Effects of Activation Sequence on the Local Recovery of Ventricular Excitability in the Dog

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SUMMARY **We measured refractory periods at ventricular sites in the dog heart during drive from ventricular test sites and during combined drive from the test sites and other ventricular sites or the atrium. We found that ventricular activation sequence caused significant alterations on refractory period. Combined drive from two sites which resulted in fusion complexes was associated with shorter refractory periods at the test sites than those observed during drive at the test sites only. Our findings are explicable because earlier excitation and consequent earlier recovery of surrounding areas during fusion beats exerts an electrotonic influence on test sites.**

LOCAL recovery properties and the time sequence of ventricular excitation are factors that determine the waveform in the electrocardiogram. Recovery properties normally are organized in such a manner that refractory periods are longer in endocardial than epicardial layers and in apical than in basal areas.[1] The sequence of excitation influences the T wáveform because it determines the sequence of onset of repolarization. It is also possible that the sequence of excitation affects local recovery properties. Subthreshold currents alter transmembrane action potentials; anodal currents reduce and cathodal currents prolong the action potential duration. Such currents are present during ventricular repolarization and their spatial distribution depends on activation sequence as well as on intrinsic recovery properties.[2, 3] Electrotonic interactions during repolarization have been demonstrated for Purkinje tissue by Weidmann[4] and in the atrioventricular node and at the Purkinje-papillary junction by Mendez and co-workers.[5, 6] Evidence of electrotonic interaction between the sinus node and adjacent cells during repolarization was obtained by Miller and Strauss,[7] and Cranefield and Hoffman[2] have demonstrated propagated repolarization in ventricular fibers of dog and cat.

The study reported here was carried out to determine whether the activation sequence affected the time required for recovery of excitability at local sites in the intact dog ventricle. We measured refractory periods during ventricular activation which resulted from stimulation at test sites. These measurements were compared to refractory periods at the same sites during activation by impulses initiated at the test sites and at other sites whose activation resulted in fusion complexes. There were consistent differences in refractory periods with the different sequences of activation, and these differences were compatible with electrotonic interactions during ventricular repolarization.

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Supported in part by Program Project Grant HL-13480 from the National Institutes of Health, and the Richard A. and Nora Eccles Harrison Fund for Electrocardiographic Research.

Received October 13, 1975; accepted for publication December 16, 1975.

Methods

Experiments were performed on 10 dogs anesthetized with sodium pentobarbital (30 mg/kg, iv) prior to thoracotomy. For each experiment the chest was opened in the midline and the heart was suspended in a pericardial cradle. The sinus node was crushed and the heart was paced at a cycle length of 400 msec through bipolar hook electrodes attached to the right atrial appendage. Unipolar hook electrodes also were placed in the superficial ventricular muscle at the left ventricular apex and either the region of the pulmonary conus, the left ventricular base, or both sites. Ventricular electrodes were paired with a needle electrode in the lateral chest wall for ventricular pacing.

Atrial stimulation was continued during ventricular pacing to minimize the occurrence of conducted supraventricular excitation. Ventricular activation due exclusively to ventricular stimulation was obtained by simultaneous stimulation of atria and ventricles. Ventricular activation due to both supraventricular and ventricular stimulation was obtained by delaying the ventricular stimulus with reference to atrial stimulation. Activation due to stimulation of two ventricular sites also was produced and in this condition the atria were stimulated simultaneously with one of the ventricular sites. Various delays in ventricular stimulation with reference either to atrial stimulation or stimulation at another ventricular site were employed to produce activation patterns due to both stimuli. These conditions of cardiac stimulation will be referred to as "fusion" drive. Effectiveness of ventricular stimulation at the site at which the refractory period was being evaluated during fusion drive was evaluated in terms of the QRS waveform in a vertical body surface lead.

