Nonuniform Distribution of Vagal Effects on the Atrial Refractory Period

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ABSTRACT

ALESSI, R., M. NUSYNOWITZ, J. A. ABILDSKOV AND G. K. MOE. Nonuniform distribution of vagal effects on the atrial refractory period. Am. J. Physiol. 194(2): 406–410. 1958.—The refractory period (RP) was measured at several points on the right atrial surface in anesthetized dogs. Under control conditions with vagi cut the values recorded at various points varied by no more than 40 msec. During stimulation of the vagus nerves, singly or together, the RP varied widely. At some points marked effects were observed, while at others little or no effect was apparent. Reflex excitation of the vagi, induced by increased arterial pressure, yielded similar results. It was concluded that the effects of vagal stimulation are not uniformly distributed.

It is a safe assumption that the well-known propensity of the atria to fibrillate during vagal stimulation is related to abbreviation of the refractory period (1). Whatever the basic mechanism of fibrillation, it is probable that nonuniformity of the refractory period, allowing some fibers to escape excitation while closely adjacent fibers are discharged, would tend to facilitate and perpetuate the irregularity. It also seems likely that alterations of the refractory period induced by nerve stimulation might be nonuniform, for each atrial muscle fiber can hardly be supplied with its individual twig of cholinergic or adrenergic nerve (2). The following experiments were conducted to determine whether or not the effects of vagal stimulation are uniformly distributed over the atria.

METHODS

Two sets of experiments were conducted. In the first series, the effects of direct electrical stimulation of the vagi were assessed; in the second series reflexly elicited vagal effects were studied.

Direct Vagal Stimulation. Dogs of both sexes, weighing from 8 to 15 kg, were anesthetized with sodium pentobarbital. Under artificial respiration the chest was opened in the mid-line and the heart was cradled to give wide exposure of the antero-lateral surface of the right atrium and its appendage. Bipolar stimulating electrodes (S1) and recording electrodes were placed near the tip of the appendage. The heart was driven at frequencies just sufficient to overcome the sinus pacemaker. A monopolar electrode of stainless steel wire 100 micra in diameter, mounted on a light spring to maintain constant contact with the atrial surface, was used to determine the moment of activation (R1) at various test points, and to deliver the test stimuli for estimation of the refractory period (RP) at those points. The cathodal test stimulus (S2) was triggered at variable intervals after each sixth driving stimulus (S1).

At each atrial point tested, the response (R1) to the driving stimulus was first recorded from the monopolar electrode and displayed on a cathode ray oscilloscope with a calibrated sweep speed of 1 cm/50 msec. The sweeps were triggered by the S1 stimulator. The position of the beam was adjusted horizontally so that the moment of activation (R1) at the test point was accurately aligned with the left vertical axis of the oscilloscope scale. Con-
stancy of position of the electrode was assumed when the configuration and temporal position of $R_I$ remained constant. This was checked at frequent intervals during a series of measurements. For estimation of the RP the monopolar electrode was switched from its recording channel to the $S_2$ stimulator circuit, and the responses recorded from the bipolar electrodes were used to indicate the success or failure of the test stimuli. The position of the $S_2$ shock artifact on this record, relative to the left axis of the scale ($R_1S_2$ interval), was recorded by direct measurement from the oscilloscope scale, with a reading error of no more than 5 msec. At each placement of the electrode the diastolic threshold stimulating voltage, using “square” waves of 2 msec. duration, was determined. Estimates of RP were then made with test voltages of 1.5 times the threshold value. In some experiments rough excitability recovery curves were constructed at stimulus intensities up to 7 times threshold.

Both vagi were exposed in the neck. Bipolar stimulating electrodes were applied to each nerve caudal to a crushed area, and the nerves were submerged in paraffin oil. Stimuli were square pulses of 1 msec. duration applied at a frequency of from 1.25 to 10/sec. The stimulus intensity was 20 volts, demonstrated to be supramaximal in terms of cardiac slowing in each experiment. In most experiments both vagi were stimulated simultaneously. In others combined stimulation was compared with individual stimulation of right and left vagus at the same frequencies.

