J. Physiol. (1972), **226**, *pp.* 491–510
With 11 *text-figures*
Printed in Great Britain

AN EXPERIMENTAL ANALYSIS OF THE TACHYCARDIA THAT FOLLOWS VAGAL STIMULATION

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(*Received 17 April 1972*)

SUMMARY

1. Postvagal tachycardia, the transient increase in heart rate that follows the sinus bradycardia elicited by vagal stimulation, was investigated in thirty chloralosed cats. Maximum postvagal tachycardia was elicited by stimulation at frequencies of 20–60 Hz with train durations of 30–90 sec. A positive correlation was demonstrated between the magnitudes of postvagal tachycardia and of the preceding sinus bradycardia.

2. Postvagal tachycardia was not affected by either spinal transection at C7 or by administration of propranolol (1·5 mg/kg I.V.), but was abolished by the administration of atropine (2·0 mg/kg I.V.).

3. Postvagal tachycardia was observed to follow the vagal bradycardia induced reflexly either by administration of phenyldiguanide (100–300 μg I.V.) or by stimulation of the aortic depressor nerve.

4. In the isolated atria-vagus preparations from six rabbits a positive correlation was demonstrated between the magnitude of postvagal tachycardia and of the preceding bradycardia elicited by vagal stimulation.

5. Continuous intracellular recordings were obtained from four sinu-atrial node pace-maker cells in the isolated atria-vagus preparation of the rabbit before, during and after vagal stimulation. During postvagal tachycardia the slope of the diastolic prepotential, the maximum diastolic potential, threshold potential and the overshoot were found to be increased; these changes are different from those found in pace-maker cells during adrenergic activation.

6. These findings demonstrate that postvagal tachycardia is not mediated by sympathetic adrenergic mechanisms, but suggest that it is dependent upon the preceding vagal bradycardia and may be related to an increase in net sodium influx into pace-maker cells initiated by the hyperpolarization of the pace-maker cell membrane during and immediately after vagal stimulation.

INTRODUCTION

Many physiological systems are known to respond to the application and to the removal of a stimulus by transiently 'overshooting' the steady-state level that is eventually achieved (Burton, 1939). A physiological overshoot that has attracted many investigators since it was first described (Hunt & Harrington, 1897) is postvagal tachycardia, the brief increase in heart rate that follows the bradycardia elicited by vagal stimulation (Copen, Cirillo & Vassalle, 1968).

In the course of experiments aimed at providing a mathematical model of the vagus-heart rate system (Chess & Calaresu, 1971) it became obvious that, although the amount of experimental work on postvagal tachycardia is very large, there is no well demonstrated physiological mechanism that accounts for this phenomenon. Postvagal tachycardia has been attributed to several factors: simultaneous excitation of sympathetic fibres the effect of which outlasts that of the vagus (Warner & Russell, 1969); depolarization of sympathetic nerve endings by the acetylcholine produced by vagal stimulation (Leaders, 1963); liberation of catecholamines from intracardiac adrenergic neurones or chromaffine cells innervated by vagal fibres (Kottegoda, 1953; Jacobowitz, 1967; Copen *et al.* 1968; Vassalle, Mandell & Holder, 1970); paradoxical excitation of pace-maker cells by acetylcholine (Misu & Kirpekar, 1968; Iano & Levy, 1971); 'some inherent myogenic property of cardiac muscle' (Raper & Wale, 1969).

In view of this diversity of experimental results suggesting different mechanisms for postvagal tachycardia and of the lack of well controlled quantitative studies that permit an accurate empirical description of this phenomenon a systematic analysis of the possible mechanisms of postvagal tachycardia was attempted. This paper describes the chronotropic responses to vagal stimulation in the cat in which a sinus rhythm was maintained in all experiments to ensure that the heart beat always originated in the physiological pace-maker. In addition, the influence of changes in the parameters of stimulation was accurately assessed. Furthermore, the effect of several experimental manipulations on the magnitude of the vagal chronotropic responses was studied. Finally, in an attempt to obtain information on the changes in the electrical properties of pace-maker cells during postvagal tachycardia, continuous intracellular recordings were obtained from sinu-atrial node pace-maker cells in the isolated atria-vagus preparation of the rabbit, the preparation of choice in the study of pace-maker potentials, before, during and after vagal stimulation.

METHODS

Whole animal preparation

Results were obtained from thirty adult cats anaesthetized with α-chloralose (60 mg/kg I.V.) after ethyl chloride and ether induction. Arterial pressure was monitored by a Bionix F-300 transducer connected to a catheter in the right femoral artery. Respiration was monitored as a change in temperature of tidal air in the tracheal cannula. The right femoral vein was cannulated for the injection of drugs. The electrocardiogram (lead II) was continuously monitored with subcutaneous needle electrodes. Rectal temperature was maintained at 37° C ± 0·2 by a heating pad connected to a Yellow springs Model 73 temperature controller.

Stimulation of the vagus nerve. Both carotid sheaths were exposed in the neck, and vagi and cervical sympathetic nerves were dissected free for approximately 5 cm. To prevent heart rate changes occurring as a result of stimulus spread to the cervical sympathetic nerves, the exposed portions of these nerves were removed. Both vagi were cut and were kept in pools of 360 Medical Fluid (Dow Corning Corp., Midland, U.S.A.). The vagi were stimulated by means of bipolar stainless-steel electrodes connected to a Grass S44 Stimulator through a stimulus isolation unit. After preparation of the animals a period of at least 1 hr was allowed before starting the experiments.

Stimulus parameters. As it has been shown that the magnitude of the negative chronotropic effect of stimulation of the right vagus is greater than that of the left vagus (Brown & Eccles, 1934) only the right cervical vagus was stimulated. The effects of changing either stimulus frequency or train length on heart rate responses were studied while the other parameters were held constant. Frequencies of 5, 10, 20, 40, 50, 60, 80 and 100 Hz were applied with pulse durations of 0·1 msec and a train length of 30 sec. Train lengths of 5, 10, 20, 30, 60 and 90 sec were applied while frequency and pulse duration were held at 40 Hz and 0·1 msec. A pulse duration of 0·1 msec was used to avoid the possibility of stimulating sympathetic cardio-acceleratory fibres which have been described to travel in the vagus nerve and to be excited by stimuli of more than 0·1 msec in duration (Misu & Kirpekar, 1968). The amplitude of the stimulating pulses was kept constant at a level (1–8 V) that elicited a maximum bradycardia without loss of sinus rhythm (approximately 50% of control heart rate); this ensured that observed changes in heart rate were due to effects on the physiological pace-maker of the heart, the sinu-atrial node. Parameters were changed in random order. A series of experimental runs was retained only when, at the end of the series, repetition of the first run produced a response that was similar in amplitude and time course to the initial response, indicating that the nerve-electrode contact impedance had not changed.