Ventricular stimuli at the test sites were cathodal and of twice threshold voltage. We measured refractory periods by introducing a premature stimulus after each three or four basic drive stimuli; the cycle length of the initial premature stimulus was shorter than that required for a propagated response. Cycle length of the premature stimulus then was increased by steps of 1 msec until the earliest propagated response occurred. The cycle length of that stimulus was taken as the refractory period. Measurements during activation from a single ventricular site were alternated with

measurements during fusion drive, and 10 measurements were made for each of the two activation orders being compared. To minimize the effects of previous premature beats and of varying local cycle lengths with the new activation order, a minimum time of 30 seconds was allowed to elapse after the activation sequence was altered. We determined diastolic threshold voltage before obtaining each set of 10 paired measurements.

Prior to measurement of refractory periods, one-half the initial dose of sodium pentobarbital was repeated and a slow intravenous infusion of 500 ml of 5% dextrose in water containing 240 mg of pentobarbital was begun. Repeated measurements of the refractory period at one test site then were made with the same activation order until five successive measurements varied by only 2 msec or less. The large anesthetic dose was empirically observed to be associated with stable refractory periods and probably acted by reducing variations in effects of the sympathetic nervous system on ventricular recovery.

Results

In all experiments, fusion drive with one or more of the stimulus time patterns resulted in shorter refractory periods than those observed during activation from ventricular stimulation. Maximal shortening occurred when ventricular stimulation at the test site was as late as possible with respect to the other stimulus involved but still effective in producing fusion drive. When the delay between stimuli responsible for fusion drive was reduced, the influence of the fusion drive on refractory periods also was reduced. Further reduction in the delay between stimuli for fusion drive eliminated the effect on refractory periods.

An example of findings from an individual experiment is shown in Figure 1. Refractory periods measured at the ventricular site during ventricular drive and during each condition of fusion drive are shown in Figure 2. The measurements during ventricular drive and fusion drive with each of the three delays were made alternately; there was increasing reduction in refractory period with increasing delay of the ventricular stimulus during fusion drives.

Similar findings were obtained in all experiments and are summarized in Table 1, which shows average values for the series of refractory periods during ventricular drive and during fusion drives, with the stimulus time phase which resulted in the maximal difference of refractory periods in individual experiments. Differences cannot be considered the maximal ones achievable, because the number of refractory period measurements carried out and the time required precluded testing all possible time phases of stimuli used to produce fusion drives. In all experiments, however, fusion drives caused reduction in refractory periods, and for 12 of the 16 sets of observations the maximal effects observed had P values of <0.005. Most of the fusion drives were produced by stimulation of the atrium and ventricular test sites, but in three experiments fusion drive from two ventricular sites yielded similar results; this indicates that

