Role of the Sinus Node In the Mechanism of Cholinergic Atrial Fibrillation

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ABSTRACT

The effect of suppressing sinus rhythm on initiation and maintenance of atrial fibrillation was studied in 17 thoracotomized and artificially ventilated dogs. Atrial fibrillation was evoked by methacholine chloride applied to the right atrium followed by light mechanical stimulation. Episodes of fibrillation were compared before and after sinus rhythm had been suppressed by injection of absolute alcohol or concentrated sodium pentobarbital into the sinus node artery. Sixty-nine control episodes of atrial fibrillation lasting $6.00 \pm 0.36$ (SE) minutes were easily initiated while the heart was in sinus rhythm. The response to methacholine application plus mechanical stimulation depended on the type of escape rhythm obtained after suppression of sinus rhythm. In ten dogs with no sign of supra A-V junctional pacemaker activity, only 50% of the attempts to initiate fibrillation were successful. The 26 episodes of atrial fibrillation observed in this group of animals lasted $0.65 \pm 0.23$ (SE) minutes. In seven other dogs, supra A-V junctional pacemaker activity persisted after injection into the sinus node artery. In these seven dogs, 60% of the attempts at initiating fibrillation were successful, and the 18 episodes of fibrillation lasted $4.44 \pm 0.30$ (SE) minutes. In dogs showing no supra A-V junctional pacemaker activity, a more sustained arrhythmia was obtained after recovery of sinus node activity or during electrical pacing of the right atrial appendage. These observations suggest that the sinus node participates in the mechanism of cholinergic atrial fibrillation and that an equivalent role can be played by a natural or artificial supra A-V junctional pacemaker.

ADDITIONAL KEY WORDS
atrial pacemakers electrical pacing A-V junctional escape rhythms methacholine sinus node perfusion

The mechanism of atrial fibrillation is still poorly understood. Experimental studies (1-4) have emphasized the importance of cholinergic stimulation in initiating and sustaining this arrhythmia. This cholinergic fibrillating influence has been related to a shortening of the refractory period of atrial tissues, a faster rate of membrane repolarization and a variable degree of homogeneity in the excitability of atrial fibers (5-7). Whether pacemaker inhibition, also a cholinergic effect, plays a contributory role has not been fully determined. In the dog, cooling or crushing of the sinus node has been reported to facilitate atrial fibrillation elicited either by intravenously administered acetylcholine or by vagus nerve stimulation (8). Nahum and Hoff (9) reported that in the dog the onset of atrial fibrillation induced by acetyl-β-methylcholine applied to the right atrial surface was preceded by slowing of the heart and even sinus arrest. In man, atrial fibrillation is often associated with disease of the sinus node. Postmortem studies have revealed an injured sinus node when atrial arrhythmias had occurred during acute myocardial infarction (10). In patients with chronic atrial fibrillation, pathologic alterations were also found in the sinus node region, although a definite
Pathogenic relationship could not be established (11-13).

In contrast, Azuma et al. (14) and Shimizu et al. (15) have reported that destruction or excision of the sinus node reduced the incidence and the duration of atrial fibrillation obtained from aconitine application to the right atrium. Scherf (16) has even suggested that the sinus node is implicated in the genesis of atrial fibrillation, either as a source of ectopic impulse formation or as a region favoring multiple reentrance. Scherf et al. (17, 18) further demonstrated that cholinergic atrial fibrillation could be abolished by cooling both the sinus and A-V nodes simultaneously.

In the present study, atrial fibrillation was induced in the dog by the method described by Nahum and Hoff (9), before and after suppression of sinus rhythm. Sinus rhythm was suppressed by injection of absolute alcohol or sodium pentobarbital into the sinus node artery, using a previously described technique (19). It was found that in the absence of sinus rhythm atrial fibrillation was difficult to evoke and, when obtained, was of short duration and frequently converted to flutter. It was further observed that electrical pacing of the right atrium facilitated the induction and maintenance of atrial fibrillation.

Method

Adult dogs of either sex weighing 10 to 19 kg were anesthetized with intravenous pentobarbital (30 mg/kg). Under artificial respiration with a Harvard pump, the chest was opened through the right 5th intercostal space and the heart cradled loosely in the incised pericardium. The sinus node artery was isolated and cannulated with polyethylene tubing. Right atrial and ventricular unipolar electrograms were recorded by small silver needle electrodes, along with a peripheral lead electrocardiogram, on a Grass polygraph.

