals were operated to form a heart-rate control subgroup (HR). Each animal had cardiac pacing electrodes in addition to the coronary-occluder device. Two disc electrodes, insulated with silicone rubber except at the surface in contact with the epicardium, were permanently attached to the right ventricle of each animal. These electrodes were stimulated in the chronic closed-chest preparation via multistranded, 28-ga, silicone-rubber-insulated silver wires at an intensity 20% above the threshold necessary to capture the sinus rhythm at the desired rate. These animals were not given the usual 7–10 days of postoperative recovery but were adapted to the laboratory as rapidly as possible, in several longer adaptation sessions per day, beginning on the third day following surgery. This procedure was felt to be necessary in order to be able to have sufficient time to perform all of the experiments before the wires of the pacing electrodes broke due to the constant flexing with each heart beat. The discomfort of these animals due to insufficient postsurgical recovery appeared to cause these animals to manifest ventricular fibrillation very rapidly (within AI, see figure 2) following their first experience with coronary occlusion. Sixteen other directly comparable, unadapted control animals, which did receive sufficient postsurgical recovery, manifested ventricular fibrillation later (i.e., within AII) following their first coronary occlusion. An extended period of postsurgical discomfort may explain why two of the subjects in subgroup 4 manifested VF within AII instead of AII, although no overt behavioral signs of pain were apparent in these animals.

In group B, all 15 of the animals were unadapted (except for that small amount of adaptation gained during the recording session on the first postoperative day), and a single experimental procedure was performed on each pig 7–10 days after surgery. All of the animals were handled in an identical manner and were randomly assigned to the drug-treatment groups. These pigs were injected with either saline (S), 2 mg/kg dextropropranolol (D), 2 mg/kg racemic propranolol (R), or 0.2 mg/kg racemic propranolol (2) 5 min before being subjected to their first and only occlusion experiment.

In group C, eight of the animals were partially adapted to the laboratory; five additional unadapted controls were simultaneously studied with this group. Each of the eight animals to be adapted was taken from the vivarium and placed in the recording chamber; the recording wires were then attached, and the animal was left undisturbed for 1 hr before it was returned to its home cage. These adaptation sessions were performed daily for six consecutive days. It had been determined in group A that adaptation of more than eight days before the first occlusion (OCC) resulted in the animals' failing to manifest VF after OCC, whereas adaptation for less than four days was ineffective in preventing VF after OCC. The five unadapted control animals were not exposed to the laboratory or experimenter and were left in their vivarium cages for six additional days. Each of the eight partially (6-day) adapted animals was sub-

<table>
<thead>
<tr>
<th>Subgroup designation</th>
<th>Number of animals</th>
<th>Experimental procedure</th>
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<tbody>
<tr>
<td><strong>Group A (adaptation)</strong></td>
<td></td>
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<tr>
<td>0 (4) Nonadapted</td>
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<tr>
<td>4 (4) 4 days of adaptation prior to first occlusion experiment</td>
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<tr>
<td>8 (4) 8 days of adaptation prior to first occlusion experiment</td>
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<tr>
<td>HR (5) Electric pacing of right ventricle to maintain constant heart rate</td>
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<td><strong>Group B (beta blockade)</strong></td>
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<tr>
<td>S (3) Saline control, injected 5 min prior to occlusion</td>
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<tr>
<td>D (5) 2 mg/kg dextropropranolol, injected 5 min prior to occlusion</td>
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<tr>
<td>R (3) 2 mg/kg racemic propranolol, injected 5 min prior to occlusion</td>
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<tr>
<td>P (2) 0.2 mg/kg racemic propranolol, injected 5 min prior to occlusion</td>
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<tr>
<td><strong>Group C (adaptation plus beta blockade)</strong></td>
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<tr>
<td>C(U) (5) Saline injected, independent control</td>
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<tr>
<td>C(P) (8*) Saline injected, partially adapted (6 days)</td>
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<tr>
<td>P(P) (8*) 2 mg/kg racemic propranolol injected, partially adapted (6 days)</td>
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<tr>
<td>P(A) (4†) Animals in P(P) that proved to be completely adapted (i.e., did not manifest VF within 20 min following occlusion in succeeding control experiment)</td>
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<tr>
<td>P(U) (4†) Subjects in P(P) that proved to be partially unadapted (i.e., manifested VF within 20 min following occlusion in succeeding control experiment)</td>
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</table>

*These were the same animals, each of which received a saline control and a propranolol treatment in two occlusion experiments; the group was counterbalanced for order of experiments by subgroups P(1) and P(2).
†These subgroups, combined, made up P(P). The two subgroups were divided according to the a posteriori criterion of whether or not the subject manifested VF during the succeeding control occlusion experiment.

