THE RESPONSE OF ISOLATED RABBIT ATRIA TO ACONITINE

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The arrhythmias observed in isolated rabbit atria appear to be due to two actions of aconitine. One is an apparent direct stimulant effect on the sino-atrial node and the other is a depressant effect on the processes involved in intra-atrial propagation of impulses. Acetylcholine has either an inhibitory or stimulatory effect on atria pretreated with aconitine because it antagonizes the actions of aconitine both on the sinus node and on conduction. When conduction block is not apparent, acetylcholine depresses the atrial rate. On the other hand, when conduction is markedly depressed, acetylcholine increases the atrial rate.

Atrial flutter and fibrillation can be produced *in vivo* by the topical application of aconitine to the atria of the dog. It is believed that these arrhythmias are due to the rapid discharge of an ectopic focus of activity (Scherf, Romano, and Terranova, 1948; Prinzmetal, Corday, Brill, Oblath, and Kruger, 1952).

Acetylcholine is known to favour the establishment of atrial fibrillation after the local application of aconitine to atrial tissue. For instance, Scherf *et al.* (1948) showed that vagal stimulation converts aconitine-induced flutter to fibrillation, and Burn, Vaughan Williams, and Walker (1956) found that it was necessary to add acetylcholine to the blood to produce fibrillation with aconitine in the dog heart-lung preparation.

This study was undertaken to define the nature of atrial arrhythmias induced by aconitine in isolated mammalian atria and to determine the effects of acetylcholine on the response. A preliminary report of these results was made at the meeting of the American Society for Pharmacology and Experimental Therapeutics, Philadelphia, Pennsylvania, April, 1958.

METHODS

Male rabbits of the New Zealand strain were used. Immediately after stunning an animal by a blow on the back of the head, the thorax was opened and the entire heart removed and placed in oxygenated nutrient fluid at room temperature. The atria were carefully dissected from the ventricles by cutting along the atrio-ventricular junction and through the inter-auricular septum, and

then were cleared of adjacent tissue and suspended vertically in a muscle chamber which was located in a larger thermostatically controlled water bath. The tip of the left atrium was anchored to the tissue holder, and the tip of the right atrium was attached to a silk thread leading to a force displacement transducer. The diastolic tension was arbitrarily set at 1 g. Action potentials were obtained with two unipolar silver electrodes. One electrode was placed on the right atrial appendage, another on the left atrial appendage, and an indifferent electrode was placed in the bath remote from the atria. The action potentials and isometric contractions were recorded on a four-channel Sanborn Polyviso Recorder. The working volume of the muscle chamber was 100 ml.

The composition of the tissue medium in mmol./l. was as follows: NaCl, 119.7; KCl, 5.6; MgCl₂, 2.1; CaCl₂, 2.1; NaHCO₃, 17.0; and glucose 9.0. The pH of this fluid aerated with 95% oxygen and 5% carbon dioxide at 37.5° was 7.4.

Solutions of aconitine were prepared by dissolving 5 mg. of pure aconitine crystals (S. B. Penick and Co., New York, N.Y.) in 50 ml. of distilled water containing one drop of N hydrochloric acid. Since aconitine solutions are unstable, fresh solutions were made on the day of each experiment.

RESULTS

General Response to Aconitine

Control experiments carried out for periods of from 5 to 6 hr. showed that the spontaneous rate of the isolated atria remained the same or showed a slight progressive decrease, and that there was a one-to-one relationship between the action potentials and the isometric contractions throughout the test period (Fig. 1). When a basis for comparison had been established, aconitine was added in various concentrations. In a concentration of 0.01 γ /ml. aconitine had no effect on the atria in 6 tests. With

