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MECHANISM OF ATRIAL FLUTTER AND FIBRILLATION INDUCED BY ACONITINE IN THE DOG, WITH OBSERVATIONS ON THE ROLE OF CHOLINERGIC FACTORS

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The topical application of aconitine nitrate to the right atrial appendage in the "intact" anaesthetized dog produced atrial flutter. Premature systoles with fixed coupling preceded the development of flutter. In early stages of the arrhythmia, atrial rate was irregularly irregular. Also, the form of flutter beats was similar to that of preceding premature systoles. The fibrillatory activity of acetylcholine described by earlier workers has been confirmed. Transient atrial dissociation was seen after intravenous injection or topical application of acetylcholine. The occurrence of fibrillation in the left atrium after focal application of acetylcholine has been demonstrated, while the right atrial appendage containing the ectopic focus induced by aconitine continued to flutter. Aconitine produced slow-rate flutter in dogs treated with atropine or hemicholinium; this flutter was easily distinguishable from the sinus tachycardia produced by these drugs, by recording the electrocardiogram from a direct atrial lead from the area treated with aconitine, but not from limb lead II. The importance of these findings in the interpretation of the mechanism of atrial flutter and fibrillation is discussed.

The focal origin of impulses in atrial flutter and fibrillation induced by aconitine is well documented (Scherf & Terranova, 1949; Prinzmetal, Corday, Brill, Oblath, & Kruger, 1952; Brown & Acheson, 1952; Scherf, Schaffer, & Blumenfeld, 1953). The mechanism of impulse formation within the ectopic focus, however, still remains debatable. Scherf *et al.* (1953) believe that repetitive discharges develop in the cells affected by aconitine, while Yelnosky & Clark (1960) concluded that the arrhythmia was due to stimulation of the sinus node by aconitine. In addition, DiPalma & Schults (1950) and Dawes (1952) suggested that a localized circus movement might develop in the area treated with aconitine. No experimental evidence is available in the literature to prove or to disprove this possibility.

The conversion of atrial flutter induced by aconitine into fibrillation under cholinergic influences has been stressed by several workers (Brown & Acheson, 1952; Burn, Vaughan Williams & Walker, 1956; Lanari, Lambertini, & Ravin, 1956), but the role of acetylcholine in the production of atrial flutter is not clear. Scherf *et al.* (1953) and Scherf, Blumenfeld, Golbey, Ladopoulos & Roth (1955) showed

that aconitine did not produce flutter in dogs treated with atropine. Preliminary experiments in the present study, however, showed that aconitine regularly produced slow flutter after atropine.

The experiments reported here were designed, therefore, to determine (*a*) the nature of impulse formation within the ectopic focus induced by aconitine, (*b*) the role, if any, of cholinergic factors in the production of atrial flutter, and (*c*) the mechanism by which acetylcholine converts flutter into fibrillation. A preliminary report of part of this work has been published (Sharma, 1962).

METHODS

Mongrel dogs of either sex weighing between 10 and 18 kg were anaesthetized by intravenous injection of 35 mg/kg of pentobarbitone sodium. Under positive-pressure artificial ventilation the chest was opened by a midline incision. The pericardium was incised and the heart was cradled by stitching the pericardial flaps to the chest wall. Injections were made through a cannula inserted into the right femoral vein. Atrial flutter was induced by the topical application of a 0.05% solution of aconitine nitrate near the tip of the right atrial appendage (Scherf, 1947). Care was taken to avoid any accidental contact of aconitine with the ventricular myocardium, as this always resulted in fatal ventricular fibrillation. Arrhythmia started in less than 2 min after the application of aconitine, and reached a stable rate in about 5 min. The first observations were made, therefore, after 10 min.

Direct atrial electrograms from the sinus node, right and left atrial appendages, and conventional bipolar lead II were recorded simultaneously on a Siemen-Ediswan 4-channel oscillograph. The right vagus nerve was cut in the neck and the peripheral end was stimulated electrically (rectangular wave pulses of 0.5 msec duration, frequency 20 shocks/min and 20 V) for not more than 15 sec at a time. In some experiments the left vagus nerve was also stimulated.