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jected to two occlusion experiments, one preceded by an injection of 2 mg/kg racemic propranolol and the other preceded by a saline injection.

For purposes of data analysis, the propranolol-treatment experiments on partially adapted animals, P(P), were divided into each of two classes: P(1) and P(2), to counteract the effect of the order of experiments; and P(A) and P(U), depending upon the control response that followed. Members of P(1) received the propranolol injection before the first occlusion experiment, and those in P(2) received it prior to the second experiment. The animals in P(A) did not manifest VF within 20 min after coronary occlusion in the immediately subsequent control experiment, and those of P(U) did. This latter criterion was taken to separate the animals that were fully adapted (A) and those that were partially unadapted (U) during the previous propranolol experiment. It was necessary to subject the animals in P(2) to a third occlusion experiment so that they would have a control experiment after propranolol administration.

A third of the eight partially adapted animals was subjected to a control procedure (saline injection), in addition to the propranolol treatment, in either its first or second occlusion experiment, and together these results formed control observations, C(P), that were also partitioned into subclasses analogous to those obtained during the propranolol treatment: C(1) received the control experiment after the propranolol experiment, and C(2) received the control experiment first; C(A) did not manifest fibrillation in the occlusion experiments subsequent to the propranolol one, and C(U) did. The five independent control animals, C(U') had only one occlusion experiment, preceded by an injection of saline. Each partially adapted animal of group C functioned, therefore, as his own internal control. The additional unadapted control group was furnished to evaluate further the role of adaptation in the propranolol response.

Postmortem Examination

In order to evaluate the pathologic effects of temporary ischemia for the period of 20 min or less, five of the 12 nonfibrillating, adapted animals of group A were subjected to a final occlusion experiment that lasted only 20 min. Thirty minutes following freeing the occlusion and return of the ECG to normal, each of the animals was sacrificed and the heart removed. Full-thickness left ventricular tissue samples, 1 x 1 cm, were taken from the center of the field of perfusion of the LAD coronary artery that was distal to the point of occlusion and from the center of the field of perfusion of the left circumflex artery. The tissue was fixed in 10% buffered, neutral formalin, embedded in paraffin, cut at 6 µ thickness, and stained with hematoxylin and eosin (H & E), Movat's pentachrome, and the hematoxylin-basic fuchsin-picric acid (HBFP) stain which has been shown to be sensitive to acute myocardial ischemia.

The other seven animals, as well as the five in the heart-rate control subgroup, had final occlusions that lasted from 40 min to 24 hr. They were sacrificed four to ten days later. Only the H & E staining procedure was used in these infarcted hearts, since it was obvious that ischemic injury was present. The histological examination was done without a prior knowledge of the group of the animal (i.e., double-blind).

The hearts of the pigs of groups B and C were similarly subjected to the above staining procedures. The free-wall thickness was measured in the ischemic and control regions of the left ventricle in all of the animals because Schaper had found that in chronic preparations maintained alive for two weeks or longer, complete reabsorption of infarcted and necrotic tissue occurred, but wall-thinning persisted. He found that the ratio of the thickness of infarcted tissue divided by that of control tissue was 0.9 ± 0.05 (sd) after one week, 0.6 ± 0.05 after two weeks, and 0.5 ± 0.06 after four weeks. The effect on the pressurized (350 mm Hg) flow of saline through the LAD coronary artery was observed in all of the animals following operation of the occluder.

