Br. J. Pharmac. (1979), **67**, 3–12

EXCITATORY ACTIONS OF ACETYLCHOLINE IN THE HEART

J.H. BURN

J.H. Burn went to Cambridge in 1909 to read natural sciences and afterwards spent a brief period with H.H. Dale at the Wellcome Physiological Research Laboratory, before enlisting in the army in 1914. In December 1917 he started his medical training at Guy's Hospital and then rejoined Dale at the National Institute for Medical Research in 1920. Early researches with Dale convinced him of the importance of bioassay, and he continued with these problems when be became the first director of the Pharmaceutical Society's pharmacological laboratory in 1926. In 1937 Burn became Professor of Pharmacology in the University of Oxford, retiring from the Chair in 1959. Many pharmacologists were to take their first steps in research with Burn during this period, with an emphasis on the acquisition of practical skills. A delightful account of this period has been given by Burn himself (Burn, 1969).

Burn was a member of the British Pharmacological Society from 1931–1959, and was elected to honorary membership in 1960. From 1934–1945 he acted as the Society's secretary and treasurer, and from 1947–1958 as its foreign secretary. He has been a trustee since 1965. In December 1978 he was awarded the first Wellcome Gold Medal for his contributions to pharmacology. What follows is an account of a review lecture he gave to the staff and students of the Department of Pharmacology in Cambridge on November 15, 1978.

Most pharmacologists give the credit to Otto Loewi for the discovery that nerves can transmit their impulses chemically. In 1921 Loewi used the preparation of the frog heart introduced by Straub, in which, when the ventricles contract, the Ringer solution which they contain is driven into the aorta, and when the ventricles relax, the Ringer solution returns to the ventricles. Loewi discovered that when the vagus to the heart was stimulated, not only was the heart rate slower, but also when the Ringer solution was transfered to another heart, the beats of this heart were also slowed. Loewi concluded that the vagus, when stimulated, released a substance which had the power of slowing the heart.

However in 1907, which was 14 years earlier, W.E. Dixon, working in Cambridge, stimulated the vagi in pithed animals for 30 min and then made an extract of the heart. The extract inhibited the frog's heart, and the inhibitory effect was abolished by atropine. Dixon then went on to make the fundamental observation that 'hearts treated in an identical manner, but in which the vagus nerve had not been excited, also gave a supply of this inhibitory substance, but in a smaller degree than in the excited heart' (see Burn, 1956a). It was, however, Loewi who showed that the inhibitory substance was acetylcholine (ACh) and he demonstrated his finding on every day of the Physiological Congress in Stockholm in 1926, after which there was no longer doubt expressed as to the truth of the discovery.

The work of Dixon might, however, have been forgotten if the Royal Society of Medicine in London had not set up a triennial Walter E. Dixon Memorial Lecture to recall the contribution which he first made. Moreover it provided a strong suggestion that ACh was not only produced in the heart by the vagus.

Excitatory actions of acetylcholine on isolated auricles

It was in 1949 that the first indication was obtained that the effect of ACh in cardiac muscle was not always inhibitory (Burn & Vane, 1949). J.R. Vane was studying the properties of an antimalarial compound called Paludrine which had been prepared by Curd, Davey & Rose. The compound, being a derivative of biguanide, is now known as proguanil. Vane tested its action on the contractions of rabbit auricles. When 100 μg ACh was added to a 50 ml bath containing the auricles, the contractions were decreased in size, but recovered on washing out the ACh. The addition of 4 mg proguanil reduced the contractions to half their size, and ACh then had only a slight effect in decreasing them further. After exposure to proguanil for 65 min, the contractions stopped, and did not start again when proguanil was removed. Vane then made the striking discovery that the addition of 100 μg ACh was effective in causing a vigorous resumption of the beats. When ACh was removed, the beats stopped once more. Later, beats started again when 30 μg ACh was added. The results are shown in Figure 1. This was the first occasion when ACh was shown to have an excitatory action.