RESULTS

Genesis of atrial flutter

The different stages in the development of atrial flutter induced by aconitine are illustrated in Fig. 1. There was a latent period of 76 ± 8.43 (mean and standard error, forty experiments) before the arrhythmia started. The first abnormality noted was the appearance of premature systoles occurring singly, in pairs or in short runs of atrial tachycardia. All the single premature systoles and the first beat of a pair of premature systoles or the first beat of a short run of atrial tachycardia (which are serially numbered in Fig. 1) showed fixed coupling to the preceding sinus beat and had the same shape and amplitude. When persistent flutter resulted, the form of beats remained the same as that of preceding premature systoles.

Another important feature of the early stages of flutter was an irregularly irregular atrial rate. After a few minutes, as the effect of aconitine reached its peak, the rate became more regular.

Cholinergic factors in flutter and fibrillation induced by aconitine

The role of cholinergic influences was studied in two ways, by vagal stimulation or the injection of acetylcholine, and by giving atropine or by blocking the synthesis of acetylcholine with hemicholinium.

Effect of vagal stimulation on atrial flutter. Twenty-two observations were made in eight dogs. In sixteen instances flutter was converted into fibrillation and flutter

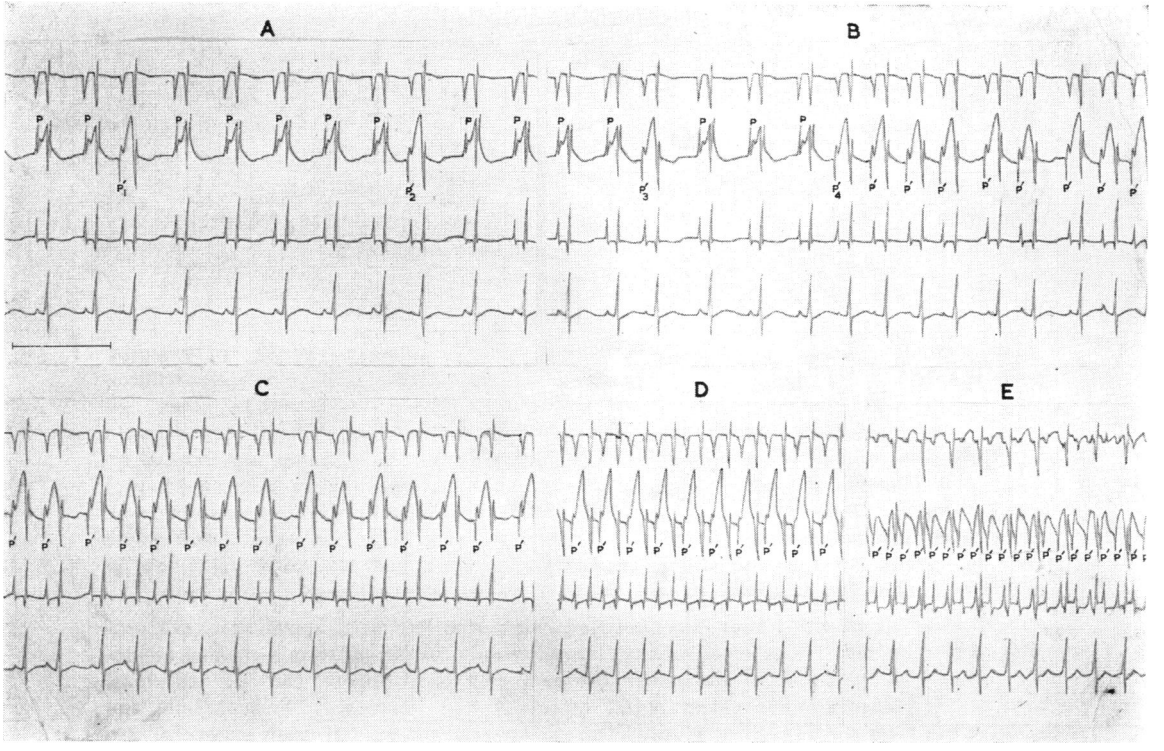

Fig. 1. Stages in the development of atrial flutter induced by aconitine. From top to bottom, electrograms from the sinus node, tip of the right atrial appendage, tip of the left atrial appendage and conventional bipolar lead II. P=sinus beats. P′=ectopic beats. A: 48 sec after topical application of aconitine to the right atrial appendage. There are two premature systoles (P'_1 and P'_2) which each show fixed coupling to the preceding sinus beat (0.36 sec). B: 54 sec after aconitine; P'_3, a premature systole with the same fixed coupling. The first beat of the tachycardial episode (P'_4) also shows the same fixed coupling and is of the same shape and amplitude. The atrial rate is irregularly irregular. C: 68 sec after aconitine; slow flutter is present, the rate is still irregular and the shape and amplitude of the beats are very like the isolated premature systoles. D: 80 sec after aconitine; the flutter rate is 200 beats/min and quite regular. E: 96 sec after aconitine; the flutter rate is 402 beats/min. Time scale, 1 sec.

