Auricular Fibrillation Induced and Maintained in Animals by Acetylcholine or Vagal Stimulation

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Animals pretreated with appropriate doses of anticholinesterase agents develop auricular flutter and fibrillation following injection of acetylcholine or vagal stimulation. Depression of normal sinoauricular nodal function facilitates induction of fibrillation. During fibrillation, additional injections of acetylcholine, or vagal stimulation, increase the rate of fibrillation. Atropine in small doses reverts the fibrillation to normal sinus rhythm.

The production of auricular fibrillation by acetylcholine is well recognized. This type of fibrillation is not constant in occurrence and is transient. The present report describes a method for consistently producing auricular fibrillation of extended duration by the use of acetylcholine or vagal stimulation in animals treated with anticholinesterase agents. The report describes a means of regulating the rate of fibrillation, the role of normal sino-auricular node function in the initiation of the fibrillating state, and the abolition of fibrillation by atropine.

Methods

Thirty-four dogs, four goats and six monkeys were used. Three types of experiments were conducted. The first type used animals anesthetized with chloralose (50 mg./Kg.) in which conventional electrocardiograms were obtained. The second type of experiment used the anesthetized animal under artificial, positive pressure respiration with the chest open at the sternal midline, and in which action potentials were obtained from electrodes applied directly to the myocardium. The third type of experiment was conducted on unanesthetized animals with normal respiration in which direct cardiac electrodes had been previously implanted using thiopental anesthesia.

The preparation of the animals for the first and second type of experiments is a well established procedure. Our method for the preparation of animals for the third type of experiment is as follows:

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RESULTS

Effect of anticholinesterase agents on the electrocardiograms. The anticholinesterase agents were given to a series of 9 dogs from which continuous conventional electrocardiograms were obtained. The total dose administered was increased by repeated injection, starting with 15 µg per Kg. and continuing with 5 µg per Kg. every five minutes. The response to each of the anticholinesterase agents was first a slowing of the sino-auricular rate, then the appearance of a partial A-V block which progressed to a complete A-V block with diminution in the amplitude of the QRS wave and frequently an increased amplitude of the T wave. At this time, some animals showed evidence of retrograde A-V conduction. This was followed by widening of the QRS complex. Occasionally, brief 10 to 30 second periods of auricular fibrillation appeared in some animals. At this time, respiratory effects of the agent usually necessitated positive pressure artificial respiration, which allowed the administration of additional amounts of the anticholinesterase agent. Then, auricular asystole invariably developed and the ventricle continued as an idioventricular, regular rhythm. Additional anticholinesterase agent resulted in further slowing of the ventricle to a rate which was frequently only 15 to 20 per minute. Additional anticholinesterase agent always resulted in the appearance of bizarre, ectopic, ventricular action potentials. Although, occasionally, an animal developed ventricular fibrillation, the usual course was a further gradual slowing of the ventricular rate until ventricular asystole occurred. There were no major differences, in this series, of these effects with TEPP, Tabun or Sarin. The major difference in these anticholinesterase agents was in the dose required to produce respiratory embarrassment and subsequent auricular asystole. In all of the foregoing experiments, artificial respiration was instituted when respiratory embarrassment became evident. As evidence that a hypoxic state did not exist, intravenous injection of 0.1 mgm. per Kg. of atropine regularly reversed all of the above-mentioned effects of the anticholinesterase agents on the heart if given just prior to ventricular asystole.

The influence of anticholinesterase agent on response of the heart to vagal stimulation and injection of acetylcholine: Figure 1 shows the effect of control injections of acetylcholine and vagal stimulation on the auricular and ventricular rates in the unanesthetized closed-chest type of experiment. The data for the figure are from one dog but are typical of the response obtained in four of six such animals. The remaining two animals failed to develop auricular fibrillation, although vagal stimulation produced a brief partial and complete A-V block which rapidly reverted to normal sinus rhythm. Two additional dogs, and one goat, developed terminal ventricular fibrillation when, or shortly after, the pericardium was opened.

