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THE EFFECT ON THE ISOLATED RABBIT HEART OF VAGAL STIMULATION AND ITS MODIFICATION BY COCAINE, HEXAMETHONIUM AND OUABAIN

By L. M. McEWEN

From the Department of Pharmacology, University of Oxford

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The experiments to be described have been concerned with the effect of vagus stimulation in the isolated heart and auricles of the rabbit. In the vagus-heart experiments the action of cocaine, of hexamethonium and of the cardiac glycoside ouabain was studied.

As recently as 1953, Obrink & Essex state that 'it has become a widely accepted fact that stimulation of the vagi does not influence an isolated heart after 20–30 min. The reason for the transient response is not known.' Not many attempts to study this question have been found in the literature. Effects of vagal stimulation on perfused isolated heart preparations have been described by a number of workers (Cullis & Tribe, 1913; Middleton, 1947; Perry and Talesnik, 1953; Perry & Reinert, 1954), but of these only Middleton made a statement concerning the length of time his preparations worked satisfactorily. He perfused the cat's heart and found that vagal effects were obtained for 6 hr provided the heart remained *in situ*, but when the heart was isolated, vagal effects were not obtained after 10–30 min. Using the method now to be described it was possible to make observations for periods up to 9 hr.

METHODS

Rabbits were anaesthetized with urethane. The vagi were cut in the neck and dissected free from the surrounding tissue to the level of the aortic arch. The thoracic contents were excised and the cardiac ends of the vagi and the heart were cleared of as much tissue as possible, but leaving the root of the lungs and a short piece of trachea still adhering to the auricles. In this way the finer cardiac branches of the vagi were undisturbed. Finally, the aorta was cannulated, and the heart perfused by the usual Langendorff method. The preparation behaved satisfactorily provided that the final cleaning took no longer than 20 min.

To prevent drugs from affecting the nerves, the perfusion fluid for the heart and the fluid bathing the stimulating electrodes were supplied by separate systems. These both included large chambers for saturating the solutions with the gas mixture. Having been warmed to a temperature of 35° C, fluid reached the heart with a pressure of 40 cm of water. The fluid supplied to the electrodes was heated by part of the water jacket which surrounded the heart. The flow of fluid

past the nerves was controlled by a tap and could be observed in a drop-chamber. The apex of the ventricles was attached by a thread to an isotonic lever writing on a smoked drum.

The heart was perfused with a solution of the following composition: NaCl 7·6 g, KCl 0·42 g, $CaCl_2$ 0·24 g, NaH_2PO_4 0·143 g, $NaHCO_3$ 2·1 g, dextrose 2·0 g, sucrose 4·5 g, distilled water 1000 ml. The addition of sucrose to the solution increased the useful life of the preparation. The solution was saturated with oxygen containing 5% carbon dioxide, and the pH when fully saturated was 7·3. The parts of the nerves near the stimulating electrodes were bathed by Krebs's solution, since it was observed that a more constant response was obtained in this way.

Stimulation. The vagi were threaded through a Perspex tunnel 1·5 mm in diameter through which warm oxygenated Krebs's solution was passed. The stimulating electrodes were platinum rings in the wall of the tunnel 2·5 mm apart (see Fig. 1).

In the experiments on ouabain it was desired to use both maximal and submaximal stimulation of the nerves. Two pairs of electrodes were used. The lower pair supplied square pulses of 0·2 msec duration and supra-maximal current strength at a frequency of 7/sec. The upper pair of electrodes supplied submaximal stimulation. It was found, however, that with ordinary submaximal currents the effects were not constant. A portion of the nerves between the upper and lower pairs of electrodes was therefore damaged by repeatedly stabbing with the point of a pin. This was continued until the slowing of the heart rate by stimulation through the upper pair of electrodes was reduced to about half. After an interval of 30 min to ensure that no further change was occurring at the point of injury, the experiments were begun using stimuli at the upper pair of electrodes similar to those applied at the lower pair but at a frequency of 23 per sec.