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FIG. 1.—Recordings from an Untreated Spontaneously Beating Isolated Rabbit Atria. Top tracing, electrograms from the right atrial appendage; middle tracing, electrograms from the left atrial appendage; bottom tracing, mechanograms. (Paper speed—25 mm./sec.). (A) Record obtained at the beginning of the observation period; (B) record obtained at the end of 5 hr.; (B) shows a decrease in the spontaneous rate and in the size of the action potentials.

higher concentrations of aconitine, a triphasic response was produced. The first phase usually consisted of a progressive acceleration of the spontaneous rate. Intermittent bouts of tachycardia were occasionally observed before the maximal rate was reached. The second phase consisted of (a) an overall slowing in the rate, (b) intermittent periods of rapid and slow activity, (c) a loss of a one-to-one relationship between the action potentials recorded from each atrium, and between the action potentials and the contractions, (d) variations in the amplitude of the contractions, and (e) variations in the amplitude and shape of the action potentials. The onset of the third phase was manifested by apparent electrical and mechanical arrest of the atria. Four atria were observed for 40 min. after activity stopped. Two atria remained quiescent throughout this period, while occasional transient bursts of electrical activity were recorded from the right atrial appendage in the other two.

The effects of aconitine on the atria were reversed by numerous replacements of the nutrient fluid.

Aconitine (0.04 $\mu\text{g./ml.}$) produced only changes described as the first phase. When the concentration was increased to 0.16 $\mu\text{g./ml.}$ the first and second phases were always seen and in 2 of 4 experiments the third phase was also observed. A concentration of 1 $\mu\text{g./ml.}$ always produced a triphasic response (Fig. 2). The results are summarized in Table I.

Site of Action of Aconitine

A second series of experiments was carried out to determine whether aconitine acts during the initial phase by establishing an ectopic pacemaker, or by an action involving the sino-atrial node. To distinguish between these possibilities, experiments were conducted on isolated left atria, which did not beat spontaneously but responded to electrical stimuli. When stimulation was discontinued, the

TABLE I

EFFECTS OF GRADED DOSES OF ACONITINE ON ISOLATED RABBIT ATRIA

To estimate onset the aconitine response was considered to have begun when the atrial rate was increased by at least 20 beats/min.

No. of Atria	Aconitine $\mu\text{g./ml.}$	Control Rate Beats/Min.		Incidence of Response	Onset of Response Min. Range	Max. Rate Beats/Min.		Incidence of Arrest	Time for Arrest—Min.	
		Mean	Range			Mean	Range		Mean	Range
6	0.01	140	128–160	0/6				0/6		
4	0.02	130	120–140	2/4	5–10	340	320–360	0/4		
5	0.04	126	120–140	5/5	4–35	288	210–380	0/5		
4	0.16	145	120–160	4/4	2–10	365	340–420	2/4	50	40–60
5	1.0	135	120–160	5/5	1–5	360	300–480	5/5	36	20–52

FIG. 2.—*Response of Isolated Rabbit Atria to Aconitine.* Top tracing, electrograms from the right atrial appendage; middle tracing, electrograms from the left atrial appendage; bottom tracing, mechanograms. (Paper speed—50 mm./sec.). (A) Control; (B, C) records taken 2 and 4 min. after the addition of aconitine ($1.0 \mu\text{g./ml.}$) show the tachycardia characteristic of the first phase of the aconitine response; (D, E, F, G) records taken 6, 8, 10, and 15 min. after aconitine show interference with the propagation of action potential which characterizes the second phase of the aconitine response; (H) shows electrical and mechanical arrest of the atria characteristic of the third phase.

FIG. 3.—*The Effect of Hexamethonium on Aconitine-induced Atrial Tachycardia.* Top tracing, electrograms from the right atrial appendage; middle tracing, electrograms from the left atrial appendage; bottom tracing, mechanograms. (Paper speed—25 mm./sec.). (A) Control; (B) tachycardia induced by nicotine ($9 \mu\text{g./ml.}$); (C) record taken 10 min. after the removal of nicotine and the addition of hexamethonium chloride (0.06 mg./ml.); at this time, nicotine ($9 \mu\text{g./ml.}$) was again added to the bath; (D) block of the nicotine response by hexamethonium; (E) record taken after 9 washes and the subsequent addition of hexamethonium chloride (0.06 mg./ml.); at this time aconitine (0.04 mg./ml.) was added to the bath; (F) record taken 15 min. later shows hexamethonium does not block the tachycardia induced by aconitine.