Figure 1 (graph 3) shows that following administration of TEPP the slowing of both auricular and ventricular rate as well as the A-V dissociation is manifest with lower doses of acetylcholine and occurs with lower voltage vagal stimulation than was obtainable in the control period. This is presumably the result of the ability of the anticholinesterase agent to inactivate cholinesterase and thereby prevent rapid destruction of acetylcholine in tissues, rather than enhancement of the acetylcholine effect on myocardial tissue. The experiments further showed that following 60 to 80 µg. of TEPP the injection of 1 to 10 µg. of acetylcholine or subsequent stimulation of the vagus would result in the induction of auricular fibrillation. The rate of the auricular action potentials was initially 1200 to 1500 per minute. The ventricular rate at this time was inconstant and in some animals was high (above 200 per minute), while in others the ventricular rate was between 30 and 50 per minute. The auricular rate gradually decreased over the subsequent 2 to 10 minutes to approximately 600 per minute, following which it abruptly reverted to normal sinus rhythm. As the dose of TEPP was further increased (fig. 1, graph 5), A-V dissociation occurred. Initial stimulation of the vagus produced auricular asystole which persisted. Additional vagal stimulation and injections of acetylcholine were without effect on the stable
AURICULAR FIBRILLATION

Fig. 1. Influence of peripheral vagus stimulation (5 second interval at each voltage level) and injected acetylcholine before and following increasing total dose of TEPP. Arrows indicate occurrence of auricular fibrillation. "A" and "V" refer respectively to auricle and ventricle. Points on graphs represent maximum response following each manipulation. In graph 5 a persistent auricular asystole developed, otherwise heart rates were permitted to return to control values following each manipulation.

A small dose of atropine reversed the effect of TEPP (fig. 1, graph 6) so that the auricle and ventricle again responded in a normal manner to vagal stimulation of sufficiently greater voltage and to increased doses of acetylcholine. In two additional dogs, control injection of acetylcholine (40 to 100 µg. per Kg.) initiated auricular fibrillation of 10 to 20 seconds duration. Following the administration of TEPP (60 µg. per Kg.), injection of acetylcholine (1 µg. per Kg.) resulted in auricular fibrillation which gradually slowed over the subsequent 14 to 20 minutes, and then abruptly reverted to normal sinus rhythm. Additional experiments were designed to demonstrate the influence of acetylcholine as well as atropine on existing auricular fibrillation. The results indicated that the rate of fibrillation was increased by the injection of acetylcholine and decreased by the injection of atropine. Usually, acetylcholine would initiate auricular fibrillation when administered in a dose of 1 to 5 µg. per Kg. to a dog which had been treated with TEPP (60 to 100 µg. per Kg.) or moderate doses of Sarin or Tabun. The initial auricular rate was approximately 2000 per minute. This high auricular rate gradually decreased over a 6 to 10 minute period to a stable rate of 600 to 800 per minute following which the auricle abruptly reverted to normal sinus rhythm. The fibrillating state could then be reinduced by injection of acetylcholine (1 to 5 µg. per Kg.). When the rate of fibrillation then decreased to 600 to 800 per minute, additional doses of acetylcholine (1 to 5 µg. per Kg.) immediately increased the rate to 2000 to 2400 per minute. By proper continu-
ous infusion of acetylcholine the fibrillating state could be maintained for indefinitely long periods of time. Auricular fibrillation was maintained in one of the dogs for a period of 150 minutes. In a similar manner auricular fibrillation could be induced and subsequently maintained by electrical stimulation of the distal end of the cut right vagus nerve. Furthermore, the fibrillation could also be induced with injected acetylcholine and then perpetuated with electrical stimulation of the vagus nerve.

In contrast to the effect of acetylcholine in increasing the rate of the auricular fibrillation, the administration of atropine resulted in a prompt decrease in the rate of the fibrillation. Atropine given intravenously in a dose of 1 μg. per Kg. resulted in a prompt reduction in the rate of the fibrillation. In two animals (1 dog and 1 goat), this dose of atropine immediately reverted the fibrillating state to normal sinus rhythm. Repeated attempts to reinduce the fibrillating state either by vagal stimulation or by the injection of acetylcholine were unsuccessful. These results indicated that even 1 microgram per Kg. of atropine was sufficient to prevent fibrillation. In a single subsequent dog experiment, the injection of pentobarbital (10 mg. per Kg.) resulted in prompt reversion of the fibrillation to normal sinus rhythm. This suggests the existence of a significant atropine-like effect of this barbiturate. Bohr and Helmendoch have also shown that sodium pentobarbital decreases the effect of vagal stimulation on the heart. Since pentobarbital is a frequently used laboratory anesthetic agent, it may account for the failure of some investigators to observe the induction of auricular fibrillation following the administration of acetylcholine to animals treated with anticholinesterase agents.