Spinal cord transection. To investigate the possibility that the postvagal tachycardia was due to reflex sympathetic discharge elicited by the arterial hypotension which accompanies vagal bradycardia, the magnitude of the tachycardia was compared in animals before and after the sympathetic input to the heart was isolated from supraspinal influences by transection of the spinal cord at C7.

Reflex bradycardia. To ensure that changes in heart rate were due solely to activity in vagal fibres to the heart, in some animals reflex vagal bradycardia was elicited in spinal cats transected at C7, by I.V. administration of 100–300 μg phenyldiguanide (Abbott Laboratories, North Chicago, Ill.), an amide derivative that causes marked hypotension, bradycardia and a transient inhibition of respiration in the intact but not in the vagotomized animals (Dawes & Mott, 1950). In addition, in some of these animals the central end of the cut aortic depressor nerve was stimu-

lated for 10–30 sec with low intensity (4–8 V) high frequency (80–100 Hz) pulses to obtain bradycardia and hypotension (Douglas & Schaumann, 1956).

Autonomic blockade. To determine whether postvagal tachycardia was mediated by adrenergic or cholinergic mechanisms the effects of blocking either or both systems were investigated. The cardiac β-adrenergic receptors were blocked by the administration of DL-propranolol (Inderal, Ayerst Laboratories, Montreal), in two equal doses of 0·75 mg/kg I.V. given 20 min apart. Propranolol, in doses of 0·75 mg/kg infused over a 30 min period, has been shown to abolish completely the increase in heart rate produced by stimulation of the right stellate ganglion in chloralosed cats (Black, Duncan & Shanks, 1965). Muscarinic receptors were blocked by the administration of atropine (atropine sulfate, Merck Inc., Montreal) in a dose of 2 mg/kg I.V. (de Vleeschhouwer & Heymans, 1967). At this dose it is unlikely that atropine had any blocking effect on nicotinic receptors as it has been shown that this drug can block receptors on autonomic ganglia only in doses greater than 4 mg/kg (Fink & Cervoni, 1953).

Data acquisition and analysis. Heart period was measured by a method previously described (Chess & Calaresu, 1969) and displayed, after digital-to-analogue conversion (Hewlett-Packard 580A A/D converter), with arterial pressure and respiration on a Grass 7 Polygraph. Heart period was also recorded on a Philips Analog 7 magnetic tape recorder and later converted into digital form for further computation on a PDP 12A computer (Digital Equipment, Maynard, Mass.). As analysis of the time course of the negative chronotropic response to stimulation of the vagus nerve revealed that the steady-state level of heart rate was preceded by an overshoot, the magnitude of bradycardia was computed from the mean of twenty heart periods just before stimulation ended. The estimation of the magnitude of the response during this period was more representative than a value obtained at peak bradycardia for stimulus trains greater than 30 sec. Prestimulus (control) heart rate was estimated from the mean of twenty heart periods just before the onset of stimulation. The magnitude of postvagal tachycardia was calculated from the mean of ten heart periods taken before and after the shortest post-stimulus heart period. Negative and positive chronotropic responses were usually expressed in beats per minute below (bradycardia) and above (postvagal tachycardia) the control rate; however, the responses to changes in stimulus parameters were expressed as percentages of the maximum response which occurred over the range of parameters used. Analysis of variance and Student's t test were used to determine the level of significance.

Isolated heart–vagus preparation

To investigate the mechanism of postvagal tachycardia at the cellular level, experiments were attempted on the isolated atria-vagus preparation of the rabbit. To our knowledge, recordings of cardiac pace-maker potentials have not been recorded in the cat; the rabbit was chosen as it had previously been used to record pace-maker activity (Paes de Carvalho, de Mello & Hoffman, 1959; Toda & West, 1965; Toda, 1968) and postvagal tachycardia had been demonstrated in the rabbit (McEwen, 1956; Toda, Fujiwara & Shimamoto, 1964).

Thirty-three adult New Zealand white rabbits were anaesthetized with urethane (1·6 g/kg I.V.), and after thoracotomy a block of tissue which included the vagi, heart and lungs was removed to a primary bath containing physiological solution at 30° C oxygenated with 95 % O_2–5 % CO_2. The atria and the vagus nerves were separated from other tissues and placed in the recording organ bath where they were perfused at a rate of 2–3 ml./min. The organ bath and a gravity reservoir feeding into it were oxygenated with 95 % O_2–5 % CO_2 and the bath was maintained at $30 \pm 0{\cdot}5°$ C by thermostatic control as it has been shown that at this temperature

stimulation of the vagus nerve to the isolated rabbit heart produces maximum bradycardia (Toda *et al.* 1964). The composition of the physiological solution was (g/l.): NaCl 7·6, KCl 0·42, $CaCl_2$ 0·24, NaH_2PO_4 0·143, $NaHCO_3$ 2·1, dextrose 2·0, sucrose 4·5 (McEwen, 1956). The internal surface of the right atrium was exposed by a method previously described (Paes de Carvalho *et al.* 1959) and the preparation was pinned to a paraffin block in the organ bath. The vagus nerves were kept in the physiological solution and lifted above the surface for stimulation. The electrical stimulus to the right vagus was a 10 sec train of 20 Hz, 1·0 msec pulses (Toda *et al.* 1964) delivered by means of bipolar stainless-steel electrodes connected to a Grass S44 Stimulator through a stimulus isolation unit. Transmembrane action potentials were continuously recorded from true pace-maker cells in the sinu-atrial node before, during and after vagal stimulation, by means of flexibly mounted ultramicro-electrodes (Woodbury & Brady, 1956). Three criteria were used to confirm that the recording electrode was located in a true pace-maker cell. First, the electrode was positioned by visual microscopic control in the tissue of the superior vena cava just rostral to the orifice of the inferior vena cava (Paes de Carvalho *et al.* 1959). Secondly, true pace-maker cells always depolarized in advance of atrial cells (approximately 50 msec) as indicated by the simultaneously recorded surface atriogram. Thirdly, these cells had a low maximum diastolic potential (approximately -60 mV) and exhibited a spontaneous diastolic depolarization which proceeded to threshold (Hoffman & Cranefield, 1960).