Results

Adapted Animals of Group A

Figure 2 illustrates the effects of LAD coronary occlusion on the ECGs of the animals that manifested VF. Two distinct periods of arrhythmia (A1 and AII) occurred in most of the pigs, with a normal sinus

![Figure 2](https://circ.ahajournals.org/)

Critical periods of arrhythmia following occlusion of the left anterior descending coronary artery in the conscious pig. The lower four rows of traces are continuous with the upper four. Traces from standard ECG leads I, II, and III and from a pericardial lead (P C.) at the border of the zone of perfusion of the distal portion of the occluded artery are shown before (PRE), during, and 8 min after (POST) occlusion. The time scale above the traces begins with occlusion (OCC) and ends with disocclusion (DISOCC) and electrical defibrillation. The shaded portion indicates the two periods of arrhythmia and represents the median range in minutes of all the intervals obtained in all the occlusion experiments of the subjects of Group A. PVC = premature ventricular contraction; AE = aberrant excitation resulting from decremental conduction; TACH = ventricular tachycardia; VF = ventricular fibrillation. Calibration for traces: 500 µV and 1 sec.
rhythm reinstated between them. The arrhythmias were all typical of those following acute coronary occlusion and consisted of short trains of ventricular extrasystoles with occasional single aberrant beats also presumed to be of ventricular origin. Depolarizations without P-waves (PVC) and beats with normal P-R intervals but aberrant R waves (AE) were generally recorded in the same animals. Only two animals of the group manifested VF during AI.

Table 2 shows the results obtained from subgroups 0, 4, and 8. Each animal in subgroup 0 had six to ten consecutive occlusion experiments in which the VFL was less than the 20 min maximum period of occlusion; that is, VF occurred within AI or AII following OCC. The mean VFL for each animal in these initial experiments is shown at the left in Table 2. In the first experiment subsequent to these, VF failed to occur within 20–60 min following OCC; that is, the VFL in each animal was dramatically lengthened. The animals in subgroup 4 had four prior adaptation experiments before their first OCC experiment and, as a consequence, manifested only two to four consecutive OCC experiments in which their VFL was less than 20 min. The pigs in subgroup 8 had eight prior adaptation experiments, and as a result, showed a dramatically long VFL following their first OCC.

All pigs failed to manifest VF within 20 min to 24 hr after OCC in at least one experiment. Since actual OCC experiments were thought to be adaptive, the number of the experiment in the successive series before VF first failed to occur was measured and found to be highly dependent upon the amount of prior adaptation. A negative linear Pearson product-moment correlation coefficient of \( r = -0.92 \) indicated that the more prior adaptation an animal had had, the fewer consecutive, temporary OCC experiments before VF failed to occur in one of them. In addition, the mean number of OCC experiments that were necessary before VF failed to occur was found to be significantly different among the three subgroups \( (P < 0.01) \).

In seven of the 12 animals, when VF did not occur within the expected AII period, the occlusion was maintained from 40 min to 24 hr (1440 min) with no subsequent occurrence of VF (animals 1, 2, 3, 5, 10, 11, and 12). In the other five animals, when VF had not occurred within 20 min, the LAD occlusion was released so that the cumulative effects of the previous OCC experiments on the myocardial tissue could be assessed histologically (animals 4, 6, 7, 8, and 9).

It was noted in the animals of subgroups 0, 4, and 8 that a significant reduction in heart rate (HR) occurred during the non-VF experiments as compared to the ones in which VF did occur. For this reason, electric pacing of HR was carried out in a control subgroup. Figure 3 shows the results obtained. A high

