Brit. J. Pharmacol. (1958), 13, 415.

A DEPRESSANT EFFECT OF ACETYLCHOLINE ON THE IDIO-VENTRICULAR PACEMAKER OF THE ISOLATED PERFUSED RABBIT HEART

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(RECEIVED JULY 2, 1958)

In Langendorff preparations of the isolated rabbit heart, excision of the atria and severance of the atrio-ventricular bundle resulted in the onset of idio-ventricular rhythm at a rate much below the original sino-atrial rate. The mean decrease in rate in twelve experiments was 61%. Acetylcholine perfusion decreased the idio-ventricular rate still further. Physostigmine augmented this effect while atropine prevented or abolished it. Similar results were obtained with a rat heart. The results indicate that a cholinergic receptor mechanism is present at pacemaker sites in mammalian ventricles. However, when rabbit ventricles, beating under atrio-ventricular nodal rhythm or idio-ventricular rhythm, were cooled to the point of cardiac arrest, acetylcholine failed to cause reappearance of the cardiac beat.

Acetylcholine has been shown to affect the transmembrane atrial resting and action potentials in a number of species (Cranefield and Hoffman, 1958), and these effects present a basis for understanding the actions of this substance on rate, conduction velocity, and refractory period of atrial tissue. Effects on the transmembrane action potential of the frog ventricle have also been demonstrated, but experiments in which the effect of acetylcholine on the mammalian ventricular action potential was examined have proved negative (Brooks, Hoffman, Suckling, and Orias, 1955). Further, the generally accepted fact that the ventricle does not receive cholinergic innervation has been suggested as a reason for these negative results (Hoffman and Suckling, 1953; Brooks *et al.*, 1955). The experiments presented here indicate that acetylcholine can decrease the idio-ventricular rate of the isolated perfused rabbit and rat heart, and that this effect is augmented by physostigmine and is reversed or prevented by atropine. The effect of acetylcholine on isolated rabbit ventricles cooled to the point of cardiac arrest was also examined. The results of 19 experiments are described.

METHODS

Rabbit hearts were set up for Langendorff perfusion at 38°. The perfusion fluid used was that described by McEwen (1956). The heart rate was measured from the electrocardiogram, which was recorded by leads from the two ventricles. In a few early experiments, heart rate was counted with the aid of a stop-watch by observing ventricular contractions on a kymograph drum. For the production of atrio-ventricular nodal rhythm the atria and interatrial septum were excised at the level of the atrio-ventricular groove. In the experiments on idio-ventricular rhythm, the atrio-ventricular bundle was severed by an additional cut in the inter-ventricular septum. Drugs employed were added to the bottles containing the perfusion fluid. Drug concentrations are expressed as g./l. of the base. When hearts were cooled, this was accomplished by the gradual adding of tap water and ice to the water-bath in which the perfusion coil was immersed. The rate of cooling was about 1°/min.

RESULTS

Idio-ventricular Rhythm and the Effect of Acetylcholine

Table I shows the change in rate produced in the isolated perfused rabbit heart by excision of the atria and severance of the atrio-ventricular bundle. In 12 experiments, the mean heart rate dropped from 166 to 66 beats/min., a mean decrease in rate of 61%. In each of six of these experiments acetylcholine proved capable of decreasing the idio-ventricular rate still further and the magnitude of the effect increased with

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TABLE I

THE CHANGE IN HEART RATE PRODUCED IN THE ISOLATED, PERFUSED RABBIT HEART BY EXCISING THE ATRIA AND SEVERING THE ATRIO-VENTRICULAR BUNDLE

Sino-atrial Rate (Beats/Min.)	Idio-ventricular Rate (Beats/Min.)	Difference (Beats/Min.)	% Decrease
120	47	73	61
138	90	48	35
145	38	107	74
152	40	112	74
160	76	84	53
165	41	124	75
170	76	94	55
170	102	68	40
172	29	143	83
181	95	86	48
190	51	139	73
234	102	132	57
Mean 166	66	101	61
Range 120–234	29–102	48–143	35–83