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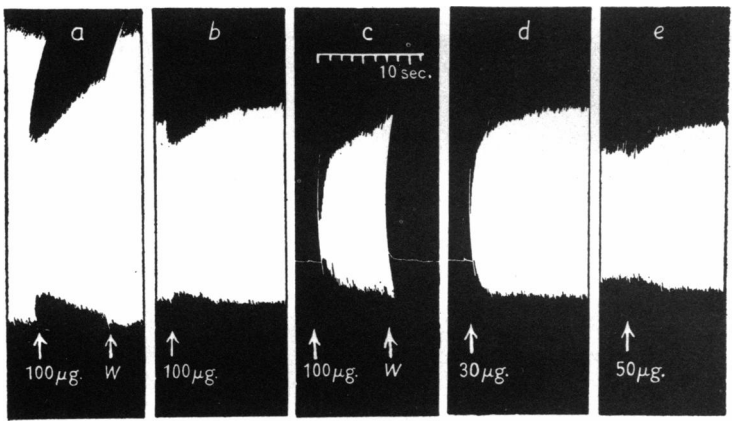

Figure 1 (a) Spontaneous contractions of rabbit auricles showing inhibitory effect of adding 100 μg acetylcholine (ACh) to bath (50 ml). At W, fluid changed in bath. (b) Record taken 20 min after addition of 4 mg proguanil to bath; inhibitory action of 100 μg ACh was greatly reduced. (c) After exposure to proguanil for 65 min the contractions stopped and remained absent when proguanil was removed; they started again when 100 μg ACh was added to the bath, and stopped again when it was removed. (d) The contractions were restarted by adding 30 μg ACh which was left to act for 10 min before it was removed. (e) The contractions continued at smaller amplitude, but were augmented by 50 μg ACh. (From Burn & Vane, 1949; reproduced by permission of *J. Physiol.*)

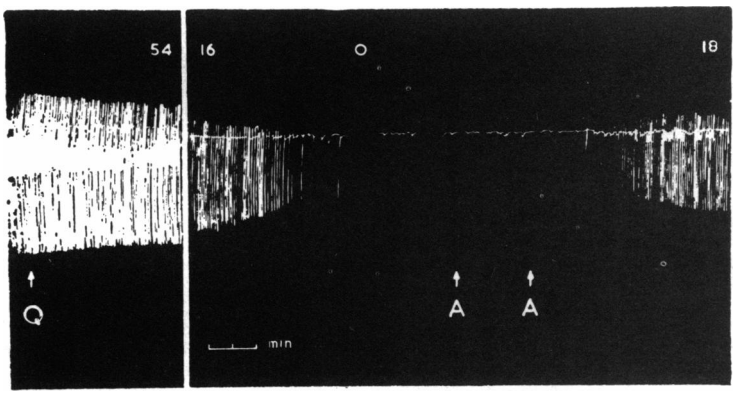

Figure 2 Contractions of rabbit auricles in 35 ml bath. At Q, 0.5 mg quinidine sulphate was added which caused arrest of contractions in 30 min. At A, 30 μg acetylcholine (ACh) was added. Figures above the record are rates of beating. (From Briscoe & Burn, 1954a.)

Stimulant effect of acetylcholine in the presence of quinidine

In a search for an explanation of the reversal of the action of ACh by proguanil, experiments were performed some years later to see if quinidine would act in the same way (Briscoe & Burn, 1954) (Figure 2). Again the contractions declined and stopped and again ACh caused them to start again. When quinidine had caused the atria to stop, they started again when the amount of K^+ in the bath fluid was reduced from 5.6 mM to 1.4 mM. This suggested that the arrest of the atria by quinidine might be due to a diminished permeability of the cell membrane to K^+, so that in the presence of quinidine, the K^+, which must escape through the cell membrane after a contraction in order to repolarize the membrane, could not escape. It could escape when the gradient of K^+ concentration across the membrane was made steeper by reducing the external K^+. This suggestion at once explained the action of ACh in restarting the contractions stopped by quinidine, because, as Harris & Hut-

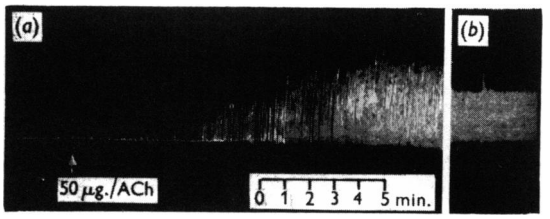

Figure 3 Isolated auricles of rabbit. Tyrode, bath 75 ml, 28°C. Auricles suspended in bath on previous day; the record begins 1 h after the beat stopped. (a) Addition of 50 μg acetylcholine (ACh) starts the beat again; (b) 1 h later. (From Bülbring & Burn, 1949; reproduced by permission of *J. Physiol.*)

ter (1956) showed, ACh increases the permeability of the cell membrane for K^+, so that the exit of K^+ is facilitated and repolarization is once more achieved.