at the control rate returned within 5 sec after vagal stimulation was stopped. The highest rate of fibrillation recorded was 1,620 beats/min, while the usual range was 720 to 1,400 beats/min. In the remaining six experiments, vagal stimulation increased the flutter rate (up to 600 beats/min) but did not result in fibrillation. During vagal stimulation both atria fibrillated simultaneously. The fibrillatory activity of vagal stimulation was completely blocked by atropine sulphate (0.1 mg/kg, intravenously).

Effect of injected acetylcholine on atrial flutter. Acetylcholine chloride (0.2 to 1.0 mg in 1 ml. of 0.9% saline, given intravenously over 5 sec) converted flutter into fibrillation in all the thirty experiments performed in fourteen dogs. The highest rate of fibrillation recorded was 3,960 beats/min, while the usual range was 1,800 to 2,760 beats/min.

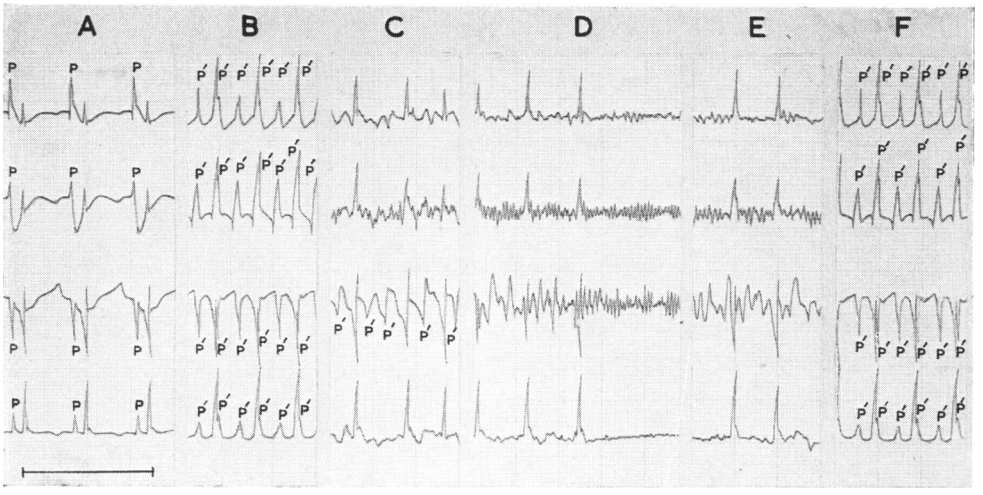

Fig. 2. Effect of intravenous acetylcholine on atrial flutter. Records as in Fig. 1. P=sinus beats. P′=flutter beats. A: control; the heart rate is 132 beats/min. B: 20 min after the topical application of aconitine to the right atrial appendage; flutter at 400 beats/min with 2 : 1 atrioventricular block is present. C: 8 sec after the intravenous injection of 500 μg of acetylcholine chloride (in 1 ml. of 0.9% saline during 5 sec). The right atrium is fibrillating at 1,820 beats/min while the left atrium is still fluttering at 400 beats/min. D: 12 sec after the injection of acetylcholine; the left atrium is now fibrillating, but the rate in the right atrium is somewhat higher (2,340 beats/min) than in the left (1,980 beats/min). E: 26 sec after the injection of acetylcholine, the fibrillation rate has decreased in both atria. F: 46 sec after acetylcholine, atria flutter at the control rate has returned. Time scale, 1 sec.