Data acquisition and analysis. Action potentials were recorded continuously on a Philips Analog 7 magnetic tape recorder and later played back through a Tektronix 564 oscilloscope from which they could be photographed. Measurement of membrane parameters was taken before stimulation, when the cardiac slowing was maximum and at the height of postvagal tachycardia. The parameters measured were cycle length, maximum diastolic potential, threshold potential, overshoot and slope of diastolic prepotential (Toda, 1968).

RESULTS

Whole animal preparation

Time course of the vagal chronotropic responses. Stimulation of the cut right cervical vagus nerve with 40 Hz, 0·1 msec pulses for 60 sec elicited a decrease in heart rate which reached a maximum 10–15 sec after the onset of stimulation (overshoot) and gradually declined to a steady-state level at approximately 35 sec. After cessation of stimulation the heart rate quickly returned to and then exceeded the prestimulus level. Maximum postvagal tachycardia and return of heart rate to control values occurred approximately 15 and 90 sec after the end of stimulation. Arterial hypotension was also elicited by vagal stimulation; it reached a maximum at approximately 45 sec and then decreased to a steady-state level at approximately 50 sec after onset of stimulation. At the end of stimulation the arterial pressure returned to and, like heart rate, exceeded the prestimulus level; this hypertension reached a maximum at approximately 25 sec, and within 90 sec returned to control level. The characteristic time course of changes in heart rate and in arterial pressure before, during and after vagal stimulation in a typical experiment is shown in Fig. 1.

Parameters of vagal stimulation. Experiments designed to investigate the effect of varying either frequency or train length while the other stimulus parameters were maintained constant, demonstrated that for each of the parameters studied there were values at which the chronotropic responses were maximal. When the frequency of stimulation was varied the results shown in Fig. 2 were obtained. Within the range of frequencies studied analysis of variance revealed significant differences in the magnitudes of both the negative chronotropic responses ($F = 5{\cdot}49$, $P < 0{\cdot}001$) and the positive chronotropic responses ($F = 4{\cdot}69$, $P < 0{\cdot}001$). By inspection of

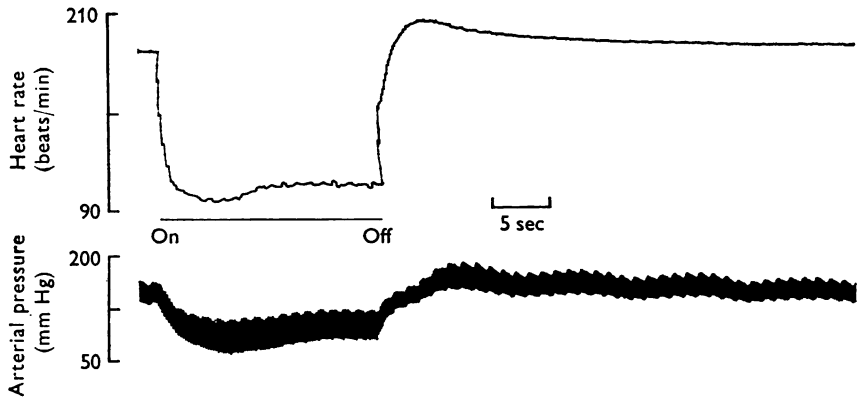

Fig. 1. Time course of the characteristic changes in heart rate and in arterial pressure during and after stimulation of the right cervical vagus with 40 Hz, 0·1 msec pulses for 60 sec. Note the postvagal tachycardia after discontinuation of the stimulus.

Fig. 2 it is apparent that maximum vagal bradycardia occurred between stimulus frequencies of 10 and 50 Hz and maximum postvagal tachycardia occurred between 20 and 60 Hz. At frequencies below or above these ranges the magnitude of the responses was decreased.

When the length of the train of stimulation was varied the results shown in Fig. 3 were obtained. The magnitude of the bradycardia varied significantly over the range of train lengths studied ($F = 6{\cdot}08$, $P < 0{\cdot}01$) and was maximum for stimulus trains of 10, 20 and 30 sec. The magnitude of postvagal tachycardia also varied over the range of train lengths studied ($F = 11{\cdot}79$, $P < 0{\cdot}001$). It increased considerably as the train length was increased from 5 to 30 sec and remained at the same level for trains of 30, 60 and 90 sec ($P > 0{\cdot}10$ for differences between 30, 60 and 90 sec).

As the experiments just described showed that maximum chronotropic responses could be obtained using a frequency of stimulation of 40 Hz and a train length of 30 sec, all subsequent experiments were done using these parameters of stimulation.

Relation between the magnitudes of postvagal tachycardia and of the preceding bradycardia. In the investigation of the effect of changes in the parameters of stimulation on the magnitude of the chronotropic responses a correlation between the magnitude of the postvagal tachycardia and that

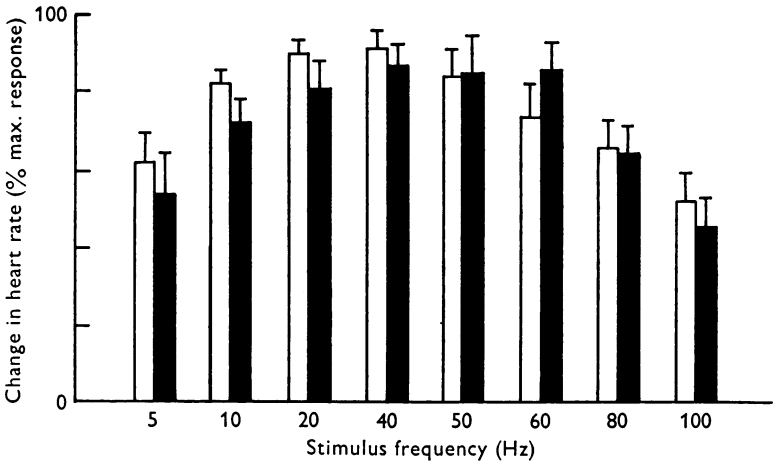

Fig. 2. The effect of changing the frequency of stimulation of the right cervical vagus on the magnitudes of bradycardia (open blocks) and of postvagal tachycardia (filled blocks) in eight animals. Stimuli were 0·1 msec pulses of constant amplitude for 30 sec. Changes in heart rate are expressed as % of the maximum response. Vertical bars indicate S.E. of mean.

