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THE ACTION OF CHOLINE DERIVATIVES ON ISOLATED RABBIT AURICLES WHEN ARRESTED BY PALUDRINE

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The observation made by Sachs (1937), that minute doses of acetylcholine would stimulate the beat of isolated hearts, has now been repeated by many workers (Spadolini and Domini, 1940; Rothberger and Sachs, 1938; McDowall, 1946; Haney and Lindgren, 1945). Of these Spadolini and Domini were the first to postulate that this action was due to the liberation of an adrenaline-like substance from either ganglia or chromaffin tissue in the heart. Hoffmann *et al.* (1945) and McNamara, Krop, and McKay (1948) confirmed that an adrenaline-like substance is liberated from the heart muscle by acetylcholine.

In a previous paper from this laboratory (Burn and Vane, 1948), it was shown that if isolated rabbit's auricles were exposed to the action of paludrine, then the inhibitory effect of acetylcholine was gradually changed to a stimulation. This change was accompanied by a gradual reduction in the rate and amplitude of beat. Within 7–38 min. after the paludrine had been added the beat of the auricles stopped, usually abruptly, and the auricles remained quiescent even when left in fresh Ringer-Locke solution for periods of up to an hour. During this time acetylcholine restarted the beat. These observations have now been extended to include other acetylcholine-like compounds and adrenaline.

RESULTS

The auricles of a freshly killed rabbit were dissected clean of fat and ventricular tissue; they were then suspended in well-oxygenated Ringer-Locke solution at 29° C., so that the contractions were recorded on smoked paper by a light straw lever, an upstroke representing systole.

Acetyl-β-methylcholine.—Fig. 1*a* shows the normal inhibitory effect of acetylcholine and acetyl-β-methylcholine on the beat. Paludrine (8 mg.) was added to the bath of 75 ml. Ringer-Locke solution. The amplitude and rate of beat steadily declined until they were about 60 per cent of the

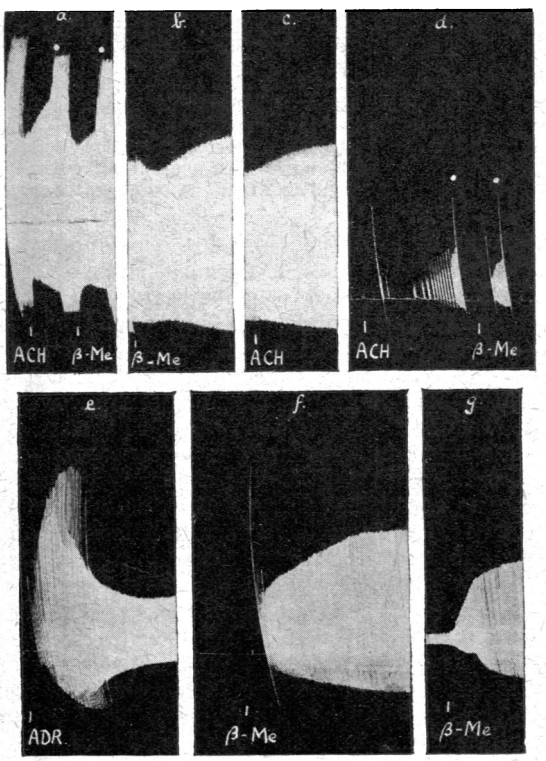

FIG. 1.—Isolated rabbit auricles. (*a*) Acetylcholine (40 μg.). Acetyl-β-methylcholine (20 μg.). Between (*a*) and (*b*) paludrine (8 mg.). (*b*) Acetyl-β-methylcholine (40 μg.) and (*c*) Acetylcholine (40 μg.). Both caused stimulation. The beat stopped. (*d*) Beat restarted with acetylcholine (200 μg.). Stopped when washed out. Restarted with acetyl-β-methylcholine (200 μg.). Stopped when washed out. (*e*) Restarted with adrenaline (20 μg.). Irregular beat, which stopped when washed out. (*f*) Restarted with acetyl-β-methylcholine (100 μg.). Continued beating regularly and with larger amplitude than when started with adrenaline. (*g*) Stimulated by acetyl-β-methylcholine (100 μg.). Dots above record indicate that the bath was changed to fresh Ringer-Locke solution.