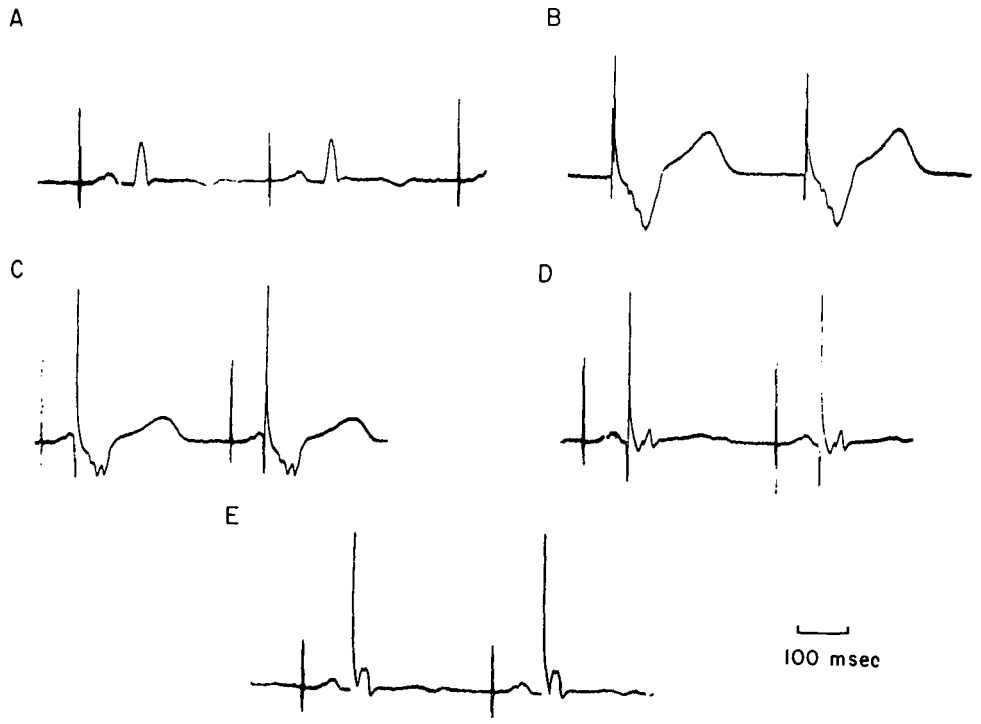

FIGURE 1 *Electrocardiograms from electrodes in the neck and left hindlimb showing evidence of varied ventricular activation orders. Panels A and B, respectively, show records during atrial drive and drive from a site at the left ventricular apex. Panels C, D, and E show records during drive of the atrium and left ventricular apex with stimulation of the latter site delayed 70, 90, and 100 msec with reference to atrial stimulation. QRS waveforms in the records shown in panels C, D, and E differ from that during atrial drive alone, and provide evidence of effective stimulation of the ventricular site.*

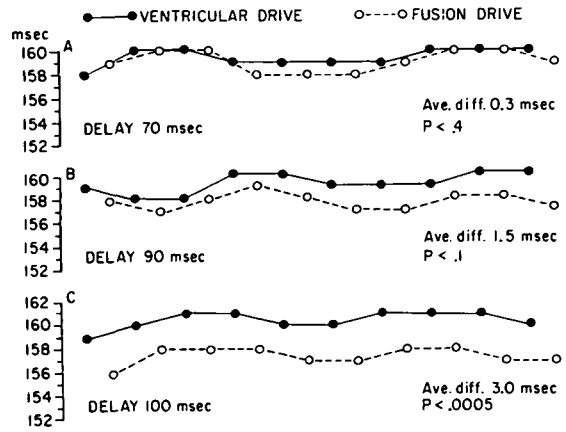

FIGURE 2 *Refractory period values at the left ventricular apical site from the same experiment illustrated in Figure 1. Three sets of 10 paired observations are shown during ventricular drive and drive with stimulus patterns resulting in fusion beats. With the drive consisting of fusion beats in which ventricular stimulation was delayed by 70 msec, refractory periods were not significantly different from those during ventricular drive. Drives consisting of fusion beats in which ventricular stimulation was delayed by 90 and 100 msec showed increasing reduction of refractory periods with respect to those measured during ventricular drive.*

effects are not unique for supraventricular excitation. Test sites usually were at the left ventricular apex, but in three experiments other test sites at the pulmonary conus or left ventricular base also showed refractory period shortening with fusion drives.

Discussion

The effects of activation sequence on ventricular refractory periods demonstrated by this study were small but statistically significant. Use of deep anesthesia and the interval permitted to elapse between alteration of activation yielded unusually stable refractory periods during the sets of 20 measurements. Further, the technique of alternating measurements during two activation orders furnished evidence of different refractory periods with differing sequences of activation even when the values associated with one activation sequence varied.

The study does not provide direct evidence for the mechanism responsible for alteration of recovery properties by activation sequence, but the findings are consistent with electronic interactions. During fusion drives, areas surrounding the site at which refractory periods were being evaluated necessarily were activated earlier than during the ventricular drive from the test site only. Since activation time is one determinant of recovery time, earlier activation can be expected to result in earlier recovery of areas outside the test site during fusion drives. The expected electrotonic effect of earlier recovery outside the test site would be a reduction in the refractory period at that site.