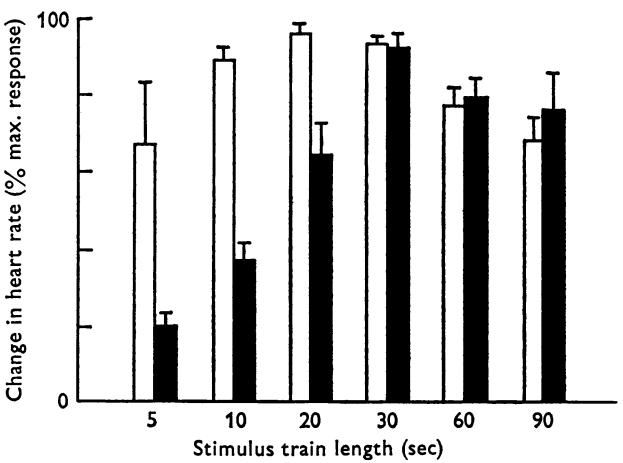

Fig. 3. The effect of changing the length of the train of stimulation on the magnitudes of bradycardia (open blocks) and of postvagal tachycardia (filled blocks) in seven animals. Stimuli were 40 Hz, 0·1 msec pulses of constant amplitude. Changes in heart rate are expressed as % of the maximum response. Vertical bars indicate S.E. of mean.

of the preceding bradycardia became apparent. The possibility of this correlation was therefore systematically investigated in ten animals. In each animal the magnitudes of the chronotropic responses to ten runs of vagal stimulation (40 Hz, 0·1 msec pulses of different amplitude for 30 sec) were obtained and correlation analysis was done. It was shown that for each animal there was a significant positive correlation between the magnitudes of postvagal tachycardia and of the preceding bradycardia (r values between 0·73 and 0·98, $P < 0·001$). Characteristic experimental results from one animal are presented in Fig. 4. Because of the existence of this relation between bradycardia and tachycardia it was considered essential, in

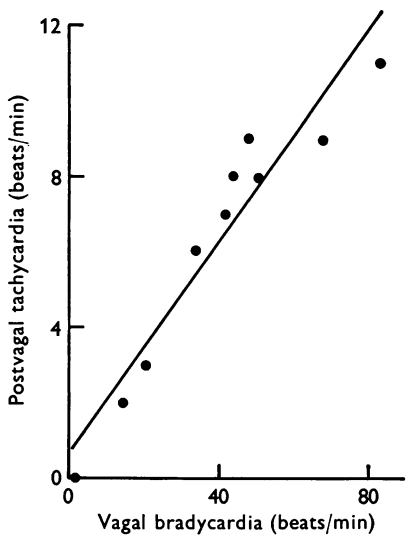

Fig. 4. Relation between the magnitudes of postvagal tachycardia and of the preceding bradycardia. Stimuli were 40 Hz, 0·1 msec pulses of different amplitudes for 30 sec. The line of best fit is fitted to ten experimental points from a representative animal ($r = 0·96$, $P < 0·001$).

making comparisons between magnitudes of postvagal tachycardia before and after the experimental procedures to be described later, to obtain similar magnitudes of bradycardia before and after the experimental variable under investigation.

Chronotropic responses to vagal stimulation in spinal animals. It has been suggested that postvagal tachycardia may be due to increased sympathetic discharge secondary to the arterial hypotension observed during vagal stimulation and that the effect of sympathetic activity to the heart, masked by the bradycardia during vagal stimulation, is manifest only on discontinuing the stimulus to the vagus (Copen *et al.* 1968). To test this

hypothesis, a series of experiments was done in nine animals in which the supraspinal input to preganglionic sympathetic neurones was eliminated by transection of the spinal cord at the level of the seventh cervical vertebra.

Control values of heart rate during and after vagal stimulation (40 Hz, 0·1 msec for 30 sec) were established. The vagus was then stimulated after spinal section using the same parameters of stimulation, at an amplitude such that the bradycardia obtained was of the same magnitude as that before spinal section. Under these experimental conditions the magnitude of the postvagal tachycardia was not significantly altered by spinal section ($P > 0{\cdot}10$). These results are shown in Fig. 5.

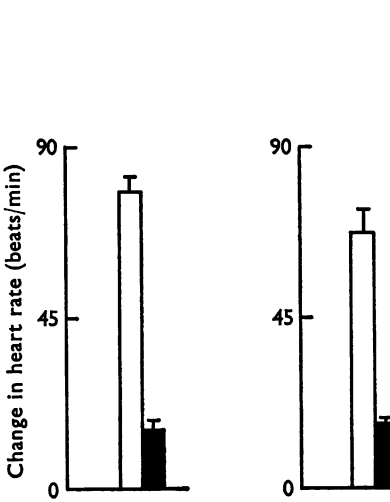

Fig. 5

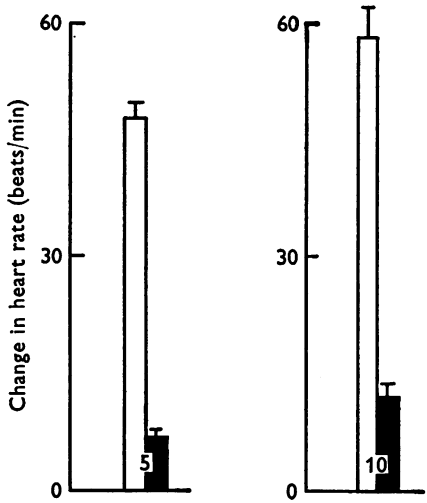

Fig. 6

Fig. 5. The effect of transection of the spinal cord at C7 on the magnitudes of vagal bradycardia (open blocks) and of postvagal tachycardia (filled blocks) in nine animals. On the left control changes in heart rate, on the right changes after spinal section. Stimuli were 40 Hz, 0·1 msec pulses for 30 sec. Vertical bars indicate S.E. of mean.

