J. Physiol. (1949) 108, 104–115 547·435·292

THE RELATION BETWEEN THE MOTOR AND INHIBITOR ACTIONS OF ACETYLCHOLINE

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(*Received* 22 *March* 1948)

Recently different workers have recorded the stimulant action of acetylcholine in cardiac tissue. Sawaya (1939), working on the heart of the crustacean *Callinectes danae*, observed that acetylcholine increased the heart rate, and that acetylcholine is present in the heart of this species. Hoffmann, Hoffmann, Middleton & Talesnik (1945) have shown that the isolated cat heart, after treatment with atropine, responds to acetylcholine in a manner similar to that in which it responds to adrenaline. They observed that the increased contractions were accompanied by the appearance of an adrenaline-like substance in the perfusate. The perfusate relaxed the isolated intestine and the hen's rectal caecum, while it stimulated the frog heart. This observation has been confirmed by McNamara, Krop & McKay (1948). Hoffmann *et al.* found that nicotine abolished the stimulating action of acetylcholine; they concluded that acetylcholine acted on either ganglia or chromaffin tissue liberating an adrenaline-like substance which was responsible for the acceleration. McDowall (1946) also observed that acetylcholine stimulated the cat heart after atropine, and recorded a brief stimulant action of very small doses of acetylcholine in the heart not treated with atropine. In cat hearts in which the A.V. bundle was not divided, acetylcholine caused increased contraction of the ventricle. McDowall explained the stimulating action of acetylcholine as being in the main an effect on the ventricle in which it increased the force of contraction.

Observations of a reversed action of the vagus on the heart have been made by several observers. In 1910 Dale, Laidlaw & Symons described an increase of heart rate in the cat caused by vagal stimulation after the administration of tropine, nicotine, hordenine methiodide or curare. They observed that when the stimulation stopped, the rate dropped, giving the impression that the usual inhibitory effect was delayed. They were uncertain whether the phenomenon was due to a reversal of the function of fibres normally inhibitory in effect, or to the presence in the vagus of accelerator fibres which normally were masked.

In the course of examining the pharmacological action of the antimalarial substance known as paludrine (N′-*p*-chlorophenyl-N^5-*iso*propyl biguanide), its effect on the heart was determined. Under its influence cardiac tissue was found to be so modified that doses of acetylcholine, which previously produced inhibition, produced stimulation. The circumstances of this change led to observations on smooth muscle also, which indicate a relation between the motor and inhibitor effects which acetylcholine produces.

Results

Isolated rabbit auricles. The auricles of the rabbit heart when dissected free from other tissue contract spontaneously for several hours in well-oxygenated Ringer-Locke solution. They maintain activity better at 29° than at 37° C.

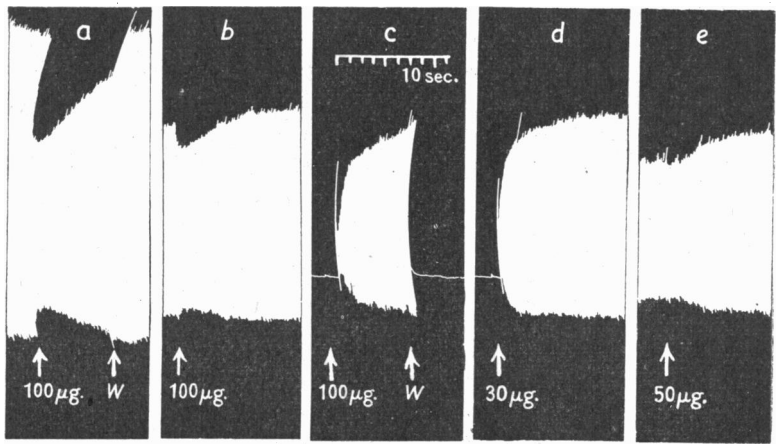

Fig. 1. (*a*) Spontaneous contractions of rabbit auricle, showing inhibitory effect of adding 100 μg. acetylcholine to bath (50 ml.). At *W*, fluid changed in bath. (*b*) Record taken 20 min. after addition of 4 mg. paludrine to bath; inhibitory action of 100 μg. acetylcholine was greatly reduced. (*c*) Exposure to paludrine for 65 min. has stopped the contractions; they are restarted by addition of 100 μg. acetylcholine, and stop again when this is removed. (*d*) The contractions are restarted by 30 μg. acetylcholine; this was left to act for 10 min. before it was removed. (*e*) The contractions continued, and were augmented by 50 μg. acetylcholine.