Isolated auricles. The heart and the vagi were removed from the animal and prepared in the usual way. Finally, the ventricles were cut away, and the auricles and nerves mounted on a special holder which incorporated the electrodes (Fig. 2). The auricles were then placed in a 50 ml. bath containing the same solution as that used for perfusion of the Langendorff heart, to which penicillin and streptomycin were added at concentrations of 10 mg/100 ml. The temperature was 29° C. Oxygen with 5% carbon dioxide was supplied through a sintered glass tube. The nerves were threaded through a Perspex tunnel in which the stimulating electrodes were mounted. Since a fresh supply of the solution, oxygenated and warmed, flowed slowly down the tunnel, it was impossible for drugs placed in the bath to affect the nerves at the point of stimulation.

RESULTS

Experiments on the isolated heart

The effects of stimulation of the vagi were uniform over periods of 2–3 hr. When maximal stimuli were applied to the nerves, a frequency of 2 impulses/sec was sufficient to slow the heart slightly. 20 impulses/sec usually stopped the beat for about 10 sec, after which occasional beats would occur. No increase in effect was produced if the frequency of stimulation was increased beyond 30 impulses/sec.

At the end of stimulation the heart rate and amplitude often increased as shown in Fig. 3. This effect was greater in some hearts than in others, and it tended to disappear after the heart had been perfused for a few hours. Acceleration of the heart was not seen before the end of stimulation.

Cocaine. When cocaine was added to the fluid perfusing the heart in a concentration such as 5×10^{-6} g/ml. of the hydrochloride, the phase of acceleration which followed stimulation was increased: and in hearts in which no acceleration was previously seen, cocaine caused the acceleration to appear,

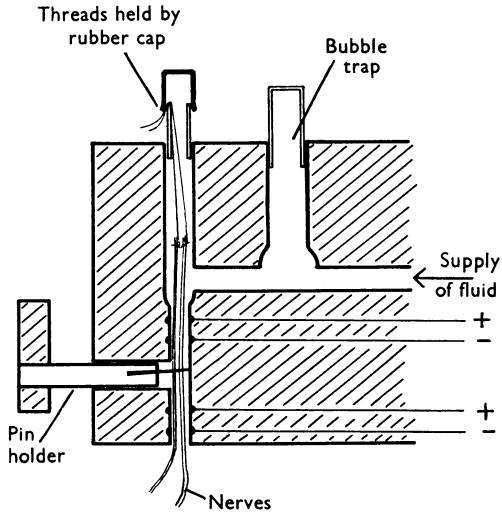

Fig. 1.

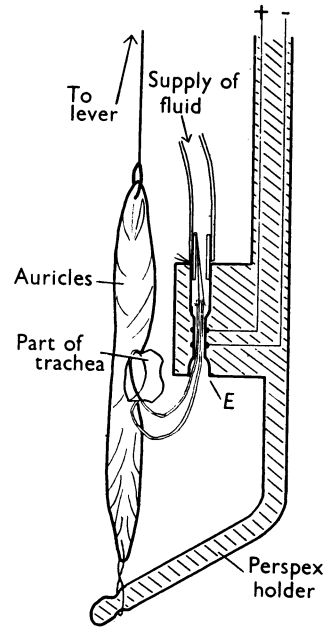

Fig. 2.

Fig. 1. The stimulating electrodes. The nerves were secured by threads attached to their cut ends. They were damaged by the pin mounted in its holder. Maximal stimuli applied to the upper pair of electrodes were conducted to the heart only by nerve fibres not damaged by the pin. All the nerve fibres were excited by the lower pair of electrodes.

Fig. 2. Diagram of the holder for the isolated auricles and the stimulating electrodes. The nerves were secured in the tunnel *E* containing the electrodes by the threads attached to their cut ends.

