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QUINIDINE AND ANTICHOLINESTERASES ON RABBIT AURICLES

BY

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In a recent paper Burn and Kottegoda (1953) have described the action of eserine on the isolated auricles of the rabbit heart. They found that in most preparations low concentrations decreased the rate of beating, though in some they increased the rate. Higher concentrations always decreased the rate and finally arrested the contractions, and then the auricles were found to be electrically inexcitable. The effect on the amplitude of contraction was different, for low and medium concentrations increased it; high concentrations, however, reduced it. It was stated that the effect of eserine was likely to be that of an anticholinesterase, since DFP had a similar action on the rate leading to arrest of the beat, while neostigmine in high concentration had a different action, increasing the rate, neostigmine being known (e.g., from the work of Riker and Wescoe, 1946) to have an action of its own in addition to its action as an anticholinesterase.

In the following account we describe some experiments which appear to relate the action of high concentrations of eserine to the action of quinidine, and we describe also the effect of various other anticholinesterases on the auricles. We have also examined the effect of hexamethonium and of cocaine and procaine on the action of eserine.

METHODS

All observations have been made on auricles dissected from the heart of a freshly killed rabbit so as to be free from other tissue. The auricles were suspended in a bath of Locke's solution at 29° C., vigorously aerated with oxygen. The capacity of the bath was 35 ml. The Locke's solution contained in 1 litre 9 g. NaCl, 0.42 g. KCl, 0.24 g. $CaCl_2$, 0.5 g. $NaHCO_3$ and 2 g. dextrose. The contractions were recorded by a straw lever against the pull of a light spring. The natural frequency of the lever was much greater than the highest rate of contraction.

RESULTS

Action of Quinidine.—We have examined the action of quinidine in the light of the observation of Dawes (1946) that it is a substance which has been shown to reduce the action of acetylcholine (ACh) in all forms of muscle. Thus in the presence of quinidine not only the stimulant action of ACh on the frog rectus and the rabbit intestine is reduced, but also the inhibitory action on the rabbit auricles.

A small concentration of ACh (0.16×10^{-7} g./ml.) caused a slight inhibition of rabbit auricles as shown in Fig. 1. When this concentration was applied in the presence of eserine 10^{-6} g./ml., the contractions were arrested. This effect of eserine was described by Webb (1950). The contractions were resumed when the bath fluid was changed. The arrest was produced a second time by the same concentrations of eserine and ACh, and quinidine (1.5×10^{-5} g./ml.) was then added. The contractions began again. This result was con-

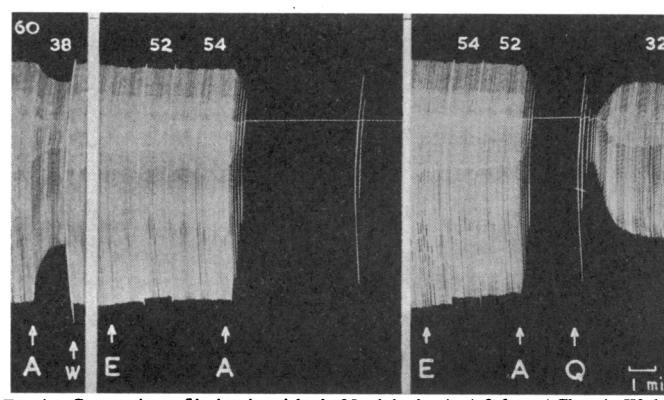

FIG. 1.—Contractions of isolated auricles in 35 ml. bath. At A 0·6 μg. ACh. At W the bath fluid was changed. At E, eserine 10^{-6} g./ml. At Q, 0·5 mg. quinidine sulphate was added. The numbers above the record are the rate per min.