Fig. 6. Magnitudes of reflex vagal bradycardia (open blocks) and of postvagal tachycardia (filled blocks) in spinal animals transected at C7. The numbers of animals are shown in the insets. On the left changes in heart rate elicited by stimulation of the aortic depressor nerve, on the right changes elicited by I.V. administration of phenyldiguanide. Vertical bars indicate S.E. of mean.

Reflex vagal stimulation in spinal animals. It has been suggested that postvagal tachycardia may be due to excitation, during electrical stimulation of the vagus nerve, of aberrant sympathetic fibres travelling in the vagus (Misu & Kirpekar, 1968). As removal of the cervical sympathetic

nerve would not have assured that all sympathetic cardioacceleratory fibres travelling in the vagus had been removed, a series of experiments was done in spinal cats in which vagal excitation was induced reflexly either by administration of phenyldiguanide (ten cats) or by stimulation of the central segment of the cut aortic depressor nerve (five cats). Administration of phenyldiguanide (100–300 μg I.V.) elicited a bradycardia of approximately 10 sec duration which was followed by a short lasting tachycardia that disappeared after approximately 30 sec. Similar results were obtained by stimulation of the aortic depressor nerve: a marked bradycardia persisted for the duration of the stimulation and was followed by a tachycardia which lasted 15–20 sec. These results are shown in Fig. 6.

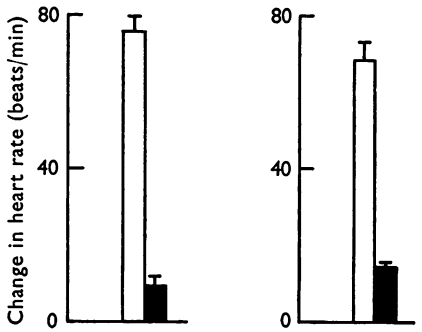

Fig. 7. The effect of administration of propranolol (1·5 mg/kg I.V.) on the magnitude of postvagal tachycardia in eleven animals. On the left control changes in heart rate, on the right changes after propranolol. Stimuli were 40 Hz, 0·1 msec pulses for 30 sec. Vertical bars indicate S.E. of mean.

The effect of beta-adrenergic blockade on the chronotropic responses to vagal stimulation. In view of the suggestion that postvagal tachycardia is mediated by an adrenergic neural mechanism (Copen *et al.* 1968), the effect of administration of the β-adrenolytic agent propranolol on the chronotropic responses to vagal stimulation was investigated in eleven cats. Control values of heart rate during and after vagal stimulation (40 Hz, 0·1 msec, for 30 sec) were obtained in these animals before administration of propranolol. After administration of this drug the stimulation was repeated using the same parameters of stimulation and a pulse amplitude that produced a bradycardia of the same magnitude as that obtained in the control stimulation. Under these experimental conditions it was shown that the magnitude of the postvagal tachycardia was not altered by the drug ($P > 0{\cdot}70$). The results of these experiments are shown in Fig. 7.

The effect of atropine administration on the chronotropic responses to vagal stimulation. As the results with propranolol strongly suggested that the

postvagal tachycardia was not mediated by an adrenergic mechanism and in view of the demonstration that either vagal stimulation or acetylcholine can elicit cardio-acceleration in the atropinized heart (Dale, Laidlaw & Symons, 1910; Hoffmann, Hoffman, Middleton & Talesnik, 1945), a series of experiments was done to investigate the influence of atropine administration on the response to vagal stimulation. In ten animals it was shown that when stimulus parameters used to obtain the control responses (bradycardia $90{\cdot}8 \pm 4{\cdot}4$, tachycardia $14{\cdot}9 \pm 1{\cdot}6$) were repeated after the administration of atropine, both bradycardia and postvagal tachycardia were abolished by the drug. However, in five of the animals the intensity of the stimulus to the vagus was greatly increased (from 0·1 msec to 0·5 msec, from 0·6–8·0 to 10–30 V), and it was possible to elicit a tachycardia of small magnitude ($6{\cdot}2 \pm 0{\cdot}6$ beats/min), which was abolished by propranolol. The tachycardia began 5–8 sec after the onset of stimulation and was maintained for 10–15 sec after the end of the stimulus. A small increase in arterial pressure accompanied the tachycardia. Records of a typical experiment in this series are shown in Fig. 8.

Isolated atria-vagus preparation of the rabbit

Our attempts at maintaining the isolated atria-vagus preparation in a viable state were rarely successful, an experience reported by previous workers (S. M. Middleton, 1947; Öbrink & Essex, 1953; Toda & Fujiwara, 1963; West, T. C. 1971, personal communication). In thirty-three experiments it was possible to obtain only six preparations in which stimulation of the vagus nerve elicited a decrease in heart rate.

Chronotropic responses to stimulation of the vagus nerve. Stimulation of the right vagus nerve with 20 Hz, 1·0 msec pulses of different amplitudes for 10 sec produced a decrease in heart rate of $45 \pm 4{\cdot}4$ beats/min (mean ± S.E. of mean) which was followed approximately 25 sec after the end of the stimulus by a postvagal tachycardia of $5{\cdot}0 \pm 0{\cdot}9$ beats/min. Heart rate returned to the prestimulus level approximately 55 sec after cessation of stimulation. These results and the record of a typical experiment are shown in Fig. 9.

In addition, as it had been found that the magnitudes of postvagal tachycardia and of the preceding bradycardia in experiments on whole animals were positively correlated (cf. Fig. 4), the possibility of a similar correlation in the isolated atria-vagus preparation was investigated in nineteen experimental runs from the six preparations. To reduce any influence of prestimulus heart rate on the magnitude of the chronotropic responses, vagal bradycardia and postvagal tachycardia were converted to per cent changes from the control rate. Statistical analysis showed that there was a significant positive correlation between the magnitude of