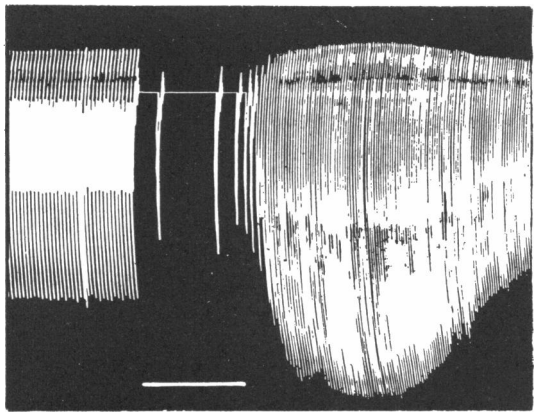

Fig. 3. Rabbit heart. Langendorff perfusion. Both vagi were stimulated by maximal impulses 20 times/sec for 10 sec as shown by white line.

as shown in Fig. 4. Larger concentrations of cocaine reduced both the inhibitory and stimulating effects of the vagi. When the concentration of cocaine blocked the effects of the nerves completely (e.g. 2×10^{-5} g/ml.), the hearts frequently developed abnormal rhythms, and the amplitude of beat was reduced. Vagal inhibition was usually restored within 25 min after perfusing without cocaine, but the phase of acceleration often remained potentiated for a long time. The effect of the injection of 2–4 μg acetylcholine (ACh) into the cannula, initially sufficient to slow the heart rate by 50% during a 10 sec count, was not abolished by cocaine in concentrations which abolished the effect of vagal stimulation.

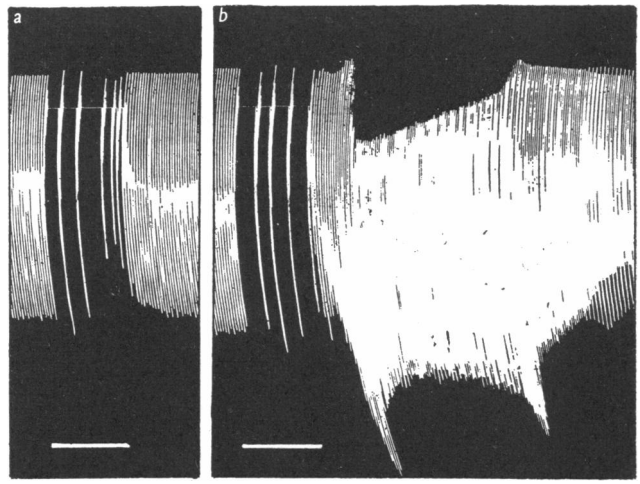

Fig. 4. Rabbit heart. Langendorff perfusion. Both vagi were stimulated with maximal stimuli 20 times/sec for 10 sec as shown by white lines: (*a*) before, and (*b*) during, perfusion with 3 μg/ml. cocaine.

Hexamethonium. In the hearts in which vagal stimulation caused inhibition followed by acceleration, perfusion with hexamethonium (2×10^{-4} g/ml.) abolished the inhibition within 10 min but not the acceleration, as shown in Fig. 5. This was observed ten times in six preparations. It was unaffected even if the concentration of hexamethonium in the perfusing fluid was increased tenfold, when any sympathetic ganglia in the heart were almost certainly blocked. The acceleration was still seen if the nerves were stimulated in a region which had been at the level of the larynx before the preparation was removed from the rabbit. The effect might, however, have been due to the stimulation of postganglionic sympathetic nerve fibres present in the parts of the vagi which originally lay high in the neck.

Perry & Talesnik (1953) found that the inhibiting action of small doses of

ACh on the perfused cat's heart was abolished by hexamethonium, and concluded that ACh acted not directly on the cardiac muscle but rather on the ganglia. A series of observations was therefore made in which the effect of hexamethonium on vagal stimulation and on the effect of ACh was studied.

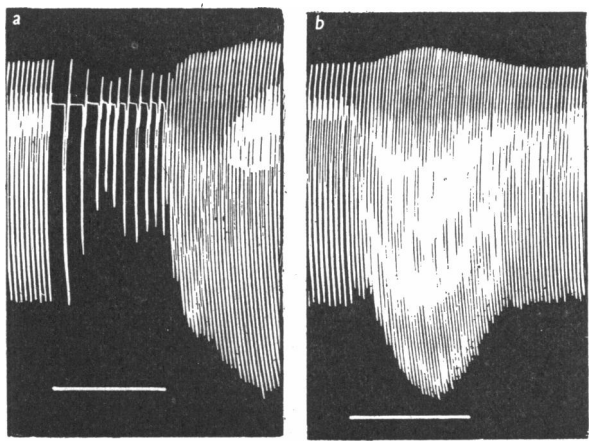

Fig. 5. Rabbit heart. Langendorff perfusion. Both vagi were stimulated with maximal impulses 20 times/sec for 10 sec as indicated by the white lines: (*a*) before hexamethonium; (*b*) after perfusion with 0·2 mg/ml. hexamethonium for 15 min.

