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THE MODIFICATION OF THE ACTION OF OUABAIN IN CARDIAC TISSUE BY ESERINE AND ACETYLCHOLINE

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Various papers have been published which indicate some relation between the action of cardiac glycosides such as strophanthin or ouabain, on the one hand, and that of acetylcholine (ACh), on the other. Thus Gremels (1937) showed that a small amount of ACh, which alone was without effect on the amplitude of the contractions of the isolated frog heart (Straub preparation), exerted an inhibitory effect of long duration in the presence of strophanthin. Recently Baker (1953) has observed a similar phenomenon in the human foetal heart perfused by the Langendorff method. The inhibitory effect of a small amount of ACh became steadily greater when ouabain was added to the perfusion fluid in a concentration of 0.2 μg./ml., the inhibition affecting both rate and amplitude.

The mode of action of the cardiac glycosides is still obscure; and, in view of increasing evidence that the cardiac rhythm is maintained by the local formation of ACh (Bülbring and Burn, 1949; Burn and Kottegoda, 1953; Briscoe and Burn, 1954; Burn and Walker, 1954; Burn, 1953, 1954), it was decided to make further observations to see if the action of ouabain on the isolated heart was modified by the presence of eserine or of ACh.

METHODS

The heart was excised from a freshly killed rabbit and perfused with Ringer–Locke solution by the Langendorff method. The apparatus used is shown in Fig. 17 of *Practical Pharmacology* (Burn, 1952). The temperature of perfusion was 36–37° C. The solution contained 1 mg./ml. dextrose. The amplitude, rate, and coronary flow were recorded. In experiments on atria, these were dissected and suspended in Locke's solution containing dextrose (2 mg./ml.) at 29° C.; they were aerated with a brisk stream of oxygen.

RESULTS

The Effect of Eserine on the Action of Ouabain.—Observations were first made to see if the presence of eserine sulphate modified the chain of events which followed perfusion of the heart with ouabain.

The ordinary effect of ouabain when perfused through the heart is to cause a slight decline in the amplitude of contraction followed by an increase during the early period. Irregularities then develop and the amplitude slowly declines until the beat is arrested. The precise moment at which this happens is often difficult to determine, and Baker (1947) measured the toxicity of ouabain by finding the time which elapsed until the amplitude was reduced to half its value when perfusion with ouabain began. He observed that, when ouabain was perfused through the heart in a concentration of 0.4 μg./ml., the mean survival time measured in this way was 38 min., the variation in 6 hearts being from 32–51 min.

A series of hearts was therefore perfused with ouabain alone in a concentration of 0.4 μg./ml. Other hearts were perfused with Locke's solution containing eserine sulphate for 30 min., and then with Locke's solution containing eserine and ouabain 0.4 μg./ml. Three concentrations of eserine were used, 1.5×10^{-6} g./ml., 4.5×10^{-6} g./ml., and 1.3×10^{-5} g./ml. In all experiments the time was taken by Baker's method just described. The results are given in Table I. The mean time for ouabain alone was 42.5 min., which was similar to the time of 38 min. found by Baker (1947). The presence of 1.5×10^{-6} eserine made no appreciable difference, but its presence in the higher concentrations prolonged the mean time to 106 min. and to 76 min. respectively. The prolongation of the time was, however, not evident in all the hearts, and when the eserine concentration was 4.5×10^{-6} g./ml. the times were outside the range for ouabain alone in six out of 11. In one of these the prolongation was more than 200 minutes, when the experiment was discontinued. Similar results were obtained when eserine was used in concentration 1.3×10^{-5} g./ml.; five of the results were outside those obtained with ouabain alone. The

TABLE I
RABBIT HEARTS PERFUSED WITH 0·4 μG./ML. OUABAIN, WITH AND WITHOUT ESERINE
Figures are the times in min. from beginning perfusion with ouabain until the amplitude was reduced to half.

Ouabain Alone	Perfusion with Eserine followed by Ouabain + Eserine. Eserine concentration:—		
	$1·5 \times 10^{-6}$ g./ml.	$4·5 \times 10^{-6}$ g./ml.	$1·3 \times 10^{-5}$ g./ml.
41	63	28	77
29	36	56	96
25	35	171	108
25	50	98	102
23	44	161	57
65		55	92
23		71	89
27		200+	45
59		114	48
85		49	79
63		161	46
53			
34			
Mean 42·5	45·6	106·0	76·0

mean results for the two higher concentrations of eserine differed significantly from those with ouabain alone. The standard error of the results with ouabain alone was 5.65; for the results with eserine 4.5×10^{-6} g./ml. it was 17.81, and for the results with eserine 1.3×10^{-5} g./ml. it was 7.1. P was therefore less than 0.01 in both cases.