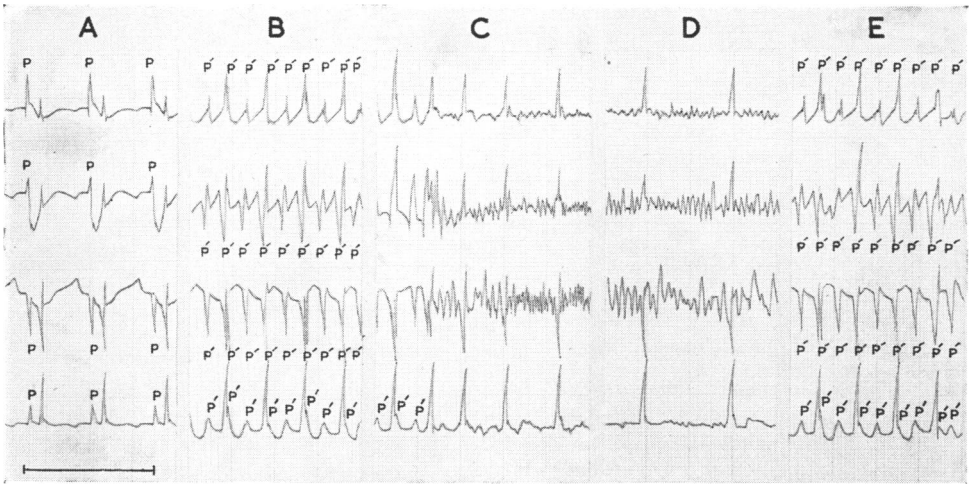

Fig. 3. Effect of intraventricular injection of acetylcholine on atrial flutter. Records as in Fig. 1. P=sinus beats. P′=flutter beats. A: control, the heart rate is 132 beats/min. B: 30 min after application of aconitine the flutter rate is 400 beats/min. C: 3 sec after injection of 500 μg of acetylcholine chloride directly into the left ventricle. Note the simultaneous development of fibrillation in both atria. Atrial dissociation did not occur. The maximum fibrillation rate recorded is 2,760 beats/min. D: 22 sec after acetylcholine; the fibrillation rate has decreased. E: 54 sec after acetylcholine; flutter at the control rate has returned. Time scale, 1 sec.