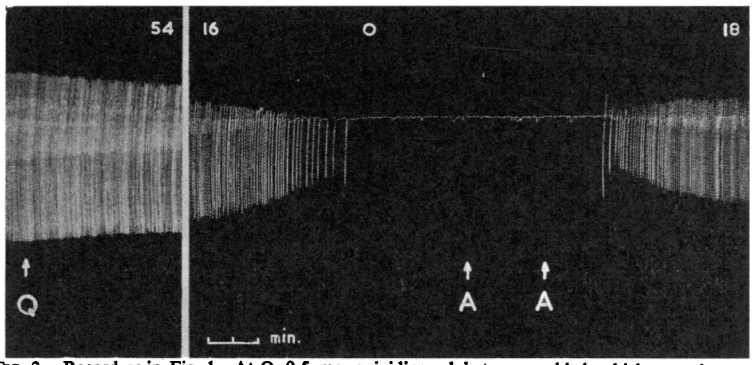

FIG. 2.—Record as in Fig. 1. At Q, 0·5 mg. quinidine sulphate was added, which caused arrest in 30 min. At A, 30 μg. ACh was added.

sistent with the view that quinidine reduced the action of ACh on the auricles so that arrest of the beat was no longer maintained.

A second experiment illustrated in Fig. 2 was carried out in which quinidine (1.5×10^{-5} g./ml.) was added to the bath containing freshly dissected auricles. When this was allowed to act, both amplitude and rate of beat were reduced, until at the end of 30 min. the contractions stopped. When ACh (2×10^{-6} g./ml.), was added the contractions began again after a latency of about 3 min. This result was obtained in 8 out of 8 trials on 6 auricles; it was consistent with the view that quinidine reduced the action of ACh endogenously produced, so that the contraction could not be initiated, or could not be conducted. When the concentration of ACh was raised by adding it to the bath the contractions began again. The amount of ACh which was added before the beat began again varied widely from 5 μg. to 2.25 mg.; the mean amount in 9 experiments was 0.42 mg., or a concentration of 1.2×10^{-5} g./ml.

A variant of this experiment, illustrated in Fig. 3, was also carried out. Quinidine was added as before and the drum was stopped while it reduced the amplitude and the rate; the rate was initially 78 per min. and fell to 48 per min. ACh (0.4×10^{-7} g./ml.) was then added; the rate briefly slowed and then the amplitude and the rate both increased, the rate to 66 per min. A further addition of ACh (1.5×10^{-7} g./ml.) was followed by inhibition and arrest of the contractions. Thus in the auricles after treatment with quinidine a stimulant action of ACh was observed in one concentration and an inhibitory action in higher concentration. This experiment was repeated three times with the same result.

The Action of Eserine.—We discovered that when the contractions of the auricles were arrested by a high concentration of eserine the addition of ACh was followed by resumption of the beat as shown in Fig. 4. In this experiment the eserine concentration was 3.4×10^{-4} g./ml. The beat was arrested in 5.5 min. ACh was then added in small amounts, and the beats began again when the concentration was 0.3×10^{-6} g./ml. After further additions of ACh the rate fell from 42 to 32, and

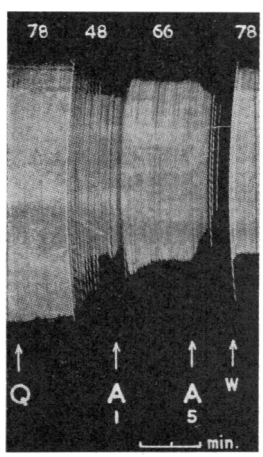

FIG. 3.—Record as before. At Q, 0·5 mg. quinidine sulphate added. The drum was stopped and started again when the rate had fallen to 48. At A_1, 1 μg. ACh was added, and the rate rose to 66. At A_5, 5 μg. ACh was added.

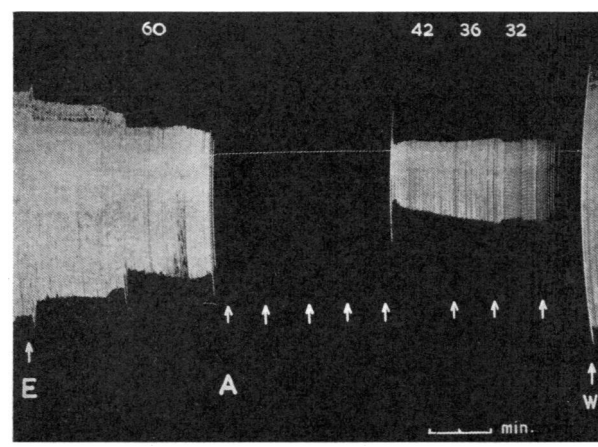

FIG. 4.—Addition of 12 mg. eserine depressed the amplitude and arrested the contractions. At the arrows, ACh was added, 0·1 μg., 0·5 μg., 1·0 μg., 3·5 μg., 5 μg. The contractions began again. Further additions were 1 μg., 5 μg. and 10 μg.

when the concentration reached 10^{-6} g./ml. the beat stopped again. Thus, as under the influence of quinidine, so under the influence of eserine it was possible to observe a stimulant action of ACh in one concentration and an inhibitory action in a higher concentration. The beat was resumed after the addition of ACh in 13 out of 14 trials in 10 auricles, but the amount of ACh used was much less than when the beat was arrested by the action of quinidine, the mean amount being only 10 μg. or a concentration of 0.3×10^{-6} g./ml.