FIG. 4.—*Persistence of Electrical Activity in Atria Exposed to Aconitine.* Top tracing, electrograms from the right atrial appendage; middle tracing, electrograms from tissue in relatively close proximity to the sinus node; bottom tracing, electrograms from the left atrial appendage. (Paper speed—25 mm./sec.). (A) Control; (B) shows aconitine-induced tachycardia; (C, D, E, F) show the persistence of electrical activity in tissue near the sinus node and the progressive failure of the action potentials to be propagated to the appendages.

FIG. 5.—*Effects of Acetylcholine in Aconitine-induced Tachycardia.* Top tracing, electrograms from the right atrial appendage; middle tracing, electrograms from the left atrial appendage; bottom tracing, mechanograms. (Paper speed—25 mm./sec.). (A) Control; (B) tachycardia induced by aconitine; (C) shows the slowing of the rate immediately after the addition of acetylcholine (0.5 μ g./ml.); (D) shows partial recovery of the rate 15 min. after acetylcholine was added; (E) shows complete recovery of the rate 20 min. after acetylcholine; (F) shows slowing of the rate after a subsequent addition of acetylcholine (0.5 μ g./ml.); (G) shows recovery of the rate 5 min. after acetylcholine was added.

FIG. 6.—*The Effects of Acetylcholine in Atria Arrested by Aconitine.* Top tracing, electrograms from the right atrial appendage; middle tracing, electrograms from the left atrial appendage; bottom tracing, mechanograms. (Paper speed—25 mm./sec.). (A) Control; (B) shows aconitine-induced tachycardia; (C) shows arrest of the atria; (D) shows the immediate restarting of the atria by acetylcholine (0.05 $\mu\text{g./ml.}$); (E) shows arrest of the atria after a single replacement of the bath fluid.

atria stopped contracting. Aconitine (0.04 $\mu\text{g./ml.}$) produced no detectable electrical or mechanical activity in these atria during a 1 hr. observation period. At the end of the hr. the nutrient fluid was replaced, and after 5 min. the concentration of aconitine in the bath was increased to 1.0 $\gamma\text{/ml.}$ Even so, no activity was observed in the next 25 to 30 min. The results suggest that aconitine was not establishing an ectopic pacemaker and so was affecting the sino-atrial node directly or indirectly.

It was possible that aconitine accelerated the atria by stimulation of sympathetic ganglia or atrial chromaffin tissue. The presence of sympathetic ganglia or chromaffin tissue in rabbit atria has been demonstrated by Kottogoda (1953), who reported that nicotine produced an increase in the rate of isolated atria which could be abolished by ganglionic blocking agents. It was found in 5 experiments that hexamethonium chloride (0.06 mg./ml.) or tetraethylammonium chloride (0.3 mg./ml.), which were shown in each test to block the tachycardia induced by nicotine (9 $\mu\text{g./ml.}$), did not modify the atrial tachycardia induced by aconitine. The results of one of these experiments are shown in Fig. 3.

It can be seen in Fig. 2 that conduction is impaired during the second phase of the aconitine response.

The inability of the atria to conduct impulses well was found to be the cause of the relative inactivity of the atria during the third phase. This was determined in 4 experiments in which action potentials were recorded from each atrial appendage and from tissue in close proximity to the sino-atrial node. Fig. 4 shows that action potentials were recorded from the region of the node when no activity was recorded from the appendages.