Stimulant effect of acetylcholine in the absence of drugs

Experiments were carried out by Dr Edith Bülbring in which the isolated auricles were suspended in a bath of Tyrode solution at 28°C aerated by a mixture of 95% O_2 and 5% CO_2, and were allowed to contract until the contractions finally stopped (Figure 3). The contractions continued during the first day but sometimes stopped during the night, when the temperature fell to that of the room, to begin again when the temperature was raised to 28°C the next morning. Many preparations ceased to beat after 20 to 30 h, but some continued for 48 h. Sometimes the beat became gradually smaller and smaller, while in other experiments it stopped abruptly. Sometimes the beat became irregular and periods of arrest alternated with periods of regular activity before final arrest.

The addition of ACh to the bath was found to restart the contractions in about two-thirds of the experiments (Figure 3, Bülbring & Burn, 1949). In this experiment the beat had ceased for 1 h; when the ACh was added (1 μg/ml) the beat began after 2 min. The arrest of the atria in this case was shown by Goodford (1959) to be due to a steady fall in the intracellular K^+, so that the gradient from inside to outside again became insufficiently steep for repolarization. But when ACh was added, the permeability for K^+ was increased and repolarization was once more achieved. The atria resumed their contractions.

The synthesis of acetylcholine by the auricles

In 1946 Comline had shown that when the auricles of the rabbit heart are treated with ice-cold acetone and converted to an acetone-dried powder, they contain a system which acetylates choline and forms ACh in amounts up to 90 μg g^{-1} h^{-1}. ACh synthesis was therefore measured by the method described by Feldberg & Mann (1946) for brain tissue in which the ACh formed from choline in the presence of ATP (to supply energy) and of citrate (to supply acetyl groups) was estimated.

When acetone-dried powder from 32 freshly excised auricles was examined, it was found that 1 g powder synthesized an amount of ACh varying from 20 to 75 μg in 75 min, the mean figure being 40.2. Similar observations made on auricles kept in an organ bath until they ceased to contract, gave a mean figure of 14.9. Restarted auricles gave a mean figure of 37.0. No connection was observed between the concentration required and the length of time since the beat stopped. The way in which the beat started however was related to the duration of the arrest. If the period of arrest was only 5 to 15 min, the auricles resumed a regular beat at once; if the period was 1 h or more, the auricles began by beating irregularly.

These, and further results showed that there appeared to be a correspondence between the synthesizing power and the functional state of the auricle, and the results furnished a basis for the view that ACh synthesis played an important part in maintaining the cardiac rhythm.

Electrical changes in auricles on cooling

Jean Marshall & Vaughan Williams (1955) obtained new evidence for the excitatory action of ACh. They recorded the tension in the auricles by means of a transducer, and recorded the action potential of the left auricle through a pair of electrodes placed near the tip; a second pair of electrodes was placed at a point in the right auricle which acted as a pacemaker. The solution flowing through the bath was then cooled. When the temperature dropped to 20°C or less, both the electrical and the mechanical activity ceased. In the majority of experiments, however, there remained very small action potentials at the pacemaker which were not propagated, and which caused no tension change.

Keeping the temperature constant, they added small amounts of ACh to the bath, and then observed that after a latent period, ordinary action potentials took off from the small pacemaker potentials, and these larger potentials were accompanied by changes of tension. After a further short interval these action potentials were propagated to the left auricle, so that normal activity of the auricles was restored. The effect persisted for some time after the ACh was washed out of the bath and then disappeared, leaving behind the small pacemaker potentials only.

This series of changes produced by ACh could be observed repeatedly even when the concentration of ACh required was, in some experiments, as low as 10^{-9} M.

Philpot & Vaughan Williams compared the synthesizing power of tissue taken from the pacemaker region with that from other parts of the same auricle. They examined 15 auricles in this way and found that the choline acetylase activity of the pacemaker tissue was greater than that of the other parts. The mean result was that the pacemaker tissue was 2.7 times more active.

Experiments on the perfused rabbit heart

Other experiments furnished additional evidence for the original finding of Dixon in 1907. The isolated rabbit heart was perfused with a limited volume of Locke solution which contained eserine (4 μg/ml). As the perfusate left the heart it was collected below and returned by a stream of oxygen to the reservoir in the warming bath above the heart. Thus the same perfusing fluid was kept circulating through the heart for 40 min. The perfusate was then examined for ACh, by testing samples of the perfusate on the dorsal muscle of the leech. The perfusate was found to contain a substance which stimulated the leech muscle and was antagonized by tubocurarine. A further examination was made of the substance which stimulated the leech by tests on the frog heart, the cat blood pressure and the frog rectus (Briscoe & Burn, 1954b).