The results are shown in Table 1. In five of the ten observations in Table 1, hexamethonium, although blocking vagal stimulation completely, reduced the action of ACh by less than 10%. In four of the observations hexamethonium diminished the action appreciably, but three of these observations were made in the same heart. Thus of the six hearts used, only in two did hexamethonium reduce the action of ACh significantly, and in no case did it abolish the action. This is illustrated in Fig. 6. Fig. 6*a*, *b* shows the abolition of vagal stimulation by hexamethonium, while Fig. 6*c*, *d* shows that the slowing caused by 2 μg ACh was not appreciably affected. During a 10 sec count the rate was reduced by 58% in the control period and by 56% in the presence of hexamethonium. The observation in Fig. 6*c* was made immediately after that in Fig. 6*a*, and that in Fig. 6*d* was made immediately after that in Fig. 6*b*.

Ouabain. Preliminary experiments showed that ouabain at a concentration of 0·2 μg/ml. in the perfusing fluid usually had no effect on the heart, while a concentration of 0·3 μg/ml. blocked the inhibitory action of the vagi, and produced a rapid and irregular heart rate within 45 min. Potentiation of the inhibitory action of the nerves was, however, demonstrated when 0·25 μg/ml. of ouabain was perfused. In this concentration ouabain reduced the heart rate, and after a latent period of 10 min or more, increased the amplitude of beat, though sometimes these effects were small.

TABLE 1. Effect of hexamethonium (2×10^{-4} g/ml.) on inhibition of heart rate produced by ACh at a time when inhibition by vagal stimulation was blocked

Expt.	Dose of ACh (μg)	Percentage rate reduced: Control	Percentage rate reduced: In presence of hexamethonium
1	3	64	18
	6	79	59
	9	79	46
2	2	58	56
	1	45	35
3	2	39	41
	8	96	88
4	5	54	25
5	7	82	90
6	5	80	73

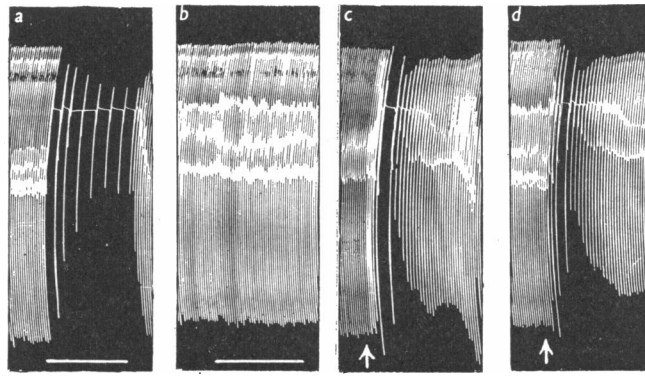

Fig. 6. Rabbit heart. Langendorff perfusion. Both vagi were stimulated at (*a*) and (*b*) with maximal stimuli 20 times/sec for 10 sec as indicated by the white lines. At (*c*) and (*d*) after injection of 2 μg ACh; (*b*) and (*d*) were taken consecutively after 25 min perfusion with hexamethonium (2×10^{-4} g/ml.).

Table 2 shows the results of ten experiments with 0·25 μg/ml. of ouabain. Observations were made in thirty-three preparations and positive results were obtained in almost all. As more experience was gained, increasing care was taken to establish that variations in the response to stimulation did not occur spontaneously. The results given in Table 2 were those of the last ten experiments in which the effect of both submaximal and of maximal stimulation were seen to be nearly constant during 50–60 min, not varying more than 10% from the mean value. Only when the preparation was thus stabilized was the effect of perfusing ouabain examined. The figures in Table 2 for the control periods were mean figures calculated from ten observations. When ouabain was infused the effect of stimulating the vagus slowly increased, and the figures in the table show the highest values reached. When maximal

stimulation was used, potentiation was observed in six out of ten experiments, the mean change for all ten experiments being an increase of 19%. When submaximal stimulation was used vagal stimulation was potentiated in all experiments, the mean increase being 34%. The rate of increase was sometimes

TABLE 2. Effect on vagal slowing of ouabain (0·25 μg/ml.) in the perfusion fluid. Figures are the percentage by which the rate was reduced.