When the contractions were recorded as in Fig. 1*a*, the effect of adding acetylcholine to the bath was to reduce the amplitude and the rate (in this experiment from 92 to 72 per min.). The amplitude slowly returned towards its original height, which was fully regained when the bath was washed out with fresh Ringer-Locke solution. Paludrine was then added to the bath (4 mg. in 50 ml.), with the result that the amplitude and frequency of the contractions slowly declined, and after 20 min. became as shown in Fig. 1*b*. Addition of acetylcholine at this point had much less inhibitory action, and indeed the inhibition gave place to

a small increase in amplitude. There was no change of rate. The bath was emptied and refilled with Ringer-Locke solution containing the same amount of paludrine, and the decline in amplitude continued until the auricles stopped 65 min. after first being exposed to it. The paludrine was then removed and the bath refilled with Ringer-Locke solution. The auricles remained still during the next 40 min. until acetylcholine was added to the bath in a dose of 100 μg., the resulting concentration being 2×10^{-6}, when the auricles began to beat again after a latent period of 90 sec. (see Fig. 1*c*). The beat continued while the acetylcholine remained in the bath, but it ceased promptly when the solution in the bath was changed to fresh Ringer-Locke. The beat began a second time when 30 μg. acetylcholine was added (Fig. 1*d*), and the auricles were then left in this solution for 10 min. On washing out after this longer period the beat persisted, though with smaller amplitude than in the presence of acetylcholine. Addition of 50 μg. acetylcholine (Fig. 1*e*) augmented the amplitude, but did not alter the rate.

The arrest of the natural contractions by paludrine was usually abrupt, as shown in Fig. 2*c*, though sometimes it was gradual, the amplitude steadily diminishing to zero. When the spontaneous contractions were restarted by the addition of acetylcholine, the first contraction was usually large; this can be seen in Fig. 1*c*. The subsequent contractions then started with a small amplitude and steadily increased.

Transition from inhibition to stimulation by acetylcholine. The experiment described shows that under the influence of paludrine the spontaneous contractions of the auricles slowly decline until they cease altogether, and that the auricles are then in a condition in which the effect of acetylcholine is to stimulate them. Acetylcholine will cause resumption of contractions, and, when the contractions are resumed, will augment their amplitude. It is important to follow the gradual change in the response to acetylcholine. When paludrine first affects the auricles, the inhibition produced by a given dose of acetylcholine becomes smaller; a point is then reached where there is a transient inhibition followed by augmentation, as illustrated in Fig. 2*a* and *b*. The augmentation became the dominant feature of the effect, and preliminary inhibition was seen only when a large concentration of acetylcholine was applied. Thus there was no sudden change from inhibition to augmentation; at first both small doses and large produced inhibition proportional to the size of the dose; the inhibitory effects then became smaller and preceded a phase of augmentation; later still the smaller doses produced simple augmentation, and the larger doses transient inhibition followed by augmentation. When, under the continued influence of paludrine, the auricles stopped altogether, acetylcholine restarted the contractions.

Transition from stimulation to inhibition. The reverse change from a stimulating to an inhibiting effect can be observed in preparations in which the