The Effects of Acetylcholine on the Aconitine Response

During the first phase of the aconitine response, when the spontaneous rate was greater, acetylcholine (0.5 to 2.0 $\mu\text{g./ml.}$) depressed, for varying periods of time, the fast auricular rates induced by aconitine. A subsequent addition of acetylcholine was not as effective in depressing the rate and the effect did not last as long (Fig. 5). This antagonism by acetylcholine was seen when the concentration of aconitine in the bath was 0.04 or 1.0 $\mu\text{g./ml.}$ Acetylcholine (0.05 to 2.0 $\mu\text{g./ml.}$) also antagonizes the depressant effects of aconitine on conduction. This can be seen in Fig. 6. In most instances, restarting of the atria was characterized by chaotic electrical activity and irregular contractions.

Physostigmine sulphate (0.05 $\mu\text{g./ml.}$) added during the third phase of the aconitine response

produced after a latent period of 4 to 15 min. the same effect as added acetylcholine. The latent period was presumably the time required to build up an effective concentration of acetylcholine. Atropine sulphate (0.1 to 0.2 $\mu\text{g./ml.}$) abolished the effects of acetylcholine or physostigmine. The results indicate that atropine can block the excitatory effects of endogenous acetylcholine in atria pretreated with aconitine. Webb (1950) showed that the depressant effect of endogenous acetylcholine could also be blocked by atropine; it was therefore assumed that pretreating atria with atropine would reveal whether or not the acetylcholine normally present in the atria plays a role in the genesis or maintenance of the aconitine response. In tests on 6 auricles, aconitine (0.04 to 1.0 $\mu\text{g./ml.}$) was added to the bath 10 min. after the addition of atropine (0.1 to 0.2 $\mu\text{g./ml.}$). The aconitine response was not modified. These experiments strongly suggest that the acetylcholine normally present in isolated atrial tissue is not at all concerned with the response of rabbit atria to aconitine.

DISCUSSION

The experimental evidence obtained in this study indicates that aconitine-induced arrhythmias in isolated rabbit atria are due to stimulation of the sinus node and to interference with the propagation of impulses. The tachycardia observed after aconitine is attributed to an effect on the pacemaker, since isolated left atria which are presumably devoid of sinus nodal tissue do not respond to aconitine in concentrations that stimulate the paired atria. Furthermore, this effect on the pacemaker appears to be a direct action and not due to a nicotine-like stimulation of sympathetic ganglia of chromaffin tissue, since the response to aconitine is unaltered by ganglionic blocking drugs.

The depressant effects of aconitine on conduction are shown by the loss of a one-to-one relationship between the action potentials recorded from each atrial appendage and by the persistence of electrical activity in tissue in close proximity to the sinus node at a time when action potentials cannot be recorded from either appendage. This effect on conduction occurs with higher doses of aconitine than are required to cause atrial tachycardia and can be

reversed by washing out the aconitine or by acetylcholine. The ability of acetylcholine to facilitate intra-atrial conduction when intra-atrial block is present has long been recognized (Lewis and Drury, 1923; Drury, 1925-26).

When atria depressed by aconitine were reactivated by acetylcholine, the electrical activity and contractions were irregular. It is evident that aconitine has a disproportionate depressant effect on the ability of atrial cells to conduct impulses, as shown by notching of the action potentials and the appearance of action potentials from one part of the atria when they are not being recorded from another part. Therefore, the arrested myocardium can be considered to be comprised of cells which have been depressed to varying degrees by aconitine. It would be expected that myocardial cells so depressed would vary at any one instance in their ability to propagate an impulse after the addition of acetylcholine. Under these conditions, re-entry of impulses is favoured. Furthermore, because of the marked acceleration of the repolarization process due to acetylcholine (Brooks, Hoffman, Suckling, and Orias, 1955; Hoffman and Suckling, 1953), the myocardial units would be capable of responding to greater rates of stimulation, therefore electrograms consisting of irregular action potentials could be expected.

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