### Table 2

**Ventricular Fibrillation Latency Following Coronary Occlusion Is Modified by Adaptation to the Laboratory in the Pigs of Group A**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Ventricular fibrillation latency (min) in initial experiments (VFL ≤ AII)</th>
<th>Prior* adaptation experiments</th>
<th>Consecutive* occlusion experiments (VFL ≤ AII)</th>
<th>Subsequent occlusion experiments (VFL &gt; 20 min)</th>
<th>Duration of terminal occlusion experiment (min)</th>
<th>Postmortem histological results</th>
<th>Wall thickness ischemic + control (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.0</td>
<td>11.0–13.3</td>
<td>0</td>
<td>6</td>
<td>1†</td>
<td>40</td>
<td>+</td>
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<td>2</td>
<td>12.1</td>
<td>9.8–14.8</td>
<td>0</td>
<td>7</td>
<td>1†</td>
<td>60</td>
<td>+</td>
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<tr>
<td>3</td>
<td>10.9</td>
<td>9.0–16.0</td>
<td>0</td>
<td>10</td>
<td>1†</td>
<td>60</td>
<td>+</td>
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<tr>
<td>4</td>
<td>12.3</td>
<td>9.7–14.0</td>
<td>0</td>
<td>7</td>
<td>2</td>
<td>20§</td>
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<tr>
<td>5</td>
<td>18.0</td>
<td>17.7–18.3</td>
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<td>2</td>
<td>1†</td>
<td>60</td>
<td>+</td>
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<td>&gt;20.0</td>
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<tr>
<td>11</td>
<td>&gt;60.0</td>
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<td>1†</td>
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<tr>
<td>12</td>
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<td>0</td>
<td>1†</td>
<td>1440</td>
<td>+</td>
<td>0.5</td>
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</table>

*Correlation coefficient = -0.92.

†H & E = hematoxylin and eosin; HBFP = hematoxylin-basic fuchsin-picric acid.

‡The first occlusion experiment in which VF failed to occur was terminal.

§The LAD coronary artery was never occluded in these subjects for more than 20 min.

+ = signs of ischemic injury; - = not different from controls.
average HR was encouraged in these subjects by not allowing them time to calm down after placing them in the apparatus. When HR was adjusted (paced) after the animals had been adapted to the same rate as occurred during their first occlusion experiment (unadapted), it had no effect on lowering VFL. Cutaneous shocks to the hind limbs (100 V, 1 msec) once every 10 sec for 5 min prior to and during the OCC period did, however, lower the VFL of these adapted subjects (shock). Terminal OCC experiments in these same pigs (terminal) did not result in VF within 24 hr, a finding that suggests that the VFL during the adapted and paced conditions was much larger than the 20-min minimum that could be measured at that time.

Unadapted, Propranolol-Treated Animals of Group B

Figure 4 shows the results from the animals of group B, which received no laboratory adaptation and only one occlusion experiment. Subgroup R received 2 mg/kg of racemic propranolol, a treatment that reduced their mean VFL (P < 0.01) compared to that of the saline-injected control subgroup, S. As seen in subgroup 2, a smaller dose of racemic propranolol (0.2 mg/kg) had no effect on VFL. A dose of 2 mg/kg of dextropropranolol also had no effect on VFL, as shown by subgroup D, a finding that suggests that beta blockade, per se, is the dominant effect that results in the shortening of VFL.

No statistically significant differences in resting HR were observed between any of the subgroups; however, within a subgroup, when HR was measured in each animal before and after the drug was injected, it was found that 2 mg/kg racemic propranolol reduced resting HR from 130 to 111 beats/min (P < 0.01). The dextropropranolol and lower dose of racemic propranolol had no significant effect on HR within the subgroups. When resting HR was increased in response to brief electric shocks (100 V, 1 msec, 50 Hz, 0.5-sec train), 2 mg/kg propranolol treatment resulted in a reduction of the evoked increase in HR from a mean of 24 ± 8% to 12 ± 2% (P < 0.02). The dextropropranolol and 0.2 mg/kg racemic propranolol had no significant effect on heart rate.