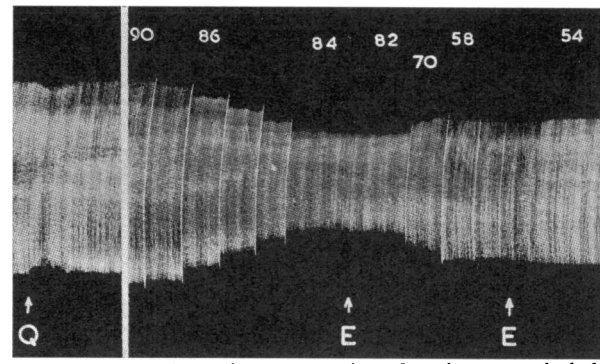

FIG. 6.—To show that a low concentration of eserine antagonized the depression of the amplitude by quinidine but accelerated the depression of the rate. Q = 0·5 mg. quinidine; E = 100 μg. eserine.

Effect of ACh in the Presence of Eserine.—The observation, that when the contractions stopped in the presence of a high concentration of eserine the addition of ACh was followed by resumption of the beat, showed that the contractions did not stop because of an accumulation of ACh. We examined the effect of ACh in the presence of different concentrations of eserine. As Fig. 1 shows, the concentration of 10^{-6} g./ml. greatly intensified the inhibitory action of ACh. A concentration of 2×10^{-4} g./ml. which slowed the rate in one experiment from 66 to 40 per min. abolished the inhibitory action of a similar amount (1 μg.) of ACh, while a concentration of 5×10^{-4} g./ml. which slowed the rate to 16 per min. reversed the action of 1 μg. ACh so that the rate was accelerated from 16 to 29 per min.

Synergism between Quinidine and Eserine.—On the assumption that eserine would cause an accumulation of acetylcholine we at first thought that eserine would antagonize the action of quinidine in causing arrest of the beat. In almost all preparations the contrary was observed and the two substances worked together. Thus in Fig. 5

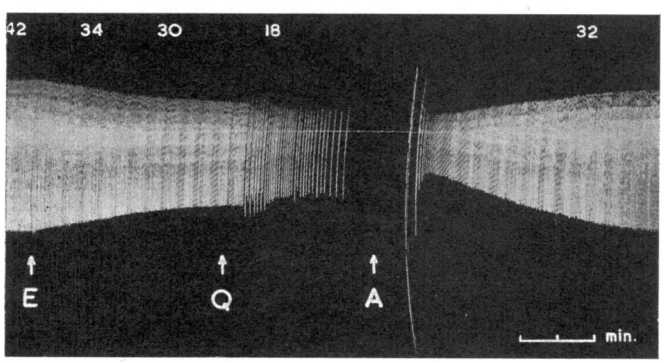

FIG. 5.—To show the synergism between eserine and quinidine in causing arrest of the beat. Eserine alone in a concentration 10^{-4} g./ml. does not arrest the beat, and 0·5 mg. quinidine alone arrests it only after 20–30 min. Together they caused arrest as shown. 100 μg. ACh then caused resumption of the beat.

eserine was added in concentration 10^{-4} g./ml. The rate fell, but the amplitude was not greatly reduced; after the addition of quinidine the beat stopped in 3 min., beginning again after the addition of ACh. In other experiments quinidine was added first; the addition of eserine in a concentration which alone would not lead to arrest of the beat shortened the time before the arrest. There were, however, experiments in which antagonism was shown. In two of these, one of which is illustrated in Fig. 6, the addition of quinidine was followed by a fall in rate and amplitude; the addition of eserine accelerated the fall in rate, but increased the amplitude.