Perfused hearts driven electrically

In a series of experiments by Margaret Day the amount of active material was determined not only when the heart was beating spontaneously but also when it was driven by electrical stimuli applied near the apex of the ventricle (Table 1). The period of perfusion was always 40 min and the period of spontaneous beating preceded in one experiment and followed in the next, the period in which the heart was driven. The mean results of 10 experiments showed that when beating spontaneously the amount of ACh liberated was 0.27 μg (estimated on the frog heart) compared with 0.97 μg when driven electrically. Again the mean results of 10 experiments showed that when the estimations were made on the frog rectus, the amount of ACh liberated when the hearts were beating spontaneously was 0.44 μg, while when they were driven electrically the amount was 1.1 μg.

There was also evidence of the release of an adrenaline-like substance as well as ACh. In addition to adrenaline there was a larger amount of noradrenaline. Five extracts were mixed and chromatographed side by side with a mixture of both amines. In one experiment the activity corresponded to 1.4 μg noradrenaline and 0.24 μg adrenaline (Day, 1956).

Table 1 Amounts of active material liberated by isolated rabbit hearts during perfusion for 40 min expressed as μg acetylcholine (ACh)

When beating spontaneously Estimated on		*When driven electrically* Estimated on	
Heart	*Rectus*	*Heart*	*Rectus*
0.1	0.4	3.0	3.1
0.1	0.25	0.45	0.48
0.07	—	3.2	1.4
0.73	0.9	0.33	0.44
0.05	0.4	0.33	1.0
0.2	0.6	0.4	1.6
0.15	0.25	0.75	—
0.3	0.24	0.25	0.6
0.25	0.2	0.55	0.5
0.7	0.7	0.4	0.8
Mean 0.27	0.44	0.97	1.1

From Day (1956); reproduced by permission of *J. Physiol.*

Cholinesterase inhibitors in the heart-lung preparation

Since the isolated rabbit heart was found to liberate ACh, we expected that the heart-lung preparation of the dog would also liberate it, and that the addition of an inhibitor of cholinesterase to the blood would slow the rate. Burn & Walker (1954) found that this occurred, as illustrated by the effect of eserine seen in Figure 4. The fall in rate was from 118 per min to 32 per min. The concentration of eserine was 1.5×10^{-5} M, but smaller concentrations still caused a large fall. We found that the effect of inhibitors of cholinesterase in slowing the spontaneous heart rate were much greater than the effects of infusion of ACh.

The double action of the vagus

The evidence clearly shows that ACh can have two opposing effects, and it is not surprising that stimulation of the vagus can also have two opposing effects. Figure 5 shows an experiment carried out by Burn & Rand (1958) in which the isolated atria were set up with the right vagus nerve attached. When cooled to a temperature at which the spontaneous contractions stopped, one pulse applied to the vagus would not cause them to start again, but two pulses would do so. When the contractions had continued for 5 min, then without change of temperature, one pulse would arrest the contractions. After 5 min arrest, two pulses would start the contractions again, and so on. These observations were repeated no less than 30 times in the course of a single experiment.

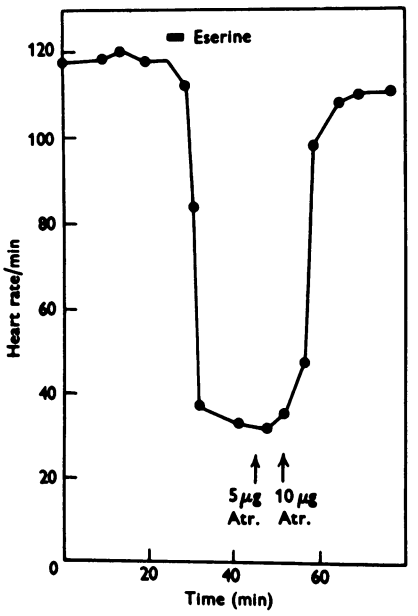

Figure 4 Heart-lung preparation of dog. The record of the rate shows the effect of adding eserine sulphate to the reservoir of blood so that the concentration was 1.5×10^{-5} M. The rate fell from 118 to 30 per min. When 5 and 10 μg atropine sulphate were added, the rate returned to about the initial value. (From Burn & Walker, 1954; reproduced by permission of *J. Physiol.*)

The action of anticholinesterases on heart block

Heart block is sometimes considered a disadvantage because it is taken as indicating a deficient output. In the heart-lung preparation of the dog the output can be measured directly and a study with this preparation showed that when the atria were contracting at a rate greater, for example, than 170/min the ventricular output fell, although the ventricles were still following each atrial beat. Only when the ventricles ceased to respond to each atrial beat, did the ventricular output rise.