Atrial fibrillation was induced by 2 drops of a solution of methacholine chloride (1 mg/ml) applied to the anterior surface of the right atrium, followed by one to four light strokes of the area with the blunt edge of a surgical instrument. These manipulations were repeated in the same animal during normal sinus rhythm and after suppression of sinus rhythm obtained by injection of 1 ml of absolute alcohol into the sinus node artery in ten dogs and 1 ml of concentrated (60 mg/ml) sodium pentobarbital in seven other dogs.

In four dogs, after suppression of sinus node activity, the right atrium was driven at a rate close to the control sinus rate. For this purpose square-wave pulses (2 msec, 3 to 5 volts) were delivered from a Grass S4 stimulator through small bipolar silver electrodes placed on the right atrial appendage. Application of methacholine and mechanical stimulation were repeated before, during, and after electrical pacing of the right atrium.

Results

As a control procedure, atrial fibrillation was induced repeatedly in all animals at the start of the experiment. All attempts at initiating atrial fibrillation were successful. From two to nine episodes of fibrillation were recorded in any one dog, for a total of 69 control observations. The right atrial electrogram showed characteristic fine fibrillation waves and the limb lead electrocardiogram an undulating base line between the irregularly spaced ventricular complexes. An interval of 3 minutes of stable sinus rhythm was allowed between episodes. The average duration of these 69 periods of fibrillation was 6.00 ± 0.36 (SE) minutes. Successive episodes of arrhythmia in each dog were remarkably similar in duration and waveform. Exceptionally, episodes of atrial fibrillation converted to atrial flutter—once in one dog and three times in another.

Different Types of Escape Rhythms after Suppression of Sinus Rhythm.—To determine the effect of suppressing sinus rhythm on the characteristics of atrial fibrillation, absolute alcohol or sodium pentobarbital was injected into the sinus node artery. This procedure resulted in immediate sinus arrest and the emergence of escape rhythms of atrial, A-V junctional, or ventricular origin. The term A-V junctional rhythm refers to rhythms which appear to originate in the region of the A-V junction and is used in preference to the designation A-V nodal rhythm, as suggested by Pick and Langendorf (20). As will be described later, attempts to produce atrial fibrillation gave different results depending on the presence or absence of residual atrial
(supra A-V junctional) pacemaker activity. The animals were therefore divided into two groups on this basis.

In ten dogs (group 1) there was no electrocardiographic evidence of persistent supra A-V junctional pacemaker activity. Tracings from these dogs are illustrated in Figure 1. Control sinus rhythms are shown on the left-hand side, and rhythms obtained after injections of alcohol or pentobarbital into the sinus node artery are shown on the right-hand side. In the commonest type of escape rhythm (A), the P wave was masked by the QRS complex and atrial and ventricular activation occurred simultaneously, as verified from the simultaneously recorded atrial and ventricular electrograms. This rhythm was observed in five dogs—in three after injection of alcohol and in two after injection of pentobarbital. The next commonest type of escape rhythm is shown in B. The P wave was either biphasic or of a polarity opposite to that of the sinus P wave; this rhythm occurred in three dogs after injection of alcohol. In a third type of A-V junctional escape rhythm, observed in one dog after injection of alcohol, the QRS complex preceded the P wave (C). An escape rhythm of ventricular origin (D) was seen in one dog after injection of pentobarbital.

In the remaining seven dogs (group 2), pacemaker activity of supra A-V junctional origin was betrayed by persisting characteristic P waves after injection into the sinus node artery (Fig. 2). In two animals, after injection of alcohol, an atrial rhythm was observed (A). The P wave configuration was slightly altered, the rate was regular but slower than the control sinus rhythm, and the PR interval was slightly reduced. In another animal, after injection of pentobarbital, a slow atrial
Figure 2
Electrocardiographic tracings illustrating control periods of sinus rhythms on the left and escape rhythms in dogs of group 2 on the right. In A, a slow atrial rhythm is seen. In B, atrial beats alternate with A-V junctional beats. In C, the second QRS is preceded by a P wave. In D, the third and last QRS complexes are preceded by P waves. The P waves in these tracings were believed to originate above the A-V junctional region. A and D were taken in lead aVR and B and C lead II.

rhythm alternated with an escape A-V junctional rhythm (B). This latter escape rhythm is similar to that shown in Figure 1A. Three dogs showing this same type of junctional escape rhythm (one after alcohol injection and two after pentobarbital injections) had occasional atrial beats. An example is illustrated in Figure 2C. The P wave was slightly altered but the PR interval was of normal duration. One animal, after pentobarbital, had a ventricular escape rhythm interrupted by occasional atrial beats. The tracing is shown in Figure 2D.