The influence of normal sinoauricular nodal activity on the induction and maintenance of acetylcholine induced auricular fibrillation: Although these experiments demonstrated the ability of acetylcholine to induce and maintain the state of auricular fibrillation in animals “sensitized” with moderate doses of anticholinesterase agent, about 25 per cent of the animals failed to develop the fibrillating state. In these animals, usually the auricle became refractory to injected acetylcholine and to vagal stimulation although the ventricle responded by a brief period of asystole and subsequent marked slowing of the rate. Additional experiments were designed to study the influence of persistent sinoauricular nodal activity on the failure to induce fibrillation with acetylcholine.

In open-chest experiments, the area on the right auricle containing the sinoauricular node could be easily located by application of a tip of ice having a diameter of about 3 mm. Only when placed directly on the S.A. node, was there immediate slowing and occasional brief auricular arrest. This area corresponded to that described by Puech and co-workers as containing the sinus node in normal dogs. Experiments were then conducted in which the response of the auricle to injected acetylcholine and to nodal cooling was determined both before and after the administration of anticholinesterase agent. Representative results shown in figure 2 indicate that neither cooling of the node nor the injection of acetylcholine alone induced the fibrillating state in the doses used. Cooling of the node and simultaneous injection of acetylcholine resulted in induction of the fibrillating state both before and after the use of Tabun as the anticholinesterase agent. As an additional means of studying the influence of the normal node on the induction of auricular fibrillation, the S-A node was located with the ice probe and this area was crushed by clamping repeatedly in a small hemostat. The clamping procedure resulted immediately in some irregularity in the auricular rhythm and on one occasion resulted in a brief period of flutter and fibrillation. Application of the ice probe to the area of the crush no longer influenced the auricular rate. No area could be located with the ice probe on the right auricle or in the area of the superior or inferior vena cava lying within the pericardium which would decrease the rate or influence the rhythm of the auricle, although apparently a new pacemaker had become active. The injection of acetylcholine (5 to 20 μg. per Kg.) resulted in prompt initial slowing of the auricular rate and then a 2 to 3 second period of
auricular flutter or fibrillation. Following the injection of Tabun as the anticholinesterase agent, acetylcholine in a dose of 1 microgram per Kg. resulted in auricular fibrillation of several minutes duration after which it reverted to a normal rate and rhythm. Similar results were obtained in four dogs and in one monkey.

Influence of direct electrical stimulation on the auricle in facilitating the induction of auricular fibrillation with acetylcholine: Acetylcholine injected in a dose of 1 mg. per Kg. failed to produce auricular fibrillation in three dogs, two monkeys and one goat which had been bilaterally vagotomized and arranged for the open-chest type of experiment. The auricle showed refractoriness to this dose of acetylcholine although the ventricle responded by marked slowing. Larger doses of acetylcholine irregularly produced the fibrillating state in the auricle. At this time, it was believed that even in the presence of persistent nodal function, if the fibrillation could be induced it would be perpetuated by the presence of acetylcholine. This was demonstrated by initiating the fibrillation with electrical stimulation applied directly to the midportion of the right atrium.

Control periods of stimulation (6 volts, 20 per second) of 1 to 10 second duration applied directly to the auricle in the TEPP treated animal in the absence of acetylcholine initiated an auricular flutter or fibrillation which invariably reverted to normal sinus rhythm within 1 to 3 seconds following cessation of the stimulus. This has also been reported by Sherf and co-workers. However, if acetylcholine (1 mg. per Kg.) was injected, and 10 to 20 seconds later a one second period of electrical stimulation was applied to the surface of the auricle, a fibrillating state was induced. The rate of fibrillation gradually but irregularly slowed over the subsequent 5 to 20 minute period and then abruptly reverted to normal sinus rhythm.