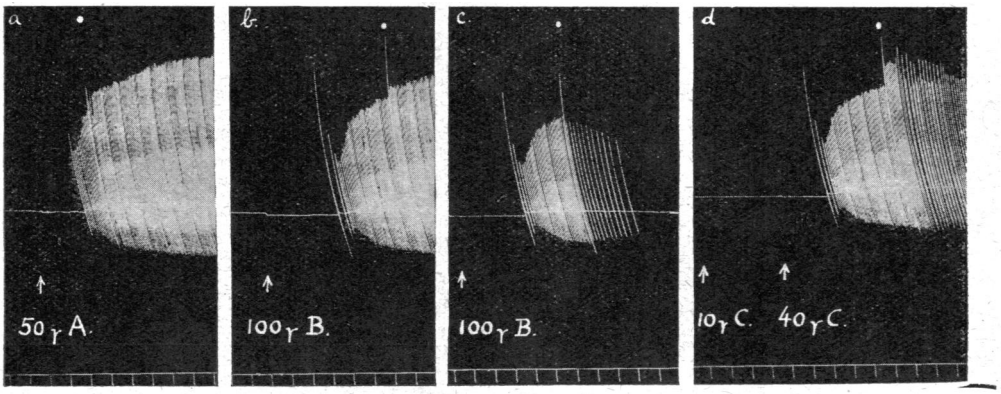

FIG. 2.—Isolated rabbit auricles stopped by paludrine (8 mg.) in 18 min. (*a*) Restarted with acetylcholine (50 μg.) and stopped again with paludrine (8 mg.) in 4 min. (*b*) Restarted with Bovet's acetal compound (100 μg.). Stopped in 4 min. with paludrine (8 mg.). (*c*) Restarted with Bovet's acetal compound (100 μg.). Stopped when washed out. (*d*) Restarted with carbaminoylcholine (50 μg.). Irregular and slow after washing out. Time 30 sec. Dots above record indicate that the bath was changed to fresh Ringer-Locke solution.

initial amplitude (Fig. 1*b*). At this point acetyl-β-methylcholine caused a transient inhibition, followed by a stimulation, of the beat. After washing out, acetylcholine also augmented the beat (Fig. 1*c*). There was no change in rate during these stimulations. The bath was replaced by Ringer-Locke solution containing the same concentration of paludrine; the beat of the auricles soon stopped. The arrest was not gradual, but abrupt. Both acetylcholine and acetyl-β-methylcholine restarted the contractions of the auricles (Fig. 1*d*); these stopped when the fluid in the bath was changed to fresh Ringer-Locke solution. Acetyl-β-methylcholine was again used to restart the beat (Fig. 1*f*). It was allowed to act for a longer time than before (Fig. 1*d*), and when the bath was washed out the auricles did not stop beating but the beat became gradually much weaker. Acetyl-β-methylcholine once more stimulated the beat (Fig. 1*g*). It has been repeatedly noticed in this, and in previous work, that if the substance which initiated the beat was removed within 90 sec. of the first contraction, then the beat stopped almost immediately (see Figs. 1*d* and 2*c*). If the substance was allowed to act for a longer time (2–5 min.) then washing out only reduced the beat, and if the drug was left in the bath for more than 5 min. washing out had little effect on the beat.

Adrenaline.—Adrenaline also restarted the beat, as is shown in Fig. 1*e*. The difference between the effect of adrenaline and that of a choline derivative (Fig. 1*f*, acetyl-β-methylcholine) should be noted. Contractions started with adrenaline quickly reached a large amplitude, but then diminished to a small value. When the contractions were started with an acetylcholine-like compound, after the first few beats the amplitude of the contractions steadily increased to a much larger value than that obtained with adrenaline.

Bovet's acetal compound.—Bovet (1944) introduced a new compound, which he claimed had the muscarine-like properties, but no nicotine-like properties of acetylcholine. The formula of Bovet's acetal compound is shown below:

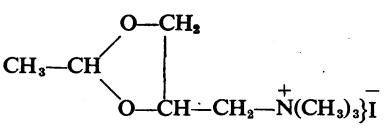

Fig. 2 shows that this, as well as acetylcholine, would restart the auricles which had been stopped with paludrine. The auricles were started with acetylcholine (Fig. 2*a*) but did not stop when washed out. Paludrine was added again, and the beat stopped in 4 min. The bath was washed out, and then Bovet's acetal compound restarted the contractions (Fig. 2*b*). Paludrine again stopped the beat in 4 min.; Bovet's acetal compound again restarted it. This time the bath was washed out within 90 sec. of the initial contraction, and the beat stopped almost immediately.