In order to avoid severe ventricular slowing and hypotension during the measurements of RP, the vagus was stimulated at frequencies insufficient to cause atrio-ventricular block. Data obtained from several atrial points were considered comparable only if the effects of vagal stimulation on heart rate remained relatively constant during the time required for the several observations.

Reflex Vagal Stimulation. Dogs were anesthetized with morphine sulfate, 4 mg/kg followed by alpha-chloralose, 50 mg/kg. After exposure of the heart the animals were heparinized, a femoral artery was cannulated and attached to a pressure reservoir, and preheparinized blood from a donor dog was slowly infused into a femoral vein. Blood was allowed to accumulate in the arterial pressure reservoir. Electrodes were placed on the right atrium as in the previous series. At each selected atrial point the RP was assessed at two or more levels of arterial pressure, ranging between 80 and 150 mm Hg. The pressure levels were set by altering the pressure applied to the arterial reservoir. At each level of pressure the spontaneous heart rate was recorded as an index of the intensity of reflex vagal discharge.

RESULTS

In all experiments it was found that, although the initial RP values at various points differed by no more than 40 msec., the effects of vagal stimulation were much more pronounced at some points on the atrium than at others. At many points no significant effects could be demonstrated with the parameters of vagal stimulation used. There was no apparent relationship between the initial control RP at various points and the degree of shortening resulting from vagal stimulation. Selected examples from three experiments are illustrated.

![Fig. 1. For explanation see text.](http://ajplegacy.physiology.org/)
in figure 1, in which the refractory periods ($R_0S_2$ intervals) obtained with test stimuli of 1.5 times threshold are plotted against the frequency of supra-maximal stimuli applied to the vagi.

At the point $B$ on the atrial map (top segment of fig. 1) stimulation of both vagi caused no measurable abbreviation of the refractory period at 2.5 and 5.0 cps, and had only a minimal effect at 10/sec. Point $A$ exhibited a slightly greater effect, and point $C$ a very marked effect of vagal stimulation.

In the middle graph of figure 1 the curves $L$, $R$ and $L + R$ represent observations taken at a single point during stimulation of left vagus alone, right vagus alone, and both vagi together, respectively. Here it is apparent that the whole effect of vagal stimulation at this point was the result of right vagal activity. In the bottom part of figure 1 are recorded observations made in another animal. At the point represented in the chart the right vagus exerted only a slight effect, while the left was responsible for almost all the effect of bilateral stimulation. In other cases, not illustrated, each vagus exerted moderate effects which were summated when both nerves were stimulated simultaneously.

Although it has been established that vagal stimulation shortens the absolute RP without greatly altering the shape of the excitability recovery curve (3), it was considered possible that the nonuniform distribution of effects described above might have been fortuitous, resulting from the arbitrary selection of a single multiple of threshold for the estimation of RP. Accordingly, three experiments were performed in which excitability recovery curves were constructed at each atrial point and at each frequency of vagal stimulation. To make certain that spontaneous changes were minimal, all determinations were made in duplicate. Determinations of duplicate values at any given combination of vagal stimulus frequency and $S_2$ stimulus intensity were not made sequentially, but were separated by observations made at other stimulus parameters. The curves were considered satisfactory if the duplicate values so obtained agreed within 10 msec.

Curves taken at three different atrial points in one experiment are plotted in figure 2. Segment $A$ shows the curves (as averages of the duplicate observations) recorded at point $A$ on the accompanying map without vagal stimulation ($O$), and during stimulation of both vagi at 1.25 ($V_1$), 2.5 ($V_2$) and 5.0 cps ($V_3$). Relatively little vagal influence was apparent.

Figure 2B shows the effects recorded at a second atrial point, $B$ on the diagram. At this point, considerably greater effects of vagal stimulation were apparent. At the third point (fig. 2C), vagal effects were very pronounced, even at the lowest stimulus frequency ($V_1$). Estimation of the RP was difficult during vagal stimulation at 2.5/sec. ($V_2$) because of the frequent occurrence of flutter or fibrillation when early shocks were applied to the atrium.
When the vagi were stimulated at 5/sec., fibrillation occurred with every early \( S_2 \) stimulus, and no reliable estimate of RP could be made.