Action of Neostigmine.—The action of neostigmine shown in Fig. 7 resembled that of eserine only in the effect of low concentrations on the rate; this slowed progressively to a mean value of 65% of the initial rate in the presence of molar concentrations 10^{-7}, 10^{-6}, and 10^{-5}. In higher concentrations the rate declined less and even accelerated above the initial rate. The amplitude was not appreciably affected by any concentration of neostigmine. Neostigmine was the only substance tested which did not cause arrest of the beat in high concentrations. Even in the presence of a concentration as high as 10^{-2}M, the rate and amplitude were not diminished. Although high concentrations of neostigmine did not arrest the contractions, they reduced the inhibitory action of ACh as shown in Fig. 8.

Action of DFP.—In the presence of molar concentrations of 10^{-6} and 10^{-5} DFP the rate decreased to 93 and 67% of the initial rate as shown in Fig. 7. In the presence of 10^{-4}M and 10^{-3}M the

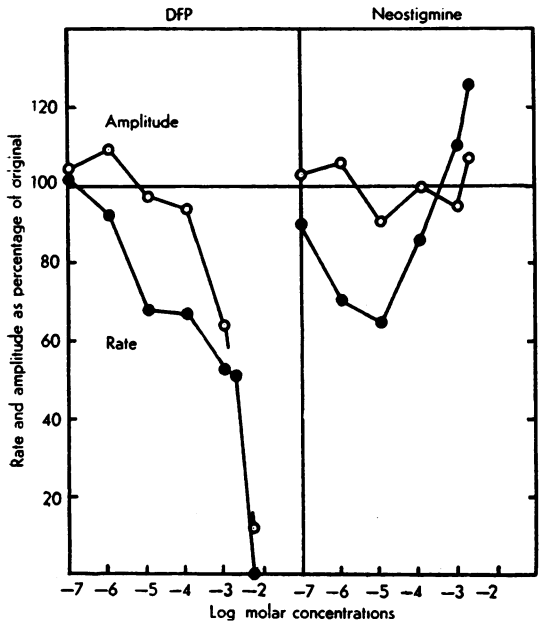

FIG. 7.—Effects of DFP and of neostigmine on amplitude and rate of rabbits' isolated auricles. Ordinates: rate and amplitude as a percentage of the initial, taken as =100. Abscissae: logarithms of the molar concentrations. Open circles, amplitude. Closed circles, rate. The effect was that recorded at the end of 30 min.

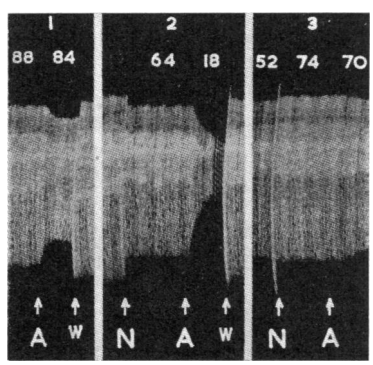

FIG. 8.—To show reduction of inhibitory action of ACh by high concentrations of neostigmine. (1) A=0·6 μg. ACh. (2) N=10^{-6}M neostigmine; A=0·6 μg. ACh. (3) N=10^{-3}M neostigmine: A=0·6 μg. ACh.

rate declined a little but not much further. In the presence of 4×10^{-3}M the contractions were arrested. The amplitude was little affected in the presence of concentrations up to 10^{-4}M, but above this the amplitude was greatly reduced, so that it fell to zero with the rate.

Action of Other Anticholinesterases.—The mean results with three other anticholinesterases are given in Table I. Results with Nu 683 (the dimethyl carbamate of (2-hydroxy-5-phenylbenzyl)-trimethylammonium bromide) were of interest because this

TABLE I

ACTION OF ANTICHOLINESTERASES ON RATE AND AMPLITUDE OF RABBITS' ISOLATED AURICLES

Effects are measured as percentage of initial value after 30 min.