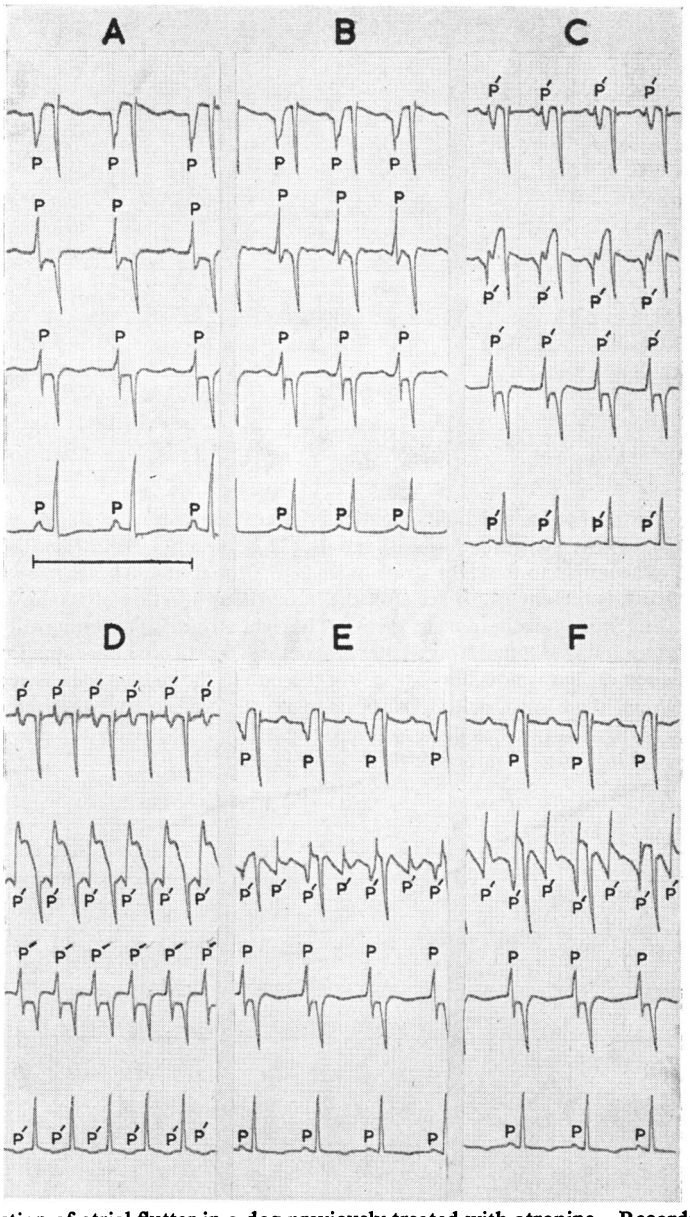

Fig. 4. Production of atrial flutter in a dog previously treated with atropine. Records as in Fig. 1. P=sinus beats. P′=flutter beats. A: control; the heart rate is 126 beats/min. B: 10 min after the intravenous injection of 1 mg/kg of atropine sulphate (in 20 ml. of 0.9% saline during a period of 5 min). The heart rate has increased to 165 beats/min. C: 108 sec after the topical application of aconitine to the right atrial appendage. The heart rate has increased to 180 beats/min, and the beat is ectopic as seen from the reversal of the wave form in the right atrial record. D: 152 sec after aconitine, the rate has increased to 265 beats/min. To differentiate the slow flutter from the sinus tachycardia caused by atropine, the area treated with aconitine was clamped for 1 min. E: immediately after the release of the clamp, the area treated with aconitine shows flutter at 272 beats/min; elsewhere sinus tachycardia at 164 beats/min is present. F: 5 min after the release of the clamp, the flutter rate has increased to 310 beats/min, while the sinus tachycardia rate is still 162 beats/min. Time scale, 1 sec.

After intravenous injection of acetylcholine, atrial dissociation was seen in that the right atrium fibrillated 5 to 10 sec earlier than the left atrium. A typical experiment is illustrated in Fig. 2. Since no dissociation was seen after vagal stimulation, it was possible that it was due to the arrival of acetylcholine in the right atrium earlier than in the left atrium. To test this possibility, acetylcholine was injected directly into the left ventricle so that it reached both the atria simultaneously through the coronary arteries. The two atria now fibrillated simultaneously (Fig. 3). Figs. 2 and 3 are recorded from the same animal, atrial dissociation being apparent in Fig. 2 but not in Fig. 3. Atrial dissociation was also seen after focal application of acetylcholine (5% solution in 0.9% saline) to the right or the left atrial appendage. The fibrillation was confined to the area treated with acetylcholine ; elsewhere flutter was recorded.

Effect of atropine on established atrial flutter. In six dogs flutter was produced with aconitine, and atropine sulphate (1.0 mg/kg) was then injected intravenously. This produced a decrease in flutter rate from 446 ± 18 to 310 ± 12 beats/min (means and standard errors). The rhythm continued to be ectopic and could be easily distinguished from sinus tachycardia induced by atropine in the recording from the direct atrial lead from the area treated with aconitine, but not in that from limb lead II.