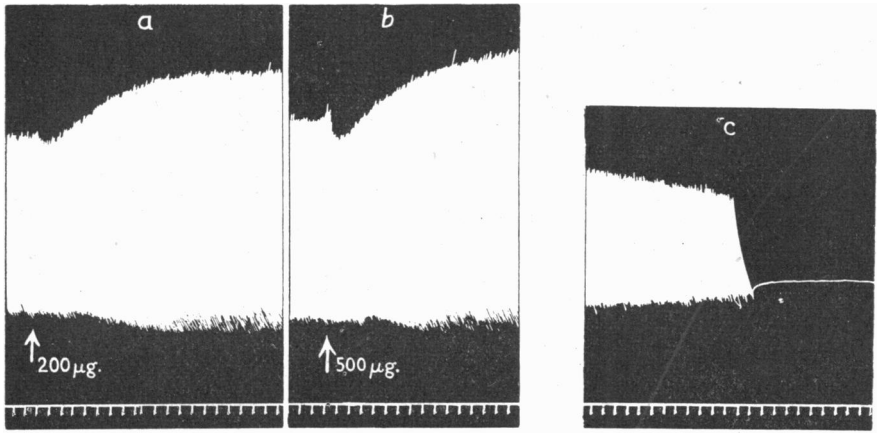

Fig. 2. (*a*) Auricles were exposed to paludrine; the spontaneous contractions diminished until at the point shown 200 μg. acetylcholine caused the rate to drop from 64 to 60 per min., but the amplitude to increase. (*b*) The addition of 500 μg. acetylcholine caused an initial diminution followed by augmentation of amplitude without change of rate. (*c*) The abrupt arrest of the auricles by paludrine. The rate fell from 44 to 40 to 36 per min. before the stop. Time 10 sec.

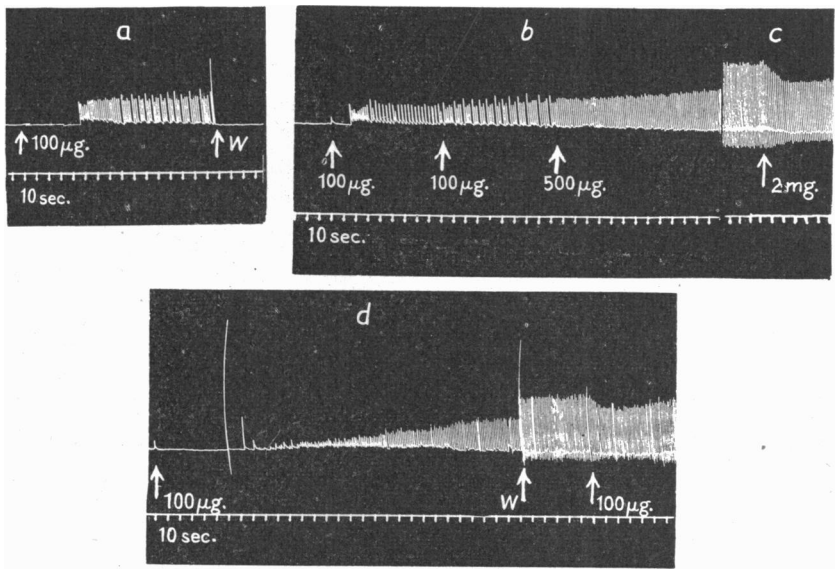

Fig. 3. (*a*) Auricles were arrested by exposure to paludrine. After remova of paludrine they were restarted by addition of 100 μg. acetylcholine. They stopped when it was washed out. (*b*) Auricles restarted by 100 μg. acetylcholine; the rate and amplitude were increased by a further addition of 100 μg. acetylcholine, and still more by 500 μg. acetylcholine. (*c*) When the amplitude increased further, the addition of 2 mg. acetylcholine caused diminution and slowing. (*d*) In another experiment the auricles were restarted by 100 μg. acetylcholine; this was removed by washing out at *W*. The beat continued for 12 min., and then a further addition of 100 μg. caused inhibition.

spontaneous contractions have been first stopped by paludrine. For example Fig. 3*a* shows a resumption of contractions when 100 μg. acetylcholine was added to the bath after the auricles had been still for 1 hr. When the acetylcholine was washed out, the contractions stopped again. Fig. 3*b* shows further resumption of contractions when acetylcholine was added and a steady increase in their rate, regularity and amplitude, as the concentration of acetylcholine in the bath was increased. After 10 min. the bath was washed out and the auricles then continued to beat as shown in Fig. 3*c*. At this point, addition of 2 mg. acetylcholine caused the ordinary inhibitory response. Thus in this experiment smaller doses restored spontaneous contractions and increased them, but when they were established a larger dose caused inhibition.