Induction of Atrial Fibrillation after Injections into the Sinus Node Artery.—The manipulations used to obtain control episodes of atrial fibrillation were generally unsuccessful in eliciting the phenomenon in the ten dogs (group 1) in which sinus rhythm had been completely suppressed. An example is given in Figure 3. In A, during control sinus rhythm, the application of methacholine and two light strokes with the blunt edge of an instrument on the right atrium resulted in atrial fibrillation. In B, in the same dog after A-V junctional rhythm was established, atrial fibrillation failed to develop despite repeated attempts to evoke it. In dogs of this group, 50% of attempts to initiate atrial fibrillation were unsuccessful. In the remaining 50%, atrial fibrillation appeared only after application of a large quantity of methacholine (8 to 10 drops) to the right atrium and repeated mechanical stimulation. When it was evoked, atrial fibrillation persisted for a mean duration of only $0.65 \pm 0.23$ (SE) minutes. The atrial
SINUS NODE AND ATRIAL FIBRILLATION

The effect of suppressing sinus node activity was also demonstrated by injection of alcohol in two dogs and of pentobarbital in two others, during a control episode of atrial fibrillation. One of these experiments is illustrated in Figure 4. Atrial fibrillation initiated during sinus rhythm ceased abruptly a few seconds after the injection of 1 ml of absolute alcohol into the sinus node artery. After a brief period of arrest, an escape rhythm appeared, but atrial fibrillation did not occur.

A return of sinus node activity was seen in three dogs of group 1 within 30 to 60 minutes after injection of pentobarbital. Two of these animals had presented A-V junctional escape rhythms, the other a ventricular escape rhythm. The recovery of sinus activity in these dogs was in the form of a normal sinus rhythm in one dog and in the form of sinoatrial block in another; the third dog manifested occasional sinus beats of the type illustrated in Figure 2C. The average duration of 24 episodes of atrial fibrillation was of $4.15 \pm 0.33$ minutes. After suppression of sinus rhythm, 19 attempts at initiating atrial fibrillation were unsuccessful. Atrial fibrillation initiated in 9 instances lasted an average of $0.24 \pm 0.05$ minutes. After recovery of sinus activity, 65% of attempts at

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**FIGURE 3**

Low speed record of the atrial electrogram of a dog during sinus rhythm, A. Atrial fibrillation was easily induced by applying 2 drops of methacholine, arrow, and two light strokes to the right atrium. After sinus rhythm suppression, B, the application of methacholine, arrow, and repeated mechanical stimulation of the atrium failed to elicit the arrhythmia. Time scale corresponds to 10 seconds for recording at low speed and 1 second for faster recording shown in insert.

electrogram in the dogs of group 1 generally showed a coarse fibrillation rather than the usual fine oscillations observed during the control period. In 12 instances occurring in seven dogs, this type of fibrillation changed into atrial flutter of variable duration.

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**FIGURE 4**

Recording of the right atrial electrogram (RA), the right ventricular electrogram (RV), and the electrocardiogram (ECG), during atrial fibrillation. The injection of 1 ml absolute alcohol into the sinus node artery (arrow) was followed by a sudden disappearance of fibrillation, a brief arrest of the heart, and an escape rhythm of atrial origin.

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TABLE 1

Comparison of Episodes of Atrial Fibrillation in Four Dogs during Control Sinus Rhythm, after Suppression of Sinus Rhythm, and during Electrical Pacing of the Right Atrium

<table>
<thead>
<tr>
<th></th>
<th>Sinus rhythm</th>
<th>After suppression of sinus rhythm</th>
<th>During right atrial pacing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Episodes of atrial fibrillation</td>
<td>18</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>Duration of atrial fibrillation (min) (mean ± se)</td>
<td>4.25 ± 0.38</td>
<td>0.26 ± 0.07</td>
<td>3.05 ± 0.37</td>
</tr>
<tr>
<td>Unsuccessful attempts</td>
<td>0</td>
<td>12</td>
<td>2</td>
</tr>
</tbody>
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initiating atrial fibrillation were successful, and the episodes lasted 3.04 ± 0.23 minutes.