Appearance of Irregularity.—In addition to recording the time to half the initial amplitude, other measurements were made; thus the time at which irregularity was first seen was recorded, and also the time at which the rate became much faster. Finally, the time was recorded at which the coronary flow began to accelerate. The mean times are given in Table II, from which it appears that the

TABLE II
PERFUSION OF RABBIT HEARTS WITH OUABAIN 0·4 μG./ML.

Eserine g./ml.	Mean Times (min.)		
	To Irregularity	To Quickening of Rate	To Increase in Coronary Flow
0	19	21	23
$1·5 \times 10^{-6}$	33	32	30
$4·5 \times 10^{-6}$	77	58+	59+
$1·3 \times 10^{-5}$	40	41	47

mean time for irregularity was similar to the mean time to quickening of the rate and to the increase in coronary flow. These times were lengthened by all concentrations of eserine, the greatest effect being produced by the concentration 4.5×10^{-6} g./ml. In one of the hearts no quickening of rate was observed, so that the mean figure excludes a high value.

When the time to the occurrence of irregularity was expressed for each heart as a percentage of the time to half amplitude, the mean for ouabain alone was 45%, and for ouabain plus eserine in concentration 4.5×10^{-6} g./ml. was 57%. Thus in the presence of eserine the occurrence of irregularity was delayed. The difference between 45 and 57% was just about significant at the 5% level. For $P = 0.05$, t should have been 2.074, whereas it was 2.025. This calculation, however, did not include one high value. All measurements therefore supported the main conclusion that the toxic action of ouabain on the heart was diminished in the presence of eserine.

Experiments on Atria.—Burn and Kottegoda (1953) have shown that when eserine acts on isolated atria in a concentration between 10^{-4} and 10^{-3} g./ml. the contractions stop. Briscoe and Burn (1954) have given reasons for supposing that this arrest is due to a failure of conduction, and that the action of eserine in this high concentration is not that of an anticholinesterase but is an action resembling that of quinidine. They think that quinidine and high concentrations of eserine both compete with ACh formed in the heart for receptors on which that ACh normally acts. Eleven experiments were carried out on isolated atria to see if the presence of ouabain affected the amount of eserine necessary to arrest the contractions.

In some experiments the action of eserine alone was tested first by adding it to the bath in amounts of 1 or 2 mg., waiting for 15 min. between each addition, until arrest occurred. The amount of eserine was recorded, and the bath was then changed repeatedly during the next hour to remove the eserine as thoroughly as possible. Ouabain was added to the bath so that the concentration was 0.5×10^{-6} g./ml.; eserine was then added as before. When the atria were again arrested the amount of eserine was recorded, the bath changed, and eserine alone was repeated. In other experiments ouabain was added at the beginning so that the amount of eserine required to stop the atria in its presence was determined first, and so on. The results are given in Table III. In expts. 1–4 eserine was tested

TABLE III
AMOUNT OF ESERINE SULPHATE (MG.) NECESSARY TO ARREST THE CONTRACTIONS OF RABBITS' ISOLATED ATRIA IN THE ABSENCE AND PRESENCE OF OUABAIN ($0·5 \times 10^{-6}$ g./ml.)

Expt.	Ouabain				Expt.	Ouabain		
	0	+	0	+		+	0	+
1	15	10	—	—	5	16	—	—
2	10	7	10	7	6	5	—	—
3	12	6	—	—	7	13	—	—
4	24	6	8		8	16	16	—
					9	8	20	10
					10	14	16	10
					11	10	10	—
	15·2	7·2	9·0	7·0		11·7	15·5	10·0

alone, then in the presence of ouabain, then again alone, and in expt. 2 once again in the presence of ouabain. In expts. 5–11 eserine was first tested in the presence of ouabain, and so on. In the presence of ouabain less eserine was required. The difference was well shown in expts. 2, 9, and 10. The mean figures of expts. 1–4 and of expts. 5–11 also show it. When all experiments were taken together the mean amount of eserine required to arrest the atria in the absence of ouabain was 14.1 mg., whereas in the presence of ouabain it was 9.86 mg. The standard errors of these figures were 1.61 and 1.06 respectively, so that $t=2.21$ and $P=0.04$. The conclusion was drawn that ouabain reduced the amount of eserine required to arrest the atria.