Molar Concn.	Nu 683		Nu 1250		BW 284C51	
	Rate	Amplitude	Rate	Amplitude	Rate	Amplitude
10^{-7}	98·5	103	75	104	86	100
10^{-6}	65·5	111·5	80	112	86	98
10^{-5}	69	111	135	126	96	98
10^{-4}	75	109	157	112	103	99
10^{-3}	74·5	58·5	143	84	101	81

substance was shown by Hawkins and Gunter (1946) to be a highly specific inhibitor of pseudocholinesterase, while results with BW 284C51 (the dimethobromide of 1:5-di(*p*-N-allyl-N-methylaminophenyl)-pentan-3-one) were interesting because this substance was shown by Austin and Berry (1953) to be a specific inhibitor of true cholinesterase. Nu 1250 (the N-*p*-chlorophenyl-N-methylcarbamate of *m*-hydroxyphenyltrimethylammonium bromide) was found to be a rather less specific inhibitor of true cholinesterase (Hawkins and Mendel, 1949). Each inhibitor was tested on 4 or 5 auricle preparations.

All three inhibitors failed to affect the amplitude in concentrations less than 10^{-3}M, and in this concentration Nu 683 depressed the amplitude more than did the others. At a concentration of 10^{-6}M the rate was slowed to 66% in the presence of Nu 683, a lower figure than was observed with the other two inhibitors. At concentrations of 10^{-5}M, 10^{-4}M, and 10^{-3}M there was no further fall in rate in the presence of Nu 683, while in the presence of Nu 1250 the rate was accelerated above the initial value, and in the presence of BW 284C51 the rate returned to the initial value. At a concentration of 2×10^{-3}M, 3 out of 5 auricles were arrested in the presence of Nu 683, and 1 out of 4 in the presence of Nu 1250. In the presence of BW 284C51 auricles were arrested only in concentrations as high as 8×10^{-3}M and 10^{-2}M.

In the presence of concentrations 10^{-4}M and 10^{-3}M, the inhibitory action of ACh on the auricles was reduced by all three anticholinesterases.

Observations with Hexamethonium.—In the auricles endogenous ACh may be liberated from nerve endings originating in ganglia or may be formed in non-nervous tissue. The action of nicotine on the auricles which is partly inhibitory and partly stimulant is abolished by hexamethonium (Kottegoda, 1953). Some observations were therefore made to see whether the action of eserine in slowing or arresting the contractions, or of neostigmine and Nu 1250 in increasing the rate of contractions,

was modified in the presence of hexamethonium. The results are given in Table II. This shows that hexamethonium did not modify the slowing of the rate caused by eserine (10^{-5} g./ml.). Further, hexamethonium did not affect the arrest of the auricles which occurred in the presence of a high concentration of eserine (4×10^{-4} g./ml.); nor the resumption of the beat which followed the addition of ACh. The acceleration of the rate by neostigmine (10^{-3}M) and by Nu 1250 (10^{-4}M) was also unaffected by the presence of hexamethonium.

TABLE II

EFFECT OF HEXAMETHONIUM ON ACTION OF ANTICHOLINESTERASES ON RATE OF AURICLES

The effect is expressed as the percentage of the initial value

Substance and Concn.	Concn. Hexamethonium	No. of Auricles	No. of Observations	Mean Effect on Rate
Eserine 10^{-5} g./ml. ..	—	4	6	73%
,, ,, ..	3×10^{-4} g./ml.	4	6	76%
Eserine 4×10^{-4} g./ml.	—	2	5	Arrest
,, ,,	3×10^{-4} g./ml.	2	5	Arrest
Neostigmine 10^{-3}M ..	—	4	6	143%
,, ,, ..	10^{-4} g./ml.	4	6	148%
Nu 1250 10^{-4} ..	—	2	2	116%
,, ..	10^{-4} g./ml.	2	2	121%

Hexamethonium did not modify the rate of auricles which had not been exposed to the action of other substances; this observation was made in 3 preparations using a concentration of 3×10^{-4} g./ml. Hexamethonium in this concentration, however, raised the rate of auricles previously exposed to eserine. It was rarely possible to remove all eserine by repeated change of the bath fluid, and the residual slowing was antagonized by hexamethonium in 9 auricles, the mean rise in rate being 20%. We believe this to be due to a very weak atropine-like action which hexamethonium possesses, an action too weak to modify the effect of ACh either on the auricles or in the heart-lung preparation (as observations recently made here by one of us have shown), but perhaps explaining the inhibition of the action of ACh in the isolated and perfused cat heart described by Perry and Talesnik (1953). This action, one thousand times weaker than that of atropine, can, however, be seen to lessen the action of eserine in the heart-lung preparation.