TABLE II

THE EFFECT OF INCREASING THE ACETYLCHOLINE CONCENTRATION (WITH AND WITHOUT PHYSOSTIGMINE) ON THE IDIO-VENTRICULAR RATE OF THE ISOLATED, PERFUSED RABBIT VENTRICLE

Heart rates are given in beats/min. ACh=acetylcholine. The results on each horizontal line all refer to one experiment. The letters in brackets relate results from the same experiment. (1) Indicates that the response was prevented by atropine 10^{-7} during subsequent ACh perfusion; (2) that the response was reduced by atropine 10^{-7} during continued ACh perfusion; (3) that the effect was prevented by atropine 10^{-6} during subsequent ACh perfusion; (4) that the results were obtained in the presence of atropine 10^{-7}.

ACh Conc.	Without Physostigmine		Physostigmine 10^{-7}		Physostigmine 10^{-6}	
	Control Rate	% Decrease after ACh	Control Rate	% Decrease after ACh	Control Rate	% Decrease after ACh
10^{-7}	40 (a)	8				
	76	13	63	37	53	91 (1)
	95 (b)	34 (2)				
			82 (c)	100 (1)		
10^{-6}	36 (a)	17				
	63	17			53	100 (2)
	90	32			82	100 (2)
					60	100 (1)
10^{-5}	32 (a)	44				
	76 (b)	63 (3)				
	80 (b)	54				
	85 (c)	49 (4)				
	90 (c)	52 (4)				
					38	100 (3)

increasing concentrations. Physostigmine, which by itself reduced the rate slightly, markedly augmented the effect of acetylcholine. Cardiac arrest, not seen with a 10^{-5} concentration of acetylcholine, was observed with a 10^{-7} concentration of acetylcholine in the presence of physostigmine 10^{-7}. In concentrations of 10^{-7} and 10^{-6}, atropine prevented or abolished the acetylcholine effect even in the presence of physostigmine. These results are summarized in Table II. Similar results with acetylcholine, physostigmine and atropine were observed in one experiment with the isolated perfused rat heart after excision of the atria and severance of the atrio-ventricular bundle.

TABLE III

THE EFFECTS ON RHYTHMICITY OF PROGRESSIVE COOLING OF THE ISOLATED, PERFUSED RABBIT VENTRICLE

(2) This result was obtained from the heart with suffix (1) following a second period of cooling after rewarming to 38°.

	Temperature at which Contractions Ceased	Q_{10} for Heart Rate (38–28°)
Atrio-ventricular nodal rhythm	6·2	2·1
	6·8	
	12·5	2·0
	14·0	
	14·8	
Idio-ventricular rhythm	13·5	2·3 (1)
		2·9 (2)
	17·2	3·3
	18·0	2·7
	Mean 12·9	2·6
	Range 6·2–18·0	2·0–3·3

The Effects of Cooling

In eight hearts, with atria excised (atrio-ventricular nodal rhythm), three of which had the atrio-ventricular bundle severed (idio-ventricular rhythm), progressive cooling resulted in cardiac arrest at a mean temperature of 12.9° (range 6.2 to 18.0). The Q_{10} for heart rate (the increase in heart rate for a rise of temperature of 10°) measured in five of these hearts over the temperature range 38° to 28°, had a mean value of 2.6 (range 2.0 to 3.3).

Table III summarizes the results. Acetylcholine in concentrations of 10^{-7}, 10^{-6}, and 10^{-5} failed to cause the reappearance of the cardiac beat during cardiac arrest at the low temperatures. Acetylcholine (10^{-7}, 10^{-5}), administered at low temperatures, but not low enough for cardiac arrest, produced cardiac slowing or cardiac arrest in each of three experiments.

DISCUSSION

The excision of the atria and section of the atrio-ventricular bundle to establish ventricular pacemaker activity in the isolated rabbit heart is an unphysiological procedure. However, it provided a series of hearts in which ventricular pacemaker activity, at rates well below the original sino-atrial rates, could be studied. The exact location of the pacemaker was unknown, but on anatomical grounds it could not have been atrial or nodal.