Figure 6 shows the course of the fall in output resulting from the rise in ventricular rate as the ventricles followed the artia up to 285/min. The output at this rate was almost nil. When block began and the ventricular rate fell, the output promptly rose. In most experiments the output after the recovery was greatest when the block was 3:2, and diminished slightly as the block increased to 2:1.

After the observations in the top part of the figure were made, neostigmine was then added to the blood reservoir to make a concentration of 4.5×10^{-6} M. Some minutes later the stimulation was repeated, but as the figure shows in the middle part, block was now present at the lowest rate applied. Although the atria were driven as before up to 590/min, the ventricular rate did not rise above 160/min and the output remained nearly parallel to this rate.

Atropine was then injected and the various stimulations were applied for the third time. The changes in ventricular rate and output were now very similar to those in the top part of the figure. The addition of the cholinesterase inhibitors eserine, neostigmine, paraoxon and Nu 683 in molar concentrations from 10^{-6} to 10^{-5} M acted with great regularity in 21 experiments as described. But infusions of ACh did not act with regularity and were often ineffective in lowering the rate of stimulation at which block was seen (Burn, Vaughan Williams & Walker, 1955).

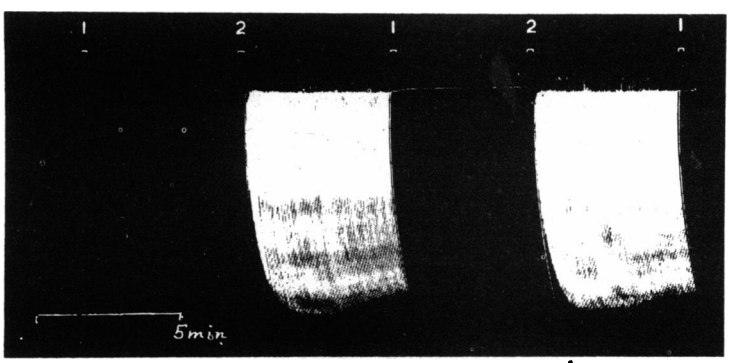

Figure 5 Experiment showing the double action of the vagus on isolated rabbit atria. When the bath temperature fell to 17°C the contractions stopped. At '1', a single pulse was applied to the vagus. It had no effect. Then, at '2', two pulses at 1 s interval were applied, and the contractions then began. After 5 min, one pulse was applied to the vagus and the contractions stopped. After 5 min more, two pulses were applied and the contractions began again, to be arrested 5 min later by one pulse. These effects were repeated 30 times. The rabbit had been given reserpine. (From Burn & Rand, 1958; reproduced by permission of *J. Physiol.*)

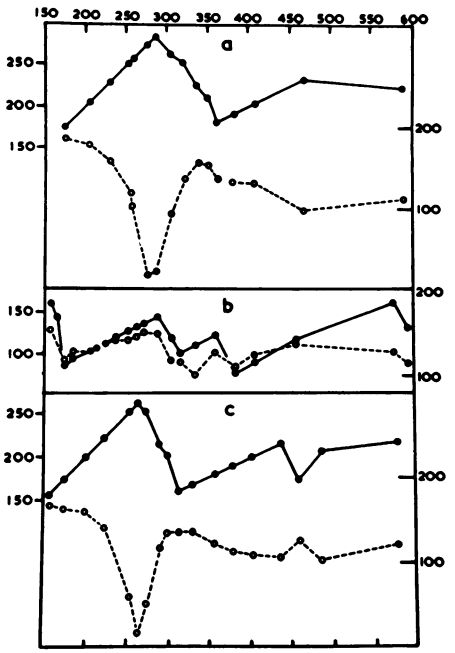

Figure 6 Heart-lung preparation. On the abscissa scale is the rate per min at which stimuli were applied to the right auricle. On the left hand ordinate scale is the rate per min of the ventricles. The relation of auricular rate to ventricular rate is shown as a continuous line. On the ordinate scale on the right hand side is the systemic output per 15 s. The relation of auricular rate to outflow is shown as a broken line. (a) Shows control observations. (b) Shows observations in the presence of neostigmine 4.5×10^{-6} M. (c) Shows observations after the injection of 50 μg atropine. (From Burn, Vaughan Williams & Walker, 1955; reproduced by permission of *Br. Heart J.*)

Fibrillation

In 1913 Mines cut out a ring of muscle from the auricles and ventricles of the heart of a tortoise. The ring did not include the pacemaker, and Mines saw that after a few stimuli, a contraction began which travelled round and round the ring.