Observations on Cocaine and Procaine.—Cocaine hydrochloride in a concentration of 5×10^{-6} g./ml. abolishes the peristaltic reflex in the isolated guinea-pig intestine, and we found that it abolished the action of nicotine on the auricles. We therefore used this concentration in the observations on eserine. By itself cocaine produced some increase of the rate in occasional preparations, but commonly a decrease. This made it difficult to determine the effect of cocaine on the action of eserine. In one experiment in which eserine 10^{-6} g./ml. was used, a concentration likely to exert an anticholinesterase action only, eserine applied for 20 min. reduced the rate to 70% of the initial value, eserine and cocaine together reduced it to 76%, and cocaine alone to 88%. The initial rates were almost the same in each case. Thus cocaine may have reduced the effect of eserine, but did not abolish it. Other experiments gave similar inconclusive results.

We observed that higher concentrations of cocaine (4×10^{-5} g./ml.) and of procaine led to arrest of the beat, but the addition of ACh was not followed by resumption of beating.

DISCUSSION

The results obtained may be considered in the light of the hypothesis that some of the effects of quinidine on cardiac muscle are due to its property of diminishing the action of ACh. This diminution, we may suppose, would be due to quinidine competing for receptors on which ACh acts. When the auricles are arrested by ACh in the presence of a low concentration of eserine, the addition of quinidine to the bath causes the beats to start again. This can clearly be explained as a diminution of the action of ACh, which is no longer able to maintain the state of arrest.

When quinidine is added to the bath containing auricles recently set up the rate and amplitude decline and the beat stops. This action can only be due to a diminution of the action of ACh if the rate and force of the beat depend on the local formation of ACh, as Bülbring and Burn (1949) suggested. The fact that the auricles arrested by quinidine begin to beat again when ACh is added to the bath is in accordance with the suggestion. We may therefore suppose that quinidine arrests the auricles by occupying more and more of the receptors on which the intrinsic ACh acts, until the beat stops. When more ACh is added, some of the quinidine molecules are dislodged from the receptors, and the beat begins again.

Further support for the view that quinidine causes arrest of the beat by diminishing the action of intrinsic ACh comes from the observations with eserine. If quinidine occupies receptors on which ACh acts, other substances which combine with these receptors should also arrest the beat. Now eserine combines with cholinesterase, and ACh combines with cholinesterase, so it is reasonable

to expect that eserine would have some affinity for receptors on which ACh acts. Our results show that high concentrations of eserine arrest the beat just as quinidine does, and that the addition of ACh then starts the beat again. In causing arrest of the beat eserine and quinidine were found to be additive in their action.

Arrest of the beat by eserine might be thought to be caused by accumulation of ACh. Experiments by one of us (S.B.) with U. Trendelenburg showed that the amount of ACh present in auricles stopped by eserine was not greater than that found in normal auricles. Moreover, the resumption of the beat when ACh was added proves that the arrest was not due to excess. Further, neostigmine did not act like eserine; the highest concentrations of neostigmine did not arrest the beat.

Arrest of the beat by eserine is perhaps related to a failure of conduction for the following reasons. The effect of eserine is like that of quinidine, and quinidine has long been known to depress conduction. Rothschuh and Bammer (1952) have observed that eserine diminishes the rate of conduction in a strip of frog ventricle, while neostigmine increases it. As already mentioned, neostigmine did not cause arrest of the auricles. Auricles arrested by eserine were found by Burn and Kottegoda (1953) to be electrically inexcitable. The resumption of the beat after the addition of ACh may be explained by a restoration of conduction. Prinzmetal, Corday, Brill, Oblath, and Kruger (1952) showed that infusion of ACh into the dog prevents the failure of conduction which otherwise occurs when the heart is driven electrically at very high rates. Their records show that the conduction time shortens under the influence of ACh. Burgen and Terroux (1953) state, on the other hand, that in isolated cat auricles ACh slows the rate of conduction, basing their conclusions on the action of carbachol. Our colleague, Dr. E. M. Vaughan Williams, has observed that the rate of conduction in rabbit auricles is quickened by ACh. It is probable that both effects can be obtained. Whether the arrest of the beat by quinidine and by eserine is in fact due to a failure of conduction must be determined by other methods.