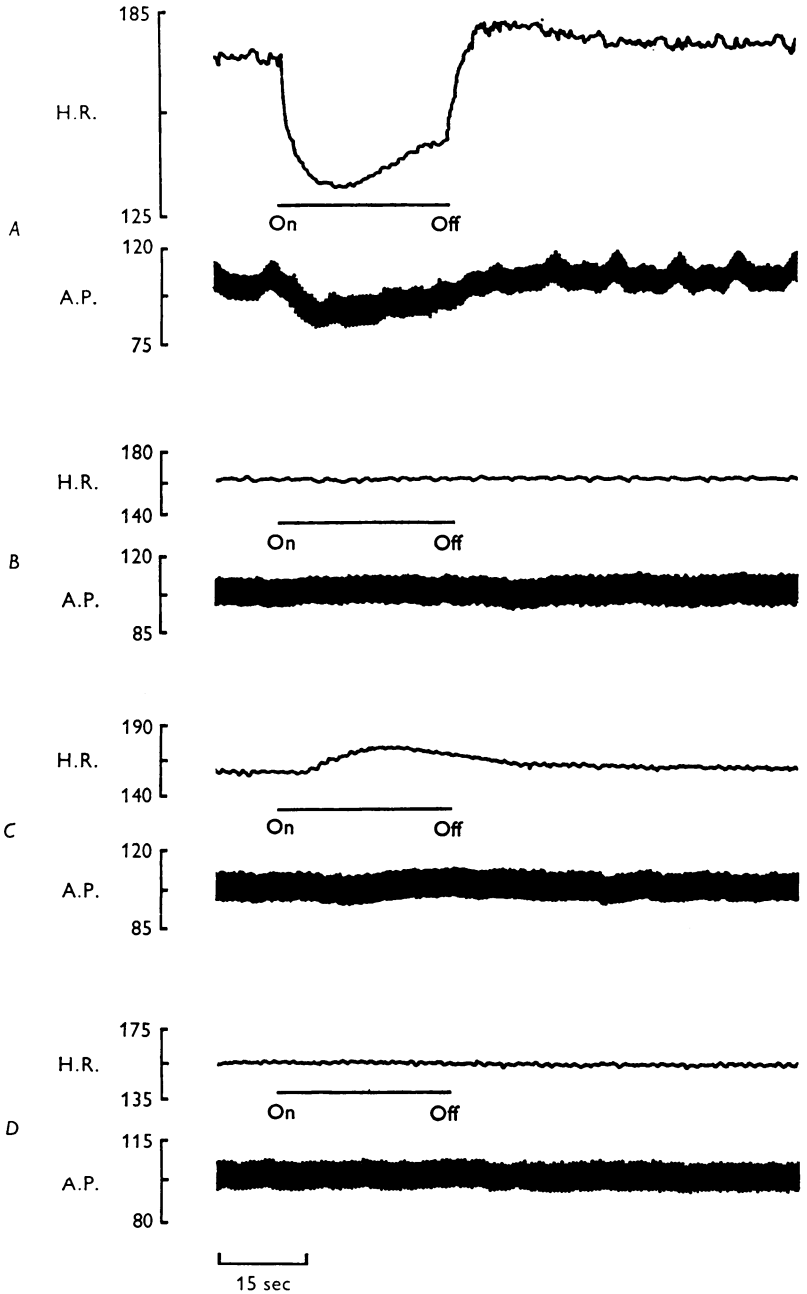

Fig. 8. For legend see facing page.

postvagal tachycardia and the magnitude of the preceding bradycardia ($r = 0{\cdot}72$, $P < 0{\cdot}001$). These results are shown in Fig. 10.

Electrical activity of pace-maker cells. Transmembrane action potentials of true pace-maker cells were recorded before, during and after stimulation of the vagus nerve in the isolated rabbit atria-vagus preparation. It was reasoned that if postvagal tachycardia was due to an adrenergic mechanism the changes in the pace-maker action potentials during this period would be the same as those seen during sympathetic tachycardia. Data were obtained from four preparations in which the recording micro-electrode remained in the same pace-maker cell for the duration of the run. Changes

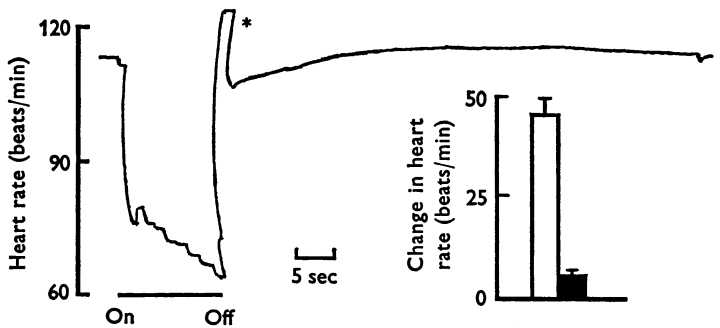

Fig. 9. Bradycardia and postvagal tachycardia elicited by stimulation of the right vagus nerve to the isolated atria of the rabbit. Time course of the changes in heart rate during and after stimulation of the vagus with 20 Hz, 1·0 msec, 6 V pulses for 10 sec. An extrasystole after the end of the stimulus caused the writing pen to go off scale (*). Note the postvagal tachycardia. In the inset magnitudes of bradycardia (open blocks) and of postvagal tachycardia (filled blocks) in six animals. Stimulus parameters same as above. Vertical bars indicate S.E. of mean.

in the characteristics of action potentials of true pace-maker cells during the bradycardia were similar to those observed previously (Toda & West, 1965). However, in these cells the following changes with respect to pre-

Fig. 8. The effect of administration of atropine and of propranolol on the bradycardia and postvagal tachycardia elicited by electrical stimulation of the right cervical vagus. In each panel: top tracing, heart rate (H.R., beats/min); bottom tracing, arterial pressure (A.P., mm Hg). *A*, control response to vagal stimulation (40 Hz, 0·1 msec, 4 V pulses for 30 sec). *B*, response to vagal stimulation (same parameters as A) after administration of atropine (2 mg/kg I.V.) *C*, response to strong vagal stimulation (40 Hz, 0·5 msec, 25 V pulses for 30 sec) after administration of atropine: note the tachycardia. *D*, response to strong vagal stimulation (same parameters as *D*) after administration of atropine and propranolol (1·5 mg/kg I.V.): note the disappearance of the tachycardia shown in *C*.