Partially Adapted, Propranolol-Treated Animals of Group C

In order to test the effects of propranolol in adapted animals, group C was given adaptation experience that, based on the results of group A, would be insufficient to produce the prevention of VF following OCC. If 2 mg/kg propranolol was deleterious, then a shortened VFL should occur, and if propranolol was beneficial, as Kahn et al.9 suggested, then prevention of VF should occur in these animals. The matrix in table 3 shows the probability level of the significance of the difference between the mean VFL of the propranolol treatment group and its controls. When experimental and control measures were made in the same animal, a two-tailed, correlated-measures t-test was used.15 It will be noted that the mean VFL obtained in the 2 mg/kg propranolol-treated experiments, P(P), is significantly different from that obtained in C(P), the correlated-measures control (i.e., the same animal was used in both experiments, with and without propranolol). However, the mean VFL of P(P) is not significantly different from that of the five unadapted, independent control animals, C(U'), who did not receive propranolol.
In order to assess the degree of adaptation in the animals immediately after the propranolol treatment, and to determine whether or not there was an effect of the order of experiments in the animals used as their own controls, the P(P) and C(P) results were subdivided and analyzed. As previously described, P(P) was divided into two groups determined by the results of the control occlusion experiment given 24 hours after the propranolol-treatment occlusion experiment. If the animal manifested VF within 20 min following occlusion, it was indicative that during the previous propranolol-treatment occlusion experiment, the animal was still partially unadapted [P(U)]. If the animal did not manifest VF within 20 min following occlusion, it was a sign that the animal had received sufficient adaptation to prevent VF during the preceding propranolol-treatment occlusion experiment [P(A)]. The VFL of subgroup P(U) was found to be significantly different from that of its correlated-measures control, C(U). In contrast, the adapted group, P(A), manifested a VFL that was not significantly different from its correlated-measures control, C(A). When P(U) was compared to the wholly unadapted group, C(U'), there also was dramatic shortening of VFL (fig. 5). Thus, in the partially unadapted animals, propranolol was deleterious, as it was in the wholly unadapted animals of group B, but in the sufficiently adapted pigs, propranolol had no effect on VFL. The comparison of the results of P(A) and C(A) suggests that adaptation counteracts the deleterious effects of the propranolol. The order of experiments did not influence the response to propranolol, since the VFL in P1 and P2 were not statistically different, nor were they in their correlated-measured controls, C1 and C2.

The average resting HR was measured for each animal in the group during the 60 sec before each coronary occlusion was performed. No statistically significant differences were found between the means of any of the independent or correlated groups. Even the mean HR of the unadapted control group, C(U'), was not statistically significantly different from any of the subgroups of the partially adapted subjects. However, when HR was increased from the resting level in response to brief electric shocks (100 V, 1 msec, 50 Hz, 0.5-sec-train duration), propranolol treatment (2 mg/kg) resulted in a decrease of the evoked increase in HR from a mean of 35 ± 10% to 12 ± 2% (P < 0.01).

**Histological Results**

The five animals shown in table 2 which never received LAD coronary occlusion for more than 20 min were subjected to careful histological analysis, using a double-blind experimental procedure. The animals were sacrificed with an overdose of pentobarbitis 30 min after the final occlusion experiment ended. Figure 6 illustrates the results obtained. Once

Figure 4

Ventricular-fibrillation latency (in min) following coronary occlusion in the animals of group B (one occlusion experiment performed in wholly unadapted subjects). The subgroup mean, bracketed by its standard deviation, is shown for each drug treatment. S = saline; D = 2 mg/kg dextropropranolol; R = 2 mg/kg racemic propranolol; C = 0.2 mg/kg racemic propranolol. The number in each subgroup is indicated at the bottom of each bar.
Ventricular Fibrillation Latency

Table 3

Matrix of Significance Levels (P) of Two-tailed t-tests Performed between the Means of the Ventricular Fibrillation Latency (VFL) of Each Subgroup* of Group C

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>P(P)</th>
<th>P(U)</th>
<th>P(A)</th>
<th>P(1)</th>
<th>P(2)</th>
<th>C(P)</th>
<th>C(U)</th>
<th>C(A)</th>
<th>C(U')</th>
</tr>
</thead>
<tbody>
<tr>
<td>VFL (in min)</td>
<td>7.1</td>
<td>2.2</td>
<td>11.9</td>
<td>6.8</td>
<td>7.3</td>
<td>12.7</td>
<td>10.5</td>
<td>14.8</td>
<td>13.4</td>
</tr>
<tr>
<td>Std. deviation</td>
<td>7.4</td>
<td>1.2</td>
<td>8.0</td>
<td>8.9</td>
<td>7.0</td>
<td>5.0</td>
<td>3.2</td>
<td>6.1</td>
<td>5.3</td>
</tr>
<tr>
<td>Group size</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

*Subgroup abbreviations: P = 2 mg/kg racemic propranolol; C = saline control; (P) = partially adapted, partitioned into subgroups as follows: (A) = adapted according to criterion of no VF within 20 min after OCC in control experiment that followed propranolol experiment; (U) = unadapted according to criterion of VF within 20 min after OCC in control experiment that followed propranolol experiment; (1) = received propranolol experiment followed by control experiment; (2) = received control experiment followed by propranolol experiment. (U') = unadapted independent control.