Before leaving the discussion of the action of quinidine, reference should be made to a view put forward by one of us (Burn, 1953) that there is an optimal concentration of ACh for cardiac activity and that amounts less than this or more than this cause diminished activity. This hypothesis suggests that the usual inhibitory action of ACh is an effect of excess. The hypothesis may be illustrated by reference to Figs. 3 and 4, where both the stimulant and the inhibitory action of ACh is seen, the first in low concentration and the second in higher concentration. This interpretation, however, ignores what is shown in Fig. 3 and what was seen in several experiments, that the low dose of ACh produced a further slowing of the rate as its immediate effect, this phase being transient and giving place to increased rate and amplitude. That is to say, the ordinary inhibitory action of ACh was detectable as a preliminary to the stimulant action, and therefore the inhibitory action on the rate cannot be considered as the effect of excess.

Burgen and Terroux (1953) have found that the resting potential of the cat auricle is increased by ACh, a result which agrees with the finding of Gaskell (1887) that the demarcation potential of the auricle of the turtle was increased by vagus stimulation. These observations suggest that the inhibitory action of externally applied ACh is due to hyperpolarization, and they do not fit with a conception that added ACh has both a stimulant and an inhibitory action on the rate. Eserine, on the other hand, in low concentration can accelerate the rate of the isolated auricles, though it usually retards it; similarly it can accelerate the rate of the perfused frog heart. Thus it would appear that changes in the concentration of endogenous ACh due to inhibition of cholinesterase may modify the rate in either direction.

The idea that ACh may stimulate in low concentration and inhibit in high concentration may, however, apply to its action on conduction and also on the force of the beat. In some of our experiments a low concentration of eserine was found to antagonize the depressant action of quinidine on the amplitude of contractions, though collaborating with it in reducing the rate. This would suggest that ACh may have a double action on the force of the beat.

The gradual decline in the rate and amplitude of the contractions of auricles exposed to proguanil until the beat is arrested (Burn and Vane, 1949) is probably similar to the action of quinidine, since in both cases the beat is resumed on addition of ACh. We found that cocaine, procaine, and atropine, which Dawes (1946) showed to act like quinidine on the electrically driven auricles, also had a similar action on those beating spontaneously, though when arrest was caused by these substances the addition of ACh was not followed by resumption of the beat.

The effect of other anticholinesterases, including neostigmine, was similar to eserine in one respect only, that low concentrations, 10^{-7}M and 10^{-6}M (or, for DFP, 10^{-6}M and 10^{-5}M) decreased the rate.

We think it likely that this action was an uncomplicated anticholinesterase action and indicated that the contracting auricles were forming ACh and that this formation was controlling the rate. We attempted to discover whether the formation took place in ganglion cells by testing the effect of hexamethonium and cocaine. Hexamethonium did not modify the action of eserine (10^{-5} g./ml.). Cocaine did not appear to modify the effect of eserine (10^{-6} g./ml. or 10^{-5} g./ml.). It was, however, more difficult to be certain of this, since cocaine alone caused some slowing of the rate. On the whole, the evidence was not in favour of a nervous origin for the ACh.

If the beat is initiated by the formation of ACh at the pacemaker, the generally accepted myogenic origin of the heart beat put forward by Gaskell and Engelmann long ago would indicate a myogenic origin for the ACh.

Summary

1. Quinidine, which diminishes the action of ACh in all forms of muscle, causes the rate and amplitude of the beat of rabbits' isolated auricles to diminish until the contractions cease. If ACh is added to the bath, the contractions are resumed. A further addition of ACh may inhibit the contractions.

2. If eserine is added to the bath containing contracting auricles, when the concentration reaches a point between 10^{-4} and 10^{-3} g./ml. the contractions cease. If ACh is added to the bath, the contractions are resumed. A further addition of ACh inhibits the contractions.

3. Quinidine and eserine appear to act as synergists in causing arrest of the auricles.

4. Low concentrations of other anticholinesterases, neostigmine, DFP, Nu 683, Nu 1250, BW 284C51, act like eserine in slowing the rate.

5. Higher concentrations of neostigmine increase the rate, and do not cause arrest of the beat; neostigmine does not modify the amplitude.

6. DFP depresses both rate and amplitude in high concentrations and causes arrest.

7. Reasons are given for regarding the action of quinidine and of high concentrations of eserine in arresting the auricles as perhaps due to an effect on conduction which is reversed by ACh.

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