In another similar experiment (Fig. 3*d*) the change from a stimulant to an inhibiting action was observed without increase of dose. The quiescent auricles began to beat when 100 μg. acetylcholine was added to the bath. The beat steadily increased in amplitude, and after 12 min., when the bath was changed, the auricles continued to beat at the rate of 52 per min. When 100 μg. acetylcholine was added, the effect was to diminish the amplitude.

Removal of paludrine. During the observations described, paludrine was not added to the bath after the auricles had once stopped. It was therefore evident that paludrine would diffuse out of the tissue so that the contractions might resume without the addition of acetylcholine. This was tested by washing out the bath several times after the auricles had stopped; it was found that the auricles did in fact resume contractions. A series of observations was therefore made in which, from the moment the auricles ceased to beat, the bath was washed out at 10 min. intervals. The time until the auricles began to beat again was measured. This series was compared with another series in which acetylcholine was added to the bath 7 min. after each wash-out and left in contact

TABLE 1. Time elapsing until contractions resumed

Controls	100 μg. ACh added for 3 min.	250 μg. ACh added for 3 min.
41	20	20
54	34	37
70	49	43
73	55	46
76	58	47
80	60	59
81	—	—
200	—	—
Mean 84	46	42

for 3 min. The results are given in Table 1. The number of additions of acetylcholine necessary to restart the contractions varied from 2 to 6, and all 12 auricles to which these additions were made started within 60 min. When no acetylcholine was added so that the resumption of contractions was due to removal of paludrine, only 2 of the 8 auricles began to contract in this time.

Even when the high figure of 200 min. in the first column was excluded (though there was no reason for excluding it) the significance of the difference between the means in the first and third columns was 3·3. That acetylcholine was responsible for restarting the auricular contractions was in any case proved by the observations in which the contractions stopped when acetylcholine was washed out.

Electrical stimulation. When the auricles were arranged so that they could be stimulated electrically (Dawes, 1946), and when the contractions were arrested by the addition of paludrine to the bath, it was found that they contracted in response to stimulation, but the amplitude was small, being equal to the amplitude at the point when the spontaneous contractions ceased.

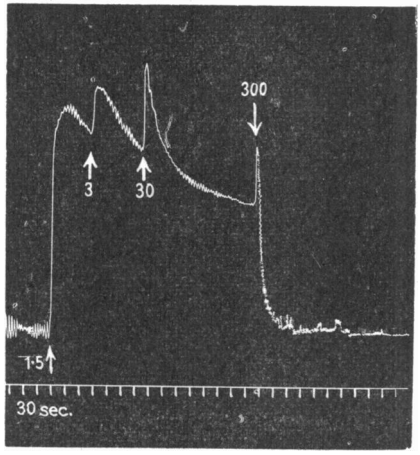

Fig. 4. Rabbit jejunum. Tyrode solution. Changes due to additions of acetylcholine to the bath, the figures being the amount in μg. per ml. of fluid in the bath present after each addition. 300 μg./ml. caused inhibition.

General properties of paludrine. The general properties of paludrine will be described by one of us (J.R.V.) elsewhere, but it may be remarked at this point that it depresses the action of acetylcholine in those tissues in which the action of acetylcholine is stimulant or motor. Thus paludrine depresses the stimulant action of acetylcholine on the isolated frog rectus and on the isolated guinea-pig intestine. Paludrine depresses the effect of vagal stimulation on the contractions of the cat's intestine. Thus a substance which depresses the stimulant action of acetylcholine in smooth muscle and in skeletal muscle was found to convert the inhibitory action of acetylcholine on the heart to a stimulant action by a gradual process. This suggested that a double effect of acetylcholine might be observed in other tissues, and that an inhibitory effect of acetylcholine in smooth muscle might be observed by increasing the concentration.

Observations on the intestine. Loops of rabbit intestine were isolated and suspended in a bath of Ringer-Locke, or of Tyrode solution aerated with oxygen + 5% CO_2. The observations, which were made in about 20 loops from different rabbits, are illustrated in Fig. 4. The addition of acetylcholine (the bromide was the salt used) to make a concentration of 1·5 μg./ml. caused contraction of the loop. Further additions of acetylcholine to raise the concentration by 3, then by 30 and finally by 300 μg./ml. were then made as shown. After the final addition there was a momentary contraction followed by inhibition almost to the base line. Some sign of an inhibition was also seen after raising the concentration by 30 μg./ml.