In 1920 Sir Thomas Lewis, being influenced by this work, obtained results in the dog which led him to put forward the theory that fibrillation resulted from a wave of excitation travelling continuously around the base of the great veins at a sufficiently slow rate to find the tissue in front of it always excitable. This wave Lewis described as a circus movement. In 1921 Lewis, Drury & Bulger made the additional observation to the work of Mines, that a high rate of stimulation not only diminishes the conduction velocity but shortens the refractory period as well.

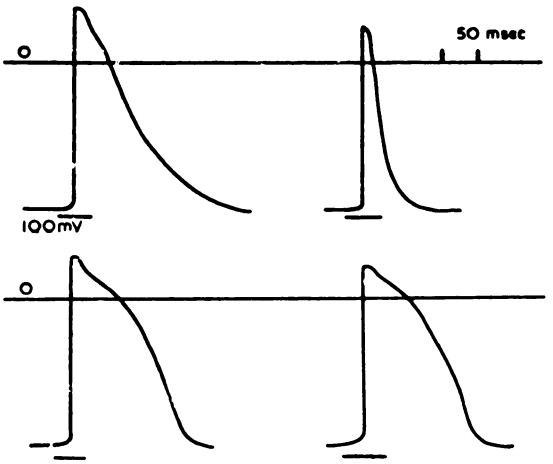

Figure 7 To show the effect of acetylcholine (ACh) on the action potential. The upper records are from the atria and the lower records from the ventricles. Records on the left are control records; those on the right are in the presence of ACh. (Drawn from records of Hoffman & Suckling (1953) and reproduced by permission of *Am. J. Physiol.*)

The electrical changes which occur in the heart are shown in Figure 7, which are records made by Hoffman & Suckling (1953). They show the action potentials of the atria (above) and of the ventricles (below). On the left are control records, and on the right records in the presence of acetylcholine which greatly shortens the duration of the action potential in the atria, but has no effect on the duration of the action potential in the ventricles.

Atrial fibrillation

An important contribution to the observations on atrial fibrillation was made by Andrus & Carter (1930). They showed that during vagal stimulation of the dog heart, fibrillation could be produced by a single shock applied early in the relative refractory period following a preceding contraction. About 10 years later Wegria & Wiggers (1940) produced ventricular fibrillation by one localized induction shock also applied during the relative refractory period. They called the brief time in which this was possible the vulnerable period; it coincided with the rapid fall of threshold during the period of returning excitability.

Experiments on the heart-lung preparation of the dog In one of our experiments on the heart-lung preparation we found that the atria were fibrillating. We had given ACh by intravenous infusion, and this had produced a slight fall in blood pressure, but no

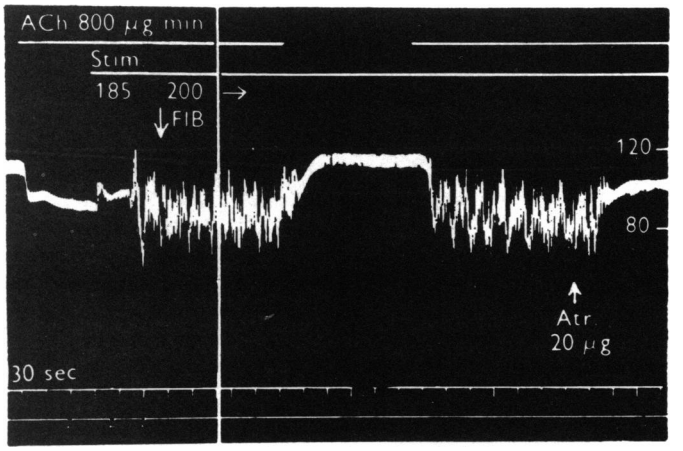

Figure 8 Dog heart-lung preparation. Blood pressure in aorta. At first the effect of infusing acetylcholine (ACh) into the superior vena cava at a rate of 800 μg/min caused a slight fall in blood pressure. Then stimulation was applied to the right auricle at the rate of 185/min and then at the rate of 200/min; this rate caused fibrillation as shown by the ECG. The fibrillation continued for 15 min until the ACh infusion was stopped. The fibrillation started again when the ACh infusion was turned on. The injection of atropine (Atr.) stopped the fibrillation. (From Burn, 1956b).

other change. Next we stimulated the right atrium at 200 per min, and to our surprise auricular fibrillation began and persisted. We prepared a burette containing ACh, and we could drive the solution into the superior vena cava at a slow uniform rate, often at 1 mg per min. The electrodes were applied to the tip of the right auricle and were held in place by a spring and did not pierce or damage the auricle. In these conditions there was no leakage of blood from the system and the concentration of any substance introduced into the blood was known. Each dog was anaesthetized in a roomy box into which ether was pumped, and was then injected with a solution of chloralose intravenously.