	Maximal stimulation			Submaximal stimulation		
Expt.	Control period	In presence of ouabain	Change	Control period	In presence of ouabain	Change
7	46	51	+5	52	78	+26
8	42	79	+37	39	100	+61
9	50	66	+16	40	68	+28
10	45	81	+36	42	69	+27
11	34	68	+34	7	67	+60
12	51	46	−5	36	44	+8
13	48	65	+17	50	84	+34
14	38	86	+48	35	74	+39
15	39	46	+7	5	46	+41
16	42	40	−2	30	50	+20
Mean			+19			+34

faster for submaximal than for maximal stimulation, so that in Expts. 9, 11 and 14 the potentiation of submaximal stimulation was fully developed from 10 to 20 min before that of maximal stimulation. Recovery from the effect of perfusion with 0·25 μg/ml. ouabain usually took place within 30 min, but was incomplete; in addition, the heart never regained its original rate. The effect of ouabain is illustrated in Fig. 7.

Experiments on the isolated auricles

The isolated auricles retained their responsiveness to vagal stimulation longer than the isolated hearts. Two preparations remained beating for 42 and 48 hr, and at the end of these periods vagal stimulation was still effective (see Fig. 8*a*). When the auricular contractions became infrequent, vagal stimulation increased the rate and amplitude (Fig. 8*b*, *c*). When the auricles stopped beating it was possible to excite them by stimulating the vagi (Fig. 8*d*). The auricles were restarted by the addition of acetylcholine to the bath in a concentration of 40 μg/ml., after which the vagi again exerted an inhibitory effect (Fig. 8*e*).

DISCUSSION

Whereas it has been commonly believed that the vagal fibres remain active on the isolated heart for short periods only, it has now been found possible to maintain a preparation for 6–9 hr by using the method described. When the auricles have been dissected from the rest of the cardiac tissue, but leaving the vagi intact, preparations have lasted for 2 days.

The effect of vagal stimulation on the heart was often twofold; the heart was inhibited during the application of the stimulus, and subsequently accelerated. When acceleration did not occur it was brought out by perfusing the heart with cocaine. The phase of acceleration was not affected by perfusing with hexamethonium, which abolished the phase of inhibition. The occurrence of acceleration as part of the effect of vagal stimulation has been observed previously by several workers. Dale, Laidlaw & Symons (1910) found that