DISCUSSION

The results indicate that anticholinesterase agents used in proper doses function to prolong the normal effect of acetylcholine on the
rhythmicity of several parts of the heart. This enabled the present study to be conducted. Acetylcholine-induced auricular fibrillation possesses some of the characteristics of aconitine-induced fibrillation in that it is initially of very rapid, irregular rate which gradually slows over a period of time, and yet unlike the aconitine type, the rate does not fall progressively to that of the sinus node rate. Rather, the rate of acetylcholine-induced fibrillation tends to fall progressively to about 600 per minute and then suddenly reverts to average sinus rate. In this respect, acetylcholine-induced fibrillation simulates the type of flutter (circus movement) described by Lewis,1 which also characteristically suddenly reverts from a high regular rate to a normal sinus rate.

It is well established that the most common effect of acetylcholine on the S-A node is that of decreasing its rate of discharge. In the present experiments, acetylcholine in various doses was found to have various effects. Small doses decreased the rate of the auricle. With larger doses the pacemaker occasionally became refractory or the auricle suddenly fibrillated. When the S-A node was cooled or destroyed by crushing, acetylcholine induced fibrillation. The extremely small doses of atropine (of the order of 1 ng per Kg.) which readily reduce the rate of acetylcholine-induced fibrillation and prevent its subsequent induction are well within the average clinical dose range. The common pre-anesthetic use of drugs having the antimuscarinic type of anticholinergic action (such as atropine, scopolamine, d-tubocurarine, the synthetic atropine-like compounds, and the barbiturates) may exert an unrecognized protection against severe auricular tachycardia and fibrillation during cardiac surgery.

Studies on the isolated auricle of the rabbit and cat7 11 have suggested that acetylcholine decreases the force of contraction and shortens the refractory period. The same effects have been observed on the cold blooded heart.10 In the studies of Turner, West and Loomis,7 flutter of varying duration occasionally appeared in the auricle following electrical stimulation. Such "flutters" were more frequent in occurrence and more persistent in the presence of acetylcholine than in its absence.

At the present time, the mechanisms involved in the acetylcholine type of fibrillation are concerned with the following possibilities. In the absence of normal sinus node control of the auricle, acetylcholine may increase the rate of one or more ectopic foci. The shortening of the refractory period by acetylcholine could make available additional direct or even devious tracts for the passage of an impulse. It would, therefore, be theoretically possible to increase the rate of a fibrillation or flutter by only a shortening of the refractory period without having any effect on conduction velocity.

Existing methods for inducing auricular fibrillation or flutter in the experimental animal involve either the "crushing" technique or the local application or injection of large amounts of acetylcholine (5 per cent solution) to the area of the sinus node.11 The significance of the role of the vagal effect and its chemical mediator (acetylcholine) presents a possible normal physiological mechanism that may be significant in the initiation and maintenance of clinical auricular fibrillation. The further demonstration of the ease by which the fibrillation can be induced when sino-auricular nodal function is depressed and when acetylcholine is protected from destruction by prior administration of an anticholinesterase agent suggest the following: Clinically, the presence of high vagal tone and the presence of some inflammatory or degenerative pathological interference with sinoauricular nodal function would suffice to initiate recurring episodes of auricular fibrillation. Such a fibrillating state should be readily reverted to normal rate and rhythm with small doses of atropine.

In anticholinesterase poisoning, the possibility of cardiac arrhythmias occurring as a result of accumulation of acetylcholine may be of some significance. With the additional presence of a high vagal tone the incidence of auricular arrhythmias may be expected to be high. In severe anticholinesterase poisoning interference with respiratory function appears and necessitates positive pressure artificial respiration. However, in the light of the results reported here, adequate oxygenation of the blood does not prevent the development of the
cardiac arrhythmias described; these are controllable by atropine.

SUMMARY AND CONCLUSIONS

Auricular fibrillation can be induced in the intact heart by the intravenous administration of acetylcholine to unanesthetized normal dogs, goats and monkeys. By the use of proper anesthetic agents, and by the protection of injected acetylcholine from destruction through the use of anticholinesterase agents, injected acetylcholine or vagal stimulation produce auricular fibrillation of extended duration. The effect of acetylcholine or vagal stimulation on such a pre-existing fibrillation is to increase the rate of the fibrillation, and the fibrillation can be perpetuated by repeated injection of acetylcholine or by vagal stimulation. The effect of atropine on the fibrillating auricle is to slow the rate of fibrillation. Small doses of atropine revert the auricle to normal rate and rhythm and prevent its subsequent induction by acetylcholine. The significance of the presence of acetylcholine plus depressed sino-auricular nodal function as the controlling factors for the development of auricular fibrillation are presented.

REFERENCES

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