As Figure 8 shows, the infusion of ACh by itself did not cause fibrillation, but when stimulation was applied fibrillation began. Then when ACh was no longer infused, the fibrillation stopped although the stimulation continued. Later when the infusion of ACh was resumed during the stimulation, the fibrillation began again. It was arrested by the injection of atropine.

We had discovered that auricular fibrillation could be regularly produced by stimulating the atrium during the infusion of ACh. The stimulus necessary could be very brief. Thus in one experiment in which the ECG was recorded and the shocks given were simultaneously recorded, we found that the fibrillation began with the seventh shock.

Earlier workers had produced auricular fibrillation by rapid stimulation alone without infusion of acetylcholine. Thus Rothberger & Winterberg had done this in 1910, and Lewis, Feil & Stroud had done it in 1920. We ourselves also did this when we used fish-hook electrodes which pierced the auricle, but after changing to electrodes which did not damage the auricle, we failed, in the absence of ACh to produce fibrillation at rates as high as 1200 per min. The combination of acetylcholine infused at a steady rate intravenously with very brief stimulation was always successful, as we found in 11 consecutive experiments in some of which fibrillation was produced and arrested several times. The amount of acetylcholine infused varied from 40 μg/min to 3.2 mg/min.

Cessation of fibrillation The cessation of fibrillation when the infusion of ACh is stopped is shown in Figure 9. In (a) the ECG shows the fibrillation of the atria and the occurrence of 3 ventricular contractions. In (b) the infusion was arrested and the fibrillation of the atria became more like flutter; the number of ventricular contractions increased. Finally in (c) the normal rhythm returned.

Means of arresting fibrillation in the artia It is evident that the effect of ACh upon the action potential is to accelerate the rate of repolarization, that is to make the downstroke much steeper. Burgen & Terroux (1953) suggested that the action of ACh is to make the membrane more permeable to potassium, since the downstroke is determined by exit of potassium from the cell. Hence the maintenance of atrial

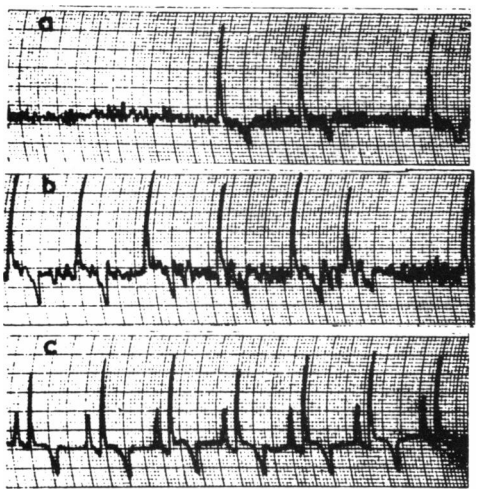

Figure 9 ECG of heart-lung preparation. (a) Shows auricular fibrillation produced by stimulation at 190 per min in the presence of eserine 2×10^{-6} M and during the infusion of acetylcholine (ACh) at 3.5 μg per min. The stimulation was stopped but the fibrillation persisted so long as the infusion of ACh continued. (b) Shows that after the infusion of ACh was stopped, the fibrillation became more like flutter and the number of ventricular complexes increased. (c) Shows the return to normal rhythm.

fibrillation appears to be linked to the diminution in membrane resistance to potassium, so that there is an increased rate of loss of potassium.

In a series of experiments in which we established atrial fibrillation in the dog heart-lung preparation, we infused a solution of KCl slowly at a constant rate. We found that this infusion arrested the fibrillation after amounts from 50 to 410 mg had entered the blood, the mean amount being 227 mg. Since the system contained 1 litre of blood, the concentration was raised from 5 to 8 mEq/l. Not only was fibrillation arrested in spite of continuous infusion of ACh but further attempts to restart fibrillation all failed.