Observations on the uterus. Experiments were performed using one horn of the non-pregnant uterus of the rat suspended in Tyrode solution aerated with oxygen and 5% CO_2. An example of the results is shown in Fig. 5, in the first part of which acetylcholine added to produce a concentration of 12 μg./ml. caused a greater contraction than had occurred spontaneously before; the addition of a high concentration then caused inhibition. When the acetylcholine was removed, the uterus remained fairly quiescent, but (Fig. 5*b*) contracted when the concentration was raised to 12 μg./ml. As the contractions declined, the concentration was raised further by 100 μg./ml. and there were renewed contractions. The high concentration of 1000 μg./ml. then caused inhibition. After the acetylcholine was washed out, contractions were resumed. At this point, as shown in Fig. 5*c*, the addition of a concentration, namely 12 μg./ml., which previously caused contraction, now caused inhibition.

In other experiments it was observed that during the initial period of observation, the gradual raising of the concentration of acetylcholine in the bath even to 1000 μg./ml. did not cause inhibition. Thus additions were made of 25, 60, 250 and 1000 μg./ml. respectively. The first caused a sharp rise in tone and increased amplitude of rhythm; the succeeding additions caused a slight rise in tone, but there was no sign of inhibition. When the bath was washed out the uterus relaxed, and then resumed its rhythm. When now the concentration was raised to 125 μg./ml. followed after 8 min. by a further rise of 1000 μg./ml., the first caused stimulation, but the second caused an inhibition like that seen in Fig. 5*a*. The different effect of the high concentration of 1000 μg./ml. on the same muscle at the two times indicated in the first place that the inhibitory effect was not due to any change of tonicity or pH, and that it was facilitated by the previous exposure of the muscle to a high concentration of acetylcholine. This facilitation was repeatedly observed, as in Fig. 5*c*.

Observations on the aorta. The blood vessels are composed of tissue which is normally relaxed by acetylcholine. It is known that this is not the only response which can be obtained, for when the vessels of the rabbit ear are perfused with Ringer solution, after 24 hr. the injection of acetylcholine causes vasoconstriction. Observations were made on the aorta, spirals being cut from

the aorta of the freshly killed rabbit. These were suspended in a bath of Ringer-Locke solution and left for 24 hr. at room temperature, the lever being weighted

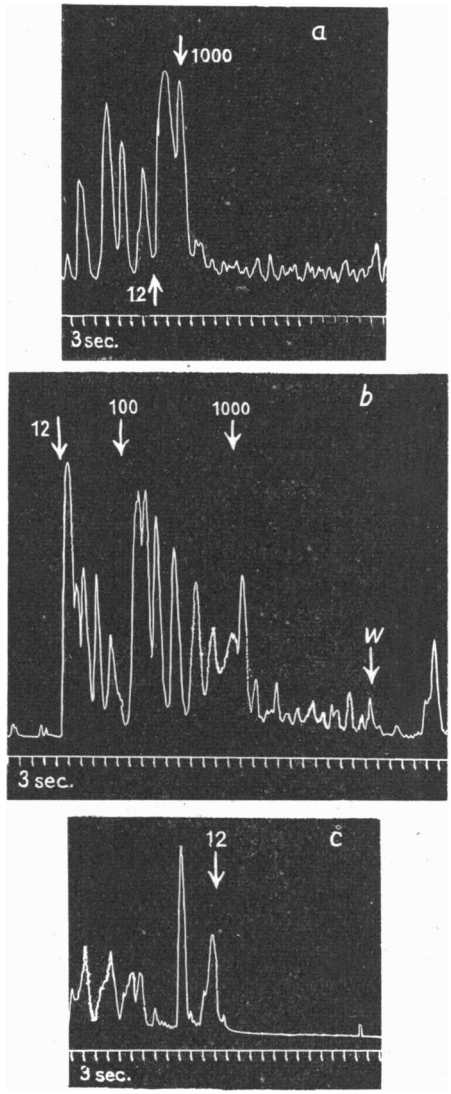

Fig. 5. Rat uterus. Tyrode solution. (*a*) Stimulation by addition of 12 μg./ml. acetylcholine. Inhibition by 1000 μg./ml. (*b*) Continuation of (*a*), showing stimulation by 12 and by 100 μg./ml. but inhibition by 1000 μg./ml. (*c*) Continuation of (*b*) showing inhibition by 12 μg./ml.