Experiments with ACh and Ouabain.—Since eserine has been shown to have a quinidine-like action as well as an anticholinesterase action, experiments were carried out on the isolated rabbit heart to see if ACh itself would modify the action of ouabain in the same way as eserine. In a series of 6 hearts, Ringer-Locke containing ouabain 0.4 μg./ml. and ACh 0.1 μg./ml. was perfused and the time was observed until the amplitude was reduced to half the initial value. The results shown in Table IV indicate that these hearts behaved like

TABLE IV

RABBIT HEARTS PERFUSED WITH 0·4 μG./ML. OUABAIN AND ACh 0·1 μG./ML.

Figures are the times in min. from beginning perfusion with ouabain until the amplitude was reduced to half.

Ouabain and ACh Together	Ouabain and ACh following 30 min. Perfusion with ACh Alone
31	150
27	121
33	45
25	57
66	54
19	85
	160
	53
	53
	72
Mean 33·5	85

those perfused with ouabain alone. Further experiments were then made in which hearts were first perfused with ACh for 30 min. and then with ouabain plus ACh, as had been done in the experiments with eserine. In these circumstances the time to half-amplitude was outside the range for ouabain alone in 4 out of 10 preparations. These results also appear in Table IV. The difference between the mean for ouabain alone, given in Table I, and the mean value for ouabain and ACh following 30 min. perfusion with ACh alone was significant, P being less than 0.01. In the experiment in Table IV in which the time was 160 min., a change at that point from ouabain and ACh to ouabain alone caused a rapid reduction of the amplitude in 5 min. Thus evidence was obtained that the effect of eserine in diminishing the toxic action of ouabain was probably due to its anticholinesterase action.

DISCUSSION

The work described in this paper was carried out to test one aspect of the hypothesis put forward by Bülbring and Burn (1949) that the heart beat is maintained by the local formation of ACh. The cardiac glycosides have an important effect on cardiac rhythm and force of contraction, and if the rhythm and contraction are controlled by the local production of ACh the effect of a cardiac glycoside like ouabain should be modified by ACh and especially by a change in the amount of ACh locally produced.

The results show that the simultaneous perfusion of ouabain and ACh has less toxic effect on the isolated heart than the perfusion of ouabain alone. The effect of the ACh was similar to the effect—described by Baker (1947)—of an increase in the amount of potassium in the perfusion fluid, with the difference that the ACh diminished the toxic effect of the ouabain only when the heart was exposed to the action of the ACh for 30 min. before it was acted on by the two substances together. The main result, however, was that eserine in a concentration of 4.5×10^{-6} g./ml. appreciably diminished the toxicity of ouabain. Recent work in this department by Dr. Heather Shelley has shown that, when the effect of eserine is determined on the cholinesterase in the atria of the rabbit heart, a concentration of 5×10^{-6} g./ml. causes 82% inhibition. It is probable that this amount of eserine is acting only as an anticholinesterase when perfused with ouabain through the heart, and that the diminution of the toxicity of the ouabain is due to the rise in the concentration of the ACh produced locally.

In the observations on the atria, eserine was added in much higher concentrations until the contractions were arrested. The evidence of Briscoe and Burn (1954) indicates that at this point eserine is exerting a quinidine-like action quite apart from its anticholinesterase action. In the presence of ouabain less eserine was required to arrest the atria. The relationship between ouabain and eserine was thus the opposite of the relationship in the perfused heart when low concentrations of eserine were employed, and this difference adds

to the probability that in the perfused hearts it was the anticholinesterase action of eserine which diminished the toxicity of ouabain.

The mechanism of the action of the cardiac glycosides is still unknown, but these results suggest that it may be related to the part played by ACh in maintaining the cardiac rhythm.

Summary

1. When the isolated rabbit heart is perfused with ouabain (0.4 μg./ml.) the toxic effect of the ouabain is diminished by the presence of eserine sulphate (4.5×10^{-6} g./ml.) in the perfusion fluid.

2. If acetylcholine is added to the perfusion fluid instead of eserine, a similar diminution in the toxic action of ouabain is observed.

3. When the isolated atria of the rabbit are beating spontaneously in a bath, the amount of eserine sulphate required to cause arrest of the beat is diminished in the presence of ouabain (0.5×10^{-6} g./ml.).

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