†Nonsignificant. Since most of these multiple t-tests were not significant, modification of the α level was not necessary. Those tests which did not meet the homogeneity of variance criterion were also found not significant by the Mann-Whitney U-test.*

‡These t-tests did not meet the homogeneity of variance criterion. However, the compared groups did show significantly different variances (P < 0.01, two-tailed F-test). The actual values of the variance suggest that adaptation increases variance.

the pathological results were determined, the code was broken, and the results revealed that no significant changes had occurred in the tissue from the field of perfusion of the LAD coronary artery (experimental, E) compared to that of the field of the circumflex artery in the same heart (control, C). Comparisons were also made between the E tissue from the previously ischemic hearts and tissue from the E and C regions of the myocardium of two sham-operated preparations in which no occlusions had been made because of damage to the external hydraulic tubes of the occlusion device. Scattered regions of the E blocks revealed a few positive HBFP-stained cells, but the number of such cells was not quantitatively different from that observed in the C block from the same preparation or from the E or C blocks of the sham-operated controls. The H & E- and HBFP-stained tissue revealed occasional "wavy" fibers that were thought to be overstretched fibers not yet recovered and contracting. Some of these fibers showed positive HBFP stains, but most did not. All of the results are consistent with reversible ischemia or ischemia produced during the expiration of the animal. No inflammatory cells (granulocytes) or extravasated red blood cells, characteristically seen in acute infarction, were noted in the tissue from the hearts not occluded for more than 20 min. This finding suggests that the effects of ischemia completely reversed following release of the coronary artery.

All of the animals in which the occlusion was maintained for more than 20 min showed the typical patterns observed in infarcted tissue, and considerable wall-thinning had occurred in the tissue by ten days following the occlusion (table 2).

Discussion

This study showed that reduction of psychological stress in pigs by daily laboratory-adaptation experiences alone, a repeated series of temporary coronary-occlusion episodes in the recording chamber, or a combination of both, had a dramatic effect on preventing the occurrence of VF within the expected second arrhythmia period (AII, 9-14 min) following occlusion of a major coronary artery.

The results in group B animals, all of whom were unfamiliar with the laboratory, did not confirm the findings of Khan et al., who observed in dogs that a 0.1 mg/kg dose of racemic propranolol had a protective effect against VF after coronary occlusion. We confirmed their finding that the larger dose of propranolol is less beneficial than the smaller, but they stated that the larger dose had no effect, whereas we found it to be deleterious in our unadapted and par-

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Ventricular-fibrillation latency in the partially adapted animals of group C. Pigs were adapted for six days until their resting heart rates lowered and they appeared calm. Each pig was then subjected to a saline control dose or a 2 mg/kg dose of racemic propranolol 5 min before occlusion of the LAD coronary artery. The mean and standard deviation of the control, C(P), and propranolol, P(P), results are shown at the left. The P(P) experiments were divided into two subclasses that depended upon whether VF occurred, P(U), or did not occur, P(A), in the succeeding control experiment. C(U°) is an independent, wholly unadapted, control group.

Partially adapted animals. If their control group had shown the same incidence of VF as their low-dose racemic-propranolol-treatment group, as in our series, then their interpretation regarding propranolol would be similar to ours. It is possible that their failure to consider the psychological variable of reactivity to the laboratory in their subjects is the reason for their findings8, 16 differing from ours. Alternatively, the difference in species might alter the pharmacologic effects or even the psychological effects, since dogs frequently have been pets, already accustomed to indoor surroundings and human contact.

The results obtained in group C animals (partially adapted to the laboratory) show that adaptation to the laboratory increases the mean VFL in animals treated with large doses of propranolol. When a cardio-active drug such as propranolol is studied in conscious animals, its effects on VFL may be misinterpreted un-
blockade was found not to lengthen VFL.