so as to stretch the spiral. The next day the bath was warmed to 37° and oxygenated. A concentration of 1 mg./ml. acetylcholine then regularly caused

contraction of the aorta. When the concentration in the bath was raised to 10 mg./ml., relaxation occurred, though it was not complete. The effect is illustrated in Fig. 6.

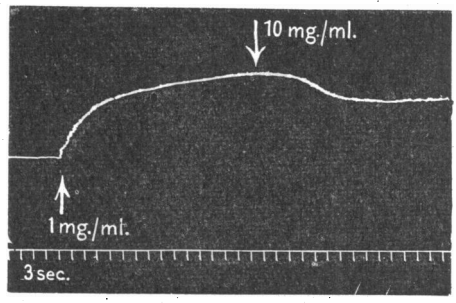

Fig. 6. Spiral cut from rabbit aorta, left in Ringer-Locke solution overnight until relaxed. Contraction caused by 1 mg./ml. acetylcholine; some relaxation by addition of 10 mg./ml.

Discussion

By exposing the isolated auricles of the rabbit to a constant concentration of paludrine we have been able to observe not only a change in the response to acetylcholine from inhibition to stimulation, but also that the change is gradual. It has occurred together with a gradual reduction in amplitude of the contractions, leading to complete arrest. When the auricles have thus stopped beating, we have observed that the addition of acetylcholine started the contractions once more, and that when the acetylcholine was removed, the contractions again stopped. At this point the presence of acetylcholine, so far from causing inhibition, initiated the automatic rhythm. In some experiments it was observed that each further addition of acetylcholine increased both the rate and the amplitude of the spontaneous contractions. Later, again when paludrine had diffused out of the auricular tissue, the normal inhibitory action returned, sometimes in response to a large dose, sometimes in response to a dose which shortly before had been used to restart the contractions.

Hitherto, in its relation to heart muscle, acetylcholine has been generally regarded as the transmitter of the inhibitory influence of the vagus nerve. Within the last few years, however, various observations have suggested that acetylcholine also has another quite different function in the heart. In his search for substitutes for quinidine Dawes (1946) pointed out that substances which prolong the refractory period, such as procaine, quinidine and quinine are substances which depress the action of acetylcholine in many different tissues. They reduce its effect on the rate and amplitude of heart muscle and on the movements of the isolated intestine. Now the refractory period is the time required for the muscle to reload its contractile mechanism, and as has been shown by Dawes

(1946) and studied quantitatively by de Elio (1947), the addition of acetylcholine shortens this time. From these observations there emerged the suggestion that acetylcholine is not merely concerned in the humoral transmission of vagal impulses, but is present in heart muscle, playing an important part in the processes responsible for spontaneous contraction. The observation of Comline (1946) that the auricles contain the enzyme system which synthesizes acetylcholine in relatively large amounts conforms with this suggestion.

Though our acceptance of this as a hypothesis sprang from the work of Dawes, and gained support from that of others in this department (Dews & Graham, 1946; de Elio, 1947, 1948; Stephenson, 1948), the hypothesis is not new. It was put forward by Abdon (1945) as a result of observations on the presence of an acetylcholine precursor in the heart of the rabbit. Abdon spoke of 'the general appearance of tissue acetylcholine in many places where it could not have the function of a humoral transmitter', and said that 'the breakdown and formation of precursor belong to the normal metabolism of cardiac muscle'.

