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CHOLINE ACETYLASE ACTIVITY IN THE ATRIA AND ITS POSSIBLE RELATION TO THE MAINTENANCE OF THE MEMBRANE POTENTIAL

BY

J. H. BURN AND A. S. MILTON

From the Department of Pharmacology, University of Oxford

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Choline acetylase is present in rabbit atria and forms acetylcholine continuously. It is known that the contractions of isolated atria cease when the temperature falls to a point below 20° C., and that they can be started again by acetylcholine, which causes a rise in the transmembrane potential. The question may therefore be asked whether the transmembrane potential is normally kept sufficiently high for impulses to be propagated by the acetylcholine which is formed within the atria. The effect of lowering the temperature on choline acetylase activity has therefore been studied. The activity was found to decline much more steeply below 20° C. than between 33° and 20°. The Q_{10} rose to 7.8. This finding is consistent with the view that one function of choline acetylase activity is to maintain the transmembrane potential.

Studies on the isolated atria of the rabbit heart have shown that, as a result of cooling, the contractions are arrested and can then be restarted by the addition of acetylcholine (Marshall and Vaughan Williams, 1956). There is evidence that acetylcholine acts by raising the membrane potential (Marshall, 1957). Acetylcholine can be extracted from atria, and choline acetylase and cholinesterase are present in them. That the choline acetylase is not inactive, and that it is constantly forming acetylcholine, was shown by Burn and Kottegoda (1953), who found that eserine in concentrations from 10^{-6} to 10^{-5} g./ml. slowed the rate of contraction. Day (1956) found that the isolated rabbit heart perfused with Locke solution liberated acetylcholine into the perfusion fluid. The suggestion has therefore arisen that one of the functions of acetylcholine thus formed is to maintain the membrane potential at sufficient height and so to make possible the propagation of the impulses from the pacemaker.

Since cooling the atria to a temperature below 20° causes arrest of the atrial contractions, we have investigated the effect of cooling on the synthesis of acetylcholine to see if the synthesis declined at the point at which the contractions stopped.

METHODS

The methods used were similar to those previously described (Milton, 1959a, b).

Enzyme Preparation.—Acetone powders of rabbit atria which had been minced were prepared as follows. After washing the tissue in a mortar with a large volume (approx. 300 ml.) of cold dry acetone (−15°) which was rapidly poured off through a Buchner filter funnel, the tissue was ground up with several further additions of acetone until all the material had been transferred to the Buchner filter in the form of a fine powder. After drying on the filter paper for 5 min. the powder was transferred to a desiccator and evacuated over P_2O_5. After standing at 4° for 24 hr., the powder was removed from the desiccator, weighed and suspended (40 mg./ml.) in cysteine saline solution (6 mg. of neutralized cysteine hydrochloride/ml. 0.9% NaCl).

The solution was stored at −15° (for a minimum period of 24 hr.). Just before use it was thawed and centrifuged at 3,000 rev./min.

Incubation System. — The incubation system contained the following reagents (Hebb, personal communication). Co-enzyme A, (400 units/ml.), 0.05 ml.; 30% KCl, 0.04 ml.; 4% choline chloride, 0.04 ml.; 1.2% $MgCl_2.6H_2O$ 0.08 ml.; acetyl phosphate (10 mg./ml.), 0.14 ml.; (−)-cysteine HCl (neutralized; 30 mg./ml.), 0.12 ml.; phosphotransacetylase (2.5 mg./ml.), 0.10 ml.; eserine sulphate (1 mg./ml. in 4% NaCl), 0.10 ml.; water, 0.23 ml. to a total volume of 0.90 ml.

The co-enzyme A preparation was diluted with distilled water in those experiments in which different concentrations were required [0.05 ml. of co-enzyme A (400 units/ml.)=5 units enzyme/mg. of atrial acetone powder]. It was prepared by a modification of the method described by Hebb (1955). Acetyl

phosphate obtained commercially was suspended in distilled water. Phospho-transacetylase, which was also supplied by the Worthington Biochemical Corporation, U.S.A., was suspended in 0.02 N-$KHCO_3$.