Carbaminoylcholine.—Fig. 2*d* shows that carbaminoylcholine also restarted the contractions. When the bath was changed, the beat became slow and irregular and did eventually stop.

Choline.—Choline restarted the beat. Fig. 3 shows the record of an experiment in which acetylcholine (100 μg.) started the rhythm (Fig. 3*a*). The bath was washed out twice and the contractions stopped in 4 min. Choline (5 mg.) had no effect, but choline (50 mg.) added 90 sec. later restarted the beat (Fig. 3*b*). When the bath was washed out, the contractions again stopped; they were restarted with acetylcholine (50 μg.).

Benzoylcholine.—Benzoylcholine, which is said to have only nicotine-like properties, failed to restart the contractions of the auricles.

Nicotine.—Nicotine was added (1, 10, and 50 mg.), but this did not restart the beat of the quiescent auricles. After the nicotine had been washed out, two doses of acetylcholine (100 μg.) were needed to restart the contractions, the force of which was much weaker than before the nicotine (Fig. 3*c*). The beat stopped when the bath was changed, but restarted again with acetylcholine (100 μg.) and became more regular on addition of acetylcholine (200 μg.). Nicotine (0.5, 1, and 2 mg.) also failed to restart the contractions of the auricles of which Fig. 2 is the record.

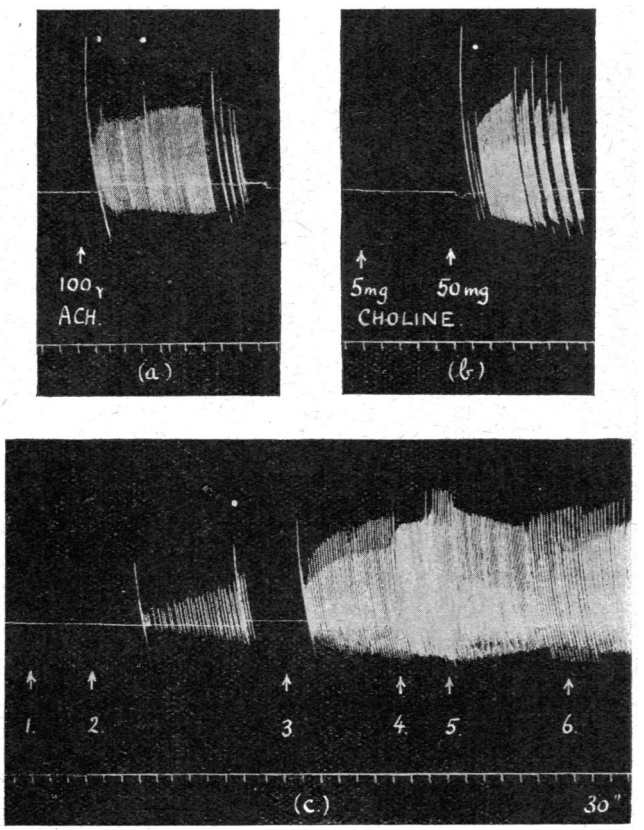

FIG. 3.—Auricles stopped with paludrine (8 mg.) in 26 min. (*a*) Restarted with acetylcholine (100 μg.); stopped on washing out. (*b*) Restarted with choline (55 mg.); stopped on washing out. Between (*b*) and (*c*), restarted with acetylcholine (50 μg.), but failed to restart with nicotine (1, 10, and 50 mg.). Washed out. (*c*) Acetylcholine (100 μg. at 1 and 2), started beat, but not so well as in (*a*). Beat stopped when bath was washed out; restarted with acetylcholine (100 μg. at 3) and improved with acetylcholine (200 μg. at 4). Atropine (500 μg. at 5) made the beat irregular and the amplitude decreased. The beat seemed to improve when more acetylcholine was added (1 mg. at 6). Time 30 sec. Dots above record indicate that bath was changed to fresh Ringer-Locke solution.

Atropine.—Atropine (500 μg.) added to the bath (Fig. 3*c*) made the beat irregular and the amplitude gradually decreased. The rhythm improved, however, when more acetylcholine was added (1 mg.). The effect of atropine on auricles restarted with acetylcholine depended upon the time at which it was added. If the atropine was added within 90 sec. of the initial contraction, then the beat stopped immediately. This is recorded in Fig. 4. Acetylcholine restarted the beat, which stopped when the bath was changed. The contractions were again started with acetylcholine, but stopped immediately atropine was added. After the bath had been washed out, although acetylcholine did not restart the beat, adrenaline did, and the contractions were unaffected by atropine.