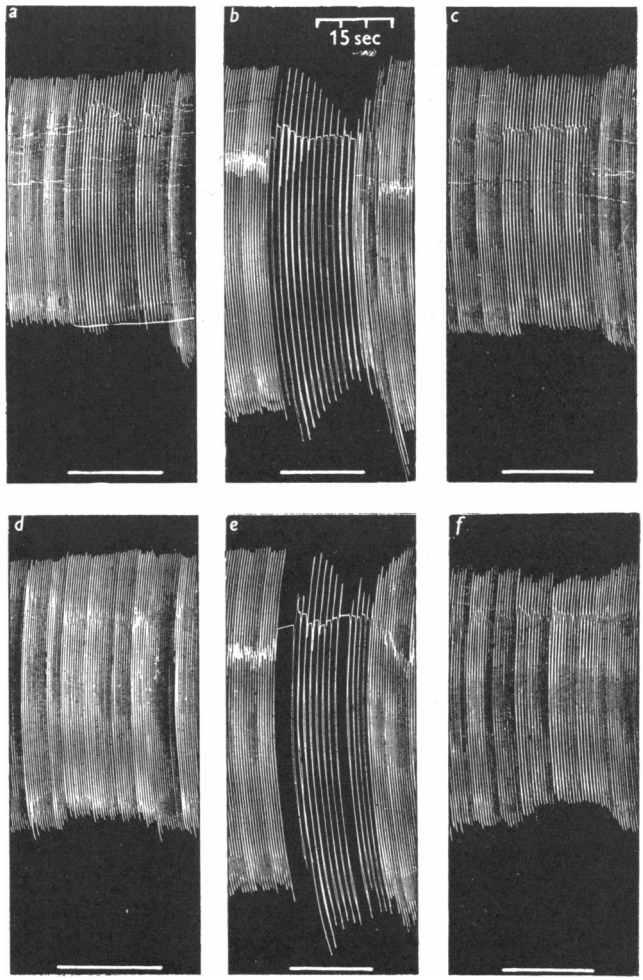

Fig. 7. Rabbit heart. Langendorff perfusion. Both vagi were stimulated at (*a*), (*b*) and (*c*) with maximal stimuli 7 times/sec and at (*d*), (*e*) and (*f*) with submaximal stimuli 23 times/sec for 15 sec, as indicated by the white line; (*a*) and (*d*), controls; (*b*) and (*e*), 35 min after perfusion with ouabain (0·25 μg/ml.); (*c*) and (*f*), 20 min after perfusion with ouabain was discontinued.

when cats, anaesthetized with paraldehyde, were injected with 1–2 mg nicotine, stimulation of the vagus no longer caused inhibition, but when repeated caused acceleration. Since this acceleration might have been due to the stimulation of sympathetic fibres running for part of their course with the

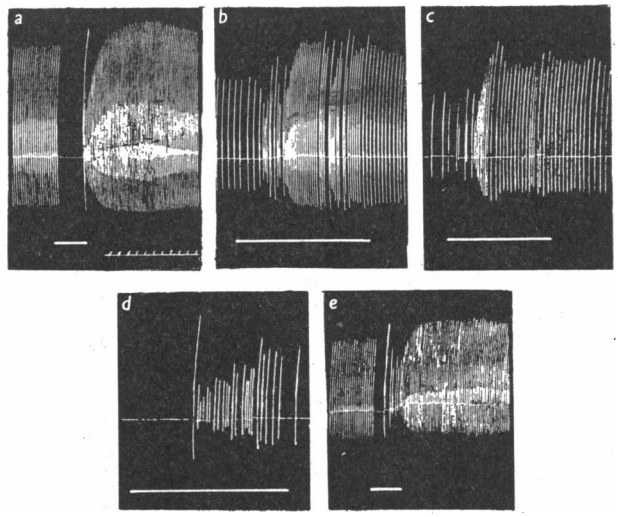

Fig. 8. Isolated rabbit's auricles. Record started when auricles had been continuously beating for 41 hours at 29° C. Stimulation of both vagi with maximal stimuli at 20/sec as indicated by white lines. Time marker 5 sec. (*a*) Stimulation for 10 sec; (*b*) stimulation for 1½ min, 40 min later (auricular beat had been very slow for the last 20 min); (*c*) stimulation for 1 min, 10 min later; (*d*) stimulation for 1½ min, 4 min later after auricles had stopped beating for 2 min; (*e*) stimulation for 10 sec, 5 min later, after auricular beat had been restarted by acetylcholine 40 μg/ml. in bath.

vagus, they prepared cats in which they removed the superior and inferior cervical and the stellate ganglia allowing periods up to 31 days for degeneration. The degeneration of the nerves arising in these ganglia did not modify the phenomenon.

In 1936 Jourdan & Nowak described the occurrence of cardiac acceleration when the vagus was stimulated in the dog after giving it atropine: their results led them to conclude that the accelerator fibres were not sympathetic in origin but came from the cells of the vagus nucleus; they considered that the presence of these accelerator fibres was a further example of the artificiality of the anatomical division of the autonomic system into sympathetic and parasympathetic trunks. Kabat (1939) obtained cardiac acceleration in the atropinized dog by stimulation of the intracranial roots of the vagus. Middleton, Middleton & Toha (1949) observed an acceleration at the end of vagal stimulation of the perfused cat heart (without atropine) and demonstrated the

presence of an adrenaline-like substance in the perfusate. The observations which are described in the present paper agree with the findings of these authors, though they provide no evidence of the origin of the accelerator fibres. The observations make clear, however, that if these are regarded as aberrant sympathetic fibres, such aberrant fibres are usually if not always present.