The findings that acetylcholine decreased the rate of ventricular pacemaker discharge, that physostigmine augmented this effect and that atropine prevented or abolished it clearly indicate the presence of a receptor mechanism for acetylcholine in the rabbit and rat ventricle. A report by Baker (1953) on the slowing of the idio-ventricular rate produced by acetylcholine in the isolated perfused human foetal heart suggests that a similar mechanism exists in the human ventricle.

Additional support for the idea that acetylcholine has a depressant effect on the rate of discharge of ventricular pacemakers comes from experiments on the isolated perfused rat heart. In this preparation, as in the rabbit heart, atrial excision and severance of the atrio-ventricular bundle caused a marked drop in rate consistent with the appearance of an idio-ventricular pacemaker.

However, in experiments on isolated perfused rat hearts beating under sino-atrial rhythm, acetylcholine and physostigmine perfusion for 8 min. caused immediate cessation of the beat without the establishment of an idio-ventricular pacemaker (Benforado, 1959). Such an effect points to inhibition of all cardiac pacemakers by acetylcholine.

Although it is generally accepted that the ventricles do not receive cholinergic innervation, this fact cannot be a basis for suggesting (Hoffman and Suckling, 1953; Brooks *et al.*, 1955) that the ventricular cell membrane is incapable of responding to acetylcholine. Numerous examples of responsiveness of non-innervated tissues to transmitter agents are available. For acetylcholine one of the best studied examples is the nerve-free gillplate of *Mytilus edulis* (Bülbring, Burn and Shelley, 1953). The responsiveness of nerve-free placental vessels to adrenaline was demonstrated by von Euler (1938).

That the isolated rabbit ventricle can liberate acetylcholine during perfusion is evident from the experiments reported by Day (1956). When recirculated perfusion fluid from rabbit hearts, with and without the atria excised, was assayed on the frog heart and on the frog rectus, two-thirds of the acetylcholine liberated was found to derive from the ventricles. This endogenous acetylcholine, which may play a rôle in impulse production in the ventricle, could account for the decrease in idio-ventricular rate with physostigmine described in the rabbit ventricle. Such effects on rate have also been noted in rabbit atria (Burn and Kottegoda, 1953).

In the experiments on cooling of rabbit ventricles during atrio-ventricular nodal and idio-ventricular rhythm, the Q_{10} values for changes in rate obtained over the range 38–28° were of the same order as those reported for isolated rabbit auricles (30 to 20°) by Marshall and Vaughan Williams (1956). In their experiments on auricles, acetylcholine was able to restart contractions after arrest at low temperatures. No such action was seen in the ventricles. The results in the atria were explained by the action of acetylcholine in increasing the resting membrane potential of cooled auricles, allowing non-conducted pacemaker potentials to be propagated. Confirmatory experiments, showing that acetylcholine increased the low diastolic membrane potential of cooled auricles, were reported by Marshall (1957). The negative results in the present experiments on the ventricles may have been due to the fact that pacemaker potentials were absent during cardiac arrest at low temperatures. However, since acetylcholine has no effect on the membrane potential of ventricular tissue there may be an alternative explanation. This is that it is unable to restore conduction in the cooled ventricle as it does in the cooled auricle. It would be misleading to infer from this that acetylcholine is without any effect on ventricle tissue. The present experiments indicate a depressant effect on pacemaker sites in the ventricle. Taken together with reports on ventricular arrhythmias induced by acetylcholine (Scherf and Schott, 1953) and the well-known effects of acetylcholine and vagal stimulation on the rate of discharge of atrial and atrio-ventricular nodal pacemakers, there appears to be a general responsiveness of cardiac pacemakers to this substance. However, the failure of Weidmann (1956) to observe any effect of acetylcholine on the rate of diastolic depolarization in Purkinje fibre pacemakers (species not specified) makes such a generalization untenable. The presence of an effect of acetylcholine on the action potential of frog ventricle and its absence in mammalian ventricle indicates a species difference. Such a difference may also exist between mammalian hearts. Differences in the effect of acetylcholine on the atrial resting membrane potential of various mammalian species (Cranefield and Hoffman, 1958) support this idea.

This research was done during the tenure of a Life Insurance Medical Research Fund Fellowship, New York, N.Y. I wish to thank Professor J. H. Burn for suggesting the problem and for the opportunity of working in his laboratory.

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