Effect of previous treatment with atropine on the production of atrial flutter. Six dogs were treated with atropine sulphate (1.0 mg/kg, intravenously) as before, and aconitine was applied to the right atrial appendage 30 min later. Aconitine regularly produced slow flutter after atropine ; this was easily distinguishable from the sinus tachycardia induced by atropine in the record from the direct atrial lead from

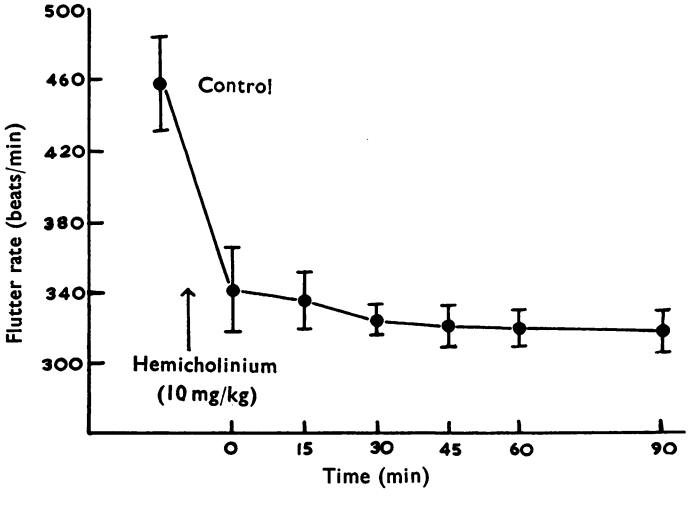

Fig. 5. Effect of hemicholinium on atrial flutter. Ordinate, flutter rate in beats/min. Abscissa, interval between the injection of the drug and measurement of rate. Each point is the mean value of five experiments; the vertical lines are standard errors. The drug was injected intravenously, dissolved in 20 ml. of 0.9% saline, slowly over a period of 10 min.

the area treated with aconitine. Also, when the area treated with aconitine was isolated from the body of the atrium with a clamp, flutter continued in the isolated area while elsewhere sinus tachycardia was recorded (Fig. 4).

Effect of hemicholinium on established atrial flutter. In five dogs, atrial flutter was produced with aconitine, and hemicholinium (10 mg/kg, intravenously) was injected to block synthesis of acetylcholine. This produced a marked decrease in flutter rate but the rhythm, as after atropine, continued to be ectopic. The results of all the five experiments are presented in Fig. 5.

DISCUSSION

The mechanism of atrial flutter and fibrillation induced by aconitine has been studied extensively (Scherf, Romano, & Terranova, 1948 ; Scherf & Terranova, 1949 ; Brown & Acheson, 1952 ; Prinzmetal *et al.*, 1952 ; Scherf *et al.*, 1953 ; Lanari *et al.*, 1956). All these workers agree that the arrhythmia is due to ectopic focal discharge, and that the impulses spread out from the focus in a radial direction. The cooling and clamping experiments of Scherf *et al.* (1948) and the high-speed film and direct atrial lead studies of Prinzmetal *et al.* (1952) are fully consistent with the focal origin of this arrhythmia. These studies, however, reveal nothing about the mechanism of impulse formation within the ectopic focus. It has been suggested by these workers that the mechanism of impulse formation could be repetitive discharge from the cells affected by aconitine.

The radial spread of impulses cannot be explained on the basis of circus movement theory as outlined by Lewis (1920), but as suggested by DiPalma & Schults (1950), Dawes (1952) and Tenney & Wedd (1954) the arrhythmia could be due to localized circus movement in the area treated with aconitine. Such a localized circus movement would behave as a rapidly discharging focus, and impulses would spread out radially from it.

Evidence in support of re-entry mechanism

According to Mines (1913) the development of re-entrant excitation requires a short refractory period and prolonged conduction time. Aconitine produces both these effects on atrial myocardium (Wedd & Tenney, 1953 ; Tenney & Wedd, 1954 ; Dawes & Vane, 1956 ; Yelnosky & Clark, 1960). In the present study, premature systoles with fixed coupling to the preceding sinus beat occurred in all the experiments. This could be due to the development of local blocks in the area treated with aconitine. Also, the similarity of the form of flutter beats and premature systoles has been demonstrated. Most workers agree that premature systoles with fixed coupling originate by a re-entry mechanism (Schmitt & Erlanger, 1928 ; DiPalma & Schults, 1950 ; Barker, 1952 ; Langendorf, Pick & Winternitz, 1955), while others (Scherf, 1959 ; Scherf & Schott, 1959) believe that they are due to repetitive discharge. The irregularly irregular rate of the arrhythmia in early stages would support the re-entry mechanism, since repetitive discharges are known for their clock-like regularity of rate (Dawes & Vane, 1951).