The clear dependence of reduction of the refractory period with fusion drive on the time phase of stimuli at the two sites suggests that earlier activation and consequent earlier recovery of an area near the test site are required to affect the refractory period. This is consistent with the decreasing influence of electrotonic interaction with increasing distance. Weidmann[8] determined the length constant for resting Purkinje tissue in the longitudinal axis to be 2 mm. The space constant during repolarization for intact ventricular muscle with both longitudinal and transverse electrotonic interactions has not yet been determined, although the relatively high membrane resistance near the end of the plateau phase suggests that the space constant is larger than in the resting condition.[8]

TABLE 1 *Average Values of Serial Refractory Period Measurements during Activation from the Test Site and Combined Activation from the Test and Another "Fusion" Site*

Experiment no.	Test site	Fusion site	Delay	Average RP ventricular drive	Average RP fusion drive	Average difference	P
1	LV apex	Atrium	110	199.1	195.8	3.3 ± 0.48	<0.0005
	LV apex	Pulmonary conus	65	198.2	194.4	3.8 ± 0.45	<0.005
	LV apex	Atrium	140	170.9	166.1	4.8 ± 0.79	<0.0005
2	LV base	Atrium	140	155.4	151.8	3.6 ± 0.52	<0.0005
	LV apex	LV base	30	155.1	149.9	5.2 ± 0.42	<0.0005
3	LV apex	Atrium	100	160.4	157.4	3.0 ± 0.47	<0.0005
	LV apex	Pulmonary conus	55	155.3	153.6	1.7 ± 0.48	<0.005
4	LV apex	Atrium	110	151.7	146.9	4.8 ± 0.92	<0.0005
5	LV apex	Atrium	90	182.6	179.0	3.6 ± 2.3	<0.1
6	Pulmonary conus	Atrium	100	189.7	187.5	2.2 ± 0.79	<0.025
7	LV apex	Atrium	125	165.3	162.3	3.0 ± 1.25	<0.025
8	LV apex	Atrium	95	172.7	171.4	1.3 ± 0.48	<0.025
9	LV apex	Atrium	90	146.2	143.8	2.4 ± 0.52	<0.005
10	Pulmonary conus	Atrium	150	140.0	138.2	1.8 ± 1.03	<0.1
	LV apex	Atrium	85	165.7	163.4	2.3 ± 0.67	<0.005

LV = left ventricle; RP = refractory period. Values of delay, refractory periods, and differences are in milliseconds. Delay refers to delay of the test site stimulus with reference to stimulation of the "fusion" site.

Whatever the underlying mechanisms, the study reported here furnishes evidence that recovery properties in the intact ventricle are subject to the influence of excitation sequence. This supports previous evidence obtained by Cranefield and Hoffman[2] that ventricular repolarization has features of a propagated process. It also provides a possible explanation for some features of the body surface electrocardiogram. The magnitude of the alteration in refractory period which is caused by activation sequence was small in this study but represents only alterations at the test sites. Alteration of recovery properties at other sites by activation sequence is probable and the cumulative effect of such alterations is likely to be capable of affecting the electrocardiographic waveform. In particular, the failure of the QRST area to be independent of activation sequence may reflect the failure of recovery properties to remain independent of activation order.[9, 10]