The immediate difficulty of the hypothesis was to explain why, if acetylcholine played a part in promoting the cardiac rhythm, the ordinary effect of adding acetylcholine was to arrest it. Paludrine shared the properties of substances like quinidine, procaine and some others, of prolonging the refractory period of rabbit auricles when tested by Dawes's method, and also of diminishing the response of other tissues such as the frog rectus, the rabbit intestine and the rabbit auricles to acetylcholine. Since the application of paludrine was found to lead to arrest of contractions which, however, could be restarted by the addition of acetylcholine, we supposed that paludrine must gradually depress the response of the tissue to acetylcholine as it depressed that of the isolated intestine, so that the amount normally synthesized became increasingly ineffective, and finally incapable of causing contraction to take place. Under these conditions, in which the threshold for acetylcholine is raised, the addition of acetylcholine to the bath might have the effect of increasing the concentration in the tissue to this threshold and thereby producing a contraction.

How then were we to regard the normal inhibitory action of acetylcholine on the heart, which, when the heart was depressed by paludrine, gradually became an augmentation? Since the process was reversed when paludrine was slowly removed, we conceived the idea that acetylcholine when externally applied might have two actions in an excitable tissue with rhythmic activity in which acetylcholine synthesis was proceeding. If the amount synthesized was less than the amount to which the muscular elements were capable of responding, as in the normal intestine, or in auricular tissue rendered insensitive to acetylcholine by paludrine, then the added acetylcholine would cause stimulation. On the other hand, inhibition would follow the addition of acetylcholine if the amount synthesized was the full amount to which the muscle elements were capable of responding, as in the normal auricles, or if the tissue was saturated

by previous additions. This view was tested by examining the effect of acetylcholine on the intestine, and on the uterus. In both of these tissues the enzyme system for acetylcholine synthesis is present (for the uterus, see Reynolds, 1939, and Emmens, MacIntosh & Richter, 1943). It was found that in preparations in which the effect of low concentrations of acetylcholine was stimulation, the addition of excess of acetylcholine caused inhibition.

In the intestine inhibition was produced by 250–300 μg./ml. acetylcholine bromide. In some preparations of the uterus, concentrations as high as 1 mg./ml. did not at first cause inhibition, though a second exposure to this concentration did so; in other preparations, after one or two exposures to a high concentration, it was sufficient to put in the bath 12 μg./ml. to observe inhibition.

A few experiments were made on the smooth muscle of the blood vessels. In the body acetylcholine causes dilatation, that is to say, inhibition. We found that if a spiral of rabbit aorta was allowed to relax when suspended in a bath, concentrations of 1 mg./ml. caused contraction, and ten times this concentration caused inhibition. Since further observations are being made on blood vessels, discussion of this admittedly slender evidence will be omitted, except to say that it too gives support to the hypothesis. The point we believe to be new in the present work is the suggestion that the double action, stimulation in low concentration, inhibition in high, is the key to the difference in the effect of vagus stimulation on the heart and on the intestine. It implies that in a tissue such as the heart (and perhaps the blood vessels) in which the normal action of acetylcholine is inhibitory, there exists a mechanism in which the production of acetylcholine is a normal feature and in which that production is at such a level that the addition of more acetylcholine causes inhibition.

SUMMARY

1. The action of acetylcholine on isolated rabbit auricles is gradually changed by exposure to paludrine from an inhibitory action to a stimulant action. This change proceeds *pari passu* with a diminution in the size of the spontaneous contractions.

2. When exposure to paludrine is continued, the auricles are arrested, and if paludrine is removed they remain still for long periods. Spontaneous contractions are restarted by exposure to acetylcholine. They stop once more when acetylcholine is removed.

3. When the auricles are restarted by acetylcholine, further additions augment the rate and amplitude of contractions. As paludrine diffuses out of the tissue, a point is reached at which the inhibitory action of acetylcholine is seen once more.

4. The action of paludrine is to be explained in the light of the fact that it depresses the response of other tissues such as the frog rectus and rabbit intestine to acetylcholine, and lengthens the refractory period of the auricles.

5. When the isolated rabbit intestine is exposed to high concentrations of acetylcholine, the tone and rhythm is inhibited. This is also true of the rat uterus.

6. A hypothesis is put forward to relate the stimulant and inhibitor actions of acetylcholine.

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