The origin of the deleterious effect on VFL by large doses of propranolol is also not known. Propranolol does not seem to affect the coronary flow in the ischemic zone, so it probably is not acting directly on collateral-circulation mechanisms. It is possible that the large, but incomplete, blocking dose of racemic propranolol produces nonuniform inactivation of beta receptors throughout the heart, which in turn results in dispersion of refractoriness to the normal beta stimulation via tonically-active sympathetic nerves.

This might then result in further instability in both ischemic and adjacent nonischemic zones, due to regional variation of conduction velocity and refractoriness in myocardial cells, a phenomenon associated with lowered thresholds for the initiation of VF by re-entry. The effect of adaptation could then be to lower the sympathetic tone and thereby reduce the contrast in the regional variations, an action which would tend to restore the electrical stability.

Gamble and Cohn recently suggested that the mechanism of re-entry is more important than that of

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**Figure 6**

Hematoxylin-basic fuchsin-picric acid (HBFP) stained tissue from previously ischemic (experimental, E) and nonischemic (control, C) regions of the myocardium. All photomicrographs show the region of greatest positive HBFP-stained cells observed in the myocardium. The E blocks of tissue were taken from the center of the field of perfusion of the LAD coronary artery that was distal to the point of occlusion, and the C blocks were taken from the center of the field of perfusion of the unoccluded left circumflex coronary artery in the same animal. All pictures are of the subendocardial region and were photographed through a green filter that makes the positively stained tissue and red blood cells appear black. POS HBFP = tissue made ischemic for 1 hr; NEG HBFP = tissue made ischemic for less than 20 min during two occlusion experiments but removed after four days; this heart showed no traces of positive HBFP-stained cells; E-SHAM = tissue from an operated animal that received no occlusion experiments; E-3 OCC = tissue made ischemic for less than 20 min during three occlusion experiments and removed 30 min after the last one; E-12 OCC = tissue made ischemic for less than 20 min during 12 occlusion experiments and removed 30 min after the last one; C-SHAM = control tissue from same heart as E-SHAM; C-3 OCC = control tissue from same heart as E-3 OCC; C-12 OCC = control tissue from same heart as E-12 OCC.
automaticity in the generation of arrhythmias following acute myocardial infarction. They also showed that a dose of 0.2 mg/kg propranolol is ineffective against the re-entry type of arrhythmias. This latter observation extends an earlier one by Moran et al., who showed that adrenergically-induced arrhythmias were antagonized by another beta-receptor blocking agent, dichloroisoproterenol, but had no effect on the spontaneous arrhythmias that followed myocardial infarction. Arrhythmias that are produced by direct electrical stimulation of cardiac nerves also are not affected by large doses of beta-receptor blocking agents. All of these observations support our finding that propranolol has no beneficial effect on VFL.

Scherlag et al. have strongly suggested that re-entry, and not automaticity, is indeed, the mechanism underlying arrhythmias associated with early, but not late infarction. Furthermore, they suggest that the site of origin of the ventricular arrhythmias is from the ventricular and Purkinje tissue within and not at the borders of the ischemic zone. Presumably, the 2 mg/kg dose of propranolol and the nonadapted psychological state operate upon this mechanism in such a way as to reduce the VFL.

For many years reduced HR has been thought to increase vulnerability to VF after coronary occlusion, and a recent series of studies by Epstein et al. and by others have shown that reduced HR does not increase dispersion of refractoriness in the ischemic areas, lower the threshold for the electrical initiation of VF, or increase incidence of arrhythmias. The reduced HR in the unadapted pigs, observed during the 2 mg/kg dose of propranolol, probably does not account for the reduction in VFL after coronary occlusion. Furthermore, the lack of correlation between HR and VFL in the heart-rate control animals in group A and in the partially adapted subjects in group C suggests that HR is not a critical variable associated with initiation of VF.

In summary, our findings underscore the role of psychological stress in the determination of the electrophysiological consequences of acute ischemia. In addition, the possibility that psychological factors influence the effects of a drug intervention is demonstrated. These studies have important implications for the explanation of conflicting data among animal studies performed under different conditions of consciousness or laboratory adaptation.

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