In the seven dogs (group 2) showing persistent supra A-V junctional pacemaker activity after injection into the sinus node artery, 60% of all attempts at initiating atrial fibrillation were successful. The mean duration of the episodes of fibrillation was 4.44 ± 0.30 minutes. This value is shorter than that obtained during control experiments with an intact sinus node but much longer than that noted in the dogs of group 1. Atrial fibrillation transformed into atrial flutter once in three dogs of group 2.

Effects of Right Atrial Pacing on Atrial Fibrillation.—The above results suggest that the phenomenon of atrial fibrillation is closely related to the presence of atrial (supra A-V junctional) pacemaker activity. This conclusion was tested during experiments in four dogs with complete suppression of sinus rhythm; pacemaker activity was replaced by electrical stimulation of the right atrium at a rate similar to that of the control sinus rate, and atrial fibrillation was attempted. The results of these experiments are presented in Table 1 where the number of successful attempts and the average duration of the episodes of atrial fibrillation are compared in the same dogs before and after sinus rhythm suppression and during right atrial pacing. Regular electrical stimulation of the right atrium appeared to facilitate the induction of atrial fibrillation and increased appreciably the mean duration of the episodes of fibrillation. An experiment carried out in one of these dogs is illustrated in Figure 5. A shows a short segment of atrial fibrillation obtained during sinus rhythm. B shows an A-V junctional escape rhythm (left) resulting from the injection of pentobarbital into the sinus node artery; atrial flutter (right) resulted from methacholine application and mechanical stimulation. In C, it is seen that electrical pacing of the right atrium allowed atrial fibrillation to be elicited easily. In five experiments in two dogs, atrial fibrillation terminated within 18 ± 5.79 (se) seconds after interrupting right atrial pacing, a result similar to that obtained from suppressing sinus node activity as in Figure 4.

Observations on the Occurrence of Atrial Flutter.—Atrial flutter characterized by rapid regular atrial activity similar to the example illustrated on the right in B, Figure 5, was observed only four times in two dogs during control experiments. These four episodes of flutter lasted from 1.6 to 6 minutes. In group 1, after complete suppression of sinus rhythm, atrial flutter was observed 12 times in seven dogs. The duration of the periods of atrial flutter varied from 0.05 to 28.5 minutes. In group 2, with persistent supra A-V junctional pacemaker activity, three episodes of atrial flutter lasting 5.5, 11, and 55 minutes were noted in three dogs.

The greater incidence of atrial flutter after sinus rhythm suppression may be significant. An area of injury is known to favor the establishment of a circus movement in atrial tissue (21). This factor did not, however, prevent the occurrence of atrial fibrillation when a residual atrial pacemaker was present or when the right atrium was paced. Considering the circus movement hypothesis, it is conceivable that a focus of automatic activity on the path of a
Recordings of an atrial electrogram (a) and of a ventricular electrogram (v). In A, the heart was in sinus rhythm (left) and atrial fibrillation was easily induced (right). In B, after sinus rhythm suppression, the heart showed an A-V junctional rhythm (left) and the use of methacholine and mechanical stimulation produced an atrial flutter (right). In C, the right atrium was paced artificially (left), and this allowed atrial fibrillation to be induced easily (right).

Circulating wave could disrupt a wave front sufficiently to disorganize a potential flutter into fibrillation.

To verify that the above observations were related to an effect directly on the sinus node and not to injury to the atrial myocardium, a ligature was secured about the sinus node artery about halfway along its course up the right atrial epicardium. The subsequent injection of alcohol into the proximal artery caused an area of discolorated myocardium but did not affect the character or duration of control fibrillatory episodes.