References

1. Burgess MJ, Green LS, Millar K, Wyatt R, Abildskov JA: The sequence of normal ventricular recovery. Am heart J **84:** 660-669, 1972
2. Cranefield PF, Hoffman BF: Propagated repolarization in heart muscle. J Gen Physiol **41:** 633-649, 1958
3. Wood EH, Heppner RL, Weidmann S: Inotropic effects of electric currents. Circ Res **24:** 409-445, 1969
4. Weidmann S: The electrical constants of Purkinje fibers. J Physiol (Lond) **118:** 348-360, 1952
5. Mendez C, Mueller WJ, Merideth J, Moe GK: Interaction of transmembrane potentials in canine Purkinje fibers and at Purkinje fiber-muscle junctions. Circ Res **24:** 361-372, 1969
6. Mendez C, Moe GK: Some characteristics of transmembrane potentials of AV nodal cells during propagation of premature beats. Circ Res **19:** 993-1010, 1966
7. Miller HC, Strauss HC: Measurement of sinoatrial conduction time by premature atrial stimulation in the rabbit. Circ Res **35:** 935-947, 1974
8. Weidmann S: Effect of current flow on the membrane potential of cardiac muscle. J Physiol (Lond) **115:** 227-236, 1951
9. Simonson E, Schmitt OH, Dahl J, Fry D, Bakken EE: The theoretical and experimental basis of the frontal plane ventricular gradient and its spatial counterpart. Am Heart J **47:** 122-153, 1954
10. Abildskov JA, Burgess MJ, Millar K, Wyatt R: New data and concepts concerning the ventricular gradient. Chest **58:** 244-248, 1970

The Relationship between Age and Relaxation of Vascular Smooth Muscle in the Rabbit and Rat

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SUMMARY **Relaxation of rabbit and rat aorta by isoproterenol decreases with increasing age, whereas such responses caused by nitroglycerin or sodium nitrite are not age-dependent. In the present study, we sought to determine whether this relationship also exists in pulmonary arteries and portal veins. As was the case with the aorta, isoproterenol-induced relaxation of pulmonary arteries decreased as the animal aged; relaxation by nitroglycerin was minimally altered. Aging did not influence responses of rabbit and rat portal veins to isoproterenol or nitroglycerin. If the responses of these blood vessels are characteristic of the responses of other vascular smooth muscles to vasodilators, then this study suggests a difference in the manner by which arteries and veins age. We also confirmed that isoproterenol-induced relaxation of rabbit and rat aorta markedly decreases with increasing age and that the responses of rat aorta to nitroglycerin are independent of age. Because of the agonist used to contract the tissues prior to drug-induced relaxation, the results of the first series of experiments with nitroglycerin on rabbit aorta were at variance with our earlier findings. When KCl was used to contract the aortas, the mean effective dose (ED_{50}) obtained for nitroglycerin for tissues from 2-year-old rabbits was 8 to 19 times larger than that obtained from 2-month-old rabbits. This ratio dropped to 4 when the tissues were contracted with histamine. Since KCl and histamine contract rabbit aorta by different mechanisms, this finding suggests that, in addition to a specific loss in β-receptor activity, increasing age results in an alteration in the contraction-relaxation process of rabbit aortic tissue.**

RELAXATION of rabbit and rat thoracic aorta mediated by β-receptors decreases with increasing age of the animal.[1, 2] The biochemical mechanism responsible for this phenomenon is unknown, although Ericsson and Lundholm[3] have presented evidence suggesting that, at least in rat aorta, the defect is in the adenylate cyclase-cyclic AMP system.

Our present experiments began with the idea of defining the relationship between vascular relaxation and aging in blood vessels other than the aorta. Relaxation of rabbit and rat pulmonary arteries induced by isoproterenol decreased with increasing age, whereas responses to nitroglycerin did not depend on the age of the animal. In contrast, the responses of rabbit and rat portal veins to both isoproterenol and nitroglycerin were not related to the age of the animal. This suggests that at least one facet of the aging process may differ in arterial and venous smooth muscle.

During the course of this investigation we reexamined the relationship between age and drug-induced aortic relaxation. This reexamination was important because we used animals obtained from colonies other than those used for our earlier studies. As the rabbits and rats aged, their aortas lost the ability to relax in response to isoproterenol; sodium nitrite, as well as nitroglycerin, produced maximal relaxation which was independent of age. These findings were in agreement with our previous observations.[4, 5] In addition, we now have demonstrated that the mean effective dose (ED_{50}) of nitroglycerin for aortas from older but not younger rabbits is

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Presented in part at the Second International Symposium on Vascular Neuroeffector Mechanisms, Odense, Denmark, August 1975.

Received April 23, 1975; accepted for publication December 31, 1975.