The water used for making up solutions was obtained by glass distillation of tap water which had previously been demineralized using a Permutit demineralizing plant.

The reagents listed above were pipetted into suitable glass tubes and incubated for 15 min., to initiate the formation of acetyl co-enzyme A.

After 15 min., 0.1 ml. of the enzyme preparation to be tested was added to the incubation mixture. The tubes were corked and the incubation then allowed to proceed for 1 hr. As controls, similar tubes were set up which contained the same reagents, and 0.1 ml. of boiled enzyme. These controls were used in the assay when matching the test solutions against standard acetylcholine solutions.

After exactly 1 hr., the reaction was stopped by adding 6 drops of B.D.H. Universal indicator and 0.2 ml. 0.33 N-HCl and 1.0 ml. of phosphate buffer M/15. Frog Ringer solution (4 ml.) was added to each tube and the contents boiled for 1 min. After cooling, the volume was made up to 10 ml. with frog Ringer solution and stored at −15°.

The acetylcholine content was then assayed on the isolated frog rectus abdominis muscle preparation. The standard acetylcholine solutions were made up with addition of the control solutions in amounts corresponding to the amounts of test solution used.

Results

Temperature and Choline Acetylase Activity.—The results of five experiments to determine the effect of temperature on the formation of acetylcholine by choline acetylase present in acetone-dried powders from rabbit atria are given in Table I. For comparison, the results in each experiment were expressed in relation to the activity at 33° taken as 100, and the values so obtained are shown in Table II. The mean results showed that at 21° the activity was 43% of that at 33°, but that at 17° it had fallen to 21%, and at 13° to 9%. Thus the fall in activity was steep below 21°.

From these results the Q_{10} was calculated for each rise of temperature, and the values are shown in Table III, together with values for rabbit brain calculated from work of Milton (1958). Normal values for the Q_{10} of biological reactions are from 1.0 to 2.5, and this held for choline acetylase activity between 21° and 41°. Below 21°, however, the Q_{10} values steadily increased, being 6.2 between 17° and 21°, and 7.8 between 13° and 17°. The estimates for choline acetylase in atria were very similar to those in brain at corresponding temperatures.

TABLE I

THE EFFECT OF TEMPERATURE ON THE SYNTHESIS OF ACETYLCHOLINE BY RABBIT ATRIA

The values show the amount of acetylcholine synthesized in μg./g. of acetone powder/hr.

Temp. (°C.)	Expt. No.				
	1	2	3	4	5
13	—	5	38	19	31
17	—	50	78	62	44
21	125	88	138	81	100
25	131	137	169	119	138
29	175	206	288	150	187
33	238	231	350	194	238
37	300	—	—	—	—
41	323	—	—	—	—

TABLE II

CHOLINE ACETYLASE ACTIVITY

The results are expressed in relation to those at 33° taken as being 100.

Temp. (°C.)	Expt. No.					Mean
	1	2	3	4	5	
13	—	2·2	10·9	9·8	13·0	9·0
17	—	21·6	22·3	19·5	18·5	20·5
21	52·5	38·1	39·4	41·8	42·0	42·6
25	55·0	59·3	48·3	61·3	58·0	56·4
29	73·5	89·2	82·3	77·3	78·6	78·6
33	100	100	100	100	100	100
37	126	—	—	—	—	126
41	136	—	—	—	—	136

TABLE III

THE Q_{10} OF CHOLINE ACETYLASE FROM RABBIT ATRIA COMPARED WITH VALUES FOR CHOLINE ACETYLASE OBTAINED FROM RABBIT BRAIN

Temp. (°C.)	Atria	Temp. (°C.)	Brain
13–17	7·8		
17–21	6·2	17–22	6·7
21–25	2·0	22–27	3·1
25–29	2·3		
29–33	1·8	32–37	1·6
33–37	1·8		
37–41	0·7		