The nonuniform distribution of vagal effects described above gives no assurance that similar conditions obtain during physiologic vagal discharge. It was therefore considered essential to study the influence of reflex activation of the vagi on the RP at various points on the atrium. Graded reflex activation of the vagi was accomplished in three experiments by raising the arterial pressure step-wise as described under Methods.

At the lowest level of arterial pressure the spontaneous rate was recorded as an index of vagal activity. The atrium was then driven at a slightly higher constant rate while the RP was estimated, using an \( S_2 \) stimulus of twice threshold intensity. Observations of RP (always at the same driven frequency) and of spontaneous rate were then repeated at each higher pressure level, followed by repeat measurements at control pressure. Similar observations were made at several points of placement of the \( S_2 \) electrode.

Nonuniformity of vagal effects was apparent in all experiments. Figure 3 illustrates the range of values obtained at 14 such points in one experiment. Refractory periods at each level of pressure are plotted against the average cycle lengths observed when the heart was allowed to beat spontaneously at the corresponding pressure. It should be emphasized that in order to eliminate the effect of cycle length upon the RP (4) the measurements of RP were all made at the same driven frequency. The data include numerous points at which little or no abbreviation of the RP was observed as the pressure was raised, and others at which the reflex vagal effects were pronounced. At those points which exhibited intense vagal effects, the application of an early \( S_2 \) stimulus often resulted in atrial fibrillation which frequently persisted until the arterial pressure was temporarily reduced. Fibrillation never followed the early \( S_2 \) stimuli at other points.

**DISCUSSION**

The results described demonstrate that the effects of vagal stimulation upon atrial refractory period are not uniformly distributed throughout the atrial muscle. It is probable that fibers immediately adjacent to vagal postganglionic endings are exposed to relatively high concentrations of the cholinergic mediator and are profoundly affected, while fibers more remote from sites of acetylcholine liberation are influenced to a much lesser degree.

The data do not permit an estimate of the dimensions of 'active' areas, nor of the distance between them. In one experiment a larger

**FIG. 3.** Relation between RP (\( R_0S_2 \) interval) and intensity of reflexly induced vagal discharge at 14 randomly selected points on the right atrial surface in a single animal. For explanation see text. All observations made at the same basic driving frequency (160/min.).
'pinch' electrode, with an effective contact area of about 0.5 x 1.0 mm, was tested at several positions on the atrial surface. Differences in the RP at different points were also apparent with the larger electrode.

The distribution of 'active' points appeared to be random. On the assumption that vagal endings might be more concentrated in the region of the S-A node than in the atrial appendage, comparisons were made with electrode placements in these areas. Active and inactive points were located in both, but the data are not sufficiently numerous to permit quantitative comparison.

It is possible that the observed differences do not represent random scattering of cholinergic endings within the atrial muscle, but rather a random distribution of sensitivity to acetylcholine. This, however, is unlikely; points which were profoundly influenced by stimulation of one vagus were on occasion unaffected by the other, as illustrated in figure 1B and 1C.

It is also possible that the differences observed with direct vagal stimulation resulted from failure to excite all the cardiac efferent fibers. The criterion of maximal cardiac slowing at a given vagal stimulus frequency does not guarantee that all fibers mediating effects upon the refractory period were also excited by the same stimulus. The physiological significance of the changes is, however, emphasized by the observation that reflexly induced vagal stimulation yielded equivalent data. It may be concluded that, whether or not reflex vagal discharge plays upon all the endings mediating effects upon the RP, the net result of such discharge is not uniformly distributed over the atrial muscle. It follows that an early ectopic impulse generated during a period of vagal stimulation is bound to be propagated along an irregular wave front as the impulse encounters areas in varying states of excitability. The likelihood of fibrillation must be enhanced by such irregularity.

REFERENCES