The observations with hexamethonium throw light on the conclusions of Perry & Talesnik (1953) that the inhibitory action of ACh in the isolated heart is exerted on the ganglion cells in the heart, and not on the heart itself. They observed that doses (e.g. 6 mg) of hexamethonium which blocked the effect of vagal stimulation also blocked the action of ACh. In the present experiments hexamethonium was perfused in a concentration (2×10^{-4} g/ml.) just sufficient to abolish the effect of vagal stimulation; this concentration did not appreciably diminish the effect of ACh in five out of ten experiments and did not abolish it in any. Perry & Reinert (1954) found that hexamethonium when perfused through the heart of the cat in a concentration of 3×10^{-4} g/ml., and when perfused through the heart of the guinea-pig in a concentration of 5×10^{-4} g/ml. abolished the inhibitory effect of ACh. This may have been due to an atropine-like action of such high concentrations. Armitage & Kopera (unpublished) have observed in this laboratory that doses of 20–40 mg hexamethonium diminished the secretion of saliva produced in the cat by intravenous infusion of carbachol to the same extent as 1 μg atropine.

Numerous authors have suggested that the cardiac glycosides, such as ouabain, potentiate the effect of vagus stimulation on the heart. Gremels (1935) produced evidence of this in the cat under chloralose, and similar observations have been made by Abdon, Hammarskjöld & Nielsen (1938) Abdon & Nielsen (1938) and Perry & Reinert (1954). The sites of action might be two. The potentiation might occur at the ganglia around which the preganglionic vagal fibres end: and an action here would conform with the observations of Konzett & Rothlin (1952) and of Perry & Reinert (1954) that cardiac glycosides potentiated the action of preganglionic stimulation and of ACh on the superior cervical ganglion. Alternatively, the potentiation might occur at the postganglionic terminations in the heart muscle. An attempt has been made in the present experiments to distinguish between these two sites by using both maximal and submaximal stimuli. Potentiation of maximal stimulation was presumed to occur in the heart muscle only, and not at the ganglia. Submaximal stimulation was obtained by destroying a proportion of the preganglionic fibres and stimulating the remainder maximally. The potentiation of this stimulation might be expected to occur at the ganglia. If the terminations of each preganglionic vagal fibre made contact with several ganglion cells, each cell could receive nerve endings from several fibres. When only a few preganglionic fibres were excited, the rest having been destroyed, some of the ganglion cells would then receive subthreshold stimuli. Therefore

a substance which lowered the threshold would cause an increased response to submaximal stimulation, while the response of the preparation to maximal stimulation would remain unaffected. Evidence for this conception has been given by Eccles (1935) in the superior cervical ganglion, and by Whitteridge (1937) in part of the cat's ciliary ganglion.

In fact, potentiation of both kinds of stimulation was observed when ouabain was perfused, but the potentiation of submaximal stimulation occurred more rapidly and was greater than that of maximal stimulation. The results therefore gave strong support to the view that potentiation of vagal effects is one of the more important properties of the cardiac glycosides, and that the site of this potentiation might be both the ganglia themselves and also the postganglionic terminations.

SUMMARY

1. An isolated rabbit vagus nerve-heart preparation has been described which worked effectively for periods from 6–9 hr. An isolated rabbit vagus nerve-auricle preparation has also been described which worked for 2 days.

2. Stimulation of the vagi produced two effects. As long as stimulation was continued, the heart rate was slowed. When stimulation ended, a phase of increased heart rate and amplitude followed. This effect was greater in some preparations than in others.

3. Cocaine, when present in concentrations which did not affect the inhibitory action of the vagi, increased the phase of acceleration which followed.

4. In the presence of hexamethonium, stimulation of the vagi caused only acceleration of the heart. When hexamethonium was perfused through the heart in a concentration sufficient to abolish the inhibitory action of the vagi, the effect of small amounts of acetylcholine was never abolished and was appreciably reduced in only five of the ten experiments.

5. The effect on vagal stimulation of perfusing the heart with ouabain was determined. Both submaximal and maximal stimulation was applied. The effect of both forms of stimulation was potentiated, but the rate of onset and the magnitude of the potentiation of submaximal stimulation was greater than that of maximal stimulation.

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