Effect of Acetylcholine on Synthesis in Cooled Atria.—The effect of acetylcholine on synthesis in atria which had been arrested by cooling was also studied. This was done because Bülbring and Burn (1949) found that, when an acetone powder was prepared from atria which had ceased to beat after many hours in the bath, the addition of acetylcholine to the powder increased the rate of acetylcholine synthesis. These findings have been confirmed by Milton (1959b). The first experiments were carried out using amounts of co-enzyme A which varied from 0 to 2.5 units/mg. of powder, and synthesis was compared in the presence of 300 μg. of acetylcholine/g. powder with synthesis in its absence. The results are shown in Table IV. It will be seen that the added acetylcholine had an inhibitory effect which decreased as the co-enzyme A concentration increased.

TABLE IV

THE RELATION OF CO-ENZYME A CONCENTRATION TO THE INHIBITORY ACTION OF ACETYLCHOLINE ON A CHOLINE ACETYLASE PREPARATION FROM ATRIA STOPPED BY COOLING

Co-A, co-enzyme A; ACh, acetylcholine; temperature 37°.

Co-A (units/ mg. Powder)	ACh Added (μg./g. Powder)	ACh Synthesized (μg./g./hr.)	Change
0	0	100	—
0	300	50	−50%
0·625	0	222	—
0·625	300	175	−21%
1·25	0	317	—
1·25	300	275	−13%
2·50	0	375	—
2·50	300	325	−13%

In the next experiments the amount of co-enzyme A was kept constant but low at 0.625 unit/mg. of powder, while the acetylcholine concentration was varied. The results in Table V show that the inhibitory action was proportional to the amount of acetylcholine added. When large amounts of co-enzyme A were used (5 units/mg.) the synthesis was practically unaffected by acetylcholine. Finally, experiments were done in which powder from cooled atria was incubated at 15° instead of at 37°. Again acetylcholine caused inhibition of synthesis in the absence of co-enzyme A.

Choline Acetylase Activity of Rabbit Ventricle.—Acetone-dried powders of rabbit ventricle were prepared and the choline acetylase activity was measured. For comparison duplicate tubes were set up containing acetone powders prepared from rabbit atria. The incubation was carried out at 37° and the results are shown in Table VI. The synthesis in ventricle was very small indeed.

Effect of Atropine.—If the acetylcholine formed in the atria maintains the transmembrane potential, it might be thought that atropine should abolish the potential. We carried out experiments in which atria were cooled until they ceased to beat, and then observed the restarting of the beat

TABLE V

RELATION BETWEEN AMOUNT OF ACETYLCHOLINE ADDED AND INHIBITION OF SYNTHESIS IN ATRIA. STOPPED BY COOLING

Concentration of co-enzyme A, 0·625 unit/mg. Incubation at 37°.

Acetylcholine Added (μg./g. Powder)	Acetylcholine Synthesized (μg./g./hr.)	% Change
0	158	—
12·5	154	−2·6
31·2	144	−9·2
62·5	117	−26·4
125·0	54	−65·8

TABLE VI

CHOLINE ACETYLASE ACTIVITY OF RABBIT VENTRICLE COMPARED WITH THAT OF RABBIT ATRIA

	Acetylcholine Synthesized μg./g. Acetone Powder/hr.	
	Ventricle	Atria
	19	275
	12·5	232
	44	300
Mean	25	269

on gradual warming. The addition of atropine sulphate in concentrations up to 10^{-5} g./ml. had no effect on the restarting of the beat.