Discussion

Cholinergic stimulation is known to favor the establishment and perpetuation of atrial fibrillation. The technique used in the present experiments to induce atrial fibrillation in dogs was first introduced by Nahum and Hoff (9), who placed small filter paper pledgets soaked in methacholine on the right atrial epicardi-
um. These authors noted that when atrial arrhythmia failed to develop after methacholine application, light stimulation of the atrium sufficed to evoke it. In the present series of experiments, we used this technique routinely in an attempt to maintain uniformity in the mode of onset of arrhythmia. Nahum and Hoff noted that fibrillation persisted for 3 to 5 minutes after the pledgets were removed from the atrial surface. This is comparable to the duration of the fibrillatory episodes obtained in our experiments and corresponds to the duration of action of methacholine, which is hydrolyzed at a rate approximately equal to one-third that of acetylcholine (22). As the effect of methacholine disappears, atrial fibrillation ceases, since, as stated by Moe and Abildskov (6) "independent survival of fibrillation is possible only in the presence of adequate cholinergic discharge."

The results described in this paper indicate that the presence of an atrial (supra A-V junctional) pacemaker may also be a necessary condition for initiation and maintenance of atrial fibrillation obtained in the dog by cholinergic stimulation. During sinus rhythm, atrial fibrillation was easily initiated. The intranodal injection of substances having a suppressive effect on sinus node function brought about prompt arrest of atrial fibrillation, and during A-V junctional and ventricular rhythms, it was difficult, if not impossible, to initiate fibrillation. The fibrillation obtained under these latter conditions lasted less than a minute, was of lower frequency (coarse fibrillation), and frequently converted to atrial flutter. On the other hand, when atrial activation still preceded ventricular activation, as it did during escape rhythms of supra A-V junctional origin, after recovery of sinus node activity, and with right atrial pacing, atrial fibrillation could be initiated, and persisted for periods only slightly shorter than during normal sinus rhythm. The high number of unsuccessful attempts recorded does suggest, however, that atrial susceptibility to fibrillation is related to the presence of an intact sinus node.

One cannot be certain that sinus node activity was completely abolished by the substances injected into the sinus node artery. Usually, however, the rate of sinus node impulse formation was sufficiently reduced for escape rhythms to emerge at a much slower rate. A dominant atrial escape rhythm was observed in three dogs, and persistent supra A-V junctional pacemaker activity was evidenced by the occurrence of occasional atrial beats in three others. The exact site of origin of atrial activity was not determined in our experiments. Atrial escape rhythms during vagal stimulation have been shown to arise from either the sinus node region itself or from the upper interatrial septum (23). Sinus activity may also persist despite extensive destruction and excision of the node (24). Finally, alterations in the shape of the P wave are unreliable indicators of the site of origin of atrial depolarization and may reflect only the destruction of normal pathways of atrial conduction (25). Pentobarbital presumably caused depression rather than destruction of the sinus node, and consequently recovery of sinus node activity was observed in three dogs, along with a change in susceptibility to atrial fibrillation. The use of pentobarbital could be criticized since it is known to have vagolytic properties (26). However, perfusion of the sinus node region with this substance did not prevent the occurrence of atrial fibrillation whenever a supra A-V junctional pacemaker was present.

The hypothesis that the sinus node may participate in the genesis of atrial fibrillation is supported by a number of reports in the literature. Nelson and Smith (27) observed sinus venous impulses in the frog heart during acetylcholine perfusion, which apparently initiated momentary fibrillation in adjacent atrial areas and led eventually to a sustained and generalized arrhythmia. In the dog, Scherf et al. (18) demonstrated that acetylcholine-induced atrial fibrillation could be stopped by simultaneously cooling the sinus and A-V node regions and that the arrhythmia recurred upon rewarming. More recently, it was reported (28) that the injection of acetylcholine into the canine sinus

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node artery easily induced atrial fibrillation when the heart was in sinus rhythm but not at all during A-V nodal rhythm. Azuma et al. (14) reported that suppression of the normal sinus impulse by local application of trichloroacetic acid or by ligation or clamping of the sinus node region either prevented or shortened the duration of atrial fibrillation obtained from aconitine and from rapid electrical stimulation of the right atrium. Sano et al. (29) observed that in strips of rabbit right atrium bathed in a low potassium medium, a sinus action potential usually preceded spontaneous atrial fibrillation and that in quiescent strips of left atrial muscle under the same conditions, fibrillation could be obtained by electrical stimulation simulating sinus node impulses. Recently, Azuma et al. (30) also reported that aconitine-induced atrial fibrillation could be restored by electrical pacing after sinus node destruction in isolated rabbit atria. These observations, similar to those described in the present study, suggest that repetitive stimulation of the atria facilitates the initiation and maintenance of atrial fibrillation.

References
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