Effect of adrenaline on acetylcholine response.—The beat of the auricles was stopped with paludrine and restarted with acetylcholine. After 10 min. the acetylcholine was washed out; the auricles were left for a further 20 min. The amplitude of the beat was then as shown at the beginning of Fig. 5. Adrenaline increased the amplitude and rate of beat to approximately the value previous to treatment with paludrine, and at this point acetylcholine (10 μg.) inhibited both the amplitude and rate of beat. Without any adrenaline in the bath, acetylcholine (10 μg. and 50 μg.) had no effect on the amplitude, whilst acetylcholine (100 μg.) caused a slight increase. This was accompanied by a decrease in rate. The in-

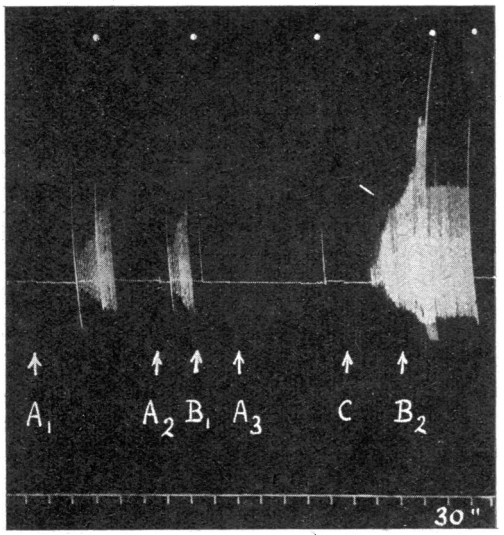

FIG. 4.—Isolated rabbit auricles. The beat was stopped with paludrine (8 mg.) in 27 min. A_1 Started with acetylcholine (50 μg.). Stopped when washed out. A_2 Restarted with acetylcholine (50 μg.). Stopped on the addition of atropine (500 μg. at B_1). A_3 Acetylcholine (50 μg.) failed to restart the beat, but adrenaline (20 μg.) at C started the contractions, which were unaffected by atropine (500 μg. at B_2). Time 30 sec. Dots above record indicate that the bath was changed to fresh Ringer-Locke solution.

hibition with acetylcholine (10 μg.) was repeated in the presence of adrenaline.

DISCUSSION

The possibility that acetylcholine has an integral function in the mechanism of the heart beat has already been discussed in a previous paper (Burn and Vane, 1948). In this paper it was shown that when the beat of isolated rabbit auricles was stopped with paludrine, it could be restarted with acetylcholine. It is interesting to note that Eurico-Paes and Soares (1940) found that chick-embryo heart-cultures which had ceased to beat could be restarted with a mixture of adrenaline and acetylcholine, but not with either drug separately, and that Singh, Sehra, and Singh (1945) published a record of the resumption of contractions of a frog's heart when acetylcholine was added.

It has now been shown that adrenaline and several acetylcholine-like compounds will also restart the beat of auricles stopped with paludrine. That the mechanism of this resumption cannot be due to the release of an adrenaline-like substance (described by Hoffmann *et al.*, 1945; McNamara *et al.*, 1948) is suggested for the following reasons:

(*a*) The release of an adrenaline-like substance is a nicotine-like action of acetylcholine. Nicotine did not restart the beat of the auricles; nor did benzoylcholine, which is said to have only nicotine-like properties. Bovet's acetal compound, however, which he claims has no nicotine-like action, restarted the beat.

(*b*) When the beat was restarted with adrenaline, in most cases the amplitude was initially stimulated, but then declined and remained small (Fig. 1*e*). Contractions restarted with acetylcholine-like compounds, however, steadily increased to a large value (Fig. 1*f*).

As would be expected, atropine prevented the start of the beat with acetylcholine, but not with adrenaline. If atropine was added to the auricles which had just started to contract under the influence of acetylcholine, then the contractions stopped; if the addition of atropine was delayed until about 5 min. after the contractions had started, then it only slowed the beat or had no effect. The same effect could be obtained by washing out; if the bath was washed out within 90 sec. of the first contraction, then the beat stopped; if the bath was not washed out until about 5 min. after the first contraction, then the beat continued.

Thus the mechanism of contraction seems to be both initiated by, and dependent upon, the added acetylcholine for the first few minutes; the beat then becomes independent of the added acetylcholine. This is interesting in view of the work of Abdon and his collaborators, who found that acetylcholine was present in the form of a precursor in cardiac tissue and suggested that there was a constant breakdown to, and reformation from, acetylcholine. They postulated that this "precursor was necessary for the contractions of the cardiac muscle" (Abdon and Hammarskjöld, 1944; Abdon, 1945).