DISCUSSION

In three different conditions acetylcholine has been found to restart the contractions of the atria. Marshall and Vaughan Williams (1956) found that, when the atria were cooled, the contractions ceased at a temperature below 20°. In most experiments they were still able to record action potentials at the pacemaker and, when these were present, they found that the addition of acetylcholine caused contractions to begin again. Marshall (1957) found that the process of cooling caused a fall in the transmembrane potential and that contractions ceased when it became less than 60 mV. She showed that the addition of acetylcholine caused a rise in the potential and that the restarting of the contractions was due to this rise. The action of acetylcholine is probably due to its effect in increasing the permeability of the membrane to potassium (Harris and Hutter, 1956) and so allowing the transmembrane potential to move nearer to the potassium equilibrium potential. At the higher potential an impulse is able to depolarize because the rate of entry of sodium ions is faster; as Weidmann (1955) has shown, when the transmembrane potential is too low, the rate of sodium entry is too slow to depolarize.

An earlier observation was that of Bülbring and Burn (1949), who allowed atria to contract until they stopped after being left in the organ bath for 30 to 40 hr. They showed that acetylcholine restarted the beat, and this result was confirmed by Holtz and Westerman (1955). Goodford (1959) determined the intracellular potassium and found that, when contractions stopped, it had fallen to a point at which the transmembrane potential could not have been more than 60 mV. The restarting of the contractions by acetylcholine could therefore be attributed to a rise in the membrane potential.

Later Briscoe and Burn (1954) found that atrial contractions were also arrested by quinidine,

and that again acetylcholine would restart them. When the contractions were arrested by quinidine, Armitage (1957) found that they could also be started again by reducing the external potassium concentration to one-half. This indicated that the arrest by quinidine was due to a fall in the membrane potential, and made it likely that the restarting by acetylcholine was due to the rise in the membrane potential.

Thus in the three conditions of atrial arrest there is good reason to believe that the action of acetylcholine in restarting the beat was due to the rise in membrane potential which it caused. The choline acetylase system in the atria can synthesize acetylcholine, and, as already stated, there is clear evidence that it is constantly being formed. The acetylcholine so formed could then have as one of its functions the maintenance of a normal transmembrane potential, and, if this were so, a fall in temperature which causes atrial arrest should cause a fall in acetylcholine synthesis.

We found that the activity of the choline acetylase at 21° was still about 40% of that at 33°. Below 21°, however, the activity fell very rapidly, being down to 20% at 17°, and down to 10% at 13°. Thus in the temperature range in which atria ceased to beat the choline acetylase activity was greatly affected by temperature. Whereas in the range from 33° to 21° the Q_{10} was 2.0, in the range from 21° to 17° it was 6.2 and from 17° to 13° it was 7.8. These observations support the view that the arrest of the atria by cooling is due to the reduction of choline acetylase activity, as a result of which insufficient acetylcholine is formed to maintain the transmembrane potential above 60 mV.

When atria arrested by cooling were restarted by the addition of acetylcholine to the bath, atropine abolished the effect (Marshall and Vaughan Williams, 1956). Similarly when they were restarted by vagal stimulation (Burn and Rand, 1958) atropine also abolished the effect. The restarting of atria by warming was not affected by atropine, and hence it might be concluded that warming did not restart the atria by increasing acetylcholine synthesis. However, it is quite possible that acetylcholine produced within the atria is acting at a point where it is inaccessible to the blocking action of atropine. Thus it is well known that certain parasympathetic effects, those of the vagus and the pelvic nerves on the muscular walls of the small and of the large intestine and of the bladder, are highly resistant to atropine. It is possible that studies with the electronmicroscope will throw more light on the location of the structures containing choline acetylase.

In the ventricles, choline acetylase activity was found to be negligibly small, from which it may be concluded that acetylcholine can exert no similar control over the membrane potential in ventricular muscle. The difference between atria and ventricles in this respect is parallel to the difference in the effect of acetylcholine on the action potential. In the atria, acetylcholine shortens the action potential whereas in the ventricles it has no effect.

If the conclusion concerning the function of acetylcholine formed in the atria is correct, we may have a clue to the reason for the formation of acetylcholine in the gill plates of *Mytilus edulis* where it maintains ciliary movement. It is possible that here also acetylcholine maintains the membrane potential sufficiently high for the impulses to be propagated which give rise to ciliary contraction.

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