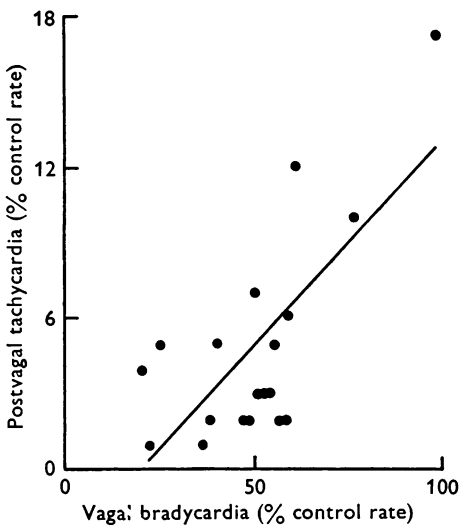

Fig. 10

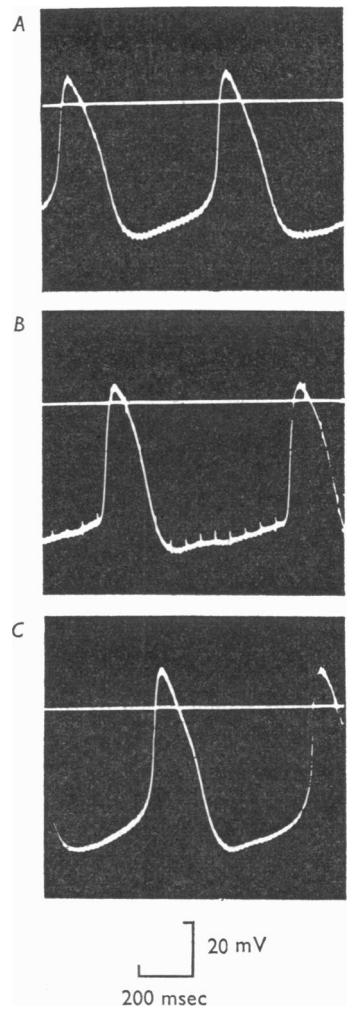

Fig. 11

Fig. 10. Relation between the magnitudes of postvagal tachycardia and of the preceding bradycardia in the isolated atria-vagus preparation rabbit in six animals. Stimuli were 20 Hz, 1·0 msec pulses of different amplitudes for 10 sec. The line of best fit is fitted to nineteen experimental points ($r = 0{\cdot}72$, $P < 0{\cdot}001$).

Fig. 11. Action potentials of true pace-maker cells of the isolated rabbit heart. *A*, before vagal stimulation. *B*, during vagal stimulation that elicited bradycardia (20 Hz, 1 msec pulses for 10 sec). *C*, after vagal stimulation during postvagal tachycardia. In all figures the horizontal line is zero potential; the shock artifact can be seen in *B*. Note hyperpolarization and increased overshoot in *C*.

stimulation values, were observed during postvagal tachycardia: cycle length was shorter, maximum diastolic potential was greater (hyperpolarized), threshold potential was greater, overshoot was increased, slope of the diastolic prepotential was greater. In contrast to these observations are the previous findings that stimulation of cardiac sympathetic nerves or administration of noradrenaline produce only an increase in the slope of the diastolic prepotential (Toda & Shimamoto, 1968). Characteristic pace-maker potentials and values of mean changes of the membrane parameters during and after vagal stimulation are shown in Fig. 11 and Table 1.

TABLE 1. Changes in magnitude of membrane parameters of pace-maker cells in the rabbit during and after vagal stimulation (20 Hz, 1 msec, for 10 sec). Values are mean changes from prestimulus levels ($\pm$ S.E. of mean), $n = 4$

	Changes in magnitude of parameter	
	During stimulation	After stimulation
Cycle length (msec)	$+133{\cdot}0 \pm 28{\cdot}5$	$-18{\cdot}0 \pm 5{\cdot}0$
Maximum diastolic potential (mV)	$+7{\cdot}0 \pm 0{\cdot}6$	$+5{\cdot}7 \pm 0{\cdot}3$
Threshold potential (mV)	$+7{\cdot}0 \pm 0{\cdot}6$	$+3{\cdot}3 \pm 0{\cdot}9$
Overshoot (mV)	$-2{\cdot}3 \pm 0{\cdot}9$	$+1{\cdot}3 \pm 0{\cdot}3$
Slope of diastolic prepotential (mV/sec)	$-17{\cdot}0 \pm 4{\cdot}5$	$+14{\cdot}7 \pm 2{\cdot}6$

$P < 0{\cdot}05$ for all changes with respect to control values.

DISCUSSION

It was apparent from a review of the literature on postvagal tachycardia that, although much attention had been paid to the presence or absence of this phenomenon after a number of experimental manipulations, little attention had been paid to either the parameters of vagal stimulation or to the magnitude of the vagal chronotropic responses before and after these manipulations. Because of this lack of controlled conditions of stimulation it had not been possible to draw unequivocal interpretations of the experimental results of these previous studies. In this study, in contrast to previous experiments, great care was taken to determine quantitatively the effect of changing parameters of stimulation on the magnitude of the chronotropic responses to vagal stimulation. After these preliminary experiments it was therefore decided to use a frequency of 40 Hz and a train length of 30 sec as maximum postvagal tachycardia was elicited by using these stimulus parameters. In addition, a stimulus pulse duration of 0·1 msec was used to avoid the possibility of stimulation of sympathetic post-ganglionic fibres travelling in the vagus as it has been shown that these fibres are excited only by stimulus pulses longer than 0·1 msec (Misu & Kirpekar, 1968). Furthermore, the amplitude of the stimulus pulse was

adjusted so that maximum bradycardia was elicited without loss of sinus rhythm (approximately 50% of control heart rate); by maintaining a sinus rhythm, a precaution that was not attended to by previous investigators, measured changes in heart rate during or after vagal stimulation could only be due to effects on the physiological pace-maker of the heart, the sinu-atrial node. Finally, the findings of a correlation between the magnitudes of postvagal tachycardia and of the preceding bradycardia (cf. Fig. 4) prompted us to use similar magnitudes of bradycardia before and after the experimental procedures employed to investigate possible mechanisms of postvagal tachycardia. This ensured that if any changes in the magnitude of postvagal tachycardia were observed, they were due exclusively to the experimental variable under investigation.