Adenosine triphosphate Another substance which may arrest fibrillation is adenosine triphosphate, as Figure 10 shows, though we have not found records of its action in patients. In an experiment in which a heart was fibrillating in a solution containing normal concentrations of potassium and calcium, the addition of adenosine triphosphate in a concentration of 2.5×10^{-5} M converted fibrillation to tachycardia; when the solution was changed to one without ATP, the fibrillation returned. But with a higher concentration of ATP (6.4×10^{-5} M) fibrillation reverted to a normal rhythm. These changes were repeated twice more with the same results. In Figure 10 the fibrillation had continued for 17.5 min before 0.1 mg ATP

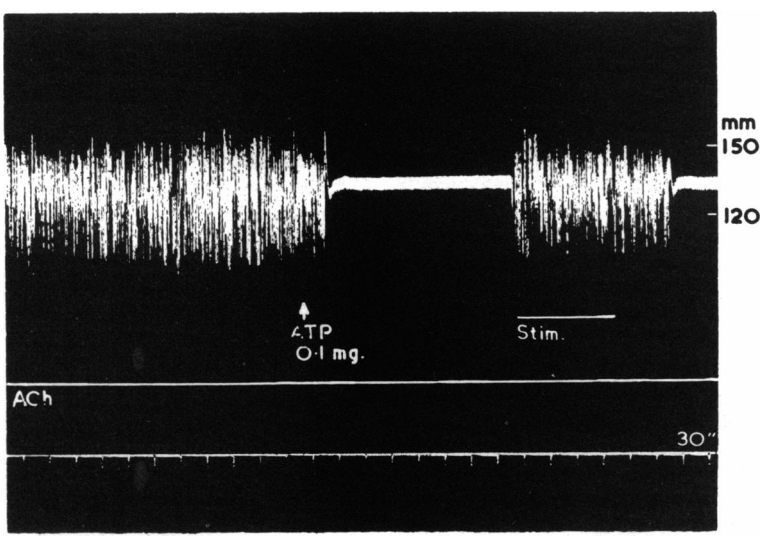

Figure 10 To show the effect of adenosine triphosphate (ATP) in stopping atrial fibrillation. Infusion of acetylcholine (ACh) at 0.6 mg/min at 11 h 39 min. Stimulation at 11 h 41 min to 11 h 43 min caused fibrillation. The record begins at 11 h 55 min, showing the continuance of fibrillation. At 12 h 01 min, 0.1 mg ATP was injected and the fibrillation was arrested within 30 s although the infusion of ACh continued. When stimulation was applied again for 2 min, fibrillation began again but stopped within 1 min after the stimulation ended. (From Burn, 1956b).

was injected. It arrested the fibrillation; when stimulation was repeated 4 min later, the fibrillation lasted for only 1 min after the stimulation stopped.

It is worth recording that similar experiments were performed without difficulty in rabbit atria. When these were isolated from other tissue and stimulated electrically they were found to fibrillate provided that ACh was present in the bathing fluid. But this fibrillation was observed only when the concentration of K^+ was reduced to one quarter of normal, and when the temperature was 37°C. When the temperature was reduced to 27°C, the fibrillation was arrested but returned at 37°C.

Ventricular fibrillation

It might be expected that since ACh is so important a factor in atrial fibrillation, it would be equally important in ventricular fibrillation. This is not so. Observations were made on rabbit isolated hearts perfused through the aorta, the fluid passing through the coronary vessels. Platinum electrodes were hooked into the left ventricle, and the perfusion was carried out at 34°C.

It was found: (1) that stimulation while ACh was added to the perfusion fluid did not cause fibrillation. (2) That removal of glucose from the perfusion fluid caused fibrillation. This sometimes happened after stimulation, but sometimes spontaneously without stimulation. In both cases fibrillation was arrested when glucose was replaced in the perfusion fluid. (3) That addition of the following metabolic inhibitors to the perfusion fluid caused fibrillation on stimulation: dinitrophenol, sodium azide and monoiodoacetate. The fibrillation produced by these substances was persistent, but was reversible in the case of dinitrophenol and of sodium azide when they were removed from the perfusion fluid. These metabolic inhibitors have been shown to shorten the action potential. (4) That diminishing the oxygen supply caused fibrillation. In these experiments the Tyrode solution was gassed with 95% O_2 and 5% CO_2. A series of hearts were first stimulated using this mixture, and then each heart was gassed with a mixture containing 47.5% O_2 and 47.5% N_2 and was then stimulated again. Stimulation with the second mixture produced fibrillation, whereas stimulation with the first mixture did not produce fibrillation. (5) That reducing the K concentration in the perfusing fluid resulted in hearts fibrillating on stimulation only.

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(*Received January 15, 1979.*)