Evidence against repetitive discharges

Repetitive discharges occur in cardiac muscle under certain experimental conditions. Dawes & Vane (1951) and Brooks, Hoffman, Suckling & Orias (1955) produced them by applying an extra stimulus during a vulnerable phase of the preceding beat. In the present study, aconitine produced flutter in the normally beating atria without the application of any extra stimulus. Under these circumstances how could repetitive discharge begin ? Dawes & Vane (1951) also studied the mechanism of repetitive discharge and observed that it originated in a single cell, but later when they studied this problem by multipoint recordings they concluded that a re-entry mechanism was involved (Dawes, 1952).

Aconitine also produces negative after-potentials (Segers, 1942), and attempts have been made to explain the mechanism of repetitive discharges on this basis. These potentials are oscillatory and, once they reach the threshold value, repetitive firing takes place (Arvanitaki, 1940). It is, however, difficult to comprehend how such a mechanism could produce single premature systoles. A premature systole has a short refractory period (DeBoer, 1920 ; DiPalma, 1955), and it would be expected that subsequent oscillatory potentials would be similarly propagated, as seen in Arvanitaki's experiments on nerve (Arvanitaki, 1940). Also, if negative after-potentials were responsible for producing premature systoles, these should be much more frequent during the vulnerable phase of the atrial excitability curve. Brooks *et al.* (1955) showed that the vulnerable phase in the dog occurred 100 to 150 msec after the atrial beat. In the present study, the coupling intervals varied between 240 and 380 msec, an interval in the atrial cycle when normal threshold of excitability exists.

Role of cholinergic influences

Scherf *et al.* (1953, 1955) observed that aconitine did not produce flutter in atropinized animals. The present results show that aconitine regularly produced slow flutter after atropine. Similar results were obtained with hemicholinium. The inability of Scherf *et al.* to demonstrate flutter after atropine may have been due to the fact that they did not record from direct atrial leads. As shown in Fig. 4, the flutter waves in lead II are positive and cannot be distinguished from sinus tachycardia, but the difference is very clear in recordings from the direct atrial lead from the area treated with aconitine.

These results show that acetylcholine is not essential for the initiation or maintenance of atrial flutter, because aconitine regularly produced flutter after administration of atropine or of hemicholinium. Acetylcholine does, however, play a part in determining the rate of ectopic rhythm, as shown by the marked decrease in flutter rate after atropine or hemicholinium. This disagrees with the hypothesis of Torchiana & Angelakos (1961) that acetylcholine may be concerned in the development of all types of atrial arrhythmias.

The conversion of atrial flutter induced by aconitine into fibrillation under cholinergic influences in the dog has been described by Brown & Acheson (1952), Scherf *et al.* (1953), Burn *et al.* (1956) and Lanari *et al.* (1956). The present experiments

confirm the fibrillatory activity of acetylcholine. Prinzmetal *et al.* (1952) suggested that atrial premature systoles, tachycardia, flutter and fibrillation were all due to single ectopic foci, and that the rate of discharge determined the type of the arrhythmia developed. In the present study, it was possible to produce localized fibrillation in the left atrium under focal cholinergic influence while the right atrium containing the ectopic focus continued to flutter. This phenomenon of atrial dissociation cannot be explained on the basis of a unitary concept of atrial arrhythmias. This suggests that fibrillation is due to the development of multiple re-entries under cholinergic influence, and is not due to an increase in the rate of discharge of the ectopic focus. This agrees with the hypotheses of Burn (1957) and of Moe & Abildskov (1959).

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