The demonstration that postvagal tachycardia was not altered by spinal cord transection establishes that this positive chronotropic response is not due to a reflex activation of the sympathetic input to the heart by the arterial hypotension elicited by vagal stimulation. These observations are in agreement with the report that postvagal tachycardia was still present in dogs in which arterial pressure had not been allowed to fall during vagal stimulation by electrically driving the ventricles (Copen *et al.* 1968). In addition, the observation of the existence of postvagal tachycardia in the isolated atria-vagus preparation of the rabbit (cf. Fig. 9) conclusively establishes that postvagal tachycardia is not due to a reflex mechanism mediated by the central nervous system. It is remarkable, however, that the magnitude of postvagal tachycardia was not reduced by spinal transection, as it has been demonstrated that a decrease in arterial pressure can elicit a reflex increase in heart rate (Glick & Braunwald, 1965) which is associated with an increase in the electrical activity of the inferior cardiac nerve (Green & Heffron, 1968). The basis for the absence of this response in the present study was not investigated; however, it may be suggested that the sympathetic response was inhibited by the presence of acetylcholine, as it has been recently demonstrated that stimulation of cholinergic muscarinic receptors on sympathetic fibres inhibits the release of noradrenaline (Löffelholz & Muscholl, 1969).

The finding of the existence of postvagal tachycardia in animals in which bradycardia was elicited reflexly by administration of phenyldiguanide or by stimulation of the aortic depressor nerve (cf. Fig. 6) unequivocally eliminates the possibility that postvagal tachycardia is due to simultaneous activation of sympathetic fibres during direct electrical stimulation of the vagus nerve as has been suggested previously (Warner & Russell, 1969).

The persistence of postvagal tachycardia after the administration of propranolol, a drug which has been shown to eliminate the tachycardia

elicited by sympathetic stimulation (Black *et al.* 1965), excludes the possibility that this phenomenon is mediated by the liberation of noradrenaline by any of the mechanisms suggested by previous investigators: activation of sympathetic fibres in the vagus (Warner & Russell, 1969); excitation of intracardiac adrenergic neurones or chromaffine cells innervated by vagal fibres (Kottegoda, 1953; Jacobowitz, 1967; Copen *et al.* 1968; Vassalle *et al.* 1970); depolarization of sympathetic nerve endings by the acetylcholine liberated during vagal stimulation (Leaders, 1963). This interpretation is given substance by the demonstration that the electrical changes in pace-maker cells of the rabbit atrium during postvagal tachycardia (cf. Fig. 11, Table 1) are different from those observed during the tachycardia elicited by adrenergic activation (Toda & Shimamoto, 1968). Although our demonstration of the persistence of postvagal tachycardia in the presence of propranolol is in agreement with previous reports (Misu & Kirpekar, 1968; Raper & Wale, 1969; Iano & Levy, 1971), the possibility exists that a cardioacceleratory transmitter other than noradrenaline may be released during vagal stimulation from sympathetic neurones or chromaffine cells. This is unlikely, however, as all known sympathetic transmitters (noradrenaline, adrenaline, dopamine) exert their positive chronotropic effect by activation of cardiac β-adrenergic receptors (McDonald & Goldberg, 1963; Shanks, 1966).

The demonstration that both vagal bradycardia and postvagal tachycardia were blocked by the I.V. administration of atropine clearly indicates that postvagal tachycardia cannot be elicited independently of vagal tachycardia. This finding is at variance with those of previous investigators (Dale *et al.* 1910; Jordan & Nowak, 1936; Middleton, Middleton & Toha, 1949). However, the results of these authors were confirmed by the demonstration that a tachycardia could be elicited in atropinized animals, by increasing the intensity of the stimulus to the vagus nerve; this response was abolished by propranolol (cf. Fig. 8) and may be interpreted as having been caused either by the release of large amounts of acetylcholine which diffused away from cholinergic synapses and depolarized nearby sympathetic nerve endings or chromaffine cells, or by the release of catecholamines from sympathetic fibres travelling in the vagus which had been excited by the increased stimulus amplitude and pulse duration. This phenomenon, which may account for increases in heart rate seen by other investigators because of lack of control of stimulus parameters, should be considered an artifact of vagal stimulation and not a physiological mechanism of postvagal tachycardia or evidence for a 'cholinergic link' (Burn, 1971) in the release of noradrenaline.

The observation of postvagal tachycardia in the isolated heart preparation of the rabbit excludes the possibility that this cardioacceleration

is due, in the intact animal, to the mechanical stretch of sinu-atrial node pace-maker cells by an increase in right atrial pressure during and after vagal stimulation (McDowall, 1956; Pathak, 1959).

The changes in membrane parameters during postvagal tachycardia in the isolated rabbit heart, to our knowledge never reported before, are different from those previously reported during sympathetic tachycardia in this species (Toda & Shimamoto, 1968) and clearly demonstrate that postvagal tachycardia is not due to an adrenergic mechanism. Furthermore, it is suggested, on the basis of the increased magnitudes of the maximum diastolic potential and of the overshoot of these action potentials (cf. Fig. 11*c*; Table 1), that postvagal tachycardia may be related to an increase in the net influx of sodium ions. This suggestion appears reasonable as it has been established that an increase in the magnitude of the maximum diastolic potential of mammalian pace-maker cells increases the activity of a carrier system for sodium ions, which is manifested as an increase in the amplitude of the overshoot of the action potential (Weidmann, 1955), and that an increase in the activity of this system may lead to an increase in heart rate (Trautwein, 1963).

In summary, it has been demonstrated both in intact cats and in isolated atria-vagus preparations of the rabbit that postvagal tachycardia can be observed only if vagal bradycardia is present, and that there is a correlation between the magnitudes of these two chronotropic responses. In addition, it has been shown that postvagal tachycardia is not due to an adrenergic mechanism, as indicated by its persistence after spinal transection and after administration of propranolol, and by its presence after reflexly induced vagal bradycardia. Furthermore, the intracellular recordings of action potentials of pace-maker cells in the rabbit suggest that postvagal tachycardia is due to an intrinsic property of pace-maker cells which may be related to an increase in the activity of a sodium carrier system initiated by the hyperpolarization of the pace-maker cell membrane during and immediately after vagal stimulation. Finally, although it has not been conclusively established that postvagal tachycardia is an intrinsic response of sinu-atrial node pace-maker cells in the cat as the intracellular data were obtained from rabbits, the similarity of the correlation between the magnitudes of postvagal tachycardia and of the preceding bradycardia in the intact cat and in the isolated rabbit's heart suggests that the same cellular mechanism is responsible for postvagal tachycardia in both preparations.

This work was supported by the Ontario Heart Foundation. Mrs G. Chang, Mrs M. Kaibara and Mrs C. J. Kuo provided technical assistance in certain parts of this work. Mr K. LaBelle provided help in the computer analysis of data.

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