After treatment with paludrine, the normal inhibitory effect of acetylcholine could be restored by first increasing the force and rate of beat with adrenaline. This suggests that the action of acetylcholine may depend upon the rate of metabolism of the myocardium, or the actions of adrenaline and acetylcholine on the heart are interdependent after treatment with paludrine.

SUMMARY

1. Various drugs have been tested to find whether they will restart the contractions of isolated rabbit auricles, previously stopped with paludrine.

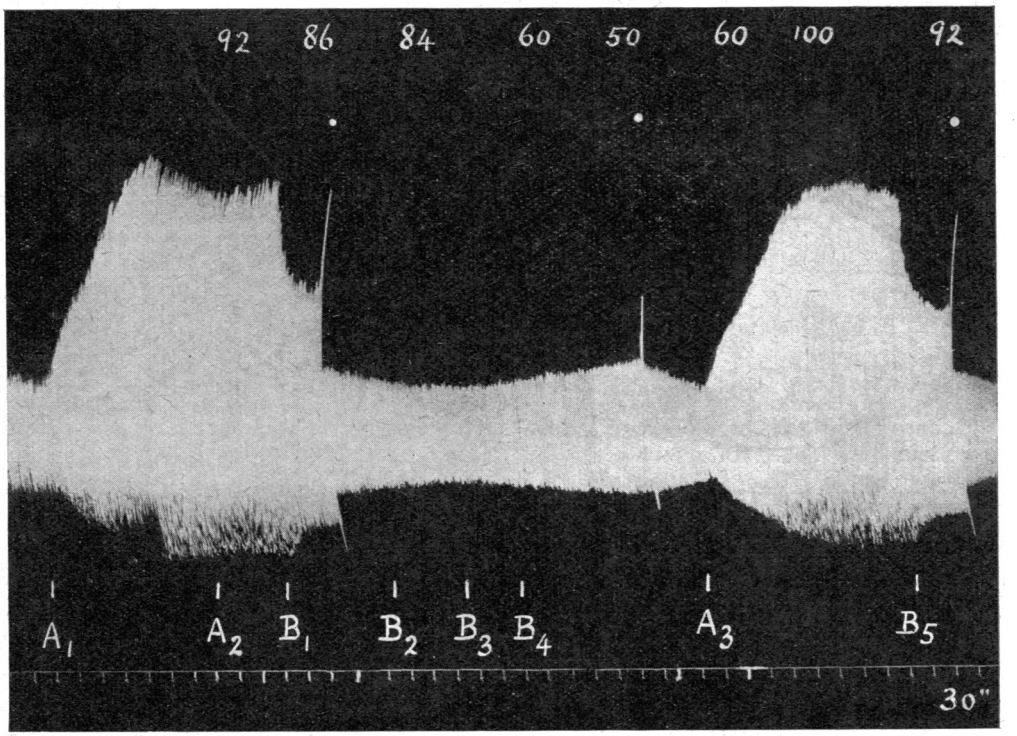

FIG. 5.—After exposure to paludrine the beat of the auricles stopped, but was restarted with acetylcholine (100 μg.). This was washed out after 10 min. Auricles were left for 20 min. At A_1, adrenaline (20 μg.) increased the rate and amplitude. Further adrenaline (20 μg. at A_2) had no effect, but acetylcholine (10 μg. at B_1) caused inhibition of beat. After washing out, the amplitude returned to its previous value, and at this stage acetylcholine (10 μg. at B_2 and 50 μg. at B_3) gave no inhibition of amplitude, although the rate was decreased. Acetylcholine (100 μg. at B_4) caused slight augmentation of amplitude. After washing out, adrenaline (20 μg. at A_3) again increased the rate and amplitude; acetylcholine (10 μg. at B_5) again inhibited the beat. Figures along top indicate rate of beat per min. Time 30 sec. Dots above record indicate that the bath was changed to fresh Ringer-Locke solution.

2. Of these drugs, choline, carbaminoylcholine, acetyl-β-methylcholine, Bovet's acetal compound, and adrenaline all restarted the beat.

3. The beat was not started by nicotine or benzoylcholine.

4. After treatment with paludrine the normal inhibitory effect of acetylcholine could be restored by first adding adrenaline to the auricles.

5. The results are discussed.

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